PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF *LAWSONIA INERMIS* L. LEAF

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Micromorphological characters for *Lawsonia inermis* L. are not reported. The leaves are frequently used as a herbal remedy for an array of human disorders including wounds, ulcers, strangery, cough, bronchitis, lumbago, hemi crania, leucoderma, scabies, boil, hepatopathy, spleenopathy, ophthalmic conditions, falling of hairs and jaundice. The main constituents in the plant are carbohydrates, glycosides, tannins, phenolic compounds and gums and mucilage. It is characterized by the presence of distinct midrib from lamina, which is broadly shallow on adaxial side and convex on abaxial side. Mesophyll tissue is differentiated into palisade cells and spongy parenchyma. Vascular bundle is small and collateral but not prominent. It has a single layered polygonal epidermal cells containing cuticle on outer layer only. It also consists of unicellular covering trichome. Diacytic stomata are present on both the surface. The leaf is dorsiventral as oblong palisade cells are present below the upper epidermis and absent on lower epidermis. The abaxial epidermis is also very thin and distinct. The ground tissue of the midrib is parenchymatous and homogeneous. The cells are circular or angular and compact. Tannin is seen in some of the cells. The vascular strand is single, small, collateral and hemispherical in shape. It consists of a thick horizontal band of xylem and fairly wide band of phloem. Xylem elements are narrow, angular, thin walled and somewhat diffuse. Phytochemical investigation of leaf shows total ash (14.60 %), acid insoluble ash (4.50 %), and water soluble ash (3.0 %). Loss on drying is (4.5 %). Alcohol soluble extractive value and aqueous extractive value were (3.8 % w/w and 5.0 % w/w), respectively. The percent practical yields of alcohol and aqueous extract percent yield were found to be 12.34 % and 15.50 %. In alcoholic extract and aqueous extract carbohydrate, glycosides, tannins, phenolic compounds and gums and mucilage were present in good quantity and saponins, alkaloids, phytosterols, fixed oils, fats, proteins, amino acids, volatile oils were absent.

Key words: *Lawsonia inermis* L, henna, red henna, phytochemical investigation.

INTRODUCTION

India, the richest floristic regions of the world, has got a source of plants and their products since antiquity. Man uses them as food and medicine as per his

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desires. Among the entire flora, estimated 2,500,000 higher plant species on earth, only 35,000 to 70,000 species (less than 1 %) have been used for medicinal purpose (Ponnu *et al*., 2003). There are plenty of chances to find out a new compound derived from plant (Farnsworth, 1988).

Lawsonia inermis L. (*Lythraceae*) is a much-branched shrub that grows in Middle East of Africa is commonly known as Mendi in Gujarati, Henna in Hindi and Egyptian henna. There are three types of henna like Neutral henna, Red henna and Black henna. Neutral henna, a green powder that smells like freshly cut grass, is neither henna nor neutral. It is *Cassia obovata*, contains anthraquinones, particularly chrysophanic acid, a remarkable anti-fungal, anti-microbial and anti-bacterial compound. *Cassia obovata* does not color hair. Red henna, a green powder that smells like hay, is *Lawsonia inermis,* commonly known as henna. The leaves of the henna plant have a red-orange dye molecule: lawsone, a naphthaquinone compound. Henna will stain your hair red-orange; but this stain is translucent and will combine with your natural color. Black henna, a green powder that smells like frozen peas, is neither black nor henna. It is indigo, *Indigofera tinctoria.* (Vaidya, 2000; Wallis, 2001; Trease and Evans, 1983; Bhattacharjee, 2003).

The different species of *Lawsonia inermis* L. are *Lawsonia alba* and *Lawsonia spinoza/spinosa. Lawsonia alba* and *Lawsonia spinoza* are misleading older names for *Lawsonia Inermis L.* When henna is a small and immature plant, it has low dye content and is spineless; when mature, it develops spines and higher dye content. Henna plants undergo this change when they are 3 years old. When western botanists saw juvenile and mature henna plants, they thought they were seeing two species, and gave them different botanical names. *Lawsonia* also has different colors of flowers. The plants varieties with white flowers are sometimes called *var. alba*, but they are used for dye as the plants with yellow, pink and red flowers. Henna is a small tree or large shrub, growing to six meters high. It has lateral branches with leaves that grow in pairs. Henna is a juvenile plant for the first two years. The leaves do not have high lawsone content, and the branches do not have thorns. In mature plants, thorns develop at the leaf buds during dormant phases. The henna fruits ripen at the end of the summer. In each henna fruit there is an average of 40–45 seeds. Geographically henna is distributed in Egypt, Arabic countries, Persian countries, India, Pakistan, the USA (Florida), and China, Sudan (Kokate, 2001).

The leaves are generally useful in treatment of that troublesome and painful affection called as “burning of foot”. For this purpose the fresh leaves are beaten into paste with vinegar or limejuice and applied as poultice to the soles of the feet. The flowers are intellect promoting cardio-tonic, refrigerant, febrifuge and tonic. The seeds are antipyretics, constipating, and are useful in intermittent fever, insanity, amentia, dysentery, diarrhea and gastropathy. The roots are bitter, diuretics, emmenogogue, abortifacient, are useful in dyspepsia, leprosy, skin

disease, amenorrhea, dysmenorrhea, and premature graying of hairs. The leaves are useful in wounds, ulcers, cough, bronchitis, leucoderma, scabies, boil, hepatopathy, spleenopathy, ophthalmic conditions, falling of hairs and jaundice. Plant henna extract is used as hair growth stimulators for the treatment of dandruff and as hair colorant or dye. Mechanism of action appears to be done by acceleration of blood circulation, activation of dermal papilla and increase nutrition to the hair follicles. Henna is a perennial shrub native to Northern Africa and cultivated in the Tropical regions of America, Egypt, India, and other parts of Middle East. Also known as El-henna, Egyptian priest, Mignonette tree, the species is sometimes classified as *Lawsonia alba* Lam., or *Lawsonia ruba*. Reaching the height of 6 meters, it has fragmented white and rose-red flowers. Dried powdered henna contains 0.5 to 1.0 % Lawsone, the chief content responsible for the dying properties of plant. The main component of henna is lawsone that is a naphthaquinone derivative (Wallis, 2001; Evans, 1983; Panigrahi and Sahu, 1998; Chatterjee, 2000). There is no record for pharmacognostical and preliminary phytochemical work on such potential drug; hence, present work was undertaken.

MATERIALS AND METHODS

Collection and identification. The proposed material for study was identified and submitted as *Lawsonia inermis* L. leaf, and it was authenticated by Dr. Bimal Desai, Botanist, Department of Biotechnology, CGBIBT, Gopal Vidyanagar, affiliated to Veer Narmad South Gujarat University, Surat. The voucher specimen no. VCJ/01/10072009. The leaves were collected, washed with water, dried in sunlight and stored properly. The dried leaf was powdered and passed through the sieve no. 60. Coarse powder was used for phytochemical work.

Morphological studies. The morphological characters like condition, type, size, shape, apex, margin, base, surface, color, odor and taste of *Lawsonia inermis* L. leaf, were studied (Wallis, 2001).

Microscopical studies. The required samples of *Lawsonia inermis* L. leaf were sectioned with the help of fresh blade. The sections were first cleared with chloral hydrate and then stained with Phloroglucinol and concentrated HCL. Sections were also stained with Iodine solution (I-KI) for starch. Photographs were taken with Magnus MLX Binocular microscope.

Physicochemical constants *Ash Values***.** Ash values were used to determine the quality and purity of the crude drugs. Procedure given in Indian Pharmacopoeia was used to determine the different ash values such as total ash and acid insoluble ash. Alcohol soluble and water soluble value was also determined as per procedure given in Indian Pharmacopoeia (Indian Pharmacopoeia, 1985).

Phytochemical analysis. The dried powder material was extracted with ethanol and water successively in a soxhlet apparatus. The extracts were filtered

while hot and concentrated under reduced pressure. The practical and % yields of the extracts were calculated. The concentrated ethanolic and aqueous extracts of the leaves were subjected to qualitative chemical test for the identification of various active constituents (Harbone, 1988; Mohmmad, 1994; Agrawal, 2000; Divakar, 2002).

RESULTS

Morphological characters. The leaf of *Lawsonia inermis* L. is short, smooth, compound, ovate-lanceolate, acute, symmetrical, entire, pinnate, opposite, sweet smelling, characteristics or bitter in taste and varies in length, Lawsone is mainly present in the marginal vein or petiole in large quantity (Nadhkarni, 2004; Bhattacharjee, 2003; Panigrahi and Sahu, 1998; Kokate, 2001). Fig. 1 shows a photograph of *Lawsonia inermis* L.

Fig. 1. Photograph of *Lawsonia inermis* L.

Microscopic characters *Anatomy of leaf*. The leaf of *Lawsonia inermis* L. is short and smooth. The midrib is distinct from the lamina. It is broadly shallow on the adaxial side and convex on the abaxial side. It has a single layered polygonal epidermal cells containing cuticle on outer layer only. It also consists of unicellular covering trichome. Diacytic stomata are present on both the surface. The leaf of *Lawsonia inermis* L. is dorsiventral as oblong palisade cells are present below the upper epidermis and absent on lower epidermis. The abaxial epidermis is also very

thin and distinct. The ground tissue of the midrib is parenchymatous and homogeneous. The cells are circular or angular and compact. Tannin is seen in some of the cells. The vascular strand is single, small, collateral and hemispherical in shape. It consists of a thick horizontal band of xylem and a fairly wide band of phloem. Xylem elements are narrow, angular, thin walled and somewhat diffuse.

Lamina. The lamina is uniformly flat with even surface. Both adaxial and abaxial epidermal layers are thin and distinct. The mesophyll tissue is differentiated into palisade and spongy parenchyma. It consists of about 4 to 6 layers of small, thin walled, vertically oblong and polyhedral parenchymatous cells. The cells are compact. Some of the cells are filled with dark tannin content. The vascular bundles of the lateral veins are small and not prominent. They are collateral with a thin adaxial patch of xylem and abaxial rest of phloem. The leaf margin is narrower than the middle part.

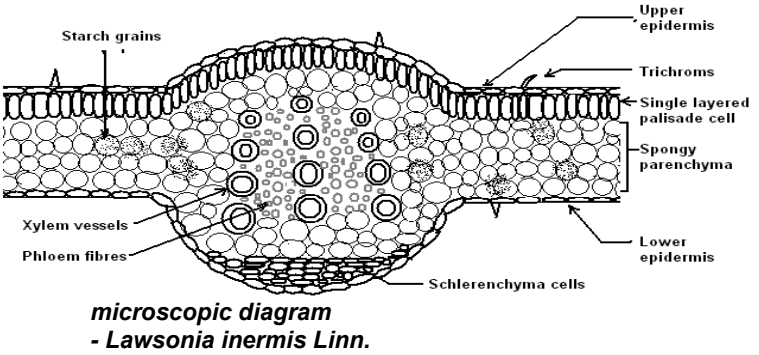

Fig. 2. Photograph of T.S of *Lawsonia inermis* L. leaf.

Epidermal tissue. In paradermal section of the lamina, the epidermal layer appears as flat polygonal mat of cells which are variable in shape and size, they are polyhedral, and the anticlinals walls are thin, straight with the deposition of cuticle. Some of the epidermal cells are smaller and have dark tannin content. The stomata are present on both surfaces. The abaxial epidermal cells are larger and their walls are slightly thicker and wavy. Stomata are abundant. The stomata are Dicytic type. Each stoma is surrounded by two subsidiary cells the long axis of which is perpendicular to the long axis of stoma pore. The stomata are elliptical with wide opening.

Petiole. In cross sectional view, the outline of the petiole is elliptical with adaxial groove along with upper part and ovate, without adaxial grooves along the lower part of the petiole. The surface of the petiole is even and smooth. The epidermal layer is thin and very distinct. The ground tissue is homogeneous and parenchymatous, the cells are thin walled and compact. Some of them contain

tannin (Nadhkarni, 2004; Bhattacharjee, 2003; Panigrahi and Sahu, 1998; Kokate, 2001). Figure 2 shows a photograph of T.S of *Lawsonia inermis* L. leaf.

Physicochemical parameters. Physicochemical parameters are total ash (14.60 %), acid insoluble ash (4.50 %), and water soluble ash (3.0 %). Loss on drying was found to be (4.5 %) w/w. Alcohol soluble extractive value and aqueous extractive value were 3.8 % w/w and 5.0 % w/w respectively. The % yields of alcohol and aqueous extract was found to be 12.34 % and 15.50 % (Karnick, 2002; Evans, 1983; Anonymous, 2000; Indian Pharmacopoeia 1996; Becket and Setnlake, 1983).

In alcoholic extract and aqueous extract carbohydrate, glycosides, tannins, phenolic compounds and gums and mucilage were present in good quantity and saponins, alkaloids, phytosterols, fixed oils, fats, proteins, amino acids, volatile oils were absent (Indian Pharmacopoeia, 1996; Mukherjee, 2002; Agrawal, 2007, Arunmugam and Murugesh, 1995).

DISCUSSION

Lawsonia inermis L. leaves are frequently used as a herbal remedy for an array of human disorders including wounds, ulcers, cough, bronchitis, leucoderma, scabies, boil, hepatopathy, spleenopathy, ophthalmic conditions, falling of hairs and jaundice. The main constituents in the plant are carbohydrates, glycosides, tannins, phenolic compounds and gums and mucilage.

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CONCLUSIONS

In these present investigations, various pharmacognostical standization parameters such as macroscopy, microscopy, and preliminary phytochemical screening were carried out which could be helpful in authentification of ***Lawsonia inermis* L.** The result of the present study will also serve as reference material in the preparation of herbal monograph.

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