FEDERATION OF HOLISTIC HEALTH & TRADITIONAL MEDICINES
International Journal of Unani and Traditional Medicine (IJUTM)
Volume: I, Issue: I
2024

Pharmacognostical Characterization of *Lawsonia inermis* Linn. Leaves

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Abstract:

Traditional herbal medicine has been serving a crucial function in primary healthcare on a global scale. Recent research indicates that studies on traditional herbal medicine have been rising steadily each year since 1990, accompanied by a corresponding increase in global citations over this timeframe. The unproven legacy of these herbal medicines is now being proved on scientific lines. Recent uptrend in conducting researches in herbal systems of medicine have resulted in increased demand of herbal medicinal plants in the global phytomedicine market. As the demand increased chances of misidentification and adulteration of crude drugs have also escalated, which could potentially endanger the health and safety of the consumers. Therefore, proper identification and pharmacognostical evaluation of crude medicinal plants is required. In this regard pharmacognostical evaluation holds immense significance in the realm of medicinal plants and hence this study was initiated on Lawsonia inermis Linn., to evaluate the pharmacognostical features for proper identification like finding various morphological and microscopic characteristics of the leaves. Various leaf constants (Stomatal index, Stomatal number, Palisade ratio and vein-islet number, Vein termination number) were also analysed. Proper pharmacognostic identification of Lawsonia inermis may be instrumental in conducting further researches on this plant.

Keywords:

Pharmacognostical identification, Lawsonia inermis Linn., Leaf microscopic constants, Henna, Natural Drug Adulteration

Introduction:

Lawsonia inermis L. (synonym *Lawsonia alba*), belonging to the Lythraceae family, this plant stands as the sole species within its genus. The origin of human awareness regarding the distinct henna stain is believed to have stemmed from observations of domesticated animals, such as sheep and goats, grazing on this particular plant. Despite consuming it regularly, the animals displayed no adverse effects, except their staining their tongues red. This peculiar phenomenon piqued the curiosity of our

curiosity of our ancestors, leading to a longstanding fascination with the plant. Over time, through observation, experience and scientific investigation, henna has revealed a plethora of pharmacological effects. With the wild henna species exhibiting high genetic diversity in Africa, it is highly probable that the discovery of henna occurred within the continent during the late Holocene warming period (*Boubaya et al.,* 2013.)

Vernaculars:

Urdu: Henna, Mehndi
Hindi: Henna, Mehndi
Sanskrit: Kuravaka, Mendika, Raktagarbha
Bengali: Mehedi, Mendi, Shudi
Malayalam: Mailanchi, Mailanji, Mail-linshi
Marathi: Mendi; Odiya: Momjaathi
Tamil: Aivanam, Marithondi, Marudhani, Maruthondri
Telugu: Goeranta, Iveni, Kuravamu
Arabic: al Henna, Hinna
Chinese: Zhi jia hua, Zhi jia ye, Zhou jia mu
English: Egyptian privet, Henna, Mignonette tree, Samphire
French: Henné, Reseda
German: Hennastrauch
Greek: Arkan, Fakuliyun
Spanish: Alcana, Alheña (Akbar, 2020).

Taxonomical Classification of *Lawsonia inermis*:
(Moutawalli *et al.,* 2023)

Kingdom	**Plantae**
Subkingdom	**Viridiplantae**
Infrakingdom	**Streptophyta**
Superdivision	**Embryophyta**
Division	**Tracheophyta**
Subdivision	**Spermatophytina**

Class	**Magnoliopsida**
Superorder	**Rosanae**
Order	**Myrtales**
Family	**Lythraceae**
Genus	**Lawsonia**
Species	**Inermis**

***Ethno-botanical uses*:** Over the past few years, henna body art has gained global popularity, transcending its traditional use during weddings and festivals and has transformed from a customary adornment to an exotic fashion statement. In addition to its traditional use as hair dye and body art, henna has also piqued the interest of researchers to investigate its therapeutic uses mentioned in various folk medicines, including the Unani system of medicine. *Lawsonia inermis* is used as traditional or folk medicine throughout the world and has been linked to various biological activities, such as hepatoprotective, immunomodulatory, antihelminthic, antitrypanosomal, antifungal, antibacterial, virucidal, antiparasitic, anti-inflammatory, analgesic, and anticancer effects (Ali, B. H. *et al.,* 1995; Ahmad and Beg, 2001; Aqil *et al.,* 2006; Raja *et al.,* 2009; Akter *et al.,* 2010; Zohourian *et al.,* 2011).

***Therapeutic uses*:** A traditional Unani compound formulation containing *Lawsonia inermis, Olea europea,* and *Nigella sativa* is said to alleviate eczema symptoms, such as itching, burning, redness, and swelling, by as much as 80% in human patients. (Nawab et al., 2008). It's use has also been reported by eminent Unani Scholars in Jarb o Hikka(Scabies), Juzaam(leprosy), Bars(leukoderma), Iltehaab e Mafasil(arthritis), Zarba o Saqta(boils and bruises), Harq o Salq(burn and scald), Amraz e Kabid(liver ailments), Aatishak(syphillis), Usr e Baul(dysuria), Suda(headache), Waja ul Warik(hip pain), Amraz-e-Azfaar(nail diseases), Izam e Tehaal(splenomegaly), Qulaa e Dehan(stomatitis), Khashoonat e Halaq(sore throat).
(Ibn Sina,1931; Kabiruddin,1955; Ibn Baitar,1985; Kirtikar and Basu,1984; Ghani,1888; Tariq,2004; Alvi MH, 1913; Khan H, 1880; Hakim A ,1991; Khan A,1996; Panda H, 2013).

***Need of the study*:** The rising trend of using henna for body art and hair coloring, along with its growing importance as a subject for research, has resulted in the widespread adulteration of commercial products. Adulterated henna, especially when mixed with PPD (Paraphenylene diamine) in dyes, can lead to serious side effects.(Almeida *et al.,* 2012). Because additions go unreported and researchers often base their conclusions on compromised raw materials, this approach has impeded quality of research. Future work based on unreliable data will be equally problematic if researchers are unsure about "what is in the box." This will raise doubts about both the research's assumptions and results (Gupta *et al.,* 1986). To explore and utilise the therapeutic potential of henna, it must be ideally identified. Therefore, present studies were carried out to determine the macro and micro-morphological characters, and quantitative parameters to evaluate pharmacognostic properties of the leaves of *Lawsonia inermis* Linn.

Material and Methods:

***Collection and authentication of medicinal plant*:** Fresh leaves *Lawsonia inermis* Linn., were obtained from the herbal garden of the department of Ilmul Advia (Unani pharmacology), Aligarh Muslim University, Aligarh. The leaves were identified in the department's pharmacognosy lab based on morphological features and were verified by the lab's in-charge. For future reference, a voucher number [SC-394/24] was submitted to the Ibn Baitar Museum of the department of Ilmul Advia.

***Preparation for test samples of leaves of selected test drug*:** Fresh leaf samples were cleaned of dirt and other contaminants by washing under water.
Microscopic examination was done using standard method as per Unani Pharmacopeial Guidelines (Afaq S.H.,1994; Anonymous,2007).

***Macroscopic analysis*:** Fresh foliage was assessed for a range of detailed morphological and organoleptic characteristics including shape, size, color, odor, taste, leaf margin, apex, texture, presence or absence of petiole, leaf arrangement and more, following established textbook guidelines. (Anonymous, 2006; Evans, 2009).

***Microscopic analysis*:** To prepare the transverse sections of the leaves, mature fresh leaves were collected and rinsed with water. Thin sections were then sliced from the middle of the leaf blade, including the midrib, and immersed in water to preserve moisture. Subsequently, they were boiled in a chloral hydrate solution for 5-6 minutes to eliminate chlorophyll presence. The resulting fine, thin, and intact transverse leaf sections were transferred to a watch glass filled with water, followed by another watch glass containing 2-3 drops of safranin stain. After a while (30-45 seconds), the sections were retrieved and rinsed with water to remove excess stain. Any residual stain was cleared using ethanol. These leaf sections were then mounted on slides, covered with a cover slip, and examined under a microscope. High-quality staining agents like Safranin and Fast Green, in addition to glycerin and DPX, were employed, all of which were of analytical grade. Microphotographs were captured using an OPTIKA binocular digital microscope B-290, adjusting the magnification as necessary.

Leaf parameters were documented using a camera lucida and stage micrometre. This included observation of epidermal cells, examination of stomatal structure, distribution, and type, as well as analysis of trichome structure and distribution on the fresh leaves.

Leaf surface study:

***Stomatal number*:** Stomatal number (SN) or stomatal density refers to the mean count of stomata observed within a 1 mm square area of the leaf, encompassing both the upper and lower epidermis.

***Stomatal length and Osteolar length*:** They were quantified utilizing the software provided by the OPTIKA B-290 microscope. Total stomatal length and total osteolar length were thus recorded.

***Stomatal index*:** The percentage of number of stomata relative to the total count of epidermal cells, where each stomata is treated as an individual epidermal cell, is calculated. (Evans 2009).

***Procedure*:** To ascertain the stomatal number and stomatal index in both the upper and lower epidermis, thin sections were finely removed from fresh young leaves using forceps, a razor, and transparent adhesive tape. These sections were then affixed onto slides using glycerin and inspected under an OPTIKA binocular digital microscope B-290 at 40X magnification. The stomatal count per square millimetre was recorded to determine the stomatal number. To ensure precision and reliability, five readings were taken for each sample, and the slides were photographed for documentation.

Stomatal Index was calculated as per following formulae:

$$\textit{Stomatal Index}: = \frac{(S \times 100)}{(S + E)}$$

S = Quantity of stomata per unit area

E = number of ordinary epidermal cell in same unit area

***Vein islet number*:** Vein islet number refers to the count of individual areas or regions enclosed by veins (vein islands) within a specified area of a leaf, typically measured per square millimetre. These vein islets often correspond to the spaces between the network of veins on the leaf surface.

***Veinlet termination number*:** It refers to the count of ends or terminations of veinlets within a specific area of a leaf. It represents the number of points where veinlets terminate or branch out within a defined region, often measured per square millimetre. It is usually measured from the region of midrib up to the leaf margin.

***Procedure*:** To ascertain vein islet and vein termination numbers, sections of the leaf lamina between the midrib and margin were cut into small pieces approximately 1-3 mm square. These sections were then immersed in a concentrated solution of chloral hydrate and boiled for 15 minutes until the pieces lost coloration. The resulting transparent fragments were then transferred onto a glass slide and examined under a microscope at a 10X magnification. Vein islets were counted within a 1mm square area.

Additionally, veinlet terminations were also counted within the same square. To ensure precision and reliability, five readings were taken for each vein islet and vein termination number, and the slides were photographed for documentation.

***Palisade ratio*:** Palisade ratio is defined as the average number of Palisade cells below single upper epidermal cell (Evans 2009). It provides insight into the shape and arrangement of these cells, which can impact their efficiency in capturing light for photosynthesis. This parameter is crucial for identifying and describing leaf-based medicinal substances and remains consistent within a specific plant taxon. (Khan *et al.*, 2016).

***Procedure*:** Small leaf fragments (1-2mm) grown under full sunlight were selected and treated by boiling in a solution of 200% chloral hydrate for clarification. These clarified fragments were then mounted and examined using a microscope. Initially, a number of clusters consisting of four upper epidermal cells were brought into focus. Subsequently, a slight adjustment of the fine focus allowed for the underlying palisade cells to come into focus within the same area of the four epidermal cells. The palisade ratio was determined by dividing the number of palisade cells by 4. To ensure accuracy, five readings were taken from various leaf fragments to establish a reliable average (Evans 2009; Kokate 2020).

Results and Discussion:

***Macromorphology*:** The findings from both macroscopic and organoleptic evaluation indicate that Lawsonia inermis Linn. is a branched shrub or small tree, reaching approximately 4-5 meters in height. Its leaves are small and simple, arranged oppositely, exhibiting a reticulate venation pattern with smooth margins. They are elliptical to broadly lanceolate in shape, nearly sessile, measuring about 1.5 to 5 centimetres in length and 0.5 to 2 centimetres in width. The leaves appear dull green to dark green, are glabrous, with short petioles, and possess an acute or obtuse apex with a tapering base. They impart a sweet, slightly astringent taste and emit an aromatic odor. The inflorescence forms a terminal panicle (raceme). (Fig.1a, b, c, and Table 1).

The morphological findings of *Lawsonia inermis* were found to be in co-ordination with standard textbook (Standardization of Single Drugs of Unani Medicine, Central Council for Research In Unani Medicine).

a) Abaxial surface b) Adaxial surface c) Opposite phyllotaxy

Fig.1 Morphological characters of leaf of *Lawsonia inermis* Linn.

***Microscopic Analysis*:** The transverse section reveals a clear distinction between the midrib and lamina. The midrib appears broad and shallow on the upper side, while it appears convex on the lower side.T.S. of the midrib shows an upper and lower epidermis covered by thick cuticle. The epidermis consists of single layer of mostly cubicle cells. Below the epidermis 2-4 layers of collenchyma and 3-4 layers of parenchyma cells are present. The collenchyma cells are circular or elliptical in shape while the parenchyma cells are round with intercellular spaces. Mesophyll is differentiated into single layer of palisade tissue and 2-3 layers of spongy parenchyma. The palisade cells are long, thick and filled with chloroplasts. Anomocytic or Ranunculaceous type of stomata, with 3-4 subsidiary cells surrounding them are present on both the surface. Fig 2a, b, Fig 3, Fig 4. The findings of *Lawsonia inermis* were found to be in co-ordination with standard textbook (Standardization of Single Drugs of Unani Medicine, Central Council for Research In Unani Medicine).

***Quantitative Microscopy*:** The quantitative examination reveals that the count of epidermal cells ranges from 180 to 200, stomata number is 32-56, stomatal index is 15.09-21.87 for abaxial surface. Stomatal length is 0.03µm while osteolar length is 0.02µm. Vein islet number of the leaves was found to be between 2-3 per sq.mm, vein termination number was between 6-8 per sq.mm and palisade ratio was found to be 3.12-3.50. Refer Table 2.

***Leaf Constants*:**

STOMATAL NO	**32-56**
STOMATAL INDEX	**15.09-21.87**
PALISADE RATIO	**3.12-3.50**

Table.1. Organoleptic features of leaves of *Lawsonia inermis* Linn

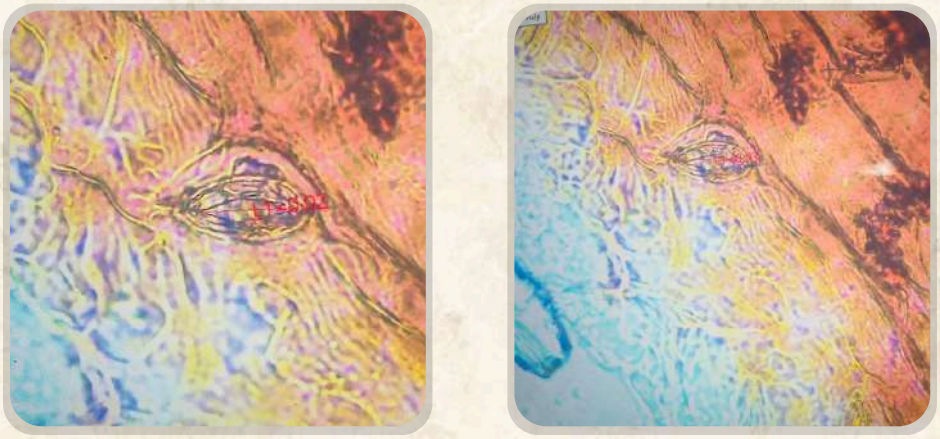
Parameters	Leaves of *Lawsonia inermis* Linn
Leaf type	Simple
Phyllotaxy	Opposite
Petiole	Subsessile
Stipule	Absent
Shape	Elliptical/broadly lanceolate
Size (l*b) cm	1.5-5/0.5-2
Venation	Reticulate

Apex	Acute/obtuse
Base	Cuneate
Margin	Entire
Glands at leaf base	Absent
Trichomes	Absent
Surface appearance	Smooth
Odour	Aromatic (crushed)
Taste	Sweet/astringent

Table.2. Quantitative Parameters of the leaves of *Lawsonia inermis* Linn.

Sl. No.	Parameters	*Lawsonia inermis* Linn.
1.	stomata no.	32-56
2.	epidermal cell no.	180-200
3.	stomatal index	15.09-21.87
4.	Vein islet number	2-3
5.	Vein termination number	6-8

Microscopic characteristics of *Lawsonia inermis* Linn. leaves



a) L1 Osteolar length=0.02µm b) L2 Stomatal length=0.03µm

Fig.2. T.S. of leaf of *Lawsonia inermis* showing anomocytic stomata

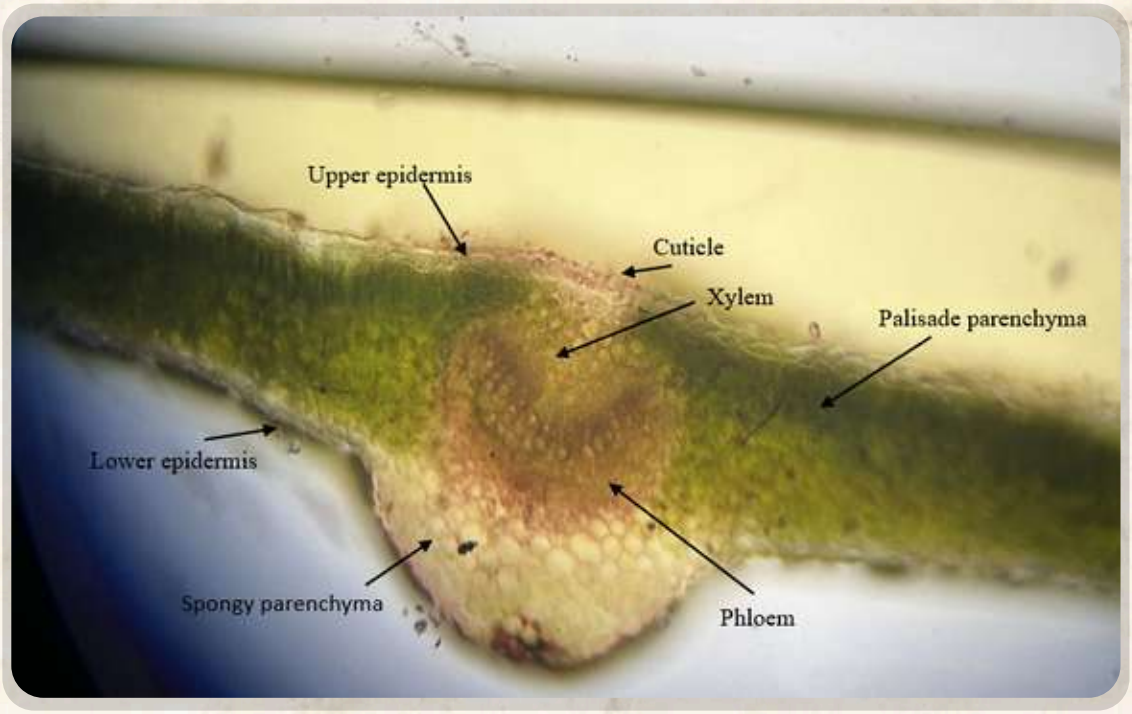


Fig. 3. T.S. of leaf of *Lawsonia inermis* showing micromorphological features.

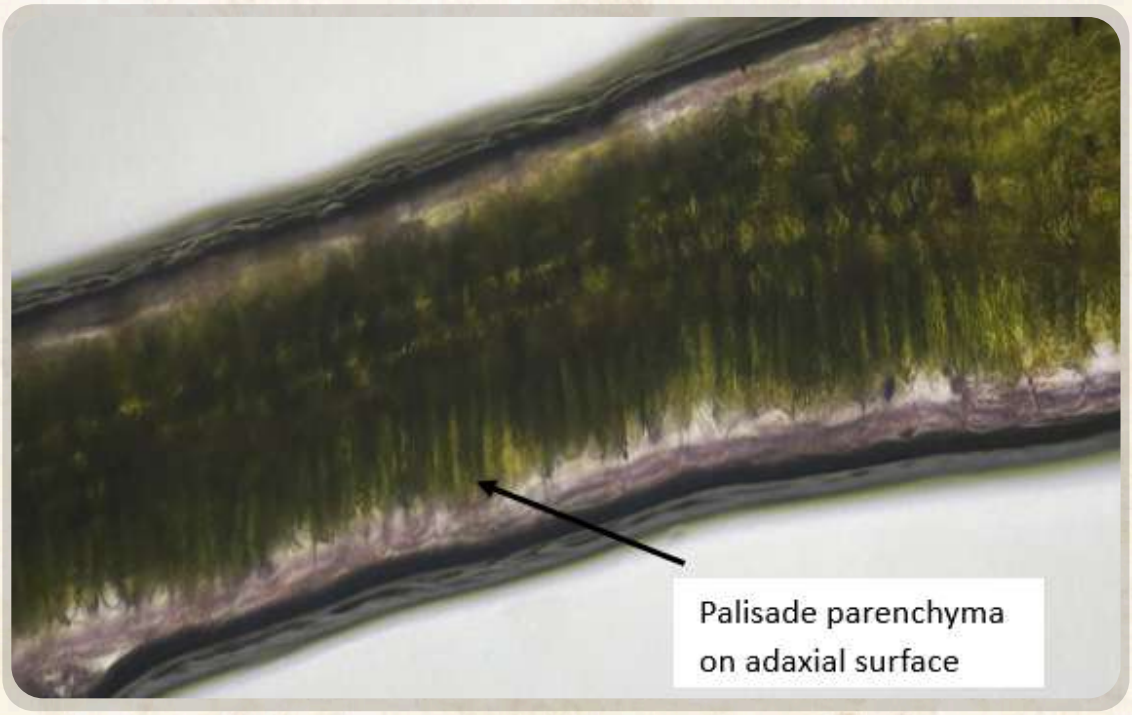


Fig. 4. T.S. of leaf of *Lawsonia inermis* showing palisade parenchyma on adaxial surface.

Discussion:

Despite the widespread availability and accessibility of modern medicine, a significant portion of the global population continues to place their trust in traditional medicinal systems. According to the WHO (2002), approximately 80% of the world's population relies on medicinal plants for treating various ailments, with even higher rates observed in African countries. In the United States, more than 40% of the population opts for complementary and alternative medicines, which also includes botanical dietary supplements. WHO has documented approximately 21,000 plants utilized for medicinal purposes worldwide, with around 2,500 species found in India, which is

known as the largest producer of medicinal herbs and often referred to as the botanical garden of the world.

As the reliance on herbal medicines continues to grow globally, it is pertinent that authenticity of research should be more firmly established and hence more focus should be given to drug identification, which serves as the cornerstone for further research endeavors. The pharmacognostic parameters examined in this study, including stomatal index, stomatal number, palisade ratio, vein-islet number, vein termination number, as well as morphological and microscopic characteristics, shall provide a robust foundation for future in-depth research on *Lawsonia inermis*.

Conclusion:

This research aims to establish a set of pharmacognostic criteria for *Lawsonia inermis* Linn. leaves. The examination revealed that the leaves of *L. inermis* are small, sub-sessile, arranged oppositely, with entire margins, and shaped elliptically to broadly lanceolate, displaying distinctive microscopic characteristics. This investigation serves as a preliminary step towards conducting comprehensive chemical profiling studies in future.

Acknowledgement:

The authors would like to take this opportunity to acknowledge the continuous support and guidance from the faculties of the Department of Ilmul Advia, Faculty of Unani Medicine Aligarh Muslim University.

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