Advances in Bioresearch
Adv. Biores., Vol 7 (4) July 2016: 102-106
©2016 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV Value 8.21 [2014]

Advances in Bioresearch

ORIGINAL ARTICLE

Preliminary Pharmacognostic Standardization of leaves of *Lawsonia inermis* Linn. (Lythraceae)

Bhumika Yogi * and Ashutosh Mishra
*Department of Pharmaceutical Chemistry, Hygia Institute of Pharmaceutical Education and Research, Lucknow-206020
Department of Pharmaceutical Chemistry, Acharya Narendra Deo College of Pharmacy, Babhnan, Gonda - 271313
Email: bhumi.is.yogi@gmail.com

ABSTRACT
Lawsonia inermis, belonging to the Lythraceae family, is present more or less throughout India and north Africa including Egypt and Ceylon. Its common names are Mehadi, Henna and Nil Madayantika. The leaves of Lawsonia inermis are also used to treat disease of spleen. A study on Lawsonia inermis leaf sample extracted the air-dried leaf powder with different solvents such as petroleum-ether (60-80°C), benzene, chloroform, ethanol and sterile water. Preliminary phytochemical analysis was done long with measurement of the leaf constants, fluorescence characteristics and extractive values. Quantitative estimation of total ash value acid insoluble ash and water –soluble ash may serve as useful indices for identification of powdered drug. Histochemical studies which reveal rows of cylindrical palisade cells and vascular bundles may also serve as useful indices for identification of the tissues. These studies suggested that the observed pharmacognostic and physiochemical parameters are of great value in quality control and formulation development of Lawsonia inermis

Key words: *Lawsonia inermis leaves, macroscopy, microscopy, pharmacognostic standardization, phytochemical screening*

Received 11/01/2016 Accepted 18/04/2016

How to cite this article:
B Yogi and A Mishra. Preliminary Pharmacognostic Standardization of leaves of *Lawsonia inermis* Linn. (Lythraceae). Adv. Biores. Vol 7 [4] July 2016: 96-101. DOI: 10.15515/abr.0976-4585.7.4.96101

INTRODUCTION

The plant, *Lawsonia inermis* (of family Lythraceae), commonly known as Mendi in Gujarati, Henna in Hindi is used in many ayurvedic formulations like *Madayantyadi Curna*. The medicinal potential of *Lawsonia inermis* has been known to traditional systems of medicine for a while now with its leaves being broadly utilized. The use of the plants, plant extracts, and pure compounds isolated from natural sources has always provided a foundation for modern pharmaceutical compounds. The astringent stem-bark of *Lawsonia inermis* is used for cure of various skin diseases, isoplumbagin, inflammation and jaundice [1]. The leaves have a bitter bad taste with diuretic effects which are useful in the treatment of hemicranias, headache bronchitis, ophthalmia, sores, amenorrhea, scabies, lumbago, boils and diseases of the spleen. Henna leaf powder is used for staining hair, nails, beard and favor the growth of the hair [2]. There are numerous reports on the living space, land conveyance, and morphological characters of the plant. However, no work has been carried out on the leaves of this plant, which contain potentially useful ethnomedicinal drugs. In this manner, the present study was attempted to think about the pharmacognostic parts of *Lawsonia inermis* leaves.

MATERIALS AND METHODS

PLANT MATERIALS

Fresh leaves were collected from the *Lawsonia inermis* plant growing in the medicinal garden of Hygia Institute of Pharmaceutical Education and Research, Lucknow, U.P., India in the month of January and February and identified by an expert taxonomist in Department of Taxonomy & Pharmacognosy, Central

Institute of Medicinal and Aromatic plants. The plant specimens were authenticated (Ref. No. LI-1) and the leaves were washed in running water and air-dried. The fresh leaf was then studied for pharmacognostic evaluation, including examination of morphological and microscopic characteristics and some preliminary phytochemical evaluation.

INSTRUMENTATION AND TECHNIQUES

Leaf specimens were cut into rectangular pieces that incorporated the midrib and a portion of the lamina. For paradermal sections, specimens measuring 0.05 cm^2 were cut out from the midrib portion of the lamina. The leaf specimens were fixed and embedded in paraffin blocks, [3] took after by dehydration, infiltration, and sectioning [4] and finally staining and photographing of the sections [5]. Photography was done by using a Radical RXL-4T microscopic unit. Descriptive features were matched with those included in standard anatomical books [6, 7]. Air-dried leaves were powdered utilizing a homogenizer and the leaf powder was considered as drug. The leaf powder and the extracts of the powder in various solvents were analyzed under ordinary day light and in UV-light (254 nm). The fluorescence was determined according to the methods of Chase and Pratt [8]. The total ash, water-soluble ash, and acid-insoluble ash content was determined by employing standard methods of analysis as described [9] in the Indian Pharmacopoeia (1966). Quantitative determinations of the powdered drug such as physicochemical constants [10], fluorescence [11] and a preparatory phytochemical screening [12, 13] were done.

MORPHOLOGICAL CHARACTERISTICS OF LEAF

Lawsonia inermis L. (Lythraceae) is a small tree or glabrous much-branched 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 morphological studies revealed the leaves to be sessile 1.3-3.2 cm by 0.6-1.6 cm, broadly lanceolate, obtuse, opposite, often mucronulate, elliptic or ovate narrow at base entire [14-17] [Figure 1].

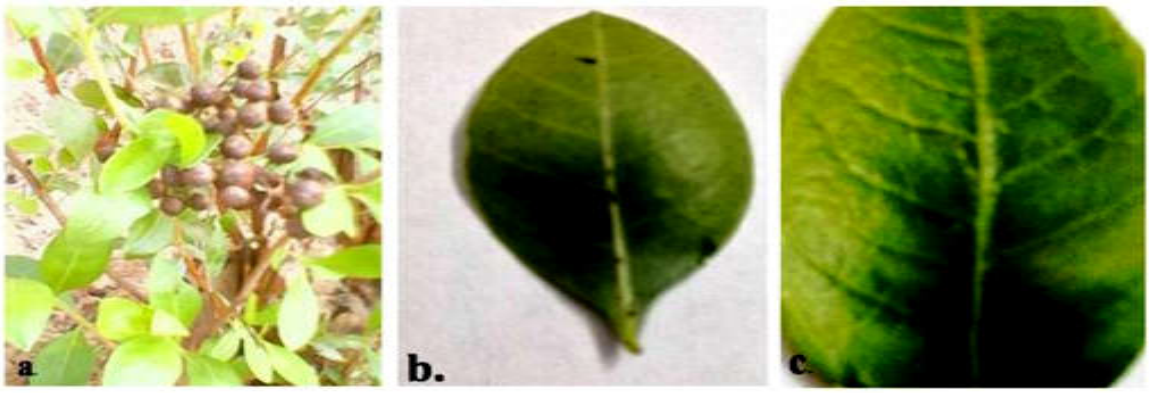

Figure 1: Morphology of *Lawsonia inermis* leaf

[**a -** A twig with oppositely arranged sessile leaves; **b -** Broadly ovate or elliptical, cottony, pubescent when young and glabrous on maturity; **c -** Portion of the lamina showing venation pattern]

MICROSCOPIAL CHARACTERISTICS OF LEAF

Transverse sections through the midrib demonstrated an upper and lower epidermis that was externally covered with a thick, striated cuticle, a few epidermal cells on both lower and upper surfaces followed by 2-4 layers of collenchymatous cells that were angular thickening thin-walled and isodiametric with intercellular spaces. The xylem consisted mostly of vessels and tracheids, and a thin strip of cambium was present between the xylem and phloem tissues. Phloem fibers were available in phloem zone. Rosette and prismatic crystals of calcium oxalate were likewise present alongside the parenchymatous zone.

The lamina which was dorsiventral with the mesophyll was seen to be differentiated into a palisade and spongy tissue. The upper and lower epidermises were secured remotely with a thick, striated cuticle. Anomocytic stomata were circulated on both surfaces. The mesophyll were composed of 2-4 layers of spongy parenchyma and 1-3 layers of palisade tissue with oil globules. Underneath the upper epidermis were three rows of elongated, closely arranged, palisade parenchyma. Spongy parenchyma tissues were almost oval to circular elongated with intracellular spaces. Mesophyll traversed by vascular strands made out of xylem encompassed by phloem with a patch of sclerenchymatous fiber on abaxial side. Central cells were irregular in shape and vascular bundles were also present scattered in this region; the details are shown in Figure 2.

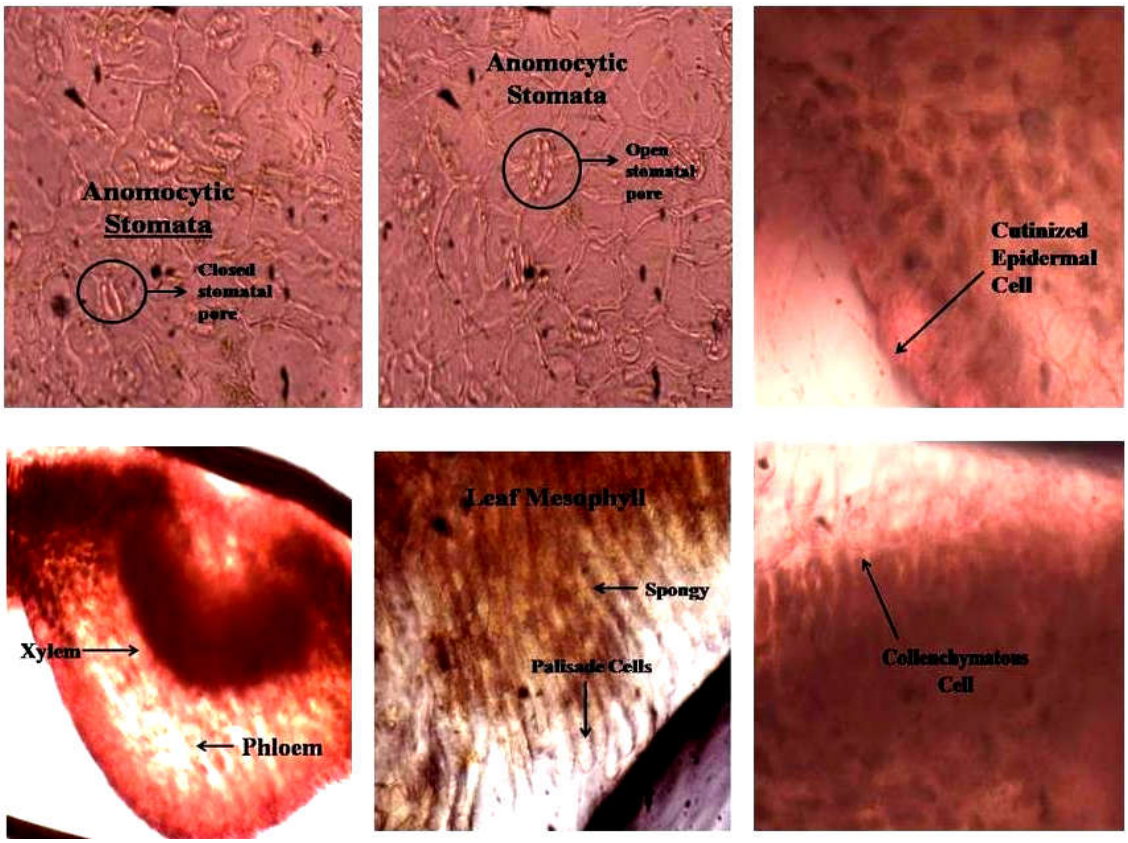

Figure 2. Microscopy of *Lawsonia inermis* leaf

RESULTS

The present study highlights the consequences of an extensive study on the microscopic parameters including the gross anatomical features, leaf constants, cellular composition, tissue organization, and cellular inclusion of the leaf. The lamina which was dorsiventral with the mesophyll, was seen to be separated into a palisade and spongy tissue. The upper and lower epidermis were covered externally with a thick, striated cuticle. Underneath the upper epidermis were three rows of elongated, closely arranged, palisade parenchyma. Spongy parenchyma tissues were almost radially elongated with intracellular spaces. Central cells were irregular in shape; laticifers and vascular bundles were also present scattered in this region; the details are shown in

Table 1: Leaf constant of *Lawsonia inermis* leaf

Leaf constant	Value
Stomatal index	14
Stomatal number	8
Palisade ratio	7.8
Vein-islet number	32.2
Vein termination number	11.9

Table 2: Ash value of powdered leaf of *Lawsonia inermis*

Type of ash	Ash value (%)
Total ash	**10.2**
Acid-insoluble ash	**1.3**
Water-soluble ash	**1.7**

Table 3: Fluorescence of *Lawsonia inermis* leaf powder in different solvents

Treatment	Under visible light	U.V. light (short wavelength; 254 nm)
Powder as such	**Brown green**	**No change**
Powder + 1N NaOH (aqueous)	**Light green**	**Green**
Powder + 1N NaOH (ethanolic)	**Pale green**	**Light green**
Powder + 1N HCl	**Brownish green**	**Green**
Powder + 50% HNO_3	**Dark brown**	**Geen**

Table 4: Preliminary phytochemical screening of leaf powder of *lawsonia inermis*

Phytochemicals	Petroleum ether extract (60-80°C)	Chloroform extracts	Ethanol extracts	Water extracts
Alkaloids	-	-	-	-
Sugars	-	-	+	-
Phenols	-	+	+	+
Flavonoids	-	-	+	-
Saponons	-	-	-	+
Steroids	+	+	+	+
Terpenoids	+	+	+	-
Tannins	+	-	+	+
Fatty acids	-	-	-	-
Glycosides	+	+	+	+

'+' = presence of the compound**; '-' =** Absence of compound

DISCUSSION

By excellence of their photosynthetic machinery, leaves serve as a sink for several metabolites and as an important source of several bioactive compounds. The macroscopic and microscopic evaluation of leaves of *Lawsonia inermis*, the quantitative estimation of leaf constants, ash values, and fluorescence, and preliminary phytochemical screening of the leaf powder would be of considerable use in the identification of this drug. Empirical knowledge about medicinal plants plays a vital role in primary health care and has great potential for the discovery of new herbal drugs. These findings may be useful to supplement existing information with regard to the identification and standardization of Lawsonia inermis, even in the powdered form of the plant drug, to distinguish it from substitutes and adulterants. These studies also suggested that the observed pharmacognostic and physiochemical parameters are of great value in quality control and formulation development. All in all, the present study might be valuable to supplement data with respect to its recognizable proof and, institutionalization, and in completing further research and revalidation of its utilization in the Ayurvedic System of Medicine.

ACKNOWLEDGMENTS

The authors are thankful to the Management of Hygia Institute of Pharmaceutical Education and Research, Lucknow, U.P. for providing laboratory facilities.

REFERENCES

1. Evans, W.C. (2005). Trease & Evans Pharmacognosy. Saunders an Imprint of Elsevier pp. 41-7, 244
2. Kirkland, D., Marzin, D. (2003). An assessment of the genotoxicity of 2-hydroxy-1,4 naphthoquinone, the natural dye ingredient of Henna. Mutation Re, 537: pp. 183-199.
3. Johanson, (1940). Plant Microtechnique. Mc Graw Hill Book Co, New York p.323
4. Sass, J.R. (1940). Elements of Botenica Microtechnique. Mc Graw Hill Book Co, New York p. 275
5. O'brien, T.P., Feder, N. (1964). Mc Cull ME. Protoplasma. 59 pp. 364–73
6. Easu, K. (1979). Anatomy of Seed Plants. John Wiley and Sons; New York p. 550
7. Fahn, A.(1974) Plant Antomy. Oxford, England: Pergamous Press; p. 611
8. Chase, C.R., Pratt, R. (1949) Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. Am Pharm Assoc.; 38: 324–31
9. The Indian Pharmacopoeia. New Delhi: Govt. of India Publication;.1996 Anonymous.
10. Corner, E.J.H. (1976) The Seeds of Dicotyledons. London: Cambridge University Press;
11. Brindha, P.; Sasikala, P.; Purushothamam, K.K. (1981) Bull Med Ethno Bot Res.; 3: 84–6
12. Lala, P.K. (1993) Lab Manuals of Pharmacognosy. Calcutta: CSI Publishers and Distributors
13. Kokate, C.K., Purohit AP, Gokhale SB. (2005). Pharmacognosy. CBS Publishers and distributors; p. 254.
14. A Dictonary of India Raw materials and industrial products. 'The Wealth of India', pp. 25–26
15. Nandkarni, A.K. (2000) Indian Materia Medica. pp. 730–31.

16. Rastogi, R.P., Mehrotra, B.N. (1999). Compendium of Indian Medicinal Plant, Central Drug Research Institute Lucknow, National Institute of Science. New Delhi, pp. 483-84
17. Kokate, C.K. (1994). Practical Pharmacognosy. Vallabh Prakashan, pp. 115–21