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Phytochemical High Performance Thin Layer Chromatography based Estimation of Lawsone in *Lawsonia inermis* (Henna) obtained from Two Natural Habitats and Dye Products Collected from Local Market

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Abstract

The present study elucidates the preliminary phytochemical analysis and High Performance Thin Layer Chromatography (HPTLC) fingerprint analysis of lawsone extract of the leaves of *Lawsonia inermis* (henna) and evaluates their regions and products based quantitative estimations. The lawsone content has been evaluated quantitatively in two plants samples collected from Rewa and Bhopal and one commercial henna powder procured from open market of Bhopal city. Here we aimed to optimize high performance thin layer chromatography for the determination of lawsone. The chromatography development was carried out on the TLC silica gel 60 F254 aluminum plate and good resolution was achieved with toluene: ethyl acetate: formic acid (22:16:2) as mobile phase. Lawsone detected was carried out densitometrically at 254 nm by absorption mode and a linear regression coefficient was obtained with Y=133.527+13.470 × R=0.99092 ± 6.43%. The methods were applied are validated with respect to specificity, linearity, accuracy precision and robustness, and also find out the linear calibration come with respect to the concentration and regression coefficient. The developed method was validated as percentage relative standard deviation of peak areas was found to be which indicates the suitability of system for the further validation parameters. There was the presence of the peak at R_f 0.4 in the placebo, indicates the method is specific as none of the recipients interfered with the analytes of interest. The peak purity of Lawsone was achieved from 560-1865 Au.

Keywords: Phytochemical; *Lawsonia inermis* (henna); Densitometrically; Aluminum plate

Introduction

The name henna also refers to the dye prepared from the plant and the art of temporary body art based on dyes it's also know mehndi [1]. Henna "*Lawsonia inermis*," also known as henna, the henna tree, the mignonette tree, and the Egyptian privet and the sole species of the Lawsonia genus. The English name "henna" comes from Arabic ḥinna pronounced as "henna". Henna is a traditional medicinal plant [2]. The use of henna as medicinal plant and also for body painting is described in the Ebers papyrus [3].

Henna can be used in a wide variety of ways, including its dye form, as well as in aqueous extracts, tinctures, and salves, composed of the bark, seeds, or leaves. This versatility makes henna a very valuable element in traditional medicines, particularly Ayurvedic practice. In India as a part of Hindu and Sikh weddings, henna is applied during wedding ceremonies. It is a common practice among Indians, particularly elderly ones, to dye their hair using Henna. Our culture though traditional henna many women may work together during a large wedding, where in hundreds of guests have henna applied to their body parts. This particular event at a marriage is known as the Mehndi Celebration or Mehndi Night, and is mainly held for the bride [4]. Various physiological factors, such as skin type and temperature, hormone levels, and stress affect the appearance of mehendi on people. After the dried paste is scraped off the skin, air oxidation or perspiration can further darken the stain over the next 48 hours [5,6].

Classification of Henna

Kingdom:	Pantae
Order:	Myrtales
Family:	Lythraceae
Genus:	*Lawsonia*
Species:	*L. inermis*

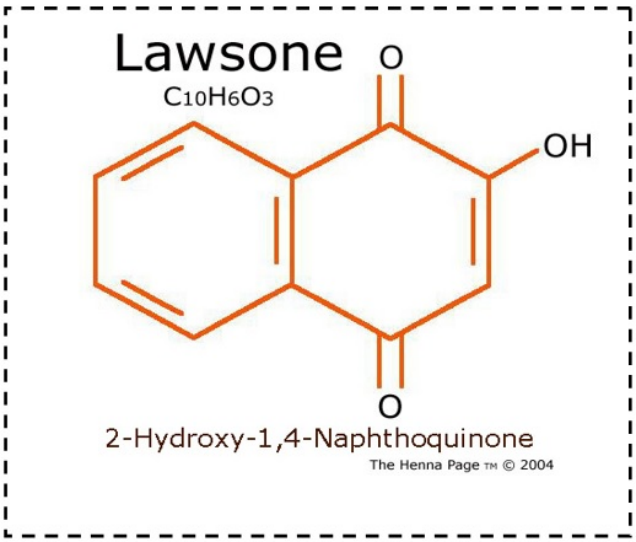

Figure 1: Chemical Structure of lawsone compound.

Figure 2: Photographic views of *Lawsonia inermis*.

"Henna" is understood by people around the world in many different ways. The majority of people probably associate henna with the dark-red/brown dye for hair and skin that is traditionally used in Eastern cultures, but the name also applies to the flowering plant from which that dye is derived. Henna can be used in a wide variety of ways, including its dye form, as well as in aqueous extracts, tinctures, and salves [7]. HPTLC was the methods used estimation of lawsone compound which quantify through the HPTLC analysis.

Henna, *Lawsonia inermis* contain a red orange pigment lawsone the molecules of which is also known as hennotannic acid. The name and molecular structure of lawsone show its congeniality to naphthalene [8]. The phytochemistry of henna was largely studied and revealed interesting information. Already in 1920, the dye principle was known. Lawsone, $C_{10}H_6O_3$, the colouring matter contained in henna leaves, is fixed well by wool, silk and tenaciously by the skin [9]. In lawsone two oxygen are attached to the naphthalene carbon at position 1 and 4 to form 1,4 naphthoquninone and a hydroxyl group is present at position 2 [8]. Its molecule contains 10 carbon, 6 hydrogen's and 3 oxygen's ($C_{10}H_6O_3$), giving a total molecular weight of 174.16 atomic units of mass [10].

Henna is known to be dangerous to people with glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency), which is more common in males than females. Infants and children of particular ethnic groups, mainly from the Middle East and North Africa, are especially vulnerable. Though user accounts cite few other negative effects of natural henna paste, save for occasional allergic reactions, pre-mixed henna body art pastes may have ingredients added to darken stain, or to alter stain color. The health risks involved in pre-mixed paste can be significant [11]. The green leaves of *Lawsonia inermis*, a small tree that grows in warm, arid regions of the world such as India, Pakistan, and Northern Africa and all other countries (Figures 1 and 2).

Henna contains natural ingredients which are vital for nourishment of hair [12]. It has a bond with the hair structure as it serves to penetrate, cleanse and thicken the hair shafts thus improving its quality. It also has great dandruff fighting ability. Henna is mainly used as a coloring agent. Nature has been a rich source of therapeutic agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources based on the uses of these plants in traditional medicine. Henna is one such plant commonly known as Persian Henna or *Lawsonia inermis*, a bushy, flowering tree, commonly found in India. The most common forms for medicinal benefits, and the high concentration of chemicals and nutrients in the plant gives it anti-inflammatory, hypotensive, antibacterial, astringent, and antiviral effects, among many others.

The antioxidant capacity of henna has not been widely studied, the oil has been proven to be an astringent, which has led some people to use henna juice and oil on the skin to reduce the signs of aging and wrinkles, as well as unsightly appearance of scars and other blemishes. This is complemented by the antiviral and antibacterial effects that can protect the body's largest organ is the skin. Henna oil is used for rheumatic and arthritic pains. Ground leaves are applied to sore flints to ease rheumatism. The juice of the medicinal plant can be applied to the skin for headaches, and the henna oil is applied to hair to prevent it from graying [13,14].

In the present investigation, we elucidated preliminary phytochemical analysis and High Performance Thin Layer Chromatography (HPTLC) based quantitative analysis of lawsone in *Lawsonia inermis* and evaluates qualitative and quantitative status of lawsone in L. inermis.

Materials and Methodology

The study was design to make the comparative analysis of the quantity of the lawsone compound founds in henna (Mehndi). Henna powdered dried leaves from samples of *Lawsonia inermis* L., Lythraceae, were obtained from suppliers of plant materials from different places of Madhya Pradesh. Total three samples were collected. Two leaves samples of plant henna were collected from Bhopal and Rewa respectively. And one packed powdered sample (Amena) is collected from local market of Bhopal. Plant material reference standard used *Lawsonia inermis*. The Samples were identified and deposited into the botanical Herbal Garden of MPCST (Bhopal). Samples of henna were obtained from the market and confirmed by morphological analysis (Table 1).

S N	Location	Sample ID
1	Bhopal, Madhya Pradesh	Sample-I (Bhopal)
2	Rewa, Madhya Pradesh	Sample-I (Rewa)
3	Open Market of Bhopal	Sample-I (Open Market)

Table 1: Collected henna samples and there assigned ID.

Sample preparation

Henna powdered dried leaves of each sample taken (1 g) and were separately mixed with methanol (10 ml) and mixed in shaker for overnight. The mixture was centrifuge at 10,000 rpm for 10 minutes. The supernatant was collected in a fresh tube and was 10 times diluted with methanol before use again further dilutes the sample.

Standard preparation

Stock solution of lawsone was prepared by accurately weighing 10 mg of 2 hydroxy 1,4 naphthoquinone (lawsone), dissolving it in methanol. The mixture was collected 1 ml and dissolved in 10 ml making up the volume with methanol.

Quantification of lawsone in methanolic extract of Lawsonia interims using high performance thin layer chromatography

The HPTLC system (CAMAG, Muttenz, Switzerland) consisted of (I) a Linomat 5 sample applicator using 100 µl syringes connected to a nitrogen tank (II) an Automatic Developing Chamber 2 containing twin trough chamber of 20 × 10 cm (III) TLC Plate Heater III; (IV) TLC scanner 3 linked to winCATS software.

Sample application

The standard was taken in syringe 50 µl loaded the syringe in linomate-5 through which the sample is loaded in TLC plate (1-8 µl). 8 Tracks of each standard applied according to the band with 8 mm, and application volume was 1-8 µl and gas flow 150 nl/s. Then after loading the standard in TLC plate again was the syringe 2 times wash by methanol. Sample is taken in syringe 20 µl 2 tracks of each samples applied 1-2 µl. Total 14 tracks of samples and standards are applied in TLC plate (Table 2).

Appl. position	Appl. Volume	Vial	Sample ID	Active
15.0 mm	1 µl	1	Std1	Yes
28.0 mm	2 µl	1	Std2	Yes
41.0 mm	3 µl	1	Std3	Yes
54.0 mm	4 µl	1	Std4	Yes
67.0 mm	5 µl	1	Std5	Yes
80.0 mm	6 µl	1	Std6	Yes
93.0 mm	7 µl	1	Std7	Yes
106.0 mm	8 µl	1	Std8	Yes
119.0 mm	1 µl	2	Sample-I (Bhopal)	Yes
132.0 mm	2 µl	2	Sample-I (Bhopal)	Yes
145.0 mm	1 µl	3	Sample-II (Rewa)	Yes
158.0 mm	2 µl	3	Sample-II (Rewa)	Yes
171.0 mm	1 µl	4	Sample-III (Open Market)	Yes
184.0 mm	2 µl	4	Sample-III (Open Market)	Yes

Table 2: Sample application.

Development of optimum mobile phase

The composition of the mobile phase for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity. After sample loading plate were developed to 10 × 10 cm Twin Through chamber before developing pre-saturated for 20 minute through filter paper. Chamber fill up 10 ml of developing solvent toluene: ethyl acetate: formic acid (22:16:2). Plates were developed into 8 cm. The developed plate allowed dry 5 Minutes by hair drier.

Evaluation

The plate was dried then inspected under a D2 white lamp at 254 nm web scanned at 254 nm with using D2 white lamp. Spectra scan web absorbed performed on plate and common spectra scanned web obtained by CAMAG Scanner 3 and analyzed by winCATS software (Table 3).

Rf	Substance	Maximum wavelength
0.04	Lowsone	461 nm
0.04	Lowsone	288 nm
0.04	Lowsone	286 nm
0.04	Lowsone	285 nm
0.04	Lowsone	284 nm
0.04	Lowsone	283 nm
0.04	Lowsone	280 nm
0.04	Lowsone	283 nm
0.04	Lowsone	292 nm
0.04	Lowsone	291 nm
0.03	Lowsone	294 nm
0.03	Lowsone	292 nm
0.04	Lowsone	289 nm
0.04	Lowsone	289 nm

Table 3: Web scanned at 254 nm with using D2 white lamp.

Results and Discussion

The qualitative and quantitative estimation of henna samples were carried out using HPTLC method. Various different concentration of sample was applied to the TLC plate were used to quantification on impact regional variations of lawsone. The chromatographic conditions in particular the developing solvents were carefully optimized and then formulation samples were analyzed scanned at 254 nm using D2 white lamp the result observed good separation for all compounds. A reference marker compound of lawsone was separated on the plate for the authentication of each sample.

Sample I Bhopal, Sample II Rewa, Sample III Open market Bhopal.

The specificity of the method was ascertained by analyzing. Various different concentration of standard was made and also scanned for comparisons. Bands were scanned at D2 white lamp. Different peaks and bands can be observed in the graph (Figure 3) and concentration calculated can be observed.

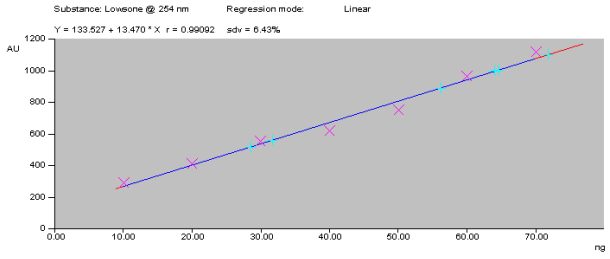

Figure 3: Standard plot of standard of lawsone (pink in colour) and sample of *Lawsonia inermis*.

The spots for lawsone in samples were confirmed by comparing the Rf and spectra of the spot with that of standard. The peak purity of lawsone was assessed by comparing the spectra at three different levels, i.e., peak start, peak apex, and peak end positions of the spot. WinCATs and integration software 1.4 was used to calculate the amount of lawsone. Under the chromatographic conditions described in procedure the Rf for lawsone was found to be 0.4. For positive identification, the sample must exhibit bands with chromatographic characteristics, including colors and Rf values similar to those of reference marker compound. It was observed that lawsone appeared at Rf 0.4 in samples which is similar to the Standard Lawsone.

Developed and validated HPTLC method for estimation of Lawsone in henna was found to be simple, accurate, precise and robust. Different mobile phase was tried and good resolution was achieved

with toluene: Ethyl acetate: formic acid (22:16:2 v/v/v). Sample preparation was optimized by using methanol as solvents. Lawsone was detected at 0.04 Rf value at 254 nm by absorbance mode [15]. The developed method was validated as percentage relative standard deviation of peak areas was found to be which indicates the suitability of system for the further validation parameters. There was the absence of the peak at R_f 0.04 in the placebo, indicates the method is specific as none of the excipients interfered with the analytes of interest. The peak purity of Lawsone was achieved 1000.

Total 3 plants sample were applied during quantification of lawsone. Each sample were applied in duplicate of them track 1, having sample 1 µl, however they were in another track is 2 µl. sample 1 track 1 occupied 560.14 Au, sample 1 track 2 occupied 1004.50 Au, sample 2nd track 1st occupied 517.47 Au, Sample 2nd track 2nd occupied the areas of 996.31 Au, similarly samples 3 track 1st occupied 1100.48 Au sample 3rd track 2nd occupied area of 1865.16 Au. Above absorption depicted that all sample are within the range of standard accept sample 3 track 2, having the out of range of the standard (Tables 4-6, Figure 4).

Sample ID	Sample application Volume (µl)	Maximum position (Rf)	Maximum height (AU)	Area (AU)
Standard	1 (µl)	0.04	32.6	292.18
	2 (µl)	0.04	39.3	412.92
	3 (µl)	0.04	47.8	553.62
	4 (µl)	0.04	49.4	617.47
	5 (µl)	0.04	63.9	749.66
	6 (µl)	0.04	82.9	963.25
	7 (µl)	0.04	99.1	1117.13
	8 (µl)	0.04	86.8	890.19
Sample I	1 (µl)	0.04	57.7	560.14
	2(µl)	0.04	88.8	1004.50
Sample II	1 (µl)	0.03	51.3	517.47
	2 (µl)	0.03	79.0	996.31
Sample III	1 (µl)	0.04	103.7	1100.48
	2 (µl)	0.04	165.0	1865.16

Table 4: TLC plate scanned at 254 nm using D2 white lamp.

Substance	Rf	X(Average)	CV[%]	n	Regression remark
Std. lawsone	0.04	56.17 ng	0.000	1	Linear
S.B. lawsone	0.04	48.17 ng	48.430	2	Linear
S.R. lawsone	0.03	46.28 ng	54.316	2	Linear
S.M. lawsone	0.04	71.79 ng	0.000	1	Linear

Table 5: Calibration results per analysis.

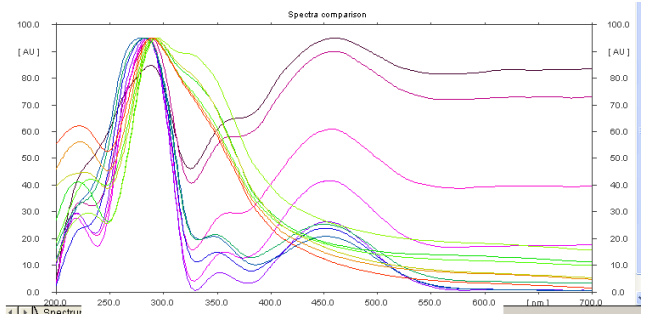

Figure 4: Spectra scan for all tracks for lawsone scanned 254 nm wavelength.

All standard and all sample were detected at 0.04 Rf value and wavelength 254 nm by absorption mode (Tables 6 and 7) and a linear regression coefficient was obtained Figure 5 with Y=133.527+13.470 × R=0.99092 ± 6.43%. After comparison of the all unknown samples with standards it has found that the average concentration of the standard solution is 0.999 µg/µl. plants samples collected from Human Herbal Heath Care Garden (HHHCG) achieved the concentration 1.91 µg/µl in the crude extract of the plants as 1 µg/ µl. The plants samples which were collected from Rewa showed the concentration 1.77 µg/µl in the crude extract of the plants as 1 µg/µl. However, the 3rd sample was collected from open market make Amina Herbal Hair Powder (coded as sample 3rd). Quantitative the lawsone is 3.76 µg/µl. The similar type of research work has been carried out by many scientist [15,16] for the quanti ication of *Lawsonia inermis* is compared our result with this research studied it has concluded that our results as well as methods applied are validated with respect to specificity, linearity, accuracy precision and robustness. We also find out the linear calibration come with respect to the concentration and regression coefficient.

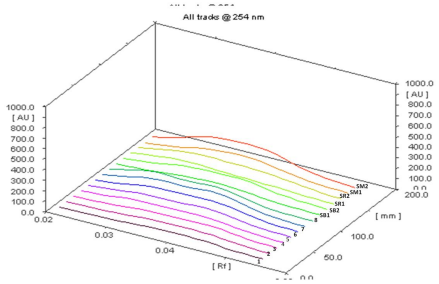

Figure 5: 3D graph for specifity for 1-Lawsone standard 1 µg/µl, 2-Lawsone standard 2 µg/µl, 3-Lawsone standard 3 µg/µl, 4-lawsone standard 4 µg/µl, 5-Lawsone standard 5 µg/µl, 6-Lawsone standard 6 µg/µl, 7-lawsone standard 7 µg/µl, 8-Lawsone standard 8 µg/µl, 9-Sample Bhopal 1 µg/µl, 10-Sample Bhopal 2 µg/µl, 11 Sample-Rewa 1 µg/µl, 12-Sample Rewa 2 µg/µl, 13-Sample Market 1 µg/µl, 14-Sample Market 2 µg/µl.

Vial	Rf	Amount	Arera	X (cal)
1	0.04	10.00 ng	292.18	-
1	0.04	20.00 ng	412.92	-
1	0.04	30.00 ng	553.62	-
1	0.04	40.00 ng	617.47	-
1	0.04	50.00 ng	749.66	-
1	0.04	60.00 ng	963.25	-
1	0.04	70.00 ng	1117.13	-
1	0.04	-	890.19	56.17 ng
2	0.04	-	560.14	31.67 ng
2	0.04	-	1004.50	64.66 ng
3	0.03	-	517.47	28.50 ng
3	0.03	-	996.31	64.05 ng
4	0.04	-	1100.48	71.79 ng
4	0.04	-	1865.16	>77.00 ng

Table 6: Amount of standards and samples on TLC plate.

S. N.	Sample ID	Concentration
1	Standard	0.999 µg/ µl
2	Sample I (1)	1.91 µg/ µl quantified by HPTLC
3	Sample I (2)	3.43 µg/ µl quantified by HPTLC
4	Sample II (1)	1.77 µg/ µl quantified by HPTLC
5	Sample II (2)	3.40 µg/ µl quantified by HPTLC
6	Sample III (1)	3.76 µg/ µl quantified by HPTLC
7	Sample III (2)	6.38 µg/ µl quantified by HPTLC

Table 7: Final quantified lawsone compound in plants samples analyzed.

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References

1. Bailey LH, Bailey EZ (1976) A concise dictionary of plants cultivated.
2. Kamal M, Jawaid T (2010) Pharmacological activities of Lawsonia inermis Linn. A review. Int Jour Biomed Res.
3. Papyrus of Eber Cal (1912) State J. Med. 19: 219.
4. Jones CW, Catherine A (2004) Cassia Obovata Henna for Hair. Retrieved 5 May 2013.
5. Saksena J (1979) Art of Rajasthan. Henna and loor decoration. Dehli (Sundeep).
6. Brady D (2013) FDA: Beware of "black henna" tattoos The Style Blog. The Washington Post.
7. Dweck AC (2002) Natural ingredients for coloring and styling. Int J Cosmetic Sc 24: 287-302.
8. Westermarck E (1972) Marriage ceremonies in Morocco. Curzon Press, London, UK.
9. Tommasi G (1920) Henna (Lawsonia inermis) Chemical constitution of lawsone. II -Gazzetta Chimica Italiana 50: 263.
10. Maunang MP, Bhavna RS, Nilesh CG, Prateek HP, Shweta SV (2013) Method development for Lawsone estimation in Trichup herbal hair powder by high performance thin layer chromatography. J Adv Pharm Technol Res 4: 160.
11. Brauer E, Raphael P (1993) The Jews of Kurdistan. Detroit (Wayne State University Press).
12. Biringanine G, Chiarelli MT (2006) A validation protocol for the HPTLC standardization of herbal products: Application to the determination of acteoside in leaves of Plantago palmata Hook. f.s. Talanta 69: 418-424.
13. Yogisha S, Samiulla DS, Prashanth D, Padmaja R, Amit A (2002) Fitoterapia Temporary Tattoos and Henna/Mehndi. Food and Drug Administration. 73: 690.
14. Raupp P, Hassan JA, Varughese M, Kristiansson B (2001) Henna causes life threatening haemolysis in glucose-6-phosphate dehydrogenase deficiency. Archives of Disease in Childhood 85: 411.
15. Dhiman A, Sharma K, Goyal J, Garg M, Sharma A (2012) Determination of Lawsone Content in fresh and dried leaves of Lawsonia inermis Lin. and its quantitative analysis by HPTLC. J of Pharmacu and Scie Inov 1: 17.
16. Patel NM, Mehta FA, Shah DA, Chhalotiya UK (2015) Development and Validation of HPTLC Method for Estimation of Saroglitazar in Bulk and Pharmaceutical Dosage Form. Austin J Anal Pharm Chem 2: 1035.