AN OVERVIEW OF *LAWSONIA INERMIS* L.: A NATURAL DYE PLANT

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ABSTRACT: The leaves of *Lawsonia inermis* L**.** contains lawsone (2-hydroxy-1, 4-naphthaquinone) chief constituent responsible for dyeing property of the plant. Lawsone reacts with keratin in skin and hair thus henna is used for colouring hair, skin and leather. Henna is also an important medicinal plant of Indian Systems of Medicine. This beneficial plant is not widely utilized hence this review is to explore the hidden potentials of *Lawsonia inermis* L.

***Keywords*:** *Lawsonia inermis* L., lawsone, phytoconstiuents

INTRODUCTION:

Lawsonia inermis L., commonly known as Henna or Mehendi belongs to the family Lythraceae. *Lawsonia alba* and *Lawsonia spinosa* are the older names for *Lawsonia inermis L. (*Kokate, 2001). *The plant* is a native of North Africa and South-west Asia and is widely cultivated as an ornamental as well as dye plant in India, the Middle East and the African coast of the Mediterranean Sea (Zafar *et al*., 2006). *Lawsonia inermis* L. is naturalized in India, common in dry jungles and used as hedges. *Lawsonia inermis* L. prefers drier conditions so it is commercially cultivated in many areas including Rajasthan and Tamil Nadu (Lee, 2008).

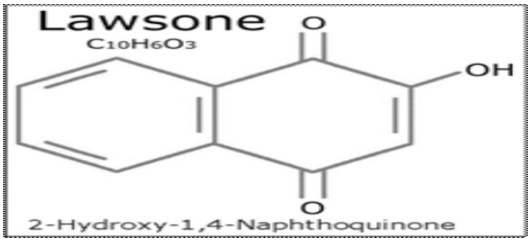

Plate 1: Structure of Lawsone

Lawsonia inermis L. is 6-12 m tall shrub or small tree with greyish-brown bark and quadrangular young branches. Older plants sometimes have spine-tipped branches. Leaves are decussate, opposite, simple and entire, sub-sessile; shortly petiolate; stipules minute; blades are elliptical to oblong or broadly lanceolate, 1.08.8 cm X 0.54.0 cm, cuneate at base, acute to round at apex. Inflorescence is large, pyramidal, terminal panicle up to 25 cm long with many flowers. Flowers bisexual, regular, tetramerous, sweet-scented; pedicel 24 cm long; calyx with up to 2 mm long tube and spreading, ovate lobes 2-3 mm long; petals orbicular to obvate, 1.5-4 mm X 4-5 mm, usually whitish, sometimes reddish; stamens 8, inserted in pairs on the rim of the calyx tube, filaments 45 mm long; ovary superior, 4 celled, style erect, up to 5 mm long, stigma head-shaped. Fruit a globose capsule 48 mm in diameter, purplish-green, indehiscent or opening irregularly, many seeded. Seeds are tetra-angular, 33 mm long with thick seedcoat (Grubbe, 2005). In transverse section, the shape of the petiole is cylindrical with small wings on its lateral sides (Kshirsagar and Vaikos, 2009). *Lawsonia inermis* L. can be propagated by seeds. Vegetative propagation is by cuttings. *Lawsonia inermis* L. grows on any type of soil, from light loam to clay loam, but does best on heavy soils which are retentive of moisture. Lawsone is the chief constituent responsible for the dyeing properties of the plant. Dried powdered leaves of henna contain 0.5-1.5% lawsone, traditionally used to produce colour fast orange, red and brown dyes (Muhammad and Muhammad, 2005).

Phytochemical constituents: *Lawsonia inermis* L., contains a red-orange pigment, lawsone, also known as 2 hydroxyl 1, 4 - naphthoquione or hennotannic acid (Plate 1). Industrial classifications describe lawsone as Natural Orange 6 and C.I. 75480. The name and molecular structure of lawsone show its congeniality to naphthalene. Its molecule contains 10 carbons, 6 hydrogens and 3 oxygens ($C_{10}H_6O_3$), giving a total molecular weight of 174.16 atomic units of mass. Pure lawsone is an orange powder, insoluble in water, with a melting point higher than 192ºC and optical absorption maximum of 452 nm. The size of the lawsone molecule, approximately 6.5x5x1.3 Å, compares to that of naphthalene (Bonev, 2003). Besides lawsone other constituents present are gallic acid, glucose, mannitol, fats, resin (2 %), mucilage and traces of an alkaloid. Analysis of leaf showed 8.97% moisture, 14.84% ash and 10.21% tannins. Flowers yield an essential oil (0.01-0.02%) and consist mainly of α and β-ionones and resin. Seeds contain moisture (10.6%) proteins (5%), carbohydrates (33.62%), fibers (33.5%), fatty oils (10-11%) composed of behenic acid, arachidic acid, stearic acid, palmitic acid, oleic acid, linoleic acid and 4.75% ash. The unsaponified matter contains waxes and colouring matter (Kardar, 2005). Phytochemicals reported in *Lawsonia inermis* L. are listed in Table 1.

Ethnobotanical uses: Henna plant is widely cultivated as a dye plant in India. Use of henna as a natural dye is related with cultural practices, rituals, arts and crafts, fabrics and for personal embodiment (Aggarwal *et al*., 1959). It has been in use as a hair colour and for the decoration of soles of the feet and palm of hands. Henna is widely used in cosmetology for its dyeing properties, due to the strong binding of lawsone to hair, which probably can be attributed to the reaction of thiol group with keratin. It is also used in the form of shampoo and hair lotion (Zafar *et al*., 2006). *Lawsonia inermis* is a natural colorant with antimicrobial effect. It is environmental friendly as it can exhibit better biodegradability and have a higher compatibility with the environment (Ahmed and Muhamad, 2010). 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 and cardiac disease. It is used for alleviating jaundice, skin diseases, venereal diseases, smallpox and spermatorrhoea (Chaudhary *et al*., 2010). **Root** is considered as a potent medicine for gonorrhoea and herpes infection. Decoction of the root generally in combination with prepared indigo as a powerful abortifacient. The **bark** is applied in the form of a decoction to burns and scalds. **Flowers** are very fragrant and used to extract a perfume. An infusion of the flowers is a valuable application to bruises. **Seeds** are deodorant. Powdered seeds with real ghee (clarified butter) are effective against dysentery. Seeds in powdered form are good medicine for liver disorders and associated problems (Chaudhary *et al*., 2010). This plant has been described in Charaka Samhitaa for the treatment of epilepsy and for dyeing grey hair. In Sushruta Samhitaa it has been recommended as a remedy for malignant ulcers. The Ayurvedic Pharmacopoeia of India indicated the use of leaves in dysuria, bleeding disorder, prurigo and other obstinate skin diseases (Borade *et al*., 2011).

Pharmacological activities

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 (Chaudhary *et al*., 2010).

Antibacterial activity: The ethyl acetate extract of *Lawsonia inermis* L. was found to be the most active against all the bacteria in the test system. Quinonic compounds from henna were studied *in vitro* for antimicrobial properties (Chaudhary *et al*., 2010).

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* (Chaudhary *et al*., 2010). The antifungal effect of chloroformic, methanolic and aqueous extracts of henna leaves was tested against on *Malassezia sp.* The results demonstrated that henna has antifungal activity against *Malassezia.* In addition aqueous extract is more effective on *Malassezia* than methanolic and chloroformic extracts (Fariba *et al*., 2010).

Antidiabetic activity: Ethanol (70%) extract of *Lawsonia inermis* showed significant hypoglycaemic and hypolipidaemic activities in alloxan induced diabetic mice after oral administration. The feeding of 0.8 g/kg of *Lawsonia inermis* extract decreased the concentration of glucose, cholesterol and triglycerides to normal. Methanol (95%) extract of leaves of *Lawsonia inermis* showed significant *in vitro* antihyperglycemic effect (Chaudhary *et al*., 2010).

Antifungal activity: Aqueous extracts of leaves of *Lawsonia inermis* were tested for the antifungal potential against eight important species of *Aspergillus. 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 (Chaudhary *et al*., 2010). Henna has antifungal activity against *Malassezia.* In addition aqueous extract is more effective on *Malassezia* than methanolic and chloroformic extracts (Berenji *et al*., 2010). The Algerian *Lawsonia inermis* plant has antifungal activity that can be related to the presence of lawsone in the leaves plant (Rahmoun *et al*., 2012).

Anti-inflammatory activity: Isoplumbagin and lawsaritol, isolated from stem bark and root of *Lawsonia 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% inhibition in comparison with controls. Isoplumbagin showed significant anti- inflammatory activity similar to that of phenylbutazone (Verghese *et al*., 2010).

Antioxidant effect: Chloroform extract of leaves of *Lawsonia inemris* had shown the highest activity (87.6 %) followed by á-tocopherol (62.5 %) by using FTC method and based on TBA method significant activity (55.7 %) compared to α-tocopherol (44.4 %). 2-hydroxy-1, 4- naphthoquinone (HNQ; lawsone) is the main ingredient of *Lawsonia inermis*. HNQ inhibits the production of superoxide anion and substrate oxidation more potently than hydrogen peroxide. The IC_{50} value of HNQ with phenanthridine oxidation by aldehyde oxidase was 9.3±1.1µM, which in excess of 15 fold of maximal plasma concentrations of HNQ, indicating a high degree of safety margin (Chaudhary *et al*., 2010). 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. 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 (Borade *et al*., 2011).

Antiparasitic activity: Polar, non-polar and alkaloidal extracts of various parts of these species were evaluated *in vitro* in an antiparasitic drug screening. Antimalarial, leishmanicidal, trypanocidal, antihelminthiasis and antiscabies activities were determined. Among the selected plants, *Lawsonia inermis* L. showed interesting trypanocidal activities (Makhija *et al*., 2011).

Antiviral activity: The ethanol soluble fraction of *Lawsonia 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 (Makhija *et al*., 2011).

Cytotoxic activity: 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 (Borade, 2011).

Hepatoprotective activity: Alcoholic extract of the bark of *Lawsonia inermis* showed hepatoprotective effect against the carbon tetrachloride induced elevation in serum marker enzymes (GOT & 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 *Lawsonia alba* bark. The hepatoprotective activity of the ethanolic extract of the dried leaves of *Lawsonia inermis* and its fractions (petroleum ether, ethyl acetate, butanol and butanone fractions) was evaluated against CCl_4 induced hepatotoxicity in mice. The ethanolic extract & its fractions reduced the total bilirubin content & SGOT, SGPT & SAL activities, and reduced liver weight compared to LIV-52 (control). The aqueous extract of *Lawsonia inermis* possesses hepatoprotective activity against paracetamol induced hepatotoxicity (Chaudhary *et al*., 2010).

Immunomodulatory effects: 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. Naphthoquinone fraction obtained from leaves showed significant immunomodulatory effect (Makhija *et al*., 2011).

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. 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 *Lawsonia 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 fibers and fibroblasts (Makhija *et al*., 2011).

***In vitro culture*:** Hairy root culture was established by a co-culture method using leaf and *Agrobacterium rhizogenes* NCIB 8196. Lawsone production (0.13% dry weight) has been seen only in hairy root tissues incubated in dark and cultured in ½ MS and MS media (Zafar *et al*., 2006). Multiple shoots were induced in apical and axillary meristems derived from mature explants of *Lawsonia inermis* on Murashige and Skoog (1962) medium supplemented with 0.25 mg/l 6-benzylaminopurine (BA), 0.25 mg/l Kinetin (Kn), 0.5 mg/l ascorbic acid and 3 % (w/v) sucrose. Rooting was readily achieved upon transferring the micro-shoots onto MS basal semi-solid medium supplemented with 0.25 mg/l indole-3-butyric acid (IBA) after ten days of culture (Rout *et al*., 2001). Callus from leaf explants (young and old) of field grown plants of *Lawsonia inermis* L. was initiated on MS medium supplemented with different concentrations of 2, 4 - dichlorophenoxy acetic acid (2, 4 - D) (0.1-1.0 mgL) used singly or in combination with coconut milk(CM), polyvinyl pyrrolidone (PVP), adenine sulphate (AS) and casein hydrolysate (CH) (Phirke and Saha, 2010). Different coloured callus were obtained from henna tissue culture on MS supplemented with different combinations and concentrations of hormones 0.5 mg/L 2,4-D and 0.5 mg/L IAA, 2.0 mg/L NAA and 2.0 mg/L BAP, 0.5 mg/L NAA and 0.5 mg/L BAP, 1.5 mg/L NAA and 1.5 mg/L BAP, and also 0.5 mg/L IAA and 0.5 mg/L BAP and thus production of important secondary metabolites *in vitro* was possible (Rahiman and Taha, 2011).

Table 1: Phytochemicals in *Lawsonia inermis* L. (Makhija *et al*., 2011)

Compounds	Plant Parts
Napthoquinone derivatives	
Lawsone (2-hydroxy 1,4-naphthoquinone)	Leaves
1,3-dihydroxy naphthalene, 1,4-napthaquinone, 1,2-dihydroxy-4-glucosylnaphthalene	Leaves
Isoplumbagin	Stem bark
Phenolic conpounds	
Lawsoniaside (1,3,4-trihydroxynaphthalene 1,4-di-β-D-gluco-pyronoside), Lalioside (2,3,4,6-tetrahydroxyacetoxy-2-β-D-glucopyranoside) Lawsoniaside B (3-(4-O-a-D-glucopyranosyl-3,5-dimethoxy) phenyl-2E-propenol), syringinoside, daphneside, daphnorin, agrimonolide 6-O-β-D-glucopyranoside, (+)-syringaresinol O-β-D-glucopyranoside, (+)-pinoresinol di-O-β-D-glucopyranoside, syringaresinol di-O-β-D-glucopyranoside, isoscutellarin	Bark, Leaves
Terpenoids	
3β, 30-dihydroxylup-20(29)-ene (hennadiol), (20S)-3β, 30-dihydroxylupane, Lupeol, 30-*nor*-lupan-3β-ol-20-one, betulin, betulinic acid, lawnermis acid (3β-28β-hydroxy-urs-12,20-diene-28-oic acid) and its methyl ester	Bark, Seeds
Sterols	
Lawsaritol (24β-ethycholest-4-en-3β-ol)	
Stigmasterol and β-sitosterol	Roots, Leaves
Aliphatic constituents	
3-methyl-nonacosan-1-ol, n-tricontyl n-tridecanoate	Stem bark
Xanthones	
Laxanthone I (1,3 dihydroxy-6,7 dimethoxy xanthone), Laxanthone II (1-hydroxy-3,6 diacetoxy-7-methoxyxanthone), Laxanthone III (1-hydroxy-6-acetoxy xanthone)	Whole plant
Coumarins	
Lacoumarin (5-allyoxy-7-hydroxycoumarin)	Whole plant
Flavonoids	
Apigenin-7-glucoside, apigenin-4-glycoside, luteolin-7-glucoside, luteolin-3-glucoside	Leaves
Essential oil	
-(Z)-2-hexenol, linalool, α ionone, β ionone, α-terpineol, terpinolene, δ-3-carene and γ-terpineol	Leaves
Other chemical constituents	
Hennotannic acid, glucose, gallic acid, amino acid	
Trace metal – Cu, Ni, Mo, V, Mn, Sr, Ba, Fe and Al	
Minerals – Na_2O, CaO and K_2O	Whole plant

CONCLUSIONS :

Herbal cosmetics have growing demand in the world market. The demand of herbal medicines is increasing rapidly due to

their comparatively lesser side effects. There is now growing scientific evidence that these plants possess a vast and complex arsenal of active ingredients (photochemical) which are able to calm or smooth the skin also actively restore, heal and protect the skin.

In India henna plant has been used as herbal dye to decorate hands, feet, hair etc. Henna is natural herbal dye and it tends to be softer. Natural dyes are now a days in demand not only in textile industry but in cosmetics, leather, food and pharmaceuticals. Henna also has a wide range of medicinal properties and there is an increasing awareness among people towards this plant due to their non-toxic properties, fewer side effects, more medicinal value. This versatile plant is the source of various types of chemical compounds. The extensive survey of literature revealed that *Lawsonia inermis* L. is not only a dye plant, but it is also an important indigenous medicinal plant as it possesses antioxidant, antimicrobial, antidiabetic, anticancer, anti-inflammatory, antiparasitic, antidermatophytic properties, anticancer, antiviral, wound healing, immunomodulatory, hepatoprotective, tuberculostatic, antifertility, protein glycation inhibitor properties. For the welfare of mankind further evaluation needs to be carried out in order to explore the practical clinical applications of *Lawsonia inermis* L.

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