

LAWSONIA INERMIS L. (HENNA): A COMPREHENSIVE REVIEW OF ITS THERAPEUTIC POTENTIAL, PHYTOCHEMISTRY, AND RISKS OF CHEMICAL ADULTERATION

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ABSTRACT

Henna, scientifically known as *Lawsonia inermis L.* or *Lawsonia alba* is a plant used in various industries for the purpose of beautification, prevention as well as for therapeutic effects. It has a wide range of Phytochemicals and essential oils present in it. The main chemical constituent called Lawsone giving it the distinct color with odor and an astringent fragrance. Due to the presence of Lawsone, henna leaves acquire dyeing property that leaves a reddish orange color on application on skin or hair. It also has proven to have therapeutic properties like Anti-microbial, Termiticidal and Anti-Alzheimer's property. In recent years, more importance is given towards standardization and evaluation of herbal marketed products, as different companies use different active constituents for its formulations which are not claimed on the label and hence regulatory issues and its efficacy and safety concerns arise. Henna has also known for showing non-toxic and eco-friendly nature due to which it is used in dyeing industries. Comparative analysis of various henna products

from different regions, its bioactive properties, adulterations and safety are some of the points focused in the following review article. One of the possible chemical adulterants for henna is Para-phenylenediamine, that is added to enhance the dyeing property of henna, but is a Carcinogen.

KEYWORDS: Henna, Lawsone, Therapeutic, Adulterant, Para-phenylenediamine.

INTRODUCTION

Recently, there has been an increasing demand for plant-based products in many of the developing countries. These products are being categorized as medicinal products, Nutraceuticals and Cosmetics. The need for standardization and authentication of these products has highly increased.^[1] *Lawsonia inermis* L., commonly known as Mehndi or Henna is an example of such products.

The Henna plant is a native to Middle East, North Africa and the Indian subcontinent.^[2] *Lawsonia inermis* L., belongs to the Lythraceae family popularly known for its significant color potential. *Lawsonia inermis* L. is a shrub standing 2-6m tall and has a whitish bark. The bright green, desiccated leaves are slightly crinkled, 2-4cm in length, hairless, entire and pinnately veined. The leaves have a distinct odor, with uncharacteristic flavors and little astringency. The flowers also contain odor, are white to pink in color and arranged in big pinnacles. The fruit is small, globular, capsular and reddish, containing several angular seeds in each compartment.^[2] The species is also known as *Lawsonia alba* Lam. or *Lawsonia ruba*. *Lawsonia* was named after Isaac Lawson, an 18th century Scottish army doctor, a friend of Linnaeus; and *inermis* in Greek means unarmed or without spines.^[3]

Lawsonia inermis L. (Henna) has Therapeutic, Medicinal, Beautifying effects. Natural Henna is being used in Egypt, Africa and India for dying hair since thousands of years. Henna is a very valuable element for traditional medicines, like Ayurveda. In India, as a part of Hindu, Sikh and Muslim weddings, as well as in the Middle East, Henna is applied during wedding ceremony. This particular event is called as Mehendi Night.^[4] At times, application of henna in form of extract or paste containing adulterants can show moderate to severe side effects. The above-mentioned properties of henna are determined by using various parameters such as the physical characteristics, physicochemical properties, medicinal properties using analytical methods. Back then, when animals used to chew the leaves of henna, their mouths were stained with orangish tint, if humans tried clearing it with their hands, it might have stained their hands leading to the discovery of henna leaves.^[5]

SCIENTIFIC CLASSIFICATION

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida

Order:	Myrtales
Family:	Lythraceae
Genus:	<i>Lawsonia</i>
Species:	<i>Inermis</i>



Fig 1: Leaves of *Lawsonia inermis* L. (Henna).^[4]

CHEMICAL CONSTITUENT

Lawsonia inermis L. contains an orange-red colored pigment known as **Lawsone** $C_{10}H_6O_3$ (2-Hydroxy-1,4-Naphthoquinone), the molecules of Lawsone are called as Hennotannic acid. The name and structure show similarity between Lawsone and Naphthalene. In lawsone two oxygen are attached to the naphthalene at carbon 1 and carbon 4 to form 1,4 naphthoquinone and a hydroxy group present at carbon 2. The lawsone molecule contains 10 carbons, 6 hydrogens and 3 oxygens in total. The molecular weight of lawsone is 174.16 atomic units of mass.^[4]



Fig 2: Chemical Structure of Lawsone compound.^[4]

TRADITIONAL USES OF *Lawsonia inermis* L. (Henna)

Various studies show that various plant parts of henna are recognized for varied pharmacological activities such as, ethanolic extract of dried and powdered leaves, aqueous extract of dried and powdered leaves, whole plant extract with methanol and water show antioxidant activity. Further, chloroform extract of dried leaves, aqueous and alcoholic extract of dried and powdered leaves show anticarcinogenic activity. Crude extract of fresh and dried leaves shows antimicrobial property. Leaves extract and bark extract show antifungal activity. Ethanolic extract of leaves are virucidal. Crude ethanolic extract of leaves show a number of Pharmacological activities such as anti-inflammatory, analgesic, and antipyretic activity. Aqueous and hydroalcoholic extract of leaves are Nootropic agents that mainly improve human thinking, learning and enhances memory.^[6] Leaves of *Lawsonia inermis* L. provides an important cosmetic dye. *Lawsonia inermis* L. (Henna) leaves were extensively used for centuries in the Middle East, the Far East and Northern Africa as dye for nails, hands, hair and textile. Henna is also used in treating skin problems, headache, jaundice, amebiasis and enlargement of the spleen. Whole plant, roots, fruits, stem, leaves, barks, inflorescence, rhizome, bulbs, latex, seeds, flowers and oil were used in different ailments.^[7]

PHYTOCHEMICAL COMPOSITION

The preliminary screening of two different henna powders shows 10 secondary metabolites present in the leaves such as, alkaloids, flavonoids, glycosides, tannins, carbohydrates, saponins, sterol, quinines, resins and fats/lipids, having importance in medicinal and industrial sciences.^[3] Lawsone, a reddish orange dye compound, is the primary active ingredient responsible for its coloring properties and medicinal benefits. The main components which are phenols, O-H, C=O and alkenes C=C can be found in both the henna powders extracted using methanol and ethyl acetate as solvents. These are the main components present in Lawsone structure.^[8] These compounds contribute to henna's therapeutic efficacy by acting as natural antioxidants and antimicrobial agents.

PHYSICOCHEMICAL PROPERTIES

The appearance of the powder is green in color with a distinctive odor which is typical for henna, and a bitter taste. Physiochemical investigation of leaves is shown in the table given below.

Table 1: Physiochemical investigations on henna leaves.

Sr. No	Physiochemical properties	Percentage (%)
1.	Total Ash	14.60%
2.	Acid insoluble ash	4.50%
3.	Water soluble ash	3.0%
4.	Loss on drying	4.5%
5.	Alcohol soluble extractive value	3.8%
6.	Aqueous extractive value	5.0%

Phytochemical analysis of the aqueous root extracts of the plant indicates the presence of reducing sugars, tannins, flavonoids, saponins, and alkaloids.^{[9][10][1]}

INHIBITIVE ACTION OF *Lawsonia inermis* L. (Henna)

A study explores the use of henna extract *Lawsonia inermis* L. as a natural corrosion inhibitor for aluminum alloy 5083 (AA5083). Unlike synthetic inhibitors, that are often expensive and harmful to the environment and toxic to living beings, henna provides an eco-friendly solution. The effectiveness of extracts prepared using ethyl acetate and methanol is assessed through weight loss analysis, electrochemical techniques, and surface analysis (SEM/EDS). The presence of heterocyclic compounds with conjugated double bonds in the henna extract found using FTIR contributes to its corrosion resistance. Results indicate that henna acts as a mixed-type inhibitor, mainly affecting the cathodic process, with lawsone being the key component responsible for its inhibitive properties.^[11]

G6PD Deficiency

Glucose-6-Phosphate Dehydrogenase is an enzyme, which helps red blood cells to work properly by preventing reactive oxygen species from building up. G6PD deficiency is seen more in males than females. It is an X linked deficiency, due to the mutations seen on X chromosome. Males are the ones with exclusive hemolytic crisis and anemia, whereas females are heterozygous, meaning that they are carriers without suffering any consequences. Thus, henna can be dangerous for male population. It has also been reported that some ethnic groups of Middle East and Africa are more vulnerable to it particularly infants and children.^[4] Extended applications of henna in people with G6PD deficiency will cause oxidative hemolysis.^[12]

ANALYTICAL TECHNIQUES FOR ANALYSIS OF HENNA

1. TLC Profiling of Henna leaf Extract

TLC profiling of leaf extracts of Egyptian and Nigerian Henna show 9 different bands ranging between R_f value of 0.20 to 0.86.^[3] These detected compounds show different colorations such as Yellow for Flavonoids, Orange for Alkaloids, Green for Chlorophyll and Purple for Anthocyanin, etc. The comparison of these two variants of Henna show almost similar R_f values ranging from 0.2 to 0.8.^[3] Even though the plants are taken from two different locations, this study indicates the similar phytochemicals present in them.

2. Dye uptake value (DE)

The dye uptake value at various soaking time analyzed using HPLC. The DE value at immediate and 30 mins soak time gives variably lower dye uptake value, whereas the DE value at greater than 4hrs soak time shows slight decrease in the dye uptake. The study suggests that the optimum soak time for maximum release of Lawsone for better color is 4 hours.^[5]

3. HPLC analysis

Analysis of henna sample is carried out using HPLC method, where sample of varied concentration are analyzed at 254nm by injecting two different sample application volumes. The presence of Lawsone is detected by comparing the AUC of sample peaks with the AUC of standard peak.^[4] This analysis shows about 1.62% of Lawsone present in the leaf powder.^[5]

Another Qualitative and Quantitative analysis of three alkaline extracts of henna leaves using HPLC, show the effect of the dyeing temperature on color strength. This analysis reveals three different peaks found at three different intervals of times show a difference of 0.3 each. This indicates that the increase in dyeing temperature increases the intensity of the color. After 24hrs the intensity the color reaches an exhaustion point and starts to fade way.^[12]

4. Essential Oil Composition analysis by GC-MS

Essential oil composition of *Lawsonia inermis* L. is determined using GC-MS analysis with help of HP-5MS column.^[13]

The nine compounds identified show 80.8% of total oil content. The major component is Eugenol (17.61%), followed by Hexadecenoic acid (15.07%), Phytol (10.17%), alpha-

terpineol (8.36%), Etherphenylvinyl (6.72%), 1,3-indandione (6.60%), Oxirane-tetradecyl (6.20%), Cis-hexahydro-8a-methyl, 8- [2H, 8H]-nephthalenedione (5.60%) and Linalool (4.23%).^[13]

THERAPEUTIC PROPERTIES OF *Lawsonia inermis* L. (Henna)

1. Antimicrobial and Antibacterial activity

The antimicrobial and antibacterial properties of plant extracts depend on numerous factors such as the environmental and climatic conditions, the solvent that is used for the extraction, the protocol followed for the extraction and the test concentrations.^[14]

The ethanol extract of 20 plant species is studied for the treatment of pathogenic diseases caused by both gram-positive and gram-negative bacteria. Among all the 20 plant species tested, the ethanolic extract of *Lawsonia inermis* L. shows the highest antibacterial activity.

Different pathogenic strains tested in-vitro confirms antimicrobial activity as the growth of strains is inhibited by the aqueous, methanol, and chloroform crude extracts of *Lawsonia inermis* L. leaves.^[15]

Ethanol, ethyl acetate and n-hexane extract of *Lawsonia inermis* L. leaves is studied for their antibacterial potential (1000 µg/ml) where both the gram-positive and Gram-negative bacterial strains such as *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Enterococcus faecalis* are tested using disc diffusion assay. This study reports that all extracts possess the antibacterial activity against the tested bacteria. Amongst the tested extracts, highest antibacterial effect is seen in ethanol extract, followed by ethyl acetate and n-hexane.^[7]

2. Termiticidal activity

The biocide activity of *Lawsonia inermis* L. is tested using various concentration of leaf pastes on treated and untreated wood against field termite species. The termiticidal activity is expressed as % wood weight loss.

The weight loss of less than 10% for bio deteriorated wood sample is considered as excellent or highly resistant according to the ASTM D 2017-81. More than 10% of wood weight loss can cause significant damage to the mechanical properties of the wood. The % weight loss is compared to the decay resistance class ASTM D 2017-81.^[16] This study presents the positive effect of termiticidal activity of *Lawsonia inermis* L. on the treated wood.

3. Anti-Alzheimer activity

The methanolic extract of seeds of *Lawsonia inermis* L. is assessed for its antioxidant properties with respect to Cholinesterase inhibition and neurotoxicity, where henna shows inhibition over cholinesterase. It shows that henna is a strong inhibitor for both acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE); and it shows metal chelating properties as well.^[17]

4. Antioxidant and Anti-inflammatory properties

A study reveals that methanolic extract of henna is effective in increasing the antioxidant enzymes, hepatic glutathione reductase, superoxide dismutase, and catalase activities. The study is conducted to determine the radical scavenging activity and antioxidant by performing the experiment on *Lawsonia inermis* L. seeds. Amongst the extracts tested to determine the antioxidant activity of henna; the ethanolic extract of *Lawsonia inermis* L. seeds is an efficient antioxidant as compared to aqueous extract, petroleum ether extract and dichloromethane extract, as ethanolic extract consists higher concentration of phenolic and flavonoids compounds.^[15]

Another study on methanol extract of *Lawsonia inermis* L. flowers shows anti-inflammatory activity against 5-lipoxygenase due to the high amounts of total phenolic compounds present in it. Also, the crude ethanolic extract of the *Lawsonia inermis* L. shows anti-inflammatory, analgesic, and antipyretic activity in rats.^[18]

5. Wound healing and skin health

A study shows that the water and chloroform extracts of henna leaves are effective against the growth of microorganisms that cause infections on burn wound. The ethanolic extract accelerates the healing process.^[15]

According to Unani physicians, the plant consists anti-inflammatory as well as analgesic activities. It is known to be used in the treatment of boils, bruises, burns and scald. The extracted plant oil shows healing effect on the test group with a full re-epithelialization and re-appearance of skin appendages with well-organized collagen fibers and no inflammatory cells; but same effect is not seen on the untreated group.^[18]

CHEMICAL ADULTRANT IN *Lawsonia inermis* L. (Henna)**PPD adulteration**

(PPD) p-phenylenediamine is an amine, with chemical formula $C_6H_4(NH_2)_2$. It is a highly toxic, white colored substance which on coming in contact with air oxidizes to give reddish brown color. On contact with the skin, it causes contact dermatitis, hemolytic anemia, hepatic and renal failure. It is known to give permanent black color when applied to skin, hair, fabric and leather, hence it is commonly used in Henna to enhance the color. PPD is used in oxidative Hair colors under the permissible limit of 4.0%. But the use of PPD in henna is unacceptable above the limit of 9.0 % due to its adverse effects.^{[19] [20]}

In a Study conducted using HPLC, PPD is found in most of the examined henna samples, containing an appreciable amount ranging between 9.1-88%, which can be considered as a toxic adulterant. The high concentration of PPD can cause the hazard since PPD is known to have a toxic and carcinogenic effects.^[20]

ANALYTICAL TECHNIQUES FOR DETECTION OF CHEMICAL ADULTRANT:**1. High Performance Liquid chromatography (HPLC)**

HPLC is widely used to analyze the chemical composition of henna and detect various adulterants such as PPD and other synthetic dyes. In this technique the compounds are separated based on their chemical properties and by measuring the concentration. HPLC can efficiently differentiate between the natural compounds of henna, such as lawsone, and synthetic additives like PPD.

Assessment of PPD in *Lawsonia inermis* L. with the use of HPLC shows altered composition of the sample by presence PPD. The PPD standard shows retention time at 4.49 minutes which when compared to test samples shows presence of high concentration of PPD in all Black henna samples used for test.^[19]

2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR identifies the functional group in compounds by measuring their absorption of infrared radiation causing the functional groups to vibrate and stretch. The peaks observed in the sample's spectrum show resemblance when compared to the spectrum of standard PPD, where the amino group (-NH) is a key factor in determining the presence of PPD. This is used to distinguish between natural henna and samples adulterated with PPD.^[21]

3. Gas Chromatography - Mass Spectrometry (GC-MS)

This is a highly sensitive technique that combines both the features gas chromatography and mass spectrometry to separate, identify and quantify the volatile compounds in a sample. GC-MS can be used to detect the low levels of henna adulterants in the products.

The extracts of *Lawsonia inermis L.* leaves prepared using chloroform, ether, methanol, and ethyl acetate analyzed on GC Clarus 500 Perkin Elmer system and connected to Mass-spectrometry, shows the retention time of PPD in samples between 2.53 to 2.63minutes. The abundance of PPD between 2.33 to 37.04 % is seen in samples labelled as Black Henna, whereas samples labelled as red henna show absence of PPD.^[22]

CONCLUSION

As the world is now opting for non-toxic plant-based products, the rise of drug-resistant pathogens has made the traditional medicines gain importance. *Lawsonia inermis L.* (Henna) offers an efficient alternative to modern drug due to its wide range of pharmacological activities, therapeutic effects and benefits. This plant has various phytochemicals that can treat a wide range of diseases. Ethnopharmacological studies on henna are on the rise. The extensive research on *Lawsonia inermis L.* demonstrates its significant pharmacological and therapeutic potential. However, while in vitro and in vivo studies support its efficacy, clinical trials are still needed to validate these effects in humans. The analysis of *Lawsonia inermis L.* is necessary as to know the purity and quality of the plant that is being used. Given its rich phytochemical composition, *Lawsonia inermis L.* remains a valuable subject for future research in pharmacology and traditional medicine. The chemical adulteration of *Lawsonia inermis L.* remains a significant concern, with various synthetic chemicals being added to improve color, texture, consistency and reduce production costs. However various bioanalytical techniques such as HPLC, FTIR, GC-MS have provided effective procedures to identify and detect these chemical adulterants. These techniques not only provide consumer safety but also contribute to the high quality of henna products.

PROSPECTS OF FUTURE RESEARCH

More research is needed to better understand the mechanism through which henna works. It's also essential to establish its safety and efficacy profiles in a clear and reliable way. Developing standardized formulations will be crucial for its clinical settings. Furthermore, exploring innovative delivery systems, such as nanoparticles and liposomes, could improve the bioavailability of henna's active compounds and allow for more targeted delivery. Efforts

should be made on creating new formulations of henna extracts for both topical and internal use to understand and enhance the bioavailability and effectiveness of its active compounds. Additionally, clinical trials could be conducted to assess the safety and potential benefits of henna extracts in treating various human diseases. Exploring and identifying new drug targets based on henna's properties would also be a valuable step forward. To unlock the full medicinal potential of henna, extensive research is needed to explore its use in treating various diseases. Given its wide range of therapeutic properties, this plant warrants special attention from scientists and researchers aiming to develop groundbreaking treatments. However, more in depth studies are necessary to uncover the hidden benefits of *Lawsonia inermis* L. and its potential application for improving human health.

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