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Analytical Estimation of Secondary Metabolites In Lawsonia Inermis Leaves

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ABSTRACT

Lawsonia inermis (Lythraceae) commonly called as Henna or Mailanchi is known for its cosmetic properties. Henna is an important source of phytochemicals of immense medicinal and pharmaceutical significance such as alkaloids, naphthoquinone derivatives, aliphatic components, tannins, phenolic derivatives, coumarins, xanthones, flavanoids, therefore an effort to compile all the information reported on its phytochemical and pharmacological activities, so that interest could be diverted towards this dye herb, for the treatment and relief from various ailments and diseases. The resultant extracts were analyzed by thin layer chromatography (TLC), column chromatography, ultraviolet spectroscopy (UV), Infrared (IR) spectroscopy, and High performance liquid chromatography (HPLC) techniques UV analysis was done by diluting the extracts with their respective solvents. 2 to 3 peaks were observed for extracted samples at 212 nm, 238.60 nm and 283.30 nm in methanol extract, 241 nm in chloroform extract, 238 nm in chloroform: methanol extract and 272.20 nm in ethyl acetate extract respectively. In HPLC compounds methanol extract is carried out for HPLC separation, and mainly three peaks were observed at 2.057, 2.522, 4.133 min retention times, which is the confirmation of presence of three separated compounds.

Keywords: Lawsonia inermis, HPLC,UV, IR.

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INTRODUCTION

The plant Lawsonia inermis (Lythraceae) commonly called as Henna or Mailanchi is known for its cosmetic properties. Henna is an important source of phytochemicals of immense medicinal and pharmaceutical significance such as alkaloids, naphthoquinone derivatives, aliphatic components, tannins, phenolic derivatives, coumarins, xanthones, flavanoids, gallic acid, hennot annic acid and mannitol which are effective as immunomodulators and other allied agents.

A medicinal herb is different from botanic term "Herb". It refers to any plants used for medicinal purposes. For example, a medicinal herb can be a real herbal plant, a shrub, other woody plant, or a fungus. The used part may be the seeds, berries, leaves, barks, roots, fruits, or other parts of a plants, or mushroom, which may be considered "herbs" in medicinal or spiritual use.

Henna is an important source of phytochemicals of immense medicinal and pharmaceutical significance such as alkaloids, naphthoquinone derivatives, aliphatic components, tannins, phenolic derivatives, coumarins, xanthones, flavanoids, gallic acid, hennotannic acid and mannitol which are effective as immunomodulators and other allied agents.

An ointment prepared from the leaves was used to cure ulcers and wounds. The astringent stem bark of *L. inermis*is traditionally used in India for the treatment of jaundice, enlargement of liver and spleen. It has been reported to posses' anti-inflammatory, antimicrobial and anti cancer activity. One of the major active constituents of leaves of Lawsonia inermis are flavanoids. Leaves and flowers are also better sources for the management of various ailments; leaves are useful for the treatment of diarrhea, dysentery, leprosy, scabies and boils, and the flowers are used in cephalalgia, burning sensation, sardiopathy, anemia, insomnia and fever.[2]

MATERIALS AND METHODS

Materials

Methanol, Chloroform, Ethyl acetate, Acetonitrile, HPLC water.

Methods

Soxhlet extraction method:

Leaves of Lawsonia inermis were collected, shade dried at room temperature for 3 days and ground in a manual mill and sieved with 2mm copper sieve to form uniform powder. 100 g of dried powdered drug was weighed and filled in the thimble of Soxhlet apparatus. After that the thimble was fixed with the round bottom flask, and the assembly was attached to the condenser. And the paraffin wax was put at the joints of the assembly for the easy removal of the assembly at the completion of the extraction procedure. Then the solvent for extraction (chloroform) was

filled for the removal of chlorophyll followed by methanol. After completion of the extraction procedure the extract was filtered using Whattman filter paper and then concentrated at 45°C. Dried extracts were kept at 20°C until further test were carried out.

Solvent system: Chloroform, methanol

Drug solvent ratio: 1:5

Time of extraction: 16 hours

Temperature for extraction: 60-70^{0}C

Maceration method:

The leaves were shade dried at room temperature and ground in a manual mill and sieved with 2mm copper sieve to form uniform powder. The sieved powder was used for evaluation and extraction purpose. The powder of Lawsonia inermis leaves was extracted by maceration method using different solvents (methanol, chloroform, ethyl acetate, chloroform-methanol). Dried extracts were kept at 20°C until further tests were carried out.

RESULTS AND DISCUSSION

The powder of leaves was extracted with methanol, chloroform, chloroform: methanol and ethyl acetate by maceration method individually.

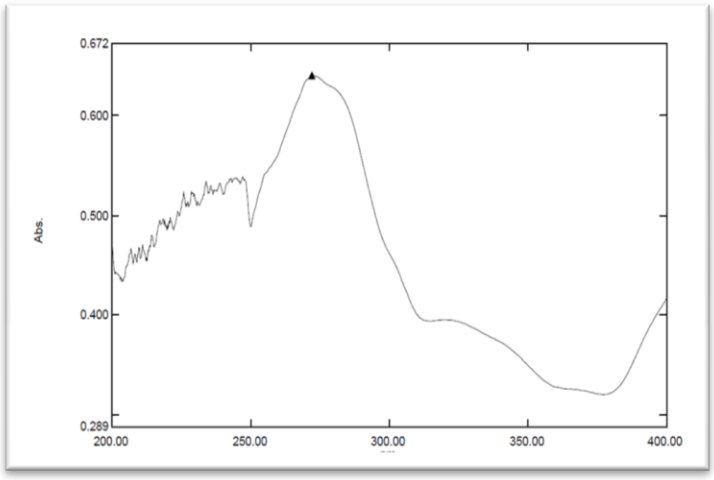
The resulted extracts were tested for phytoconstituents by preliminary phytochemical constituents in Table1. Different leaves extracts of *Lawsonia inermis* showed presence of secondary metabolites such as alkaloids, flavonoids, tannins, steroids, terpenoids, glycosides and phenolic compounds.

UV analysis was done by diluting the extracts with their respective solvents. 2 to 3 peaks were observed for extracted samples at 212 nm in Figure 1, 238.60 nm and 283.30 nm in methanol extract, Figure 2,241 nm in chloroform extract, Figure 3,238 nm in chloroform: methanol extract and 272.20 nm in ethyl acetate extract respectively.

Table1: Phytochemical tests of leaves of Lawsoniainermis:

Phytoconstituents	M E	Chl E	Chl M E	EA E
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	-	-	-
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Glycosides	+	+	+	+
Phenols	-	-	-	-

M E = methanol extract, Chl E = chloroform extract, Chl M E = chloroform-methanol extract, EA E = ethyl acetate extract, "+" = present, "-" = absent



No	Wave length	Absor bance	Description
1	212.00	0.510	Methanol
2	238.60	0.421	Methanol
3	283.30	0.192	Methanol

Figure1: UV spectrum of methanol extract(leaf)

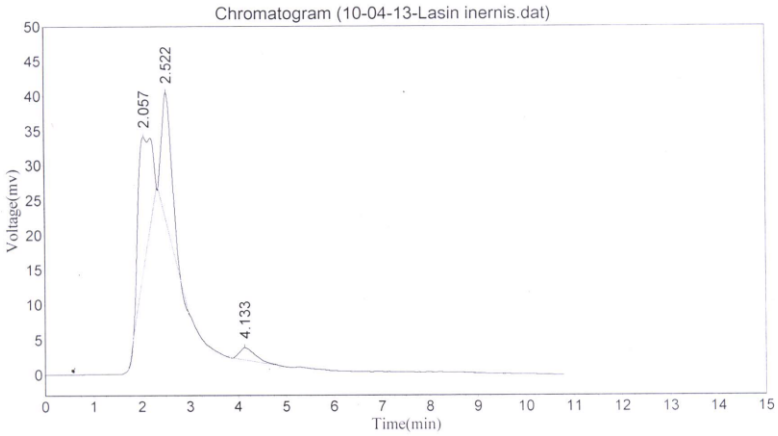
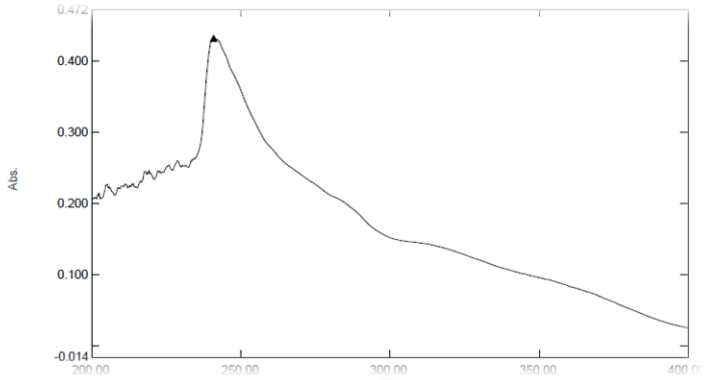
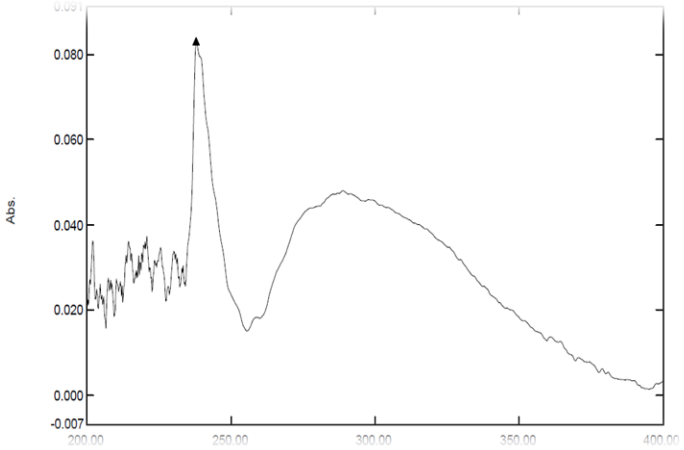


Figure2: UV method of Chloroform extract (leaf



No	Wavelength	Absorbance	Description
1	241.00	0.431	LI Chloroform

Figure 3: UV method of chloroform: methanol extract (leaf)



No	Wavelength	Absorbance	Description
1	238.00	0.083	Chloroform:methanol

Figure 4: UV spectrum of ethyl acetate extract

Then the samples were examined for the confirmation of bond formation between the groups by using IR spectroscopy Figure 5,6, and results were proved the presence of different functional groups such as alkanes, alkenes, ketones, esters, lactones, aromatics, symmetric nitro compounds, asymmetric nitro compounds, ethers, chalcones and aurones etc.

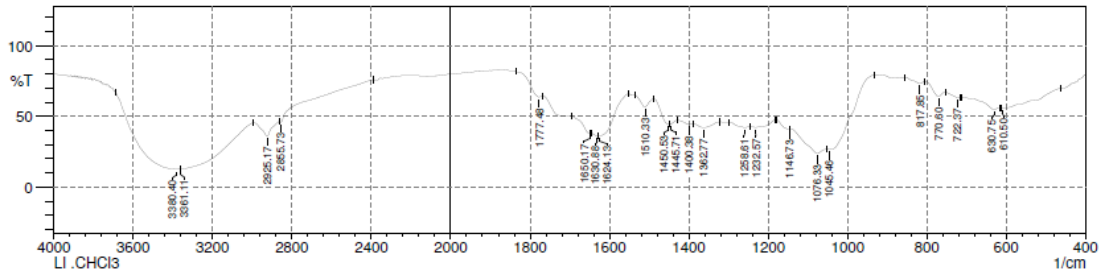


Figure5: IR method of chloroform extract (leaf)

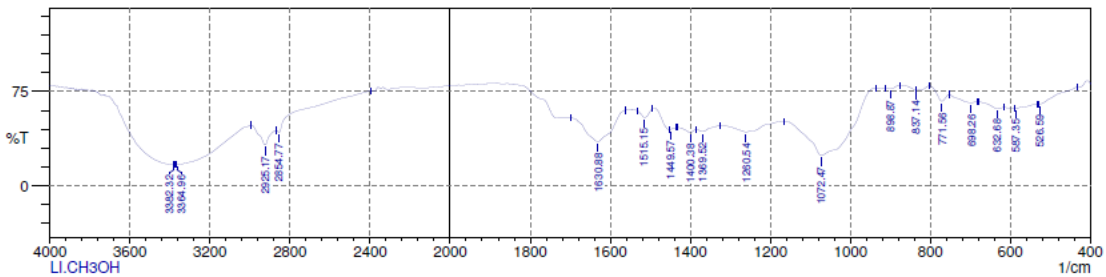
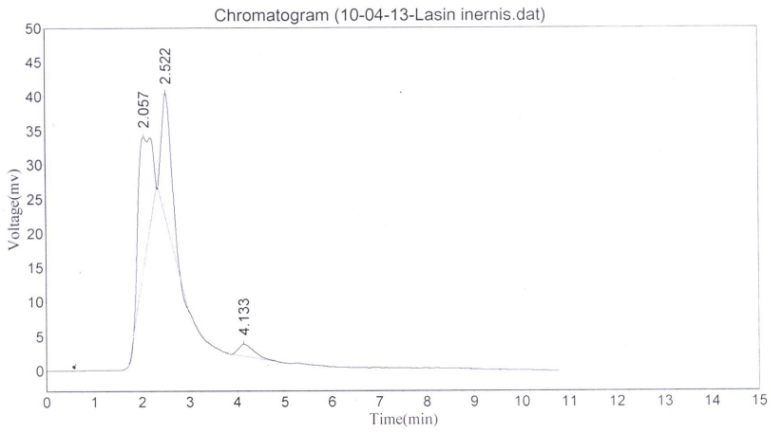


Figure 6: IR method of methanol extract (leaf)

The present study concludes that *Lawsonia inermis* may be useful as antibacterial agent due to the presence of secondary metabolites like tannins. It can be used in combination with other natural drugs.

For further confirmation of compounds the methanol extract is carried out for HPLC separation with mobile phase potassium di hydrogen phosphate + di potassium hydrogen phosphate buffer pH adjusted with 6 and methanol with the ratio of 50:50% v/v at the wavelength 254nm, Figure 7, and mainly three peaks were observed at 2.057, 2.522, 4.133 min retention times, which is the confirmation of presence of three separated compounds.



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Peak No	Peak ID	Ret Time	Height	Area	Conc.
1		2.057	19022.578	347034.406	53.7595
2		2.522	18556.305	259217.984	40.1558
3		4.133	1742.173	39278.504	6.0847
Total			39321.056	645530.895	100.0000

Figure7: HPLC method of Methanol extract (leaf)

CONCLUSION

The result of present study supports the traditional usage of the studied *Lawsonia inermis* and suggests that some of the plant extracts possess compounds used in treating various ailments. Wherein a detailed research work in the characterization and standardization is required for this potential plant for developing its various formulations, which can ultimately be beneficial for human beings as well as animals. It can be used in combination with other natural drugs. As the leaves extracts of the plant confirmed the presence of compounds with HPLC, UV and IR

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