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A PHARMACOLOGICAL AND TOXICOLOGICAL REVIEW OF *LAWSONIA INERMIS*

Mounica Ponugoti

Department of Pharmacology, Hindu College of Pharmacy, Guntur - 522002, Andhra Pradesh, India.

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Correspondence to Author:

Mounica Ponugoti

Assistant Professor,
Department of Pharmacology,
Hindu College of Pharmacy,
Guntur - 522002, Andhra Pradesh,
India.

E-mail: mounicaponuganti@gmail.com

ABSTRACT: Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs. *Lawsonia inermis* L. is commonly known as Henna or Mhendi and abundantly available in tropical and subtropical areas. Henna belongs to lythraceae, also known as the loosestrife family. Ancient history of India describes its diverse uses and also plays appreciable role in Ayurvedic or natural herbal medicines. Henna has been used cosmetically and medicinally for over 9,000 years. Currently, there is a renewed interest in henna due the wide range of its pharmacological activities, safety and availability. The present attempt is to review and compile updated information on various aspects of *Lawsonia inermis* (Linn), a plant used all over the world. It gives a complete view of its pharmacological actions such as analgesic, anti-inflammatory, antipyretic, antiarthritic, antibacterial, antifungal, antiviral, antimalarial, antidiabetic, abortifacient, hepatoprotective, antioxidant, anticancer, antifertility, antiulcer, diuretic, wound healing, protein glycation inhibitory, enzyme inhibitory, antitypanosomal, anticoagulant, antisickling, nematocidal, molluscicidal, immunomodulatory, nootropic and tuberculostatic actions along with toxicological studies.

INTRODUCTION: Many of today's modern drugs have their origin in traditional plant medicine¹. The therapeutic efficacies of many indigenous plants for various diseases have been described by practitioners of traditional herbal medicines. Natural products are a significant source of synthetic and traditional herbal medicine and are still the primary health care system². Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems.

There exists a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic (Traditional Indian Medicine), Siddha, Unani and Chinese medicine³.

The present attempt is to review and compile updated information on various aspects of *L. inermis* Linn. a plant used all over the world. This plant is commonly known as Henna or Mhendi and abundantly available in tropical and subtropical areas. Ancient history of India describes its diverse uses and also plays appreciable role in Ayurvedic or natural herbal medicines⁴.

Lawsonia inermis Linn (Lythraceae) is a perennial plant commonly known as Henna, belongs to lythraceae, also known as the loosestrife family. Henna is cultivated by many farmers for cosmetic and pharmaceutical purposes, it belongs to the group of plants that are popular in nature and all parts of the plant (root, stem, leaf, flower pod and seed) are of great medicinal important⁵. It is native

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to North Africa and South East Asia, and often cultivated as an ornamental plant throughout India, Persia, and along the African coast of the Mediterranean Sea⁶.

Common names:⁷

English	: Henna, Samphire, Cypress shrub
Sanskrit	: Mendi, Mendika, Timir, Rakigarbha
Telugu	: Goranta, kormni
Hindi	: Mehndi
Malayalam	: Mailanchi
Tamil	: Maruthani
Oriya	: Benjati
Kannada	: Mayilanchi
Bengali	: Mehedi

Morphology: *Lawsonia inermis* is a glabrous branched shrub or small tree (2 to 6 m in height). Leaves are small, opposite, entire margin elliptical to broadly lanceolate, sub-sessile, about 1.5 to 5 cm long, 0.5 to 2 cm wide, greenish brown to dull green, petiole short and glabrous acute or obtuse

Traditional Uses:

TABLE 1: TRADITIONAL USES

Plant Parts	Traditional Uses (as/in)
Leaves ¹³⁻¹⁶	Bitter, astringent, acrid, diuretic, emetic, edema, expectorant, anodyne, anti-inflammatory, constipating, depurative, liver tonic, haematinic, styptic, febrifuge, trichogenous, wound, ulcers, strangury, cough, bronchitis, burning sensation, cephalalgia, hemicranias, lumbago, rheumatalgia, inflammations, diarrhoea, dysentery, leprosy, leucoderma, scabies, boils, hepatopathy, splenopathy, anemia, hemorrhages, hemoptysis, fever, ophthalmia, amenorrhoea, falling of hair, greyness of hair, jaundice.
Flower ¹³	Cardiotonic, refrigerant, soporific, febrifuge, tonic, cephalalgia, burning sensation, cardiopathy, amentia, insomnia, fever
Seed ¹³	Antipyretic, intellect promoting, constipating, intermittent fevers, insanity, amentia, diarrhea, dysentery and gastropathy.
Root ¹³	Bitter, depurative, diuretic, emmenagogue, abortifacient, burning sensation, leprosy, skin diseases, amenorrhoea, dysmenorrhoea and premature graying of hair.

Chemical Constituents:

Leaves: 2-Hydro xy-1, 4-naphthoquinone (HNQ; Lawsone) is the principle natural dye contained at 1.0 -1.4 % in the leaves of Henna¹⁷. Other related compounds present in the leaves are: 1, 4dihydro xynaphthalene, 1,4-naphthoquinone, 1,2-dihydroxy-glucyloxy naphthalene and 2-hydroxy-1,4-diglyucyloxy naphthalene. Flavonoids (luteolins, apigenin, and their glycosides). Coumarins (esculetin, fraxetin, scopletin). Steroids (β -sitosterol)¹⁸. The leaves of *Lawsonia inermis* also reported to contain soluble matter tannin, gallic acid, glucose, mannitol, fat, resin and mucilage².

apex with tapering base. New branches are green in colour and quadrangular, turn red with age. Young barks are greyish brown, older plants have spine-tipped branchlets. Inflorescence has large pyramid shaped cyme. Flowers are small, numerous, aromatic, white or red coloured with four crumbled petals. Calyx has 0.2 cm tube and 0.3 cm spread lobes. The fruits are small, brown globose capsule, opening irregularly and split into four sections with a permanent style. Seeds have typical, pyramidal, hard and typical seed coat with brownish colouration⁸⁻¹⁰.

Cultivation: Henna grows better in tropical savannah and tropical arid zones, in latitude between 15° and 25° N and S, produces highest dye content in temperature between 35 - 45 °C. The optimal soil temperature range for germination is 25 - 30 °C. Henna leaves are very popular natural dye to colour hand, finger, nails and hair. The dye molecule, lawsone is the chief constituents of the plant; its highest concentration is detected in the petioles (0.5-1.5 %) ^{11, 12}.

Bark: Bark contains naphthoquinone, isoplumbagin, triterpenoids, Hennadiol, aliphatics (3-methyl nonacosan-1-ol)¹⁸.

Flower: Flowers on steam distillat ion gave an essential oil (0.02 %) rich in ionones (90 %) in which β -ionones predominated¹⁸.

Root: Aqueous root extract of *L. inermis* contains alkaloids, saponins, steroids, cardiac glycosides, flavonoids, tannins and reducing sugars¹⁹.

Pharmacological Activities: Several researchers have reported the different pharmacological activities of *L. inermis* which are discussed below.

Analgesic and Antipyretic Activity: The ethanolic extract of leaves of *Lawsonia* showed significant analgesic as well as antipyretic activity. The fixed oil obtained from seeds were screened for pharmacological activity both *in-vitro* and *in-vivo*. It was concluded that seed oil is devoid of behavioral and CNS effects and failed to produce any effect on isolated tissue though it possess significant analgesic activity²⁰.

Anti-Inflammatory Activity: Methanol extract of *Lawsonia inermis* flowers showed a good anti-inflammatory activity against 5-Lipoxygenase (IC₅₀=49.33mg/L) compared to references. It may be interpreted that the greatest anti-inflammatory activity was due to the high amounts of total phenolic compounds²¹.

Isoplumbagin and lawsaritol, isolated from stem bark and root of *L. inermis* screened for anti-inflammatory activity against carrageenan induced paw edema in rats. The results showed that isoplumbagin exhibited significant activity, was compared to that of phenylbutazone²².

Butanol and chloroform fractions showed potent anti-inflammatory, analgesic and antipyretic effects that aqueous fraction of crude ethanol extract of *L. inermis* in a dose dependent manner. Leaves showed significant anti-inflammatory effect with some active principles²⁰.

Antiarthritic Activity: Aqueous and ethanol leaf extract demonstrated anti-arthritic activity, as reflected by a reduction in paw oedema, paw diameter and body weight loss in both Freund's adjuvant-induced and formaldehyde-induced arthritis mice models, at doses of 200 and 400 mg/kg p.o., respectively. In this study, an oral dose of 10 mg/kg of diclofenac sodium was used as the positive control²³.

Anti-ulcer Activity: Aqueous, ethanol and chloroform leaf extracts showed a strong anti-ulcer activity in pylorus ligation- and aspirin-induced rats when compared to ranitidine, the positive control. In addition, significant reductions (p.o. = 0.001) in gastric acid secretions, total acidity and ulcer index were observed²⁴. Aqueous, ethanolic and chloroform extracts produced significant activity against acute and chronic gastric ulcers in two rat models at doses of 200 and 400 mg/kg p.o. when

compared to the negative control gum acacia (2%, w/v). Sucralfate (250 mg/kg) served as the positive control. Aqueous, ethanolic and chloroform extracts were found to reduce ethanol-induced ulcers by up to 81, 94 and 88%, respectively, and cold-restraint stress-induced ulcers by up to 56%, 30% and 56%, respectively²⁵.

Ethanolic leaf extract showed antiulcer activity in indomethacin-induced gastric ulcers in pylorus ligation rat models by reducing the ulcer index for all three doses (100, 200 and 400 mg/kg p.o.) tested²⁶.

Antidiabetic activity: The ethanolic extract of leaves of *Lawsonia inermis* linn (400mg/kgBW) in alloxan induced diabetic rats showed significant hypoglycaemic activity after oral administration²⁷. Ethanolic extract of *Lawsonia inermis* (500mg/kg body weight) significantly decreased level of blood glucose in streptozotocin induced diabetic rats²⁸.

Ethanol (70 %) extract of *L. inermis* showed significant hypoglycemic and hypolipidemic activities in alloxan induced diabetic mice after oral administration. The feeding of 0.8 g/kg of *L. inermis* extract decreased the concentration of glucose, cholesterol and triglycerides to normal. Methanol (95 %) extract of leaves of *L. inermis* showed significant *in-vitro* antihyperglycemic effect²⁹.

Antibacterial activity: Antibacterial activity of aqueous, methanol extracts of Yemeni henna (*Lawsonia inermis*) leaves were tested against three bacterial species including (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*) using agar diffusion and minimum inhibitory concentration (MIC) as a determination method. Preliminary phytochemical screening revealed the presence of Alkaloids, Quinones, Glycosides, Tannins and saponins. The methanolic extract displayed a potential antibacterial activity against all the bacterial species, than the aqueous extract.

The maximum activity was observed in methanolic extract against *Staphylococcus aureus* at inhibition zone of about (27 ± 1 mm) and minimum activity was observed in aqueous extract against *Escherichia coli* at inhibition zone of about (8.6 ± 1.2). MIC values for all the existing extracts at a concentration of 2.5mg/ml at *Staphylococcus*

aureus and 10 mg/ml at *Pseudomonas aeruginosa*³⁰. Ethanolic extract of Lawsonia leaves were investigated for antimicrobial property using Agar well diffusion method. It was found to inhibit the growth pattern of *A. niger*, *F. oxysporum*, *Streptococcus sp* and *S. aureus*³¹.

Ethanolic extract of *Lawsonia inermis* was investigated for antimicrobial activity against different life threatening pathogenic microorganisms. It was found to possess good antibacterial properties over a wide range of disease causing gram positive (*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus fusiformis*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*) as well as gram negative (*Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexneri*, *Vibrio cholera*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*) bacteria³².

Antioxidant, antibacterial activities and phytotoxic potentials of commonly commercialized henna's dried leaves (HL) and processed powder (HP) were investigated and the antimicrobial potential evaluated using a range of microorganism strains demonstrated that *Bacillus subtilis* (ATCC6633) was the most sensitive bacteria to both HL and HP extracts with MBC $\approx 165.8 \pm 3.7$ $\mu\text{g/ml}$ (HP) and 454.3 ± 42 $\mu\text{g/ml}$ (HL)³³.

Methanolic leaves extracts of *Lawsonia inermis* Linn inhibit the growth of micro organisms (Gram positive; *B. subtilis*, *S. aureus* and *S. epidermidis* and Gram negative; *E. coli*, *S. flexneri*, *P. aeruginosa* bacteria) in a dose dependent manner using disc diffusion method. The presence of flavonoids and glycosides as major constituents of the plant leaves that are commonly known to possess antimicrobial activity³⁴.

Antibacterial activity of aqueous, ethanol, methanol, ethyl acetate and chloroform extracts of *Lawsonia inermis* Linn. leaves were tested against reference bacterial strains (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, *Vibrio cholerae*, *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus*) and clinical isolates (*Staphylococcus aureus* and Amp C β -lactamases producing *Proteus mirabilis*). The

solvent extracts of *L. inermis* leaves exhibited profound antibacterial activity against the bacterial pathogens tested. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts for Gram positive species were between 34-64 μg and 39-74 μg . MIC and MBC of the extracts for Gram negative species were between 46-71 μg and 51-75 μg . The role of the plant material was apparent in inhibiting the growth of clinically important AmpC β -lactamases producing *Proteus mirabilis*, which have developed resistance to commonly prescribed antibiotics used for treating infections caused by uropathogens³⁵.

Lawsonia inermis displayed noteworthy antimicrobial activity against both gram positive and gram negative bacterial strains used in the study. The minimum value of MIC for different bacterial strains ranged from 2.31 mg/ml to 9.27 mg/ml. At 1x MIC of each bacterial isolate, 3log₁₀ decrease in CFU was recorded after 6 hours of drug exposure and no growth was observed in almost all tested bacteria after 24 hours of exposure³⁶.

Ethanol extracts of 20 plants species used by Yemeni traditional healers to treat infectious diseases were screened for their antibacterial activity against both gram positive and gram negative bacteria. The ethyl acetate extract of *L. inermis* was found to be the most active against all the bacteria in the test system^{37,38}.

Henna samples from different regions of Oman demonstrated antibacterial activity against a wide range of different bacterial strains with the highest antibacterial activity being demonstrated against *P. aeruginosa* organisms³⁹.

Henna leaves extracts showed considerable antimicrobial activity almost on all of the tested microorganisms (*S. aureus*, *Bacillus* spp., *K. pneumoniae*, *Proteus* spp., *E. coli*, *P. aeruginosa*, and *Enterococcus* spp. with the exception of aqueous extract which showed the least effect on most bacterial samples tested⁵. *In-vitro* antibacterial activities of the aqueous extract, fractions of ethanol extract and fractionation residue of the leaves were investigated against *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus pneumoniae*, *Pseudomonas*

aeruginosa, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Salmonella typhi* and *Shigella dysenteriae* using agar-disc diffusion method. The aqueous extract, the fractions and the fractionation residues all showed antibacterial activities against the test isolates⁴⁰.

Different concentrations of aqueous, methanol and chloroform crude extracts of *Lawsonia inermis* leaf were bioassayed *in vitro* for its bioactivity to inhibit the growth of 6 human pathogenic fungi and 4 types of bacteria. The growth of all pathogens was inhibited to varying degree by increasing the concentration of extract. Aqueous extract showed superior activity followed by methanol and chloroform⁴¹.

The antibacterial activity of methanolic extract of *L. inermis* was investigated by agar well diffusion method using *S. aureus* (MTCC 087), *E. coli* (MTCC 729), *K. pneumoniae* henna may be effective in the management of wound infections. (MTCC 432), *P. aeruginosa* (MTCC 1688) and *P. mirabilis* (MTCC 425)⁴².

Primary invaders of burn wounds *viz* *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Fusarium oxysporum* and *Aspergillus niger* were treated with aqueous and chloroform extract obtained from leaves of *L. inermis*, using *in-vitro* agar incorporation and well diffusion methods. Extract inhibit growth pattern of all microbes except *C. albicans*. Overall, study suggested that henna may be effective in the management of wound infections¹.

Crude extracts of fresh dry leaves and seeds of henna were investigated for their antimicrobial activity against three standard strains. Henna dry leaves demonstrated the best *in-vitro* antimicrobial activity and in particular against *Shigella sonnei*⁴³. Out of forty-five species of 29 plant families used in the traditional medicine by Iranian people showed antibacterial activities against eleven bacterial species, henna showed strong activity against *Bordetella bronchiseptica*. These findings indicated that *L. inermis* can be used in the treatment of bacterial infections⁴³.

Genotoxic studies on main constituent of henna suggested that it was a weak bacterial mutagen for *Salmonella typhimurium* strain TA98 and was more

mutagenic for strain TA2637. It suggested that hydroxyl naphthaquinone have no genotoxic risk to the consumer¹⁷.

Antifungal Activity: *L. inermis* leaves extract showed a fungicidal effect against *Trichophyton mentagrophytes* and *Candida albicans*⁴⁴. It was reported that the sensitivity of dermatophytes toward henna was strong in *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, *T. violaceum*, *T. verrocosum*, *T. schoenleinii*, *Epidermophyton floccosum*, *Microsporum ferrugineum*, *M. canis* and *sporotrichum schenckii*⁴⁵. The effect of aqueous and methanolic extract of henna using 25 µl of the extracts against *C. albicans* and *Microsporum* was examined and confirmed³⁷.

Aqueous extract of leaves of *L. inermis* was tested for the antifungal potential against eight important species of *Aspergillus* which isolated from sorghum, maize and paddy seed samples. *A. flavus* recorded high susceptibility and hence solvent extracts *viz.*, petroleum ether, benzene, chloroform, methanol and ethanol extract of the plant showed significant antifungal activity. These finding suggested that henna extract could be used as alternative source of antifungal agents for protection of plants or crops against fungal infection⁴⁶.

During screening of barks of 30 plant species for activity against *Microsporum gypseum* and *Trichophyton mentagrophytes*, only *L. inermis* extract exhibited absolute toxicity. The extract showed broad fungitoxic spectrum when tested against 13 other dermatophytes. Further the fungi toxicity of the extract remained unaltered at high temperature on autoclaving and after long storage⁴⁷.

Ethanol, methanol and aqueous extracts of leaves of *L. inermis* showed defensive mechanism against spore germination of *Drechslera oryzae*. In another research by Natarajan and Lalitha, the activity of ethanol, ethyl acetate and hexane extracts of *L. inermis* were tested on 5 strains each of *Tinea rubrum* and *Tinea mentagrophytes*. All these extracts showed significant antidermatophytic properties *in-vitro*⁴⁸.

Antiviral Activity: Henna definitely has an antiviral effect that became clear by its action on warts, whitlow and herpes simplex. Henna was tried traditionally in many times especially on the warts which are resistant to cryo (Nitrogen liquid) treatment and prove effective on giant wart measuring 1.5x1.5cm on a child thumb which was resistant to all forms of treatment, at last the child referred to the plastic surgeon for operation, “we tried Henna on it, applied every other day over night and in few weeks it disappeared completely”. Henna was found very useful especially on multiple warts. On warts Henna applied as paste. The second proven and successful effect of henna on viral infections was after its application to herpes it was noticed that; it dried the vesicles at the site early, prevent ulceration and crust formation and it prevents secondary infection. This anti-viral effect of Henna is very promising and should be explored further; it could be used as treatment for AIDS. It is natural, cheap, and it looks to have no side effect even when taken by oral route⁴⁹.

The ethanol soluble fraction of *L. inermis* fruits displayed highly potent activity against Sembiki forest virus (SFV) in swiss mice and chick embryo models exhibiting 100 to 65 % activities after 10 to 25 days of virus challenge⁵⁰.

Antimalarial Activity: *L. inermis* is a potential antimalarial drug, having high in vitro and in vivo antiplasmodial activity. The *in vitro* combination of *L. inermis* and *T. diversifolia* (1:1) extracts against *P. falciparum* showed the highest synergy with IC₅₀ of 0.4370.02 mg/mL and 2.5570.19 mg/mL against *P. falciparum* Chloroquine sensitive (D6) and resistant (W2) strains respectively. This study also indicates that, combination of *L. inermis* and *T. diversifolia* could serve as a potential antimalarial drug candidate in combination⁵¹.

Antimalarial activity of *Lawsonia inermis* is also explained by Oladele AT. 87 Traditional medicine practitioners (TMP's) were interviewed for medicinal plants used in the management of malaria in the Yoruba folklore in south western Nigeria. A total of 21 plant species used by TMP's in the management of malaria infections were identified which includes *Lawsonia inermis* Linn.⁵²

Antileishmanial Activity: Methanolic extract of the plant material at concentrations from 100 - 500 µg/ml was tested *in vitro* to get % inhibition activity on *L. tropica* KWH23 promastigotes in comparison with negative control and Amphotericin-Bat 12-24 hours, whereas *in vivo* antileishmanial activity was checked against *L. tropica* infected Albino mice. For *Lawsonia inermis* bark, mean % inhibition in extracellular promastigotes at four different concentrations (100 µg/ml, 125 µg/ml, 250 µg/ml, and 500 µg/ml) at 24th hour were 98.02 0.06, 98.70 1.09, 99.41 0.00 and 100.00 0.00 respectively, whereas after 8 weeks, mean lesion size decreased from 0.81 ± 0.20mm to 0.10 ± 0.11mm (p < 0.01) and % cure rate against intracellular amastigotes at dose 75mg/kg was 98.022 (95% C.I = 96.13-98.09) in Albino mice⁵³.

Antifertility Activity: Ethanol extract prepared from the powdered seeds of *L. inermis* failed to show significant antifertility activity. However in subsequent studies it was observed that the powdered leaves of henna, when administered as suspension or incorporated into the diet inhibited the fertility of rats. The infertility induced appeared was found to be permanent⁵⁴.

Tuberculostatic Activity: The tuberculostatic activity of henna was tested *in-vitro* and *in-vivo* using Lowenstein Jensen medium, the growth of *Tubercle bacilli* from sputum of *Mycobacterium tuberculosis* was inhibited by 6µg/ml of the herb. *In-vivo* studies on guinea pigs and mice showed that the plant at a dose of 5 mg/kg body weight led to a significant resolution of experimental tuberculosis following infection with *M. tuberculosis* H37Rv⁵⁵.

Anticancer Activity: Methanolic extract of *Lawsonia Inermis* bark showed cytotoxicity activity when exposed to lymphocyte cells having IC₅₀ values of 25.105 µg/ml (95% C.I = 15.55-33.83)⁵³. The chloroform extract of *Lawsonia inermis* flowers exhibited a higher tannins content (148.5±1.5mg CE/kg of dry mass), has the stronger anticancer activity with IC₅₀=21mg/L. The methanol extracts was weaker with less growth inhibition of HCT-116 cells with IC₅₀=50mg/L. We can deduce that chloroform is a better solvent for

more extraction of anticancer compounds from *L. inermis* as compared to other solvents²¹.

Chloroform extract of Mehndi leaves showed cytotoxicity on HepG2 and MCF-7 (hormone dependent breast cancer cell line) with an IC₅₀ value of 0.3 and 24.8µg/mL respectively. The findings revealed that ethanolic extract of Mehndi/Henna increased the life span of DLA tumor bearing mice, enhanced the antioxidant status and reduced the lipid profile⁵⁶.

The antitumour activity of *L. inermis* leaf extract was studied on 7,12-dimethylbenzanthracene (DMBA) induced 2-stage skin carcinogenesis and B16F10 melanoma tumour model using swiss albino mice. Topical application of *L. inermis* leaf extract at a dose level of 1000 mg/kg body weight was found to be effective in reducing the number of the papillomas⁵⁷.

The antitumour activity of *Lawsonia inermis* was investigated by constituting peritonitis carcinomatous with Ehrlich ascites cells. The animals were divided to three groups and *Lawsonia inermis* extract and tap water were given to mice for 5 day, all of animals were decapitated by cervical dislocation and their liver tissues were sampled to measure reduced glutathione (GSH) level. Mean survival times (MST) and Average survival times (AST) were calculated; peritoneal liquid pH was measured; Ehrlich Ascites Carcinoma (EA C) cells were counted with hemocytometer. As the result, the longest life period was detected on the group which was given 10 mg/kg/day *Lawsonia inermis*⁵⁸.

Lawsonia inermis can destroy cancer cells by induction of apoptosis due to decreasing of intracellular H⁺ ion level or increasing intracellular free radicals and H₂O₂ levels in cancer cells as a result of oxidative effect or not. 70 Swiss albino mice females are used and divided them into four groups. Group 1 was given only tap water. Group 2 was given only *L. inermis*. Group 3 was given Ehrlich Ascites tumor (EAT) tap water and Group 4 was given EAT + *L.inermis*. At the result of this study the thickness of subcutaneous lipid tissue, diameters of gluteal mass, the pH levels of cells gluteal mass, the GSH levels at the liver tissue samples and the MDA levels of the liver tissue

samples of these groups were measured. This study showed and that, *L.inermis* can be used as a supplementary agent for cancer treatment⁵⁹.

The anticarcinogenic activity of chloroform extract *L. inermis* leaves was carried using microculture tetrazolium salt assay on the human breast (MCF-7), colon (Caco-2), liver (HepG2) carcinoma cell lines and normal human liver cell lines (Chang Liver). The preliminary results showed that the henna extract displayed the cytotoxic effects against HepG2 and MCF-7 and IC-value of 0.3 and 24.85µg/ml respectively⁶⁰.

Essential oil from the leaves of Mehndi/Henna also exhibited strong cytotoxicity on HepG2 with an IC₅₀ value of 24µg/mL in MTT assay⁶¹.

Modulatory effect of Mehndi/Henna leaf extract on drug metabolising enzymes was investigated by Dasgupta *et al.*, (2003). Effect of 200 and 400 mg/kg bw of 80% ethanolic extract of the fresh leaves on drug metabolizing phase I and phase II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase and lipid peroxidation in the liver of 7 weeks old Swiss albino mice was investigated. Anti-carcinogenic potential of Mehndi/Henna leaf extract was also studied adopting the protocol of benzo (a) pyrene-induced forestomach and 7, 12 dimethylbenz (a) anthracene (DMBA)-initiated and croton oil-promoted skin papillomagenesis.

Outcomes of the primary result reveals the 'duel-acting' nature of mehndi/henna leaf as only phase II enzyme activity was induced associated with detoxification of carcinogen in liver of mice whereas the activity of phase I enzyme was inhibited. Significant inhibition of tumor burden was observed in both the studied model and reduced tumor incidence was observed in both the doses signifying the cancer chemopreventive potential of mehndi/henna⁶². Different constituents isolated from the leaves of *L. inermis* were tested for their antioxidant activity using ABTS. The IC₅₀ value of henna constituents are p-coumaric acid (2.6 mM), cosmosiin (2.9 mM) apiin (1.6 mM) respectively. These fundings depicted that all isolated compounds exhibited antioxidant activity comparable to that of ascorbic acid (2.5 mM)⁶⁷.

Hepatoprotective Activity of Henna: The aqueous extract of *Lawsonia inermis* was administered orally to the rats with hepatotoxicity induced by paracetamol. Silymarin was given as reference standard. The plant aqueous extract was effective in protecting the liver against the injury induced by Paracetamol in rats. This was evident from significant reduction in serum enzymes alkaline aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Acid Phosphatase (ACP), Protein and Bilirubin⁶⁸.

The ABTS [2,2-azino-bis (3-ethyl benzthiazoline-6-sulfonic acid)], free radical scavenging assay depicted that all isolated compounds from henna exhibited antioxidant activity in an *in vitro* study comparable to that of ascorbic acid⁶⁹.

Alcoholic extract of the bark of *L. inermis* showed hepatoprotective effect against the CCl₄. Extract cause elevation in serum marker enzymes (GOT and GPT), serum bilirubin, liver lipid peroxidation and reduction in total serum protein, liver glutathione, glutathione peroxidase, glutathione-S-transferase, glycogen, superoxide dismutase and catalase activity^{70,71}.

The hepatoprotective activity of the ethanolic extract of the dried leaves of *L. inermis* and its fractions (petroleum ether, ethyl acetate, butanol and butanone fractions) was evaluated against CCl₄ induced hepatotoxicity in mice. The ethanolic extract and its fractions significantly reduced the total bilirubin content and aspartate aminotransferase or serum glutamic oxaloacetic transaminase Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Oxaloacetic Transaminase (SGPT) and Serum Alkaline (SAL) activities, and reduced liver weight compared to Liver Care (LIV-52) used as control⁷².

Anthelmintic activity: *L. inermis* have shown no statistically significant effect on the survival of adult parasites at the concentrations tested, and the few mortality cases recorded were not dose-dependent ($P < 0.05$). It did not inhibit the hatching of eggs of *Haemonchus contortus*, significantly, at all tested concentrations⁷³.

Diuretic activity: Aqueous and ethanolic extracts of *Lawsonia inermis* leaves showed diuretic activity in rats at a dose of 250mg/kg and

500mg/kg orally. The ethanolic extract shown more activity compared to aqueous extract⁷⁴.

Abortifacient Activity: Methanol extract of roots of *L. inermis* was most effective in inducing abortion in mice, rats and guinea pig. The effect apparently was dosage dependent. The results of the whole animal experiments support the methanol extract effectiveness as an abortant due to its maternal and foetal toxic effects⁷⁵.

It was confirmed that the use of *Lawsonia inermis* to induce first trimester abortions, prevent and treat postpartum haemorrhage in traditional medicine and suggest that uterotonic activity involving the beta-adrenergic pathway may be the mechanism⁷⁶.

Molluscicidal Activity: The molluscicidal activity of leaf, bark and seed of henna against *Lymnaea acuminata* and *Indoplanorbis exustus* were studied. Seed powder was more toxic than leaf and bark against *L. exustus*¹⁴.

Binary combinations of henna seed with *Cedrus deodara Roxb* and *Azadirachta indica* A Juss oil, or powdered *Allium sativum*, or *Zingiber officinale* rhizome oleoresin was more toxic to snails *L. acuminata* and *I. exustus* than their single treatment. The highest increase in the toxicity was observed when henna seeds powder and *C. deodara* oil (1:1) were tested against both the snails. The combination with neem oil was also more toxic than their individual components and other combinations⁷⁷.

Nematicidal Effect: A suppressive effect was obtained by *L. inermis* against *Meloidogyne incognita* development. Henna reduced tomato root gall numbers, number of the egg-laying females and rate of the nematode reproduction, when tomato and henna were grown together. Also, same reduction in the nematode biological processes was found, when tomato plants were grown in soil containing root exudates of henna, but with less amount. When henna was grown alone, root gall index and the rate of nematode production reduced to 75% and 99%, respectively, compared with those of tomato grown alone⁷⁸.

Antitrypanosomal Activity of Henna: Crude methanolic extract of leaf of *L. inermis* showed *in vitro* activity against *Trypanosoma brucei* at

concentration of 8.3 mg/ml of blood in mice but not *in-vivo*. The treatment tends to ameliorate the disease condition, but did not affect the level of parasitaemia and packed cell volume⁷⁹.

Anticoagulant Effect: Lawsone and its oxazine derivatives isolated from leaves of *L. inermis* had proven to be potential anticoagulant agent⁸⁰.

Wound Healing Effects: Ethanolic extract of henna leaves and lawsone exhibited significant wound healing activity on rat excision and incision wound models. It was reported that the topical application of ethanolic extract as well as lawsone were more effective than the same given by oral route.⁸¹

Ethanol extract of the plant (200 mg/kg) was used to evaluate the wound healing activity on rats using excision, incision and dead space wound models. Topical application was made in the case of excision wound model. Whereas, oral treatment was done with incision and dead space wound model. Extract of *L. inermis* showed high rate of wound contraction, a decrease in the period of epithelialization, high skin breaking strength, a significant increase in the granulation tissue weight and hydroxyproline content⁸².

Histological studies of the tissue showed increased well organized bands of collagen, more fibroblasts and few inflammatory cells when compared with the controls which showed inflammatory cells, scanty collagen fibres and fibroblasts. These findings suggested the use of *L. inermis* in the management of wound healing². Chloroform and aqueous extracts of leaves of the plant were capable of inhibiting the growth of microorganisms that are involved in causing burn wound infections¹.

Immunomodulatory Effect: Methanol extract of henna leaves at 1 mg/ml concentration had displayed immunostimulant action as indicated by promotion of T-lymphocyte proliferative responses. Seven compounds were isolated adopting the lymphocyte transformation assay (LTA)-guided fractionation of the total methanolic extract of henna leaves⁶⁷.

Naphthoquinone fraction obtained from leaves *L. inermis* showed significant immunomodulatory effect⁸³.

Nootropic activity: The effect of acetone soluble fraction of petroleum ether extract of *L. inermis* leaves was investigated on memory, anxiety and behaviour mediated *via* monoamine neurotransmitters using elevated plus maze and passive shock avoidance paradigms. The extract exhibited prominent nootropic activity, potentiated clonidine induced hypothermia and decreased lithium induced head twitches. However, the haloperidol induced catalepsy was not modified⁸⁴.

Protein inhibitory activity glycation: Ethanol extract of the plant tissues was evaluated *in-vitro* for protein glycation inhibitory activity using the model system of bovine serum albumin and glucose. The extract and its components showed significant effect on protein damage induced by a free radical generator during *in-vitro* assay system⁸⁵.

It was found that the alcoholic extract, lawsone and gallic acid showed significant inhibition of Advanced Glycated End Products (AGEs) formation and exhibit 77.95 %, 79.10 % and 66.98 % inhibition at a concentration of 1500 µg/mL, 1000 µg/mL and 1000 µM respectively. *L. inermis* constituents were confirmed to be glycation inhibitors⁸⁶.

Antisickling Activity: Aqueous extract of leaves of *L. inermis* was found to inhibit sickling and to increase the oxygen affinity of HbSS blood⁸⁷.

Enzymes Inhibitory Activity: The ethanol extract of *L. inermis* L. leaves and lawsone tested for trypsin inhibitory activity showed an IC₅₀ value of 64.87 and 48.6µg/ml, respectively⁸⁸.

Miscellaneous:

Anticataleptic Activity: Significant action was obtained by treating haloperidol-induced catalepsy in mice with an aqueous extract of henna. A reduction in cataleptic scores was found and an increase in superoxide dis-mutase activity was measured at a dose of 400 mg/kg⁸⁹.

Anticlastogenic Activity: A methanol extract (2.5 mg/kg) of henna demonstrated *in vivo* anticlastogenic effects in a rat bone marrow cell micronuclei test in which clastogenicity was induced by sodium arsenite⁹⁰.

Allelopathic Activity: Antioxidant, antibacterial activities and phytotoxic potentials of commonly commercialized henna's dried leaves (HL) and processed powder (HP) were investigated. The extracts showed allelopathic activity on seed germination, stem and root growth of wheat (*Triticum aestivum* L.) and canary grass (*Phalaris canariensis* L.) when assessed *in-vitro*. Canary grass was the most sensitive species to HL and HP. Both extracts were effective inhibitors of germination, stem and root growth in a dose-dependent manner. These findings could support the potential applications of HL and HP extracts as natural herbicide³³.

The leaf extract of *Lawsonia inermis* L. showed allelopathic effect on the seed germination and growth performance of pulse crops *viz* *Vigna radiata* (L), Hepper., *Vigna mungo* (L) Hepper and *Arachis hypogaea* L.⁹¹.

Toxicological Studies: The use of henna is widespread and has been used for centuries as generally considered harmless since prehistoric time to date. Opinion of The European Commission (EC) (2013) during the meeting of the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) intended for consumers concerning *Lawsonia inermis* (henna) concluded that *Lawsonia inermis* is not irritant for the rabbit skin and eye and was considered not irritating on human skin after Repeated Insult-Patch Tests (RIPT)⁹². However, seed and root extracts of *Lawsonia* produced certain toxidromes which are reported below.

Acute toxicity studies were carried out with ethanol extract of *Lawsonia inermis* (300 mg/Kg) to one group and other group with equal volume of vehicle (DMSO) daily through subcutaneous route for 2 weeks by subcutaneous injection. The animals were continuously observed for signs of toxidromes such as aggression, sedation, rising fur, increased respiration, altered cardiac rate, excitation, convulsion, stupor, vomiting, etc. or death in first 2 hours and then after 24 hours. No sign of toxidrome were observed during *in vivo* toxicity evaluation in mice at 300 mg/kg concentration³⁶.

Acute toxicity profile of leaves extract of *L. inermis* using albino wistar rats of both sex was

determined with dose pattern of 100mg, 500mg, 1000mg and 2000mg /kg/b.w for 72 hours. After determining the lethal dose sub-acute study was conducted with dose 200mg, 500mg and 1000mg /kg/b.w. for 14 days. After 14 days blood was taken for various biochemical test and animals were scarified, heart, liver, kidney and spleen were histologically studied. The histopathological and biochemical results showed that no pathological and haematological changes were observed with dose level of 200 and 500mg/kg/b.w. when compared with the control group⁹³.

The effect of aqueous extract of *Lawsonia inermis* seeds was carried out to study the acute and sub-chronic toxicological effects on rats. The 78.57 mg kg⁻¹ of the extract administered for 4 weeks, caused body weight gain to rats and a significant decrease on hematological parameters and potassium concentration. Also there was a significant increase in the AST, ALP, total protein, albumin and urea concentrations with no obvious histopathological changes. 78.57, 392 and 785.7 mg/kg/day administered orally to rats for 1 week, caused an increase in AST, ALP and total protein concentrations. 785.7 mg kg⁻¹ of the extract caused an increase in the ALT activity and a decrease in the potassium concentration. 78.57 and 785.7 mg kg⁻¹ of the extract caused an increase in urea and cholesterol concentrations, while 392 and 785.7 mg kg⁻¹ of the extract had caused hepatocytic necrosis, dilatation of the renal tubules and desquamation of the intestinal epithelium.⁹⁴

The lethal dose of the aqueous root extract was determined to ascertain its safety using Wistar rats. Five groups of rats (4 rats per group; including two pregnant females) were administered intra peritoneally (i.p.) doses of the stock aqueous root extract (0.3 g/ml) volumes corresponding to 200,400, 800, 1200 and 1600 mg/kg Body Weight (BW). The control group was given 0.5 ml of distilled water and observed for 7 to 10 days. Various clinical symptoms (physiological changes) like dizziness, loss of appetite, partial paralysis, temporary amnesia and spontaneous abortion in the included pregnant females; were visibly observed in groups treated with 800 to 1600 mg/kg, while groups with 200 to 400 mg/Kg BW and the control remain active and healthy. No mortality was recorded in any of the groups. Conclusions from

the result indicate delayed toxicity after ip route administration of the extract at various concentrations. It demonstrated that the aqueous root extract of the *L. inermis* although, it is active in inducing spontaneous abortion; is slightly toxic and is safe for therapeutic purposes within the dose range.

This activity could be related to the various phytochemical compounds proven to be present in the aqueous extract in preliminary phytochemical analysis.⁹⁵

It was also reported that the use of henna in people with glucose 6 phosphate (G6PD) deficiency produced haemolytic anaemia and acute renal failure. An important chemical ingredient of henna is lawsone (2-hydroxy- 1, 4 naphthoquinone), constituting about 1% by weight of the crushed leaves. The structure and redox potential of lawsone is similar to that of one of the naphthalene metabolites, 1, 4 naphthoquinone, a potent oxidant of G6PD – deficient red cells. In vitro observations indicate that lawsone, is capable of inducing oxidative injury to G6PD normal red cells, and even more so to G6PD and acute renal failure was observed due to nephrotoxic effect of the henna.⁹⁶⁻⁹⁹

CONCLUSION: *Lawsonia inermis* is not only a colouring agent, but a universal herbal medicine with diverse pharmacological activity spectrum. This versatile medicinal plant is the unique source of various types of chemical compounds, which are responsible of the various activities of the plant. Although crude extracts from leaves of plant have medicinal applications from time immemorial, modern drugs can be developed after extensive investigation of its bioactivity, mechanism of action, pharmacotherapeutics and toxicity after proper standardization and clinical trials. At present the global scenario is changing towards the use of non-toxic plant products having traditional medicinal use, development of modern drugs from *L. inermis* should be emphasized for the control of various diseases.

Further evaluation needs to be carried out on *L. inermis* L. in order to explore the concealed areas and their practical clinical applications, which can be used for the welfare of the mankind.

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