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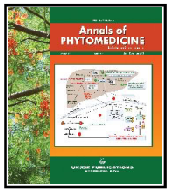

Review Article : Open Access

Phytopharmacological investigation on *Lawsonia inermis* L.: A comprehensive review

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Abstract

India is the supplier and has a rich heritage of medicinal plants. *Lawsonia inermis* L. (Henna) is frequently used for its phytoconstituents in traditional Indian medicines. Various phytochemicals that henna contains are lawsone (hennatonic acid), tannins, lalioside, lawsoniaside, lawsoniaside B, hennadiol, laxanthones I, II, III, lacoumarin, scopoletin, flavone, glycosides, esculetin, fraxetin, betulin, betulinic. Many animal studies were done using henna extracts as per OECD guidelines. *L. inermis* has many pharmacological actions such as antibacterial, antifungal, anticancer, antidiabetic, antiviral, antiparasitic, tuberculostatic, anti-inflammatory, wound healing, antimalarial, immune stimulant and anthelmintic. This article elaborates on the morphological, traditional, phytochemical and pharmacological features of *L. inermis* with its toxicological studies. It is a possible source of natural compounds for developing medications for certain disorders. Henna is used in industries for manufacturing of hair care products.

1. Introduction

Herbal medicines have wide biological and medicinal activities as they are in huge demand in different countries. Herbal medicines which contain natural substances promote good health and support wellness (Prasathkumar *et al.,* 2021). To combat disease, medicinal plants are used as well as they are part of human society from ancient times (Manoharacharya and Nagaraju, 2016). Trends in herbal medicines focus on research on the pharmacological action of herbs, for confirmation of the claims in official books (Rana and Chauhan, 2022). The purpose of writing this review is to consolidate the recent information including various biological activities of *L. inermis*. This plant is very common and also used in the whole world (Ahmed and Beg, 2001). Researchers are interested in henna because of its diverse pharmacological properties (Aqil *et al.,* 2005). *L. inermis* is a perennial plant. The taxonomy of *L. inermis* is as follows: Kingdom, Plantae; Division, Magnoliophyta; Class, Magnoliopsida; Order, Meyrtales; Family, Lythraceae; Genus, *Lawsonia;* Species, *Lawsonia inermis* L. It is used as a traditional product with religious importance mainly for medicinal and cosmetic purposes (Warrier, 2021). Henna is widely used as a cosmetic ingredient for coloring hair, skin and nails (Bhuvaneswari *et al.,* 2021). Henna has been widely used in Africa, Asia and many other continents of the world. This plant is grown in India, North America and Southern Asia. *L. inermis* plant leaves are dried and finely powdered, this green-colored powder is used as henna. The paste made of powdered leaves of *L. inermis* is applied on the skin as protection against the sun in North Africa. In India, henna is cultivated in Gujarat for hair dye, in Madhya Pradesh for hair coloring, and making perfume oils, in Punjab for hair dye and in Rajasthan as body art (Kannahi and Vinotha, 2013). The plant grows better in tropical savannah and tropical arid zones at temperature of 35-45°C and produces the highest dye content (Supian and Osman *et al.,* 2023). For optimum germination (73%) of seeds, 25-30°C is favorable soil temperature. *L. inermis* is a small tree or branched glabrous shrub, grow up to a height of 6.5 to 23 feet. Popularly its leaves are useful but other parts such as stem, roots, bark, flowers and seeds have many medicinal uses. It is used to colour the hair, skin and nails which is worldwide known as cosmetic agent. Leaves are elliptical in shape or broadly lanceolate, acute or obtuse measuring 1.3-3.2 cm by 0.6-1.6 cm, respectively. Flower character is white or rose-coloured, pedicels short and slender. Capsules are of 6 mm diameter, supported by persistent calyx and tipped and slightly veined outside. Seed capsules are red, about the size of a pea, globose with numerous tiny pyramidal, pitted brown seeds. Henna is grown in homes since it does not need specific equipment, can be found nearby residential areas and does not require skilled labor. Compared to other crops, it is a reliable crop during droughts as a source of income. In 2003-2004, India became the largest producer of henna though unable to meet the demand globally. The twigs and leaves are trimmed annually or semi-annually. For use of *L. inermis* as hair dye and skin stain, their leaves are sorted, pulverized, sieved and grind to a fine powder ((Rojas-Sandoval, 2017). The *L. inermis* is known by different names such as Hindi, heena; Marathi, mendi; Sanskrit, medika and dvivratna; Urdu, mehendi and English, egyptian privet.

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2. Plant parts of *L. inermis*

Henna as a whole plant and its parts including roots, stems, flowers, barks, leaves, pods and seeds are quite useful (Hassaballa and Ahmed *et al.,* 2022). Lawsone is a principal natural dye about 1-1.4% present in leaves. Besides these, various flavonoids are also present like apigenin, and luteolins. Coumarins present in henna are fraxetin, scopoletin and esculetin. Steroid like β-sitosterol is also present (Chaudhary *et al.*, 2010). Dark brown-colored essential oil is obtained on the steam distillation of its flowers. This essential oil has a strong fragrance and contain α-ionones and β-ionones, resin and nitrogenous compound (Muheyuddeen *et al.,* 2023). Seeds consist of carbohydrates (33.62 %), fatty oils (10-11 %), proteins (5.0%), arachidonic acid, oleic acid, palmitic acid and stearic acid (Chaudhary *et al.,* 2010). The aqueous extract of the root contains cardiac glycosides, saponins, flavonoids, steroids and tannins (Makhija, 2011). The bark extract of *L. inermis* found to contain phenylbutazone, lawsaritol and isoplumbagin (Nesa *et al.,* 2014).

3. Chemical constituents

3.1 Naphthoquinone derivatives

The most important and active compound in *L. inermis* is lawsone. It is a red-orange pigment that binds to proteins, especially in keratin, giving henna its characteristic staining properties. It is responsible for imparting color to skin, hair, and nails. Lawsone showcases antibacterial, antiviral and antifungal properties. Moreover, it has been studied for its potential anti-inflammatory effect and its role in wound healing. Lawsone's versatility has sparked interest in its potential for pharmaceutical and medicinal applications (Kapadia *et al.,* 2013).

3.2 Tannins

They are polyphenolic compounds that contribute to the dyeing process by aiding in the binding of lawsone to proteins. Tannins also have astringent properties and help darken the color over time. They are valuable in combating oxidative stress and promoting overall wellness. Their antimicrobial properties add an extra layer of protection, underlining the multifaceted nature of phytochemicals present in *L. inermis* (Maslovaric *et al.,* 2021).

3.3 Gallic acid

This is another polyphenolic compound found in *L. inermis*. It is well-known for its anti-inflammatory and antioxidant effects. By scavenging free radicals and reducing inflammation, gallic acid contributes to cellular health and mitigates various chronic conditions. This compound showcases the plant's potential to contribute to preventive healthcare through natural means (Elansary *et. al.,* 2020).

3.4 Terpenes and terpenoids

L. inermis contains α-pinene and β-pinene which exhibit antimicrobial and anti-inflammatory properties (Al-Snafi and Ali Esmail, 2019).

3.5 Polysaccharides

L. inermis contains polysaccharides, vitamins (vitamin C) and minerals (calcium) that can contribute to health benefits (Singh and Luqman, 2014).

3.6 Flavonoids

The flavonoids derived from *L. inermis* encompasses a spectrum of compounds, notably including apigenin, apigenin-7-glucoside, apigenin-4-glycoside, apigenin-4'-O-β-D-glucopyranoside, luteolin, luteolin-7-glucoside, luteolin-3-glucoside, kaempferol, quercetin, catechin, *etc.* (Ezzat *et al.,* 2021 and Karaoðlan *et al.,* 2023).

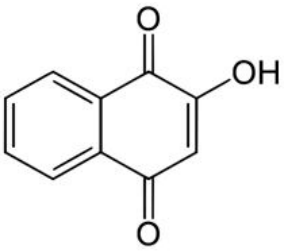
3.7 Essential oils

The essential oil extracted from leaves of *L. inermis* possess two major components, *i.e.,* caryophyllene and eugenol. It contributes to anti-inflammatory and antioxidant activity respectively (Leela and Singh, 2020).

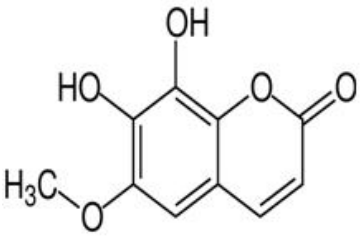
Methanolic extract of leaves was found to contain the number of phytochemicals employing GC-MS chromatogram as follows: squalene; 9,12,15-octadecatrienoic acid; pentadecanoic acid; vitamin E; hexadecanoic acid; 9,12-octadecadienoic acid; stigmast-5-En-3-Ol; 5-hydroxymethylfurfural; phytol; methyl ester; methyl stearate; phenol; 2,6-bis (1,1-dimethylethyl); stigmasterol; acetate; docosane; methyl tetradeconate; dianhydromannitol; hexadecanoic acid; methyl 18-methylnonadecanoate; 1,2 benzenedicarboxylic acid, *etc.* (Dev *et al.,* 2016). Six compounds were identified by GC-MS analysis of leaves extract of *L. inermis*, they are 2H-pyran-2,6(3H)-dione; 4H-pyran-4-one; 2,3-dihydro-3,5-dihydroxy-6methyl; 1,4-naphthalene-dione 2-hydroxy; benzene; alpha-D-glucopyranoside methyl; n-hexadecenoic acid (Abdelrahman *et al.,* 2020). Some of the phytochemical extracted from *L. inermis* by different techniques are listed in Table 1.

Table 1: Botanical components: Extraction and analysis overview

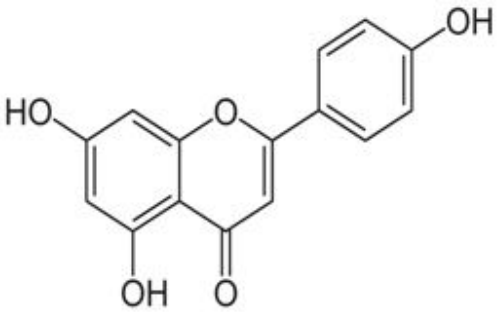
Part of plant	Extraction method	Analytical method	Chemical constituent	References
Leave	Maceration	Colorimetric analysis.	Lawsone 2.39-2.99%	Sakarkar *et al.,* 2004
	Decoction	UV-visible spectrophotometer	Flavonoids, coumarins, naphthalene andgallic acid derivatives	Fatima Alem, 2021
Whole plant	Soxhlet method	1. HPLC method 2. Gas chromatography	1,2,3,4,5,6-hexachloro-cyclohexane (HCH), J-isomere	Alem *et al.,* 2020
	Cold maceration	Thin layer chromatography	Tannins, flavonoid, alkaloids, glycosides, steroids, phenols, anthraquinones	Danzarami *et al.,* 2016



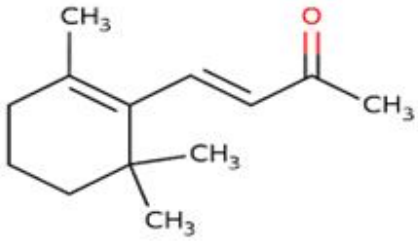
Lawsone



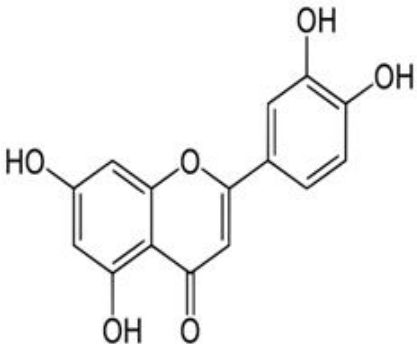
Fraxetin



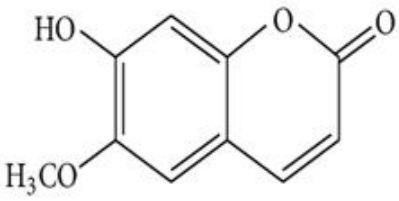
Apigenin



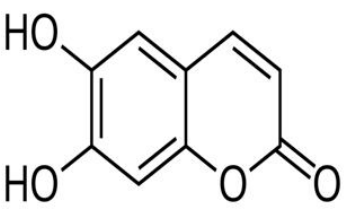
β- ionone



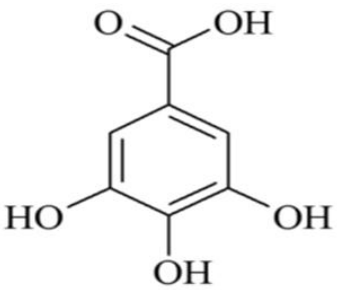
Luteolin



Scopoletin



Esculetine



Gallic acid

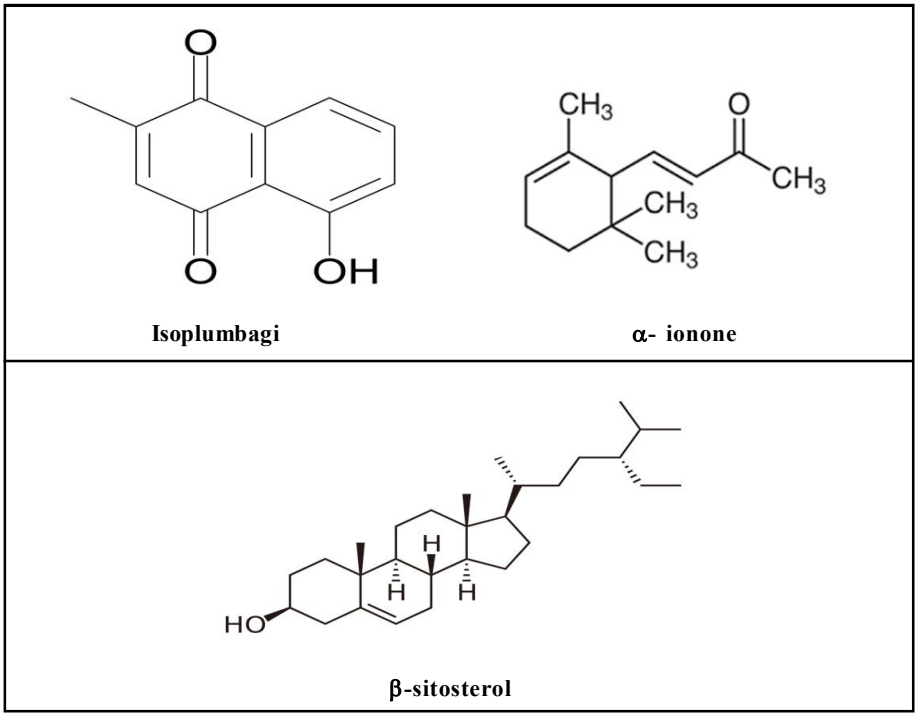


Figure 1: Structures of phytoconstituents present in *L. inermis* (Chaudhary *et al.*, 2010).

Table 2: Cataloguing the diverse pharmacological activities explored in *L. inermis*

Pharmacological activity investigated	Extract used	*In vitro/in vivo* study performed	Dose range tested	Results	References
Wound healing	Petroleum ether, chloroform, ethanolic and aqueous extract of leaves, standard lawsone. Ointments prepared from above extract for topical application	Rat incision, and excision model Wistar rats were selected for the study	For oral administration: ethanolic extract 200-260 mg/kg, lawsone 50 mg/kg. Topical application: ethanolic extract ointment (30%) and lawsone ointment (0.1%)	Compared to oral treatment, topical application was more efficient.	Sakarkar *et al.*, 2004
	Ethanolic extract of leaves	The study was conducted on horses from local Arabian breed, cutaneous incisions were made on neck regions	Ethanolic extract in concentration of 5%, 10%, 15% and 20%	20% ethanolic extract showed better healing.	Towfik *et al.*, 2015
Antimicrobial	Aqueous, ethanolic, and acetone extract of leaves	*In vitro* assay	40 mg/ml	MIC value 2.5-10 mg/ml.	Rao *et al.*, 2016
	Methanol, ethanol extract of levaes	Well diffusion method	62.5-1000 µg/ml	Methanolic extract was more effective.	Rex *et al.*, 2019
	Ethanolic extract of leaves	Bore well assay method using agar as nutrient	150 mg/ml	Extract shows antimicrobial activity.	Naseem and Farrukh, 2015
	Aqueous, and methanolic extract of leaves	Bore well assay method using agar as nutrient	250 mg/ml	Synergistic effect with fluconazole.	Al-Rubiay *et al.*, 2008
	Methanolic, chloroform, and aqueous leaf extract	Antifungal activity against fungus	0.25-4% v/v	Antifungal activity against Malassezia was observed.	Ahmed and Beg, 2001

	Aqueous, ethanol, chloroform, and petroleum ether extracts of leaves	*B. cereus, B. subtilis, S. aureus, E. coli, P. vulgaris,* and *P. aeruginosa* inhibition by disc diffusion assay	400 mg/kg	Maximum inhibition was 3.8 cm.	Akter *et al*., 2010
Hypoglycaemic	Hydroalcoholic extract	Antidiabetic activity in alloxan induced model	100-800 mg/kg	800 mg/kg dose reduces blood glucose to normal level.	Singh *et al*., 2015
Antioxidant	Aqueous and methanolic extracts	Scavenge free radicals, and inhibit lipid peroxidation	125, 250, 500, and 1000 µg	Antioxidant activity was observed.	Guha *et al*., 2011
	Hydroalcoholic leave extract	Formaldehyde-induced model of male wistar rat.	200, and 400 mg/kg	Antiarthritic activity found.	Ali *et al*., 1995
Anti-inflammatory, analgesic, antipyretic and anti-diarrhoeal	Leaves ethanolic extract, chloroform, butanol and isolated lawsone	Effect for reducing inflammation was observed	0.25-2.0 g/kg	Anti-inflammatory, analgesic and antipyretic effects.	Al-Snafi *et al*., 2022
	Ethanolic extract of leaves	Effect for reducing inflammation	20 mg/kg 500 mg/kg	Analgesic and antidiarrheal activity.	Sultana and Khosru, 2011
Hepatoprotective	fraction of seeds using ethanolic (90%) and ethyl acetate solvents 50% ethanolic bark extract	Hepatoprotective activity in CCl_4-induced toxicity in rats CCl_4-induced oxidative stress models	200-400 mg/kg 250 and 500 mg/kg	Validated use of *L. inermis* seeds prevention of microsomal lipids peroxidation.	Kumar *et al*., 2017 Ahmad *et al*., 2000
Diuretic	Leaves extract in ethanol and water	Lips chitz method of diuretic	250 mg/kg, and 500 mg/kg	Diuresis effect.	Reddy *et al*., 2011
CNS activity	Ethanol, dichloromethane, aqueous and petroleum ether extract of seeds	Strychnine-induced convulsions models.	100, 200 and 400 mg/kg	Altered body functions.	Jacob *et al*., 2010
CNS depressant	Methanolic extract of bark	Hole cross test, and open field test.	300 and 500 mg/kg orally	CNS depressant activity.	Nesa *et al*., 2014
Antiparasitic	Petroleum ether extract of leaves	*In vitro* anthelmintic activity.	25-75 mg/ml	Anthelmintic activity noted.	Wadekar *et al*., 2016
Antiviral	Methanolic extract of leaves	Inactivation of model virus (bacteriophage MS2 DSM 13767)	0.03125 mg/ml to 1mg/ml	As sanitizer during covid 19 pandemic.	Majiya and Galstyan, 2023
Anticancer	Alcoholic extract of leaves	Isolated lawson tested on MTT assay on HeLa, and IMR-32 cell lines.	500, 1000, 1500, 2000 µg/ml	Lawsone proves to have anticarcinogenic activity.	Singh and Luqman, 2014
Antifungal activity	Bark extract	Tested against *Trichophyton mentagrophytes* and *Microsporum gypseum*	Maximum inhibitory dilution of 1:30 W/V 1: 10 W/V	Fungistatic at fungicidal activities were observed.	Singh and Pandey, 1989
Antiurolithiasis activity	Bark extract using methanol	Tested in albino Wistar male rats	Orally 300 and 500 mg/kg	Methanolic extract of *L. inermis* bark proves antiurolithiasis activity.	Patel and Shah, 2017

Anthelmintic activity	Chloroform, ethanol and water extract of leaves	Investigated against adult *Ecinia fetida*	10, 20, 50, 100 mg/ml	Aqueous extract shows better anthelmintic activity.	Wadekar *et al*., 2016
Anticonvulsant activity	Chloroform, ethanol and water extract of leaves	Tested by electro shock method in mice	20 mg/kg	Chloroform extract shows significant activity.	Wadekar *et al*., 2016
Ameliorative effect	Methanolic extract of leaves	level of paras-eataemia, PCV, erythrocyte osmotic fragility was observed	125, 250, 500 mg/kg	Antitrypanosonal effect.	Tauheed *et al*., 2016
Nootropic effect	Leave extract with petroleum ether	Assessed using passive shock avoidance and elevated plus maze	100 mg/kg	Prominent noot-ropic effect.	Iyer *et al*., 1998

4. Pharmacological activity

A wide spectrum of biological activities of the *L. inermis* have been studied and validated using diverse advanced pharmacological methods. A brief explanation is given in Table 2.

5. Toxicological studies

Henna is widely used in cosmetics and also as a dye for hair (Rubio *et al.,* 2022). One of the uses of henna is antimicrobial, it is also directly applied on affected areas like dandruff, eczema, scabies, infections and wounds (Zaidi *et al.,* 2023). The leaf extract exhibits biocide action against both fungus and termites (Adedeji *et al.*, 2017). Leaf extracts have a cytotoxic effect on vero cells (Manuja *et al.*, 2021). The skin and eyes of rabbits are not irritated by *L. inermis,* nor in human skin (European Commission. Directorate General for Health and Consumers, 2013). The use of herbal remedies is linked to mutagenicity, carcinogenicity and hepatotoxicity as per the toxicological research published in medical journals (Towfik *et al.,* 2015). Acute toxicity tests were performed using 300 mg/kg of ethanol extract through subcutaneous injection for two weeks. In the first two hours and then again after 24 hours, the animals were regularly observed for clinical symptoms like hostility, drowsiness, increasing fur, increased breathing, vomiting, cardiac rate, alteration in behavior, convulsions or mortality (Gull *et al.,* 2013). The plant and its parts contains substantial antioxidant, anti-inflammatory, hepatoprotective, antibacterial, analgesic and adaptogenic qualities, as reported in the literature, making it safe to use (Zaidi *et al.,* 2023). The methanolic extract of plant roots is used as cosmetic product and against malaria but shows abortifacient activity (Aguwa, 2008; Esteki and Miraj, 2016). People having glucose 6-phosphate (GPD) deficiency has some negative effect of henna paste such as allergic reactions (Raupp *et al.,* 2001). Additional ingredients are added to pre-mix henna paste for better staining of skin (palm) and hair. Consumers are advised to take natural henna paste than pre mix powder as it is safe, some pastes have synthetics like carmine, silver nitrate, disperse orange dye, pyrogallol and chromium (Chauhan and Chauhan, 2021).

6. Conclusion

L. inermis is a universal herbal medicine that acts as coloring agent and also as a product with diverse pharmacological activity. It is a unique source of various phytochemicals and thus obtained the title of versatile medical plant. Crude extract from the leaves has many therapeutic uses in traditional times. Being a source of various phytochemicals, the plant possesses wound healing, anti-inflammatory, antibacterial, analgesic, antipyretic, antidiarrhoeal and hepatoprotective activity. At present traditional organic products are preferred over inorganic products. Different formulations can make by using different parts of *L. inermis* for the management of different diseases but still there is a need to extrapolate more delivery system to have proper effect. For the welfare of mankind, further evaluation is needed on *L. inermis* to see the practical clinical applications.

Conflict of interest

The author declares no conflicts of interest relevant to this article.

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