IN VITRO CALLOGENESIS AND PHYTOCHEMICAL SCREENING OF LEAF AND CALLUS OF *LAWSONIA INERMIS* L.: A MEDICINAL PLANT

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ABSTRACT: Henna or Mehndi (*Lawsonia inermis* L.) powder is used for colouring hairs, nails and beard. Beside the colouring pigment henna plant also produces many secondary metabolites which show medicinal properties. The objective of the study was to standardize the protocol for *in vitro* callus induction in *Lawsonia inermis* L. using leaf explants. Callus induction was observed maximum in MS medium supplemented with 2, 4-D in combination with coconut milk (CM). The preliminary phytochemical analysis was performed showed the presence of alkaloids, anthraquinone, carbohydrates, glycosides, phenols, flavonoids, tannins, saponins and essential oils in both leaf and callus powders of *Lawsonia inermis* L. which were then confirmed with the help of TLC.

Keywords: *Lawsonia inermis* L., Callus, Phytochemicals, Auxins, TLC

INTRODUCTION

Lawsonia inermis L. is a native of North Africa and South-West Asia. Henna is commonly cultivated as ornamental and dye plant in India, the Middle East and along the African coast of the Mediterranean Sea (Zafar *et al.*, 2006). Mehndi powder is extensively used as a hair dye and also used traditionally to decorate hands and feet.

Plant contains a many pharmacologically active compounds which are used in traditional system of medicine since Vedic era. Synthetic drugs show many adverse effects therefore medicinal plants are explored for the synthesis of commercial drugs which are of herbal origin (Mansa *et al.*, 2017). Due to increased demand for henna there has been indiscriminate uprooting of plants and mass collection of henna leaves by various small scale and large scale industries which has made the plant less conspicuous. The present work includes i) Callogenesis (*in vitro* callus induction) to study the presence of phytochemcials; ii) Comparative phytochemical analysis (leaf and callus) to study the presence or absence of phytochemicals in leaf and callus of *Lawsonia inerims* L.; iii) The presence of phytochemicals in leaf and callus of *Lawsonia inerims* L. were confirmed with the help of TLC.

MATERIALS AND METHODS

Callus Induction

- **Collection of plant material:** Healthy leaves with petiole of henna were collected from Kalyan (Maharashtra, India).
- **Explants preparation:** The disease free and healthy leaf explants were selected for *in vitro* callus induction. The explants were treated with Tween 20 and 1% Bavistin solution to remove dust particles from the surface of leaves and also it minimizes the fungal infection. Then it was treated with 1% $HgCl_2$ with occasional shaking and then washed with the st. double distilled water. Pretreated explants were transferred to 70% alcohol for 30 seconds and then transfer it to blotting papers to remove the moisture.
- **Media selection:** Pre-treated leaf explants were inoculated on basal MS medium (Murashige and Skoog, 1962), MS medium fortified with 2, 4-D, NAA, BAP, Kinetin, Zeatine (0.1 – 1.0 mg/l) used singly and various combinations of 2, 4-D and coconut milk (CM); Adenine hemisulphate (AS); Polyvinyl pyrrolidone (PVP), etc. The cultures were maintained at 25 ± 2ºC under 16 hr photoperiod obtained from cool white fluorescent lamps. 10 explants were inoculated in each combination of PGR's with one explant per test tube. Each experiment was carried out in triplicates. The explants were observed at regular intervals for callus induction, development and also for its color and texture. The calli were subcultured at 4 weeks of intervals.

Preliminary Phytochemical Screening: Qualitative chemical examination of the dried leaf and callus powder of *Lawsonia inermis* L. revealed the presence or absence of various plant constituents in different chemical extracts. The observations were recorded in + (present) or – (absent). The tests were performed according to Khandelwal (1998) and Kokate (2007).

Preparation of crude extract: 1gm of *Lawsonia inermis* L. leaf and callus powder were extracted separately in 10 ml of five different solvents (toluene, ethanol, ethyl acetate, methanol and water) overnight. The mixture of plant material and solvent was filtered through Whatman filter paper No. 1. The filtrate was then evaporated on boiling water bath until dry and then the extracts were stored in refrigerator for further studies.

Phytochemical analysis by TLC:

- **Preparation of Extracts for TLC:** The precise method of extraction depends on the plant material being extracted and on class of compound that is being isolated. Extract preparation for various phytochemicals was carried according to Wagner and Bladt (1996).
- **Stationary phase:** Pre – coated silica gel G60 F254 TLC plates (Merck) were used as stationary phase.
- **Solvent systems**: The solvent system for separation of active constituents varies according to the phytoconstituents to be separated (Wagner and Bladt, 1996). The solvent systems for the respective constituents were prepared. The chromatogram was developed in pre-saturated twin trough chamber (10 cm x 10 cm) for 25 minutes.

RESULTS AND DISCUSSIONS

Callus Induction: Leaf explants with petiole when inoculated on basal MS medium and MS medium fortified with NAA, BAP, Kinetin, Zeatine used singly showed enlargement or did not show any response. Leaf explants with petiole inoculated on MS medium with 2, 4-D (0.1 – 1.0 mg/l) gave rise to non-embryogenic white, green, brown or black coloured callus. The callus produced was less to moderate amount. Leaf explants with petiole inoculated on MS medium fortified with 2,4-D (0.5 mg/l) and AS (0.4 - 0.5 mg/l) in combinations gave rise to nodular white, brown coloured callus in less to moderate amount.

Leaf explants with petiole inoculated on MS medium fortified with 2, 4 –D (0.5 mg/l) and CM (5 – 15 %) showed non-embryogenic white, green or brown coloured callus in moderate to good amount. Leaf explants with petiole inoculated on MS medium fortified with 2, 4 –D (0.5 mg/l), AS (0.5 mg/l), PVP (0.5 mg/l) and CM (10%) in different combinations gave rise to non-embryogenic white, green or brown coloured callus. The callus produced was in less to moderate amount (Table 1 and plate 1). In henna, different coloured callus were obtained from tissue culture, when explants inoculated on MS supplemented with different combinations and concentrations of auxins and cytokinins (Rahiman and Taha, 2011). Callus obtained from various explants like, shoot tip and leaflets on MS + 2, 4 – D and MS + IAA in different concentration in *Lawsonia inermis* L. coloured callus was obtained (Paiker and Kandir, 2018).

Table 1 Effect of plant growth regulators on leaf explants (young and old) inoculated in MS medium for the initiation of callus

PGR(mg/l) + Addenda	Concentration of PGRs (mg/l)	% of callus induction (%)	Response intensity & colour of callus	Texture of callus
MS medium	-	0	-	-
MS+2,4-D	0.2	26.67	bgw+	Friable
	0.3	43.33	bgw+	
	0.4	56.67	bgw++	
	0.5	83.33	bgw++	
MS+2,4-D + AS	0.5+0.4	23.33	nB+	Friable
	0.5+0.5	63.33	nbw++	
MS+2,4-D + PVP	0.5+0.5	33.33	nbw+	Friable
MS+2,4-D + CM	0.5+ 5%	66.67	b++	Friable
	0.5+ 10%	93.33	bgw+++	
	0.5+ 15%	53.33	b++	
MS+2,4-D+ AS + PVP + CM	0.5+0.5 +10%	73.33	bw ++	Friable

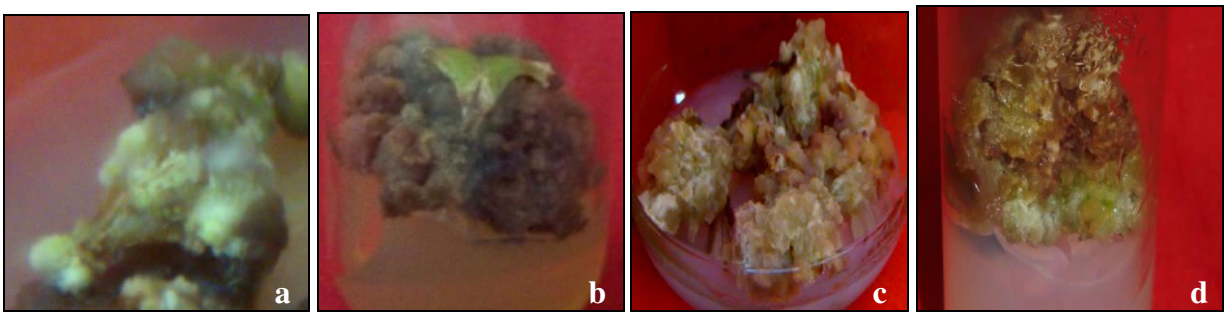

Plate 1: *In vitro* callus induction from leaf explants of *Lawsonia inermis* L. on MS medium fortified with a) 2,4-D (0.5 mg/l); b) 2,4-D (0.5 mg/l) + AS (0.5 mg/l); c) 2,4-D (0.5 mg/l) + CM (10%); d) 2,4-D (0.5 mg/l) + AS (0.5 mg/l) + PVP (0.5 mg/l) + CM (10%)

Preliminary Phytochemical Screening: Preliminary phytochemical analysis of leaf powder extracts from *Lawsonia inermis* L. showed the presence of alkaloids, anthraquinone, proteins, carbohydrates, glycosides, phenols, flavonoids, tannins, saponins and essential oils (Table 2).

Preliminary phytochemical analysis of callus powder extracts from *Lawsonia inermis* L. showed the presence of alkaloids, anthraquinone, carbohydrates, proteins, glycosides, phenols, flavonoids, tannins, saponins and essential oils (Table 2).

The phytochemical analysis with the crude extract of *Lawsonia inermis* L (Henna plant) gives various residues or components such as Tanins, flavonoids, alkaloids, terpenoids, Saponins, cardiac glycosides, glycosides, reducing sugars, phlobatanins, steroids, phenolic, aminoacids, proteins, quinines (Rao *et al*., 2016).

Table 2: Preliminary Phytochemical analysis of leaf from *Lawsonia inermis* L.

Test	To		E		EA		M		Aq	
	L	C	L	C	L	C	L	C	L	C
Alkaloids	+	+	+	+	+	+	+	+	+	+
Anthraquinone	-	-	+	-	+	-	+	+	+	+
Proteins	-	-	+	+	-	-	+	-	-	-
Carbohydrates	-	-	+	+	-	-	+	-	+	+
Glycosides (Killer-Kinliani Test)	+	+	+	+	+	+	+	+	+	+
Phenols	-	-	-	-	-	-	-	-	+	+
Flavonoids	-	-	+	+	-	-	+	+	+	+
Tannins	-	-	+	+	-	-	+	+	-	+
Saponins	-	-	-	-	-	-	-	-	+	+
Essential oils	-	-	+	+	-	-	+	+	-	-
Starch	-	-	-	-	-	-	-	-	-	-

+ = Present; - = Absent; To = Toluene; E = Ethanol; EA = Ethyl Acetate; M = Methanol; Aq = Aqueous; L = Leaf; C = Callus

Phytochemical analysis by TLC:

TLC fingerprints shows the presence of alkaloids, anthracene derivatives, arbutin derivatives, bitter principles, cardiac glycosides, essential oils, lignans, naphthoquinones, pungent principles, saponins, triterpenes and valepotraites in leaf powder of *Lawsonia inermis* L. (Plate 2). TLC fingerprints shows the presence of alkaloids, anthracene derivatives, arbutin derivatives, bitter principles, cardiac glycosides, essential oils, lignans, naphthoquinones, pungent principles, triterpenes and valepotraites in callus powder of *Lawsonia inermis* L. (Plate 2). The phytochemical screening of *Lawsonia inermis* L. revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, quinines, resins and sterols (Wagini *et al*., 2014).

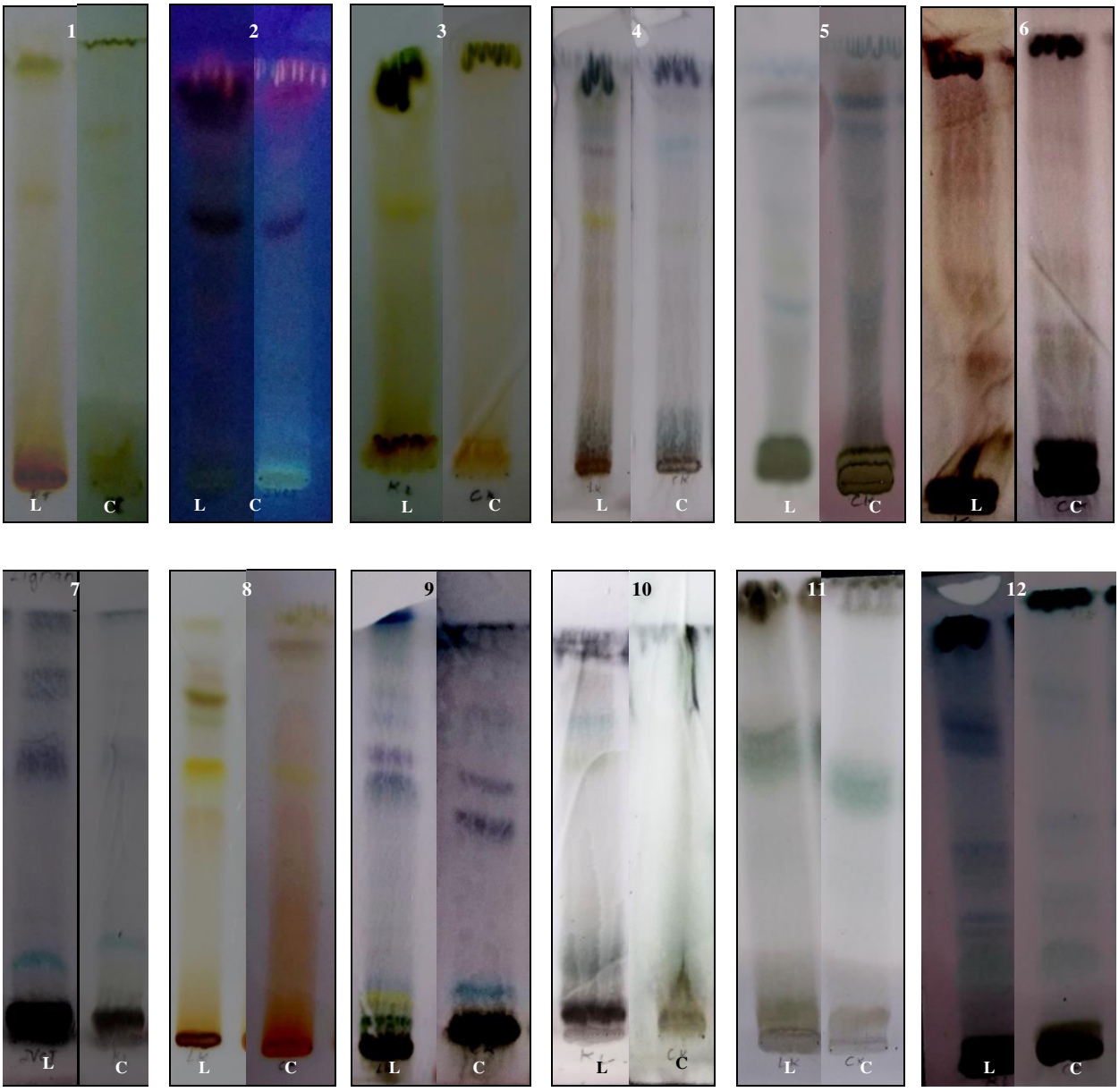

Plate 2: TLC fingerprints of phytochemical constituents in leaf and callus powders of *Lawsonia inermis* L. 1: Alkaloids; 2: Anthracin; 3: Arbutin derivatives; 4: Biiter principles; 5: Cardiac glycosides; 6: Essential oil; 7: Lignans; 8: Naphthaquinone; 9: Pungent principles; 10: Saponins; 11: Triterpenes; 12: Valepotriates

CONCLUSION

In the present study good amount of callus was obtained from leaf explants of *Lawsonia inermis* L. inoculated on MS medium fortified with 2, 4-D (0.3, 0.4 or 0.5 mg/l) + CM (10%). Comparative preliminary phytochemical analysis of leaf and callus powder extracts from *Lawsonia inermis* L. showed the presence of alkaloids, anthraquinone, carbohydrates,

glycosides, phenols, flavonoids, tannins, saponins and essential oils. The present study gives enough information regarding various phytoconstituents present in the extracts of *Lawsonia inermis* L. and also helps in generating basis for the quality control, correct identification and standardization of *Lawsonia inermis* L. The results of primary phytochemical screening were further confirmed by the results of TLC analysis. Different coloured bands symbolized the presence of specific phytoconstituents. Therefore the preliminary investigation will help in standardization parameters, for quality control of the powdered drug of herbal origin.

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