

Review Article

Lawsonia inermis Linnaeus: A Phytopharmacological Review

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ABSTRACT

Lawsonia inermis L. is a much branched glabrous shrub or small tree, cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine. The plant is reported to contain carbohydrates, proteins, flavonoids, tannins and phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthones and fatty acids. The plant has been reported to have analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, anti-fertility, tuberculostatic and anticancer properties. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products. This review gives a bird's eye view mainly on the pharmacognostic characteristics, traditional uses, phytochemistry and pharmacological actions of the plant.

Keywords: Flavonoids; Henna; Pharmacological action; Phenolic compounds.

INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization.^[1] There exits a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic (Traditional Indian Medicine), Siddha, Unani and Chinese medicine. According to the World Health Organization, 2003 about 80 % of the population of developing countries being unable to afford pharmaceutical drugs rely on traditional medicines, mainly plant based, to sustain their primary health care needs. ^[2] 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. ^[3-4]

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 medicines.^[5]

Lawsonia inermis Linn.

Genus Lawsonia bears one species, *L. inermis (Henna, Mhendi, Shudi, Madurang, Mendi, Manghati, Madayantika and Goranti)*^[6-7] till date, having different synonyms as *alba*

*Corresponding author: Mr. Gagandeep Chaudhary, Lecturer, Department of Pharmacognosy, S. D. College of Pharmacy, Barnala, Punjab, India; Tel: 09463509193 E-mail: gdchaudharym.ph@gmail.com and *spinosa* belonging to family Lythraceae. It is a biennial dicotyledonous herbaceous shrub. A native of North Africa and South-West Asia, the plant is now widely cultivated through out the tropics as an ornamental and dye plant.

A much branched glabrous shrub or small tree (2 to 6 m in height). Leaves are small, opposite in arrangement along the branches, sub-sessile, about 1.5 to 5 cm long, 0.5 to 2 cm wide, greenish brown to dull green, elliptic to broadly lanceolate with entire margin, petiole short and glabrous and acute or obtuse apex with tapering base. Young branches are green in colour and quadrangular which turn red with age. Bark is grevish brown, unarmed when young but branches of older trees are spine tipped. Inflorescence is a large pyramid shaped cyme. Flowers are small, about 1 cm across, numerous, fragrant, white or rose coloured with four crumbled petals. Calyx is with a 0.2 cm tube and 0.3 cm spread lobes. Fruit is a small brown coloured round capsule. Fruit opens irregularly and splits into four sections at maturity and is many seeded. Seeds are about 3 mm across, numerous, smooth, pyramidal, hard and thick seed coat with brownish coloration.^[7-9]

ETHNOBOTANICAL USES

Henna has been used cosmetically and medicinally for over 9,000 years. Traditionally in India, mehndi is applied to hands and feet. Henna symbolizes fertility. Its use became popular in India because of its cooling effect in the hot Indian summers. Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrheoa, leprosy,

fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent.^[10-12] Henna **leaf** has an orange-red due and leaf

Henna leaf has an orange-red dye and leaf paste or powder is widely used for decorating hands, nails and feet with patterns. It is also used as a hair dye. It is used for alleviating jaundice, skin diseases, venereal diseases, smallpox and spermatorrhoea. Flowers are very fragrant and used to extract a perfume, which is used as base for local scents. An infusion of the flowers is a valuable application to bruises. Decoction of the flowers is describes as an emmenagogue. Seeds are deodorant. Powered seeds with real ghee (clarified butter) are effective against dysentery. Seeds in powered form are good medicine for liver disorders and associated problems. The **bark** is applied in the form of a decoction to burns and scalds. It is given internally in a variety of affections, such as jaundice, enlargement of the spleen, calculus, as an alternative in leprosy and obstinate skin affections. Root is considered as a potent medicine for gonorrhoea and herpes infection. Root is astringent may be pulped and used for sore eyes. Pulped root may also be applied to the heads of children for boils. Cambodians drink a decoction as a diuretic. Decoction of the root generally in combination with prepared indigo as a powerful abortifacient. The root is supposed to be useful in treatment of hysteria and nervous disorders. [10-12]

CHEMICAL REVIEW

The principal colouring matter of henna is lawsone, 2hydroxy-1:4 napthaquinone ($C_{10}H_6O_3$, m.p.190° decomp.) besides lawsone other constituents present are gallic acid, glucose, mannitol, fats, resin (2 %), mucilage and traces of an alkaloid. Leaves yield hennatannic acid and an olive oil green resin, soluble in ether and alcohol. Flowers yield an essential oil (0.01-0.02 %) with brown or dark brown colour, strong fragrance and consist mainly of α - and β - ionones; a nitrogenous compound and resin. Seeds contain proteins (5.0 %), carbohydrates (33.62 %), fibers (33.5 %), fatty oils (10-11 %) composed of behenic acid, arachidic acid, stearic acid, palmitic acid, oleic acid and linoleic acid. The unsaponified matter contains waxes and colouring matter. The root contains a red colouring matter. Phytochemicals reported in *L. inermis* L. are listed in Table 1 with their structures.

BIOLOGICAL REVIEW

Although this plant has been widely used in various symptoms and diseases, however few pharmacological studies have been reported.

Antidiabetic activity

Ethanol (70 %) extract of *L. inermis* showed significant hypoglycaemic and hypolipidaemic 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. ^[32] Methanol (95 %) extract of leaves of *L. inermis* showed significant *in-vitro* antihyperglycemic effect. ^[33]

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 leaves. ^[34] Naphthoquinone fraction obtained from leaves *L. inermis* showed significant immunomodulatory effect. ^[35]

Hepatoprotective activity

Alcoholic extract of the bark of L. inermis showed hepatoprotective effect against the carbon tetrachlorideinduced 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. The results suggest hepatoprotective and antioxidant activity of extract of L. alba bark. Pretreatment of rats with the extract also inhibited the peroxidation of microsomal lipids in a dose-dependent manner. ^[36-38]The hepatoprotective activity of the ethanolic extract of the dried leaves of L. inermis and its crude fractions (petroleum ether, ethyl acetate, butanol and butanone fractions) was evaluated against CCl₄ induced hepatotoxicity in mice. The ethanolic extract and its fractions reduced the total bilirubin content and SGOT, SGPT and SAL activities, and reduced liver weight compared to LIV-52 (control). [39-40]

Antioxidant effect

Modulator effect of 80 % ethanol extract of leaves of henna on drug metabolising phase I and phase II enzymes, antioxidant enzymes, lipid peroxidation in the liver of Swiss Albino mice. The hepatic glutathione S-transferase and DTdiaphorase specific activities were elevated above basal level by L. inermis extract treatment. With reference to antioxidant enzyme the investigated doses were effective in increasing the hepatic glutathione reductase (GR), superoxide dismutase (SOD) and catalase activities significantly at both the dose levels. Reduced glutathione (GSH) measured as non-protein sulphydryl was found to be significantly elevated in liver. Among the extrahepatic organs examined (forestomach, kidney and lung) glutathione S-transferase and DTdiaphorase level were increased in a dose independent manner. [41] Chloroform extract of leaves of Lawsonia inemris had shown the highest activity (87.6 %) followed by α -tocopherol (62.5 %) by using FTC method and based on TBA method significant activity (55.7 %) compared to α tocopherol (44.4 %). [42] Total phenolic compound was 2.56 and 1.45 mg tannic per mg of Henna dry matter as extracted with methanol and water respectively. In effect of different concentrations of methanolic extract of henna in comparison with synthetic antioxidant. ^[23, 43] 2-hydroxy-1, 4naphthoquinone (HNQ; lawsone) is the main ingredient of L. inermis. During the oxidation of 100µM phenanthridine by guinea pigs aldehyde oxidase formation of superoxide anion (SO_2) and hydrogen peroxide (H_2O_2) at 6-10 % and 85-90 % resp. HNQ inhibits the production of superoxide anion and substrate oxidation more potently than hydrogen peroxide.the IC₅₀ value of HNQ with phenanthridine oxidation by aldehyde oxidase was 9.3 $\pm 1.1 \mu$ M, which in excess of 15 fold of maximal plasma concentrations of HNQ, indicating a high degree of safety margin. ^[44]

Antibacterial activity

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* L. was found to be the most active against all the bacteria in the test system. ^[45] Quinonic compounds from henna were studied *in-vitro* for antimicrobial properties. ^[46] Genotoxic studies on lawsone suggested that it was a weak bacterial mutagen for *Salmonella typhimurium* strain TA98

and was more clearly mutagenic for strain TA2637. Overall, the weight of evidence suggested that henna and hydroxy napthaquinone possess no genotoxic risk to the consumer.^[47] Aqueous extract of leaves of *L. inermis* showed the significant antibacterial effect against.^[48] Aqueous, methanol and chloroform crude extracts of leaf showed the *in-vitro* antimicrobial activity to inhibit the growth of 6 human pathogenic fungi and 4 types of bacteria in dose dependent manner.^[49-51]

Antifungal activity

During screening of barks of 30 plant species against Microsporum gypseum and Trichophyton mentagrophytes, only L. inermis L. extract exhibited absolute toxicity. The extract showed broad fungitoxic spectrum when tested against 13 ring worm fungi. Further the fungitoxicity of the extract remained unaltered at high temperature on autoclaving and after long storage. ^[52] The leaves of L. inermis L. were also found to exhibit strong fungi toxicity and non-phytotoxicity. The minimum effective dose against test organism was found to be 1000ppm. [53] Ethanol, methanol and aqueous extract of leaves of L. inermis are involved in defensive mechanism against spore germination of *Drechslera oryzae*. ^[54] Lawsone isolated from the leaves of *L. inermis* has shown significant antifungal antibiotic effect. [55] 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 extracts of the plant showed significant antifungal activity. ^[56] Essential oil obtained by hydro-distillation from leaves of L. inermis growing in Iran were analysed by GC-MS and showed an antifungal activity.^[57] Ethanol extract of leaves of L. inermis showed significant antifungal effect against phytopathogenic fungi. Ethanol extract could be used as alternative source of antifungal agents for protection of plants or crops against fungal infection. [58]

Antiviral activity

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 challenge.^[59]

Antitrypanosomal activity

Crude Methanolic extract of leaf of *L. inermis* showed *invitro* 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 pack cell volume. ^[60]

Antiparasitic activity

During an ethnopharmacological survey of antiparasitic medicinal plants used in Ivory Coast, 17 plants were identified and collected. Polar, non-polar and alkaloidal extracts of various parts of these species were evaluated *invitro* in an antiparasitic drug screening. Antimalarial, leishmanicidal, trypanocidal, antihelminthiasis and antiscabies activities were determined. Among the selected plants, *L. inermis* L. showed interesting trypanocidal activities.^[61]

Molluscicidal activity

L. inermis showed significant molluscicidal activity. ^[62] *Antidermatophytic activity* The antidermatophytic 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*. ^[63] *Tuberculostatic activity*

The tuberculostatic activity of henna was tested *in-vitro* and *in-vivo*. On Lowenstein Jensen medium, the growth of *Tubercle bacilli* from sputum and of *Mycobacterium tuberculosis* H37Rv was inhibited by 6 μ g/ml of the herb. *In-vivo* studies on guinea pigs and mice showed that the herb at a dose of 5 mg/kg body weight led to a significant resolution of experimental tuberculosis following infection with *Mycobacterium tuberculosis* H37Rv. ^[64]

Antifertility activity

Ethanol extract prepared from the powdered seeds of *L. inermis* L. failed to show any antifertility activity. However in subsequent studies it was observed that the powdered leaves of when administered as suspension or incorporated into the diet inhibited the fertility of rats. The fertility induced appeared to be permanent. ^[65]

Analgesic activity

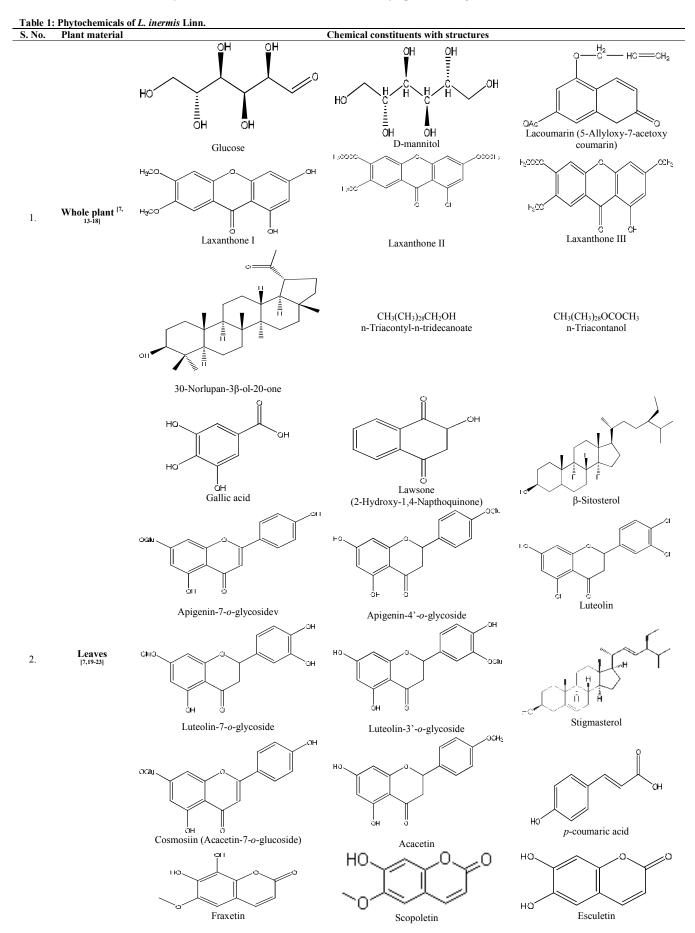
The ethanol extract of 25 plants commonly used in traditional Arab system of medicine for treatment of pain, fever and rheumatism were investigated for their analgesic and antipyretic activities. The extract of leaves of henna showed significant analgesic as well as antipyretic activity. ^[66] The fixed oil obtained from seeds were screened for pharmacological activity both *in-vitro* and *in-vivo*. It was concluded that seed oil is devoid of behavioural and CNS effects and failed to produce any effect on isolated tissue though it possess significant analgesic activity. ^[67]

Anti-inflammatory activity

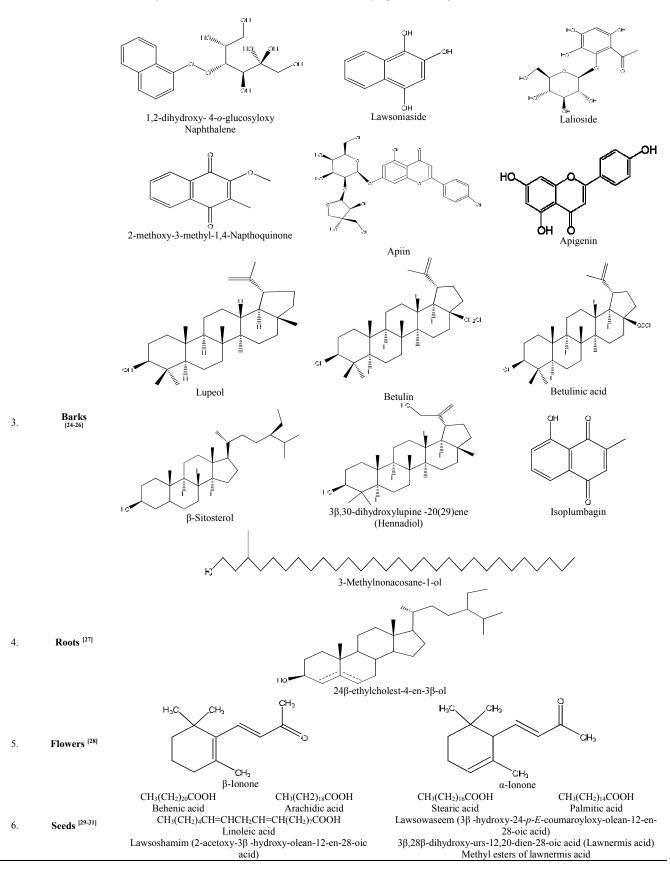
Isoplumbagin and lawsaritol, isolated from stem bark and root of *L. inermis* L. showed anti- inflammatory activity against Carrageenan induced paw oedema in rats. The compounds phenylbutazone, isoplumbagin and lawsaritol at the oral dose of 100 mg/kg exhibited 61, 60 and 40 percent inhibition in comparison with controls. Isoplumbagin showed significant anti- inflammatory activity similar to that of phenylbutazone. ^[68] Butanol and chloroform fractions showed more potent anti-inflammatory, analgesic and antipyretic effects than aqueous fraction of crude ethanol extract of *L. inermis* in a dose dependent manner. ^[69] Leaves showed significant anti-inflammatory effect with some active principles. ^[70-71]

Cytotoxic activity

Isoplumbagin exhibited up to a 1000 fold range of differential sensitivity, which represents distinct fingerprint of cellular responsiveness. At concentration of 10.5-10.8 M, the compound typically produced LC50 - level responses against a majority of the melanoma and colon cancer cell lines as well as against several of the non- small cell lungs, colon, CNS, and renal cell lines. Isoplumbagin showed an interesting profile of cytotoxic activity. ^[72] Chloroform extract of leaves of L. inermis displayed the cytotoxic effects against liver (HepG2) and Human breast (MCF-7) with IC₅₀ values of 0.3 and 24.85µg/ml by microculture tetrazolium salt assay (MTT).^[42] CAT assay, a zone of inhibition test of bacterial growth and colony-forming efficiency test of transformant Escherichia coli strains that express mammalian catalase gene derived from normal catalase mice (Cs^a) and catalase-deficient mutant mice (Cs^b), Ames mutagenicity assay and H₂O₂ generation assay are carried out.



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Lawsone generated H_2O_2 slightly in phosphate buffer system and was not mutagenic in Ames assay using TA98, TA100 and TA102, both in the absence and presence of metabolic activation. Lawsone exposure inhibited the growth of both Cs^a and Cs^b strains in a dose-dependent manner. Oxidative stress probably arises when napthoquinone part in lawsone reduced to a semiquinone by enzymatic systems.^[73]

Antisickling activity

Aqueous extract of leaves of L. inermis was found to inhibit sickling and to increase the oxygen affinity of HbSS blood.

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 effects. [75]

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, respectively.^[76]

Memory and behaviour effectiveness

L. inermis showed significant effect on memory and behaviour mediated via monoamine neurotransmitters.^[77] 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 alone.^[78] Anticoagulant effect

Lawsone and its oxazine derivatives isolated from leaves of L. inermis had proven to be potential anticoagulant agent.^[79]

Wound healing effects

Chloroform and aqueous extracts of leaves of the plant were capable of inhibiting the growth of microorganisms that are involved in causing burn wound infections. [80-81] Ethanol extract of the plant (200 mg/kg/day) was used to evaluate the wound healing activity on rats using excision, incision and dead space wound models. Extract of L. inermis when compared with the control and reference standard animals: a 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 content. 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. [82]

Protein glycation inhibitory activity

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 in *in-vitro* assay system. 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 1500µg/mL, 1000µg/mL and 1000µM respectively. L. inermis, compounds 1 and 2 were found to be glycation inhibitors with IC₅₀ 82.06±0.13µg/mL, 67.42±1.46µM and 401.7±6.23µM respectively.^[83]

SAFETY EVALUATION

Most of the toxicological studies report that toxic effects due to the use of herbal medicine are associated with hepatotoxicity. Other toxic effects of the kidney, nervous system, blood and cardiovascular system, as well as mutagenecity and carcinogenicity have also been published in medical journals. Therefore, numerous advance biological experimental techniques have been used as standard safety test prior to the efficacy study. From the literature it has been that L. *inermis* L. exhibited noted significant hepatoprotective, antioxidant, antiinflammatory, antibacterial, analgesic and adaptogenic effects indicating that it is a safe substance to be used as a drug ordinarily.

DISCUSSION

Medicinal plants have provided copious leads to combat diseases, from the dawn of civilization. The extensive survey of literature revealed that L. inermis L. is highly regarded as a universal panacea in the 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. Hence extensive investigation is needed to exploit their therapeutic utility to combat diseases. A drug development programme should be undertaken to develop modern drugs with the compounds isolated from henna. 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. As the global scenario is now 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. Henna imbibing a tremendous potential deserves a special attention of the scientific fraternity to emerge as a milestone for medical science of this millennium due to its various medicinal uses. 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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