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Proceeding of

Multidisciplinary International Conference on GREEN EARTH: A PANORAMIC VIEW

12th and 13th January 2018

Organized by

DEPARTMENT OF BOTANY
VPM's B. N. Bandodkar College of Science, Thane

In collaboration with

Birbal Sahni Institute of Palaeosciences, Lucknow
Bombay Natural History Society (BNHS), Mumbai

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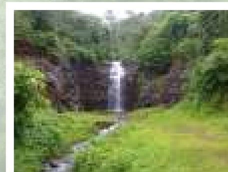
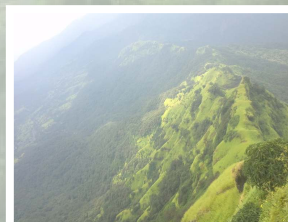
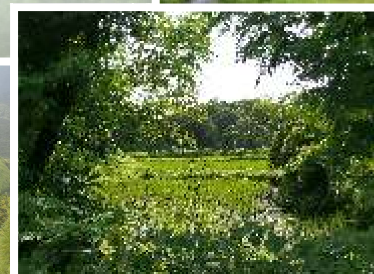

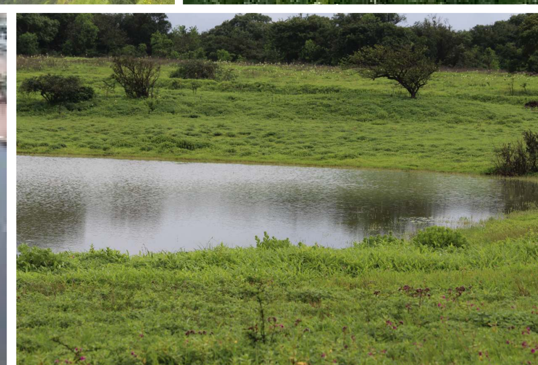

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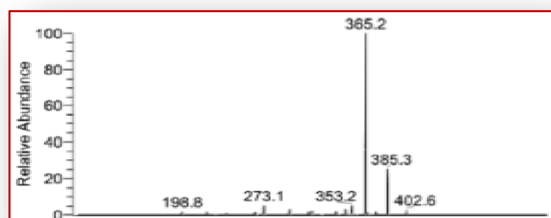

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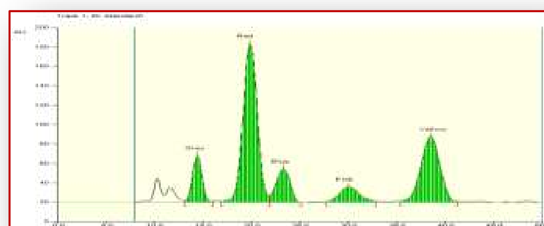

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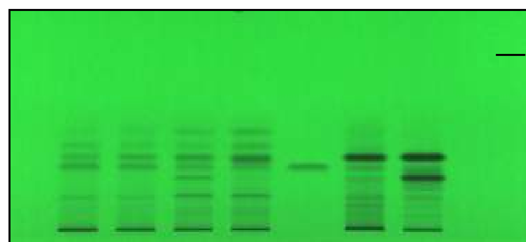

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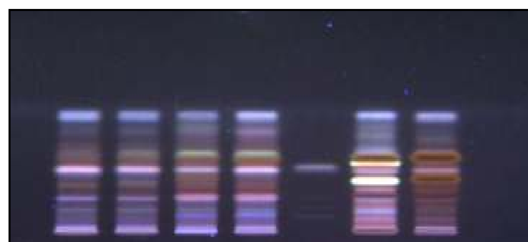

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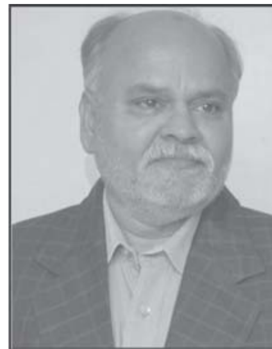


Chairman's Message

I have an immense pleasure in presenting this volume of proceeding on Multidisciplinary International Conference on Green Earth: A Panoramic View under the auspices of VPM's B. N. Bandodkar College of Science, Thane on 12th and 13th January 2018.

Vidya Prasarak Mandal has organized conferences at National as well as International levels with themes associated to current world scenario. And this year too an interesting conference concerning our Mother Earth is arranged. The themes of the conference revolve around role of biological sciences in helping mankind towards fulfillment of every need. Revolving around the central theme of green planet, the conference will focus on past and work on present trends to build better eco friendly technologies in the future. The world today is busy churning out ideas to conserve and preserve the natural resources on Mother Earth. And this conference aims at strengthening those ideas with this small step. It will help delegates and participant to rethink development in earth friendly ways.

The best thing about the conference is being held at an international platform which has attracted delegates from around the world. Varied themes have helped us in receiving good quality research papers from diverse fields and sources. The work done by various researchers will also motivate inquisitive minds in making the intricate decisions needed to ensure a healthy planet for generations to come. The Conference will open up many avenues and opportunities through the contribution of eminent scientist and delegates participating from around the world.

I wish this conference all the success.

Dr. Vijay Bedekar
Chairman
Vidya Prasarak Mandal, Thane

Convener's Message

हिरवे हिरवे गार गालिचे
हरित तृणांच्या मखमालीचे ।

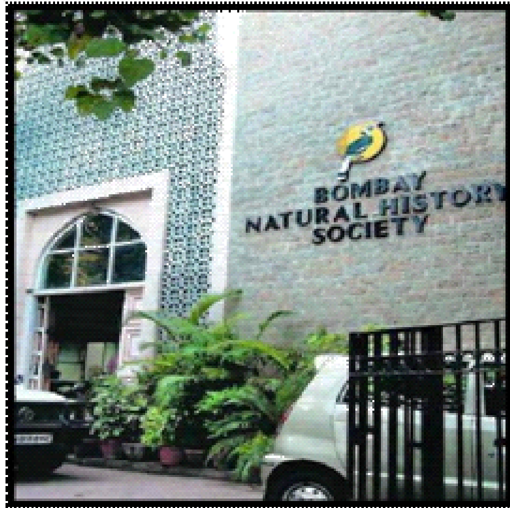
From childhood I have heard this poem and fallen in love with the " Green Earth" described in the poem.

The earth was really green then, with thick forests spread all over. Now where have this greenery vanished? Due to interference of Homo sapiens in the ecosystem, the total balance is lost. Due to mass cutting forests, we have lost our green earth, but in addition due to heavy pollution, hunting, acquiring forest land, modern life style, we are interfering the balance of our own ecosystem.

To express our thoughts, our research, in the field VPM's B. N. Bandodkar College of Science felt to give opportunity to scientists and that is how the conference got the title "Multidisciplinary International Conference on Green Earth: A Panoramic View." This conference will touch upon topics like Agricultural Science, Bio Sciences, Bio Physics, Enviro-management, Green Chemistry and humanities.

We are very happy that understanding the importance of the topic we got overwhelming response from you all and received many full length research papers. We are happy to give the compilation of these research papers in your hands in the form of proceeding.

Dr. Madhuri K. Pejaver
Convener



Bombay Natural History Society is one of the oldest and largest non-government organization (NGO) of India, actively involved in wildlife research, conservation and nature education since 1883. It is founded by eight enthusiast naturalist (six Englishmen and two Indians) who came together for the study of Natural History by forming a society. Initially the work of BNHS started with monthly meetings, exchange of notes, collection and exhibition of specimens. Later it widened the perspective in all fields of nature. In 125 years of existence, BNHS is committed for the cause of conservation of India's natural wealth, protection of environment and sustainable use of natural resources for the balanced and healthy development of future generations. It supports many research efforts in Indian wildlife and conservation through grants and publishes the four monthly journal, Journal of the Bombay Natural History Society (JBNHS) and a quarterly magazine, Hornbill. Many prominent naturalists including ornithologist Salim Ali and S. Dillon Ripley have been associated with it. The BNHS logo is the great hornbill, inspired by a great hornbill named William, who lived on the premises of the Society from 1894 until 1920.

Dr. Deepak Apte (Director, BNHS) has been working with BNHS since 1993. He did M.Sc. in Zoology and Ph.D. in Marine Ecology from University of Mumbai. Prior to becoming the Director, he was the Chief Operating Officer and has previously worked as a Principal Scientist. Dr Apte is active researcher in various programmes related to conservation action and advocacy. He established a full-fledged Marine Conservation Programme in BNHS, apart from various conservation initiatives in terrestrial habitats across India. Dr Apte will now be responsible for managing the overall working of BNHS, including its future course of action, based on the in-house Strategic Initiatives. He aims to strengthen and expand the research and conservation action of BNHS by inviting efforts from scientists, conservationists and young researchers.

Dr. Deepak Apte
Director of Bombay Natural History Society

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तिथि **Dated: 05th December, 2017**

I am glad to know that **'*VPM's B. N. Bandodkar College of Science, Thane*'** is organizing the ***"Multidisciplinary International Conference on Green Earth: A Panoramic View"*** in collaboration with '*Birbal Sahani Institute of Palaeosciences, Lucknow*' and '*Bombay Natural History Society (BNHS), Mumbai*' in their renowned institute on 12th and 13th January, 2018 .

The theme selected for this International Conference is of utmost importance in today's context to share insights on unraveling the past, deciphering the present and predicting the future of green earth and to reinforce regional and global accountability towards planet earth. I am sure that eminent speakers and researchers will definitely deliberate number of issues related to conservation of nature, environmental sustainability and on conference broad theme. This conference would be useful to Scientists, academicians, researchers and students participating from all over the world.

I extend my best wishes to organizers and participants and wish the conference all success.

(Dr. Gaurav Sharma)
वैज्ञानिक-घ/Scientist-D

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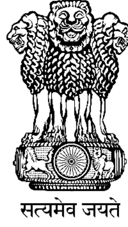

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Dr. S V C Kameswara Rao, Ph.D
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Message

I am happy to learn that Department of Botany, B. N. Bandodkar College of Science of Vidya Prasarak Mandal (VPM),Thane is organizing a "Multidisciplinary International Conference on Green Earth: A Panoramic View" in collaboration with Birbal Sahani Institute of Palaeosciences, Lucknow and Bombay Natural History Society (BNHS), Mumbai and that the proceedings is being published on this occasion.

The theme chosen for the Conference is of topical interest. We only have one planet and we should all do our part to ensure that it's protected by combining our modern ways of living with a deeper respect and understanding of what nature provides. Environmental conservationists understand that the way we live is a reflection of how we feel about the natural world, and our everyday habits show how much we truly value all the things that the Earth gives us. There is much to do when it comes to rebuilding and protecting what's left of natural resources and the biodiversity within our ecosystems. Environmental conservation is an umbrella term that defines anything we do to protect our planet and conserve its natural resources so that every living thing can have an improved quality of life. Talking to other people about environmental conservation will make more people recognize the environmental issues we face. The government and many institutions play a big part in how we make use of the Earth's resources, but they aren't the only ones who determine the impact we have on the planet. With so many of us on this Earth, we can make all the difference in the world. In this context, this Conference is timely.

I congratulate the Organisers for providing a platform for this interaction through this Conference. I have no doubt that the suggestions made by the speakers for making the mother earth greener will be well taken seriously and implemented by the concerned authorities.

I wish the Conference a great success.

Date: 05-12-2017

(S.V.C Kameswara Rao)

Indian Space Research Organisation

Organizing Secretary's Message

On behalf of the organizing committee, I would like to welcome all the delegates to the Multidisciplinary International Conference on Green Earth: A Panoramic View organized by Department of Botany, VPM's B.N. Bandodkar College of Science, Thane Maharashtra India, on 12th and 13th January, 2018.

A tiny speck in the extraordinarily enormous universe, Earth has myriad facets however it has the unique condition that supports life. Whether it is flora or fauna, the fact is that it is an abode for all of us. However, a sea change has taken place in the last few decades as resource extraction continues to shake the geography and economic future of the land. Thus it is essential to share insights on unraveling the past, deciphering the present and predicting the future of green earth.

Special thanks to Birbal Sahani Institute of Palaeosciences, Lucknow and Bombay Natural History Society (BNHS) Mumbai for collaborating with us. I would also like thank our sponsors for their contribution. I extend warm welcome to our distinguished guests and delegates contributing in Multidisciplinary International Conference on Green Earth: A Panoramic View.

In the conference, we will scrutinize how to reinforce regional and global accountability towards planet earth, to mitigate the pressure between economic and environmental sustainability and to correlate humanities with scientific values to strengthen and uphold harmony.

For any kind of stability, sustainability and worthwhile conservation efforts we should be well versed with our green earth. This conference is a genuine reflection of scientific, academic, and social contribution. It will be a focused approach and collective effort where multiple institutions, laboratories, researchers, academicians and students will share their work on a common platform. This conference will not only allow us to meet and greet people from different walks of life but also provide a unique forum for exchange of ideas and opportunities.

Every language has its own name for our planet. Our planet is called "Earth" in English, "Prithivi" in Hindi and Marathi, "terra" in Italian and Portuguese, "terre" in French, "dünya" in Turkish. Let us all join hands to infuse new enthusiasm so that our "Earth" remains our home to all of us.

Dr. Moitreyee Saha
Organizing Secretary

From the Editorial Desk

Green Earth not only takes care of our essential needs but also reminds us the existence of life. Flora and fauna stand as an evidence of balanced nature, yet resource extraction continues to shake the geography and economic future of the land. Sustainable utilization and use of natural resources need knowledge about their diversity. Properly placing down all such knowledge shared by authors into one manuscript will indisputably aid in the betterment of humanity.

The editorial team celebrates the event of bringing out this conversant proceeding on the occasion of this prestigious Multidisciplinary International Conference on "Green Earth: A Panoramic View" organized under the aegis of VPM's B. N. Bandodkar College of Science, Thane. The overwhelming response to our call-for- papers indicates the popularity of this conference and confirms that our management has become the world-wide forum for all aspects of science, technology and humanities. We have received extended full-length research papers from researchers covering all the aspects concerned with Green Earth themes. We have received papers from India as well as Abroad. The extended research papers have gone through a proper reviewing process with the help of experts.

With a sense of pride and satisfaction, we would like to express that with all the efforts and contributions put in by the editorial team, the proceeding has come alive. We congratulate the speakers and authors for making this proceeding resourceful and informative. We specially express appreciation to all the authors of the research papers for giving a generous amount of time and effort.

We hope that this conference would be a successful endeavor with full support and involvement of experts and participants. We are obliged to our Patron and Convener for their guidance and encouragement. Let us aim to support sustainability in all aspects of living through this mega event. We wish all the success for this International Conference.

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- VPM’s London Academy for Education and Research
- VPM’s Academy of International Education and Research
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- VPM’s Institute of Distance Education
- VPM’s Centre for Career and Skilled Development
- VPM’s Council of Senior Scientists

SECTION I
INVITED TALKS

Birbal Sahni Institute of Palaeosciences, Lucknow: A Window into past Life, Ecosystems and Climates

Sunil Bajpai
Birbal Sahni Institute of Palaeosciences, Lucknow

The Birbal Sahni Institute of Palaeobotany (BSIP), Lucknow was established in 1946 with an aim to decipher the evolutionary history of plant kingdom through the study of fossil plants. Recently, to provide the necessary thrust on multidisciplinarity and an integrated approach within the overall framework of Earth System sciences, this globally known institute was renamed Birbal Sahni Institute of Palaeosciences. The newly renamed BSIP is now well placed to carry out multidisciplinary research in the vast field of palaeosciences including palaeobotany, palaeozoology, palaeoclimatology, palaeobiogeography, palaeogeography, geochemistry, palaeomagnetism, palaeoethnobotany, and numerous other related aspects. Several additional areas/ facilities are now being developed in the institute, including ancient DNA studies, Archaeobiology, Aeropalynology etc. The induction of these analytical facilities has enabled BSIP to delve deeper into multiple dimensions of palaeosciences with a focus on the evolution of biotas, environments, climates and ecosystems through time. Research activities deal with data ranging in age from Archaean to Recent (3200 million years before present to 400 AD) for deciphering the past plant life, evolution, palaeoclimate, palaeoecology and palaeobiogeography, archaeobotany and dendrochronology and allied aspects. Currently, the thrust areas of research at BSIP include 1. Early life, contemporary environments and evidence from Indian Precambrian basins, 2. Phanerozoic terrestrial and coastal ecosystems, 3. Marine micropalaeontology with a focus on high resolution biostratigraphy, sea level changes, palaeo-oceanographic and palaeoclimatic events, 4. Organic petrology including characterization of solid fossil fuels in terms of depositional setting and utilizational aspects, 5. Quaternary palaeoclimate reconstructions, vegetation dynamics and sea level changes, 6. Domestication of plants, early farming and ecosystem dynamics during Holocene and Anthropocene, 7. Geochronological and geochemical studies for high resolution dating, regional stratigraphic correlation, palaeoclimatic, tectonic and provenance studies, 8. India-Asia collision and Himalayan uplift and their signatures in palaeobotanical and associated biota.

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Earth Observations for Assessment of Soil Resources

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Introduction

Soil is one of the most important non-renewable natural resources. It is in fact at the heart of terrestrial ecology and is vital to the very existence of life. However, there is evidence to show that a majority of our soils are undergoing degradation at an unacceptable rate with risk of jeopardizing our food security for the future generations. The soil resources of India though rich & diverse, show deficiency of NPK, secondary nutrients and micro nutrients, which has become a limiting factor in augmenting food productivity. Moreover, in some parts of the country, intensive agriculture has caused second generation problems in respect of nutrient imbalance. That includes greater mining of soil nutrients depleting soil fertility, decline of water table, decreasing organic carbon content, and overall deterioration in soil health.

It is therefore imperative that we manage and conserve the soil resources judiciously to meet the growing need for food, fodder, fiber and fuel. For this purpose, we must have an in-depth knowledge about different soils, their morphology, physical and chemical properties, behaviour, kind and degree of problem and their extent and distribution on the landscape. Soil survey and mapping procedures facilitate generation of relevant information and help us plan strategies for their conservation and management. The conventional soil surveys are tedious, time consuming and expensive. In this respect, the modern technology of space borne remote sensing, now operationally used for studying soil resources, proved to be a powerful tool, because it enables to study resources in spatial domain in time and cost effective manner.

As such, from last several decades remote sensing has become an invaluable tool in soil research because of its ability to nondestructively analyze soil properties and characterize the heterogeneity of the soil in spatial and temporal domain. Survey of literature reveals that satellite data of LANDSAT MSS / TM, SPOT, IRS-LISS-I, II, III and PAN and IKONOS etc. were used to map soils at different scales from 1: 250000 to 1: 12500 scales.

The data obtained from the remote sensors are interpreted as a function of soil properties. Therefore, spectral reflectance of soils plays an important role in extracting information on different types of soils and in subsequent applications in soil mapping, land degradation mapping and monitoring, soil fertility management & watershed management. In recent times, the data interpretation techniques have undergone a tremendous change on par with satellite capabilities in terms of spatial, radiometric, temporal and spectral resolutions. Further, now with the advent and availability of high-resolution satellite data from various Indian satellites like Resourcesat-2, Cartosat-1/2, new vistas have been opened up for micro-level planning. Realising all this, here presented an overview of applications of remote sensing in soil related studies.

Spectral reflectance of soils

Understanding the principles of spectral reflectance of soils is fundamental to any applications of RS in soils (Ravisankar and Sreenivas, 2011). The soil reflectance data can be acquired in the laboratory or in the field using spectro-radiometer instrument. It enables to understand the relationship between the physical and chemical properties of soil and soil reflectance. The most important soil properties that influence the reflectance are soil texture, moisture content, structure and iron oxide content (Hofer, 1978; Stoner and Baumgardner, 1981). These factors are interrelated and, the spectral reflectance of soil is a cumulative property of combination of these factors (Baumgardner *et al.* 1985 and Irons *et al.* 1989). The shape and nature of a soil reflectance curve depends upon the physical and chemical properties of soils. The important physical properties are soil colour, soil texture, structure, soil moisture, surface conditions / roughness etc. The chemical properties of soils affecting reflectance includes soil mineralogy, organic matter, salinity, carbonates etc.

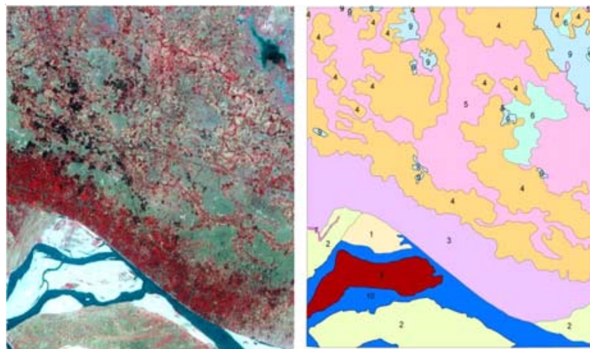
The study of spectral reflectance of soils has ability to provide non-destructive rapid prediction of soil physical, chemical and biological properties under laboratory conditions, for sensing soil organic matter content in the field and for the discrimination of major soil types from satellite data and hyper spectral data. In India, spectral libraries were developed using specto-radiometer with spectral range of 350 nm -2500 nm for about 600 pedons and 2500 surface and subsurface soils covering all major soil orders in major physiographic units in the country (NBSS & LUP, 2005). Such hyper-spectral libraries will be of immense use for characterization of soils.

Applications of remote sensing in soil studies

Remote sensing techniques have an important role to play in soil & land degradation mapping, monitoring of degraded lands, soil moisture assessment, soil fertility, soil-water conservation measures and soil suitability studies.

Soil Mapping

The remote sensing techniques are being routinely used in inventorying and monitoring of soil resource in India and abroad. The soil mapping comprises of identification, description and delineation of different kinds of soils based on physiography, climate and vegetation of the area, confirmation through field work and laboratory data and depicting on a standard base map. In this, the RS techniques have reduced fieldwork to a considerable extent and soil boundaries are more precisely delineated than in conventional methods (Rao *et.al*., 2004). Further, the stereo data obtained from RS has also proved useful in identification of different landforms, which have got close relationship with the soils associated with them. Moreover, soil scientists delineate bodies of soils accurately on the landscape by directly examining less than one-thousandth of the soil below the surface.



They can do this because of the validity of the *soil-landscape model* - a powerful paradigm, which enables soil scientists to make accurate predictions about the soils (Hudson, 1992). This soil-landscape model has now become the guiding paradigm for soil survey all over the world with the use of remote sensing techniques and ground observations.

The soil mapping procedure usually follows the approach of satellite data interpretation, soil-landscape delineation, soil survey & soil sample collection, soil sample analysis for physical & chemical properties, legend preparation, soil correlation, soil classification and finalization of maps (Rao *et.al*., 2004, Wadodkar and Ravisankar, 2011). In India, National Bureau of Soil Survey and Land Use Planning in co-operation with respective State agencies prepared soil map on 1:250,000 scale of entire country in India using satellite imagery and published them at 1:500,000 scale. The soil mapping on the scale of 1:50,000 have also been carried out by various agencies in the country under various projects for district level planning. However, soil information on larger than 1:50,000 scale is available for limited area, in the country.

Land Degradation mapping

Mapping and monitoring of degraded lands with remotely sensed data proved to be comparatively easy because of distinct manifestation of various types of degraded lands on remotely sensed data. The repetitive nature of RS satellites was exploited to monitor the effect of reclamation and conservation measures in these degraded lands. In India, several case studies and operational projects were successfully carried out to derive information on various aspects of degraded lands using the satellite data such as, salt affected soils, eroded soils (wind & water), water logging, ravinous lands, shifting cultivation, impact assessment of soil conservation program in watershed and impact of aquaculture in coastal areas.

Soil Moisture Studies

Soil moisture is an important input parameter in number of land surface and atmospheric processes. Remote sensing techniques (both optical and microwave) by virtue of their large area coverage, frequent revisit capability enabling repeated estimates on regular basis are highly suitable for soil moisture estimation. The microwave remote sensing techniques can prove very promising for soil moisture studies in view of day/night viewing, all weather capability of microwave and its strong sensitivity to soil moisture variations and penetration through crop cover and surface soil layers. Generally, microwave backscattered radiation is influenced by frequency, incidence angle, surface roughness and dielectric constant of targets & their interactions which modify this relationship.

Soil fertility assessment

The main application of RS in soil fertility is primarily in spatial assessment of soil fertility in terms of nutrient deficiency represented by poor crop growth & in preparation of soil fertility maps. The components of soil fertility that can be addressed with RS include organic carbon, NPK and micronutrients (Fe, Mn, Zn, Cu) levels. Amongst plant nutrients, nitrogen is one of the most important factors in maximizing the crop yields and economic returns to farmers. The spatial variation in nitrogen content has been addressed using crop vigor as a proxy indicator, spatial interpolation of soil analytical data using RS data as guiding force for interpolation (Ravisankar and Sreenivas, 2011).

Another main application of RS data is in preparation of soil fertility maps that helps in reducing frequency of

field observations. Historically, intensiive grid sampling has been the preferred method for estimating fertilty levels in soil. Grid samples, however, are extremely labor intensive, costly, and may not give a true spatial representation of the variability of soil fertility. Remote sensing has the potential to overcome these problems, when we follow stratified random sampling approach (Wadodkar *et. al.*, 2011). Here, initially segmentation of study area is done using remote sensing, digital elevation model (DEM), topographical data based on variations in physiography, land use / land cover, soils, parent material, & other physical features. Later, in this stratified mapping units random samples are collected in the field, taken to a laboratory for physical & chemical analysis, and examined using a number of geo-statistical, mathematical interpolation and graphical procedures.

The integration RS with geo-statistical techniques helps in soil fertility assessment. And when high resolution RS data (Resourcesat-1/2 and Cartosat-1/2) is used such studies can be done at group of villages / village level on 1:10,000 scale.

Conclusions

The application of remotely sensed data for study of soil resources is varied and is being operationally used in India for soil and land degradation mapping & monitoring and watershed management. However, its use in soil fertility studies and village level developmental planning and action plan preparation is not wide-spread. High- resolution satellite data is very useful in identifying suitable sites for taking up soil & water conservation measures and in preparation of action plan maps at village level. Looking at its significance, the remote sensing data need to be employed more on regular basis for mapping and monitoring soils and crops along with ground observation. The integration of soil maps with geo-referenced cadastral (village map), natural resource and utility maps in GIS environment will help in preparation of suitable action plans for sustaining soil health & soil management in a more efficient manner.

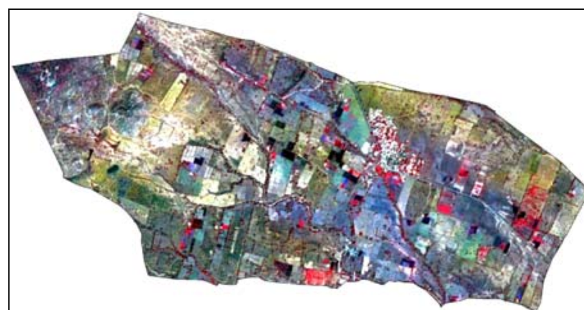


Figure 2. False colour composite of Resourcesat1 LISS-IV + Cartosat Pan merged data of village Gudi Malkapur, district Nalgonda, AP

Genomic analysis of cis regulatory elements and its experimental validation in model plant *Arabidopsis thaliana* reveals spacer distance between ACGT *cis* regulatory elements governs differential gene expression in response to salicylic and abscisic acids

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Control of gene expression at transcriptional level depends on a variety of interactions mediated by the core promoter, sequence specic DNA-binding proteins, and their cognate promoter elements. Multiplicity among promoters is an outcome of the presence of different*cis* regulatory elements, their variation in copy numbers, position in context to TATA box and the spacer length between two adjacent *cis* elements. Copy number and length of the spacer is critical in determining the expression of the gene. We have done *in silico* analysis on various *cis*- elements like ACGT, AAAG and GT. One of the prominent group of *cis* acting elements in plants are ACGT *cis* elements, has been established as a functionally important inregulation of gene expression in synergy with other *cis*-elements . An ACGT core has been shown to be involved in abs*cis*ic acid (ABA), salicylic acid (SA), and light response, anaerobiosis. In our study a genome-wide comparison of the frequency of occurrence of two ACGT elements without any spacers as well as those separated by spacers of dierent length from 0 to 30 base pair was carried out (Mehrotra *et al.* 2012). In the rst step, the frequency of occurrence of the *cis*-element sequences across the whole genome was determined by using BLAST tool. In another approach the spacer sequence was randomized before making the query. As expected, the sequence ACGTACGT had maximum occurrence in *Arabidopsis thaliana* genome. As we increased the spacer length, one nucleotide at a time, the probability of its occurrence in genome decreased. This trend continued until an unexpectedly sharp rise in frequency of (ACGT) N25 (ACGT). The frequency of occurrence of two ACGT motifs separated by a spacer of 25 bp to be 62 while for 5 bp, the occurrence was 72. The observation that larger size motif occurs in higher number suggests its directed evolution in *A. thaliana* genome.

We also did an *in-silico* comparative analysis on occurrence of ACGT across four plant species (two monocotyledonous species – *Oryza sativa* and *Sorghum bicolor*, and two dicotyledonous– *A. thaliana* and *Glycine max)* and analyze conservation among them with respect to spacer distance between them (Mehrotra *et al* 2013). Using data generated from *Arabidopsis thaliana* and *Oryza sativa*, we also identified conserved regions across all spacers and possible conditions regulating gene promoters with multiple ACGT *cis*-elements. Our data indicated specific predominant spacer lengths between co-occurring ACGT elements. Sequence specificity data clearly revealed a preference for G at the first and C at the terminal position of a spacer sequence.

Abiotic and biotic stresses adversely affect growth and productivity of plants. In response to these challenges plants adapt themselves through various mechanisms which are controlled by the molecular, physiological and cellular processes. Various phytohormones like ABA, SA, jasmonic acid (JA) and ethylene tend to play a key role in helping plants to adapt themselves to these adversities. Various molecular approaches can be used to improve growth and productivity of plants, genetic engineering being one of the most important strategies. Use of constitutive promoter is quite common and important practice in plant genetic engineering where stress responsive genes can be over expressed under the control of such constitutive promoters but suffering from several drawbacks like stunted growth, delayed germination, seed dormancy, photobleaching at the same time. The plausible solution to such problems could be the use of inducible promoters. We tried to test the inducibility of PP2C-like promoter from *A. thaliana.* Our genome wide analysis of *A. thaliana* revealed a unique genetic arrangement of ACGT motif in the protein phosphatase 2C (PP2C) like promoter (AT5G59220). It has three ACGT elements in close vicinity. They are so positioned that they are separated by a spacer of 30 base pair and 5 base pair respectively from each other. First we cloned this full length promoter then its deletion variants viz. 900 base pair, 500 base pair, 400 base pair and NRM were constructed to investigate the activity of PP2C like promoter in the presence of phytohormones like ABA,SA and JA(Bhalothia *et al.* 2013). Further, considering the fact that PP2C like promoter has ABA and SA response elements positioned in close proximity, the study could also give an insight in to the relation between abiotic and biotic stress mediated responses. In another study we indicated that the full length and 900 bp promoter-reporter constructs of PP2C-like

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shown that ACGT (N30) ACGT genetic architecture is essential for the promoter to be induced in response to abs*cis*ic acid. The synergistic and antagonistic effects of *cis* elements were observed. AACA is a positive regulatory element in endosperm and is known to act as negative regulatory element in other tissues. In this study AACA, have been found to negatively regulate the expression of reporter gene EGFP in both induced and under uninduced stress conditions (Bhalothia *et al.* 2013).

A promoter consisting of TATA box is sufficient to transcribe a gene at its basal level. This is much evident with ACGT and GT *cis* -elements, where both copy number and spacer length are responsible for high or low expression of gene. GT element acts as negative regulator on increasing its copy number with increase in spacer length. We reported decrease in gene expression with an increasing copy number of adjacent GT motifs (Mehtotra *et al*. 2005). But unlike GT elements, an increase in copy number and spacer length to 5, 10, and 25 in case of ACGT element leads to an enhanced gene expression of the â-glucuronidase (gus A) reporter gene. Our data showed that two ACGT elements separated by 25 base pair (bp) are induced by ABA. However, when separated by 5 nucleotides, they are induced by SA in transgenic tobacco.

Another important class of *cis* element is AAAG, having core sequence of T/AAAAG. Dof proteins are a large family of transcription factors which are known to bind to this core sequence. The core to which Dof proteins bind has a consensus AAAG or ACTTTA sequence. These motifs have been over represented in many promoters. We performed a genome wide analysis of AAAG repeat elements increasing the spacer length from 0 to 25 (Mehrotra *et al.* 2014). Similar analyses were done with CTTT motifs. We report unusual high frequency of AAAGN7CTTT in A. thaliana genome. We also conclude that there is a preference for A/G nucleotides in spacer sequence between two AAAG repeats.

Exploration of natural promoter arrangements and the concept of *cis* engineering have enabled fine tuning of single or multiple transgene expression in response to variations in the chemical, physiological and environmental stimuli and in the form of bidirectional promoters. It also provided a unique answer to various problems in crop improvement. We have been also working on designing of synthetic promoter, can be constructed by arranging different *cis*-regulatory elements individually or in combination (Mehrotra *et al.* 2011). We believe that our research would be helpful in designing various stress inducible promoters in plants where specific interactions could be governed.

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Conservation and Consolidation of Green Earth: A crucialpillar for livelihoods

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The worthy development of social and economic life largely depends on the effective preservation, conservation and consolidation of mother earth. A serious contemporary problem is posed by pollution and environment degradation. The changes which we are experiencing today necessitate new solutions to maintain an ecological balance. The negative impacts of this lack of balance can be detrimental to humanity at large if appropriate actions are not taken on time. It is therefore necessary to come up with ideas and implement projects for the conservation of the environment to reduce the pressure over rare species and other natural resources. Without proper socialization, education, sensitization and exchange of information on the importance of a green planet, it will be difficult to combat the environmental challenges.

The attempt to conserve the planet has been ongoing since many years. Nonetheless, new and more detrimental environment issues are surfing such as global warming and climatic changes. As a matter of fact, many societies take it as an excuse to harm environment in the name of development and modernity or for sustaining the livelihoods of its vulnerable population Nevertheless, we all know that it is most often the greediness of the capitalist modes of production for profit making are behind the devastation of the planet earth.

The time has come for strong resolutions and above all the real commitment of all countries and societies for respecting nature and making judicious use of same. Meetings of leaders at international forums on issues of environment and green earth are important but not sufficient as we find that many societies do not respect the decisions taken to preserve nature and mother earth. Pressure groups should voice out their opinions and academicians are the ones who can help to make a change in the mentality of leaders who rule and who make regional, national and international decisions related to environment and green economy. The proper dissemination of the outcomes of workshops and seminars and international conferences on Green earth should be properly channelled to the concerned authorities to initiate effective policies and programmes to sustain environment with a view to ensuring the livelihoods of humanity through the effective agricultural development.

Motherland O Motherland of mine, Sweet is thy beauty Sweet is thy fragrance around thee we gather as one people as one nation in peace, justice and liberty: beloved mother earth may god bless thee forever and ever (Adapted from the National Anthem of Mauritius).

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The role of Botanic Gardens in plant conservation: A special reference to AJC Bose Indian Botanic Garden, Howrah.

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Abstract: In the present world of vanishing plant species and genera due to anthropogenic and natural causes; the Botanic Gardens act to play a greater role in the protection and maintenance of the germplasm of many promising rare, endemic and threatened taxa and their wild progenitors of both native and exotic species. Currently, the global network of botanic gardens conserves an astounding array of plant diversity, holding nearly 105,600 species and about 13,200 species at risk of extinction. However, at the very beginning almost all the botanic gardens in the world were established solely with a different purpose such as helping the academic study and usage of medicinal plants or to promote learning and to glorify God. The world's first botanic gardens were the gardens of Italy in the 16th and 17th centuries. During the latter part of the 16th and early 17th century the scenario of botanic gardens experienced a change in usage mainly purpose related to the exploration, by world powers, of promising plant taxa. During this period, important gardens such as Royal Botanic Garden, Kew (1759), Real Jardin Botanico de Madrid, Spain (1755), Royal Botanic Garden, Calcutta (1787) and Pamplemousse Botanic Garden in Mauritius in (1735) were set up. At present there are about 1775 gardens and arboreta around the world in 148 countries. In the last 30 years botanic gardens have seen as a revival as scientific institutions due to the emergence of conservation movement. Further, gardens are finding new uses for traditional research tools such as herbarium specimens and historical photographs, which are increasingly being used to obtain information on past plant behavior and climate change that are not easily undertaken anywhere.

History of gardens in India dates back to 1000 - 800 B.C. as the 'Charaka Samhita' describes the cultivation of medicinally important plants in and around Ashrams. A book on the gardens of ancient India by Sarangadhara (1283-1301) elucidates different aspects of garden development. The scenario of the gardens changed in India during the Mughal period where they introduced a lot of fruit yielding and ornamentally important trees from Central Asia and Persia.

The Indian Botanic Garden (IBG), Howrah formerly known as 'Company Bagan', then Royal Botanic Garden, Kolkata, at present AJC Bose Indian Botanic Garden (AJCBIBG), Howrah, is one of the best landscaped gardens in the world. It was established by Col. Robert Kyd in 1787 as East India Company's measure to wade off the re-occurrence of famine by introducing many economic and useful crop plants from abroad as the Bengal was reeling through the aftermath of 'Great Bengal Famine' and subsequent failure of crops. The garden with an area of 273 acres and being a living repository of 1377 species of plants possesses 25 divisions and 24 interconnected lakes, and the lakes are connected to the Ganges through sluices for the regular inlet and outlet of water. The garden is a unique place of learning & rich array of curiosity and occupies matchless attractions like the 'Great Banyan Tree', a living wonder in the plant kingdom; the Large Palm House containing rich collection of palms including *Lodoicea maldivica* (the Double Coconut palm) etc. Economic plants like Tea, Rubber, Mahogany, Cinchona, Sugarcane, and Jute etc were made their first trial in this garden and later on cultivated to different parts of the country. Similarly, Cardamom, Pepper, Nutmeg, Cotton, Tobacco, Indigo, Coffee, Sago, Teak, etc were also introduced here for the first time. Many eminent botanist were served as superintendent of this garden like William Roxburgh (Father of Indian Botany), Nathaniel Wallich, William Griffith, Thomas Anderson, George King, David Prain, A.T.Gage etc. from time to time. Three decades ago (from 1987 onwards) launched a shift in paradigm in AJCBIBG's conservation pattern, a major deviation from the past concept, well before the Rio Earth Summit in 1992. To commensurate with the directions of Govt. of India as well as Convention on Biological Diversity (CBD), AJC Bose Indian Botanic Garden, Howrah has adopted and implemented a well planned decision of introducing Rare, Endangered and Threatened (RET) indigenous plant species, in the 'Annual Action Plans', and to multiply and conserve them so as to check the loss of species as well as to re-introduce them in the wild. This is mainly due to the loss of many key species from the wild, because of habitat loss a number of species is being pushed annually into the IUCN list of threatened plants. As the result, at present this garden serves as a living repository of plants of a country and also of selected exotic species, and a "safe abode" for the rare, endemic and threatened plants.

Key words: Botanic Garden, Company's Bagan, Conservation, RET species, Exotic, Indigenous.

Introduction

In the present scenario, the Botanic Gardens act as the last resort for the preservation and maintenance of rare and endangered species. Botanic gardens possess exclusive skills for conserving plant variety across the taxonomic range. The global network of botanic gardens conserves an astounding array of plant diversity, holding about 105,600 species, amounting to 30% of species diversity, 59% of plant genera, 75% of land plant families, and 93% of all vascular plant families. It also harbours about 13,200 species at risk of extinction, equating to just over 41% of the world's known threatened flora (Ross *et al*., 2017). However, at the very beginning almost all the botanic gardens in the world were

established solely with a different purpose. The world's first botanic gardens were the gardens of Italy in the 16th and 17th centuries. The botanic garden of the University of Pisa which was created by Luca Ghini in 1543 ranks first. Subsequently other Italian Universities followed the same like Padua (1545), Firenze (1545), Zurich (1560), and Bologna (1547). These gardens were established with a view of helping the academic study of medicinal plants. By the 16th century these gardens lead to the development of gardens in other parts of Europe like Leiden (1577), Paris (1597), Oxford (1621), Uppsala (1655), Edinburgh (1670), Berlin(1679) etc..The University of Oxford botanic garden was the first garden established in the United Kingdom in 1621 with a mission to promote learning and to glorify God (Heywood, 1983).

During the latter part of the 16th and early 17th century the scenario of botanic gardens experienced a change in usage. The botanic gardens that arose in what are today technologically lesser developed countries came initially for purpose related to the exploration, by world powers, of promising plant taxa (Brockway, 1979). This was the age of exploration and beginning of international trade. Important gardens such as Royal Botanic Garden, Kew, Real Jardin Botanico de Madrid, Spain (1755), Royal Botanic Garden, Calcutta (1787) and Pamplemousse Botanic Garden in Mauritius in (1735) were set up. These gardens created almost solely to receive and cultivate commercial crop such as Clove, Tea, Coffee, Bread fruit, Cinchona, Oil palm as well as Coco plants. It was during this time that Para Rubber was introduced to Singapore, Teak and Tea to India and Bread fruit, Pepper and Star fruit to Caribbean (Thacker, 1979).

At present there are about 1775 gardens and arboreta around the world in 148 countries and many more are under construction like the one in Oman occupies an area of 420 ha. and would become one of the largest in the world when completed containing a unique fog forest. In the last 30 years botanic gardens have seen as a revival as scientific institutions due to the emergence of conservation movement (http:/www.bgci.org/resources/history).

Further, botanical gardens have a matchless set of resources that allows hosting significant climate change research projects not easily undertaken elsewhere. Gardens are finding new uses for traditional research tools such as herbarium specimens and historical photographs, which are increasingly being used to obtain information on past plant behavior. Additional work on invasive species and comparative studies of responses to climatic variation are providing insights on important ecological, evolutionary, and management questions. With their large collections of plant species from throughout the world and excellent herbaria, botanical gardens are well positioned to expand their current activities to continue to provide leadership in climate change research and education (Primack *et al*., 2009)

History of gardens in India dates back to 1000 - 800 B.C. as the 'Charaka Samhita' describes the cultivation of medicinally important plants in and around Ashrams. Further, in olden times 'Vaticas' or 'Tapovanas' used to develop and nourish around Ashrams where 'Rishis' and 'Munis' used to pray and meditate. A book on the gardens of ancient India by Sarangadhara (1283-1301) elucidates different aspects of garden development and maintenance (Anonymous, 1994).

The scenario of the gardens changed during the Mughal period where they introduced a lot of fruit yielding and ornamentally important trees from Central Asia and Persia. Feroz Shah Tughlak (1351-1388) beautified Delhi and adjacent areas by planting trees in nearly 1200 gardens he established. They also established a large number of pleasure gardens in Agra, Lucknow, Rajasthan, Kashmir etc. Some of the famous Mughal gardens are still maintained like Mughal gardens in New Delhi (Now a part of Rashtrapathi Bhavan), Shalimar Bagh, Nishat Bagh, Cashme Shahi of Kashmir etc. In southern part of India gardens developed during the rule of Hyder Ali and his son Tipu Sultan (1760-1799) of which garden at Srirangapatnam, Bangalore, Malavalli are worth to mention here. The famous Lalbagh Garden in Bangalore was established by Hyder Ali and subsequently maintained by Tipu Sultan and later by Dr. Nathaniel Wallich during the British period (Choudhury and Pandey, 2007).

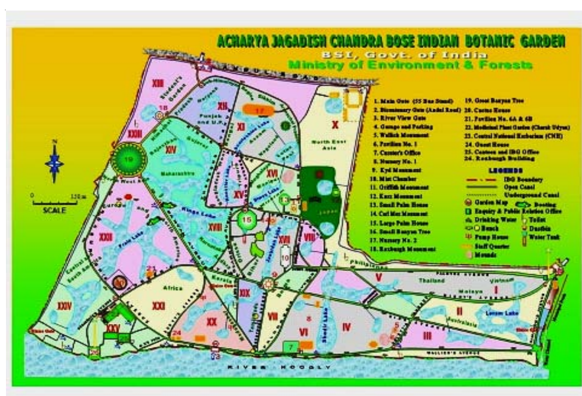
Indian Botanic Garden, Howrah

The Indian sub-continent is an important centre of origin and diversity for nearly 160 domesticated plant species of economic importance, more than 350 species of their wild allies, and over 800 species of ethno-botanical interest. India has only 2.5 per cent of the world's land area, yet it harbours 7.8 per cent of the world's recorded species. It possesses a great variety of ecological habitats and ecosystems including forests, grasslands, wetlands, coastal and marine ecosystems, and desert ecosystems etc. Therefore, it is not surprising that India is considered as one of the 17 'mega diverse' countries of the world in terms of biodiversity. Besides, India contains all or part of three Global Biodiversity Hotspots (Desai *et al*, 2011).

The Indian Botanic Garden (IBG), Howrah formerly known as 'Company Bagan', the Royal Botanic Garden, Kolkata, at present AJC Bose Indian Botanic Garden (AJCBIBG), Howrah, is one of the best landscaped gardens in the world. The original history of IBG is almost similar to the Kew Garden in England which is situated on the bank of the river Thames, a few miles away from London. The Kew garden is about 50 years younger to IBG, Howrah which owes its interests in the Botany of Royalty but the latter has been established with economic and scientific aims. The

Kew garden started initially with a meagre area of 15 acres of land in 1841 and during the course of time it grew further under the hands of a well-known botanist Sir William Hooker, the first director of Royal Botanic Garden, Kew and now it possesses 288 acres. The Royal Botanic Garden, Kolkata, on the contrary, situated on the bank of river Hooghly a few kilometres away from Kolkata, initiated in a vast tract of about 300 acres of land and it was regarded as the largest and one of the oldest botanic gardens in the world till the middle of 19thcentury and now occupies an area of 273 acres.

It was only due to the immense wish and untiring effort of a keen horticulturist Col. Robert Kyd, the then superintendent of the Hon'ble East India Company's dockyard and secretary to the military board at Fort William of the Bengal infantry, a decision for setting up a botanic garden in Kolkata was evolved. During this time Bengal was reeling through the aftermath of 'Great Bengal Famine' and subsequent failure of crops. East India Company was in search of adequate and effective measures to wade off the re-occurrence of famine. Col. Kyd in his historical letter on first January 1786 suggested to Sir. John Macpherson, then the officiating governor General of India, concerning the merit of establishing a Botanic Garden in Calcutta. Without much delay permission for setting up a Botanic Garden in Kolkata was given to Kyd's proposal with the approval of Court of Directors in England, the present site of the garden then measuring about 310 acres below Kid's private garden in Shalimar was acquired (Biswas,1938) .



A map of AJC Bose Indian Botanic Garden, Howrah

At present, the garden with an area of 273 acres and being a living repository of 1377 species of plants possesses 25 divisions and 24 interconnected lakes, and the lakes are connected to the Ganges through sluices for the regular inlet and outlet of water. The garden is a unique place of learning & rich array of curiosity and occupies matchless attractions like the 'Great Banyan Tree', a living wonder in the plant kingdom; the Large Palm House containing rich collection of palms including *Lodoicea maldivica*(the Double Coconut palm); Branching palm (*Hyphane thebaica*) introduced from Egypt; The century Palm (*Corypha macropoda*) ; The Giant Water Lily (*Victoria amazonica*) brought from Amazon river; The queen of flowering trees (*Amherstia nobilis*) a native of Burma; The mountain rose or Venezuelan rose (*Brownea* sp.); The Baobab tree or Kalpavriksh (*Adansonia digitata*) native of Africa; The Rosogolla tree (*Chrysohyllum cainito*); The Cannon ball tree (*Couroupita guianensis*); The African Sausage tree (*Kigelia pinnata*) and the mad tree (*Pterigota alata var. irregularis*); The 'Candle Stick Tree' (*Permentiera cereifera*) etc., are a few to mention.

The 'Great Banyan Tree'- a living wonder in the plant kingdom

Besides, there are important sections like National Orchidarium containing 2000 sets of indigenous as well as exotic orchids; about 40 species of Bamboos in Bambusetum; more than 140 cultivars of Bougainvilleas in Bougainvillea section. Cactarium containing more than 100 species of Cacti and Succulents; Pinetum having rich collection of Pinus and Gymnosperms. Jasminum section holding about 30 species of Jasmines; there are rich collections of legumes in arboretum including 'TYPE' trees. Palmetum possessing about 120 species of palms; Pandanetum having many rare and endemic Pandanus, valuable germ plasm collection of Lotus, water lilies mostly belong to the family Euryalacea, Nelumbonaceae, Nymphaeaceae and many other interesting and bizzarre trees and plants, etc. Propagation of tree species are mainly done in Nursery No.II, the annuals and flowering plants are multiplied and conserved in Nursery No.I. Further, Fern, Succulent, Foliage and seed sections are housed in Nursery No.I. The plant introduction section is actively involved in introduction of new species and varieties.

Col. Kyd being the first honorary superintendent of the garden was instrumental in introducing many exotic plants. He continued as superintendent till his death in 1793. Dr.William Roxburgh, the Companies Botanist in Madras, was appointed as the first official Superintendent in 1793. Roxburgh was the first to draw up a catalogue of 3,500 plants then growing in the Garden. Later it was published as **"Hortus Bengalensis"** in two volumes by Rev.Dr. William Carey in 1814. Roxburgh's monumental work on Indian Plants rightly earned him the title of "Father of Indian Botany".

William Roxburgh died at Edinburgh in 1815 and a monument was erected in 1882 on the northern side of the Great Banyan Tree to perpetuate his memory. Afterwards, various luminaries like H.T. Colebrook, Sir Buchanan Hamilton, James Hare and Thomas Casey held the charges of the Garden from 1813 to 1816.

In 1817, Nathaniel Wallich, an efficient and energetic botanist, was appointed as the Superintendent of the Garden. He was the long serving superintendent in the history of Indian Botanic Garden and held office until 1846. Dr.Wallich died in 1854; his immense contribution to Botanical science always memorable and especially his very rich collection of plant specimens and the famous matchless catalogue known as ' **Wallich's Catalogue'** kept at Central National Herbarium, Kolkata.

Dr. George King who took charge as superintendent in 1871 becomes the re-maker of the garden (The present landscape of the garden was made by him). He took the charge when the garden was in most unpromising state. Dr. King's strong willpower and deep commitment turned the garden into the present form. For acquiring such great achievement Dr. King has been knighted as 'SIR' by the queen and later he was known as Sir. Dr. George King. He is also the founder director of Botanical Survey of India in 1890.

Lt. Col. David Prain succeeded Sir. George King, who, after 26 years of meritorious service, retired in 1897. Before Sir David left India in 1904, he sketched out a geographical plan of garden divisions in accordance with which future plantings were to be regulated. Sir David Prains plan with slight modifications, continued to be carried on by his successors. Lt. Col. A.T.Gage succeeded Sir David as superintendent of the Garden in 1906. A catalogue of non-herbaceous phanerogams cultivated in the Royal Botanic Garden, Calcutta, prepared during Lieutenant- Colonel A.T. Gage's time and was published with the objective of facilitating the exchange of plant materials with other institutions. Mr. Calder succeeded Col. A.T.Gage in 1923. During Mr. Calder's absence on leave from India Dr.J.M.Cowan acted as superintendant from 12th July 1926 to 20th November, 1927. In 1937, K.P.Biswas, the first Indian, joined as Superintendent of the Garden and remained until 1955.

Introduction of milestone species in the Garden

Indian Botanic Garden in Howrah undoubtedly played a significant role in introducing, multiplying and distributing many commercially important plants from various parts of the world. Introduction of some of the notable species like **Tea, Cinchona, Rubber, Mahogany** etc. directly influenced the welfare of people and economic development of the country. Experimental cultivation of the above mentioned species were first made in this garden in trial plots and later on released to various parts of the country for commercial cultivation.

Commercial introduction & multiplication

The garden has served as a platform for acclimatization and distribution of a number of economic and ornamental plants of Agri- Horticultural importance of Indian and foreign origin. Some of the important species are:

Amherstia nobilis (the Queen of flowering trees) introduced from Burma in 1826 by Nathaniel Wallich; *Allamanda* sp. in 1803 by W. Hamilton; *Bougainvillea* sp. in 1803 by W. Hamilton; Garden Croton in 1798 by C. Smith; Australian pine in 1798; *Crescentia cujete* (Calabash tree) in 1795 by W. Hamilton; *Lodoicea maldivica* (the Double Cocunut) in 1874 by Sir George King; Passion flower in 1797; Chinese rose in 1794 by William Roxburgh; *Victoria amazonica* (the Giant Water lily) in 1873 by Sir George King. The foregoing examples are only a few of them. A large number of economic and spice species like Cardamom, Cinchona, Cinnamon, Coffee, Cotton, Indigo, Nutmeg, pepper, Clove, Sugarcane, potato, Sago, teak etc., and other species used as forage, fodder, oil, fruit, fibre, timber and ornamental plants were first introduced into this historic Garden. Multiplication of most of the introduced species carried out in the Garden itself and distributed to different parts of the country for commercial cultivation.

A shift in paradigm

The historic Earth Summit (United Nations Conference on Environment and Development –UNCED) held in Rio de Jeneiro in Brazil from 3rd June to 14th June 1992 and the subsequent opening of Convention on Biological Diversity(CBD) for discussion and signature of participating countries made a new start towards redefinition of measures for conserving world biodiversity. The CBD especially emphasized the need of conserving world threatened species and the role Botanic Gardens (*ex-situ* conservation) can play in conserving thousands of vanishing Rare, Endangered and Threatened plant species from their natural habitats.

In view of the international and national conservation scenario and urgent need of the hour, AJC Bose Indian Botanic Garden (AJCBIBG), Howrah has also made an immediate shift in strategy of Introduction of plants in the line of conservation, a major deviation from the past concept, to commensurate with the directions of Govt. of India as well as CBD**.**

The pattern of Change in plant Introduction & conservation

Well before the Rio Earth Summit i.e. the way back in 1987 AJC Bose Indian Botanic Garden, Howrah has adopted and implemented a well planned decision of Introducing Rare, Endangered and Threatened (RET) indigenous plant

species, in the 'Annual Action Plans', prior to their extinction from the wild and to multiply and conserve them so as to check the loss of species. This is evident from the introduction of plants in AJCBIBG for the past 30 years i.e. from 1987-2017.

After the Rio Convention in 1992 the trend of introducing RET species in AJCBIBG has increased many folds and annually a large number of exclusive RET species being collected from the wild and introduced and multiplied for the purpose of effective conservation. In the last nine years i.e. from 2008 -2017 AJCBIBG officials concentrated only in the collection of Endemic, Endangered & Threatened (EET) species. This is mainly due to the loss of many key species from the wild, because of habitat loss a number of species is being pushed annually into the IUCN list of threatened plants. Nevertheless, the mission of introducing threatened species from the phytogeographically and ecologically fragile Western Ghats, Eastern Ghats, North East India and Andaman & Nicobar Islands continues to be a major target of AJCBIBG under the Annual Action Plans.

Conclusion

The global network of botanic gardens conserves an astounding array of plant diversity, holding about 105,600 species, amounting to 30% of species diversity, 59% of plant genera, 75% of land plant families, and 93% of all vascular plant families. It also harbours about 13,200 species at risk of extinction, equating to just over 41% of the world's known threatened flora. Gardens are also finding new uses for traditional research tools such as herbarium specimens and historical photographs, which are increasingly being used to obtain information on past plant behavior. Additional work on invasive species and comparative studies of responses to climatic variation are providing insights on important ecological, evolutionary, and management questions.

The Indian Botanic Garden (IBG), Howrah, currently Acharya Jagadish Chandra Bose Indian Botanic Garden (AJCBIBG) possessing a brilliant past in the history of plant conservation, and presently it is taken as a centre of conservation of plant resources from their extinction. This garden serves as a living repository of plants of a country and also of selected exotic species, and a "safe abode" for the rare and endemic plants. As a result it houses the germ plasm collection of selected economic, ornamental and medicinal plants and their wild progenitors. The garden also act to promote regional and national educational programmes on conservation and international exchanges in order to generate awareness about the value of trees and other curious, beautiful, interesting plants with delightful landscaping and display. As a whole, this garden acts as a data bank of information, a national repository, and documentation on holdings of the Indian Botanic Gardens.

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The integration of mind and hearts of human interactions in a cosmological construct for Green Earth

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A multidisiplinary approach to appreciate the duality of existence and its connectivity with man-man interaction within the core space and intra-action (the observer-player) within the sub-space as the civilizing outcome. Our Green Earth. This will provide us a basis to understand what is inwardly hidden and outwardly manifest.

The interaction of various spheres (alam) and its effect on man the manager-leader responsibility will be discussed to further realise the significance of multi-spheres integration on man-man activities and man-creator imperatives. Thus making reasonably visible the connectivity of human interactions within a panoramic construct. Agricultural activities as the key man-man activities and best practices as clearly stated in agro-ecological approach as in the case of SRI (sustainable resources intensification) will be discussed in detail. The uphill roads for SRI adoptions by our team in Malaysia will be presented as an example of the real challenges to achieve Green Earth.

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Studies on the Faunal diversity with *s.r.t.* Mammalian Diversity and their conservation in India

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Abstract : India is very rich in terms of faunal diversity due to its unique biogeographic location, diversified climatic conditions and enormous ecodiversity and geodiversity. The Indian landmass is bounded by the Himalaya in the North, the Bay of Bengal in the East, the Arabian Sea in the West, and Indian Ocean in the South. In this paper, the efforts were made to provide status of faunal diversity with special reference to mammalian diversity and their conservation in India, from studies conducted, past literature, museum records and other lesser-known sources of information. About 15,66,353 faunal species belongs to different faunal groups were recorded from throughout the world, in that about 1,00,693 species belongs to different faunal groups were receorded from India, which is 6.42% of world faunal species. The synthesis suggests that 427 Mammals species representing 48 families and 14 orders are known from India, out of 5,853 species belongs to 154 families and 29 orders from the World, which is 7.29% of world mammal species. In addition to this the valid 338 subspecies of Mammals were also recorded from India.

Indian mammals are found in all types of habitat, from the snowy heights of the Himalaya to the plains, and show all types of adaptation, *viz.* arboreal, fossorial, volant, aquatic, etc. The 14 orders of mammals reported so far form the India are Scandentia, Chiroptera, Primates, Pholidota, Carnivora, Proboscidea, Sirenia, Soricomorpha, Erinaceomorpha, Perissodactyla, Artiodactyla, Lagomorpha, Rodentia and Cetacea, as against 29 orders known so far throughout the world. In India, chiropterans represents maximum number of species (118) followed by rodents (101) and these two taxa jointly constituting 51.40% of the total Indian mammal species. Indian carnivores are also diverse having 60 species followed by artiodactyls (36), cetaceans (33), soricomorphs (30), primates (23), lagomorphs (11), perissodactyls (5), scandents (3), erinaceomorphs (3), pholidots (2), proboscidean (1) and sirenian (1). Of the 427 Indian mammals, the conservation status of 392 species have been evaluated in the IUCN Red List of Threatened Species by group experts. However, the IW(P)A and CITES evaluated, only 245 and 146 species respectively. As per the IUCN Red List category, 4 species are Extinct, 8 (Critically Endangered), 41 (Endangered), 46 (Vulnerable), 27 (Near Threatened) and 266 (Least Concern); IW(P)A' Schedule I (77), II (82), III (10), IV (5) and V (71), and CITES' Appendix I (70), II (55) and III (21). Of the 427 mammal species, 44 species are endemic and 4 species are extinct from India *i.e. Acinonyx jubatus,* Cheetah; *Bos javanicus,* Banteng; *Dicerorhinus sumatrensis,* Sumatran Rhinoceros and *Rhinoceros sondaicus,* Javan Rhinoceros.

Conserving what we have today is hampered by lack of management measures including outreach and our ability to predict what would live in India and lack of data relating changes in biodiversity to those of environment. Most of the Mammalian species of India are given protection in different Schedules of Indian Wildlife (Protection) Act, 1972, listed in Appendixes of CITES and in IUCN Red List of Threatened Species. Beside protection and awareness, still hunting and poaching going on in different parts of India and illegal wildlife products trade is recorded. The experts of Zoological Survey of India helps the enforcement agencies *i.e.* Forest Department, Police Department, Custom and Wildlife Crime Control Bureau in identification of seized wildlife material. In recent times, however, many species are facing catastrophic decline in number, so much so that several species have become scarce or rare, and have been given asylum in reserve forests, Wildlife Sanctuaries, National Parks and Biosphere Reserves *etc*. Hence, adequate and systematic knowledge of Indian mammalian fauna through taxonomic, distributional and biological studies are necessary, so that ways for their conservation or control may be devised.

Key words: Fauna, Mammals, diversity, conservation, India.

Introduction

India is very rich in terms of faunal diversity due to its unique biogeographic location, diversified climatic conditions and enormous ecodiversity and geodiversity. The Indian landmass is bounded by the Himalaya in the North, the Bay of Bengal in the East, the Arabian Sea in the West, and Indian Ocean in the South. In this paper, the efforts were made to provide status of Faunal diversity with special reference to Mammalian diversity and their conservation in India, from studies conducted, past literature, museum records and other lesser-known sources of information. Indian mammals are found in all types of habitat, from the snowy heights of the Himalaya to the plains, and show all types of adaptation, *viz.* arboreal, fossorial, volant, aquatic, *etc.* Studies on Indian mammals can be considered to have started as early as the eighteenth century, when the tenth edition of *Systema Naturae* by Karl von Linnaeus was published (1758), which provided binomial names to many Indian mammals. Knowledge about mammals of India is very old. Descriptions of some mammals are available in the 'Vedas' and even in Pre-Vedic edicts. Authorities of the 'Ayurvedic' and 'Unani' systems of medicine were aware of the medicinal values of some

products obtained from certain species of mammals. The montane ecosystem of the Himalaya, the mangrove ecosystem of the Sundarban, the desert ecosystem of the Thar desert of Rajasthan and Gujarat, the insular ecosystem of Andaman & Nicobar Islands, and the Lakshadweep, the cave-ecosystem of various caves such as the Siju Cave in Meghalaya, and other caves, the tropical rain forest ecosystem of north-eastern India and Western Ghats, *etc.*, are all to be found in the Indian Union. All these ecosystems have their characteristic mammalian fauna. Besides, the tropical deciduous forests of central India, the semi-arid tracts of peninsular India, the Gangetic plains of northern and eastern India- all have their own peculiar mammalian fauna. It is encouraging to note that mammalian fauna of all the ecological zones of India have been worked out to a greater or lesser extent.

Descriptions of some mammals are available in the 'Vedas' and even in Pre-Vedic edicts (Rao, 1957; Bhaduri, Triwari and Biswas, 1972). Authorities of the 'Ayurvedic' and 'Unani' systems of medicine were aware of the medicinal values of some products obtained from certain species of mammals. Mughal emperors Babur and Shahjahan took much interest in game animals. However, elaborate studies on Indian mammals have been conducted only during the last two centuries or so. Though description of Indian mammals are available in standard literature, yet there are many whose status is not clear and lack distributional, behavioural and ecological information. Many species and subspecies are based on small variation, with considerable amount of overlap. Also, the distributional pattern of many species are changing rapidly, commensurate with the man-made changes in their habitats. Over ten thousand publications, including books and articles, the latter mostly scattered in a number of journals and periodicals, are available on Indian mammals.

The work done in recent years on mammals have been mostly on taxonomy, distributions, status, ecology, ethology, biology, population control, morphology, anatomy, tricho-taxonomy, cyto-taxonomy, *etc*. Knowledge about mammals of India is very old. It is impossible to give coverage to the vast literature on Faunal diversity of India within this short communication. However, an attempt has been made in this paper to highlight some of the important literature that have been published so far in widely scattered journals and periodicals, for the facility of mammalogists, and to ensure a proper understanding of this large and economically important group.

Results and Discussion

About 15,66,353 faunal species belongs to different faunal groups were recorded from throughout the world, in that about 1,00,693 species belongs to different faunal groups were receorded from India (Anon, 2017), which is 6.42% of world faunal species (Table 1).

Table 1: Status of Faunal (Animal) species of World and India

Kingdom	Phylum	No. of Species			% in India
		World (living + fossil)	World (living)	India	
Protista	Phylum Protozoa	36,400	36,400	3,510	9.64
Animalia	Phylum Mesozoa	122	122	10	8.02
	Phylum Porifera	11,055	8,838	545	6.16
	Phylum Cnidaria	17,702	11,522	1,396	12.12
	Phylum Ctenophora	199	199	19	9.55
	Phylum Platyhelminthes	29,488	29,487	1,738	5.89
	Phylum Rotifera	2,049	2,049	466	2.24
	Phylum Gastrotricha	828	828	162	19.56
	Phylum Kinorhyncha	196	196	10	5.10
	Phylum Nematoda	25,043	25,033	2,914	11.63
	Phylum Acanthocephala	1,461	1,330	301	22.63
	Phylum Sipuncula	156	156	41	26.28
	Phylum Echiura	198	198	47	23.73
	Phylum Annelida	17,426	17,388	1,024	5.89
	Phylum Onychophora	187	183	1	0.53

	Phylum Arthropoda	**13,02,809**	**12,57,040**	**75,528**	**6.00**
	Subphylum Chelicerata	1,15,992	1,13,773	5,945	5.23
	Subphylum Crustacea	73,141	67,735	3,796	5.61
	Subphylum Hexapoda	10,80,760	10,63,533	65,409	6.15
	Subphylum Myriapoda	12,010	11,999	378	3.15
	Phylum Phoronida	**16**	**16**	**3**	**18.75**
	Phylum Bryozoa (Ectoprocta)	**11,652**	**6,186**	**327**	**5.29**
	Phylum Entoprocta	**186**	**186**	**10**	**5.37**
	Phylum Brachiopoda	**7,390**	**392**	**8**	**2.04**
	Phylum Chaetognatha	**186**	**170**	**44**	**25.88**
	Phylum Tardigrada	**1,335**	**1,167**	**30**	**2.57**
	Phylum Mollusca	**1,18,062**	**84,978**	**5,189**	**6.11**
	Phylum Nemertea	**1,368**	**1,368**	**6**	**0.43**
	Phylum Echinodermata	**20,550**	**7,550**	**777**	**10.29**
	Phylum Hemichordata	**162**	**139**	**14**	**10.07**
	Phylum Chordata	**89,955**	**71,526**	**6,573**	**9.08**
	Class Pisces	37,172	34,362 3,324	9.70	
	Class Amphibia	8,007	7,667	388	5.06
	Class Reptilia	16,123	10,450	572	5.47
	Class Aves	11,241	10,357	1,340	12.93
	Class Mammalia	15,969	5,853	427	7.29
	Total (Animalia)	**16,64,289**	**15,29,953**	**97,183**	**6.35**
	Grand Total (Protista + Animalia)	**17,00,689**	**15,66,353**	**1,00,693**	**6.42**

The synthesis suggests that 427 Mammals species representing 48 families and 14 orders are known from India **(Table 2)**, out of 5,853 species from the World, which is 7.29% of world mammal species. In addition to this the valid 338 subspecies of Mammals were also recorded from India (Sharma *et al.,* 2015). The 14 orders of mammals reported so far form the India are Scandentia, Chiroptera, Primates, Pholidota, Carnivora, Proboscidea, Sirenia, Soricomorpha, Erinaceomorpha, Perissodactyla, Artiodactyla, Lagomorpha, Rodentia and Cetacea, as against 29 orders known so far throughout the world. In India, chiropterans represents maximum number of species (118) followed by rodents (101) and these two taxa jointly constituting 51.40% of the total Indian mammal species. Indian carnivores are also diverse having 60 species followed by artiodactyls (36), cetaceans (33), soricomorphs (30), primates (23), lagomorphs (11), perissodactyls (5), scandents (3), erinaceomorphs (3), pholidots (2), proboscidean (1) and sirenian (1) (Sharma *et al.,* 2015). In the mammalian fauna of India, as stated above, genera such as *Anathana* (Scandentia), *Latidens* (Chiroptera), *Nilgiritragus* (Artiodactyla), *Platacanthomys*, *Cremmomys* and *Diomys* (Rodentia) are indigenous to India.

Sl. No.	**Class Mammalia**	**India**	
		Genera	Species
1.	Order Proboscidea	1	1
2.	Order Sirenia	1	1
3.	Order Scandentia	2	3
4.	Order Primates	6	23
5.	Order Rodentia	45	101
6.	Order Lagomorpha	3	11
7.	Order Erinaceomorpha	2	3
8.	Order Soricomorpha	11	30
9.	Order Chiroptera	39	118
10.	Order Pholidaota	1	2
11.	Order Carnivora	35	60
12.	Order Perissodactyla	3	5
13.	Order Artiodactyla	25	36
14.	Order Cetacea	23	33
	Total	**197**	**427**

Table 2: Status of Mammal species in India

Status survey of some of the endangered species of Indian mammals have been conducted *viz.,* Bonnet Monkey, *Macaca radiata*, Lion-tailed Macaque, *Macaca Silenus*, Crab-eating Macaque, *Macaca fascicularis*, Rhesus Macaque, *Macaca mulatta*, Hanuman Langur, *Presbytis entellus*, Golden Langur, *Presbytis geei*, John's Langur, *Presbytis johni*, Capped Langur, *Presbytis pileatus*, Phayre's Leaf Monkey, *Presbytis phayrei*, Hoolock Gibbon, *Hylobates hoolock*, Malabar Civet, *Viverra megaspila*, Tiger, *Panthera tigris*, Indian Lion, *Panthera leo persica*, Indian Elephant, *Elephas maximus*, Great one-horned Rhinoceros, *Rhinoceros unicornis*, Indian wild Ass, *Equus hemionus khur*, Pygmy Hog, *Sus salvanius*, Musk Deer, *Moschus moschiferus*, Swamp Deer, *Cervus duvauceli*, Thamin, *Cervus eldi*, Hangul, *Cervus elaphus hanglu*, Indian wild Buffalo, *Bubalus bubalis*, Blackbuck, *Antilope cervicapra*, Himalayan Tahr, *Hemitragus jemlahicus*, Nilgiri Tahr, *Hemitragus hylocrius*, Markhor, *Capra falconeri*, Hispid Hare, *Caprolagus hispidus*, Gangetic Dolphin, *Platanista gangetica*.

Of the 427 Indian mammals, the conservation status of 392 species have been evaluated in the IUCN Red List of Threatened Species. However, the IW(P)A (Indian Wildlife (Protection) Act, 1972) and CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) evaluated, only 245 and 146 species respectively. As per the IUCN Red List category, 4 species are Extinct, 8 (Critically Endangered), 41 (Endangered), 46 (Vulnerable), 27 (Near Threatened) and 266 (Least Concern); IW(P)A' Schedule I (77), II (82), III (10), IV (5) and V (71), and CITES' Appendix I (70), II (55) and III (21) (Sharma *et al.,* 2015). Of the 427 mammal species, 44 species are endemic and 4 species are extinct from India *i.e. Acinonyx jubatus,* Cheetah; *Bos javanicus,* Banteng; *Dicerorhinus sumatrensis,* Sumatran Rhinoceros and *Rhinoceros sondaicus,* Javan Rhinoceros. Altogether 77 species of mammals have been included in schedule I of the Indian Wild Life (Protection) Act, 1972, as amended till date. Out of these, some 25 species appear to be highly threatened. These are Lion-tailed Macaque (*Macaca silenus*), Phayre's Leaf Monkey (*Presbytis phayrei*), Chinese Pangolin (*Manis pentadactyla*), Malayan Sun Bear (*Helarctos malayanus*), Red Panda (*Ailurus fulgens*), Ratel (*Mellivora capensis*), Malabar Civet (*Viverra civettina*), Desert Cat (*Felis silvestris*), Caracal (*Caracal caracal*), Marbled Cat (*Pardofelis marmorata*), Golden cat (*Catopuma temminckii*), Indian Lion (*Pathera leo persica*), Snow Leopard (*Uncia uncia*), Indian Wild Ass (*Equus hemionus khur*), Pygmy Hog (*Porcula salvania*), Musk Deer (*Moschus moschiferus*), Thamin (*Rucervus eldi*), Four-horned antelope (*Tetracerus quadricornis*), Wild Yalk (*Bos mutus*), Indian Wild Buffalo (*Bubalus arnee*), Himalayan Tahr (*Hemitragus jemlahicus*), Markhor (*Capra falconeri*), Bharal (*Pseudois nayaur*), Hispid Hare (*Caprolagus hispidus*) and Small Travancore Flying Squirrel (*Petinomys fuscocapillus*).

It is encouraging to note that mammalian fauna of all the ecological zones of India have been worked out to a greater or lesser extent. Hardwicke collected in West Bengal**,** Uttar Pradesh and Kashmir, during 1778 to 1823, and prepared numerous drawings of mammals through local artists. A number of species were also described by him. So far as the Indian Union is concerned, Hodgson collected in the Darjeeling district of West Bengal from where he also described some species of mammals. Jerdon made extensive explorations in southern and central India, Kashmir and various hill stations of the Himalaya. Result from his extensive collecting work appeared in his book mentioned earlier. Blyth took considerable interest in scientific collection of mammals in and around Calcutta, during mid-nineteenth century, and published many papers. Blanford collected a number of specimens from southern and central India. Other important explorations made on mammals of various areas up to 1900 and studies made thereon are: Assam by W. Griffith and J. McClelland; Andaman & Nicobar Islands by Tytler, J. Barbe, and W. L. Abbot; south-eastern part of Bihar by S. R. Tickell; Khasi Hills (Meghalaya) by R. W. Frith; central India by Whitehead; Dharward (Karnataka) by W. Elliot; Kashmir by C. H. Stockley, W. L. Abbot, F. Stoliczka, J. Scully, Baron von Hugel and A. E. Ward; Kumaon and Mussoorii (Uttar Pradesh) by Tytler, T. Hutton and Stwart; Pune (Maharashtra) in Western Ghats by A. L. Adams; Punjab by Bitrel and Dunn; Southern India, including Madras By W. H. Sykes and Health; Travancore (Kerala) by H. Furguson, and Tripura by J. Barbe, *etc.*

The 'Mammal Survey' of India and adjoining areas was started by the Bombay Natural History Society from 1911 with a view to modernising taxonomic knowledge of Indian mammals on the basis of freshly Killed material from selected localities at different seasons, with detailed field data on habitat, behaviour, etc. Areas explored during this surveys are Arunachal Pradesh (Mishmi Hills), Assam, Bihar (in part), Gujarat (Kutch, Palanpur, Kathiawar), Himachal Pradesh (Kangra, Chamba), Jammu & Kashmir (Islamabad district), Karnataka (Coorg, Dharwar), Kerala (Travancore), Madhya Pradesh (East Khandesh, Berar, Nimar, Gwalior), Maharashtra (Koyana valley, poona), Nagaland (Naga Hills), Orissa (in Part), Punjab (Gopalpur), Sikkim, Tamil Nadu (Palni Hills, Coimbatore, Nilgiri, Madura, Nelliampathy) and West Bengal (in part). Altogether 25,000 specimens were collected by the 'Mammals Survey'. The most prominent among the collectors who made untiring efforts to procure such a large collection are: C. Primrose, C. A. Crump, C. McCann, G. C. Shortridge, H. W. Wells, N. A. Baptista, Ryley O' Brain, and others. To make this large collections, special assistance

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was also received from different persons throughout the country. Some material by competent authorities resulted in a number of excellent publications which appeared in the various issues of the journal of the Bombay Natural History Society, during 1912 to 1929.

With the inception of the Zoological Survey of India, a number of surveys were conducted by this department in diverse types of ecological zones, to procure fresh material of mammals for study. The areas covered by this department are Andaman & Nicobar Islands (some major islands only), Andhra Pradesh (Nagarjuna Konda, Adilabad district), Assam, Arunachal Pradesh (in Part) and West Bengal. Some specimens have also been received by the department from different joint expeditions with foreign institutes. The material obtained from the surveys of the above areas, along with the collection of the Royal Asiatic Society of Bengal, the Indian Museum, part of the 'Mammals Survey' specimens, received in this department, and other stray specimens obtained by way of donations from various sources, have been studied. Some important State Fauna of mammals completed by the scientists of the Zoological Survey of India are those of Manipur, with special reference to rodents (Roonwal, 1950); undivided Assam (Kurup, 1966); Goa (Agrawal, 1973); Tripura (Agrawal and Bhattacharyya, 1977); Orissa (Das *et al.*); Jammu & Kashmir (Chakraborty, 1983); Rajasthan (Ghose); Namdapha National Park, Arunachal Pradesh (Saha, 1985), and Andaman & Nicobar Islands (Bhattacharyya, 1975, Saha, 1980, Das, 1980, Chaturvedi, 1980). Collection and study of material have also been done on cave fauna: Siju Cave, Garo Hills, Meghalaya (Kemp, 1924) and Borraguhalu Cave, Andhra Pradesh (Das). Considerable interests have also been taken in the study of high altitude mammals of northern and north-eastern India (Khajuria and Ghose, 1970, Ghose and Saha, 1981, Mahajan and Mukherjee, 1974, Ghosh, 1981). The Mangrove fauna of the Sundarban has been worked out by Mandal and Ghose (1989).

Biological resources have traditionally been a major source of food for local inhabitants and of major economic value in terms of commercial exploitation. Ecosystems and biodiversity of India have been exploited since long time but it is only in the last century that the rate of exploitation has increased dramatically, due mostly to the increase in the human population. Except for some of the Andaman-Nicobar Islands, no pristine area exists today. At the end of the last century or in the beginning of this century, very few areas of India remained unaffected, whereas most were partially deteriorated and a few were severely affected. The fauna of the India have been adversely affected owing to combined effect of habitat destruction, fragmentation, hunting, poaching, illegal trade, grazing, deforestation, hybridization, random use of pesticides, landslides, cloud bursts, floods, construction of Hydroelectric Projects and others. Himalayan glaciers are shrinking at a faster pace causing changes in the water dynamics of perennial rivers in the region affecting faunal components.

Conserving what we have today is hampered by lack of management measures including outreach and our ability to predict what would live in India and lack of data relating changes in biodiversity to those of environment. Most of the Mammalian species of India are given protection in different Schedules of Indian Wildlife (Protection) Act, 1972, listed in Appendixes of CITES and in IUCN Red List of Threatened Species. Beside protection and awareness, still hunting and poaching going on in different parts of India and illegal wildlife products trade is recorded. The experts of Zoological Survey of India helps the enforcement agencies *i.e.* Forest Department, Police Department, Custom and Wildlife Crime Control Bureau in identification of seized wildlife material. To monitor changes in land use practices especially in areas of endangered animals. The present need is sincere, collective efforts and action oriented strategies for conservation of Biodiversity and different ecosystems of India are required from all levels and also efforts to restore degraded ecosystems to be accelerated. In order to control losses in biodiversity and restore degraded ecosystems, we need to have a full assessment of the magnitude of such losses that stem from the utilization of biodiversity, and the factors that promote the unsustainable use of biotic resources. An important aspect is to strengthen the measuring, monitoring and management of biodiversity and evaluate our conservation strategies in the light of human needs for biomass with coordinated approach to sustain protection of both areas and diversity as seen in totality. Some species have become so numerous as to cause great strain on agriculture and forestry. Some others are causative agents of human and veterinary diseases. Hence, control of their population has become a necessity. In recent times, however, many species are facing catastrophic decline in number, so much so that several species have become scarce or rare, and have been given asylum in reserve forests, Wildlife Sanctuaries, National Parks, Biosphere Reserves *etc*. Hence, adequate and systematic knowledge of Indian mammalian fauna through taxonomic, distributional and biological studies are necessary, so that ways for their conservation or control may be devised.

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Impact of climate change in forest diseases of Nepal

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The increasing of concentrations of Green house gases (GHG) in the atmosphere is responsible for global climate change. The accelerated boost in temperature and CO2 concentration were reported over the last 100 years resulting climate change was due to Anthropological reasons such as Industrial Revolution and burning of fossil fuel and land-use changes like deforestation. Global increase in average temperature was observed since the mid-20thcentury and the growth rate in CO_2 concentration was increasing rapidly since 2000. The atmospheric temperature of Nepal has been rising consistently after the mid1970s with higher rate than the global average and the warming has found obvious in the high altitudes of Nepal Himalayas. It has suggested that the impacts of climate change may be intense at high elevations and in regions with complex topography in mid-hills of Nepal. With the increase of global mean temperature exceeds warming of 2-3 degree Celsius 20% -30% of plants and animal species are likely to be at high risk of extinction. As Nepal is rich enough in biodiversity that has over 6500 species of flowering plants, over 1500 fungi species, over 350 lichen species. Out of those about 370 species of flowering plants are considered to be endemic to Nepal, which are highly affected by climate change. The impact of climate change causes the vulnerable to forests and biodiversity and many species are in the state of threatening. Changing environment will affect the microclimate surrounding plants and the susceptibility of plants to infection. However, dramatic changes are noted in the magnitude of disease expression in a given pathosystem. Thus, it is important to know the patterns of climate change and its impact on both host and pathogen that would make easy to forest disease management.

Forest and Climate Change

Padma Shri Jadav Payeng
(Forest Man of India)

Padma Shri **Jadav Molai Payeng** born in 1963 is a Mishing tribe environmental activist. He is a forestry worker **at Plant for Planet and Peace (PPP),** from Jorhat, India. He has done a great service to mankind by working towards saving the environment.

Moved over by the sight of more than hundred snakes dying one after the other due to severe heat on the deserted sandbar, he singlehandedly decided to plant life on eroded island. In April 1979 he started his task by sowing the seeds and shoots offered by villagers. Over the course of several decades, he planted and tended trees on a sandbar of the river Brahmaputra and turned it into a forest reserve. It now boast of thousands of trees including bamboos, many endangered animals, including one-horned rhinos, Royal Bengal tigers, vultures and migratory birds. A herd of around hundred elephants reqularly visits and stays in forest for six months every year. His thirty-six years of hardship and dedication has helped him create a forest. The forest, called Molai forest after Jadav Molai Payeng, is located near Kokilamukh of Jorhat in Assam. It encompasses an area of about 1,360 acres per 550 hectares.

In 2015, he was honored with Padma Shri, the fourth highest civilian award in India for his contribution towards society. In October 2013, he was honored at Indian Institute of Forest Management during their annual event Coalescence. Film maker Aarti Shrivastava acknowledged the life and works of Jadav Payeng into a film documentary called "Foresting life" in 2013. Padma Shri Jadav Payeng was also honored by the School of Environmental Sciences at Jawaharlal Nehru University on 22nd April 2012 for his remarkable achievement. JNU vice-chancellor Sudhir Kumar Sopory honored Jadav Payeng by naming him as "**Forest Man of India**".

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SECTION II
RESEARCH PAPERS

Theme - I
Agricultural Sciences

Effect of Carbendazim Seed Treatment on Seed Mycoflora, Seed Germination and Vigour Index of Green Gram (CV. S-8)

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Abstract: Seed treatment through chemicals is safest, cheapest and most effective means of controlling most seed borne pathogens. Keeping this in view, the present research work was undertaken to evaluate the efficiency of five seed dressing fungicides for their relative efficacy against seed mycoflora and seed germination and vigour index of Green gram. Cultivar S-8 shows the response to the carbendazim treatment in relation to its seed mycoflora, seed germination and vigour index. It was found that as concentration of carbendazim increases, there was decrease in seed mycoflora where as increase in seed germination and vigour index. At 0.5% of carbendazim seed treatment it shows minimum seed mycoflora with 00% as compared with control with 70%. At the same concentration there was increase in seed germination which was 90% as compared with control with 55%. At the concentration vigour index was 800 as compared with control with 150.

Keywords: Fungicide, Carbendazim, Green gram (CV. S-8)

Introduction

Fungicides are biocidal chemical compounds used to kill fungi or fungal spores (Haverkate, *et. al.,* 1969). A fungistatic inhibits their growth. Fungi can cause serious damage in agriculture, resulting in critical losses of yield, quality, and profit. Gray *et. al.* (1983) studied the potential exposure of commercial seed- treating applicators to the pesticides carboxinthiram. Fungicides are used both in agriculture and to fight fungal infections in animals. Chemicals used to control oomycetes, which are not fungi, are also referred to as fungicides, as oomycetes use the same mechanisms as fungi to infect plants. Fungicides are frequently applied to seed to protect from disease. Farmers may be exposed to fungicides through both personal application and use of treated seed. Bradley (2008) reported that *B. pumilus* protected against stand and yield losses in soybean in North Dakota, but not as consistently as chemical seed treatments. Rathod and Pawar (2013) studied the seed treatment on soybean cultivar.

Materials and Methods

Carbendazim fungicide was used for this study. Different concentrations of fungicide made were from 0.1% to 0.5% and applied to the seeds of Green gram Cv. S-8. The effect on seed mycoflora, seed germination and vigour index was recorded. The vigour index was determined by multiplying the percent germination with the sum of the root and shoot length.

Vigour index = (Root length in cm + Shoot length in cm) x germination (%).

Result and Discussion

Cultiver S-8 shows the response to the carbendazim treatment in relation to its seed mycoflora, seed germination and vigour index. The results are given in Table No.1. It was found that as concentration of carbendazim increases, there was decrease in seed mycoflora where as increase in seed germination and vigour index. At 0.5% of carbendazim seed treatment it shows minimum seed mycoflora with 00% as compared with control with 70%. At the same concentration there was increase in seed germination which was 90% as compared with control with 55%. At the concentration vigour index was 800 as compared with control with 150.

Conclusion

Effect of carbondazim fungicides was observed on seed mycoflora, seed germination and vigour index of Legume Cv. S-8. The fungicide used for seed treatment was carbendazim, ranging from 0.1-0.5% concentrations. The carbendazim was found to be inhibitor which reduce percent seed mycoflora and increase percent seed germination and vigour index significantly.

Acknowledgement

I express my deep sense of gratitude and indebtedness to my dynamic, dedicated, kind hearted and enthusiastic guide Principal and Reader Dr. Muley S. M., Head, P.G. Department of Botany, Shivaji Mahavidyalaya, Udgir. I must mention special thanks for his valuable guidance, constant encouragement and concise suggestions in completion of this research paper.

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Table 1: Effect of Carbendazim seed treatment on seed mycoflora, seed germination and vigour index of Green gram (Cv. Green gram S-8).

Conc. (%)	**Seed mycoflora (%)**	**Seed germination (%)**	**Vigour index**
0.00 (Control)	70	55	150
0.1	67	65	180
0.15	65	67	198
0.2	50	70	245
0.25	40	78	290
0.3	25	80	300
0.35	15	83	510
0.4	10	85	650
0.45	05	88	700
0.5	00	90	800
S.E ±	8.15	3.42	72.32
C.D. at 5%	**18.41**	**7.72**	**163.44**

Biological Control of Seed Borne Fungi of Black Gram

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Abstract: Biological control is an environmentally sound and effective means of reducing or mitigating pests, pest effects, pathogenic fungi, Bacteria and Viruses through the use of natural enemies. An experiment was conducted to assess the reduction in association of seed borne fungi and enhancement of germination of black gram (*Vigna mungo* L.) using two varieties S-1-1 & T-9. The seed mycoflora of different varieties of black gram was screened by standard blotter paper, agar plate and seed washates methods. Among these three methods, the standard blotter paper was found to be as in less incubation; there was higher percent incidence of seed mycoflora. In this study, *in vitro* potential of *Trichoderma harzianum* and *Bacillus subtilis* were evaluated against two fungal pathogens of black gram by dual culture method.

Keywords: Black gram, *Trichoderma species,* dual culture method & seed health, testing methods.

Introduction

Seed is the basic unit in crop production technology. It plays an important role in the healthy crop production. The seeds carry a heavy load of microorganisms which are capable of causing severe diseases and considerable loss of the yield. These microorganisms enter into the seedcoat; cotyledons and embryonic parts of the seed from field and also proliferate the infection during the ill storage condition. These seeds were employed to detect the external and internal seed-borne fungi associated with the seed by using standard blotter paper method and agar plate method as recommended by ISTA (1966). Seeds are the important carriers of plant pathogens, like fungi, bacteria, viruses etc. It is observed from the literature that microbes during their association with seeds both in the field as well as in the storage cause different abnormalities in the seeds (Neergaard, 1977).

The terms "biological control" and its abbreviated synonym "bio-control" have been used in different fields of biology, most notably entomology and plant pathology. Biopesticides and their use in the control of plant diseases has become a modern trend in agriculture, which is termed as biological control. Biological control of various plant pathogens using different bioagents has been attempted and found effective. Such biocontrol agents are found to be belonging to different groups of plants and microbes besides, which have been reported to be highly potent, economical and ecofriendly for the successful control of seed borne pathogens and plant pathogens. Biological control plays an important role in biological control of plant diseases. The strains of fungal genera *Trichoderma* species have shown biocontrol activity against damping off disease in several crops (Hader *et al*., 1983, Adams, 1990).

Material S And Methods

Dual culture technique: Dual culture technique for fungi was given by (Hang and Hoes, 1976). It consists of growing the test organism and pathogenic organism on the same plate. This can be done by the following procedure. Pour 20 mL of melted cooled (45-50^{0}C) PDA medium in each petriplates. Allow the medium to solidify. Place 9 mm mycelial growth disc cut from the margin of the actively growing colonies of pathogenic culture (*Aspergillus flavus, Fusarium oxysporum* and *Alternaria tennis)* near the periphery on one side of the PDA plate. Now place another disc of 9 mm of test organism (*Trichoderma harzianum* and *Trichoderma viride),* on the other side of same plate opposite to the first disc i.e. at an angle of 180^{0}C. Petriplates were incubated at 28± 1^{0}C. .

Results and Discussion

The *Trichoderma* species including *T. harzanium and T. viride* were tested against *Aspergillus flavus* Link, *Fusarium oxysporum* Schlecht and *Alternaria tenuis* Ness. by dual culture technique. The petriplates were incubated with test fungi and biocontrol agents. The zone of inhibition was measured in relation to its growth and results were recorded.

Effect of Fungal species on the growth of *Aspergillus flavus* Link.

Table 1. show that the growth of *Aspergillus flavus* was 4.0 cm in presence of *Trichoderma harzianum,* while 4.7 cm in the presence of *Trichoderma viride.* The percent growth of inhibition of *Trichoderma harzianum* and *Trichoderma viride* were 46.66% and 42.00% respectively. The growth of *Aspergillus flavus* on control plate was 8.5 cm. It is clear that *Trichoderma harzianum* inhibit the maximum growth of *Aspergillus flavus* as compared to *Trichoderma viride.*

Effect of *Trichoderma* species on the *Fusarium oxysorum* Schlecht.

The growth of *Fusarium oxysporum* Schlecht. in presence of *Trichoderma harzianum* was 2.9 cm and

percentage growth of inhibition was 58.86. In presence of *Trichoderma viride* growth of fungus was 3.5 cm and percentage growth of inhibition was 41.66. The growth of *Fusarium oxysorum* on control plate was 6.0 cm from the Table 2. So, it is clear that *Trichoderma harzianum* inhibits the maximum growth of *Fusarium oxysorum* as compared to *Trichoderma viride.*

Effect of *Trichoderma* species on the growth of *Alternaria tenuis* Ness.

Table 3. reveal that growth of *Alternaria tenuis* in presence of *Trichoderma harzianum* was 2.0 cm and percentage growth inhibition was 50.00 while in the presence of *Trichoderma viride* growth was 3.0 cm and percentage of growth inhibition was 25.00. The growth of *Alternaria tenuis* on control plate was 4.0 cm. It is clear that percentage of growth inhibition was maximum by *Trichoderma harzianum.*

Conclusion

Seed act as a carrier for transport of seed borne pathogens harmful to the plants. The abundance of seed viability of seed borne fungi is responsible for loss in seed viability and poor growth of the crop, which brings about varied pathogenic effects on plants. Biological control is an ecology-conscious, cost effective and sustainable alternative method in disease management. As reported by many researchers that *Trichoderma spp.* produces different antibiotics which suppress the disease causing pathogens. The ability of fungal trichodermin, trichonitrin, glycodin and viridin suppress to seed borne pathogens could be of significant agronomic importance. We conclude by describing future prospects for using biological control to limit the damage of seed borne pathogens in both conventional and organic agriculture.

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Table 1: Effect of *Trichoderma* species on the growth of *Aspergillus flavus* Link. in Dual culture

Sr. No.	Fungal antagonists	Growth of *Aspergillus flavus* against fungal species in cm	% of growth inhibition
1	***Trichoderma harzianum***	4.0	46.66
2	***Trichoderma viride***	4.7	42.00
3	Control	8.5	-
	S.E. ±	0.89	2.35
	C.D. at p=0.01	8.99	160.60
	C.D. at p=0.05	3.90	29.86

Table 2: Effect of *Trichoderma* species on the growth of *Fusarium oxysorum* Schlecht. in Dual culture

Sr. No.	Fungal antagonists	Growth of *Fusarium oxysorum* against fungal species in cm	% of growth inhibition
1	***Trichoderma harzianum***	2.9	58.86
2	***Trichoderma viride***	3.5	41.66
3	Control	6.0	-
	S.E. ±	0.84	6.26
	C.D. at p=0.01	8.34	398.51
	C.D. at p=0.05	3.61	79.56

Table 3: Effect of *Trichoderma* species on the growth of *Alternaria tenuis* Ness. in Dual culture

Sr. No.	Fungal antagonists	Growth of *Alternaria tenuis* against fungal species in cm	% of growth inhibition
1	***Trichoderma harzianum***	2.0	50.00
2	***Trichoderma viride***	3.0	25.00
3	Control	4.0	-
	S.E. ±	0.46	8.86
	C.D. at p=0.01	4.56	564.02
	C.D. at p=0.05	19.17	112.61

Inhibitory Effects of *Trianthema Portulacastrum* L. (Aizoaceae) on Crop Seedlings and Detection of its Allelochemicals through 'Fourier Transform Infrared Spectroscopic Analysis (FT-IR)'

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Abstract : *Trianthema portulacastrum* L. is a succulent annual herb. It is native to several countries (including Africa, north and south America) ranging from tropical to sub-tropical zones and also present in many other countries as an introduced species. It is widely distributed in India. In grows in different habitat, including cultivated land as a weed. *Trianthema portulacastrum* has been reported as a dominant weed in seasonal crops (Sesame, jute) of the study area (Burdwan, West Bengal). In present work allelopathic effect of Trianthema was tested on sesame and jute seed germination and seedling growth. The aqueous extract and leachate of whole plants, in different concentration gradients (1:2.5, 1:5, 1:10 and 1:20 w/v) along with a control set were used to conduct the experiment and found to have significant inhibitory effects on germination and seedling growth. In the second part of work, FT-IR analysis of methanolic extract of leaf sample was done to detect the allelochemicals responsible for the adverse effect of Trianthema. Results showed that leaf contains a wide range secondary metabolites, such as phenolic compounds (gallic acid, caffeic acid and ferulic acid), Anthraquinones and different aliphatic hydrocarbons (alkane, alkene and alkyne). FT-IR spectrum revealed characteristic wave number for each functional groups of several compounds present in test sample.

Keyword: *Trianthema portulacastrum*, allelochemical, FT-IR, secondary metabolites.

Introduction

The phenomenon of allelopathy is known for over 2000 years (Rice, 1992). Molisch (1937) coined the term "Allelopathy" from two Greek words "allelo" and "pathy" meaning "mutual harm", but he described allelpathy as both beneficial and harmful biochemical interaction between organisms. "The allelopathic compounds are secondary plant metabolites, including a variety of compounds, which are released from plants into the environment by means of four ecological processes: volatilization, leaching, decomposition of plant residues in soil and root exudation" (Chang-Hung Chou). In many plants secondary metabolites plays an important role in defence (Mazid *et al*., 2011). In naturally occurring environment distribution and abundance of a particular species may depend on the allelopathic interaction within the plant community. Allelopathy can be a significant factor in the adaptation of a new species in different places as an invasive species (Chou, 1999; Mallik, 2003; Field *et al*., 2006; Inderjit *et al*., 2006; Zheng *et al*., 2015; Cheng and Cheng, 2015).

Materials and Methods

Preparation of Test solutions for germination Test: Whole plant extract and leachate of the weeds was used in different concentration gradient as test solution. Experimental procedures conducted by Putman & Duke (1978), Datta & Ghosh (1987), Acharya (1998), Kadir (2001), Ghosh (2006), Sutradhar *et al*., (2017) was followed.

Freshly collected whole plants of T. Portulacastrum were collected from fields, washed thoroughly in distilled water to remove all the soil particle. For the extract, 100 gm of whole plant, including the root portion was grinded properly with distilled water and filtered through Whatman (No. 1); made to 250 ml using distilled water. This forms the stock solution (1:2.5). Similarly for leachate 100 gm of fresh plant material was soaked in distilled water for 72 hours and filtered. Leachate solution was made up to 250 ml with distilled water to get the stock of leachate solution (1:2.5). Different concentration gradients (1:5, 1:10, 1:20 w/v) were prepared for both the solution by subsequent dilution of the stock with distilled water.

Germination Test: Mature and healthy seeds of test crop plant (locally cultivated variety) were used to conduct germination tests. Germination tests for sesame and jute seeds were performed in the month of April- May and June respectively. Crop seeds were cleaned properly and were allowed to germinate in different concentration of test solutions (1:2.5, 1:5, 1:10 and 1:20 w/v) of *T. portulacastrum,* separately for extract and leachate in petri dishes on filter paper. . All the sets were kept in room temperature (± 32° C) under normal light during the day and dark at night. Distilled water was used in control set.

Sample preparation for FT-IR analysis: Mature and healthy plants of *Trianthema portulacastrum* L. (Aizoaceae) including roots were collected from cultivated fields of test crop plant. Washed thoroughly in distilled water to remove all the adhere particles. Plant materials are air dried under shed for 20-25 days. After that 10 gm of the dried plants were extracted in 100 ml of 95% methanol. The resultant extract was filtered with the help of a vacuum filter and the

filtrate was defatted using hexane. The methanolic portion was dried and dissolved in distilled water for analysis.

FT-IR spectra Analysis: The methanol extract of the plant was mixed with KBr salt using a mortar pestle and compressed into thin tablets and IR spectra and peaks were recorded on a Perkin Elmer FT-IR (model RX1) spectrometer between 4000-400 cm^{-1}. Each analysis was repeated two times for confirmation (Banerjee *et al*., 2016; Das *et al*., 2017).

Results and Discussions

Initially germination percentage, liner length of radicles and plumules were recorded, subsequently length of roots and shoots of seedlings were recorded. Important changes during the development of seedlings under the influence of test solutions and control set, like formation of root hairs, lateral roots and leaf emergence, growth of main roots and abnormalities were also observed and recorded.

Results of the experiments revealed that aqueous extracts and leachates of *Trianthema portulacastrum* has severe allelopathic effects on seed germination and growth of root and shoot of sesame and jute seedlings.

Effects on Germination%, Mean root length & Mean shoot length: First germination was seen in control set after 3, 5 days of sowing in sesame and jute respectively. It was revealed that aqueous extract and leachate in the high concentration i.e. 1:2.5 w/v almost completely inhibit (2% and 0%) the germination of sesame seeds. In case of jute these values varies in between 32-36% in present work. **Fig 1** represents the effects of Trianthema on germination % of sesame (Fig I,a) and jute (Fig I,b) in extract and leachate solution. Germinated seeds in test solutions showed further growth with retarded radicle and plumule growth. In **Fig 2(a)** we can see that, in highest concentration (1:2.5 w/v) of extract and leachate radical growth of sesame was totally reduced. In lowest concentration (i.e 1:20 w/v) it was 6.899 mm (extract) and 1.538 mm (leachate) respectively. Jute seedlings showed highest mean root length (27.76 mm) in 1:20 w/v (i.e lowest concentration of test solution) but lower than the control set (32.94 mm) and lowest mean root length (3.12 mm) in 1:2.5 w/v (highest concentration of leachate solution). After germination of sesame seeds no plumule growth was found in 1:2.5 w/v sol of extract and leachate **(Fig 3, a)**. Highest value for mean shoot length of sesame (6.267 mm) was found in 1:20 w/v (extract) and lowest value was 0.357 mm in 1:5 w/v (leachate). In comparison with control set (35.29 mm) highest mean shoot length of jute seedlings was 32.25 mm in 1:20 w/v (leachate) and lowest was 9.5 mm in 1:5 w/v test solution of extract.

FT-IR analysis: FT-IR analysis of methanolic extract of leaf sample revealed that leaf contains several secondary metabolites, including phenolic compounds, anthraquinones and aliphatic hydrocarbons. These secondary metabolites are potent allelochemicals in several plants. FT-IR spectrum revealed the presence of phenolics (presence of phenolic O-H) with characteristic wave number at 3387.00 cm^{-1} (**Fig 4**). Anthraquinones, presence of hydrocarbon (C-H) stretch in the spectrum with characteristic wave number at 2922.16 and 2850.79 cm^{-1} supports the efficacy of the plant as an allelopathic plant. Spectrum also showed the peak of Alkyne stretch (with characteristic wave number at 2108.94cm^{-1}), Alkene (C=C) stretch (with characteristic wave number at 1631.78 cm^{-1}), Alkane (-C-H) bending (with characteristic wave number at 1398.39 and 1365.60 cm^{-1}). Results supports the efficacy of the plant as a potent alleopathic one.

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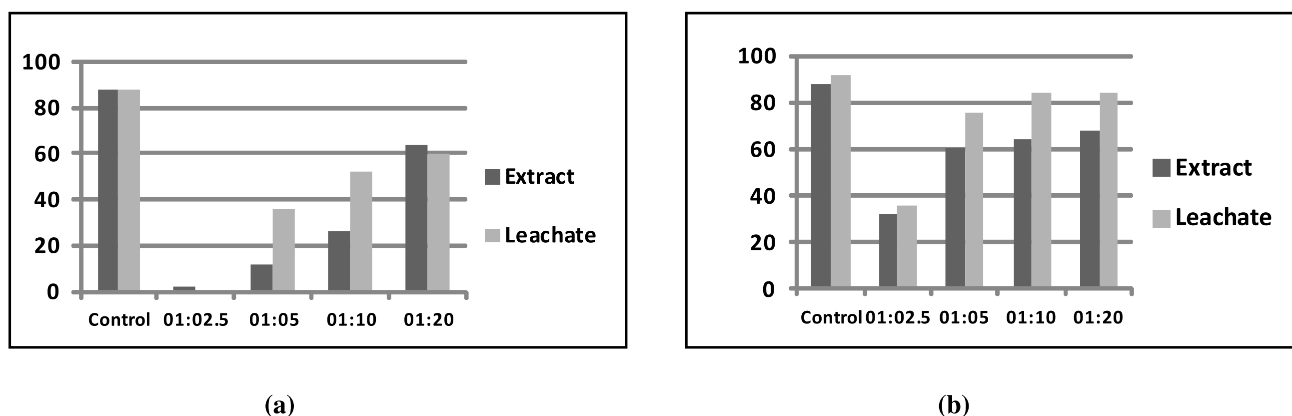
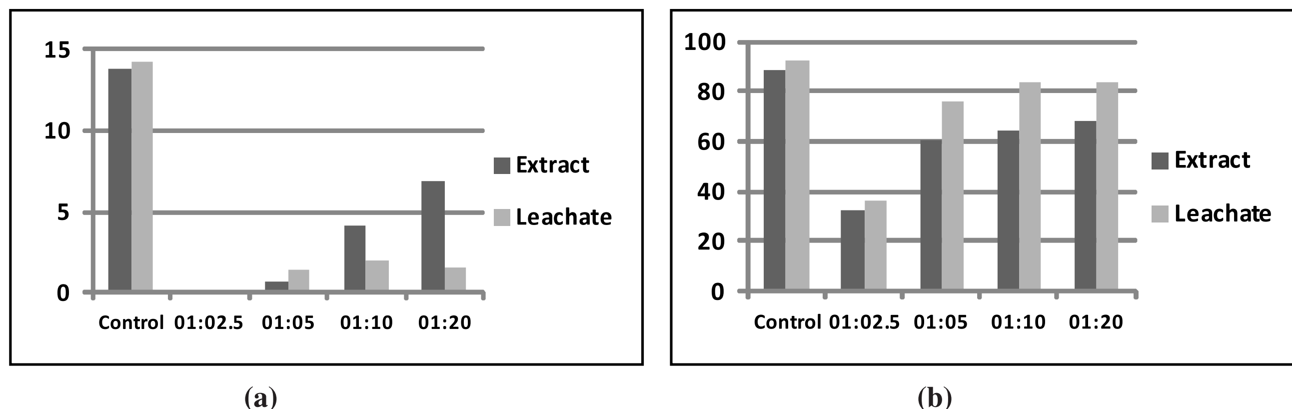
Figure 1: Effect of *Trianthema portulacastrum* L. extract and leachate on Germination % of (a) sesame and (b) jute.



(a) (b)

Figure 2: Effect of *Trianthema portulacastrum* L. extract and leachate on Mean root length (mm) of (a) sesame and (b) jute



(a) (b)

Figure 3: Effect of *Trianthema portulacastrum* L. extract and leachate on Mean shoot length (mm) of (a) sesame and (b) jute

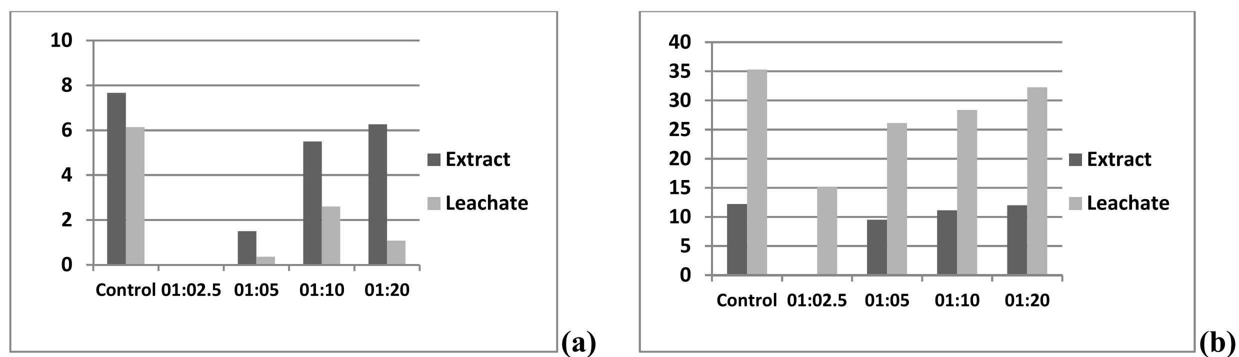
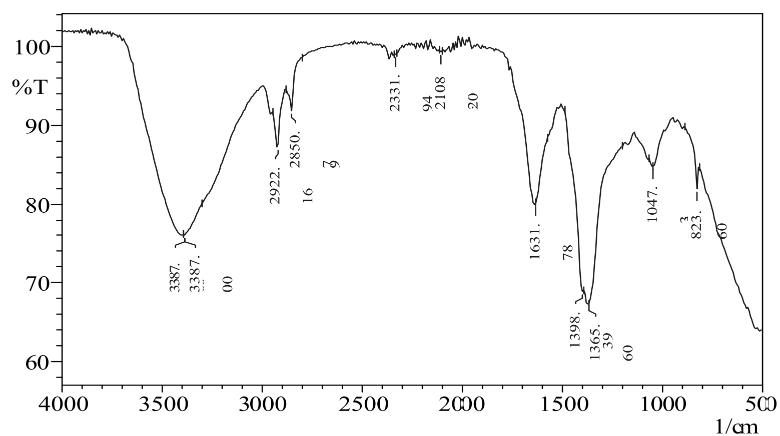


Figure 4 : FT-IR spectrum of methanolic extracts of leaves of *Trianthema portulacastrum* L.



Remedial Attempt To Improve Soil For Cultivation

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Abstract: Plants need salts to complete their metabolic processes. Too much of salt affects adversely on plant growth, whereas some soils are deficit in salts. The attempt is made to remediate soil with leachate obtained from fish decomposition for the growth of wheat. The soil was treated with leachate obtained from fish decomposition and was analysed for its physico – chemical properties. Wheat was sown in the treated soil, taking soil from the nearby land piece as a control. In first set, the salt treatment was initiated before germination and in second set it was introduced after germination. The effect was seen in terms of growth and photosynthetic pigment. Treated soil was proven to improve growth when the salt stress was initiated before germination.

Keywords: chlorophyll, organic carbon, nitrogen, antifungal

Introduction

Soil is very important natural resource that cannot be renewed in human timescale (Jenny 1980). The nature of the soil is changing and it depends on various activities like irrigation, land use pattern, crop, use of fertilizer etc. The soil needs to be remediated as per the requirement of the soil and crop.

Soil is a major source of nutrients to plants and serves as a medium to grow. Due to increasing soil pollution, soil gets contaminated and loses its vital nutrients like Phosphorus (P), Magnesium (Mg), Potassium (K), Nitrogen (N) etc. and also increases soil salinity. The soil needs to be replenished before a next crop is taken. To remediate soil the compost, generally cow-dung and the chemical fertilizers are applied as per the requirement of the soil. Application of vermicompost and goat droppings is also very effective (Zucco *et al*., 2015, Osuhor *et al*., 2002).

Fish waste generated in local markets is one of the major problems. The industrial fish waste is often treated and is converted into to animal feed and several other by-products. But the waste generated at local market creates nuisance. The proper management of this fish waste is necessary. The present work focuses on the anaerobic decomposition of fish and use of its leachate to remediate soil so as to see whether it can help in improving salt tolerance by the crop.

Materials and Methods

Preparation of leachate: The fish waste including fish viscera, fillets, scales were collected from the local market and were digested anaerobically. The digester was designed as shown in fig.1. The initial culture was provided with a small quantity of jiggery.

Treatment of soil: The fish leachate obtained was used to treat soil. The soil was collected from the nearby land piece. Approx. 5 kg soil was mixed with 50 ml of fish leachate and left for incubation for a month. Both soil samples, i.e. control (Untreated) and treated were analysed for their physicochemical properties.

Pot assay: The pot assays were performed where wheat was sown in the pots. Two sets of pots were made. Set 1 was watered with saline water of 10 mM, 20 mM and 30 mM concentrations respectively from the beginning of the seed germination. Set 2 was watered with saline water of salinity 10 mM, 20 mM and 30 mM respectively after germination of seeds. The different growth parameters of plant were studied.

Antifungal and antibacterial assay: The fish leachate was also studied for its activity against soil flora and two plant pathogenic fungi i.e. *Fusarium* and *Curvularia* by disc diffusion method so as to understand whether it is affecting normal soil flora and can it be used as antifungal agent against plant pathogen.

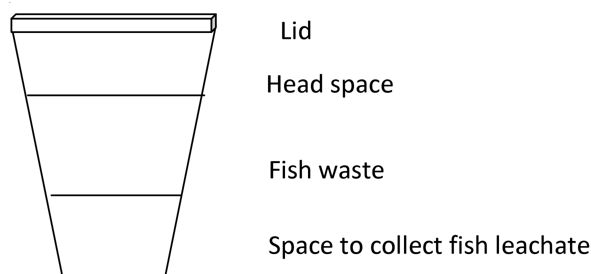

Fig 1. Design of a digester to decompose fish waste

Results and Discussion

Physicochemical parameters

The soil was analysed for its physicochemical parameters and the results were as shown in the table 2. The pH has become slightly less than control which helps in

dissolution of mineral nutrients. Irrigation with water containing high carbonates and bicarbonates turn the agricultural soil alkaline. The nutrients like nitrogen, potassium, calcium, magnesium and sulphur are available within soil pH 6.5 to 8. The reduced pH towards neutrality indicates that soil becomes more suitable for these main nutrients. The micronutrients such as boron, copper, manganese, nickel and zinc are also available at low pH ranging from 5 – 7 (Bolan and Brennan, 2011).

Conductivity of the soil was also found to be increased (Table 2) from 1.10 dsm"1 to1.33 dsm"1Conductivity is a function of the amount of electrolytes (charge carrying particles) in the solution. The more electrolytes the higher the conductivity. EC denotes the presence of salts. Very high conductivity increase the salt stress however slight increase in the current shows the availability of salts is been increased. However, it fails to tell which salts contribute conductivity. Higher conductivity hinders the absorption of plant nutrients due to increased osmotic pressure whereas lower conductivity may severely affect plant health and yield (Anon, 2002). The present value shows that the given conductivity levels are critical to salt sensitive plants (Kadam, 2016).

Chlorides were estimated for both treated and untreated soil. The treated soil showed slightly higher chlorides than untreated. This could be because of several minerals leached from the fish waste during decomposition. Electric conductivity also could be the function of several ions released during decomposition procedure.

There was slight increase in the concentration of organic matter and organic carbon which was more in treated soil. The nitrogen content was also slightly higher in treated soil. The carbon: nitrogen ratio was also reduced in treated soil.Ideally the carbon to nitrogen ration for compost is expected to be 25 – 30:01. The higher values indicate that there is lesser amount of nitrogen to support microbial growth. Lesser C:N ratios may result in stinking odour due to excess ammonia. The leachate that was used to treat the soil has C:N 21.94:1 indicating more amounts of nitrogen. Similarly, the nitrogen concentration of leachate was 1.0501% (table 1). Generally the C:N ration should be 10:1 in aerated soil.The ratio is reduced in treated soil because of the higher nitrogen present in the leachate. Studies on possible interactions between salinity and N fertilization have focused mainly on establishing optimal N application under saline conditions, rather than on its direct prevention of salinity hazards. Nutrient uptake in saline soils might be low due to high concentrations of cations and anions which might compete with the uptake of nutrient ions (Fageria *et al*., 2011). Chen *et al*. (2010) found that N content in cotton plant tissues increases when N fertilization is increased under medium salinity levels (2.4 to 7.7 dS m"1), but is not influenced at the high salinity level of 12.5 dS m"1 . Nadian *et al*. (2012) showed that the negative effect of salinity (4 to 8 dS m"1 in the irrigation water) on sugarcane yield and root growth is partially alleviated when the rate of N fertilizer is increased from 200 to 400 kg ha"1. However, the higher rate of N fertilizer also improved K uptake and increased leaf proline content. In other study, Garg *et al*. (2006) found that increased N application, up to a rate of 60 kg ha"1, to Indian mustard plants irrigated with saline water (2–10 dS m"1) increased shoot concentrations of N, P and K and decreased Na concentration. The plants also displayed higher photosynthetic rates and more efficient N metabolism resulting in improved plant growth and seed yield. It was found in his experiment that the increase in N levels enhanced stomatal conductance and chlorophyll, malondialdehyde, and water content in the plant tissues.Similar results were found with chickpeas grown in a recycled nutrient solution, when increasing nitrate level up to 11 or 18 mM NaNO3 at salinity levels of 12 or 36 mM NaCl increased the chickpeas yield (Tavori *et al*., 2004). Edelstein *et al.* (2009) studied the interaction between fertilization and salinity (1 to 6 dS m"1 in the irrigation water) on foliage biomass of vetiver (*Vetiveria zizanioides*) grown in pots in a heated greenhouse. They found a significant, negative linear regression (Y = 1.6 – 0.08X, r2 = 0.6) between soluble NO_3 " and soluble Cl" concentrations in the plant foliage, indicating that increasing NO_3 fertilization can decrease the uptake of Cl by the plant. By keeping all these findings in mind the leachate with more nitrogen is used to remediate saline stress.

The treated soil also showed increased phosphate concentration which is one of the essential micronutrient for plant growth.

Pot assay

Set I: It is the set of treated and untreated soil watered with saline water right from the sowing of the seeds. The root length and shoot length was seen more at the saline treatment 10 mM in treated as well as untreated pots where as the growth was stunted with increased saline treatment in either cases (fig. 2a). It suggests that the salts were less in the soil which was compensated by saline treatment. The growth was comparatively more in treated pots than untreated when watered with saline solution suggesting that treatment with leachate has increased the salinity tolerance though it was not as good as control of treated and untreated soil.

The chlorophyll concentration also was found to be more in treated pots in control set which was watered with normal water. As the saline stress increased, the decrease in cholorophyll a, b and total chlorophyll was seen in both treated and untreated pots (fig 3a). However the chlorophyll is comparatively more in treated pots than untreated pots.

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Set II: It is the set of treated and untreated soil watered with saline water after germination of the seeds. Treated soil pots watered with normal water grew more than untreated soil showing more root length and shoot length (fig. 2b). However, further saline treatment in treated pots stunted growth. The untreated set has showed more growth when watered with saline water after germination.Treated soil showed decreasing chlorophyll content with increasing salt stress.

The results of pot assay can be concluded as if the irrigation water is saline, then treatment with leachate improves salt stress. But if the salinity stress is introduced after germination then better growth is seen untreated pots and the growth is in proportional to the salt stress. However, further investigation is needed to prove these findings.

Antimicrobial assay

The assay was performed to see whether leachate affects on the normal soil flora. There was no growth inhibition even after treatment with leachate indicating no negative impact on soil microflora. The potential of the leachate was also tested to see whether it can be used as antifungal agent against two plant pathogenic fungi, viz. *Curvularia* sp. and *Fusarium* sp. The growth inhibition was seen in the area treated with leachate.

Conclusion

The fish can be decomposed anaerobically by using specially designed digester. The leachate thus obtained has potential to be used as a mean for the treatment of soil. It also can be used as preventive measure against plant pathogen. Further study is required in this field.

Acknowledgement

The authors are grateful to the Principal, D.G. Ruparel College for providing support to carry on the research. We are also thankful to the Head, Department of Zoology, D.G. Ruparel College for her moral support. We thank to Mr. Mayur Kale, Mr. Rohit Inkar and Ms. Shruti Koli for helping us in designing the digester.

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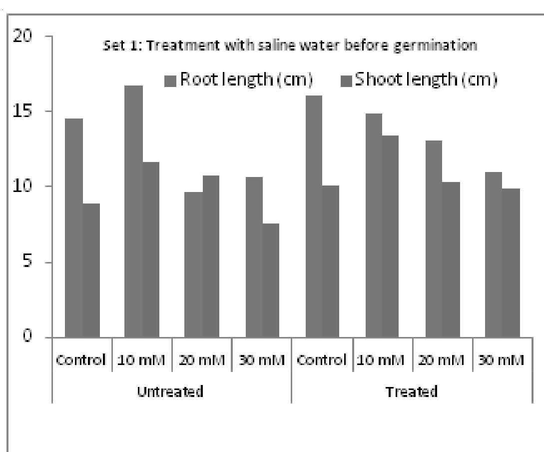
Table 1: Physicochemical parameters of leachate

Parameter	Result
pH	8.5
Total Nitrogen	1.0501%
Available potassium	25.15%
Available phosphorus	5.374 mg/l
Cation Exchange capacity	1.0073 meq/100g
Total carbon	23.04%
Organic matter	40.1%
Chlorides	620 mg/l
Conductivity	777 ìs
C/N ratio	21.94

Table 2: Physicochemical parameters of soil

Parameters	Before treatment	After treatment
pH	8.23	7.98
conductivity	123.8 ìs	150 ìs
Chlorides	1.10 dsm¯1	1.33 dsm¯1
Organic matter %	4.497	4.5944
Carbon %	2.609	2.665
Nitrogen %	0.26	0.30
Phosphorus mg/l	2.6	6.13
C/N ratio	10.03	8.88



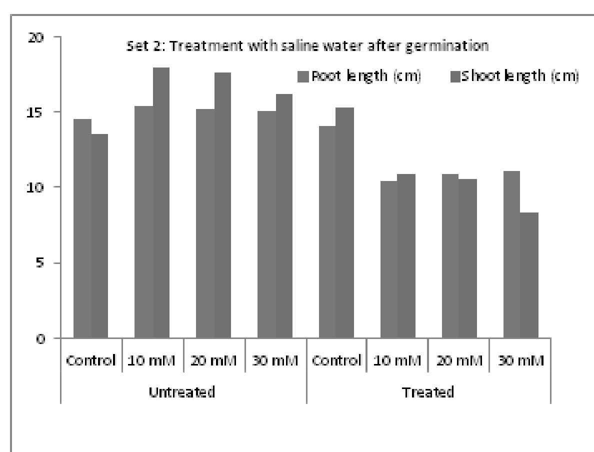
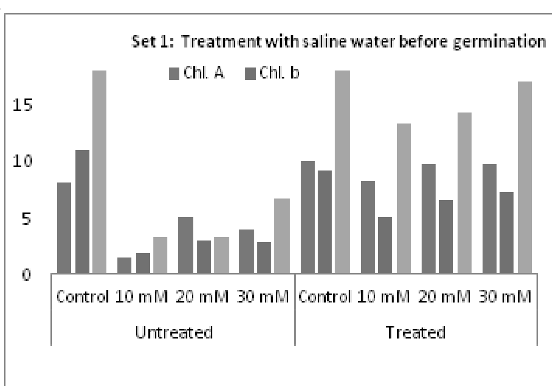


Fig 2a

Fig 2b

Results of pot assay indicating root length and shoot length



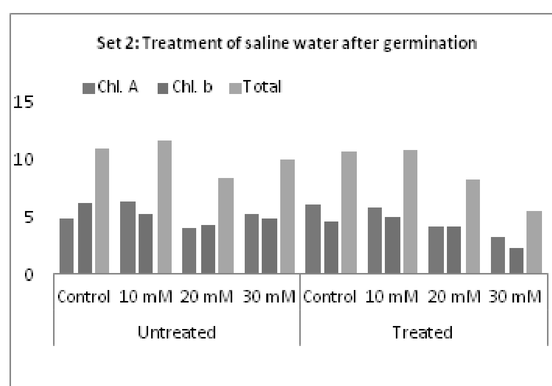


Fig 3a

Fig 3b

Results of pot assay indicating chlorophyll content variation

Effect of Sewage Irrigation on Soil as Abiotic Component and Roots as Biotic Component on *Raphanus sativus* (L.) Domin, Cultivated along Railway Tracks in Mumbai

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Abstract: The green vegetable growers along the railway tracks use polluted sewage water from nearby industries for irrigation of their farms. This highly polluted sewage water shows adverse effects on edible vegetables. Different solid wastes like corroded railway tracks, cement beds, wires, tins, railway poles are disposed along the agricultural farms since many years. The selected area was near Vikroli railway station, one of the eastern suburbs along the central railway main line. The research work proposed here basically aims to investigate soil as abiotic factor and roots of *Raphanus sativus* (L.) *Domin*. As biotic factor . That showed high levels of heavy metal toxicity caused by Iron and Chromium as a result of phenomenon of biosorption caused heavy metal pollution analysed through Atomic Absorption Spectroscopy (AAS) and Flame photometry. The most significantly, high levels of alkali metals like Sodium and Potassium were also present in sewage samples, which can lead to dangerous portions leading to biomagnifications in the trophic levels and subsequent risk of enterance in the food-chain and thereby of global concern.

Keywords: Biosorption, Atomic Absorption Spectroscopy (AAS), Flame photometry, biomagnifications.

Introduction

India is developing country.After 1991 LPG policy (Liberalization, *Privatization & Globalization) helps to increase the industrialization on large scale. More* number of people settles in small Different anthropogenic activities always creates problems for environment natural cycles & trees. Due to these anthropogenic activities human health also has hazardous effect. . Due to more population, there is large demand of basic needs for all. The demand of fresh vegetables is more from nearest wholesale market in such mega cities. Mumbai city is intra-connected with local railway lines. Huge areas were occupied by the railway ministry to build railway tracks. The narrow width farms along these railway tracks are used by farmers for cultivating vegetable crops. As told by farmers, the farms are transferred in the name of the farmers on contract by Railway Ministry of India.

The farmers along the railway tracks use polluted sewage water of industries for irrigation of their farms. This highly polluted sewage water shows adverse effects on the plants. (Khan *et al*, 2007). In one more study, different kinds of solid wastes like corroded railway tracks, cement beds, wires, railway poles, etc. are disposed along the agricultural farms since many years. These materials get degraded & corroded since many years. Such solid wastes are increasing every year & are neglected there of. The selected study area is near Vikhroli railway station (one of the eastern suburbs along the Central Railway main line) besides the railway tracks. The area of the farm is approximately 3 acres. The source of sewage water i.e. gutter flows through this farm, which is the only source of irrigation to farmers. The Godrej industry & Cipla pharmaceutical company are located near the farm. In all these conditions, farmers have been cultivating vegetables using sewage water since many years. These reasons were strong enough to narrow down on abiotic parameter analysis of heavy metals & alkali metals in these agriculture farms of Vikhroli railway tracks.

The study area comes under the zone no. 8 of Indian railways i.e. Central Railway (Mumbai).

The coordinates of the study area are as follows:

Latitude: - 19.109935 / 19°6’35.77” N

Longitude: - 72.928393 / 72°55’42.22” E

Latitude: - 19.108026 / 19°6’28.89" N

Longitude: - 72.927887 / 72°55’40.39" E

The study area is flanked by the Cipla Pharmaceuticals & Godrej Factories. The area is spread across 3 acres which is registered in the name of a farmer Mr. Krishna Kumar Chauhan since last 16 years. He grows various vegetable crops like *Raphanus sativus* (Radish), *Spinacia oleracea* (Spinach), *Amaranthus viridis* (Matth), *Trigonella* spp. (methi)

Materials and Methods

The samples collected from the field under study were brought to the laboratory and preserved for analysis. The collected samples were filtered with Whatman filter no-41to remove dust and particulate matter. Qualitative tests for heavy metal detection were performed. The physical parameters like Ph, electrical conductivity, turbidity were measured. The quantitative estimation using Atomic Absorption Spectroscopy (AAS)(iCE3000) was carried out,

for which sample was prepared by diacid digestion. Alkali metals like Sodium and Potassium were determined using flame photometer. Display readings were plotted as graph to determine unknown concentration of the sample.

Result and Discussion

Estimated Ph values of soil and water samples were 7.56 for soil and 7.75 for water samples in dry season. During wet season the values were 7.29 for soil and 7.41 for water. Thus all the samples were slightly alkaline.

Discussion

The investigation of the effects of sewage irrigation on soil and cultivated crop i.e *Raphanus sativus* (root) was carried out for two heavy metals, Iron and Chromium using atomic absorption spectroscopy, whose samples were collected from study area near Vikhrol i railway station. The estimation of alkali metals like Sodium and Potassium were also carried out for sewage water. Initially, it was hypothesized to detect the possible contamination of heavy metals through sewage in soil, and from soil to crop which would cross the permissible limit. But the analysis of results revealed different contamination levels in dry and wet seasons. The study revealed that the interactions of every biotic and abiotic component with Iron and Chromium as heavy metals are different. Khan (2007) also concludes that sewage irrigation led to elevated levels of heavy metals in soil and in edible part of food crops. Different researchers all overfound varied results of metal concentrations in plants under different conditions with respect to permissible levels .The preliminary chemical tests did not reveal the presence of any heavy metal in the samples but it was logically expected to contain trace amounts of heavy metal in it owing to the apparent conditions. To confirm this, a quantitative estimation by Atomic Absorption Spectroscopy was also carried out. The pH of the sewage in both wet and dry season was found to be slightly alkaline Iron content in sewage was very higher than permissible limit which means high contamination in sewage water. Surprisingly, chromium content was completely absent in sewage. In case of soil, Chromium was found to be much within the standard permissible limit indicating its least contamination. On the other hand, Iron was found to be on a higher side but still within the standard permissible limit.

In case of crop i.e. *Raphanus sativus* (root) Iron was within the standard ermissible limit, but Chromium was detected more than standard permissible limit in wet season. In dry season there is no accumulation of Chromium in crop. So it revealed that the amount of Chromium is more in wet season than dry season. It could be due to the plant not being able to evade chromium uptake in wet season as compared to that in the dry season. There can be many reasons attributed to this with respect to the physiological or anatomical aspects of the plants. In the estimation of Sodium and Potassium by flame photometry, they were found to be tremendously higher than the standard permissible limit. It clearly depicts how the effluents discharged in the soil and the crops adversely affect the components of the ecosystem..

Conclusion

The overall fact that comes into picture after carrying out this study is that the biotic and abiotic factors of the ecosystem under consideration is badly affected due to the discharge of the effluents and the sewage from the factories surrounding it. Following is the gist of the various parameters and their revelations that were highlighted in this work.

The pH level of the sewage that was discharged from the factories was found to be slightly alkaline.

Heavy metals were detected to be almost absent in the preliminary qualitative tests.

However, trace amounts of iron and chromium were found to be present in the soil, water and plant root samples.

Quantification of the heavy metals using AAS revealed the presence of trace amounts of iron and chromium in all the samples in varying proportions, but overall more than the standard permissible limit, indicating its alarming bioaccumulation in the ecosystem.

The most significantly high levels of alkali metals, sodium and potassium were found to be present in the sewage samples, which can lead to dangerous proportions of biomagnifications in the trophic levels, ultimately degrading the health of the various factors of the ecosystem.

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Table 1: Estimations of different parameters from water and soil sample

Parameters	Wet season	Dry season
Iron content in water sample	2.86 mg/gm	11.5 mg/gm
Iron content in soil sample	11.8 mg/gm	47.9 mg/gm
Chromium content in soil sample	0.05mg/gm	0.03mg/gm
Iron content in *Raphanus* roots	0.02mg/gm	0.1mg/gm
Chromium content in *Raphanus* roots	0.02mg/gm	Zero mg/gm
Sodium content of sewage sample	480mg/lit	600mg/lit
Potassium content in sewage sample	20mg/lit	80mg/lit

Study of Succulents Plants Growing in Gandhinagar, Gujarat, Ahmedabad

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Abstract: Succulence is an adaptive response to drought, rapid drainage in rocky and sandy soil, high evaporation in windy, hot environments and in salty or alkaline habitats. There are probably more than five thousand species worldwide. Succulent plants are increasingly popular among plant collectors, home gardeners and professional landscapes for colorful leaves, sculptural shapes, simple care, etc. Succulents are widely used for the indoor gardening as well as outdoor gardening for their outstanding appearance. The visits were conducted across various gardens; nursery besides theses also visited some people who has a personal collection of cactus and succulent. During field work the photos of each succulent were taken along with habit, stem, leaves and other floral parts. The study reported 10 species of succulent plants from Gandhinagar.

Keywords: Diversity, Succulent, Gandhinagar

Introduction

Succulent plants have a global distribution and represented in nearly all habitat type. Over 30 botanical families have succulent plant species, ranging from tiny annuals plants to huge tree (IUCN, 1997).

The "Succulent Karoo" of South Africa and Namibia boasts the richest succulent flora of Earth. Mexico is the country with the highest diversity of cactus in the American continent (Ortega and Hector, 2006). More than 60 species are listed in the red Data Book of the International Union for the Conservation of Nature (IUCN) (IUCN, 2003). Many of these species has an outstanding biological, cultural and economical importance. Several species of cactus are among the most dominant plants in different vegetation types, where they interact with a large variety of animal and plant species (Hector *et al*., 2003).

All the succulent evolved from other related plants growing in a normal environment by adaptation to the changing climatic conditions of their habitat, especially the regularity and amount of rainfall. This process of adaptation varied in every family and doubtless many plants succumbed in the struggle for survival. Water is essential for the growth and life of all vegetation, including the succulents, which have mastered the art of economizing water (Rudolf, 1980). In geological times, the earth's climate changed becoming drier as the mountains were pushed up to create rain shadows and deserts. Other plant families adapted similarly to these conditions and there arte thousands of succulent species (Edwards and Donoghue, 2006).

Succulent plants are increasingly popular among plant collectors, home gardeners and professional landscapes for a number of reasons. With their colourful leaves, sculptural shapes and simple care, succulents are beautiful yet forgiving plant for pots (Debra, 2010). Succulents are highly diverse. The present study shows diversity in the succulents. They are highly ignored by taxonomist in Gujarat just because many of them are ornamental. Nowadays succulent are used in outdoor and indoor gardening at various place like malls, industries, colleges, hospitals and gardens in the city of Ahmedabad. So there is urgent need to have documentation of such ornamental groups also.

Materials and Methods

The study deals with the diversity of succulent plants growing in Gandhinagar, Gujarat. Succulents are highly diverse. The present study shows diversity in the succulents. They are highly ignored by taxonomist in gujarat just because many of them are ornamental. So there is urgent need to have documentation of such ornamental groups also. Field notes were taken to have information on habit, habitat and characteristics of succulents. All the specimens were identified with the help of available literature (Anderson, 2012, Mary, 2000, Qureshimatva, 2016, Rudolf, 1980, Scott, 2012, Shah, 1978).

Results and Discussion

In the present study 10 genera and 10 species belonging to 10 families have been reported from the study area. In the present study 5 succulents were reported as indigenous and endemic to India. Other succulents were introduced from the Madagascar, Southern Africa, Brazil, Europe, Mexico, Tropical America, etc.

Study of Succulents plants of Gandhinagar, Gujarat, Ahmedabad.

1. *Adenium obesum*

Common Name: Red sandalwood, Desert rose

Plant Family: Apocynaceae

Habit: A small, evergreen, treelet grown in pots.

Stem: Thick gouty stem of slow growth rapidly diminishing to short stout branches.

Leaves: Spirally arranged, clustered, near the tips, simple, entire, leathery.

Flowers: Bright rosy oleander like flowers. Petals 5 in number, typically red to pink coloured having a whitish blush at the throat.

Flowering and Fruiting time: March- April.

Significance: It is mostly grown in pots as an indoor bonsai. It is propagated by stem cuttings.

2. ***Agave americana***

Common Name: Ketki, Vilayati kumvar, Century Plant

Plant Family: Agavaceae

Habit: A large, perennial herb.

Leaves : Radical in a massive rosette , long , broad, thick, prickly at the Margins, sharp – pointed.

Flowers: Flowers only once during life, at any time after many years, 3-6m tall scape wiith large scape and bracts, yellowish green, Borne erect on many horizontal branches. Some flowers are modified into flower - bulbils. Perianth funnel – like with short tube, segments 6. Stamens 6, filaments exserted, anthers versatile. Ovary inferior 3 – celled, ovules many, stigma 3 lobed.

Flowering and Fruiting time: January – June

Significance: Fibers are obtained from the leaves. Leaves are used as a fodder during scarcity.

3. ***Agave salmiana***

Common Name: Giant Century plant

Plant Family: Agavaceae

Habit: Xerophytic rosette succulent with low water requirements.

Stem: Reduced underground stem.

Leaves: Dark green leaves, slightly bluish at their base. The leaves are corrugated and folded towards the apex. Their margins possess sharp pointed spines.

Flowers: Yellow in colour when buds.

Flowering and Fruiting time: July – August

Significance: It is mainly used for decoration purpose because of its ornamental foliage.

4. *Aloe vera*

Common Name: Kuwarpathu

Plant Family: Liliaceae

Habit: Succulent

Stem: Very short on which vase like leaves is arranged in rosette Manner.

Leaves: Radical, very fleshy, broad at base, narrowed from base to apex, pale green with distant horny prickles on the margins.

Flowers: Small tubular yellowish orange. Perianth about an inch or more in length, segment 6, concave. Stamens 6, filaments slender, Anthers versatile. Ovary superior, 3-celled, ovules many, styles exerted, stigma small.

Flowering and Fruiting time: August - December

Significance: Aloe are used as a stomachic and puragative. It is regarded as valuable in the treatment off piles and rectal Fissures. The mucilage is colling and used for poultice for inflammations. The Whole plant is often kept hanging in houses and stables to drive away mosquitoes.

5. *Beaucarnea recurvata*

Common Name: Pony Tail Palm as they are not palms, or even closely related to palms.

Plant Family: Asparagaceae

Habit: An evergreen perennial with a remarkable expanded caudex for the purpose of storing water inside.

Stem: Palm–like and swollen at the base. The swollen trunk is basically a water storage structure enabling. These plants to survive long periods without any water. This water storing capacity is makes these plants 'succulents'.

Leaves: Strap – shaped, recurved and leathery produced in tufts at the terminal end of the stem.

Flowers: Flowers are borne on a large branched inflorescence which bear thousand of minute pale yellow to cream colored flowers.

Flowering time: In spring

Significance: It is generally popular as an ornamental plant, often grown as a household or outdoor plant in temperate climate garden. It is one of the most popular plants in cultivation, for both pot culture and landscaping, throughout the world. It is best known for its response to sound waves, growing faster in loud populated areas.

6. ***Bryophyallum delagoense***

Common Name: Live–Forever, Mother of Millions

Plant Family: Crassulaceae

Habit: A large succulent herb

Stem: Erect, cylindrical

Leaves: Simple, all fleshy, cylindrical, pale green to pale

brown leaves with dark green patches and a shallow groove on the upper surface, with small bulbils at tips.

Flowers: Scarlet, in large racemes, drooping or pendulous in terminal cymes. Calyx tubular, 5-10mm long. Corolla tube to 30 mm long, lobes obovate and about a third as long, salmon- coloured to scarlet.

Flowering and Fruiting Time: February-March

Significance: Cultivated in the gardens.

7. ***Bryophyllum pinnatum***

Common Name: Pan–Futi, Life - plant, Ghamari, Zakhme-Hayat

Plant Family: Crassulaceae

Habit: A succulent herb

Stem: Obtusely 4-angled

Leaves: Variable, decussate, the lower simple, the upper 3-5 or 7-foliate, petiole long, united by a ridge round the stems. Leaflets ovate or elliptic, crenate.

Flowers: Flowers drooping with opposite stout branches. Calyx inflated, cylindric, 4-fid, teeth triangular, green striated red. Corolla reddish–purple, swallon at the base, constricted in the Middle, with 4 triangular lobes above. Stamens 8, in 2 series, epipetalous, filaments green at the base, pinkish, below the anthers, hastate, black. Hypogynous scales 4, free or connate with the carples. Carpels 4, narrowed into the long, green styles. Ovules many.

Flowering and Fruiting Time: January - February

Significance: Cultivated in the gardens. Leaves produce adventitious buds when placed in moist soil. The juice of the leaves is used in diarrhea, dysentery and applied on bruises for its antiseptic and astringent properties.

8. ***Cissus quadrangularis***

Common Name: Hadsankal, Devils backbone, Veldt Grape

Plant Family: Vitaceae

Habit: A succulent herb

Stem: Long, fleshy, deep green, glabrous, young branches sharply angular or winged, tendrils long, simple.

Leaves: Deciduous, ovate or reniform sometimes 3-7 lobed, denticulate, base rounded, stipules ovate.

Flowers: Calyx cup – shaped petals 4, ovate- oblong, hooked at apex, disk 4- lobed. Stamens 4, anthers introrse. Ovary bilocular, cells 2-ovuled, style short.

Flowering and Fruiting Time: June – July

Significance: It is cultivated as hedge plant as well as in gardens. The young stems eaten in curries. The juice of the stem is used in scurvey. The pulp of the stems employed on broken and fractured bones and swellings. It is also useful in eye diseases and chronic ulcers.

9. ***Cissus rotundifolia* Vah**

Common Name: Peruvian Grape lvy, Venezuelan Treebin, succulent grape

Plant Family: Vitaceae

Habit: A Perennial shrub vine

Stem: The stems of the *Cissus rotundifolia* are green.

Leaves: Alternate, simple, fleshy, waxy, toothed and of a deep green color. They are about 6-8 cm in diameter and are slightly heart shaped at the base. The tendrils appear opposite of the young foliage.

Flowers: The small flowers can be either whitish or greenish.

Flowering time: July to October

Significance: The *Cissus rotundifolia* can be used for food purposes. The tiny berries (which are either obovoid or ellipsoid in shape) of the plant are edible and are about half an inch in length, with a color that can be purple or red. Also, the foliage of the *Cissus rotundifolia* can be cooked and consumed as a vegetable.

10. ***Euphorbia neriifolia***

Common Name: Vaad thor

Plant Family: Euphorbiaceae

Habit: A large, fleshy, much - branched shrub.

Stem: Branches rounded, some what verticillate with pairs of sharp, stipular spines arising from low conical truncate, distant spirally arranged tubercles.

Leaves: Fleshy, alternate, obovate, rounded at the apex, smooth, narrow at base, sessile deciduous.

Flowers: Involucers 3-nate, forming small, shortly pedunculate solitary or twin cymes, the central flower is male, sessile, appearing first, the 2 lateral flowers bisexual, pedicellate, lobes large, erect, fimbricate, glands transversely oblong, bracteoles many.

Flowering and Fruting time: March-April

Significance: Common everywhere and often planted for hedges.

Conclusion

We have found total 20 plant species belonging to belonging to 11 different species.Above 10 plants of Botanical Herbal Garden, Gandhinagar were studied were studied im lab as well as on field. They were under strict

observation and their vertical and horizontal sections are studied deeply.

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Growing of Amaranth by Hydroponic Technique

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Abstract: Today our numbers are increasing day by day. There is demand for food and space. To meet the food requirement there is tremendous use of pesticides and chemical fertilizers. The plants are also irrigated with contaminated water. This has affected our health. In order to overcome the said problems the current project was undertaken where by soilless cultivation was carried out using Hydroponic techniques. For the study Amaranth the leafy vegetable from family Amaranthaceae was taken, as this plant is rich in protein, calcium, vitamins etc. The Amaranth plant is also known for its therapeutic potentials in curing cancer, malaria, intestinal disorder etc.

The seedlings were grown in coco peat and the saplings were transferred in to a nutrient rich container. The plants grew in short period of time and healthy too. The pH of the solution was maintained to 5.5 – 6.5. This hydroponically grown plant was compared with the Amaranth plant grown in soil. The result obtained favoured soilless cultivation rather than the plant grown in soil. Thus the attempt to grow Amaranth using hydroponic technique was significant. It yielded safe and nutrient rich leafy vegetables even during off seasons.

Keywords: Amaranth, Amaranthaceae, Hydroponics.

Introduction

Men depend on Mother Nature for food, clothing and shelter. The food should be rich in protein, carbohydrate, vitamins and minerals for better health. To meet these requirements we consume leafy vegetables like spinach, methi, amaranth etc. The leafy vegetables are grown by the farmers in many places so that it reaches the market in proper time. The plants are irrigated by contaminated water as a result it affects our health. One of the leafy vegetable that is in great demand is Amaranth next to spinach. Amaranth is botanically known as *Amaranthus cruentus* (family-Amaranthaceae) (Almeida, 2001). Amaranth is a multipurpose crop whose leaves and grains are tasty and of high nutritional value, additionally it can be cultivated as an ornamental plant. The genus *Amaranthus* has received considerable attention in many countries because of high nutritional value of some species that are important sources of food, either as vegetable or grain (Esther Njeri Muriuki, 2015). In order to grow healthy amaranth crop hydroponic techniques is used in current study. Hydroponics is the practice of growing plants with their roots suspended in water containing mineral nutrient. In other words it is soil less cultivation of plants (Echeverria, Laura Perez 2008).

Materials and Methods

Plant materials: The Amaranth seeds were purchased from Namdeo Umaji shop at Byculla. The seeds were sown in small containers containing cocopeat. It was covered with a net to avoid the damage of saplings.

Hydroponic set up: A plastic container with a lid was taken. The lid was cut in such a way that the small pots can be placed within. The pots were filled with brick pieces as a support for the saplings. The sides of the container were covered with black paper to avoid the light.

Nutrient solution: The nutrient solutions were prepared for healthy growth of plants. It is given in table 1 (Murashige and Skoog, 1962, Munoz and Hector, 2005).

Methodology:

The container was filled with water and 1ml of the stock nutrient solution was added to it. It was then mixed thoroughly. The pH of the water was maintained in between 5.5 to 6. The lid was fixed on the box. The pots filled with brick pieces were inserted into the cut portion. The saplings grown in the cocopeat were transplanted into these pots. It was allowed to grown in proper sunlight. The plants were monitored daily for its growth of leaves as well as roots. The nutrients solution was added every week and the pH of the water was checked. The photograph was taken for evidence.

Results and Discussion

The Amaranth seeds took 10 – 12 days for germination. After few days the saplings were transplanted into the pots filled with brick pieces. In hydroponics the growth of root as well as the growth of plant is faster than normal soil growth. The plant produce by this method shows bigger leaves in a shorter period of time. The leaves were dark in colour and were devoid of any infection. The wastage of nutrient and water were minimized. The plant received the right amount of nutrient at the right time. The Amaranth grew organically- without pesticide and preservative.

Conclusion

In country like India, soil less culture can be used to improve yield and quality and ensure food security of our country. Growth of plant without utilizing land as it is a fast depleting source. It can become a household hobby and

can enjoy the veggies any time of the year without contamination. Progress has been rapid and results obtained have proved that this simple technology is conventional.

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Improvement In Methylene Blue Adsorption of Coir Pith By Anaerobic Microbial Treatment

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Abstract: The discharge of dyes is a major concern due to both hazardous and aesthetic reasons. The textile industry is one of the major sources of dye effluents. Methylene blue, a basic dye, is a commonly used dye and is known to have harmful effects on human health. Coconut coir pith is a low cost agricultural waste obtained during extraction of coconut fibres from husks. It is light, spongy, porous, highly hygroscopic and has a large surface area. In the present study, use of pith as an adsorbent of methylene blue was investigated in a batch process. Coir pith was found to be a promising adsorbent of the dye. Further, the pith was subjected to microbial treatment under anaerobic conditions. There was a considerable increase in the dye adsorption property of microbial treated pith. The effect of parameters such as pH, temperature, amount of adsorbent and time was studied for both untreated and treated pith. FTIR analysis of the dye adsorbed pith was carried out. Surface area of pith was determined by BET analysis.

Keywords: Coir pith, methylene blue, adsorption, microbial treatment.

Introduction

Most of the dyes used in plastic, dyes, textile, paper and pulp industries are synthetic and toxic in nature. The discharge of dyes is a major concern due to both hazardous and aesthetic reasons. The textile industry is one of the major sources of dye effluents. Methylene blue, a basic dye, is a commonly used dye and is known to have harmful effects on human health.

As the control of water pollution has become an increasing importance in recent years, conventional methods for treating dye-containing wastewaters such as membrane filtration, reverse osmosis, coagulation and flocculation are not economically feasible. Low-cost treatment methods have, therefore, been investigated for a long time (Mohammed *et al*., 2014).

Many researchers have proved the capability of agricultural solid wastes as adsorbents to remove many types of pollutants including dyes. Agricultural solid wastes are effective adsorbents for cationic and anionic dyes. Natural agricultural wastes can remove cationic dyes better than the anionic dyes (Salleh *et al*., 2011).

The agricultural solid wastes from cheap and readily available resources such as bagasse, seed shells, fruit peels, cane pith, coir pith, fruit husk and coconut shells have been investigated for the removal of numerous dyes from aqueous solutions. The basic components of the agricultural materials include celluloses, hemicelluloses, lignin, lipids, proteins, simple sugars, water, hydrocarbons and starch, containing a variety of functional groups with a potential sorption capacity for various pollutants. Agricultural waste products are used in the natural and modified form (Gisi *et al*., 2016).

Sintered coconut husk has been used as an effective adsorbent for the removal of Rhodamine B, Methylene B, and ammonium ion (Dabwan *et al*., 2015) Activated carbon prepared from coir pith and coconut shells has also been used for dye adsorption (Santhy *et al*., 2006). However, preparation of activated carbon and sintered products is not economically feasible and the process itself employs the use of hazardous chemicals. Coconut coir pith is a low cost agricultural waste obtained during extraction of coconut fibres from husks. It is light, spongy, porous, highly hygroscopic and has a large surface area. It makes up about 70% of weight of coconut husks.

In the present investigation efficacy of coir pith as an adsorbent of aqueous solution methylene blue was studied. Cowdung has been used for generation of biogas in anaerobic digesters since a long time. A microbial consortium enriched from cowdung was used for anaerobic treatment of the pith with an aim to enhance dye adsorption property.

Materials and Methods

Preparation of anaerobic consortium: The consortium of organisms for treatment of the pith was enriched from cowdung slurry. Incubation was carried out for a period of two months in an anaerobic bottle assembly (Fig.1) using coconut husks for enrichment. The anaerobic bottle assembly consists of a sealed glass bottle with silicone tubing, as a gas outlet, that passes through an air trap.

Preparation of bioadsorbent: Coconut coir pith was procured from the local market in Mumbai. Dry coconut husks were collected and the pith was separated from the fibres. The pith was soaked in de-ionized water for two days for removal of coloured extract and washed till clean water was obtained (Etim *et al*., 2016). It was then air-dried for three days and stored in an air tight container. The pith was subjected to anaerobic microbial treatment using the enriched consortium prepared as above for a period of 30 days. Untreated pith

was used as control. Both treated and control pith samples were pulverized and sieved through a mesh of 100um.

Preparation of methylene blue: Methylene blue from HiMedia was used for the adsorption study. 1.0 g of methylene blue was dissolved in D/I water and volume was made up to 1 litre using a volumetric flask to obtain a final concentration of 1g L^{-1}. This was used as the stock solution. The ëmax was determined as 650nm. Standard curve was plotted in the range from 0.2-1.0 mg L^{-1} of methylene blue (Fig 2). UV- Vis spectrophotometer (Shimadzu UV-1700 Pharmaspec) was used for recording absorbance.

Batch Adsorption experiments: 50 mL of aqueous solution of methylene blue at a concentration of 50mg L^{-1} was taken in a 250 mL Erlenmeyer flask (Etim et.al., 2016). Required dosage of adsorbent was added and the flasks were shaken at 200rpm on a mechanical shaker at ambient temperature for the desired time period. After adsorption the supernatant was separated by centrifugation at 3500rpm for 10 mins and absorbance was recorded for determining the residual amount of dye.

The effect of dosage of coir pith was studied with different amounts ranging from 0.5 to 2.0 g of pith shaken in dye solution at pH 8.0 for 15 minutes. For optimization of time 0.1 g of pith was shaken for time interval from 5-25 mins at pH 8.0. To study effect of pH, adsorption over a range from 2.0 -9.0 pH values was investigated with 0.1g of pith and 15 mins of shaking time. Effect of temperature was studied from 20^0C – 60^0C with a dosage of 0.1 g, pH 8.0 and shaking time of 15 mins.

The percentage removal of dye was calculated by using the following formula:

$$\% \text{ Removal} = \frac{(Ci - Cf) \times 100}{Ci}$$

where Ci and Cf are initial and final concentrations of methylene blue respectively in mg L^{-1}.

Fourier Transform Infra Red Analysis: After adsorption pith was separated by centrifugation and dried. The samples of both raw and dye loaded pith were mixed with KBr and analysed by FTIR on Shimadzu IR Prestige 21 using DRS-8000 attachment.

BET (Brunauer-Emmett-Teller) Analysis: Surface area of the coconut pith was analysed using BET analyser (Quantachrome Nova 1200e). Adsorption of N_2 at subcritical temperatures (BET) is the routine technique for the determination of the specic surface area of dispersed solids (Ardizzone *et al*., 1999). The pith sample was evacuated at 60^0C for 18 hours before nitrogen adsorption and desorption.

Results and Discussion

Effect of various parameters on adsorption: The effect of variables like adsorbent dosage, time of adsorption, pH of dye and temperature of adsorption was investigated for the adsorption of methylene blue on coconut coir pith.

Adsorbent dosage: The dosage of pith determines the amount of surface area and ion exchangeable sites available for adsorption of the dye methylene blue. As the dosage increases from 0.5g to 2.0g there is an increase in the amount of dye removed from the aqueous solution. As seen in Fig.3 there is a marked increase in adsorption from 0.5 to 1.5g of pith but a negligible increase is seen thereafter. At every dosage the pith that is subjected to treatment by anaerobic microbial consortium gave better adsorption. 1.5g of treated pith with an intial dye concentration of 50mgL^{-1} at pH 8 and shaking time of 15 mins achieves 99.5% adsorption.

Time of adsorption: An increase in contact time between dye and pith resulted in increase in the adsorption. Maximum adsorption i.e. 99.36% was obtained by microbial treated pith after 25 mins at pH 8.0 and dosage of 0.15g (Fig 4). However, most of the adsorption was completed within 15 mins. Hence, for further experiments 15 mins of adsorption time was maintained.

pH: The pH is one of the most important operating variables in the adsorption process because the values of pH affect the surface and binding capacity of adsorbent due to exchange of H+ ions with adsorbate (Ratan, 2016). There was a sharp increase in adsorption as pH increased from 2.0 to 3.0. Percentage removal increased from 97.8 to 99.2 for treated pith and from 96.6 to 98.4 for control pith as pH increased from 2.0 to 8.0. (Fig.5). Lower adsorption at pH 2.0 could be due to the competition between excess of H+ ions and dye cations for binding to the adsorption sites on the pith.

Temperature of adsorption: As seen in Fig 6 there is an increase in adsorption with increase in temperature. Microbial treated pith showed an increase from 98.68% to 99.44% as temperature increased from 20^0C to 60^0C. In this experiment also treated pith exhibited better adsorption capacity.

FTIR and BET analyses: Fourier transform infrared spectra (FTIR) were used to investigate the changes in vibrational frequency in the functional groups of the adsorbents (Figure 7). The broad band at 3356 cm^{-1} corresponds to O – H stretching vibrations. The band at 2939 cm^{-1} represents symmetric or asymmetric C – H stretching vibration of aliphatic acids. The bands at 1608 cm^{-1} and 1512 cm^{-1} are due to the aromatic C – C stretching in phenyl ring of lignin. The absorption band at 1447 cm-1 is assigned to methoxy group of lignin. Peaks at 1105 cm^{-1} and 1058 cm^{-1} correspond

to C-OH of secondary and primary alcohols respectively (Suksabye *et al*., 2007; Jeyagowri *et al*., 2015). The disappearance of peaks at 1328 cm^{-1} and 1251 cm^{-1} and the shifts in the wave length of most peaks showed the involvement of a number of functional groups in the dye binding process taking place at the surface of the pith.

The BET technique was used to determine surface area of the coconut pith. The BET surface area of coconut pith is 6.68m^2/g. This indicates the large surface area available on the pith for dye adsorption. The irregular and porous structures on surface of the adsorbent play an important role in adsorption (Ratan, 2016).

Conclusion

Adsorption studies conducted on coir pith reveal it to be a promising adsorbent of the cationic dye methylene blue. Further, the pith subjected to microbial treatment under anaerobic conditions proved to be a better adsorbent under all conditions. It was capable of 99.54% adsorption under optimum conditions. This could be due to the microbial action on the pith that led to an increase in porosity which increased the availability of large number of adsorption sites. The FTIR spectra indicated a number of changes in peaks, which demonstrate the complex nature of the adsorbent coir pith. Coconut pith, that is obtained as an agricultural waste during processing of coir has tremendous potential for textile wastewater management.

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Figure 1: Anaerobic bottle assembly

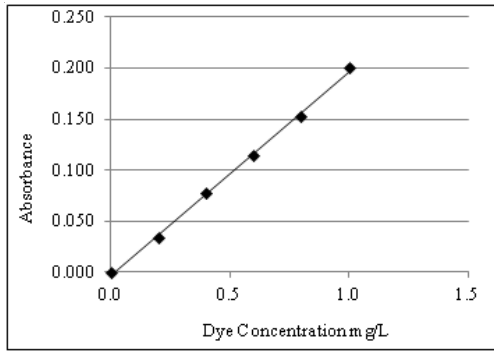


Figure 2: Standard plot of Methylene blue at 650nm

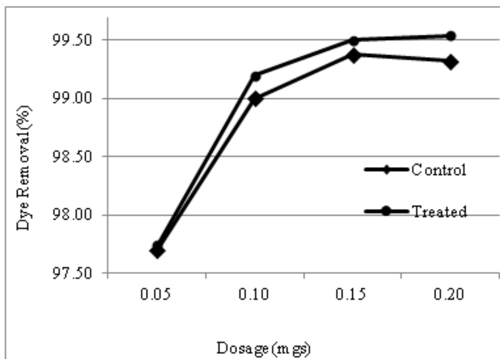


Figure 3: Effect of adsorbent dosage on adsorption of dye on control and microbial treated pith.

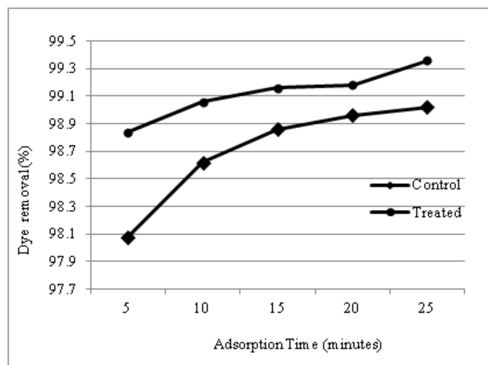


Figure 4: Effect of adsorption time on adsorption of dye on control and microbial treated pith

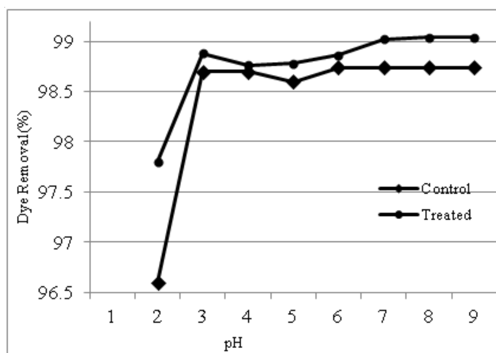


Figure 5: Effect of pH on adsorption of dye on control and microbial treated pith.

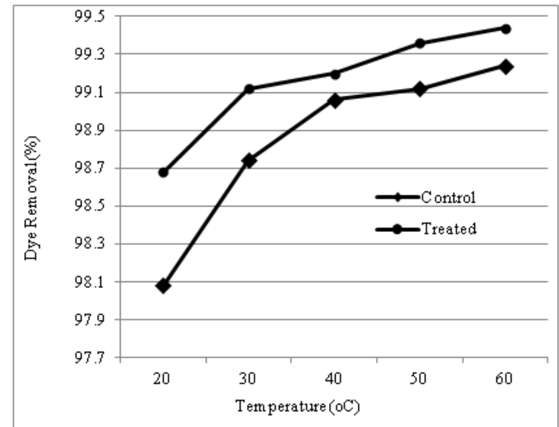
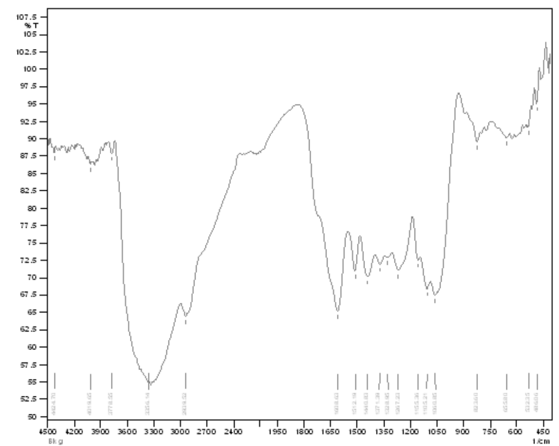


Figure 6: Effect of pH on adsorption of dye on control and microbial treated pith.



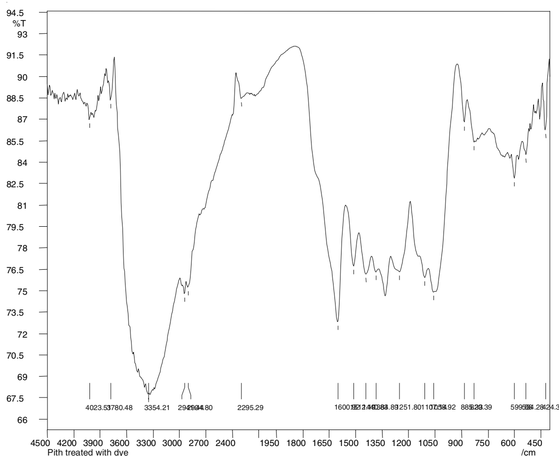


Figure 7: FTIR spectra of Microbial treated pith a) without dye and b) loaded with dye.

Allelopathic Impact of Local Plants on Seed Germination, Seedling Growth and Alteration in PGR of Pacifica Cherry Halo Vinca (Periwinkle)

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Abstract: The present study was aimed to determine the allelopathic impact of aqueous leaves extract of local plants *Viz. Terminalia catappa, Terminalia arjuna, Ficus Benghalensis, Ficus religiosa, Ficus racemosa, Mangifera indica* on *Vinca pacifica Rose* synergistically. Allelopathic impact of aqueous extract of matured and fallen leaves were studied on seed germination and seedling length measurement (cm), also the alteration in the quantity of PGR (Plant growth regulators) were estimated with the help of HPLC analysis. The seed germination and seedling length was monitored consistently at the intervals of 3 days whereas HPLC analysis of seedlings for PGR constituents was carried out on 13th day stage of growth. The results revealed that percentage germination and seedling length decreases with increasing concentrations of experimental plant extract (5%, 10%, 15%, 20% and 25%) significant results Pd"0.05were found at 15%, 20% and 25%. Matured leaves extract had a greater allelopathic impact than that of fallen leaves extract. Correspondingly the HPLC analysis too revealed greater suppression in PGR constituents by matured leaves extract in contrast to fallen leaves extract.

Keywords: Allelopathy, Aqueous extract, Pacifica Cherry Halo Vinca (Periwinkle), percentage germination, PGR (plant growth regulators), seedling length

Introduction:

Allelopathy is natural visible fact, in this the bio-chemicals produced by an organism shows the positive or negative impacts on development of an another organisms. In plants, these bio-chemicals are referred as allelochemicals. It includes secondary metabolites and phytochemicals like flavonoids, terpenoids, phenolic compounds, saponins, alkaloids, long chain fatty acid structures etc. (Ding, *et.al.*, 2016). Allelochemicals differs along with species, organs, and tissues (Ambika, 2013). These allelochemicals may exhibit constructive or destructive impact on targeted individual (Vaithiyanathan, 2014). Allelochemicals enters in environment via weathering of plants or by processes like volatilization, root exudation, leaching, and decomposition of plant residues (Putnam and Weston, 1986). Allelopathy has received a special attention in literature, due to its potentials to explain the domestic success of invasive plant (Hierro and Callaway, 2003). Many workers have proposed the practical applications of these allelochemicals in agricultural sector as natural herbicides to control weeds (Mahmood *et al* 2010).

Some workers have also mentioned the involvement of medicinal plants as a allelopathic plants causing effectiveweed management. (Abdul Raoof *et al*., 2012) has recorded the allelopathic effects of aqueous extracts of different parts of *T. cordifolia* (willd) Miers on some weeds. (Ahmad *et al*., 2013) reported the allelopathic effect of *Azardirecta indica* (Neem) and *Eucalyptus citroides* on wheat. Lakpat Singh Rawat, 2016 reported the allelopathic performance of medicinal plants such as *Asparagous racemosa, Ocimum sanctum*, *Valiriona wallichii*, *Picrorhiza kurroa* on traditional oil seeds and pulse crops of central Himalaya. Chandra *et al*., (2012) reported allelopathic effect of Ashwagandha against the germination and radicle growth of *Cicer arietinum* and *Triticum aestivum*.

In contrast, till date little work is carried out for allelopathic effect on medicinal plant itself, from surrounding environment. Keeping in view the paucity of information the present study was carried out on one of the medicinal plants namely Vinca, family of Apocynaceae, a perennial evergreen herb. It is well-known for anti-cancer activity in the medicinal world (Shambaditya Goswami, 1998). There are numerous varieties available commercially. The present study deals with allelopathic impact of local plants such on one of the varieties of Vinca, known as Pacifica Cherry Halo Vinca (Periwinkle).

Materials and Methods

In the present study, matured and fallen leaves of all the experimental plants i.e. *Terminalia catappa, Terminalia arjuna, Ficus Benghalensis, Ficus religiosa, Ficus racemosa, Mangifera indica* were collected from the campus of S.S. and L.S. Patkar College, Goregaon (w), Mumbai, similarly the matured and fallen leaves of *Pacifica Cherry Halo Vinca (Periwinkle)* plant was also collected, as a source of auto-allelopathy. The leaves were washed with clean water and allowed to shade dry naturally, grinded in electric grinder to make a fine powder.25g. of powders of each variety was added in 100 mL of distilled water (D.W) and kept overnight and filtered through muslin cloth followed by filter paper (whatman No.1) after 24 hours of soaking at room temperature Singh *et.al*, (1989).

Experimental set up: The seeds of Pacifica Cherry Halo Vinca (Periwinkle) were obtained from a reputed seed distributer, Ratanshi Agro-horticulture Byculla, Mumbai.

Plantlet grown from it was identified and authentification of cultivar variety was done from, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth College of forestry, Dapoli, Ratnagiri (M.S.) India. The allelopathic impact was studied by following the method of Raoof *et al*., (2012) and T.vaithiyanathan, (2014).To study the synergistic impact, all the extracts were mixed with each other in equal proportion. Further dilutions were prepared to get 5% 10%, 15%, 20% and 25% of concentration (Singh *et al*., 1989). Seeds and filter paper were moistened with 5 mL of each 5% 10%, 15%, 20%, 25%, concentration of aqueous extract. Distilled water was used as control. Experiment was set up in triplicates, 10 seeds were kept in each sterile Petri dish and allowed to germinate for next 10-12 days.

HPLC analysis for PGR (plant growth regulators) determination:

To determine the quantity of IAA, ABA, and GA3 validated method of Tang, *et al*., (2011) was followed. 100 mg. seeds were treated with experimental plant extracts separately for matured and fallen leaves. Seeds were allowed to germinate in the Petri dish for 12days. After 12 days the treated seedling samples were collected and frozen in liquid nitrogen, stored at -70°C. These samples were then homogenized in 80% cold aqueous methanol (5 mL) and left to stand overnight in darkness at 4°C. The extracts were centrifuged at 5000 r/min at 4°C for 15 mints. Supernatant was collected. Precipitate was extracted three times with fresh cold methanol. The total methenolic extract was dried on rotator evaporator and dissolved in 10 ml of cold methanol. IAA, ABA, and GA3 were quantified by injecting the extract into reversed phase HPLC withmethenolic gradient in 0.6% acetic acid.

Instruments and reagents: HPLC machine used**,** Shimadzu LC-2010 HT (auto sampler) standards of IAA, ABA, and GA3 were procured from Loba chemie. Pvt. Ltd.107, Woden house road, Jahangir villa, Mumbai-400005.

Chromatographic conditions: Chromatographic column LCGC ® Qualisil BDS C18, 4.6×150 mm, 5 micron was used with mobile phase methanol-0.6% ethenoic acid, gradient elution. Column temperature 35°c, sample size 10µl, flow rate 1 ml/min. Ultraviolet detection at 254 nm.

Detection of PGR: Quantitative evaluation of IAA, ABA, and GA3 were carried out by ITA LABS PVT. LTD FORT Mumbai.400001.

Statistical analysis: The differences between standard and experimental samples were studied by Student t test (unpaired). The result were considered statistically significant when Pd"0.05.statistical analysis was done using© 2017 GraphPad software, Inc. with Quick Calcs.

Results and Discussion

Seed germination is very vital phase in life cycle of plants. It comprises various environmental factors. The alteration in metabolic activities of plant initiates with it. In the present work the seed germination and seedling length were affected because of allelopathic impact of aqueous extract, when compared with control. As the concentration of extract was increased the seed germination and seedling growth both were decreased. The higher concentrations of aqueous extract such as 20% and 25% (Pd" 0.1, Pd"0.05) were more effective than the lower concentration. In comparison it was noticed that matured leaves extract was more allelopathic than fallen leaves extract. Similar results were noticed by Flaviana Maluf Souza *et al*., (2010) while studying Allelopathic potential of bark and leaves of *Esenbeckialeio carpa* Engl. (Rutaceae)

HPLC analysis:

In the present study HPLC analysis of aqueous extracts of local plants showed their allelopathic impact on PGR of *Pacifica Cherry Halo Vinca (Periwinkle).*The study reveals that matured leaves extracts has more allelopathic impact on PGR, it has been noticed that the quantity of IAA was increased significantly at Pd"0.05 (Table:5) as compared to control. Similar results were found by Soltys *et al*., (2012) while studying the effect of cyanamide (CA) as anallelochemical on tomato root growth. Thus accordingly significant decrease in level of ABA (Pd"0.05) was noticed (Table 5).

The fluctuation in the GA3 (Pd"0.05) (Table 6) levels was observed when aqueous extracts of fallen leaves were applied. These results suggest that, endogenous hormones may exhibits dependence as well as interactive effects on growth of seedlings, and their adaptability to allelochemicalsstress, Yang*et al*., (2008) in the same way Fang Cheng and Zhihui Cheng, (2015) have reported that allelochemicals can alter the contents of plant growth regulators or induce imbalances in various phytohormones, which inhibit the growth and development of plants.

Conclusion

The present study revealed the response of medicinal plant towards allelopathic environment of surrounding local plants, in terms of seed germination, seedling length (cm) and alteration in PGR.It is observed that local plants can hinder in the morphology and physiology of medicinal plants. It implies that medicinal plants too, cannot escape from the effect of Allelopathy. Since till date no study is yet carried out for allelopathic impact on medicinal plant itself. There is little literature available with this line of thought. Hence this novel approach is taken into consideration, to provide new magnitude to the Allelopathy and medicinal plants.

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Table 1: Allelopathic effect of matured leaves aqueous extract of experimental plants on percentage (%) seed germination of Pacifica Cherry Halo Vinca (Periwinkle)

Interval in days	Effect of Extract concentrations on seedling length.(cm)					
	5%	10%	15%	20%	25%	control
3	73.83	53.33	33.33	43.33	10.00	86.66
6	83.33	80.00	50.00	56.66	30.00	86.66
9	83.33	83.33	50.00	56.66	30.33	90.33
12	86.66	83.33	53.33	56.33	33.33	90.33
SD	5.772	14.530	9.027	6.665*	10.671*	
SEM	2.886	7.265	4.513	3.332	5.335	

Level of significance:* = pd≤0.1, ** = pd≤0.005, *** = pd≤0.01 and NS= not significant

S.D- standard deviation, SEM- Standard Error of mean.

Table 2: Allelopathic effect of fallen leaves aqueous extract of experimental plants on percentage (%) seed germination of Pacifica Cherry Halo Vinca (Periwinkle)

Interval in days	Effect of Extract concentrations on seedling length. (cm)					
	5%	10%	15%	20%	25%	control
3	56.66	40.00	06.00	00.00	00.00	60.00
6	90.00	83.33	60.00	46.66	40.00	93.33
9	90.00	83.33	60.00	46.00	40.00	93.33
12	90.00	86.00	60.00	46.00	40.00	93.33
SD	16.670	22.145	27.000	23.222*	20.000**	
SEM	8.335	11.072	13.500	11.6110	10.000	

Level of significance:* = pd≤0.1, ** = pd≤0.005, *** = pd≤0.01 and NS= not significant

S.D- standard deviation, SEM- Standard Error of mean

Table 3: Allelopathic effect of matured leaves aqueous extract of experimental plants on length of seedlings (cm) of Pacifica Cherry Halo Vinca (Periwinkle)

Interval in days	Effect of Extract concentrations on seedling length.(cm)					
	5%	10%	15%	20%	25%	control
3	0.470	0.230	0.080	0.160	0.030	0.676
6	3.380	2.660	1.380	1.423	0.556	4.853
9	3.803	3.073	1.926	1.956	0.803	5.210
12	3.840	3.250	2.010	2.116	0.803	5.213
SD	1.615	1.404	0.890	0.886	0.364*	
SEM	0.807	0.702	0.445	0.443	0.182	

Level of significance:* = pd≤0.1, ** = pd≤0.005, *** = pd≤0.01 and NS= not significant

S.D- standard deviation, SEM- Standard Error of mean

Table 4: Allelopathic effect of fallen leaves aqueous extract of experimental plants on length of seedlings (cm) of Pacifica Cherry Halo Vinca (Periwinkle)

Interval in days	Effect of Extract concentrations on seedling length.(cm)					
	5%	10%	15%	20%	25%	control
3	0.446	0.330	0.036	0.000	0.000	0.89
6	4.610	4.136	2.743	1.576	0.880	4.713
9	3.736	3.250	2.010	2.116	0.803	5.213
12	4.800	4.493	2.953	1.710	0.796	4.770
SD	2.0218	1.888	1.329	0.929	0.414*	
SEM	1.010	0.944	0.664	0.464	0.207	

Level of significance:* = pd≤0.1, ** = pd≤0.005, *** = pd≤0.01 and NS= not significant
S.D- standard deviation, SEM- Standard Error of mean

Table 5 : HPLC analyses for, Allelopathic effect of aqueous extract of matured leaves of experimental plants on PGR of Pacifica Cherry Halo Vinca (Periwinkle)

Concentration of matured leaves extract. (%)	Quantity of plant growth regulators PGR in (ppm)		
	GA3	IAA	ABA
5%	239.00	5.13	0.58
10%	210.49	4.13	0.56
15%	99.27	6.52	0.56
20%	42.57	7.47	0.77
25%	44.55	7.57	0.79
CONTROL	144.48	0.20	2.04
SD	92.4812	1.5013**	0.1173**
SEM	41.3591	0.6714	0.0525

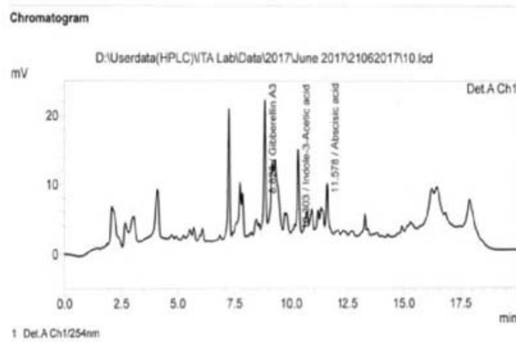
Level of significance:* = pd≤0.1, ** = pd≤0.005, *** = pd≤0.01 and NS= not significant
S.D- standard deviation, SEM- Standard Error of mean

Table 6: HPLC analyses for, Allelopathic effect of aqueous extract of fallen leaves of experimental plants on PGR of Pacifica Cherry Halo Vinca (Periwinkle)

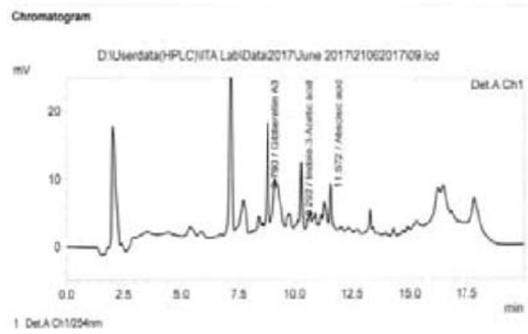
Concentration of Fallen leaves extract. (%)	Quantity of plant growth regulators PGR in(ppm)		
	GA3	IAA	ABA
5%	355.66	3.49	0.31
10%	82.64	3.70	0.26
15%	65.39	3.43	0.25
20%	88.16	3.87	0.24
25%	52.36	5.48	0.35
CONTROL	380.28	3.69	0.24
SD	127.5850**	0.8488	0.0466
SEM	57.0577	0.3790	0.0208

Level of significance:* = pd≤ 0.1, ** = pd≤0.005, *** = pd≤0.01 and NS= not significant
S.D- standard deviation, SEM- Standard Error of mean

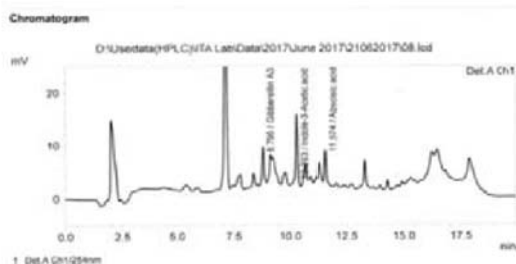
Figure 1: Chromatogram of matured leaves aqueous extract of experimental plants on PGR of Pacifica Cherry Halo Vinca (Periwinkle)



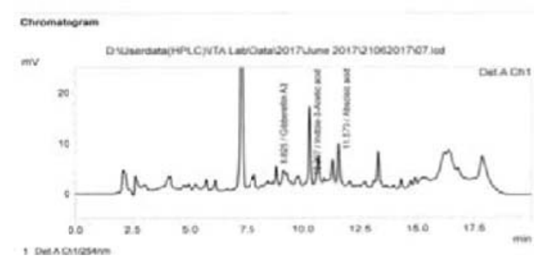
Allelopathic effect of 5% concentration



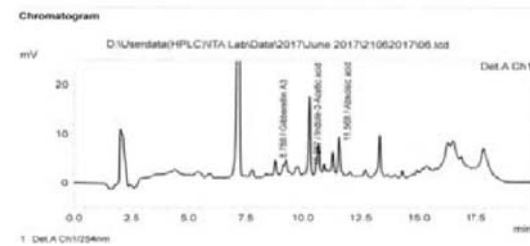
Allelopathic effect of 10% concentration



Allelopathic effect of 15% concentration

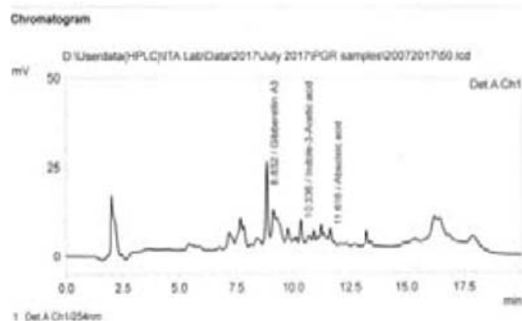


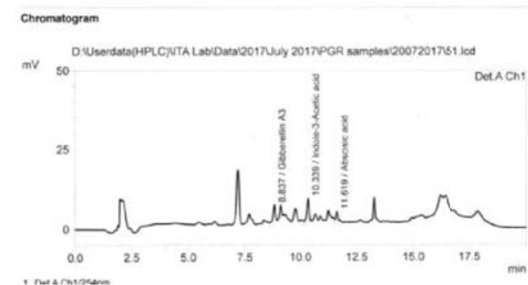
Allelopathic effect of 20% concentration



Allelopathic effect of 25% concentration

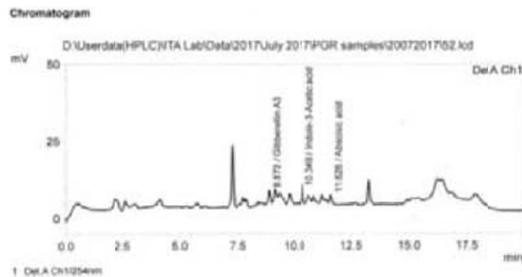
Figure 2: Chromatogram of fallen leaves aqueous extract of experimental plants on PGR of Pacifica Cherry Halo Vinca (Periwinkle)

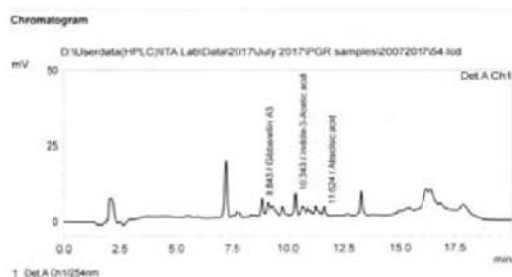




Allelopathic effect of 5% concentration

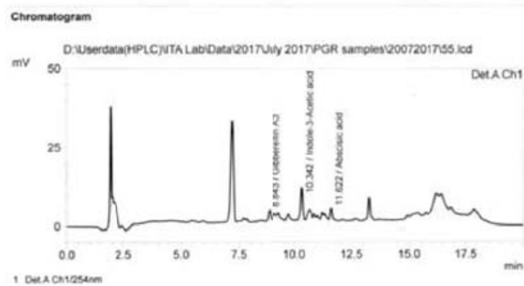
Allelopathic effect of 10% concentration





Allelopathic effect of 15% concentration

Allelopathic effect of 20% concentration



Allelopathic effect of 25% concentration

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Theme II
Bio - Sciences

Detection of Adulterants In Chilli By Thin Layer Chromatography and Chemical Test

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Abstract: The genus *Capsicum* belongs to the family Solanaceae. Chilli occupies an important place in Indian diet. Some varieties of chillies are famous for red colour because of the pigment 'capsanthin,' others are known for biting pungency attributed to 'capsaicin. Chillies are rich in vitamins, especially in vitamin A and C. It has anti-bacterial, anti-carcinogenic, analgesic and anti-diabetic properties. Chili is used as vegetable in cuisine in many parts of world. Sudan-1 yellow and Sudan Red colour is added to chilli powderas an adultrants. Those colours are responsible for making tumors in the liver and bladder and finally cancer. Therefore the aim of the present study was to bring awareness to public on the important subject of adulteration in chili by performing Thin Layer Chromatography and chemical tests.

Keywords: Chili, Standard, Thin Layer Chromatography, Adulteration.

Introduction

Capsicum is commonly called as "Chillies." It belongs to family Solanaceae. The genus *Capsicum* consists of about 25 wild and 5 domesticated species (George and Giraddi, 2007). Among the spices consumed per head, dried chilli constitutes a major share. India is not only the largest producer but also the largest consumer of chilli in the world. India contributes about 36% to the total world production. In India, chillies are grown in almost all the state throughout the country. Andhra Pradesh is the largest producer of chilli in India and contributes about 26% to the total area under chilli, followed by Maharashtra (15%), Karnataka (11%), Orissa (11%), Madhya Pradesh (7%) and other states contributing nearly 22% (Karpate and Saxena, 2009).

Some varieties of chillies are famous for red colour because of the pigment 'capsanthin. Capsaicin, one of these naturally occurring phytochemicals, is the major pungent constituent of hot chili peppers of the genus Capsicum, which are extensively used as a food additive (Lin *et al*., 2013). It has been shown that capsaicin is involved in several physiological and pharmacological effects. The capacity of capsaicin to suppress the growth of these cancer cells is primarily mediated through induction of apoptosis. Additionally, the activities associated with capsaicin-induced anti-cancer effects include the arrest of cell cycle progression, regulation of transcription factor expression, and suppression of growth signal transduction pathways (Lin *et al*., 2013).

Adulteration in food is normally present in its most crude form. Adulterants may be intentionally added to more expensive substances to increase visible quantities and reduce manufacturing costs, or for some other deceptive or malicious purpose. Most of the locally branded spices were found containing harmful substances like white chilli powder is dyed with 'Sudan red', an artificial dye to turn it into an expensive red chilli powder; whole red chillies are adulterated with coal tar to enhance their appearance (Jaiswal *et al*., 2016).

Therefore the aim of the present study was to bring awareness to public on the important subject of adulteration in chili by performing Thin Layer Chromatography and chemical tests.

Materials and Methods

Collection of plant materials: Dried chili was purchased from local market, Thane (Maharashtra).

Market samples collection: 4 different brands of chilies were purchased from local market, Thane (Maharashtra) and labeled as sample 1, sample 2, sample 3 and sample 4.

Drying and powdering of materials: Chilies were dried in oven at 40^{0}C for 30 mins and then grind, powdered and stored in air tight container.

Extract preparation: 0.1 gm of each sample was mixed with 10 ml of methanol separately. The filtrate was evaporated to 3 ml and used for TLC (Wagner and Bladt, 1996).

Chromatographic plate: Silica gel 60 F_{254}- precoated TLC plates (Merk, Germany)

Solvent system: Toluene: Ethyl acetate (70:3)

Water soluble coal tar test: Take a glass full of water. Sprinkle the chilli powder to the glass tumbler (Abhirami and Radha, 2015).

Oil soluble coal tar test: Take 2 grams of the sample in a test tube, add few ml of solvent ether and shake. Decant ether layer into a test tube containing 2 ml of dilute Hydrochloric acid (1 ml HCL plus 1 ml of water). Shake it (Abhirami and Radha, 2015).

Rhodamine B: Take 2 grams sample in a test tube, add 5 ml of acetone (Abhirami and Radha, 2015).

Brick Powder test: Brick powder settles fast chilli powder settles slowly when put in water (Dixit, 2015).

Red Colour dye: Sprinkle some Chilli powder on the surface of water in a glass beaker. Artificial colorants will descend as coloured streaks (Dixit, 2015).

Red lead salts test: To a sample of chilly powder, dil. Nitric Acid was added. The solution was filtered and two drops of potassium iodide were added into it (Mansuri, 2015).

Results and Discussion

R_f value for dry and wet chillies and marketed sample1, sample 2, sample 3 and sample 4 was shown in table 1. Chemical test was done to determine possibility of presence of adulterants. When water soluble coal tar color and Rhodamine B test was done all the four samples gave positive test results which means they are adulterated. When oil soluble coal tar color was done all the four samples gave negative test results which means they are not adulterated with oil soluble coal tar color as seen in Table 2. Abhirami and Radha, 2015 showed similar results when brick powder and red color dye test was done the all four samples gave positive results which means samples are adulterated with brick powder and red color dye. Red lead salt test was done all the samples except sample 1 shown positive test which means samples 2,3,4 are adulterated with red lead salt and sample 1 was not adulterated (Dixit, 2015). When brick powder test was done all the four samples gave positive test results which means they are adulterated as seen in Table 5. Similar test results were seen by Mansuri, 2015.

Conclusion

Adulteration is commonly practiced in both branded and unbranded foods in daily life. From local market to the hyper market adulteration is prevalent everywhere. Majority of adulteration in India is Intentional adulteration and it affects the people of all the age group. Even today many people in India are unaware about adulteration and its harmful effects. From present work, it was concluded that red lead salt, brick powder, red color dye, water soluble coal tar color, oil soluble coal tar color, Rhodamin B are harmful adulterants that are added in various spices to enhance the color of spices. When such types of harmful chemicals are ingested in our body then it leads to various types of diseases. Read the labels of the chili powder packet, while purchasing commercial brands. Test which everyone can do at their home are available to check the amount of adulteration in a product. So there is a great need to make them aware about these tests.

Acknowledgment

Authors are thankful to Department of Botany, VPM's B. N. Bandodkar College of Science, Thane for providing the laboratory facilities. We are also thankful to DBT-Star College Scheme for providing the instruments and chemicals required for the research work.

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Table 1: Thin Layer Chromatographic analysis of chili samples

CHILI SAMPLES	Rf value
Dry chili	0.07, 0.15, 0.30, 0.46, 0.57
Wet chili	0.09, 0.14, 0.22, 0.33
Sample 1	0.03, 0.08, 0.13, 0.29, 0.50, 0.79
Sample 2	0.06, 0.12, 0.18, 0.23, 0.31, 0.43, 0.50, 0.64
Sample 3	0.06, 0.23, 0.33, 0.42, 0.61, 0.76, 0.83
Sample 4	0.10, 0.23, 0.28, 0.44, 0.48, 0.71, 0.83, 0.98

Table 2: Chemical tests to detect adulteration of chilli powder

ADULTERANT	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
Water soluble coal tar Test	+	+	+	+
Oil soluble coal tar test	-	-	-	-
Rhodamine B	+	+	+	+
Brick powder	+	+	+	+
Red color dye	+	+	+	+
Red lead salt	-	+	+	+
Brick powder	+	+	+	+

Positive (+) ; Negative (-)

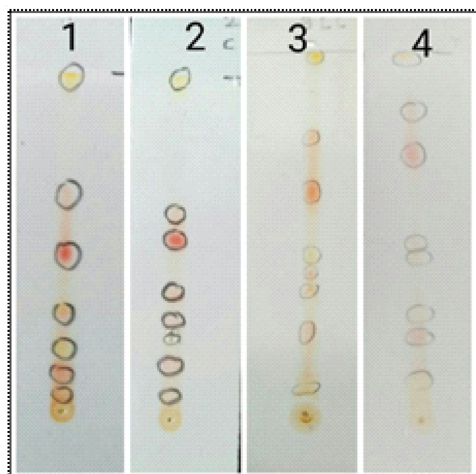

Plate 1: TLC of marketed samples

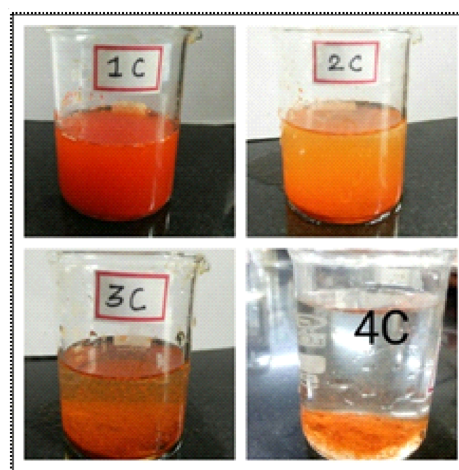

Plate 2: Water soluble coal tar test of marketed samples

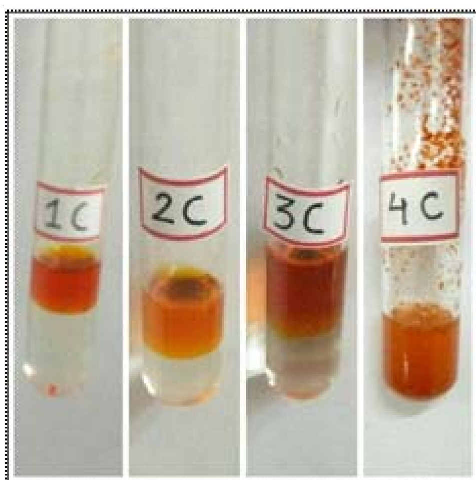

Plate 3: Oil soluble coal tar test of marketed samples

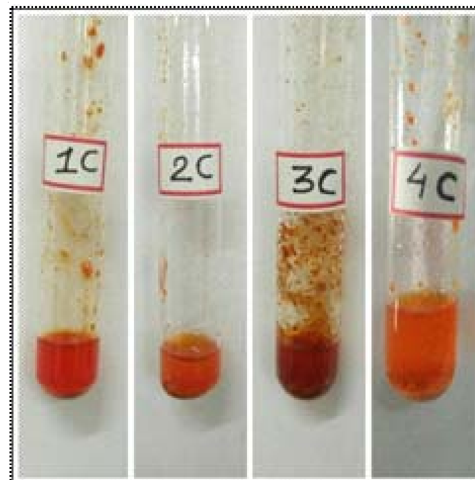

Plate 4: Rhodamine B test of marketed samples

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Pharmacognostical and Phytochemical Studies of *Ailanthus excelsa* and *Simarouba glauca*

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Abstract: Medicinal plants are the important source for their therapeutic remedies in various ailments. *Ailanthus excelsa* is a large deciduous tree commonly known as 'Mahanimb'. *A. excelsa*is a tree of rapid growth and is called tree of heaven. The plant has antitumor, antiviral, antimalarial, antileukemic and antibacterial activities. *Simarouba glauca* is commonly known as 'Laxmitaru' or 'paradise tree'. This is evergreen tree grows to a height of 12-15 m with large circular crown. The bark and leaf extract of *Simarouba* is well known for its different types of pharmacological properties such as hemostatic, anthelminthic, antiparasitic, antidysentric, antipyretic and anticancerous. Plants have limitless ability to synthesize substances mainly secondary metabolites. The present study provides pharmacognostical and physicochemical details of the plant. Present study summarizes the pharmacological and phytochemical studies of leaf, stem and stem bark of *Ailanthus excelsa* and *Simarouba glauca* and these observations will enable to standardize the botanical identity of the drug in its crude form.

Key Words: Pharmacognostical, Phytochemical, *Ailanthus excelsa*, *Simarouba glauca*.

Introduction

Plants are the most important parts of all living organisms. They provide different types of products like fruits, bark, leaves and medicines. Near about 80% compounds of plant origin are used as medicine (WHO, 1993). Medicinal plants constitute a source of raw materials for both traditional system of medicine and modern medicine. In many developing countries, a large proportion of the population relies heavily on traditional practitioners, who are dependent on medicinal plants to meet the primary health care needs. Although modern medicines are available, herbal medicines have often retained popularity for historical and cultural reasons. Since the usage of these herbal medicines has increased, the issues are regarding their safety, quality and efficacy.

Plants from Simaroubaceae are known to contain compounds with highly oxygenated triterpenes and quassinoids. Initially the compounds of such chemical nature were known by the term "quassin" after the physician "Quassi" who used the bark of plants from this family for the treatment of fever. Studies on quassinoids have shown their promising role as therapeutic agents as an antitumor, antiviral, anti-inflammatory, antiamoebic, antimalarial, insecticidal, antitubercular, anticancer, antiulcer and herbicidal, etc. (Lavhale and Mishra,2007)

Ailanthus excelsa Roxb. belonging to the family Simaroubaceae and commonly known as "Mahanimb". The plant is distributed in Asia, north Australia and is indigenous to central and southern India. *Ailanthus excelsa* is a large deciduous tree, 18-25 m tall; trunk straight 60-80 cm in diameter; bark, light grey and smooth, becomes grey-brown and rough on large trees, aromatic, slightly bitter. Leaves alternate, pinnately compound; leaflets 8-14 or more pairs, long stalked, ovate with unequal base, edge coarsely toothed and often lobed. *Ailanthus excelsa* is really a plant of heaven (Kumar *et. al.,* 2010). *A. excelsa* bark is bitter, astringent, anthelmintic and mainly useful in diarrhea and amoebic dysentery and known to have antiviral (Rashed *et al*., 2013) antibacterial (Shrimali *et al*., 2001), antifungal (Joshi *et al*., 2003) and antitumor (Said *et al*., 2012) activity.

Simarouba glauca is commonly called as Lakshmi taru, it is evergreen tree which grows to a height of 12 - 15 m with large circular crown. The root system is shallow suitable for mountain soils. Stem is up to 9 m in height with 40 - 50 cm in diameter. It has finely cracked and grey colored outer bark while inner bark is creamy in color. Leaves are pinnately compound with 3 - 21 leaflets oblong and often notched or smooth at apex. *Simarouba* is one of the important medicinal plants with wide use. The bark and leaf extract of *S. glauca* is well known for its different types of pharmacological properties such as hemostatic, anthelmintic, antiparasitic, antidysentric, antipyretic and anticancerous (Patil *et al*., 2011). Joshi and Joshi (2002) speculated that the chemicals present in leaf, fruit and seed of *S. glauca* are known to possess the medicinal properties such as analgesic, antimicrobial, antiviral, astringent, stomachic, tonic, vermifuge. The oil obtained from this plant is largely used in preparation of bakery products, vegetable oil and is free from bad cholesterol. Wood is light and generally insect resistance, hence useful in making light furniture (Sharanya *et al*., 2016).

Materials and Methods

Plant matrial: Fresh plant material of *Ailanthus excelsa* and *Simarouba glauca* (leaf, stem and bark) was collected and used for determination of moisture content. Fresh leaves were used for various parameters like stomatal

index, palisade ratio, vein-islet number and vein-termination number of apical, middle and lower regions. Plant material (leaf, stem and stem bark) were dried in an oven at 80°C and then powdered using a grinder. This powdered material was used for further analysis like ash content (acid soluble and acid insoluble ash) and phytochemical analysis.

Leaf microscopy: Fresh leaves of *Ailanthus excelsa* and *Simarouba glauca* were taken for determination of stomatal index and comparing the apical, middle and lower regions of only lower surface of leaf as stomata were found to be absent on upper surface of leaf. For moisture content 1g of fresh plant material (leaf, stem and stem bark) was taken and dried in an oven at 80°C till the constant weight was obtained.

For palisade ratio, vein-islet number and vein-termination number, fresh leaves were first divided into apical, middle and lower region and boiled in water for 10 minutes to remove the water soluble elements and fix the cells and then leaves were decolorized by keeping in pure methanol for 24 hours followed by boiling in 80% lactic acid for 10-15 minutes to achieve the transparency (Rajendra *et al*., 1977).

Physico-chemical analysis: About 1 g of dried powder of leaf, stem and stem bark of *A. excelsa* and *S. glauca* were accurately weighed and were transferred to three different silica crucibles and kept in muffle furnace for 1 hour at 550°C till a white carbon free ash was formed. The silica crucible were cooled at room temperature and weighed. The percent total ash content was then calculated for each plant powder. The ash obtained was used for determination of acid soluble and acid insoluble ash.

Phytochemical studies

Preparation of plant extract: The preliminary phytochemical study was carried out to check the presence of different phytoconstituents in different extracts. The extracts were prepared by taking 3g of powdered plant material (leaf, stem and stem bark) of *A. excelsa* and *S. glauca* in 30 ml of distilled water/ ethanol/ methanol and benzene separately and kept in water bath for 2 hours at 60°C. The extract was filtered using filter paper. Filtrate was used for further preliminary phytochemical screening for various secondary metabolites.

1. **Test for tannins:** 1ml of lead acetate was added in 1ml of each of the above aqueous filtrate. Formation of white precipitate indicates the presence of tannins.
2. **Test for flavanoids:** In 1ml of filtrate, 1ml of aqueous NaOH and 1ml of aqueous HCl was added. Formation of yellow coloration indicates the presence of flavonoids.
3. **Test for terpenoids:** In 1ml of filtrate, 2ml of chloroform and few drops of conc. H_2SO_4 were added. Reddish brown coloration indicates presence of terpenoids.
4. **Test for saponins:** In 1ml of filtrate, 2ml of distilled water was added. Formation of persistent frothing on shaking indicates presence of saponins.
5. **Test for cardiac glycosides:** In 1ml of filtrate, 2ml of glacial acetic acid followed by one drop of alcoholic $FeCl_3$ and few drops of concentrated H_2SO_4 was added. Formation of greenish blue coloration indicates presence of cardiac glycosides.
6. **Test for steroids:** In small quantity of filtrate, 5ml of chloroform was added. From this solution 1ml was taken and few drops of conc. H_2SO_4 were added. Formation of brown ring indicates presence of steroids.
7. **Test for phenols:** In 1ml of filtrate, 2ml of 10% lead acetate was added. Formation of brown precipitate indicates presence of phenols.
8. **Test for anthocyanide:** In 1ml of filtrate, 5ml of dilute HCl was added. Formation of pale pink coloration indicates presence of anthocyanide.
9. **Test for phlobatannins:** In 1ml of filtrate, few drops of 1% HCl was added. Formation of red precipitate indicates presence of phlobatannins.
10. **Test for alkaloids:**
    a. In 1ml of filtrate, 1ml of Dragendroff's reagent was added. Formation of turbid orange coloration indicates presence of alkaloids.
    b. In 1ml of filtrate, 1ml of Mayers's reagent was added. Formation of whitish yellow precipitate indicates presence of alkaloids.

Results and Discussion

The quantitative microscopic studies revealed the values like stomatal index, palisade ratio, vein-islet number and vein-termination number. For quantitative microscopic studies apical, middle and lower part of leaf were compared and the results obtained are presented in Table 1. The results shows the minor difference in the values of stomatal index, palisade ratio and vein islet number from apical, middle and lower regions of *Ailanthus excelsa* and *Simarouba glauca* leaf. However, vein termination number was found to be little high in lower region of leaf as compared to upper and middle region of *A. excelsa,* whereas there was minor difference in vein termination number of upper, middle and lower regions of *S. glauca* leaf.

Physico-chemical analysis of powder reveals the moisture content (loss on drying), ash content.

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Ash values used to determine quality and purity of crude drug. Acid insoluble ash indicates the presence of various impurities like carbonate, oxalate and silicate. Ash values can be used as reliable tool for detecting adulteration. These studies help in the identification of the plant materials.

The higher percentage of total ash content may be due to presence of calcium oxalate crystals (Handrall *et al.*, 2010). The ash content values represent the presence of inorganic salts in crude drug. The results of physico-chemical analysis are presented in Table 2.

Presence of classes of secondary metabolites may be a useful indicator of both efficacy and potential toxicity hence test for the presence of phytochemical classes were performed using aqueous, ethanolic, methanolic and benzene extract of powdered sample of *Ailanthus excelsa* and *Simarouba glauca* (leaf, stem and stem bark). *Ailanthus excelsa* shows the presence of secondary metabolites like tannins, flavonoids, terpenoids, phenols and alkaloids. While saponins, steroids, anthocyanide and phlobatannins were found to be absent, cardiac glycosides were found to be present only in ethanolic and methanolic extract of leaf. The results obtained are presented in Table 3. *Simarouba glauca* shows the presence of secondary metabolites like tannins, flavonoids, terpenoids, phenols and alkaloids. While saponins, steroids, anthocyanide and phlobatannins were found to be absent whereas cardiac glycosides were found to be absent only in aqueous extract of stem and stem bark and in methanolic extract of stem. The results obtained are presented in Table 4. Physicochemical screening plays a vital role in the pharmacological and chemical study of the medicinal plants. It may suggest feasible pharmacological effects of its extracts or fractions in comparison of identified phytochemicals group, highlighting a close relationship with its main therapeutic uses.

Ethno medically the plant is being used in the treatment of various disease conditions. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal pharmacopoeia, pharmacognostics and standards must be established. For the pharmacological study of novel drug, the information regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extract. Preliminary phytochemical investigations are useful to isolate the pharmacologically active principles present in the plant.

Conclusion

The various specific pharmacognostical characters of *Ailanthus excelsa* and *Simarouba glauca* can be used as a diagnostic tool for the correct identification of the plant drug and also to detect the authenticity, adulteration of this medicinally useful plant if any. The preliminary phytochemical analysis showed the presence of various phytoconstituents like alkaloid, flavonoids, phenols, terpenoids, tannins, which may contribute to the different pharmacological activity of this plant.

Acknowledgments

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Table 1: Values of quantitative microscopic studies from *A. excelsa* and *S. glauca* leaf

Regions ⇨ Parameters ⇩	***Ailanthus excelsa***			***Simarouba glauca***		
	Apical	Middle	Lower	Apical	Middle	Lower
Stomatal index	11.76	13.26	12.19	17.72	18.07	17.14
Palisade ratio	5.5	6	5.75	6.5	7.25	6.75
Vein-islet number (per square mm)	25.71	28.42	27.06	16.91	17.59	15.56
Vein-termination number (per square mm)	37.89	40.59	45.33	39.91	42.62	41.94

Table 2: Values of physico-chemical analysis from *Ailanthus excelsa* and *Simarouba glauca* leaf, stem and stem bark

Plant parts ⇨ Parameters ⇩	***Ailanthus excelsa***			***Simarouba glauca***		
	Leaf	Stem	Stem bark	Leaf	Stem	Stem bark
Moisture content %	75	64	71	67	50	62
Dry Matter %	25	36	29	33	50	38
Ash content %	8	3	7	4	3	3
Acid Insoluble Ash %	4	2	2	1	2	1

Table 3: Results of phytochemical analysis of *Ailanthus excelsa* leaf, stemand stem bark

Chemical class	DW extract			Ethanolic extract			Methanolic extract			Benzene extract		
	Leaf	stem	Stem bark	leaf	stem	Stem bark	Leaf	Stem	Stem bark	Leaf	Stem	Stem bark
Alkaloids a. Dragendroff's reagent b.Mayer's reagent	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -
Anthocyanide	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	+	-	-	+	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	-	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+

Table 4: Results of phytochemical analysis of *Simarouba glauca* leaf, stem and stem bark

Chemical class	DW extract			Ethanolic extract			Methanolic extract			Benzene extract		
	Leaf	stem	Stem Bark	leaf	stem	Stem Bark	Leaf	Stem	Stem Bark	Leaf	Stem	Stem Bark
Alkaloids a. Dragendroff's reagent	+	+	+	+	+	+	+	+	+	+	+	+
b.Mayer's reagent	-	-	-	-	-	-	-	-	-	-	-	-
Anthocyanide	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	-	-	+	+	+	+	-	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	-	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	-	+	+	-	+	-	-	-

Paleontological Survey of Pranaitha Godhavari Valley Tahsile Sironcha Dist Gadchiroli

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Abstract: Life has existed on earth for about 3200 million years and during this vast span of time an enormous variety of plants and animals has existed. Fossils are the remains of plants and animals that are preserved in the rocks close to their original shape.

Sironcha is a tahsil situated in the pranhita - godavari Valley. It comprises under gondwana region. The present report embodies the results of paleontological survey of fossils. During survey we visited 18 sites of fossils at sironcha tahsil and we found different type of fossil fishes, Dinosaur bones, Petrified wood. These survey of fossils useful for paleontologist as well as geologist.

Keywords: Sironcha, pranhita - godawari Valley, fossil fishes, dinosaur bones, petrified wood.

Introduction

The rock of Gondwana Region represent the sedimentation during upper palaeozoic and Mesozoic have been studied several geologist. Sironcha area comprises under the gondwana region. The terms Gondwana is a introduced by H-BMedicot in 1972 in a manuscript report after the ancient tribal Dravidian of second kingdom in central India. Feistmantal in1876 reported that Gondwana comprises rock with coal seams, Fossil, Plant& animals .The masozoic Gondwana Formation of Maharastra is best exposed in lranhita- Godavari Valley.

The present report embodies the result of paleonpological survey of pranhita Godavari valley in Sironcha area of Gadchiroli district. kota Formation which is named after the village kota is situated at the eastern bank of pranhita river in the Adilabad dist. of Telangana.

Materials and Methods

The survey was carried out in parts of pranhita gaodavari basin. During survey. The survey period was jun 2015 to September 2016. Sironcha is located at the 18.83^{0} N 79.93 E^{0}.It has an average elevation of 118 meteres (390) feet. During the summer month survey period temperature ranges 28^{0} C to 48^{0} C.

Observation

During the study period we collected fossil of fish, scale of fishes ,dinosaurs' bone, petrified wood .Fossils are of paradepedium fish species. By using different literature we concluded its dinosaurus bone.

Result and Discussion

The paleontological study were carried out by collection of different types of fossils in pranhita gadavari vally of gadchiroli district. Survey of areas was done from June 2015 to September 2017. During survey we visited different fossil ferrous locality. We visited Sironcha, Borgudam, Chitur, Wadadham, Ankisa, Laxmipur, Nandigao, Koppela, Kottapalli, Amdelli, Rangayyapalli, Kishtaraopad, Kota Bamni. During survey we collected fossil fishesh, fragments of fossil fishesh, dinosaur vertebra column, dinosaur fragment, petrified wood. The most common and dominant occure fauna is formation of fossil fishesh and dinosours. The fossils fishes assign lower jurassic age to the kota formation. The fossils are kept and proceed for detailed study. Mejority of fossils where satisfactory idenfied by using different literature.The fossil fishesh is paradepedium, lepidodedron and tetragonolepsis was found and bones were the dinosours.

The similer finding where done by the previous worker. King (1881) was the pioneer worker who mapped the pakel groups of rocks in parts of chandrapur and gadchiroli district. Battacherya in 1980 has worked on the dopositional patern in lames stone of kotaformation. This information will be significant to the paleontologist.

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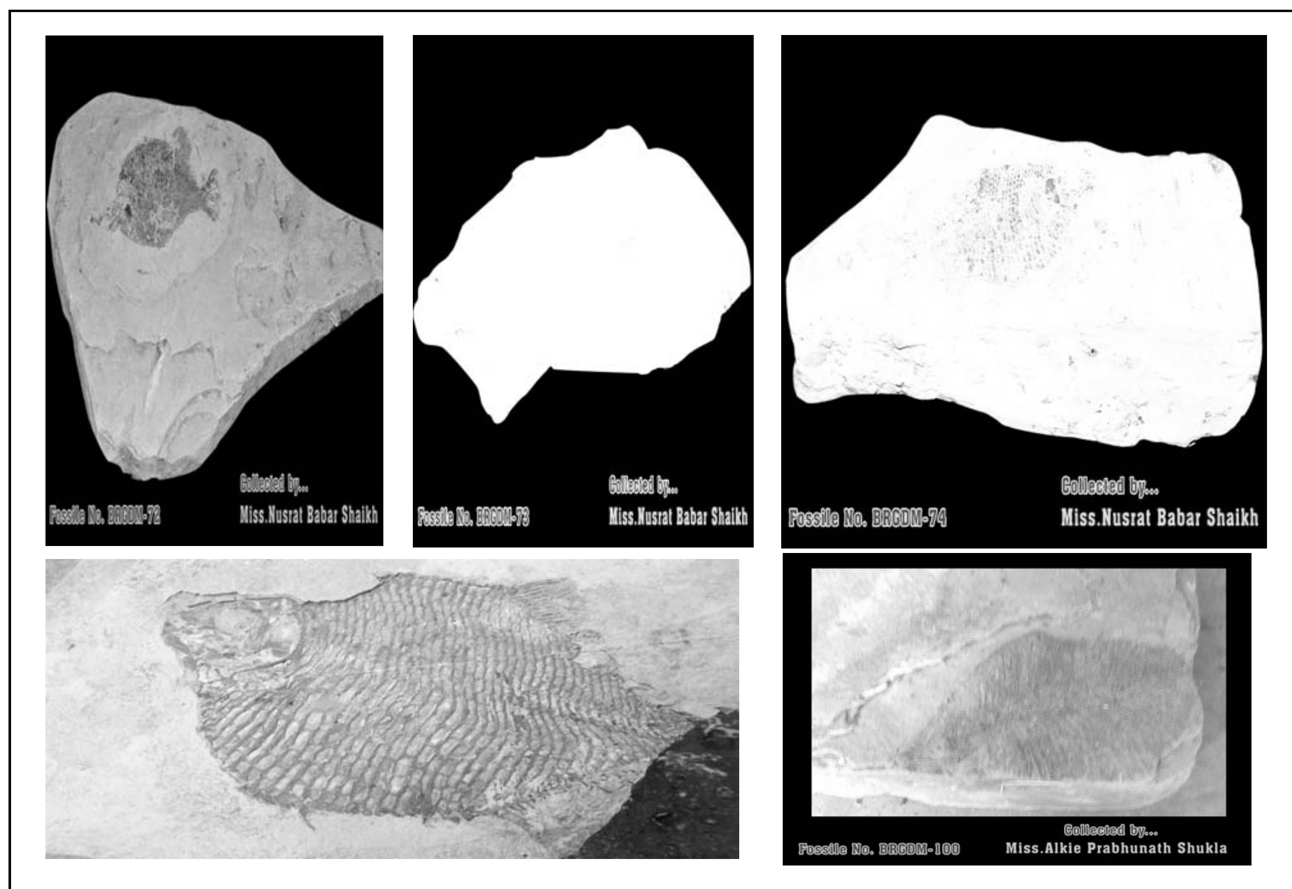
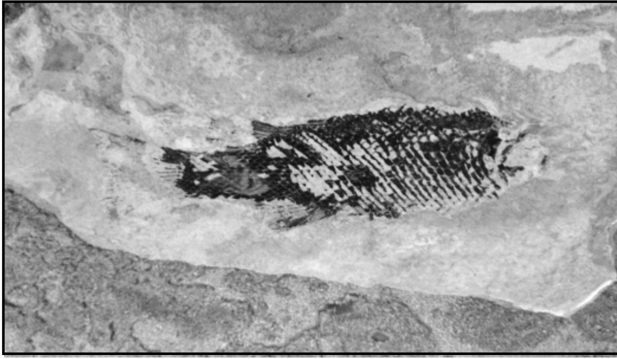
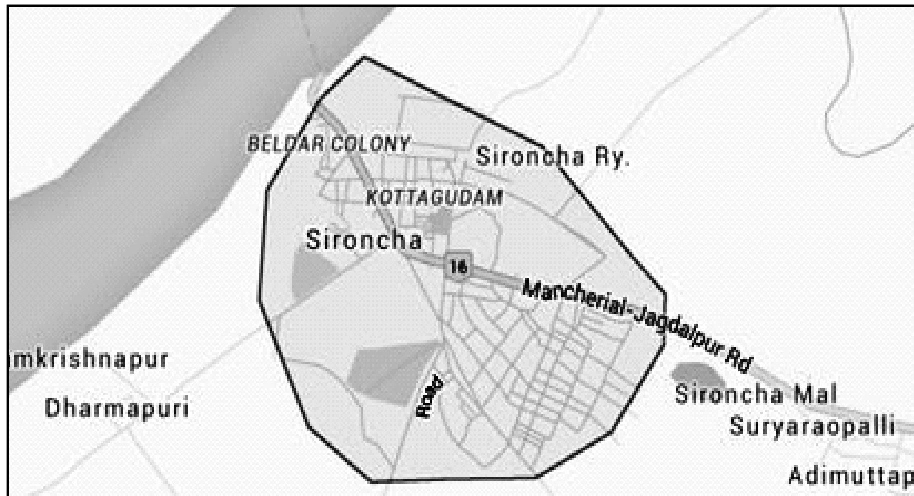
Fossil Fishesh





Map of Sironcha



Observation on Medicinal Uses of Trees Found from Jawaharlal Nehru Ayurvedic Udhyan, Gandhinagar, Gujarat, India

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Abstract: The term "medicinal plant" Include various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses.The word "Herb" has been derived from the Latin word, "herba" and an old French word "herbe". Now A days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non-woody plant. Earlier, the term "herb" was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities.The Jawaharlal Nehru Ayurvedic Udhyan is spread over 30 acres. Once the meditional properties of wild plat is organized by an established medicine system like allopathic , ayurvedic. The main reason for this undesirable situation is that as on today "Many of the plant base pharmaceutical entrepreneurs produce that raw from the wild rather than cultivating them. Jawaharlal Nehru Udhyan there are 240 onwards plant species and 200 onward medicinal plants are growing in the Udhyan. It is situated behind Ministers bunglow , Gandhinar , Gujarat. The paper highlights the less known medicinal uses and Taxonomic Description of 46 plants. The objective of the study was to document the knowledge of medicinal plants, to sensitize and create awareness among the local people about the importants of medicinal plant resources of Gujarat , India.

Keywords: Medicinal Uses , Trees, Ayurvedic Udhyan

Introduction

Plants have been used for medicinal purposes since prehistoric period. Ancient Unani manuscripts, Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically.

Traditional systems of medicine has been widely practised on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.

Among ancient civilisations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practised in India.

Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants.

As per data available over three-quarters of the world population relies mainly on plants and plant extracts for their health care needs. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It has been estimated, that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China, the contribution is as much as 80%. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world.

Medicinal plants such as Aloe, Tulsi, Neem, Turmeric and Ginger cure several common ailments. These are considered as home remedies in many parts of the country. It is known fact that lots of consumers are using Basil (Tulsi) for making medicines, black tea, inpooja and other activities in their day to day life.

Medicinal plants are considered as a Richresources of ingredients which can be used in drug development either Pharmacopoeial, non- pharmacopoeial or synthetic drugs. Moreover, some plants are considered as important source of nutrition and as a result of that they are recommended for their therapeutic values. Some of these plants include ginger, green tea, walnuts, aloe, pepper and turmeric etc. Some plants and their derivatives are considered as important source for active ingredients which are used in aspirin and toothpaste

etc.Apart from the Medicinal Uses, herbs are also used in natural dye, pest control, food, perfume, tea and so on. Many Herbal are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxins. These are also known as 'blood cleansers'. Certain herbal improve the immunity of the person, thereby reducing conditions such as fever.

Over the past two decades, there has been a tremendous increase in the use of herbal medicine; however, there is still a significant lack of research data in this field. Therefore since 1999, WHO has published three volumes of the WHO monographs on selected medicinal plants.

➤ **Study Area**

The Government Herbal Botanical Garden inspires human society to plant the trees and taking care of all matters. Government Herbal Botanical Garden is situated in sector 20, Gandhinagar. Thus Government Herbal Botanical Garden gives us inspiration to protect the plants and to know its uses. There are various plants planted according to different themes like planets, constellations.

Present paper indicates 100 constellations with 100 plant- species show importance of individual. In the ancient time our elderswere directly or indirectly connected with the trees. Theybelieved that plants are useful for different purposes in life.Recently people believe in astrology, so they can care and protect the plants regularly.

Methodology

Such regions were frequently visited and plants observed and photos taken of the medicinal plant species . According to Bentham and Hooker all the selected plant species are arranged and presented with colour photo graphs, Botanical name , Local name, Family and Uses given in the present research paper. Plants were identified by using flora of Gujarat state by (Shah-1978) and the text book of systematic botany by R. N. Sutaria (1958) and other standard book.Some important information of various medicinal plants collected from Yogi Sir. We reported certain species by the photo graphs and uses of the medicinal plants.

Result and Discussion

Total 10 medicinal plants different8 families recorded. This paper Botanical name , Family , Local name and Uses are taken. Scientific studies may elaborate the prospect of growing more and more medicinal plants successively. By proper management of medicinal plants remarkable improvement may be made on the earning of foreign exchange for the country. Certain industries based on the medicinal plants may be developed which will not only be economically viable but also help in the economic up liftman of the nation. On the basis of distribution of some medicinal plants it is observed that, there is a good scope for commercial exploration of some pharmaceutically important medicinal plants. The authors are very optimistic about the reclamation of waste and uncultivated land in the state for turning them into innumerable medicinal plant gardens. Fig is shown Density of Different Species Of Trees , Shrubs , Herbs and Climbers Of Medicinal Plants , which are growing in the study area. List of Biodiversity of Medicinal Plants is given in the table. Plate 1 and Plate 2 shows images of different species of the study area.

1. *Annona reticulata*

Local Name: Ramphal, Bullock's heart

Family: Annonaceae

Habit: Tree

Chemical Constituent: Annotemoyin-2, reticulatain-2, motrilin, cherimolin-1, cherimolin-2.

Medicinal Uses: A root decoction is taken as a febrifuge, while fragments of the root bark are packed around the gums to relieve toothache. The bark is very astringent and the decoction is taken as a tonic and also as a remedy for diarrhea and dysentery.

2. *Polyalthia longifolia*

Local Name: Asopalav, mast tree, false ashok

Family: Annonaceae

ISBN : 978-81-923628-5-4

Habit: Tree

Chemical Constituent**:** *P. longifolia* mainly contains diterpenoids, alkaloids, tannins and mucilage. The chief components of the plant are 0-methylbulbo capnine-N-oxide, polyfothine, N-methylnandigerine-N-oxide.

Medicinal Uses: *P. longifolia* are well known as folk medicines for thw treatment of septic infection, coughing, hepatomegaly, hepatospleenomegaly, diarrhea and cancers.

3. *Phyllanthus emblica*

Local Name: Aamla

Family: Phyllanthaceae

Habit: Tree

Chemical Constituent**:** ascorbic acid (vit.C), anti-oxidant, ellagitannins, emblicanin-A-B, Punigluconin and pedunculagin,punicafolin and phyllanemblinin A, phyllanemblin, pollyphenols.

Medicinal Uses: Aamla fruit is sour and as tringent in taste, with sweet, bitter and pungent secondary tastes. Aamla˜ s qualities are light and dry the post digestive effect is sweet and its energy is cooling. Aamla is used to revitalizing potency and the digestive system, rejuvenating longevity, treat constipation, reduce fever, purity the blood, reduce cough, alleviate asthma, strengthen the heart, benefit the eyes, stimulate hair growth, enliven the body and enhance intellect.

4. *Cascabela peruviana*

Local Name: Yellow oleander, Lucky nut, Pili karen

Family: Apocynaceae

Habit: Tree

Chemical Constituent**:** Phytosterolin, glucosides, ahouain,kakilphin, thevetin,neriifolin, acetylneriifolin, thevefolin, theveneriin, cerebrin, peruvoside, apigenin-5-methyl ether, theveside, viridoside, perusitin, polyhydroxy-dinormonoterpenoids, apiosylglucosides, hesperitin-7-glucoside.

Medicinal Uses: All parts of the plant are poisonous. Milky juice of the plant is also highly poisonous. Bark and seeds are cardiac tonic and strong cardiac stimulant. Bark is cathartic powerful febrifuge and emetic, useful in various kinds of intermittent fever.Seed are abortifacient, used for suicidal and homicidal purposes. Seeds oil is emetic and purgative and anti-bacterial and anti-fungal properties.

5. *Ziziphus jujuba*

Local Name: Indian jujube, Bor, Mota bor

Family: Rhamnaceae

Habit: Tree

Chemical Constituent**:** Triterpenoids, two sterols, six flavonoids, 3-cerebrosides.

Medicinal Uses: Anti-fungal, anti-bacterial, anti-spastic, anti-fertility, hypotensive, anti-nephritic, cardio-tonic, anti-oxidant, immunostimulants, fruits helpful for chronic constipation and jaundice, treated colds, flu and coughing.

6. *Bixa orellana*

Local Name: Sindoori, Lipstick tree, Annattoo

Family: Bixaceae

Habit: Tree

Chemical Constituent**:** farnesyl acetate, occidentalol acetate, spathulenol and ishwarane.

Medicinal Uses: feverish infections including gonorrhea, dysentery and hepatitis, protect the liver, reduce cholesterol, snake bites, insects, protects the skin against the ultraviolet rays of the Sun, skin tonic, to heal skin conditions and diabetes.

7. *Ficus religiosa*

Local Name: Pipado, Bodhi tree, Scaredfig

Family: Moraceae

Habit: Tree

Chemical Constituent: Phenol, tannins, sterolds, alkaloids and flavonoids, â-sitosteryl-D-glycoside, vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupen-3-one.

Medicinal Uses: astringent, hemostatic, anti-inflammatory and laxative, burn injuries and inflammatory swellings, wounds, skin diseases, constipation, digestive, asthma, cooling, hemorrhages, diabetes, diarrhea, gonorrhea, leucorrhea, cracked feet, anal fistula, aphthous sores, menorrhagia, nervous disorders, metrorrhagis, blood dysentery, bleeding piles, hematuria, hemorrhages.

8. *Gossipium herbacium*

Local Name: Castor

Family: Malvaceae

Habit: Tree

Chemical Constituent**:** Hemigosypol, quercimetitrin, gossypol, caryophyllene, pinene, limonene.

Medicinal Uses: Headache, pain killer, wound healing, constipation, liver related problems, uterus related problems, Breast discomforts, Dysurea, urine related problems, fever especially malaria, blood related disorders, General body weakness, poisoning.

9. *Murraya koenigii*

Local Name: Kadi-patti, Mitho-Limbdo

Family: Rutaceae

Habit: Tree

Chemical Constituent: Mahanimbine, girinimbine, murrayanine, murrayazoline, murrayacine, sucrose.

Medicinal Uses: Anti-oxidant, anti-noiciceptive, lipid lowering, alzheimer's disease, anti diabetic and islet protective, gastrointestinal disorders, anti-cancer, hepato protective, anti-bacterial.

10. *Citrus limon*

Local Name: Lemon, Limbu

Family: Rutaceae

Habit: Tree

Chemical Constituent: Vitamin C, essential nutrients, polyphenols, terpenes, tannins and citric acid.

Medicinal Uses: Anti-septic, anti-biotic, anti-viral, protection of the liver, kidneys, bladder and pancreas, digestion, diet, protection of the cardio vascular systems, blood circulation, blood purifier, skin protection, hair, nails, eyes, teeth, mouth, throat, joint and tendon pains, inflammations.

Checklist of Trees Found From Jawaharlal Nehru Ayurvedic Udhyan, Gandhinagar

Sr. No.	Scientific Name	Local Name	Family	Habit
1	*Annona Reticulata*	Ramphal	Annonaceae	Tree
2	*Polyalthia Longifolia*	Asopalav	Annonaceae	Tree
3	*Phyllanthus Emblica*	Aamla	Phyllanthaceae	Tree
4	*Cascabela Peruviana*	Yellow oleander	Apocynaceae	Tree
5	*Ziziphus Jujuba*	Bor	Rhamnaceae	Tree
6	*Bixa Orellana*	Sindoori	Bixaceae	Tree
7	*Ficus Religiosa*	Piparo	Moraceae	Tree
8	*Gossipium Herbacium*	Castor	Malvaceae	Tree
9	*Murraya Koenigii*	Mitho-Limbdo	Rutaceae	Tree
10	*Citrus Limon*	Limbu	Rutaceae	Tree

Conclusion

In Botnical Herbal Garden, Gandhinagar we have found total 100 plant species belonging to 58 families out of which 45 Trees, 19 Shrubs, 16 Climbers, 13 Herbs, 5 Succulents, 1 Grasses. And we didn't find cultivation of any rare species in the garden.

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

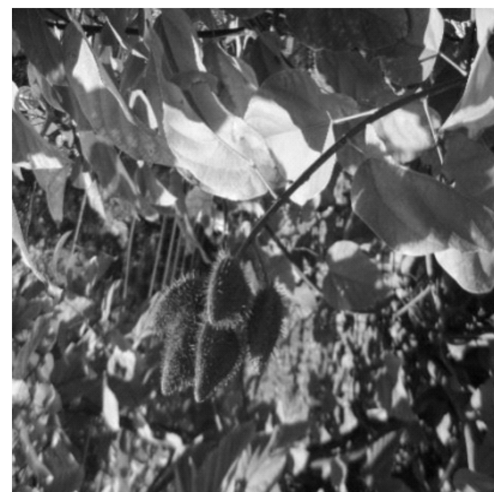

Figure 6

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Figure 7

Figure 8

Figure 9

Figure 10

Floristic Study of Shahapur Taluka (Tehsil), Thane District, Maharashtra

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Abstract: The present work provide the first systematic account of the flora from the adjoining forest region of Gokulgaon village, Shahapur taluka, Thane district. It is a part of Konkan region which share biodiversity of western ghat upto certain extent. During this study, various species of monsoon ephimerals, shrubs & trees were recorded. Among them few plants were found to be dominant as frequent in appearances e.g. *Tectona grandis, Carissa carandus, Madhuca indica* etc. Thus the current study of floristic diversity can serve as primary data for fuether research pertaining to various aspects of biodiversity.

Keywords: Shahapur taluka, flora, biodiversity

Introduction

Shahapur is the largest tehsil in Thane district. Shahapur is surrounded by Western Ghats (Sahyadri). The Mahuli fort is the largest peak of shahapur. Shahapur is located at 19.45'N 73.33'E.The selected study area is village forest of shahapur taluka. Gokulgaon village is located in shahapur tehsil. Gokulgaon village is 16-17 km away from the shahapur tehsil headquartor. The forest type of tehsil is "Tropical Moist Deciduous Forest" according to Revised classification of India forest types by (Champion & Seth, 1968). The typical soil derived from the deccan trap is the black cotton soil contains high aluminia and carbonates of calcium & magnesium with variable amount of potash. The rainy season last from June to September & average rainfall is about 3000mm. The main river of tehsil is Bhatsa, Tansa & kalu river. Bhatsa and Tansa are main reservoir. Shahapur tehsil is highest population of Katkari and Thakar (Kamble *et.al.*2009) tribes. The study area has Katkari & Thakar tribes which make their homes using the stem of Karvi plant (*Strobilanthes* spp).

The Vegetation is tropical moist deciduous forest. Major tree species in forest is Ain (*Terminalia tomentosa*), Savar (*Bombax ceiba*), Moha (*Madhuca indica*), Sag (*Tectona grandis*), Mango (*Mangifera indica*), Khair (*Acacia catechu*), Palas (*Butea monosperma*), Karvand (*Carissa carandas*) etc. (Ghate *et al*., 2009).

Methodology

The study area selected to explore the forest of gokulgaon village, Shahapur Taluka, Thane district, Maharashtra, India. The survey of forest was done during the month of April to October. The field data such as name of trees, shrub, habit, colour of flower were recorded. The first visit was accompanied in April month. After that study area was visited in every month upto October. The listing of plants was done with help of various floras (Cook, 1967) and experts taxonomist. The photographs were taken. The identification of plants was done by doing morphological study using various floras (Almenda, 2010; Chandore, 2015; Kakade, 2014).

Conclusion

The study area was explored from botanical point of view for first time. The documented data inclusive of information about herbs, shrubs, tree species found in summer and monsoon season. The most dominant species was *Carissa carandas, Madhuca indica, Gloriosa superba, Tectona grandis, Curcuma pseudomontana, Butea monosperma, Strobilanthus callosa,* etc. These species are involved in socioeconomic and cultural aspects of many local tribal communities. The tribal people consider *Madhuca indica* (Moh) as god tree; it yields edible fruit and flowers. The fruits of mohare used to make vegetable while seed oil used for cooking purpose. The *Strobilanthus spp* (karvi) used for making walls of huts. The ripe fruits of *Carissa carandas* and *Ziziphus rugosa* are sold by tribal womens in markets of Mumbai suburbs as 'Ranmeva' that helps to improve the economic status of tribal people. The flowers of *Gloriosa superba* is used to worship as local deity. This botanical data involving listing of plants can serve as primary data of a given area. Shahapur tehsil is rapidly developing area located near Mumbai. Therefore there are likely chances of threat to various valuable species. The present work can indirectly help in assessment of impact of urbanization on existing flora of tehsil.

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Table 1: List of plants of study area as follows.

Sr. No.	Botanical Name	Family	Common Name
1	*Abelmoschus manihot* L.	Malvaceae	Ranbhendi
2	*Abrus precatorius* L.	Fabaceae	Gunj
3	*Acacia catechu* (L.)Willd.	Mimosaceae	Khair
4	*Acacia nilotica* (L.)Willd. Ex Delile	Mimosaceae	Babhul
5	*Acacia pennata* (L.)Willd.	Mimosaceae	Shembi
6	*Alternanthera sessilis* (L.) R.Br.	Amaranthaceae	-
7	*Amaranthus spinosus* L.	Amaranthaceae	Math
8	*Amorphophallus commutatus* (Schott) Engl.	Araceae	Shevla
9	*Ampelocissus latifolia* (Roxb.) Planch.	Vitaceae	Wild grapes
10	*Achyranthus aspera* L.	Amaranthaceae	Aghada
11	*Adhatoda vasica* Nees	Acanthaceae	Adulsa
12	*Argemone mexicana* L.	Papaveraceae	Pivladhotra
13	*Alysicarpus heyneanus* Wight &Arn.	Fabaceae	
14	*Azadirachta indica* A. Juss.	Meliaceae	Kadunimb
15	*Bambusa bamboo* L.	Poaceae	Bamboo
16	*Barleria prionitis* L.	Acanthaceae	Katekoranti
17	*Bauhinia purpurea* L.	Caesalpiniaceae	Rakt-chandan
18	*Bauhinia racemosa* Lam.	Caesalpiniaceae	Apta
19	*Bauhinia malabarica* Roxb.	Caesalpiniaceae	Korla
20	*Bombax ceiba* L.	Bombaceae	Katesavar
21	*Borassus flabellifer* L.	Palmae	Tad
22	*Breynia retusa* L.	Euphorbiaceae	Cup-bashi plant
23	*Butea monosperma* (Lamk.) Taub.	Fabaceae	Flame of forest,palas
24	*Calotropis gigantea* (L.) W. T. Aiton	Asclepidaceae	Rui
25	*Carissa carandas* L.	Apocynaceae	Karvand
26	*Carvia callosa* (Nees) Bremek Syn. *Strobilanthes callosa* (Nees)	Acanthaceae	Karvi

27	*Cassia tora* L.	Fabaceae	Takla
28	*Celosia argentea* L.	Amaranthaceae	Kurdu
29	*Costus speciosus* (Koen.) J. E. Smith	Costaceae	Peva
30	*Cleome viscose* L.	Capparidaceae	Pivlitilvan
31	*Cleome rutidosperma* DC.	Capparidaceae	Gulabitilvan
32	*Cordia dichotoma* Forst. f.	Boraginaceae	Bhokar
33	*Chromolaena odorata (*L.)R.M.King	Asteraceae	Bitter bush
34	*Cucurbita maxima* Duchesne	Cucurbitaceae	
35	*Curculigo orchioides* Gaertn.	Hypoxidaceae	Kali musli
36	*Curcuma pseudomontana* J.Graham, Cat. Pl.	Zingiberaceae	Hill turmeric
37	*Cuscuta reflaxa* Roxb.	Convolvulaceae	Amarvel
38	*Cynodon dactylon* (L.) Pers.	Poaceae	Durva
39	*Cyperus rotundus* L.	Cyperaceae	Barikmotha
40	*Dioscorea bulbifera* L.	Dioscoreaceae	Kadukaranda
41	*Eleusine indica* (L.) Gaertn.	Poaceae	
42	*Emblica officinalis* Gaertn.	Phyllanthaceae	Avla
43	*Ensete superbum* Roxb.	Musaceae	Ran kel
44	*Ficus benghalensis* L.	Moraceae	Vad
45	*Ficus racemosa* L.	Moraceae	Umber
46	*Ficus religiosa* L.	Moraceae	Pimple
47	*Gloriosa superba* L.	Liliaceae	Agnishikha
48	*Grewia tilifolia* Vahl.	Malvaceae	Dhaman
49	*Guruga pinnata* Roxb.	Burseraceae	Kakad
50	*Helicteres isora* L.	Sterculiaceae	Murudsheng
51	*Holarrhena antidysentrica* (L.)Wall.	Apocynaceae	PandharaKuda
52	*Hyptis suaveolens* L.	Lamiaceae	Bush mint
53	*Impatience balsamina* L.	Balsamianaceae	Terda
54	*Indigofera astragalina* DC.	Fabaceae	Ranmethi
55	*Impatience minor* (DC.) Bennet.	Balsamianaceae	Chhotaterda
56	*Lantana camara* L.	Verbenaceae	Ghaneri
57	*Leea microphylla*	Vitaceae	Gajkarna
58	*Madhuca indica* (J.Koning) J. F. Macbr.	Sapotaceae	Moh
59	*Momordica dioica* Roxb. Ex Willd.	Cucurbitaceae	Kartoli

60	*Mangifera indica* L.	Anacardiaceae	Mango
61	*Macuna pruriens* L.	Fabaceae	Khajkuiri
62	*Malachra capitata* (L.)L.	Malvaceae	Ran bhendi
63	*Neuracanthus sphaerostachyus* Dalzell	Acanthaceae	Golgonda
64	*Physalis minima* L.	Solanaceae	Kapalphodi
65	*Plumeria rubra* L.	Apocynaceae	Pandharachapha
66	*Pongamia pinnata* (L.)Pierre	Fabaceae	Karanj
67	*Portulaca oleracea* L.	Portulaceae	Ghol
68	*Persicaria glabra* (Willd.)M.Gomez.	Polygonaceae	Sheral
69	*Ricinus communis* L.	Ephorbiacae	Castor
70	*Sesamum indicum* L.	Pedaliaceae	Sesam
71	*Smithia sensitive* Aiton	Fabaceae	Kavla
72	*Solanum virginianum* L.	Solanaceae	Kateringni
73	*Sphaeranthus indicus* L.	Asteraceae	Gorakhmundi
74	*Syzygium cumini* (L.)Skeels.	Myrtaceae	Jamun
75	*Tamarindus indica* L.	Caesalpiniaceae	Tamarind
76	*Tectona grandis* L.f	Verbenaceae	Teak
77	*Terminalia arjuna* (Roxb.) Wight & Arn	Combretaceae	Arjun
78	*Terminalia cebula* Retz.	Combretaceae	Hirda
79	*Terminalia tomentosa* (Roxb.)Wight & Arn	Combretaceae	Ain
80	*Thespesia populnea* (L.)Sol. Ex Correa	Malvaceae	Paraspimple
81	*Trichodesma indicum* (L.)Sm.	Boraginaceae	Chhotakalp
82	*Ziziphus jujuba* Mill.	Rhamnaceae	Ber
83	*Ziziphus rugosa* Lam.	Rhamnaceae	Wild ber

Curculigo orchioides

Gloriosa superba L.

Curcuma pseudomontana

Dioscorea bulbifera L.

Helicteres isora L.

Madhuca indica

Breynia retusa L.

Grewia tilifolia

Ziziphus rugosa

Carissa carandas

Holarrhena antidysentrica

Fruits of *Z. rugosa*

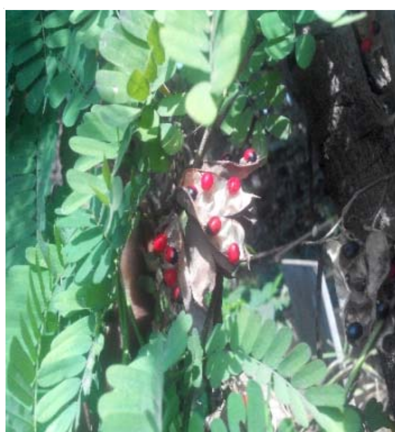
 ISBN : 978-81-923628-5-4

Costus speciosus

Fruits of *Momordica dioica*

Tectona grandis

Bauhinia malabarica



Abrus precatorius

Neuracanthus sphaerostachyus

Cordia dichotoma

Fruits of *Madhuca indica*

Tribal home made by dry
stems of *Strobilanthus* spp.

Present Avifaunal Diversity And Allied Ecology of Bhandup Pumping Station

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Abstract: Since long, Bhandup Pumping Station has been the sewage dumping area of Mumbai City. This site is a mosaic of salt marshes, mangroves, wetlands and trees. It is also home to several migratory and residential birds, despite being located amidst the busy suburb of Bhandup in Mumbai. The present research was conducted to study the ecology of this area during the period of January 2016 to January 2017. Seasonal variations were recorded in avifaunal diversity as also sediment and water quality parameters. Avifaunal diversity showed an increase during post-monsoon period along with increase in salinity of water and increase in dissolved oxygen. Values of Nitrates, Nitrites increased and Phosphates showed a decrease in post-monsoon and pre-monsoon seasons. High values of phosphates were recorded during the monsoon season along with decrease in dissolved oxygen and salinity of water. The study creates awareness about the unique bird habitat which is threatened at present by various anthropogenic disturbances of locals.

Keywords: Wetland, Avifauna, Seasonal variations, Physico-chemical parameters

Introduction

Bhandup Pumping Station is a wetland surrounded by mangroves, salt marshes and trees. It is surprising to find numerous resident as well as migratory birds in this area amidst dense human population and pollution of the suburb. Wetlands provide home to a huge diversity of wildlife such as birds, mammals, fish, frogs, insects and plants (Buckton, 2007). Birds prefer specific types of habitats. Within a habitat, birds choose from the forest floor and low bush to the middle level and high canopy (Monga, 2003). Under stress of abnormal conditions birds are frequently driven out of their accustomed habitats in search of a living and are met with as stragglers far beyond their normal range (Ali, 2002). Hence it is imperative to conserve the bird habitats. Many bird species require mixed habitat types (Grimmett, Inskipp, and Inskipp). Habitat preference amongst birds depends on several biotic and abiotic factors.

Manikannan, *et al*, 2012, studied the factors affecting waders' population in the Great Vedaranyam Swamp of Point Calimere Wildlife Sanctuary, while Pawar, 2012, studied species diversity in Mangroves of Uran (Raigad), Navi Mumbai. Chaudhari-Pachpande and Pejaver, 2016, have done a preliminary study on Birds of Thane Creek.

Present study was undertaken to document existing avifaunal diversity and know the water and sediment quality parameters of study area.

Materials and Methods

Bhandup Pumping Station is geographically located at 19.14 degree North and 72.93 degree East co-ordinates and near Bhandup, an eastern suburb in Mumbai.

Observation of Birds: Study site was visited every 15 days and birds were observed by NIKON 8x40 CF binoculars, photographed using NIKON D 3200 camera and identified with the help of Guide Book by Ali, 2002. Taxonomy of the birds was studied with the help of Grimmett, Inskipp and Inskipp, 2003. Standardised common and scientific names were referred from Manakadan, R. and Pittie, A. (2001).

Water Sampling: Water sampling was done by immersing labelled plastic bottles in the water at the study site during high tide and low tide. Analysis was carried out for parameters as Nature of water, pH, Salinity, Dissolved Oxygen, Nitrite-nitrogen, Nitrate-nitrogen, Phosphate, COD and BOD following the Standard Methods for the Examination of Water and Wastewater, American Public Health Association (APHA), 1998.

Sediment Sampling: Sediment samples were collected by spatula and transferred to labelled plastic bags. Sediments were air dried and prepared soil was analysed for parameters as Sediment colour, pH, Water retention capacity, Soil composition including percentage of sand, silt and clay, Organic carbon, Organic matter, Total Nitrogen and Phosphorus; following the Soil and Water Testing Methods, 1983.

Results and Discussions

Avifaunal diversity was maximum post monsoon. Birds were identified till species level and avifauna belonging to 12 orders, 21 families, and 56 species were recorded. Rahmani *et al*, 2014, studied birds of Maharashtra and have confirmed the presence of near threatened birds as per IUCN guidelines. Some of the near threatened birds as per the IUCN guidelines were observed in the present study area. They are the Lesser Flamingos (*Phoeniconaias minor*), Painted Storks (*Mycteria leucocephala*), Black-headed Ibises (*Threskiornis melanocephalus*), Black-tailed Godwits (*Limosa limosa*) and River Terns (*Sterna aurantia*).

The pH indicates the health and productivity of the wetland. In acidic pH of water, aquatic plants and animals cannot survive which results inversely on avifaunal

diversity. pH of the water in the present study area (Table 1) implied alkaline nature of the water.

Salinity increased during pre-monsoon and post-monsoon seasons, showing a decrease during monsoon (Table 1), due to the rain water, run offs from land drainage as also wave action. Salinity is vital for survival of fish, frogs, benthic fauna which are consumed by waders.

Dissolved Oxygen [DO] (Table 1), imply that DO was more during the pre and post-monsoon and less during monsoon. Above values show that more DO was recorded when more birds were present. Low DO indicates pollution due to domestic sewage / effluents which increases demand on oxygen consumption.

Nitrates and Nitrites showed a decrease during monsoon and increase during pre and post monsoon seasons (Table 1). Low values during monsoon due to mixing of water from land drainage and increase during pre and post monsoon seasons supports good productivity of the wetland.

High values of Phosphates (Table 1) in the monsoon point out the organic pollution due to rain water run-offs, as also toxic land-water drainage from the neighbouring area. High phosphate correlates with high BOD of the study area.

Chemical Oxygen Demand [COD] (Table 1) showed decrease during monsoon and high values during pre and post-monsoon seasons.

Pre and post-monsoons showed a higher Biochemical Oxygen Demand [BOD] (Table 1) than monsoons as organic decay of mangrove leaves as also evaporation increase the demand for oxygen in water.

BOD and DO are important indicators about pollutants in the water body. Values of COD were greater than the values of BOD implying presence of organic pollution.

Sediment analysis (Table 2) showed that the sediment was blackish in colour. Average pH value for all three seasons shows alkaline quality of the sediment and good water holding capacity. Nature of the sediment was found to be sandy- silty- clayey. Black soil with good water holding capacity and organic carbon content as also organic matter supports good productivity and faunal diversity.

Conclusion

Present study area, a resilient bird habitat is under threat due to ever expanding metropolis. It needs to be protected from people driving vehicles inside the study area as it scares away the birds, residents dumping plastics and domestic waste and cutting trees which results in fragmentation of the habitat. There is need to increase local participation and create awareness about conservation of this important ecological resource.

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Table 1: Water quality analysis at Bhandup Pumping Station

Sr. No.	Parameter	Average values at high &low tides	Average values at high &low tides	Average values at high &low tides
	Season	**Pre-monsoon**	**Monsoon**	**Post-monsoon**
1	pH	8.35	7.6	9.4
2	Salinity (ppt)	46	1.75	43
3	Dissolved Oxygen (mg/l)	5.0	0.6	7
4	Nitrite-nitrogen (No2-N: mg/l)	0.05	0.12	0.67
5	Nitrate-nitrogen (NO3-N:mg/l)	0.58	0.38	0.11
6	Phosphate (PO4-P: mg/l)	0.92	0.81	1.48
7	COD (mg/l)	71	30	59
8	BOD (mg/l)	15.5	11.75	16.05

Table 2: Sediment Quality Analysis at Bhandup Pumping Station

Sr. No.	Parameter	Sediment Sample	Sediment Sample	Sediment Sample
	Season	**Pre-monsoon**	**Monsoon**	**Post-monsoon**
1	Sediment Colour	Blackish	Blackish	Blackish
2	pH	7.8	7.2	8.5
3	Water retention capacity	61.55	67.10	71.43
		Soil Composition (%)		
4	Sand	41.0	40	42
5	Silt	28.0	26	30
6	Clay	31.0	34	28
7	Organic Carbon (%)	2.67	0.37	2.61
8	Organic Matter (%)	4.60	0.63	4.49
9	Total Nitrogen (mg/100gm)	270	35	266
10	Phosphorus (mg/100gm)	8.54	4.9	8.32

Study of Anti-Genotoxicity of Sonneratia Alba J. Smith. Fruit Extract

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Abstract: Genotoxins are involved in the chromosomal aberrations causes pathogenesis of several chronic degenerative diseases. The mature fruit powder extracts (0.5% and 1.0%) of *Sonneratia alba*. were tested for antigenotoxic potential by *Allium cepa* root tip meristem cells test. The treatment of 0.5% and 1.0% of extracts of fruits and 0.75ppm.HgCl2 and & 7% H_2O_2 were applied to onion root tips with Pre. Post, and Simultaneous treatments. It was noticed that fruit extract of *S.alba* the mitotic index was induced due to Pre. Post, and Simultaneous treatments. The chromosomal aberrations were decreased in response to 0.5 and 1 % fruit extract and were significant in response to pretreatment of (0.5 and 1%). Thus the aqueous fruit extract of *S.alba* have anti-genotoxic potential against 0.75ppm.HgCl2 and & 7% H_2O_2 induced chromosomal aberrations in *Allium cepa* root tip.

Keywords: Antigenotoxic; Cancer; carcinogens; Chromosomal aberrations

Introduction

One of the important mangrove plant is *Sonneratia alba* J.Simth, which is tree to 20 m broadly spreading evergreen plant belongs to the family Sonneratiaceae (now Lythraceae) and genus *Sonneratia* (Little *et al*., 2004). Previous biological investigation showed that, it has antidiabetic property (Morada *et al*., 2011), cytotoxic and antimicrobial activity (Milon et al., 2012) and nutritional value (Patil *et al*., 2012).

In the past few years' considerable advancement were made in the natural products endowed with anti-mutagenic and anti-carcinogenic properties. Many natural products referred to as dietary chemo preventive compounds offer a great potential in the fight against cancer through a different range of mechanisms including antioxidant, anti-mutagenic activity, enzyme modulation, gene expression, apoptosis etc.(Flora *et al*., 2001), (Webb and Ebeler, 2004), (Miadokova *et al*., 2008).

To the best of our knowledge, no research on the antigenotoxicity properties of fruits of *S. alba* has been carried out. Hence, it was thought worthwhile to the study, antigenotoxic potential of fruit extract of *S. alba.*

Materials and Methods

Plant collection and extraction: -Fresh Fruits of *Sonneratia alba* were collected from the creek of Kharda at Malyai village near to Devgad. Creek Kharda belongs to Taluka Devgad, Dist. Sindhudurg. State Maharashtra. Devgad (16^0 23' N, 73^0 21' E; p. 2,493), 8m above Sea level. It was oven dried and powdered .Fruit powder of *Sonneratia alba* (2g) was subjected to extraction, using 100ml. D.W. boiled for 10min.Extract was filtered through using Whatman no. 1 filter paper. The 0.5 and 1 % extracts were used to treat the *Allium cepa* to evaluate the anti-genotoxic effects of *S.alba* plant extract. Equal sized *A. cepa* Onion bulbs (3 to 4gm) carefully unscaled and placed on top of test tubes filled with D.W and allowed to germinate in Laboratory. Separate two sets of equal sized germinated onion bulbs were taken and placed, on a test tubes containing 7% H_2O_2 and 0.75ppm $HgCl_2$ for one hour. After one hour onion bulbs placed on test tube containing 7% H_2O_2 and 0.75ppm $HgCl_2$ were removed and their root tips were cut and fixed in the Carnoy's fluid (Ethanol : Acetic acid , 3:1). The plant extracts were applied as pre, post and simultaneous treatments by using method of (Sharma and Vig, 2012).

Results and Discussion

The effect of Pre, Post and Simultaneous treatments showed dose dependent protective effect against 7% H_2O_2, 0.75ppm HgCl2, induced chromosomal aberrations. Fruit powder extracts of *Sonneratia alba* results in decrease in mitotic index as compare to positive control, (table 1). The Physiological aberrations and Clastogenic aberrations were decreased in response to 0.5 and 1% fruit powder extracts (Table 2, 4).The percent inhibition of chromosomal aberrations were more significant in response to pretreatment of (0.5 and 1%) of *S.alba*. (Table 3, 5).The reduction in percentage of chromosomal aberrations in extract treated groups before, after and simultaneous due to fruit extract of *S.alba* indicates its anti-genotoxic potential.

Conclusion

The aqueous extracts *S. alba* fruits has antigenotoxic potential and have protective effect against the oxidative damage induced by H_2O_2, and chromosomal aberrations caused by $HgCl_2$. Further investigation is required for isolating the possible bioactive constituents responsible for such activities.

Acknowledgment

Authors are thankful to local people and fishermen who helped to complete this work. Also express our gratitude towards to respected teachers, friends and family members.

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Table 1: Effect of Pre, Post and Simultaneous treatments of 7% H_2O_2, 0.75ppm $HgCl_2$, and fruit extracts of *Sonneratia alba* on Mitotic index

Sr. No.	Concentrations	M, I	% of M.I in 7% H_2O_2						% of M.I in 0.75 ppm $HgCl_2$					
		%	I		II		III		I		II		III	
			0.5	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5	1
1	N.C (D.W)	75.40	51.95	46.66	40.95	51.48	42.33	40.43	41.31	41.85	41.33	38.68	37.44	42.37
2	P.C (H_2O_2)	88.74	-	-	-	-	-	-	-	-	-	-	-	-
3	P.C,($HgCl_2$)	69.76	-	-	-	-	-	-	-	-	-	-	-	-

I-Pre, II-Post and III- Simultaneous treatments, N.C- Negative control, P.C- Positive control M.I-Mitotic index.

Table 2: Effect of Pre, Post and Simultaneous treatments of H_2O_2 on extracts of *Sonneratia alba* -on physiological and clastogenic aberrations in root tip cells of *Allium cepa*

	NC	PC	0.5% Treatment			1% Treatment		
			I	II	III	I	II	III
Physiological aberrations (PA)								
C-Mito	-	05	03	02	03	03	03	05
Delayed anaphase	01	03	02	06	03	03	04	01
Laggards	01	03	-	03	02	02	01	01
Stickiness	05	06	03	-	03	01	03	01
Vagrants	-	04	01	01	02	-	01	-
Total PA	07	21	09	12	13	09	12	08

Clastogenic aberrations (CA)								
Bridges 02	03	02	02	04	03	03	02	
Rings -	01	-	-	01	02	03	01	
Breaks 01	06	05	04	01	-	-	03	
Total CA 03	10	07	06	06	05	06	06	
Total aberrations	10	31	16	18	19	14	18	14

Table 3: Effect of Pre, Post and Simultaneous treatments of aqueous extracts of *Sonneratia alba* on percent inhibition of genotoxicity induced by H_2O_2 in root tip cells of *Allium cepa*.

Sr. No.	Types of Aberrant cells	NC	PC	0.5%			1%		
				I	II	III	I	II	III
1	No of cells with (PA)	07	21	09	12	13	09	12	08
2	PI of PA	-	-	85.71	64.29	57.14	85.71	64.29	92.86
3	No of cells with (CA)	03	10	07	06	06	05	06	06
4	PI of CA	-	-	42.86	57.14	57.14	71.43	57.14	57.14
5	(PA± CA)	-	-	-	-	-	-	-	-
6	PI of (PA± CA)	-	-	127.47	121.43	114.28	157.14	121.43	140.00

I-Pre, II-Post and III- Simultaneous treatments, PI- Percent inhibition, PA-Physiological aberrations, CA- Clastogenic aberrations, PI=a-b/a-c x 100. Where a - number of aberrant cells induced by Positive control, b – number of aberrant cells induced by plant extract and c – number of aberrant cells induced by negative control.

Table 4: Effect of Pre, Post and Simultaneous treatments of Mercuric chloride on extracts of *Sonneratia alba* -on physiological and clastogenic aberrations in root tip cells of *Allium cepa*

	NC	PC	0.5% Treatment			1% Treatment		
			I	II	III	I	II	III
Physiological aberrations (PA)								
C-Mito	-	06	04	03	02	03	03	03
Delayed anaphase	01	03	03	02	03	05	04	03
Laggards	01	03	03	01	01	02	03	02
Stickiness	05	07	02	04	03	02	02	03
Vagrants	-	05	01	01	01	-	01	01
Total PA	07	24	13	11	10	12	13	12
Clastogenic aberrations (CA)								
Bridges	02	04	01	03	-	02	01	06
Rings	-	02	01	01	06	02	01	-
Breaks	01	06	03	03	01	02	03	01
Total CA	03	12	05	07	07	06	05	07
Total aberrations	10	36	18	18	17	18	18	19

I-Pre, II-Post and III- Simultaneous treatments. NC- Negative control (Distilled water) PC- Positive control (0.75 ppm Mercuric chloride)

Table 5: Effect of Pre, Post and Simultaneous treatments of aqueous extracts of *Sonneratia alba* on percent inhibition of genotoxicity induced by 0.75ppm $HgCl_2$ in root tip cells of *Allium cepa*.

Sr. No.	Types of Aberrant cells	NC	PC	0.5%			1%		
				I	II	III	I	II	III
1	No of cells with (PA)	07	24	13	11	10	12	13	12
2	PI of PA	-	-	64.70	76.47	82.35	70.54	64.70	92.86
3	No of cells with (CA)	03	12	05	0.7	0.7	06	05	07
4	PI of CA	-	-	77.77	55.55	55.55	66.66	77.77	55.55
5	(PA± CA)	-	-	-	-	-	-	-	-
6	PI of (PA± CA)	-	-	142.47	132.02	137.90	137.20	142.47	126.09

I-Pre, II-Post and III- Simultaneous treatments, PI- Percent inhibition, PA-Physiological aberrations, CA- Clastogenic aberrations, PI=a-b/a-c x 100. Where a - number of aberrant cells induced by Positive control, b – number of aberrant cells induced by plant extract and c – number of aberrant cells induced by negative control.

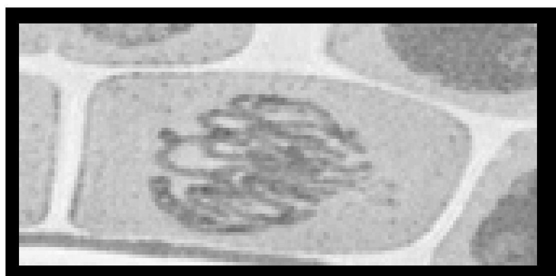

Fig - 1. Prophase

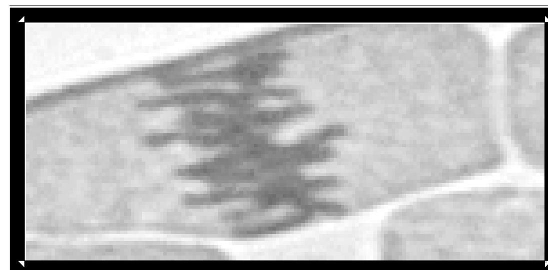

Fig - 2. Metaphase

Fig - 3. Anaphase

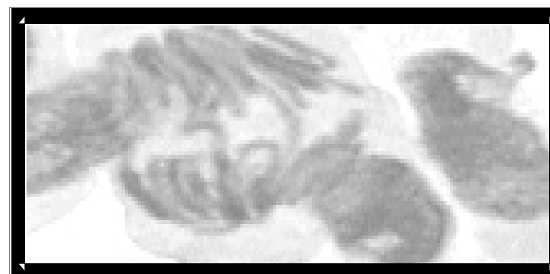

Fig - 4

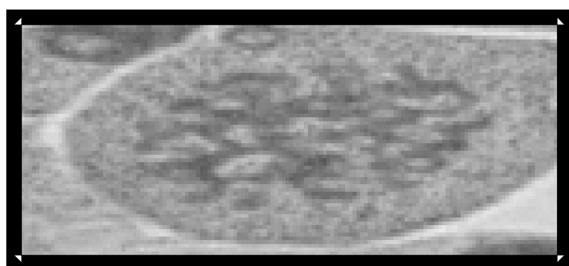

Fig - 5

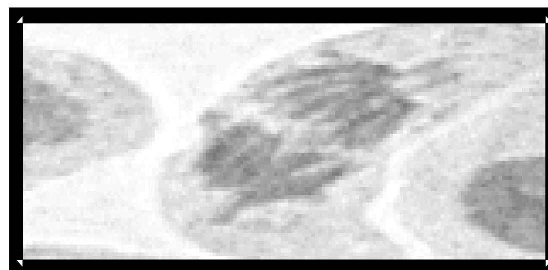

Fig - 6

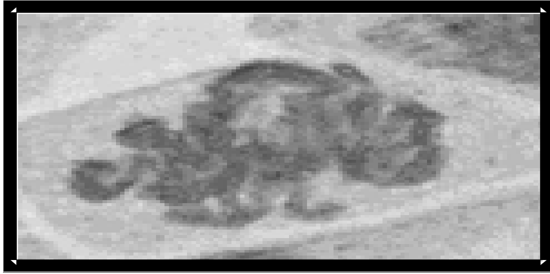

Fig- 7

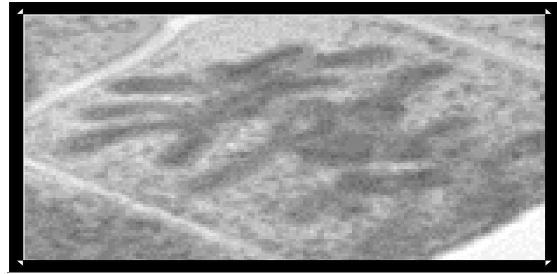

Fig - 8

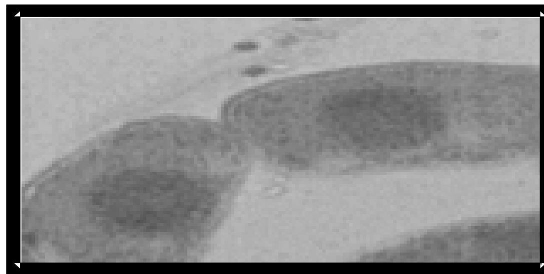

Fig - 9

Fig-1) Normal Prophase, 2) Metaphase, 3) Anaphase 4) Bridge in Anaphase 5) Abnormal prophase 6) Abnormal. Anaphase 7) C-Mitosis. 8) Abnormal Metaphase 9) Cells treated with plant extract

Antioxidant Activity of Leaves From Field Grown Plants and *In Vitro* Seedlings of *Lawsonia inermis* L.

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Abstract: Phenolic compounds and flavonoids from medicinal plants possess strong antioxidant activity. In the present study total phenol content, flavonoid content and relative antioxidant capacities of leaves of field grown plants and *in vitro* seedlings of Lawsonia inermis L. were determined. The antioxidant activity of the aqueous and methanol extracts of leaves of field grown plants and *in vitro* seedlings of *Lawsonia inermis* L. were evaluated by several *in vitro* systems of assay, namely, total antioxidant activity, nitric oxide scavenging activity and DPPH assay. *In vitro* seedlings of *Lawsonia inermis* L. obtained from Jodhpur (Rajasthan) showed higher phenolic content, flavonoid content and antioxidant activity than Kalyan (Maharashtra).

Keywords: DPPH, nitric oxide scavenging activity, *in vitro* seedlings

Introduction

Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species (ROS). Reactive oxygen species (ROS) are continuously generated inside the human body which includes superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide (Ottolenghi, 1959). Overproduction of ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA (Farber, 1994). Even though our body is safeguarded by natural antioxidant defense, there is always a demand for antioxidants from natural sources. Several synthetic antioxidants, e.g., butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commercially accessible but are quite perilous and their toxicity is a problem of disquiet (Madhavi and Salunkhe, 1995). The use of these synthetic antioxidants, have been restricted in foods as they are suspected to be carcinogenic. Therefore, the importance of search of natural antioxidants has greatly increased in the recent years.

Lawsonia inermis L. (Henna) belongs to the family Lytheracae. Henna has a wide range of medicinal properties and there is an increasing awareness among people towards this plant due to their non-toxic properties, fewer side effects, more medicinal value. This versatile plant is the source of various types of chemical compounds (Phirke and Saha, 2013). Therefore, the purpose of the present study is to evaluate antioxidant activity of aqueous and methanol extracts of *Lawsonia inermis* L.

Materials and Methods

Collection of plant material: Leaves of field grown plants and seedlings regenerated *in vitro* (12 week old) of *Lawsonia inermis* L. from Kalyan (Maharashtra) and Jodhpur (Rajasthan) were collected, dried, powdered and stored in air tight containers separately. Authentication of the plant (S.H.-1533) was done at Blatter Herberium, St. Xavier's College, Mumbai and the voucher specimen was deposited there.

Preparation of crude extract: 10 mg powders of leaves and *in vitro* seedlings from *Lawsonia inermis* L. (Kalyan and Jodhpur) was extracted separately in 10 ml of distilled-water and methanol overnight. The content was filtered through Whatman filter paper No. 1. The filtrate was evaporated on boiling water bath until dry. The extracts were then stored for further use.

Total Phenolic Content: The soluble phenolic of *Lawsonia inermis* L. was estimated by Folin-Cioclteau reagent method (Slinkard and Singleton, 1977) using gallic acid as a standard phenolic compound. The total phenolic content of different extracts was measured using colorimetric Folin - Ciocalteu method. The reaction mixture consisted 0.5ml of diluted sample to which 0.5 ml of distilled water and 0.5 ml Folin - Ciocalteu reagent was added. After 3 minutes, add 2 ml of 20% Na_2CO_3 solution and place the tubes in boiling water bath for one min, cooled and the absorbance was measured at 760 nm. Standard graph was prepared by using different concentration of gallic acid.

Total Flavonoid Content: The soluble flavonoid of *Lawsonia inermis* L. was estimated by aluminium chloride colourimetric method (Woisky and Salatino, 1998) using quercetin as a standard flavonoid compound. The flavonoid content of aqueous and methanolic extracts from twigs, *in vitro* seedlings of *Lawsonia inermis* L. (Kalyan and Jodhpur) were measured using a modified colorimetric method. 0.5ml of sample was mixed with 0.5 ml of 2% $AlCl_3$ and incubated for 10 mins and the absorbance was measured at 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

Total Antioxidant Activity: 0.1ml of extract was combined in Eppendorf tube with 1 ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium

molybdate). The tubes were capped and incubated in thermal block at 95°C for 90 minutes. After cooling to room temperature; the absorbance of the aqueous solution of each was measured at 695 nm against blank (Shirwaikar, *et al*., 2006).

Nitric oxide radical scavenging activity: Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological *p*H spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions that can be estimated by use of Griess reagent. Scavenger of nitric oxide competes with oxygen leading to reduced production of nitric oxide (Sreejayan, 1997). Sodium nitroprusside (5 mM) in phosphate buffered saline (PBS) was mixed with 3.0 ml of different concentrations (1000-5000 ìg ml^{-1}) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150 min. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance at standard solutions of potassium nitrite, treated in the same way with Griess reagent. The percentage scavenging of nitric oxide of *Lawsonia inermis* L. and standard Ascorbic acid was calculated using the following formula:

NO Scavenged (%) = **(A cont - A test)/A cont × 100**

Where A cont is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts.

DPPH free radical scavenging activity

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH, Hi-media) free radical according to the method described by Blois (1958). DPPH reacts with an antioxidant compound which can donate hydrogen and reduce DPPH. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 1.0 ml of aqueous and methanolic extracts of twigs and *in vitro* seedlings at different concentrations (10-100 µl/ml) of *Lawsonia inermis* L. After 30 min incubation period at room temperature in the dark, the absorbance of the resulting mixture was measured at 517 nm.

Control contained all the reagents except the extract. Thus the mixture of 1ml methanol and 1ml DPPH solution was used as control. L- ascorbic acid (1-100 µg/ml) was used as reference standard.

The scavenging activity was estimated based on the percentage of DPPH radical scavenged. The antioxidant activity percentage (inhibition percentage) was calculated using the expression below:

AA (%) = [(control absorbance – sample absorbance)/(control absorbance)] × 100

Statistical analysis

All the assays were carried out in triplicates. Experimental results were expressed as mean ± standard error (SE) of three parallel measurements. All the experiments were performed in triplicates. Data were analyzed statistically. Significance of difference among the treatment means was tested by Two way ANOVA followed by a post hoc test called Tukey's Test (Zar, 2005).

Results and Discussion

Total phenolic content: The total phenolic contents was examined in aqueous and methanol extracts of twig, *in vitro* seedlings from *Lawsonia inermis* L. (Kalyan and Jodhpur) using the Folin-Ciocalteu's reagent expressed in terms of gallic acid equivalent (the standard curve equation: For aqueous, $y = 0.0195x - 0.0282$, $r^2 = 0.9859$ and for methanol $y = 0.0206x - 0.0589$, $r^2 = 0.9939$). The total phenolic content in aqueous extract of twig, *in vitro* seedlings of *Lawsonia inermis* L. (Kalyan and Jodhpur) was 13.17, 22.83 µg /mg respectively and in methanol extract it was 24.67, 33.80 µg/mg respectively (Table 1).

Two way ANOVA test showed significant difference in phenol content of aqueous and methanol extracts from leaves (field grown plants) and *in vitro* seedlings of *Lawsonia inermis* L. (Kalyan and Jodhpur). A post hoc Tukey's test showed significant difference in phenol content in aqueous and methanol extracts of *Lawsonia inermis* L. (Kalyan and Jodhpur). It also showed significant difference in leaves (field grown plants) and *in vitro* seedlings procured from Kalyan and Jodhpur.

In the present investigation, *in vitro* seedlings of *Lawsonia inermis* L. (Kalyan and Jodhpur) showed maximum phenolic content in aqueous and methanol extract. Phenolic compounds are known to be powerful chain breaking antioxidants. They may contribute directly to the antioxidative action (Bendary *et al*., 2013).

Total flavonoid content: The amount of flavonoids in the plant extracts can directly correlate with its antioxidant activity (Melinda *et al*., 2010). The total flavonoid contents was examined in aqueous and methanol extracts of leaves, *in vitro* seedlings from *Lawsonia inermis* L. (Kalyan and Jodhpur) using aluminium chloride method expressed in terms of quercetin equivalent (the standard curve equation: For aqueous, $y = 0.0058x - 0.004$, $r^2 = 0.99$ and for methanol $y = 0.0065x - 0.036$, $r^2 = 0.9865$). The total flavonoid content in aqueous extract of twig, *in vitro* seedlings of *Lawsonia*

inermis L. (Kalyan and Jodhpur) was 20.13, 26.13µg /mg respectively and in methanol extract it was 25.13, 34.13µg/mg respectively (Table 1).

Two way ANOVA test showed significant difference in flavonoid content of aqueous and methanol extracts from leaves (field grown plants) and *in vitro* seedlings of *Lawsonia inermis* L. (Kalyan and Jodhpur). A post hoc Tukey's test showed significant difference in flavonoid content in aqueous and methanol extracts of *Lawsonia inermis* L. (Kalyan and Jodhpur). It also showed significant difference in leaves (field grown plants) and *in vitro* seedlings procured from Kalyan and Jodhpur.

In the present investigation, *in vitro* seedlings of *Lawsonia inermis* L. (Kalyan and Jodhpur) showed maximum flavonoid content in aqueous and methanol extract.

Total antioxidant capacity: Total Antioxidant capacity of *Lawsonia inermis* L. is shown in Table 1. In this assay methanol extract of *in vitro* seedlings of *Lawsonia inermis* L. from Jodhpur was found to have higher activity than Kalyan. The phosphormolybdenum method was based on reduction of MO (VI) to MO (V) by the antioxidant compound and the formation of green phosphate/ MO (V) complex at acidic pH. The extracts demonstrated electron donating capacity and thus they may act as radical chain terminators, transforming reactive free radical species into stable non reactive products (Dorman *et al.*, 2003).

Nitric oxide scavenging method: Nitric oxide scavenging activity of aqueous and methanol extract of leaves, *in vitro* seedlings from *Lawsonia inermis* L. (Kalyan and Jodhpur) was determined. The IC_{50} value of aqueous extract of leaves, *in vitro* seedlings powder of *Lawsonia inermis* L. was found to be 3.93 mg/ml, 3.66 mg/ml and 3.68 mg/ml, 3.59 mg/ml for Kalyan and Jodhpur respectively (Figure 1, Table 2). The IC_{50} value of methanol extract of twig, *in vitro* seedlings powder of *Lawsonia inermis* L. was found to be 3.48 mg/ml, 2.82 mg/ml and 3.58 mg/ml, 3.34 mg/ml for Kalyan and Jodhpur respectively (Figure 2, Table 2). Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals (Kumaran and Karunakaran, 2007).

DPPH free radical scavenging activity: DPPH free activity of aqueous and methanol extract of leaves, *in vitro* seedlings from *Lawsonia inermis* L. (Kalyan and Jodhpur) was determined. The IC_{50} value of aqueous extract of leaves, *in vitro* seedlings powder of *Lawsonia inermis* L. was found to be 76.41 µg/ml, 68.04 µg/ml and 68.68 µg/ml, 65.29 µg/ml for Kalyan and Jodhpur respectively (Figure 3, Table 3). The IC_{50} value of methanol extract of leaves, *in vitro* seedlings powder of *Lawsonia inermis* L. was found to be 69.83 µg/ml, 67.08 µg/ml and 58.63 µg/ml, 65.15 µg/ml for Kalyan and Jodhpur respectively (Figure 4, Table 3).

Conclusion

In the present study, total phenolic content and flavonoid content was found to more in methanol extract as compared to aqueous extract *Lawsonia inermis* L. (Kalyan and Jodhpur) when compared with standards. *In vitro* seedlings of *Lawsonia inermis* L. (Kalyan and Jodhpur) showed maximum total phenolic and flavonoid content in aqueous and methanol extract. Aqueous and methanol extracts of *Lawsonia inermis* L. (Kalyan and Jodhpur) showed potent antioxidant activity, nitric oxide scavenging radicals, reducing power activities and DPPH free radical scavenging activity when compared with standard ascorbic acid. The present study showed that *Lawsonia inermis* L. is a natural dye yielding plant and it can also be used as easily accessible source of natural antioxidant.

Acknowledgement

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Table 1: Total phenolic content, total flavonoid content and Total Antioxidant Activity of *Lawsonia inermis* L.

Sr. No.	Extracts	Total Phenolic content (µg/mg)		Total flavonoid content (µg/mg)		Total antioxidant activity (µg/mg)	
		Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
1.	Leaves (Kalyan)	13.17 ± 0.25	24.67 ± 0.38	20.13 ± 0.25	25.13 ± 0.38	38.81 ± 0.57	41.03 ± 0.15
2.	*In vitro* seedlings (Kalyan)	22.83 ± 0.38	33.80 ± 0.30	26.13 ± 0.38	34.13 ± 0.30	51.53 ± 0.25	56.30 ± 0.42
3.	Leaves (Jodhpur)	14.50 ± 0.28	28.07 ± 0.36	23.46 ± 0.28	26.13 ± 0.36	48.19 ± 0.19	50.19 ± 0.57
4.	*In vitro* seedlings (Jodhpur)	23.75 ± 0.66	34.08 ± 0.47	27.47 ± 0.66	36.47 ± 0.47	53.53 ± 0.61	62.97 ± 0.42

Values are mean of three determinants. Mean ± S. E.

Table 2: IC_{50} value of Nitric oxide scavenging activity and DPPH free radical scavenging activity of *Lawsonia inermis* L.

Sr. No.	Extracts	**Nitric oxide scavenging activity** (mg/ml)		**DPPH free radical scavenging activity** (µg/ml)	
		Aqueous	Methanol	Aqueous	Methanol
1.	Leaves (Kalyan)	3.93	3.48	76.41	69.83
2.	*In vitro* seedlings (Kalyan)	3.66	2.82	68.04	67.08
3.	Leaves (Jodhpur)	3.68	3.58	68.68	58.63
4.	*In vitro* seedlings (Jodhpur)	3.59	3.34	65.29	65.15

Values are mean of three determinants.

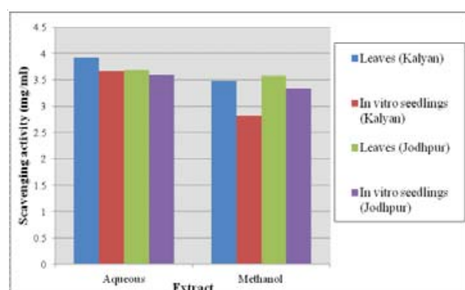

Figure 1: Nitric oxide scavenging activity *of Lawsonia inermis L.*

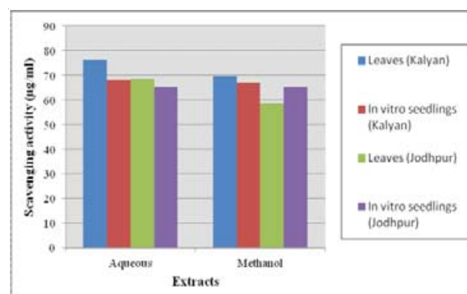

Figure 2: DPPH free radical scavenging activity *of Lawsonia inermis L.*

Antidiabetic Study of *Butea Monosperma* (lamk.) Taub. Plant Parts

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Abstract: In the present study, STZ induced diabetic rats were treated with aqueous slurry of *Butea monosperma* (Lamk.) Taub. leaf, bark and flowers to determine their anti-hyperglycaemic activity. Reduction in blood glucose level was observed after 14 days administration of aqueous slurry of *Butea monosperma* (Lamk.) Taub. leaf, bark and flowers in STZ induced diabetic rats. The aqueous slurry of leaf, bark and flowers of *Butea monosperma* (Lamk.) Taub. showed similar effect, the aqueous slurry of *Butea monosperma* (Lamk.) Taub. leaf showed 18.42% reduction in blood glucose level, the aqueous slurry of *Butea monosperma* (Lamk.) Taub. bark showed 23.95% reduction in blood glucose level and the aqueous slurry of *Butea monosperma* (Lamk.) Taub. flowers showed 33.52% reduction in blood glucose level. The treatment with flowers of *Butea monosperma* (Lamk.) Taub. showed most significant reduction in the blood glucose level in streptozotocin induced rats as compared to the leaf and bark of *Butea monosperma* (Lamk.) Taub.

Keywords: *Butea monosperma, Antidiabetic, Streptozotocin* (STZ), *Glibenclamide* (GBC)

Introduction

Plants have served a valuable starting material for drug development. Plants are now occupying important position in allopathic medicine, herbal medicine, homeopathy and aromatherapy. Medicinal plants are the sources of many important drugs of the modern world. *Butea monosperma* (Lamk.) Taub. commonly known as Flame of forest, belongs to the family Fabaceae11. Different species of Butea found in India are Butea parviflora, Butea purpurea, *Butea monosperma* and Butea superba. It is said that this tree is a form of Agnidev, God of Fire. *Butea monosperma* (Lamk.) Taub. has various healing effects which are seen in treatment of many diseases. It showed anti-inflammatory property, antistress activity, anti-diarrhoeal potential, anti-cancer property. So STZ induced diabetic rats were treated with aqueous slurry of *Butea monosperma* (Lamk.) Taub. leaf, bark and flowers to determine their anti-hyperglycaemic activity.

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million worldwide. Diabetes has been a clinical model for general medicine. The primary defect in fuel metabolism results in widespread, multi-organ complications that ultimately encompass virtually every system of the body and every specialty of medicine. It has been said that to know diabetes is to know medicine and health care. Although from a clinical standpoint this may be true, our increasing knowledge of the pathophysiology of the syndrome, together with the mechanisms of long-term complications, has placed diabetes research at the frontier of immunology and molecular biology (Joshu, 1991).

According to World Health Organisation (WHO) – "Any plant and its organs containing any substance that can be used therapeutically, or can be used as raw material for chemical pharmaceutical synthesis" is classified as drugs (WHO, 1977). Herbal drugs are playing an important role in health care program in developing countries. This is because they are being cheap and locally available. There is a general belief amongst the consumers globally that herbal drugs are safe because they are natural. However evidences suggest otherwise. The mere fact that a product is "natural" may not signify that the product is safe. Although limited evidence suggests that adverse effect associated with use of herbal drugs occur to a lesser extent than allopathic drugs. They do occur though usually mild and only affecting a small number of people. Recent evidence suggests that some of the herbs considered to be safe over the last many decades have proven to be associated with health hazards. Herbal remedies can act either as agonistic or antagonists that potentiate some drug therapies. Therefore an understanding of herbal drug is an essential pre-requisite for effective herbal therapeutics. The advancement of technology has enabled the scientist to detect minute amount of carcinogenic and toxic chemicals in these herbs and evaluate potentially hazardous effects of some of the herbs which have been used in traditional medicine since centuries (George, 2011).

Toxicology can be defined as that branch of science that deals with poison, and a poison can be defined as any substance that causes harmful effect when administered, either by accident or design to a living organism. Acute toxicity is usually defined as the adverse changes occurring immediately or a short time following a single or short period of exposure to a substance or substances or as adverse effects occurring within a short time of administration of a single dose of a substance or multiple doses given within 24 hrs. An adverse effect is "any effect that results in functional impairment that may affect the performance of the whole organism or that reduce the organ's ability to respond to an additional challenge". Consequently, a

chemical that enters the organism via the oral route during a restricted time and produces any adverse effect with little delay is orally and acutely toxic. However, the term acute oral toxicity is most often used in connection to lethality and LD50 determinations (Walum, 1998).

In the present study, toxicity of leaf, bark and flowers powder of *Butea monosperma* (Lamk.) Taub. was evaluated in Albino Swiss female mice after its oral administration according to OECD guideline 420. For efficacy study streptozotocin (0.05g/kg body weight) induced diabetic model was used to study the hypoglycaemic activity of the *Butea monosperma* (Lamk.) Taub. at a dose (0.3g/kg body weight). The final dose for the study was decided based on preliminary toxicity dose response study and this activity was compared using reference drug Glibenclamide (GBC) at dose (0.0005g/kg body weight).

Protocol approval (PB101006-01) was taken from the Institutional Animal Ethics Review Committee of Ramnarain Ruia College, Matunga, Mumbai and the study was performed according to the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the use and care of experimental animals.

Materials and Methods

Plant material: The dried powder of *Butea monosperma* (Lamk.) Taub. leaf, bark and flowers each was soaked in distilled water to get aqueous plant slurry to be used in the study.

Animals: In toxicity study, Albino Swiss female mice were procured form Bharat Serum Limited, Thane. Their body weights were in the range between 35 - 45g. In efficacy study, Albino Wistar rats (female) of body weight ranging 150-200g were procured from Bharat Serum Limited, Thane.

Maintenance of animals

The animals were housed in polyurethane cage. The cages were provided with the rice husk bedding and were cleaned daily. Animals were acclimatized for 7 days and were provided with drinking water ad libitum. The animal room was maintained at ambient temperature of 30 to 32°C and relatively humidity of 60 to 70%. The rate of air exchange is continuous through a system of inlet of fresh air and outlet of bad air. The lighting is controlled by a times switch to give 12 hrs continuous light and 12 hrs continuous dark cycles. The animals were provided with drinking water ad libitum and fed on commercially available rat feed supplied by Amrut feed (Table 1, Table 2 and Table 3).

Experimental Design

Efficacy study using standard protocol (Table No. 4) was approved under OECD guideline 420. Following are the different groups used in study.

- **Group 1**: Normal control

  2ml distilled water received daily for 14days.

- **Group 2**: Streptozotocin control (Diabetic)

  2ml distilled water received daily for 14days.

- **Group 3**: Glibenclamide treated rats

  GBC was given (500µg/kg body weight in 2ml distilled water) daily for 14days.

- **Group 4**: *Butea monosperma* (Lamk.) Taub. leaf treated group

  Aqueous slurry of *Butea monosperma* (Lamk.) Taub. leaf was given (300mg/kg body weight in 2ml distilled water) daily for 14days.

- **Group 5**: *Butea monosperma* (Lamk.) Taub. bark treated group

  Aqueous slurry of *Butea monosperma* (Lamk.) Taub. bark was given (300mg/kg body weight in 2ml distilled water) daily for 14days.

- **Group 6**: *Butea monosperma* (Lamk.) Taub. flowers treated group

  Aqueous slurry of *Butea monosperma* (Lamk.) Taub. flowers was given (300mg/kg body weight in 2ml distilled water) daily for 14days.

Results and Discussion

Statistical analysis of the results

The plasma glucose levels for various treated groups were statistically compared with the 0.0hr and 24.0hrs of the same group and with corresponding level of diabetic control group.

- In normal control group mean plasma glucose level of 0.0hrs and 24.0hrs showed no significance with plasma glucose level of 7th day, 10th day and 15th day(Table No. 5 and Fig. 1).
- In GBC treated group showed significant reduction in plasma glucose level on 10th day and 15th day and showed no significance on 7th day when compared to 0.0hr and showed significant reduction in plasma glucose level on 15th day but showed no significance on 7th and 10th day with 24.0hrs(Table No. 5 and Fig. 1).
- In *Butea monosperma* (Lamk.) Taub. leaf treated groups showed significant reduction in plasma glucose level on 7th day, 10th day and 15th day when compared to

0.0hr and showed no significance on 7th day, 10th day, 15th day when compared with 24.0 hrs(Table No. 5 and Fig. 1).

- In *Butea monosperma* (Lamk.) Taub. bark treated groups showed significant reduction in plasma glucose level on 7th day, 10th day and 15th day when compared to 0.0hr and showed significance on 15th day when compared with 24.0hrs but showed no significance when compared with 24.0hrs with 7th day and 10th day(Table No. 5 and Fig. 1).

- In *Butea monosperma* (Lamk.) Taub. flowers treated groups showed significant reduction in plasma glucose level on 7th day, 10th day and 15th day when compared to 0.0hr and 24.0hrs (Table No. 5 and Fig. 1)

Discussion

In the present study, *Butea monosperma* (Lamk.) Taub. leaf, bark and flowers showed significant reduction in total cholesterol and urea as compared to STZ control group (Table No. 6 and Table No. 7). Similar effect was observed in *Euphorbia hirta* Linn. (Kumar *et al*., 2010), in aqueous extract of *Cynodon dactylon* Pers. (Jarald *et al*., 2008), in aqueous extract of *Costus pictus* leaves (Jayasri *et al*., 2008) and alcoholic extract of *Tribulus atatus* (Tantawy and Hassanin, 2007). The plant parts of *Butea monosperma* (Lamk.) Taub. showed increase in the liver glycogen when compared to STZ control group, similar effect was observed in methanolic extract of *Lippia nodiflora* L. and in aqueous leaf extract of *Basella rubra* (Nirmala *et al*., 2011).

Conclusion

In the present study, results obtained for the efficacy studies showed that the aqueous slurry of *Butea monosperma*(Lamk.) Taub. leaf, bark and flowers were effective in controlling the plasma glucose level showing good hypoglycemic property. The effect of *Butea monosperma* (Lamk.) Taub. flowers was the best. It can be concluded that *Butea monosperma* (Lamk.) Taub. may be useful in treating diabetes mellitus with no visible signs of toxicity indicating a high margin of safety. The plant parts (leaf, bark and flowers) of *Butea monosperma* (Lamk.) Taub. exhibited anti-hyperglycemic activity comparable to the standard drug Glibenclamide.

Acknowledgement

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Table 3: Dose regimen for acute toxicity study of *Butea monosperma* (Lamk.) Taub.

Parameters	Group 1(Control)	Group 2(Leaf)	Group 3(Bark)	Group 4(Flowers)
Sex	Female	Female	Female	Female
Doseg/kg of body weight	-	2.0	2.0	2.0
No. of mice	6	6	6	6
Total volume administered/ animal (ml)	2.0*	2.0	2.0	2.0

Note: *Control group was given distilled water treatment

Table 4: Protocol for efficacy study

Animal model	Albino Wistar Rats
Sex	Female
Weight of animals	Between 150-200g
No. of dose groups	Six
Animals per group	Six
Dose of Streptozotocin	50mg/kg
Route of Streptozotocin administration	I.P. (Intra peritoneum)
Vehicle of Streptozotocin administration	0.1 M Citrate buffer pH 4.5 for injection
Volume of vehicle for Streptozotocin	0.2 ml per animal
Route of drug administration	Oral
Dose volume	2.0 ml per animal
Vehicle for oral dose	Distilled water
Volume of blood to be collected	0.2 ml at each sampling point
Test sample	Aqueous slurry of *Butea monosperma* (Lamk.) Taub. leaf, bark and flowers
Study duration	Acclimatization for 14 days before dosing and 14 days observation period after Streptozotocin i.p
Parameters for evaluation	Estimation of plasma glucose, proteins,cholesterol, blood urea, liver glycogen

Table 5: Treatment regimen for various groups of animal under study

Days	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	Normal control	**STZ or diabetic control**	**STZ + GBC**	**STZ + *Butea monosperma* (Lamk.) Taub. leaf**	**STZ + *Butea monosperma* (Lamk.) Taub. bark**	**STZ + *Butea monosperma* (Lamk.) Taub. Flowers**
	Induction of diabetes before dosing					
Day 0	No treatment with STZ	All the rats were treated with STZ (50mg /kg body weight) i.p. to induce diabetes				
Day 0	Normal rats	After 72hrs of STZ induction diabetes was confirmed by plasma glucose levels. Blood samples were collected at 0.0hr (just before dosing) by ROP (Retro – orbit puncture) technique and rats with plasma glucose levels > 250mg/dl were selected. All diabetic rats were divided into 5 groups, 6 rats per each group				
Day 1		After induction of diabetes, treatments were given to diabetic animals				
Day 1	Single oral dose of distilled water (2.0ml)	Single oral dose of distilled water (2.0ml)	Single oral dose of 500µg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)
Day 1	Monitoring of plasma glucose level (ROP technique) for 0.5hr, 1.0hr, 2.0hrs, 4.0hrs and 6.0hrs					
Day 2	Monitoring of plasma glucose level (ROP technique) for 24hrs blood sample					
Day 2	Single oral dose of distilled water (2.0ml)	Single oral dose of distilled water (2.0ml)	Single oral dose of 10mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)
Day 3 to Day 6	Single oral dose of distilled water (2.0ml)	Single oral dose of distilled water (2.0ml)	Single oral dose of 10mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)
Day 7	Monitoring of plasma glucose level (ROP technique)					
	Single oral dose of distilled water (2.0ml)	Single oral dose of distilled water (2.0ml)	Single oral dose of 10mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)
Day 10	Monitoring of plasma glucose level (ROP technique)					
Day 10 to Day 14	Single oral dose of distilled water (2.0ml)	Single oral dose of distilled water (2.0ml)	Single oral dose of 10mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)
Day 15	Single oral dose of distilled water (2.0ml)	Single oral dose of distilled water (2.0ml)	Single oral dose of 10mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)	Single dose of 300mg/kg body weight (2.0ml)
Day 15	Monitoring of plasma glucose level (ROP technique) and other biochemical: Total Protein, Urea, Total Cholesterol					
Day 15	Sacrificed and liver removed for the estimation of Liver Glycogen					

Keywords: N – Normal control group, STZ – STZ control group, GBC – GBC treated group,

Table 6: Mean plasma glucose levels (mg/dl) of normal control and treated diabetic (STZ) ratswith different groups

Groups	0.0hr	24.0hrs	7th Day	10th Day	15th Day
N	90.83*±5.98	92.50*±6.83	96.67*±6.65	96.50*±3.94	100.50*±10.52
STZ	299.33±18.52	285.67±29.45	298.50±33.64	296.83±29.29	306.33±15.02
GBC	302.50±5.68	287.67±12.89	297.00±9.94	276.83±13.17	249.83*±24.09
L	286.83±19.80	262.33±34.92	251.83*±30.98	241.33*±30.70	234.00*±31.83
B	309.00±19.74	275.17±19.33	263.00*±18.85	249.33*±26.20	235.00*±27.28
F	305.83±14.78	276.67±18.30	236.17*±11.44	215.83*±7.31	203.33*±4.59

Values are Mean ± S.D. of six determinants, values are statistically significant at p* 0.05 (when compared with diabetic control); p*<0.05=significant

Table 7: Biochemical parameters in experimental animal after 14days treatment

Parameters	N	STZ	GBC	L	B	F
Liver Glycogen (mg/g)	29.98±1.28*	27.58±1.50	38.58±1.41*	30.83±0.75*	31.50±2.43*	35.00±1.30*
Plasma Protein (mg/dl)	6.67±0.14*	8.12±0.61	6.82±0.11*	7.35±0.22*	7.32±0.15*	6.90±0.14*
Urea (mg/dl)	14.57±3.18*	44.50±2.83	25.92±2.84*	32.75±1.41*	35.28±3.06*	35.32±3.89*
Total Cholesterol(mg/dl)	100.67±2.84*	280.46±2.28	182.12±0.24*	132.50±0.71*	123.75±0.72*	125.67±2.47*

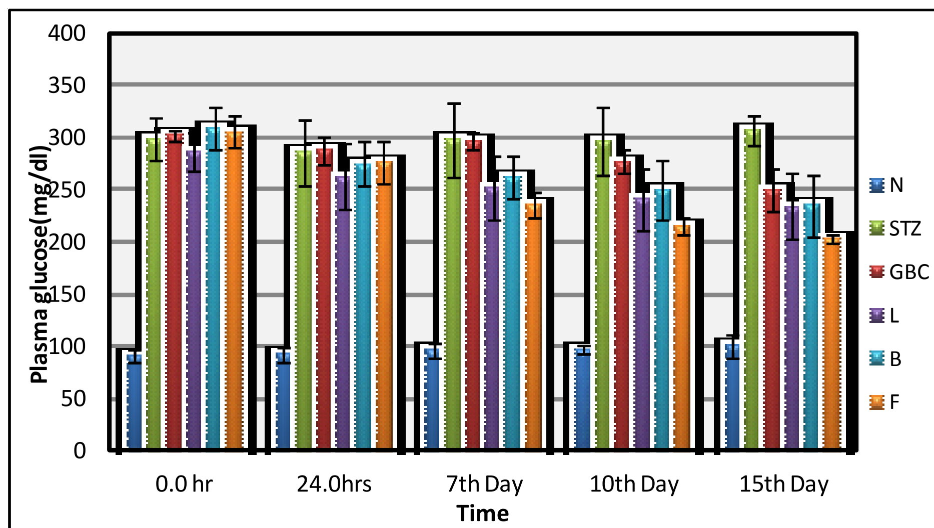
Values are Mean ± S.D. of six determinants

"*" - Significant values

Keywords: N – Normal control group, STZ – STZ control group, GBC – GBC treated group,

L – Leaf treated group, B – Bark treated group, F – Flowers treated group

Figure 1: Plasma glucose levels (mg/dl) of normal control and treated diabetic (STZ) ratswith different groups



Keywords: N – Normal control group, STZ – STZ control group, GBC – GBC treated group,

L – Leaf treated group, B – Bark treated group, F – Flowers treated

Qualitative Estimation of Adulterants In Turmeric By Chemical Tests and Thin Layer Chromatography

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Abstract: *Curcurma longa* is a rhizomatous herbaceous perennial plant belonging to the family *Zingiberaceae*. It has been used in India for thousands of years as a spice and medicinal herb. It is mildly aromatic and has pungent and bitter flavor. It has powerful anti-inflammatory effects and is a very strong anti-oxidant. *Curcumin* leads to various improvements that should lower your risk of heart disease. It is an ingredient of curry powders, and also used to give mustard its characteristic color. It is used place of saffron to provide color and flavor. This study aim to detect the adulterants in turmeric by Thin Layer Chromatography and chemical tests.

Key Words: Turmeric, Standard, Thin Layer Chromatography, Adulterants

Introduction

Turmeric is the root stalk of a tropical plant *Curcurma longa* belonging to *Zingiberaceae* family. One of the main components of the spice is a substance called *curcumin* which has potentially healing properties. *Curcumin* is a polyphenolic compound from *Curcuma longa* is known to posses various therapeutic activities (Kamble *et al*., 2015). The spice is used in lots of Asian dishes, mustards and pickles. Turmeric has been used for many thousands of years in Chinese and Indian Ayurvedic medicine fro condition including heart bun, diarrhea, stomach bloating, colds, fibromyalgia and depression (Chattopadhyay, 2004). The three coloring components of curcumine are 3 yellow diferuloylmethane derivatives curcumine, demethoxycurcumine and bisdemethoxycurcumine. Curcumin constitutes 3.14 % of powdered turmeric, having variations in content among the species of *Curcuma longa*. Some 34 essential oils are present in turmeric, among turmerone, germacrone, atlantone and zingiberene are major constituents. Turmeric root powder is used in food industry as color and also in spice and often both color and flavor are characteristic for the product, as in the case of mustard or curry.

Adulteration means the addition of ingredients which are not permitted in food. They are added because of business profit only. Adulterated foods are harmful for human health as they contain the unauthorized food ingredients (Chattopadhyay, 2004). Turmeric may be adulterated with, Lead chromate, (which adds color as well as weight to it, being heavier, Metanil Yellow dye or any starch based items like flour or rice powder or even industrial starch. Lead chromate; it is one of the most toxic salts of lead. It can cause anemia, paralyses, mental retardation and brain damage in children and abortion in pregnant women (Abhirami and Radha, 2015). Therefore the aim of the present study was to detect the adulterants in turmeric by Thin Layer Chromatography and chemical tests.

Materials and Methods

Collection of plant materials: Dried and wet rhizomes were purchased from local market, Thane (Maharashtra).

Market samples collection: 4 different brands of turmeric powders were purchased from local market, Thane (Maharashtra) and labeled as sample 1, sample 2, sample 3 and sample 4.

Powdering of materials: Dried rhizomes were grind, powdered and stored in air tight container.

Extract preparation: 1 gm of each sample [wet and dry rhizome and 4 marketed samples] was mixed with 20 ml of methanol separately. The filtrate was evaporated to 5 ml and used for TLC (Wagner and Bladt, 1996).

Chromatographic plate: Silica gel 60 F_{254}- precoated TLC plates (Merk, Germany)

Solvent system: n-butanol : n-propanol : glacial acetic acid : water (30:10:10:10)

Metanil Yellow Test: Add a few drops of HCl to turmeric in water. Instantly a violet colour appears (Dixit, 2015).

Other aniline dyes: Take some turmeric powder in a test-tube and add water to make a solution. Add 1 to 2 ml of rectified spirit. An immediate separation of yellow colour in the rectified spirit will indicate the presence of added dyes (Dixit, 2015).

Metanil yellow Test: Take a sample of turmeric powder. Add 1N H_2SO_4 to the extract (Abhirami and Radha, 2015).

Yellow clay: Take sample of turmeric powder with water and allow to stand for some time (Abhirami and Radha, 2015).

Detection of chalk powder: A small amount of given sample was taken in a test tube and about ml of dil. HCl was added to it (Mansuri, 2015).

Results and Discussion

The study was concluded to evaluate the adulteration in turmeric powder. The data collected from the chromatogram was recorded and calculated in table 1. Chromatogram of sample 4 showed only one spot which had Rf value different than the standard (Plate 1).

Chemical test of all the four samples of turmeric showed positive test results for metanil yellow and yellow clay (Plate 2). In table 2 the all the four samples gave a negative test results which means they are not adulterated with metanil yellow. Sample 1 gave a negative test result for other aniline dyes which means it is not adulterated and remaining three samples gave a positive testwhich means they are adulterated with the other aniline dyes (Plate 3). Similar test results were observed in test done by Dixit, S., 2015. All the four samples gave a negative test result for chalk powder (Plate 4) which means they are not adulterated with the chalk powder which was confirmed by Mansuri, 2015.

Conclusion

The turmeric samples were adulterated with metanil yellow, other aniline dyes, yellow clay, chalk powder which are harmful adulterants that are added to enhance the color and taste of spices. Adulterated foods are harmful for human health as they contain the unauthorized food ingredients. Adulterated products can be identified by doing easy test and procedure which can be done at home. For a buying non adulterated turmeric powder the ingredients on the packet should be checked along with the AGMARK, FPO and ISI labels.

Acknowledgment

Authors are thankful to Department of Botany, VPM's B. N. Bandodkar College of Science, Thane for providing the laboratory facilities. We are also thankful to DBT-Star College Scheme for providing the instruments and chemicals required for the research work.

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Table 1: Thin Layer Chromatographic analysis of turmeric samples

TURMERIC SAMPLES	Rf value
Dry turmeric	0.18, 0.41, 0.61
Wet turmeric	0.30, 0.46, 0.58
Sample 1	0.07, 0.16, 0.23, 0.46
Sample 2	0.06, 0.11, 0.27, 0.40, 0.65
Sample 3	0.05, 0.07, 0.11, 0.24, 0.59, 0.92
Sample 4	0.88

Table 2: Chemical tests to detect adulteration of chilli powder

ADULTERANTS	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4
Metanil yellow	+	+	+	+
Yellow clay	+	+	+	+
Metanil yellow	-	-	-	-
Other aniline dye	-	+	+	+
Chalk powder	-	-	-	-

Positive (+); Negative (-)

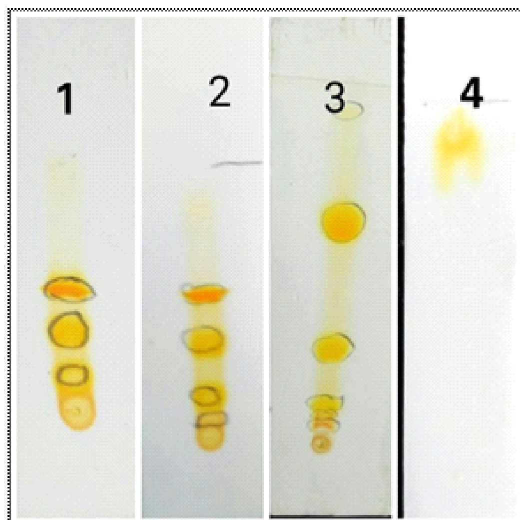

Plate 1: TLC of marketed samples

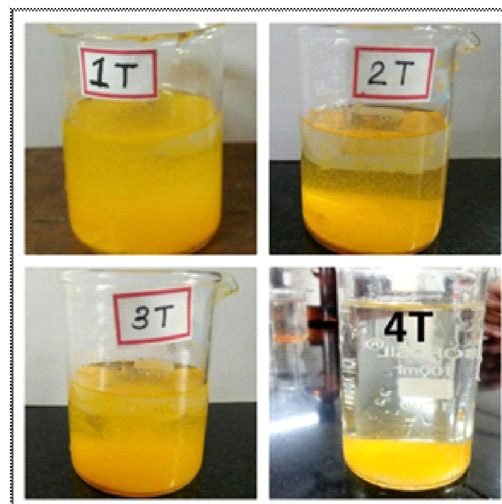

Plate 2: Yellow clay test of marketed samples

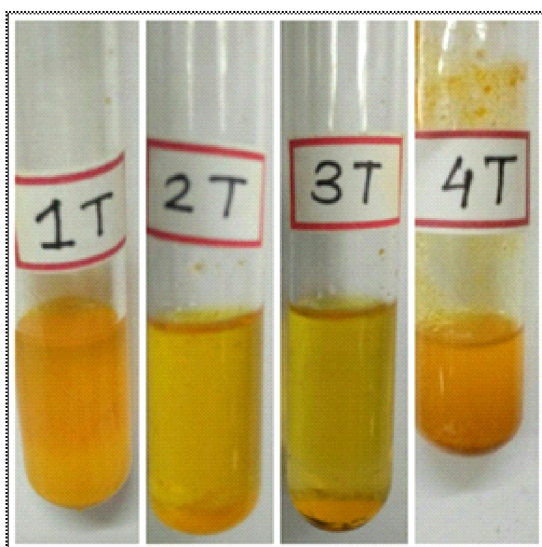

Plate 3: Aniline dye test of marketed samples

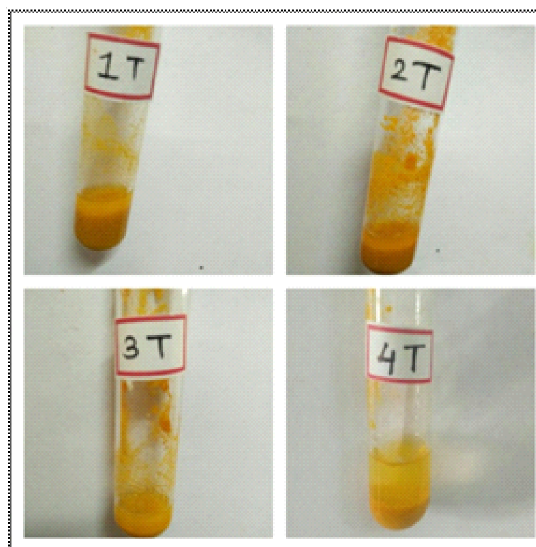

Plate 4: Chalk powder test of marketed samples

Antidandruff Activity of Leaves from Field Grown Plants of *Lawsonia inermis* L.

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Abstract: Medicinal plants with therapeutic qualities which are useful in treating various ailments. *Lawsonia inermis* L. (henna) is an important medicinal plant of Indian Systems of Medicine. Dandruff is a common scalp disorder in human being. It is caused by *Pityrosporium ovale* and *Candida albans*. In the present study antidandruff activity was studied using aqueous and methanol extract of leaf from field grown plants of *Lawsonia inermis* L. (Kalyan and Jodhpur) against *Malassezia furfur* using agar well method. MIC of these samples were also evaluated.

Key word: *Lawsonia inermis* L., antidandruff, MIC

Introduction

Dandruff is a common scalp disorder affecting half of the pubertal population of any ethnicity in both the genders (most prevalent in male population) between the age group of 20 and 60 years (Ravichandran *et al*., 2004). It is generally a major cosmetic problem that causes very great public health concern both in developed and developing countries (Sunenshine *et al*., 1998). The causative agents of dandruff belong to the group of scalp commensal lipophilic yeasts of the genus, *Malassezia* (Barenji *et al*., 2010). It is also caused by *Pityrosporium ovale* and *Candida albans* (Sureshkumar *et al*., 2010).

Medicinal plants have been used in traditional treatment of skin diseases worldwide. In India, herbal medicines have been the basis of treatment and cure for various diseases in traditional methods practiced, such as Ayurveda, Unani and Siddha. The rich availability and easy access to these medicinal plant resources have made them almost inevitable in the healthcare practices. Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of lower side effects. Unlike the synthetic drugs medicinal plants have the ability to control antibiotic resistant microorganisms (Sharma *et al*., 2011).

Lawsonia inermis L. is a branched glabrous shrub or small tree, cultivated for its leaves (Chaudhary *et al*., 2010). This plant is used all over the world and is commonly known as Henna or Mehndi. Genus *Lawsonia* bears one species, *Lawsonia inermis* (Henna, Mehndi, Madayantika, etc.) belonging to family Lythraceae (Kirtikar and Basu, 1933). Henna leaf and other parts of the plant are also used in the indigenous systems of medicine (Chopra *et al*., 1956).

Hence, the present study was undertaken with the preparation of different solvents extracts of *Lawsonia inermis* and determination of antidandruff activity of its leaf extracts by agar well diffusion method.

Materials and Methods

Collection of plant material: Leaves of field grown plants of *Lawsonia inermis* L. were collected from Kalyan (Maharashtra) and Jodhpur (Rajasthan). Authentication of the plant (S.H.-1533) was done at Blatter Herberium, St. Xavier's College, Mumbai. The specimen voucher was deposited in the Blatter Herberium, St. Xavier's College, Mumbai.

Collection of micro-organism: To check anti-dandruff activity dandruff culture *Malessazia furfur* (MTCC No. 1374) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh.

Media: Sterile Sabouraud's dextrose broth and media supplemented with olive oil (1%) was used.

Preparation of crude extract: 3 gm powders of leaf from field grown plants of *Lawsonia inermis* L. (Kalyan and Jodhpur) was extracted separately in 50 ml of distilled-water and methanol overnight. The content was filtered through Whatman filter paper No. 1. The filtrate was evaporated on boiling water bath until dry. The evaporated solvent so obtained was weighed. Both crude extracts were dissolved in sterile distilled water at a concentration of 100 mg/ml and extracts were stored at 4°C in airtight bottles until further use.

Minimum Inhibitory Concentration (MIC) assay: Various concentration of aqueous and methanol extract of leaf from *Lawsonia inermis* L. (Kalyan and Jodhpur) ranging between 10 and 100 mg/ml were introduced into different test tubes. Each tube was inoculated with an overnight culture of *Malessazia furfur* diluted to give a final concentration of approximately 10^7/ml. The tubes were incubated at 37°C for 24 h. The least concentration of the plant extract that did not permit any visible growth of the inoculated test organism in broth culture was recorded as the MIC.

Agar well diffusion method: Sterile Sabouraud's dextrose agar supplemented with olive oil plates were prepared for all extracts, 1 ml inoculum of *Malessazia furfur* was mixed in sterile Sabouraud's dextrose agar butts and pour it on sterile petriplates. After solidifying the plates four wells

approximately 5 mm diameter were prepared with the help of sterile borer. The equal volume (50µl) of aqueous and methanol and distilled water extract of leaf powder (Kalyan and Jodhpur) were poured into the wells. The plates were incubated at 37°C for 24 hrs and zone of inhibition was observed.

Results and Discussions

The aqueous and methanol extract of leaf from field grown plant of *Lawsonia inermis* L. (Kalyan and Jodhpur) showed minimum inhibitory concentration (MIC) at 60 mg/ml (Table 1).

The zone of inhibition for aqueous extracts of leaf from *Lawsonia inermis* L. (Kalyan and Jodhpur) was found to be 17.33 mm and 18.67 mm respectively, however for methanol extract 18.33 mm and 20.33 mm against *Malessazia furfur* respectively (Table 2).

Conclusion

In the present study, aqueous and methanol extract of leaf from field grown plants of *Lawsonia inermis* L. (Kalyan and Jodhpur) at different concentrations suppressed the growth of the tested bacteria at varying degrees. Methanol extract of these samples showed more inhibition towards the tested microorganism as compared to that of water. Dandruff is a major cosmetic problem that causes immense anxiety both in men and women. In most of the cases, hair fall is associated with this problem. Synthetic formulations are effective but herbal remedies are the most sought after alternative to combat this. Thus, attention has been paid to plant-derived antifungal compounds based on the knowledge that plants have their own defense systems against fungal pathogens.

Acknowledgments

Authors are thankful to Botany Department, VPM's B. N. Bandodkar College of Science, Thane for providing the laboratory facilities.

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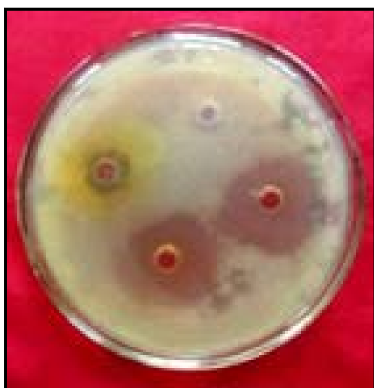
Table 1: Minimum inhibitory concentration (MIC) of leaf of Lawsonia inermis L. against *Malessazia furfur*

Sr. No.	Samples	Concentration (mg/ml)
	Aqueous extract	
1.	Leaf powder (Kalyan)	60 mg/ml
2.	Leaf powder (Jodhpur)	60 mg/ml
	Methanol extract	
3.	Leaf powder (Kalyan)	60 mg/ml
4.	Leaf powder (Jodhpur)	60 mg/ml

Table 2: Antimicrobial activity for aqueous and methanol extracts of leaf from *Lawsonia inermis* L. (Kalyan and Jodhpur) against *Malessazia furfur* by agar well method

Sr. No.	Samples	Zone of inhibition (mm)
	Aqueous extract	
1.	Leaf powder (Kalyan)	17.33±0.10
2.	Leaf powder (Jodhpur)	18.67±0.32
	Methanol extract	
3.	Leaf powder (Kalyan)	18.33±0.32
4.	Leaf powder (Jodhpur)	20.33±0.40

Values are mean of three replicates. Mean ± SE



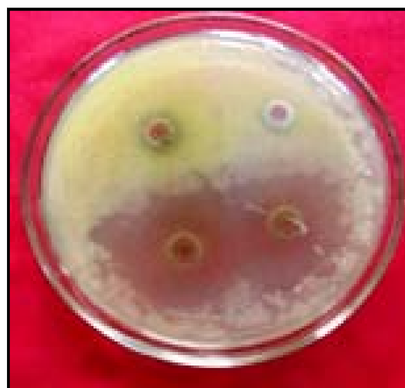


Plate 1: Zone of ihibition of against *Malessazia furfur*

a) Methanol extract of Leaf (Kalyan) of *Lawsonia inermis* L.

b) Methanol extract of Leaf (Jodhpur) of *Lawsonia inermis* L.

Antioxidant activity of *Spinacia oleracea* L. from three Different Regions

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Abstract: *Spinacia oleracea* Linn. (Family: Amaranthaceae), is a green leafy vegetable that came originally from south-west Asia and is now grown in most parts of the world. Spinach is packed with vitamins such as C, A and E, minerals like Mg, Mn, Fe, Ca and folic acid. Spinach is also rich in carotenoids, beta-carotene and lutein. It is a good source of bioflavonoid, quercetin and many other flavonoids. In the present work, comparative study of phytochemical, antioxidants and flavonoid content in spinach from three different areas is carried out.

Keywords: Spinach, Antioxidant, Flavonoid, Phytochemicals.

Introduction

Spinach (*Spinacia oleracea* L.) is an edible flowering plant in the family Amaranthaceae (Mane, 2015). Spinach is an important agricultural crop not only because of its economic importance, but also for the nutritional values of its leaves, mainly due to the fact that they are an excellent source of nutrients, phytochemicals, antioxidants and flavonoids (Kaur and Kapoor 2001, Sardas 2003). Spinach is increasingly becoming important in health because of its micronutrients and phytochemicals. It has an additional advantage of being low in calories.

Nowadays, phytochemicals and antioxidants in plants are raising interest in consumers for their roles in maintaining human health. Phenolic and flavonoids are known for protective effect against cardiovascular diseases, cancer and other diseases (Kaur and Kapoor 2001, Sardas 2003).

The leaf of this annual plant is used as major ingredient in Indian cuisine mainly due to its therapeutic values. A cool climate is best for growing spinach. During periods of warm temperatures and long days, plants are likely to produce seed stalks before making desirable foliage growth. Spinach is fast growing and short lived plant. It matures within 7 weeks (Evans *et al*., 1986). The purpose of this study is to determine the antioxidant activity, flavonoid contents and the essential phytochemicals present in spinach and evaluate the potential benefits.

Materials and Methods

Collection of plant material: The plant material *Spinacia oleracea* L. was collected from three different regions i.e. local market (Thane), sewage area (Kalwa station) and one grown as control. The plant material was collected in polythene bags and washed twice with tap water to remove any debris. The clean material was then air dried, grind and stored in air tight containers separately.

Extractive value: 5 gm of powders from *Spinacia oleracea* L. was extracted separately in 100 ml of distilled-water and ethanol overnight separately.

Preparation of crude extract: 10 mg powders from *Spinacia oleracea* L. was extracted separately in 10 ml of distilled-water overnight. The content was filtered through Whatman filter paper No. 1. The filtrate was evaporated on boiling water bath until dry. The extracts were then stored for further use.

Total Flavonoid Content: The soluble flavonoid of *Spinacia oleracea* L. was estimated by aluminium chloride colourimetric method (Woisky and Salatino, 1998) using quercetin as a standard flavonoid compound. The flavonoid content of aqueous extracts of *Spinacia oleracea* L. were measured using a modified colorimetric method. 0.5ml of sample was mixed with 0.5 ml of 2% $AlCl_3$ and incubated for 10 mins and the absorbance was measured at 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

Total Antioxidant Activity: 0.1ml of extract was combined in Eppendorf tube with 1 ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in thermal block at 95°C for 90 minutes. After cooling to room temperature; the absorbance of the aqueous solution of each was measured at 695 nm against blank (Shirwaikar, *et al*., 2006).

Results and Discussion

The commonly consumed green leafy vegetable *Spinacia oleracea* L. collected from three different places selected for the present study contains substantial amount of phytochemicals. They showed presence of alkaloids, glycosides, steroids, flavonoids, tannins etc. (Table 1)

Extractive value: Aqueous extract of *Spinacia oleracea* L. from home grown plants showed maximum extractive value as compared to sewage irrigated and marketed sample (Table 2).

Total flavonoid content: The total flavonoid content in aqueous extract *Spinacia oleracea* L. was 2.41, 5.86 and 2.41 ìg /mg in home, market and sewage respectively (Table 3). The amount of flavonoids in plant extracts can directly correlate with its antioxidant activity (Melinda *et al*., 2010).

Total antioxidant capacity: Total antioxidant capacity of *Spinacia oleracea* L. is shown in Table 3. In this assay aqueous extract of *Spinacia oleracea* L. from home grown plants showed higher activity than sewage irrigated and marketed sample.

Conclusion

The market Spinach shows greater amount of phytochemicals, antioxidants and flavonoids content than the home grown and sewage one. This is due to the fulfillment of parameters required for proper growth of the plant. The sewage spinach showed better results than the home grown. As the content of the sewage water helped to increase the phytochemical and the antioxidants of the plant. Even if nutrients present in the water helped the plant to show better results, but can be harmful for the human body if consumed. Hence, sewage spinach is not recommended. Home grown showed least results as the parameters for the proper growth of the plant cannot be fulfilled at home.

Acknowledgment

Authors are thankful to Department of Botany, VPM's B. N. Bandodkar College of Science, Thane for providing the laboratory facilities. We are also thankful to DBT-Star College Scheme for providing the instruments and chemicals required for the research work.

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Table 1: Phytochemical constituents in *Spinacia oleracea* L.

Phytochemical constituents	Aqueous			Methanol			Toulene		
	Market	Sewage	Home	Market	Sewage	Home	Market	Sewage	Home
Acid compounds	+	-	-	+	+	+	+	+	+
Aleurone grain	+	+	+	-	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	-	+
Anthraquinone	-	+	+	-	-	-	-	-	-
Amino acid	-	-	-	-	-	-	-	-	-
Protein	-	-	-	-	-	-	-	-	-
Carbohydrate	-	+	+	-	+	+	-	+	+
Starch	-	-	-	-	-	-	-	-	-
Fats & Fixed oils	+	+	-	-	+	+	+	+	+
Glycosides	+	-	+	-	-	-	-	-	-
Mucilage	-	-	-	-	-	-	-	-	-
Phenols	-	-	-	-	-	-	+	-	+
Flavonoids	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	-	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	+	+	+
Essential oils	-	+	-	-	+	-	-	-	-
Resins	-	-	-	-	-	-	-	-	-

Key: '+' positive, '-' Negative

Table 2: Extractive value of *Spinacia oleracea* L.

Sample	Overnight Soaking Method	
	Aqueous	Ethanol
Home	12.9±0.28	13.2±0.92
Market	10.4±0.69	10.2±0.89
Sewage	11.5±1.6	8.6±0.2

Values are mean of three determinants. Mean±SD

Table 3: Total Antioxidant Activity and Total flavonoid content of *Spinacia oleracea* L.

Extracts	Total flavonoid content (µg/mg)	Total Antioxidant Activity (µg/mg)
Home	2.41±0.0	6.12± 0.0
Market	5.86±0.0	5.46±0.0
Sewage	2.41±0.0	6.12±0.0

Values are mean of three determinants. Mean±SD

Study of Quantitative Phytochemistry and Antioxidant Potential of Two Allied Species of Pink Cassia

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Abstract: *Cassia javanica* Linn. and *Cassia nodosa* Buch.-Ham. ex Roxb. are popular garden plants from family Caesalpiniaceae. They are grown for beautiful pink blossoms which appear during summer season. Medicinal properties of these plants are meagrely known. Recently pre-clinically leaves of these plants are proved to be antidiabetic. Morphologically *C. javanica* and *C. nodosa* are very much similar. However their efficacies as hypoglycaemic drugs differ from each other. Therefore in present work, dried leaflet powders of two plants were subjected to detail phytochemical analyses. It involved qualitative and quantitative phytochemical studies. As primary phytochemical screening of plants revealed presence of certain antioxidants, antioxidant potential of both materials was measured by three types of assays, *viz*. Total antioxidant, DPPH and Total Phenolic method. Qualitatively both plants were found to be similar in phytochemical composition while in quantitative assays of certain secondary metabolites were with significant variations. The results of quantitative phytochemistry went concurrent with antioxidant activity of *Cassia* species.

Keywords: Pink Cassias, Antidiabetic, Antioxidants, DPPH assay.

Introduction

Cassia javanica is non-native of India, whereas *Cassia nodosa* is indigenous to Eastern Himalaya. These plants are well acclimatized and reported in varied places of India especially in gardens of metropolitan cities. Both these plants provide timber which is of commercial importance. The previous literature gives very meagre information about the medicinal properties of these plants. *Cassia nodosa* leaves are fatal to livestock. They are refrigerant, purgative and used in poulticing boils. Whereas the medicinal use of *Cassia javanica* leaves is not yet documented. The pods are in use and possess purgative and haemoagglutination actions (Kirtikar, and Basu, 2001; Anonymous, 1992).

Generally plants are considered to be reservoirs of potentially useful chemical compound. They could serve as newer lead and clues for modern drug design. Herbal formulations today are the symbol of safety in contrast to synthetic drugs. The phytochemicals and bioactivity of plants are desirable to know for synthesis of compounds with specific activities to treat various non-chronic and chronic ailments. Hence the present study dealt with qualitative phytochemistry of these plants. Further antioxidant potential of water extracts of said plants has also been explored.

Materials and Methods

For present investigation, authentic samples of leaves of *Cassia javanica* Linn. and *Cassia nodosa* Buch.–Ham. ex Roxb. were collected from the cultivated plants growing in gardens of Bangalore of Karnataka state, Pune, Mumbai and Thane districts of Maharashtra. The botanical identity of the collected samples was confirmed using the standard herbaria at Botanical Survey of India (BSI), Pune and Blatter Herbarium of St. Xavier's College, Mumbai (Accession No. *Cassia javanica* - BSI Kumavat-1 and *Cassia nodosa* - Blat. 15780). Freshly collected leaf drug samples were subjected to artificial drying and ground into powder. The powdered drugs were stored in closed, airtight, labelled containers. Silica bags were put into containers to keep the drug free from moisture.

In qualitative phytochemical screening, known quantities of dried powders were subjected to extraction using water, ethanol and chloroform as solvents. The obtained concentrated extracts were further tested for different chemical constituents (Khandelwal, 2004; Harborne, 1998). In quantitative phytochemical evaluation, certain important secondary metabolites were estimated using dried pounded samples of the drugs. For saponins, initially the frothing property of an aqueous decoction of plant materials was measured in terms of foaming index (Mukherjee, 2002). Later saponin was extracted and estimated by the method described by Rajpal, (2001). Similarly, quantification of other biomolecules *viz*., alkaloids, anthraquinone glycosides, flavonoids**,** phytosterols and tannins was done (Kosalec, *et al.*, 2004; Kulkarni and Apte, 2000; Goad and Akihisa, 1997; Burroughs and Whiting, 1960).

For antioxidant assays only water extracts of two *Cassia* species were utilized. The soluble phenolics were estimated by Folin-Cioclteau reagent method (Slinkard and Singleton, 1977). Total Antioxidant Activity was measured by standard method described by Shirwaikar, *et al*. (2006). The antioxidant activity of water extracts was also evaluated on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH, Hi-media) free radical

according to the method described by Blois (1958).

Results and Discussion

Preliminary phytochemical screening of plant extracts showed that both plants are similar in phytochemical composition. They exhibited presence of various important secondary metabolites such as alkaloids, tannins, glycosides, steroids, terpenoids, flavonoids and saponins (Table 1). Both plants found to contain significant amounts of saponins, alkaloids and anthraquinone glycosides. While flavonoids, phytosterols and tannins were also in considerable range (Table 2). These plants had been proved to be antidiabetic in activity which may be due to single active biomolecule or due to synergistic effect of many chemical constituents. *Cassia nodosa* is more effective crude drug than *Cassia javanica* (Kumavat, *et al.*, 2012). This difference in bioactivity may be due to difference in contents of phytochemicals which has been documented through this study. The present work also gives an idea that water extract of *Cassia nodosa* proved to be stronger in antioxidant potential compared to other species (Table 3).

Conclusion

Cassia javanica and *Cassia nodosa* showed excellent phytochemical potential. Although morphologically and phytochemically *Cassia javanica* and *Cassia nodosa* are very much similar but their crude drugs can be differentiated on the basis of significant difference in contents of anthraquinone glycosides. Currently available synthetic antioxidants like Butylated hydroxy anisole (BHA), Gallic acid, Butylated hydroxyl toluene (BHT), etc. have bad effect on human health. Besides traditionally used natural antioxidants of fruits and vegetables, the crude drugs of allied species of Pink cassia can serve as novel source of antioxidants for preparation of drugs and cosmetics.

Acknowledgments

Authors are thankful to Botany Department and Library of VPM's B. N. Bandodkar College of Science, Thane for providing the necessary facilities.

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Table 1: Qualitative phytochemical screening of *Cassia javanica* and *Cassia nodosa*

Plant constituent test	*Cassia javanica* extracts			*Cassia nodosa* extracts		
	Water	Ethanol	Chloroform	Water	Ethanol	Chloroform
Alkaloids	+	+	+	+	+	+
Tannins	+	+	-	+	+	-
Cardiac glycosides	+	+	+	+	+	+
Cyanogenetic glycosides	+	+	+	+	+	+
Anthraquinone glycosides	+	+	+	+	+	+
Steroids	-	+	+	-	+	+
Sterols	+	+	-	+	+	-
Terpenoids	-	+	-	-	+	-
Flavonoids	-	+	-	-	+	-
Saponins	+	-	-	+	-	-

Key: '+' Present, '-' Absent

Table 2: Quantitative estimations of phytochemicals of *Cassia javanica* and *Cassia nodosa*

Phytochemicals	*Cassia javanica*	*Cassia nodosa*
Foaming Index	166.66	100
Total Saponins	9.88% ±0.25	9.52% ±0.27
Total Alkaloids	5.03% ±0.35	4.08% ±0.28
Anthraquinone glycosides	6.03% ±0.25	0.945% ±0.31
Flavonoids	1.46% ±0.40	1.36% ±0.35
Total Phytosterols	0.64% ±0.25	1.37% ±0.37
Total Tannins	0.21% ±0.35	0.29% ±0.32

Values are mean of three determinants. Mean ± S. E.

Table 3: Total Antioxidant Activity, Total phenolic content and DPPH free radical scavenging activity of *Cassia javanica* and *Cassia nodosa*

Sr. No.	Water Extracts	Total antioxidant activity (gm/100 gm)	Total Phenolic content (gm/100 gm)	DPPH free radical scavenging activity (in %)
1.	***Cassia javanica***	1.581±0.25	1.6±0.25	74.00±0.30
2.	***Cassia nodosa***	2.317 ±0.30	1.79 ±0.38	79.70±0.40

Values are mean of three determinants. Mean ± S. E.

GC-MS Analysis of *Lagenaria Siceraria* (Molina) Standl. Vegetable Peel Waste

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Abstract : India is second major producer of fruits and vegetables in world. Peel waste is a serious problem to processing industries and pollution monitoring agencies. Waste utilization is the one of the important and challengeable job around the world. The effect of resources depletion and environmental concerns have triggered new regulations and growing awareness throughout the world, thus promoting use of more and more fruit and vegetable waste to obtain by-products with health benefits. Thus, in present study an attempt was made to bring utilization of peel waste of *Lagenaria siceraria* (Molina) Standl. The aim of the study was to investigate the presence of phytochemical compounds from *Lagenaria siceraria* (Molina) Standl. peel waste using methanolic extract by GC MS method. 22 bioactive phytochemical compounds were identified in the methanolic peel extract of *Lagenaria siceraria* (Molina) Standl. The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula. Various phytoconstituents identified were found to possess biological and pharmacological activity such as antimicrobial, antifungal, anticancer, antioxidant properties. Recycling of fruit and vegetable waste is one of the most important means of utilizing it in a number of innovative ways yielding new products.

Keywords: *Lagenaria siceraria* (Molina) Standl., Phytochemical compounds, GC MS analysis

Introduction

"Let food be thy medicine and medicine be thy food" was the famous dictum proclaimed by Hippocrates about 2500 years ago. Recently many scientific studies supported the above fact. It appears that diet containing some phytochemicals also termed as bioactive molecules, can provide protection against various chronic diseases like cancer, atherosclerosis, thrombosis, etc and also impart other health benefits (Wildman, 2001). Food of plant origin is capable of contributing appreciable quantities of nutrients, including proteins needed by both children and adults (Okaka *et al*., 2002). Fruits and vegetables form the rich source of bioactive molecules. Over 10,000 bioactive phytochemicals have been identified in different fruits and vegetables which are integral part of the human diet (Wise, 2001).

India is the second major producer of fruits and vegetables in the world. It contributes 10% of world fruit production. According to India Agricultural Research Data Book 2004, the total waste generated from fruits and vegetables comes to 50 million tons per annum (Uchakalwar *et al*., 2014).

Due to the high consumption of vegetables and fruits, peel waste are generated in large quantities in big cities. Peel waste which are highly perishable and seasonal, is a problem to the processing industries and pollution monitoring agencies (Chacko *et al*., 2014). These wastes if not disposed correctly are seen to cause serious environmental problems such as water pollution, unpleasant odors, explosions and combustion and greenhouse gas emissions (Roy *et al*., 2014). Thus, in the present study an attempt has been made to bring about the utilization of the vegetable peel waste for mankind.

Material and Methods

Collection of Sample : The vegetable of *Lagenaria siceraria* (Molina) Standl. used in the present study was collected from the local market of Kalyan. These vegetable was identified from the Department of Botany, Agharkar Institute, Pune. The peels were washed properly under running tap water to remove dust particles. The peels were then shade dried for 5 days and once the moisture was reduced the peels were then completely dried in an oven at 50°C.The dried peels were then powdered using grinder and stored in air tight bottles.

Preparation of sample for GC/MS study: About 5 grams of the peel powder of *Lagenaria siceraria* (Molina) Standl. was soaked in 50 ml methanol. Extraction was done in orbital shaker at 100 rpm (25°C) for 24 hours, followed by filtering of the extract using Whatman filter paper No.1. The residue was removed and supernatant was used for further analysis.

GC-MS analysis: The dried peel powder of *Lagenaria siceraria* (Molina) Standl. was analyzed using GC/MS (Shimadzu capillary GC-quadrupole MS system QP 5000). The sample was injected into the GC-MS on a 30 m glass capillary column with a film thickness of 0.25 µm (30 m × 0.25 mm) with helium as carrier gas at 1 ml/min constant flow mode. GC temperature programme was 50˚C - 300˚C at 10˚C/min. The mass spectra were recorded in electron ionization mode at 50 eV.

Result and Discussion

GC-MS analysis of methanolic extract of *Lagenaria*

siceraria (Molina) Standl. peel

Gas Chromatography and Mass spectroscopy analysis of compounds was carried out in methanolic peel extract of*Lagenaria siceraria* (Molina) Standl.shown in Table-1. GC-MS analysis of methanolic peel extract of *Lagenaria siceraria* (Molina) Standl. revealed the presence of 22 phytoconstituents. The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula.

In the present investigation a variety of compounds have been detected including 9-Octadecenoic acid (0.22), Hexasiloxane (0.31), 1-Monolinoleoylglycerol (0.55), 2,5-Dihydroxyacetophenone (0.56), Gamolenic acid (4.54), Arachidonic acid (1.16), 5-Bromo-8-[(4-hydroxybenzylidene) amino] quinoline (3.14),Oxazepam ditms (1.45), Spirost-8-en-11-one (2.54), Estra-1,3,5 (10)-trien-17a'-ol (1.21), Octasiloxane (0.77), 9-Hexadecenoic acid (1.38), Heptasiloxane (1.41), 9,10 Secocholesta-5,7,10 (19)-triene-1,3-diol(0.54), Digitoxin (0.65), Cis-Vaccenic acid (2.20), Ethyl iso-allocholate (1.60), Pentadecanoic acid (5.20), Dibutyl phthalate (29.93), 3, 12- Oleandione (1.27), Oleic acid, eicosyl ester (0.12)and Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5a)- (0.36) with retention time 4.44, 5.79, 5.85, 5.936.46, 7.46, 8.31, 9.18, 9.87, 10.25, 10.70, 10.98, 11.25, 11.91, 12.04, 12.58, 12.93, 14.33, 14.57, 15.02, 15.42 and 15.91 respectively. Irrespective of the amount or concentration (high or low) in which these compounds were found to be present, almost all these compounds have been reported to possess some pharmacological or the other biological activity.

Conclusion

Recycling of fruit and vegetable waste is one of the most important means of utilizing it in a number of innovative ways yielding new products. The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. Thus the GC-MS analysis of methanolic extract of *Lagenaria siceraria* (Molina) Standl. peel showed a highly complex profile, containing approximately 22 components. This study further may be useful to explore the pharmacological and biosynthetic activity of the peel.

Acknowledgment

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Table 1: GC MS analysis of methanolic extract of *Lagenaria siceraria* (Molina) Standl. peel

Sr. No.	Name of Compound	RT	% Area (min)	Formula	Molecular weight
1.	9-Octadecenoic acid	4.44	0.22	$C_{28}H_{44}O_4$	444
2.	Hexasiloxane	5.79	0.31	$C_{12}H_{38}O_5Si_6$	430
3.	1-Monolinoleoylglycerol trimethylsilyl ether	5.85	0.55	$C_{27}H_{54}O_4Si_2$	498
4.	2,5-Dihydroxyacetophenone, bis (trimethylsilyl) ether	5.93	0.56	$C_{14}H_{24}O_3Si_2$	296
5.	Gamolenic acid	6.46	4.54	$C_{18}H_{30}O_2$	278
6.	Arachidonic acid	7.46	1.16	$C_{20}H_{32}O_2$	304
7.	5-Bromo-8-[(4-hydroxybenzylidene) amino] quinoline	8.31	3.14	$C_{16}H_{11}BrN_2O$	326

8.	Oxazepam ditms	9.18	1.45	$C_{21}H_{27}C_1N_2O_2SiO_2$	430
9.	Spirost-8-en-11-one	9.87	2.54	$C_{27}H_{40}O_4$	428
10.	Estra-1,3,5 (10)-trien-17a'-ol	10.25	1.21	$C_{18}H_{24}O$	256
11.	Octasiloxane	10.70	0.77	$C_{16}H_{50}O_7Si_8$	578
12.	9-Hexadecenoic acid	10.98	1.38	$C_{16}H_{30}O_2$	254
13.	Heptasiloxane	11.25	1.41	$C_{14}H_{44}O_6Si_7$	504
14.	9,10 Secocholesta-5,7,10 (19)-triene-1,3-diol	11.91	0.54	$C_{30}H_{52}O_3Si$	488
15.	Digitoxin	12.04	0.65	$C_{41}H_{64}O_{13}$	764
16.	Cis-Vaccenic acid	12.58	2.20	$C_{18}H_{34}O_2$	282
17.	Ethyl iso-allocholate	12.93	1.60	$C_{26}H_{44}O_5$	436
18.	Pentadecanoic acid	14.33	5.20	$C_{17}H_{34}O_2$	270
19.	Dibutyl phthalate	14.57	29.93	$C_{16}H_{22}O_4$	278
20.	3, 12- Oleandione	15.02	1.27	$C_{30}H_{48}O_2$	440
21.	Oleic acid, eicosyl ester	15.42	0.12	$C_{38}H_{74}O_2$	562
22.	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5a)-	15.91	0.36	$C_{29}H_{50}O_2$	430

Figure 1: Mass spectrum of different compounds identified in methanolic extract of *Lagenaria siceraria* (Molina) Standl. peel by GC-MS analysis

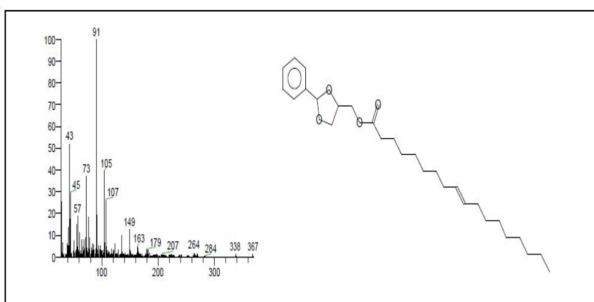

Figure 1.1: GC MS Spectrum of 9 Octadecenoic acid

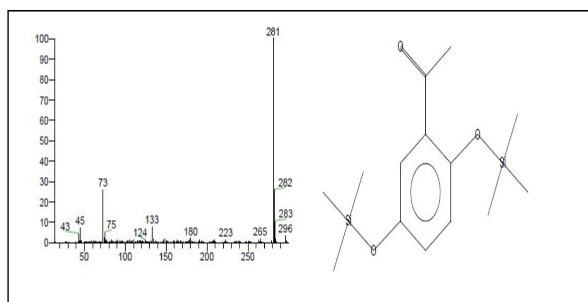

Figure 1.2:GC MS Spectrum of 2,5- Dihydroxyacetophenone

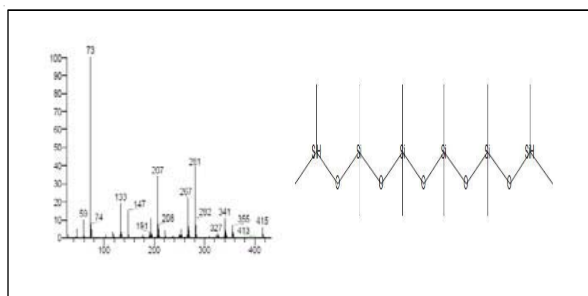

Figure 1.3:GC MS Spectrum of Hexasiloxane

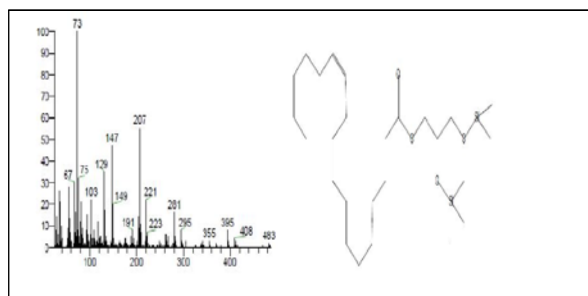

Figure 1.4:GC MS Spectrum of 1- Monolinoleoylglycerol trimethylsilyl ether

 ISBN : 978-81-923628-5-4

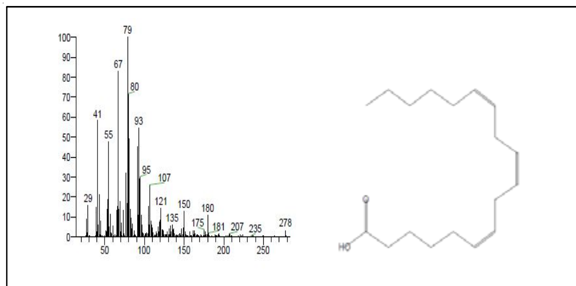

Figure 1.5:GC MS Spectrum of Gamolenic acid

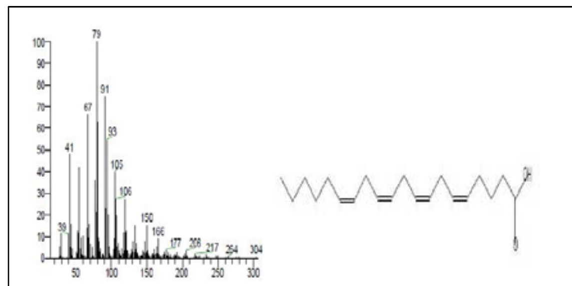

Figure 1.6:GC MS Spectrum of Arachidonic acid

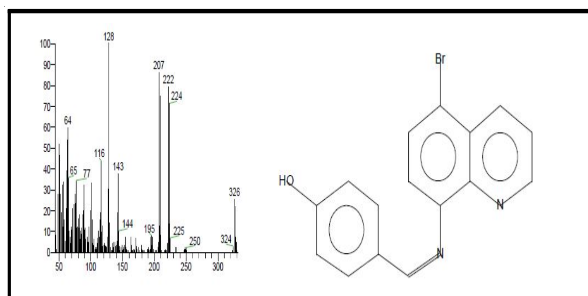

Figure 1.7:GC MS Spectrum of 5-Bromo-8-[(4-hydroxybenzylidene) amino] quinoline

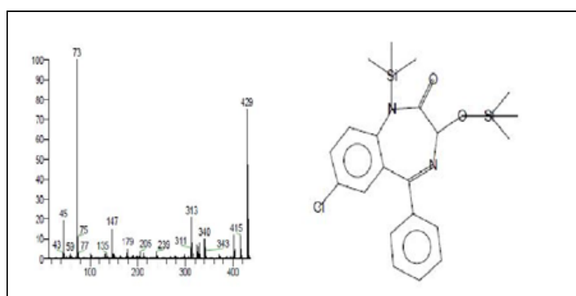

Figure 1.8:GC MS Spectrum of Oxazepam ditms

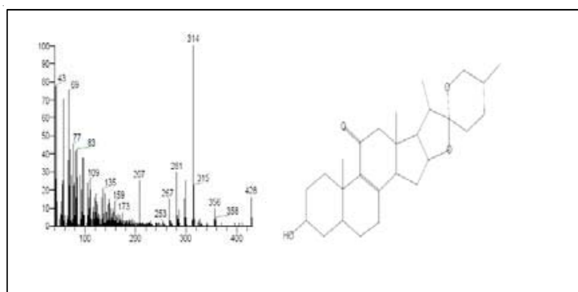

Figure 1.9:GC MS Spectrum of Spirost-8-en-11-one

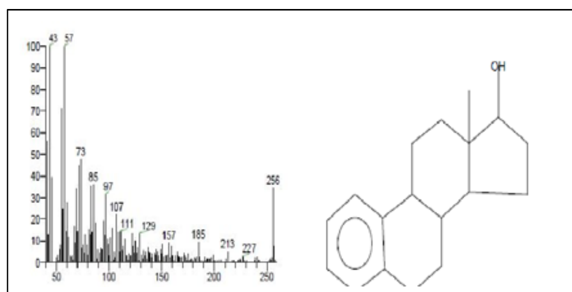

Figure 1.10: GC MS Spectrum ofEstra-1,3,5 (10)-trien-17a'-ol

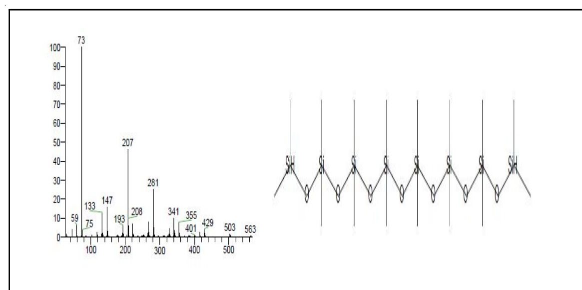

Figure 1.11:GC MS Spectrum of Octasiloxane

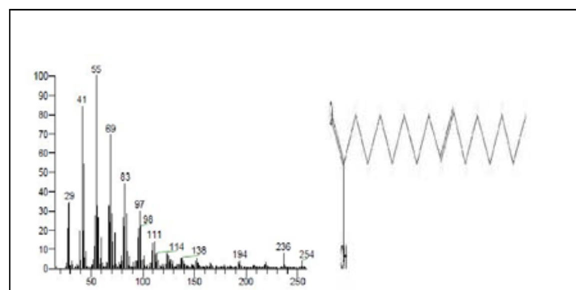

Figure 1.12:GC MS Spectrum of 9-Hexadecenoic acid

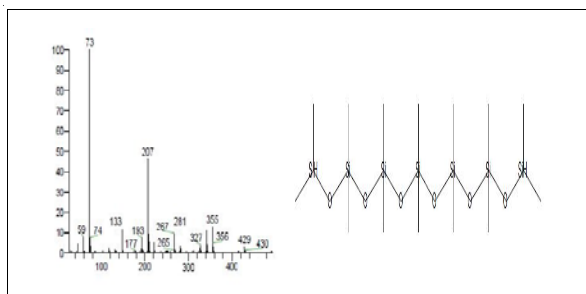

Figure 1.13:GC MS Spectrum of Heptasiloxane

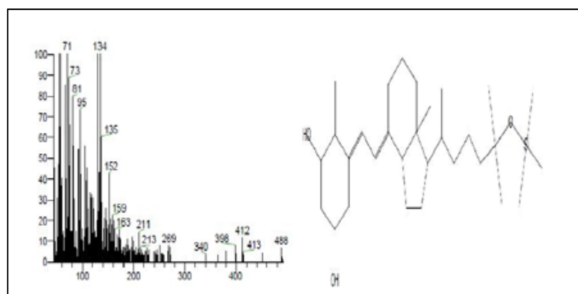

Figure 1.14:GC MS Spectrum of 9,10 Secocholesta-5,7, 10 (19)-triene-1,3-diol

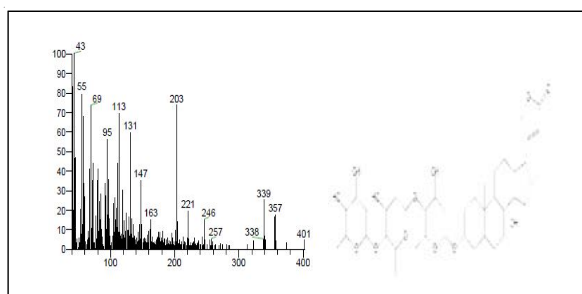

Figure 1.15: GC MS Spectrum of Digitoxin

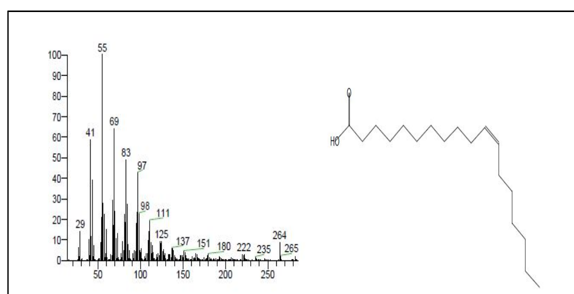

Figure 1.16: GC MS Spectrum of Cis-Vaccenic acid

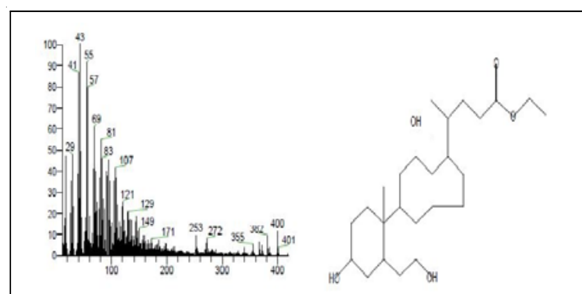

Figure 1.17:GC MS Spectrum ofEthyl iso-allocholate

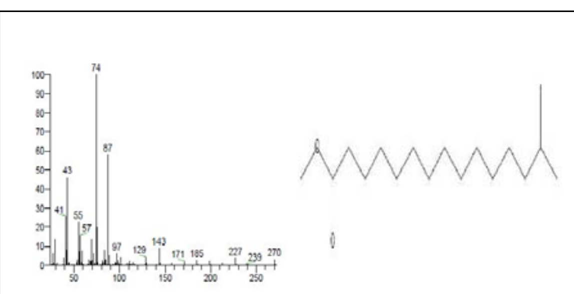

Figure 1.18:GC MS Spectrum of Pentadecanoic acid

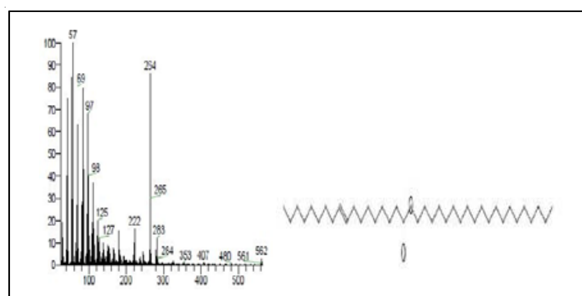

Figure 1.19:GC MS Spectrum of Oleic acid, eicosyl ester

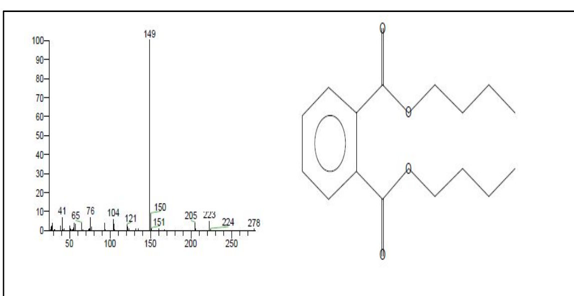

Figure 1.20:GC MS Spectrum of Dibutyl phthalate

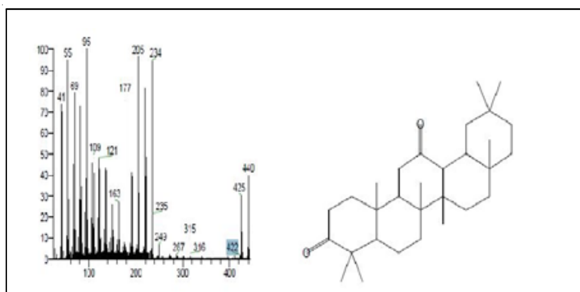

Figure 1.21:GC MS Spectrum of 3,12-Oleandione

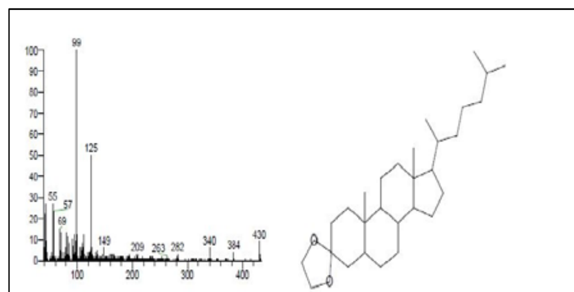

Figure 1.22:GC MS Spectrum of Cholestan-3-one, cyclic 1,2-ethanedityl aetal, 5(a)-

Pharmacognostical Evaluation of *Ficus Retusa* Stem

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Abstract: *Ficus retusa* L. belonging to family Urticaceae (Moreaceae), known as chinese banyan. It is a huge tropical deciduous, evergreen tree with obovate dark green shiny leaves, a chunky trunk with conspicuous aerial roots. The bark is grey to dark brown, bitter taste, cylindrical shape and rough irregularly cracked. This present study has been undertaken to establish the pharmacognostical standards for the stem drug. The study comprises of macroscopy, microscopy, histochemistry, physicochemical parameters, fluorescence analysis, preliminary phytochemistry along with antimicrobial study. The phytochemical studies showed the presence of starch, reducing sugars, proteins, tannins, steroids, terpenoids and flavonoids. These metabolites are the active constituents of *Ficus retusa* stem and they may be responsible for its pharmacological activites.

Keywords: *Ficus retusa* stem, Pharmacognosy, Phytochemistry.

Introduction

Ficus retusa L. belonging to family Urticaceae (Moreaceae), known as chinese banyan is distributed throughout western peninsular and also in chota Nagpur, Bihar, Central India, Andaman, Sundribuns, Malaya islands and Australia. It is a huge tropical deciduous, evergreen tree with obovate dark green shiny leaves, a chunky trunk with conspicuous aerial roots.

Ficus retusa is used as aphrodisiac, burning sensations, leucoderma, “tridoaha”, leprosy, itching, biliousness and diseases of the blood. It mainly contains sterols*, terpenoids, glycosides, flavonoids,* polyphenols, proteins, carbohydrate, etc. (Chauhan *et.al*. 2013). Though the plant shows different medicinal uses it is still under ignorance. Therefore this present study was under taken to put forth the pharmacopoeial standards. In the present study macroscopy, microscopy, histochemistry, physicochemical parameters, fluorescence analysis, preliminary phytochemistry along with antimicrobial study were carried out.

Material and Methods

For the present investigation the stem of *Ficus retusa,* was collected from Maharashtra. The sample of *Ficus retusa* stem was collected from Xavier’s villa, Khandala. The collection was done with prior permission of the concerned authorities. The stem was collected from the mature trees which were about to flower. These samples were authenticated for their botanical identity with the help of Blatter’s Herbaria St. Xavier’s College. The specimens were deposited in Botany Research Laboratory M.V.L.U. college Voucher specimen no. MV/FR/001, MV/CT/002, MV/GA/003, MV/TP/004.

Storage of plant material: The collected plant material was brought to laboratory and air dried. The dried material was grinded in mixer and stored in air tight container containing silica filled bags to keep them free from moisture. It was labeled properly. Some of the sample was also preserved in Formalin: Acetic acid: Alcohol solution (F.A.A.) in the ratio of 6.5 (40%): 2.5:100 (50%).

Macroscopy and microscopy: The authenticated plant sample was studied for its macroscopy in details using standard methods (Anonymous, 2001; Mukherjee 2002; Wallis 1985).

Transverse hand cut sections of authenticated samples were taken and made permanent with suitable stains. Photomicrographs were taken of the permanent preparations (Khandelwal 2004; Ruzin 1998; Jackson 1990; John 1958). The histochemical analysis for the cell contents were performed using standard techniques (Krishnamurthy 1988; Jensen 1962). The powdered drugs were soaked in aqueous solution of chloral hydrate and mounted in 50% glycerin for microscopical studies. For the measurement of cell contents like starch grains, fibers, vessels, calcium oxalate crystals, stone cells, etc. occular and stage micrometer were used (Anonymous, The Ayurvedic Pharmacopoeia of India, 2001; Jackson and Snowdow 2005; Khandelwal 2004; Wallis 1985; Iyengar 1974).

Physico-chemical and florescence properties: The physicochemical parameters like ash values (total ash, acid insoluble ash, water soluble ash), extractive values were done (Anonymous, 2006; Khandelwal 2004; Handa 1999; Kokate 1999). The fluorescence response of powdered drugs exposed to U.V. radiations was studied using the procedure given by Kokoski and Kokoski 1958; Chase and Pratt 1949.

Phytochemical and antimicrobiological studies: For phytochemical investigations, a known quantity of dried powder was extracted with alcohol and water. These extracts were tested for different constituents (Edeoga 2005; Rajpal 2001; Kokate 1999; Brain and Turner 1975; Peach and Tracy, 1955). Antimicrobial activity was performed using disc

diffusion method. For antibacterial activity standard nutrient agar media was used. Disc of 6mm diameter were prepared from whatmann filter paper no. 1 with the help of punch press. The disc were then sterilized by autoclaving it. Few of these discs were soaked in absolute alcohol and were used as standard disc or control disc. Remaining discs were soaked in plant extract for 48 hrs so that maximum amount of plant extract was impregnated on each disc. These discs were used for antimicrobial activity.

About 0.1 ml of 18 hrs old culture was placed in each plate with a pasteur pipette. The plates were then rotated to spread the inoculum uniformly. Then the impregnated discs were placed on the media with a sterile forceps. 3-4 discs impregnated with plant extract and the control disc at the centre. The discs were then pressed gently on the surface so that they could not shift from their position subsequently and were firmly affixed to the plate. All this operation was carried out aseptically. The plates were then incubated, bacterial plates at 37°C for 24 hrs and fungal plates at 30-35°C for 36 hrs. The experiment was performed in triplets and the average zone of inhibition was recorded (Nair and Bhide, 1996; Kavitha *et.al.*, 2004; Mukherjee 2002; Khan *et. al.*, 2001).

Result and Discussion

Macroscopy and Microscopy: The shape of stem in *Ficus retusa* is circular. The outer surface of the stem showed presence of lenticles and was smooth in appearance. The colour is light brown to grey. It is fibrous in fracture. The stem does not show any characteristic taste. Macroscopical observations of stem of *Ficus retusa* have not been reported so far (Figure 1a & 1b).

The stem showed the presence of Phellum which was 4-5 layered, rectangular compactly arranged cells, phellogen and phelloderm is undifferentiated. Secondary cortex made up of 7-8 layers of chlorenchymatous cells compactly arranged, filled with chloroplast. It is followed by 12-13 layered polygonal parenchymatous cells. They are filled with starch grains and latex. Just below the cortex, pericycle fibres are seen which have polygonal to pentagonal cells. Pericycle fibres are followed by few layers of phloem cells. Phloem is followed by secondary xylem which is interrupted by medullary rays. 3-4 layers of cambium cells are present inbetween phloem and secondary xylem, which is rectangular compactly, arranged thin walled cells. Medullary rays are uniseriate to multiseriate, Xylem vessel is mostly unilateral but at some places multilateral vessels are also seen. Xylem is followed by pith. Pith is made of parenchymatous cells which are smaller towards the periphery and larger towards the centre. They are filled with starch grains and prism shaped calcium oxalate crystals (Figure 2a, 2b & 2c).

Histochemical studies of stem showed the presence of starch, lipids, tannins, proteins, calcium oxalate crystal, cellulose, mucilage and pectin. The powder study of *Ficus retusa* stem is brown, non aromatic coarse and fibrous in texture. Microscopically the powder shows cork cells which were in fragments of suberized hexagonal to polygonal frequently measuring 22.84- 28.55-.34.26 µm in length and 28.55- 34.26-39.97 µm in breadth. Cortical cells were thin walled, polygonal measuring 22.84-28.84 µm in length and 17.13 - 22.84 in breadth. Tannin filled cells were irregular in shape with brown tannin content measuring 22.84-34.26-45.68 µm in diameter. Starch grains were simple and compound types, round in shape measuring 3.42 – 5.71-6.84-9.29 µm in diameter. Calcium oxalate crystals were prism shaped, ranging from 22.84-28.55-34.26 µm in length and17.13 -22.84-28.55 µm in breadth. Fibres were lignified, elongated measuring 74.23- 137.04 µm in length (Fig: 3a, 3b, 3c, 3d & 3e).

Physico-chemical and florescence properties: The physic-chemical parameters like ash value, acid insoluble and water soluble values, not more than 8.72 % w/w, 1.56 % w/w, 2.5 % w/w while extractive value was put forth for stem. These parameters are essential for the determination of quality and purity of the crude drugs. (Table 1 & 2) The observations of the fluorescence analysis for the stem are quite distinct and can be used to detect the adulteration (Table 3).

Phytochemical and antimicrobiological studies: The preliminary phytochemical analysis was carried out with a known quantity of dried power. Water and Alcoholic extracts were prepared. These two extracts were tested for different constituents and complied in Table 4. The ethanolic extract of the stem of *Ficus retusa* was tested for their antimicrobial activity against 14 common pathogenic bacteria. The ethonalic extract of stem of *Ficus retusa* showed activity against *Klebsiella pneumonia, Salmonella paratyphii, Corynebacteria diphthariae, Pseudomonas aerogenosa, Proteus vulgaris, Bacillus subtilus, Bacillus cereus, Sarcina lutea*. The results are shown in Table 5.

Conclusion

The present work deals with the macroscopical and microscopical structure, histochemical analysis, powder study, physico-chemical, fluorescence studies, phytochemical investigations of *Ficus retusa* stem.

Anatomical observations were confirmed in powder studies. This will be a reliable parameter to identify the authentic samples from the adulterant. The physico-chemical standards for ash values and extractive values are put forth for the stem will be useful in laying pharmacopoeial standards. Powdered stem showed characteristic fluorescence. This can be used for the quick identification of the authentic drug from adulterants.

Phytochemical analysis showed the presence of starch, reducing sugars, proteins, tannins, steroids, terpenoids and flavonoids. This exhibits chemical profile of the plant to which we can relate its therapeutic activities. Antimicrobial studies shows activity against a wide range of bacteria and fungi, suggesting that the alcoholic extract may serve as a broad spectrum antibiotic. It is also interesting to note that most parts of the tree are effective versus more than 50% strains of bacteria.

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Table 1: Ash value of *Ficus retusa*

Ash values	Stem
Total ash % w/w not > than	8.72
Acid insoluble ash %w/w not > than	1.56
Water soluble ash % w/w	2.57

Table 2: Extractive values

Sr. No.	Name of plant	Plant part	Alcoholic Extractive value in %	Water extractive value in %
1	*Ficus retusa*	Stem	23.75	25

Table 3: Fluorescence studies of *Ficus retusa* stem

Sr. No.	Test	Stem	
1.	Powdered crude drug + reagent	Day light	UV light
2.	Powered crude drug mounted in nitrocellulose in amyi acetate	Brown	Greenish
3.	Powered crude drug + 1N NaOH in Methanol	Dark brown	Dark brown
4.	Powered crude drug + 1N NaOH in methanol, dried and mounted in nitrocellulose in amyl acetate	Dark brown	Dark brown
5.	Powered crude drug + 1 N conc HCl	Brown	Dark brown
6.	Powered crude drug + 1N HCl in methanol, dried and mounted in nitrocellulose in amyl acetate	Dark brown	Pale yellow
7.	Powered crude drug + 1N NaOH in water	Dark brown	Dark brown
8.	Powered crude drug + + 1N NaOH in water, dried and mounted in nitrocellulose in amyl acetate	Brown	Black
9.	Powered crude drug + 1N HNO_3 in water	Brown	Pale yellow
10.	Powered crude drug + 1N H_2SO_4 in water	Brown	Pale yellow
11.	Powered crude drug +Picric Acid	Brown	Dark brown
12.	Powered crude drug + Acetic acid	Dark brown	Yellowish green
13.	Powered crude drug + Liq. Ammonia	Dark brown	Black
14.	Powered crude drug + Alcohol	Dark brown	Pale yellowish
15.	Powered crude drug + Petroleum ether	Dark brown	Pale yellowish

Table 4: Preliminary Phytochemical Screening of *Ficus retusa* Linn.

Sr. No.	Plant constituent test	Stem	
		W	**E**
1.	Test for starch.	+	+
2.	Test for reducing sugars. a. Fehling's test. b. Benedict's test.	 + +	 + +

3.	Test for mucilage.	+	+
4.	Test for amino acids.	-	-
5.	Test for proteins.		
	a. Biuret test.	+	+
	b. Xanthoproteic test.	+	+
	c. Million's test.	+	+
6.	Test for Alkaloids.		
	a. Mayer's test.	-	-
	b. Wagner's test.	-	-
	c. Dragendorff's test.	-	-
	d. Hager's test.	-	-
7.	Test for Tannins.		
	a. Ferric chloride test.	+	+
	b. Lead acetate test.	+	+
	c. Potassium dichromate test.	+	+
8.	Test for Cardiac glycosides.	-	-
9.	Test for Anthroquinone glycosides.	-	-
10.	Test for Cyanogenetic glycosides.	-	-
11.	Test for steroids.		
	a. Salkowski test.	+	+
	b. Libberman Burchard test.	+	+
12.	Test for Terpenoids.	+	+
13.	Test for Flavonoids.	+	+
14.	Test for Saponins.		
	a. Foam test.	-	-
	b. Haemolysis test.	-	-

Note :- W- water, E- ethanol , + present , - absent.

Table 5: Antibacterial activity of *Ficus retusa*

Sr. No.	Name of organisums	Stem
1.	*Klebsiella pneumoniae*	2.24±0.007
2.	*Salmonella paratyphi B*	0.95±0.011
3.	*Corynebacterium diphthariae*	0.9±0.019
4.	*Pseudomonas aerogenosa*	0.9±0.01

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5.	*Proteus vulgaris*	1.01±0.005
6.	*Escherichia coli*	-
7.	*Shigella sps*	-
8.	*Salmonella paratyphii A*	-
9.	*Streptococcus pyogenes*	-
10.	*Salmonella typhi*	-
11.	*Staphylococcus aureus*	-
12.	*Bacillus subtilus*	0.75±0.011
13.	*Bacillus cereus*	0.75±0.011
14.	*Sarcina lutea*	0.76±0.011

Fig.1a *Ficus retusa* tree

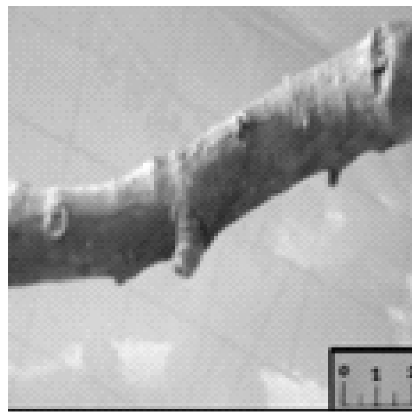

Fig.1b. *Ficus retusa* stem

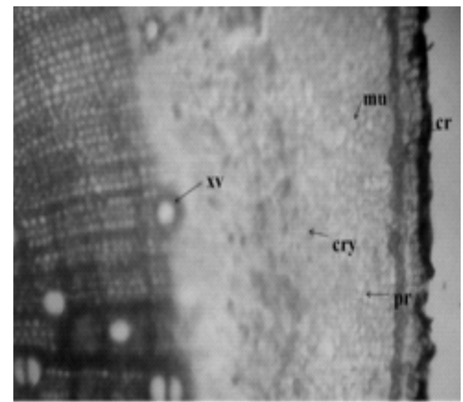

Fig. 2a. T.S of *Ficus retusa* stem

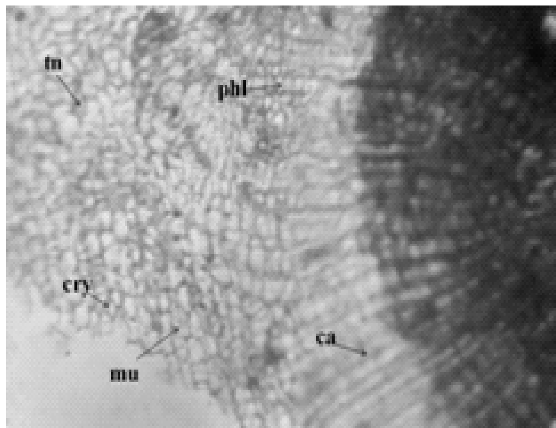

Fig 2b. T.S of *Ficus retusa* stem

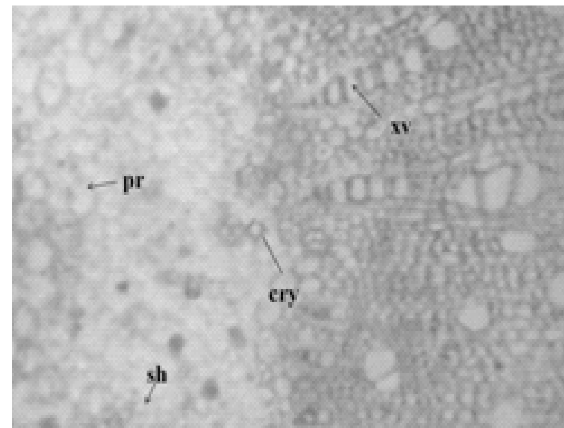

Fig. 2c. T.S of *Ficus retusa* stem

xy- xylem, pr- pith, cry- calcium oxalate crystals, sh- starch grains, phl-phloem, tn- tannin filled cells, Mu- mucilage cells, cr- cork cells, pr- parenchyma cells

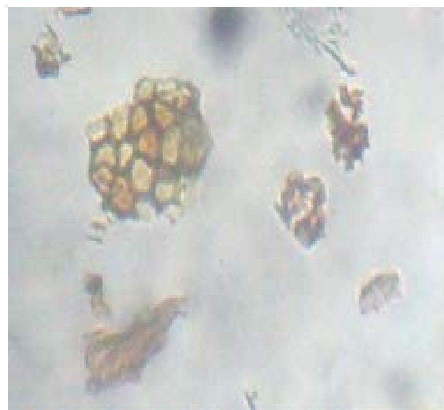

Fig 3a. cork cells

Fig. 3b. cortical cells.

Fig. 3c. fibres.

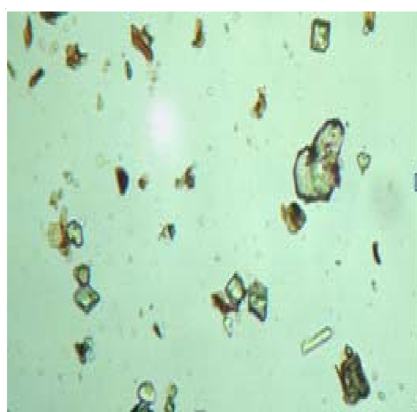

Fig. 3d. calcium oxalate crystals & starch grains.

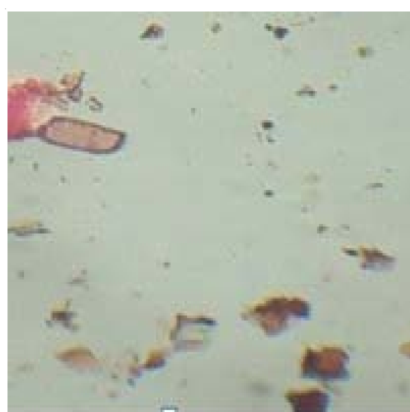

Fig 3e. tannin filled cells.

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Estimation of Chlorophyll Content in Plant Leaves from Polluted and Non-Polluted regions

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Abstract: Chlorophyll is basically a group of green pigments produced by green plants that convert sunlight into energy via photosynthesis. Leaf chlorophyll concentration act as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. In the present study, five plant species were analysed for chlorophyll content from polluted and non - polluted regions. Estimation of chlorophyll content was done by analyzing the extracted sample using colorimetric method which shows the difference in chlorophyll content analysed from polluted and non-polluted region.

Keywords: Chlorophyll, colorimeter, polluted plants, non -polluted plants.

Introduction

Chlorophyll is the green pigments of chloroplasts taking part in photosynthesis. Chlorophyll a is present in all higher plants, Chlorophyll-b is seen in higher plants and algae and Chlorophyll-c, d and e are mainly confined to some algae (Ragland *et al*., 2014). It is difficult to estimate the effects of air pollutants because the organisms are exposed to wide range of uncontrolled variables (parasites, weather conditions and complex mixture of pollutants). On the physiological and morphological point of view, the plants from polluted sites shows important changes especially regarding their colors, shapes, leaf length, width, area and petiole length. Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers and roots. The chlorophyll plays an important role in plant physiology and it can act as nutrition in declined blood sugar conditions, detoxification, digestion, excretion and decreasing allergens.

The chlorophyll production is mainly depended on penetration of sunlight and it is the main source of energy for plant. The proportion of chlorophyll-a to chlorophyll-b depends on light intensity to which the leaves are being exposed. Even in same species, it is higher in shaded plants than in plant exposed to full sunlight (Ragland *et al*., 2014). The chlorophyll pigments show characteristic absorption spectra. Chlorophyll-a shows maximum absorption at 435nm, 670nm and 680nm while chlorophyll-b shows maximum absorption at 480nm and 650nm. Absorption maxima of pigments indicate the quality of light useful for photosynthesis (Ragland *et al*., 2014).Present paper records the chlorophyll content of different plant leaves. Difference between the chlorophyll content in five different plant sps. from polluted and non-polluted region were studied. This work was an experimental study and objective of study was to analyze total chlorophyll content plant leaves in polluted and non-polluted areas.

Materials and Methods

Present study was carried out at Thane which is located in the Thane Tehasil of Thane district, Maharashtra during the month of September 2017. A total of five different plant species were selected twice from two different region representing polluted and non-polluted areas. Sampling was done by once only. Both the samples of plants were collected and subjected to standard chemical procedures for determination of chlorophyll content.

Chlorophyll analysis : 0.50 gram of leaf sample was finely cut and gently mixed using a clean pestle and mortar. To this homogenized leaf material, 1 ml of 80% acetone was added. The materials was further grinded gently. The get separated with the help of funnel, cotton and muslin cloth. Filtrate was collected separately and analysed for estimation. The color absorbance of the solution was estimated by a colorimeter using 645 and 663nm wavelength. The readings were further calculated using Arnon's method.

Formula,

$$\text{mg Chlorophyll a / gm tissue} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg Chlorophyll b / gm tissue} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{mg total Chlorophyll /gm tissue} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

Results and Discussion

The chlorophyll content in plants varies depending on the effect of various environmental factors on them.

Pollution is one of the environmental factor which can influence the change in chlorophyll content in the plants. A comparative study was done between leaf samples of five plants *viz Terminalia catapa, Azadirachta indica, Mangifera indica, Saraca asoca, Ficus religiosa.* from polluted and non-polluted areas.

The non-polluted area sample of *Terminalia catapa* showed more total chlorophyll content (9.4) as compared to its polluted area sample (8.8). The non-polluted area sample of *Azadirachta indica* showed maximum total chlorophyll content (18.2) as compared to the polluted area sample (11.9). The non-polluted area sample of *Mangifera indica* showed maximum total chlorophyll content (11.6) as compared to polluted area sample (9.4). In the non-polluted area sample of *Saraca asoca* the highest concentration of total chlorophyll content (50.4) was observed as compared to polluted area sample (7.3). The non-polluted area sample of *Ficus religiosa* showed higher total chlorophyll content (14.7) as compared to its polluted area sample (8.3).

The present study revealed that in all the selected plants, the chlorophyll content was high in non-polluted area plants in comparison to polluted area plants. A significant difference in presence of total chlorophyll content was observed between the plant samples of polluted and non-polluted areas.

Conclusion

Chlorophyll content can be used as measurement of robustness of plants canopy and the rate of photosynthesis as well. This study will be helpful to do research in chlorophyll content analysis of various plants species and study the vegetation cover area. Also the effect of pollution on chlorophyll content is an important point of study. Continuous monitoring of chlorophyll content is required to maintain and check the health of plants. The chlorophyll pigments are an indicator of oxygen production and carbon sequestration.

Acknowledgment

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Table 1: Estimation of chlorophyll content of different plant species from polluted and non polluted regions

Sr. No.	Name of Plant *sps.*	Area	Chlorophyll a	Chlorophyll b	Total Chlorophyll
1.	*Terminalia catapa*	Polluted Area	2.94×10^{-4}	5.92×10^{-4}	8.87×10^{-4}
		Non-Polluted Area	3.14×10^{-4}	6.28×10^{-4}	9.43×10^{-4}
2.	*Azadirachta indica*	Polluted Area	3.64×10^{-4}	8.29×10^{-4}	11.93×10^{-4}
		Non-Polluted Area	6.66×10^{-4}	11.56×10^{-4}	18.22×10^{-4}
3.	*Mangifera indica*	Polluted Area	3.95×10^{-4}	7.74×10^{-4}	9.39×10^{-4}
		Non-Polluted Area	6.86×10^{-4}	11.93×10^{-4}	11.69×10^{-4}
4.	*Saraca asoca*	Polluted Area	2.60×10^{-4}	4.73×10^{-4}	7.33×10^{-4}
		Non-Polluted Area	18.07×10^{-4}	32.33×10^{-4}	50.39×10^{-4}
5.	*Ficus religiosa*	Polluted Area	2.74×10^{-4}	5.55×10^{-4}	8.30×10^{-4}
		Non-Polluted Area	10.10×10^{-4}	19.50×10^{-4}	14.79×10^{-4}

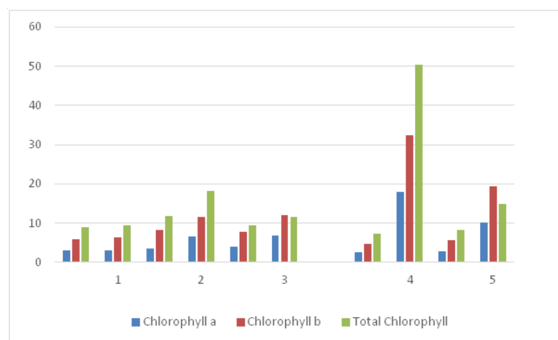

Plate 1: List of plants grown in polluted area

A: *Terminalia catapa*, B: *Azadirachta indica*, C: *Mangifera indica*, D: *Saraca asoca*, E: *Ficus religiosa*

Plate 2: List of plants grown in un-polluted area

A: *Terminalia catapa*, B: *Azadirachta indica*, C: *Mangifera indica*, D: *Saraca asoca*, E: *Ficus religiosa*

Phytochemical Marker Based Quality Evaluation of Indian Spice Mixtures Using High Performance Liquid Chromatography

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Abstract: Indian foods are popular world over for their flavours especially the use of spice mixtures. Several spice mixtures are available for use in Indian recipes. Some of the most common ones available are *Garam Masala, Goda Masala, Pav Bhaji Masala* etc. There are no standards available for these spice mixtures and manufacturers market them as per their own standards complying only to the food standards of FSSAI.

The current paper is High Performance Liquid Chromatography based evaluation of two spice mixtures; *Garam Masala* and *Goda Masala*. HPLC fingerprints have been developed for some popular marketed mixtures and compared. The chromatographic patterns have been evaluated on the basis of specific phytochemical standards (Anethole, Eugenol, piperine etc.) and the content of these phytochemical markers have been compared for different spice mixtures. A quality control parameter based on chromatographic fingerprints has been suggested for the spice mixtures.

Keywords: HPLC, Quality control, Spices, Fingerprint pattern

Introduction

Garam Masala and *Goda Masala* are delectable blends of common spice powders that lend the final touch of flavors to many Indian cuisines. Their ingredients are powders of common spices, mixed together in specific proportions to give a subtle and unique flavour to foods. *Garam masala* is one of the 'must have' spice-mixtures in a typical Indian kitchen. These *"masalas"* are available in ready-to-use powders, branded by many companies and available at many retail stores around street corners. With people mostly avoiding grinding spices at home, the consumption of these ready-to-use, pre-packed masala brands is significantly high amongst commercially available, branded food items. Many companies are reaping the benefits of rising demands and many stores have dedicated shelves to stock branded masala packets (Shailajan *et. al.*, 2011 and 2016).

There is no single standard masala recipe and therefore, the ingredients differ by region and according to individual preferences. The branded *Garam masala*, however, consists of a mixture of coriander, cumin, cardamom, cloves, black pepper, cinnamon and nutmeg. Additional ingredients may include turmeric, saffron, fennel seeds, ginger, garlic, mustard seeds, mace, star anise, tamarind, fenugreek and bay leaves. The branded goda masala generally consists of Coriander Seeds, Dry Coconut, Cumin seeds, Sesame Seeds, Black Pepper, Caraway Seeds, Poppy Seeds, Turmeric, Black Cardamom, Bay Leaf, Compounded Asafoetida, Cinnamon, Clove, Mace, Black Stone Flower, Cardamom, Nagkeshar, Refined Edible Vegetable Oil, Iodised edible Salt.

There have been reports of variations in organoleptic characteristics of branded *masala* claimed to be containing the same ingredient spices (Ashok, 2013). The variations are existent mainly due to lack of proper quality standards except, for the food safety standards (NPCS, 2017). These branded *masalas*, though are safe for consumption, do not tickle the organoleptic senses to the same extend, owing to the difference in the proportions of their ingredients. It was therefore, felt necessary to evaluate the chromatographic fingerprints of these *masalas*, in an effort to suggest chromatographic quality of these spice mixtures (FSSAI).

Different marketed spice mixtures were evaluated for the content of *trans*-anethol and eugenol; two major phytochemicals that give characteristic flavour to the spice mixtures. Simple and convenient isocratic Reverse Phase High Performance Liquid Chromatographic method has been developed for the evaluation. Sample processing has been standardized to be applicable for different matrices. It is felt that, the method could be easily adopted by manufacturers for fast and reliable quantitative evaluation of quality control measures of the spice mixtures. Additionally, some physical characteristics of the spice mixtures have also been evaluated. The results of HPLC analysis have been compared with those from Thin Layer Chromatographic evaluation of the same samples. Attempt has been made to suggest quality parameters for the spice mixtures, in order to standardise their organoleptic potentials.

Materials and Methods

Reagents and Chemicals

Pure standard of *trans*-Anethole ($C_{10}H_{12}O$ – purity 99%), Eugenol ($C_{10}H_{12}O_2$ - purity 99%) were procured from the Sigma Aldrich Laboratories India Pvt. Ltd and Piperine

($C_{17}H_{19}N_{03}$ - purity 95%) was procured from the Synthite Industries India Pvt. Ltd. The chemical structures of *trans*-Anethole, Eugenol and Piperine are illustrated in Figure. 1. Methanol, Acetonitrile (HPLC grade), Ammonium Dihydrogen Phosphate, Toluene, Ethyl acetate were purchased from Merck Ltd., Mumbai, India. Ultra pure (Milli-Q) water of 18.2 MΩ resistances and 0.22µ membrane filtered was obtained from Millipore water purication system (Molsheim, France). Absolute alcohol (AR grade) was obtained from Changshu Yengyan Chemicals (China). All other chemicals used in the study were of analytical reagent (AR) grade.

HPLC Instrumentation and chromatographic conditions

The Jasco HPLC system (Jasco Corporation, Japan) used consisted of an isocratic pump (Jasco PU-980 intelligent HPLC pump) and an auto-sampler (Jasco AS-2057 plus intelligent sampler) with temperature control system. The HPLC system was attached with a UV-Visible detector (Jasco UV 970 intelligent UV/VIS detector). Data acquisition was done by Jasco-Borwin version 1.5 (Built 14 - HSS-2000 data acquisition software, Jasco Corporation, Japan). For spectral confirmation of analytes, a Photo Diode Array detector (Jasco MD-910 Multiwavelength detector) with Jasco-PDA™ version 1.5 data acquisition software were used. A Precisa XB 120A monopan balance (Germany) was used to weigh the standards and samples. The pH was measured by using a PHAN µp controlled pH analyzer (LABINDIA, Thane, India). Additionally, a Multi-tube vortex shaker (XT 520, Neolab, Mumbai, India), and a centrifuge (Remi R-8CDX, Mumbai, India) were used. Calibrated auto-pipettes from Finnpipettes® F3 (10-100 µL and 100-1000 µL variable range, Thermo Scientific, Finland) and Eppendorf model research (0.5-10 µL variable range, Hamburg, Germany) were used for spiking and solvent dispensing.

Chromatographic separations for *trans*-Anethole, Eugenol, Piperine were carried out on a Cosmosil® Reverse phase C_{18} column (5 µm particle size, 4.6 X 150 mm ID) at room temperature. The mobile phase was 0.25 M Ammonium Dihydrogen Phosphate: Acetonitrile (40:60, *v/v*) and filtered through 0.45 µ nylon membrane filter paper (Millipore) and degassed in ultra sonic bath (Ultra sonic cleaner, *Trans*-O-sonic, D120 IH, Mumbai, India) prior to use. The flow rate of mobile phase was set at 1.0 mL min^{-1} and the injection volume was 20 µL. Detection was carried out at a wavelength of 260 nm. Total chromatographic run time was 20 minutes.

Preparation of stock and working standard (calibration standard) solutions

Two sets of *trans*-Anethole, Eugenol and Piperine stock solutions were prepared by independent weighing considering the purity of the reference standards (*w/v*). One set was used to prepare calibrant working solutions while the other set was used to prepare quality control (QC) working solutions in the diluent. *trans*-Anethole, Eugenol and Piperine stock solution (1000 ppm) were prepared in methanol. The diluent used was Methanol (1:1, *v/v*) for *trans*-Anethole, Eugenol and Piperine to prepare, by serial dilution, 100 ppm, 10 ppm, 1 ppm working stock solutions. All stock and working solutions were stored at 2-8°C and brought to room temperature prior to use. From standard stock solutions, concentrations of 10, 50, 100, 500, 1000, 2500, 5000, 10000 ng/mL for *trans*-Anethole and Piperine and 100, 500, 1000, 2500, 5000, 10000 ng/mL for Eugenol were prepared by diluting previously prepared working stock solution up to 10 mL with diluent volume in separate 10 mL volumetric flasks.

Preparation of crude drug from various samples containing *trans*-Anethole, Eugenol and Piperine

The various marketed masala powder; *Everest Garam Masala* (Batch No-54 N/1940 and Batch No-E5874 30/12/16), *Everest Pav Bhaji Masala* (Batch No-E54N/3028 and Batch No-E13D/2835), *Bedekar Goda Masala* (Batch No-UU717021) were sourced from local market, Mumbai. A in-house processed *Garam Masala* was prepared as per mixture of standardised ingredients. Fennel Seeds (*F. vugare*), Star Anise fruits (*I. verum*) were purchased from local market, Sion, Mumbai. Later, the seeds were powdered using an electric grinder and sieved through a BSS sieve (Mesh No. 85). The sieved powder was stored in commercially available airtight polypropylene containers.

Processing of samples (Sample Clean-up and extraction)

500 mg of each sample was accurately weighed and transferred to a 10 mL volumetric flask directly with butter paper. 10 mL methanol was added to it and sonicated for 30 minutes to extract the *trans*-Anethole, Eugenol, Piperine from the various powders. The final volume was made up to the mark and centrifuged for 10 minutes at 3000 rpm. The supernatent solution was filtered with 0.45 ì nylon membrane using a syringe filter. Three injections (20µL) of each sample were injected in HPLC system.

Identification test was carried out by TLC to detect the *trans*-Anethole, Eugenol, Piperine according to monograph for Lavang *(Syzigium aromaticum),* Saunf *(Foeniculum vulgare)* mentioned in Indian pharmacopoeia (2014).

Proximate analysis

From the cultivation of plants to the final product, there are several factors which influence the quality of plant based products. The importance of quality control of plant raw materials has been a topic of much debate and concern among herbal practitioners. Establishing quality of medicinal raw materials involves making use of physical and chemical tests (Peter, 2001).

Proximate analysis is one such quality control parameter that provides valuable information pertaining to the quality of medicinal raw materials and their preparation. Proximate analysis values help in defining the quality of plant raw materials.

Proximate analyses were carried out according to the procedure of Association of Official Analytical Chemist (AOAC, 1990). This constitutes the foreign organic matter, total ash content, acid insoluble ash, water soluble ash, loss on Drying (LOD), ethanol soluble extractable matter and water soluble extractable matter.

Results and Discussion

Mobile Phase Development

The mobile phase, 0.25 M Ammonium Dihydrogen Phosphate: Acetonitrile (40:60, *v/v*) in isocratic elution with a flow rate of 1 mL/minute resulted in good resolution, symmetrical peak shape of *trans*-Anethole, Eugenol and Piperine and short analysis time per run. All three samples exhibited the UV maximum absorption at 260 nm corresponding to the standard *trans*-Anethole, Eugenol and Piperine. All the reference compounds were resolved in the optimized HPLC condition and were eluted within 20 minutes. The retention time was 7.95, 10.60 and 17.15 minute for Eugenol, Piperine and *trans*-Anethole standards respectively as. The suitability of the solvent system was decided by cost, sensitivity of the assay and time required for the analysis. A chromatogram of mixture of standards *trans*-Anethole, Eugenol and Piperine has been shown in Figure 3.

Method application and Estimation of *trans*-Anethole, Eugenol and Piperine from different spices

The developed method was used as an application to determine the presence of *trans*-Anethole, Eugenol and Piperine from various marketed and in-house prepared spices. The detection of 10 ng/mL for *trans*-anethole and piperine (Table 1), suggesting the sensitivity of the method. From the above results, it can be said that *trans*-Anethole, Eugenol and Piperine showed a broad range of linear detection and was also highly sensitive method for the analysis of three markers form various spices. The slope, intercept and correlation coefficient are tabulated in Figure 2.1, 2.2 and 2.3. Representative chromatograms of Eugenol, Piperine and *trans*-Anethole have been shown in Figure. 3.1 and 3.2. The developed method was applied for the estimation and quantitation of *trans*-Anethole, Eugenol and Piperine from methanolic extract of various Indian spice mixtures.

Mixtures of different spices containing *trans*-Anethole, Eugenol and Piperine as one of their phytochemical constituents were evaluated for the presence of *trans*-Anethole, Eugenol, Piperine by using reverse phase HPLC method. Individual peaks ware observed for Eugenol, Piperine and *trans*-Anethole at 7.95 min., 10.60 min. and 17.15 min. (the retention time) in the samples of all spices. There was no interaction between *trans*-Anethole, Eugenol, Piperine with other excipients present in the marketed formulations. The ratios of Eugenol, Piperine and *trans*-Anethole were found to be distinctive in *Garam Masala, Goda Masala* and *Pav-Bhaji Masala*. Eugenol is maximum in *Pavbhaji masala* and minimum in *Goda masala*. Piperine is maximum in *Garam masala* and minimum in *Goda masala*. *Trans*-Anethol is maximum in *Pavbhaji masala* and minimum in *Goda masala*. The ratios of Eugenol : Piperine : *trans*-Anethole are listed in Table 2.1 and 2.2. The Proximate analysis results of the products are represented in Table 3. Similar results are obtained in TLC visualised at visible light after derivatization with Vanillin-sulphuric acid reagent (Figure 4).

Conclusion

The present study brings out significant variations in Eugenol, Piperine and *trans*-Anethole content in marketed spice mixtures. The three spice mixtures evaluated show distinctive ratios of Eugenol, Piperine and *trans*-Anethole in them. The HPLC method reported in this work can be applied for effective quality control and to reduce batch to batch variations in the spice mixture products.

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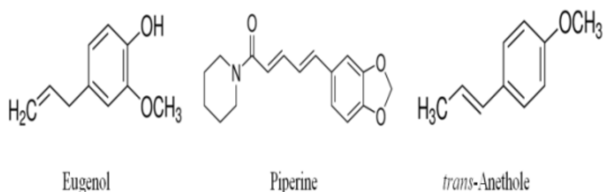

Figure 1: Structure of Eugenol, Piperine and trans-Anethole

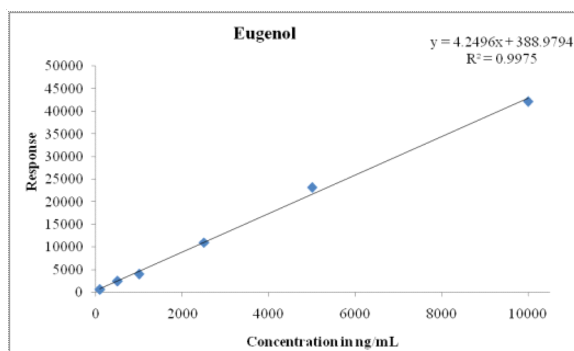

Figure 2.1: Calibration curve of Eugenol

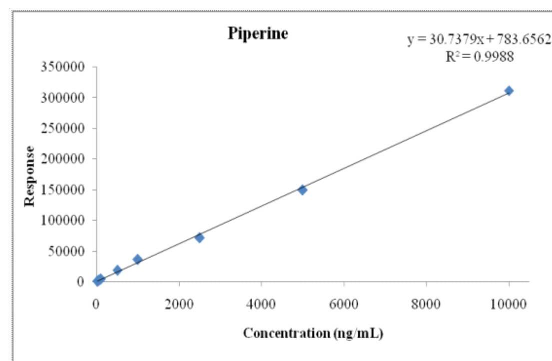

Figure 2.3: Calibration curve of Piperine

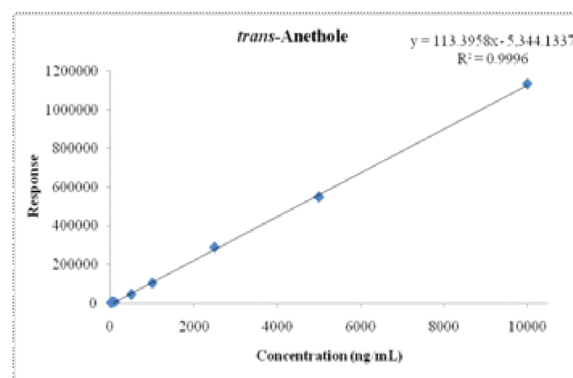

Figure 2.4: Calibration curve of trans-Anethole

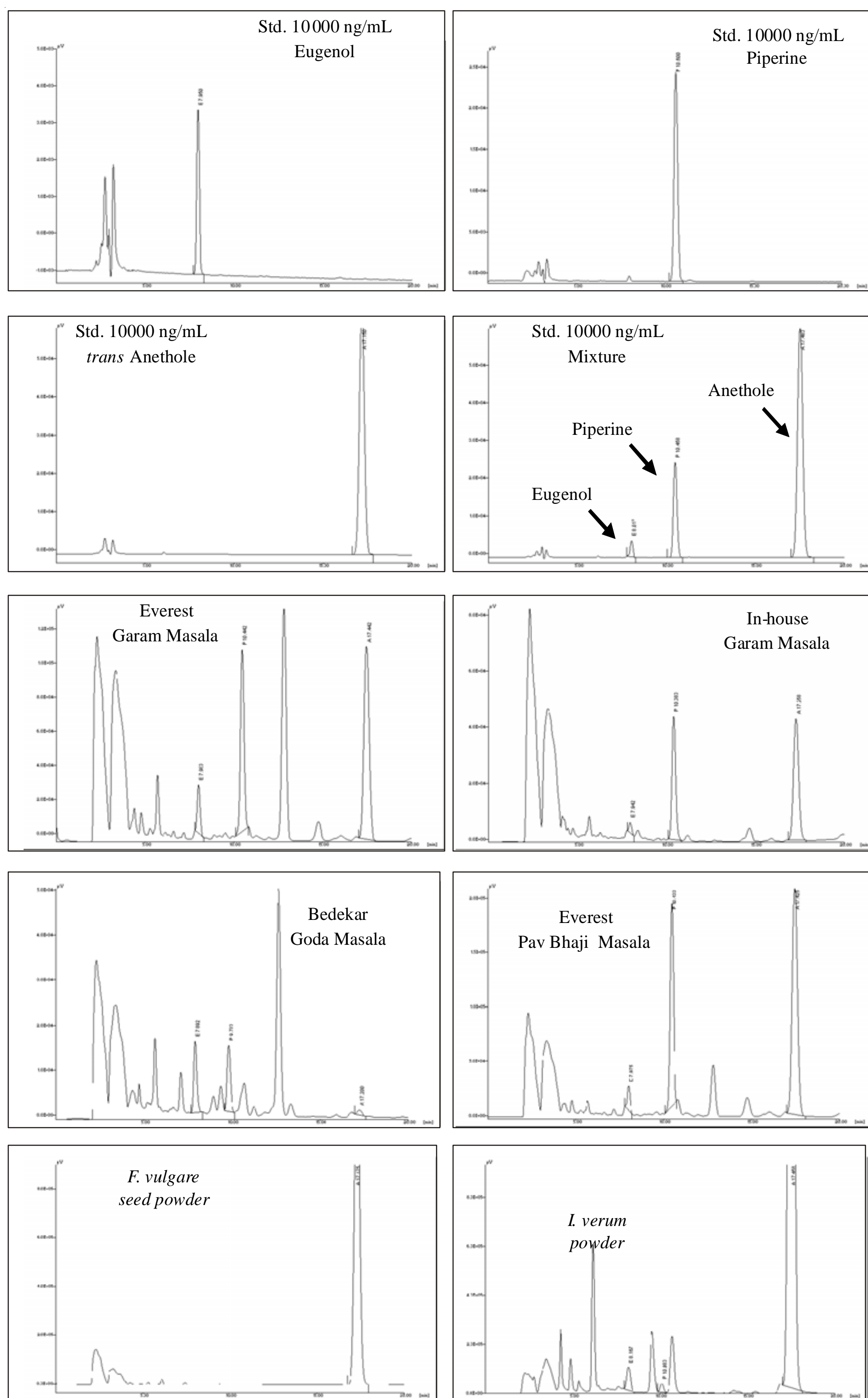

Figure 3.1: Representative HPLC chromatograms of various Indian spices

Note: Three distinct peaks corresponding to **A.** Eugenol (R.T. 3.4 min.), **B.** Piperine (R.T. 3.7 min.) and and **C.** *trans*-Anethole (R.T. 3.9 min.)

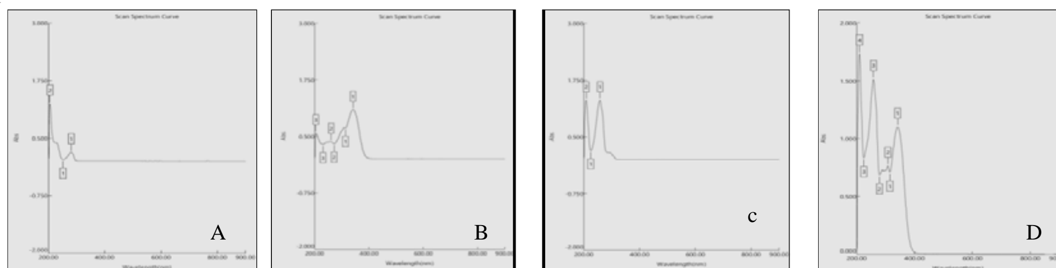

Figure 3.2: Representative UV-Vis spectra of A. Eugenol, B. Piperine C. trans-Anethole and D. their mixture (10 ppm)

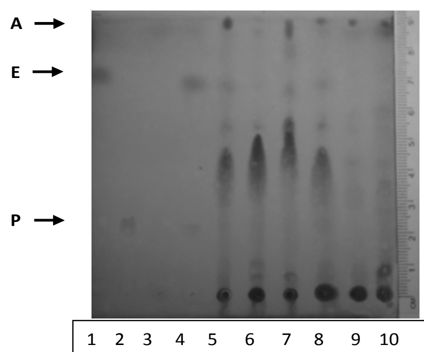

Figure 4: Representative TLC plate of various spice mixture and visualized at visible light after derivatization with Vanillin-sulphuric acid reagent (E: Eugenol, P: Piperine and A: trans Anethole)

Lane 1	Eugenol
Lane 2	Piperine
Lane 3	*trans*-Anethole
Lane 4	Mixture of Eugenol, Piperine and *trans*-Anethole
Lane 5	EVEREST *Garam Masala*
Lane 6	In-house *Garam Masala*
Lane 7	BEDEKAR *Goda Masala*
Lane 8	EVEREST *Pav Bhaji Masala*
Lane 9	*F. vulgare* seed powder
Lane 10	*I. verum* powder

Table 1: Summary of system suitability parameters and chromatographic conditions for Eugenol, Piperine and *trans*-Anethole (10000 ng/mL)

Summary of system suitability parameters for Eugenol, Piperine and *trans*-Anethole (10000 ng/mL)			
	Eugenol	Piperine	*trans*-Anethole
No. of theoretical plates (N)	16220.52	17348.27	19937.33
Retention Time	7.95	10.6	17.15
Capacity Factor	953	1271	2057
Asymmetry factor	1.13	1.11	1.04
Resolution	0.00	8.73	17.25
Summary of chromatographic conditions for Eugenol, Piperine and *trans*-Anethole (10000 ng/mL)			
LOD	50.00 ng/mL	5.00 ng/mL	5.00 ng/mL
LOQ	100.00 ng/mL	10.00 ng/mL	10.00 ng/mL
ULOQ	10000.00 ng/mL	10000.00 ng/mL	10000.00 ng/mL
Regression equation	y = 4.2496x + 388.9794	y = 30.7379x + 783.6562	y = 113.3958x - 5,344.1337
Coefficient of determination (r^2)	0.9975	0.9988	0.9996
HPLC Column	Cosmosil® Reverse phase C_{18} column (5 µm particle size, 4.6 X 150 mm ID)		
Mobile Phase	0.25 M Ammonium Dihydrogen Phosphate: Acetonitrile (40:60, v/v)		
Run time	10.00 Minutes		
Flow Rate	1 mL/ Minute		
Injection Volume	20 µL		
Detector	Jasco UV 970 intelligent UV/VIS detector		
Wavelength	260 nm		

Table 2.1: Estimation of Eugenol, Piperine and *trans*-Anethole from spices

Content of Eugenol, Piperine and *trans*-Anethole (Mean ± SD, n=3)			
Sample	Eugenol	Piperine	*trans*-Anethole
	mg/g		
EVEREST *Garam Masala*	**2.575 ± 0.014**	**1.702 ± 0.052**	**0.742 ± 0.033**
In-house *Garam Masala*	**0.274 ± 0.002**	**0.717 ± 0.053**	**0.281 ± 0.009**
BEDEKAR *Goda Masala*	**1.590 ± 0.013**	**0.236 ± 0.013**	**0.005 ± 0.001**
EVEREST *Pav Bhaji Masala*	**1.963 ± 0.005**	**2.936 ± 0.062**	**1.380 ± 0.008**
F. vulgare seed powder	**Not Detected**	**Not Detected**	**8.351 ± 0.144**
I. verum powder	**11.761 ± 0.024**	**0.482 ± 0.030**	**12.269 ± 0.211**

Table 2.2: Ratio analysis of Eugenol: Piperine: *trans*-Anethole from spice mixtures

Sample	**Eugenol**	**Piperine**	***trans*-Anethole**
EVEREST *Pav Bhaji Masala*	31	47	22
In-house *Garam Masala*	22	56	22
BEDEKAR *Goda Masala*	87	13	0.2

Table 3: Summary of Proximate Analysis of various products

Summary of Proximate Analysis
(The results are expressed in terms of percentage) (Mean ± SD, n=3)

Proximate Analysis Parameter	EVEREST *Garam Masala*	IN-HOUSE *Garam Masala*	EVEREST *Pav Bhaji Masala*	*Goda Masala*	*F. vulgare* seed powder (Fennel)	*I. verum* powder (Star anise)
Total Ash Content (% TAC)	10.13 ± 1.12	9.57 ± 1.77	10.611 ± 0.33	6.57 ± 0.96	8.89 ± 0.31	2.43 ± 0.18
Acid Insoluble Ash Content (% AIAC)	16.757 ± 1.11	10.033 ± 1.45	17.790 ± 1.69	14.427 ± 1.15	0.80 ± 0.15	20.553 ± 1.22
Water Soluble Ash Content (%WSAC)	8.433 ± 1.01	15.570 ± 0.88	12.413 ± 1.30	21.280 ± 1.11	1.39 ± 0.14	22.817 ± 1.50
Loss on drying (% LOD)	9.931 ± 0.99	12.370 ± 1.24	11.415 ± 3.16	5.623 ± 0.56	7.77 ± 0.34	11.879 ± 1.20
Water Extractable Matter (% WEM)	23.107 ± 1.56	37.240 ± 1.53	21.3877 ± 1.11	29.510 ± 0.82	13.10 ± 1.59	10.680 ± 0.94
Ethanol Extractable Matter (% EEM)	22.067 ± 2.96	22.507 ± 3.07	20.627 ± 0.96	21.533 ± 1.68	14.38 ± 0.23	11.267 ± 1.32

Standardisation of Quality Parameters For "*Avala supari*" UsingHigh Performance Liquid Chromatography

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Abstract: "*Avala Supari*" is an Indian Gooseberry (*Phyllanthus emblica*) based mouth freshener. It is prepared by sun dried *P. emblica* fruits mixed with flavouring spices. '*Avala Supari*" is popular as a digestive and has astringent action giving a "feel-fresh effect" in the mouth.

The preparation is marketed by several manufacturers but its quality standards are not specified except for the broad food safety guidelines. The current work reports quality standards for "*Avala supari*" using phytochemical (Gallic Acid) based HPLC method. The gallic acid content in different marketed product is compared along with physical parameters.

The results have been discussed and a quality standard has been proposed for manufactures to practice.

Keywords: HPLC, Quality control, *P. emblica*, Gallic Acid

Introduction

Amala or *Avala* are fruits of *Phyllanthus emblica* Linn. (Family: Euphorbiaceae) also known as Indian Gooseberry. These are consumed as raw fruits or processed into various forms of food products. It is a medium-sized deciduous tree found throughout India (Zang, 2000). Traditionally, the fruit is useful as an astringent, cardiac tonic, diuretic, laxative, liver tonic, restorative, antipyretic, anti-inflammatory, hair tonic, and digestive medicine (Zhang, 2000; Perianayagam, 2004). The fruit contains two hydrolysable tannins Emblicanin A and B, of which Emblicanin A, on hydrolysis gives gallic acid (Ghosal *et al*, 1996, Zhang *et al*, 2001). Gallic acid is a phenolic compound with phenolic groups that serve as a source of readily available hydrogen atoms (Robards, 1999; Nikolic, 2006). "*Avala Supari*" is a traditional form of mouth freshener made using dried fruits of *P. emblica*. The fruits of *P. emblica* are dried and mixed with salt and spices to make a granular mixture. The product is available freely and is popular for its astringent effect with a "clean feeling" in mouth and digestive properties.

Though "*Avala Supari*" can be made at home, it requires sun drying of the fruits. Branded "*Avala Supari*" is available and is popularly consumed. The quality of the final product can highly vary depending on the source of the fruit and the proportion of condiments added. The shelf life of the final product primarily depends on the extend of drying of the fruit (Prajapati *et al*, 2011). Though the marketed product is regulated by the guidelines of food safety, there are no standards for its quality and organoleptic characteristics. It was therefore, felt necessary to evaluate the chromatographic fingerprints of "*Avala Supari*", in an effort to suggest chromatographic quality of this traditional mouth freshener.

Marketed "*Avala Suapri*" is evaluated for the content of gallic acid. HPLC estimation of gallic acid from the fruits of *P. emblica* has been reported (Biswas *et al*, 2012). In this study simpler and more convenient isocratic Reverse Phase High Performance Liquid Chromatographic method has been developed for the evaluation. Sample processing has been standardized to be applicable for the dry granular matrix. It is felt that, the method could be easily adopted by manufacturers for fast and reliable quantitative evaluation of quality control measures of the product. Additionally, some physical characteristics of "*Avala Supari*" have also been evaluated. The results of HPLC analysis have been compared with those from Thin Layer Chromatographic evaluation of the same sample. Attempt has been made to suggest quality parameters for "*Avala Supari*", in order to standardise the organoleptic characteristics.

Materials and Methods

Reagents and chemicals: Pure standards of Gallic acid ($C_7H_6O_5$) (Purity 97.5%) were procured from Sigma Aldrich (St. Louis, MO, USA). The chemical structure of Gallic acid is illustrated in Figure. 1. Methanol, Acetonitrile (ACN) (HPLC grade), 10 mM KH_2PO_4, Ortho-phosphoric acid (OPA) were purchased from Merck Ltd., Mumbai, India. Ultra pure (Milli-Q) water of 18.2 M&! resistances and 0.22µ membrane filtered was obtained from Millipore water purication system (Molsheim, France). All other chemicals used in the study were of analytical reagent (AR) grade.

HPLC Instrumentation and chromatographic conditions: The Jasco HPLC system (Jasco Corporation, Japan) used consisted of an isocratic pump (Jasco PU-980 intelligent HPLC pump) and an auto-sampler (Jasco AS-2057 plus intelligent sampler) with temperature control system. The HPLC system was attached with a UV-Visible detector (Jasco UV 970 intelligent UV/VIS detector). Data acquisition was done by Jasco-Borwin version 1.5 (Built 14 - HSS-2000 data acquisition software, Jasco Corporation, Japan). For spectral confirmation of analytes, a

Photo Diode Array detector (Jasco MD-910 Multiwavelength detector) with Jasco-PDA™ version 1.5 data acquisition software were used. Chromatographic separations for Gallic Acid were carried out on a Cosmosil® Reverse phase C_{18} column (5 µm particle size, 150 X 4.6 mm ID) from Cosmosil Ltd. at room temperature. The mobile phase was 10mM KH_2PO_4: ACN + 0.05% OPA and filtered through 0.45 µ nylon membrane filter paper (Millipore) and degassed in ultra sonic bath (Ultra sonic cleaner, Trans-O-sonic, D120 IH, Mumbai, India) prior to use. The flow rate of mobile phase was set at 1.0 mL min^{-1} and the injection volume was 20 µL. Detection was carried out at a wavelength 210 nm. Total chromatographic run time was 10 minutes.

Preparation of stock and working standard (calibration standard) solutions: Two sets of Gallic Acid stock solutions were prepared by independent weighing considering the purity of the reference standards (*w/v*). One set was used to prepare calibrant working solutions while the other set was used to prepare quality control (QC) working solutions in the diluent. Gallic acid stock solution (1000 ppm) was prepared in methanol. The diluent used was 10 mM KH_2PO_4: ACN (95:05, *v/v*) + 0.05% OPA for Gallic acid to prepare 100 ppm, 10 ppm, 1 ppm working stock solutions. All stock and working solutions were stored at 2-8°C and brought to room temperature prior to use. From standard stock solutions, concentrations of 10, 50, 100, 500, 1000, 2500 and 5000 ng/mL were prepared by diluting previously prepared working stock solution up to 10 mL in separate volumetric flasks.

Preparation of crude drug from various samples containing Gallic Acid: The marketed *'Avala Supari'* manufactured by P. D. Vaidya and sons (Batch No. 07 and 23) and 3 different *Amalaki* powder products [(**M1**) manufactured by Divya Pharmacy, Batch No. B AMC028; (**M2**) manufactured by Omkar Ayurved Mandir, Batch No. 97 and (**M3**) Vivek Herbal Products (Lot No. 03) and *Triphala Churna* manufactured by Divya Pharmacy, Batch No. A-TPC020] from local market vendor, Sion, Mumbai. Later, *Avala Supari* was powdered using an electric grinder and sieved through a BSS sieve (Mesh No. 85). The sieved powder was stored in commercially available airtight polypropylene containers.

Processing of samples (Sample Clean-up and extraction): 500 mg of each sample was accurately weighed and transferred to a 10 mL volumetric flask directly with butter paper. 5 mL methanol was added to it and sonicated for 30 minutes to extract the Gallic acid from the various products. The final volume was made up to the mark and centrifuged for 10 minutes at 3000 rpm. The supernatent solution was filtered with 0.45 ì nylon membrane syringe filter. Three injections (20 µL) of each sample were injected in HPLC system.

Proximate analysis: Proximate analyses were carried out according to the procedure of Association of Official Analytical Chemist (AOAC., 1990). This constitutes the foreign organic matter, total ash content, acid insoluble ash, water soluble ash, loss on Drying (LOD), ethanol soluble extractable matter and water soluble extractable matter.

Identification test was carried out by TLC for Gallic acid according to monograph for *Amalaki* mentioned in Indian pharmacopoeia (IP, 2014) (Figure 4).

Results and Discussion

Mobile phase development

The primary aim of the present study was to separate curcumin from various formulations containing turmeric extracts and powder. This included mobile phase selection, flow rate optimisation and column type. In this experiment, mixtures of several mobile phases were tried to separate peak of Gallic acid from other interfering peaks.

The mobile phase, 10 mM KH_2PO_4: ACN (95:05, *v/v*) + 0.05% OPA in isocratic elution with a flow rate of 1 mL/minute resulted in good resolution, symmetrical peak shape of Gallic acid and short analysis time per run. All the samples exhibited the UV maximum absorption at 210 nm corresponding to the standard Gallic acid. All the reference compounds were easily resolved in the optimized HPLC condition and were eluted within 10 minute. The retention time was 4.26 minute for standard as well as the samples. The suitability of the solvent system was decided by cost, sensitivity of the assay and time required for the analysis. A chromatogram of standard Gallic acid has been shown in Figure 3.

Method application and Estimation of Gallic acid from *Avala supari and Amalaki* powder

The developed method was used as an application to determine the presence of Gallic acid from various marketed products. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 5 ng/mL and 10 ng/mL for Gallic acid (Table 1), suggesting the sensitivity of the method. From the above results, it can be said that Gallic acid showed a broad range of linear detection and was also highly sensitive method. The slope, intercept and correlation coefficient were found to by 87.7415, 2363.873 and 0.9913. Calibration curve of Gallic acid has been shown in Figure. 2. The developed method was applied for the estimation and quantitation of Gallic acid from methanolic extract of *Avala supari* and *Amalaki* powder.

Different products containing *P. emblica* as one of their ingredient were tried for the presence of Gallic acid in it using reverse phase HPLC method. Different extraction techniques were tried for the *'Avala Supari'* and *Amalaki* powder. A single peak was observed at the same retention time in the samples of all products (Figure 3). There was no interaction between Gallic acid with other excipients present in the analysed samples. Among the various products *'Avala Supari'* in the form of whole form showed the maximum content of Gallic acid. The content of marker was found to

be least in *Amalaki* powder (**M3**). The assay results of the products are represented in Table 2. Similar results are obtained in TLC visualised at 254 nm (Figure 4).

The importance of quality control of plant raw material has been a topic of much debate and concern among herbal practitioners. Establishing quality of medicinal raw materials involves making use of physical and chemical tests. Proximate analysis is one such quality control parameter that provides valuable information pertaining to the quality of medicinal raw materials and their preparation. Data on moisture content, presence of foreign matter, ash value, extractive values help in defining the quality of plant raw materials. The Proximate analysis results of the products are represented in Table 3.

Conclusion

The present study brings out significant variations in Gallic acid content in *'Avala Supari'* and *Amalaki* powder. The label claims are not fully specific to the actual Gallic acid content. Thus, the method reported in this work can be applied for effective quality control and to reduce batch to batch variations in the final product.

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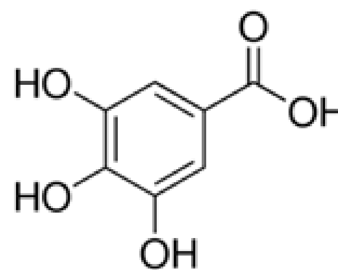

Figure 1: Structure of Gallic acid

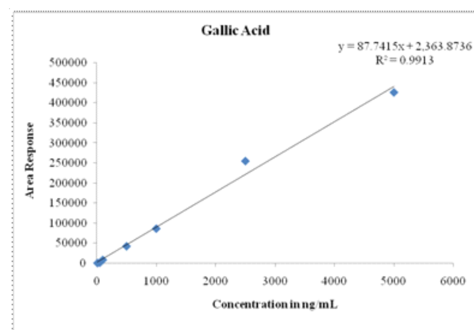

Figure 2: Calibration curve of Gallic acid

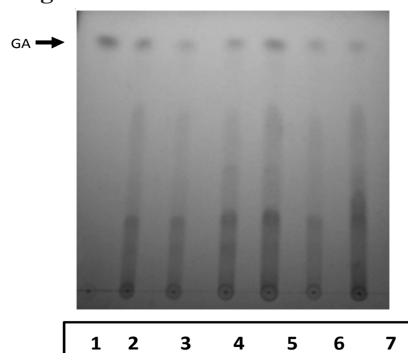

Figure 4: Representative TLC plate of various products containing P. emblica visualized at 254 nm [Lane 1: Gallic acid standard 100 ppm, Lane 2. Avala supari, Lane 3. Avala supari powder, Lane 4. Amalaki powder (M1), Lane 5. Amalaki powder (M2), Lane 6. Amalaki powder (M3) and lane 7. Triphala churna

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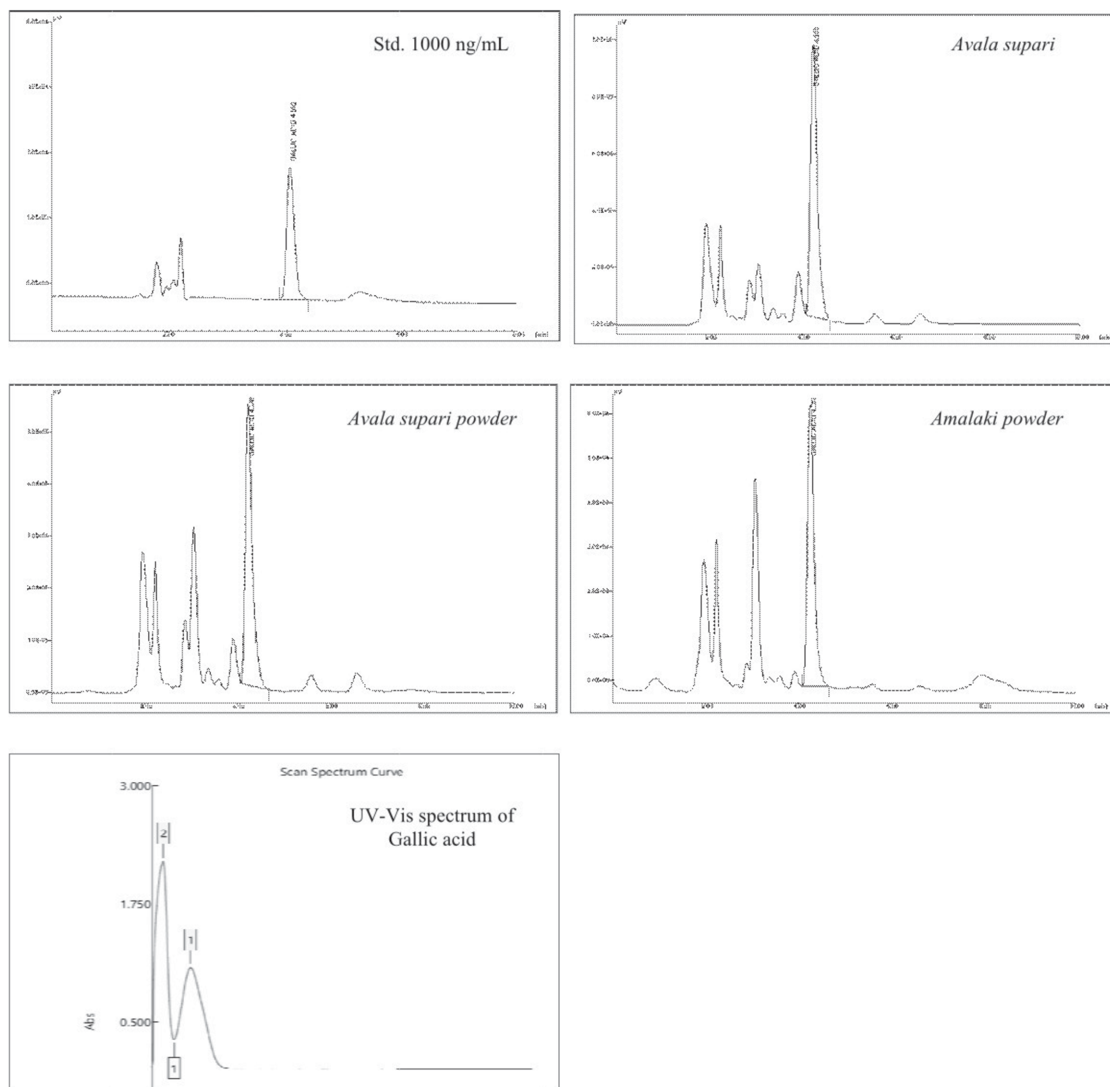

Figure 3: Representative HPLC chromatograms of various products containing P. emblica and representative UV-Vis spectrum

Table 1: Summary of system suitability parameters and chromatographic conditions for Gallic acid

Summary of system suitability parameters for Gallic acid (1000 ng/mL)	
No. of theoretical plates (N)	5808.43
Retention Time	4.26
Capacity Factor	511
Asymmetry factor	1.33
Resolution	Not applicable
Summary of chromatographic conditions for Gallic acid	
LOD	7.50 ng/mL
LOQ	10.00 ng/mL
ULOQ	5000.00 ng/mL
Regression equation	$y = 87.7415x + 2363.87$
Coefficient of determination (r^2)	0.9913
HPLC Column	Cosmosil® Reverse phase C18 column (5 µm particle size, 150 X 4.6 mm ID)
Mobile Phase	10 mM KH_2PO_4: ACN (95:05, v/v) + 0.05% OPA
Run time	10.00 Minutes
Flow Rate	1 mL/ Minute
Injection Volume	20 µL
Detector	Jasco UV 970 intelligent UV/VIS detector
Wavelength	210 nm

Table 2: Estimation of Gallic acid from various products containing *P. emblica*

Content of Gallic acid (Mean ± SD, n=3, N.D.: Not Detected)	
Sample	mg/g
***Avala Supari* (Batch 1)**	20.092 ± 0.01
***Avala Supari* Powder (Batch 1)**	11.601 ± 0.09
***Amalaki* Powder (M1)**	5.673 ± 0.21
***Amalaki* Powder (M2)**	8.994 ± 0.03
***Amalaki* Powder (M3)**	10.320 ± 0.06
Triphala Churna	9.021 ± 0.03

Table 3: Summary of Proximate Analysis of various products

Summary of Proximate Analysis (The results are expressed in terms of percentage)				
Proximate Analysis Parameter	*Avala Supari* (Batch 1)	*Avala Supari* (Batch 2)	*Avala Supari* Powder (Batch 1)	*Avala Supari* Powder (Batch 2)
Foreign Organic Matter (% FOM)	0.01 ± 0.01	0.21 ± 0.34	NA	NA
Total Ash Content (% TAC)	23.06 ± 0.78	25.86 ± 1.42	35.13 ± 0.43	34.00 ± 2.94
Acid Insoluble Ash Content(% AIAC)	12.57 ± 2.97	12.83 ± 3.02	13.10 ± 3.72	11.57 ± 11.61
Water Soluble Ash Content(%WSAC)	5.30 ± 1.97	4.07 ± 3.00	8.33 ± 1.97	9.70 ± 1.47
Loss on drying(% LOD)	7.06 ± 2.44	3.75 ± 1.49	8.20 ± 1.87	6.11 ± 2.37
Water Extractable Matter(% WEM)	47.32 ± 4.37	41.76 ± 1.64	52.17 ± 2.29	48.67 ± 6.15
Ethanol Extractable Matter (% EEM)	14.99 ± 0.94	6.72 ± 0.40	13.80 ± 0.32	8.75 ± 1.14

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Comparative Evaluation of Curcumin in Marketed Topical Formulations Using High Performance Liquid Chromatography

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Abstract: Curcumin is a major constituent of several topical formulations based on turmeric and its therapeutic benefits. It is a major bio-active phytochemical in herbal formulations used in wound healing and skin enhancers. The content of curcumin varies depending on the source and the formulation. The current work is a chromatographic evaluation of curcumin content in various herbal formulations using HPLC. Different marketed formulations have been compared for their curcumin content. The extraction method has been optimised for different matrices of the formulation. The results have been compared on the basis of targeted use of herbal formulations and their label claims.

Keywords: HPLC, Quality control, Curcumin, Topical formulations

Introduction

Medicinal plants, have been in use since time immemorial for treatment of illnesses (Yunes *et al.*, 2001; Firmo *et al.*, 2011). Approximately 30% of all drug molecules are still sourced directly or indirectly from plants (Koehn, Carter, 2005). Turmeric, (*Curcuma longa* (family: Zingiberaceae), is rich in curcuminoid pigments (curcumin, demethoxycurcumin, bisdemethoxycurcumin, and cyclocurcumin) of which curcumin is the major component. Curcumin has been reported to be antiparasitic, antispasmodic, anti-inflammatory, antioxidant and anticancer in bioactivity (Lin, Lin, 2008; Silva Filho *et al.*, 2009; Almeida, 2006; Swanson *et al.*, 2010).

During routine laboratory analysis, it was observed that the curcumin content in various marketed formulation varies significantly. Some of them failed to meet the label claims. Several curcumin based topical formulations are sold as "Over-The-Counter" (OTC) products with varying label claims. It was therefore, found necessary to evaluate different marketed formulations for their curcumin content and record the variability and their compliance to label claims.

Simple and convenient isocratic Reverse Phase High Performance Liquid Chromatographic method has been developed for the evaluation. Sample processing has been standardized to be applicable for different matrices. It is felt that, the method could be easily adopted by manufacturers for fast and reliable quantitative evaluation of curcumin for quality control measures. The results of HPLC analysis have been compared with those from Thin Layer Chromatographic evaluation of the same samples.

Materials and Methods

Reagents and chemicals: Pure standards of curcumin ($C_{21}H_{20}O_6$) (purity > 89.49%) were procured from the Gelnova

Preparation of stock and working standard (calibration standard) solutions: Two sets of Curcumin stock solutions were prepared by independent weighing considering the purity of the reference standards (*w/v*). One set was used to prepare calibrant working solutions while the other set was used to prepare quality control (QC) working solutions in the diluent. Curcumin stock solution (1000 ppm) was prepared in Acetonitrile: Ammonium acetate buffer (pH 4.5, 0.1M, 50:50, *v/v*). The diluent used was 0.1% Formic acid: Acetonitrile (4:6, *v/v*) for Curcumin to prepare, by serial dilution, 100 ppm, 10 ppm, 1 ppm working stock solutions. All stock and working solutions were stored at 2-8°C and brought to room temperature prior to use. From standard stock solutions, concentrations of 10, 50, 100, 300, 750, 1000, 1500 and 2000 ng/mL were prepared by diluting previously prepared solution up to 10 mL with diluent volume in separate 10 mL volumetric flasks.

Preparation of crude drug from various samples containing curcumin: The various marketed topical formulations and turmeric powder [UBTAN face pack, manufactured by Paru enterprises (Batch No. 001), VICCO® Turmeric Skin Cream manufactured by VICCO® Laboratories (Batch No. N 0371), Turmeric Beauty Mask Pentasoft, (Batch No. B 01), manufactured by Niral Cosmetics, Fem Bleach Turmeric Herbal Cream Bleach, manufactured by DABUR® India Ltd., (Batch No. BM 0046), Rambandhu turmeric powder, manufactured by Empire spices and food Ltd., (Batch No. 06HH061), and in-house processed turmeric powder prepared from fresh dried rhizomes of turmeric. The rhizomes of *Curcuma longa* Linn. (Family: Zinziberaceae) were purchased from local market, Mumbai. The dried plant material (rhizome) was then placed in a preset oven and incubated at 37°C ± 5°C. Later, it was powdered using an electric grinder and sieved through a BSS sieve (Mesh No. 85). The sieved powder was stored in commercially available airtight polypropylene containers.

Processing of samples (Sample Clean-up and extraction): 500 mg of each sample was accurately weighed and transferred to a 10 mL volumetric flask directly with butter paper. 10 mL methanol was added to it and sonicated in ultrasonic bath for 30 minutes to extract the Curcumin from the various formulations and powders. The final volume was made up to the mark and centrifuged for 10 minutes at 3000 rpm. The supernatent solution was filtered with 0.45 ì nylon membrane syringe filter. Three injections (20 µL) of each sample were injected in HPLC system.

Identification test was carried out by TLC for curcumin according to monograph for *Haridra* mentioned in Indian pharmacopoeia (IP, 2014).

Results and Discussions

Mobile phase development

The mobile phase, 0.1% formic acid in water: ACN (40:60, *v/v*) in isocratic elution with a flow rate of 1 mL/ minute resulted in good resolution, symmetrical peak shape of curcumin and short analysis time per run. All the samples exhibited the UV maximum absorption at 425 nm corresponding to the standard curcumin. All the reference compounds were easily resolved in the optimized HPLC condition and were eluted within 10 minute. The retention time was 3.9 minute for standard as well as the samples. The suitability of the solvent system was decided by cost, sensitivity of the assay and time required for the analysis. A chromatogram of standard curcumin has been shown in Figure 3.

Method application and Estimation of curcumin from different marketed topical formulations containing *Curcuma longa*

The developed method was used as an application to determine the presence of curcumin from various marketed topical formulations of varied matrices. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 5 ng/mL and 10 ng/mL for curcumin (Table 1), suggesting the sensitivity of the method. From the above results, it can be said that curcumin showed a broad range of linear detection and was also highly sensitive method. The slope, intercept and correlation coefficient were found to by 103.54, -4864.1 and 0.9927. Calibration curve of Curcumin has been shown in Figure. 2. The developed method was applied for the estimation and quantitation of curcumin from methanolic extract of various topical formulations containing *C. longa*.

Different formulations containing *C. longa* as one of their ingredient were tried for the presence of curcumin in it using reverse phase HPLC method. Different extraction techniques were tried for the formulations with varied matrices. A single peak was observed at the same retention time in the samples of all formulations (Figure 3). There was no interaction between curcumin with other excipients present in the marketed topical formulations. Formulations of varied matrices like cream, powder, face pack and bleach were tried for the presence of curcumin. Among the various formulations Rambandhu Turmeric Powder in the form of powder showed the maximum content of curcumin. The content of marker was found to be least in VICCO Turmeric Skin Cream amongst all the formulations used. The curcumin could not be detected in Turmeric Beauty Mask and Fem Bleach (Turmeric Herbal Cream Bleach) amongst the formulations evaluated. This could be attributed to the high pH of the formulation making curcumin unstable. The assay results of the formulations are represented in Table 2. Similar results are obtained in TLC visualised at 254 nm.

Conclusion

The present study brings out significant variations in curcumin content in marketed formulations. The label claims are not fully specific to the actual curcumin content. Thus, the method reported in this work can be applied for effective quality control and to reduce batch to batch variations in the final product.

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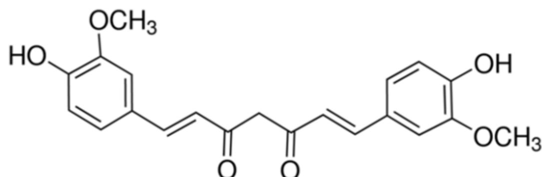

Figure 1: Structure of curcumin

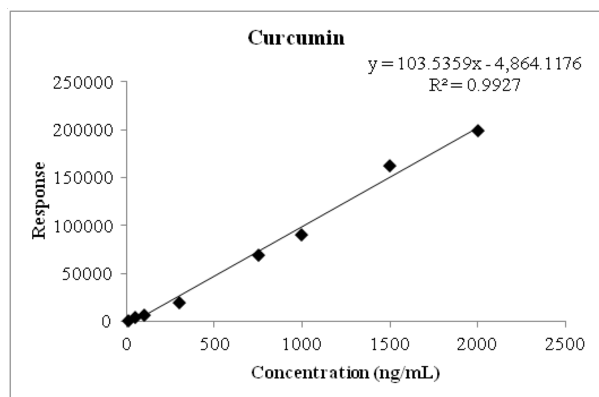

Figure 2: Calibration curve of curcumin

Table 1: Summary of system suitability parameters and chromatographic conditions for curcumin

Summary of system suitability parameters for curcumin (1000 ng/mL)	
No. of theoretical plates (N)	6182.64
Retention Time	3.9
Capacity Factor	474
Asymmetry factor	1.37
Resolution	1.33
Summary of chromatographic conditions for curcumin	
LOD	5.00 ng/mL
LOQ	10.00 ng/mL
ULOQ	2000.00 ng/mL
Regression equation	y = 103.5359x - 4,864.1176
Coefficient of determination (r^2)	0.9927
HPLC Column	Cosmosil® Reverse phase C_{18} column (5 µm particle size, 150 X 4.6 mm ID)
Mobile Phase	0.1% Formic acid: Acetonitrile (4:6, v/v)
Run time	10.00 Minutes
Flow Rate	1 mL/ Minute
Injection Volume	20 µL
Detector	Jasco UV 970 intelligent UV/VIS detector
Wavelength	425 nm

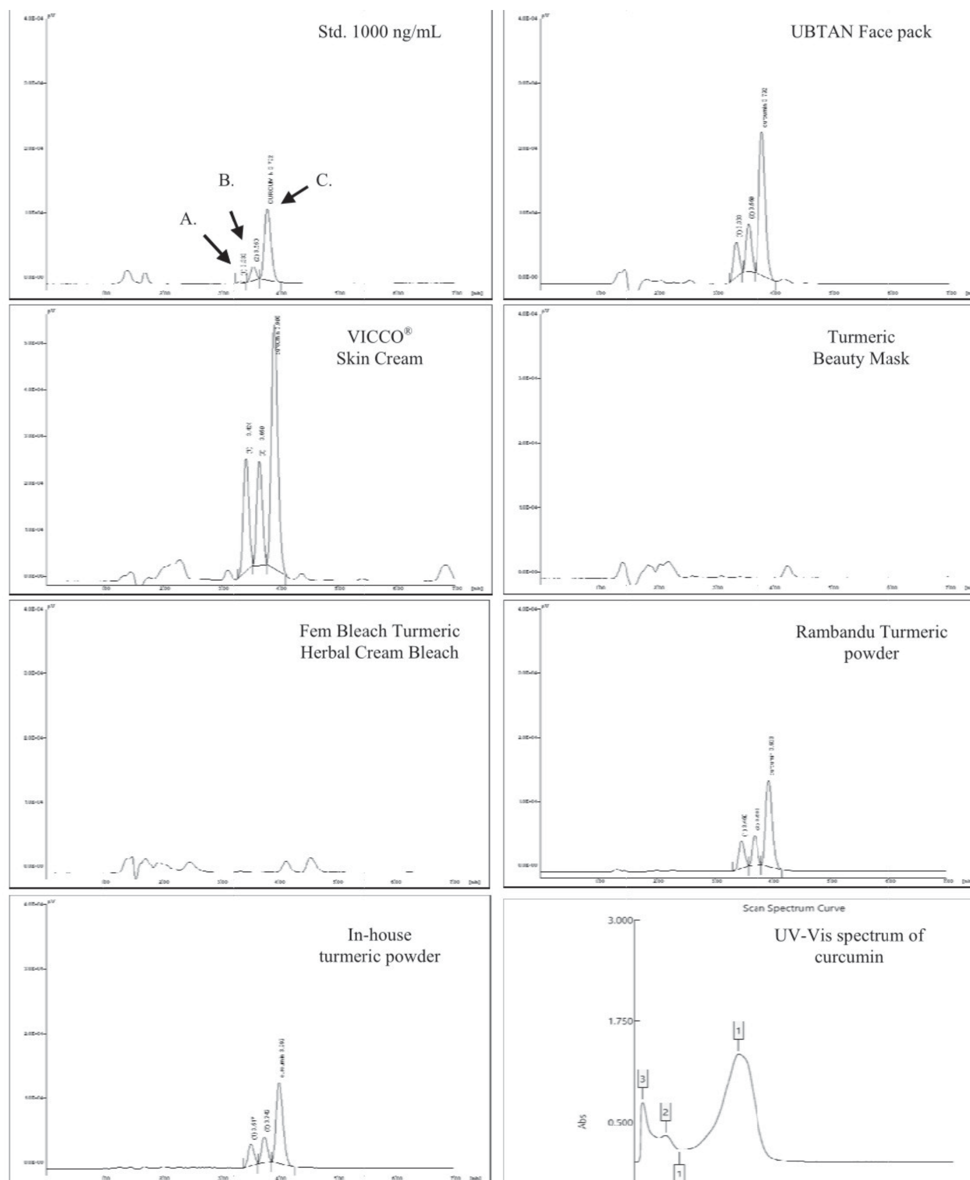

Figure 3: Representative HPLC chromatograms of various topical formulations and powder containing C. longa and representative Uv-Vis spectrum

Note: Three distinct peaks corresponding to A. Bis demethoxy curcumin (R.T. 3.4 min.), B. Demethoxycurcumin (R.T. 3.7 min.) and C. Curcumin (R.T. 3.9 min.)

Table 2: Estimation of curcumin from various marketed topical formulations

Content of Curcumin(Mean ± SD, n=3, N.D.: Not Detected)	
Sample	**mg/g**
UBTAN face pack	0.343 ± 0.002
VICCO® Turmeric Skin Cream	0.083 ± 0.001
Turmeric Beauty Mask (Pentasoft)	N.D.
Fem Bleach Turmeric Herbal Cream	N.D.
Rambandu Turmeric Powder	20.091 ± 0.229
In-house Processed Turmeric Powder	19.228 ± 0.130

High Performance Liquid Chromatographic Evaluation of Food Supplements Containing Beta Carotene

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Abstract: Beta Carotene is a major constituent of food supplements because of its health benefits as the precursor for Vitamin A. Several beta carotene based food supplements are available in the market with various claims. These food supplements use beta carotene from various sources. The current paper describes a chromatographic method to evaluate beta carotene from marketed food supplements. The extraction method has been standardised to suite different matrices of the food supplements. The beta carotene content is compared between food supplements from different manufactures and the results are discussed.

Keywords: HPLC, Beta carotene, Quality control, food supplements

Introduction

For many communities in developing countries, the major source of vitamin A in the diet is through dietary carotenoids especially beta carotene. The carotenoids derive their name from the major pigment in the roots of carrot, *Daucus carota*. Caratinoids are among the most widespread and important pigments in living organisms and is found throughout the plant kingdom. Structurally, Carotenoids have a chain of 40-carbon atoms. The hydrocarbon carotenoids are known as carotenes, while their oxygenated derivatives are known as xanthophylls (Britton, 1995). Carotenoids have antioxidant properties and help sequestering toxic radicals.

β-carotene is a provitamin A, being converted to vitamin A in the human body and is necessary for the proper functioning (Goodman, 1984). It is an antioxidant and is clinically recommended to improve eyesight, the condition of the skin and nails, as well as to protect the body against free radicals (Burri 1997; Paiva and Russell 1999; Palozza 1998; Palozza *et al.* 2001). β-carotene affects the immune system by increasing the counts of T cells (Fryburg *et al*. 1995). High doses of carotenes have been reported to be harmful in heavy smokers (Omenn *et al*. 1994). Therefore, the quantitative determination of beta carotene is important for evaluating the nutritional values and quality of fresh foods, processed food products and dietary supplements for health benefits in humans.

During routine laboratory analysis, it was observed that the â-carotene content in various marketed dietary supplements varies significantly. It was therefore, found necessary to evaluate different marketed formulations for their â-carotene content and record the variability and their compliance to label claims. Simple and convenient isocratic Reverse Phase High Performance Liquid Chromatographic method has been developed for the evaluation. Sample processing has been standardized to be applicable for different matrices. It is felt that, the method could be easily adopted by manufacturers for fast and reliable quantitative evaluation of β-carotene for quality control measures. The results of HPLC analysis have been compared with those from Thin Layer Chromatographic evaluation of the same samples.

Materials and Methods

Reagents and chemicals: Pure standards of Beta Carotene ($C_{40}H_{56}$) (purity > 97 %) were procured from the Sigma Aldrich (St. Louis, MO, USA). The chemical structure of β Carotene is illustrated in Figure. 1. Methanol, Acetonitrile, Chloroform, Triethylamine, Diethyl Ether (HPLC grade) were purchased from Merck Ltd., Mumbai, India. Ultra pure (Milli-Q) water of 18.2 MΩ resistances and 0.22μ membrane filtered was obtained from Millipore water purication system (Molsheim, France). All other chemicals used in the study were of analytical reagent (AR) grade.

HPLC Instrumentation and chromatographic conditions: The Jasco HPLC system (Jasco Corporation, Japan) used consisted of an isocratic pump (Jasco PU-980 intelligent HPLC pump) and an auto-sampler (Jasco AS-2057 plus intelligent sampler) with temperature control system. The HPLC system was attached with a UV-Visible detector (Jasco UV 970 intelligent UV/VIS detector). Data acquisition was done by Jasco-Borwin version 1.5 (Built 14 - HSS-2000 data acquisition software, Jasco Corporation, Japan). For spectral confirmation of analytes, a Photo Diode Array detector (Jasco MD-910 Multiwavelength detector) with Jasco-PDA™ version 1.5 data acquisition software were used. A Precisa XB 120A monopan balance (made in Germany) was used to weigh the standards and samples. The pH was measured by using a PHAN μp controlled pH analyzer (LABINDIA, Thane, India). Additionally, a Multi-tube vortex shaker (XT 520, Neolab, Mumbai, India), and a centrifuge (Remi R-8CDX, Mumbai, India) were used. Calibrated auto-pipettes from Finnpipettes® F3 (10-100 μL

and 100-1000 µL variable range, Thermo Scientific, Finland) and Eppendorf model (0.5-10 µL variable range, Hamburg, Germany) were used for spiking and solvent dispensing.

Chromatographic separations for β carotene were carried out on a Cosmosil® Reverse phase C_{18} column (5 µm particle size, 150 X 4.6 mm ID) from Cosmosil Ltd. Japan at room temperature. The mobile phase was 0.5% Triethylamine in Methanol: Acetonitrile: Chloroform (40:40:20) (*v/v/v*) and degassed in ultra sonic bath (Ultra sonic cleaner, Trans-O-sonic, D120 IH, Mumbai, India) prior to use. The flow rate of mobile phase was set at 1.0 mL min^{-1} and the injection volume was 20 µL. Detection was carried out at a wavelength of 450 nm. Total chromatographic run time was 10 minutes.

Preparation of stock and working standard (calibration standard) solutions : Two sets of β Carotene stock solutions were prepared by independent weighing considering the purity of the reference standard. One set was used to prepare calibrant working solutions while the other set was used to prepare quality control (QC) working solutions in the diluent. â Carotene stock solution (1000 ppm) was prepared in Diethyl Ether. The diluent used was Mobile Phase 0.5% Triethylamine in Methanol: Acetonitrile: Chloroform (40:40:20, *v/v/v*) for â Carotene to prepare, by serial dilution viz. 100 ppm, 10 ppm, 1 ppm working stock solutions. All stock and working solutions were stored at 2-8°C and brought to room temperature prior to use. From standard stock solutions, concentrations of 10000, 7500, 5000, 2000, 1000, 500 and 100 ng/mL were prepared by diluting previously prepared solution up to 10 mL with diluent volume in separate 10 mL volumetric flasks.

Preparation of crude drug from various samples containing β carotene: The vegetables, fruits and nutraceutical supplements were obtain from local market (Papaya, carrots, Lady finger, Green Chili, Spinach, Red Chili, Dry red Chili, Capsicum and Oxidon Plus manufactured by Softech Pharma Pvt. Ltd. (batch No. OX PS 025) and Carofit+ manufactured by Ajanta Pharma Limited (Batch No. G00117E) were purchased from local pharmacy shop.

Processing of samples (Sample Clean-up and extraction): 500 mg of each sample was accurately weighed and transferred to a 10 mL volumetric flask directly with butter paper. 5 mL Diethyl ether was added to it and sonicated for 30 minutes to extract the Beta Carotene from the various food and dietary supplements. The final volume was made up to the mark and sample was extracted overnight by using rotatory shaker. Further the samples were centrifuged for 10 minutes at 3000 rpm. The supernatent solution was separated and evaporated to dryness using Low Volume Evaporator. After evaporation the sample was reconstituted with mobile phase. All samples were filtered with 0.45 µ nylon membrane syringe filter and 20 µL aliquot of each sample were injected in HPLC system.

Results and Discussions

Mobile phase development

The primary aim of the present study was to separate β Carotene from vegetables, fruits and dietary supplements. This included mobile phase selection, flow rate optimisation and column type. In this experiment, mixtures of several mobile phases were tried to get better response for â carotene from sample noise.The mobile phase, 0.5% triethylamine in Methanol: Acetonitril: Chloroform (40:40:20, *v/v/v*) in isocratic elution with a flow rate of 1 mL/minute resulted in good resolution, symmetrical peak shape of â carotene and short analysis time per run. All the samples exhibited the UV maximum absorption at 450 nm corresponding to the standard β carotene. All the reference compounds were easily resolved in the optimized HPLC condition and were eluted within 10 minute. The retention time was 7.8 minute for calibration standards as well as for test samples. The suitability of the solvent system was decided by cost, sensitivity of the assay and time required for the analysis. A representative chromatogram of standard β carotene has been shown in Figure 3.

Method application and Estimation of β carotene from different dietary supplements containing β carotene

The developed method was used as an application to determine the presence of *beta carotene* from various marketed formulations, vegetables and fruits. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 5 ng/mL and 10 ng/mL for *beta carotene* (Table 1), suggesting the sensitivity of the method. From the above results, it can be said that *beta carotene* showed a broad range of linear detection and was also highly sensitive method. The slope, intercept and correlation coefficient were found to by 219.91, 3028.9 and 0.9986.

Calibration curve of β Carotene has been shown in Figure. 2. The developed method was applied for the estimation and quantitation of β carotene from diethyl ether extract of various formulations, vegetable and fruits, dietary supplements containing â Carotene.

Various dietary supplements containing β Carotene as one of the chief ingredient were tried for the presence of β Carotene in it using reverse phase HPLC method. Different extraction technique has been carried out for the formulations with varied matrices. A single peak was observed at the same retention time in the samples of all formulations (Figure 3). There was no interaction between β Carotene with other excipients present in the various dietary supplements. Vegetables, fruits and dietary supplements with varied matrices were tried for the presence of β Carotene. Among the samples dry red chili sample showed the maximum

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content of β Carotene. The content of marker was found to be least in unripe (green) papaya. The assay results of the formulations are represented in Table 2.

Conclusion

The present study brings out significant variations in β Carotene content in marketed dietary supplements, vegetables and fruits. Thus, the method reported in this work can be applied for effective quality control and to reduce batch to batch variations in the final product.

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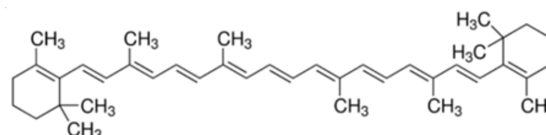

Figure 1: Structure of β Carotene

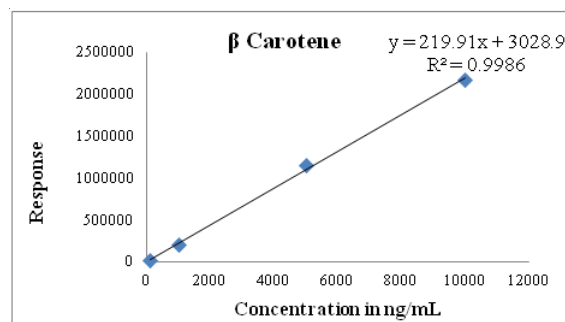

Figure 2: Calibration curve of β Carotene

Table 1: Summary of system suitability parameters and chromatographic conditions for β Carotene

Summary of system suitability parameters for â Carotene (1000 ng/mL)	
No. of theoretical plates (N)	7078.42
Retention Time	7.867
Capacity Factor	943.00
Asymmetry factor	1.22
Resolution	Not applicable
Summary of chromatographic conditions for â Carotene	
LOD	50.00 ng/mL
LOQ	100.00 ng/mL
ULOQ	2000.00 ng/mL
Regression equation	$y = 219.911x + 3028.9$
Coefficient of determination (r^2)	0.9986
HPLC Column	Cosmosil® Reverse phase C_{18} column (5 µm particle size, 150 X 4.6 mm ID)
Mobile Phase	0.5% triethylamine in methanol:acetonitril:chloroform (40:40:20) (*v/v/v*)
Run time	10.00 Minutes
Flow Rate	1 mL/ Minute
Injection Volume	20 µL
Detector	Jasco UV 970 intelligent UV/VIS detector
Wavelength	450 nm

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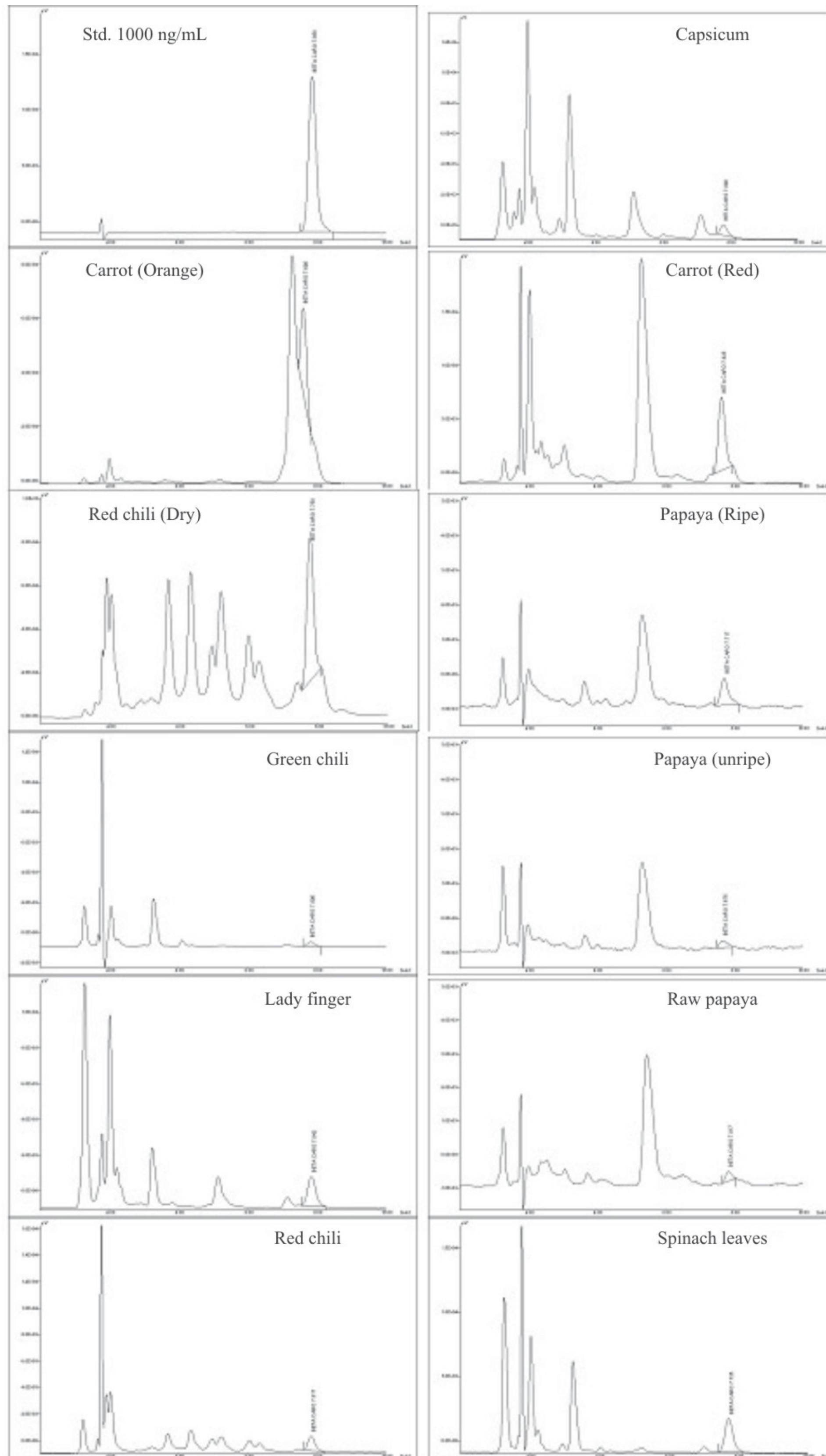

Figure 3: Representative HPLC chromatograms of various dietary supplements containing β Carotene

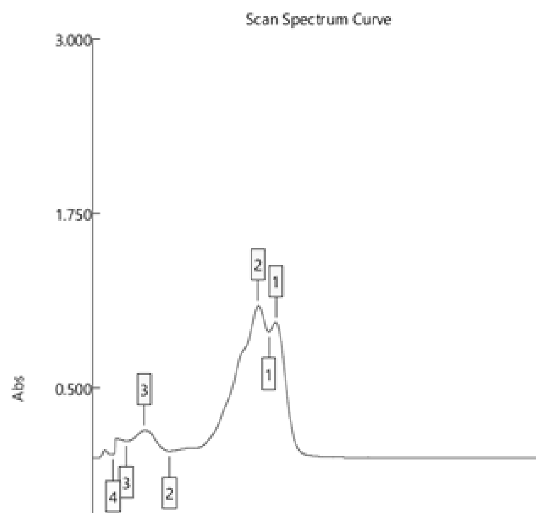

Figure 4: representative UV-Vis spectrum of β Carotene

Table 2: Estimation of β Carotene from various products

Content of β Carotene (Mean ± SD, n=3, N.D.: Not Detected)			
Samples	**Local Name**	**Botanical Name**	**µg/g**
papaya (unripe)	*Papaya*	*Carica Papaya*	**0.365**
papaya (ripe)	*Papaya*	*Carica Papaya*	**8.776**
Papaya (raw)	*Papaya*	*Carica Papaya*	**0.616**
Carrot (Red)	*Gajar*	*Raphanus sativus var*	**78.617**
Carrot (Orange)	*Gajar*	*Daucus Carota subsp. Sativus*	**297.535**
Lady finger	*Bhendi*	*Abelmoschus esculentus*	**21.046**
Capsicum	*Shimla Mirch*	*Capsicum annuum*	**6.265**
Green chili	*Hara Mirch*	*Capsicum frutescens*	**36.434**
Spinach leaves	*Palak*	*Spinacia oleracea*	**364.006**
Red chili	*Lal Mirch*	*Capsicum annuum L*	**139.294**
Red chili (dry)	*Sukha Lal Mirch*	*Capsicum annuum L*	**7844.346**

Marker Based Standardization of an Ayurvedic Plant *Mimosa pudica* L.

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Abstract: *Mimosa pudica* L. (Mimosaceae) commonly known as touch-me-not is reported in treatment of menorrhagia, dysfunctional uterine bleeding, and urinary tract infections etc. It is reported for various pharmacological activities like antiulcer, anti-inflammatory, anticancer activity etc, and has been prescribed by folk practitioners. *Mimosa pudica* is also a part of various traditional formulations like *Samangadi Churna, Pushyanuga Churna* etc.

Concentration of secondary metabolites and other biochemical markers in plant tend to change due to environmental factors. Thus standardization is an integral step in maintaining the therapeutic potency of herbal drugs. In present study, in order to standardize the extract, phytochemical and proximate analysis of *Mimosa pudica* (whole plant) has been carried out along with its chromatographic evaluation. HPTLC fingerprint has been developed as a quality assurance tool.

Further, a HPTLC method was developed and validated to quantitate content of rutin from *Mimosa pudica.*

The data can be recommended for quality control of *Mimosa pudica* can also be used for detection of rutin from other biological matrices. The variation in content of rutin implies the effect of regional and climatic variation on marker content.

Keywords: HPTLC, *Mimosa pudica*, Rutin, Validation.

Introduction

Mimosa pudica L. (Mimosaceae) short prickly plant distributed throughout India (Quality standards of Indian medicinal plants, Joseph *et al.*, 2013, Pandey & Pathak, 2010). The whole plant of *Mimosa pudica* is traditionally important. It is one of the ingredients used in many ayurvedic formulations like *Samangadi Churna, Pushyanuga Churna, Gangadhar Churna, Kutajavaleha* etc which are reported for gynecological disorders, piles, diarrhea etc (Ayurvedic Pharmacopoeia of India). Apart from traditional literature it is scientifically reported for various pharmacological activities like anti diabetic, antioxidant, wound healing, leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, asthma, leucoderma, and fatigue and blood diseases, urinary infections etc (Joseph *et al*., 2013, Azmi *et al*., 2011). The pharmacological and therapeutic activity is due to presence of several classes of secondary biologically active metabolites like alkaloid, glycoside, flavonoid, terpenoids and tannins, gallic acid, mimosine, norepinephrine, ascorbic acid, mimosinic acid etc (Shaikh *et al*., 2016; Shailajan *et al*., 2016, Joseph *et al*., 2013; Tamilarasi and Ananthi, 2012; Jadhav, 2012 and Quality standards of Indian medicinal plants, 2011). These secondary metabolites play a pivotal role and serve to be useful in many areas.

Thus the aim of the study is to standardize *M. pudica* in terms of physicochemical parameters, phytochemical and chromatographic evaluation. Due to varied application of these secondary active metabolites one such biologically active biomarker Rutin, flavonoid glycoside reported for anti-inflammatory, antihepatotoxic, antiulcer, anti-oxidant and reduction in low density lipoprotein (LDL) oxidation (Jain *et al*., 2009) was quantitated using validated chromatographic technique from *Mimosa pudica.* The data generated in the current study can be used as a quality control tool for the use of authentic sample of *Mimosa pudica* which is one of the key ingredients in many herbal formulations.

Materials and Method

Plant material: *Mimosa pudica* was collected from Manglore and authenticated by Agharkar Research Institute, Pune (Authentication No. ARI 10-75). Samples were shade dried for 7 days, then dried at 37±2ºC for week, powdered in a mixer grinder, sieved through 85mesh (BSS) and stored in air-tight containers.

Reference standards and chemicals: Rutin (e"95% purity, Figure 1) was procured from Sigma aldrich. All the chemicals used were of analytical grade and were procured from Merck speciality Pvt Ltd. Mumbai, India

Preparation of standard solution: 10.0 mg of standard (rutin) was accurately weighed and transferred to 10.0 mL standard volumetric flask. The content was initially dissolved in minimum quantity of methanol, sonicated and then diluted up to the mark with methanol. The stock solution of 1000.0µg/mL was used to prepare working solutions of 100.0µg/mL, 10.0µg/mL and 1.0 µg/mL.

hysicochemical and Phytochemical evaluation: The physicochemical parameters of the *M. pudica* (whole plant)

such as foreign organic matter, loss on drying, ash content (total, acid insoluble and water soluble) and extractive values were determined using standard pharmacopoeial methods (Indian pharmacopoeia, 2010; Khandelwal, 2008; Jadhav, 2012 and Nair, 2007). Similarly the qualitative phytochemical screening of some major class of secondary metabolites (flavonoids, tannins, glycosides, alkaloids and resins) was carried out by performing preliminary phytochemical test as per the reported method (Khandelwal, 2008).

Optimization of Extraction Conditions

Extraction of phytochemical constituent from *M. pudica* (whole plant): The powder sample (1.0 g) was extracted with ethanol (10.0 mL), vortex mixed for a minute and kept for overnight extraction followed by filtration through whatmann filter paper no. 1. The filtrate was subjected to HPTLC analysis for development of a phytochemical fingerprint and for separation and quantitation of rutin.

Optimized chromatographic conditions for phytochemical fingerprint and quantitation of rutin: Chromatographic separation of the phytochemical constituents was achieved on TLC plates (E. Merck) precoated with silica gel 60 F_{254} (0.2 mm thickness) on aluminium sheet support. To develop HPTLC fingerprint of *M. pudica* (whole plant), the sample (10.0 µL) was applied to the plate as a band of 7.0 mm wide and at a distance of 12.0 mm from the edges. Each plate was developed up to a distance of 85.0 mm in CAMAG twin trough glass chamber pre saturated with the mobile phase for 20 min. After development, the plate was dried in a current of air at room temperature. The plate was derivatized using 1% Anisaldehyde Sulphuric reagent and dried in oven preset at 110 ^{o}C. All measurements were performed at 22 ± 1°C. Plate was photo-documented at 254 nm (before derivatization), 366 nm and 550 nm (after derivitization, Figure 3).

To separate rutin from *M. pudica* (whole plant), the sample (10.0 µL) and rutin standard (100.0 µg/ mL) were spotted on TLC plates as bands of 7.0 mm wide and at a distance of 12.0 mm from the edges of similar instrumental conditions. The plate was developed up to a distance of 85.0mm in CAMAG twin trough glass chamber pre- saturated with mobile phase for 20min. The plate was scanned and photo-documented at 254nm.

Method validation: The HPTLC method was developed and validated as per ICH guidelines.

Results and Discussion

Medicinal plants have made its niche in traditional system of medicines since ancient era (Ganeshpurkar and Saluja, 2017). Collection of these medicinal plants at proper time is very important in terms of their bioactive marker as the variation in these phytomarkers can impair its therapeutic efficacy. The therapeutic activity is due to the presence of several classes of secondary biologically active metabolites like alkaloid, glycoside, flavonoid and tannins, resins, glycosides etc. On preliminary screening of these secondary metabolites, flavonoids, tannins, alkaloids, glycosides, resins were found to be present as per their chemical tests (Khandelwal, 2008). Proximate parameters such as foreign organic matter, loss on drying, ash values (total, acid insoluble and water soluble) and extractive values (ethanol soluble and water soluble) of *Mimosa pudica* whole plant were determined (Table 1). The water soluble extractive value was found to be maximum. This suggests the presence of more polar components in the *Mimosa pudica*. Standardization of medicinal plants is essential in order to assess its quality, based on the concentration of their active biomarkers (Shailajan *et al.*, 2017 and Kamboj, 2000). Thus HPTLC, a quality control tool was used as a standardization parameter fingerprint was developed using toluene: ethyl acetate: methanol: formic acid and rutin was quantified using ethyl acetate: methanol: formic acid: water. The R_f of rutin was found to be 0.47 under optimized chromatographic conditions and content was found to be 0.62873±0.00066 mg/g.

Conclusion

The developed validated method is simple, precise, accurate and sensitive and can be used as quality-control check for plant extracts or poly-herbal combination containing *M. pudica* which will aid in standardization and prevent its adulteration, as there are many species of *M. pudica* which are deliberately or in-deliberately substituted with the other low quality and morphologically similar medicinal plants which tend to reduce the therapeutic efficacy (Shailajan *et al.*, 2016). These methods can be applied to other plant raw materials containing the same phytochemical marker. The exact time and methods of harvesting, drying, storage and processing have an effect not only on morphological part but also in content of phytochemicals. This method can be used as quality control tool for the quality evaluation of *M. pudica* and formulation containing *M. pudica* as one of the ingredient.

Acknowledgement

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Table 1: Results of physicochemical parameters of *M. pudica* (Whole plant)

Parameters		Results
	Foreign organic matter	0.3361±0.0364
	Loss on drying	10.8499±0.7166
Ash content	Total	5.7892±0.1841
	Acid insoluble	2.2149±0.4132
	Water soluble	0.2184±0.0378
Extractive value	Ethanol soluble	14.0733±0.4962
	Water soluble	18.5332±2.5092
Values are (% Mean± S.D., n=3); (Ayurvedic Pharmacopoeia of India; Jadhav, 2012 and Nair, 2007)		

Table 2: Results of Phytochemicals in *M. pudica* (Whole plant) detected as per preliminary test

Phytochemical Constituents	Tests	Observation	Inference
Flavonoids	Ethanolic extract +increasing amount of NaOH	Yellow precipitate was observed	+
	Ethanolic extract + Lead acetate	Yellow precipitate was observed	+
Tannins	Aqueous extract + 5% $Fecl_3$	No deep blue colour was observed	—
	Aqueous extract + $K_2Cr_2O_7$	Red precipitate was observed	+
	Aqueous extract + Lead acetate	White precipitate was observed	+
Alkaloids	Ethanolic extract + Wagner's reagent	Orange brown precipitate was observed	+
Glycosides	Ethanolic extract + 1.0 mL H_2O + NaOH	Yellow coloration	+
Essential Oils	Ethanolic extract + drops of Vanillin Sulphuric acid	No white crystals	—
Resins	Boiled aqueous extract + conc. H_2SO_4	Reddish brown colour was observed	+

(+) : present; (-) : absent (Khandelwal, 2008)

Table 3: Results for estimation of Rutin from *Mimosa pudica* using HPTLC

Sample	Content of rutin (mg/g)*
Mimosa pudica	0.62873±0.00066
*Mean±SD, n=3	

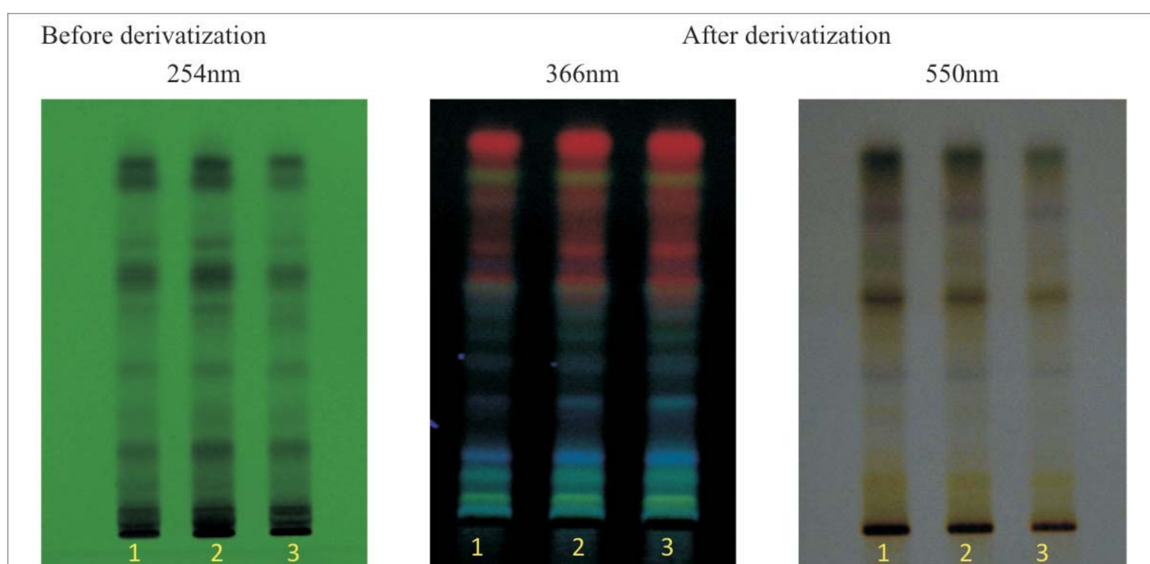

Figure 1: Phytochemical fingerprint of *Mimosa pudica* (Whole plant)

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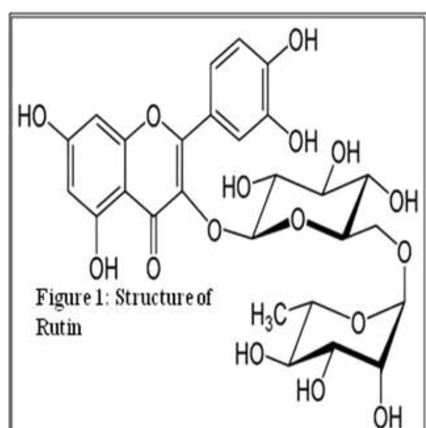
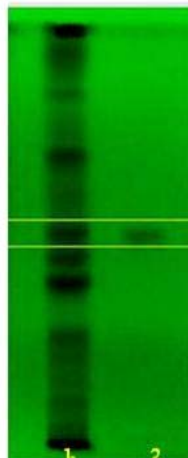


Figure 1: Structure of Rutin



Track details: Track 1: *Mimosa pudica* (Manglore), Track 2: Rutin (100ppm).

R_f -: 0.47

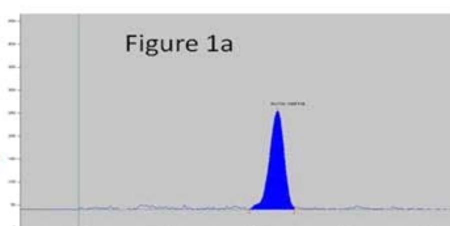


Figure2: Estimation of Rutin

Modern Approach Towards Standardization of A Traditional Ayurvedic Formulation *Pushyanuga churna* A Need of The Hour

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Abstract: *Pushyanuga churna* (PC) is an Ayurvedic formulation composed of 25 plant ingredients and 01 mineral described in AFI for its use in various female reproductive disorders. Owing to its therapeutic efficacy, it is prepared and marketed by different manufacturers. But, as there is paucity of scientific data on its standardization and quality control parameters which may lead to undesired quality and variation in its consistency, standardization of this formulation using modern bioanalytical techniques is required.

Hence, in the current research work, PC was purchased from different manufacturers available in the market. Quality control parameters which include preliminary phytochemical analysis and physicochemical analysis were established. Chromatographic separation was achieved to develop a HPTLC fingerprint for different manufacturers of PC. Further, a simple, rapid, accurate and sensitive HPTLC method was developed for the estimation of two therapeutically potent biomarkers *viz.* gallic acid and bergenin simultaneously using a toluene: ethyl acetate: methanol: formic acid as mobile phase. The developed method was validated as per ICH guidelines.

The data obtained after scientific evaluation of PC can be adapted to laying down new pharmacopoeial standards to be followed in the preparation of the formulation for reproducible batch-to-batch consistency.

Keywords: - HPTLC, *Pushyanuga churna*, Gallic acid, Bergenin

Introduction

Under the parasol of traditional system of medicine, ayurvedic medicines have gained a lot of popularity all over the globe due to its curative properties and minimal side effects (Singh and Pande, 2016). Ayurvedic formulations have active constituents which are not known and are used as phyto-pharmaceutical agents in various traditional formulations (Ranjana *et al.,* 2016). Traditional formulations are combinations of more than one herb that work synergistically to achieve greater therapeutic efficacy (Sharma *et al.,* 2009). Hence, standardization of herbal formulation is essential to assure its safety, efficacy and concentration of chemical constituents for their bio-potency (Ahmad *et al*., 2006).

Different ayurvedic formulations have been reported to treat various female reproductive disorders like *Pathadi Kwatha, Ashokaristha* etc. *Pushyanuga Churna* is one such ayurvedic polyherbal formulation composed of twenty-five plant ingredients and one mineral described in Ayurvedic Formulary of India (AFI-I, 2003). Ayurvedic texts prescribe it for various female reproductive disorders like Asrgandha (menorrhagia), Svetapradara (leucorrhoea), Rajodosa (Menstrual disorders), Arsa (Piles) and Yonidosa (disorders of female genital tract) (AFI-I, 2003). Owing to its clinical efficiency, PC is being prepared and marketed by different manufacturers like Dabur, Baidyanath, Arkashala, Dhootpapeshwar, Patanjali and kottakkal etc. As the formulation contains twenty-five plant ingredients, collection of authentic plant parts becomes difficult, also pre-and post-harvesting conditions, manufacturing process may affect its quality and this inturn may curtail its therapeutic potency.

Therefore, in the current research work, marketed formulation of *Pushyanuga Churna* was subjected to quality evaluation in terms of physicochemical parameters, phytochemical evaluation and chromatographic characterization in terms of its marker content. Polyherbal formulation *Pushyanuga Churna* may contain many secondary active metabolites like ursolic acid, â-sitosterol and lupeol (Shailajan *et al.,* 2017) which are responsible for its therapeutic efficiency. Bergenin and gallic acid which are reported to possess various pharmacological activities have been quantitated from *Pushyanuga churna.* The research data may provide substantial information to the manufacturers and help in scientific evaluation of the traditional formulation *Pushyanuga churna.*

Material and Methods

Marketed samples: Marketed samples of *Pushyanuga churna* were purchased from six different manufacturers available in the market Marketed PC-1, Marketed PC-2, Marketed PC-3, Marketed PC -4, Marketed PC-5 and Marketed PC-6.

Chemicals and reagents: Chemicals of HPLC grade were purchased from Merck Specialties Pvt. Ltd, Mumbai. Reference standards, gallic acid (e” 98% purity) was procured from Sigma Aldrich, Steinheim, Germany and bergenin (e” 97.0% purity) was procured from Chengdu Biopurify Phytochemicals Ltd, China. 10% methanolic sulphuric acid as derivatizing reagent was prepared according to described method (Reich and Schibli, 2006).

Quality control parameters

Organoleptic evaluation: The organoleptic characters of the marketed formulations were carried out based on reported methods (Wallis, 2004; Tripathi *et al.,* 2013).

Physico-chemical evaluation: Physico-chemical evaluation of marketed formulations were carried out using parameters like pH, loss on drying, total ash, acid insoluble and water-soluble ash content, alcohol and water soluble extractive content using standard pharmacopeial method (Indian Pharmacopeia, 2010).

Preliminary phytochemical screening: Ethanolic extracts of *Pushyanuga churna* were subjected to preliminary phytochemical screening for evaluation of phytoconstituents using reported method (Khandelwal, 2008).

Determination of physical characteristics: Samples were subjected to physical characteristic parameters like bulk density, tap density, Haussner ratio and Carr's index as per the reported methods (Awasthi *et al.,* 2014; Chamoli *et al*., 2013).

HPTLC - Instrumentation and optimized conditions

Optimization of extraction technique from different marketed formulations of PC:. To the accurately weighed 1g of each marketed PC, hydroalcohol was added in ratio of 2:8 v/v, vortexed for 5 minutes and kept standing overnight. It was filtered through Whatman filter paper No. 1 and the filtrate (10 µL) was then used for HPTLC analysis.

HPTLC-Fingerprint: Chromatographic separation was achieved on silica gel $60F_{254}$ precoated HPTLC plates. Samples were spotted using the CAMAG Linomat 5 sample spotter (CAMAG Muttenz, Switzerland) equipped with syringe (Hamilton, 100µL). For the development of fingerprints, plate was developed in a glass twin trough chamber (CAMAG) pre-saturated for 20 mins with toluene: ethyl acetate: formic acid as mobile phase. The plate was derivatized in 10% methanolic sulphuric acid. Densitometric scanning was performed using CAMAG TLC Scanner 4 at 254nm and CAMAG Reprostar 3 was used for photodocumentation (Table 5).

Chromatographic evaluation of phytochemical markers: For simultaneous estimation of the biomarkers gallic acid and bergenin from marketed formulations of PC, a validated method as per ICH guidelines was used (Singh and Shailajan, 2016).The statistical analysis of the results obtained was done using Microsoft Excel 2007.

Result and Discussion

Quality evaluation of herbal formulations is imperative to justify their acceptability in modern system of medicine (Shinde *et al*., 2009). Due to unavailability of rigid quality control profiles, herbal formulations lack consistency and quality (Choudhary and Singh, 2011). All marketed PC were subjected to organoleptic, physico-chemical, preliminary phytochemical and chromatographic evaluation to evaluate the uniformity in results with different manufactures of *Pushyanuga Churna*.

Quality control evaluation

Organoleptic evaluation of the marketed formulations of PC showed slight variation in its color and texture this may be due to the variation in collection conditions of raw materials, storage and its preparation. Results have been summarized in table 1.

Physico-chemical parameters of marketed formulation of PC showed loss on drying within the acceptance limits (Table 2). Higher values of moisture content in marketed 3 and marketed 5 samples showed susceptibility for bacterial, fungal or yeast growth as compared to marketed PC 1, marketed PC 2, marketed PC 4 and marketed PC 6 sample (Madhav *et al.,*2011). Total ash content of marketed formulations was found to be within acceptance limit except marketed PC 4 and marketed PC 6. Higher content of total ash in marketed PC 4 and marketed PC 6 formulations may be due to contamination, substitution or adulteration (Tripathi *et al*., 2013). The results of extractive value showed all marketed formulations contain more amounts of polar compounds (Shailajan *et al*., 2017).

Preliminary phytochemicals screening showed the presence of flavonoids, phenolic compounds alkaloids, saponins, terpenoids and glycosides in marketed PC which are considered to be responsible for its therapeutic activity (Table 3).

The results of physical properties (Table 4) of marketed PC showed broad range of variation which may be due to different and improper preparation, grinding method, storage and packing of finished product (USP, 2015). The variation in particle properties of marketed PC revealed that marketed 2 and marketed 4 have better flow properties, high solubility due to smaller car's Index value.

Chromatographic fingerprint of hydroalcoholic extracts of the marketed PC showed variation in fingerprint profile (Figure 1). The observed variation might be due to the variation in the quality of ingredients or unavailability of the authentic ingredients and post processing methods utilized during the preparation of the formulation.

For simultaneous estimation of gallic acid and bergenin from marketed formulations of *Pushyanuga churna,* a validated method reported by our group was employed (Singh and Shailajan, 2016). Chromatographic evaluation

using mobile phase toluene: ethyl acetate: methanol: formic acid gave the best resolution of bergenin and gallic acid from the other components of the hydroalcoholic extract of marketed PC.

Therapeutically important biomarkers gallic acid and bergenin have been reported to possess various pharmacological activities like anti-oxidant, anti-inflammatory, hepatoprotective, neuroprotective and analgesic activity (Sajeeth. *et al*., 2010; Tatiya *et al*., 2011; Patel *et al*., 2012). Based on the concentration of bioactive markers, formulations can be selected having maximum content supporting its efficacy. The maximum content of gallic acid and bergenin were found in Marketed PC 2 (2.346 ± 0.026 mg/g) and Marketed PC 4 (2.283 ± 0.175 mg/g) respectively (Table 7).

Conclusion

The method followed can be designed as a standard protocol to assure the quality of marketed ayurvedic formulation *Pushyanuga Churna.*

Industries manufacturing *Pushyanuga Churna* can use marker-based standardization to assure quality and enhance the therapeutic value of the formulation with reference to the content of phytochemical marker. These standardized parameters should be followed during different stages of preparation and processing of *Pushyanuga Churna*. This would increase global acceptance of ayurvedic formulations.

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Table 1: Organoleptic evaluation of different marketed formulations

Parameters	MarketedPC 1	Marketed*PC* 2	MarketedPC 3	MarketedPC 4	MarketedPC 5	Marketed PC 6
Appearance	Powder	Powder	Powder	Powder	Powder	Powder
Color	Light brown	brown	Dark brown	Dark brown	Reddish brown	Light brown
Odor	Musty	Musty	Musty	Characteristic	Musty	Aromatic
Taste	Bitter	Bitter	Bitter	Slightly bitter	Bitter	Bitter
Texture	Fine powder	Moderately fine	Moderately fine	Fine powder	Fine powder	Fine powder

Table 2: Physio-chemical Evaluation

Parameters	**Marketed PC 1**	**Marketed *PC* 2**	**Marketed PC 3**	**Marketed PC 4**	**Marketed PC 5**	**Marketed PC 6**
pH	5.3±0.21	4.9±0.22	4.7±0.25	5.1±0.23	4.8±0.21	4.8±0.27
LOD (NMT 11%)	6.68±0.38	6.75±0.54	7.62±0.385	6.20±0.24	8.51±0.16	3.04±0.21
Total Ash (NMT 15%)	13.70±0.16	12.33±0.74	11.65±0.14	16.26±0.34	12.63±0.36	16.52±0.22
Acid insoluble ash(NMT 4%)	1.32±0.12	2.59±0.42	1.44±0.21	3.64±0.19	2.54±0.35	6.27±0.28
Water soluble Ash	4.39±0.18	2.94±0.38	8.07±0.48	6.59±0.59	8.93±0.74	4.63±0.25
Alcohol soluble extractive (NLT 12%)	13.72±0.61	13.47±0.58	11.45±0.84	9.13±0.54	12.20±0.15	9.70±0.58
Water soluble extractive (NLT 13%)	65.76±0.52	67.98±0.23	66.57±0.52	59.79±0.58	65.23±0.25	48.19±0.25

Table 3: Preliminary Phytochemical Screening

Phytochemical constituents	Test performed with	Inference					
		Marketed PC 1	Marketed PC 2	Marketed PC 3	Marketed PC 4	Marketed PC 5	Marketed PC 6
Flavonoids	$(CH3COO)_2Pb$ solution	Present	Present	Present	Absent	Present	Present
	Increasing amount of NaOH solution	Absent	Absent	Absent	Present	Absent	Absent
Phenolic compounds	$K2Cr_2O_7$solution	Present	Present	Present	Present	Present	Present
	$KMnO_4$ solution	Present	Present	Present	Present	Present	Present
Alkaloids	Wagner's reagent	Present	Present	Present	Absent	Present	Present
	Mayer's reagent	Present	Present	Present	Absent	Present	Present
Saponins	Water with vigorous shaking	Present	Present	Present	Present	Present	Present
Terpenoids	Chloroform and carefully addition of concentrated H_2SO_4	Present	Present	Present	Present	Present	Present
Glycosides	Water and NaOH	Present	Present	Present	Present	Present	Present

Table 4: Physical characteristic of different marketed formulation

Parameters	MarketedPC 1	MarketedPC 2	MarketedPC 3	MarketedPC 4	MarketedPC 5	Marketed PC 6
Bulk density (g/ml)	0.357 ± 0.003	0.455 ±0.012	0.400 ±0.009	0.455 ±0.010	0.398± 0.015	3.336 ±0.200
Tap density (g/ml)	0.556 ± 0.029	0.556 ±0.066	0.553 ±0.033	0.553 ± 0.018	0.556± 0.075	5.004 ±0.003
Haussner ratio	1.556 ± 0.018	1.222 ± 0.017	1.389 ±0.011	1.222 ±0.024	1.396± 0.025	1.500± 0.002
Carr's index	35.714 ±0.049	18.182±0.016	28.000±0.027	18.182±0.012	28.417±0.028	33.333±0.185

Table 5: Optimized chromatographic conditions for fingerprint and quantitation of biomarkers of *Pushyanuga Churna*

Parameters	**Specifications**	
	Fingerprint	Quantitation
Stationary Phase	Merck silica gel $60F_{254}$HPTLC pre-coated plates	
Sample Applicator	Camag Linomat 5	
Development distance	85 mm	
Derivatization	10% Methanolic sulphuric acid reagent	——
Densitometric scanner	Camag scanner 4 Software winCATS planar chromatography manager software version 1.4.7 Lamp	
Wavelength	366 nm, 254nm	254nm
Photo documentation	Camag Reprostar 3	

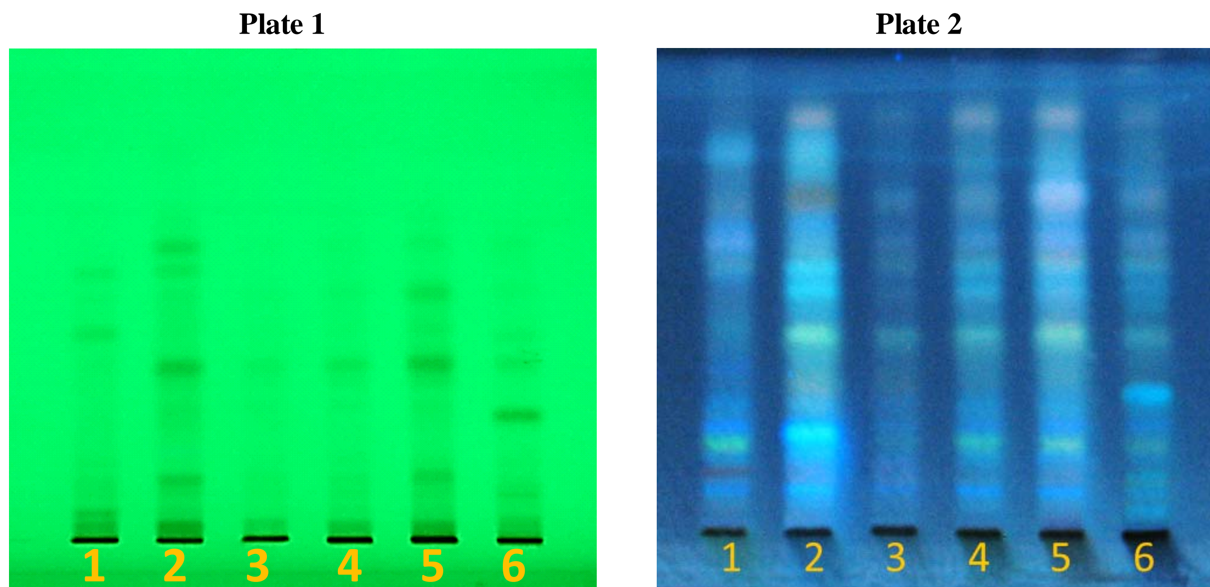

Figure 1: HPTLC-Fingerprint plate photo

Track details:
1. Marketed PC 1
2. Marketed PC 2
3. Marketed PC 3
4. Marketed PC 5
5. Marketed PC 4
6. Marketed PC 6

Table 7: Content of bergenin and gallic acid in the marketed formulations

Sample	Gallic acid	Bergenin
	Concentration (mg/g) Mean ± SD, n=3	
Marketed PC 1	0.537 ±0.108	0.801±0.109
Marketed PC 2	2.346 ± 0.026	1.950 ± 0.150
Marketed PC 3	0.945 ± 0.017	0.922 ± 0.028
Marketed PC 4	2.078 ± 0.028	2.283 ± 0.175
Marketed PC 5	0.417 ± 0.019	0.016 ± 0.027
Marketed PC 6	1.470 ± 0.025	0.114 ± 0.179

Comparative Evaluation of Kaempferol and Genistein from Three Species of *Flemingia*

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ABSTRACT: *Flemingia* genus is an important source of medicinal natural products, particularly flavonoids and steroids. Despite a long tradition of use of some species, the genus has not been explored properly. Hence, in the current study, an attempt has been made to standardize extracts of aerial and underground parts of *Flemingia tuberosa, Flemingia vestita* and *Flemingia strobilifera* and thus compare them in terms of phytochemical constituents and physicochemical parameters.

Chromatographic technique was used to develop fingerprints for the extracts that can be used as an identity parameter for the plants. Further a HPTLC method was developed and validated as per ICH guidelines for quantification of pharmacologically active biomarkers kaempferol and genistein from the plant extracts wherein the content of both the markers were found to be maximum in the tubers of *Flemingia vestita* which can be attributed to the change in climatic and edaphic conditions.

The extracts were found to be safe in albino Swiss mice upto 2g/kg body weight. The data generated forms the first comparative analysis of these species and can be compiled as a monograph for the same.

Keywords: *Flemingia tuberosa*, *Flemingia vestita*, *Flemingia strobilifera*, kaempferol, genistein, HPTLC, safety

Introduction

Flemingia is a genus of flowering plants in the legume family, fabaceae. Out of 105 species of the genus *Flemingia*15 species occur in India (Anonymous, 1993). An exhaustive survey of literature revealed that little information is available on 15 species. A thorough literature survey on *Flemingia* reports that 5 species have been investigated pharmacologically. Among these, *Flemingia chappar* and *Flemingia strobilifera* have been exhaustively explored for their antimicrobial and antioxidant activity while *Flemingia vestita* has been reported to possess anthelmintic activity due to the presence of isoflavones (Gahlot *et al*., 2011). *Flemingia macrophylla* is known to possess anxiolytic and hepatoprotective effect due to flavonoids (Gahlot *et al*., 2011).

In this project, work has been done on three species of *Flemingia viz., Flemingia tuberosa*, *Flemingia vestita* and *Flemingia strobilifera*. *Flemingia tuberosa* and *Flemingia vestita* are endemic to Western ghats and North east of India respectively while *Flemingia strobilifera* is found in many regions of Maharashtra, Sind, Rajputana, Bengal, South India and Andamans ranges of India (Prasad *et al*., 22011; Duthie, 1994; Nipunage *et al*., 2016).

Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries thus making it important to screen in order to identify the new sources of therapeutically and industrially important compounds (Ghurde and Malode, 2014). Therefore it is extremely important to standardize these drugs based on their marker compounds using suitable chromatographic technique in order to identify morphological and geographical variations (Shailajan *et al.,* 2014). The available literature on phytochemical reports of the genus reveals that it comprises mainly flavonoids (Ghalot *et al*., 2011). Tubers of *Flemingia tuberosa* has been reported to cure dysentery and vaginal discharge while *Flemingia vestita* has been known to be consumed by locals in North-east due to its traditionally known therapeutic activities and *Flemingia strobilifera* is used in the treatment against insomnia, pyrexia, gastrointestinal problems, etc. (Basu, 1993; Ghalot *et al*., 2011). Kaempferol and genistein are bio-markers reported for similar activities (Singh and Kumar, 2013; Lin and Lin, 2000). Thus the current research work aims in comparative study between three species of genus *Flemingia* with respect to their chemoprofiling and safety in term of its use in efficacy studies.

Materials and Methods

Chemicals and reagents:

Kaempferol (98 % purity) and Genistein (98% purity) (Figure 1) was procured from Sigma Aldrich chemical company (Steinheim, Germany). All the chemicals used were of HPLC grade and analytical grade reagents were procured from Merck Specialities Pvt. Ltd.

Plant sample:

Flemingia tuberosa, *Flemingia strobilifera* and *Flemingia vestita* was collected and authenticated by Shivaji University (Kolhapur), Agharkar Research Institute (Pune) and North-Eastern Hill University (Shillong) respectively. The samples were thoroughly washed, cleaned and shade dried for a week. The material was then packed in absorbent paper, oven dried at 37°C for three days, powdered using a mixer-grinder and sieved through BSS mesh number 85.

Physicochemical evaluation:

Foreign organic matter, ash content (total, acid insoluble and water soluble) and extractive values were determined for all the three species of *Flemingia* and compared using standard Pharmacopoeial methods (Indian Pharmacopoeia, 2010; Mukherjee, 2007).

Phytochemical evaluation:

Phytochemical screening of some major groups of secondary metabolites was carried out by performing preliminary phytochemical tests as per the methods reported (Khandelwal, 2008.) and further the plant was subjected to phytochemical evaluation by successively soxhlet extraction with various organic solvents in order to analyze the percent extract of major class of compounds present in the plant raw material as per the method reported (Harborne, 2007). The extract was standardized in terms of content of major phytochemical groups by spectroscopy. The content of total flavonoids (Marinova *et al*., 2005), total phenolics (Rajopadhye and Upadhye, 2013), total alkaloids (Sreevidya and Mehrotra, 2003), total saponins (Uematsu *et al*., 2000) and total terpenoids (Ghorai *et al*., 2012) were estimated using respective methods for the three species.

Chromatographic characterization: Extraction of phytochemical constituents from *Flemingia tuberosa, Flemingia strobilifera* and *Flemingia vestita*:

The powdered plant material (1.0 g) was extracted with ethanol (10.0 mL), vortex mixed for 1 min and sonicated for 20 min followed by filtration through Whatman filter paper No. 1. The filtrates were subjected to HPTLC analysis for the development of a phytochemical fingerprint and for separation and quantitation of kaempferol and genistein.

Preparation of standard stock solutions:

Standard stock solution of kaempferol (1000 ìg/mL) and genistein (1000 ìg/mL) were prepared in methanol. Serial dilution of the stock solution in methanol was carried out in order to prepare calibrant/ quality control samples.

Optimized chromatographic conditions for phytochemical fingerprint and quantitation of markers

The HPTLC system details and the chromatographic conditions are mentioned in the table 1.

Safety Evaluation

Safety of ethanolic extract of the plants was conducted in albino Swiss mice. The mice were fasted for 4 hours prior dosing and approximately 2 hours post dosing, water is provided *ad libitum.* The animals were orally administered with the ethanolic extract of the plants. The animals were observed individually during the first 30 min for all reflexes, periodically during the first 48 h with special attention given during the first 4 h (short-term toxicity) and daily thereafter for a total of 14 days (long-term toxicity).

Statistical Analysis

Microsoft Excel-2007 was used to determine mean, standard deviation (SD), relative standard deviation (RSD) and mean difference during the analysis.

Results and Discussion

Quality evaluation of herbal medicine is an important and basic requirement for the herbal drug industry (Shailajan et al., 2015). In the current research work, three species of *Flemingia* genus were compared in order to establish discernible characteristics among the species. It was observed that in the preliminary studies carried out, the water soluble extractive value was found to be more as compared to ethanol soluble extractive value suggesting the presence of more polar components in all the species. Among the plants *Flemingia vestita* was found to contain highest amount of polar components (Table 2A). *Flemingia strobilifera* was found to have lesser loss of drying value suggesting lesser moisture content. These values also supplement the scrubby habitat of *Flemingia strobilifera* In preliminary phytochemical evaluation (Table 2B), flavonoids, phenolics and terpenoids were found to be present in the methanolic extract of *Flemingia tuberosa*, whereas alkaloids and saponins were found to be absent. *Flemingia vestita* and *Flemingia strobilifera* showed the presence of all these class of compounds. Soxhlet extraction revealed that the plants are rich in quaternary alkaloids and N-oxides followed by, fats and waxes, terpenoids, phenolics and alkaloids. Phytochemical evaluation of the plants is also used as a quality control tool in the medicinal plants research (Harborne, 2007).

Chromatographic fingerprints represent the chemical integrities of the plant and hence form an identity of the plant. HPTLC fingerprint was developed from ethanolic extracts of the three species of *Flemingia* (Figure 2).

The genus was observed to be rich in flavonoids. Based on these observations, two potent flavonoids, *viz.*, kaempferol and genistein, which have been reported to possess various therapeutic activities, have been quantitated from these plants. HPTLC methods for characterization of *Flemingia tuberosa*, *Flemingia vestita* and *Flemingia strobilifera* in terms of marker content have been developed and validated as per ICH guidelines (Shailajan and Joshi, 2011). Kaempferol and genistein were separated into well resolved bands and comparative estimation from the three species of *Flemingia* was carried out. Presence of kaempferol and genistein in samples was confirmed by Densitometric scanning and spectral

confirmation. The content of kaempferol and genistein was found to be more in *Flemingia vestita* as compared to *Flemingia tuberosa* and *Flemingia strobilifera* (Table 3).

Safety of ethanolic extracts of *Flemingia tuberosa, Flemingia vestita* and *Flemingia strobilifera* was established in albino Swiss mice using acute toxicity study. The extracts were found to be safe following OECD guidelines 420.

Conclusion

Comparative chemoprofiling of species of *Flemingia* established in this project work can be used for the correct identification and differentiation of *Flemingia*. All the species of *Flemingia* were found to be a rich source of kaempferol and genistein. These methods can also be applied to various plant matrices and polyherbal formulations containing kaempferol and genistein. Using such validated methods, *these species* with established quality control and chromatographic conditions can be utilized in herbal industries.

Flemingia vestita is cultivated in the north eastern hilly regions of India and consumed. The results of the current study also propose the cultivation and usage of *Flemingia tuberosa* and *Flemingia strobilifera* in and around Maharashtra for their therapeutic and nutraceutical properties. Hence, cultivation of these plants can be encouraged for its commercial use.

Acknowledgement

We would like to thank Prof. S. R. Yadav for helping us in identifying *Flemingia tuberosa* in wild.

Conflict of Interest

Nil

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Figure 1: Structure of (a) Kaempferol (b) Genistein

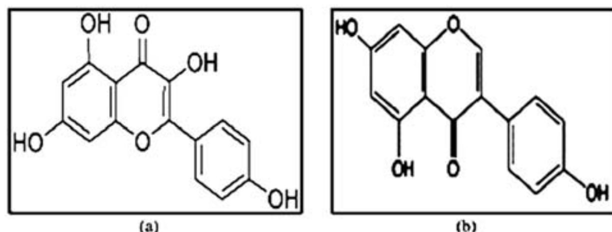

Table 1: Chromatographic conditions

Parameters	Description		
	Fingerprint	**Kaempferol estimation**	**Genistein estimation**
System	CAMAG TLC Scanner 4 supported by win CATS software version 1.4.7 equipped with CAMAG Linomat 5 sample spotter and CAMAG Reprostar 3 system for photo-documentation		
Stationary Phase	Merck Silica gel 60 F_{254} HPTLC pre-coated plates		
Plate size	9.0 cm x 10.0 cm	11.0 cm x 10.0 cm	
Mode of separation	Normal phase		
Mobile phase	Toluene : ethyl acetate	Cyclohexane: ethyl acetate: formic acid	Toluene: ethyl acetae: acetone:: formic acid
Development chamber	Camag twin trough chamber		
Chamber saturation	15 min		
Sample applicator	Camag Linomat V		
Syringe	Hamilton, 100.0 µL		
Band width	7.0 mm		
Space between the bands	7.0 mm		
Distance from the edges of the plate	14.0 mm		
Rate of sample application	150 nL/sec		
Development distance	85.0 mm		
R_f	—	0.41	0.34
Densitometric scanner	Camag Scanner IV equipped with win CATS Planar Chromatography manager software version 1.4.7		
Lamp and wavelength	Before derivatization: Deuterium (254 nm), Mercury (366 nm); After derivatization: Mercury (366 nm), Tungsten (540nm)		
Derivatizing reagent	10% Methanolic Suphuric acid, 1% Anisaldehyde reagent	—	

Table 2A: Proximate analysis

Parameters		Results		
		Flemingia tuberosa	***Flemingia vestita***	***Flemingia strobilifera***
Foreign organic matter	0.388 ± 0.004	0.462 ± 0.006	0.297±0.004	
Loss on drying	9.679 ± 0.074	9.269 ± 0.098	7.767±0.139	
Ash content	Total	5.447 ± 0.117	3.230 ± 0.019	5.407±0.038
	Acid insoluble	4.808 ± 0.015	0.780 ± 0.014	4.497±0.060
	Water soluble	1.120 ± 0.026	1.892 ± 0.027	2.051±0.040
Extractive value	Ethanol soluble	5.2949 ± 0.096	7.248 ± 0.122	1.465±0.008
	Water soluble	15.523 ± 0.083	24.384 ± 0.430	10.427±0.176
Phytochemical fractions	Fats and waxes	2.314 ± 0.024	1.679 ± 0.027	1.965±0.013
	Moderately polar extract	2.235 ± 0.033	3.714 ± 0.064	2.086±0.017
	Polar extract	6.816 ± 0.014	4.699 ± 0.086	7.767±0.058
	Basic extract	0.503 ± 0.002	0.324 ± 0.005	0.844±0.005
	Crude fiber	82.530 ± 0.489	83.697 ±0.789	84.667±0.577

Table 2B: Phytochemicals screening

Phytochemical constituents	Tests	Observation	Inference		
			Flemingia tuberosa	***Flemingia vestita***	***Flemingia strobilifera***
Flavonoids	Methanolic extract + Lead acetate	Yellow precipitate	Present		
Tannins	Aqueous extract + 5% $FeCl_3$	No deep blue-black color	Present		
Alkaloids	Methanolic extract + Mayer's reagent	Precipitate forms	Absent	Present	
Terpenoids	Methanolic extract + $CHCl_3$ + conc. H_2SO_4	Reddish brown coloration at interface	Present		
Saponins	Methanolic extract + water	Persistent foam	Absent	Present	

Figure 2: Chromatographic fingerprint of aerial and underground parts of Flemingia vestita, *Flemingia tuberosa* and *Flemingiastrobilifera*

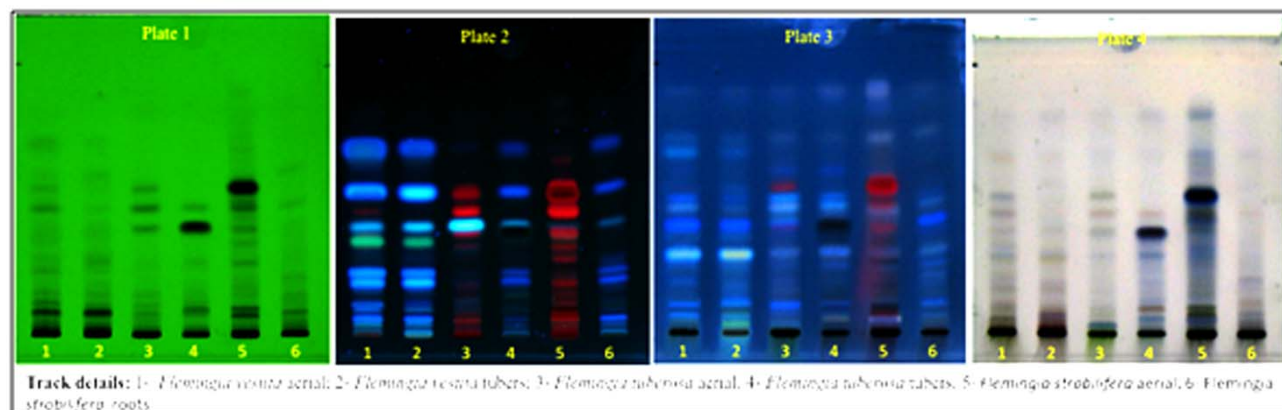

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Table 3: Content of kaempferol and genistein in different species of Flemingia

Samples		Concentration of kaempferol in mg/g (mean ± SD, n = 7)	Concentration of genistein in mg/g (mean ± SD, n = 7)
Flemingia tuberosa	Aerial	0.24	0.11
	Tubers	0.76	0.62
Flemingia vestita	Aerial	1.23	0.67
	Tubers	1.17	0.89
Flemingia strobilifera	Aerial	0.49	0.37
	Roots	0.55	0.40

Use of Bioactive Markers for Quality Evaluation of *Mustakarishta*: An Ayurvedic Formulation

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Abstract: *Mustakarishta*, an ayurvedic formulation involving the fermentation of *Mustaka* (*Cyperus rotundus*) by *Dhatki* (*Woodfordia fruticosa*) flowers, is widely used to treat digestive impairments including dyspepsia, piercing pain and gastro-enteritis. The formulation is readily available OTC but due to lack of pharmacopoeial standards and variations in marker constituents, it may not have desired quality and require standardization.

Hence in the present research work, *Mustakarishta* was prepared in-house as per Ayurvedic formulary of India and compared with marketed formulation in terms of qualitative and quantitative parameters. Extraction of the formulation was standardized using petroleum ether and reconstituted in ethanol for chromatographic characterization.

HPTLC fingerprint was also developed in order to standardize the formulation. A simple rapid method was further developed and validated as per ICH guidelines for the extraction of potential bioactive markers betasitosterol and lupeol. The content was estimated from standardized extracts of *Mustakarishta* and was observed to be higher in the in-house formulation.

The variation of marker content evaluated in fermented and matured samples also showed positive effect of maturation. Thus, the current standardization of *Mustakarishta* can be used as baseline data by manufacturing units to maintain consistency of the formulation.

Keywords: *Mustakarishta*, HPTLC, Simultaneous Estimation, *Dhataki*

Introduction

Ayurvedic system of medicine is the oldest medical system existing since 900 B.C. (Kadam, 2012). In many cases it is more effective than modern drugs with relatively less side effects (Jerald, 2007). Ayurvedic drugs are of various types which can meet the diverse requirements in the treatment of human illness such as *arishta* and *asavas*. These formulations are also found to be superior to tinctures for absorption in the gut since they are partly digested and has been reported to have better properties and functions than any other mode of drug preparation because of the combination of different types of medical materials and their transformations (Sabu and Haridas, 2015). *Arishtas* are made by soaking drugs (coarse powder or decoctions called *kashayas*) in a solution of sugar or jaggery and fermented for a specific period of time to generate alcohol which facilitates the extraction of the active principles contained in the drugs (Selvan and Priya, 2015).

Mustakarishta is an *Arishta* prescribed in Ayurveda for loss of appetite. Various plant ingredients in this formulation are *mustaka (Cyperus rotundus), marica (Piper longum), methi (Trigonella foenum-graecum), sunthi (Zingiber officinale), chitrakmool (Plumbago zeylanica), lavanga (Syzygium aromaticum), sveta jiraka (Cuminum cyminum)* and *yavani* (*Trachyspermum ammi)* Phadtare, *et al*, 2012). It is also reported to be used in the treatment of dyspepsia, digestive impairment, malabsorption syndrome and gastroenteritis with piercing pain.

Literature survey reveals that *Woodfordia fruticosa* (Linn.) Kurz. flowers (Dhatki, Lythraceae), *Madhuca indica* J.F. Geml flowers or yeast has been used traditionally as inoculum for Ayurvedic biomedical fermentation process (Chaudhary *et al*, 2011).The exact role of the *Woodfordia fruticosa* flowers in *asavas* and *arishtas* is still elusive, but it has been suggested that the microflora may bring about enzymatic bioconversions of extracted phytochemical constituents of plants which in turn modulates bioavailability or efficacy of the formulation (Bhondave *et at*., 2013).

Although formulation and their individual ingredients have been reported to be highly curative, their standardization is a must which will help to ensure quality, safety and efficacy of these herbal medicines (Hema *et al*., 2013).

Hence the aim of the present study was to develop a method for the standardization of the Ayurvedic formulation *Mustakarishta* in terms of chromatographic analysis. Beta-sitosterol and lupeol are well known biomarkers reported for a range of activities including hepatoprotection, nephroprotection and anti tumor properties. Hence, based on the reported chemical compositions of the ingredients, these two markers have been used in the standardization of the formulation.

Materials and methods

Herbal plant materials of *mustaka, marica, methi, sunthi, chitrakmool, lavanga, shvetjiraka, yavani* and marketed formulation *Mustakarishta* (MF) were purchased

from local markets. Plant materials were authenticated by Dr. Sunita Shailajan, Herbal Research Lab, Ramnarain Ruia College. *Woodfordia fruticosa* flowers were collected from Lonavala and authenticated. They were shade dried and stored under appropriate conditions.

Preparation of *Mustakarishta*

All plant materials except *mustaka* rhizomes were pulverized to make coarse powder passing through BSS mesh 44 to get uniform powder. *Mustaka* rhizomes were cleaned and soaked overnight in distilled water. Decoction of the resulting mixture was prepared to get one fourth of the initial volume and allowed to cool. *Guda, yavani, sunthi, marica, lavanga, methi, chitrakmool* and *sveta jiraka* were added. Fermentation jars were cleaned and smeared with pure cow's ghee procured from the local market. Dried flowers of *Woodfordia fruticosa* were added as prescribed in the Ayurvedic Formulary of India (AFI, 2003). The fermentation jars were sealed using double layer of muslin cloth and wax. Formulations were allowed to ferment for 30 days (FM) and stored for maturation in amber color bottles in the dark for 30 days (MM).

Preparation of standard solutions

A working stock solution of lupeol and betasitosterol (1000.0 ìg/mL each) was prepared by dissolving 10.0 mg of accurately weighed standard in methanol and diluting it to 10.0 mL.

Sample preparation for Chromatographic analysis

1 mL of the *Arishta* was extracted in 5 mL petroleum ether overnight. On the next day, the immiscible petroleum ether layer was separated and filtered in a clean glass beaker. In case of *Woodfordia fruticosa* (WF) and *Cyperus rotundus* (CR) samples, 0.5 g of accurately weighed samples were extracted overnight in 5 mL of petroleum ether and filtered on the next day. The filtrate was evaporated at 70 ºC to dryness and reconstituted in 0.5 mL ethanol. This prepared sample was spotted on the TLC plates. Similar extraction was used in the case of marketed formulation and for fermented and matured samples.

Optimized Chromatographic conditions for estimation of biomarkers

Chromatographic separation was achieved on TLC plates pre-coated with silica gel 60 F254 (E. Merck) of 0.2 mm thickness with aluminium sheet support. Samples (10.0 ìL) were spotted using CAMAG Linomat 5 sample applicator (Camag Muttenz, Switzerland) equipped with syringe (Hamilton). Plates were developed in a twin trough chamber (CAMAG) pre-saturated for 20 minutes with toluene: methanol and scanned with Camag TLC Scanner 4 conjugated with winCATS software version 1.4.7. The plate was derivatized with 10 % methanolic sulphuric acid reagent and scanned at 366 nm using mercury vapor lamp. Camag Reprostar 3 system was used for photo documentation at 366 nm post derivatization. The experimental condition was maintained at 22 ± 2 ˆC. The method has been validated as per ICH guidelines (Shailajan et al., 2017) and the content of markers namely betasitosterol and lupeol was estimated using regression analysis.

Results and Discussion

Standardization is an essential factor for the preparation of Ayurvedic formulations in order to assess their quality based on the concentration of chemical and bioactive markers present in the formulation (Shailajan *et al*., 2010). Hence, in the current research work, popular modern bioanalytical technique such as HPTLC has been used to standardize the formulation. Moreover the developed and validated method has been applied for the estimation of betasitosterol and lupeol from the formulation.

The method has been further applied as a fingerprint to access the changes in the chemical integrities of the prepared formulation at definite time intervals (figure 1). It was observed that prepared *mustakarishta* and marketed formulation displayed bands which were present in the main ingredient *mustaka* and the fermenting agent *Woodfordia fruticosa* (Argade and Pande, 2016). There were more numbers of phytochemicals being extracted in the prepared *mustakarishta* with gradual increase in incubation period from 15 days to 30 days. The concentrations of the phytoconstituents further increased on allowing the formulation to mature for 30 days in a controlled condition. In comparison to the prepared formulation, very few bands were observed in marketed formulation.

Similar studies have been carried out for quantitation of markers from traditional formulations such as *Pushyanuga churna* (Shailajan et al., 2017), *Jawarish – e – amla – sada* (Shailajan et al., 2015), *Eladi gutika* (Shailajan et al., 2011) etc, wherein standardization has been achieved using the basis of chromatography. Validated method was applied for the quantitation of reported biomarkers betasitosterol and lupeol from the processed samples (Shailajan et al., 2017) (figure 2). It was observed that both markers were found to be present in *Woodfordia fruticosa* and *Cyperus rotundus* samples. Marketed sample of the formulation showed presence of betasitosterol (0.026 ± 0.002 mg/ mL) but lupeol was found to be absent. Similar results were also obtained from the fermented sample of *Mustakarishta,* where in, the content of betasitosterol was found to be higher (0.040 ± 0.005 mg/ mL) in comparison to the marketed sample.

The presence of markers, betasitosterol and lupeol in the matured sample of the formulation suggests positive chemical changes in the constituency of the formulation

during maturation. The absence of lupeol in fermented samples in comparison to the presence of the same in matured samples might suggest that lupeol is present even in fermented samples but might be below the detection limit of the method. The increase in the content of betasitosterol and lupeol in the matured samples also suggest self generation of these markers during maturation which can indirectly be related to the increase in efficacy of the formulation post maturation.

The content of betasitosterol and lupeol was found to be 0.137 ± 0.008 mg/ mL and 0.022 ± 0.002 mg/ mL respectively. The 3D overlay of the plate is given in figure 3 and the content of the markers has been tabulated in table 1.

Conclusion

The observations of the current research work suggest that, standard operating procedure for the preparation of *Mustakarishta* can be followed by the manufacturers to reduce the variation and discrepancies of the markers in the formulation. The results of marker quantitation also highlighted that the optimal method for preparing *mustakarishta* would be fermentation for 30 days followed by a maturation period of 30 days in the dark. The developed extraction method may also be used for the extraction of above mentioned markers from other liquid based biological matrices.

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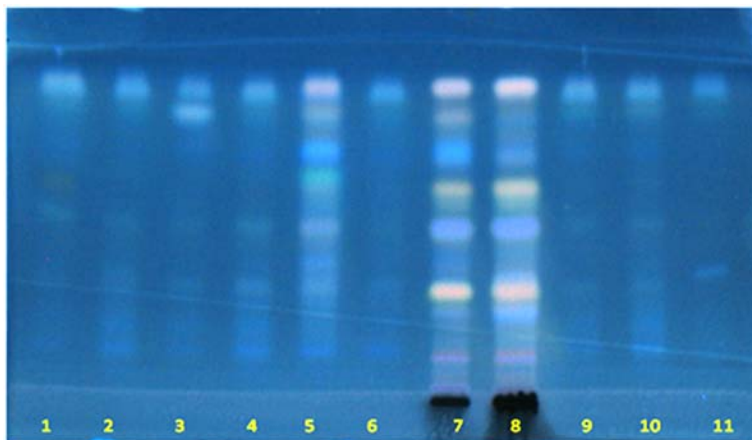
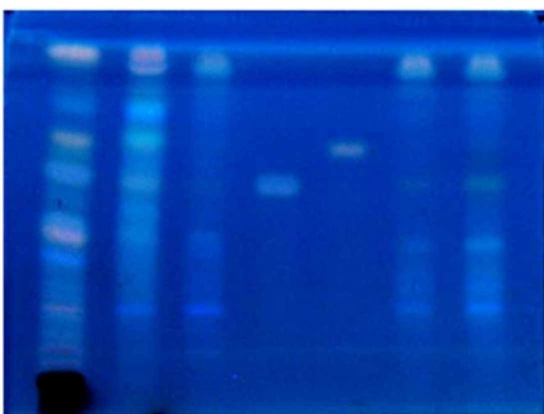
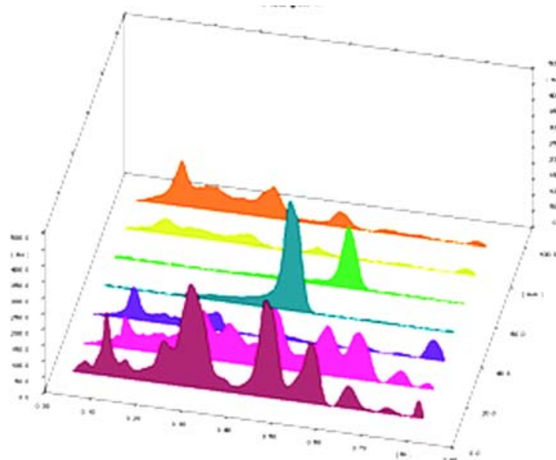
Figure 1: Stages of *Mustakarishta* preparation in comparison with WF, CR and Marketed formulation



Track details:
Track 1: 'O' day Kwath
Track 2: 15days Fermentation
Track 3: 21days Fermentation
Track 4: 30 days Fermentation
Track 5:*Cyperus rotundus*
Track 6: Marketed Formulation
Track 7: *Woodfordia fruticosa* powder
Track 8: *Woodfordia fruticosa* flowers
Track 9: 30 days Maturation
Track 10: 21 days Maturation
Track 11: 15 days Maturation

Figure 2 and 3: Plate photo and 3D overlay at 366 nm





Track Details:
Track 1:*Woodfordia fruticosa* flowers
Track 2: *Cyperus rotundus*
Track3: Marked Formulation
Track 4: Betasitosterol
Track 5: Lupeol
Track 6: 30 days Fermentation
Track 7: 30 days Maturation

Table 1: Content of betasitosterol and lupeol

Sample	Betasitosterol	Lupeol
	Concentration (mg/ml) Mean ± SD, n=3	
***Woodfordia fruticosa* flowers**	0.927±0.018	1.601±0.029
Cyperus rotundus	0.609±0.025	1.653±0.012
Marketed Formulation	0.026±0.002	-
30 days Fermentation	0.040±0.005	-
30 days Maturation	0.137±0.008	0.022±0.002

An Overview of Cultivation and Applications of *Sesbania bispinosa* (Jacq.) W. F. Wight

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Abstract: *Sesbania bispinosa* (Jacq.) W. F. Wight is a common weed, belonging to family Fabaceae, but has an extreme importance as cover crop, fuel wood, green manure plant and bio-fertilizer crop. It is a neglected legume with great potentials. It has great adaptability, resistance to pests and diseases. Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past. Herbal medicines are promising choice over modern synthetic drugs. The plant has been used as a medicine by traditional healers. It has several applications in traditional systems as well as in modern research. The plant has tremendous potential with respect to research and its applications in the dyeing, nutraceutical and pharmaceutical industry. Some of the applications of *Sesbania bispinosa* (Jacq.) W. F. Wight have been reported in the present paper. The plant needs further extensive study for its therapeutic effect which can be used for the welfare of mankind.

Keywords: *Sesbania bispinosa*, Green manure, Soil fertility, Natural dye, Phytochemical.

Introduction

India is a country of different traditional culture and tribes and follow different customs and systems of medicine. It is generally established that over 6000 plants in India are used for traditional, folk and herbal medicines, representing about 75% of the medicinal needs of the third world countries (Dubey *et al.*, 2004). There is the need to conserve and multiply the natural resources, which are used in different indigenous systems of medicines in India. Among native people, in addition to information shared generally, there is specialized knowledge held by a few, who have expertise in soils, plants, animals, crops, medicines and rituals. The present paper deals with one of such neglected weed *Sesbania bispinosa* having tremendous commercial and medicinal potential. *Sesbania bispinosa* (Jacq.) W. F. Wight., belonging to family Fabaceae is a common weedy, annual to biennial shrub found all through the Indian territory. It is commonly known as Dhaincha or Ran-shevri or Chinchani or Itkata. The other synonyms are *Aeschynomene bispinosa* (Jacq.), *Coronilla aculeate* Willd., *Aeschynomene surattensis* Wight and Arn., *Sesbania aculeatus* Poir., *Sesbania aculeata* Willd. (Pers.), *Aschynomene aculeata* Schreb. Common English names are Prickly sesban, Dunchi fibre, Sesban pea, Danchi, Canisa, etc. The plant has many applications in traditional medicine and in Ayurveda. The plant has great potential as forage crop, cover crop, green manure and in various drugs. The plant is reported as trypsin and chymotrypsin inhibitor. Medicinally, leaves and seeds are used to treat ringworm, other skin diseases, and wounds. Ayurveda has recommended the use of plant as for urine infection, stomach ailments, for microbial infections, diabetes, to improve the health of eyes, etc. A systematic study of this plant is needed for the same purpose (Almeida, 1998). In the present paper, cultivation practices, details of various parts of this plant with respect to traditional, ayurvedic uses and uses for other purposes are studied.

Morphology

It is a biennial crop, much branched, tall shrub attaining the height of 3-4 metres.

Roots: Roots are white, profusely branched tap root with considerably large number of nodules of Rhizobia associated with it.

Stem: Much branched, branches nearly horizontal, spiny or prickly. Stem is semi-woody, red to purplish in colour.

Leaves: Pinnately compound, 1-1.5 feet long and nearly 10" broad, stipulate. Leaflets forming upto 50 pairs, 10-20 mm long and 3-3.5 mm wide, linear, oblong, obtuse apex and acute base, petiolules very short.

Inflorescence: 3-4 flowered axillary, drooping racemes with slender peduncle.

Flowers: Irregular, 1.6-1.8 mm long, bisexual, complete, hypogynous, short, slender pedicels, in September-October. Flowers are mainly pollinated by bees.

Calyx: 5 sepals, gamosepalous, short, 3-4 mm long, membranous.

Corolla: 5 petals, papilionacious, yellow with pink to purple tinge on petals, 10-12 mm long.

Androecium: Staminal tube is short, the filament length above it are 2-4 mm long.

Gynoecium: Monocarpellary superior ovary, style 2-3 mm long, stigma capitate.

Legumes: Pods 15-20 cm long, straight, slightly beaked, constricted between the seeds, 2-4 mm wide and glabrous,

containing 20-50 seeds, 5 mm apart. It is self-pollinating and ripe pods shatter to release the seeds.

Seeds: 1-1.5 mm wide, 2-2.5 mm long, pale brown to greenish black.

Reasons for Cultivation

It is a common weed in much part of coastal India and almost neglected legume. But since it has high potential as a cover crop, a green manure plant, as fire wood resource and as a Nitrogen fertilizer to the soil, it is now cultivated in many parts. In Vietnam, it is grown in rice fields (Anonymous, 2002). It has high ability to compete with other weeds and can grow very tall in a short period. It grows very rapidly. It normally spreads on the ground and forms a profuse growth very soon. Cultivation is normally by seeds or seedlings. Seed viability is very high. If the seeds are dried in sun, they can be protected from fungal attack and from grain pest evil. Such seeds are stored in normal containers at room temperature for 41 years have retained 24% viability (https://cals.arizona.edu/fps/sites/cals-/sesbania.pdf).

Pests and Diseases

Major fungal diseases are not yet reported. It is attacked by many insects but not much damage has been noted. The most commonly observed pests are-

1. Caterpillars of *Azygophleps scalaris*- They do not damage the epidermis but bore in the pith region of root and stem. This makes the plant weak. Such plants should be uprooted to destroy the pest.
2. Larvae of Leaf webber, *Stringlina scitaria* normally attack young leaves, twist them and reside within them. This affects the photosynthesis. 0.1 percent BHC or Dieldrin can be used to control them.
3. Caterpillars of *Thypsidra successara* and *Amsacta moorei* are also found on leaves. They can be controlled by BHC or DDT.
4. Beetles and bugs may also attack the pods (Anonymous, 1980).

Growth Conditions

Climate: It can grow well in tropical and subtropical climatic conditions. Very commonly it is grown in India, Pakistan, and Vietnam etc. Cultivation and natural growth are found in Africa, Southeast Asia, China, West Indies and other tropical countries. Its trials of cultivation have been successful in Italy (Khare, 2016).

Soil: *Sesbania bispinosa* is tolerant of soil alkalinity even up to a pH 10, and grows in almost all soil conditions. It can grow well in water logged, saline soils or wetlands. But can also grow in sandy, alkaline, wastelands, barren lands or in deserts (Anonymous, 1980). The Cultivation is beneficial to soil because of two reasons-

1. It produces nodules profusely in the roots that fix the atmospheric nitrogen to a greater extent and increase the fertility of the soil.
2. In the soil, it releases a large amount of organic matter that is degraded by microorganisms in the soil. The partially degraded organic matter includes gums, mucilage and resins. These substances bind the soil particles and form soil aggregates. This results in increasing the tillers in the crops and water holding capacity of the soil, (https://cals.arizona.edu/fps/sites/cals——/sesbania.pdf).

Altitude: It can grow well from sea shores to the heights of 1200 m from mean sea level.

Rainfall: It prefers the rainfall of 550-1100 mm per year. But can grow in dry conditions. It is highly resistant to drought.

Ayurvedic Medicinal Uses

Herbs are natural, effective, less expensive, have less side effects and can be taken safely (Hota, 2007). *Sesbania bispinosa* is used for cough, coryza, fever, diseases of eye, calculus, bile duct calculus, glycosuria, dysuria, and urinary obstructions (Khare, 2016). Leaves and flowers are prepared as poultices for external application or taken as a decoction for internal ailments (Kirtikar & Basu, 2005). Due to *Sesbania bispinosa*'s astringent properties, preparations made from it can be used against inflammation, bacterial infections and tumours. In traditional medicine, seed mixed with flour is used to treat ringworm and other skin diseases and worms (Orwa *et al.,* 2009). In the preparation of Chandanadya taila (Charak Samhita, 1000 B. C.), Stanyajananakashaya churna, Mutravirechniya churna (Bhaishajyaratnavali, 16th century, Charak Samhita, 1000 B. C.). Dosage: 3-6 grams.

Ayurvedic Properties

GUNA (Quality): Snigdh, Guru ; RASA (Taste): Madhur ; VIPAK (Metabolism): Madhur ; VIRYA (Potency): Shith ; PRABHAV (Impact): Vatahara

Phytochemical Constituents

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*., 2005; Chahlia, 2009). Gohel and Pandya (2015) showed that the stem of *Sesbania bispinosa* reveals presence of carbohydrates, phytosterols, fixed oils, flavonoids, phenolic compounds and saponins in different extracts. Misra and Siddiqui (2004) have isolated and patented phytosterols and showed the presence of pinitol from the leaves of *S. bispinosa*. Momin and Kadam (2012) have studied the extractive values of root, stem and

leaves of *S. bispinosa* in different solvents. Khare (2016) have mentioned that different root and stem extracts show presence of palmitic acid, linolenic acid, oleic acid, poriferasterol, glycosides, pinitol and sucrose.

Extraction of Natural Dye

Environment activists support the use of natural colorants as they are seen to be using renewable resources causing minimum pollution and having low risk to human health. Aqueous extract of *Sesbania bispinosa* yields shades of cream, beige, yellow and browns using chemical mordant such as copper sulphate, alum, ferrous sulphate and potassium dichromate. Alum being alkaline in nature yields lightest shades and ferrous sulphate gave darkest shades (Vankar *et al.,* 2005). The coloring efficiency of its dye is high for silk with Fe, Cu, Cr used as a mordants (Swami *et al.,* 2012). Application of this dye contributes in attaining a safe, eco-friendly and green environment.

Dietary Supplement

Sesbania bispinosa (Jacq.), is rich in metals such as K, Na, Ca, Fe, Mg, and P. and has macro minerals in ample amount, It can be used as a good natural dietary supplement as well as for therapeutic uses and might play an important role in the maintenance of human health (Parab *et al.,* 2016).

Other Uses

1. **Fire wood:** As it is a fast growing, it is a good source of high yield of firewood. The plant gives the yield twice a year. The yield can be 15 tons per year. The wood is of low density with Specific gravity 0.3.
2. **Pulp:** Stem is potential source of paper pulp and construction material. It is less studied than gums. It has short fibres of length 0.96mm, good bleaching capacity. Pulp is equivalent to that of birch, one of the best wood sources for pulp.
3. **Fibre:** Stem fibres are having jute like properties and can be used for fishing nets, gunny bags and sails.
4. **Gums:** Seeds are smaller than those of guar. But their composition is nearly the same. Seeds contain 30-42% of the gum. It is viscous, water soluble, galactomannan (Mannose: Galactose in the proportion 5:1), mucilage, very low protein, oil and fibres.
5. **Green manure:** The crop is grown before rice and is harvested for use when rice is transplanted.
6. **Forage:** Since the leaves are a good source of proteins and carbohydrates, it can be a good source of fodder. But its fodder value should be checked.

Current and Future Plan of Work

Sesbania bispinosa (Jacq.) W. F. Wight belonging to family Fabaceae was collected in the month of June to August from Smt. C. H. M College campus in Ulhasnagar of Thane district. The plant was identified at Blatter herbarium, St. Xavier's College, Mumbai. The voucher specimens were deposited in St. Xavier's College Herbarium and Botany department of Smt. C. H. M College for further reference. The accession number for *Sesbania bispinosa* (Jacq.) W. F. Wight is SYJ1. The ayurvedic medicinal uses, phytoconstituents and other uses were studied. As a part of future plans, it is proposed to isolate and characterize the phytoconstituents from the plant.

Conclusion

Various studies on *Sesbania bispinosa* (Jacq.) W. F. Wight indicates that the plant is a neglected medicinal plant and has tremendous potential with respect to research and its applications in the dyeing, nutraceutical and pharmaceutical industry.

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Phytochemical Screening and Thin Layer Chromatography of Crude Extract of *Sauromatum Venosum* (Ait.) Schott. Tuber

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Abstract: The present study was aimed to investigate the medicinally active substances present in petroleum ether, chloroform, acetone and water tuber extract of *Sauromatum venosum* (Ait.) Schott. The dried powder of tuber was extracted successively in petroleum ether, chloroform, acetone and water and tested for the presence of different phytoconstituents. The phytochemical screening was carried out by various qualitative and quantitative methods. Qualitative phytochemical determination by chemical methods detected the presence of carbohydrate and glycosides, protein and amino acids, alkaloids, phenolic compounds & flavonoids, phytosterols, saponins and terpenoids. In the quantitative analysis, carbohydrates, protein, alkaloids, flavonoids, saponins and phenol were extracted by using the standard chemical protocol and the results showed that the percentage of carbohydrate was 55.49%, protein 16.48%, alkaloids were 14.9 ±0.83g/100gm, Flavonoids 3.73±0.05g/100gm, saponin 27.6± 0.90 g/100gm and phenol 0.02±0.0 gm./100gm. Using thin layer chromatography (TLC) components like alkaloids and flavonoids were separated from mixture. The Rf. values of the developed spots in the different solvent systems were noted 0.22, 0.26, 0.30, 0.33, 0.43 and 0.61 for alkaloids. For flavonoids Rf values were 0.14 and 0.21.Presence of medicinally important bioactive compounds in *Sauromatum venosum* depicted that it has great potential for becoming a future drug.

Keywords: *Sauromatum venousm* (Ait.) Schott.var. *guttatum* Kunth., Bioactive compounds, Phytochemicals, Primary and Secondary metabolites

Introduction

In India, the importance of Ethno medicine in treating various ailments and ill- effects on health has been recognized by health care experts in the sector of indigenous system of medicine across the rural parts of India. There are thousands of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very essential to know the value of the plant. The rich knowledge base of countries like India in medicinal plants and healthcare has lead to the keen interest by pharmaceutical industries to use this knowledge as a resource for research and development in the pursuit of discovering novel drugs. However, in India several plants are used for various aspects in the form of crude form without scientific evidence of efficacy. At this juncture, it is of interest to determine the scientific basis for the traditional use of the plants (Shah and Gilani, 2010). Many plants harvested as wild in India are used by local people as medicine-next to their source of food, shelter and various livelihood needs. Thin layer chromatography is a basic chromatographic technique that is used to separate mainly non-volatile compounds. TLC supports the identity of a unknown compound in a mixture when the Rf. value of that compound is compared with the Rf. value of a known compound. Retention factors are general characteristics value and are changed depending on the polarity of the mobile and stationary phase (Stoddard *et al*., 2007).

Sauromatum venousm (Ait.) Kunth.var. *guttatum* Schott. belongs to the family Araceae and is locally known as “Sanp Ki Booti” and commonly called as “Snake Plant” (Nabi Shah *et al*., 2014). It is a shade loving plant and found in Melghat region of Amravati District. It`s corm is a condensed form of rhizome consist of solid, stout, fleshy underground stem. It contains heavy deposits of food material. Plants are reputed to be capable of neutralizing the action of snake venom and their antivenom activity has been related to certain chemical compounds identified in the plants (Pereira *et al*., 1994). In folk medicines, the tuber is used as stimulating poultice in snake bite. The present attempt has been made to evaluate the phytochemical constituents of different extracts and thin layer chromatography of *Sauromatum venosum* tuber.

Materials and Methods

Collection and Identification of Plant Material: The fully matured healthy plant material i.e. tuber of *Sauromatum venosum* (Ait.)Schott.was collected from Melghat region, District-Amravati during the rainy season. The plant material was identified with the help of standard floras (Sharma *et al*., 1996). The material was thoroughly washed; air dried under shade and powdered by the help of mechanical process. The coarse powder of bulb was stored in airtight container for further studies

Preparation of Plant Extract: Soxhlet method was used with petroleum ether, chloroform, acetone and distilled water as solvent systems for the extraction of crude compounds

Phytochemical Analysis : Phytochemical tests were performed for the screening and identification of bioactive chemical constituents in the tuber. The dried extracts from

petroleum ether, chloroform, acetone and distilled water solvents were used for the phytochemical screening. All the plants were studied on the basis of qualitative (preliminary) and quantitative analysis. The tests were performed by using the standard methods described by Raman, 2006 and Khandelwal, 2007 and Carbohydrate, protein, phenol were estimated following (Thimmaiah, 2002), alkaloid, flavonoid, saponin (Krishnah, 2009).

Thin layer chromatographic (TLC) analysis: Quantified extract of alkaloid and flavonoid was subjected to TLC studies. 20×20 cm TLC plate precoated with silica gel 60GF254 (Merk, India) was used. Then the plate was marked with the pencil softly 1.5 cm far from the both bottom and top. Glass capillaries were used to spot the sample on the TLC plate on the pencil marked bottom line, allow each spot to dry completely before applying the next with the help of hair dryer. Repeat the application of drops until the marks are dark coloured. Then the solvents Toulene : Methanol (86:14) for alkaloid and Toulene: Ethyl acetate: Formic acid (50:40:10) for flavonoid was taken about 20ml in the chamber. The spots were visualized by exposure of the plates to Dragendorff's reagent for alkaloid and Ammonium hydroxide for flavonoid. Different bands were observed and corresponding Rf values are determined.

Results and Discussion

Yield Extract (%): The tuber extracts of *Sauromatum* were weighed and the amount obtained from petroleum ether, chloroform, acetone and aqueous extract of tuber are 0.9gm, 0.5gm, 1.0gm and 8.9 gm. respectively. The percentage of yield was calculated and presented in table-1.

Preliminary Phytochemical Screening: In the present study, primary and secondary metabolites were qualitatively and quantitatively analyzed using tuber of *Sauromatum*. The presence and absence of the phytoconstituents in the various extracts of tuber are listed in the Table 2. The various extracts of tuber have revealed the presence of carbohydrate, protein and amino acids, cardiac glycosides, alkaloids, flavonoids, phenolics and tannins, steroids, triterpenoids and terpenoids (Table 2).The results showed that that almost all the phytoconstituents are present in aqueous extract except sterols. Carbohydrates, Protein and amino acids which constitute the major edible part of the plant were found to be present in all the four extracts. Along with the primary metabolites, the secondary metabolites and other phytochemical constituents of medicinal plants account for their medicinal value. Plants containing glycosides used as cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia (Bhandary *et al*., 2012), found to present only in acetone and aqueous extracts of tuber absent in rest of the extracts. Alkaloids are one of the diverse groups of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase (Ranjith kumar *et al*., 2010), also shows cytotoxic activity (Nobori, *et al*., 1994) indicated the presence in all the four extracts. Flavonoids and tannins are the phenolic compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of *Sauromatum* (Robards *et al*., 1999) and show strong anticancer activities (Yadav and Agrawala,2011; Bhandary *et al*., 2012). Phenolic compounds were found to be present only in aqueous and acetone extract and tannins were found to present dominantly in all extracts except chloroform. Steroids has shown the strong presence in non-polar solvent i.e. petroleum ether and chloroform and absent in polar solvents i.e. acetone and aqueous extract. Saponin showed the positive results only in aqueous and acetone extracts. Terpenoids were present in all the four extracts. The quantitative estimation of primary and secondary metabolites in *Sauromatum* tuber revealed that the percentage of carbohydrate content was found high (55.49%), followed by Protein (16.48%) whereas the percentage of other secondary metabolites like alkaloids were found to be 14.9± 0.83g/100g, flavonoids 3.73±0.05g/100g and saponin 27.6±0.90g/100g. High carbohydrate and protein content in tuber might be considered as very good source of energy and value aided food. Saponins found to be present in acetone and aqueous extract which are known for formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al*., 2000; Okwu, 2004). Flavonoids are known to remove LDL from blood by increasing the LDL, the presence of high saponins might play a role in the cardioprotective potential of the plant (Bhandari *et al.,* 2012). The quantitative analysis showed maximum quantity of saponin, alkaloids and flavonoids in tuber.

TLC Profiling: Rf. values obtained from thin layer chromatographic analysis are listed in the Table 4. For TLC study of alkaloid extract of tuber solvent system Toulene:Methanol (86:14) was usedand 6 spots were visible and the Rf values were 0.22,0.26,0.30,0.33,0.43 and 0.61 respectively. Whereas, for flavonoid extract the solvent system Toulene: Ethyl acetate: Formic acid (50:40:10) was used and it showed two spots with Rf. values were 0.14 and 0.21 respectively (Table 4 and Plate 1).

Conclusions

The present study showed the presence of medicinally important bioactive compounds in various extracts of *Sauromatum venosum* tuber which may be contribute medicinal as well as physiological properties to the plant studied. TLC analysis of the phytochemicals showed the good sensitivity and separation of alkaloids and flavonoids from mixture. Therefore the extract from the tuber could be seen as a good source for useful drugs. These findings may also lead to the further isolation, purification,

characterization of the active compounds using chromatographic and spectroscopic techniques responsible for the activity of this plant.

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Table 1: The percentage of yield of different extracts of *Sauromatum venosum* tuber

Sr. No.	Solvent	Color and Nature of extract	Yield of the extract (in gm)	Percentage of yield (%w/w)
1	Petroleum ether	Yellow &sticky	0.9	1.46
2	Chloroform	Light yellow &powdery	0.5	2.72
3	Acetone	Light yellow &sticky	1.0	5.10
4	Aqueous	Yellow &dry	8.9	26.56

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Table 2: Preliminary phytochemical analysis of *Sauromatum venosum* Schott. Tuber

Plant constituents	Tests	Tuber extract			
		P E	C H	AC	AQ
Carbohydrates	Fehlings test	+	+	+	+
	Benedict's Test	+	+	-	+
Cardiac Glycosides	Killer-Killiani Test	+	-	+	+
Anthraquinone	Modified Borntrager's	-	-	+	+
Protein & amino acids	Ninhydrin Test	-	-	-	+
	Xanthophorin Test	+	+	+	+
Alkaloids	Dragendorff's Test	+	+	+	+
	Wagners Test	+	+	+	+
Flavonoids	Shinoda Test	-	-	-	+
	Lead Acetate Test	+	+	+	+
Phenolics& Tannins	Ferric Chloride Test	-	-	+	+
	Lead Acetate Test	+	-	+	+
Phytosterols	Salkowaski Test	+	+	-	-
Triterpenoids	Libermann-Burchards test	++	+	+	+
Saponin	Foam test	-	-	+	+
Terpenoids	Terpenoids test	++	+	+	+

PE- Petroleum ether; CH-Chloroform; AC-Acetone; AQ-Aqueous+ -Presence; - -Absence

Table 3: Quantitative Phytochemical analysis of *Sauromatum venosum* Schott.

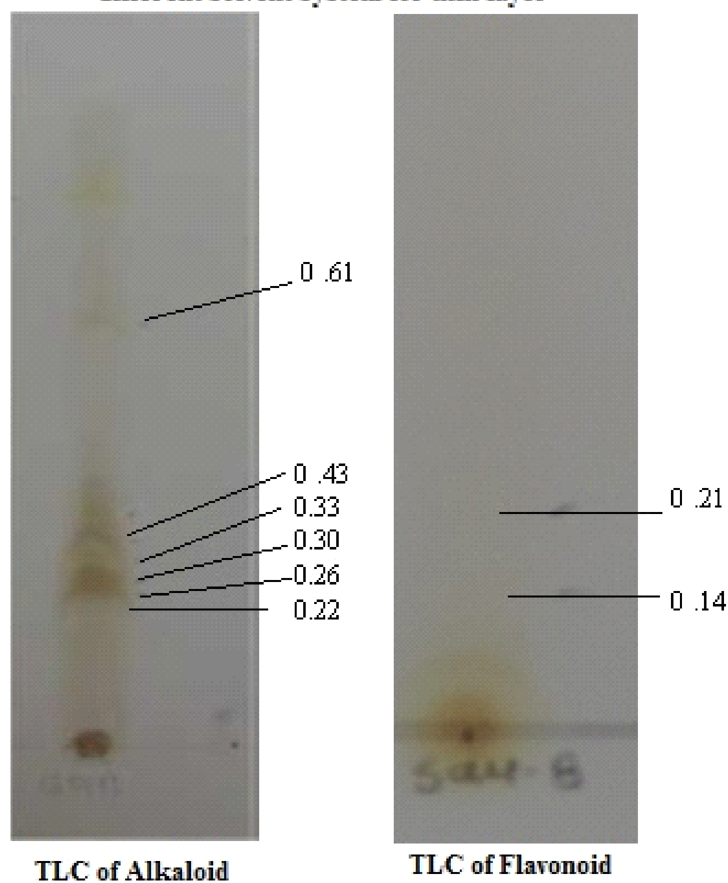
Sr. No.	Phytochemicals	Corm
1	Carbohydrate (%)	55.49
2	Proteins (%)	16.48
3	Alkaloids (g/100 gm)	14.9± 0.83
4	Flavonoids(g/100gm)	3.73±0.05
5	Saponin (g/100 gm)	27.6±0.90

Where results are depicted as mean ± SD of three determinants

Table 4: Phytochemical analysis of tuber by Thin Layer Chromatography

Sr. No.	Chemical name	Solvent system	Plant part	No. of Spots	*Rf* values	Spray Reagent
1	Alkaloid	Toulene:Methanol	Tuber	6	0.22	Dragendorff's reagent
					0.26	
					0.30	
					0.33	
					0.43	
					0.61	
2	Flavonoid	Toulene: Ethyl acetate: Formic acid	Tuber	2	0.14	Ammonium Hydroxide
					0.21	

Photo plate 1: Separation of compounds by using different solvent system for thin layer



TLC of Alkaloid **TLC of Flavonoid**

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Mutagenic Effects of Gamma Irradiations on Seed Germination and Seedling Growth of Mustard *Brassica Juncea* (L.) Czern & Coss

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Abstract: In the present investigation the effect of gamma rays on seed germination and seedling growth of two genotypes of mustard (*B. juncea*) was analysed. The seeds of two genotypes viz. Bio-902 (Pusa jaikisan) and SNM-217 were irradiated with gamma rays at different doses 800Gy, 1000Gy and 1200Gy.Sensitivity of gamma irradiation was observed on seed germination and growth parameters such as seed germination rate (%), seedling height (shoot length and root length) and seedling vigour (vigour index). Our findings confirmed that, with the increase of doses of gamma irradiation there was gradual decrease in the seed germination and seedling growth. This indicates the effectiveness of gamma rays on the germination rate, seedling height and seedling vigour. It was found that germination rate was minimum at 1200 Gy as compare to control and other doses in Bio-902 and SNM-217 (76.66% and 92.00%) respectively. Seedling vigour index was found to be a minimum in 1200 Gy as compare to control and other doses in both genotypes 1245.72 and 1498.68 respectively.

Key words: *B. juncea,* Gamma rays, Mutation, seed germination, seedling height, seedling vigour.

Introduction

Indian mustard [*Brassica juncea* (L.) Czern & Coss] is one of the most economically important oil seed crops in India (Kaushik and Agnihotri, 2000). It is considered as one of the most important vegetable oil and protein meal crop in Indian subcontinent. To fulfil the ever increasing demand of oil seed yield, oil yield and oil quality needs to be improved. Yield is one of the most important economic characters and is the product of multiplicative interaction of contributing characters. Hence, the important objective in mustard improvement is oriented to develop varieties which have high yielding potential (Labana and Banga, 1984). There is need to develop *B. juncea* with '00' characteristics for its better adaptability for consumption (Malode *et al.,* 1995). There is compelling need to increase and stabilize the productivity of Indian mustard (Meena *et al.,* 2015). The other objectives are oriented to develop new varieties with wider adaptability, early maturity, disease resistance and high oil content along with high yield potential. To enhance the production of any crop, a good variability should be present in the primary gene pool (Kumar *et al.,* 2013; Kumar *et al.,* 2015). Genetic variation plays a critical role in developing well-adapted improved cultivars. Since, there is limited genetic variability in primary gene pool of *Brassica juncea*; the various tools to generate new genetic variability shall be employed. However, cross breeding has restricted usages due to limited genetic variability in nature (Sestili *et al.,* 2010).

Mutation breeding is one of the alternative methods for plant breeders as it provides the chance of obtaining some desired traits that do not exist in nature or got lost during the evolution. The most used mutagen in mutation breeding technique is physical mutagen, which leads to break on DNA double strand and have a high energy radiation application such as ultra-violet (UV) light, Gamma rays and neutrons (Ulukapi and Nasircilar, 2015).

Gamma rays are the most energetic form of electromagnetic radiation, can be useful for the alteration of physiological characters. These radicals can damage or change important components of plant cells. They have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf *et al.,* 2004). Gamma irradiation as a mutagen can induce useful as well as harmful mutation in plants (Micke and Domini, 1993; Gupta, 1996).

Materials and Methods

In the present investigation, physiological similar seeds of mustard cultivated variety Bio-902 (Pusa Jaikisan) were obtained from PKV Nagpur and SNM-217 a yellow seeded variety developed at Department of Botany, G.V.I.S.H. Amravati. The seeds of both the variants were irradiated with 800Gy, 1000Gy, 1200Gy doses of gamma rays from ^{60}Co source at Bhabha Atomic Research Centre, Mumbai.

The seed germination and seedling growth experiments were undertaken at Cytology and Genetics laboratory, Dept. of Botany, G.V.I.S.H. Amravati. For seed germination experiments 50 seeds of each dose along with control were kept in petri-dishes on blotting paper in triplicates. The emergence of radical was taken as indication for germination of seeds. Germination percentage was calculated by counting the germinated seeds and total number of seeds sown. Further percent over control and reduction over control was also calculated.

$$Seed\ germination\ (\%) = \frac{No\ of\ seedsgeminated}{No.of\ seeds\ sown} \times 100$$

For seedling growth experiments 50 seeds were placed in three replication for each dose along with control arranged in slots on combs placed in a plastic tray containing water as shown in photographs The set up was controlled by light duration conditions. On the thirteenth day the shoot length and root length were measured on a graph paper in centimetres (cm). Later the seedling height and seedling vigour index was calculated by following formula.

Seedling height (cm): Seedling height (root and shoot length) was measured on 13th day after sowing in slots.

Seedling vigour index

Seedling vigour index = Seed germination (%) in slots x seedling height.

Results and Discussion

From the observations in Table 1 and Table 2 it is revealed that Bio-902 shows maximum seed germination percentage at control. Different doses of gamma rays showed gradual reduction in seed germination percentage. Minimum reduction was found in 1200Gy (76.66%). SNM-217 also showed the same trend in the seed germination percentage. Maximum was recorded in the control (98.00%) and minimum recorded in 1200Gy (92.00).

Seedling height in Bio-902 was found to be a maximum at control (26.46 cm) which was highly reduced at 1200Gy (16.25 cm). Similarly in SNM-217 showed highest seedling height in control (27.69 cm) and lowest at 1200Gy (16.29 cm) (Fig. 1 & 2).

Seedling vigour index of Bio-902 was found to be maximum at control (2348.16) and reduced to (1245.72) in 1200Gy treatment. In SNM-217 it was (2713.62) at control and gradually reduced to (1498.68) at 1200Gy.

Many research workers studied the effect of gamma rays irradiation at different doses on different crops with respect to seed germination percent and seedling growth. Dhulgande *et al.,* (2015) in Pea; Anbarasan *et al.,* (2013) in Sesame; Satpute and Fultambkar,(2012) in Soybean; S. M. and Seetharaman, (2014) in garden bean; Ariraman, *et al.,*(2014) in Pigeon pea; Sarada *et al.,*(2015),in coriander; Bolbhat, *et al.,* (2012) in Horse gram; Bhosale and More, (2014) in *Withania somnifera* showed decrease in seed germination percentage and seedling growth with the increase in the doses of gamma rays irradiation.

Conclusions

Results obtained showed that with the increase in doses of gamma rays irradiation there was gradual reduction in seed germination percentage, seedling height and seedling vigour index in both the variants *viz*. Bio-902 and SNM-217. This indicates that gamma rays (physical mutagen) showed its effectiveness at different doses (800Gy, 1000Gy and 1200Gy) on seed germination percentage, seedling height and seedling vigour index parameters in mustard.

Acknowledgement

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Table 1: Effect of Gamma rays on Seed germination in Mustard (*B. juncea*)

Variety	Doses (Gray)	No. of seeds/ replication	Germination (%)	Percent over control	Reduction over control
Bio-902	Control	50	96.00	100.00	0.00
	800	50	88.00	91.66	-8.34
	1000	50	78.66	81.93	-18.07
	1200	50	76.66	79.85	-20.15
SNM-217	Control	50	98.00	100.00	0.00
	800	50	98.66	100.67	0.67
	1000	50	94.00	95.91	-4.09
	1200	50	92.00	93.87	-6.13

Table 2: Effect of Gamma rays on seedling growth of mustard (*B. juncea*)

Variety	Doses (Gray)	No. of seeds/ replication	Shoot length (Cm)		Root length (cm)		Seedling height (cm)	Vigour Index
			Range	Mean	Range	Mean		
Bio-902	Control	50	6.89 - 7.17	7.02 (±0.08)	17.50 - 21.08	19.44 (±1.04)	26.46	2348.16
	800	50	5.58 - 5.84	5.74 (±0.08)	13.76 - 16.75	15.35 (±0.86)	21.09	1855.92
	1000	50	5.18 - 5.32	5.23 (±0.04)	11.62 - 13.23	12.61 (±0.49)	17.84	1403.29
	1200	50	4.76 - 5.03	4.93 (±0.08)	10.34 -12.73	11.32 (±0.72)	16.25	1245.72
SNM-217	Control	50	7.64 - 8.10	7.88 (±0.13)	19.58 - 20.01	19.81 (±0.12)	27.69	2713.62
	800	50	6.20 - 6.23	6.22 (±0.01)	16.40 - 17.38	16.93 (±0.28)	23.15	2283.97
	1000	50	5.35 - 5.60	5.51 (±0.08)	12.31 - 14.15	13.46 (±0.57)	18.97	1783.18
	1200	50	5.00 - 5.46	5.52 (±0.13)	10.06 - 11.90	11.04 (±0.53)	16.29	1498.68

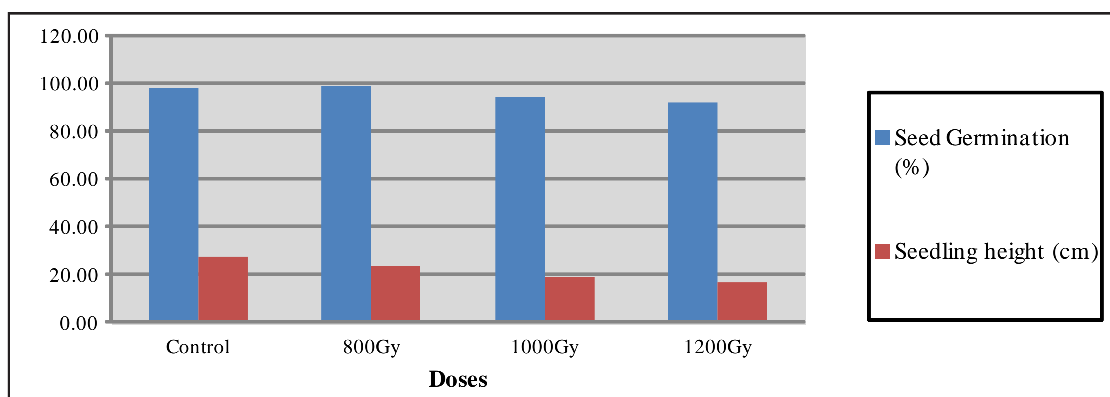

Fig.1: Effect of Gamma rays on Seed germination and Seedling height of Mustard Var. Bio-902

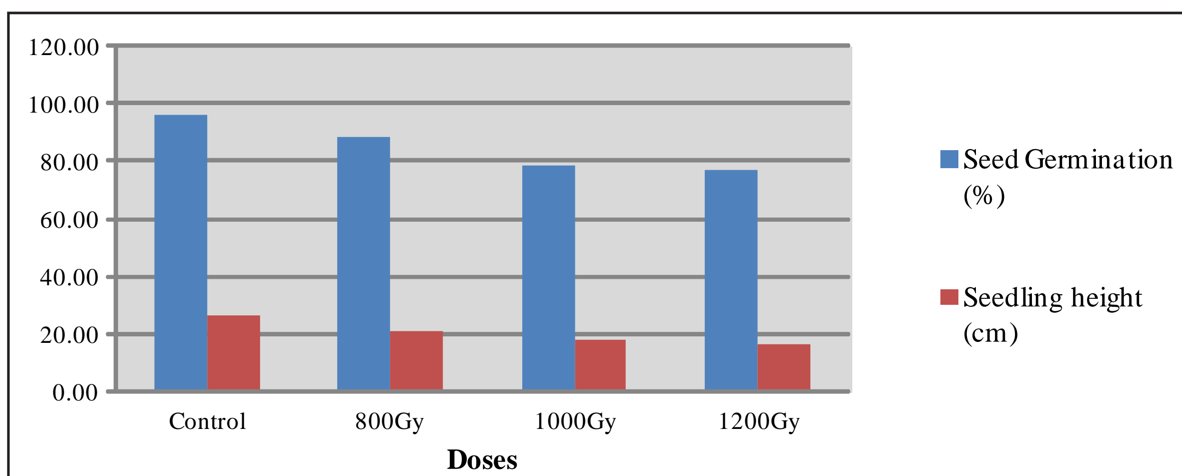

Fig. 2: Effect of Gamma rays on Seed germination and Seedling height of Mustard Var. SNM -217

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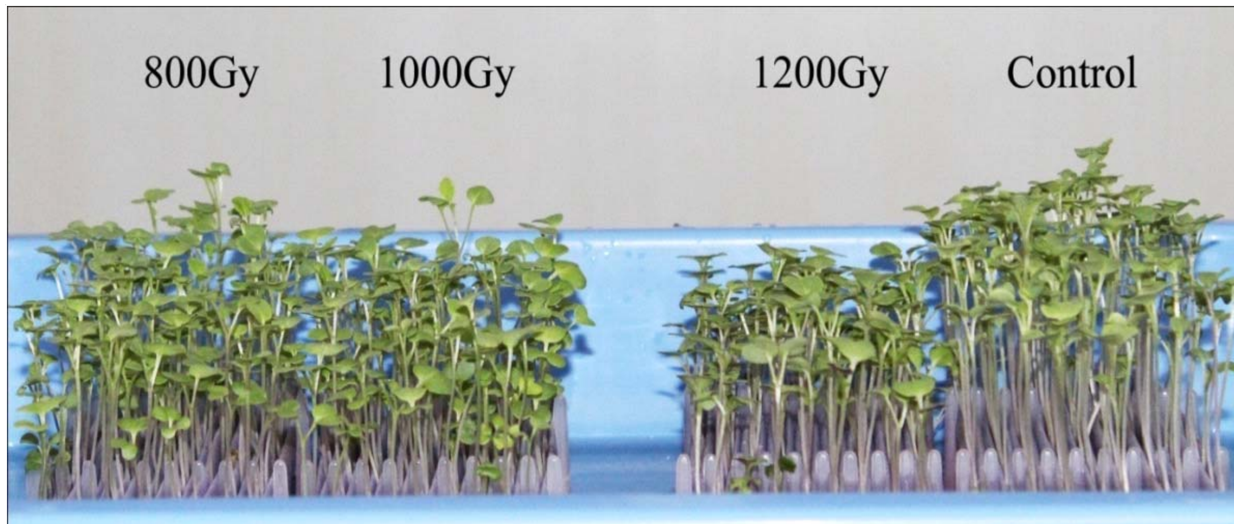

Plate 1: B. juncea Bio-902 showing variations in Shoot length at different doses of gamma irradiation

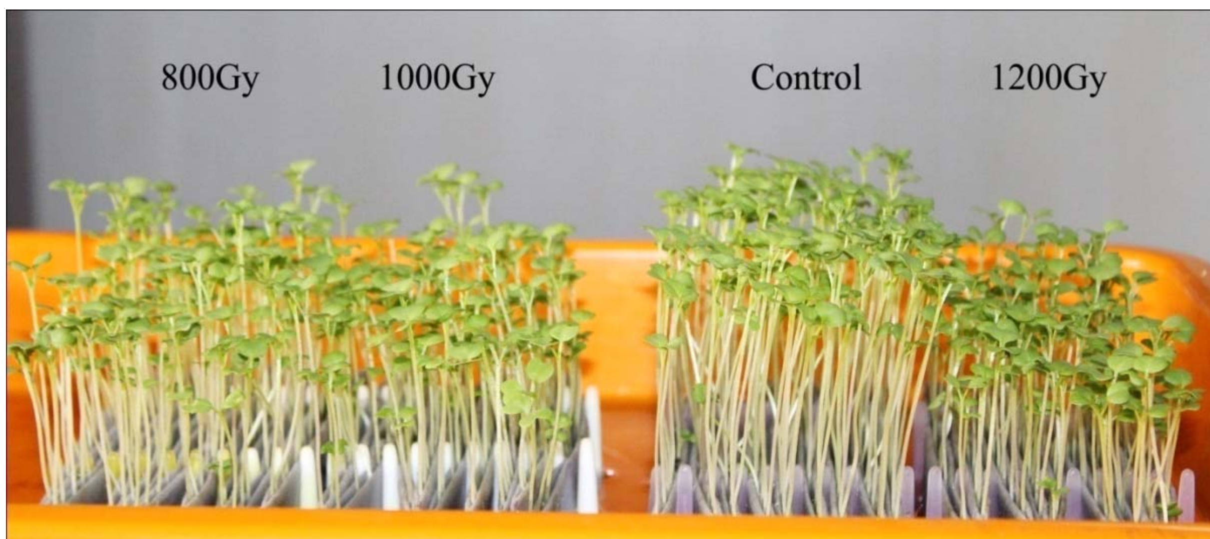

Plate 2: B. juncea SNM-217 showing variations in Shoot length at different doses of gamma irradiation

Determination of LD 50 Value for SA, EMS And Gamma Radiations In *Brassica juncea* Var. Kranti And Study of Its Effect on Seedling Growth

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Abstract: The present study was conducted to determine the dose of mutagen at which lethality to the seedlings is 50 percent. For that purpose, uniform seeds of *B. juncea* var. Kranti are subjected to chemical mutagens viz. Sodium azide and Ethyl methane sulphonate in dry and in pre-soaked in water and for gamma rays dry seeds with moisture of 6% was utilized for different doses of kR using 60Co source at BARC. Treated seeds along with non-treated control seeds are kept in three replications for seed germination and LD 50 value is determined. Accordingly seeds of three doses above LD 50 value along with non-treated control seeds were studied for shoot length and root length. Variation in the shoot length and root length is observed in almost all the treatments indicating the mutations to generate variation in the *Brassica juncea* var. Kranti.

Key words: *Brassica juncea*, Kranti, LD 50 value, Sodium azide, Ethyl methane sulphonate gamma rays

Introduction

Brassica is major oil crop of central India and it hold prominent position in terms of area under production. It account for about 62 - 68 % of total rabi crop. In country large number of different varieties of Indian mustard i.e. *Brassica juncea* is cultivated in different areas. *Brassica juncea* var. Kranti being National check can be grown in almost all part of the country because it can cope with the fluctuation in the environmental condition in early seedling stages, so it can play major role in oil crop improvement programme across the country.

In nature development of new variation is a very slow process and it take very long time while it can make happen in single generation by exposure of seeds of crop plant to mutagenic agents. It can alter the DNA contents causing changes in the genetic composition of crop plants. Chemical mutagens such as Sodium azide, Ethyl methane sulphonate and physical mutagens such as gamma radiation can be used separately or in combination. Mutagens in higher concentrations can severely damage nuclear DNA of crop and becomes lethal to the life of plant. Talebi *et al.* (2012) determined lethal dose by measuring the seed germination, seedling height, root length in Malaysian rice (cv.MR219). Sharma *et al.* (2005) observed that increase in doses of gamma rays and EMS show decrease in the seed germination, plant survival and pollen fertility in *Vigna mungo* (L.) Hepper. Kangarasu *et. al.* (2014) observed significant decrease in shoot length, leaf length and width with increase in either gamma dose or EMS as compared to non-treated control in Cassava plant. Hence, deciding mutagen and its optimum dose is the very key factor for the success in the field of plant breeding Ulukapi (2015). According to Rajarajan *et al.* (2016) determination of LD 50 values are very essential to launch any plant breeding programme. In order to produce maximum viable mutants with minimum damage to the plant LD_{50} value should be determined Gupta *et al.*. (2016). LD_{50} values differ in different varieties of plants with same type of mutagen Yadav *et. al.* (2016). Malode, (2004) observed the variation in glucosinolates and erucic acid composition in *B. juncea* (L.) Czern var. Pusa bold when exposed to mutagens.

Materials and Methods

In the present study three replication each of 50 uniform seeds of *Brassica juncea* var. Kranti obtain from PKV Nagpur, were exposed to chemical mutagen *viz.* Sodium azide and Ethyl methane sulphonate in direct (dry) and pre-soaked in water condition as well as physical mutagens *viz.* Gamma radiations. 25 seeds in each replication are used for germination study and 25 seeds of three selected doses in each treatment were kept in the slot made up of filter paper in moist condition for study of shoot length and root length along with the control seeds. In sodium azide direct treatment of 18 hour and 12 pre-soaked in distilled water then 6 hour sodium azide with constant stirring is given to seeds while in case of ethyl methane sulphonate 16 hour direct and 6 hour pre-soaked in distilled water then 6 hour in ethyl methane sulphonate with constant stirring is given to seeds. Seeds are thoroughly washed in running tap water for 3-4 times and post soaked for 1hrs. running tap water before it was kept for germination and in the slots. For radiation study seeds were exposed to gamma radiation at BARC Mumbai. The changes in seed germination is observed and LD_{50} value is determined and shoot length and root length of selected dose were observer and compare on 15th day with the control seeds which are not treated to any mutagen.

Results and Discussion

Germination of seed is indication of its viability, which

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is greatly affected by mutation as the dose of mutagen increases. In present study it is observed that seed germination is affected more in the Ethyl methane sulphonate than gamma radiation and Sodium azide, both in dry and pre-soaked in water condition as there is drastic decrease in the seed germination at higher doses in EMS (Fig. 1c and 1d). There is wide range of variation in both shoot length and root length in 16 hour dry EMS treatments (Fig.2c) and in gamma radiation treatments (Fig.2e). As increase in dose of chemical and physical mutagens there is gradually decrease in germination and shoot and root length. EMS found to be a potent mutagens in terms of toxicity as compared to SA and gamma radiations in Brassica for seed germination. Reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009). Many workers obtained decreased germination with EMS, Chandrashekar and Reddy (1971) in maize; Fowler and Stefansson, 1972; Kothekar (1978) in *Solanum nigrum*, Kashikar (1980) in *Petunia hybrida*; Afsar *et al*.,1980 and Malode (1995) in *Brassica carinata* ; Padavai and Dhanavel, 2004; Singh and Kole, 2005). Variations generated in root and shoot length in all mutagens used, gamma radiation and EMS are better in terms of variation it induce in the shoot and root length as compared to SA. The reduction in length of root and shoot was attributed to the effects of mutagens on the physiological system (Gaul, 1977) such a reduction in length of root and shoot arising out of mutagenic treatments was previously reported in crop plants (Reddy and Gupta, 1989; Amarnath and Prasad, 1998; Uma and Salimath, 2001).

Conclusions

Induce mutation play an important role in changing in the genetic composition of crop plants. It is great tool in hands of plant breeders to develop new varieties. For that purpose determination of LD_{50} value (50 % lethal dose) of mutagenic agent i.e. dose at which survivability of seedling is 50 per cent is necessary to select doses for Brassica mutation breeding programme.

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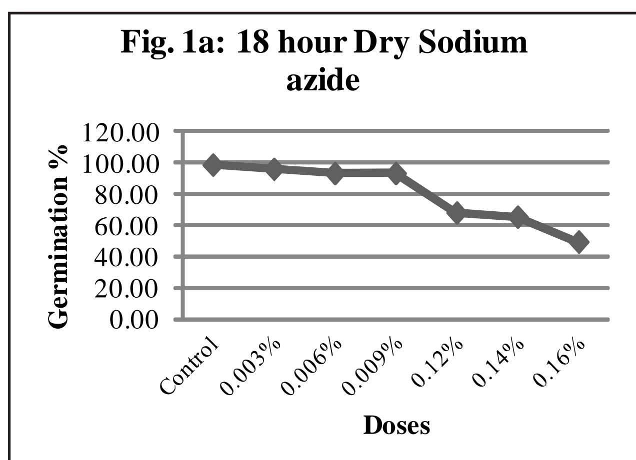

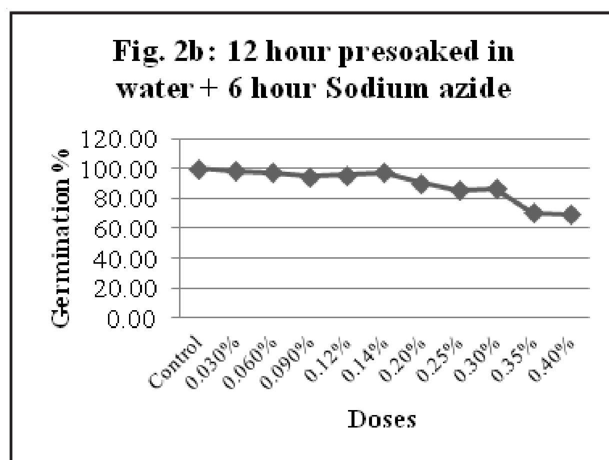

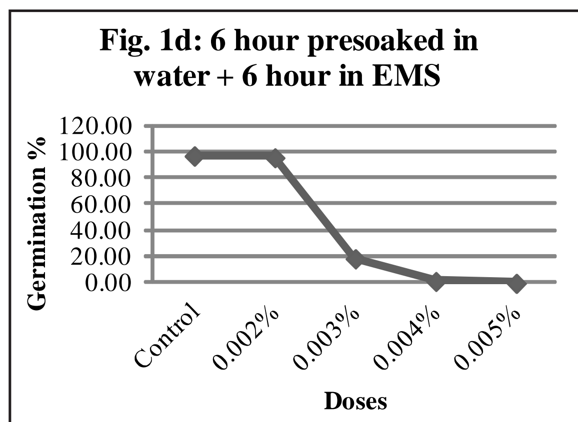

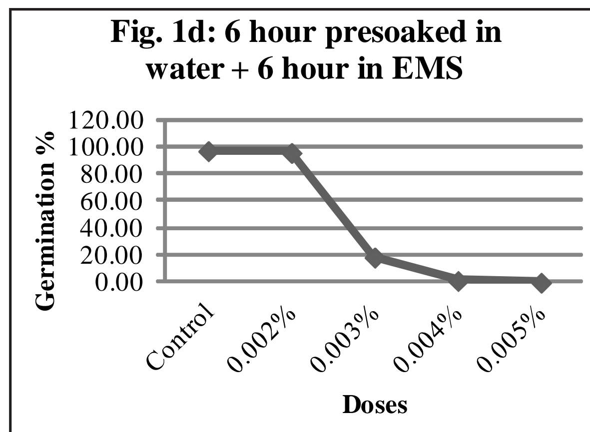

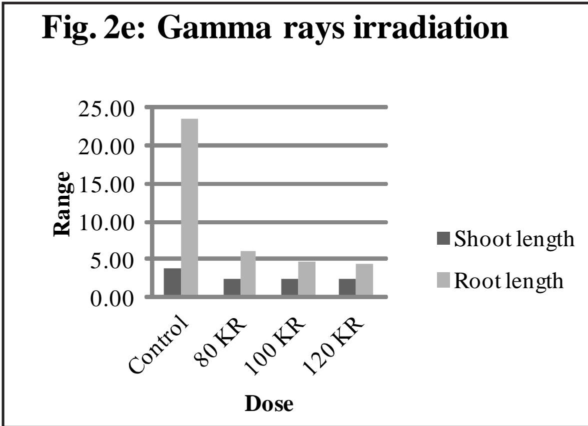
Fig. 2e: Gamma rays irradiation
25.00
20.00
15.00
10.00
5.00
0.00
Range
Control
80 KR
100 KR
120 KR
Dose
Shoot length
Root length

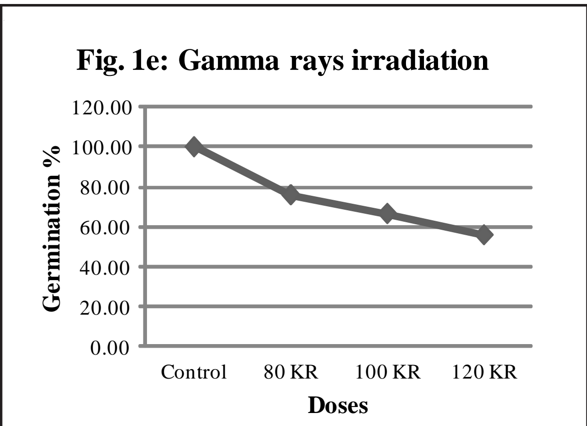
Fig. 1e: Gamma rays irradiation
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Germination %
Control
80 KR
100 KR
120 KR
Doses

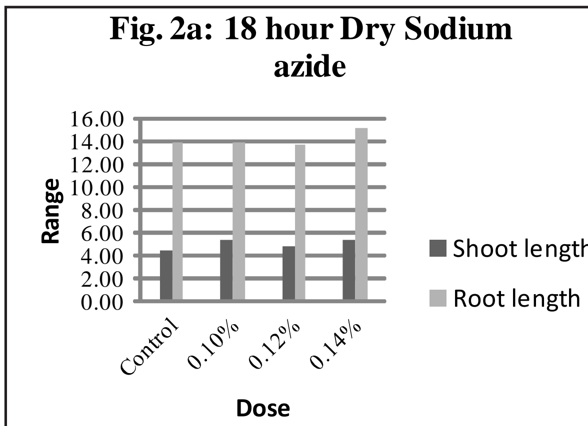
Fig. 2a: 18 hour Dry Sodium azide
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Range
Control
0.10%
0.12%
0.14%
Dose
Shoot length
Root length

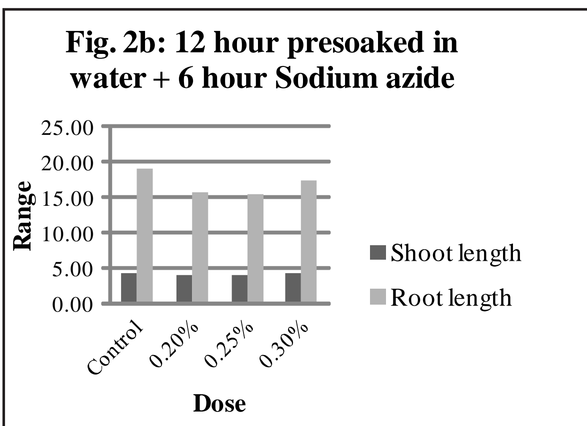
Fig. 2b: 12 hour presoaked in water + 6 hour Sodium azide
25.00
20.00
15.00
10.00
5.00
0.00
Range
Control
0.20%
0.25%
0.30%
Dose
Shoot length
Root length

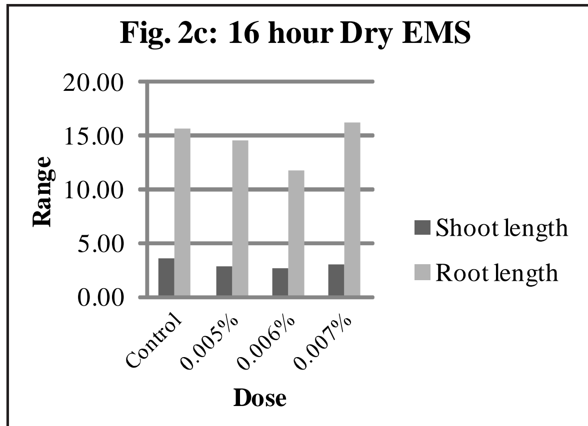
Fig. 2c: 16 hour Dry EMS
20.00
15.00
10.00
5.00
0.00
Range
Control
0.005%
0.006%
0.007%
Dose
Shoot length
Root length

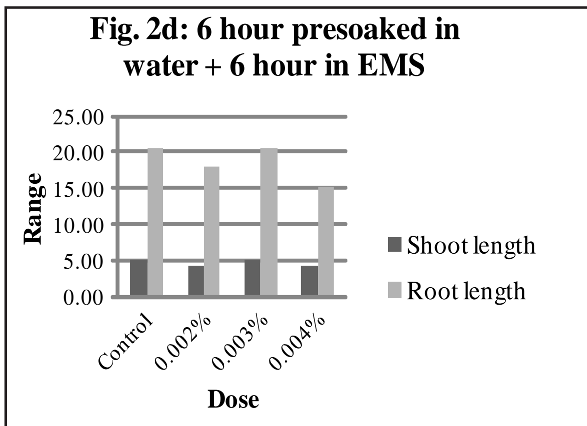
Fig. 2d: 6 hour presoaked in water + 6 hour in EMS
25.00
20.00
15.00
10.00
5.00
0.00
Range
Control
0.002%
0.003%
0.004%
Dose
Shoot length
Root length

Induced Mutation Studies in *Linum usitatissimum* Var. PKV NL – 260 With Reference To Mitotic Abnormalities

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Abstract: Mutation breeding plays an important role in crop improvement. Its effectivity on chromosome and gene mutation was studied by observing effect of mutagens on the mitotic chromosomes. In present investigation effects of dry and pre-soaked in water seed treatments by using two chemical mutagens Sodium azide (SA) and Ethyl Methane Sulphonate (EMS) as well as Irradiation treatment by Gamma radiation on mitotic chromosomes has been observed. Mitotic abnormalities in the form of clumped metaphases, anaphase bridges, laggards, clumped prophases, precautious chromosomes were recorded. All doses are effective in order to affect mitotic chromosomes; whereas maximum abnormality was recorded in 0.03% Dry seed SA treatment, 0.03% Pre-soaked seed EMS treatment and 400Gy in irradiation treatment.

Keywords: Mutation, Sodium azide, EMS, Gamma rays and Mitotic abnormalities.

Introduction

Linum usitatissimum is one of the oldest oilseed and fibre crop cultivated by humans. Linseed oil is mostly used for industrial purpose due to high linolenic acid content and hence not preferred as cooking oil as it become oxidized rapidly. Many attempts were made in order to increase yield and lower rancidity (α - linolenic acid content) of oil and many varieties were produced. In present study *L. usitatissimum* var. PKV NL – 260 is used. *L. usitatissimum* var. PKV NL – 260 is characterized by early maturing (avg. 105 days), moderately resistant to *Alternaria*, powdery mildew and bud fly, avg. yield 963Kg/hectare, 1000 seed weight 7.6 Kg with about 38% oil (Badere, 2014).

Cytological studies proved to be an effective tool in order to predict efficiency of mutagen and mutations. Mitotic abnormalities denote ability of mutagen to affect mitotic chromosome behavior. In present investigation, study on effectivity of different doses of three mutagens - Sodium azide (SA), Ethyl Methane Sulphonate (EMS) and gamma radiations on seeds of *L. usitatissimum* var. PKV NL – 260 was carried out.

Materials and Methods

1. **Chemical Mutagens treatment :** Two types of treatments were given

    - Dry Seed Treatment : Physiologically similar seeds were directly treated with mutagen solutions of different concentrations for 18Hrs.
    - Pre-soaked Seed Treatment : Physiologically similar seeds were firstly soaked in distilled water for 18hrs. and then treated with mutagen solutions of different concentrations for 6 hrs.

    For both treatments concentrations of mutagens (SA and EMS) used was – 0.01%, 0.02% and 0.03%. All treatments were given at 24±0.5^{0}C in Remi orbital shaking incubator at Cytology and Genetics laboratory, Dept. of Botany, GVISH, Amravati.

2. **Physical Mutagen treatment -** Seeds were treated with gamma rays with following doses of 400Gy, 500Gy, 600Gy, 700Gy, 800Gy, 900Gy at Mutation Breeding Section, NA and BTD, BARC, Mumbai. All treatments were given in triplicates.

The root tip is the first thing to emerge during germination process. For the cytological study, 50 seeds were kept in Petri dish lined with moist blotting paper. Actively emerging radicals were taken as the criteria for germination. When these radicles become of suitable length (about 0.8-1.2 cm) they are collected and stored in Cornoy's fixative I for about 24Hrs. and then in 70% alcohol till their use in cytology. Squashes ware made according to the method prescribed by Darlington and La Cour (1962) Paraffin bee wax was applied to the edges of coverslip to seal the slide. Semi-permanent slide was screened under compound microscope for cytological study. Cytological important slides were made permanent in Acetic acid, n-Butanol series. After observations, Photographs were taken by CMOS Camera on Zeiss microscope.

Results and Discussion

Data on effect of different concentrations of Dry Seed treatment, Pre-soaked seed treatment and irradiation treatments on mitotic chromosomes and frequencies of mitotic abnormality are tabulated in table 1, 2 and 3 resp. In control no abnormal cells were observed in all three treatments (Plate-I). Frequencies for abnormal cells in 0.01%,

0.02% and 0.03% concentrations of Dry seed SA treatment recorded were 0.5%, 1.1% and 2.6% respectively. Whereas for 0.01%, 0.02% and 0.03% concentrations of Dry seed EMS treatment frequencies of abnormal cells recorded were 1.2%, 1.9% and 1.2%, respectively (Table 1). The highest abnormality percentage was recorded in 0.03% Dry seed SA treatment (2.6%). In case of Pre-soaked Seed treatments, frequencies for abnormal cells in 0.01%, 0.02% and 0.03% concentrations of Pre-soaked seed SA treatment recorded were 1.4%, 1.2% and 2.3% respectively. Whereas for 0.01%, 0.02% and 0.03% concentrations of Pre-soaked seed EMS treatment frequencies of abnormal cells recorded were 2.4%, 2.4% and 2.6 %, respectively (Table 2). The highest abnormality percentage was recorded in 0.03% Pre-soaked seed EMS treatment (2.6%).

Radiation treatment shows frequencies of abnormal cells – 2.4%, 1.6%, 1.2%, 1.5%, 1.4% and 1.4% in doses of 400Gy, 500Gy, 600Gy, 700Gy, 800Gy and 900Gy respectively(Table 3). Highest abnormality percentage was recorded in 400Gy Irradiation treatment (2.4%). The treatment shows reduction in mitotic index with increase in dose except for 800Gy, same results were reported by Eroglu *et al.,* (2007) in *Hordeum vulgare*. Clumped metaphase arises due to the arrest of cell cycle at metaphase followed by apoptosis and ultimately intermingling of chromosomes with each other. Clumped metaphase is a one of the outcome of inactivation of spindle apparatus connected with the delay in division of centromere (Somashekare *et al.,*1984), while according to Deysson, (1968) clumped metaphases may lead to polyploidy and cells thus formed degenerate without further division.

Lagging chromosomes and fragments are observed due to the formation of acentric chromosomal fragment during exchange or chromosomal breaks (Verma *et al*., 2012). The anaphasic bridges may be formed due to paracentric inversions and stickiness; it also might have resulted due to disturbances at cytochemical level (Evans 1962; Sinha 1967). Double bridges might be due to fusion between broken ends of chromosome. Malode in 1995 suggested that the ultimate sources of all heritable variations to select from are mutations. Kumar and Rai in 2007 suggested that induction of cytological disturbances in the mitotic as well as meiotic cells is of great value, as it results in genetic damage that is handed over to the next generation.

Effectivity of mutagen in order to create phenotypic variants with reference to cytological study was reported earliar by many workers in different plants (Kumar and Srivastava, 2010) in Safflower; Gunasekharan and Pavadai (2013) in *Arachys hypogaea* (L.) var. VRI–2; Pavadai, Gnanamurthy and Dhanavel (2013) in *Glycine max* (L.) Merr.; Girija, Gnanamurthy and Dhanavel (2013) in *Vigna unguiculata* (L.); Ahirwar and Verma (2015) in *Phlox drummondii*. Many workers also reported combining effect of mutagens to generate mitotic abnormalities Reddy and Annadurai (1992) in *Lens culinaris* Medik by combining treatments of EMS and Gamma rays as well as Sodium azide and Gamma rays; Kumar and Srivastava (2013) in *Sesbania cannabina* by combining treatments of Sodium azide and Gamma rays, Shelke (2013) in *Brassica campestris* with the use of chemical mutagens EMS and sodium azide and More (2016) in *Brassica napus* L. cv. Exel by using chemical and physical mutagens.

Conclusions

In present work, mutagenic treatments induce changes in mitotic chromosomes in *Linum usitatissimum.* Both chemical and physical mutagens induce different frequencies of mitotic index, abnormal chromosomes viz. single and multiple bridges, laggards, precautious chromosomes, clumped metaphases, clumped anaphases and sticky chromosomes. Frequencies of abnormal cells in 18hrs dry 0.03% SA and 18hrs.PSW + 0.03% SA and 18hrs. PSW + 0.03% showed significant increase in abnormal cells. Gamma rays of 400 Gy. showed marked increase in abnormal cells as compared to other dose. This indicates that sodium azide, ethylmethane sulphonate as well as gamma radiations can be utilized for quality and quantitative variation improvement in *Linum usitatissimum* breeding programme.

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Table 1 - Effect of different concentrations of sodium azide and Ethyl Methane Sulphonate Dry Seed treatments on mitotic chromosomes in *L. usitatissimum* var. PKV NL – 260

Treatment	TCO	NDC	MI	AB	AB %
18hr. Dry Control (SA)	1954	193	0.1	0	0
18hr. Dry 0.01% SA	1030	297	0.28	5	0.5
18hr. Dry 0.02% SA	1240	236	0.19	13	1.1
18hr. Dry 0.03% SA	1172	269	0.22	31	2.6
18hr. Dry 0.01% EMS	1690	182	0.11	21	1.2
18hr. Dry 0.02% EMS	1072	180	0.16	20	1.9
18hr. Dry 0.03% EMS	1704	126	0.07	21	1.2

SA – Sodium Azide, EMS - Ethyl Methane Sulphonate, TCO - Total Cells Observed; NDC- No. of Dividing Cells; MI- Mitotic Index; AB - Abnormality; AB % - Abnormality percent.

Table 2: Effect of different concentrations of sodium azide and Ethyl Methane Sulphonate Presoaked Seed treatments on mitotic chromosomes in *L. usitatissimum* var. PKV NL – 260

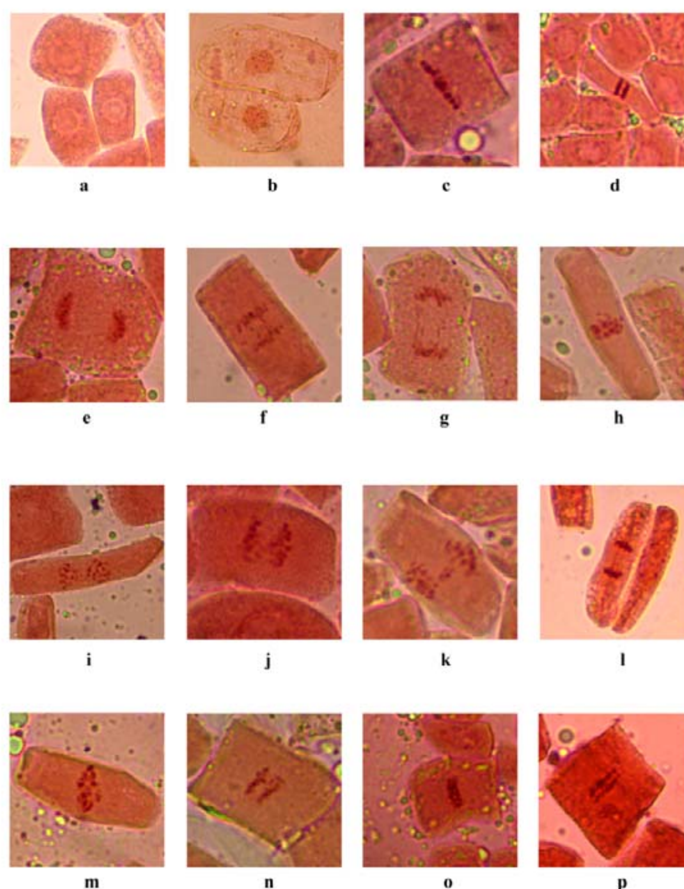
Treatment	TCO	NDC	MI	AB	AB %
18hr.PSWControl (SA)	992	90	0.09	0	0
18hr. PSW + 6hr.0.01% SA	1264	246	0.19	17	1.4
18hr. PSW + 6hr. 0.02% SA	1499	311	0.2	15	1
18hr. PSW + 6hr. 0.03% SA	**1045**	**226**	**0.21**	**24**	**2.3**
18hr. PSW + 6hr.0.01% EMS	1031	153	0.15	25	2.4
18hr. PSW + 6hr.0.02% EMS	1372	128	0.09	33	2.4
18hr. PSW + 6hr.0.03% EMS	**1696**	**145**	**0.08**	**27**	**2.6**

SA – Sodium Azide, EMS - Ethyl Methane Sulphonate, TCO - Total Cells Observed; NDC- No. of Dividing Cells; MI- Mitotic Index; AB - Abnormality; AB % - Abnormality percent

Table 3: Effect of different doses of gamma rays on mitotic chromosomes in *L. usitatissimum* var. PKV NL – 260

Treatment	TCO	NDC	MI	AB	AB %
Control	1753	208	0.12	0	0
400Gy	1261	112	0.09	30	2.4
500Gy	1642	135	0.08	27	1.6
600Gy	1856	83	0.05	23	1.2
700Gy	1374	87	0.06	20	1.5
800Gy	1931	271	0.14	26	1.4
900Gy	1608	145	0.09	23	1.4

Gy- Gray, TCO - Total Cells Observed; NDC- No. of Dividing Cells; MI- Mitotic Index; AB - Abnormality; AB % - Abnormality percent.



a to e - Normal Mitotic Phases; a - Interphase, b - Prophase, c - Metaphase, d - Anaphase e - Telophase. f to p - Mitotic Abnormalities; f,g - Anaphasic Bridges and Laggards, h- Clumped Prophase, i,j, - Multiple Chromosomal Bridges, k,l - Double Chromosomal Bridges, m - Clumped Metaphase, n - Laggard in Anaphase, o,p - Sticky Anaphases.

Plate I - Microphotograph showing different mitotic stages and Mitotic abnormalities in root tips of Linum usitatissimum var. PKV NL - 260

Ethyl Methane Sulphonate Induced Early Flowering Mutations in *Brassica napus* L. Cv. Excel

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Abstract: Cultivation of *Brassica napus* cv. Excel takes more than 81 days to flowering and more than 180 days to mature which is not suitable for the Vidarbha region having short winter days and does not fit in the crop cycle of Vidarbha agroclimate. Hence, induced mutation programme undertaken to obtain early flowering mutant of *B. napus* cv. Excel. Pure and homogenous seeds of *Brassica napus* L. cv. excel. were exposed to different concentrations/doses of chemical and physical mutagens i.e., Ethyl methane sulphonate (EMS), Sodium azide (SA) and gamma radiations. Dry and pre-soaked water seeds in DNA – synthetic phase were used for the mutagenic treatments. Different concentrations of mutagens were determined on the basis of LD_{50} seed germination studies. Mutagen treated seeds were used to raise M_1 generation. M_1population was harvested plant wise and M_2population was raised with the use of seeds of M_1population. M_2population was screened for early flowering mutation. M_3population was screened to confirm true breeding nature of early flowering mutant's progeny in successive generations. 18hrs. dry EMS 0.004% induced single early flowering plant which breed true in M_2, M_3 and M_4 generations. Early flowering true breeding mutant of *B. napus* which flowered in 54 days where as control flowered in 81 days. Mutant also had higher oleic acid (38.03%) and linolenic (14.66%) while lower percentage of erucic acid (0.03%). Control had 34.76% Oleic acid, 10.31% linolenic and0.28% erucic acid. Oil percentage increased to 52.56% and defatted meal contains 38.08% protein has been found to be increased in seeds of early flowering mutant, over control (40.38% oil and 34.56% seed protein). Glucosinolate content found to be decreased in early flowering mutant (16.29µmole/gm.) over control (18.34µmole/gm.). This mutant is characterized canola quality "00" characteristics with high oil and protein percentage hold good potential of cultivation in Indian agroclimatic condition.

Key Words: *Brassica napus* L. cv. Excel, Sodium azide, Ethyl methane sulphonate, Gammaradiations, Early flowering mutant.

Introduction

India has fifth largest vegetable oil economy in the world and second largest agricultural commodity after cereals occupying 14% of gross cropped area. India ranks third in rapeseed-mustard production all over the world after China and Canada with 12 per cent of world's total production. India holds a leading position in rapeseed-mustard economy of the world with 2nd and 3rd rank in area and production respectively. This crop accounts for near about one-third of the oil produced in India, making it the country's key edible oilseed crop. Due to the gap between domestic availability and actual consumption of edible oils, India has to resort to import of edible oils with an expected demand for edible oils at more than 20 mt in 2014-15. About 6.8 m ha is occupied under rapeseed- mustard 2006-07 and nearly 30.7% area is rainfed. The Indian cultivars, due to high content of erucic acid and glucosinolates, have limited preference in international market (Kumar *et al*., 2009).

B. napus is amphidiploids and is comparatively of recent origin (Olsson, 1960). It is uncertain to maintain if *B .napus* is found wild or not, since wild forms of this crop are difficult to find (Hinata and Prakash, 1984). However,if wild *napus* exists, it must be a European-Mediterranean species that originated in the area of overlap between *B. oleracea* and *B. campestris*(McNaughton, 1976). Though the origins of *B. napus* are ambiguous, these are proposed to involve natural inters pecific hybridization between thetwo diploid species, *B. oleracea* (n = 9) and *B. rapa* (n = 10) (U, 1935). The parental origins of *B. napus* were also investigated using six microsatellite markers located in the chloroplast genome by Allender and King (2010).

Mutagenesis is used to induce genetic variability in a great number of crops because of some reasons such as – it is simple, relatively cheap to perform, applicable to all plant species and equally useful on small and large scale (Swaminathan, 1995; Siddiqui and Khan, 1999). Essential three phases of mutation breeding are generation of genetic variability, selection of useful genotypes and comparative tests to demonstrate superiority of selected genotypes. Mutation induction is an effective tool to improve the genetic variation available to plant breeders, particularly for traits with a very low level of genetic variation (Szarejko and Forster, 2007).

Brassica napus is the crop of cold region they required very low temperature for their growth and development. It is not the traditional crop of these regions and requires long photo period for flowering, cool temperature for a longer period for its better growth and development which restrict cultivation of *B. napus* L. in India. Attempts to introduce the exotic types in India failed due to their extreme late

maturity. Cultivation of *B. napus* in Indian ecological condition is a difficult task. The acclimatization of exotic *B.napus* L. lines to traditional mustard growing areas has very limited scope for commercial exploitation in India as they are highly photo and themo-sensitive and requires a specific long days for flowering. *Brassica napus* cv. Excel has good yield potential with "00" quality characteristics and tolerant to insect and pest. Keeping this view, present investigation was undertaken to produce early flowering line in *B. napus* cv. excel through induced mutations which can be substantially suitable to in tropical and sub-tropical regions of India especially in Vidarbha region.

Materials and Methods

Pure and homogenous seeds *Brassica napus* L. cv. excel. Initially multiplied at Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati for 10 years in selfed condition were exposed to different concentrations/doses of chemical and physical mutagens i.e., (EMS), (SA) and gamma radiations. Dry and pre-soaked water seeds in DNA – synthetic phase were used for the mutagenic treatments. Different concentrations of mutagens were determined on the basis of LD_{50} seed germination studies. Dry Seeds were treated with EMS concentrations of 0 (control), 0.004%, 0.006% and 0.008% (v/v) for 6 hrs. period. Twelve and eighteen hour pre-soaked water seeds were treated with EMS concentration of 0 (control), 0.04%, 0.06%, 0.08% and 0 (control), 0.03%, 0.06%, 0.09% (v/v) solutions respectively for 6 hrs. period. Treatments of sodium azide used were 0 (control), 0.005%, 0.007% and 0.009% (w/v) solutions for dry seeds, 0 (control), 0.03%, 0.06%, 0.09% (w/v), 0 (control), 0.04%, 0.06%, 0.08% (w/v) solutions for 6 hrs. period and seeds used were twelve and eighteen hour pre-soaked seeds in DNA – synthetic phase respectively. Seeds were rinsed for 30 min. with running tap water to completely remove mutagens. Dry seeds were exposed to gamma radiations doses of 100 Gyr., 200 Gyr., 300 Gyr., 400Gyr., and 500 Gyr. All the treatments along with control were carried out in triplicates.

Mutagen treated seeds were used to raise M_1 generation. M_1population was harvested plant wise and M_2population was raised with the use of seeds of M_1 population.Total 12297 M_2 plants of EMS treated were grown from M_1 plants on plant to row progeny basis in the field at a distance of 30 cm. between rows and 10 cm. between plants in winter season at experimental field at Govt. Vidarbha Institute of Science and Humanities, Amravatiand Farmers field at Village Masdi Dist. Amravati and screened for morphological mutations. The mutants affecting flowering, maturity, seed yield and other morphological character were identified and selfed. M_2 population was screened for early flowering mutation. Selection was made from M_2 plant with early flowering including yield parameters with quality background for study breeding nature of mutant. $M_3$and M_4population was screened to confirm true breeding nature of early flowering mutant. Biochemical parameters analyzed as oil percentage Soxhlet method. Total protein content in the individual plant seeds was estimated by following micro-Kjeldhal method (AOAC, 1970) using Markham steam distillation apparatus and Fatty acids, oil, protein and Glucosinolate by Near Infra-Red Spectroscopy (NIRS).

Results and Discussion

M_2 population was screened for early flowering mutation of EMS, SA and gamma radiations early flowering mutation was recorded in EMS treatments; therefore data of only EMS treatment is discussed. Only a single early flowering mutant plant with a frequency of 0.07% was recorded from 0.004% concentration of 18hrs. Dry EMS. Treatment remaining all concentrations used fail to induce early flowering mutation (Table 1, Fig. 1, Plate I). In M_3 generation early flowering mutant shows true breeding (Plate II). Single plant obtained in EMS in M_2 generation of the invention has been bred true in successive generations. Line shows that improvement over the preceding generation in one or more desired characteristics. The induction of early flowering mutations using physical or chemical mutagens involving cultivars having desired traits can be used to produce *B. napus* early flowering line of the invention.

In M_2 generation of 18hrs, dry EMS treatment this mutant was found to initiate flowering after 54 days which was 23 days earlier than control (77 days) while, in M_3 generation it was found to give 50% flowering after 54 days which was 27 days earlier than control (81 days). Control *Brassica napus* cv. Excel had a height of 169±1.13 cm. In M_1 generation plant height was decreased to 147 cm. and further more decreased in M_3 generation to143±0.72 cm. Control had 5 ± 0.36 branches with 68±2.53 siliquae on main axis, 4.9 ± 0.20 cm long siliqua with 4 ± 0.04 mm radius. This mutant shows increased in branches per plant (6) in M_1 and M_2 generation. Number of siliquae on main axis were decreased in M_2 (59) and M3 (56±2.99) generation as compared to control (68±2.53). Near about equal increased in length and breadth of siliqua was noticed in M_2 (6.2 cm. long siliqua having 5 mm. of radius) and M_3 (6.1 ± 0.24 cm. long siliqua having 5 ± 0.04 mm. of radius) generation over control. Control had 22 ± 1.89 seeds per siliqua while seeds per siliqua enhanced to 31 seeds per siliquae in M_2 generation and 29 ± 1.40 seeds per siliqua in M_3 generation. These can be attributed to better conversion of ovule in to seeds with early flowering. In control 185±19.27 siliquae per plant were noted which was increased in M_2 generation (208) and decreased in M_3 generation (158 ± 16.72) of early flowering mutant. Weight of 1000 seeds of *Brassica napus* cv. Excel was 2.61 ± 0.06 gm. This improved to 3.10 gm and 3.28 ± 0.11 gm in M_2 and M_3 generations, respectively. Early flowering

mutant recorded 50% flowering in 54 days as compared to parental line 81 days; which was 27 days earlier which bred true in successive mutation generation after selfing was found to gives 12.33 gm of yield per plant; 36.20% seed oil and 42.81% seed protein in M2 generation and 11.71±1.42 gm of yield per plant with 40.2% seed oil and 39.6% seed protein in M3 generation which is higher than control (4.96 ± 0.71 gm seeds per plant with 37% seed oil and 44.8% seed protein). Control plant had Blackish brown seed coat colour which changes to luster black in M_2 and M_3 generation (Table 2).

Biochemical analysis of Early flowering mutant in M_3 generation (NIRS)

Data on fatty acid analysis on NIRS of M_3 mutants tabulated in Table 4.32 shows that mutant early flowering B. napus L. cv. Excel had higher oleic acid (38.03%) and linolenic (14.66%) while lower percentage of erucic acid (0.03%) than control (34.76% Oleic acid, 10.31% linolenic and 0.28% erucic acid. Oil (52.56%) and seed protein(38.08%) has been found to be increased in seeds of early flowering mutant, over control (40.38% oil and 34.56% seed protein). Glucosinolate content found to be decreased in early flowering mutant (16.29 µmole/gm) over control (18.34 µmole/gm) (Table 3, Fig. 3).

Induction ofearliness is the most important objective of mutation breeding in some crops. Early maturing mutants in rice (Wang *et al*., 1991), castor (Ankineedu*et al*., 1968), and soybean (Kawai *et al*., 1991) have been released as varieties for commercial cultivation. In oil seed *Brassica* crops several early flowering/ maturity have been reported (Pawar*et al*., 1991 and Malode, 1995). Early flowering mutants in *Brassicas* have been previously obtained by many workers like Sathawane (1993); Malode, (1995); Landge and Khalatkar (1995); Shelke (2014) and Ambavane *et al.* (2015).

Conclusions

The average height of Control and early flowering mutant was 169 cm and 143 cm respectively. In field trials, early mutant line showed dwarfism and better yield than conventional varieties 11.72 gm. per plant. The M_3 selections from M_2which bred true exhibits similar characters flowered in 41 days and attained maturity synchronously and were unique and distinct for these characters. This selection depicted a distinct advantage of 27 days earliness. The true breeding behavior of these lines was confirmed in M_4 generation. The additional criteria used was seed yield (11.71 g per plant) and oil content (above 52.56 %). The fatty acid quality parameters viz. Oleic acid (38.03%), Linoleic acid (14.66%) and Erucic acid (0.03%) at 7.71% seed humidity and glucosinolate content (16.29µmole/g) and protein (38.08%) was also analyzed by NIRS which was found to be better than parent. Large scale trial at farmer's field at Masdialso confirms the fact that these plants can be grown in Vidarbha regions as a commercial quality crop. In future multi-location field trials at different ecological condition to tropical and sub-tropical regions having varied temperature condition (range of about 4°C to 46°C). This line could be suitable for other places in India as well as other countries like Australia, Bangladesh, China, Canada, Europe and USA.

Table 1: Data on frequencies of early flowering mutants induced by EMS in M_2 generation of *B. napus*. L. cv. Excel

Mutants ↓	**18hrs. Dry EMS.**			**12hrs. PSW +6hrs. EMS.**			**18 hrs. PSW + 6hrs. EMS.**	
Treatments →	**0.004%**	**0.006%**	**0.008%**	**0.04%**	**0.06%**	**0.08%**	**0.03%**	**0.06%**
Total plants screened / harvested	1453	1650	1506	1624	1420	1536	1428	1680
Total No. of M_2 mutations	11 (0.75)	6 (0.41)	00	15 (0.92)	15 (1.05)	7 (0.46)	5 (0.35)	1 (0.06)
Early flowering mutant	1 (0.07)	00	00	00	00	00	00	00

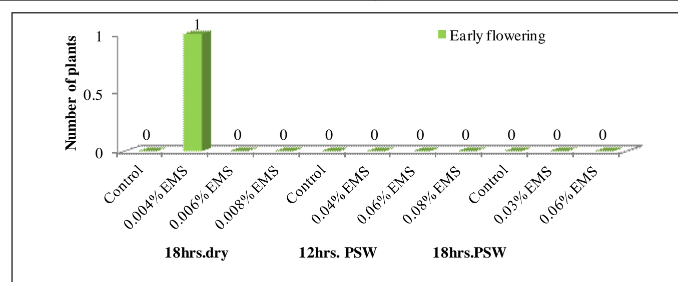

Fig. 1: EMS induced early flowering mutant M_2 generation of *B. napus* L. cv. Excel

Table 2: Data on effect of mutagen on morphological and biochemical characteristics of B. napus L.cv. Excelin M2 and M3 Generation

Parameters	Control (Mean ± SE)	Early flowering Mutant	
		M_2 generation	M_3 Generation (Mean ± SE)
Days to flower 50% plants	81	55	54
Plant height (cm.)	169±1.13	147	143±0.72
Number of branches	5 ± 0.36	6	6 ± 0.65
Siliquae on main axis	68±2.53	59	56±2.99
Length of siliqua (cm.)	4.9 ± 0.20	6.2	6.1± 0.24
Breadth of sixth siliqua (mm.)	4 ± 0.04	5	5 ± 0.04
No.of seeds /siliqua	22 ± 1.89	31	29± 1.40
Siliquae / Plant	185±19.27	208	158 ± 16.72
Single seed weight (gm)	0.0030±0	0.0037	0.0039±0
100 seed weight (gm.)	0.27±0.005	0.31	0.33± 0.01
1000 seed weight (gm.)	2.61 ± 0.06	3.10	3.28± 0.11
Yield / plant (gm.)	4.96 ± 0.71	12.33	11.71 ± 1.42
Seed colour	Blackish brown	Luster black	Luster black
Oil content (%)	37.4	36.20	40.2
Seed protein (%)	44.8	42.81	39.6

Table 3: Range in fatty acids composition, oil, protein and glucosinolate of B. napus cv. Excel early flowering mutant in M_3 generation (NIRS)

Type of sample	Fatty acid composition (%)				Oil (%)	Protein (%)	Glucosinolate (µmole/g)
	H2O	C18:1	C18:3	C22:1			
Control	6.15	34.76	10.31	**0.28**	40.38	34.56	**18.34**
Early flowering mutant	7.71	38.03	**14.66**	0.03	**52.56**	38.08	16.29

H_2O – Moisture, **C18:1** – Oleic acid, **C18:3** – linolenic, **C22:1-** erucic acid.

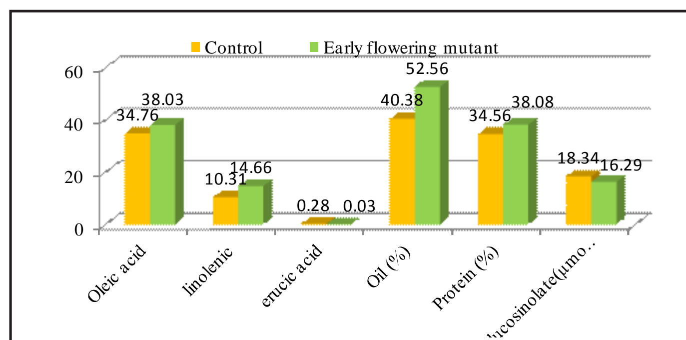

Fig. 2: Range in fatty acids composition, oil, protein and glucosinolate of *B. napus* cv. Excel early flowering mutant in M_3 generation (NIRS)

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A. Photograph of Early flowering mutant at first day

B. Early flowering mutant after few days of growth

C. Early flowering mutant at harvesting stage

Plate I: Photograph showing early flowering mutant of *Brassica napus* L. cv. Excel in M_2 generation.

A.

B.

C.

D.

E.

F.

A. - Early flowering mutant progeny. **B.** and **C.** - Different views of early flowering mutant progeny at flowering stage. **D.** and **E.** - Different views of early flowering mutant progeny at maturity stage. **F.** - Early flowering mutant progeny at harvesting stage.

Plate II: Photograph showing early flowering mutant progeny of *Brassica napus* L. cv. Excel in M_3 generation.

Phytochemical Screening and Antioxidant Activity of Various Extract of *Hypnea musciformis*

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Seaweeds, constituting an important renewable marine resource, grow in the shallow water of sea wherever suitable substrata are available.Seaweeds are important primary producers of organic compounds regarded to the base of food chain in the oceans. It has been used as antioxidant, anti-mutagen, anticoagulant and antitumoragent. The red seaweed, *Hypnea musciformis* is bushy species with apical hooks abundantly found on intertidal rocks and red algae are distinguished by their color which is pinkish red. Considering the medicinal importance of seaweeds, the present investigation was carried out on qualitative phytochemical screening and DDPH radical scavenging activity, total antioxidant activity (FRAP method), reducing power assay and total phenol content of various extracts of *Hypnea musciformis*were determined. Phytochemicals were extracted using solvents like aqueous, ethanol and HCL. Phytochemical analysis showed positive results for the presence of glycosides, saponins, tannins, carbohydrates, proteins, flavonoids, phenolic compounds and steroids. Among the various extracts methanolic extracts revealed better DPPH activity, ethanolic extracts showed highest phenolic content and good reducing power and benzene extract found to have highest total antioxidant activity.

Key Words: *Hypnea musciformis,* DDPH radical scavenging activity, reducing power assay, antioxidant activity, phenolic content.

Introduction

From the ancient times, the seaweeds have been closely associated with human life and are being exclusively used in numerous ways. They used as a source of food, feed, fertilizer, medicine and chiefly for economically important phycocolloids (Champman, 1981). In recent years, many marine resources have attracted attention in the search for bioactive compounds to develop new drugs and health foods. Today, seaweed is part of the habitual diet in many Asian countries. There is popularly increase in consumption of seaweed in western cultures, due to the influx of the Asian cuisine as well as notional health benefit.Seaweeds are the extraordinary sustainable resources in the marine ecosystem which have been used as a source of food, feed and medicine. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from algae (Dhargalkar and Neelam, 2005). Algae play most important role in aquatic environment because they are the "primary producers'' of organic matter in aquatic ecosystem because of their photosynthetic activity. Animal life in water mainly depends on algae. They form the primary source of food and energy from them. Seaweeds belong to a group of plants known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient, pigments and chemical composition. Seaweeds are rich in antioxidants like polyphenol, carotenoids, pigments, polysaccharides and several enzymes. Seaweed produces a diverse array of compounds that function as chemical defence systems facilitating their survival in extremely competitive environments. About 15,000 novel compounds are isolated from algae many of which have been shown to have bioactive properties (Cardozo *et al.,* 2007). Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from seaweeds and used in medicine and pharmacy (Siddhanta *et al*., 1997). Red and brown algae are traditionally consumed in the Eastern Countries as a part of daily diet. Human consumption of red algae represents 33%, while brown algae represent 66.5% mainly in Japan, China and Korea (Abd EI Mageid *et al.,* 2009). Seaweeds are nutritionally valuable as fresh or dried vegetables and certain edible macroalgae contain significant quantities of protein, lipids, minerals and vitamins (Manivannan *et al.*, 2008). Edible seaweeds area rich source of dietary fiber, minerals, and proteins (Kuda *et al.,* 2002).Seaweeds are excellent source of carbohydrate, protein enzymes, fiber, vitamins including: A, B, B1, B2, B6, niacin and C, and are rich in iodine, potassium, iron, magnesium and calcium (Raja *et al*., 2013). Like terrestrial plants, Seaweeds have protective enzymes and antioxidative molecules such as ascorbic acid, tocopherols, carotenoids, phospholipids, chlorophyll related compounds bromophenols, amino acids etc. Therefore, seaweeds are a potential source of novel antioxidants.

The red algae *Hypnea musciformis* (Wulfen) Lamouroux is pinkish red in colour with bushy thallus, which occur luxuriantly during November to April growing on intertidal rocks (Jha *et al*., 2009).

Considering immense medicinal properties of seaweeds, the present study has been carried out along the coast of Sindhudurg, Maharashtra to analyse the phytochemical constituents and antioxidant activity among the various extracts of *Hypnea musciformis*.

Material and Methods

Collection of seaweeds: The seaweed *Hypnea musciformis* was collected from different beaches of Sindhudurg districts. The plant sample was identified by referring Deodhar (1987) and Jha *et al.,* 2009.

Preparation of the sample: For the screening of phytochemicals fresh sample is used. Five grams of the fresh sample was weighed and homogenized with 50 ml of water, HCL (1%) and ethanol separately. The extracts was boiled for 1 hour, cooled and filtered. The filtrate was used to screen for the presence of phytochemicals using standard procedure (Harborne, 1973).

Preparation of the organic extract of the sample for antioxidant analysis: The shade dried sample of five seaweeds ground to coarse powder. Twenty gram of powder weighed and wrapped in Whatmann No.1 filter paper and successively extracted with 200ml of different solvents such as petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol with their increasing order of polarity by soxhlation for 12-24 hours. The Extracts were analysed for the antioxidant activity using standard procedure (Thoudam *et al*., 2011).

Dpph Radical Scavenging Activity:

The radical scavenging effect of seaweed extracts was determine by using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) method. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 ml of the solution mixed with 1.0 ml of seaweed extracts in methanol with different concentrations (0.5-2.5 mg/ml). The reaction mixture was mixed thoroughly and kept in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Blank is methanol, control is DPPH without incubation. BHT and BHA were used as references (Liyana-Pathiranan and Shahidi, 2005). The percentage of antioxidant activity was calculated by the following formula,

$$\% \text{ scavenging} = (OD_{Blank} - OD_{sample})/OD_{Blank} * 100$$

Total Antioxidant Activity (frap Method):

Total antioxidant activity of five selected seaweeds was determined by FRAP (ferric reducing antioxidant potential) assay. The stock solutions included 300 mM acetate buffer (3.1g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCL, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 30 ml acetate buffer, 3 ml TPTZ, and 3 ml $FeCl_3.6H_2O$. The temperature of the solution rose to 37^0C before analysis. Seaweed extracts (150 µL) was allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. The coloured product (Ferrous tripyridyltriazine complex) was read spectrophotometrically at 593 nm and standard curve was linear between 100 and 1000 µM $FeSO_4$. Results were expressed in µM Fe (II)/g dry mass and compared with that of BHT and BHA (Kasote *et al*., 2011). Total antioxidant activity was calculated by the following formula,

$$FRAP = O.D / Slope$$

Reducing Power Assay:

The reducing power of seaweeds extracts was assessed by using various concentrations i.e. 50-250 µL/ml of seaweeds extracts in methanol (10 mg/ml) and reference standard BHT and BHA (1 mg/ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50^0C for 20 minutes and (10%, 2.5 ml) trichloroacetic acid was added. This solution is centrifuged at 3000 rpm for 10 minutes. Then upper layer of the reaction mixture (2.5 ml) is mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%), and absorbance measured at 700 nm. DW is blank. Increased absorbance of the reaction mixture indicates the increased reducing power (Kasote *et al*., 2011).

Determination of Total Phenolic Content:

Total phenolic content in seaweeds algal extracts were determined by using modified Folin-Ciocalteu reagent method. According to (Zahin *et al*., 2009), gallic acid is a standard phenolic compound. The reaction mixture contained various concentrations of the extracts and Folin- Ciocalteu reagent. To 500 µL (10 mg/ml) of seaweed extracts in methanol, 2.5 ml of 1:10 dilution of Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (7.5% w/v) were added and mixed thoroughly and incubated at 45^oC for 15 minutes. Same procedure is followed for other seaweeds extracts with petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol respectively. The absorbance measured at 765 nm. Blank is all without the extract. The concentration of total phenolic content in the seaweed extracts was determined as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g DW) (Zahin *et al*., 2009).

Total phenolic content = O.D/Slope.

Result and Discussion

Qualitative analysis for the presence of phytochemical:

The preliminary phytochemical analysis showed the presence of phytoconstituents such asglycosides, saponins, tannins, carbohydrates, proteins, flavonoids, phenolic compounds and steroids. Different extracts of ***Hypnea musciformis*** were subjected to qualitative analytical tests for the various plant constituents such as alkaloids, flavanoids, glycosides, phenolic compound, saponins, steroids, tannins, carbohydrates, proteins and fats. Table 1 shows the phytochemical constituents of various extracts of *Hypnea musciformis.*

Table 1: Phytochemical constituents of various extracts of*Hypnea musciformis*

Sr. no.	Seaweed extracts	Phytochemical Constituents									
		1	2	3	4	5	6	7	8	9	10
1.	Aqueous extract	-	-	+	-	+	-	+	+	+	-
2.	Ethanol extract	-	+	-	+	+	+	+	+	-	-
3.	HCl extract	-	-	+	-	+	-	+	-	-	-

+ Presence;- Absence; A= Aqueous; H=Hcl

Flavonoids have been exerting antimicrobial activity, cytotoxicity, anti-inflammatory activity, enzyme inhibition, anti- allergic activity, antioxidant activity and anti-tumor activity (Tapas *et al*., 2008). Seaweeds can provide a dietary alternative due to its nutritional value and its commercial value can be enhanced by improving the quality and expanding the range of seaweed-based products (Parthiban*et al*., 2013). Phenolics compounds compound are a largest complex group of chemical constituents found in plants. They have important role as defence compound and possess several properties beneficial to humans and its antioxidant activity protect against free radical - mediated diseases (Saxena *et al*., 2013).

DPPH Radical Scavenging Activity:

DPPH has been used extensively as a stable free radical to evaluate reducing substance and also useful reagent for investigating free radical scavenging activity (Cotelle*et al*., 1996). Figure 1 indicates the DPPH radical scavenging activity of *Hypnea musciformis* in different solvent extract with various concentrations. The results showed that the methanolic extracts of *Hypnea musciformis* showed maximum potent free radical scavenging activity followed by chloroform. Petroleum ether showed less scavenging activity followed by ethanol. The DPPH radical scavenging activity increased as the concentration increased in all the extracts. Many report have been recently described the ability to scavenging DPPH free radical on seaweeds. Heo *et al*., (2006) also showed that each sea organic extract have positive effect in DPPH free radical scavenging. Ragan and Glombitza (1986) reported the radical scavenging activity of seaweeds to be mostly related to their phenolic contents.

Total antioxidant activity (FRAP assay) of *Hypnea musciformis*:

Total antioxidant activity was evaluated from petroleum ether, chloroform, ethyl acetate, ethanol and methanol extracts of *Hypnea musciformis.* Table 2 indicates the total antioxidant activity of various extracts of *Hypnea musciformis.*In FRAP assay, highest total antioxidant activity of Hypnea musciformis showed by benzene extracts followed by ethanolic extracts, ethyl acetate extracts, methanolic extracts and petroleum ether extracts showed lowest antioxidant activity. The higher the FRAP value the greater is the antioxidant activity (Chorage *et al*., 2013). Estimation of the total antioxidant activity of *Polysiphoniaurceolata*showed that the total antioxidant activity was maximum in the methanolicextract (Dhargalkar and Neelam, 2005).

Reducing power assay:

The reducing power of the different extract of the seaweeds was analysed. Increase in the O.D. determines the increase in the reducing power. Figure 2 indicates reducing power of *Hypnea musciformis*Reducing power of *Hypnea musciformis* showed highest reducing power in ethanol extracts followed by methanolic extracts, petroleum ether, chloroform extracts, benzene extracts and ethyl acetate extracts. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al*., 1995). The reducing power property indicated the *Padina sp.* and *Eucheumacottanii*extracts shows higher antioxidant compounds in the seaweeds (Yen and Chen 1995).

Total phenolic content:

Total phenolic content was evaluated from petroleum ether, chloroform, ethyl acetate, ethanol and methanol extracts of *Hypnea musciformis.* Figure 4 indicates total phenolic content of various extracts of *Hypnea musciformis.*Ethanol extracts of *Hypnea musciformis* showed highest phenolic contents and petroleum ether extract, shows lowest phenolic contents. A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical-scavengers (Kumar *et al*., 2008). Total phenolic compounds found in plants, have several biological effects including antioxidant, antiapoptosis, anti-aging, anti-carcinogen and considered for their important dietary roles as antioxidant and chemoprotective agents (Han *et al*., 2007).

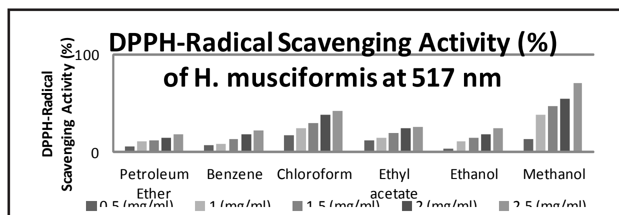

Figure 1: DPPH-Radical Scavenging Activity (%) of various extracts Hypnea musciformis

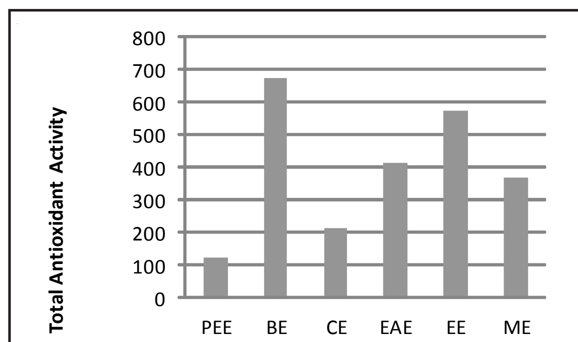

Figure 2: **Total antioxidant activity (FRAP) of seaweeds at 593 nm**

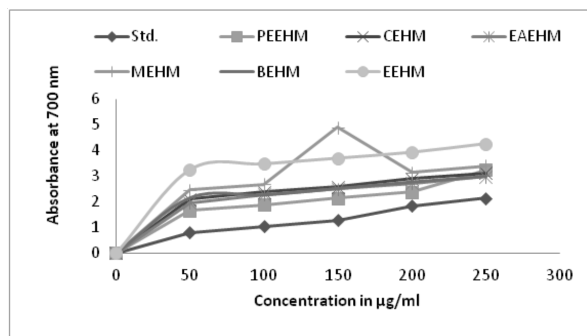

Figure 3: Reducing power of standard and different solvent extracts of Hypnea musciformis

Std.- standard; PEEHM- petroleum ether extract of *H. musciformis*; CEHM- chloroform extract of *H. musciformis*; EAEHM- ethyl acetate extract of *H. musciformis*; MEHM- methanol extract of *H. musciformis*; BEHM- benzene extract of *H. musciformis*; EEHM-ethanol extract of *H. musciformis*.

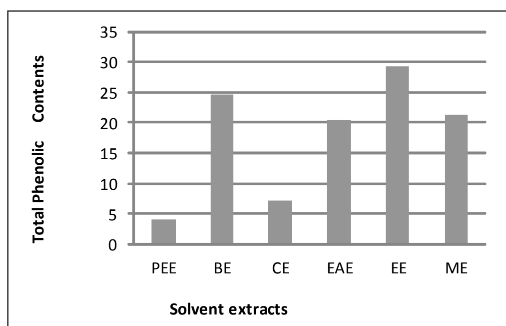

Figure 4: Total phenolic contents (mg gallic acid/100 g dried seaweed)

PEE- petroleum ether extract; BE- benzene extract; CE- chloroform extract; EAE- ethyl acetate extract; EE- ethanol extract; ME-methanol extract

Conclusion

From the study it may be concluded that *Hypnea musciformis* is good source of phytochemicals. Methanolic extract showed the highest DPPH activity, benzene extract showed highest total antioxidant activity and ethanolic extract revealed higher reducing power and total phenolic content among various organic extracts of *Hypnea musciformis.* Like commercial antioxidants (vitamin E and BHT) *Hypnea musciformis* is promising seaweed for its antioxidant activity which can contribute to human health should be explored in future studies.

Acknowledgment

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Biosurfactant Production From *Pseudomonas* Sp Isolated From Petrol Pump Soil

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Abstract: *Pseudomonas* sp. was isolated from diesel contaminated soil. The ability of the isolate to produce biosurfactant was determined by using mineral salt medium with different carbon substrates like coconut oil, sesame oil, diesel oil and hexadecane. Biosurfactant production in culture broth was assessed using emulsification index and surface tension analysis. Emulsification index ($E_{24\%}$) for coconut oil, sesame oil, diesel oil and hexadecane broths was found to be 80%, 54%, 68% and 62% respectively. The surface tension of the cell free broths was found to be reduced to 30.25 mN/m, 35.40 mN/m, 32.45 mN/m and 46.24 mN/m in coconut oil, sesame oil, diesel oil and hexadecane containing broths from 62.4 mN/m, after five days of growth. The yield of crude biosurfactant was maximum in medium supplemented with sesame oil (1.31 g/L) as compared to coconut oil (0.85g/L), diesel oil (0.39 g/L) and n-hexadecane (0.21g/L).

Keywords: Biosurfactant, n-hexadecane,emulsification index, surface tension, *Pseudomonas* sp.

Introduction

Surfactants and emulsifiers are widely used in the pharmaceuticals, cosmetics, petroleum and food industries (Haddad, 2008; Nayak *et al.*, 2009). Most of these compounds are of petroleum origin, which are not easily biodegradable and can be environmentally hazardous. Biosurfactants are heterogeneous group of surface-active compounds produced by a wide variety of microorganisms (Kumar, 2008). Microorganisms have been reported to produce several classes of biosurfactants such as glycolipids, lipopeptides, phospholipids, neutral lipids or fatty acids and polymeric biosurfactants (Cooper, 1986; Kosaric, 1993). These compounds are produced during the growth of microorganisms on water-soluble and water-insoluble substrates (Cooper and Zajic, 1980). Biosurfactants forms an emulsion with hydrocarbons thereby facilitating increased contact between hydrocarbons and the degrading bacteria. Biosurfactants are readily biodegradable and hence are environment friendly when compared to the chemical biosurfactants. These can be produced in large amounts by microorganisms and at least in some instances, they might replace the traditional synthetic surfactants (Banat *et al.*, 2000; Sarubbo *et al.*, 2006).

Materials and Method

Enrichment, isolation and identification of bacterium:

A standard enrichment technique was used to isolate hydrocarbon degrading microorganisms from petrol pump soil. 5.0 grams of soil sample was transferred to 250 ml flask containing Bushnell-Haas medium with 2% (vv^{-1}) diesel oil as carbon source. Flasks were incubated at room temperature on a rotary shaker (200 rev min^{-1}) for 5 days. After 5 days 0.5 ml of the culture was transferred to fresh medium and re-incubated for further 5 days. After 5 cycles of such enrichments, 0.1 ml of culture was plated on Bushnell-Haas medium plates containing 0.5% diesel as sole carbon source. The organism was identified as *Pseudomonas* sp using biochemical tests like gram staining, oxidase test, catalase test, gelatin liquefaction test, motility test and morphological appearance.

Media and cultivation conditions:

The isolated organism *Pseudomonas* sp was grown overnight in nutrient broth at room temperature and this was used as the preinoculum at 2% (v/v). For biosurfactant production the following mineral salt medium was utilized (values expressed per 1L): KNO_3 (3 g), Na_2HPO_4 (2.2 g), KH_2PO_4 (0.14 g), NaCl (0.01 g), $MgSO_4$ (0.6 g), $CaCl_2$ (0.04 g), $FeSO_4$ (0.02 g), and 1 ml of trace element solution (TES) containing (per 1 L), 2.32 g $ZnSO_4{\cdot}7H_2O$, 1.78 g $MnSO_4{\cdot}4H_2O$, 0.56 g H_3BO_3, 1.0 g $CuSO_4{\cdot}5H_2O$, 0.39 g $Na_2MoO_4{\cdot}2H_2O$, 0.42 g $CoCl_2{\cdot}6H_2O$, 1.0 g EDTA, 0.004 g $NiCl_2{\cdot}6H_2O$ and 0.66 g KI (Makkar and Cameotra, 2002). Growth and biosurfactant studies were performed in three 500 ml flasks containing 100 ml of the medium and 2% hydrocarbons viz. coconut oil, sesame oil, diesel oil and n-hexadecane (one in each flask) at room temperature on a rotary shaker (200 rev min^{-1}) for 5 days. Growth was measured at 600 nm. Experiments were done in t*riplicates.*

Emulsification activity

Emulsification index (E_{24}) was determined using the method described by Kumar *et al* (2008), whereby hydrocarbons (n-hexadecane and diesel oil) and vegetable oils (coconut oil and sesame oil) and cell free culture broths were mixed in equal volume, vortexed vigorously for 2 *minutes and kept undisturbed for 24 hours. The emulsification activity was determined as the percentage*

of height of the emulsified layer (mm) divided by the total height of the liquid column (mm). To study the stability of emulsion, emulsified solutions were allowed to stand at room temperature and emulsification index was analyzed at different time intervals.

Surface activity

The surface tension was determined using Kruss Processor Tensiometer K12 by plate method. All measurements were made on cell-free broths obtained by centrifuging at 12000 rev min^{-1} for 20 minutes (Huszcza *et al.*, 2003; Sharma, 2009).

Extraction of crude biosurfactant

The crude biosurfactants were extracted as per the method described by Makkar and Cameotra (2002). The culture broths were centrifuged at 12000 rev min^{-1} for 20 minutes. Biosurfactant in cell-free broths was precipitated by adjusting the pH to 2.0 using 6 N HCl. Flasks were kept at 4^0C overnight. The precipitate thus obtained was pelleted at 18000 rev min^{-1} for 20 minutes, freeze dried, and weighed.

Results

Bacterial strain isolation and identification

Through four enrichment cycles, bacteria capable of utilizing diesel oil were isolated on Bushnell-Haas medium plates. This strain preliminary identified as *Pseudomonas* sp was found to be well growing on medium containing diesel oil as a carbon source. The biochemical tests found the strain as gelatin positive, oxidase positive, catalase positive, gram negative, motile rods.

Microbial growth and analysis of emulsification activity

Cells were grown in nutrient broth overnight and used as preinoculum in mineral salt medium. During the incubation period of five days, cell density was measured at 600 nm (fig.1). Per day emulsification activity was measured. Emulsification index (E_{24} value) for coconut oil, sesame oil, diesel oil and hexadecane broths was found to be 80%, 54%, 68% and 62% respectively. The values given are on 1st, 4th, and on 5th day respectively. Day wise emulsification index for culture grown on coconut oil, sesame oil, diesel oil and hexadecane is shown in fig.2. The isolate could grow well in all the oil source, however maximum growth and emulsification activity was seen with coconut oil followed by diesel.

Surface tension measurement and Biosurfactant production

The surface tension of the cell-broths was found to be reduced to 30.25mN/m, 35.40mN/m, 32.45mN/m and 46.2 mN/m in coconut oil, sesame oil, diesel & n-hexadecane containing broths from 62.4mN/m, after 5 days of growth. The yield of crude biosurfactant was 1.31g/L in medium supplemented with sesame oil, 0.35g/L in medium supplemented with coconut oil and 0.21g/L in medium supplemented with n-Hexadecane.

Discussion

Bacteria utilizing oil was isolated from petrol contaminated soil. The isolated strain, *Pseudomonas* sp was found to be well growing on Bushnell-Haas medium plates containing diesel as a carbon source. Biosurfactant production was studied using four different carbon substrates *viz.* coconut oil, sesame oil, diesel and n-hexadecane. The growth pattern studies on different hydrocarbons showed that growth of bacteria is more in sesame oil broth as compared to n-hexadecane broth. This suggests that n-hexadecane may have inhibitory effect on bacterial growth. Similar results were reported by Yin *et al.*, (2009). Emulsification studies indicate that biosurfactants were readily formed in all the oils and was quite stable till fifth day. The striking difference was seen in the pattern of emulsion formed in case of coconut oil as compared to sesame oil & n-hexadecane (Fig. 3). In case of sesame oil & n-hexadecane, emulsion was formed at the broth/oil interface while in case of coconut oil emulsion was formed at the bottom. Another important factor is density of the hydrocarbons which also play a role in emulsification. The density of the coconut oil is more (0.925g/cm^3) as compared to the density of sesame oil (0.920 g/cm^3) & n-hexadecane (0.773 g/cm^3) at 15^oC. Coconut oil & sesame oil used are vegetable oils whereas n-Hexadecane is a synthetic chemical. Thaniyavarn et al., (2006) used palm oil and found that this substrate was utilized well by *Pseudomonas aeruginosa* A41 for biosurfactant production. The biosurfactants produced by bacteria lowered the surface tension of the broths. The surface tension of the broth containing coconut oil was reduced more followed by diesel as compared to sesame oil & n-hexadecane containing broths. The yield of crude biosurfactant was more in medium supplemented with Sesame oil as compared to coconut oil, diesel and n-Hexadecane. These studies suggest that this bacterium can be used in bioremediation experiments.

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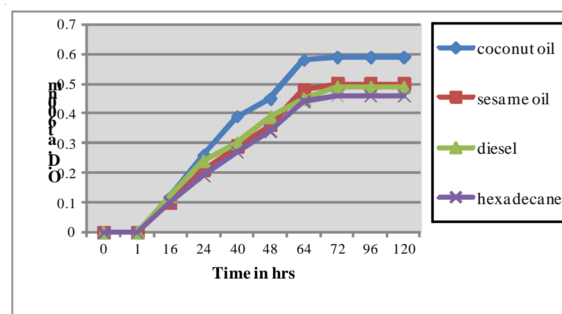


Fig.1.: Growth pattern on different hydrocarbons

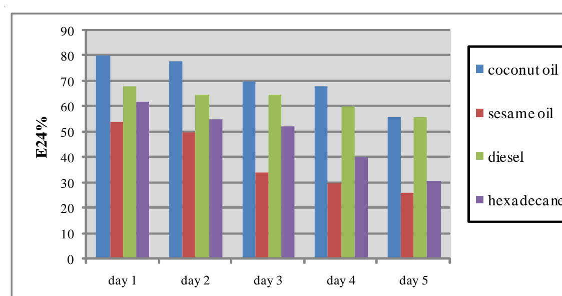
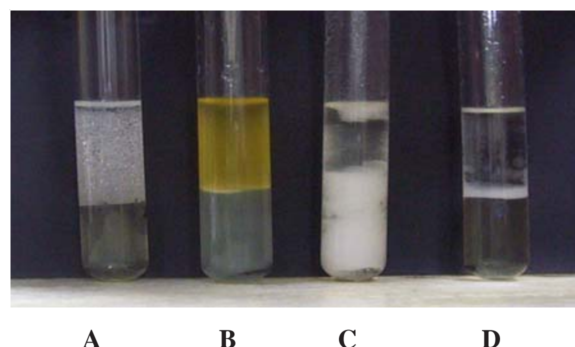


Fig.2.: Day wise emulsification index



A B C D

Plate 1: Emulsification activity showing different pattern of emulsion

A – Supernatant from n-Hexadecane broth & n-Hexadecane (1:1) after 24 hrs.

B – Supernatant from Sesame oil broth & Sesame oil (1:1) after 24 hrs.

C – Supernatant from Coconut oil broth & Coconut oil (1:1) after 24 hrs.

D – Supernatant from plane broth & Sesame oil (1:1) after 24 hrs.

Study of Hull Microfouling and Assessment of Preventive Strategies

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Abstract: Biofouling is referred to as the undesired development of microbial layers on surfaces.Biofouling can cause damage to shipping equipment, resulting in economic losses. Marine biofilms from the ship hull was collected from various locations of the ship and studied. Out of the six samples, the selected microcosm S6 was studied for various properties i.e. microbialdiversity, extracellular polymeric substance, antibacterial agent susceptibility, etc. It was primarily made up of three key organisms *Stenotrophomonas maltophilia, Acinetobacter Iwoffii and Shewanella putrefaciens*, which were found to be sensitive to benzylalkonium chloride and iron oxide nanoparticles. They also showed presence of high extracellular polymeric substances and proteins in the biofilm matrix. Hence, the biofilm forming efficiency of the isolates was affected with the amylase and protease action. This study reveals the need to develop multi-pronged approach to tackle biofouling.

Keywords: biofouling; microcosm; biofilm; nanoparticles; hull.

Introduction

A biofilm is a sessile microbial community that are irreversibly attached to a substratum and embedded in an extracellular polymeric matrix. Biofilm formation occurs when microorganisms attach to an abiotic or biotic surface, colonize, transform from planktonic form to immobilized status. Finally, the microcolony is formed and continuous proliferation of microorganisms will result in a three dimensional architecture of biofilm, which is considered as mature biofilm (Simoes, *et al.,* 2010).

Within the biofilm, bacteria are embedded in a self-produced extracellular matrix, which accounts for 90% of the biomass. The matrix is composed of extracellular polymeric substances (EPS) and carbohydrate-binding proteins, pili, flagella, other adhesive fibers and extracellular DNA. The structural components of the matrix are responsible for a highly hydrated, robust structure with high tensile strength that keeps bacteria in close proximity, enabling intimate cell-to-cell interactions and DNA exchange and also protects the biomass from desiccation, predation, oxidizing molecules, radiation, and antimicrobials (Aswathanarayana, 2012). A change in nutrient concentration, shear forces, temperature or other factors can cause either biomass production or sloughing of biofilms, which leads to increasing contamination of the water (Flemming, 2002).

Biocorrosion or microbially influenced corrosion (MIC) is the damage caused or accelerated by the presence of bacteria and other microorganisms and their metabolic activities (Miranda, 2006). Ships show a 10% higher fuel consumption caused by increased drag and frictional resistance resulting from hull and propeller fouling. Biofouling increases the frictional drag across a vessel's hull and reduces the speed that the vessel can achieve for a given power (Buhaug *et. al* 2006). The cost of dry docking and antifouling can amount to between 1 and 2 months operating profit of the ship, so an increase in the frequency with which it is done can have a significant impact on profitability (Abbott *et al.,* 2000). Vessels that have been laid up for extended periods may have accumulated a greater biomass of biofouling across a larger range of hull surfaces (Inglis *et al.,* 2012).

Formulations containing several different enzymes seem to be fundamental for a successful biofilm control strategy. Basically, proteases and polysaccharide hydrolyzing enzymes may be useful (Johansen *et al.,* 1997). Several attempts have been made to avoid biofilm formation by the incorporation of antimicrobial products into surface materials by coating surfaces with antimicrobials or by modifying the surfaces physicochemical properties (Simoes *et al.,* 2010). The decreased diffusion of antibiotics through the biofilm matrix, decreased oxygen and nutrient availability accompanied by altered metabolic activity, formation of persisters, increased production of oxidative stress and other specific molecules are identified as some of the intrinsic factors imparting resistance to the antimicrobials in biofilms. The induced resistance factors are due to the induction by the antimicrobial agent (Aswathnarayan *et al.,* 2013).

A lot of research efforts have been devoted to understand the fundamental ecology and biology of fouling environments, organisms, and communities in diverse settings. In the present study we have focussed on the types of organisms involved in biofilm formation and deleterious effects associated with their presence and some of the strategies preventing the biofilm formation, providing an insight to concerns for marine biofouling.

Materials and Methods

Various samples of marine biofilm were obtained from ship hulks (sailing in the Arabian Sea) stationed at The Gateway of India, Mumbai, Maharashtra, in sterile plastic

containers. Samples S1, S3 and S4 collected along the sides of the ships. Samples S2, S4, S6 collected from the bottom of the ships near the hull were used to isolate biofilm forming bacteria on Sterile Zobell's Marine Agar. Different isolates from these media were further identified and characterized using Gram staining and standard biochemical test. All biochemical media was supplemented with aged sea water to mimic the marine environment for selective isolation of halophilic bacteria (Al Enazi *et al.,* 2014).

Fresh cultures of the isolates and the microcosm biofilm samples were spread plated on St. Zobells Marine Agar plates. St. Standard Antibiotic disc of Chloramphenicol, Nalidixic Acid, Ampicillin, Penicillin-G and Bacitracin were placed on it. Plates were incubated at R.T. /24 hr. Zones of inhibition were observed.Fresh cultures of the isolates and the microcosm biofilm samples were pour plated on St. Zobells Marine Agar plates. Benzalkonium Chloride (BKC) (Quaternary Ammonium Salt), iron oxide and Capsaicin, antibacterial compound extracted from Green Chilies (Cut and soaked overnight) was added in cups made in the plates. Plates were incubated at R.T. /24 hr. Zones of inhibition were observed (Paraje, 2011; Guss Roeselers *et al.,* 2006; Monica *et al.,* 2014).

Results and Discussion

All the six samples (S1, S2, S3, S4, S5 and S6) of marine biofilm were enriched in St. Zobells Marine Broth incubated at R.T. / 1 week in order to enrich the microcosm biofilm forming bacteria for further analysis. Out of total 21 isolates obtained, 16 isolates were found to be Gram Negative coccobacilli, 2 isolates were Gram Negative rods, 2 isolates were Gram Positive cocci and one isolate was Gram Positive short rods.

Since the number of the isolates obtained were quite high, the microcosm studies was first subjected to different microbiological techniques for checking their sensitivity and then depending on the results obtained only the isolates obtained from one of the samples of the biofilm which gave satisfactory results were further used for analysis.

The microcosm biofilm samples S1, S2, S3, S4, S5 and S6 were subjected to St. Standard Antibiotic disc of Chloramphenicol, Nalidixic Acid, Ampicillin, Penicillin-G and Bacitracin. It was found that Sample S1 showed resistance to almost all the antibiotics except Chloramphenicol whereas sample S2 was found to be sensitive towards Chloramphenicol, Ampicillin and Nalidixic Acid. Bacitracin was found to be effective against samples S3 and S4 only. Penicillin G showed an antimicrobial effect against samples S5 and S6. Benzalkonium Chloride (BKC) (Quaternary Ammonium Salt) an antibacterial compound showed an antimicrobial effect against all the microcosm biofilm samples except S4. Capsaicin (a natural antimicrobial compound from green chilies) did not show any antibacterial effect against any of the microcosm biofilm samples. (Table 1).The microcosm biofilm samples were subjected to Iron-oxide Nanoparticles. The nanoparticle showed excellent activity against all the samples indicating an excellent control strategy.

Efficacy of biofilm removal by Amylase and Protease enzymes was studied. A measure of efficacy called Percentage Reduction was used to evaluate the efficacy to these enzymes.

Percentage Reduction = [(C-B)-(T-B)/(C-B)] x 100%

Where: B denotes, the average absorbance per tube for blank (no. biofilm, no treatment); C denotes the average absorbance per tube for control wells (biofilm, no treatment) and T denotes the average absorbance per tube for treated wells (biofilm and treatment).As per the Percentage Reduction values obtained it showed that Amylase enzyme was successful in removing the biofilm formed by the samples S1, S4, S5 and S6. While S3 was moderately affected by the enzyme, sample S2 was found to be almost resistant to the biofilm degrading efficacy of the amylase enzyme (Fig.1).The protease enzyme showed excellent biofilm removal efficacy in the range of 82% to 94% towards all the microcosm biofilm samples (Fig. 2). Protein concentration in the EPS of the microcosm biofilm was determined by the Lowry Method, It was found that samples S1, S2 and S3 had high amount of proteins in their EPS (Fig.3). Carbohydrate concentration in the EPS of the various isolate microcosm biofilm was determined by the Anthrone method, Samples S2 and S6 had very high amount of Carbohydrate in their EPS (Fig.4). The carbohydrate concentrations were higher than the protein concentration in its biofilms. These results are comparable to the work of Simoes (2003). In some studies, it was indicated that carbohydrates are the main constituents of the EPS while some studies found proteins to dominate (Zhang *et al.*, 2001; Liu *et al.*, 2003). In this study carbohydrate were found to be dominant rather than protein. Nonetheless, the EPS components of the biofilms differ in quantity; structure or nature depending on the microorganisms within the biofilm. Allison *et al.* (1998) indicated that the EPS of the biofilms is highly heterogeneous even among the same bacterial species and therefore its composition and function within the biofilms will differ.

The biofilm sample 6 collected from the ship hull was selected for further investigation as its isolates were the most predominant one and showed good growth.It also contained high amounts of carbohydrate (3,840μg/ml) and protein (133mg/ml) which is important in forming the extracellular polymeric substances. The efficacy removal of the biofilm by the Amylase and Protease enzyme of the

sample S6 was effective. It also showed high sensitivity towards the Benzalkonium chlorideand Iron oxide nanoparticle. Based on the colony characteristics, Gram character, Standard biochemical test and by using VITEK the three isolates of sample 6 were identified as S6.1 to be *Stenotrophomonas maltophilia*; S6.2 to be *Acinetobacter Iwoffiiand* S6.3 to be *Shewanella putrefaciens*.

All the isolates showed sensitivity towards Chloramphenicol, Ampicillin, Nalidixic Acid and Penicillin-G. The *Acinetobacter Iwoffii* isolate was found to be resistant towards Bacitracin. A distinctly strongantimicrobial effect of Benzalkonium chloride against all the 3 isolates samples, especially against *Shewanella putrefaciens* was observed, whereas Capsaicin did not show any antibacterial effect (Table 2). Attempts have been made to test the antifouling nature of capsaicin (8-methyl- N-vanilly-6-nonenamide) is the natural extract from chili pepper. Molina-Torres *et al.*(1999)documented that *Bacillus subtilis* growth was strongly inhibited by capsaicin. However capsaicin did not show any antibacterial effect against any of the 3 isolates samples in the present study.

Efficacy of individual isolate biofilm removal by Amylase and Protease enzymes was studied. As per the Percentage Reduction values obtained it showed that Amylase enzyme was successful in removing the biofilm formed by the *Acinetobacter Iwoffii,* while that of *Stenotrophomonas maltophilia* and *Shewanella putrefaciens* showed resistant to the activity of amylase enzyme (Fig.5).

Protease enzyme showed moderate biofilm removal efficacy towards *Shewanella putrefaciens* and *Acinetobacter Iwoffii*. While *Stenotrophomonas maltophilia* showed resistant to protease enzyme activity (Fig. 6).Enzymes have been proven to be effective for the degradation of the EPS of the biofilms (Johansen *et al.*, 1997). Enzymes remove biofilms directly by destroying the physical integrity of the EPS. In the present study, Amylase and Protease enzymes were tested for the eradication of marinebiofilms. Amylase enzyme was successful in degrading the EPS of the biofilm formed by the *Acinetobacter Iwoffii*. While *Stenotrophomonas maltophilia* and *Shewanella putrefaciens* showed resistant to the EPS degrading activity of amylase enzyme, whereas Protease enzyme showed moderate biofilm removal efficacy towards *Shewanellaputrefaciens* and *AcinetobacterIwoffii*. While *Stenotrophomonasmaltophilia* showed resistant to protease enzyme activity. The reason for the inefficiency of enzymes may be due to its incompatibility with the specific protein structural components of the biofilm EPS tested in this study. Leroy *et al.* (2007) also found the protease to be more effective for the prevention of adhesion and detachment of a *Pseudoalteromonas*sp. D14 biofilm than xylanase, amylase, cellulase and lipase.Protein concentration in the EPS of the isolates were determined by the Lowry Method, It was found that samples *Stenotrophomonasmaltophilia* had high amount of proteins in its EPS (Fig. 7). Carbohydrate concentration in the EPS of the isolates was determined by the Anthrone method, *Stenotrophomonasmaltophilia* had high amount of Carbohydrate in their EPS (fig.8)

Iron-oxide nanoparticles are a special class of metal oxide nanoparticles with unique magnetic properties and superior biocompatibility. In the present study Iron oxide nanoparticle (0.15mg/mL) showed excellent antimicrobial activity against both the microcosm biofilms as well as the individual isolates. Taylor and Webster (2009) showed that iron-oxide nanoparticles in a concentration range of 0.01 to 2 mg/mL were able to kill up to 25% of *S. epidermidis*in a 48 h old biofilm. Hence it poses as an excellent candidate for prevention and control of marine biofilms.

Conclusion

Several attempts have been made to avoid biofilm formation by the incorporation of antimicrobial products into surface materials, by coating surfaces with antimicrobials or by modifying the surfaces physicochemical properties.

Marine biofilms require a complex strategy to clean. A large diversity of both micro-foulers and the EPS secreted by them makes difficult the total prevention of the initial (reversible) adhesion. Use of Iron Nanoparticle, Quaternary ammonium compounds or Enzymes in modified form during the surface fabrication can provide a useful prevention strategy against the marine biofilm.However, such solutions require a shift of paradigms and of the point of view, away from the so much desired one-shot solutions. And although optimal solutions not always exist, the already present ones would prove effective if applied in the context of holistic approaches. This is where further research should be dedicated – in particular, for longer-term, sustainable solutions.

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Table 1: Antibacterial Activity of Std. Antibiotic, QAC, Capsaicin& Iron Oxide Nanoparticle on the microcosm biofilm samples enriched in St. Zobell's Marine Broth

ANTIBIOTICS	SYMBOL	CONC./DISC	S1	S2	S3	S4	S5	S6
Chloramphenicol	C^{30}	30mcg	36mm	38mm	37mm	40mm	46mm	44mm
Ampicillin	Ax^{10}	10mcg	-	27mm	28mm	23mm	20mm	-
Nalidixic Acid	NA^{30}	30mcg	-	20mm	-	-	-	-
Bacitracin	B^{10}	10 units	-	-	26mm	28mm	-	-
Penicillin G	P^{10}	10units	-	-	-	-	40mm	38mm
Quaternary Ammonium Compound (BKC)	BKC	50%	29mm	28mm	36mm	-	24mm	27mm
Capsaicin		Crude	-	-	-	-	-	-
Iron Oxide Nanoparticle		0.15mg/ml	26mm	40mm	36mm	37mm	26mm	36mm

Table 2: Study of Antibacterial Activity of various compounds against the selected isolates

ANTIBIOTICS	SYMBOL	CONC./DISC	S6.1	S6.2	S6.3
Chloramphenicol	C^{30}	30mcg	40mm	40mm	42mm
Ampicillin	Ax^{10}	10mcg	30mm	20mm	30mm
Nalidixic Acid	NA^{30}	30mcg	12mm	22mm	15mm
Bacitracin	B^{10}	10 units	22mm	-	25mm
Penicillin G	P^{10}		46mm	32mm	12mm
Benzalkonium Chloride	BKC	50%	12mm	30mm	45mm
Capsaicin		Crude	-	-	-
Iron Oxide Nanoparticle		0.15mg/ml	26mm	25mm	44mm

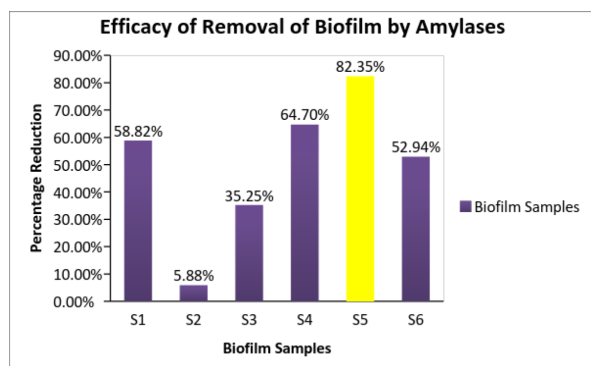

Fig. 1: Comparison of Efficacy of Removal of the microcosm biofilms by Amylases

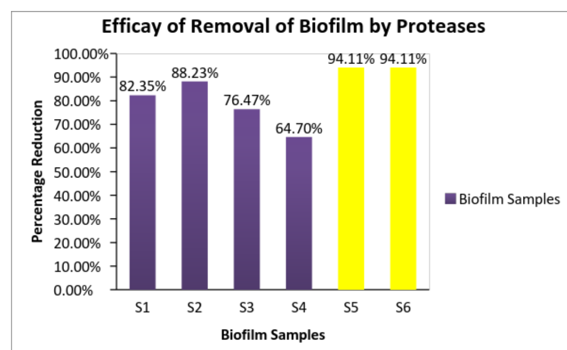

Fig. 2: Comparison of Efficacy of Removal of various microcosm biofilms by Proteases

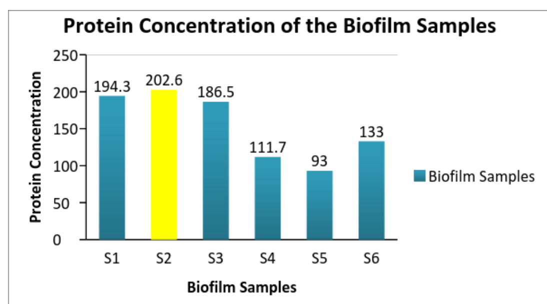

Fig. 3: Total Protein content of the microcosm biofilm samples

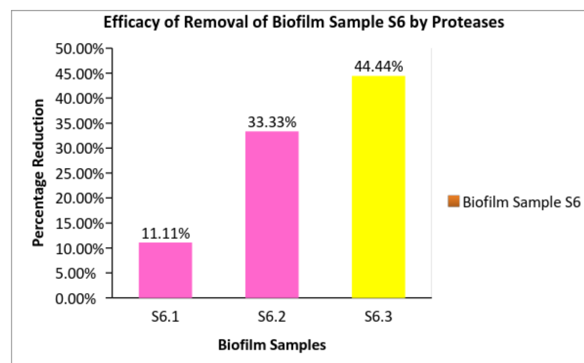

Fig. 6: Efficacy of Removal of Biofilm of the 3 selected isolates by Proteases

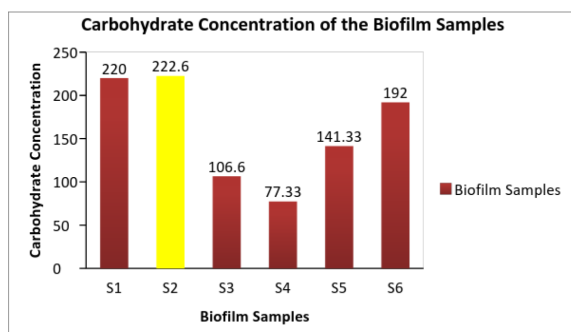

Fig. 4: Total Carbohydrate content of the various microcosm biofilm samples

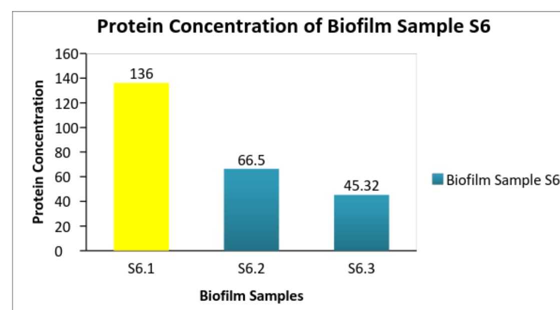

Fig.7: Protein content of the 3 isolates obtained from biofilm sample S6

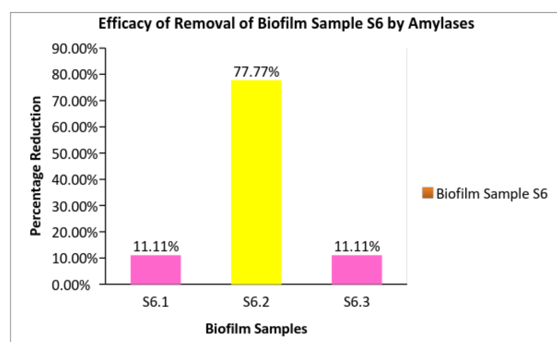

Fig. 5: Efficacy of Removal of Biofilm of the selected isoaltes by Amylases

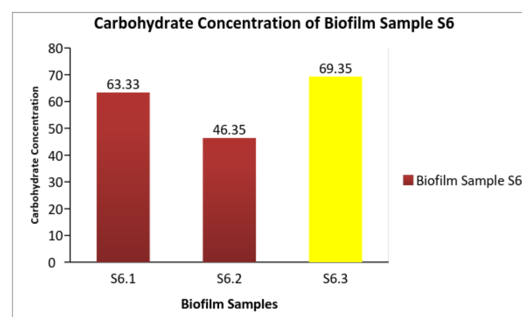

Fig.8: Carbohydrate content of the 3 isolates obtained from biofilm sample S6

Floral Diversity of Tansa Wildlife Sanctuary, Maharashtra

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Abstract: Tansa Wildlife Sanctuary consists of the catchment region of Tansa Lake in addition to the neighboring forests of Khardi, Vaitarna, Shahapur and East Wada Ranges in the Tehsil of Shahapur in Thane district. It has a rich diversity in flora and fauna. The current work was undertaken to study availability of different plant species during each season. Few plant sps. like *Tectona grandis* L. f. ,*Butea monosperma* (Lam.) Taub., *Terminalia crenulata* (Heyne) Roxb., *Carrisa caranda* L., etc were found to be dominant. The present study was found useful in recording endangered and rare plant sps. from the concerned area.

Introduction

Tansa wildlife sanctuary has rich diversity in flora. Four different location Mahuli Fort, Piwali Forest, Manas Mandir and Tansa Dam in Tansa wildlife sanctuary were selected for study. Tansa Sanctuary is home to many wild and endemic plants. The sanctuary covers an area of around 320 sq. km. Forest type is tropical moist deciduous. Rainfall ranges from 1188 mm to 2902 mm per year. Tansa vegetation includes herbs, shrubs, climbers & trees. Few plant sps. like *Tectona grandis* L.f., *Butea monosperma* (Lam.) Taub., *Terminalia crenulata* (Heyne) Roxb., *Carrisa caranda* L., etc were found to be dominant.

Methodology

The area selected for survey was Mahuli Fort, Piwali Forest, Manas Mandir & Tansa Dam which falls in tehsil of Shahapur in Thane district, Maharashtra, India. The study was done for a period of two years from 2014 to 2017. Recording of only flowering plants were undertaken with the help of various floras (Cook, 1967) and taxonomist. Identification of plants were done by doing morphological studies using reference books (Almeida, 2010; Ingalhalikar,2012; Krishan,2006 and Krishnan, 2014). Interviews were arranged with local tribes to collect information. Trees, climbers, herbs and shrubs plants belonging to forty different families were recorded. Listing was done according to their availability during three seasons i.e. monsoon, premonsoon, and post monsoon. For investigation of the area interviews and discussions with local people were undertaken to understand ethnobotanical aspects of the plants observed.

Disscussion

Total 104 plants sps. belonging to 40 different families were reported from study area. Endangered plant like *Operculina tansaensis* (Sant. & Patel. Trans. Bose Res. Inst. Calcutta xxii. (Convolv. Bomb. Addit. & Corr.) 33 (1958), a climber belonging to convolvulaceae family, and other critically endangered plants was recorded in Tansa Sanctuary region(ref).Few plants were found to be threatened, including *Curcuma pseudomontana, Erinocarpus nimmoni*,*Pterocarpus marsupium(near threatened)* etc. (ref. IUCN).Profitable plants like *Madhuka longifolia*, *Terminalia crenulata, Bridelia retusa, Tectona grandis, Butea monosperma , Carissa caranda,* etc. were also recorded. Some herbs, climber, and trees fruits useful in vegetable as well were found.

Conclusion

The present research study was useful on identifying seasonal flowering plants in Tansa sanctuary. Recordings of valuable plants, endangered and rare plant sps. was also done from the concerned area. Most of the wild plants were used as a source of income for local people. Local people are totally dependent on the forest.

Acknowledgement

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Table 1: List of plants available according to seasons (premonsoon, monsoon and post monsoon)

SR.NO	BOTANICAL NAME	FAMILY	PRE MONSOON	MONSOON	POST MONSOON
1.	*Abelmoschus ficulneus* (L.) Wight & Arn. ex Wight	*Malvaceae*	√		
2.	*Accacia catechu* (L. f.) Willd.	*Mimosaceae*	√	√	
3.	*Achyranthus aspera* L.	*Alternanthera*		√	√
4.	*Adiantum fern*	*Pteridaceae*		√	
5.	*Adina cordifolia(Roxb.)* Benth.$ Hook.f.ex B.D.Jacks.	*Rubiaceae*	√		
6.	*Aeginetia indica* Linnaeus.	*Orobanchaceae*		√	
7.	*Aerva lanata*(L.) Juss. ex Schult.	*Amaranthaceae*		√	√
8.	*Ageratum conyzoides* L.	*Asteraceae*			√
9.	*Alternanthera seesilis* (L.) R. Br.	*Amaranthaceae*	√	√	√
10.	*Alysicarpus glandulosus*	*Fabaceae*		√	
11.	*Ammania baccifera* L.	*Lythraceae*		√	
12.	*Anogeissus latifolia* (Roxb. ex DC.)	*Combrataceae*			
13.	*Argyreia sericea* Dalz. & Gibs.	*Convolvulaceae*		√	
14.	*Ariopsis peltata Nimmo*	*Araceae*		√	
15.	*Barleria cristata* L.	*Acanthaceae*	√	√	√
16.	*Bauhinia racemosa* Roxb.	*Leguminaceae*	√		
17.	*Bauhinia spp.*	*leguminaceae*	√		
18.	*Begonia crenata* L.	*Bignoniaceae*		√	
19.	*Boehmeria macrophylla* Hornem.	*Urticaceae*		√	
20.	*Bombax ceiba* L.	*Bombacaceae*	√		√
21.	*Borreria hispida*	*Rubiaceae*		√	
22.	*Bridelia retusa (L.)* Spreng.	*Acanthaceae*		√	
23.	*Burmannia coelestis* (D.Don) *Least concern*	*Burmanniaceae*			√
24.	*Butea monosperma* (Lam.) Taub.	*Fabaceae*	√		
25.	*Cactus spp.*	*Cactaceae*			
26.	*Calotropis gigantea* (L.) W.T.Aiton	*Ascelpiadaceae*	√		√
27.	*Canscora devendrae* R. Kr. Singh & Diwakar	*Gentianaceae*		√	
28.	*Carrisa caranda* L.	*Apocynaceae*	√		

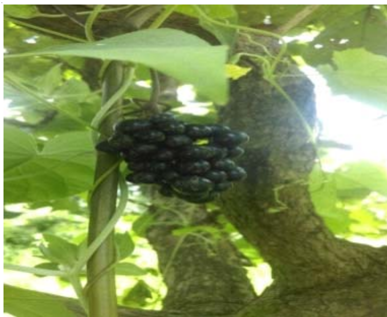
29.	*Carvia callosa* (Nees) Bremek.	*Acanthaceae*	√	√	√
30.	*Cassia tora* L.	*caesalpinidae*		√	√
31.	*Celosia cristata* L.	*Amaranthaceae*		√	√
32.	*Chlorophytum* spp	*Asparagaceae*		√	
33.	*Clitoria anua* L.	*Fabaceae*		√	
34.	*Commelina bengalensis* L.	*Commelinaceae*		√	
35.	*Crotalaria quinquefolia* L.	*Fabaceae*			
36.	*Curcuma pseudomontana* J. Graham. Vulnerable.	*Zingiberaceae*		√	
37.	*Cuscuta reflexa* Lam.	*Convolvulaceae*	√		
38.	*Cynospermum asperimum*	*Acanthaceae*	√		√
39.	*Cynotis tubrosa* (Roxb.) Schult. & Schult.f.	*Commilinaceae*		√	
40.	*Dendrolobium triangulare Muell.Arg.*	*Leguminosae*		√	
41.	*Dendropthoe falcata* (L. f.) Ettingsh.	*Loranthaceae*	√		
42.	*Dioscora pentaphyllum* L.	*Dioscoraceae*		√	
43.	*Ensete* spp.				
44.	*Erantheum roseum* (vahl) R.Br.	*Acanthaceae*			√
45.	*Erinocarpus nimonii* Nimmo ex J. Grah.	*Tiliaceae*		√	
46.	*Euphorbia hirta* L.	*Euphorbiaceae*	√	√	√
47.	*Exacum jencia*	*Gentiabaceae*			√
48.	*Ficus benghalensis* L.	*Moraceae*			
49.	*Ficus racemosa* L.	*Moraceae*			
50.	*Ficus religiosa* L.	*Moraceae*			
51.	*Gliricidia sepium* (Jacq.) Kunth ex Walp.	*Fabaceae*	√		
52.	*Gloriosa superba* L.	*Liliaceae*		√	
53.	*Grewia serrulata* L.	*Tiliaceae*			√
54.	*Haplanthodus tentaculqtus*	*Acanthaceae*			√
55.	*Helotropium indicum* L. least concern	*Boraginaceae*	√	√	√
56.	*Hemideamus indicus*(L.) R. Br	*Apocynaceae*			√
57.	*Hemigraphis latrbrosa* (Roth) Nees	*Acanthaceae*			√
58.	*Hibiscus sabdariffa* L.	*Malvaceae*	√		
59.	*Holarrhena pubescens* Wall.ex G. Don	*Apocynaceae*	√		
60.	*Holostemma ada-kodien* Schult.	*Apocynaceae*	√		
61.	*Hygrophila auriculata* Schumach.	*Acanthaceae*		√	√
62.	*Impatiens oppositifolia* L.	*Balsaminaceae*		√	
63.	*Indigofera tinctoria* L.	*Leguminosae*		√	
64.	*Ipomea carnea* Jace.	*Convolvulaceae*			
65.	*Ipomea hederifolia* L.	*Convolvulaceae*			√

66.	*Leucaena leucocephala* (Lam.)*de* Wit	*Fabaceae*			
67.	*Lindenbergia muraria*(Roxb.) Brhul.	*Scrophulariaceae*		√	
68.	*Madhuca longifolia* (J. Koing) J.F. Macbr.	*Sapotaceae*	√		
69.	*Mangifera indica* L.	*Anacardiaceae*	√		
70.	*Marsilea* sps.	*Marsileaceae*			
71.	*Milliusa tomantosa* (Roxb.) J. Sinclair.	*Annonaceae*	√		
72.	*Mitragyna parvifolia* (Roxb.) Korth.	*Rubiaceae*			
73.	*Momordica dioica* Roxb.ex Willd.	*Cucurbitaceae*		√	
74.	*Mucuna sanjappae* M.M. Aitawade, S.R.	*Fabaceae*			√
75.	*Mukia maderaspantana*(L.) M.Roem.	*Cucurbitaceae*			√
76.	*Musa balbisiana*	*Musaceae*			
77.	*Neurancanthus sphaerostqchys*	*Acanthaceae*			√
78.	*Oroxylum indicum* (L.) Benth. ex Kurz	*Bignoniaceae*		√	
79.	*Osbeckia muralis* L.	*Melastomataceae*		√	
80.	*Oxalis corniculata* L.	*Oxalidaceae*		√	
81.	*Pandanus odorifer* (Forssk.) Kunze Least concern	*Panadaceae*		√	
82.	*Phyllanthus embilca* L.	*Phyllanthaceae*	√		
83.	*Phyllanthus niruri* L.	*Phyllanthaceae*			√
84.	*Phyllocephalum scabridum* (D.C) k. Kirkman.	*Asteraceae*			√
85.	*Pinda cocanensis* (Dalzell) P.K. Mukh.	*Apiaceae*		√	
86.	*Plumbago* spp.	*Boraginaceae*			
87.	*Plumeria obtusa*	*Apocynaceae*	√	√	√
88.	*Pterocarpus marsupium* Roxb.	*Fabaceae*		√	
89.	*Rhamphicarpa fistulosa* (Honchst.) Benth.,	*Scrophulariaceae*		√	
90.	*Rungia repens* Dalzell & A. Gibson	*Acanthaceae*			√
91.	*Senecio graminii* L.	*Asteraceae*		√	√
92.	*Smithia sensitiva* Aiton Least concern	*Fabaceae*		√	
93.	*Sphaeranthus indicus* L.	*Asteraceae*	√		√
94.	*Sterculia urens* Roxb.	*Malvaceae*	√		
95.	*Striga aciatica*(L.) O. Ktze.	*Orobanchaceae*		√	
96.	*Tectona grandis* L.f.	*Lamiaceae*		√	√
97.	*Terminalia crenulata* (Heyne) Roxb.	*Combritaceae*			√
98.	*Tricodesma indicus* L.	*Boraginaceae*		√	√
99.	*Tridax Procumbens* L.	*Asteraceae*	√	√	√
100.	*Triumfetta pentrada* A. Rich.	*Tilliaceae*		√	
101.	*Vitex negunda* L.	*Verbinaceae*	√		
102.	*Vitis* sp.	*vitaceae*	√		
103.	*Wrightia tinctoria* (Roxb.) R.Br.	*Apocynaceae*	√		
104.	*Ziziphus mauritiana.* Lam.	*Rhamnaceae*	√		

Striga asiatica

Erinocarpus nimmoni

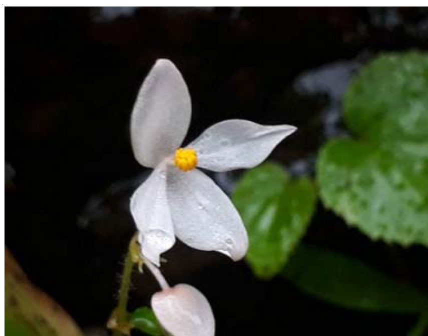
Smithia hirsuta



Vitis **spp.**

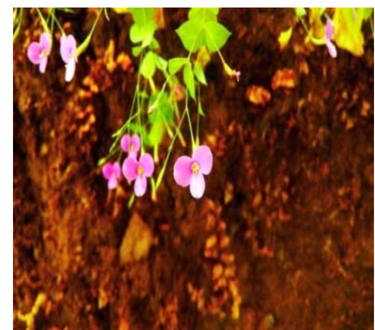
Gloriosa superba

Clitoria annua



Begonia crenata

Mucuna sanjappae



Canscora devendrae

Argyreia sericea

Trichodesma indicum

Aegintia indica

Study of The Mitotic Abnormalities Due to Mercuric Chloride on *Allium cepa* At Different Concentration

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Abstract: Humans are exposed to many chemicals as a result of the air they breathe, food and water they ingest, or occupational contacts with these chemicals. There is a need then to determine the genotoxic effects of these chemicals on living systems. Mercury was traditionally used in agricultural chemicals as a fungicide or pesticide. In the present investigation the effect of mercuric chloride and Benzene at different concentration on root tip of *Allium cepa* L. were studied. It was found that the affect of this agent increases with increasing concentration and time exposure. These characters included mean root length, numbers and appearance. The mean root length was statistically evaluated. There was a significant decrease in root length when compared to control. Various abnormalities observed mostly in anaphase with separation of chromosome that leads chromosomal anomalies.

Key words: *Allium cepa*, genotoxicity, mercuric chloride benzene.

Introduction

Carcinogenic agents are chemical compound which affect the cell at genetic level. The genetic information of all organisms resides in the individual DNA molecules or chromosomes (Siddiqui and Karkun, 2016). The use of various metals containing inorganic fertilizer has intensified over the last few decades. These fertilizers serve to supply essential nutrients and minerals for the growth and development of the crop. These heavy metals are bioavailable and tend to accumulate in the ecosystem. These chemicals can cause deleterious effects on the genetic system of living organisms (Benerjee *et al*., 2010). Domestic, industrial, and agricultural wastewaters are discharged into those rivers and streams, causing deterioration of the environment. This deterioration can cause great problems for human health as well as for aquatic flora and fauna (Athanásio *et al*., 2014).

The species *Allium cepa* presents advantages, including low raising costs, easy handling, and suitable chromosomal features. This plant bears large and few chromosomes (2n = 16), which facilitates the evaluation of chromosome damages and/or disturbances in cell division cycle, including eventual aneuploidy risks (Morais Leme and Marin-Morales 2008). Root tips of *Allium cepa* L. have been recommended as a standard for cytogenetic assay in environmental monitoring due to the correlation of these plants with mammalian and non-mammalian test systems (Nilüfer *et al*. 2008).

The aim of present study was to study genotoxic effect of mercuric chloride and Benzene at different concentration on root tip of *Allium cepa* L.

Materials and Methods

Test material: Onions bulbs were purchased from local market of Thane (Maharashtra)

Root growing: *Allium cepa* is grown in a small plastic flask, in which lower portion of onion was connected with water.

Treatment with chemicals: For test samples onion roots were grown in mercuric chloride and Benzene at different concentration (0.1%, 0.05%, 0.01% and 0.005%). Root grown in distilled water was taken as control.

Root tip cutting: Root tips were cut between 7 to 7.30 AM. After cutting, the root was transferred in to fixative so that cell division stopped at respective phases.

Washing: Firstly the root tip were washed with distilled water and then treated with 1N HCl for 10 minutes

Staining: After that root tip was stained with acetocarmine (for 10 min).

Slide preparation: The macerated and stained root tips were covered with cover slip and squashed and later viewed in a microscope.

The results of the mean root length were statistically evaluated by the analysis of variance and least significant difference (LSD) test at 5% significant level.

Results and Discussion

Genotoxic affects the cell differently depending upon the concentration of chemicals. Cell division also becomes abnormal in the presence of some chemicals which are carcinogenic. The present investigation is to study the effect of mercuric chloride and benzene at different concentration on root tip of *Allium cepa.* The root length also decreases with increasing concentration of mercuric chloride and benzene (table 1 and Plate 1). Among the chromosome anomalies caused by these chemicals were lagging

chromosome, chromosomal bridge, etc. (Plate 2).

Mercury compounds inhibit DNA replication linking the nucleus membrane and organelles in cells and also cause fragmentation in DNA. In addition, the mercury link itself to the sulpyhydrile groups found in the enzymes and protein groups, functions as an inhibitor preventing the synthesis of protein necessary for the normal cell division and delaying mitotic cycle (De Flora, 1994).

Acknowledgment

Authors are thankful to Department of Botany, VPM's B. N. Bandodkar College of Science, Thane for providing the laboratory facilities. We are also thankful to DBT-Star College Scheme for providing the instruments and chemicals required for the research work.

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Plate 1: A – Onions growing in mercuric chloride and benzene
B – Root tips of *Allium cepa* L. at 0.005% mercuric chloride solution
C – Root tips of *Allium cepa* L. at 0.005% benzene solution
D – Root tips of *Allium cepa* L. at distilled water (control)

Table 1: Root length of roots of *Allium cepa* L. at different concentration of mercuric chloride and benzene

Sr. No.	Concentration	Mercuric Chloride	Benzene	Control
1.	0.1%	NR	NR	5.16 ± 0.05
2.	0.05%	0.20 ± 0.05	0.15 ± 0.03	
3.	0.01%	1.71 ± 0.04	1.26 ± 0.06	
4.	0.005%	3.14 ± 0.06	2.72 ± 0.03	

Values are mean of three determinants. Mean ± SE (NR: No Response)

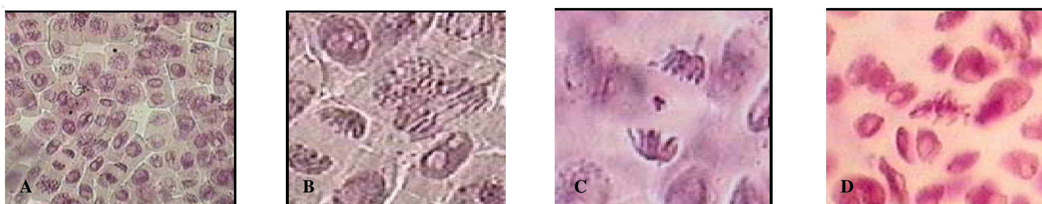

Plate 2: Effect of mercuric chloride and benzene at different concentration on mitosis of root tips of *Allium cepa* L.
A: Control; B: Chromosomal Bridge; C: Laggard at Anaphase D: Sticky Metaphase

Comparative Nutritional Analysis of Quinoa, Wheat and Rice

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Abstract: Cereals are the edible seeds or grains of the grass family, Gramineae. On a worldwide basis, wheat and rice are the most important crops, accounting for over 50% of the world's cereal production. In the present fast growing rapid changing global society, attaining good health has become a challenge for all age groups. Consumption of foods made from refined grains has increased and the physical ailments like diabetes, cardiovascular diseases, obesity and high blood pressure have been doubled. Quinoa, a *pseudocereals* is projected as a superfood, thus in the present study nutritional value of quinoa, wheat and rice were evaluated. The protein content was found to be 0.26, 0.20 and 0.40 gm/100gm and vitamin C content was found to be 13.97, 14.88 and 15.55 mg/gm in quinoa, wheat and rice respectively.

Keywords: Quinoa, wheat, rice, protein, vitamin

Introduction

Cereals are the dominant crops in world agriculture. *Cereals* are members of the grass family (*Poaceae* or *Gramineae*) and produce dry one-seeded fruits (caryopsis) which are commonly called a kernel or grain (Delcour and Hoseney, 2010). All cereals consist of a fruit coat (pericarp) surrounding the seed. The seed contains an embryo (germ) and an endosperm surrounded by a nucellar epidermis and a seed coat (testa). Some cereals, such as rice, oats, and barley retain their husk during threshing and this must be removed to produce acceptable foods for humans. *Pseudocereals*, such as amaranth, quinoa, and buckwheat are not members of the grass family, but because of the high starch content in their seeds and their use in cereal-like products. These seeds contain no gluten and are therefore suitable alternatives for people with celiac disease (Frølich and Aman, 2010). Quinoa fruits are achenes, comprised of a single seed enclosed by an outer pericarp (FAO, 2011). The quinoa seed contains a central perisperm where carbohydrate reserves are localized, surrounded by the circular oil-rich and protein-rich embryo, endosperm, and seed coat (Prego *et al.*,1998).

Cereals and cereal products are an important source of energy, carbohydrate, protein and fibre, as well as containing a range of micronutrients such as vitamin E, some of the B vitamins, magnesium and zinc. Regular consumption of cereals, specifically wholegrains, may have a role in the prevention of chronic diseases such as coronary heart disease, diabetes and colorectal cancer (McKevith, 2004).

Wheat is a major cereal crop in many parts of the world. It belongs to the Triticum family, of which there are many thousands of species (Kent and Evers 1994). Commercially, *Triticum aestivum vulgare* and *Triticum turgidum durum* (hard wheat, mainly used in pasta products) are of most importance (Macrae *et al.,* 1993). Wheat is a highly nutritious food source. Wheat is low in fat and provides complex carbohydrates, insoluble and soluble fiber, and an assortment of vitamins and minerals. Rice (*Oryza sativa*) is the second leading cereal crop and staple food of half of the world's population. On the other hand, rice is an excellent source of energy, due to the high starch content, providing also proteins, vitamins and minerals, and has low lipid content (Silva and Ascheri, 2009), but ironically it is deficient in many bioavailable micronutrients required for daily diet. It is a major contributor to dietary glycaemic load however, there is a wide variation in glycaemic and insulinaemic responses to rice as consumed. This can be largely attributed to the inherent starch characteristics of specific cultivars or the mode of post-harvesting processing. Quinoa (*Chenopodium quinoa* Willd) is known as a pseudocereal, since it is not a member of the grass family, but from Amaranthaceae family, which also produces seeds that can be ground and used as flour (Schouenlechner and Drausinger, 2010). Quinoa belongs to the group of the so called "superfoods" and has a nutritional composition that confers multiple benefits. The present study was to evaluate the nutritional value quinoa, wheat and rice.

Materials and Methods

Collection of plant materials: Flours of quinoa, wheat and rice were purchased from local market, Thane.

1. **Estimation of protein:** Protein was estimated by Lowry's method (Sadashivam, 2008). 0.1 ml of the Plant extract was added in a tube with 1.9 ml of distilled water. After that 5.0 ml of Reagent C was added to the test tubes and allowed it to stand. After 15 minutes 0.5 ml of Folin's reagent was added all the tubes. Allow it to stand for 20 minutes and took OD at 625nm.

2. **Estimation of Vitamins:** Vitamins were evaluated by DCPIC method (Sadashivam and Manickam, 2008). One gram of sample was taken and 500 ml of distilled water was added. The solution was filtered using a muslin cloth and titrated 5 ml DCPIP against it till the colour

changes to light pink. Readings were noted and vitamin C was calculated.

Results and Discussion

The nutritive values of the three cereals were represented in the Table 1 and Table 2. Protein were represented in g/100g where as Vitamins are expressed in mg/100g. The amount of protein was found to be 0.26, 0.20 and 0.40 gm/100gm in quinoa, wheat and rice respectively (Table 1). The amount of vitamin was found to be 13.97, 14.88 and 15.55 mg/gm in quinoa, wheat and rice respectively (Table 2).

Quinoa has potential health benefits and exceptional nutritional value: a high concentration of protein (all essential amino acids highly bioavailable), unsaturated fatty acids, a low glycemic index; vitamins, minerals and other beneficial compounds; it is also gluten-free (Gordillo-Bastidas *et al*., 2016). Quinoa (*Chenopodium quinoa* Willd.), is considered as a pseudocereal or pseudograin and has been recognized as a complete food due to its protein quality. It has remarkable nutritional properties and has recently been used as a novel functional food (James, 2009). Biscuits prepared with quinoa, flax seed and brown rice in 40:40:20 was superior in protein (11.27%) dietary fibre (10.4%) Potassium (242.04mg), calcium (70.73mg), Magnesium (117.15mg) and Phosphorous (239.87mg) (Sukeerthi and Singh, 2017).

Conclusion

Cereals are the dominant crops in world agriculture. They are the major source of calories and protein to the diets of humans and livestock. Proteins and vitamins were found to be high in the *Pseudocereal* quinoa. In addition to presenting high nutritional quality, quinoa is characterized by being gluten-free feature allowing to obtain a greater variety of foods more suitable and nutritious to holders of celiac disease. All this has contributed to the increase of interest and popularization of its consumption among people who seek alternative foods with high nutritional value, especially in developed countries, thus stimulating their agricultural production. Quinoa is being promoted as an alternative agricultural crop due to its stress-tolerant characteristics and marketed as a “superfood” for its nutritious qualities.

Acknowledgment

Authors are thankful to Department of Botany, VPM’s B. N. Bandodkar College of Science, Thane for providing the laboratory facilities. We are also thankful to DBT-Star College Scheme for providing the instruments and chemicals required for the research work.

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Table 1: Estimation of proteins by Lowry's method

Sr. No.	Sample	Amount of protein (g/100gm) Mean ± SE
1.	Quinoa	0.26 ± 0.33
2.	Wheat	0.20 ± 0.15
3.	Rice	0.40 ± 0.26

Values are mean of three determinants.

Table 2: Estimation of vitamins by DCPIP method

Sr. No.	Sample	Amount of vitamin (mg/100gm) Mean ± SE
1.	Quinoa	13.97 ± 0.05
2.	Wheat	14.88 ± 0.15
3.	Rice	15.55 ± 0.06

Values are mean of three determinants.

Plate 1: A: Quinoa grains; B: Wheat; C: Rice

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Isolation and identification of fungi from leaves of *Lodoicea maldivica*

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Abstract: *Lodoicea* is commonly known as the double coconut. *Lodoicea maldivica* is endemic to Praslin and Curieuse islands, Seychelles. In India it is available only in the Botanical Garden of Kolkata. There has been a decline in the area of occupancy, which is not reversible and it has been estimated that population has declined by more than 30% over three generations. The main reason behind decrease in population was exploitation and invasive pathogens. Infectious disease on the plant has lead to decaying of the plant. Therefore this study was conducted to isolate and identify the fungi associated with it. Four fungi were isolated from leaves of *Lodoicea maldivica* and identified as *Penicillium verrucosum, Aspergillus carbonarius, Mucor globosus* and Brown aspetate mycelium.

Keywords: *Lodoicea maldivica*, double coconut, fungal isolates

Introduction

Lodoicea, commonly known as the sea coconut, coco de mer, or double coconut, is a monotypic genus in the palm family. Formerly *Lodoicea* was known as Maldive coconut. Its scientific name, *Lodoicea maldivica*, originated before the 18th century when Seychelles were uninhabited (Romero-Frias, 1999). *Lodoicea* is robust, solitary, up to 30 m tall with an erect, spineless stem which is ringed with leaf scars (Calstrom, unpublished). The base of the trunk is of a bulbous form and this bulb fits into a natural bowl or socket, about 2.5 ft in diameter and 18 inches in depth, narrowing towards the bottom. The crown is a rather dense head of foliage with leaves that are stiff, palmate up to 10 m in diameter and petioles of two to four metres in length. The leaf is plicate at the base, cut one third or more into segments 4–10 cm broad with bifid end which are often drooping. A triangular cleft develops at the petiole base (Edwards *et al*., 2015).

Lodoicea maldivica, is endemic to the islands of Praslin and Curieuse in the Seychelles. There has been a decline in the area of occupancy, which is not reversible and it has been estimated that the population has declined by more than 30% over three generations. The main threats are fires, harvesting, poaching and invasive pathogens. Current levels of utilization are thought to be unsustainable and illegal harvesting of kernels is a significant additional threat (Baker and Lutz, 2011).

Materials and Methods

Collection of plant material: Diseased samples were collected from Botanical Garden of Kolkata, India, into closed paper envelops and not plastic bags to avoid creating a micro climate condition for the specimen according to Delhove and Vannière, 2013.

Isolation of fungal pathogens: Infected leaves of *Lodoicea maldivica* were used for inoculation. One centimeter of the infected leaf tissue was excised with a sterilized razor blade. Leaves were soaked in sterile saline for 5 minutes. Sterile swabs were prepared. These swabs were dipped in the above saline solution and then spread on sterile PDA plates. These PDA plates were incubated for 3 days at room temperature (25-28^{0}C).

Purification of fungal culture: After some days, the PDA plates were inspected to observe the growth of pathogens on the media. Some young mycelium emerging from tissues were sub cultured on fresh PDA media with the help of a sterile loop to obtain pure isolates of pathogens. They were checked and transferred on to new PDA media weekly and kept at room temperature (21-25^{0}C) to maintain pure cultures at all times for aiding correct identification of fungal pathogens. Isolated colonies were sub-cultured into fresh plates until pure cultures were obtained. Pure cultures obtained were identified from each variety by visual examinations and by observing under microscope. When viewed under the microscope, conidia were observed to be hyaline; single celled and cylindrical with obtuse ends.

Results and Discussion

The leaf surface of *Lodoicea maldivica* showed black spots (Plate 1). Infected *Lodoicea maldivica* leaf showed four different fungal contaminants. They were identified as *Penicillium verrucosum, Aspergillus carbonarius, Mucor globosus* and brown aspetate mycelium (Plate 2).

Infection of the fungi produces black coloured leaf spots on *Lodoicea maldivica*. Earlier reports revealed that a number of diseases were caused by different groups of fungi (Ariharan *et al*., 2016). This is the first report about the occurrence of such fungal disease on leaves of *Lodoicea maldivica* as for as the authors aware.

Conclusion

In an diseased leaf, nearly 50% of the surface area is affected. It automatically reduces the photosynthetic area of the plant. As disease advances the leaves wither off and

in turn affect the physiological activity of the plant. The yield potential of the plant will be affected because of the bacterial and fungal infection. Further work is needed to find out antimicrobial agent which inhibits the growth of the fungal disease occuring in this plant. So, preventive measures can be taken to control the disease at the earliest to avoid the economic lose and to preserve the endangered plant species.

Acknowledgment

Authors are thankful to Department of Botany, VPM's B. N. Bandodkar College of Science, Thane for providing the laboratory facilities. Authors are also thankful to Dr. Saheed Shahul Hameed from Botanical Survey of India, Kolkata for providing the plant material required for the research work.

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Plate 1: Infected leaf of *Lodoicea maldivica*

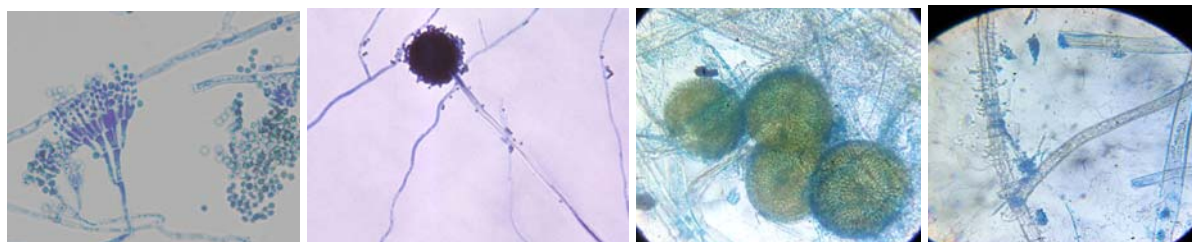

Plate 2: Microscopic morphology of fungal isolates from leaf of *Lodoicea maldivica*

Evaluation of Nutritional Analysis of Some Millets

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Abstract: Millet is a general category for several species of small grained cereal crops and is a food staple in parts of India. Milletes include Jowar (great millet), Bajra (pearl millet) and Ragi (finger millet) are the more prominent. These are available in Indian markets and in bulk areas of most natural food markets in the form of grains and also flour forms. Millet lacks gluten. In the developed countries, there is a growing demand for gluten-free foods and beverages from people with celiac disease and other intolerances to wheat. Therefore, in the present work an attempt was made to evaluate protein estimation and vitamin content in Jowar, Bajra and Ragi. The protein content was found to be 11.0, 9.0 and 14.0 gm/100gm and vitamin content was found to be 16.30, 11.90 and 11.14 mg/gm in Jowar, Bajra and Ragi respectively.

Keywords: Millet, Jowar, Bajra, Ragi

Introduction

Millet is the name given to a group of cereals other than wheat, rice, maize & barley. They are mostly tiny in size, round in shape widely grown around the world as cereal crops or grains for fodder and human food. Millets are important crops in the semiarid tropics of Asia and Africa (especially in India, Nigeria and Niger), with 97% of millet production in developing countries (McDonough *et al*., 2000).

The most widely grown millets are Jowar (great millet), Bajra (pearl millet) and Ragi (finger millet), which were important crops in India (Anonymous, 1996).Raw sorghum provides calories, carbohydrates, fat, and protein. *Sorghum* supplies numerous essential nutrients in rich content including fiber; vitamins and several dietary minerals, including iron and manganese. *Sorghum* contains no gluten, making it useful for gluten-free diets (Evans *et al*., 2010).

Bajra is the most widely grown species of millet, grown in India and Africa since prehistoric times. It is now generally accepted that pearl millet originated in Africa and that it was introduced into India from there. Bajra is well adapted to production systems characterized by low rainfall, low soil fertility, and high temperature, and thus can be grown in areas where other cereal crops, such as wheat or maize, would not survive. The Bajra (*Pennisetum glaucum*) protein has more lysine, methionine and tryptophan than other food grains. Also it is recognized as low fat diet. Bajra contains thiamin (Vitamin B1) and iron (Tornekar *et al*., 2009).

Eleusine coracana, or finger millet, is an annual herbaceous plant widely grown as a cereal crop in the arid and semiarid areas in Africa and Asia. Finger millet is especially valuable as it contains the amino acid methionine. Finger millet can be ground and cooked into cakes, puddings or porridge. In Southern parts of India, pediatricians recommend finger-millet-based food for infants of six months and above because of its high nutritional content, especially iron and calcium. Home-made ragi malt is a popular infant food and very good for health (D'Andrea *et al*., 1999).

The aim of the present work was to evaluate the protein and vitamin content in some millets (Jowar, Bajara and Ragi).

Materials and Methods

Collection of plant materials: Flours of jowar, bajra and ragi were purchased from local market, Thane.

1. **Estimation of protein:** Protein was estimated by Lowry's method (Sadashivam & Manickam, 2008). 0.1 ml of the Plant extract was added in a tube with 1.9 ml of distilled water. After that 5.0 ml of Reagent C was added to the test tubes and allowed it to stand. After 15 minutes 0.5 ml of Folin's reagent was added all the tubes. Allow it to stand for 20 minutes and took OD at 625nm.
2. **Estimation of Vitamins:** Vitamins were evaluated by DCPIC method (Sadashivam & Manickam, 2008). One gram of sample was taken and 500 ml of distilled water was added. The solution was filtered using a muslin cloth and titrated 5 ml DCPIP against it till the colour changes to light pink. Readings were noted and vitamin C was calculated.

Results and Discussion

Three millet types (Jowar, Bajra and Ragi) were used for present study for its evaluation of protein and vitamin content. The total protein contents in these three millets are shown in Table 1 and the amounts ranged from 11.0, 9.0 and 14.0 gm/100gm in Jowar, Bajra and Ragi respectively (Table 1). The amount of vitamin was found to be 16.30, 11.90 and 11.14 mg/gm in Jowar, Bajra and Ragi respectively (Table 2).

The protein content in whole grains of minor millets varied from 4.76% in Finger millet to 13.10% in Foxtail millet. Foxtail and Barnyard millet showed comparable amounts of crude protein which was highest among all the millets

(Chandel *et al*., 2014). Baebeau and Hilu (1993) found a broad range of 7.5–11.70% protein in eleven genotypes of Finger millet.

Conclusion

The present study reports the nutritional difference among the three millets (Jowar, Bajra and Ragi) in terms of protein and vitamin composition. Millets are staple food source that is not only providing major nutrients like protein, carbohydrate, fat etc. but also provide ample of vitamins and minerals. In developing country, occurrence of malnutrition and various health problems like obesity, diabetes, cancer, etc. are most prominent because of inadequate supply of nutrition. This is mainly due to the little utilized agricultural crops as food and unawareness of people and lack of knowledge to people. Millets are easily available and cheap in cost. Millets contain many major and minor nutrients like carbohydrate, good protein, fat, dietary fibre, vitamins and minerals. This study emphasized on nutraceutical properties of millets and the application of millets as alternative cereals potentially healthy to elaborate therapeutic food products like protein and energy rich diet, diet for diabetes, gluten free diet, etc.

Acknowledgments

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Table 1: Estimation of proteins by Lowry's method

Sr. No.	Sample	Amount of protein (g/100gm)Mean±SE
1.	Jowar	11.0 ± 0.06
2.	Bajra	9.0 ± 0.05
3.	Ragi	14.0 ± 0.03

Values are mean of three determinants.

Table 2: Estimation of vitamins in three millets (Jowar, Bajara and Ragi)

Sr. No.	Sample	Amount of vitamin (mg/100gm)Mean±SE
1.	Jowar	16.30 ± 0.05
2.	Bajra	11.90 ± 0.15
3.	Ragi	11.14 ± 0.06

Values are mean of three determinants.

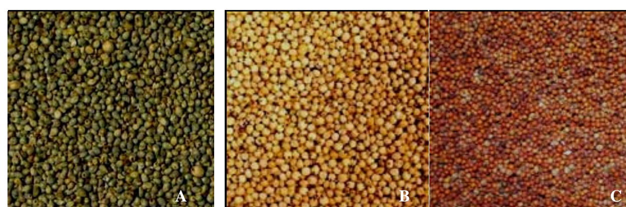

Plate 1: A: Jowar grains; B: Bajra grains; C: Ragi grains

Genome Editing And Personalized Medicine Is A Next Generation of Solid Cancer Treatment for Targeting The Cancer Epigenome

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Abstract: Cancer ismultiple genetic and epigenetic events. The cancer development and progression, tumor cells undergo abnormal epigenetic modifications, including DNA methylation, histone deacetylation and nucleosome remodeling.Currently many scientists and laboratory focus for a new method for cancer treatment and prevention such as genome editing(CRISPR-Cas9 method) and personalized medicine.Many years before the entry of CRISPR-Cas9, these previous methods were time-consuming, required higher training and were often expensive. Advantages of CRISPR-Cas9 over others like transcription activator-like effector nucleases and zinc finger nucleases are many. It is easier, faster, precise, and more cost-effective. Secondly, the personalized medicine used for the targeted treatment of solid cancer. Cancer genome sequencing has created an opportunity for precision medicine. Thus far, genetic alterations can only be used to guide treatment for small subsets of certain cancer types with these key alterations. Similar to mutations, epigenetic events are equally suitable for personalized medicine. DNA methylation alterations have been used to identify tumor-specific drug responsive markers. We review here recent work undertaken in cancer genomics with an emphasis on translation of genomic findings and treatments.

Keywords: Cancer genomics,Translation,Epigenetic modifications, CRISPR-Cas9, Personalized medicine

Introduction:

Research on solid cancer biology has provided conclusive evidence on the primary role of genetic alterations in the initiation and progression of cancer. However, the deregulation of epigenetic modifications such as DNA methylation and alterations of "histone code" are equally important oncogenic factors (Baylin SB, Schuebel KE, 2007; Jones PA, Baylin SB, 2007). Epigenetic modifications affect the packaging of chromatin and direct distinct cellular gene expression programs. They are heritable through cell division and do not involve changes in the DNA sequence (Jones PA, Baylin SB. 2007; Clark SJ. 2007). Operating at the level of chromatin structure, epigenetic mechanisms play a key role during embryogenesis, X-chromosome silencing, cellular proliferation and differentiation and in disease states (Turner BM., 2007; Baylin SB.2007; Clark SJ. 2007). They also facilitate a selective readout of the genome, thereby regulating stem cell developmental potential and cell fate. Subtle disturbances of the epigenetic framework in progenitor, differentiating or terminal cells may, besides well-known genetic alterations, promote carcinogenesis (Meissner A, 2010; Mikkelsen TS, *et al,* 2007).The dynamic and reversible nature of epigenetic mechanisms makes these processes of therapeutic relevance in many diseases including cancer.

Epigenetic modifications involve methylation of DNA and post-translationalmodification of nucleosomal histones, which contribute to a complex "epigenetic code" that superposes the nucleotide sequence to direct gene expression (Baylin, 2007; Bird, 1986; Esteller, 2007).The epigenome comprises a tissue-specific profile of DNA methylation, histone modifications, nucleosome remodeling and RNA-associated silencing **(Figure)**,which collectively result in heritable changes in gene expression. Cancer is a disease driven by progressive genetic and epigenetic aberrations that manifest as global alterations in chromatin packaging and by specific promoter changes that influence the transcription of associated genes (Fabbri *et al*., 2007; Tsai *et al*., 2010; Lujambio *et al*., 2007).

In epigenetic modifications, a cooperative interplay between DNA methylation, histone modifications and nucleosome remodeling. Evidence supports a role for small noncoding RNAs (ncRNAs) in epigenetic gene silencing. These molecules are believed to be involved indirectly *via* post-transcriptional inactivation of key epigenetic enzymes (Fabbri *et al,* 2010). The microRNA class of ncRNAs has emerged as a target of DNA methylation in cancer cells (Lujambio *et al,* 2007).Diceran endoribonuclease that cleaves hairpin microRNA precursors into short double-stranded microRNA intermediatesis required to maintain promoter hypermethylation of specific epigenetically silenced loci in human cancer cells (Ting *et al,* 2008). Promoter-directed small interfering RNAs can induce sequence-specific promoter methylation and associated gene silencing in mammalian cells (Morris *et al,* 2004).

Epigenetic modifications in cancer cells promote genomic instability and lead to silencing of tumor-suppressor genes and reactivation of endogenous retroelements (mobile DNA sequences that transpose via an RNA intermediate). However, unlike genetic anomalies, epigenetic events do not alter the DNA code and are potentially reversible. Reactivation of epigenetically silenced genes can, therefore, provide attractive therapeutic opportunities. Many promising agents have been identified that behave as inhibitors of key enzymes involved in

establishing and maintaining the epigenetic profiles of specific cell types through the process of DNA replication(Ting *et al*, 2008; Morris *et al*, 2004).

Epigenetic therapy is being more and more recognized as an effective and well-tolerated treatment of cancer. Many research data shows proof of principle that solid tumours well respond. This is also supported by preclinical data. Many Research convinced that, similarly to what has been observed for tyrosine-kinase inhibitors in cancer (Peters *et al*., 1995), we need a better selection of tumours and of patients that may benefit from these treatments. It has already been stated that epigenetic drugs and HDAC inhibitors in particular, “might be useful only in those tumours in which HDACs are directly involved in the pathogenesis” (Minucci and Pelicci, 2006).

Treatment of Cancerby genome editing (CRISPR-Cas9)-Bench to Bedside

Although gene editing was being done by laboratories many years before the entry of CRISPR-Cas9, these previous methods were time-consuming, required higher training and were often expensive. Advantages of CRISPR-Cas9 over others like transcription activator-like effector nucleases and zinc finger nucleases are many. It is easier, faster, precise, and more cost-effective.

Test new therapeutic targets.For instance, novel targets discovered using the cancer genome atlas can be assessed.

Completely knockout or enhance a gene with high specificity for research.This can make it possible to study the impact of genes on growth, division and death of cells. One can also distinguish between driver and passenger mutations. Another area is the study of tumor-microenvironment interactions *in vitro* and *in vivo.*

Can be used to enhance a gene.This is a property which sets CRISPR apart from its counterparts, as it can be employed to enhance genes as well.

Recreate the steps leading to oncogenic transformation.Thus, one can recapitulate tumor genesis, both with regard to the number of mutations and the particular order of the mutations. For example, the use of CRISPR in mouse lung was reported by two groups back-to-back in 2014, for engineering oncogenic chromosomal rearrangements in mice to create a tissue-specific model of Eml4–Alk-driven lung cancer (Maddalo *et al.*, 2014; Blasco *et al.*, 2014).

Discover targets for combination therapies.For example, one approach would be to generate a gene knockout library for a cancer cell line that normally responds well to a drug. Cells from this library that grows in the presence of low dose of the drug are sensitized to the drug therapy (in the absence of only the single gene that was knocked out). These genes can give us mechanistic insights and new targets for designing combination treatments to enhance therapy.

Understand underlying mechanisms of drug resistance.Another approach would be to grow cells in the presence of high dose of a drug. The cells that survive are resistance to the drug. Now CRISPR can be used to knock out one gene at a time, once again to generate a library. The principle is that a gene critical to drug resistance, when knocked out, will obliterate this resistance.

Personalized cancer medicine

Cancer genome sequencing has created an opportunity for precision medicine. Thus far, genetic alterations can only be used to guide treatment for small subsets of certain cancer types with these key alterations. Similar to mutations, epigenetic events are equally suitable for personalized medicine. DNA methylation alterations have been used to identify tumor-specific drug responsive markers.

Accumulation of genetic and epigenetic alterations is regarded as a major factor for cancer initiation and progression (Guo *et. al,* 2008; Guo *et. al,* 2006). The genetic structure is similar to the ‘hard drive’ of a computer, and epigenetic modifications may serve as the ‘software’ (Azad *et. al,* 2013). Precision medicine refers to individualized prevention and treatment strategies that take individual variability into account based on the underlying molecular causes and other factors, defined by geneticist (Hamburg and Collins, 2010;Collins and Varmus, 2015). The perfect ‘precision medicine’ is coupling established clinical–pathological indices with state-of-the-art molecular profiling to create diagnostic, prognostic and therapeutic strategies precisely tailored to each patient’ requirements (Hamburg and Collins, 2010; Mirnezami *et al*., 2012). Pharmacogenomics regard ‘precision medicine’ as an approach to discovering and developing medicines and vaccines by integrating clinical and molecular information (Katsnelson, 2013; Dolsten and Sogaard, 2012). Precision medicine is more about identifying individual therapies than causes. Data from the Human Genome Project have advanced the practice of personalized medicine. Knowledge of a patient’s genetic profile can help doctors select the proper medication or therapy and administer it using the proper dose or regimen (Hamburg and Collins, 2010). Targeting therapy based on aberrant genomic changes has already had an impact on the clinical care of cancer patients. Treatment of cancers with EGFR gene mutations by EGFR kinase inhibitors is a successful example (Sharma *et. al,* 2007). A total of 138 ‘mutation (Mut)-driver’ genes were discovered by cancer genome landscape in various solid cancer.

Although there are many mutations found in most tumors, it has been suggested that the average tumor contains only two to eight 'driver gene' mutations which are involved in 12 signaling pathways that regulate cell fate, cell survival and genome maintenance (Vogelstein *et. al,* 2013). Aberrant epigenetic changes occur more frequently than gene mutations in human cancers (Beggs, 2013).

It is now a well-established concept that epigenetic alterations are driver events in the pathogenesis of human cancers. In contrast to 'mut-driver genes,' a greater number of 'epi-driver genes' are abnormally expressed by aberrant epigenetic changes in human cancers (Dolsten and Sogaard, 2012). A growing number of tyrosine and serine-threonine kinase inhibitors have been developed based on the human cancer genome. These agents have been shown to possess antitumor activity and they have reached the pharmacy.

Accumulating studies of the epigenetic profile in different cancer has already found a number of 'epi-driver genes'. However, the landscape of human epigenome and human cancer epigenome is waiting to be characterized due to the technique limitations. The whole picture of 'epi-driver genes' is eagerly needed in the epigenome-based precision medicine. In the past twenty years, researchers mainly focused on promoter region regulation. More complete DNA methylomes in different cancers will help to better understand the disease mechanisms and to develop improved therapies.

Conclusion

Currently, epigenetic therapies are successfully used in the clinic to treat hematological malignancies. However, little success has been achieved in treating solid tumors. Deeper understanding of the normal physiological functions of the genetic and epigenetic regulators such as scientist and clinician will help identify more potential epigenetic targets. The combination of genetic and epigenetic markers may improve the efficiency of chemotherapy by selecting specific targeted drugs for different individuals.

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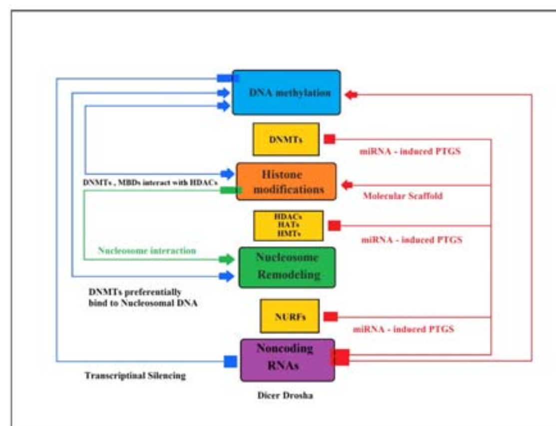

Figure: **Different faces of epigenetic modifications.**

Abbreviations: DNMT, DNA methyltransferase; HAT, histone acetyl transferase; HDAC, histone deacetylase; HMT, histone methyltransferase; MBD, methyl-CpG binding domain protein; miRNA, microRNA; NURF, nucleosome remodeling factor; PTGS, posttranscriptional gene silencing.

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Vitamin D Deficiency and Lifestyle- A Review

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Abstract: The article is review to focus on life style and vitamin D and Calcium deficiency. It includes the study of life style of the developed and underdeveloped countries from tropical and temperate regions. There is plenty of sunlight available in tropical countries. Even though the children and elderly people from these area show vitamin D deficiency and medical ailments related with calcium deficiency. The life style factors responsible for this was found to be less exposure to sunlight, use of sunscreen and food habits like less dietary intake of calcium. This causes rickets in children and osteoporosis in adults. Temperate countries located away from the equator get less amount of sunlight. Studies in tropical areas reveal that the teenagers of these areas consume very high amount of carbonated drinks. It causes dental erosion in them. The relation was also found between the serum 25(OH)D level parathyroid hormone. Excess PTH causes mineral loss. Age, oral contraceptive use, moderate alcohol consumption are some of the factors responsible for 25(OH) D.

Key Words: Serum 25 (OH) D, dental caries, physical activity, sunlight, rickets

Introduction

The main source of vitamin D is sunlight. Reduced serum 25-hydroxy vitamin D (25(OH) D) concentrations were found associated with poor nutrition and limited sunlight exposure (Ramel *et al.*, 2009). The vitamin D concentration is measured by measuring 25(OH) D level in blood serum.Vitamin D insufficiency is defined as a serum 25(OH) D < 30 ng/ml (75 nmol/L); Vitamin D deficiency: defined as a serum 25(OH) D < 20 ng/ml (50 nmol/L) (Holick et al., 2011). Vitamin D occurs in two forms. One is produced by the action of sunlight on skin (D3 or cholecalciferol) and the other is found in a limited range of foods (D2 or ergocalciferol). People living in tropical and temperate countries are suffering from Vitamin D deficiency due to lower exposure of sunlight (Ramel *et al.*, 2009; Tom *et al.*, 1999; Nowson and Margerison, 2005; Viet *et al.*, 2001). Decreased dietary intake, diminished sunlight exposure, reduced skin thickness, impaired intestinal absorption, and impaired hydroxylation in the liver and kidney are the factors causing vitamin D deficiency in older people (Omdahl *et al.* 1985, Goth and Tobin 1995, Barragry *et al.*, 1987).The importance of Vitamin D deficiency is related primarily to bone integrity and muscle strength, especially in the lower extremities. Vitamin D deficiency is found to be associated with cancer (Grant, 2015), cardiovascular diseases (Wang, 2012), infectious diseases (Camargo, 2012, Hujoel, 2012), autoimmune diseases (DeLuca, 2016) etc.

The present piece of article is a review that throws light on the different studies done in tropical and temperate regions so as to highlight the differences in their life style and availability of vitamin D.

Background

Vitamin D: Rickets was first describedin detail by Francis Glisson in 1650, but the cause of the disease was not identified untill much later. Two researchers from Untied States Elmer Mc Columm and Marguerite Davis discovered Vitamin A in cod liver oil. Their experiment in dogs first led to the thought that vitamin A cures rickets, because the dogs which do not receive cod liver oil suffering from rickets. However,they administered dogs with cod liver oil by removing Vitamin A were recovered from rickets. This led to the discovery of Vitamin D (vitamin D is fourth vitamin discovered thus it got the name Vitamin D the fourth letter of the english alphabet).Researchs continued on this substance and it was eventually discovered that a certain form of Vitamin D is created in the human body through exposure to sunlight.

The present study focuses on the relation between life style, mainly exposure to sunlight and dietary intake of vitamin D and calcium with respect to bone health. The four parts of the world with diverse climatic conditions, mainly in terms of exposure to sunlight and their developmental status are considered. Various studies throwing light on this aspects are reviewed in the current paper.

Australia: Australia is a sixth largest country having six distinct land forms. 18% of its landmass is desert. Northern part has tropical climate where as south east and south west are temperate. It is an advanced country with GDP 1.205 trillion USD. The medical journal of Australia 2002 shows that the main source of vitamin D for Australians is exposure to sunlight. Rob Daly (2012) found that one third of population in Australia is suffering from vitamin D deficiency. Some people who go to job early in the morning and come back to home at night.So those people get only very less amount of sunlight. Their major source of vitamin is fish which contain high amount of fat, milk and fortified margarine meat. Nowson and Margerison (2002) are also of opinion that the main source of vitamin for Australians is expsoured to sunlight. Their studies shows groups with dark skin, veiled women (particularly in pregnancy), their infants and older person living in residential care are suffering

from vitamin deficiency. Some experts believe that fortification as a way to increasing vitamin intake, but it was went unsuccessful, because the actual amount of vitamin D varies greatly in fortified food. Furthermore, people with restricted dietary intakes such as the elderly will not benefit greatly from fortification. Like this many attempts were done to provide vitamin D through diets but it was insufficient to correct deficiencies. Gill *et al.* 2014, in their study on vitamin D status of Australian, say that the deficiency is due to obesity and lack of physical activity.

Nigeria: Nigeria is also a tropical country having GDP of 405.10 billion US dollars in 2016 where as that of Australia was 1.205 trillion US dollars which says that this is underdeveloped country having located in the tropical region. Nutritional rickets are more common among tropical countries like Nigeria, due to the less dietary intake of calcium in diet (Tom *et al*; 1999). They found that calcium supplements with or without vitamin D therapy was more effective than supplementation with vitamin D alone in healing active rickets in cohort of Nigerian children. Vitamin D deficiency does not appear to be an important cause of rickets in these children (Okonofua *et al.* 1991). Enriching their diet with inexpensive, locally acceptable food sources of calcium may prevent rickets in such children. Pfitzner *et al.* (1998) showed that since the children play outside the home for more than 8 hrs., no vitamin deficiency is noted.They also had the same opinion that the rickets are seen among those children who had dietary calcium deficiency and not vitamin D deficiency. Studies by Robert *et al.* (2010) also supports that vitamin D concentration is higher in Nigerian women than the Caucasian women in US. This suggests that the exposure to sunlight is sufficient in Nigerians to keep their vitamin D optimum, however, because of insufficient dietary intake of calcium, there are cases of nutritional rickets in them.

Iceland: It is an arctic country where sun light is available for 3 to 5 hours only. GDP (2016) of Iceland is 20.05 billion USD, which is far less than Nigeria. Studies on Icelander populationshow dental erosion in teenagers due to the high consumption of carbonated drinks (Inga *et al*; 2003). Erosion of the labial surface of the maxillary incisors to be the most common (Lussi *et al.*2002). The recent emergence of erosion as a problem may, however reflect a change in consumption of soft drinks among young people in the past decade.The same conclusion was drawn by (Bartlett *et al*; 2002) in an investigation of erosion among adolescents in the U.K. Further refinements of the techniques as well as a search for other possible aetiological factors are necessary. Gunnarsson *et al.* (2004) say a daily intake of 15-20 microg/day during wintertime would be required to maintain normal homeostasis in Icelandic adults, which is considerably higher than present recommendations of 7-10 microg/day for adults. The 25(OH) D was found decreasing from 1 year age to 6 years of age (Thorisdottir *et al.* 2016) which says that it could be firstly due to reduced intake of vitamin D per kg of body weight. Second reason may be higher 25(OH) D at infancy may be the negative feedback which decrease the levels later. Thirdly the 25(OH) D may be over estimated due to higher concentration of C3 epimers in infancy.

Canada: This is situated in northern North America and is in arctic region. The climate is temperate to sub-arctic.It is one of the developed countries having GDP 1.53 trillion USD in 2016. It is a country which receives maximum 24 hrs sunlight during summer where as in winter nights are of 24 hours. Studies show that serum 25(OH) D changed substantially with season (Viet *et al;* 1999). The prevalence of a low 25(OH) D concentrations was greatest during the winter half of the annual vitamin D cycle (November to April).They divide the subjects based on colors to characterize the effect .The mean serum 25(OH) D levels in the non white women were not significantly lowered in either season than in white women indicating that their vitamin D status is not as severely compromized as American black women. But prevalence of vitamin insufficiency in nonwhite women are greater than white women. Those women with dark skin do not intentionally spend time to expose sunlight, because they are afraid that their skin color will get deepen due to sunlight.Therefore often they actively avoid the sun. Study by Langlois *et al.* (2010) shows that only 4% of the population was vitamin D deficient and 10% population has inadequate concentrations of 25(OH) D for bone health. Whiting *et al.* (2011) suggest that current food choices alone are insufficient to maintain 25(OH) D concentrations of 50 nmol/L in many Canadians, especially in winter.

Conclusion

Vitamin D plays a key role in maintaining bone health.Studies from different tropical and temperate countries show more vitamin D deficiencies are due to life style and food habits of the people. Less exposure to sunlight because of the indoor jobs or intentional blocking of sunlight by avoiding sun or wearing veil cause vitamin D deficiencies. Even though there is sufficient exposure to light is achieved, still the cases of poor bone health are noted which are due to poor intake of calcium as well as vitamin D supplements. The underdeveloped countries are victim of poor intake. Besides this better exposure to sunlight with improved calcium rich diet keeps their bone healthy. Icelandic population being less exposed to sunlight suffers through ill bone health. The worsened lifestyle of teenagers and insufficient exposure to sunlight have spoiled their dental health.

increased deficiency of vitamin D also cause secondary hyperparathyroidism.The only way to check vitamin D by measuring serum 25(OH)D level in blood.serum

level will not be less than 40nmol/l .serum level of 25(OH)D should be 40nmol/l in adults .There are some other medical ailments with respect to vitamin D deficiency are type 2 diabetes Mellitus,schizophrenia,cardiovascular disease,cancer,primary hypertension,stroke and renal disease ,pancreatic cancer.

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Theme - III
Bio-Physics

Growth and Photo Electrochemical Behavior of Non-stochiometric Tungsten Diselenide Single Crystals

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Abstract: The semi conductor have been used for many years as photovoltaic/photo catalytic solar energy converter, alternative green power sources, solid state lubricants as catalysts in many industrial applications. Biotic factors such as light, temperature, water, atmospheric gases combine with biotic factors (all surrounding living species). Environment often changes after some time and therefore many organisms have ability to adapt to these changes. The development of photo based material was simulated largely by the need for working out a photo electrochemical (PEC) solar energy conversion method as a new ecologically clean and inexhaustible source of energy. In the most promising variant method of converting solar energy in to electrical energy using solar cells (PEC) with semiconductor electrode. Single crystals of tungsten diselenide(WSe_2) emerged as a most efficient photo electrode material used in the PEC solar cells have been Grown by using Direct vapors transport technique (DVT) . The electrical properties of $W_{1\pm x}Se_2$ single crystal are studied and resistivity through vander-Pauw method in the High temperature range (313K – 423 K) is obtained. The vander-Pauw technique is also used to evaluate the type, mobility and carrier concentration of $W_{1\pm x}Se_2$ the single crystals of photovoltaic cells.

Keywords: Dichalcogenides, decompose, non-stoichiometry, semiconductor, resistivity

Introduction

The sun is the only source of energy for all eco-systems on earth, The incident solar radiation are useful for alternative green power source of energy A $W_{1\pm x}Se_2$ crystallize in a layered structure. The space group of these crystals is $P6_3$/mmc and the basic co-ordination unit for these structures is a trigonal prism. (Perker, R.L., 1970) The metal atom (W) at the centre of the prism is co-ordinate with six selenium atoms at the corners. A layer is composed of alternatively occupied prisms places side by side and no strong bond exists across the gap between the layers while only long range Vander walls forces hold atomic sandwiches together. This gives growth and pronounced cleavage perpendicular to c-axis. As a consequence the crystals possess facile basal cleavage, lubricity and marked anisotropy in their physical and chemical properties. Since the material of $W_{1\pm x}Se_2$ possesses a semiconducting nature, the purpose of present study of tungsten Diselenide transport properties like the resistivity (ñ), Hall coefficient (R_H), Mobility (μ) as well as carrier concentration (n).

Methodology

Since single crystals of $W_{1\pm x}Se_2$ are not available in nature, they have to be synthesized in the laboratory. There are several methods of growing single crystals. (Buckley,H.F.1951) In the recent years (Lawson,W.D.& Nielson,S.1958) There has been much interest in transition metal dichalcogenides of the form MY_2 (M=Transition metal W and Y=chalcogen Se_2) for potential use in the conversion of solar energy in to electrical or chemical energy. Very high conversion of efficiencies has been reported with tungsten diselenide for the conversion of solar energy in to electrical energy (Salvador, P.Chaparro A.M.and Mir,A.1996). It is seen (Vogt *et.al.,1989*). that variation of stochiometry in WSe_2 affect its photovoltaic performance. e.g. lowering the selenium excess during the crystal growth increases overall quantum efficiency of WSe_2 diodes.Additionally,the open circuit voltage V_{oc} could be increased by reducing the selenium excess down to 1%. Therefore there is a need for a systematic study of non-stoichiometric compound of WSe_2. Recently (Molenda, J.and Bak.T. 1993) have reported a study of electronic and electro-chemical properties of non-stoichiometric The importance of tungsten diselenide WSe_2 in the fabrication of liquid junction photo-electrochemical cells has been shown by (Aruchamy A.and Agarwal, M.K. 1992) WSe_2 Since the electrical transport properties of semi-conducting material plays an important role in deciding the photo-conversion efficiency of a solar cell fabricated with semiconductor. Therefore, one goes for growing these crystals by the method of growth from the vapour phase. Depending upon the phenomena involved, the vapour phase method is classified in the following two categories: (i) Chemical vapour transport and (ii) Direct vapour transport.

Single crystals of $W_{1\pm x}Se_2$ with x = -0.10, 0.02 have been grown using a direct vapour transport technique. A stoichiometric mixture of 99.999% pure tungsten powder and 99.95% pure selenium powder were sealed under a pressure of 10^{-5}Torr in 2.5 cm bore x 24 cm long high quality fused quartz ampoules. The sealed ampoule containing the mixture was then introduced into a two zone furnace at a constant-reaction temperature to obtain the charge of $W_{1\pm x}Se_2$. The charge so prepared was rigorously shaken to ensure proper mixing of the constituents and kept in the furnace under appropriate conditions to obtain single crystals of $W_{1\pm x}Se_2$. The growth conditions used for the synthesis are given in Table 1 and 2. The resulting crystals

from the growth were black opaque and plate like with c-axis normal of the plates.

Experimental Techniques: The resistivity measurements perpendicular to c-axis (i.e. along the basal plane) can be investigated using following techniques: (i)VanderPauw Method and (ii) Four Probe Resistivity Method. The room temperature resistivity measurements were made using Van der Pauw method. The resistivity of $W_{1\pm x}Se_2$ single crystals can be measured at different temperatures in the temperature range (313 K - 413 K) by a four probe method. The high temperature resistivity measurements were evaluated using the Four Probe Method. The four probe set up (Scientific Equipments, Roorkee, India) consists of probe arrangement, oven, constant current source, milliammeter and electronic voltmeter. Large size crystals having proper shape were used for the study of variation of resistivity with temperature using the four probe method. Many conventional methods for measuring resistivity are unsatisfactory for semiconductors because metal semiconductor contacts are usually rectifying in nature. Also there is generally minority carrier injection by one of the current carrying contacts. An excess concentration of minority carriers will affect the potential of other contacts and modulate the resistance of the material. The four probe method overcomes the difficulties mentioned above and also offers several other advantages. It permits measurements of resistivity in samples having wide variety of shapes including the resistivity of small volume within the bigger pieces of semiconductor.

Results And Discussion

Resistivity along the basal plane by van der Pauw method

The room temperature resistivity of $W_{1\pm x}$ with x = -0.10 and 0.02 single crystals, perpendicular to c - axis (i.e. along the basal plane) was investigated using van der Pauw method. The results of such measurements for representative samples are given in Table 3 and 4. Using the values of R_1 and R_2, resistivity (ρ) can be calculated. The basal plane resistivity for $W_{1\pm x}$ crystals were evaluated and are reproduced in Table 5. From Table 3, average R_1 = 5.77 Ω and R_2 = 5.71 Ω. From Table 4, average R_1 = 98.12 Ω and R_2 = 61.83 Ω.

High Temperature Resistivity Measurement Perpendicular to c-axis ($\rho_\perp$)

The high temperature resistivity measurement perpendicular to c-axis i.e. along the basal plane were carried out on single crystals of $W_{1\pm x}$ (with x= -0.10 and 0.02) using the four probe technique as described earlier. Table 6 and 7 show the variation of resistivity with temperature (313 K - 413 K) for representative samples of $W_{o\cdot 90}Se_2$ and $W_{1.02}Se_2$ respectively. In all cases it is seen that resistivity increases with decreasing temperature, thereby indicating that the samples show a classical semiconducting behavior.

Hall Effect measurements

A modification of Van der Pauw technique has been used to evaluate the type, mobility (μ) and carrier concentration (n). The phenomenon in which the production of voltage difference across an electrical conductor transverse to and electric current in a conductor placed in a magnetic field perpendicular to the current is know as hall effect. Hall Effect measurement is the only tool to provide basic electrical parameter of material. The sample $W_{1-x}Se_2$, for this measurement is kept in known magnetic field produced by an electromagnet (Type EMPS-5 Omega Electronics, Jaipur, India). This magnetic field modifies the path of electrons producing the Hall voltage. By knowing the values of difference in resistance (ΔR), magnetic field (ΔB) and the thickness of the sample t, the mobility of carriers was evaluated using the following equation

$$\mu_H = \frac{t\Delta R}{\Delta B \rho} \quad (1)$$

Where ρ is the room temperature resistivity of the samples. The Hall coefficient (R_H) and carrier concentration (n) were calculated using the following formula

$$R_H = \mu_H \times \rho \quad (2)$$

$$n = \frac{1}{R_H e} \quad (3)$$

From the sign of Hall coefficient, the nature of the charge carriers in the grown samples could be ascertained. The calculated value of R_H, mobility (μ) and carrier concentration (n) for each case are represented in Table 5. The results obtained from Hall Effect Measurement for sample are given in Table 8 and 9.

Results and Discussion

The variation of resistivity along the basal plane $\rho_\perp$ with temperature clearly brings out the classical semiconducting nature of $W_{0.90}Se_2$ and $W_{1.02}Se_2$ single crystals. Hall effect measurements shown in respective tables and figure. Following are the main results:

(1) The resistivity measurements point out that all the samples of $W_{1\pm x}Se_2$ (with x = -0.10 and x = 0.02) indicate a classical semi-conducting behavior.

(2) The off-stoichiometric crystals of tungsten diselenide show a semiconducting behavior in the temperature range 313K to 413K.

(3) The positive values of Hall coefficient for $W_{0.90}Se_2$ indicate that these crystals are p-type whereas the negative values of Hall coefficient for $W_{1.02}Se_2$ reveal their n-type character.

Conclusion

The electrical transport property measurements are carried out on $W_{1\pm x}Se_2$ (x=-0.10, x=0.02) single crystals. The experiments may be carried out on their photo electrochemical characterization. This can be done by preparing liquid junction Solar cells with them. The suitability of cells so fabricated should be decided in different electrolytes. The Photo electrochemical studies will clearly indicate the effect of non-stoichiometry on the photo electrochemical performance of WSe_2 solar cells.

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Table 1: Growth Parameters used to Synthesize Single crystals of $W_{1\pm x}Se_2$

Material	Reaction Temperature	Growth Temperature	Growth Time	Crystal Size$_{(maximum)}$	Appearance
$W_{0.9}Se_2$	1073 K	1248 K	212 hrs	8mm x 6 mm 0.2 mm	Black Opaque
$W_{1.02}Se_2$	1073 K	1248 K	212 hrs	8mm x 5 mm 0.2 mm	Black Opaque

Table 2: Structural data of $W_{1\pm x}Se_2$

Material	Lattice Parameters	
	a	c
$W_{o.9}Se_2$	12.978 ± 0.01	3.292 ± 0.001
$W_{1.02}Se_2$	12.979 ± 0.01	3.292 ± 0.001

Table 3: Measurement of Resistivity through Van der Pauw Method, (Sample: $W_{0.9}Se_{e,}$ Thickness t = 0.002 cm, Length l = 0.6cm, Breadth b= 0.3cm)

Current I_{AB} mA	**Voltage V_{DC} mV**	**Resistance R_1 ?**	**Current I_{BC} mA**	**Voltage V_{AB} mV**	**Resistance R_2 ?**
0.5	2.30	4.60	0.5	2.30	4.60
1.0	5.70	5.70	1.0	5.30	5.30
1.5	8.90	5.90	1.5	8.60	5.73
2.0	11.7	5.85	2.0	12.1	6.05
2.5	15.3	6.12	2.5	15.2	6.08
3.0	18.3	6.10	3.0	18.2	6.06
3.5	21.5	6.14	3.5	21.5	6.14
4.0	—-	—-	4.0	—-	—-

Table 4: Measurement of Resistivity through Vander Pauw Method (Sample: $\mathbf{W_{1.02}}Se_{2,}$ Thickness t=0.008cm, Length l =0.5cm, Breadth b=0.35cm)

Current I_{AB} mA	**Voltage V_{DC} mV**	**Resistance R_1 ?**	**Current I_{BC} mA**	**Voltage V_{AB} mV**	**Resistance R_2 ?**
0.5	43.2	86.4	0.5	21.5	43.00
1.0	94.3	94.3	1.0	56.6	56.60
1.5	147.8	98.53	1.5	90.8	60.53
2.0	198.3	99.15	2.0	125.8	62.90
2.5	250.0	100.0	2.5	165.2	66.08
3.0	305.2	101.73	3.0	200.0	66.66
3.5	355.0	101.40	3.5	240.4	68.68
4.0	414.0	103.5	4.0	280.8	70.20

Table 5: Result of Measurement on off-stoichiometric Crystals of $W_{1\pm x}Se\text{-}_2$ (with x=0.10, x=0.02)

Sample Examined	**Resistivity ρ Cm**	**Hall coefficient R_H Cm^3/coul**	**Mobility μ cm^2/V.sec**	**Carrier Concentration cm^{-3}**
$W_{0.9}Se_2$	0.0072	5.86	81.50	1.15 x 10^{18} (n)
$W_{1.02}Se_2$	2.82	14.22	5.04	4.71 x 10^{17} (p)

Table 6: Result of High Temperature Resistivity Measurement (Sample: $W_{0.90}$ Se_2 thickness t=0.002cm length l =0.6cm breadth b=0.3cm)

Temp (°C)	Temp T K	$\frac{1}{T}$ x 10^{-3} (k^{-1})	Resistance R k ?	Resistivity $\rho = \frac{Rbt}{l}$? cm	log ρ
40	313	3.19	8.44	8.44	0.92
45	318	3.14	8.02	8.02	0.90
50	323	3.09	7.60	7.60	0.88
55	328	3.04	7.15	7.15	0.85
60	333	3.00	6.72	6.72	0.82
65	338	2.95	6.22	6.22	0.79
70	343	2.91	5.74	5.74	0.75
75	348	2.87	5.26	5.26	0.72
80	353	2.83	4.74	4.74	0.67
85	358	2.79	4.33	4.33	0.63
90	363	2.75	3.98	3.98	0.59
95	368	2.71	3.61	3.61	0.55
100	373	2.68	3.24	3.24	0.51
105	378	2.64	2.95	2.95	0.46
110	383	2.61	2.63	2.63	0.41
115	388	2.57	2.43	2.43	0.38
120	393	2.54	2.27	2.27	0.35
125	398	2.51	2.13	2.13	0.32
130	403	2.48	2.01	2.01	0.30
135	408	2.45	2.88	2.88	0.27
140	413	2.42	1.73	1.73	0.23
145	418	2.39	1.58	1.58	0.19
150	423	2.36	1.42	1.42	0.15

Table 7: Result of High Temperature Resistivity Measurement (Sample: $W_{1.02}$ See Sample Thickness t=0.003cm length l =0.5cm breadth b=0.35cm)

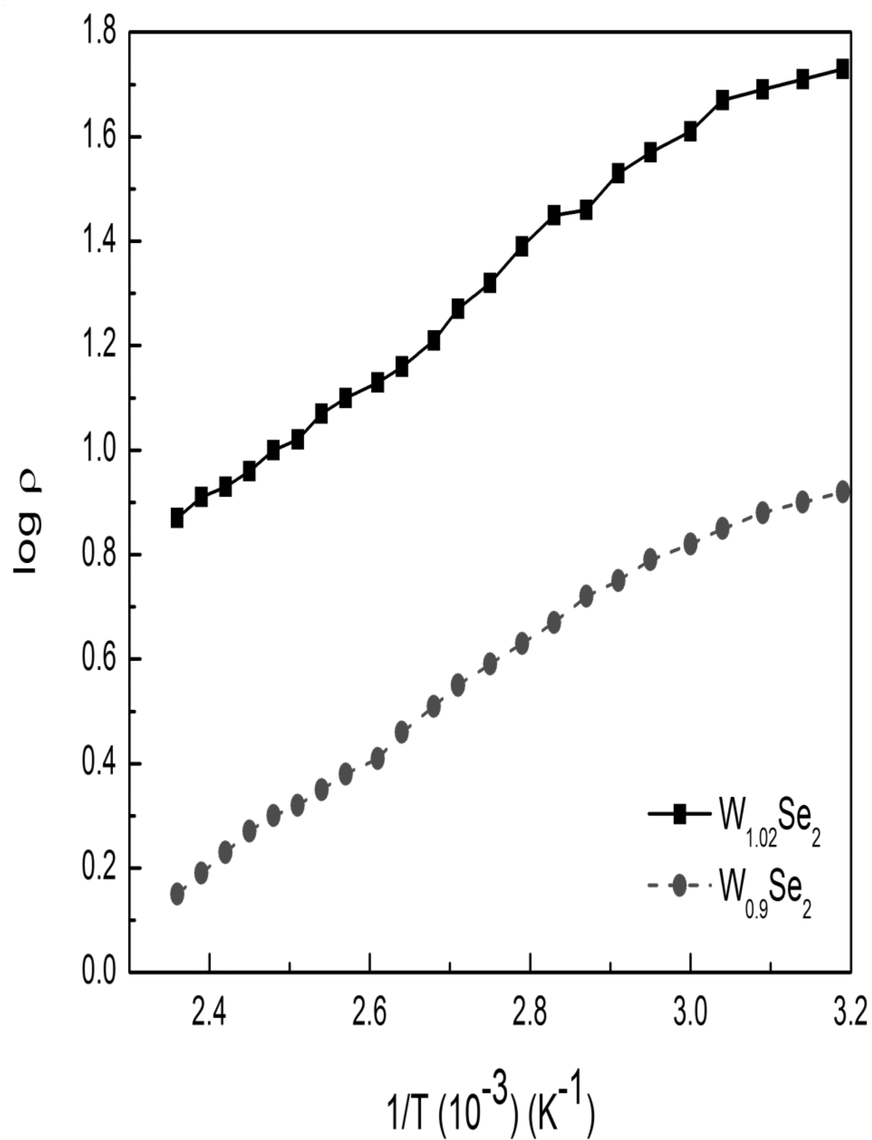
Temp (°C)	**Temp** T K	**$\frac{1}{T}$ x 10^{-3}** (k^{-1})	**Resistance** R k ?	**Resistivity** $\rho = \frac{Rbt}{l}$? cm	**log ρ**
40	313	3.19	26.0	54.60	1.73
45	318	3.14	24.7	51.87	1.71
50	323	3.09	23.7	49.77	1.69
55	328	3.04	22.5	47.25	1.67
60	333	3.00	19.5	40.95	1.61
65	338	2.95	18.0	37.80	1.57
70	343	2.91	16.2	34.02	1.53
75	348	2.87	14.0	29.40	1.46
80	353	2.83	13.5	28.35	1.45
85	358	2.79	11.8	24.78	1.39
90	363	2.75	10.0	21.00	1.32
95	368	2.71	8.90	18.69	1.27
100	373	2.68	7.90	16.59	1.21
105	378	2.64	7.00	14.70	1.16
110	383	2.61	6.50	13.65	1.13
115	388	2.57	6.00	12.60	1.10
120	393	2.54	5.60	11.76	1.07
125	398	2.51	5.10	10.71	1.02
130	403	2.48	4.80	10.08	1.00
135	408	2.45	4.40	9.24	0.96
140	413	2.42	4.10	8.61	0.93
145	418	2.39	3.90	8.19	0.91
150	423	2.36	3.60	7.56	0.87

Table 8: Result of Hall effect Measurement (Sample $W_{0.90}Se_2$ Thickness t = 0.002 cm Constant current I= 1 mA)

Magnetizing Current I Amp	**Magnetic Field B K.Gauss**	**Hall Voltage mV**	**Residtance R = V/I ?**	**Δ R**	**Δ B**	**Hall Coefficient R_H Cm^3/coul**	**Mobility U cm^2/V.sec**	**Carrier Concentration cm^3**
0.5	2.58	-30.7	6.14	0.04	2.26	3.53	49.02	1.76 x 10^{18}
1.0	4.84	-30.39	6.18	0.04	1.89	4.23	58.75	1.47 x 10^{18}
1.5	6.73	-31.1	6.22	0.04	1.55	5.16	71.66	1.21 x 10^{18}
2.0	8.28	-31.3	6.26	0.04	1.24	6.45	89.58	0.96 x 10^{18}
2.5	9.52	-31.5	6.30	0.04	0.92	8.69	120.69	0.71 x 10^{18}
3.0	10.44	-31.7	6.34	0.02	0.75	5.33	74.02	1.17 x 10^{18}
3.5	11.19	-31.8	6.36	0.02	0.52	7.69	106.82	0.81 x 10^{17}
4.0	11.71	-31.9	6.38	—-	—-	—-	—-	——

Table 9: Result Of Hall effect Measurement (Sample $W_{1.02}Se_2$ Thickness t = 0.008 cm Constant current I = 1.0 mA)

Magnetizing Current I Amp	Magnetic Field B K.Gauss	Hall Voltage mV	Residtance R = V/I ?	Δ R	Δ B	Hall Coefficient R_H Cm³/coul	Mobility U cm²/V.sec	Carrier Concentration cm³
0.5	2.58	0.78	0.78	0.03	2.26	10.61	3.76	5.89×10^{17}
1.0	4.84	0.75	0.75	0.04	1.89	17.02	6.03	3.67×10^{17}
1.5	6.73	0.71	0.71	0.02	1.55	10.32	3.65	6.05×10^{17}
2.0	8.28	0.69	0.69	0.03	1.24	19.35	6.86	3.22×10^{17}
2.5	9.52	0.66	0.66	0.02	0.92	17.39	6.16	3.59×10^{17}
3.0	10.44	0.64	0.64	0.01	0.75	10.66	3.78	5.85×10^{17}
3.5	11.19	0.63	0.63	0.02	0.52	30.76	10.90	2.03×10^{17}
4.0	11.71	0.61	0.61	—	—	—	—	—



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Acoustic Properties of Green Coconut (*Cocos nucifera* L.) Water For 2 Mhz Ultrasonic Frequency At Temperatures From 15 ºc To 70 ºc

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Abstract: The coconut water most versatile natural product and refreshing drink with electrolytes is filled with many healthy natural nutrients which can enhance the body's metabolism and immunity .Acoustic properties can characterize the interaction of the sound energy with a material. These properties are mainly influenced by temperature, composition and frequency. The temperature is increasing day by day which can be a factor to change the acoustic properties of green coconut water. The acoustic properties of green coconut water was studied using an Ultrasonic interferometer at temperature intervals of 5°C from 15°C to 70°C at constant frequency. The experimental values of Ultrasonic velocity (U) and density (ñ) were used to calculate various acoustical parameters such as adiabatic compressibility (β_{ad}), intermolecular free length (L_f) and acoustic impedance (Z).

Key Words: acoustic, ultrasonic velocity, adiabatic compressibility, intermolecular free length.

Introduction

Coconut water is mostly consumed locally as fresh in tropical areas since it deteriorates easily once exposed to air. Commercially, it is thermally processed using ultra high temperature (UHT) technology. However, coconut water loses its delicate fresh flavor and some of its nutrients during heating (Prabhakar and Mohana, 2014). Thermal treatments such as pasteurization and sterilization with the addition of preservatives are used to preserve this kind of product (Zhu *et al*., 2012). However, high temperature is required to inactivate the target enzymes (peroxidase and polyphenol oxidase), resulting in degradation of color, taste and nutritional value (Matsui *et al*., 2008). Ultrasonic velocity measurements provide qualitative information regarding the nature and strength of molecular interactions. In recent years, ultrasonic technique has become powerful and reliable tools for the study of molecular interactions in pure liquids as well as liquid mixtures (Ali and Nain, 2002).

Materials And Methods

The density (ñ) of Green Coconut (C*ocos* Nucifera L.) water was determined using volume of water at different temperatures as water is a major component which contributes to change in volume while heating. A constant temperature water bath made up of double vessel one inside the other. This arrangement provides linear heating. The water is regulated through a motor and temperature is measured using a thermometer immersed in water bath. The Ultrasonic velocity (U) in the Green Coconut (*Cocos Nucifera* L.) water has been measured using an Ultrasonic Fixed-frequency interferometer (Mittal type Model F-80) at temperature intervals of 5 °C from 15°C to 70 °C at constant frequency for the measurement of ultrasonic velocity. The liquid understudy is filled in the cell. Ultrasonic wave of known frequency (2MHz) are produced by a quartz crystal fixed at the bottom of the cell. These waves travels through the liquid under study and reflected by a movable metallic plate (plunger) kept parallel to the quartz crystal. The movement of reflector allows the determination of wavelength (l) of standing wave pattern formed within the liquid. From this measured value of 'l', ultrasonic velocity 'U' is calculated which is further employed to determine various acoustic parameters in order to study the ultrasonic behavior of liquid.

Theory

The experimental values of density (ñ) and ultrasonic velocity (U) were used to calculate various acoustical parameters such as adiabatic compressibility (β_{ad}), free length (L_f), Acoustical impedance (Z) (Mandlekar, 2014)

$$U = n\lambda \cdots \quad (1)$$

$$\beta_{ad} = \frac{1}{\rho U^2} \quad \cdots (2)$$

$$L_f = K(\beta_{ad})^{1/2} \cdots \quad (3)$$

$$Z = U\rho \; .....(4)$$

$$K = (91.368 + 0.356T) \times 10^{-8} \;\; [7].... \quad (5)$$

Where, K – Temperature dependent constant (Jacobson's constant)

Results and Discussion

Ultrasonic velocity of sound waves in a medium is fundamentally related to the binding forces between the molecules (Sahu *et al*., 2012). The variation of ultrasonic velocity with temperature is shown in the Fig. (1). The ultrasonic velocity increases from temperature 288K to 303 K but at 308 ^{0} K there is a sudden decrease in velocity followed by slight increment with temperature. The particle-particle interaction increases which can be understood by the rise in velocity.

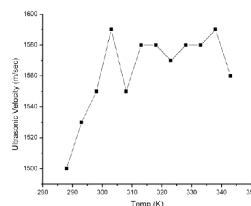

Fig.(1). Ultrasonic velocity vs. Temperature

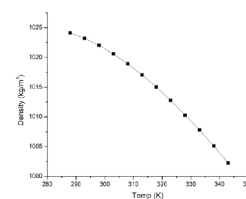

Fig.(2). Density Vs. Temperature

With the increase of temperature there is a reduction in density which tends to increase velocityIt is observed that the acoustic

impedance (Z) value increases with increase in temperature at lower temperature upto 303K which confirms the presence of molecular association as shown in fig.(3). Such increasing trends of impedance further support the possibility of molecular interaction between the components of the liquid .Further increase of temperature shows a reduction in acoustic impedance (Z).

The decreasing trend of adiabatic compressibility may be because of contraction in volume which leads to subsequent decrease in adiabatic compressibility as in fig. (4) (Thirumaran and Rajeshwari, 2011). The increase in free length is due to lose packing of the molecules which may be brought by weakening of molecular interactions shown in fig. (5) (Thirumaran and Rajeshwari, 2011).

Conclusions

The acoustical parameters in the Green coconut water suggest the weak molecular interactions in the molecules of the Green coconut water at different temperatures. Steady behavior in the acoustical parameters of the coconut water after temperature 303K suggests that coconut water is a most versatile healthy drink.

Acknowledgement

Authors are grateful to VPM'S and Mr. S. Venkatraman, Head of the Physics Department, B.N. Bandodkar College of Science, Thane.

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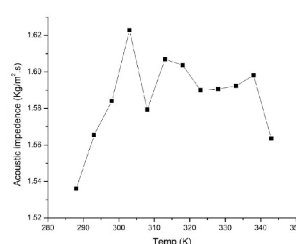

Fig.(3). Acoustic impedance Vs. Temperature

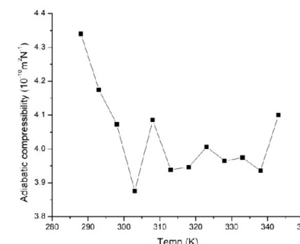

Fig.(4). Adiabatic compressibility Vs. Temperature

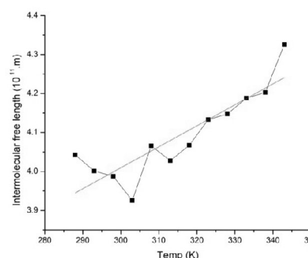

Fig.(5). Intermolecular free length Vs. Temperature

Table No.1 : For Green coconut water

$T\ ^0K$	ρ Kg/m^3	Um/sec	Z ($10^6\ Kgm^{-2}s^{-1}$)	β ($10^{-10}\ m^2N^{-1}$)	Jacobian constant K x 10^{-8}	L_f ($10^{-11}\ m$)
288	1024.1170	1500	1.5361	4.3397	194.04	4.0422
293	1023.1990	1530	1.5654	4.1750	195.82	4.0012
298	1022.0108	1550	1.5841	4.0726	197.60	3.9878
303	1020.5781	1590	1.6227	3.8757	199.38	3.9253
308	1018.9224	1550	1.5793	4.0850	201.17	4.0659
313	1017.0607	1580	1.6069	3.9385	202.95	4.0277
318	1015.0081	1580	1.6037	3.9465	204.73	4.0672
323	1012.7767	1570	1.5900	4.0057	206.51	4.1332
328	1010.3770	1580	1.5906	3.9646	208.30	4.1475
333	1007.8181	1580	1.5923	3.9746	210.08	4.1882
338	1005.1079	1590	1.5981	3.9354	211.86	4.2029
343	1002.2529	1560	1.5635	4.0999	213.64	4.3259
343	1007.6674	1580	1.5921	3.9752	213.64	4.2596

Application of Ion-selective Electrode to the Determination of Barium in Clinical Samples

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Abstract: Ion selective electrodes are the electrodes that respond selectively to a particular ion in presence of others. The application of a graphite electrode coated with polyvinyl chloride membrane containing dibenzo-24-crown-8 as the ionophore has been revealed in the determination of barium in blood and chicken bone samples. The results obtained by using ion selective electrode were compared with ICP-AES method.

Keywords: Barium, ion selective electrode, potentiometry, clinical analysis.

Introduction

Barium is a silvery-white alkaline earth metal. It is used in various alloys; in paints, soap, paper, and rubber; and in the manufacture of ceramics and glass (Tanaka, 1954). Barium occurs in nature in the combined state in the form of compounds such as barite (barium sulfate) and witherite (barium carbonate) ores. Barium sulfate is used as a radiopaque aid to X-ray diagnosis and also as a wetting agent for drilling mud in oil and natural gas industry (Breuer *et al*., 2004, Miner, 1969) while barium carbonate is used in pesticides. Exposure to barium mostly occurs in the workplace (Zschiesche *et al*., 1992, Hung and Chung, 2004, Tsai *et al*., 2011) or from drinking water (ATSDR 2007, Bowen *et al*., 2012). There may also be accidental exposure from other sources like contaminated foodstuff (Ghose *et al*., 2009) or from intentional intoxication (Downs *et al*., 1995, Jourdan *et al*., 2001, Schorn *et al*., 1991, Thomas *et al*., 1998). The health effects of the different barium compounds depend on the solubility of these compounds in water. A benign pneumoconiosis (baritosis) may result from inhalation of barium sulfate (barite) dust and barium carbonate (Newton *et al*., 1991, Penington, 1993, Tardiff *et al*., 1980, Wones *et al*., 1990). Acute oral exposure to high doses of barium has been reported to lead to gastrointestinal disturbances followed by hypokalemia, hypertension and heart rhythm abnormalities (ATSDR, 2007). Hence it is important to devise methods for the determination of barium.

Several methods have been used for the determination of barium in clinical samples such as Radioactivation analysis (Eleanor *et al*., 1957), Inductively coupled plasma–Optical emission spectrometry (ICP-OES) (Teresa Lech 2013), Inductively coupled plasma–Mass spectrometry (ICP-MS) (Jean et al 2005). However, these methods are very expensive for routine analysis and may require multiple steps for sample preparation. On the other hand, potentiometric method based on the use of ion selective electrode is a very simple, rapid and low cost method for analysis and also enables measurement of activity rather than concentration of the analyte. The current manuscript focuses on the application of a graphite coated ion selective electrode based on dibenzo-24-crown-8 as the ionophore (Gunwanti and Anita, 2015) for the determination of barium in blood and bone samples.

Materials

Analytical grade barium nitrate, sodium tetraphenyl borate (NaTPB), dibutyl phthalate (DBP) and dibutyl maleate (DBM) were obtained from Loba Chemie. High purity dibenzo-24-crown-8 (DB24C8) and o-nitrophenyl octylether (o-NPOE) were obtained from Chemical Centre, Multiwalled carbon nanotube (MWNT) was obtained from Sigma-Aldrich while tetrahydrofuran (THF) and polyvinyl chloride (PVC) were obtained from SD fine Chemicals and Chemical International respectively.

Methodology

Fabrication of Electrode

Graphite electrode was coated with a membrane made by mixing ingredients in the composition 33% PVC, 52% 2-NPOE, 7.5% NaTPB, 2% MWNT and 5.5% ionophore DB24C8 in THF solvent, dried under an IR lamp for 24 hours and then conditioned in a 1×10^{-3} M Ba^{2+} solution for 48 hours.

Electromotive Force Measurements

A digital dual channel Potentiometer Model (EQ-603) was used for the potential measurements. The potential of the barium ion selective electrode was measured against the reference saturated calomel electrode (SCE). The cell assembly is shown below:

$^{-}$SCE %%Ba^{2+} solution %membrane, graphite^{+}

Sample preparation

Blood samples were prepared by heating 1 ml of blood serum with 6 ml of concentrated nitric acid and 2 ml of

hydrogen peroxide (Teresa 2013). After cooling, the mixture was spiked with different volumes of 100 ppm Ba^{2+} and diluted up to the mark with distilled water in a 100 ml standard flask.

Chicken bones were dried under sunlight for 24 hours and then converted into ash by heating in a silica crucible on an incinerator for half an hour. 0.1 g of the ash was boiled with 15 ml concentrated nitric acid. After cooling, it was filtered and the filtrate was collected in a 100 ml standard flask, spiked with different volumes of 100 ppm Ba^{2+} and diluted up to the mark with distilled water.

Calibration curve method

The concentrations of barium in samples were determined by the calibration curve method. In this method, potential of the samples and different standard solutions of Ba^{2+} were measured against the reference saturated calomel electrode. All the potential measurements were carried out three times and the mean value was calculated. A calibration curve of the potential measured (E_{cell}) against logarithm of the concentration of standard was used to determine the concentration of barium in samples. The results obtained were compared with the ICP – AES method.

Results and Discussion

The calibration curve of E_{cell} vs. log C obtained for the standard solutions of Ba^{2+} is shown in Fig.1.The potential values for the sample solutions obtained by triplicate measurements were substituted in the equation of line to calculate the concentration of barium. The results obtained are given in Table 1. The results are found to be in good agreement with the ICP – AES method. Minoia *et al*. (1990) reported levels below 10 mg/mL while Heitland and Koster (2006) reported a median of 0.8 mg/mL of barium in blood. However, the blood samples taken in this study reported negative for the presence of barium. This may be because the persons whose blood samples were taken did not come across any exposure to barium. Traces of barium in animal bone were detected by Timm, (1932) but he could not give quantitative details. The bone samples analyzed in the present study indicated the presence of barium in the range of 0.224 ± 0.006 ppm.

Conclusions

Ion selective electrode with high selectivity and low detection limit provide a very useful tool for analysis and can serve the need of diagnostic and clinical laboratories for accurate, rapid and inexpensive devices for routine analysis.

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Table 1: Comparison of the results obtained using ion selective electrode and ICP – AES method

Sample	Volume of 100 ppm Ba^{2+} added (ml)	E_{cell} (mV)	Concentration of Ba^{2+} obtained (ppm)	
			ISE	ICP - AES
Blood 1	0.4	290.7	0.368	0.354
Blood 2	0.5	294.7	0.485	0.476
Bone 1	1.0	308.0	1.219	1.244
Bone 2	2.0	316.7	2.228	2.262

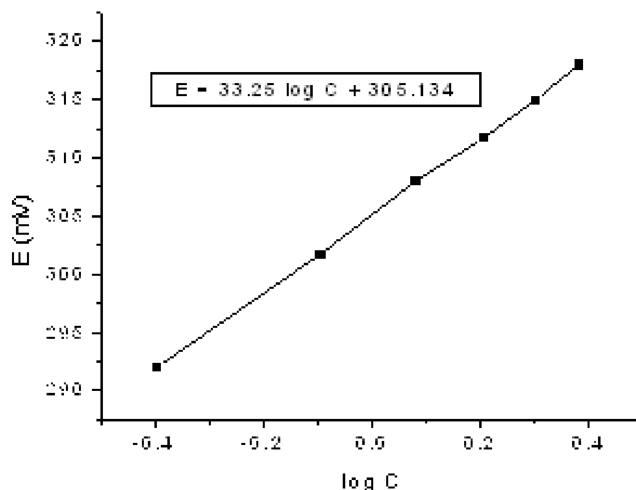

Fig. 1: Calibration curve

Theme - IV
Environment Management

Treatment of Industrial Waste Water For A Greener Earth

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Abstract: Green earth is not only our right but also our responsibility. The removal of organic compounds from waste water is necessitated as per the pollution norms, set. It makes the water harmless for the aquatic life as well as for the human being. Removal of phenolics from Industrial effluent by "Adsorption" is one of the process deployed. Regeneration of the adsorbent makes the process economical. Recovery of phenol makes it further attractive. In the present work, adsorption of phenol on activated carbon from a dilute acqueous solution(phenol concentration of 2kppm)has been studied using indigenous carbon (NVC)followed by a chemical regeneration of the carbon with 2N caustic soda solution. The objective is to provide information to evaluate the applicability and economic feasibility of this process to design a phenolic waste water treatment unit. The performance of the NVC carbon is compared with another grade from LURGI, Germany. The adsorption part focusses on Establishing "equilibrium isotherm", Batch rate adsorption study using available theoretical model (Spahn, Brauch and Schlunder), Adsorption run on a packed column in a semi continuous process to compare the fitment of theoretically predicted breakthrough curve with actually obtained one whereas the desorption part consists of, Development of a theoretical model, Desorption "equilibrium isotherm" and Batch desorption study to evaluate application of model. A column design to treat 10000litres of effluent per day is illustrated.

Key Words: Removal of phenolics, Waste water, Activated carbon, Adsorption, Desorption process, Regeneration and Recovery

Introduction

Prof. Albert Einstein once defined the environment as "Everything that isn't me.". Maintaining environment clean and healthy is everyone's responsibility. There are numerous instances even in the recent past, published in newspapers as regards to environment pollution, which are very disturbing. One wonders, whether we are really serious about ensuring a sustainable development. As a citizen (may it be individual or corporate) do we have a concern for the environment? It is best to leave that to individual's guess. In the environmental sciences, the Intergeneration Equity Principle (IEP), is well explained by the proverb, "We do not inherit the earth from our ancestors, we borrow it from our children". Every generation is under obligation to preserve its natural and cultural heritage for the enjoyment of future generations. This obligation has given rise to so many principles such as, "Sustainable Development", "The Precautionary Principle" and the "Polluter Pays Principle". The various UN conferences starting from the Stockholm Conference, 1972 to Rio to World Summit at Johannesburg in 2002, right up to the last one held in Paris in 2016, all talked about the need and responsibility of all the countries to preserve the environment to save our mother earth.

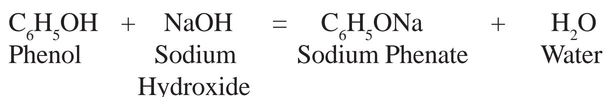
India is also a member of UN and is committed to do so. India is committed to protect the environment through the Constitution, Fundamental Rights of Citizen (Art. 14, 19, 21, 32 and 226), Fundamental Duties and the Directive Principles of State Policy (Art.48A and 48g), Federal structure, division of legislative authorities, judicial dynamism and Activism through Public Interest Litigations (PIL), and establishment of Environmental Courts. Through various landmark judgments in various PIL cases filed before it for example the cleanup of river Ganga, the famous Kanpur Tanneries case(Mehta, 1988) and the famous TN Tanneries case(Vellore Citizens Welfare Forum, 1996), the Supreme Court of India has played a very important role in making the Industry aware of their responsibilities towards the society by ensuring the environmental pollution control and telling them that, if you do not behave, we are there. The Water (Prevention and Control of Pollution) Act, 1974 and Environment Protection Act, 1986 (EPA), compel the Industry to follow the doctrine of 'Absolute Liability'. Thus to maintain a wholesome environment is an absolute necessity, the Corporate Social Responsibility (CSR) and also a responsibility of every citizen of India. Enough legislative work is done. Now is the Society's responsibility to follow the guidelines.

Development of various organic compounds for use of mankind is a blessing but also can be a curse if not handled properly. Phenol (Carbolic Acid) is one of such organic compounds. The 'Phenols' are discharged in effluents from various industries such as textiles, dye and dye intermediates, iron and steel plants, paints, petrochemical industry, oil drilling and gas extraction units, pharmaceuticals, coal washeries and refractory industries.

The EPA rules 1986 (Govt. of India**,** 1986), under the industry specific standards for emission has put a threshold limit of 5 mg/per litre (5 ppm) for phenol concentration in waste water. The present study of 'Removal of phenol from waste water' is a small step in the direction.

Materials And Methods

Selection of Process: Various processes studied by numerous researchers (Ann, 2000; Kulkarni & Kaware, 2013; Laura & Cordova, 2016) include solvent extraction, absorption, volatilization, ,chemical oxidation, ozonization, photochemical treatment, adsorption, polymerization, electro coagulation and ion exchange. Selection of method is governed by various parameters such as composition of waste, incoming feed Phenol concentration, nature of waste, availability of raw materials, process viability etc. for a given situation and also economic consideration. In the present study, adsorption process on activated carbon is selected. This method is usually suitable for lower concentrations of incoming feed (2000 ppm). The various processes deployed for regeneration of carbon are, thermal regeneration (SenthilKumar & Ramkrishnan, 2011), solvent extractions (Cooney, Nageri & Hianes, 1983; Hong & Ying, 2003; Tanthapanickakoon & Ariadejranich, 2004; Matheickal & Linden, 1998), chemical regeneration (Gupta & Sharma, 1998), yeast and sodium hydroxide method (Wenhui & Guocheng, 2017) and polymer supported catalyzed reactions using Benzoyl Peroxide solution in Toluene (Datta & Borthakur, 1988).Types of activated carbon used can be manufactured from Bagasse (Laura & Cordova, 2016), waste tyres (Tanthapanickakoon & Ariadejranich, 2004) and other materials such as wood, coal , coconut shell and polymer scrap (Cooney, Nageri & Hianes, 1983) etc. In the present study, the chemical regeneration of carbon with 2 N Caustic Soda Solution is studied. The chemical reaction is



$$C_6H_5OH + NaOH = C_6H_5ONa + H_2O$$

Phenol + Sodium Hydroxide = Sodium Phenate + Water

The Sodium Phenate thus recovered from Carbon can be further treated with Hydrochloric Acid to recover Phenol.

Equilibrium Isotherm: The equilibrium relationship between the liquid phase and the solid phase adsorbate concentration at constant temperature is called equilibrium isotherm. Each form of equilibrium isotherm can be represented by a suitable mathematical expression. Various researchers have proposed different models such as, Fruendlich Isotherm (Mcketta, 1977; Webber & Chakravorti, 1974), Langmuir Isotherm (Webber & Chakravorti, 1974), Redlich and Peterson Isotherm and Temkin Isotherm (refer figure 2.1) (Bapat, 1977). Weber and Chakravorti found that Redlich and Peterson isotherm fitted better to Phenol well to experimental data over wide range. Neretnicks (Neretnicks 1976) found Tempkins isotherm fitted better to Phenol system. It should be noted that a favourable adsorption isotherm can be a case of unfavourable isotherm for desorption. The equilibrium curve has a vast influence on the breakthrough curve. Garg, Cooney and Lightfoot has studied asymptotic behavior of the adsorption column with different types of breakthrough curves. The breakthrough curves are as shown in figure 2.2 (Bapat, 1977).

The Macro and Micro approach: Macro approach describes the design process of a column as guided by information on equilibrium capacity. Mcketta, 1977, Lukchis and Hutchins have done this exercise. Macro approach is somewhat simpler but more empirical. It helps in rapid determination and correlation of the rate data. However, the confidence in prediction of column is limited. Micro approach is a method of designing column from the first principles. Antonson and Danroff, Weber and Chakravorti, 1974, Neretnicks, 1976 and Ferrel have developed various models. Micro approach is thus more fundamental and sound. Each transport process is represented by a mathematical expression. Thus it leads to better prediction even outside the range of experimental studies. The present work examines the possibility of designing the adsorption and desorption by the micro approach.

Model for Adsorption: Basically there are three steps namely External Diffusion, Internal Diffusion and Adsorption step. The adsorption step is usually assumed to be very fast not contributing to any resistance. The external resistance is governed by applying the film theory and the intra particle transport is described by the various diffusion models. The Pore diffusion model (Neretnicks, 1976) assumes that the adsorbate is transported inside the adsorbent particle by way of molecular diffusion through pore liquid. The driving force is a concentration gradient in the pore fluid. Homogenous solid diffusion model (Rosen, 1952) assumes the adsorption process occurring at the outer surface of the adsorption particle followed by the inward diffusion of the adsorbed molecule. The driving force is concentration gradient on the solid phase. Weber and Chakravorti obtained the solution for this model to predict the theoretical curves which were compared with the experimental curves to calculate the solid phase diffusion coefficient. The agreement was not very satisfactory. Shrinking core model by Spahn, Brauch & Schlunder, 1975; Spahn & Schlunder, 1975 assumes that internal diffusion occurs through the fluid in the pores. The adsorbate is transferred by molecular diffusion inside the particle and is adsorbed in a well-defined concentration front which moves with a varying velocity from the particle surface inwards, leaving behind a used up portion of the adsorbent particle, till the whole of the particle is exhausted by saturation. The various studies in these three models suggest that a solution of a completely generalized model is very difficult to obtain.

Model for Desorption: A mathematical model based on Spahn & Schlunder, 1975 is developed with suitable changes for the desorption system Fig. 3.2 (Bapat, 1977). The various steps are visualized as

- Transport of sodium hydroxide molecule from bulk liquid to external surface of carbon particle through the surrounding liquid film.
- Internal diffusion of sodium hydroxide through pore water.
- Reaction of sodium hydroxide with phenol at the surface.
- Detachment of sodium phenate molecule from carbon surface.
- Outward diffusion of sodium phenate from interior of carbon particle to outer surface.
- Transport of sodium phenate to bulk liquid to external film.

It is argued that outward diffusion of sodium phenate from interior of carbon particle is the controlling step. The desorption is visualized as occurring along a well-defined concentration front which moves inside. A mathematical expression for mass balance on a spherical carbon particle is developed Fig. 3.2 (Bapat, 1977). The information obtained by applying the Spahn and Schlunder model for the adsorption step and a theoretical model as developed above for desorption step is used along with the experimental data to arrive at conclusions.

Experimental Techniques Adsorption:

Batch Study – (Fig. 4.1 A) (Bapat, 1977) A 250 ml glass beaker fitted with baffles and a constant speed stirrer motor assembly is used as container. A desired amount of phenol solution of non-concentration is placed in the beaker. At time t = 0 known amount of dry activated carbon is introduced in the beaker. Thorough mixing of contents is ensured. Liquid samples of 1 ml are withdrawn at regular time intervals and analyzed for the phenol content by 4 amino antipyrine method. For equilibrium tests, the experimental run was continued for 6 hours under agitation and for 4 to 8 days without stirring. Sample was drawn every 24 hours and analyzed till a constant phenol concentration was reached. The test applied is to measure very low concentration of phenol (0.1 ppm to 10 ppm). The apparatus required is a. Spectrophotometer at 510 mμ, b. Filter Photometer equipped with green filter having maximum transmission at 510 mμ, Nestler tube matched 10 ml form. The absorption of the sample with respect to a reference blank, is measured on a Spectrophotometer.

Semi continuous adsorption column – (Fig. 4.3) (Bapat, 1977). A glass column packed with activated carbon was used as fixed bed. A metering pump through rotameter was used to pump liquid of known phenol concentration at the top of the column. Samples from bottom were drawn at different time intervals and analyzed for phenol concentration.

Experimental Technique Desorption:

Batch Study - Carbon saturated with phenol was introduced to a beaker containing 2 N caustic soda solution at time t = 0. Stirring started. Samples were drawn at regular intervals until six hours and analyzed for sodium phenate concentration. Hydrochloric acid was used to neutralize phenate to phenol which was analyzed by bromate – bromide method. Desorption rate tests at higher temperature was carried out using a PEG containing bath fitted with a Jumo relay hitter assembly to maintain a constant temperature (Fig. 4.1 B). Desorption equilibrium isotherm at elevated temperature was established in a similar way.

Summary of work:

- Indigenous grade carbon NG 8/16 from Narmada Valley Corporation (NVC) and Aquaraffin BS-12 from Lurgi, Germany were studied for comparative performance.
- Carbon samples subjected to equilibrium tests to obtain isotherms.
- Batch adsorption rates test were carried out. Predicted curves matched with experimental curves to determine value of effective diffusion coefficient.
- Breakthrough curves were obtained through fixed bed and were compared with predicted curves to test predictability of the model.
- Chemical regeneration of the NVC carbon was studied in the batch desorption tests at various temperature with 2 N caustic soda solution.
- Desorption rate test at 70 degree Celsius was also carried out to study effect of temperature.
- The shrinking core model for desorption was developed.
- The model application was done to experimental results to estimate the effective diffusion coefficient for desorption.

Results And Discussion

- In adsorption step, the Fruendlich isotherm applies better than Langmuir isotherm.
- Lurgi carbon adsorbs about 10 percent more phenol than NVC carbon.
- Agreement between theory and experimental curves is much better with lurgi carbon than that with NVC carbon.

- Diffusion coefficient values estimated are lurgi carbon 8.28 * $10^{-9} m^2$ per sec. and NVC carbon 0.418 * $10^{-9} m^2$ per sec.
- Breakthrough curves for column suggest
- External mass transfer offers considerable resistance to adsorption process and cannot be neglected. However, internal diffusion transport contributes a major portion of the resistance.
- Axial dispersion factor also appears to be playing an important role.
- At room temperature only about 28 percent of the adsorbed phenol got desorbed, while at 52 degree Celsius almost 52 percent of the phenol got desorbed. No significant improvement further observed at 85 degree Celsius.
- Carbon can get regenerated totally but the outlet liquid will contain unreacted sodium hydroxide.
- Effective diffusioncoefficient for desorption in case of NVC carbon is estimated to be 0.209*10^{-9} m^2 per sec.

Column design-An Illustration: Based on the diffusion coefficient estimation and the experimental study carried out the following estimates are worked out.

- Waste water quantity for treatment – 10 Cu Meter per day
- Phenol concentration of waste water – 2000 PPM
- Final expected phenol concentration – 10 PPM
- Amount of carbon required (NVC) stiochiometrically 74 kgs.
- Packed column dimensions dia * height 0.302 m * 1.73 m
- Predicted dimensions micro approach 0.302 m * 1.94 m

Conclusions

Batch rate tests and semi continuous column data for adsorption of phenol when examined in light of shrinking core model indicates applicability of the model to the imported carbon. This model proved inadequate to explain column performance with both the grades of carbon. The discrepancy may be ascribed to axial dispersion which need to be studied.

Chemical regeneration of used carbon in a batch study when applied to theoretical developed model by using shrinking core model shows the model represents the process with good accuracy.

Acknowledgement

I wish to express my deep sense of gratitude to Prof. K. Vasudeva, Professor chemical engineering section UDCT, Matunga, Mumbai for his valuable guidance during the course of this thesis work.

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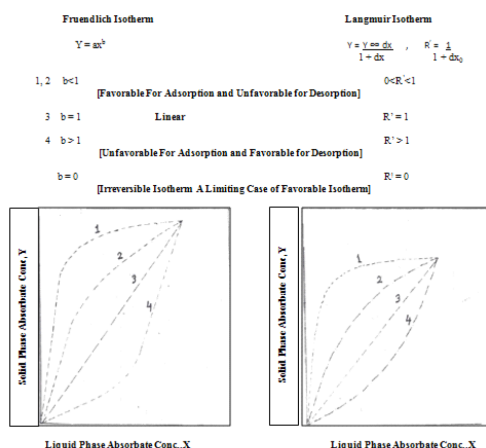

Fig 2.1 **Equilibrium Isotherms**

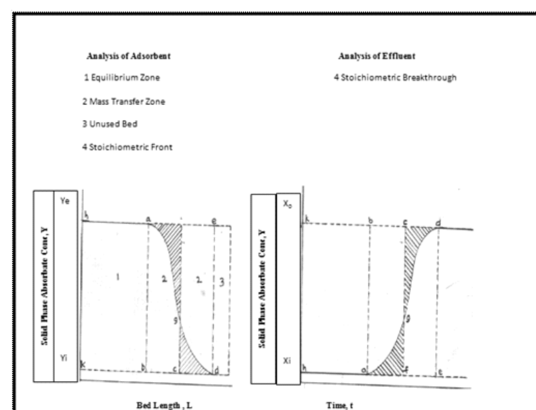

Fig 2.2 **Breakthrough Curves**

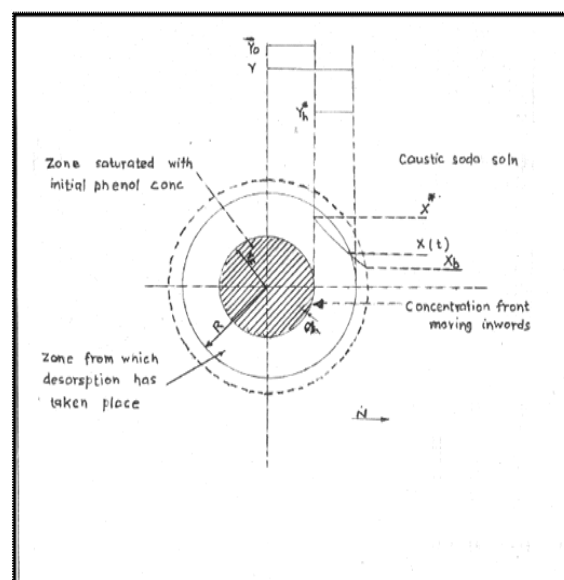

Fig 3.2 **Model for desorption rate studies**

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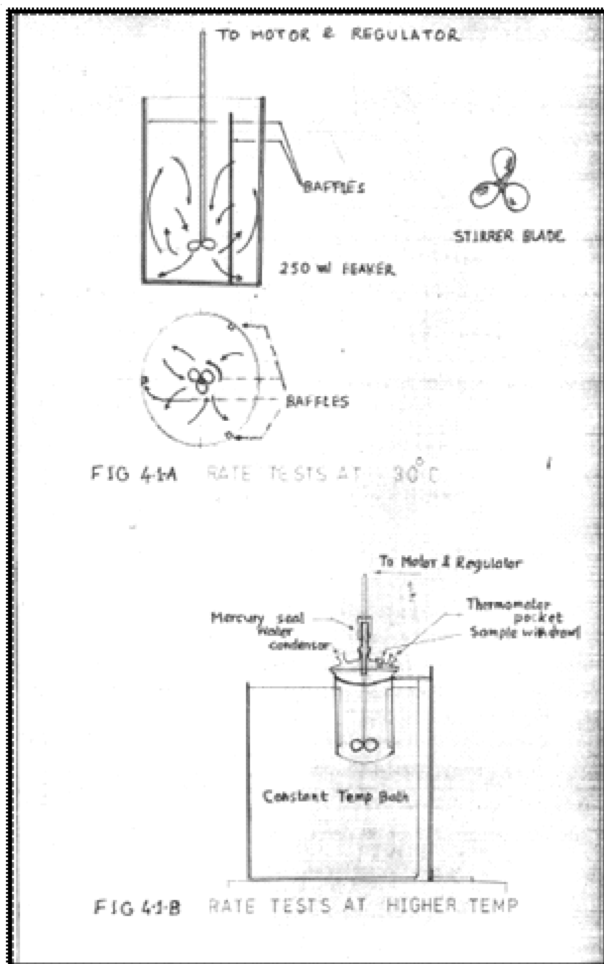

Fig 4.1 **Exptl setup for batch rate tests**

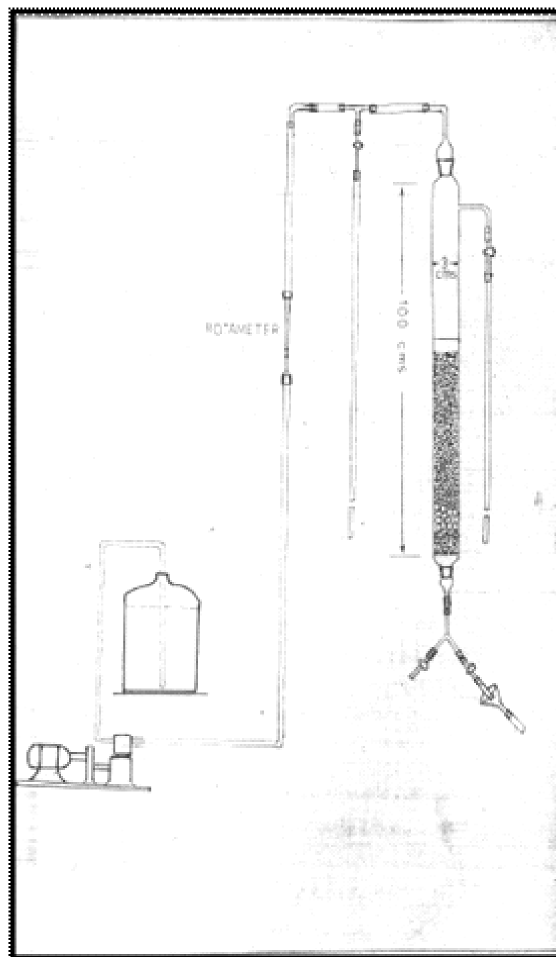

Fig 4.3 **Exptl Setup-Flow System Studies**

Estimation of Vegetation Greenness, Canopy Moisture Content and Land Surface Temperature of Mangroves Using Satellite Data: A Case Study of Sundarbans

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Abstract: The present work deals with the characterization of the mangroves of a portion of Sundarbans in terms of vegetation greenness, canopy moisture content status and land surface temperature (LST) using two Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) image datasets. Specific spectral indices, namely, Mangrove Probability Vegetation Index, Normalized Difference Wetland Vegetation Index and Shortwave Infrared Absorption Index were applied in decision tree algorithm for identifying mangrove forests, followed by creation of a mangrove mask of the study area. Vegetation greenness was estimated in terms of Normalized Difference Vegetation Index (NDVI) and canopy moisture content in terms of two indices, namely Moisture Stress Index (MSI) and Normalized Difference Infrared Index (NDII). The study showed greater vegetation greenness as well as canopy water content in the post monsoon data than the winter one. LST was found to be negatively correlated to both NDVI and NDII, but positively correlated to MSI for both winter and post monsoon. *Avicennia alba* dense communities recorded the lowest LST values as compared to the other communities in both the seasons. Overall, *Avicennia* moderately dense communities exhibited high canopy greenness as well as moisture content. It is hoped that the information obtained in the present investigation might prove to be quite useful to the mangrove ecologists and physiologists.

Keywords: mangroves, ASTER data, vegetation greenness, canopy water content, LST.

Introduction

Mangroves are highly productive ecosystems with a rich diversity of flora and fauna in the intertidal zones of tropical and subtropical coastlines (Tomlinson, 1994). Satellite remote sensing is an efficient tool that has been adopted increasingly to provide data over inaccessible areas, especially in the case of mangroves. Pixel-based classifications of various multispectral satellite imagery have been widely used for mapping mangrove forests (e.g. Rasolofoharinoro *et al.*, 1998; State of Forest Report, 2011; Giri *et al.*, 2011).

The mapping accuracy of a mangrove forest strongly depends upon the classification technique adopted (Green *et al.*, 1998). A recent work established that decision tree (DT) algorithm, a machine learning technique, could precisely and efficiently map mangrove vegetation (Liu *et al.*, 2008). Moreover, the identification of mangroves is also influenced by the terrestrial vegetations having similar spectral profiles as mangroves (Liu *et al.*, 2008). Various research groups (e.g. Zhang and Tian, 2012; Zhang *et al.*, 2013) have suggested solutions for solving this problem using multispectral data. Kumar *et al.* (2017) proposed the use of five spectral indices in DT algorithm for discriminating mangrove forests from terrestrial vegetation using hyperspectral image data. Such investigations are completely lacking in the domain of multispectral data over Sundarbans mangroves.

Literatures are also lacking in the field of canopy moisture content indices and their use in the determination of water content status of mangrove communities of the world and Sundarbans forests, *per se*. As Land Surface Temperature (LST) of dense forests would depict the canopy surface temperature, it would be quite interesting to note the variation in LST with different mangrove floristic classes. Furthermore, such investigations are totally lacking for Sundarbans mangroves. Furthermore, it would be useful to know the variation of canopy moisture status and vegetation greenness of mangroves with seasons and the relationships of canopy surface temperatures with canopy moisture and greenness. Thus, the objectives of the study were: (1) to identify mangroves from the adjoining landcover classes and create a mangrove mask using spectral information of Advanced Space borne Thermal Emission and Reflection Radiometer (ASTER) bands, (2) to determine vegetation greenness and canopy moisture status of mangroves with seasons and with mangrove communities, (3) to retrieve LST/ canopy surface temperature using ASTER thermal infrared (TIR) band and to study the variation of the same with seasons and mangrove communities, and (4) to study the relationship of LST/canopy surface temperature with vegetation greenness and canopy moisture content.

Materials and Methods

Study Area: The study area (Figure 1) covered a part

of Sundarbans including portions of both Indian as well as Bangladesh Sundarbans (nearly 2500 sq. km and extended between 21°36'6.12" to 22°9'2.60"N latitude and 88°45'15.54" to 89°1'37.59"E longitude). The study area also included some adjoining landcovers like agricultural area and terrestrial vegetation in the north.

Satellite data and ancillary data: Two sets of ASTER L1 data from two seasons (winter and post monsoon) were used, viz. one from January and the other from November 2006. In addition to the satellite data, digital database of mangrove community zonations from Coastal Zone Information System (CZIS) under 'Coastal Zones Studies' project (Ajai *et al.*, 2012) was also used.

Data processing: The 15 m ASTER visible and near infrared (VNIR) bands (Green, Red and NIR) were resampled to 30 m, while the 90 m TIR bands were resampled to 30 m in Environment for Visualizing Images (ENVI) version 5.1. Then the 14 bands [three VNIR, six shortwave infrared (SWIR) bands and five TIR bands] were layer staked and used for further analysis.

Identification, extraction of mangrove forests/ mangroves and creation of a mangrove mask for the study area: Three spectral/ vegetation indices, namely Mangrove Probability Vegetation Index (MPVI),Normalized Difference Wetland Vegetation Index (NDWVI) and Shortwave Infrared Absorption Depth (SIAD) (Kumar *et al.*, 2017) were used for enhancing the mangrove forests/ mangroves from other/ adjoining landcovers. The indices were applied on the datasets using band math tool in ENVI. The indices were used as inputs in DT classifier. The images of the three indices were stacked for highlighting the mangrove forests and the DT rules (Figure 2) for the identification of the forests were obtained after thresholding to cover all mangrove pixels. A mangrove mask was built using the classified output of DT.

Computation of canopy moisture content indices: Two indices, namely Moisture stress index (MSI) (Hunt and Rock, 1989) and Normalized Difference Infrared Index (NDII) (Hardisky *et al.*, 1983) were computed using the band math tool in ENVI. Both the indices are reflectance measurements, the former being sensitive to increasing leaf water content and the latter sensitive to changes in water content of plant canopies.

Computation of vegetation greenness: Normalized Difference Vegetation Index (NDVI) (Rouse *et al.*, 1973) was used as an indicator of vegetation greenness.

Calculation of LST from thermal infrared bands: Single channel algorithm of Jimenez-Munoz and Sobrino (2010) was used for retrieving LST from TIR 1 (band 10) of ASTER data, where input emissivity was calculated from NDVI.

MSI/ NDII/ NDVI vs. LST: Graphs were prepared to study the relationship of each of the indices with LST. Additionally, Pearson's correlation coefficients were calculated.

Mangrove community zonation classes: The mangroves of India were mapped at community level using Resourcesat-1 data of 2005-2007 time period (Ajai *et al.*, 2012). Mangroves have been classified in four density classes based on canopy closure, viz., very dense (>70% cover), moderately dense (40-70%), sparse (10-40%) and degraded (<10% cover). The mangroves of the study area of Sundarbans have been classified into seven classes of mangrove communities/ zonation (Figure 3). The zonation was available for Indian Sundarbans together with few islands of Bangladesh Sundarbans.

Determination of canopy moisture content and vegetation greenness for different mangrove communities: Box and whiskers plots of MSI, NDII, NDVI and LST were prepared in OriginPro 8 for the different mangrove communities for the two seasons.

Results and Discussion

Identification and extraction of mangrove forests/ mangroves: The DT output exhibits the mangrove forests/ mangroves in the study area (Figure 4). The result of DT algorithm indicated that the indices MPVI, NDWVI and SIAD could identify the mangrove forests. A mask was built with the DT classified output for extraction of the mangroves.

Canopy moisture content: The MSI values of the mangrove forests of majority of the islands ranged from 0.30-0.50 in the winter season, while in the post monsoon period most of the islands recorded values in the range of about 0.2-0.45, thereby indicating that the water content of the mangrove leaves were more in the post monsoon period in comparison to the winter season (Figure 5a). Though the mangroves grow in 'physiologically' dry soils, yet there was no moisture stress in the canopies. High MSI values (0.5-0.6) indicating less moisture content in the canopies was observed in parts of Gona and Sudhanyakhali of Indian Sundarbans. For most of the islands NDII values ranged from 0.3-0.5 during the winter season and 0.4-0.6 in the post monsoon time (Figure 5b). Though the common range of NDII for mesophytic green vegetation is 0.02-0.6 (Jackson *et al*., 2004), in the present study the values exceeded 0.6 for some mangrove canopies in both the seasons. Some of the areas which recorded high canopy moisture content in both the seasons included Baghmara, Chaimari, Mayadwip and Talpatti. The differences in the ranges of NDII values in the two study seasons revealed that this index might be used for mangrove canopy monitoring. The increase in the NDII values in the post monsoon data indicated to the fact that more moisture was available in the rhizophere of the

mangroves in the post monsoon time as compared to the winter season.

Vegetation greenness: Most of the mangrove forests recorded NDVI values in the range of 0.4-0.6 in the winter and 0.6-0.8 during post monsoon (Figure 5c). Thus, the NDVI values were within the range for green vegetation i.e from 0.2-0.8 (Sellers, 1985). Increase in greenness during the post monsoon time might be due to higher rates of photosynthesis of mangrove leaves. Kumar *et al.* (2015) showed higher photosynthetic rates of leaves of Lothian island mangroves (Indian Sundarbans) during the post monsoon period as compared to the values obtained during winter or summer. The common areas which recorded high NDVI values during both the seasons included Baghmara, Chaimari, northern parts of Jhilla and parts of Khatuajhuri of Indian Sundarbans and southern parts of Talpatti, portions of Kolkibari and Hilchar, Khesonkhali, Jalokathi, Mandanbaria in the western-most portions of the study area, Churkuni Gang (in the north-eastern part of the study area) of Bangladesh Sundarbans.

LST: Most of the islands recorded LST in the range of 26pC-28pC in the winter season and 28pC-30pC during the post monsoon period (Figure 5d). For the winter season high LST values were observed in parts of Baghmara, Chaimari, Gona and Chandkhali of Indian Sundarbans and southern-most parts of Talpatti, Hilchar, Kolkibari and western-most portions of Khesonkhali and Jalokathi of Bangladesh Sundarbans. During the post monsoon period high LST values were recorded in areas including parts of Kalindi, Daichi, Kachi Katta, Sajnekhali, Sudhanyakhali, Panchamukhani, Netidhopani, Matla and Chamta. Since the study area is covered with mangrove forests, hence LST at the spatial resolution of 30 m depicted the canopy surface temperature of the mangroves.

LST vs. indices: A polynomial increasing trend was observed in MSI values with increase in LST, while NDII decreased (polynomial trend) with increase in LST in both the seasons. NDVI values also decreased with increase in LST; the decrease followed a polynomial trend in the winter season, whereas a linear trend in the post monsoon period. Moreover, MSI was significantly positively correlated to LST, while NDII and NDVI were notably negatively correlated to LST for both the seasons (Pearson's correlation coefficient *r* value significant at the level of 0.01 in all the six cases) (Figure 6).

Canopy moisture content and greenness of the mangrove communities with seasons: During both the seasons mixed *Ceriops–Excoecaria–Phoenix* dense (CEP) communities exhibited highest mean/ median MSI values, while lowest mean/ median values were observed in *Avicennia* moderately dense (AMD) communities, thereby indicating that on the whole canopies of the former communities were under greatest moisture stress conditions as compared to the other communities and moisture stress was minimum in the latter (Figure 7). Fringe mangrove dense (FMD) communities showed widest range of MSI values. Overall, the range of MSI values was found to be wider in the winter season in comparison to the post monsoon period. On the contrary, for both the seasons highest and lowest NDII mean/ median values were obtained in AMD and CEP, respectively, this indicated that the water content of the canopies of the former were greatest and lowest in the canopies of the latter (Figure 7). During the winter season communities of FMD, mangrove sparse (MS) and mixed *Avicennia–Excoecaria–Ceriops* dense showed wider NDII ranges in comparison to the other communities, whereas, AMD showed widest NDII range during the post monsoon period.

During the winter season, AMD showed highest mean/ median NDVI values, followed by NDVI values of *Avicennia alba* dense (AAD) and CEP communities (Figure 7). Low mean/ median values were observed in FMD and MS; ranges of NDVI values were widest in the communities of both AMD and FMD. During post monsoon, highest mean NDVI values were obtained in AAD, followed by AMD; as in the winter season, lowest mean values were obtained in FMD and MS. Ranges of NDVI values were widest in AMD.

Canopy surface temperature of the mangrove communities with seasons: For the winter season highest mean/ median LST values were observed in AMD, while lowest mean/ median values were recorded in AAD (Figure 7). MS recorded the widest range of LST values, while the range was narrowest in AAD. During the post monsoon period highest mean/ median LST values were observed in FMD, while lowest mean/ median values were recorded in AAD (as in winter). Again, FMD exhibited the widest range of LST values. In general, the range of LST values was found to be wider in the winter season than ranges of the post monsoon period.

Conclusion

Both vegetation greenness as well as canopy water content of mangroves of the study area were found to be more in the post monsoon period than the winter season. Irrespective of the season, moisture stress increased while canopy greenness decreased with increase in LST. On the whole, *Avicennia* moderately dense communities exhibited high canopy greenness as well as moisture content. *A. alba* dense communities recorded the lowest LST values as compared to the other mangrove communities in both the seasons.

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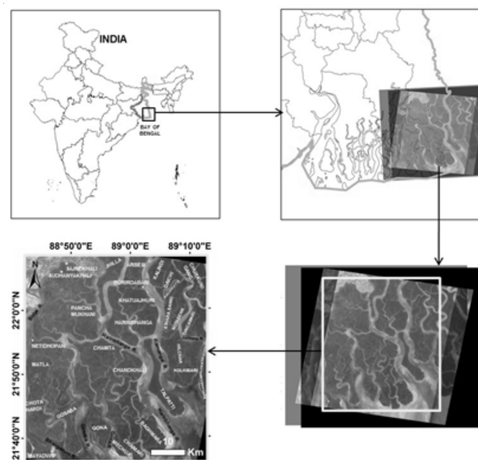

Figure 1: Index map of the study area overlaid on ASTER data

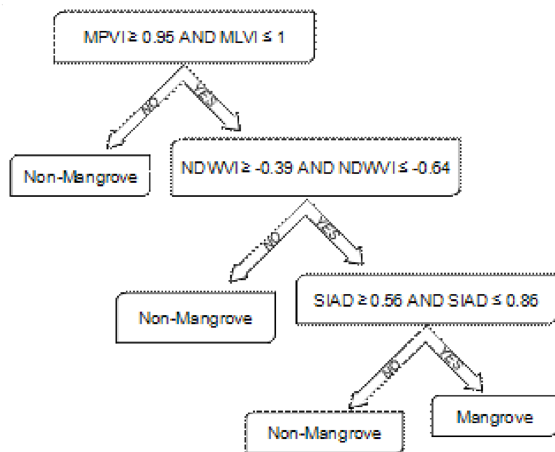

Figure 2: Rules for identifying mangrove forests of the study area using decision tree

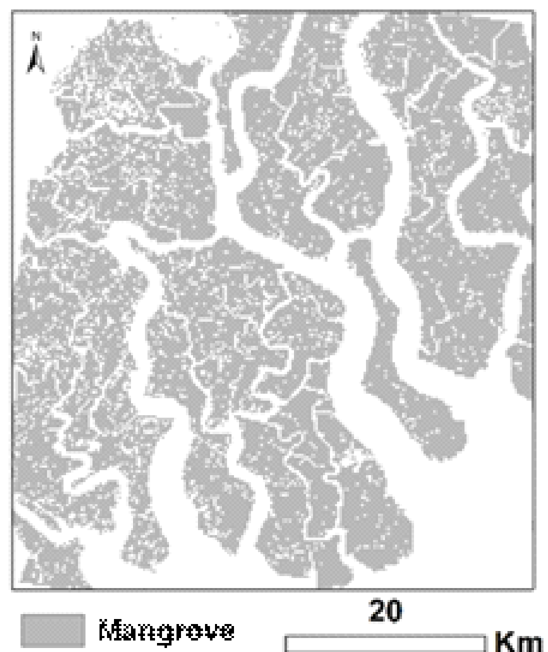

Figure 3: Decision tree output exhibiting the mangroves in the study area

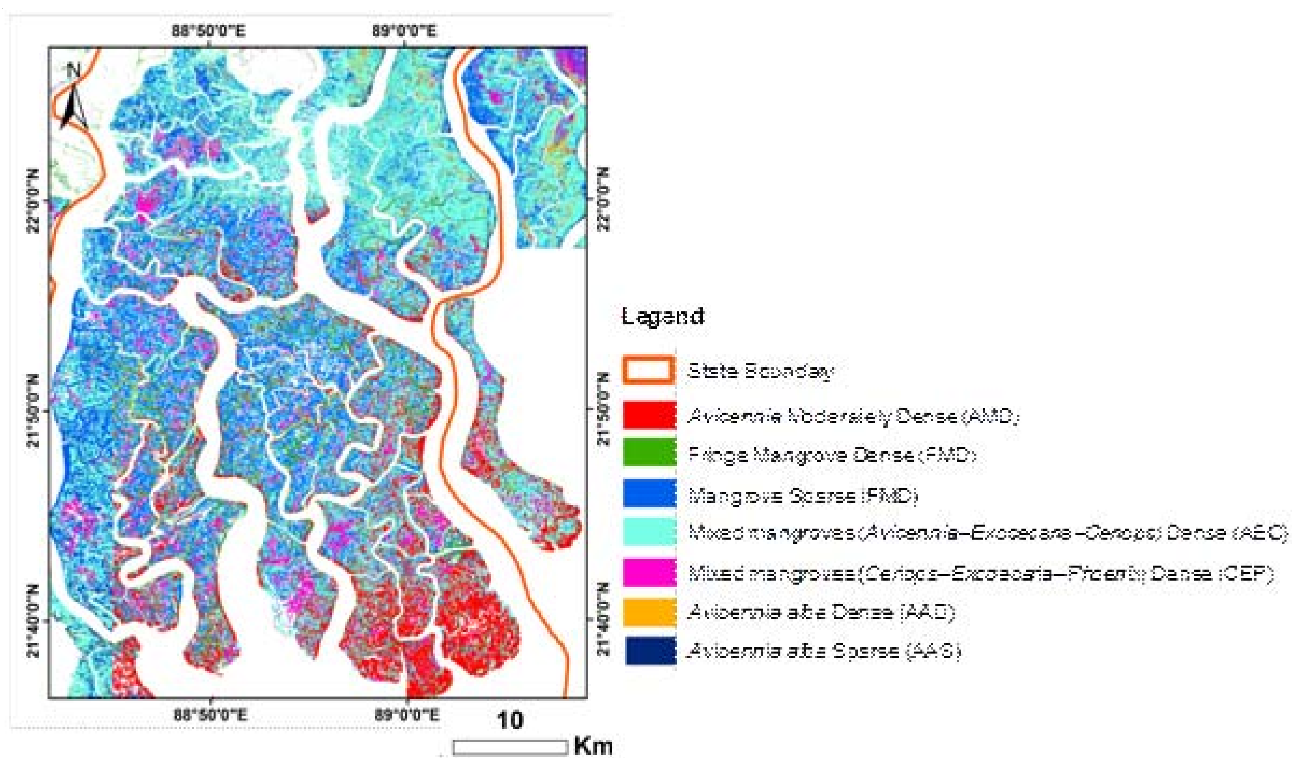

Figure 4: Mangrove communities in the study area

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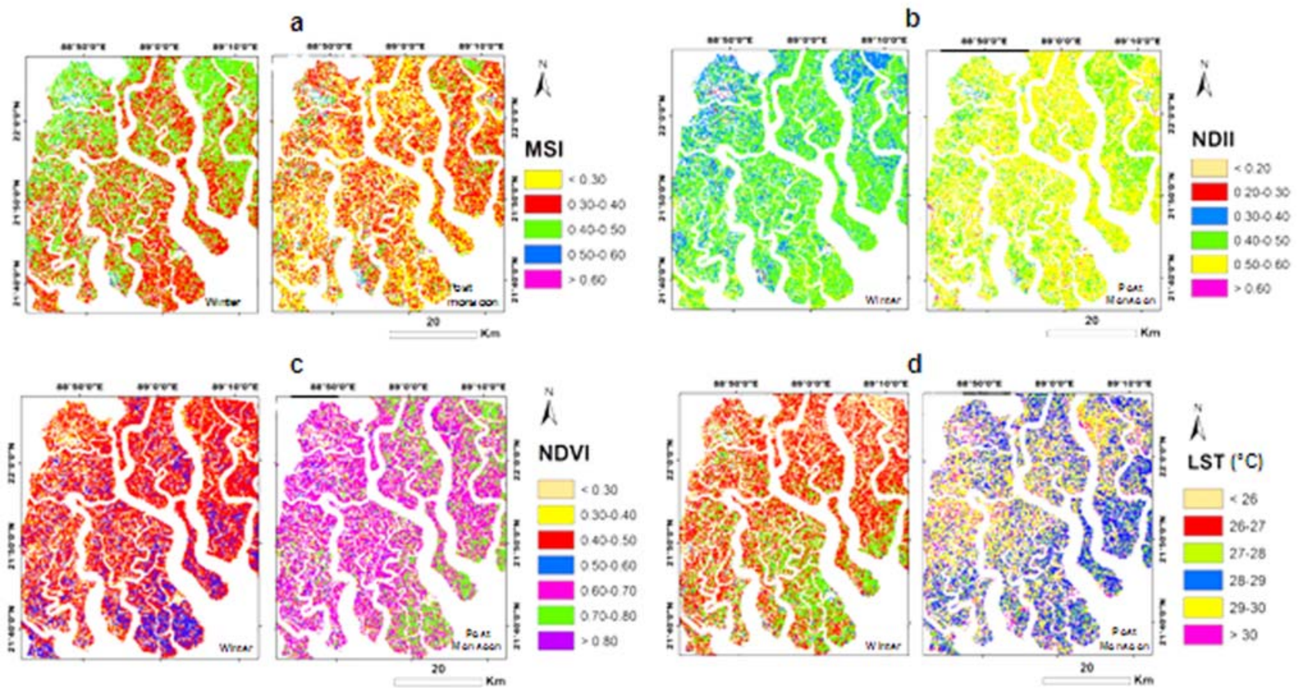

Figure 5: MSI (a), NDII (b), NDVI (c) and LST (d) for the two seasons

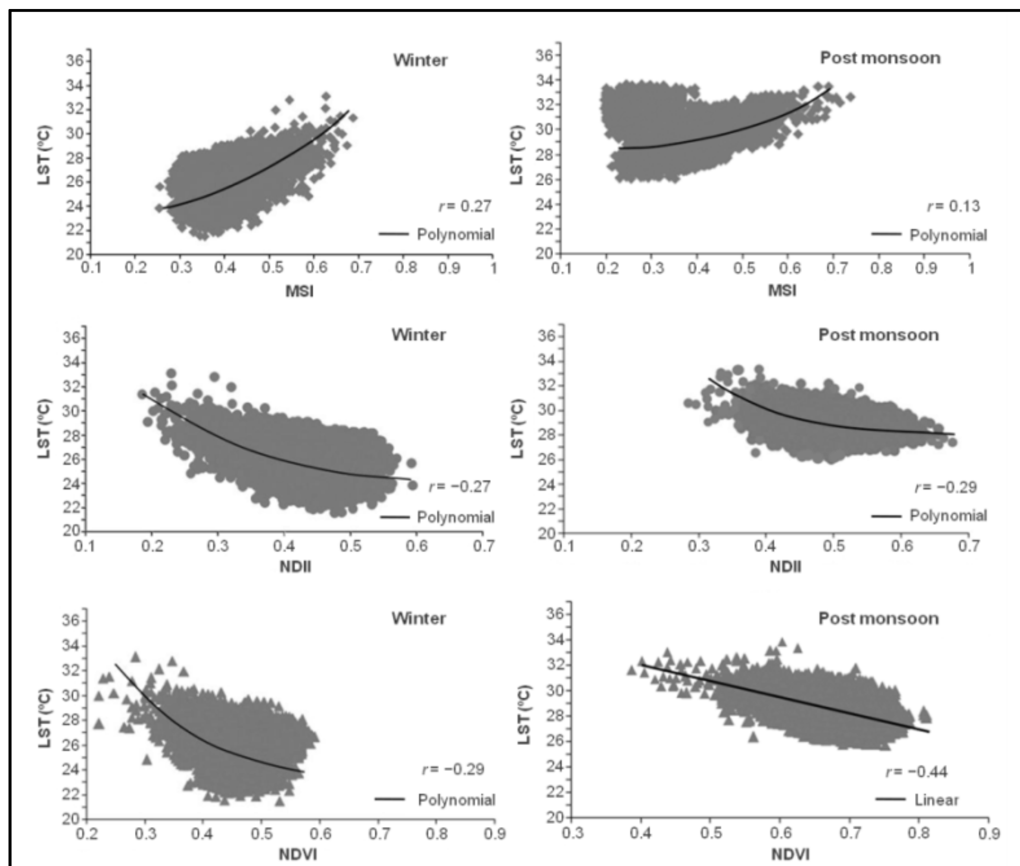

Figure 6: Graphical representations of LST vs. MSI, LST vs. NDII and LST vs. NDVI

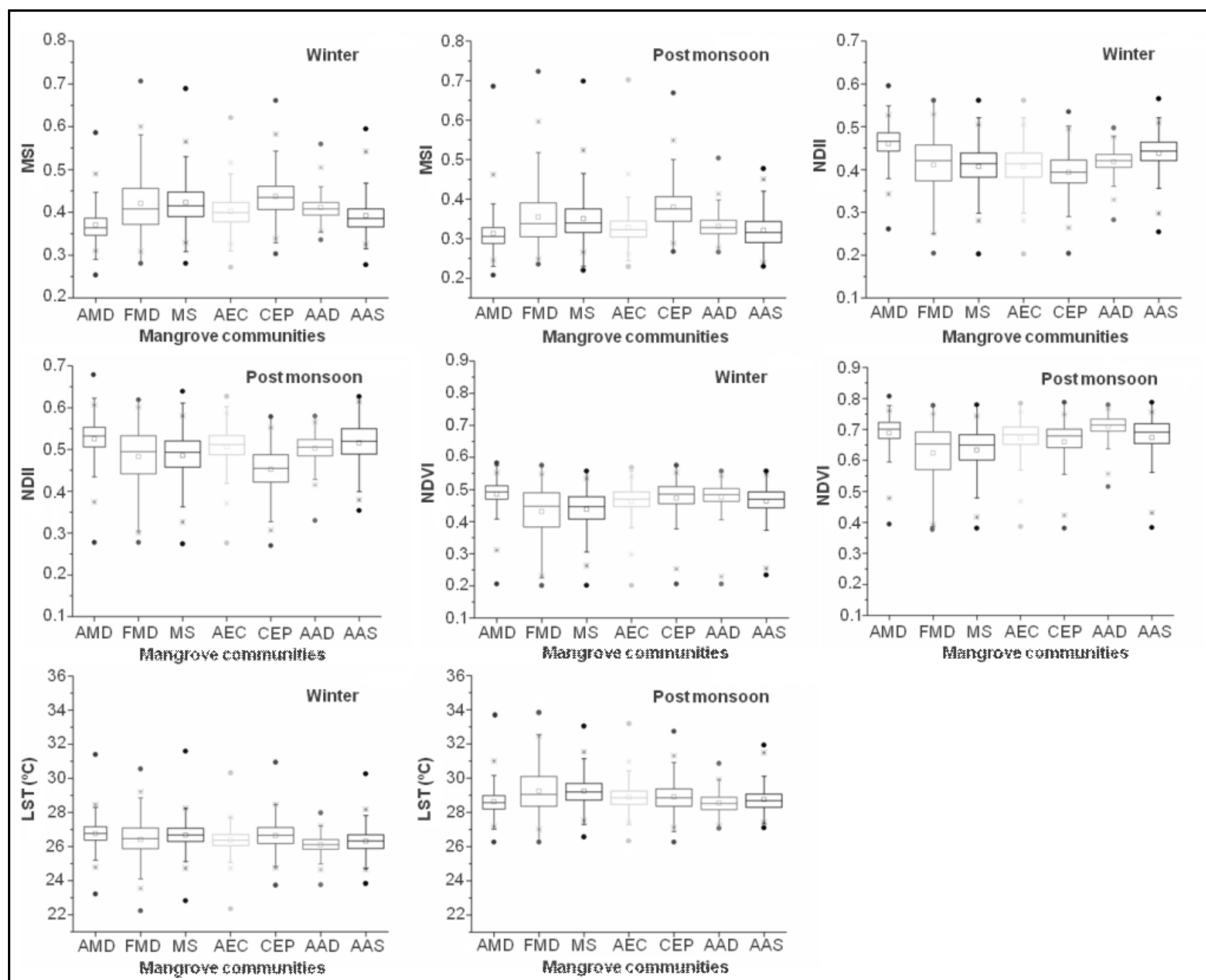

Figure 7: Box and whisker plots of MSI, NDII, NDVI and LST for the different mangrove communities for the two seasons

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Study of PAH Degradation Using Biofilm Bioreactors

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Abstract: Polycyclic aromatic hydrocarbons (PAH) are toxic to human body if consumed. Biodegradation of Polycyclic aromatic hydrocarbons is considered to be efficient and safe as compared to the existing chemical techniques. Phenanthrene and Naphthalene are the most commonly found PAHs in environment, which in large amounts are carcinogenic and hence are considered harmful. Experimental design methodology was used to optimize the bioremediation process to improve the efficiency of bioremediation. Based on the previous studies by the same authors, bioreactors were constructed to study the degradation of the PAH using bacterial consortia constructed using different combinations of 7 isolates. A comparison based on the efficiency of percentage degradation was made by using GC and COD estimations. The Gas chromatography results indicated that Consortia V showed better PAH degradation and reduction in Chemical oxygen demand than all other consortia.

Key Words: Phenanthrene, Naphthalene, Environment pollutants, PAH analysis, Bacterial PAH degradation, Bio-film Bioreactor.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants that are widely distributed diversely because they are commonly produced by many anthropogenic activities like petroleum refining, and incomplete combustion of fossil fuels, coal, gas and oil, domestic garbage, tobacco smoke, soot etc.(Xue *et. al.,* 2007; Zhu, *et. al.,* 2016). PAHs constitute of two or more fused benzene rings and are of great concern since they have detrimental biological effects. They are listed among the MPCB, US EPA and the EU priority pollutants list (Maharashtra Pollution Control Board; Environmental Protection Agency). Bioremediation of PAH is considered an effective and environmentally benign clean-up technology since it involves the partial or complete bioconversion of these pollutants to microbial biomass, carbon dioxide and water. They are considered as potent immune-suppressants which interferes with the regular function of cellular membranes as well as with the membrane-associated enzyme systems with the. Their detrimental effects are observed on host resistance, immune system development and humoral immunity. Naphthalene and Phenanthrene are the simplest PAHs and are long been used as a model compound in PAH bioremediation studies (Hussein and Mansour, 2016; Pawar, A. *et. al.,* 2010). In the environment, degradation of PAH is done by volatilization, photo-oxidation, chemical oxidation, bioaccumulation, and adsorption to soil particles. However, the most preferable and principal process is thought to be of microbial transformation and degradation.(Madhumita, *et. al.,* 2012). Biodegradation is accepted widely as the primary dissipation mechanism for most organic pollutants including PAH in the environment (Hussein and Mansour, 2016; Joan, 1999; Lyu, *et. al.,* 2014).

Compared to conventional decontamination methods, bioremediation is economical with no damaging effect on the environment. In recent studies, species of *Pseudomonas*, *Sphingomonas*, *Rhodococcus*, *Xanthomonas* and *Mycobacterium* are found to be very effective in Naphthalene and Phenanthrene degradation. Due to low water solubility and strong hydrophobic nature of PAHs, they are sparsely soluble in water but are well soluble in fats, they tend to accumulate and associate with the sediments and particulate materials in the aquatic environment (Lyu *et. al.,* 2014).

Biofilm bioreactor is one of the method in which property of the biofilm formation by microbes can be exploited for bioremediation. Efficiency of bioremediation is enhanced by the use of biofilms. Biofilm mediated bioremediation is environment friendly and cost effective option for cleaning up environmental pollutants (Mitra and Mukhopadhyay, 2016).

Different enzymatic machinery is possessed by different bacteria which are able to degrade diverse compounds. Hence one strain might be able to degrade one intermediate generated as a dead end from another strain, promoting a complete degradation and mineralization of the substance.The biofilm matrix offers a greater resistance as compared to planktonic cells tomicrobes from environmental stress, shear stress, antimicrobial agents, UV damage, desiccation, biocides, and high concentration of toxic and pollutants (Mitra and Mukhopadhyay, 2016).

The present study is aimed at isolation of PAH degrading microorganisms from soil samples which are highly contaminated with hydrocarbon waste and study mixed PAH degradation by the isolates. The standard PAHs that were used for the study were Naphthalene and Phenanthrene.

Further studies were carried to calculate the rate of PAH degradation by the selected PAH degrading isolates based on Gas Chromatography (GC) analysis in the presence of high concentration of mixed PAH as sole source of carbon. The study also includes building up a consortia using the PAH degrading isolates and using them to set up a Biofilm Bioreactor for treatment of contaminated water. This was followed by GC analysis and Chemical Oxygen Demand (COD) analysis of treated water and a comparison between the effectiveness of different consortia in degrading PAHs.

Materials And Methods

Isolation and screening of PAH degrading microorganisms and Mixed PAH degradation analysis using Gas Chromatography: For the purpose of isolation of PAH degrading microorganisms, soil samples were collected from areas contaminated with hydrocarbon waste. The sampling sites include bunk soil from local petrol pump in Chembur and Shivaji Park and soil sample surrounding railway tracks of Mumbai. The soil samples were collected in an air-tight zipper pouch and refrigerated at 4°C. The soil samples were initially enriched using Sterile Mineral Salt Medium (MSM) containing different concentrations of 99% Naphthalene and Phenanthrene as sole sources of carbon. The flasks were kept for incubation for 1 week at 30°c under shaker conditions. Isolation was carried out on Sterile Mineral Salt Agar (MSA). The solutions of Naphthalene and Phenanthrene prepared in 1M CCL_4 and were supplied on each plate by spray plate method under aseptic conditions. The plates were further incubated at 30°c. Degradation of mixture of Naphthalene and Phenanthrene(500ppm each) by PAH degrading isolates was analysed using Gas Chromatography technique. PAH from the medium was extracted using n-Hexane as a solvent. 1ìL volume of the solvent phase containing the residual PAH was injected in Gas Chromatography instrument (Varian CP 3800. Column ID – 0.53mm. Detector used – FID (Flame Ionization Detector).

Preparation of consortia and lab-scale Biofilm Bioreactor setup for treatment of water contaminated with PAH*:* Different consortia of best PAH degrading isolates were prepared using different combinations of isolated organisms for inoculation in the Bioreactor. 5 ml of each isolate (O.D.$_{600nm}$ at 1.0) was used to make a final volume of 50 ml. The Consortium which showed best degradation was further inoculated into 2000 ml of water sample of Biofilm Bioreactor. A lab-scale Biofilm Bioreactor was set up using a sterilized glass carboy containing 2000mL water contaminated with Phenanthrene and Naphthalene (2000ppm each) as sole source of carbon and it was inoculated with bacterial consortium which was prepared using PAH degrading isolates. The bioreactor was set up for a period of 2 weeks. The degree of water treatment was measured by calculating the rate of PAH degradation using Gas Chromatography.

Determination of COD: A COD assay of the treated water in biofilm bioreactor was carried out. In a 250 ml round bottom flask 2-3 porcelain pieces were added. 1ml of sample and 19ml of distilled water was added. 500mg of $HgSO_4$ was added along with 5ml of conc. H_2SO_4 and kept on ice bath for cooling. 10ml of 0.25N $K_2Cr_2O_7$ was added after mixture cools down. 25ml of solution of silver sulfate – sulfuric acid was added. Contents of the flask were mixed well and kept on ice bath for cooling. Refluxed for about 2 hours. The refluxed mixture was further cooled completely by placing it on ice bath, 100ml of distilled water was added and titrated against 0.25N Ferrous Ammonium Sulfate (FAS) using ferroin indicator. End point is the colour change of the mixture from blue green to wine red. (Sivanandan. T. P.2011)

Visualization of biofilm formation using Allison and Sutherland staining method: The slides from Biofilm bioreactor were removed and stained with a mixture of Congo red solution and 10% (v/v) Tween 80 solution which were further counterstained with 10% (w/v) Ziehl Carbol Fuchsin and finally air-dried at 37°C. These slides were further viewed under light microscope to visualize biofilm formation (Allison D. and Sutherland I. 1987).

Results and Discussion

Isolation and screening of PAH-degrading bacteria and analysis of Mixed PAH degradation using Gas Chromatography: The enriched cultures were used for isolation on sterile MSA plate by spread plate method. The sterile MSA plates were further spray-plated with Phenanthrene on the agar while Naphthalene on the lid of the plate. The plates were observed for the growth. The organisms observed to be growing on the plates were used for further studies. The isolates were obtained on the plates sprayed with 5ppm Phenanthrene and Naphthalene each and were inoculated into different sterile MSM broths containing increasing concentrations of Phenanthrene and Naphthalene. The isolates were selected according to their growth pattern in presence of high concentration of Phenanthrene and Naphthalene (500ppm each). The best isolates selected were VP50, VP29, VP30, VP33, VP35, VP41, VP10, VP13, VP06 and VP20. Residual PAH was extracted from the media containing these isolates by liquid-liquid extraction method using n-hexane as solvent. These isolates were further used to build a bacterial consortium. The PAH extracted from the media inoculated with Consortium was also analysed using Gas chromatography. The extract was further used as a sample for Gas Chromatography analysis. 1ìL of extracted sample was injected into the GC column. Consortia I(prepared using VP50, VP29, VP30, VP33, VP35, VP41, VP10, VP13, VP06 and VP20) showed a degradation rate of 93.42% and 56.7% (Figure 01), Consortia II(prepared using VP50, VP29,VP30,VP33,VP35,VP41,VP10,VP13 and VP06) showed a degradation rate of 100% and 85.02%(Figure 02), Consortia III(prepared using VP50,

VP29,VP30,VP33,VP35,VP41,VP10 and VP06) showed a degradation rate of 98.28% and 85.32% (Figure 03), Consortia IV (prepared using VP50, VP29,VP30,VP33,VP35,VP41 and VP10) showed a degradation rate of 100% and95.15% (Figure 04), Consortia V(prepared using VP50,VP29,VP30,VP33,VP35 and VP41) showed a degradation rate of 100% and 98% (Figure 05) for Naphthalene and Phenanthrene respectively. Consortia IV and consortia V were further used for inoculation in Biofilm bioreactor for water treatment. Consortia V showed best degradation in 14 days with 99.4% of Naphthalene (2000ppm) and 94.46% of Phenanthrene (2000ppm) degraded (Figure 08).

Visualization of biofilm formed by the bacterial consortia using Allison and Sutherland staining method: The slides were recovered from bioreactor and stained by Allison and Sutherland method. The slides were further viewed under light microscope at (oil immersion) 100x magnification. Biofilms were observed on slides (Figure 6) indicating that the bacterial isolates used in consortia are able to form biofilm and this would enhance the rate of PAH degradation in Biofilm bioreactor.

Analysis of treated water in Biofilm Bioreactor using Gas Chromatography*:* An aliquot of 1ml was removed after 7th day and 14th day and analysed using Gas chromatography. It was observed that by 14th day, 90.84% of Naphthalene and 72.43% of Phenanthrene was degraded using consortia IV whereas, 99.4% of Naphthalene and 94.46% of Phenanthrene was degraded using consortia V.

Determination of COD of the treated water: An aliquot of 1ml was removed at a regular interval of 24 hours for a period of 8 days from the bioreactor and was used for carrying out COD analysis. The analysis was carried out using the aliquots for a period of 8 days. The formula used to calculate COD was $\mathbf{COD(mg\ O_2\ /L\ or\ ppm)} = \frac{(A-B)\,8000}{volume\ of\ sample\ used}$. Where, A = volume of FAS required for the titration of distilled water. B = volume of FAS required for the titration of test sample. 8000 = milli equivalent weight of oxygen (8) ×1000 mL/L.

The COD results observed are presented graphically (Figure 09 and Figure 10.) which shows a downward graph indicating a decrease in COD levels during PAH degradation. This indicated the utilization of organic matter i,e, PAH during water treatment.

Conclusion

The isolates obtained from sites contaminated with hydrocarbon wastes were able to metabolize Phenanthrene and Naphthalene as sole source of carbon at a higher concentration and were able to form biofilm.The developed lab-scale Biofilm bioreactor containing consortia V among the other consortia prepared using best PAH degrading isolates was found to be better in reducing the mixture of Phenanthrene and Naphthalene and also in reducing the COD value. The isolates used in Consortia V were better in degrading mixture of Phenanthrene and Naphthalene than the isolates used in studies carried out by Parab V. and Phadke M (Parab V. and Phadke M.2017). Hence these can be used for further scale up studies on Biofilm bioreactor for water treatment. A scale-up of the biofilm bioreactor will be carried out to increase the sample load for treatment. Further studies include identification of the selected isolates. Characterization of isolates is in process, results are awaited.

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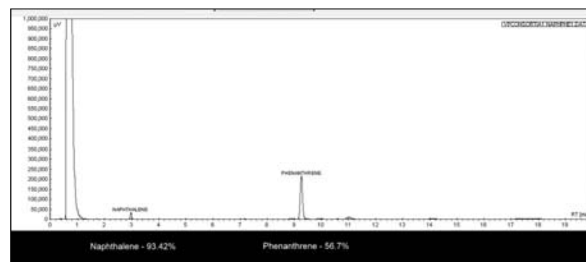


Figure 01: The chromatogram showing mixed PAH degradation by Consortia 1 (Naphthalene – 93.42%, Phenanthrene 56.

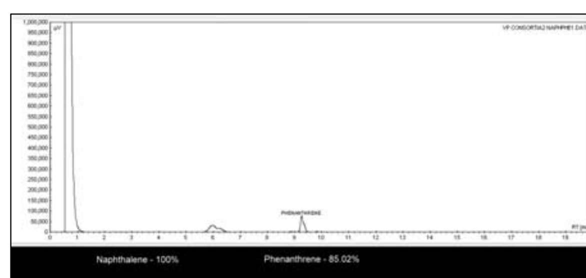


Figure 02: The chromatogram showing mixed PAH degradation by Consortia II (Naphthalene – 100%, Phenanthrene 85.02%)

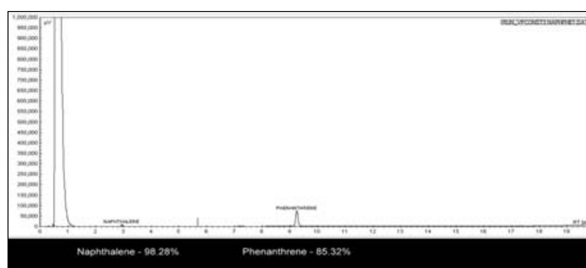


Figure 03: The chromatogram showing mixed PAH degradation by Consortia III (Naphthalene – 98.28%, Phenanthrene 85.32%)

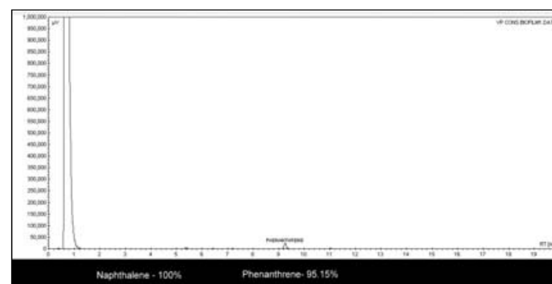


Figure 04: The chromatogram showing mixed PAH degradation by Consortia IV (Naphthalene – 100%, Phenanthrene 95.15%)

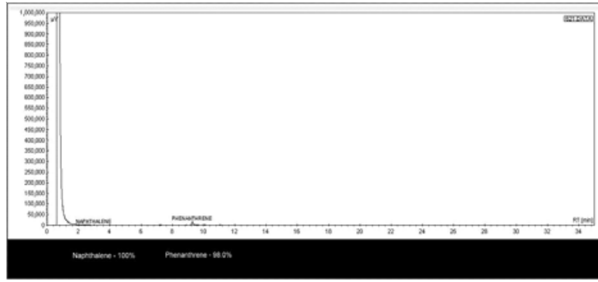


Figure 05: The chromatogram showing mixed PAH degradation by Consortia IV (Naphthalene – 100%, Phenanthrene 98.00%)

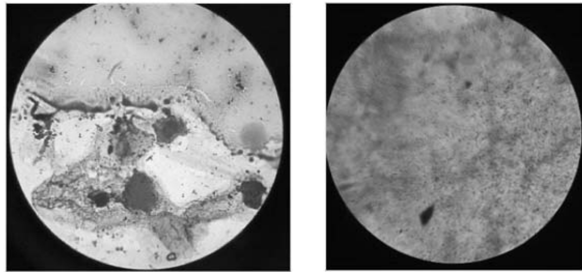


Figure 06: Allison and Sutherland staining performed to reveal the formation of Biofilm by Consortia 4 on the glass slides placed in Biofilm Bioreactor. The biofilms were observed under light microscope at 100x magnification (oil immersion).

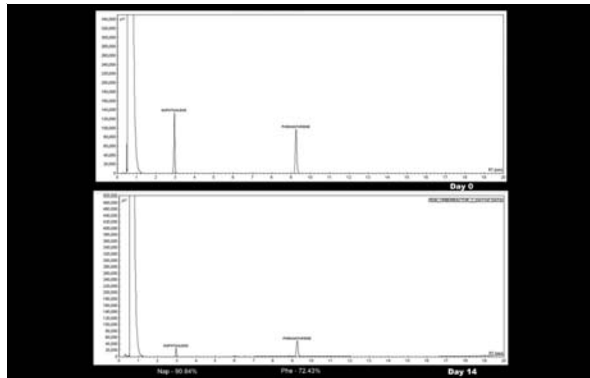


Figure 07: PAH degradation in Biofilm Bioreactor containing 2000mL of contaminated water. PAH present in water were Naphthalene (2000ppm) and Phenanthrene (2000ppm). Consortia IV was inoculated in the Bioreactor. 90.84% of Naphthalene and 72.43% of Phenanthrene was degraded within 14 days.

Key: Nap – Naphthalene **Phe** - Phenanthrene

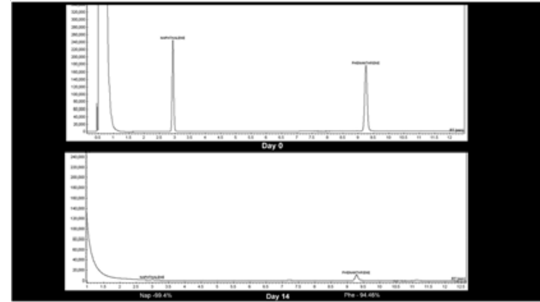


Figure 08: PAH degradation in Biofilm Bioreactor containing 2000mL of contaminated water. PAH present in water were Naphthalene (2000ppm) and Phenanthrene (2000ppm). Consortia V was inoculated in the Bioreactor. 99.4% of Naphthalene and 94.46% of Phenanthrene was degraded in 14 days.

Key: Nap – Naphthalene **Phe** – Phenanthrene

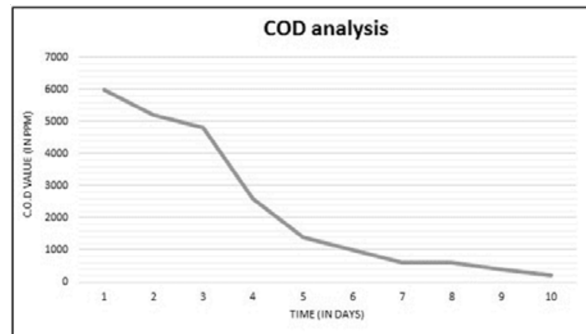
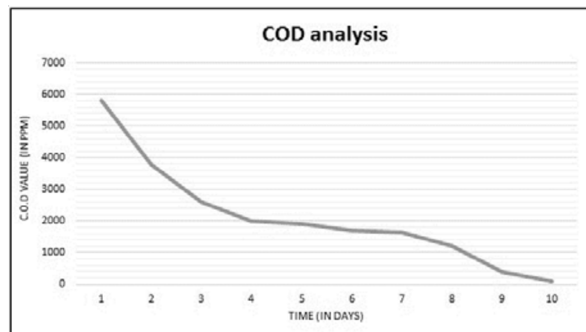


Figure 09: Chemical Oxygen Demand assay of the water being treated in Biofilm bioreactor using Consortia IV. A gradual decrease in the value of COD indicates the utilization of organic matter in the water which further indicates PAH being utilized by the bacteria in the bioreactor.



Key: ppm – parts per million.

Figure 10: Chemical Oxygen Demand assay of the water being treated in Biofilm bioreactor using Consortia V. A gradual decrease in the value of COD indicates the utilization of organic matter in the water which further indicates PAH being utilized by the bacteria in the bioreactor.

Spatial Patterns of Soil Organic Carbon Stock in Different Land Use System in Salcete Taluka of Goa

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Abstract: Soil organic carbon (SOC) stock acts as a major part of the terrestrial carbon reservoir with storage of about 1,500 Pg to 2,000 Pg C in the top layer in the world soils. Land Use Land Cover (LULC) changes directly affect the carbon sequestration rate in soil. Remote sensing, Geographical Information System (GIS) and GPS are used for spatial analysis and preparation of SOC distribution map.The present study aims to quantify SOCS of different land use and land cover classes include agriculture, mangrovesand wetlands. The study was conducted in Salcete taluka of South Goa district. Randomly soil samples were collected at a depth of 0-10cms and analyzed for SOC using Loss on Ignition (LOI) and Revised Walkley-Black method. Sentinel 2A satellite image of year 2016 was used to prepare thematic map of LULC of different land use system with ground truth data obtained from GPS coordinates. The distribution patterns of SOCS were plotted using SOC values obtained from soil health card portal and Indian Council for Agricultural Research (ICAR).The results indicated that the SOC in soils of different land use classes are significantly different. The mean SOC content in agricultural, mangroves and wetlands are 1.24% $\pm$ 0.43; 4.1% $\pm$ 1.15 and 2.16% $\pm$ 1.21 respectively. The total carbon stock of different land use systems from Salcete taluka of Goa is 5,47,626.15 tonnes.

Keywords: Soil Organic Carbon, LULC, GIS, Salcette.

Introduction

Carbon (C) sinks plays an important role in meeting the challenge of climate change. More recently scientists have been analyzed soil organic carbon to measure the net exchange of C between soil and atmosphere (Janzen, 2005). Soil is considered as the largest pool of terrestrial organic carbon in the biosphere storing more CO_2 (2,200Pg) than is contained in plants (Batjes, 1996), so small changes in the size of this flux can have a large effect on atmospheric CO_2 concentrations (Schlesinger & Andrews, 2000) and thus constitute a powerful positive feedback to the climate system. Carbon sequestration is the process of capture and long-term storage of atmospheric carbon dioxide (Roger & Brent, 2012; Watson *et al*., 2000) in soil, vegetation, oceans and geologic formation. Through the process of photosynthesis, plants assimilate carbon and return to the soil as litter and stored as soil organic matter (Negi & Gupta, 2010). Soil store 2.5 to 3.0 times higher as that stored in the plants (Post *et al*., 1990) and two to three times more than the atmospheric as CO_2 (Davidson *et al*., 2000). The first estimate of the organic carbon pool in the Indian soils done in the year 1984 and it was 24.3Pg (1 Pg = 1015 g) based on 48 soil samples (Gupta *et al., 1994*). Approximately 1500Gt of organic carbon is stored in the world's soils to a depth of 1m, with a further 900Gt between 1-2m (Kirschbaum, 2004).

Land Use and Land Cover (LULC) changes directly affect the carbon sequestration rate in soil (Lal, 2004) and other factors including climate, vegetation type, anthropogenic activities and land use land management practices (Six & Jastrow, 2002; Baker, 2007). The practices of carbon sequestration in soil help to reduce concentration of atmospheric CO_2 and in turn help plant growth as well as increasing crop yield (Shachi & Venkatramanan, 2009). The remote sensing technologies are being used for real time LULC data analysis and area estimation under different land use practices. Geographical information system (GIS) technology is useful for preparation of maps.

Materials and Methods

Study area: The study was conducted in Salcete taluka which is located in the heart of central coastal plains of South Goa district (12°39'-13°18'N and 77°22'-77°52'E), with a geographical area of 292.94 sq.km at an elevation range of 0-360m above mean sea level. In 2016,Salcetereceived an average rainfall of 2,635mm. Agriculture is one of the important economic activities of the taluka.Rice is the staple food and paddy is the principal agricultural crop (a Glance, G.A., 2000. Statistics Section. Directorate of Education, Government of Goa). The study area has been shown in Fig.1

Methodology

Remote sensing and GIS: Sentinel 2A satellite image (Source: USGS) of year 2016 was used for the LULC classification. Satellite images were analyzed using Arc GIS and ERDAS software with the help of ground truth data collected from GPS and LULC classes viz., Agriculture, Settlements, Wetlands, Mangroves and Other vegetation were identified. By using ERDAS software, LULC thematic

map and their area estimation was done by supervised classification. Agricultural, Wetland and Mangrove areas were extracted from LULC map. Interpolated map was created using SOC stock values in ERDAS software. The SOC stock map was overlayed on the extracted maps to view the distribution.

Soil Collection and Analysis: Soil samples at 0-10cm depth were collected from different LULC areas viz., Agro-ecosystems such as Paddy fields, Barren land, Coconut plantation, Cashew plantation and Current Fallow land; Wetlands; Mangroves; and Open forest areas. Bulk density of each soil sample was estimated. The collected samples were dried, crushed into pieces and were passed through 40mm sieves using electrical sieve shaker. The sieved samples were analyzed in Soil Laboratory for SOC and Soil Organic Matter (SOM) through two different methods i.e. (a) Loss on Ignition (LOI) (Storer, 1984) and (b) Revised Walkley-Black rapid titration method (Trivedi & Goel, 1986). The SOC stock was computed by multiplying the SOC values (g/kg) with bulk density (g/cm^3) and depth (cm) and was expressed in ton/ha (Joao Carlos *et al*., 2001). The flow chart of methodology has been shown in Fig. 2.

Results and Discussion

In the present study, Sentinel 2A satellite imagery of different years (2016) was classified for the land use land cover analysis. The classified image obtained after preprocessing and supervised classification which are showing the land use and land cover of the Salcete taluka.

Land Use and Land Cover map analysis

The LULC map depicts five major LULC classes which include Wetlands, Rivers, Mangroves, Settlements, Other Vegetations and Agriculture which is further classified into Paddy fields, Barren Land & Current fallow land (Fig. 3). The total geographical area of Salcete taluka is 292.94 sq.km, out of which Rivers covers 5.48sq.km (1.87%); Wetlands 5.87 (2.00%), Mangroves 3.53sq.km (1.21%); Settlements 24.85sq.km (8.48%); Other Vegetations 96.37sq.km (32.90%) and Agriculture covers 156.83sq.km (53.44%). In agricultural area, Paddy fields occupies 19.04sq.km (6.50%), Barren Land 48.25sq.km (16.47%) and Current Fallow land occupies 89.54sq.km (30.57%) of the total area of Salcete taluka (Table 1). The Land Use and Land Cover map reveals that present agriculture practices constitute 53.44% area (Table 1), whereas the other vegetation area shares 32.9% of the geographical area of the taluka. Wetlands Mangroves, Rivers and Settlement all together comprise 13.56%.

Spatial distribution patterns of SOC stock:

The soils collected from 63 sites of taluka consisting paddy fields, current fallow lands, barren lands, wetlands and mangroves which were used for laboratory testing of SOC using WB and LOI method. Results are used for spatial interpolation using IDW interpolator to generate the spatial distribution of SOC over the study region (Fig 4). The analysis reveals that the SOC ranges from 5 t/ha to 60 t/ha. It is clearly evident that are three classes of SOC stock i.e. Class I (green to lemon yellow): 5t/ha to 30t/ha, Class II: 30t/ha to 45t/ha (yellow to ochre) and Class III (orange to dark red): 45t/ha to 60t/ha with represents agricultural land, wetlands and mangroves respectively.

Class I: A very high concentration of SOC stock which ranges from 45t/ha to 60t/ha was found in mangroves areas such Curtorim, Rachol and Macazana as mangrove soils register high concentrations of carbon (Donato *et al.*, 2011) because of high rate of sedimentation and production of biomass that promote the accumulation of organic compounds in soil and generate large quantities of organic matter (Choudhury *et al.,* 2013).

Class II: Wetland areas such as Carmona, Davorlim, Bhindemol, Sirlim, Loutolim, Sarzora and Sao Jose de areal revealed high concentration of SOC stock ranging from 30t/ha to 45t/ha as wetlands accumulate high amounts of carbon in their soils because of anaerobic conditions produced by the presence of water which enhances the carbon storage in wetland soils (Meyers, *et al.,* 2016).

Class III: Agricultural land which includes paddy fields, current fallow land and barren land revealed comparatively low concentration of SOC stock ranging from 5t/ha to 30t/ha due to management practices such as fallowing, repeat cultivation, stubble burning or removal, and overgrazing which reduces SOC by reducing inputs to the soil.

Conclusion

From the present study, it has been revealed that the total carbon stock of entire Salcete taluka which includes Paddy fields, Barren land, Current fallow land, Mangroves and Wetlands is 5,47,626.15 tonnes. The methodology followed in the present study gives better representation of the spatial distribution of the SOC stock on the different land use systems of Salcete taluka of Goa. According to the present analysis, mangroves and wetlands are the potential reservoir of carbon which needs to be taken into consideration with regards to their conservation along with the upcoming developments. Present day minimum tillage methods in agriculture may contribute in enrichment of carbon reservoir of the region as paddy, barren land and current fallow land contributes 6%, 19% and 34% of the total carbon stock of the taluka. The present study can be road map for the enrichment of the carbon pools.

Acknowledgement

The present work is funded by University Grants Commission under Major Research Project. The authors are thankful to Department of Geography & Research Center and Principal, Parvatibai Chowgule College of Arts and Science (Autonomous), Margao-Goa for the infrastructural and financial facilities extended.

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Table 1: Land Use and Land Cover of Salcete Taluka

Class	Area (sq.km)	% Area
Wetlands	5.87	2.00
Rivers	5.48	1.87
Mangroves	3.53	1.21
Settlements	24.85	8.48
Paddy Fields	19.04	6.50
Current Fallow Land	89.54	30.57
Barren Land	48.25	16.47
Other Vegetation	96.37	32.90
	292.94	100

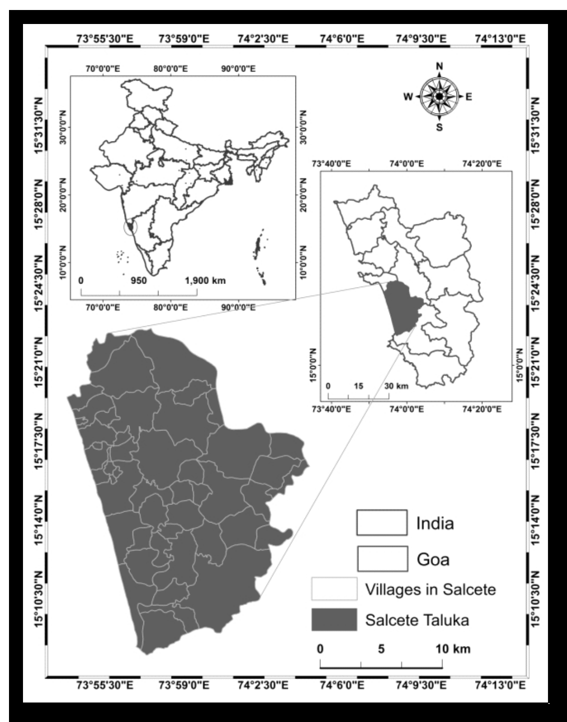

Fig. 1: Study area

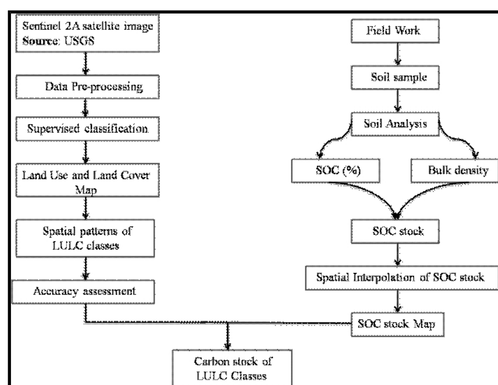

Fig. 2: Flowchart of methodology

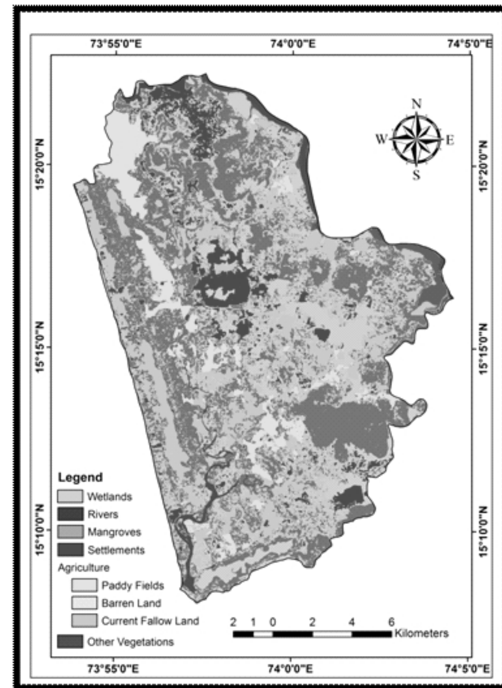

Fig. 3: Land Use and Land Cover map of Salcete Taluka

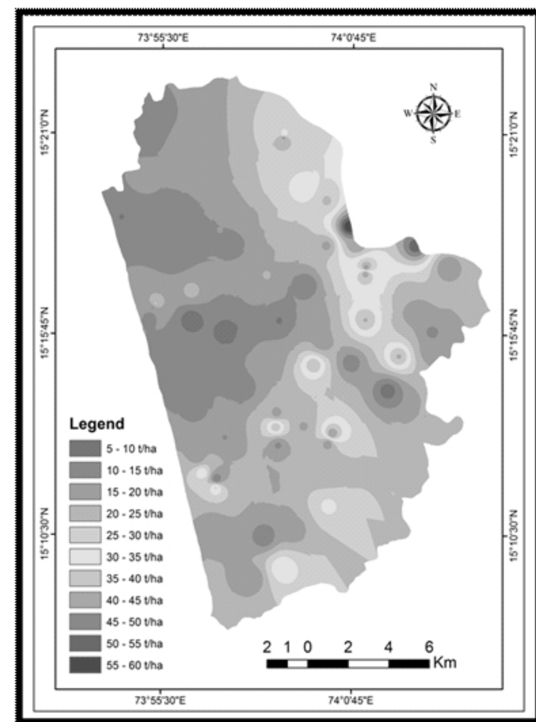

Fig. 4: SOC stock map of Salcete Taluka

Recycling of Botany Laboratory Waste Materials Into Compost

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Abstract: Our mother earth is boon to mankind. It provides food, clothing and shelter. There is tremendous increase in population which has caused pollution and depletion of natural resources. As India is a developing country there is also improvement in education sector. Many schools and colleges have come up, including the Science colleges. The Botany department utilizes plant specimens for study and later these materials are dumped into the dustbin. Thus, causing waste of materials and creating pollution. In order to utilize these plant materials it was collected and converted into compost. It took nearly 29 days for the compost formation. During this period there was change in the colour of the soil, fungal growth was observed as well as the pH of the compost was lowered from 6.5 to 6. The compost was analysed for the parameters like organic carbon, calcium and magnesium and nitrogen content. The final compost obtained was used to grow plants in Botany Department.

This project is of utmost important as it reduced the pollution, avoids landfills, prevents green house effect and also reduces cost. Thus the attempt of making our college a carbon neutral was significantly carried out. This will bring not only awareness in our college but also in other colleges and society too.

Keywords: Botany laboratory, plant waste, compost.

Introduction

Our Earth is a boon to Mankind. It nurtures us with food, clothing and shelter since ages. We are a progressing country in every field. There is also a progress in the education sector, more and more colleges and schools have come up. The Science colleges basically Botany and Zoology depend on nature for procuring the required materials for practical so that the students are aware of flora and fauna. Each day lots of plant materials are used for teaching the student during their practical. After the practical these materials are thrown into dustbins. Thus there is lot of wastage of plant specimens. The idea of utilizing these waste plant materials into compost has given rise to current work. Composting is a sustainable waste management practice that converts any volume of accumulated organic waste into a usable product. Composting is a process by which organic wastes are broken down by microorganisms, generally bacteria and fungi, into simpler forms. The microorganisms use the carbon in the waste as an energy source. The degradation of the nitrogen- containing materials results in the breakdown of the original materials into a much more uniform product which can be used as a soil amendment. Heat generated during the process kills many unwanted organisms such as weed seeds and pathogens. Advantages of composting include reduction of waste volume, elimination of heat-killed pests, and the generation of a beneficial and marketable material. Adding compost to soil increases organic matter content. This, in turn, improves many soil characteristics and allows for the slow release of nutrients for crop use in subsequent years (Bera *et. al,* 2013, Saleh Ali Tweib, *et. al,* 2011). For the present investigations the Plant materials which was used by T. Y. B. Sc. Students for practical paper II, Botany Laboratory of Mithibai College were converted into compost. This study helped in solving the wastage of plant materials obtained from nature. Further, the compost was analyzed for various physical and chemical parameters such as pH, Calcium and Magnesium, Nitrogen and Organic carbon in order to know the quality of compost. The result obtained was compared with the control soil which was used for the initiation of compost (Bhattacharyya *et.al,* 2001). The compost was tested on plants potted in the Botany Department. Thus, the above project carried out in Mithibai College, Botany Department would be an eye opener for many colleges too.

Materials and Methods

Collection of plant materials: The plant materials required for compost preparation was obtained from Mithibai College, Botany department laboratory. The materials contained leaves, stem, flowers, inflorescences of plants like rose, *Cleome rutidosperma*, *Dracena*, *Areca catechu, Vinca rosea* etc.

Conversion of plant materials into compost: A clean plastic bucket was used for compost preparation. It was filled with chopped plant materials and mixed with normal soil. They were watered at intervals. Then the materials were added on weekly bases. After three weeks the compost was ready to be used (Farhan Suraliwala, *et.al,* 2017).

Analysis of compost: The compost obtained was analysed for pH, organic carbon, Nitrogen content, Calcium and magnesium using standard methodology (Black 1965; Pawar, *et.al,* 2009).

Results and Discussion

The current investigation was undertaken to utilize the waste botanical materials into compost form rather than throwing them as landfills. The waste plant materials used

in practical were usually thrown in dustbin. It is then collected and thrown in to main garbage center. These materials remain as landfills without any use. Hence the plant materials was collected and made into compost. It showed that the rose, *Clitoria* and *Cleome* leaves degraded within 3-4 days. *Salvadora* leaves took 4-5 days to get completely decomposed. *Dracena* and Vinca got disintegrated within 5-6 days it took longer time. In case of *Areca catechu* inflorescence the flowers decomposed first while the fibers material took long time to be converted into compost. The final compost was ready in third week. During the conversion of plant material to compost there were changes in colour of soil and it also emitted characteristic odour.

As normal soil was used to initiate the compost preparation this soil was compared with the final compost. The physiochemical parameters were analyzed for both the samples which include pH, calcium and magnesium, nitrogen and organic carbon.

The pH of control soil is 6.5 which are almost near to neutral while the pH of compost is 6.0 i.e. acidic. This goes concurrent with that of increase in organic carbon. The result obtained showed an increase in calcium content in compost 0.0035mg as compared to the control soil 0.003mg. The magnesium in control is 0.003mg which decreased to 0.0002mg in compost. There is a tremendous increase in nitrogen content 602.11 kg/ha in compost whereas the control soil showed only 250.88 kg/ha. There was also an increase in Organic Carbon i.e. 0.03% in compost and 0.01% in control soil respectively (Table 1).

Conclusion

The present investigation is of great benefit to the society. It will help in utilization of botanical waste into compost rather than becoming a part of landfill. This compost will help in crop and plant cultivation. Compost has a lot of benefits like: reduce landfill space, reduce surface and groundwater contamination, reduce methane emissions, reduce transportation costs, reduce air pollution from burning waste, provide more flexible overall waste management, enhance recycling of materials and can be carried out with little capital and operating costs. Thus an attempt to make Mithibai College a carbon negative College to certain extends is fruitful.

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Table 1: Analysis of compost using different parameters for control soil and compost.

PARAMETERS	CONTROL SOIL	COMPOST
PH	6.5	6.0
CALCIUM	0.003mg	0.0035mg
MAGNESIUM	0.003mg	0.0002mg
ORGANIC CARBON	0.015%	0.03%
NITROGEN CONTENT	0.01%	0.03%
	250.88 Kg/ha	602.112 Kg/ha

A Study of Integrating Sustainability Into Business Practices Aiming For Sustainable Development

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Abstract: The United Nations Conference on Environment and Development (UNCED), held in Rio de Janeiro in 1992 also focused on integrating environmental protection with economic development to bring about sustainable development. During this Earth summit it was first highlighted that business and industry should play a crucial role in bringing about sustainable Development (United Nations Environment Programme, sustainable business, 1998). In today's world of looming environmental, economic and social challenges, businesses are making a step-change in the way they function to realize the ambition of sustainable development by incorporating suitable sustainability strategy into their business practices to ensure long term future success of the organizations. The current study aims at exploring this integration of sustainability into business practices of FMCG and service (IT and banking) sector firms in India. This is a study of 32 organisations from the above mentioned sectors for empirical and qualitative findings on sustainability integration into their business through sustainable supply chain policies, renewable energy management, waste management, water management, inclusive business practices, sustainable sourcing, identifying the various components, practices, barriers and their confrontation strategies and emergent innovations in products and processes are identified which can act as guiding light to the industry.

Keywords: Environment, sustainable development, sustainability strategy, business practices

Introduction

Sustainable Development was defined by the Brundtland Commission (WCED 1987:43) as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs." Sustainable business/marketing is a holistic approach with the aim of satisfying the wants and needs of the customers while putting equal emphasis on environmental and social issues, thus generating profit in a responsible way. This study aims to explore the current scenario of sustainability across FMCG and IT and banking service sectors in India, through empirical study and qualitative analysis of data obtained from 32 organisations about their business practices which integrates environmental sustainability and takes ecological consciousness to a next level while doing their businesses.

Objectives Of The Study

1. To understand corporate perspective on the relevance and importance of sustainable development in current market scenario and identify green branding/ marketing initiatives in this direction
2. To study the existing sustainable business practices in the select industries i.e. FMCG and service sector (IT and Banking) To identify whether there is difference between these sectors regarding sustainability practices
3. To identify the various challenges faced by the organisations while integrating sustainability into their business strategy
4. To develop Business Model archetype for sustainability integration into business practices of the respective sectors considered for the study undertaken

Literature Review

Petrini Maira, Pozzebon Marlei (2010) in their paper "Integrating Sustainability into Business Practices: Learning from Brazilian Firms" published in Brazilian Administration Review, proposes a conceptual model to facilitate incorporation of sustainability into business practices, learning from the context of companies operating in Latin America – more specifically, in Brazil – that excel in terms of sustainability initiatives. Five large companies recognized as leaders in sustainability practices were studied using the grounded theory method.

Kotler P. (2011) in his article highlights view that with the growing recognition of finite resources and high environmental costs, marketers need to reexamine their theory and practices. They need to revise their policies on product development, pricing, distribution, and branding.

Adams, Frost (2008) examines the process of developing key performance indicators (KPIs) for measuring sustainability performance and the way in which sustainability KPIs are used in decision-making, planning and performance management.

Shrivastava P. (1995) examines the implications of ecologically sustainable development for corporations. It articulates corporate ecological sustainability through the concepts of (a) total quality environmental management, (b) ecologically sustainable competitive strategies, (c) technology transfer through technology-for nature-swaps,

and (d) reducing the impact of populations on ecosystems.

Achrol P. and Kotler P. (2001) developed a three-tiered explanation of the emerging field of marketing—its sub-phenomena (consumer experiences and sensory systems), its phenomena (marketing networks), and its super-phenomena (sustainable development). Porter M. and Kramer M. (2006) propose a fundamentally new way to look at the relationship between business and society that does not treat corporate growth and social welfare as a zero-sum game.

Bocken N.M.P, Short S.W., Rana P., Evans S. (2014) introduced sustainable business model archetypes to describe groupings of mechanisms and solutions that may contribute to building up the business model for sustainability.

Methodology

The study deploys descriptive methodology, hypothesis testing and grounded theory approach for qualitative analysis. Data is collected through a structured questionnaire with 18 different questions about various aspects of integrating sustainability from 32 organisations predominantly IT, FMCG and banking firms which consciously implement sustainability into their business practices and the sampling method was Non-Probability Purposive Judgment sampling. For the purpose of data analysis, first coding was done, then reliability and validity was assessed through Cronbach's alpha, Descriptive statistics by Mean, Standard deviation, variance, hypothesis testing via Goodness of fit Chi-square test and Contingency table Chi-square test and Independent sample t-test. Numeric data entry and analysis is done through SPSS 23.0. Qualitative Analysis carried via Grounded theory approach. Grounded theory is a form of qualitative research that seeks to generate new theories by using basic elements: concepts, categories and properties. This approach rely upon following principles (Glaser & Strauss, 1967): (1) the main thrust of the method is to construct a theory, not merely to codify and analyze data; (2) as a general rule, the researcher must not define a conceptual framework before beginning research (so as to allow concepts to emerge without predefined frames); and (3) analysis and conceptualization are realized through the process of collecting data, which are constantly compared with emerging categories, thereby enriching them, establishing relationships among them or originating a new one.

Results and Discussion

1. Perception of Sustainability into business: First hypothesis about the expected understanding of sustainability among organizations is substantiated.

$H1_0$: Organizational perception of sustainability into business practices is not as per expected

$H1_A$: Organizational perception of sustainability into business practices is as per expected opinions, clear and adequate.

Test Statistics

	What do you perceive out of "Sustainabiity" in a business practice?
Chi-Square	2.792[a]
Df	3
Asymp. Sig.	.425

a. 1 cells (25.0%) have expected frequencies less than 5. The minimum expected cell frequency is 3.2.

Chi-squared value of 2.792 is not significant for degrees of freedom 3 at .05 % significance level. So there is no significant difference in the observed and expected frequencies for the various perceptions on sustainability. We therefore reject null hypotheses and accept alternative hypotheses as stated above. Thus first hypothesis is substantiated.

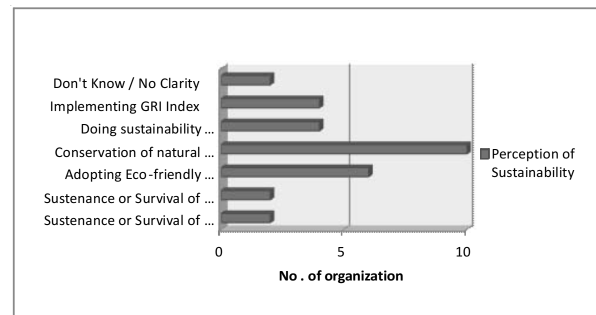

Fig.1. Perception of sustainability

2. **Integration of sustainability into business as long term competitive advantage:** The second hypothesis i.e.

$H2_0$: "Both FMCG and Service Sector firms do not have similarity in their opinion on integration of sustainability into business as a long term competitive advantage to business"

$H2_A$: "Both FMCG and Service Sector firms have similarity in their opinion on integration of sustainability into business as a long term competitive advantage to business"

Tested through independent sample t-test, hypothesis is substantiated indicating that firms whether manufacturing based or service, pay equal attention towards sustainability integration for gaining long term competitive advantage into their business.

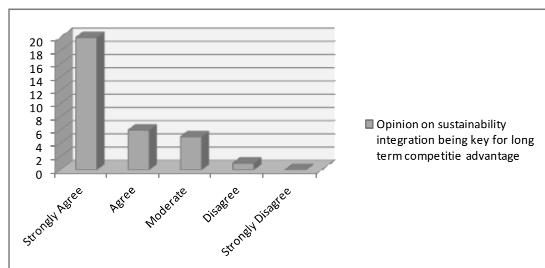

Fig.2. Opinion on sustainability integration being key for long term competitive advantage

3. **Sustainable supply-chain policy:** Hypothesis i.e. "There will be a relationship between the existence of sustainable supply-chain policy for the organization and the type of business (sector of the organization whether FMCG or service)" tested through contingency table chi-square test, is not substantiated, indicating that sustainable supply chain policy is independent of sector.

   H3$_0$: Existence of sustainable supply-chain policy and type of business are independent

   H3$_A$: There will be a relationship between the existence of sustainable supply-chain policy for the organization and the type of business (sector of the organization whether FMCG or service)

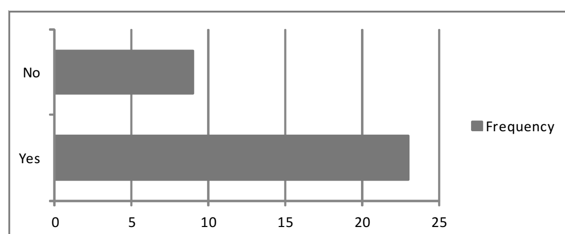

Fig.3 Existence of Sustainable Supply-chain Policy

4. **Conscious efforts in reducing firm's environmental impact by reducing its GHG emissions:** As hypothesis 4 is substantiated, i.e. "There will be difference between the sectors in the efforts of reducing their environmental impact. FMCG sector firms take more efforts in reducing its environmental impact by controlling its GHG emissions."
   Hypothesis 4 tested using t-test

   H4$_0$: There will be no difference between FMCG and Service sectors in the efforts of reducing their environmental impact
   Statistically expressed: **H4$_0$** is: $\mu_F = \mu_S$

   H4$_A$: There will be difference between the sectors in the efforts of reducing their environmental impact. FMCG sector firms take more efforts in reducing its environmental impact by controlling its GHG emissions.
   Statistically expressed: **H4$_0$** is: $\mu_F > \mu_S$

   A t-test indicated that the efforts taken for reduction of GHG emissions by FMCG sector firms are significantly different than for service sector firms.

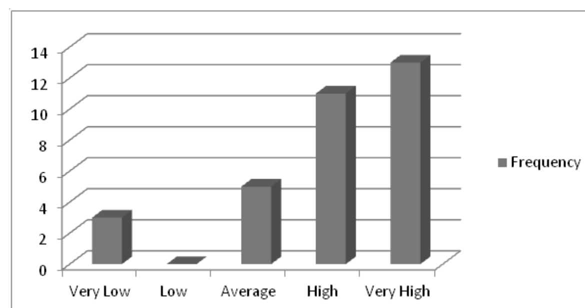

Fig.4 Efforts in reducing environment impact by reducing GHG emissions

5. **Renewable energy management:** Manufacturing firms (FMCG sector) are focusing on recycling waste and generating energy through its biomass waste, while service sector has its focus on reducing its energy consumption in its operations and trending LEED certified facilities are replacing the traditional workplaces.

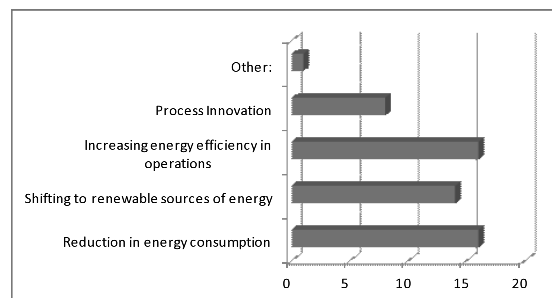

Fig.5. Measures for Renewable energy management

6. **Sustainable sourcing of raw materials:** A wide majority of firms equally opt for renewable sources and green procurement policy by working with their suppliers and having supplier's code of conduct while many giant MNCs sustainably source through backward integration for their raw materials.

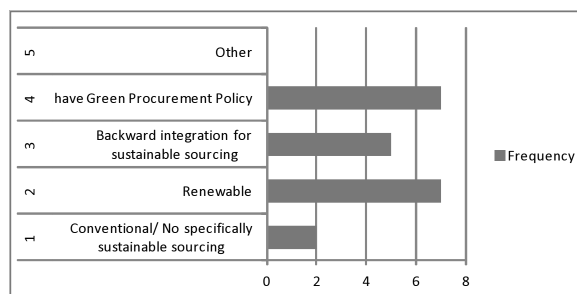

Fig.6. Sustainable sourcing practices

7. **Sustainable material management to promote recyclables:** Majority manufacturing firms adopt use of recyclable and biodegradable components in packaging or take measures to reduce usage of non-biodegradable packaging materials of their existing products while service sector is reinforcing paperless processes and focusing on electronic transactions and electronic data management.

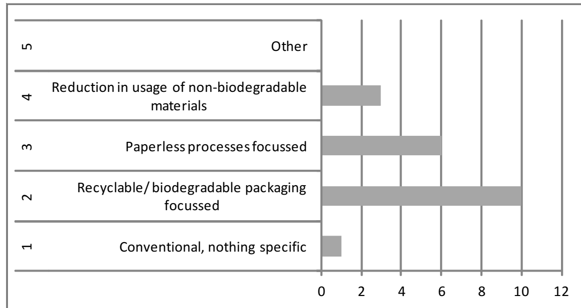

Fig.7. Material management practices to promote recyclables

8. **Barriers in sustainability integration:** Challenges in waste disposal, failure in customer communication of better price/value proposition of eco-friendly product/ services, and difficulty in communicating the need for sustainability integration across the organization are identified as top three barriers to sustainability integration in business practices.

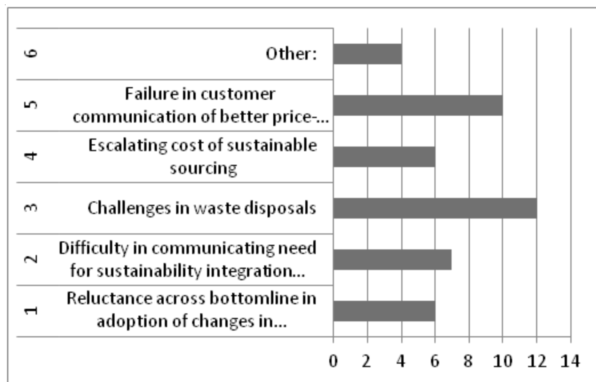

Fig.8. Barriers in sustainability integration

9. **Approaches of organisations towards barriers:** Findings to the open-ended question it was found that organisations overcome the above barriers by:

a. Carrying major process innovations for waste disposal and recycling and.

b. To improve upon customer communication, organizations are taking increasing efforts by creative media usage and reinforcing the messages with increased frequency and reach.

c. For communicating among internal stakeholders in organizations, firms adopt policy of constant enlightenment and engagement of employees in the awareness programs on sustainability integration.

10. **Product / Process innovation for sustainability:** exploration of the sixth hypothesis which is not substantiated shows that there is no difference among FMCG and service sectors in the extent of product/ process innovation.

A qualitative analysis of the different innovations reveals the following emerging themes:

FMCG (manufacturing sector)	IT and Banking (Service sector)
Light-weighing of packaging solutions	Smart and central energy management systems
Packaging from biomass waste & energy generation from biomass	Document digitization and digitized banking solutions
Supply chain efficiency innovations	Green financing services and green bonds
Life cycle assessments for eco labeling	Green IT/Data Centers
Sustainable sourcing	Low cost micro ATMs

11. **Customer and stakeholders' communication for carbon-footprint reduction:** A qualitative analysis of findings through Grounded theory approach reveals following emergent themes about how do companies carry customer and stakeholder communication for carbon-footprint reduction:

Promotion through website, mailers, newsletters, posters, signage awareness messages

Reaching consumers through conservation messaging through on package information

Tree plantation drives

Idea bank and blogs

Awareness creation and support to Clean India Mission by government

Promotion of paperless banking; mobile and e-banking among retail banking customers

Reduction in usage plastic carry-bags (communicating this by making carry-bags chargeable) for customers

Supplier engagement programmes (for B-to-B customers)

Sustainable lending businesses (for B-to-B customers)

Green Quiz

Natural capital Olympiad for students – young consumer awareness

Think Eco Act Eco fair

Free sapling sale to promote plantations

Cleanliness drives

12. **Green marketing Initiatives:** A qualitative analysis of findings through grounded theory approach reveals following emergent green marketing initiatives by leading organizations:

    Communicating sustainably sourced ingredients on packaging

    Providing sustainable choices for customer in product portfolio

    Communicating and promoting tree plantation drives

    Paperless banking platforms

    Promoting environmentally sustainable investments

    Green credit facilities

    Eco-friendly products in paper category

    Biodegradable packaging materials

    Communication about packaging material optimization and light-weighting

    Supporting and promoting sustainable farming

Conclusion

Global conglomerates better understand the significance of integrating sustainability into long term corporate objectives. Thus there are implications for many local firms as well, to take a serious consideration of sustainability and incorporate relevant strategies into its vision and mission.

To begin with their journey towards sustainability, firms can start complying with regulatory norms and follow reporting standards which provide comprehensive guidelines on various holistic aspects of sustainability.

Implications for regulatory and legislative bodies to generate greater awareness among all industrial sectors whether it is medium, small or micro enterprises. In Indian scenario, reporting standards needs to get more stringent with their regulations and periodically updated with global standards.

Adoption of sustainable supply-chain policies is strongly recommended as it not only begins with raw material supplies but spans across distributors and retailers as well to ensure sustainability across entire value chain.

Periodic employee trainings to remove reluctance across bottom line in adoption of changes in conventional practices, conduct programmes and promote awareness in alliance with regulatory bodies.

Increasing energy efficiency in operations was identified as progressive initiative towards carbon footprint reduction thus it is recommended for the companies to invest more on related technologies

As the major barrier for sustainability integration was found to be 'difficulty in communication across organizations and among customer groups' thus it is recommended that regulatory bodies need to extend their functions to awareness creating, imparting adequate knowledge, conducting requisite trainings through the organizations form merely generating regulations and performing audits.

Recommendations regarding customer communication to reduce their carbon footprint are to conduct more engaging drives, organizing contests and reward and recognition can serve as more resulting initiative.

1. Recommendations for Green marketing of their businesses firms:
2. Eco-labels
3. Eco product portfolios
4. Plantation drives
5. Paperless processes
6. Promoting environmentally sustainable investments
7. Green credit facilities
8. Eco-friendly products

    Regarding sustainable sourcing firms go for either green procurement policy which ensures collaboration with suppliers to work on production of sustainable raw materials.

    It can be concluded that the sustainable business model archetype evolved out of this study (depicted below) can be successfully emulated by organizations aspiring to integrate environmental sustainability into their business.

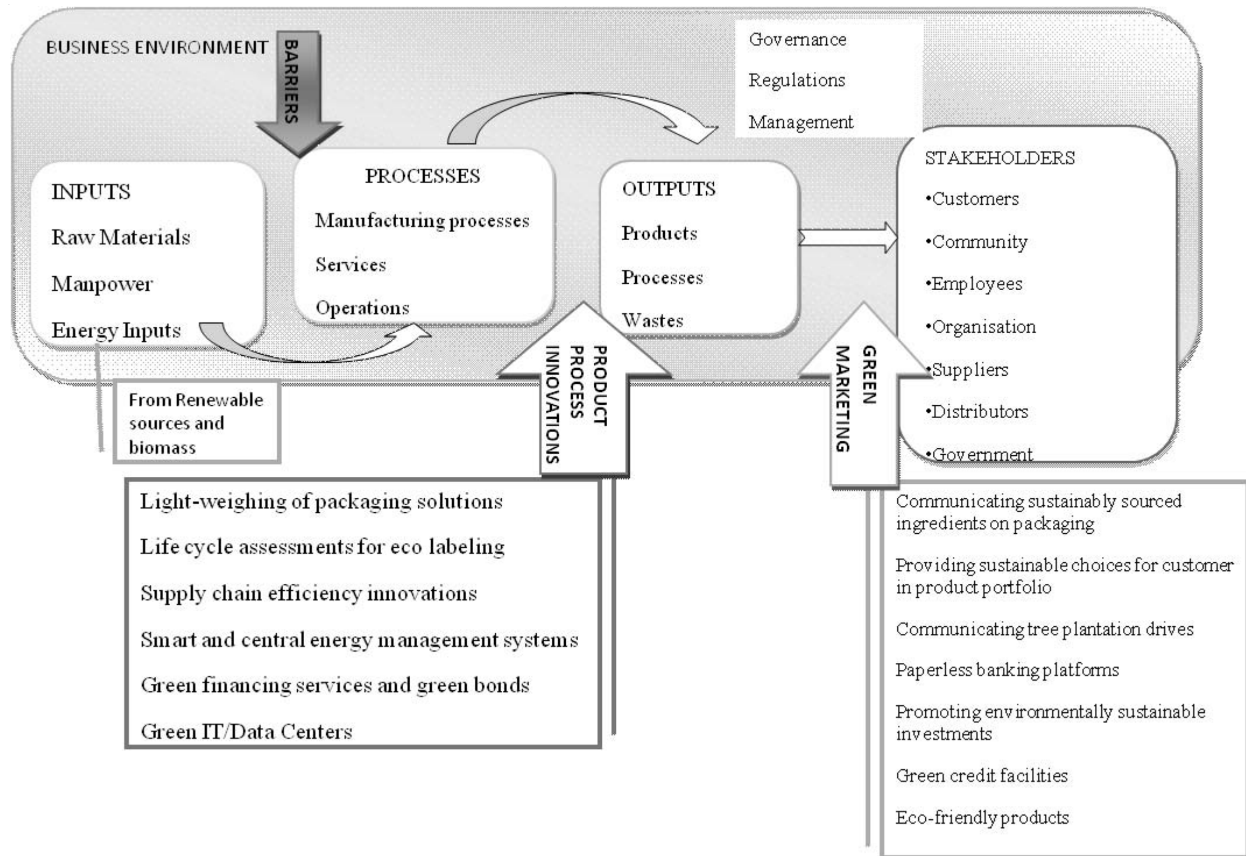

Fig.9 Sustainable business model archetype

Acknowledgement

I am thankful to Dr. V.N. Bedekar Institute of Management Studies for providing me with the resources to conduct my study. A sincere gratitude to all the respondents from multinational companies for sparing their valuable time to take an effort for responding to the questionnaire by sharing their valuable inputs and information about their company's operations for sustainability integration into their business.

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Exploratory Study To Understand The Factors Involved In Selection of Environmental Monitoring Service Provider

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Abstract: In the 21st century, fate of environment has become a critical issue in both developed and developing countries throughout the world. We know that increased societal activities and demands are changing soil, water, air, climate, and resources in unexpected ways. For successful implementation of environmental protection programme, it is essential to identify and quantify the pollution sources, pollutants, conduct baseline survey, lay down standards and build-up monitoring systems. To meet out these requirements, a competent laboratory is required with all necessary instruments, equipment, expertise, capabilities etc. Based on the guidelines and consultation of the MOEF (Ministry of Environment and Forest), the procedure for recognition of environmental laboratories which have been prepared by the CPCB (Central Pollution Control Board) .The current study of 20 organizations from chemical, engineering, agrochemical & textile industries for an exploratory study to understand the factors they consider at the time of selection of any environmental monitoring service provider. Factors such as NABL (National Accreditation board for Testing and Calibration Laboratories) accreditation, MOEF Approved laboratories, frequency of monitoring, expected services by service provider in relation of testing time, report generation time, responsiveness of service provider. Such factors have been analyzed by designing and collecting response through a structured questionnaire from the above mentioned industries. The outcome would certainly enable better understanding of its prospective customers to the environmental monitoring service providers and will help them to create a potential competitive advantage.

Keywords: Environmental monitoring, MOEF approved, NABL accreditation, environmental monitoring service provider

Introduction

Scientific and technological progress have accelerated the pace of economic growth with significant

increase in the production of food grains, development of infrastructure, agricultural crops and communication facilities, transport system but irregular, unplanned and haphazard industrial development has drastically degraded environment. It can risk human life and the very existence of mankind on this planet earth. It has degraded the environment and ecology, not only in developed and the rich countries, but also in the developing and poor countries.

Environmental Monitoring: Environmental monitoring can be defined as the systematic sampling of air, soil, water and biota in order to study and observe the environment, as well as to get knowledge from this process. (Laboratory analytical techniques series lats/9/2008-2009)

Objective Of Monitoring: Monitoring is carried out to evaluate pollution effects on man and his environment in order to identify possible causes and effect relationship between pollutant concentration, climatic changes and the health effects etc. Monitoring of the environment is considered for a number of reasons. In general, monitoring is done in order to gain information about the present levels of harmful or potentially harmful pollutants in the industrial discharges to the environment, within the environment itself that may be affected by these pollutants (Laboratory analytical techniques series lats/9/2008-2009).

Environmental Laboratory: The environmental laboratory plays a key role in assessing the status of environment comprising both biotic (flora, fauna and human being) and abiotic (soil, water and air) components. An environmental laboratory is a laboratory processing samples taken from the environmental media (air, water, soil, and biota) both from the environment as well as from sources disposing into the environment (industries, domestic and agriculture sources, automobiles etc.).The laboratories are the essential corner stones of any effective pollution control programme. The analytical laboratories provide quantitative data as well as qualitative data for better decision making purpose. For creating this valuable data with a desired accuracy and to quantify concentration of the constituents present in the samples, the laboratory should have the desired facilities and capabilities to achieve the above goal (Guidelines for recognition of environmental laboratories under the environment (protection) act, 1986 central).

Legal Provisions of Recognition of Environmental Laboratories: The need for laboratories in implementation of the various pollution control acts laid down for the protection of the environment is essential under the following sections of various acts i.e. The Water (Prevention and Control of Pollution) Act-1974, The Air (Prevention and Control of Pollution) Act-1981

The Environment (Protection) Act-1986.

Methods

<u>Population and Sample</u>

Research method- Exploratory study

Universe – Research is carried out by selecting 4 different industries whose manufacturing unit or registered offices are located in Thane and Raigad district.

Survey instrument - Questionnaire

Sampling unit - Company as whole.

Sampling size (n) - Sample size of 20 companies is considered for research.

Sampling design/ method - non probabilistic convenience sampling, Quota sampling, considering objective of research sample shall select from different industries with same sample size.

Sampling Area - Taloja ,Dombivali, Patalganga, Palghar and Lote. Data Collection Source

Primary data is collected through a structured questionnaire prepared in consultation with project guide, industrial guide and experts in this field. Data in the form of responses to the questionnaire is collected from the responsible person of the company. Respondent could be Director, Safety manager, General Manager, C.E.O, Operational manager, senior manager etc. of the company. Secondary Data existing in the form of the client list of the companies with details carried by the Mumbai Waste Management Limited was considered for study. Additional data collected from reference literature, books, journals, and e-resources.

Data Analysis And Findings

Type of environmental monitoring company does – With reference to Fig.1, majority of companies are carrying out water, air, noise and soil monitoring, as required by the type of industry.

Frequency of conducting Environmental monitoring – With reference to Fig.2, in various industries, there is different frequency of doing different types of monitoring. It is observed that majority of companies doing monitoring on monthly and quarterly basis, which is as per CPCB (Central Pollution Control Board) consent copy.

Criteria for selection of environment monitoring service provider – With reference to Fig.3, an open ended question asked here to collect the responses. Findings shows that companies for different industries are focus on list of the same parameter while selecting service provider. Among the parameters listed from the research, NABL accredited and MOEF approved laboratory is the most concerning factor among all parameters as the criteria for selection of the environment monitoring service provider. It is observed that customers are giving preference to NABL accredited and MOEF approved laboratories. Because they wanted to follow the CPCB guidelines and also companies are aware of fact that certified lab are good in technology, equipment, skillset and services. Which cover many factors that are important while selecting environmental monitoring service provider. It was also observed that customers are not much concerned for ISO certification of laboratory.

Duration of Sending quotation in response to the inquiry: With reference of Fig.4, existing service providers of the companies send quotation in response to their enquiry within a week or on the next day. Finding says that very few service provider responses after more than week. In general, response time is within a week.

On the same day of inquiry=4, On next day =7, Within a week = 8, More than a week = 1

Expected duration of revert for quotation to the enquiry – With reference to Fig.5, it is observed that as per expectation of majority of the companies, service provider should revert to their enquiry within a week or on the next day.

On the same day of inquiry=5, on next day=4, within a week=10, more than a week=1

Arrival time of the service provider for monitoring after approval of Quotation: With reference to Fig.6, it is observed that 14 out of 20 service provider came for monitoring within a week after approval of quotation.

Expected arrival time of the service provider for monitoring after the approval of Quotation: With reference to Fig.7, responses show that customers expect service to be provided within a week after approval of quotation. The current service providers of respondent are meeting the customer expectations of when to be served. Where finding says that 5customer among 20 expecting service within four days after approval of quotation, here lies the scope of improvement for monitoring laboratories.

Time taken to generate the report after testing: With reference to Fig.8, majority of service providers generate report in one week of testing. Many service providers are generating report after four days of monitoring and more than a week after monitoring.

Expect time for generating report of monitoring: With reference to Fig.9, from collected responses, it is observed customers are expecting reports of test within four days or one week of monitoring, no respondent mentioned about delivering of report as per their requirement.

NABL accreditation: With reference to Fig.10, it observed that customers are selecting service provider who is NABL accredited. It is also observed that companies are following government guidelines.

MOEF approved lab: With reference to Fig.11, it is

observed that majority of people are aware about government rules and regulations for environmental monitoring and they are selecting their service provider for environmental monitoring considering certification by NABL and MOEF approval which also justify the awareness about the requirement of environmental monitoring by industries.**Factors made company to choose their current service provider of environmental lab:** With reference to Fig.12, the factors considered by the respondent are observed. Customer is more concerned about service offered. Cost, quality of work, consistency in report are another factors recorded. Customers also consider factors such as promptness, expertise in operation, credibility and experienced staff in laboratories to carry out the tests.

Suggestions to control air and water pollution from service provider: With reference to Fig.13, from the responses it is observed that current service providers are not only providing the services but they are also providing them suggestions for controlling air and water pollution which is an extra benefit that customer is getting from the service provider. This tends to attract the customers for availing service from them again.

How do company get the reference: With reference to Fig.14, majority of companies got the reference of their existing service customer from personal reference, word of mouth, publication, pollution control board, and from website. For creating awareness of laboratory business, online and offline marketing is essential.

Result And Disscusion

Monitoring laboratories should create awareness and promote their services on every possible platform. Such as sending mails, communicating message to target customer in all possible efficient ways, printing pamphlets etc.

NABL accreditation and MOEF approval are the two most preferred parameters by companies for selecting service provider should be considered by monitoring laboratories as a competitive advantage.

Majority of respondents expect service within a week at every stage of monitoring process. Company should serve their customers within a week to their enquiry, doing monitoring and generating reports of monitoring.

Monitoring laboratory should focus on service, quality of report, consistency in report, calibration of equipment, promptness of testing, quick response to communication. As per research these are also the factors considered for selection of service provider by companies.

Conclusion

In the 21st century, the fate of the environment has become a critical issue in both developed and developing countries throughout the world. Population increases and technological advances are creating a burden on society by requiring continued expansion and concomitant resource use. Substantial evidence exists showing that such development has led to detrimental impacts on the environment. We also know that increased societal activities and demands are changing soil, water, air, climate, and resources in unexpected ways. This in turn has led to a renewed interest in protecting the environment and has focused attention on the concept of environmental monitoring.

Acknowledgment

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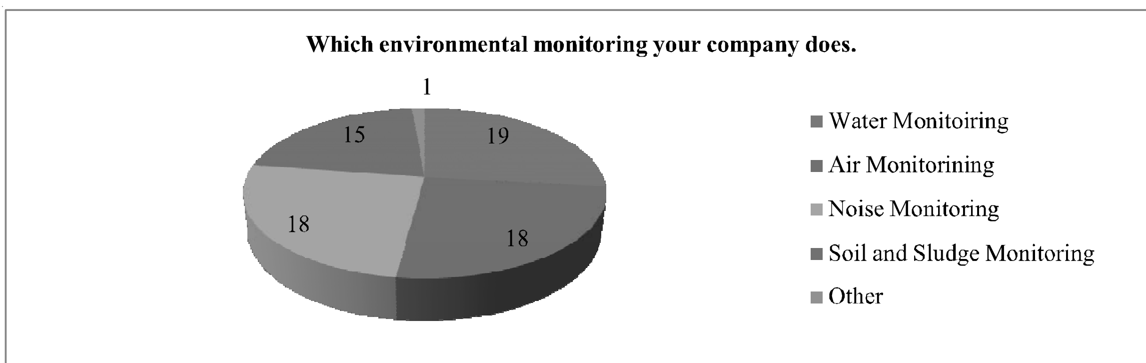


Fig 1

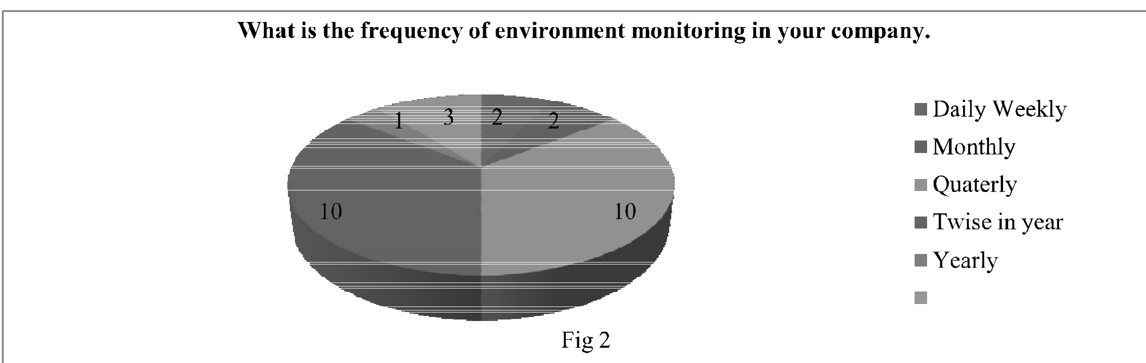


Fig 2

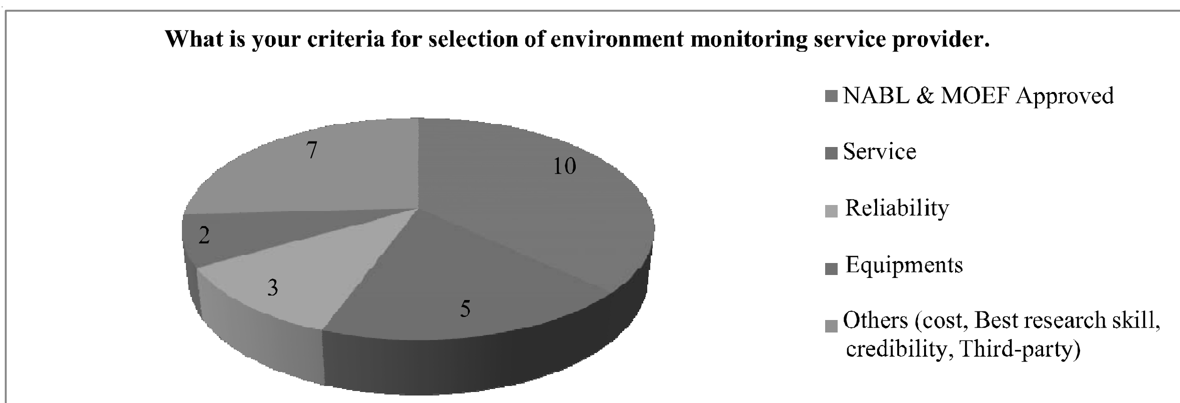


Fig 3

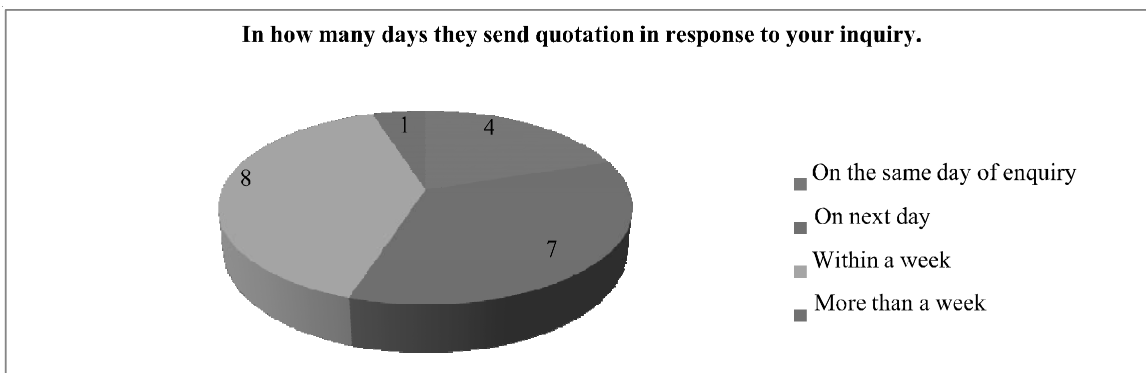


Fig 4

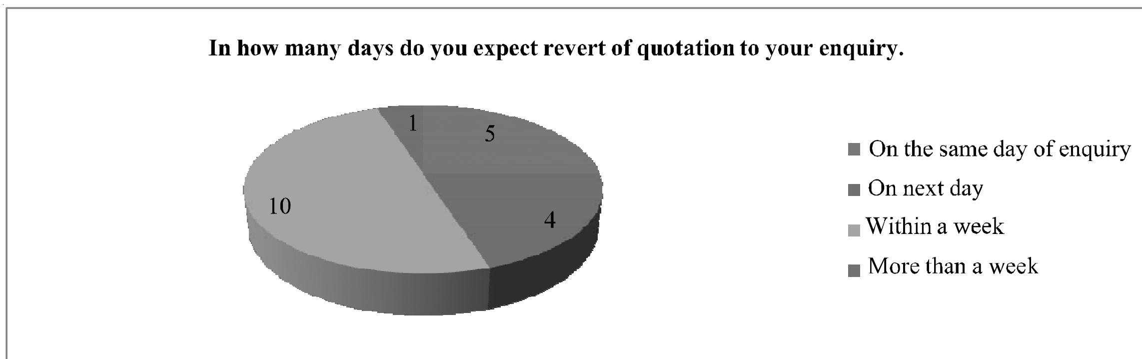


Fig 5

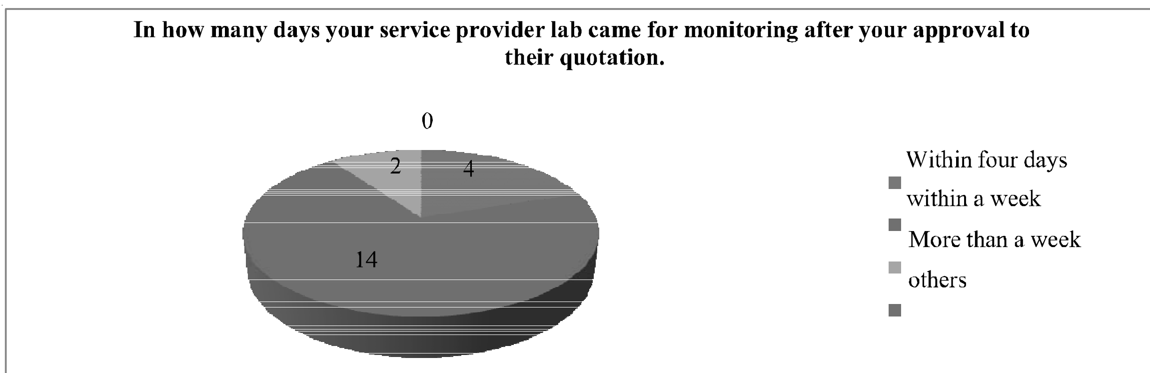


Fig 6

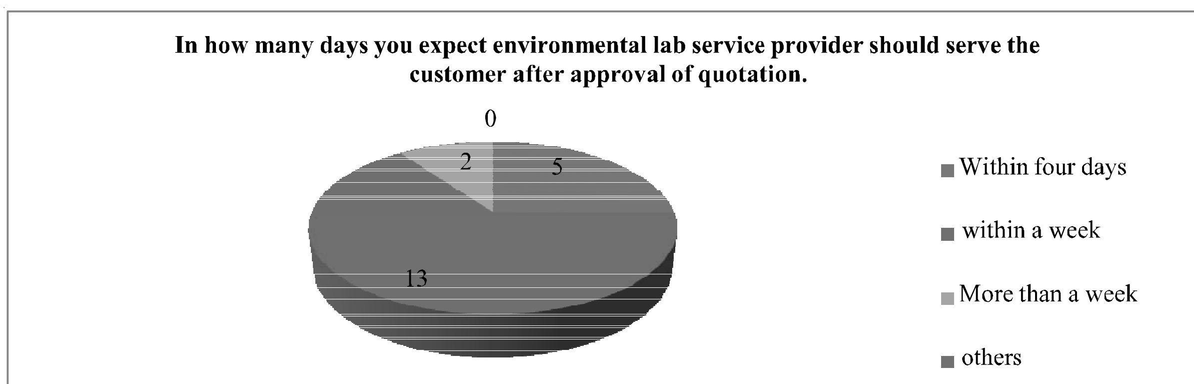


Fig 7

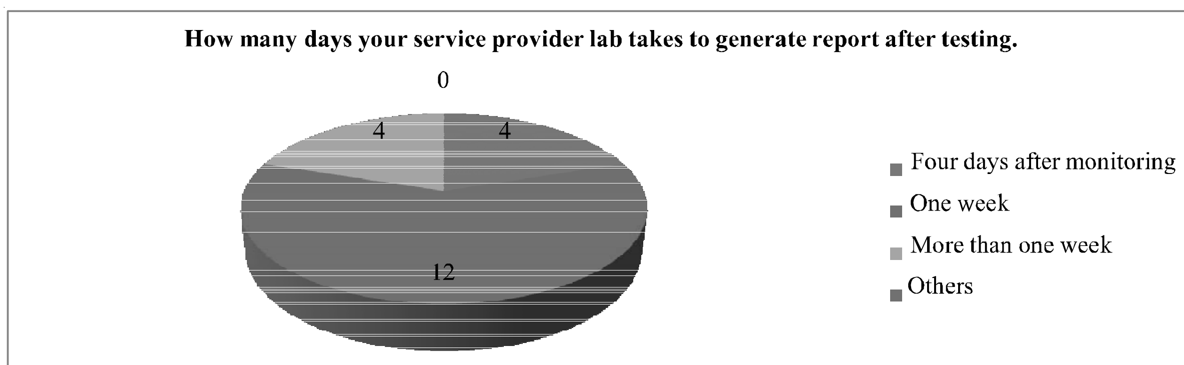


Fig 8

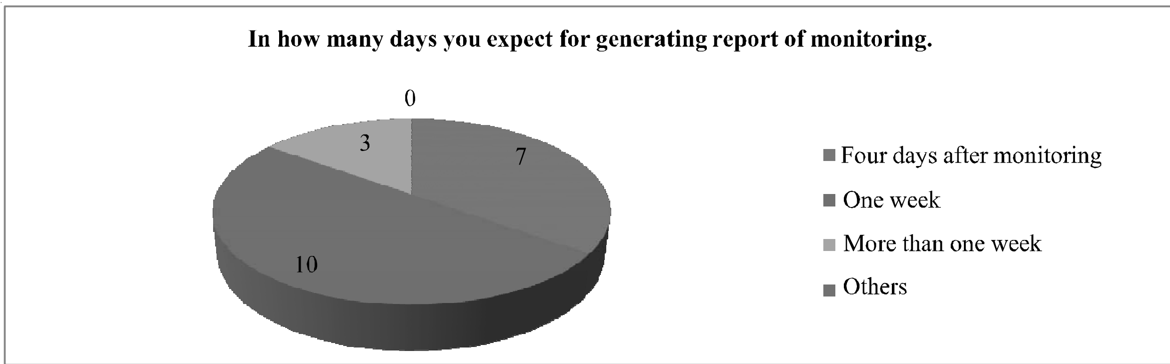


Fig 9

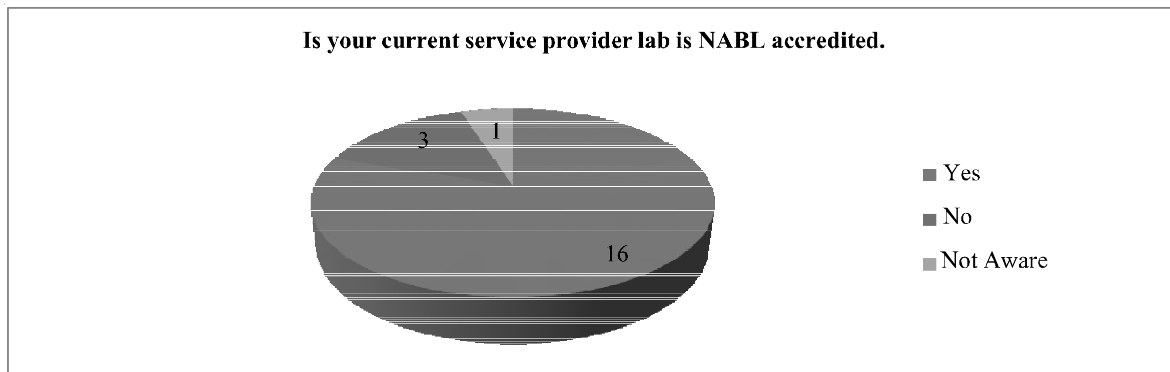


Fig 10

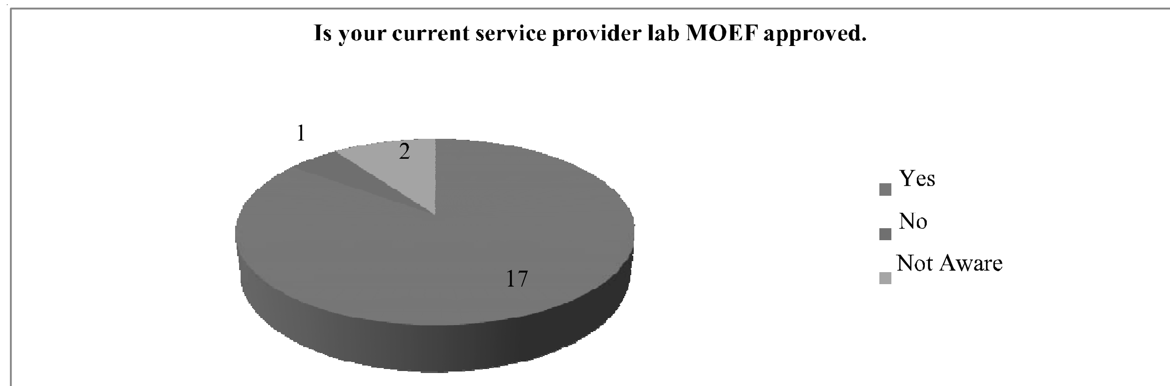


Fig 11

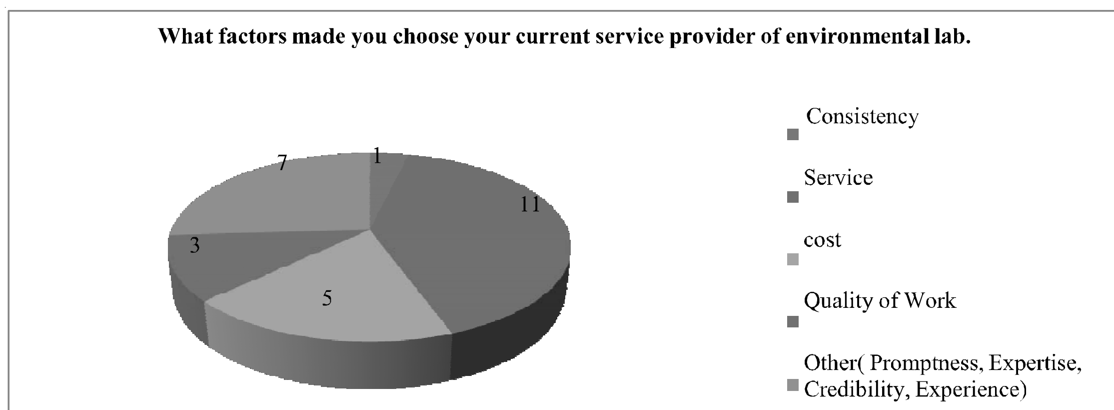


Fig 12

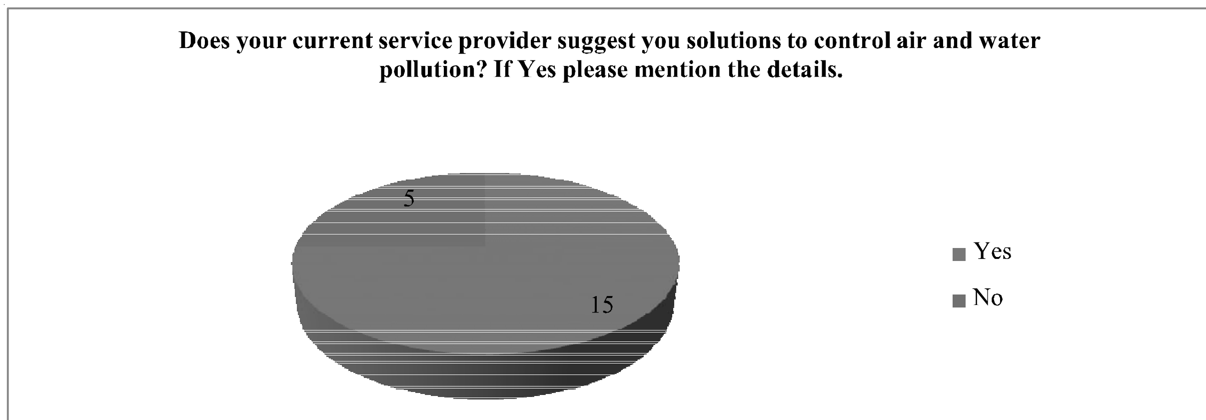
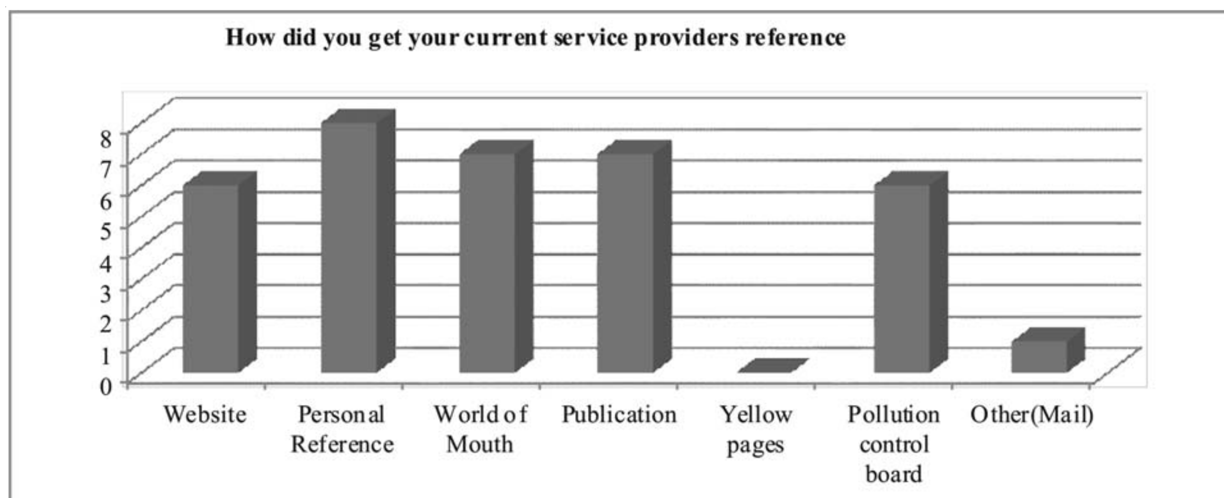


Fig 13



Study of Plant Growth Promoting Activities of Bacillus Species That Enhance The Growth of The Grey Mangrove Avicennia Marina

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Abstract: Mangroves play a very significant role in coastal cities such as Navi Mumbai where they serve as buffers between the land and sea and contribute oxygen to the polluted air. However, they are under constant threat due to urban development projects, which cause the destruction of hundreds of acres of mangroves. To compensate for such losses, mangrove regeneration programs are undertaken, in which mangrove plantlets are grown in nurseries and subsequently transferred to new areas. The growth of mangrove plantlets can be promoted by the use of PGPB (Plant Growth Promoting Bacteria) which exhibit activities that benefit the plant. The use of PGPB is well-known in agriculture and their usefulness has been reported in the case of mangrove plants too. However, growth promoting bacteria indigenous to mangrove areas in Navi Mumbai have been largely unexplored.

We have isolated three plant growth promoting *Bacillus* species from mangroves in Navi Mumbai which promote the growth of *Avicennia marina*, the dominant mangrove species found in the area. We investigated their plant growth promoting (PGP) and bio-control activities in the current study. All three isolates were found to possess multiple activities such as nitrogen fixation, phosphate solubilization, IAA production, extracellular enzyme production and anti-fungal activity. These activities were quantified and expressed in standard units. These isolates have the potential to be developed into an effective biofertilizer that can be used to promote mangrove growth in re-forestation programs undertaken in Navi Mumbai.

Key Words: Mangroves, Navi Mumbai, PGPB, *Avicennia marina, Bacillus*

Introduction

Mangroves are salt-tolerant plants of tropical and subtropical intertidal regions of the world. They are a valuable ecological and economic resource, being important nursery grounds and breeding sites for birds, fish, crustaceans, shellfish, reptiles and mammals; a renewable source of wood; accumulation sites for sediment, contaminants, carbon and nutrients; offer protection against coastal erosion and also diminish the impact of tsunamis. (Alongi, 2002; Gomes *et al*, 2010). Despite the well-known benefits of maintaining healthy mangroves, they are highly threatened ecosystems. (Gomes *et al*, 2010). The rapidly expanding coastal city of Mumbai is an ongoing example of loss of mangrove species with more than 40 per cent of its mangrove ecosystems lost in the past decade. (Hiranandani, Levitt, 2010) This is largely due to burgeoning population pressure, construction and development activities, conversion to agricultural land and fish farms besides the effect of industrial effluents (Vijay *et al*, 2005). Because mangroves are being deforested on an alarming scale, it is imperative to consider strategies for their preservation and reforestation. (Bashan, 1998; Bashan *et al*, 1989; Glick, 1995) Mangroves are regenerated by growing the seedlings in nurseries and planting the seedlings in new places. However, the survival of the saplings is variable (Bashan and Holguin, 2002; Kathiresan and Bingham, 2001). To aid in the reforestation of mangroves, inoculation of the seedlings with plant growth-promoting bacteria (PGPBs) has been suggested, similar to what has been done in agriculture and temperate forestry (Rojas *et al*, 2000).

PGPB are free-living soil bacteria that benefit the plant in various ways (Kloepper *et al*., 1989).. They may direct provide nutrients to the plant by fixing atmospheric nitrogen, solubilizing phosphorus and iron, and enhancing production of plant hormones. Additionally, they improve the plant tolerance to stresses, such as drought, high salinity, metal toxicity, and pesticide load. A group of PGPB, referred to as biocontrol PGPB, indirectly promote plant growth by preventing deleterious effects of phytopathogenic bacteria, fungi, nematodes and viruses. (Bashan *et al*, 2008)

Bacteria of diverse genera have been identified as PGPR, of which *Bacillus* and *Pseudomonas* spp. are predominant (Podile and Kishore, 2006). We have isolated three *Bacillus* strains from mangroves in Navi Mumbai, which enhance the growth of the mangrove plant *Avicennia marina,* which comprises almost 60% of the mangroves in Mumbai (Zhu *et al*, 2009). In the present study, we studied these isolates to determine their plant growth promoting and bio-control activities.

Materials and Methods:

We investigated three PGPB isolated from mangroves in Navi Mumbai for the following plant growth promoting and bio-control activities. All the media used contained 3% NaCl.

Nitrogen fixation: The isolates were tested by growing them in Glucose Nitrogen free Mineral medium (GNFMM). (San *et al*, 2011).

Phosphate solubilisation: The phosphate solubilizing ability

of the isolates was determined by inoculating the cultures on sterile Pikovaskaya's agar. Phosphate solubilizing index (PSI) was calculated as the ratio of diameter of halo (mm)/ diameter of colony (mm) (Nosrati *et al*, 2014). Quantification of the phosphate solubilisation activity was carried out by inoculating the cultures in Pikovaskaya's broth and measuring the soluble phosphate in the medium at regular time intervals by the molybdenum blue method. A standard plot of phosphorus with concentrations in the range of 20 – 100 mcg/ml was used (Woods and Mellon, 1941).

IAA production: The cultures were grown in nutrient broth containing 0.1% D, L tryptophan for 24 hours at R.T. followed by addition of Salkowsky's reagent. IAA activity was quantified by inoculating the cultures in sterile Nutrient broth containing 0.1% D, L tryptophan and measuring the IAA concentration at regular intervals by the Salkowsky's method. The concentration of IAA was determined from a standard plot of IAA with concentrations in the range of 4-20 mcg/ml. (Khin *et al*, 2012).

Extracellular enzyme production: The amylase and protease activity of the isolates were determined by growing them on starch agar plates and milk agar plates respectively. (K. Geetha *et al*, 2014). The cellulase activity was determined by growing the isolates on CMC agar, followed by flooding with 0.1% Congo red solution and washing with 1 M NaCl (Apun *et al*, 2000). To test for chitinase activity, the isolates were spot inoculated on colloidal chitin agar plates. (Chernin Leonid *et al*, 1995). In all the above cases, the enzymatic index was calculated using the formula: Enzymatic Index = Diameter of the halozone / Colony diameter in mm (Carrim *et al*, 2006; Howard et al, 2003; Ariffin *et al*, 2006)

Lipase activity: Bacteria were grown on nutrient agar amended with egg yolk. After 48 h of incubation the agar medium was flooded with saturated aqueous solution of copper sulphate ($CuSO_4$), kept for 10-15 min and observed for formation of greenish blue colour zones around their growth (Geeta *et al*, 2014).

Anti-fungal activity: The plant pathogenic fungi *Penicillium citrinium, Penicillium purpurogenum and Talaromyces purpurogenous* were first grown on PDA plates to obtain matt growth. A plug of this fungal growth was removed using a cork borer and placed in the centre of a fresh PDA plate. The bacterial isolates were then spot inoculated on the plate around the fungal plug. The plates were incubated at 28°C for 48 hours and observed for inhibition of fungal growth (Leon *et al*, 2009).

Siderophore production: The isolates were spot inoculated on CAS (Chrome azural S) agar plates and observed for an orange halo zone around their growth (Louden *et al*, 2011)

Ammonia production: The isolates were grown in peptone water; Nessler's reagent was added and the tubes were observed for development of a brown to yellow colour (K. Geetha *et al*, 2014)

Nitrate reduction: The cultures were grown in nitrate peptone broth at 28°C for 24 hours, followed by addition of equal volumes of sulphanilic acid and á- napthylamine and observing for development of a red colour (Conn and Breed, 1919)

HCN production: The cultures were spot inoculated on King's B medium containing 4.4g/l of glycine with indicator paper (Whatman filter paper soaked in 0.5% (w/v) picric acid and 2% (w/v) sodium carbonate) placed in the lid of the petri plate (Masoud *et al*, 2006). The dishes were sealed and incubated at 28°C for 48 h. Positive test is indicated by browning of the filter paper.

Salt tolerance: Each isolate was inoculated in Nutrient broth tubes containing NaCl concentrations in the 2-20% range at 2% intervals. The highest salt concentration showing growth after incubation for 24 hours was considered as the salt tolerance of the isolate.

Metal tolerance: Each culture was inoculated in sterile nutrient broth tubes containing 100-1000 ppm concentrations of copper sulphate, lead acetate and zinc sulphate at 100 ppm intervals. The tubes were incubated at 28°C for 72 hours and checked for growth. The maximum concentration of the metal salt which showed growth was considered the tolerance of the culture for the respective metal (Weigand *et al*, 2008)

Identification of the isolates: Identification of the isolates was carried out by 16S rDNA sequencing. DNA was isolated from the culture. Its quality was evaluated on 1.0% Agarose Gel. Fragment of 16S rDNA gene was amplified by PCR. The PCR amplicon was purified by column purification to remove contaminants. DNA sequencing reaction of PCR amplicon was carried out with 8F primer using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The 16S rDNA gene sequence was used to carry out BLAST with the database of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software programs (Protocol followed by Saffron Life Science, Gujarat)

Results And Discussion:

Nitrogen fixation: All three cultures showed growth in GNFMM after incubation for 48 hours, indicating that they could fix atmospheric nitrogen.

Phosphate solubilisation: The cultures N5, N13 and N14 showed phosphate solubilizing indexes of 1.89, 1.97 and 1.39 respectively on Pikovasky's agar plates. In the quantitative assay, N14 gave the highest soluble phosphate concentration of 390 ppm in 7 days (Fig.1). The ability of *Bacillus* spp. and *Vibrio* spp. to solubilize phosphate in

the marine environment is well known (Vazquez, 2000). In studies carried out by Behera *et al* (2016), Audipudi *et al* (2012), Ghosh *et al* (2012) and Vazquez *et al* (2000), several Bacillus species capable of solubilizing phosphate were isolated.

IAA production: N5 and N13 were found to be IAA producers by the qualitative test. The results of the quantitative Salkowsky's assay are shown in Fig 2. N5 showed a peak in IAA production on the second day followed by a slight decline on the 3rd day, thereafter maintaining a steady concentration of 32 mcg/ml. N13, on the other hand, showed an increase in IAA production upto the sixth day producing a concentration of 74 mcg/ml, which then remained constant. The IAA production by our isolates is significantly higher than values that have been reported so far. In studies on IAA producing mangrove bacteria by Kathiresan and Selvam (2006), Bhosale *et al* (2016), Kannahi and Dhivya (1995) and Neyser De La Torre-Ruiz *et al* (2016) a maximum IAA production of 15.7 mcg/ml was reported.

Estimation of extracellular enzyme production: All the cultures showed amylase, protease, cellulase and chitinase activity (Plates 1, 2 and 3), with the culture N14 displaying the highest enzymatic indexes of 2.74, 3.3, 4.39 and 3.56 respectively (Fig. 3). Castro et al in 2014 isolated amylase, protease and cellulase producing bacteria from a Brazilian mangrove ecosystem with enzymatic indexes ranging from 1.15-2.78, 1.05-2.53 and 1.43-2.93 respectively. Bacterial chitinase producers have been isolated from mangroves by Rishad *et al* (2016), Kannahi and Dhivya (2015) and Azevedo *et al* (2015) Greenish blue coloured zones were obtained around the growth of all three isolates indicating that they produced lipase (Plate 4).

Siderophore production: All the isolates showed the formation of an orange halo zone around them on CAS (Chrome azural S) agar plates within 24 hours of growth. The zones grew bigger with time till the entire plate turned yellow by 72 hours of incubation (Plate 5). This shows that the isolates have significant ability to produce siderophores, which play a very important role in protecting the plant from phytopathogens.

Anti-fungal activity against plant pathogens: The isolate N14 showed inhibition of growth of *Penicillium citrinium and Talaromyces purpurogenous*

Ammonia production, nitrate reduction and HCN production: All three cultures showed ammonia production and nitrate reduction**.** However none of them showed production of HCN.

Salt tolerance: The isolates N5 and N13 could tolerate upto 8% salt while N14 could tolerate upto 10% salt concentration. This outcome is of great significance because salinity is one of the harshest environmental factors that prevent a high crop yield, and most agricultural plants are sensitive to a high salt concentration in the soil. Therefore, plant growth promoting bacteria (PGPB) act as one of the most effective tools for the alleviation of salt stress (Neyser De La Torre-Ruiz *et al*, 2016).

Tolerance to heavy metals: N5 showed a low tolerance to copper and an average tolerance to lead and zinc, N13 showed good tolerance to all the three metals tested, while N14 showed low tolerance to zinc and a very high tolerance to lead. As seen in Fig. 4, all the cultures showed highest tolerance for lead. Heavy metal tolerance is a useful characteristic for PGPB, especially those that are to be used in contaminated environments such as mangroves.

Identification of the isolates: The three cultures N5, N13 and N14 were subjected to 16S rDNA sequencing at two institutes – Gujarat State Biotechnology Mission, Gandhinagar and Saffron Life Science, Navsari, Gujarat. The results obtained from both institutes revealed that N5 showed 100% sequence identity with six strains of *Bacillus cereus* and three strains of *Bacillus thuringiensis*. N13 showed 99% sequence identity with six strains of *Bacillus cereus*. N14 showed 99% sequence identity with *Bacillus subtilis subsp. inaquosorum* strain BGSC 3A28.

Conclusion:

The three *Bacillus* strains isolated from mangroves in Navi Mumbai possess a variety of plant growth promoting and bio-control activities. The results revealed that the strains are capable of fixing atmospheric nitrogen, phosphate solubilization and IAA production. They are also capable of degrading substrates such as cellulose, chitin, starch and proteins and producing siderophores. Besides, the *Bacillus subtilis* strain has anti-fungal activity. The three strains were also found to tolerate relatively high levels of salinity and heavy metal concentrations. Thus these strains can be used as effective plant growth promoting bacteria and bio-control agents to enhance the growth of mangroves as well as other plants, especially those that are affected by saline soils and presence of heavy metals.

Acknowledgements:

We thank the management and staff of the Microbiology department of Karmaveer Bhaurao Patil College, Vashi and V.E.S College, Chembur for the support and facilities provided in carrying out this work

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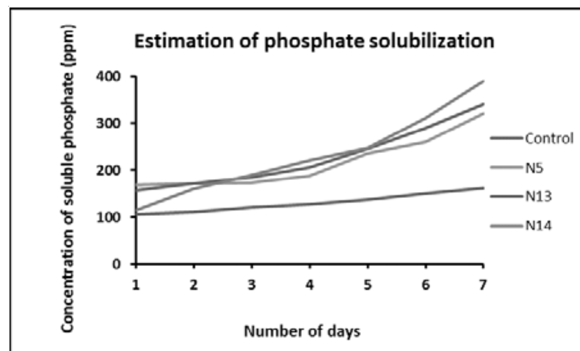

Fig1: Quantification of phosphate solubilisation by the isolates

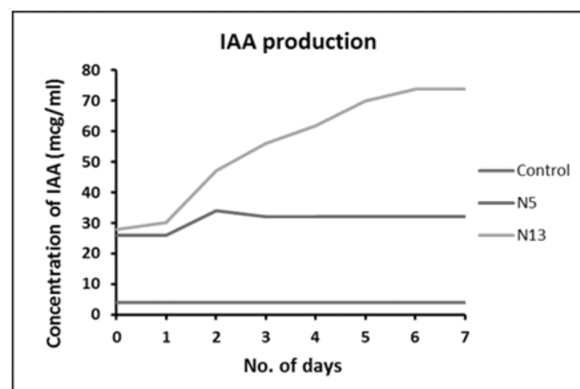

Fig 2: Quantification of IAA production by N5 and N13

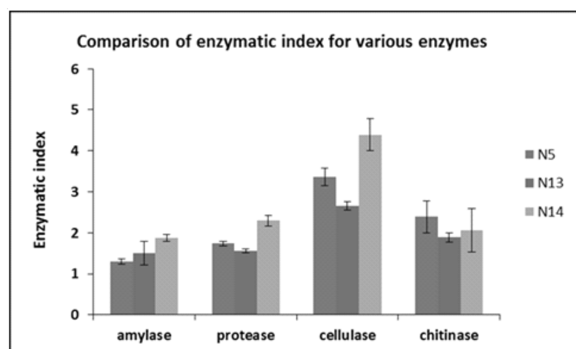

Fig. 3: Enzymatic index of the isolates for various extracellular enzymes

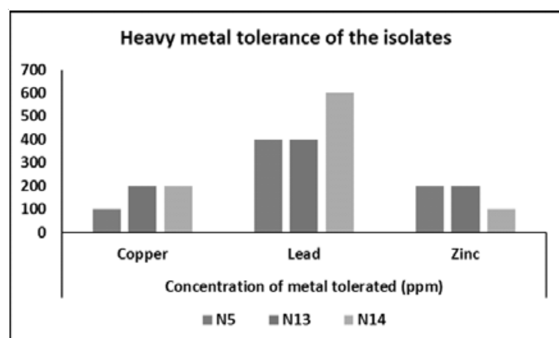

Fig. 4: Heavy metal tolerance of the isolates

Plate 1: **Amylase activity on starch agar**

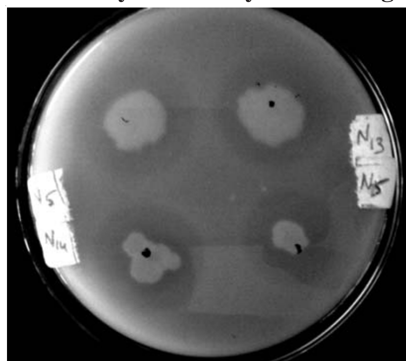

Plate 2: Protease activity on skimmed milk agar

Plate 3: Cellulase activity on Congo red cellulose agar

Plate 4: Lipase production on nutrient agar amended with egg yolk

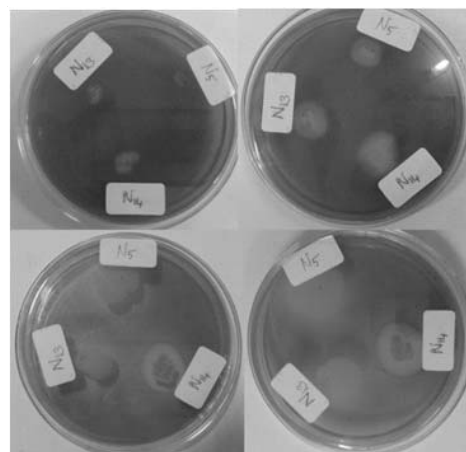

Plate 5: Siderophore production on CAS agar

Comprehensive Green Growth: A Step Towards Sustainable Development, Trends and Business Practices Globally

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Abstract: Global economies have witnessed a remarkable growth record over the past two decades; however it has been clouded by degrading environment and depletion of our natural resources. Current growth patterns have left millions of people behind on the ladder of better life; 1.2 billion people lack access to electricity, 870 million people are malnourished, and 780 million people still do not have access to clean and safe drinking water globally. Of course, poverty is both the cause and consequence of resource degradation and overuse of limited resources but does that implies growth has to be built on the expense of destroying environment and socially exploiting poor communities. Development is necessary but it will be sustainable in the long run only if it is constructed on the pillars of economic growth, environmental stewardship and social inclusion. There is a substantial scope of how the world can grow green without growing slow. This study aims at exploring how green growth is efficient and important for creating sustainable development and it suffice the needs of current population and also creates an equitable share for future generations and the global trends and business practices in sustainable development.

Keywords: Environment, Sustainable development, Green Growth.

Introduction

Growth is comprehensive only when it fosters economic, social and environmental sustainability (UN Sustainable Development Goals SDGs, 2015). Over the past 50 decades growth has come globally but largely at the expense of damaging environment to a severe extent. Economic growth propels poverty reduction, improved social outcomes like better health and education, better equality, but too often it has been clouded with environment degradation and depletion of natural resources. Sustainable development identifies growth must be comprehensive and environmentally favourable so that it builds a shared prosperity for today and equal growth prospects for future generations. (World Bank Overviewof Sustainable Development)Our current inefficient and unsustainable growth patterns are hampering the goals of sustainable development and its components of economic, social and environmental sustainability. Green growth is therefore the step forward towards sustainable development because it is clean as it reduces the pollution and prevents other hazardous consequences of environmental damage and efficient in the use of vastly depleting natural resources.Green growth is both efficient and affordable. By 2050, World's estimated population is expected to be 9 billion people and therefore the demand for good food, safe water, clean air, energy and other natural resourceswill be tremendous. Therefore properly planned actions need to be taken by both developing and developed economies in order to keep a check on robust growth.

Why Is Green Growth The Next Step Towards Sustainable Development

Green Growth Is Obligatory

Growth is generally measured in GDP and it is an important parameter for poverty reduction. Despite rapid population growth, GDP per capita in developing countries has increased by 80 percent in last 2 decades and living standards of people globally have improved significantly. A remarkable progress is visible in literacy, education, life expectancy, malnutrition, infant, child and maternal mortality. Ghana, for example, showed a great growth as compared to African average and reduced the poverty rate from 51 percent in 1990 to 30 percent in 2005.

Growth, however, need not cause income inequality. Inequality has reduced in Latin America but at the same time increased in United States of America and most of Europe. The famous economic theory of Kuznets (Andreoni, J., and A. Levinson, 2001) curve which proposes that inequality first increases and then decreases with income therefore is contradicted.Thus, economic and social pillars are inter-related but this logic does not apply well with economic and environmental components of growth. High income countries believe that growth re-distribution, which was if politically feasible, would help bring about environmental sustainability, Further, in spite of rapid growth, electricity, access to clean water, sanitation, road facilities to name a few still remain the cause of concern. As per current trend developing countries(90 percent of world's population) would consume around 50 percent of world's income by 2050. The final nail in the coffin is the rapidly growing population and aggressive steps need to be taken in order to tackle the demographic challenges of poverty, education, child and maternal mortality, employment opportunities and so on.

Thus, growth is obligatory, statutory and essential for developing the Earth as a whole on all economic, social

and environmental parameters (Copeland, 2012). Our ambitions of a developed world to live in cannot be at the cost of degrading our environment and depleting the natural resources neither creating social inequality. Growth can be enough green without being slow.

Green Growth Is Coherent

Owing to poor production, consumption and management of our natural resources, the growth patterns are highly unsustainable as compared to our current and projected population. Population growth and increase in demand for food has forced expansion of agricultural production. But poorly managed intensification has led to agrochemical and water pollution. Extensive and expansive farming has led to land degradation and deforestation. A large demand for water has led to depletion of ground water and scarcity of water. The amount of carbon dioxide emissions which gets accumulated in atmosphere is approaching the danger zone. Inexpensive and easily available energy resources are gradually depleting and therefore alternatives are searched.

Environment therefore can be thought of as a natural capital, and investing in this natural capital can be a good growth policy.For instance in China's Loess plateau, watershed restoration and reforestation led to higher value of agricultural production and declined risk of landslides and flooding. Therefore developing good policies which are both environmental and ecosystem friendly are at the heart of green growth strategies.

Green Growth Is Economical

Most of the green growth policies are cost effective and pay for it. Green policies contribute to the growth as well as boost nation's overall growth. The benefits of promoting green growth may outweigh the cost involved in its implementation. For instance, in East Asia, the estimated cost of sustainable energy path is $80 billion not more than $70 billion which the regions currently use on fossil fuels.

Importance of Green Growth Policies

China's growth rate in the past 30 years is about 10 percent per year making it from a poor country to world's second largest economy. The factors motivating China could be cost of environment degradation and the quest for new sources of growth. Countries like Ethiopia and Kenya are investing highly on green growth strategy. Many countries are trying to balance the environment and economic growth. World needs green growth to the highest level of urgency.The World Bank financed project around the lake of Gaurapiranga in Brazil is the classic example of environmental benefits of a green growth strategy. Urban renewal and slums upgrading were clinical in bringing about improved water quality which as a result provided a safe and reliable source of water supply for city of Sao Paulo.

Environmental policies have a direct benefit over the economic growth generally on long term but it can also benefit in short time economic growth. Market failures like knowledge spill overs, underinvestment in R&D and sub optimal utilization of resources have hurt both environment and economy by reducing effective use and increasing ineffective use of natural capital. For an example, urban congestion creates over the line air and water pollution but also hampers the effective usage of available resources. Green growth policies therefore are seen as tools for increasing optimal and effective utilization of natural capital.

Green Growth: Trends and Practices Globally

Green Accounting

Accounting serves two purposes; keep a score check and management purpose. In standard national accounting, GDP of the country is tracked. However, Green accounting extends to the inclusion of values of damage and depletion of natural assets under the tag of human welfare. Net savings indicate whether well-being is sustainable or not.

WAVES – Wealth Accounting and the Valuation of Ecosystem Services

The World Bank led global partnership WAVES aims at promoting sustainable development by ensuring that natural assets are safeguarded.

ITC – India's top sustainable conglomerate

ITC (Indian Tobacco Company) is pursuing various innovative strategies for environmental stewardship.

- Several of ITC's factories, premium luxury hotels and ITC Infotech Park in Bengaluru meet close to 100% of their energy requirements from renewable resources.
- ITC Grand Chola is the world's largest LEED® Platinum certified green hotel and was awarded by India's first 5 stars GRIHA (Green Rating for Integrated Habitat Assessment).
- WOW (Well-being out of waste) initiative promotes resources conservation and recycling and the importance of 'Reduce-Reuse-Recycle'

Conclusion

Sustainable growth can be achieved only when it benefits economy, society and environment as a whole. A resource effective and efficient economy can be built on the low cost. Sustainable growth focuses that growth needs to

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be both inclusive and environmentally sound to create shared prosperity for current generations and suffice the future demands. The dilemma is not whether to embrace green growth or not but to emphasize on how best it can be done. Nations are realizing the importance of various natural resources and steps are taken by government and industries to bring about a sustainable development.

Acknowledgement

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Biosorption Potential of Lead And Cadmium Resistant Stentrophomonas Sps. Isolated From Mithi River In Mumbai

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Abstract: The biosorption potential of the bacteria isolated from the downstream areas of the Mithi River and identified as belonging to the genus *Stentrophomonas*is observed experimentally in our study. The two heavy metals that the organism was subjected were Lead(Pb) and Cadmium(Cd). The species were found to show resistance for the two metals. For the biosorption studies the organism was inoculated in Lead and Cadmium in two separate aqueous medium at a concentration of 10 mg/L. *Stentrophomonassp.*showed a 49% decrease in Lead and 45% decrease in Cadmium in 24 hours.

Keywords: Mithi River, *Stentrophomonas sp*., Biosorption, Heavy metals,Resistance

Introduction

Environment pollution is a worldwide problem and has a huge potential in influencing the health of the human population (Fereidoun *et al*., 2007). Industrialization has resulted into discharge of dangerous pollutants into the water bodies. Heavy metals are one of the major components of industrial discharge. The heavy metals are non-degradable in nature hence contribute to the toxicity of the aquatic ecosystem. The most commonly found heavy metals in water include arsenic, cadmium, chromium, copper, lead, nickel, and zinc, all of which pose risks for human health and the environment (Lambert *et al*., 2000).Many methods have been developed to control water pollution, but they have all proven to be expensive. Different microbes have been proposed to be efficient and economical alternative in removal of heavy metals from water (Waisberg *et al.*, 2003).

In the present study, the genus *Stentrophomonas* was found to be resistant to Cadmium and Lead amongst numerous metal resistant bacteria isolatedand studied in the Mithi River, which is particularly subjected to huge amount of industrial waste.These species occur ubiquitously in the environment and have been isolated from a wide range of sources including water, sediment, soil, rhizosphere and plant tissues (Ryan *et al.,* 2009). It was further explored for investigating its biosorption capacity in an aqueous medium.

Materials and Methods

Collection and preparation of samples

The grab samples were collected from downstream areas of the Mithi River. 1 ml of the sample was transferred on field into sterilized broth medium. The mother culture was incubated at 37° Celsius for 24 hours.

Isolation, cultivation and identification

The mother culture was diluted using serial dilution was followed upto 10^{-7} times. The nutrient agar (Himedia) plates were used to spread the dilutions using spread plate technique. These plates were incubated at 32°C - 37°C for 24 hours. Pilot studies had already been carried out for the same using Pb and Cd. The flask having high turbidity was visually analysed and thereafter, plated and identified through its biochemical characterizationas per Bergey's Manual of Systematic Bacteriology (Holt *et al*, 2000) and subjected to biosorption experiments. All the experiments were carried out in triplicates.

Biosorption Experiments

Nutrient medium of different concentration of Cd and Pb solution was autoclaved in 250 ml side arm conical flasks containing 100 ml media. Varying pH and temperatureswas prepared for the experiment to understand the optimum pH and temperature for the activity of the organism. For pH adjustment 0.1(N) HCl and 0.1(N) NaOH solutions were used. The media was then inoculated with thelive culture of identified *Stentrophomonas sps*. After 24 hours these flasks were analysed for growth through turbidity using spectrophotometer.

Another set of conical flasks containing the metal spiked medium of upto concentration of 10mg $L^{-1}$and inoculated bacteria were run for optimal pH and temperature at, 120 rpm. After different time intervals sample was collected and centrifuged at 6000 rpm for 10 minutes. Fractions of the supernatant was analyzed for the remaining Lead and Cadmium ions which was detected using ICP-OES.

Percentage of reduction of Lead and Cadmium ions in the solution was analysed using the following equation:

$$\% \text{ Degradation} = C_0 - C_1 / C_0 \times 100$$

Where, C_0=initial metal concentration, mg/L

C_1=final metal concentration, mg/L

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Results and Discussion

Varying pH and Temperature

The hydrogen ion concentrationhas been an important parameter in biosorption studies, it shows the influence on solubility of metal ions as well as on the ionization of the fixing sites (Sassi *et al.,* 2010). Figure 1. shows that *Stentrophomonas sps.* showed better growth at pH 7 during the presence of Lead and Cadmium in the medium. At pH 6 also the growth isconsiderable. But at pH 5 and 8 the growth was not as appreciable as the former.At low pH, the cell wall ligands were closely associated with hydronium ions H_3O^+ and so restricted the biosorption (Liu *et.al*, 2009). Temperature plays a vital role in the adsorption reactions.As seen in figure 2, the temperature at whichgrowth was abundant is 26^0C, although considerable growth is observed at 37^0C. The species show no growth at too low and too high temperatures. According to the adsorption theory, adsorption decreases with increase in temperature and molecules adsorbed earlier on a surface tend to desorb from the surface at elevated temperatures (Horsfall and Spiff, 2005). Furtheron, explanations put forth byAksu and Kutsal, 1991, describe that at high temperature, the thickness of the boundary layer decreases, due to the increased tendency of the metal ion to escape from the biomass surface to the solution phase, which results in a decrease in adsorption as temperature increases.

Biosorption

As seen in figure 3, at the end of 24 hours, a considerable decrease in the metal concentrations was observed. The species showed metal sorption abilities for both Lead and Cadmium when inoculated in two separate flasks containing each metal separately. The sorption capacity of Lead was observed to be better than that of Cadmium (Figure 3). Lead is observed to be present in lower concentrations than that of Cadmium, by the end of 24 hours.

The percentage of removal of lead by *Stentrophomonas sps*. was 49% while that of cadmium was observed to be 45% after 24 hours of experimentation, figure 4.

Conclusion

Mithi River receives a lot of wastewaters hence possess a consortium of microorganisms that are surviving and working positively in the river inspite of the pollution. In our study the *Stenotrophomonas* species were isolated and identified from the downstream areas of Mithi River. The general standards laid down by CPCB for discharge of effluents allows a maximum concentration of 0.1mg/L for Lead and 2.0 mg/L for Cadmium. The biosorption experiments conducted revealed that the species thrived when subjected at an initial concentration of 10mg/L of heavy metals Lead and Cadmium. Further it also showed growth in metal stressed environments and additionally remarkable metal removal capabilities of 49% and 45 % for Lead and Cadmium respectively in 24 hours. Thus suggesting that, the Mithi River inspite of the current polluted statepossesses organisms that are capable of bioremediating the river, precisely heavy metals (Pd and Cd). A reduction in the discharge of effluents into the river and management of waste waters at source, will help the Mithi River thrive and maintain a better aquatic ecosystem.

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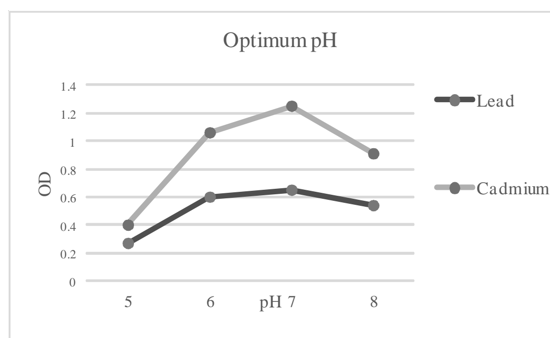

Figure 1. Optimum pH under heavy metal stress

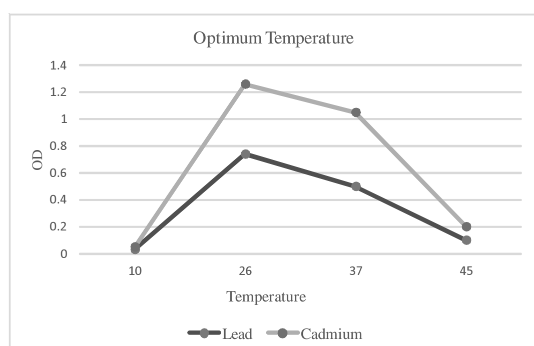

Figure 2. Optimum temperature under heavy metal stress

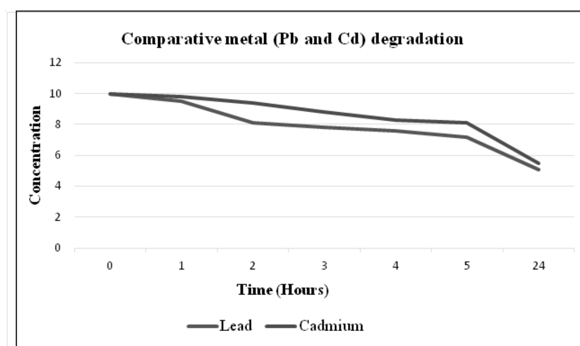

Figure 3. Comparative decrease of metal concentration (Lead and Cadmium) as seen at 24 hours

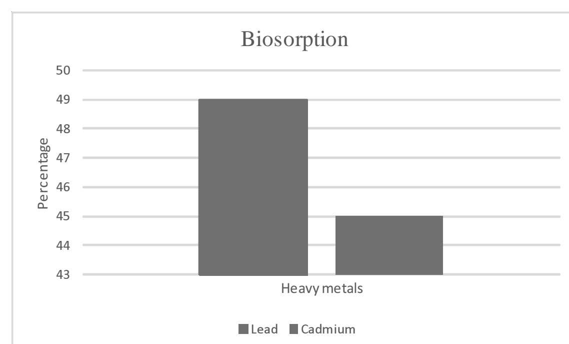

Figure 4. Percent removal of heavy metalsby *Stentrophomonas* sp.

Use of Mobile App Technology for Mangrove Research and Awareness

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Abstract: Godrej & Boyce Mfg Co Ltd is conserving thousands of acres of mangrove forest along western bank of Thane Creek (19003'31.12" N, 72056'31.54" E to 19006'31.43" N to 72056'31.54" E) since the decade of 1940s. In this rich biodiverse forest, we have recorded 16 species of mangrove and mangrove associated plants, which provides ecosystem services like prevention of coastal erosion, habitat for wildlife, livelihoods for local fisherfolk, research and education avenue for academia and regulation of local climate. The mangrove management at Godrej is based on three-pronged strategy of Research, Conservation and Awareness. To promote research and awareness on mangrove ecosystem, Godrej has developed a mobile app to identify 24 true mangrove and mangrove associate species of Maharashtra and to introduce the users to mangrove ecosystem. The app aims to empower research and academic community, Government agencies, NGOs-CBOs and other stakeholders by overcoming current limitations of trained resource persons and mangrove identification books. The app works in offline mode and hence, suitable for remote mangrove forests with zero internet connectivity. It has been downloaded by 1314 users from 33 countries so far. This paper explains process of app development, app structure, its features along with its potential uses to various stakeholders.

Keywords: mangrove ecosystem, mobile app, information technology, mangrove research, mangrove awareness.

Introduction

Mangrove ecosystem is vital lifeline for coastal states of India. It provides several ecosystem services such as prevention of coastline erosion, climate regulation, carbon sequestration, habitat for biodiversity, livelihoods for coastal community, raw material for domestic and industrial consumption, educational and recreational avenue, However, awareness about it is inadequate because of its swampy nature and remote locations. One key problem in understanding mangroves is lack of popular field guide books and inadequate resource persons to identify mangrove species.

To overcome this challenge, Wetland Management Services Department of Godrej & Boyce Mfg Co Ltd has developed a mangrove mobile app to enable interested individuals and organizations for identification of mangrove plant species. The app covers 24 true mangrove and mangrove associate species found in Maharashtra. The users have choice to identify the species based on leaf shape, flower color, taxonomic, common English and regional names of the species. Besides the identification feature, the app offers other interesting information like description of every plant species and its uses. It has a separate section on mangrove ecosystem with information on mangrove distribution, adaptations, biodiversity, threats , conservation measures and role of stakeholders. It helps the users with glossary of technical terms. The app provides optional feedback feature for users to offer suggestions for app improvement and to share relevant information. The app is available on Android, iOS, and Windows platforms.

The app is Asia's first mobile app for mangrove ecosystem and first one developed by a corporate to encourages 'citizens science', the biodiversity conservation strategy recommended world over. It is useful for researchers, teachers and students, Government agencies such as the Forest Department, Kharland or Revenue departments, NGOs and CBOs, and any other interested individuals and organizations. The app developer team has compiled useful recommendations sent by the users for its improvement through the feedback feature connected to mangroves@godrej.com email id. Based on the feedback, Godrej is in process to upscale the app to cover all true mangrove species across India in 10 regional languages for benefit of the local communities.

Materials and Methods

Following methodology was devised and applied in development and dissemination of the mangrove mobile app.

1. Data Compilation:

A) Discussions with subject experts pertaining to mangrove ecosystem, environment education, information technology

C) Finalization of geographical coverage to identify mangrove species to be covered by the app

D) Listing of true mangrove and mangrove associate species to be covered by the mobile app

E) Literature review with focus on available field guides, research reports and papers, books, conference proceedings, websites

F) Compilation of data in MS Excel format with focus on taxonomic classification, type of the plant, taxonomic name, common name, Marathi name,

leaf features, flowering and fruiting season, flower features, fruit features, root features, plant size and uses

G) Validation of the compiled data from taxonomic experts researching on mangrove ecosystem and correction of data, wherever required

2. Images Compilation:

Since the mobile app was intended to be visual covering maximum anatomical features of each species for easy identification of mangrove species by layman, compilation of good images was important step. For this, the photo stock of Wetland Management Department developed over the years was carefully examined to segregate useful images. These images were listed and categorized in an MS Excel data sheet to identify gaps. Various mangrove researchers were contacted to obtain images based on the gaps identification exercise. The last set of images not available with Godrej photostock and contacted subject experts were downloaded from Wikimedia Commons, an online media file repository making available public domain and freely-licensed educational media content (images, sound and video clips) to everyone, in their own language. All the compiled images were carefully segregated and saved into folders and sub-folders to avoid confusion during technical development of the app.

3. App Framework Development

The key objective of the proposed mobile app was to enable wide range of stakeholders to identify mangrove species and to introduce them to mangrove ecosystem. For this, making taxonomy easy and visual was important. Based on the field experience and interactions with stakeholders, three routes of species identification were finalized - leaf shape, flower color and name of the plant species. These routes were decided based on following rational

A) Each mangrove species has leaves throughout the year and can be clearly categorized into shapes such as ovate, obovate, elliptic and lanceolate for plant identification

B) Each species has distinct flowers that could be classified based on colors such as yellow, green, pink, blue, white and other

C) Each mangrove species has distinct scientific, common and regional name based on its anatomic features helpful to identify the species.

Further, a separate section to introduce the app users to mangrove ecosystem was developed. A mobile app has text limit in order to ensure maximum attention span in minimum space. This was considered during the section development.

All features of the app such as Home, About Godrej Mangroves, Mangrove Ecosystem, Identify Mangroves, Feedback, Contact Us, Glossary and Acknowledgement were listed and drafted. Detailed discussions with Godrej Infotech Limited, the company responsible for technical development of the app were held during this exercise.

4. Technical Development

Godrej Infotech Limited, a sister concern of Godrej & Boyce Mfg Co Ltd offered its technical competency in software and design development of the mobile app. Detailed discussions were conducted to discuss technology, timeline and team responsibilities. The app was developed for three mobile platforms, i.e., Android, iOS and Widows Phone. It was made available for download on respective Platform Stores, i.e., for Android, Google Play, for iOS, iTunes Store and for Windows Phone, Windows Store.

5. Pilot Testing

The app was tested with select stakeholder representatives such as researchers, teachers, students, field workers and nature enthusiasts to invite their feedback on correctness of the information and images, and user friendliness of the app. These suggestions were carefully noted and discussed by app development teams. Select suggestions were incorporated for finalization of the mobile app.

6. App Dissemination

The last, most crucial and ongoing phase in mobile app development is its dissemination. It was important to highlight the app among stakeholders for its widespread use. Towards this, an app dissemination strategy was developed. As a part of the strategy, the app was launched by the honorable Chief Minister in presence of Principal Secretary and Chief Conservator of Forests (Mangrove Cell) of Maharashtra State. This ensured adequate attention in Government departments and media. The app launch was covered by major English and Marathi newspapers such as Maharashtra Times, Loksatta, Tarun Bharat, Saamna, Lokmat, Times of India, The Indian Express, Hindustan Times, The Asian Age, Mid-Day and DNA. This helped to disseminate the app among citizens. The app announcement was circulated among relevant state, national and international level facebook groups

focusing on mangrove ecosystem , coastal wetlands, environment education, Corporate Social Responsibility, NGOs and CBOs working for nature conservation, nature photography, environmental policies, rules and regulation, citizen forums etc. Two interviews on FM radio channels facilitated mass dissemination. Further, the participants of nature trails conducted in Godrej mangroves on regular basis are encouraged to download and use the app. The app dissemination is continual activity and is guided by strategy developed by the Wetland Management Services Department.

Results and Discussion

The Godrej mangrove mobile app focusing on 24 true mangrove and mangrove associate species was launched on 12 July 2017. It covers following species: *Acanthus ilicifolius* L, *Acrostichum aureum* L, *Aegiceras corniculatum* (L) Blanco, *Avicennia marina*, *Avicennia officinalis*, *Bruguiera cylindrica* (L) Blume, *Bruguiera gymnorrhiza* (L.) Lam, *Ceriops tagal* (perr) C B Rob, *Clerodedendrum inerme* Gaertn, *Cynometra iripa* Kostel, *Derris trifoliata* Lour, *Dolichendrone spathacea* (L) K Schum, *Excoecaria agallocha* L, *Heritiera littoris*, *Kandelia candel* Druce, *Lumnitzera racemosa* Willd., *Rhizophora apiculata*, *Rhizophora mucronata*, *Salvadora persica* L, *Sesuvium portulacastrum* (L), *Sonneratia alba* Sm, *Sonneratia caseolaris* (L) Engl, *Sonneratia apetala* Buch Ham, *Xylocarpus granulatum* K D Koenig.

The App has been downloaded by 1314 users from 33 countries namely India, UAE, Malaysia, Australia, China, Indonesia, Singapore, Philipines, Thailand, Japan, USA, UK, France, Brazil, Costa Rica, Taiwan, Oman, Pakistan, Brazil, Fiji, Iraq, Kenya, Madagascar, Mauritius, Maldives, Netherlands, Nepal, Papua New Guinea, Saudi Arabia, Albania, Belgium, Italy and S Africa.

In the limited range of available mobile apps on mangrove ecosystem, Godrej app is the only India specific app and available for free of cost. Most other apps are on paid basis limiting their outreach among stakeholders. The Godrej app, once downloaded, does not require internet connection. Most mangrove ecosystems are remote in location and lack internet connectivity. This unique feature of the app makes it most suitable for field use.

Conclusion

The Godrej mangrove mobile app was first attempt in Asia for effective use of information and smart mobile technology for research and awareness of mangrove ecosystem. The number of downloads from across the world and feedback from users supports hypothesis of effectiveness of the mobile app. Based on the prominent feedback of app users from across India, Godrej plans to upscale the app to cover all true mangrove species of India in regional languages of all coastal states. The national app will be first of its kind of IT tool for participatory conservation of mangroves in the world.

Acknowledgment

Authors are thankful to Dr Mahesh Shindikar, College of Engineering Pune, for his subject expertise and images. We thank Godrej family and Mr Anup Mathew, Business Head, Godrej Construction for continual organizational support during the app development. We thank the photographer who have contributed their images to the app and their names have been listed in the Acknowledgement section of the app.

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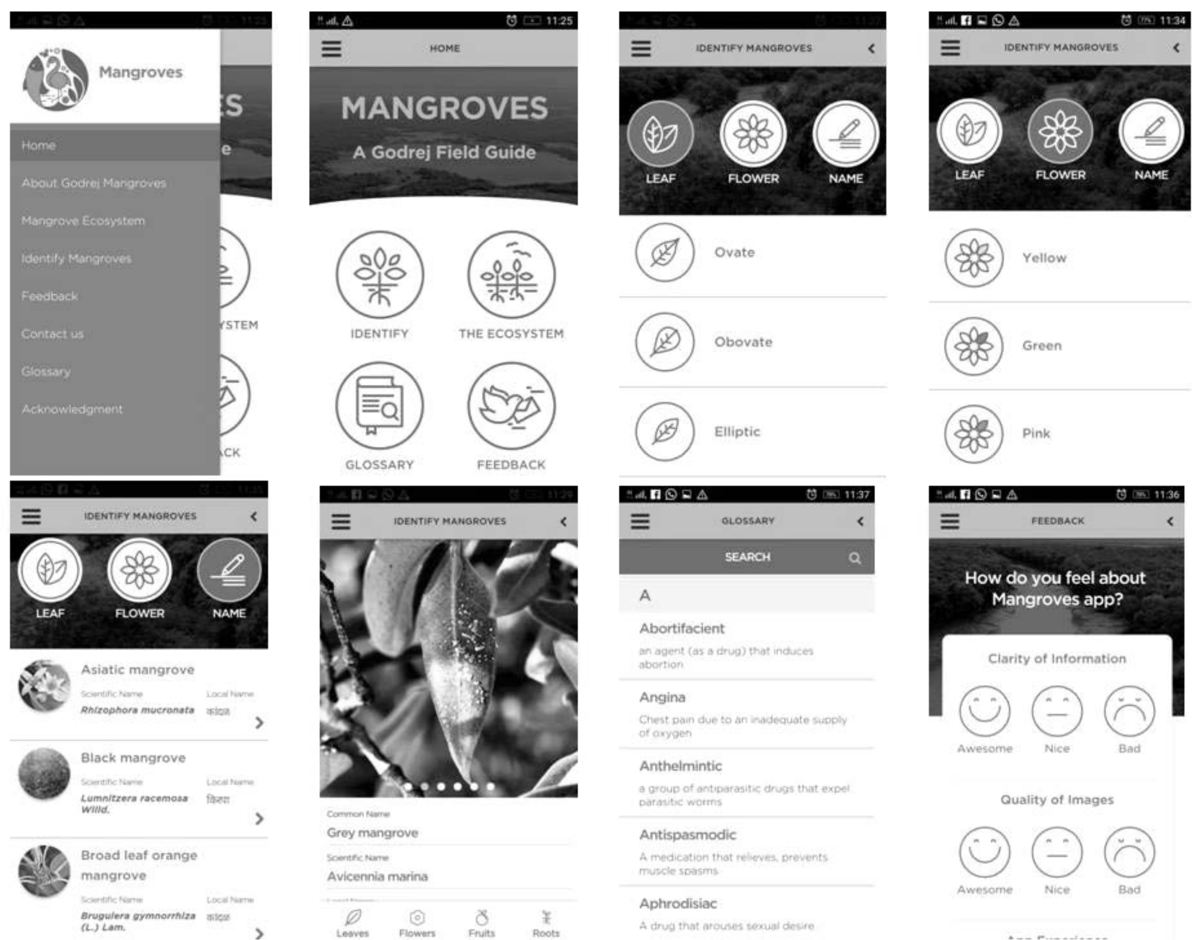
Plate 1: Key features of Godrej Mangrove Mobile App



Role of Godrej Mangroves in Climate Change Mitigation Through Carbon Sequestration

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Abstract: Godrej& Boyce Mfg Co Ltd is conserving thousands of acres of mangrove forest along western bank of Thane Creek ($19^0$03'31.12" N, $72^0$56'31.54" E to $19^0$06'31.43" N to $72^0$56'31.54" E) since the decade of 1940s. In this rich biodiverse forest, we have recorded 16 species of mangrove and mangrove associated plants, which provides ecosystem services like prevention of coastal erosion, habitat for wildlife, livelihoods for local fisherfolk, research and education avenue for academia and regulation of local climate. Annually this forest sequesters 6 lakh tons of CO_2 and its annual increment value is around 50000 tons of equivalent CO_2. This carbon sequestration service is very valuable considering high emission of CO_2in the Mumbai Metropolitan Region and its impact on climate change. This paper explains material used and methodology we carried out to measure sequestered carbon, data of past 3 years, importance of this project to the society and need to sensitize stakeholders for the mangrove conservation.

Keywords: mangrove ecosystem, Thane creek, ecosystem services, carbon sequestration, climate change

Introduction

The term mangrove is used in broad sense to refer to the highly adapted salt tolerant angiosperm plants occurring in tropical and subtropical marine coastal environments and along the banks of estuaries of creeks between lat. 30^0 N and 30^0 S. In India total 65 mangrove species belonging to 31 families have been recorded. Maharashtra has about 4.02% (186 km^2) of the total mangrove areas (4,628 km^2) in India (Forest survey of India, 2013).

Mangroves form highly reproductive ecosystem, supporting a wide variety of organisms including commercial fishery. They not only provide wood for different purposes but also give many important products such as honey, medicines etc. According to Subramanian and Sambasivam (1988), mangroves can be remedy for ozone depletion. It has been observed that mangroves protect the shore from storms, hurricanes, tsunamis etc. Mangroves supply food to marine communities via detritus food chain starting from falling mangrove leaves. The mangrove ecosystems provide a habitat for commercially important marine organisms like molluscs, prawns, crabs, fishes and act as feeding grounds for them and their juveniles. Studies have illustrated decline in fishery on loss of mangroves. The mangrove swamps are known to act as natural sewage treatment plant, when conditions are favorable and the sewage is in reasonable quantity. These days, there is growing awareness regarding importance of mangroves and attempts are being made to conserve mangrove ecosystems. However, mangroves suffer damage through various anthropogenic activities, animal grazing, by molluscan, insect pests etc.

Thane creek (Long. 72^0.55" E to 73^0.00"E and Lat. 19°.00 N to 19^0. 15"N) is important coastal ecosystem near Mumbai of which Godrej & Boyce Mfg Co Ltd is conserving thousands of acres of mangrove forest along western bank of Thane Creek ($19^0$03'31.12" N, $72^0$56'31.54" E to $19^0$06'31.43" N to $72^0$56'31.54" E) since the decade of 1940s. Thane creek extends approximately 26 Km. inside from the Arabian Sea and meets Ulhas river estuary by a minor connection near Thane city. As the riverine flow is less, the creek is tide dominated and experiences high salinity throughout the year except monsoon. However, due to growing urbanization in Thane- Mumbai region the creek receives significant quantity of fresh water in form of sewage. Thane creek's mangrove ecosystem have luxuriant mangroves along their banks. In past few decades, Thane creek ecosystem have been extensively studied for various aspects such as hydrology, sedimentology, mangroves, benthos, plankton, fishery and carbon footprints etc. Due to extensive urbanization and industrialization around Thane creek, this ecosystem is subjected to various damaging factors. Solid waste dumping, reclamation, pollution, cutting for the fuel are the main threats to mangroves in this region. To conserve mangrove along Thane creek, which is in-between Kanjur and Ghatkopar, Godrej Mangrove Center is taking enormous efforts by implementing various activities focused on three pillars.

1) Education and awareness
2) Research and development and
3) Security and infrastructure

Mumbai is India's one of the most polluted city as it experiences huge urbanization and thus obviously at the peak of air pollution. Mangroves along Mumbai acts as green lungs of Mumbai and absorbs huge amount of CO_2 release O_2 and obviously stores huge amount of Carbon in their body stock in the form of cellulose. In Godrej

mangroves we are measuring this fixed CO_2 in the form of C to know how mangroves play their important role in nature to reduce GHG and to reduce pollution in this metro city.

Materials and Methodology

The purpose of this study is to estimate the total carbon stock in the mangrove forest at Godrej, Vikhroli. Specifically, it aims to:

1) Determine the biomass and carbon density in the aboveground pools of the mangrove stands.

2) Assess the amount of carbon sequestered in the soil.

The area selected for the study consists of mangrove forest located at Vikhroli, Mumbai, Maharashtra and are under the control of Godrej & Boyce Mfg. Co. Ltd and Soonabai Pirojsha Godrej Foundation.

Photo: 1- Godrej Mangrove area along Thane creek-Locations of sample collection

As far as the land cover of the study area is concerned, it includes a mixed type of classes ranging from dense vegetation, moderate vegetation, grassland area, mud flats, water body and open area. To study the land use & land cover class of study area, Remote Sensing technique has been employed. The following are the five different land use classes obtained from the analysis:

Dense vegetation: This includes thick vegetation cover with different types of trees or plants covering land in an undefined manner. Many trees grow over only a small part of land with a dense canopy cover. The dense vegetation cover in the study area is dominated by the thick canopy cover of mangrove trees.

Moderate vegetation: This is a land use categorized under vegetation cover which represented by a land category with open type of vegetation.

This category in the study area includes mangrove trees having an open canopy cover.

Grassland: These are the areas of natural grass along with other vegetation, predominantly grass-like plants and non-grass-like herbs.

Mud-flats: These are categorized under coastal wetlands that are formed when mud is deposited by tides or rivers. They are found in sheltered areas such as bays, bayous, lagoons, and estuaries. Mudflats may be viewed geologically as exposed layers of bay mud, resulting from deposition of estuarine silts, clays and marine animal detritus.

Open area: The open area within the study area comprises of open land areas with no vegetation cover.

For deriving the number of permanent sample plots required to estimate the carbon stock present within the project area, it was necessary to collect a standard deviation of carbon stock of each stratum. This was done through a pilot sampling carried out in each stratum. Two sample plots in each stratum were visited during the pilot sampling.

Numbering the trees within the plot: Trees were numbered from the corner of the sample plot. The tree nearest to the first corner was numbered as 1st tree. Numbering was then proceeded along the row towards the other corner of the sample plot. At the end of this, next row was covered and then starting corner of the plot was covered to end tree marking. Numbering of the trees was continued in zigzag fashion (refer Fig. 2) If there was a gap or dead stock of atree was noticed, its details Carbon sequestration

was noted in the field note. Trees in research plots were numberedwerewith weather resistant, white/yellow paint.

Instruments used during this survey:

1) A calibrated measuring tape was used to measure the GBH.

2) Graduated bamboo sticks for tree height measurement.

3) Boundary demarcation of the permanent sample plot is by GARMIN eTrex VISTA Cx

4) Paint to tag trees

5) Writing material to enter recorded data

Results and Discussion

The carbon pools that can be considered for carbon stock estimation are defined as follows:

Above ground biomass (AGB): This pool includes all living biomass above the soil including stem, stump, branches, bark, seeds, and foliage. Where forest under storey is a relatively small component of the above-ground biomass carbon pool, this may be ignored so long as the methodology is used consistently throughout the inventory time series.

Below ground biomass (BGB): This pool includes all living biomass of live roots. Fine roots under, say, 2 mm diameter may be excluded as they often cannot be distinguished from soil organic matter or litter.

Dead wood (DW): All non-living woody biomass not contained in the litter, either standing, lying on the ground, or in the soil. This includes wood lying on the surface, dead roots, and stumps (usually defined as having a diameter of at least 10 cm).

Litter: All non-living biomass with a smaller diameter than that used for dead wood (say, 10 cm), lying dead, in various states of decomposition above the mineral or organic soil. This includes the litter, fumic, and humic layers. Live fine roots (of less than the diameter limit for below-ground biomass, say 2 mm) may be included here.

Soil organic carbon (SOC): Includes organic carbon in mineral soils to a specified depth chosen by the country and applied consistently through the time series. Live fine roots (of less than the chosen diameter limit for below-ground biomass) to 30 cm depth may be included here.

The pools selected for the present study were aboveground live trees, dead trees, drowned wood, belowground plant carbon, and soil carbon. These five pools likely comprise >95% of the true ecosystem carbon stock of mangroves.

The carbon stock estimation of trees i.e. for the above ground biomass & below ground biomass carbon pool wasdone by using the approved A/R CDM methodological tool: *"Estimation of carbon stocks and change in carbon stocks of trees and shrubs in A/R CDM project activities"* version 03.0.0.

As described in the tool, the change in carbon in trees wasestimated by applying one of the following methods, each applicable under its specific conditions:

(a) Stock change method: Under stock change method carbon stock in trees within the project boundary is estimated at successive points of time. Change in carbon stock in trees between two successive points of time is calculated as the difference between the two estimated stocks.

(b) Increment method: The increment method is used for the estimation of tree biomass in project when the same sample plots are measured over successive verifications. In the increment method, individual trees shall be given a unique identifier. The change in biomass of individual trees is then monitored and estimated over time.

If a tree measured at the time of the earlier verification cannot be found at the time of the later verification (i.e. the tree is missing or is dead), then its biomass on the later verification is recorded as zero.

If a new tree is found at the time of a later verification, then its biomass on the earlier verification is recorded as the biomass with the minimum biomass based on the sampling design. The new tree is found at the time of the later verification, this tree should be given a unique identifier.

(c) Baseline default method: This method is applicable only for estimation of carbon stock in trees in the baseline when:

(a) Stock change cannot be applied for lack of data; or

(b) The mean tree crown cover in the baseline is less than 20 per cent of the threshold crown cover.

From the above three methods, stock change method is adopted for the estimation of carbon stock in the present study.

Total carbon stock:

The total carbon stock is estimated by adding all the five components of the carbon pool.

The equation for total carbon stock for a given project area is as follows:

$$C_{PROJECT,t}=C_{TREE.t}+C_{DW,t}+C_{LI,t}+C_{SOC,t}$$

Where,

$C_{PROJECT,t}$: Change in the carbon stocks in project, occurring in the selected carbon pools, in *t*; t CO2-e

$C_{TREE.t}$: Change in carbon stock in tree biomass in project in year *t*, as estimated in the "Estimation of carbon stocks and change in carbon stocks of trees and shrubs in CDM project activities"; t CO2-e

$C_{DW,t}$: Change in carbon stock in dead wood in project in year *t*, as estimated in the "Estimation of carbon stocks and change in carbon stocks in dead wood and litter A/R CDM project activities"; t CO2-e

$C_{LI,t}$: Change in carbon stock in litter in project in year *t*, as estimated in the tool "Estimation of carbon stocks and change in carbon stocks in dead wood and litter in A/R project activities"; t CO2-e

$C_{SOC,t}$: Change in carbon stock in SOC in project, in year t; t CO2-e

t: *1, 2, 3, …* years counted from the start of the project activity

Substituting values in the above equation the Carbon stock in project area ($C_{PROJECT,t}$) is given in the table below:

Conclusion

This study is first of its kind in the state of Maharashtra. It highlighted remarkable contribution by a corporate house in mangrove conservation. The ecosystem services of carbon sequestration delivered by the Godrej mangroves benefit entire Mumbai Metropolitan Region which needs green lungs to absorb the Greenhouse Gases (GHGs) emitted by countless anthropogenic activates. Extrapolating the carbon sequestration services of Godrej mangroves to entire mangrove ecosystem of the Mumbai Metropolitan Region, this study makes clear case for wetland conservation for climate regulation.

Acknowledgment

Thanks to CEED-India for their valuable supportduringthis project. We thank Godrej family and Mr Anup Mathew, Business Head, Godrej Construction for continual organizational support and feedback and suggestions during this research. We thank all our departmental colleagues and on field staff for support during this project.

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Table1: Details of study area

Sr. No.	Description	Area (acres)
1	SPGF	74%
2	G&B	26%

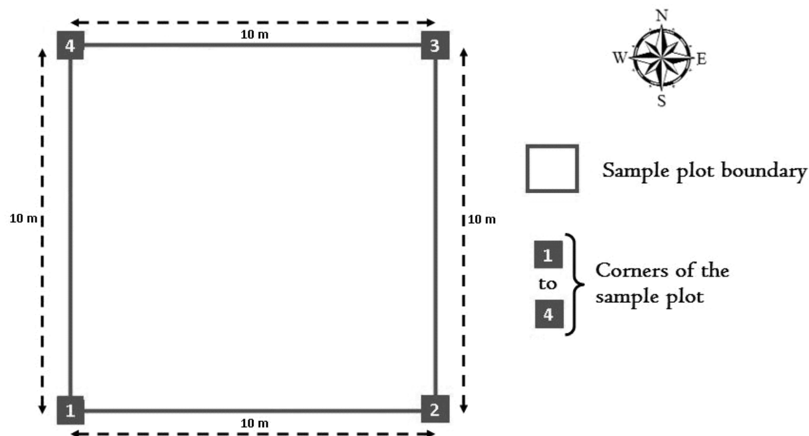

Figure 1: Layout of sample plot

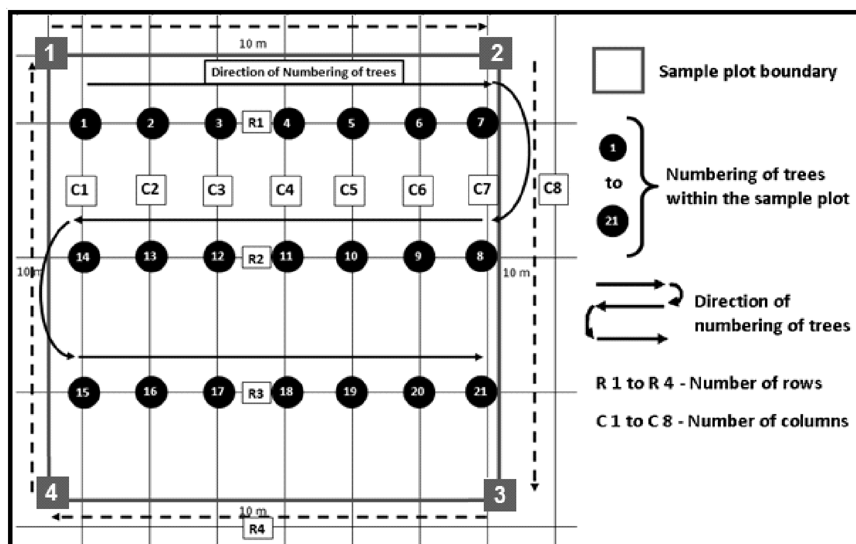

Figure 2: Numbering of trees within the sample plot

Table 2: Number of sample plots to be laid in each stratum

Sr. No.	Stratum	No. of plots
1	Dense vegetation	3
2	Moderate vegetation	7
3	Grasslands	4
4	Open area	2
	Total	**16**

Table 3: Carbon stock in project area

Project area	CTREE,ttCO2e	CDW,ttCO2e	CLI,ttCO2e	CSOC,ttCO2e	CPROJECT,ttCO2e
G&B	66284	8423	46	97221	171976
SPGF	141746	3938	21	279427	425134
Total	**208032**	**12362**	**67**	**376649**	**597112**

Theme - V
Green Chemistry

Expeditious Synthesis, Properties & Applications of Deep Eutectic Solvents

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Abstract: The research paper reports the innovative synthesis of deep eutectic solvents by the reaction of glucose, urea\ thiourea and inorganic salts. DES are providing to be increasingly promising as variable media for not only potentially 'green synthesis' but also for the better applications in terms of producing bulk product as well as for enhancing the rate of the reaction. The utilization and synthesis of such deep eutectic solvents should facilitate further development of green chemistry and green chemical synthesis.

Keyword: Deep eutectic solvents, greensynthesis, Diels-alder reaction, glucose, inorganic salts, urea.

Introduction

A large number of organic solvents are been used in the chemical synthesis at large scale and small scale as well. However these organic solvents have a high tendency to escape into the environment either through evaporation or by leakage and that's why the reduction of use of organic solvents is one goal in current efforts towards more environment friendly green chemical process. One of the options is to use water as a solvent but its use is limited because most organic compounds do not dissolve in pure water. Hence it's an initiative to develop the deep eutectic solvents that can be used as green solvent rather than using toxic solvents that has hazardous effect on environment and is much better in case of forming bulk product and reaction time is comparatively less.

Materials and Methods

Sugar + Urea/Thiourea + inorganic salts! deep eutectic solvent

Total eight deep eutectic solvents were prepared in the laboratory by consideration of mixture of compounds like urea\thiourea, glucose and inorganic salts. Three of them were taken in the ratio1:1:1 respectively i.e., 0.5g each. The inorganic salts that were used were Nicl2, Cacl2, Fecl3, and NaCl. In addition the mixtures were heated for 1 hour at water bath. The solvents that were obtained at the end of preparation were present in semisolid form. The following changes were observed:

Results and Discussion

The solvents that were prepared were used for the preparation of the Schiff base respectively and hence all the eight solvents were utilized and were compared with the Schiff base prepared in presence of chloroform. A drastic change was observed after the comparison between the two.

General Reaction: Benzaldehyde +P-Toluidine →Schiffs Base.

It took around 35 minutes to prepare Schiff base in presence of chloroform where as in presence of deep eutectic solvents, the reaction got over at 22min; the melting point was 122^0 C in presence of chloroform where as it was 105^0 C in presence of deep eutectic solvents. The yield of product was also higher for deep eutectic than chloroform. Hence DES has much better application than chloroform without any kind of ill-effect on the environment.

Conclusion

We have reported the use of low-melting mixtures of sugar, urea\thiourea, inorganic salts as a reaction media for the production of Schiff base. This nontoxic reaction media was successfully qualified as green solvent. Their application as reaction media for other organic transformations and as a substitute to ionic liquids may be envisaged.

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Table 1: Changes in the deep eutectic solvents

Time	Observations
After 10 minutes	Reaction mixture starts melting
After 30 minutes	Colour of reaction mixture changes
After 1 hour	The reaction mixture changes into semisolid form

Table 2: Comparison between deep eutectic solvent and chloroform

Name of solvent	Amount required	Melting point of product obtained	Reaction time	Yield of product
Chloroform	8.0 ml	122^0 C	35 minutes	3.8 gm
Deep eutectic solvent	1.6gm	105^0 C	26 minutes	4.7 gm

SECTION III
REPORTS

First Pre-Conference Workshop
of
Multi disciplinary International Conference on Green Earth: A Panoramic View

On 10th July 2017, Monday, Department of Botany of VPM's B.N.Bandodkar College of Science, organized **First Pre-Conference Workshop** of Multidisciplinary International Conference on Green Earth: A Panoramic View. This conference is organized in collaboration with Birbal Sahani Institute of Palaeosciences, Lucknow and BNHS Bombay Natural History Society, Mumbai and it is scheduled on 12th – 13th January 2018.

VPM's B.N.Bandodkar College of Science is one of the prospering institutes of Vidya Prasarak Mandal. The institute is committed to impart quality education in science and develop rational thinking among students to mould them in socially responsible citizens. The Pre-Conference Workshop is also contributing to the same purpose. It is intended to ignite insightful thinking about Mother Nature in a novel way. There were total 36 teachers (32 In-house & 04 Outsiders) and 275 students (269 In-house & 06 Outsider) participated in this workshop.

The workshop began with welcome address given by Dr. Moitreyee Saha (Head, Dept. of Botany & Organizing Secretary). She briefly explained the conceptualization of International Conference. Later she elaborated on various themes of the International Conference which are revolving around the idea of Green Earth. The themes were agricultural sciences involving agri-classical, agri-trends and biophysics. Another theme was biosciences including golden era, temporary era, enviro-management, green chemistry and humanities.

As a part of International Conference, a Logo Competition was conducted by Dept. of Botany in month of June for school and college students. The creative logos were judged by an External Judge Dr. Manoj Kumavat (M.D., Alternative Medicines & Member of Bombay Art Society). He has mastery is in oil painting and water colours. He had exhibited many paintings in various reputed art galleries. He selected the best logo on the basis of different parameters such as, originality, colour scheme and overall presentation. The winner of this logo competition was declared by Principal and Convener Dr. M. K. Pejaver. She briefly explained characteristic features of winner logo. **Ms. Monal Chitnis** (7th Std, Shreerang Vidyalaya, Thane) won the prize. The inaugurated winner logo will be printed on brochures, proceeding and certificates of the preconference workshop and International Conference.

After this inauguration, guest speakers of workshop Dr. Pushpa Mishra (Dept of Biophysics, University of Mumbai) and Prof. Dr. Krishna Iyer (Dept of Pharmaceutical Chemistry, Bombay college of Pharmacy Mumbai) were felicitated by hands of Principal and Convener Dr. M. K. Pejaver.

The technical session began with first invited talk of Principal Dr. Pejaver who delivered lecture on 'Australia from bird eye view'. She started the talk with general information about Australia including its geographical distribution with the help of attractive power point presentation. She gave an insight to the wide variety of natural landscapes of various cities of Australia visited by her. It included public parks, lakes, avenues, private gardens, etc. She elaborated on native flora and fauna of Australia. In fauna, she especially described variety of colourful birds with their unique characters. Some of them were white backed Magpie bird, Willie Wagtail, Wily Goose, Rainbow lorikeet, chestnut Teal, Starling, etc. Throughout the talk, she emphasized on the nature loving approach of Australian people which helps in safe-guard the biodiversity of the nation. She also highlighted the eco-friendly methods followed by all citizens, such as composting of kitchen waste, avoiding plastic use, maintaining private garden, etc. She showed wonderful scenic beauty of Australian beaches, some of which act as rich sources of iron. It was an excellent piece of information which may contribute to change mindset of all towards respecting Mother Nature for its beauty and kindness.

Second invited talk was delivered by Dr. Pushpa Mishra on the topic "Biophysics a route to serve mankind." She gave an insight about NMR technology. She elaborated about Malarial parasite *Plasmodium*, about its life cycle and how to prevent the Malaria by blocking the protein tetramers at early stage of parasite life cycle. She explains the use of NMR tool in understanding protein structure.

Third speaker was Dr. Krishna Iyer, Professor of Pharmaceutical Chemistry (Mumbai University). His topic of presentation was Pytoremediation especially designed for TYBSc students but after analyzing his audience he realized school students were also part of the crowd. He went for a simple but very interesting topic i.e. DNA discovery. His way of presentation was very innovative; he spoke about discovery of DNA sequencing and shared the technique of sequencing or decoding in very layman's term.

With his encyclopedic knowledge, memorable stories, quick wit, and meaningful insights, his speech focused on the broad implications of the latest trends in DNA decoding. He gelled with students and compelled the school students to ask questions. Indeed the school students too surprised all with interesting questions like can DNA be obtain from dead person and inserted into living human being, can disease resistant or immune child can be made in the future and so on. He also appreciated their questions by gifting them presents in the form of books and shawl. His lecture not only made the school students happy but was also appreciated by all the teachers present for the session.

Vote of thanks was proposed by Ms. Priyanka Varma followed by National anthem by T. Y. B.Sc. students.

Second Pre-Conference Workshop
of
Multi disciplinary International Conference on Green Earth: A Panoramic View

Second Pre-Conference Workshop of Multidisciplinary International Conference on Green Earth: A Panoramic View was organized by Department of Botany, VPM's B.N.Bandodkar College of Science on 11th December, 2017, Monday. This conference is organized in collaboration with Birbal Sahani Institute of Palaeosciences, Lucknow and BNHS Bombay Natural History Society, Mumbai and it is scheduled on 12th – 13th January 2018.

There were total 17 teachers (15 In-house & 02Outsiders) and 173 students (170 In-house & 03 Outsider) participant in this workshop.

The workshop began with welcome address given by Dr. V.M.Jamdhade (Joint-Organizing Secretary). He briefly explained an idea about overwhelming response received by authors in form of research papers. Approximately more than 60 research papers received by organizing committee. He also mentioned about eminent guest speaker invited for the conference from national and international research institute.

The technical session began with first invited talk by Vrisha Mitra Shree. Prakash Kale(Writer). He had developed Nakshatra botanical garden at Palghar and similar botanical garden in some colleges and cities *viz*. Sundaram, Virar, Saphale, Palghar, Koregaon. He has also published many books on Nature. His topic for the lecture was "Paryavaran, Manav ani Vanaspati yanche parasparashi aslele sambandh (Interaction between Environment, Human and Plants)". He started the talk with story of how he developed a keen interest and love towards the plants. He shared about his experiences of exploring various forests to find new species of plants and their use by the local people around the forest. He also explained that every plant is medicinally important and one should avail this property of plant. . He elaborated on importance of Forest and native flora of forest areas which he visited. Throughout the talk, he emphasized on the lack of knowledge of medicinal and commercial plants in the local and rural people around the forest and the need to safe-guard the biodiversity of the areas. It was an excellent piece of information which may contribute to change mindset of all towards respecting Mother Nature for its beauty and kindness.

Second invited talk was delivered by Mr. Suhas Naik (Programme Co-ordinator, Nehru Science Centre) on the topic "Introduction to Our Universe". Before the start of his lecture he interacted with students and asked what the questions in their mind were. Through this interactive session he made the environment lively and students enjoyed it. He gave an insight about the Planets and galaxies of Universe. He elaborated about the birth of Solar system, how the sun and planet are, their appearances, history and basic information. He explained about satellites, Meteroic shower, Comets, galaxies, Nebula, Craters in his lecture.

Third speaker was Dr. Kiran Pariya (Assistant Professor,Department of Zoology, B.N.Bandodkar College of Science). His topic of presentation was Open gene-open Mind. He spoke about Epigenesis. He discussed about active and inactive genes, Neuro-linguistic programming, Meta Model, Model M.O.N.E.Y consciousness, brain waves. He also explained benefits of meditation and alteration of genes and DNA through thoughts. The session ended with one of the meditation technique called 7 steps guide of meditation.

Students enjoyed all the lectures. They found these lectures very interesting as well as informative.

We are thankful to our Sponsorer and Well wishers...

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Glimpses of Pre-Conference Workshop

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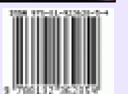

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