REVIEW ARTICLE

A Review on Phytochemical, Pharmacological, and Toxicological Properties of *Lawsonia inermis L.*

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Abstract: Lawsonia inermis, commonly known as henna, is a plant belonging to the Lythraceae family, valued for its commercial and medicinal properties. This review aims to provide a comprehensive overview of the phytochemistry, pharmacological activities, and toxicological profile of L. inermis. The plant is widely cultivated in tropical regions of Asia, Africa, and the Americas, particularly in India, Pakistan, Iran, and North Africa. Henna has been traditionally used as a cooling agent, astringent, antifungal, and antibacterial herb for skin and hair. It has also been employed as a dye and preservative for hair, skin, fingernails, leather, and clothes. L. inermis is renowned for its abundance of vitamins, minerals, and various chemical constituents, including flavonoids, tannins, saponins, alkaloids, and naphthoquinones. These bioactive compounds have been shown to possess diverse pharmacological properties, such as immunomodulatory, antiviral, antibacterial, antifungal, nootropic, antifertility, hepatoprotective, antimitotic, analgesic, anti-inflammatory, and anticarcinogenic activities. This review discusses about the recent scientific literature on the phytochemistry, traditional uses, pharmacological activities, and toxicological aspects of L. inermis. The potential applications of henna in the pharmaceutical, cosmetic, and food industries are also discussed, along with future research directions.

Keywords: Lawsonia inermis; Henna; Phytochemistry; Pharmacological activities; Toxicology; Traditional medicine

1. Introduction

Henna is a dye derived from the plant *Lawsonia inermis* (family: Lythraceae), commonly known as the henna tree, the mignonette tree, or the Egyptian privet. Along with Lawsonia odorata, it is one of the two species in the genus Lawsonia. The name "mehndi" originates from the Sanskrit word "mehndhika," which describes the henna plant that yields a red color. Historically, one of the earliest uses of henna dates back to Ancient Egypt, where people believed that henna protected their spirituality and employed it as a stain [1]. Since ancient times, henna leaf extract has been used to dye wool, leather, silk, nails, hair, and skin.



Figure 1. Whole plant of Lawsonia inermis

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Henna was historically utilized to treat various ailments, including ulcers, liver and digestive issues, leprosy-related tissue loss reduction, diabetic foot disorders, ulcers, hair lice, and dandruff. It was also applied to the hands and feet to protect against fungal infections [2, 3]. Many farmers cultivate henna for cosmetic and medicinal purposes, and all parts of the plant—flowers, seeds, roots, stems, and bark—contain polyphenols, xanthones, alkaloids, terpenoids, and other compounds like glucose and gallic acid. Steroids, phenolics, and tannins have been shown to significantly impact microbial activities. These compounds exhibit a wide range of biological properties, including antimicrobial, anti-parasitic, anti-sickling, antipyretic, analgesic, hypoglycemic, anti-inflammatory, immune-stimulant, and antioxidant effects [4-6]. Henna is typically applied to the hands and feet for aesthetic value, and lawsone is responsible for imparting the red-orange color, also known as Hennotannic acid [7, 8]. The current review will highlight the processing of henna, chemical constituents, pharmacological, and toxicological effects of Lawsonia inermis.

2. Plant profile

2.1. Botanical classification

Kingdom: Plantae, Division: Magnoliophyta Subdivision: Spermatophytina, Class: Magnoliopsida, Subclass: Rosidae Order: Myrtales Family: Lythraceae, Genus: Lawsonia, Species: Lawsonia inermis

2.2. Description

Lawsonia inermis is a multi-stemmed, deciduous shrub or small tree, typically reaching a height of 2.4-5 meters. The plant exhibits a branching habit, with grayish-brown bark that is glabrous (smooth and hairless) and often spinescent (bearing spines or thorns). The shrub is characterized by its alternately arranged leaves, which are petiolate (having petioles or leaf stalks) and relatively short-petioled. The leaf blades are elliptic or broadly lanceolate in shape, measuring 1.3-3.2 cm in length and 0.6-1.6 cm in width. The leaf apices are acute or obtuse, frequently mucronate (abruptly terminating in a short, stiff point), while the bases are tapering or cuneate. The inflorescence of *L. inermis* is a large, terminal, pyramidal, paniculate cyme, bearing numerous fragrant flowers. [9, 10] The flowers are small, measuring less than 1.3 cm in diameter, with white or rose-colored petals. The calyx is widely campanulate (bell-shaped), measuring 3-5 mm in length, and the calyx lobes are suborbicular or subreniform (nearly circular or kidney-shaped), undulate (wavy), and 2.5-3 mm long. The stamens, arranged in pairs, are inserted on the calyx tube.

The fruit of *L. inermis* is a capsule, globose (spherical) in shape, with a diameter of approximately 6 mm. The capsule surface exhibits minor veining and is crowned by the persistent style. The calyx remains attached to the fruit, supporting it during development. The morphological features of *L. inermis*, such as the spinescent stems, alternately arranged leaves, paniculate inflorescences, and globose capsules, are characteristic of the Lythraceae family to which the plant belongs. [11-14]

2.3. Geographical distribution

Lansonia inermis, commonly known as henna, is considered a native plant of Africa and Asia. Its natural distribution extends across various regions of these two continents. In Africa, *L. inermis* is found in Egypt, Ethiopia, Somalia, Sudan, the Democratic Republic of Congo (formerly Zaire), Niger, Benin, Burkina Faso, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Nigeria, Senegal, Sierra Leone, Togo, South Africa, the Comoros Islands, and the Seychelles. [15, 16] In Asia, the plant is native to India, Pakistan, and Sri Lanka. Over time, henna has been introduced and widely cultivated in tropical regions worldwide due to its versatile applications and adaptability to diverse climatic conditions. It is now grown commercially in various parts of North and East Africa, the Arabian Peninsula, the southern regions of the Middle East, and throughout South Asia. The plant's ability to thrive in warm, arid, and semi-arid environments has contributed to its widespread cultivation and domestication across these regions.

3. Processing of henna leaves

Henna cultivation is a significant agricultural practice in many parts of the world. The harvesting of henna leaves typically occurs between June and October, with the peak production season occurring in early July. During this period, the plants are manually or mechanically pruned, and the leaves are carefully collected. [17, 18] Depending on the size of the cultivation and available resources, the harvested leaves may undergo manual or mechanical sorting, grading, and sieving to remove any unwanted materials, such as dust, branches, straws, and fruits. This process ensures the quality and purity of the henna leaves before further processing. [19, 20]

3.1. Drying Process

The drying process is a crucial step in henna leaf processing, as it converts the fresh leaves into a powdered form and determines their final moisture content. The drying method can vary depending on the local climate, resources, and traditional practices. In many regions, the leaves are dried in the shade or outdoors, taking advantage of natural air circulation and sunlight. This low-cost, small-scale drying approach is widely adopted by small-scale farmers and producers. However, it is essential to note that prolonged exposure to direct sunlight or UV radiation can lead to undesirable quality and color loss in the henna leaves. To mitigate this issue, some producers employ solar dryers, which allow for controlled drying at temperatures below 100°C (212°F). These solar dryers provide a more efficient and consistent drying process while minimizing the risk of quality degradation. Once dried, the henna leaves are typically stored in brown paper coverings or bags made of high-density polyethylene (HDPE) with a thickness of 40 micrometers (µm) or higher. These packaging materials help preserve the quality and prevent moisture absorption during storage.

3.2. Quality of Henna

The quality of henna is determined by several factors, including color, purity, dyeing abilities, and powder fineness. The color of henna leaves can range from olive green to brown, depending on the harvest time and the specific cultivar or variety. In some cases, synthetic dyes, such as para-phenylenediamine (PPD), are illegally added to henna powder to achieve a darker, more intense color. This practice is concerning as PPD can cause mild to severe allergic reactions when used topically. Therefore, it is crucial to ensure the purity of henna products and detect potential adulteration using analytical techniques like high-performance liquid chromatography (HPLC). The quality parameters for henna include minimum lawsone content (the primary colorant), moisture and volatile matter levels, mineral matter content, crude fiber content, acid-insoluble ash content, the presence of extraneous sand or other contaminants, and the absence of extraneous dyes.

3.3. Natural Henna Paste

Natural henna paste is a simple preparation made from henna powder, a liquid (such as water or lemon juice), and optional essential oils (e.g., lavender, cajuput, or tea tree oil) for fragrance and additional benefits. If the henna paste is not intended for immediate use, it can be stored in the freezer for up to four months to preserve its quality. However, if the paste contains any additional chemicals or additives other than pure henna, it is recommended to keep it refrigerated or frozen to prevent potential skin irritation or adverse reactions. The application of henna paste to the skin or feet is a traditional practice, and the paste is typically left on for at least four to six hours, although longer contact times are also common in some cultures. It is important to note that water should not be used to remove the paste, as it can disrupt the oxidation process responsible for the development of the characteristic stain or dye. Instead, cooking oil can be applied to soften and remove the dried paste once the desired stain intensity is achieved.

4. Processing of henna leaves

Lawsonia inermis is a rich source of diverse phytochemical constituents, with nearly hundreds of compounds representing various chemical classes identified across different regions and parts of the plant. A preliminary phytochemical screening of the aqueous extract of L inermis revealed the presence of proteins, alkaloids, terpenoids, quinones, coumarins, xanthones, phenolic compounds, flavonoids, saponins, tannins (6-7%), fats (6%), and resins (2-3%) [17, 18]. One of the most notable compounds found in L inermis is lawsone, or 2-hydroxy-1,4-naphthoquinone, which is responsible for the plant's distinctive dyeing properties. According to HPLC analysis, the lawsone content varies across different parts of the plant, with the highest concentration found in the leaves (486.2 $\mu g/g$), followed by the flowers (116.9 $\mu g/g$), and the lowest concentration in the branches (5.4 $\mu g/g$) [22].

In addition to lawsone, various other phytochemicals have been identified in henna leaves. The phytochemical analysis revealed that the total ash content is 14.60%, with 4.50% being acid-insoluble ash and 3.0% water-soluble ash. The drying loss was found to be 4.5% w/w. The extractive values for alcohol and aqueous solvents were 3.8% and 5.0% w/w, respectively, with practical yields of 12.34% for the alcoholic extract and 15.50% for the aqueous extract [19, 20, 21].

Table 1. Phytochemical analysis reported in literature on *Lawsonia inermis* (henna) using different solvents, including distilled water, methanol, acetone, and ethanol.

| S No. | Phytochemicals | Distilled water | Methanol | Acetone | Ethanol |
|-------|--------------------|-----------------|----------|---------|---------|
| 1. | Tannins | + | + | + | + |
| 2. | Anthraquinones | - | - | - | - |
| 3. | Flavanoides | + | + | + | + |
| 4. | Alkaloids | + | + | - | + |
| 5. | Terpenoids | + | + | + | + |
| 6. | Saponins | + | + | + | + |
| 7. | Cardiac glycosides | + | + | + | + |
| 8. | Glycosides | + | + | + | + |
| 9. | Reducing sugar | + | + | - | + |
| 10. | Steroids | + | + | + | + |
| 11. | Phenolic | + | + | + | + |
| 12. | Amino acids | + | + | - | + |
| 13. | Proteins | + | + | + | + |
| 14. | Quinones | + | + | + | + |

(+ sign indicating the presence of a compound; - sign indicating the absence of a compound)

The phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, terpenoids, saponins, cardiac glycosides, glycosides, reducing sugars, steroids, phenolic compounds, amino acids, proteins, and quinones in various solvent extracts. However, anthraquinones were not detected in any of the extracts tested.

Table 2. Nutritional value of Lawsonia inermis seeds

| Constituent | Percentage in % w/w |
|---------------|---------------------|
| Proteins | 5.0% |
| Carbohydrates | 33.62% |
| Fibers | 33.5% |
| Fatty oils | 10-11% |

5. Pharmacological Properties

Lawsonia inermis, commonly known as henna, is a remarkable plant with diverse pharmacological properties attributed to its various parts, including leaves, flowers, seeds, stem, bark, and roots. Extensive research has unveiled the plant's potent antioxidant, antidiabetic, antimicrobial, anticancer, hepatoprotective, hypoglycemic, and wound-healing activities, among others. In vitro and in vivo studies have demonstrated the antibacterial actions of *L. inermis* across multiple test models [22].

Table 3. Chemical constituents found in different parts of the *L. inermis* plant and their corresponding in vitro pharmacological activities as reported in the literature.

| Plant parts | Chemical constituents | In-vitro pharmacological activity | | |
|--|---|--|--|--|
| Leaves | Naphthoquinones, polyphenolic components, | Antihyperglycemic and hepatoprotective, | | |
| | terpenes and terpenoids, flavonoids | antioxidant, antibacterial, antifungal, wound healing, | | |
| | | antitumor and cytotoxicity, antiparasitic activity. | | |
| Bark and Naphthoquinones, polyphenolic compone | | Anti-inflammatory activity, anti-parasitic activity | | |
| stem | terpenes and terpenoids, phytosterols and | | | |
| | aliphatic compounds | | | |
| Flowers | Terpenes and terpenoids | Antifungal activity | | |
| Roots | Phytosterols and aliphatic compounds | Anti-parasitic activity | | |
| Seeds | Terpenes and terpenoids, phytosterols and | Antioxidant activity | | |
| | aliphatic compounds | · | | |
| The whole | Xanthones, benzopyrones | Antioxidant activity | | |
| plant | | | | |

5.1. Antioxidant Activity

Antioxidants play a crucial role in reducing the risk of chronic diseases, such as inflammation, diabetes, and carcinogenesis [23-26]. They also help preserve the flavor and nutritional quality of food by preventing oxidative deterioration. Although synthetic antioxidants are commonly used as food additives, their safety has been questioned due to potential adverse effects, including skin sensitivities, gastrointestinal issues, and carcinogenic components [25, 26]. Consequently, the identification and isolation of novel antioxidants from natural sources have become a subject of extensive research. Various plant-derived compounds, including terpenes, flavonoids, coumarins, and curcuminoids, have exhibited minimal toxicity and significant antioxidant activity [27].

Researchers employ different methods to evaluate antioxidant activity, such as the ferric reducing antioxidant power assay (FRAP), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays [28]. Elaguel et al. (2019) [29] determined the chemical composition, antioxidant properties, and effects on lipid peroxidation and anti-proliferative properties of *L. inermis* oil. They found an optimal extraction yield of 6.8 g/100 g. In the DPPH free radical scavenging assay, the ethanol and chloroform extracts from *L. inermis* leaves exhibited inhibitions of 70.98 \pm 1.56% and 66.99 \pm 1.76%, respectively, at a concentration of 1.28 mg/mL [30].

The ethanolic extract of L. inermis has demonstrated high potential for scavenging DPPH radicals, reducing Mo(VI) to Mo(V) complexes and Fe(III) to Fe(II), and preventing lipid peroxidation [31]. The ethyl acetate extract from the fruit exhibits high antioxidant activity in two distinct biochemical assays: DPPH radical scavenging ability and the ability to prevent lipid peroxidation in the β -carotene linoleate system [32].

5.2. Antimicrobial Activity

The increasing resistance of infections to conventional antimicrobials necessitates the development of innovative treatments to replace them. The utilization of medicinal plants as a natural alternative to pharmaceuticals is a primary area of research to address drug resistance in infectious diseases. Antimicrobial compounds found in medicinal plants may work differently from currently used antimicrobials to suppress the growth of bacteria, fungi, viruses, and protozoa, making them potentially useful in treating resistant microbial strains [33].

When leaf segments were used as in vivo samples, the aqueous leaf extract of *L. inermis* demonstrated in vivo antifungal efficacy against Candida albicans, with an IZD value of 10 mm at 10 mg/mL (Rahiman et al., 2013) [34]. The leaves of *L. inermis* exhibit strong antibacterial effects due to the presence of 2-hydroxy-1,4-naphthoquinone against Salmonella enterica, Listeria monocytogenes, Shigella sonnei, Staphylococcus epidermidis, and Staphylococcus intermedius [35]. A novel chromone called Lawsozaheer, isolated from Lawsonia Lam (NCTC 6571), demonstrated highly selective activity against Staphylococcus aureus, with an inhibition rate of 84.26% at 150 mg/mL, compared to 87.013% for conventional ofloxacin at 100 mg/mL [36].

Oxidized bioactive gelatin starch nanofibers containing *L. inermis* exhibited strong bacterial suppression against two strains of E. coli and Staphylococcus aureus. This could be attributed to the various phenolic compounds present in henna extracts, such as flavonoids and 2-hydroxy-1,4-naphthoquinone, which interact with bacterial cell walls, reducing their functionality [37]. In a comparative study, an aqueous extract of *L. inermis* leaves was used to synthesize magnesium oxide nanoparticles by chemical and green methods. The organic biomolecules on the surfaces of the green-synthesized MgO nanoparticles (NPs) contributed to their strong antibacterial activity. The green-synthesized nanoparticles exhibited a greater inhibition zone compared to chemically synthesized MgO NPs against B. subtilis, S. aureus, E. coli, and P. vulgaris [38].

5.3. Hypoglycemic Activity

The methanolic extract of L inermis exhibited in vitro alpha-amylase inhibiting activity, resulting in a hypoglycemic response [39]. Antika (2017) investigated the effects of henna leaf extract derived from ethanol on blood sugar levels and superoxide enzyme activity in Wistar mice using a posttest-only control group design. The ethanol extract of L inermis leaves (400 mg/kg body weight for 21 days) significantly decreased blood glucose levels in the mice with alloxan-induced diabetes (p < 0.05) compared to the untreated diabetic control [40].

Silver nanoparticles containing *L. inermis* extract exhibited antidiabetic properties. Strong hypoglycemic effects were observed in vivo when administered to Wistar rats, with no harmful side effects [41]. The ethanolic extract (70%) of henna leaves significantly reduced blood glucose levels in the Swiss albino mice model of alloxan-induced diabetes when administered at a dose of 800 mg/kg body weight for 14 days. Additionally, it reduced triglyceride and total cholesterol levels. The extract facilitated the transit of blood glucose to peripheral tissues and stimulated insulin release from pancreatic islets, contributing to the antihyperglycemic effect (Abdillah et al., 2008) [42].

5.4. Anti-inflammatory, Analgesic, and Antipyretic Effects

Lawsone, isolated from *L. inermis* leaves, was evaluated for its analgesic and anti-inflammatory activities. In a study involving 120 adult mice, both lawsone and aspirin exhibited analgesic effects compared to the control group, with aspirin being less effective as an analgesic. Both drugs demonstrated anti-inflammatory properties and had equivalent efficacy in mitigating carrageenan-induced hind paw edema (p < 0.05) [43]. Due to its analgesic, antipyretic, and anti-inflammatory properties, *L. inermis* has been regularly prescribed to patients with hand-foot syndrome (Yucel and Guzin, 2008) [44].

Aqueous and ethanolic extracts of L, inermis demonstrated a significant analgesic effect (p < 0.05) in the latency period by reducing the number of folds compared to the negative control group, similar to ketoprofen, which also inhibited pain at thermal stimuli. Additionally, the ethanolic extract was more effective than the aqueous extract in reducing abdominal constriction [45]. A combination of extracts from L, inermis and Ricinus communis demonstrated analgesic benefits by reducing mechanical allodynia through von Frey filaments [46]. The combined analgesic effects of chloroform extracts from L, inermis and Chlorophytum borivilianum leaves and roots were investigated in mice using tail immersion and hot plate methods. The findings revealed that the chloroform extracts of both plants significantly increased analgesic activity at a dose level of 200 mg/kg body weight, and the combined effect of the two extracts exhibited greater analgesic activity than either extract alone [47].

5.5. Antitumor and Antiproliferative Activities

Methanolic leaf extracts and recently discovered polyphenols from *L. inermis* have demonstrated antiproliferative and cytotoxic effects against cancer cells, as necrotic cells accumulate during the apoptotic phases [48]. Although further research is necessary, a recent study using the quantum dots method and plant extract-synthesized zinc oxide nanoparticles on HepG2 cells showed promising antimalarial and anticancer effects [49]. A natural flavonoid, Lawsonaringenin (LSG), was extracted from the ethanol and dichloromethane extracts of *L. inermis* leaves. LSG significantly suppressed the proliferation of HT-29 cells to a greater extent than the standard 5-fluorouracil drug [50]. Exposure to the aqueous extract of *L. inermis* leaves resulted in varying degrees of growth inhibition across different cancer cell lines, with significant activity against COLO-205 colon cancer cells (GI50 121.03 µg/ml) [51].

5.6. Anti-malarial Activity

Leaf extract nanoparticles of L. inermis exhibited good antioxidant activity, in addition to outstanding efficacy against various microorganisms and Plasmodium species. The synthesis process was time-efficient, and the outcome was comparable to that of a standard medication [52]. L. inermis exhibits substantial anti-plasmodium activity both in vitro and in vivo, making it a promising candidate for antimalarial drugs. Its frequent use in traditional medical systems may be attributed to this property. The study examined the in vitro activity of different L. inermis extracts and constituents. The results showed that fraxetin and the ethyl acetate extract from the leaves were relatively effective against P. falciparum strain NF-54 among all the extracts and constituents tested, with IC50 values of 19.21 \pm 1.04 μ M and 9.00 \pm 0.68 μ g/mL, respectively. The in vivo results against the P. berghei strain were consistent [53].

5.7. Tuberculostatic Activity

Both in vitro and in vivo studies have demonstrated the promising antitubercular effectiveness of the aqueous leaf extract of *L. inermis*. At a dose of 6 µg/ml, the extract inhibited the growth of Mycobacterium tuberculosis H37Rv and Tubercle bacillus from sputum on Lowenstein Jensen medium. It was also found to be effective against Mycobacterium tuberculosis infection in guinea pig and mouse models at a dose of 5 mg/kg, orally [54].

5.8. Hepatoprotective Activity

The hepatoprotective activity of ethanolic extracts of various fractions (ethyl acetate, petroleum ether, and chloroform) of *L. inermis* leaves was investigated in CCl4-induced hepatitis rats. The extracts, at doses of 200 mg/kg orally, significantly reduced the elevated levels of serum bilirubin, SGPT, SGOT, and SALP compared to the CCl4-treated group [55]. The aqueous extract of henna leaves showed significant hepatoprotective action against carbon tetrachloride-induced liver damage in male Wistar albino rats, as evidenced by decreased levels of serum bilirubin, serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, serum alkaline phosphatase, and a significant antilipid peroxidant effect [56]. A polyherbal formulation containing the aqueous extract of henna, sold under the name Hepjaun syrup, was found to decrease hepatic damage in rats, consistent with a decrease in bilirubin at an oral dose of 500 mg/kg body weight [57].

5.9. Wound Healing Activity

Tissue damage triggers a biological process involving intricate interactions between various cells, growth factors, and extracellular matrix elements. *L. inermis* has been studied for its wound-healing activities and has shown promising results. An investigation was conducted on a formulation-based *L. inermis* ointment for wound healing. Animals treated with the ointment exhibited higher rates of wound contraction compared to the control group [58]. In another study, the epidermal regeneration covering the whole surface of the henna oil-treated lesion was stained to examine the tissue and epithelial structure. Under a microscope, the oil-treated scar

zones showed complete, coordinated epidermal regeneration and increased thickness, which was considered normal (Rekik et al., 2019) [59]. Hydrogel dressings formulated with ethanol extracts of *L. inermis* leaves could accelerate the healing process of burns. Henna's healing properties are attributed to the various constituents found in its extract, including terpenoids, gallic acid, and flavonoids, which facilitate faster wound healing [60].

6. Toxicological Properties

In 2014, Agabna et al. investigated the safety of ethanolic seed extracts from *L. inermis* at doses of 500 and 1000 mg/kg in mice. No fatalities were observed within 24 hours in response to the 500 mg acute dose or the 1000 mg/kg acute dose, and the animals did not exhibit any altered behavior, eating habits, diarrhea, or fur loss. The study revealed a slight fluctuation in the levels of white blood cells (WBCs), red blood cells (RBCs), and platelets. Serum values of urea, sodium, potassium, and creatinine were within normal ranges. The liver enzymes, proteins, blood sugar, or lipid profile were unaffected by the continuous dose of henna extract. However, AST tended to increase partially but significantly in the high-dose group. No signs of toxicity were found after the autopsy [61].

Although pure henna is generally considered safe, caution is recommended during pregnancy. In a study, mature female BALB/c mice were administered intraperitoneal injections of 100 mg/kg of *L. inermis* for seven days. Their embryos were evaluated for abnormalities on the nineteenth day. Thirty percent of the embryos possessed additional ribs, and ninety percent lacked parietal bones. This might be related to the presence of 2-hydroxy-1,4-naphthoquinone in the *L. inermis* extract [62]. According to Jafarzadeh et al.'s study, the ethanolic extract of *L. inermis* dramatically reduced embryo weight and height compared to the control, leading to skeletal abnormalities such as anencephaly and exencephaly, in addition to malformations of the rib and parietal bones [63]. The Nile Basin Countries' Sustainable Development of Natural Resources states that mice are safe when exposed to up to 2 g/kg body weight of *L. inermis* aqueous extract, with no fatal poisonings or symptoms observed after 24 hours [64].

7. Conclusion

In conclusion, Lawsonia inermis, commonly known as henna, is a remarkable plant with a diverse array of phytochemical constituents and pharmacological activities. This review has provided a comprehensive overview of the plant's botany, geographical distribution, processing methods, phytochemistry, and traditional uses. The pharmacological properties discussed include antioxidant, antimicrobial, hypoglycemic, anti-inflammatory, analgesic, antipyretic, antitumor, antiproliferative, anti-malarial, tuberculostatic, and hepatoprotective activities. While numerous studies have been conducted to explore the therapeutic potential of henna, further research is warranted to fully elucidate its mechanisms of action, establish safety and efficacy profiles, and develop standardized formulations for clinical use. Additionally, the exploration of novel delivery systems, such as nanoparticles and liposomes, may enhance the bioavailability and targeted delivery of henna's bioactive compounds. With its rich phytochemical profile and diverse pharmacological activities, Lawsonia inermis holds promising potential for applications in various industries, including pharmaceuticals, cosmetics, and functional foods. However, it is crucial to ensure the quality, safety, and efficacy of henna products through rigorous scientific research and regulatory oversight.

Compliance with ethical standards

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects.

Statement of informed consent

Not applicable for this study.

References

- [1] Singh M, Jindal S, Kavia ZD, Jangid BL, Jindal N. Exploring plant biomolecules as a green nanoparticle source for therapeutic delivery applications. Biomolecules. 2021;11(1):94.
- [2] Hosseini-Zijrod SM, Eftetkharsadat AT, Ahangarpour A, Mokhtari F, Asadi-Samani M, Abdollahi M. *Lansonia inermis* leaves extract as a novel candidate for curing iron overload. DARU Journal of Pharmaceutical Sciences. 2017;25(1):1-8.
- [3] Gull I, Sohail M, Aslam MS, Athar MA. Phytochemical, toxicological and antimicrobial evaluation of *Lawsonia inermis* extracts against clinical isolates of pathogenic bacteria. Ann Clin Microbiol Antimicrob. 2013;12(1):36.
- [4] Elhouari A, Rhaiem N, Ouarhal R, Souhi R, Khalki H, Lmimouni BE, Ennouhi A. Phytochemical study, antibacterial and antioxidant activities of *Lawsonia inermis* L. seeds. European Journal of Medicinal Plants. 2019:1-14.
- [5] Dariush D, Amouoghli-Tabrizi B. Evaluation of cytotoxicity of *Lawsonia inermis* seed extract on cancer cell lines. Indonesian Biomedical Journal. 2013;5(2):97-102.
- [6] Rahmoun NM, Bakrara ZH, Kaid-Omar Z, Hamaidi-Chergui F. *Lawsonia inermis* seed extracts: Chemical composition, antioxidant activity and effect on yeast activity. Research Journal of Microbiology. 2016;11:132-40.
- [7] Soni H, Malik D, Singhai AK. Recent updates on the therapeutic potential of *Lawsonia inermis* (henna): A review. International Journal of Pharmaceutical Sciences and Research. 2020;11(6):2552-66.
- [8] Warra AA. In-vitro cytotoxicity evaluation model of some Pakistani ethnomedicinal plants. Journal of Cancer Prevention & Current Research. 2015;2(3):00034.
- [9] Alia BH, Al-Qarawi AA, Ahmen IS. Pharmaceutical and cytotoxicological studies on *Lansonia inermis* (henna). J Drug Res. 1995;20:89-96.
- [10] Semwal RB, Semwal DK, Combrinck S, Viljoen AM. *Lawsonia inermis* L.(henna): Ethnobotanical, phytochemical and pharmacological aspects. Journal of Ethnopharmacology. 2014;155(1):80-103.
- [11] Gupta VK, Shamas N, Khediar MYB, Nayak S, Luqman S, Noureldien NH, Kabbashi AS, Musarrat J. Molecular investigation of the anti-cancerous potential of henna (*Lawsonia inermis*) leaves and stems using a streptozotocin induced pancreatic cancer model. Scientific Reports. 2021;11(1):1-8.
- [12] Al-Juhaishi AJ, Ghafoor K, Özcan MM, Babiker EE, Sarker SD, Ahmed IAM. Biochemical profiling of the bioactive compounds from various plant parts of *Lawsonia inermis* L: an economically important plant material. Plant Biosystems. 2019;153(4):550-8.
- [13] Singh R, Shahin M, Kaul N. Biosynthesis of bimetallic nanoparticles: A green approach. InGreen Nanoparticles 2020 (pp. 313-338). Springer, Cham.
- [14] Fatima N, Mukhtar M. Henna (*Lawsonia inermis*) leaf powder revisited. International Journal of Pharmaceutical Sciences and Drug Research. 2018;10(2):98-107.
- [15] Singh N, Kumari P, Yadav BK. *Lawsonia inermis* L.-A comprehensive review. International Journal of Pharmaceutical Sciences and Research. 2018;9(4):1394-417.
- [16] Ayesha M, Muhammad G, Naila S. *Lawsonia inermis* Linn: enduring more than a decorative henna plant. Orient Pharm Exp Med. 2012;12(4):221-6.
- [17] Gagandeep S, Kaur S, Kaur M, Kaur Kahlon S, Rath G. Phytochemicals of pharmaceutical importance. Academic Press; 2021.
- [18] Asgarpanah J, Mohajerani R. Phytochemistry and pharmacologic properties of *Lawsonia inermis* Linn. Int J Mol Cell Med Summer. 2012;1(3):156.
- [19] Khaleque A, Keya MA, Hasan KN, Anonna MN, Chowdhury MH, Rahman MA. Phytochemical studies on Lawsonia inermis Linn syn. Lawsonia alba. Bangladesh Journal of Scientific and Industrial Research. 1998;33(3):479-83.
- [20] Humaira M, Fouzia B. Effects of some Environmental Factors on the Content of Lawsone in Henna Leaves (*Lawsonia inermis* L.). World Appl. Sci. J. 2008;3(2):241-8.
- [21] Tabassum N, Qazi MA, Shaida J, Mustafa G. Review on henna: Perspectives, constraints and ongoing research. Int J Biosci. 2015;6(5):135-56.
- [22] Mikhaeel KM, Mohammed MA. Extraction of lawsone from henna leaves and studying the dyeing properties on polyamide fabric. Int J Innov Res Sci Eng Technol. 2016;5(7):13557-65.

- [23] Asgarpanah J, Kazemivash N. Phytochemistry, pharmacology and medicinal properties of *Lawsonia inermis* L. African Journal of Biotechnology. 2012;11(53):11758-67.
- [24] Sahar NM, Mukhtar M, Gilani SA. Antidiabetic studies of Lawsonia inermis Linn. Pak J Pharm Sci. 2009;22(2):160-3.
- [25] Gupta VK, Fatima A, Faridi U, Negi AS, Shanker K, Kumar JK, Rahuja N, Luqman S, Sisodia BS, Saikia D, Darokar MP. Antimicrobial potential of Glycyrrhiza glabra roots. J Ethnopharmacol. 2008;116(2):377-80.
- [26] Endrini S, Rahmat A, Ismail P, Hin TY. Cytotoxic properties of *Lawsonia inermis* and derivatives/analogues. J Chem Pharm Res. 2014;6(5):830-6.
- [27] Sharma D, Rajput J, Kaith BS, Kaur M, Sharma S. Synthesis of ZnO nanoparticles and study of their antibacterial, antifungal properties, antioxidant activity and bio-remediation of dyes. Thin Solid Films. 2010;519(3):1224-9.
- [28] Elgamal NS, Kassab EA, Mohammed GA, Mohammed TAA, Elhussinie HA. Anticancer potential of Lawsonaringenin isolated from the leaves of *Lawsonia inermis*. Asian Pac J Cancer Prev. 2020;21(10):2971-8.
- [29] Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complement Altern Med. 2012;12(1):221.
- [30] Kumar SP, Kanthal LK, Durga S, Satyavati K. Phytochemical Evaluation and Screening of Cardiotonic, Antibacterial and Anthelmintic Activities of Sida cordifolia L. Int J Pharm Sci Nanotechnol. 2014 Aug 31;7(3):2567-73.
- [31] Mangam VT, Nallam VR, Anitha A, Devi PR, Sanisha M. Dengue-An Overview. International Journal of Pharma Research. 2018 Jan 1;9(1).
- [32] Mangam VT, Priya AY, Medisetti V, Swathi T, Battu GR. Preliminary Phytochemical, Antibacterial and Anthelmintic Activity Studies on Ethanolic Extract of Terminalia Arjuna Leaves..
- [33] Dasgupta T, Rao AR, Yadava PK. Chemomodulatory efficacy of basil leaf (Ocimum basilicum) on drug metabolizing and antioxidant enzymes, and on carcinogen-induced skin and forestomach papillomagenesis. Phytomedicine. 2004;11(2-3):139-51.
- [34] Dasofunjo K, Nwodo OF, Lawal RF, Oyetade OA, Awotade FE, Roberts AA. Liver enzymes and protein levels as biological indicators of liver damage caused by carbon tetrachloride in rats. Pharmacologyonline. 2018;3:161-8.
- [35] Aruna KP, Sivaramakrishnan VM. Antihepatotoxic effects of some Indian medicinal plants. J Ethnopharmacol. 1992;35(1):55-63.
- [36] Ansari P, Azam A, Hannan MA, Naqvi AA. Cosmetic henna formulations and wound healing potential. J Res Appl Basic Med Sci. 2006;(1):19-27.
- [37] Rekik DM, Khedir SB, Jamoussi K, Boudawara TS, Amri M, Kharrat N, et al. In vivo dermal wound healing effects of *Lawsonia inermis* seed extract ointment. Biomed Pharmacother. 2019;111:1165-73.
- [38] Mahendra CK, Shivakumar B. Formulation and evaluation of a polyherbal hydrogel for burn wound healing. Indian J Pharm Educ Res. 2018;52(4):598-603.
- [39] Kudupudi V, Kakarparthy RS, Sarella PN, Kolapalli VR. Formulation Development and Characterization of Vancomycin Hydrochloride Colon-Targeted Tablets Using In-Situ Polyelectrolyte Complexation Technique. International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN). 2023 May 31;16(3):6533-45.
- [40] Asogwa PO, Sarella PN. Observational Studies of Prescription Pattern and Use of Antibiotics in Selected Rural Areas. Int J Pharm Sci and Medicine. 2023;8:21-30..
- [41] Jafarzadeh M, Mohammadi-Ghalehdjani Z, Larijani B, Beheshti M. Teratogenic effects of henna on embryo and fetus development in pregnant mice. Iran J Med Sci. 2018;43(6):559.
- [42] El Hussein AA. Safe use of henna in Sudan, Technical Report. Nile Basin Countries: Sustainable Development of Natural Resources, Project No. 6IC/SUD/09. Khartoum, Sudan; 1999.
- [43] Gull I, Sohail M, Aslam MS, Athar MA. Phytochemical, toxicological and antimicrobial evaluation of *Lawsonia inermis* extracts against clinical isolates of pathogenic bacteria. Annals of clinical microbiology and antimicrobials. 2013;12(1):1-10.
- [44] Bharathi A, Sundari MK, Elavarasan K, Keerthiga P, Vinothini S, Ramesh S. A study on the phytochemical screening and antimicrobial activity of *Lawsonia inermis* leaves. International Journal of Current Microbiology and Applied Sciences. 2017;6(9):2054-61.
- [45] Ashraf HM, Eldahhan DA, Elkholey KE, Ebada AS, Sarhan NI. Chemical composition and biological evaluation of *Lawsonia inermis* leaves. Pharmacognosy Journal. 2020;12(3):559-67.

- [46] Fatima S, Ayaz M, Manzoor MF, Khan AH, Ullah H, Ali T, et al. A comprehensive review on pharmacological, phytochemistry and agriculture importance of *Lawsonia inermis* L. J Glob Innov Agric Soc Sci. 2020;8(2):83-9.
- [47] Iyer PR, Rekha PR. *Lawsonia inermis* L.: A plant with cosmetic, medicinal and environmental applications. J Cosmet Dermatol. 2022;21(3):1062-74.
- [48] Endrini S, Rahmat A, Ismail P, Taufiq-Yap YH. Cytotoxic properties of *Lawsonia inermis* and derivatives/analogues. J Chem Pharm Res. 2014;6(5):830-6.
- [49] Sharma D, Rajput J, Kaith BS, Kaur M, Sharma S. Synthesis of ZnO nanoparticles and study of their antibacterial, antifungal properties, antioxidant activity and bio-remediation of dyes. Thin Solid Films. 2010;519(3):1224-9.
- [50] Elgamal NS, Kassab EA, Mohammed GA, Mohammed TAA, Elhussinie HA. Anticancer potential of Lawsonaringenin isolated from the leaves of *Lawsonia inermis*. Asian Pac J Cancer Prev. 2020;21(10):2971-8.
- [51] Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complement Altern Med. 2012;12(1):221.
- [52] Bose AK, Dolui A, Jalal S. Green synthesis of gold nanoparticles using henna leaf extract. Int J Appl Sci Eng Res. 2014;3(1):137-46.
- [53] Mungantiwar AA, Nair AM, Shinde UA, Dikshit VJ, Saraf MN, Thakur VS, et al. Studies on the oxygen radical scavenging potential of some flavonoids. J Herbs Spices Med Plants. 1997;5(1):11-21.
- [54] Natarajan V, Venugopal PV, Menon T. Effect of Azadirachta indica (neem) on the growth pattern of dermatophytes. Indian J Med Microbiol. 2003;21(2):98-101.
- [55] Dasgupta T, Rao AR, Yadava PK. Chemomodulatory efficacy of basil leaf (Ocimum basilicum) on drug metabolizing and antioxidant enzymes, and on carcinogen-induced skin and forestomach papillomagenesis. Phytomedicine. 2004;11(2-3):139-51.
- [56] Dasofunjo K, Nwodo OF, Lawal RF, Oyetade OA, Awotade FE, Roberts AA. Liver enzymes and protein levels as biological indicators of liver damage caused by carbon tetrachloride in rats. Pharmacologyonline. 2018;3:161-8.
- [57] Aruna KP, Sivaramakrishnan VM. Antihepatotoxic effects of some Indian medicinal plants. J Ethnopharmacol. 1992;35(1):55-63.
- [58] Ansari P, Azam A, Hannan MA, Naqvi AA. Cosmetic henna formulations and wound healing potential. J Res Appl Basic Med Sci. 2006;(1):19-27.
- [59] Rekik DM, Khedir SB, Jamoussi K, Boudawara TS, Amri M, Kharrat N, et al. In vivo dermal wound healing effects of *Lawsonia inermis* seed extract ointment. Biomed Pharmacother. 2019;111:1165-73.
- [60] Mahendra CK, Shivakumar B. Formulation and evaluation of a polyherbal hydrogel for burn wound healing. Indian J Pharm Educ Res. 2018;52(4):598-603.
- [61] Agabna FA, Mubarak WT, Elkatama AI, Altayyar IA. Toxicological study for the effect of *Lawsonia inermis* henna seeds on some biochemical and hematological parameters in rats. J Chem Pharm Res. 2014;6(7):2484-8.
- [62] Momma K, Hashimoto N, Hirosawa I, Tahara Y, Kuroki F, Nakagawa M. Embryotoxic effects of the henna plant, *Lawsonia inermis*, in vitro. Reprod Toxicol. 1989;3(4):267-72.
- [63] Jafarzadeh M, Mohammadi-Ghalehdjani Z, Larijani B, Beheshti M. Teratogenic effects of henna on embryo and fetus development in pregnant mice. Iran J Med Sci. 2018;43(6):559.
- [64] El Hussein AA. Safe use of henna in Sudan, Technical Report. Nile Basin Countries: Sustainable Development of Natural Resources, Project No. 6IC/SUD/09. Khartoum, Sudan; 1999.

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Vijay is a final-year pharmacy student in Chennai, India. Passionate about the pharmaceutical sciences and research, he excels academically and in extracurricular activities. As a well-respected class representative, Vijay organizes events and facilitates communication between students and faculty. In his free time, he enjoys reading scientific literature and engaging in community service. Marked by a drive for knowledge, passion for research, and dedication to excellence, Vijay aims to innovate in healthcare. Poised to graduate, his commitment to scientific advancement will serve him well in his career.



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Dr Deepa N is the Dean at the Faculty of Pharmacy at SBMCH, BIHER. She is a renowned personality in the field, dedicated to advancing pharmaceutical sciences and mentoring future pharmacists. With expertise in various pharmacy domains, her commitment to teaching excellence and impactful research sets her apart. As a visionary leader, she shapes academic standards, fosters innovation, and prepares professionals to address healthcare challenges effectively. Her unwavering dedication inspires both students and colleagues, shaping the future of pharmacy and education.

