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Review Article

AN UP-TO-DATE REVIEW ON THE PHYTOCHEMICAL AND PHARMACOLOGICAL ASPECTS OF THE *Lawsonia inermis*

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ABSTRACT

Medicinal plants are extensively used throughout the world to treat a variety of ailments because of their safety, efficacy and affordability. *Lawsonia inermis* or Henna is commonly used in folk medicine for the cure of several illnesses. Particularly, it is used as a hair and hand dye. The objective of this study is to offer the most recent information regarding the pharmacological and phytochemical profile of the medicinal plant that is backed by evidence. Only indexed research and review papers were taken into account for the present study's data gathering in order to ensure authenticity. Only articles published in English language were considered for this study. To conduct a literature survey, various databases such as Scopus, Google Scholar, PubMed, Science Direct, and MEDLINE were searched without any year limit. The following keywords are used to search the data: "*Lawsonia inermis*", "medicinal plants", "herbs", "phytochemical", "pharmacological", "*in vivo*", "*in vitro*", "Henna" and "toxicity". The study's findings showed that the plant contains a variety of potent phytochemicals, including Lawsona, linarigenin, linarisenin, luteolin, lawsochrysin, lawsoniaside, lawsonaphthoate e.tc. The fruits, leaves, and stems *of L. inermis* have all been demonstrated to have potential pharmacological effects in previous investigations. So far, its antioxidant, cytotoxic, antimicrobial, antiprotozoal, immunomodulatory, hypoglycemic, anti-inflammatory, and anticancer properties have been documented. Even though many aspects of the plant have been researched, additional study is still necessary to completely understand the mechanism behind its pharmacological actions, to confirm its medicinal efficacy, and to justify its usage in traditional medicine.

Keywords: *Lawsonia inermis*, Henna, Medicinal plants, Traditional Medicine, Pharmacological activities

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INTRODUCTION

The maintenance and improvement of human health depend greatly on medicinal plants. Natural components found in medicinal plants can aid in the treatment, mitigation, and prevention of disease (Qayyum *et al.,* 2020; Ashiq *et al.,* 2021; Qurat-ul-Ain *et al.,* 2022). The treatment of diseases and toxins by various plants has been documented by all ancient civilizations (Ashiq *et al.,* 2022). The World Health Organization (WHO) claims, the majority of the world's population relies almost entirely on complementary medicines like herbal medications since they are more affordable for patients. In the United States of America (USA), one out of every 25 prescriptions is based on herbal medication, demonstrating the importance of herbal treatment (Latif *et al.,* 2019). Given the significance of medicinal plants, the goal of this study is to provide the most recent, fact-based information on the pharmacological and phytochemical profile of the medicinal plant called *Lawsonia inermis*.

The plant *L. inermis* belongs to family Lythraceae. It is a 2-4 meters tall shrub, with smooth, elliptical, oppositely arranged leaves with dorsal placed thin veins. The flowers are delicate, having 4 sepals, white or red stamens and spear-shaped lobed. The henna fruit is a small brown capsule containing 30-40 seeds (Siddiqui *et al.,* 2003; Al-Snafi, 2019). *L. inermis* can endure extended drought and poor soil conditions and flourishes in a semi-arid, cold climate. *L. inermis* preserves green leaves in its natural habitat even over prolonged periods of drought; browsing animals that consume the last of their environment's leaves have their lips stained red-orange by the lawsone present in henna leaves (Sharma *et al.,* 2016). During a severe drought, the plant may lose its leaves and go dormant. *L. inermis* with smaller leaves is thought to be of higher quality and greater potency than with larger leaves. The leaves might be five to six times smaller in the dry season and in dry areas than they are in the rainy season or in wet areas. Wild plants are found river banks and flooded area in rocky areas they may be found in crevices at 1350 meters (Pour *et al.,* 2018).

Taxonomic classification
Kingdom: Plantae
Phylum: Tracheophytes
Class: Angiospermae
Order: Myrtales
Family: Lythraceae
Genus: Lawsonia
Species: *Lawsonia inermis*

Vernacular names and habitat: Common name of *L. inermis* is Henna or Hina in Hindi and Arabic. It is also called Egyptian privet, mignonette tree in Jamaica, French Henne, Mehndi in Urdu and Alcana in Spanish. North Africa, Asia, the Middle East, Egypt, America, and Australia are its home continents (Malekzadeh, 1968; Syeda *et al.,* 2020).

Ethnopharmacology: Traditionally, all over the world, henna is applied to hands, feet and hair due to its cooling effect and colouring or dye properties. Especially in marriages people use to apply it on skin for beautiful designing and a cooling effect. That's why it became popular in hot regions (Bashir *et al.,* 2021). The parts of *L. inermis*, including leaves, flowers, seeds, stems, bark, and roots are employed to treat numerous illnesses as these parts contain various phytochemicals. In traditional medicine, the plant is used to cure a wide range of illnesses, including inflammation, pain, rheumatoid arthritis, amoebiasis, headache, ulcers, jaundice, diarrhea, leprosy, fever, leucorrhoea, diabetes, anti-hemorrhagic, fungal infections, and cardiac and hepatic diseases. In the past, it was used to treat hair for lice and dandruff. Its oil is said to promote hair growth when applied to balding areas of the body. Further, it was used to stop tissue loss in cases of leprosy, ulcers, and diabetic foot problems. It has astringent and hypotensive properties (Kamal, 2010; Akbar, 2020). The plant is quite effective in treating dysentery. For liver illnesses and related issues, seeds in powered form are given. *L. inermis* flower extract is used to create fragrances. The bark is used to cure burns and has been given internally to treat calculus, jaundice, and spleen enlargement (Muhammad and Muhammad, 2005). The plant root has an astringent effect and is therefore used in the treatment of irritated eyes. Further, root is also regarded as a powerful treatment for herpes and gonorrhoea. The ethomedicinal uses of the plant are summarized in table 1 (Tutor and Watch, 2017).

Table 1. Pharmacological actions by different parts of *L. inermis.*

Parts	Pharmacological properties
Leaves	Cough, burning sensation, bronchitis, Hepatopathy, leprosy, Anemia, Astringent ,anti -inflammatory, Constipating , liver tonic, Dysentery, scabies, fever, falling of hair, anti- dandruff ,greyness of hair, ,leprosy, burns, boils ,diarrhea, amenorrhoea,.
Bark	Enlargement of spleen, skin disorders, leprosy, burning sensation ,jaundice.
Seeds	Amentia, constipating, diarrohea, intellect promoting, gastrophy ,intermittent fever .
Flowers	Cardiopathy, insomnia, burning sensation, refragrant agent, amentia, cardiotonic, febrifuge,
Roots	Skin diseases, burning sensation, amenorrhoea, hair growth, leprosy.

MATERIALS AND METHODS

In order to assure validity, only indexed research and review papers were considered for obtaining data. For this study, only publications written in English were taken into consideration. Numerous databases, including Scopus, Google Scholar, PubMed, Science Direct, and MEDLINE, were searched without regard to the year of publication in order to conduct the literature review. "*Lawsonia inermis*", "medicinal plants", "herbs", "phytochemicals", "pharmacological", "*in vivo*", "*in vitro*", "henna", and "toxicity" are used as search terms for the collection of the data.

Phytochemicals: A large number of phytochemicals are identified from different parts of plant, the major ones being Lawsone and lawsonoside. *L. inermis* extracts are notably rich in phenolic chemicals, such as coumarins, flavonoids, and naphthoquinones. The abundance of these constituents implies the importance of the biological activities of the plant against several diseases (Hsouna *et al.,* 2011; Shivsharan and Kothari, 2020). The lawcoumarin was isolated from whole plant. The linarigenin, linarisenin, luteolin, lawsochrysin were isolated from plant leaves (Kebedekidanemariam *et al.,* 2013). Catechin, luteolin, apigenin, genistein were isolated from stems and leaves. Tannic acid is a major constituent of the plant (Siddiqui *et al.,* 2003). The seeds contain fatty acids (10–11%) made up of behenic acid, arachidic acid, stearic acid, palmitic acid, oleic acid, and linoleic acid, as well as proteins (5.0%), carbs (33.62%), fibers (33.5%), and fatty acids (33.5%). Lawsoniaside, lawsonaphthoate, glucosyloxy naphthalene were extracted from the plant's stems and leaves. The naphthalenes are the source of naphthoquinones, which are widely present in *L. inermis* (Hema *et al.,* 2010). The essential oil produced by the tiny, white, light to dark pink blossoms has a strong scent that is attributed to the presence of β-ionone (Wong and Teng, 1995). Harmine and harmaline were isolated as alkaloids. Fe, Cd, Mn, Cr, Pb, Fe, Ni, Ca, Na, P, K, Mg and Zn were also detected in minor quantities (Boubaya *et al.,* 2011; Saeed *et al.,* 2013). Due to the abundance of pharmacologically potent chemicals in *L. inermis*, further scientific study is required to back up the claims made for why this plant is used in traditional medicine.

BIOLOGICAL AND PHARMACOLOGICAL EFFECTS

Anti-inflammatory and analgesic activity: Since *L. inermis* possesses anti-inflammatory properties, it has been recommended for various skin conditions. Rats' inflammation was greatly reduced by *L. inermis* ethanolic extract (0.25–2.0 g/kg) in a dose-dependent manner (Liou *et al.,* 2013; Raja *et al.,* 2013). Further, 3, 1-5-diphenylpenteen and methyl naphthalene carboxylates were isolated from the plant. The best anti-inflammatory effect was demonstrated by these two isolated molecules (Alia *et al.,* 1995). In an investigation, lawsone was extracted from *L. inermis* leaves, and its analgesic and anti-inflammatory properties were evaluated, A total of 120 healthy adult mice (weighing around 255 g) were used. Out of the total, 60 mice were used to calculate the LD50, 30 mice were used to calculate the anti-inflammatory test, and the final 30 mice were used to calculate the analgesic test. The analgesic impact was assessed using a hot plate, and the anti-inflammatory effect was assessed by looking at how well the substance reduced swelling and inflammation brought on by the injection of carrageenan. Aspirin and lawsone both had analgesic effects in comparison to the control group. However, lawsone had a stronger analgesic effect than aspirin. Lawsone and aspirin, on the other hand, had an anti-inflammatory effect in comparison to the control group and were equally effective in reducing carrageenan-induced hind paw edema ($p = 0.05$) (Ali *et al.,* 2022).

Antidiabetic action: Excellent antidiabetic activity was observed by silver nanoparticles of *L. inermis* extract. *In vivo* hypoglycemic against wisteria rats showed potent hypoglycemic effects and showed no toxic effects (Kalakotla *et al.,* 2019). *In vitro* alpha amylase inhibitory activity of methanolic extract of the plant elicited a hypoglycemic effect and also showed potent anti-inflammatory activity (Ashiq and Ashiq, 2021; Imam *et al.,* 2013; Munir *et al.,* 2022).

Hepatoprotective action: For many years, the plant has been utilized for its hepatoprotective action. Aqueous extract of *L. inermis* was given to albino mice that had carbon tetrachloride 1.25 ml/kg induced liver damage. A considerable reduction in liver damage was seen after administration of the plant extract. The research also indicated that the hepatoprotective effects of the plant extract were dose-dependent. Triterpenes and flavonoids found in the plant extract are responsible for this action (Hossain *et al.,* 2011). Another study of hepatoprotection against paracetamol induced toxicity in rats showed evident reduction in bilirubin, acid phosphatase and other serum enzymes. The extract controlled the amount of marker enzymes and prevented hepatotoxicity. When compared to the control, the ethanolic extract and its fractions decreased the SGOT, SGPT, SAL activity, total bilirubin concentration, and liver weight (Selvanayaki and Ananthi, 2012). Similar studies against drug-induced hepatitis showed protection against drug-induced toxicity and reduced serum leakage (Hasan *et al.,* 2016; Kumar *et al.,* 2017).

Neuroprotective effects: In an investigation, the effect of *L. inermis* extract on chronic constriction injury (CCI)-induced neuropathic pain was assessed. *L. inermis* extract (250 mg/kg and 500 mg/kg) and gabapentin (100 mg/kg) were given intraperitoneally for 14 days following the CCI operation. According to the study's conclusions, *L. inermis'* anti-oxidant and anti-inflammatory activities may have neuroprotective effects against CCI-induced neuropathic pain (Rakhshandeh *et al.,* 2021).

Antioxidant activity: Antioxidant activity is one of the major attributes of *L. inermis*. The leaves are rich source of antioxidants (Jafarzadeh *et al.,* 2015). The ethanolic extract of plant seeds had good DPPH radical scavenging activity. IC_{50} of extracts was found to higher (5.4%) as compared to the the standard ascorbic acid drug (Philip *et al.,* 2011). Aqueous and methanolic extracts were found to have a high concentration of phenolic compounds, which were found to be positively correlated with cytoprotective effectiveness against chromium-induced oxidative cellular damage, lipid peroxidation inhibition capability, and radical scavenging potential. Some of the primary phenolic components in both extracts were discovered by HPLC analysis, and they may be the source of both the extracts' antioxidant capability and their DNA and cytoprotective effects (Guha *et al.,* 2011).

Another attempt to distinguish and highlight the antioxidative potential of plant extract by study on soya bean oil stability was conducted by keeping it in storage for 16 days at 63 ^{0}C. The methanolic extract at 1400 ppm and synthetic antioxidants, including butylated hydroxyanisole and butylated hydroxytoulene showed same results (200 ppm). The good antioxidant activity was suggested owing to presence of phenolic content in the extract (Hosein and Zinab, 2007). Using the DPPH and ABTS assay, the antioxidant capacities of *L. inermis* seed extracts in hexane, chloroform, and methanol were evaluated. In the DPPH and ABTS experiments, the methanolic extract demonstrated a higher antioxidant capacity (IC_{50} = 4.6 mg/l and 3 mg/l, respectively). In chloroform or hexane extracts, no antioxidant activity was found (IC_{50} > 100 mg/l) (Rafika Chaibi *et al.,* 2017).

Antibacterial activity: The percentage of lawsone in the plant parts ranges from 0.5 to 1.5. The plant's main component, lawsone (2-hydroxynapthoquinone) is what lends its colouring abilities. When compared to a typical antibiotic, the methanol extract showed better anti-bacterial action against *Bacillus subtilis*, *Streptococcus salivarius*, *Micrococcus*, *Klebsiella pneumonia*, and *Streptococcus aureus*. The results shown that the hexane extract only efficiently inhibits *Streptococcus aureus* at high doses. It is highly efficient against *B.subtilis* and *S. salivarius* when compared to other species. The chloroform extract is only slightly more effective than the hexane extract, and it works best against *Staphylococcus epidermidis* and *Streptococcus salivarius*. The methanol extract is quite potent against all organisms when compared to other extracts (Mastanaiah *et al.,* 2011).

An extensive study on the sequentially extracted flowers, leaves and fruits of *L. inermis* in DCM, ethanol and ethyl acetate solvents showed excellent antibacterial activity against *Eschericha coli*, *Pseudomonas aeruginosa*, *Streptococcus subtilis* and *Staphylococcus aureus* in contrast to control drug Streptomycin. Except for the DCM extract of flowers, all other test extracts inhibited all of the test bacteria at a concentration of 60 mg/100 ml. The inhibitory effects varied significantly ($P > 0.05$), and the diameter of the inhibition zone ranged from (9.8 ± 0.4 mm) to (26.7 ± 0.3 mm) (Jeyaseelan *et al.,* 2012). *In vitro* antibacterial activity against various Gram positive and Gram negative bacteria showed in a dose-dependent way. The plant extract was active at the lowest tested concentration of 7.2% of 62.5 g/ml and 125 g/ml, 5% at the dosage of 250 g/ml, 75.7% at the concentration of 500 g/ml, and 92.8% at the concentration of 1000 g/ml (Arun *et al.,* 2010). The hospital-based antibacterial activity of plant extract at various quantities shown good efficacy against a variety of microorganisms, including the lab standard strain of *Pseudomonas aeruginosa* (Habbal *et al.,* 2011).

Antifungal activity: *L. inermis* is traditionally used for the dyeing of hands, feet and hair due to the presence of lawsone that reacts with protein components. But in spite of beautification, it also possesses antifungal properties, hence increasing the hair luster, preventing dandruff on the scalp and locking moisture in the skin. Skin diseases caused by fungus on hands and feet was also lessened due to presence of lawsone ingredient. Commercial lawsone displayed potentially intriguing MICs against the isolates of *Aspergillus flavus* and *Fusarium oxysporum* (12 g/mL) respectively. When compared to other extracts, the ethanol extract had the only noteworthy MIC (230 g/mL of crude extract) against the strain *F. oxysporum* (Rahmoun *et al.,* 2013). Another study of different extracts of *L. inermis* against dermatophytes strains *Trichophyton* and *Microsporum* showed that aquesous extract was most effective compared to chloroform or methanol extracts. At the highest concentration 100 mg/ml most potent antifungal action of *L. inermis* (26 mm against *M. fulvum*) was exhibited by the aqueous extract (Sharma *et al.,* 2011). Trichophyton mentagrophytes and *T. violaceum* are more susceptible to ethanol extract with a MIC of 7.5 mg/ml, according to another dermatophyton activity. However, ethanolic extract at a dosage of 10 mg/ml was found to be toxic to all Trichophyton species (Suleiman and Mohamed, 2014). Further, derivatization from lawsone to substituted napthaquinones gave compounds that showed excellent antifungal results against *Curvularia lunata* and *Fusarium oxysporum* at all concentrations i.e. 120 mg/ml, 360 mg/ml and 600 mg/ml. Even at the lowest dose of 120 mg/ml, there was a significant proportion of spore germination inhibition (Brahmeshwari *et al.,* 2012).

Wound healing properties: The *L. inermis* plant has been shown to have wound-healing qualities through traditional applications. The plant's anti-bacterial and antifungal properties make it particularly effective at healing burn wounds. The mice model with excision, incision wounds compared to placebo control experiments revealed that the extract treated animals demonstrated a faster rate of epithelialization (12.4 ±0.13, days). The histological studies of the tissue obtained from the *L. inermis* treated group showed a significant increase in collagen deposition, few macrophages, reduction in tissue edema, and more fibroblasts. Hence, it was validated from the study that the plant has potential to promote wound healing (Nayak *et al.,* 2007). In another study, the epidermal regeneration that covers the entire surface of the lesion treated with henna oil was dyed to look into the organization of the epithelium and tissue. The scar zones that had been treated with oil under a microscope revealed complete, coordinated epidermal regeneration and increased thickness, which was regarded as normal (Rekik *et al.,* 2019). A formulation based study of wound healing consisted of *L. inermis* ointment was also conducted. Animals treated with ointment demonstrated greater wound contraction (14%) than those of the control group (14%) (Yassine *et al.,* 2020). The main phytochemicals involved in wound healing are coumarins, flavonoids, tannins, and alkaloids. Additionally, the plant promotes wound healing because it contains antioxidants that lessen malondialdehyde, lower the ratio of lipid peroxidation, and reduce RNA and DNA oxidation (Daemi *et al.,* 2019).

Anti-Alzheimer activity: Recently, an extensive study has been done to estimate the *in vitro* anticholinestrase activity (R Chaibi, Romdhane, Ferchichi, & Bouajila, 2015) for treatment of cognitive impairment in patients with Alzheimer’s disease against the standard drug donepezil. The presence of oleanic acid, beta sitosterol and acetyloleonic acid indicated potent and selective anti-Alzheimer's activity and the results further verified by docking studies (Balaei-Kahnamoei *et al.,* 2021)

Antimalarial activity: The activity of petroleum ether and ethyl extracts against *Plasmodium falciparum* was investigated. Extracts showed somewhat antimalarial activity with IC_{50} of 35.5 mg/ml (Babili *et al.,* 2013). Nanoparticles of leaf extract gave excellent activity against all microbes and some species of plasmodium along with good antioxidant activity. The synthesis was time efficient and the results were comparable to the standard drug (Ajitha *et al.,* 2016). Another study was conducted against *Plasmodium berghei* and results demonstrated an excellent *in vitro* activity of *L. inermis* extract as compared to the other plants' extracts. The average chemosupressive percentage of *L. inermis* was found to be 61.57% (Deborah *et al.,* 2020).

Anticancer activity: Dalton's lymphoma ascites was induced in selected groups of mice and a dose of 180 mg/kg of plant extract was administered for 15 days and results were compared to vincristine. Light microscopy has been used to assess the histopathology of several liver segments from control and experimental animals. Cell line (DLA) generated mice's liver sections revealed structural changes to the nucleus. The main changes were loss of hepatocytes, and injury to the central vein. The *L. inermis* root extract therapy partially repairs this injury. Histopathological findings following DLA administration revealed that the treatment with an ethanolic root extract of the plant replenishes this damage (Priya *et al.,* 2011). With the advancement in technology, the nanoparticles of *L. inermis* extracts are being made and tested against several cancer cell lines (Babili *et al.,* 2013). Zinc ferrite nanoparticle of the extract was studied against breast cancer cell lines with a 25–500 µg/ml concentration range. The results showed that there is a gradual decrease in cell viability with increase in the concentration of $ZnFe_2O_4$ nanoparticles (Sarala *et al.,* 2020). Another recent study with zinc oxide nanoparticles of plant extract on hep-G cells and quantom dots method showed a significant antimalarial and anticancerous activity however, further research is still required (Amuthavalli *et al.,* 2021).

Sickle cell anemia activity: In the country of Oman, sickle cell anemia is a frequent condition. Cells with a sickle shape develop in this illness. These cells block blood vessels, which reduces the amount of oxygen carried by the blood. It has been established that *L. inermis* can prevent sickle cell development. Lawsone (2-Hydroxy-1,4-Naphthoquinone), a component of *L. inermis*, is what gives the dye its anti-sickling properties by making red blood cells more oxygen-affine. *L. inermis* contains anti-sickling properties, which have been demonstrated by combining sickle cell disease patient whole blood with aqueous and methanolic *L. inermis* extracts. Sodium bisulphite was then added to reduce the oxygen tension to 2%. As a result, the ratio of sickle cells to healthy red blood cells was assessed every 30 minutes. Sodium bisulphite was then added to reduce the oxygen tension to 2%. As a result, the ratio of sickle cells to healthy red blood cells was assessed every 30 minutes. In 84% of the analyzed samples, henna showed to slow the sickling process. Aqueous and methanolic henna extracts can both postpone sickling for roughly an hour (Kumar *et al.,* 2021).

Toxicity: A case report on a young adolescent girl with 6GDP deficiency showed nausea, vomiting and severe toxicity after ingestion of a concentrated methanolic extract of *L. inermis* (Perinet *et al.,* 2011; Sagh *et al.,* 2022). Further studies on groups showed abortifacient effects of plant extracts (Kandil *et al.,* 1996; Soker *et al.,* 2000; Ilkhanipur and Hakimian, 2013). Another aspect of the study was the induction of hemolytic anemia which leads to life threatening situations and teratogenicity (Mura *et al.,* 2009; Ilkhanipur and Hakimian, 2013). Another toxic effect of plant extract observed is allergic reaction to patients upon contact with skin (Treister-Goltzman *et al.,* 2016). Further, the Scientific Committees on Consumer Products and Consumer Safety reports to the European Commission give an overview of the extensive toxicity studies conducted on *L. inermis*, which were necessary for product registration. Henna's median oral lethal dosage was determined during an acute toxicity study to be above 2000 mg/kg b.w. in Sprague-Dawley rats, and its median dermal lethal dose was similarly over 2000 mg/kg b.w. in Wistar rats (Semwal *et al.,* 2014).

Conclusion: The miraculous herb has a large number of advantageous active pharmacological elements that are responsible for the plant's wide range of therapeutic capabilities. The rationale for its use in conventional treatments has been elucidated and supported by numerous investigations. Additionally, safety analyses necessary for the licensing of henna products indicate that *L. inermis* does not seem to have acute or chronic toxicity; nonetheless, this safety status is still under consideration. More research is required to completely understand the mechanism underlying the plant's pharmacological actions. Furthermore, it is suggested, to validate and support the use of the herb in traditional medicine as well as to demonstrate its safety and effectiveness, further exploratory studies are still needed.

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