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Determination of Lawsone by HPTLC in *Lawsonia inermis* L. Callus and Plantlets Regenerated *in vitro*

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Leaves of *Lawsonia inermis* L. (henna) contain lawsone (2-hydroxy-1, 4-naphthaquinone). The orange red dye is used for colouring hair and hands. Lawsone also exhibits antifungal, antibacterial and antioxidant activity. HPTLC technique was used for estimation of lawsone in *Lawsonia inermis* L. plantlets regenerated *in vitro* and callus derived from leaf explants of *in vitro* seedlings. The content of lawsone found was 0.057 µg/mg in plantlet regenerated *in vitro* and 0.017 µg/mg (callus powder) of *Lawsonia inermis* L.

Keywords: *Lawsonia inermis* L., Lawsone, Callus, HPTLC.

INTRODUCTION

Lawsonia inermis L. is a much branched glabrous shrub or small tree, cultivated for its leaves[1]. 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[2]. Fruit is small, brown, round capsule with many seeds. Seeds are about 3 mm across, numerous, smooth and pyramidal. Seeds have a hard and thick seed coat with brownish coloration[3]. Leaves of *Lawsonia inermis* L. (henna) contain lawsone (2-hydroxy-1,4-naphthaquinone). Lawsone exhibits antifungal, antibacterial and antioxidant activity. The orange red dye from leaf paste or powder is widely used for decorating hands, nails and feet with patterns[1].

High performance thin layer chromatography (HPTLC) is an important tool that can be used qualitatively as well as quantitatively for checking the identity of crude drugs, purity and also for quality control of the finished products[4]. In the present investigation, an attempt has therefore been made to develop a simple and reproducible HPTLC method for quantitative estimation of lawsone in dried powder of *in vitro* plantlets (two months old) and dried callus powder of leaf explants from *in vitro* plantlets of *Lawsonia inermis* L.

EXPERIMENTAL

Seeds of *Lawsonia inermis* L. selected for the present study were collected from Kalyan (Maharashtra, India). 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.

The seeds were pre-treated and inoculated on plane MS medium[5]. Callus produce from leaf explants and two months old plantlets regenerated *in vitro* were harvested, dried, powdered and stored in air-tight container.

Standard lawsone (97 % purity) was procured from Sigma-Aldrich Cheime Gmbh (Aldrich Division, Steinheim, Federal Republic of Germany). The solvents methanol, toluene, ethyl acetate and acetic acid of analytical grade purchased from Hi-media were used for analysis.

Preparation of sample: Dried powder of callus from leaf explants and *in vitro* plantlets (two months old) of *Lawsonia inermis* L. were used separately for analysis.

1 g of powder was weighed and placed in a test tube and 10 mL of 50 % methanol was added. The sample was vortexed for 10 min and left to stand overnight at room temperature (28 ± 2 °C). The extracts were filtered through Whatmann No. 41 paper (E. Merck, Mumbai, India) and the filtrate was used for experimental work.

Preparation of standard stock solutions: 10 mg of lawsone was dissolved in diluent (50 % methanol) taken in 10 mL volumetric flask. Then the volume was made up to 10 mL with diluent to obtain a stock solution having 1 mg/mL concentration of lawsone.

Calibration curve of the standard: The working standard of suitable concentration (25-100 µg/mL) was applied in triplicate on pre-coated silica gel 60 F_{254} HPTLC plates (E. Merck) of uniform thickness 0.2 mm.

Chromatography: Chromatography was performed on silica gel 60 F254 HPTLC per-coated plate (10 cm × 10 cm) of 0.2 mm thickness, for the quantification of lawsone in samples of *Lawsonia inermis* L. Samples and standard lawsone

(10 µL) were applied on the plate as 8 mm wide bands with a constant application rate of 150 nL s^{-1}, with an automatic Camag Linomat V sample applicator under a flow of N_2 gas, 5 mm from the bottom, 15 mm from the side and the space between two spots was 6 mm of the plate.

Detection and estimation of lawsone: The linear ascending development was carried out in a Camag twin through chamber (10 cm × 10 cm), which was pre-saturated with 10 mL mobile phase *i.e.* toluene: ethyl acetate: acetic acid (5: 4: 1 v/v/v), for 0.5 h, at room temperature (25 ± 2 °C). The length of the chromatogram run was up to 80 mm. Subsequent to the development; the TLC plate was air dried. Quantitative evaluation of the plate was performed in the absorption-reflection mode at 254 nm, using a slit width 6 mm × 0.3 mm, with data resolution 100 mm $step^{-1}$ and scanning speed 20 mm s^{-1} and baseline correction was used.

The source of radiation utilized was a deuterium lamp. Determination of lawsone amount in different extracts was calculated by comparison of area measured for the sample to that of the standard. Each sample was analyzed in triplicate.

RESULTS AND DISCUSSION

Calibration curve of lawsone was obtained by plotting peak area *versus* concentration applied. It was found to be in linear range (25-100 µg/mL) per spot. The peak area and concentration was subjected to linear regression analysis to calculate the calibration equation Y = 288.3X and regression coefficient (R^2) was 0.648 (Fig. 1).

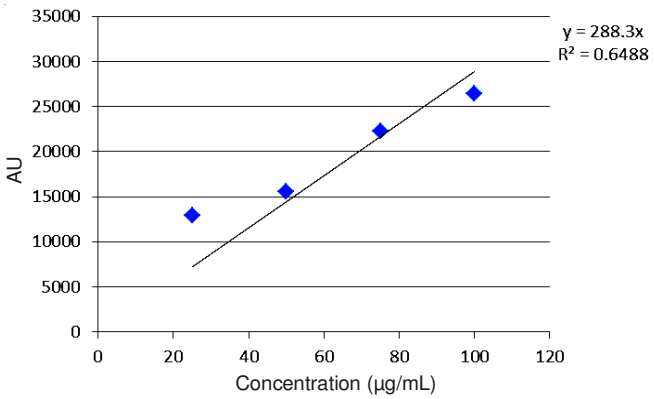

Fig. 1. Calibration curve of lawsone by HPTLC

The chromatogram showed the presence of lawsone in both callus powder and plantlets regenerated *in vitro*. The densitogram showed variation in lawsone content (Fig. 2). The lawsone content found in plantlets regenerated *in vitro* was 0.057 µg/mg (Fig. 3) and 0.017 µg/mg (callus from leaf explants) (Table-1 and Fig. 4).

TABLE-1
AMOUNT OF LAWSONE IN PLANTLETS REGENERATED *in vitro* AND CALLUS OF *Lawsonia inermis* L

Sample	Amount of lawsone present in sample (µg/mg)	R.S.D.	S.E.
Plantlet	0.057	0.029	0.55
Callus	0.017	0.054	0.31

*Mean of three values; S.D. = Standard deviation; R.S.D. = Relative standard deviation; S.E. = Standard error.

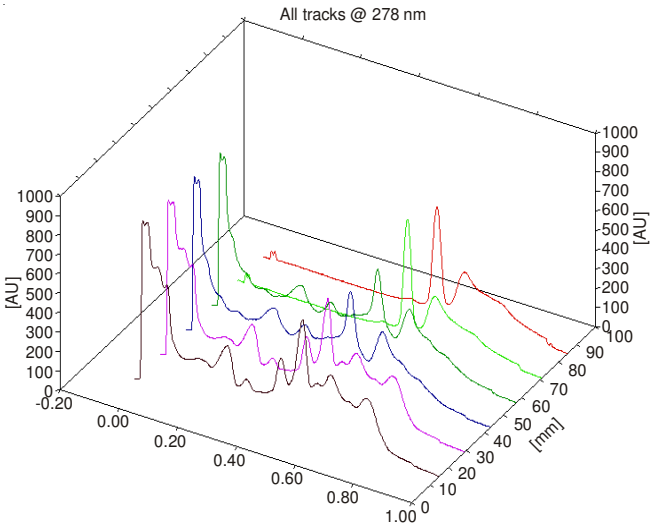

Fig. 2. Densitogram of plantlet and callus regenerated *in vitro* (3D view)

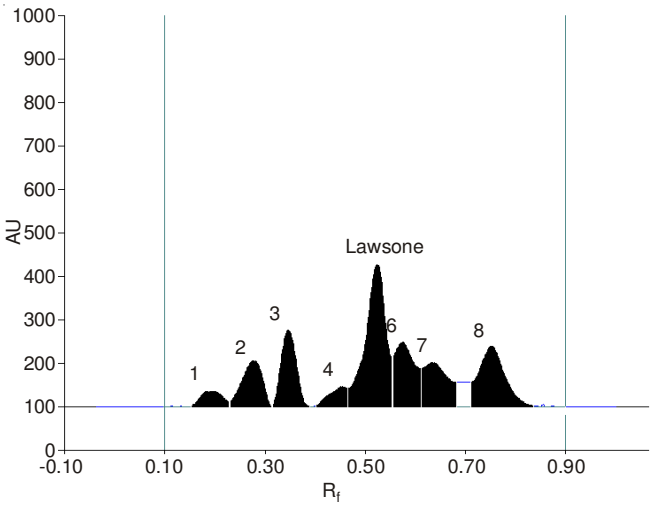

Fig. 3. Densitogram of plantlet regenerated *in vitro*

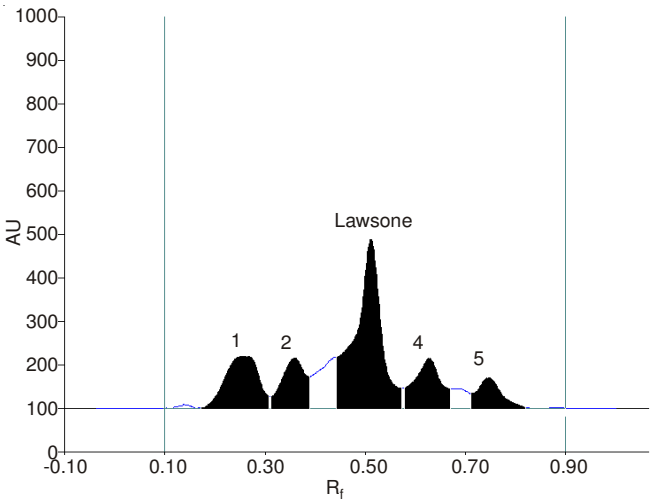

Fig. 4. Densitogram of callus

The production of lawsone (0.13 % dry weight) was observed in hairy roots tissues incubated in the dark and cultured in 1/2 MS or MS media[6]. The presence of lawsone was confirmed in the leaf callus from field grown plants of *Lawsonia inermis* L.[7]. HPTLC fingerprinting showed variation in the concentration of lawsone and was found to be highest in

plantlets regenerated *in vitro* than callus powder. Based on regression coefficient value of 0.648 (Fig. 1), the proposed HPTLC method was found to be accurate, reproducible, sensitive and simple for the estimation of lawsone in *Lawsonia inermis* L. This quantification data can be used as a diagnostic tool to identity and determine the quality and purity of the plant material. This baseline quantification can also be used to find out substitution and adulteration of genuine samples of *Lawsonia inermis* L.

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