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RESEARCH ARTICLE

Phytochemical & TLC Profile of *Lawsonia Inermis* (Heena)

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ABSTRACT

Lawsonia inermis (Heena) belongs to family (Lythraceae), is used as herbal medicine and dyeing agent since ancient times. It is cultivated as hedge plant and on large scale to obtain leaves to dye hands and hairs. The 80% ethanolic extract of L. inermis leaves is prepared and investigated for the presence of different phytoconstituents with the help of phytochemical analysis and TLC. The phytochemical analysis shows the presence of alkaloids, glycosides, hydrolysable tannins, flavanoids, steroids, proteins, carbohydrates and saponins. The TLC profile represents different retention factor value (R_f).

KEYWORDS

Lawsonia inermis, Phytochemical, Retention factor, Thin layer chromatography

INTRODUCTION

Lawsonia inermis (Heena) (Lythraceae) is a perennial plant commonly known as Heena, having different vernacular names in India viz., Mehndi in Hindi, Mendika, Rakigarbha in Sanskrit, Mailanchi in Malayalam, Muruthani in Tamil, Benjati in Oriya, Mayilanchi in Kannada and Mehedi in Bengali.[1] It is native to North Africa and South East Asia, and often cultivated as an ornamental plant throughout India, Persia, and along the African coast of the Mediterranean Sea[2] Lawsonia alba and Lawsonia spinosa are the older names for Lawsonia inermis L.[3] Lawsonia inermis is naturalized in India, common in dry jungles and used as hedges. It prefers dry conditions so it is commercially cultivated in many areas including Rajasthan and Tamil.[4]

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Botanical Description

L.inermis. is 6-12 m tall shrub or small tree with grey–brown bark and quadrangular young branches. The taxonomy detail is depicted in Table 1 and picture of plant Figure in 1 Older plants sometimes have spine-tipped branches. Leaves are decussate, opposite, simple and entire, subsessile; shortly petiolate; stipules minute; blades are elliptical to oblong or broadly lanceolate, 1.08.8 cmX 0.54.0cm, cuneate at base, acute to round at apex.

Table 1: Taxonomy of *Lawsonia inermis L.*

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Family: Lythraceae
Genus: Lawsonia
Species:L. inermis
Botanical Name: *Lawsonia inermis L.*
Common Name:Heena

Figure 1: Plant of *Lawsonia inermis L.*

Inflorescence is large, pyramidal terminal panicle up to 25 cm long with many flowers. Flower bisexual, regular tetramous, sweet-scented; pedicle 24 cm long; calyx having 2mm long tube and spreading, ovate lobe 2-3 cm long; petals orbicular to obvate, 1.5-4mmX4-5mm, usually whitish, sometime reddish; stamens 8, inserted in pairs on the rim of the calyx tube, filaments 45 mm long; ovary superior, 4 celled, style errect, up to 5mm long, stigma head shaped. Fruit a globose capsule 48mm in diameter, purplish–green, indehiscent or opening irregularly, many seeded. Seeds are tetra-angular, 33mm long thick seedcoat.[5] In transverse section, the shape of the petiole is cylindrical with small wings on its lateral sides.[6] Lawsonia inermis L. can be propagated by seeds. Vegetative propogation is by cuttings. Lawsonia inermis L. grows on the type of soil from light loam to clay. Lawsone is the chief constituent responsible for dyeing property of the plant. Dried powdered leaves of heena contain 0.5 -1.5% lawsone, traditionally used to produce fast orange and brown colour dye.[7]

Phytochemical Constituents & Medicinal Importance

The phytochemicals are those chemical compounds that occur naturally in plants and are responsible for their medicinal properties. The different constituents isolated from the different parts of plants are described Table 2.

The biological activity discovered in Lawsonia inermis are Antidiabetic activity: Ethanol (70 %) extract of L. inermis showed significant hypoglycaemic and hypolipidaemic activities.[22] Methanol (95 %) extract of leaves of L. inermis showed significant in-vitro antihyperglycemic effect.[23] Immunomodulatory activity: The methanol leaves extract of L. alba and its different fractions showed in vitro immunostimulant action promotion of T-lymphocyte proliferative responses.[24]

Naphthoquinones isolated from L. alba leaves also showed significant immunomodulatory effect.[25] Tuberculostatic activity: The tuberculostatic activity of heena was tested in-vitro and in-vivo on Lowenstein Jensen medium, the growth of Tubercle bacilli from sputum and of Mycobacterium tuberculosis H37Rv was inhibited.[26]

Antiparasitic activity: Antimalarial, leish-manicidal, trypanocidal, antihelminthiasis and antiscabies activities were determined and leaves of L. inermis showed potential trypanocidal activities.[27] Nootropic activity: The effect of acetone soluble fraction of petroleum ether extract of L. inermis leaves was investigated on memory, anxiety and behavior mediated via monoamine neurotransmitters using elevated plus maze and passive shock avoidance paradigms. The extract exhibited prominent nootropic activity, potentiated clonidine induced hypothermia and decreased lithium induced head twitches. However, haloperidol induced catalepsy was not modified.[28]

Anticoagulant effect: Lawsone and its oxazine derivatives isolated from leaves of L. inermis are proven to be potential anticoagulant agent.[29] Wound healing effects: Chloroform and aqueous extracts of leaves of the plant were capable of inhibiting the growth of micro-organisms that are involved in causing burn wound infections.[30-31] The wound healing activity on rats using excision, incision and dead space wound models. Extract of L. inermis when compared with the control and reference standard animals: a high rate of wound contraction, a decrease in the period of epithelialization, high skin breaking strength, a significant increase in the granulation tissue weight and hydroxyproline content.

Table 2: Active constituents isolated from Lawsonia inermis L.

Plant Part	Compounds	References
Napthoquinone derivatives		
Leaves	Lawsone (2-hydroxy 1,4-naphthoquinone)	8
	1,3-dihydroxy naphthalene, 1,4-napthaquinone, 1,2-dihydroxy-4-	9
	Glucosylnaphthalene	
Stem bark	Isoplumbagin	10
Phenolic compounds		
Bark, Leaves	Lawsoniaside (1,3,4-trihydroxynaphthalene 1,4-di-β-D-gluco-pyronoside),	11-12
	Lalioside (2,3,4,6-tetrahydroxyacetoxy-2-β-D-glucopyranoside)	
	Lawsoniaside B (3-(4-O-a-D-glucopyranosyl-3,5-dimethoxy) phenyl-2E-propenol), syringinoside, daphneside, daphnorin, agrimonolide 6-O-β-	
	D-glucopyranoside, (+)-syringaresinol O-β-D-glucopyranoside,	
	(+)-Pinoresinol di-O-β-D-glucopyranoside, Syringaresinol,	
	di-O-β-D- glucopyranoside, isoscutellarin	
Terpenoids		
Bark, Seeds	3β, 30-dihydroxylup-20(29)-ene (hennadiol), (20S)-3β, 30-dihydroxylupane, Lupeol, 30-*nor*-lupan-3β-ol-20-one, betulin, betulinic acid, lawnermis acid (3β-28β-hydroxy-urs-12,20-diene-28-oic acid) and its methyl ester	13-14
Sterols		
Roots, Leaves	Lawsaritol (24β-ethycholest-4-en-3β-ol)	15
	Stigmasterol and β-sitosterol	
Aliphatic constituents		
Stem bark	3-methyl-nonacosan-1-ol, n-tricontyl n-tridecanoate	16-17
Xanthones		
Whole plan	Laxanthone I (1,3 dihydroxy-6,7 dimethoxy xanthone), Laxanthone II (1-	18-19
	hydroxy-3,6 diacetoxy-7-methoxyxanthone), Laxanthone III (1-hydroxy-	
	6-acetoxy xanthone)	
Coumarins		
Whole plant	Lacoumarin (5-allyoxy-7-hydroxycoumarin)	20
Flavonoids		
Leaves	Apigenin-7-glucoside, apigenin-4-glycoside, luteolin-7-glucoside, luteolin-3-glucoside	21

Histological studies of the tissue showed increased well organized bands of collagen, more fibroblasts and few inflammatory cells when compared with the controls which showed inflammatory cells, scanty collagen fibres and fibroblasts.[32]

Antisickling activity: Aqueous extract of leaves of L. inermis was found to inhibit sickling and to increase the oxygen affinity of HbSS blood.[33] Abortifacient activity: Methanol extract of roots of L. inermis was most effective in inducing abortion in mice, rats and guinea pig. The effect apparently was dosage dependent. The results of the whole animal experiments support the methanol extract effectiveness as an abortant due to its maternal and foetal toxic effects.[34] Enzymes inhibitory activity: The ethanol extract of L. inermis L. leaves and lawsone tested for trypsin inhibitory activity showed an IC50 value of 64.87 and 48.6µg/ml, respectively.[35]

MATERIALS AND METHOD

Plant Material

L. inermis leaves were collected from the Sant Hirdayram Nagar, Bhopal, Madhya Pradesh, India. Leaves washed properly, shade dried, powdered and kept in air tight box for further use. The plant was identified and authenticated by Dr. Sumen Mishra Taxonomist, Vindhya Herbal Testing Laboratory, MFP-PARC, Bhopal.

20 gm powder of L. Inermis leaves was extracted by Soxhalet (hot extraction) method with 80 % ethanol for 18 hours and 20 gm powder was macerate with 80 % ethanol. The extract was filtered and concentrated by the help of Vacuum concentrator (DELVAC). The obtained extract is kept in moisture free container at -20˚C.

Phytochemical Analysis

Test of Alkaloids

1. Mayer's Test: Take test solution in the test tube adds the Mayer reagent (Potassium mercuric iodide solution). White or yellow precipitate indicates the presence of alkailoids.
2. Wagner's Test: Take the test solution in a test tube then add Wagner's reagent (iodine solution). Brown or reddish brown precipitate.

Tests of Glycosides

1. Raymond's Test:- Take the test solution in test tube and add 1 ml of 50% ethanol. Add 0.1% solution of dinitrobenzene in ethanol then added 2-3 drops of 20% sodium hydroxide solution . Appearance of violet color indicated the presence of Glycosides.
2. Killer Killani Test:- 2 ml of extract in a test tube add glacial acetic acid then add one drop of 5% FeCl3 with conc. H2SO4. Reddish brown color appeared at the junction of the two liquid layers and upper layer appeared bluish green.
3. Legal Test:- Take the test solution in a test tube add few drops of pyridine and a drop of 2% sodium nitroprusside then add a drop of 20% sodium hydroxide solution. Deep red color appears.

Tests for Carbohydrate

1. Molisch's Test:- 2-3 ml. extract add few drops of α- naphthol solution (20% in ethyl alcohol) then 1 ml. conc. H2SO4 added along the side of the test tubes. Violet ring was formed at the junction of two liquids.
2. Benedict's Test: To the extract add equal volume of Benedict's reagent. Heat for 5 min. Solution appears green, yellow or red.

Tests for Tannins

1. Vanillin- HCl Test: To the extract add vanillin-HCl reagent (1 g vanillin + 10 ml. alcohol + 10 ml. conc. HCl). Formation of pink or red color
2. Gelatin Test: To the extract solution add aqueous solution of gelatin. White buff color precipitate are formed

Tests for Flavanoids

1. Lead acetate test: Filter paper strip was dipped in the alcoholic solution of extract,

ammoniated with ammonia solution. Color changed from white to orange.

2. Shinoda Test: To the extract add 5 ml. 95% alcohol, few drops of conc. HCl and 0.5 g magnesium turning. Pink color observed.
3. Alkaline Reagent Test: Extracts have to be treated with a few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of falvonoids.

Tests for Resins

1. Ferric chloride test: Take the extract in test tube add alcohol with few drops of FeCl3 solution. Green color appears.
2. Turbidity Test: Extract solution (2 g of sample in methanol) add 5 ml distilled water, turbidity appears.

Test for Steroids

1. Libermann- Bur chard Test: To 2 ml. extract add Chloroform, 1- 2ml. acetic acid and 2 drops H2SO4 from the side of the test tube. First red, then blue and finally green color appeared.
2. Salkowski Reaction: To 2 ml. of extract add 2 ml. chloroform, 2 ml. conc. H2SO4. Shake well. Chloroform layer appeared red color and acid layer shows greenish fluorescence.

Test for Proteins and Amino-acids

1. Biuret Test: Take 3 ml. of extract in a test tube add 4% NaOH and 2-3 drops of 1% copper sulphate solution. Presence of red/violet coloration.
2. Precipitation test: extract then mix with absolute alcohol. White ppt.
3. Ninhydrin Test: Extract in a test tube then add ninhydrin reagent in boiling water bath for 10 min. Violet color appeared.
4. Cysteine Test: To 1 ml of protein solution in a test tube, add 2 drops of 10% sodium hydroxide solution and 2 drops of lead acetate. − Mix well and put in a boiling water bath for few minutes; a black deposit is formed with albumin, while a slight black turbidity is obtained with casein due to its lower content of sulfur. Gelatin gives negative result.

Test for Fats

1. Sudan Red test: To a test tube, add equal parts of test sample and water to fill about half full. Add 3 drops of Sudan III stain to each test tube. Shake gently to mix. A red-stained oil layer will separate out and float on the water surface if fat is present.
2. Spot test: Take a small strip of filter paper. Press a small quantity of extracts between the filter paper. Oil stains on paper indicates the presence of fixed oils.
3. Saponification test: To 1 ml of the extract add few drops of 0.5 N alcoholic potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Phenol Test

1. Ferric chloride Test: To 1 ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.

Diterpenes Test

1. Copper acetate test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for Saponins

1. Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
2. Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Thin layer Chromatography

The TLC was performed on the silica coated glass TLC plates. The TLC chambers were saturated with the solvent and after applying the sample on TLC plates they were kept for development of chromatogram. Then the separations were studied by the detecting reagents/methods. (Tab.-1)

TLC of Various Extracts

Test Solution

Alcoholic/aqueous solution of the alcoholic/aqueous extracts (20mg/ml) respectively was prepared.

Solvent System

Multiple solvent systems had been used:

a. Toluene: Ethyl acetate (93:7)

b. n-Butanol: Acetic acid: Water (4:1:5)

c. Ethyl acetate: Water: Methanol (100:10:13.5)

d. Benzene: Acetic acid (9:1)

e. Toluene: Ethyl acetate: Methanol: Acetic acid (3:4:3:1)

f. Ethyl acetate: Isopropyl alcohol: Water (65:25:10)

g. Chloroform: Ethanol:Acetic acid (94:5:1)

Procedure

Approximately 10 micro liter of sample solution was applied on the plates of uniform, thickness (0.2mm). The plates were developed in the solvent system up to a distance of 10 cm.

Visualization of SPOTS

The plates were observed under:

a. Under UV light at shorter and longer wavelength

b. Iodine chamber

c. Vanillin sulphuric acid reagent and heating the plate for 10min at 110^0C.

d. Alcoholic sulphuric acid reagent and heating the plate for 5min at 60^0C.

RESULTS

Table 3: Showing the results of phytochemical analysis of *Lawsonia inermis Linn.*

S. No	Phyto-constituents	Identification Test	Lawsonia inermis (Heena)
1	Alkaloids	a. Mayer test b. Wagner test	++ve +ve
2	Glycosides	a. Legal test b. Libberman buchard test c. salkowski test d. keller killani test	++ve -ve +ve +ve
3	Tannins	a. Vanillin-HCL test b. Gelatin test	+ve -ve
4	Resins	a. Turbidity test b. Ferric- Cl test	-ve -ve
5	Flavanoids	a. Shinoda test b. Lead acetate test c. Alkaline test	+ve -ve ++ve
6	Steroids	a. Salkowski test b. Libermann - reaction	-ve +ve
7	Amino-acids	a. Ninhydrin test b. Cysteine test	-ve -ve
8	Proteins	a. Precipitate test b. Biuret Test	+ve +ve
9	Carbohydrate	a. Molish test b. Benedict test	+ve +++ve

10	Fats & Oil	a. Sudan red b. spot test c. saponification test	+ve ++ve +ve
11	Phenol test	a. ferric chloride test	++ve
12	Diterpens	a. cooper acetate test	+ve
13	saponins test	a. forth test b. foam test	++ve -ve

Preliminary Phytochemical screening was performed for each alcoholic extract. It was noted that Heena extract contains alkaloids, glycosides, hydrolysable tannins, flavanoids, steroids, proteins, carbohydrates and saponins.

The TLC analysis is depicted in tabular form in Table 4 and picture is depicted in Figure 2.

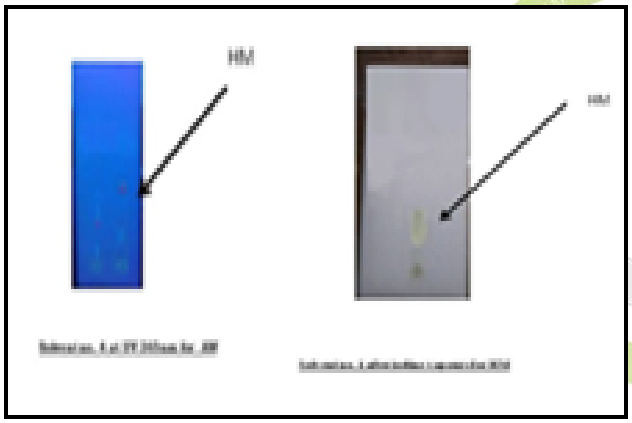

Figure 2: Showing the TLC profile of *Lawsonia inermis Linn.*

DISCUSSION

Studies have reported that leaves of *Lawsonia inermis Linn. contain* carbohydrates, proteins, flavonoids, tannins, phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthones and fatty acids.[36] Another study done revealed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids.[37] The ethanolic extracts of *L. inermis* leaves revealed the presence of alkaloids, carbohydrates, resins, saponins sterols and tannins.[38] *L.inermis* leaves showed the presence of phytochemicals like tannins, flavonoids, saponins, steroids, alkaloids and glycosides.[39] In the present study *L.inermis* leaves showed the presence the of alkaloid, glycoside, hydrolysable tannins, flavanoids, steroids, proteins, carbohydrates and saponins.

TLC of *L. inermis* performed by Agarwal and co-worker[40] on silica gel 'G' 60 F254 of 0.2 mm thickness using toluene:ethyl acetate (9:1) as solvent system and when seen under visible light showed bands with Rf 0.35, 0.60, 0.63 (all grey), under UV 366 nm shows bands with Rf 0.18, 0.26, 0.63 (all violet), 0.39, 0.61, 0.68 (all reddish violet), 0.73 (violet). On dipping in 5% Methanolic Sulphuric acid reagent and heating at 105°C for 5 min, bands appeared with Rf 0.41, 0.61, 0.70 (all grey) thus showing the different phytoconstituents present in *L. inermis*. whereas T.L.C. of alcoholic extract *L. inermis*. performed on Silica gel 'G' plate using solvent system toluene : ethylacetate (9:1) showed three spots in visible light with Rf. 0.35, 0.60 and 0.63 (all grey). Under U.V. (366 nm) seven spots appeared with Rf. 0.18, 0.26, 0.35, (all violet), 0.39, 0.61, 0.68 (all reddish violet) and 0.73 (violet). On spraying with 5% methanolic sulphuric acid regent and heating the plate at 105°C for ten minutes five grey colour spots appeared with Rf. 0.09, 0.41, 0.61, 0.70 and 0.95 as depicted by Ayurvedic Pharmacopoeia of India.[41]

In the present study the TLC profile of L. inermis leaves extract (80% ethanolic shoxhlet extract (HS)) showed three spots with solvent system of toluene: ethyl acetate (93:7) with Rf values at 0.51(green colour), 0.32(brown colour) and 0.71 (red colour) whereas L. inermis extract prepared in 80% ethanolic extract by maceration (HM)) showed one spot with solvent toluene: ethyl acetate (93:7) with Rf values at 0.35 (g). When solvent of benzene : acetic acid (9:1) two spots were observed in the HS with Rf. 0.34 (Green colour) and 0.53 (green colour) where as poor separation was observed in the HM case. The Plant active constituent's analysis is very important step as it gives the information regarding presence or absence of particular primary and secondary metabolites in the extract of various parts of plant. The phytoconstituents have important clinical use.

Table 4: Showing the Rf of TLC profile of *Lawsonia inermis Linn.*

S.No.	Solvent System	Results	HS (extract obtained by soxhalet)	HM (extract obtained by maceration)
1.	Toluene: Ethyl acetate (93:7)	No. of spots	2	1
		Rf values and color	0.51(g), 0.32(b), 0.71 (r)	0.35(g)
2.	n-Butanol: Acetic acid: Water (4:1:5)	No. of spots	Nss	Nss
		Rf values and color	Nss	Nss
3.	Ethyl acetate: Water: Methanol (100:10:13.5)	No. of spots	Nss	Nss
		Rf values and color	Nss	Nss
4.	Benzene: Acetic acid (9:1)	No. of spots	Ps	2
		Rf values and color	-	0.34(g), 0.53(g)
5.	Toluene: Ethyl acetate: Methanol: Acetic acid (3:4:3:1)	No. of spots	Nss	Ps
		Rf values and color	-	-
6.	Ethyl acetate: Isopropyl alcohol: Water (65:25:10)	No. of spots	Ps	Nss
		Rf values and color	-	-
7.	Chloroform: Ethanol:Acetic acid (94:5:1)	No. of spots	Nss	Ps
		Rf values and color	-	-

CONCLUSION

L. inermis leaves contain various active constituents, these play key role behind its therapeutics efficiency in treatment of various disorders like bronchitis, boils, scabies, amenorrhoea, splenic diseases and also used as diuretic, anticoagulant and anti-sickling agent. The key components reported in the present study are alkaloids, glycosides, hydrolysable tannins, flavanoids, steroids, proteins, carbohydrates and saponins.

The TLC profile shows three spots with solvent system of toluene: ethyl acetate (93:7) with Rf values at 0.51(green colour), 0.32(brown colour) and 0.71 (red colour) whereas L. inermis extract prepared in 80% ethanolic extract by maceration (HM)) showed one spot with solvent toluene: ethyl acetate (93:7) with Rf values at 0.35 (g). When solvent of benzene: acetic acid (9:1) two spots were observed in the HS with Rf. 0.34 (Green colour) and 0.53 (green colour) where as poor separation was observed in the HM case.

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