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Pharmacognostic assessment of *Lawsonia inermis* flowers

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Abstract
Towards authentication and quality assurance of medicinal plants, the microscopic characters, powder characteristics, behavior and florescence analysis, physiochemical evaluation, micrometric analysis and preliminary phytochemical studies of *Lawsonia inermis* Flowers were carried out. The characteristic macroscopic and microscopic features of flowers include presence of epidermal cells, parenchymal cells, spirally thickened xylem vessels, lignified fibers, oil globules, different crystals of calcium oxalate. Powder showed distinguishing fluorescent property when treated with different reagents. Physicochemical investigation revealed values for moisture content, pH, ash values and extractive values which are within the World Health Organisation standards for crude drug from medicinal plants. Micromeritic analysis of flower powder reveals fair flowability. Water and ethanol extractive values were found to be higher when compared to that of chloroform, petroleum ether and ethylacetate. Preliminary Phytochemical screening indicated the presence of alkaloids, Glycosides, Carbohydrates, Flavonoids, Tannins, Proteins, Sterols and Cardiac glycosides. It can be concluded that the information obtained from these studies may be utilized for identification and standardization of *Lawsonia inermis* flowers as a herbal remedy and also towards monograph development of it.

Keywords: *Lawsonia inermis*, physicochemical evaluation, fluorescence analysis, phytochemical screening

Introduction
Herbal plants play a significant role in preventing and treating of diseases. The World Health Organization (WHO) reported that 4 billion people (80% of the world's population) use herbal medicines for some aspect as primary healthcare medication. Herbal medicine has been documented by WHO as necessary components for primary health care and about 11% of the 252 drugs are derived from plants. Over the past decade, there has been a resurgence of interest in the exploration of medicinal plant as a source of potential herbal medicine. There is a need to advance research for the development of plant drug with the expansion of chemistry, isolation, purification and characterization of plant active compounds [1].
Lawsonia inermis Linn., is a perennial herbal medicinal plant belonging to the family Lythraceae grown as an ornamental and dye plant thorough India having different vernacular names in India viz., Mehndi in Hindi, Mendika, Rakigarbha in Sanskrit, Mailanchi in Malayalam, Muruthani in Tamil, Benjati in Oriya, Mayilanchi in Kannada and Mehedi in Bengali. The plant is reported to contain Lawsone, Esculetin, Fraxetin, Isoplumbagin, Scopoletin, Betulin, Betulinic acid, Hennadiol, Lupeol, Lacoumarin, Laxanthone, Flavone glycosides and two pentacyclic triterpenes [2]. It has been traditionally reported in use of a headache, hemicranias, lumbago, bronchitis, boils, ophthalmia, syphilis, sores, amenorrhea, scabies, diseases of the spleen, dysuria, bleeding disorder, skin diseases, diuretic, antibacterial, antifungal, anti-amoebiasis, astringent, anti-hemorrhagic, hypotensive and sedative effect [3]. Due to the presence of Pharmalogical chemical components the plant has been reported to have analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic and anticancer properties [4]. The objective of present study is to evaluate various pharmacognostical parameters such as macroscopic, microscopy, physicochemical, fluorescence and phytochemical studies of the *Lawsonia inermis* flowers.

Materials
Collection and authentication of plant material
The fresh flowers of tall shrub of *Lawsonia inermis* were collected in bulk from medicinal garden of Jayamukhi College of Pharmacy, Warangal, Telangana, India. The flowers were authenticated by Dr. P. Veera Reddy, Professor, Government Ayurvedic College, Warangal, Telangana. The flowers were separated and shadow dried.

Macromorphological Description
The *Lawsonia inermis* flowers were subjected to macroscopic studies which comprised of organoleptic characteristics *viz*. color, odor, taste, surface, shape and size of the drug.

Microscopic characteristics
Microscopic sections were cut by free hand cross-sectioning, temporary mounts of the sections of flowers were stained with saffranine, mounted with glycerine water and observed under microscope.

Powder characteristics
On the clean glass slide fine powder of *Lawsonia inermis* flowers was stained with Phloroglucinol-HCl and Sodium hypochlorite solution mounted with glycerin water. The slide was then positioned and observed under the magnifying lenses of 10x, 40x and 100x magnification of the microscope.

Behavior of *Lawsonia inermis* flowers Powder with Chemical Reagents
Behavior of *Lawsonia inermis* flowers with different chemical reagents was performed to detect the occurrence of phytoconstituents along with colour changes under ordinary daylight by standard method [5].

Fluorescence Analysis of *Lawsonia inermis* flowers
The drug powder was placed on a clean microscopic slide and 2 to 3 drops of freshly prepared reagents (acids such as 1 N HCL and 50% H2SO4; and alkaline solutions such as aqueous sodium hydroxide, alcoholic sodium hydroxide; and other solvents such as nitric acid, picric acid, acetic acid, ferric chloride, and nitric acid with ammonia) were added and mixed by gentle slanting the slide. The slides were then subjected to fluorescence analysis in the ultraviolet (UV)-light (254 nm and 365 nm) [6].

Physico-Chemical Evaluation of *Lawsonia inermis* flowers
Physiochemical parameters such as moisture content, pH, ash constants and soluble extractive values were performed according to the official method prescribed and the WHO guidelines on quality control methods for medical plants Material [7-8].

Micromeritic Evaluation of *Lawsonia inermis* flowers
The micromeritic characteristics of petal powder like Bulk density, Tapped Density, Angle of repose, Hausner's ratio and Carr's index was determined according to the official standard procedures to study the flowability of drug [9].

Preparation of extracts of *Lawsonia inermis* flowers
The dried powder material (100 g) of the *Lawsonia inermis* flowers was powdered and passed through sieve no.16. This powder material was macerated with water, ethanol, chloroform, ethyl acetate and petroleum ether for 7 days with occasional shaking. The extracts were filtered through muslin cloth, then the filtrate was evaporated under reduced pressure and vacuum dried. Extractive values were then calculated. The preliminary Phytochemical screening of the extracts was carried out [10-11].

Preliminary Phytochemical Screening of *Lawsonia inermis* flowers
The preliminary phytochemical screening was carried out on the different extracts of *Lawsonia inermis* flowers for the detection of various phytochemicals such as Carbohydrates, Alkaloids, Glycosides, Saponins, flavonoids, proteins, aminoacids, tannins, fixed oil, fats, steroids and terpenoids [10-11].

Result
Macromorphological Description
Scented Flowers *of Lawsonia inermis* are numerous small and white. Flowers are arranged in panicle, a much-branched inflorescence. Four wrinkled petals are organized on the top of the calyx tube in a bud. The results of micromorphology were depicted in Table 1. The Fresh Petals, Dried and powdered *Lawsonia inermis* flowers were illustrated in figure 1 whereas microscopy of flower is shown in Figure 2.

Fig 1: Fresh Petals, Dried and powdered *Lawsonia inermis* flowers

Table 1: Macromorphological Description *of Lawsonia inermis* flowers

S. No	Characters	Observation
Organoleptic Characters		
1.	Colour	White Inflorescence
2.	Odour	Aromatic Distinct
3.	Taste	Distinct tongue sensitizing
Quantitative Micromorphology of Fresh petals		
4.	Width	0.6-0.8cm
5.	Length	1.2-1.8cm
Extra Features		
8.	Shape	obovate
9.	Texture	Soft and smooth

Fig 2: Microscopy of *Lawsonia inermis* flowers

Powder characteristics

Some of the microscopical powder characteristic features that are seen in *Lawsonia inermis* flowers are Fragment of Epidermal cells, lignified xylem and phloem vessel, lignified fibers, parenchymatous cells with crystals and different types of Calcium oxalate crystals such as Prisamatic, Rhombic and Conglomerate crystals. Photographs detailing the microscopic features of *Gomphrena globosa* flowers are shown in Figure 3.

Fig 3: Powder Characteristics of *Lawsonia inermis* flowers

Behavior and Fluorescence analysis

Fluorescence is the significant phenomenon displayed by various chemical components present in the natural products. Some display fluorescence in the visible range in daylight. The ultraviolet light produces fluorescence in several plant materials which do not noticeably fluoresce in daylight. If the plant material is not fluorescent in nature it can show fluorescence when treated with different reagents, henceforth it can be used as an important parameter for qualitative assessment in pharmacognostical evaluation. The results of Behavior and fluorescent analysis of *Lawsonia inermis* flowers of powder and different extracts showed characteristic coloration in treatment with various chemical reagents. The results of Behavior and Fluorescence analysis of Powder of *Lawsonia inermis* flowers were described in Table 2.

Table 2: Behavior and Fluorescence analysis of Powder of *Lawsonia inermis* flowers

S. No	Treatment	Day Light	UV Light (254nm)	UV Light (365nm)
1.	Powder as such	Ochre	Brass	Black
2.	Powder + Conc. HCl	Yellow green	Olive	Black
3.	Powder + Conc.HNO_3	Rust	Lawn green	Black
4.	Powder + Conc. H_2SO_4	Burnt orange	Dark brown	Black
5.	Powder + Conc. NaOH	Sangria	Olive drab	Black
6.	Powder + Ethanol	Sepia	Dark Olive green	Black
7.	Powder + Glacial acetic acid	Old gold	Pear	Bister
8.	Powder +Dil. NaOH	Saddle brown	Yellow green	Black
9.	Powder + Picric acid	Yellow	Bright green	Bister
10.	Powder +$FeCl_3$	Black	Dark Olive	Black
11.	Powder + $NaHCO_3$	Cinnamon	Olive drab	Black
12.	Powder + 5% I_2	Tangerine	Green yellow	Black

Physicochemical parameters of *Lawsonia inermis* flowers

Loss of drying is used to evaluate the amount of volatile matter including water that is present in the plant drug. Percentage Loss on drying was found to be 20.97%. The moisture content of a drug should be minimized in order to prevent decomposition of crude drugs, either due to chemical change or microbial contamination. The percentage of moisture content ranging from 10 - 20% shows an ideal range for minimum bacteria as well as for fungal growth. Swelling index denotes the extent of polysaccharide that is present in certain drugs. It is one of the characteristic for identification of botanical drugs if swelling index defers it indicates that the powder has been adulterated or not properly stored. For the present drug the swelling factor in water after 24 hours was found to be 2.5 ml. The ability of plant material to take up water is determined by moisture sorption capacity. Higher the

moisture sorption capacity higher will be probability of bacterial or fungal contamination. In the present study moisture sorption capacity of *Lawsonia inermis* flowers was found to be 0.36/gm in 24 hours. Even after 72 hours no bacterial or fungal contamination was noted.

Ash value is a benchmark to judge the identity or purity of crude drugs. The *total ash* residue remaining after incineration which usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also contain inorganic matter added for the purpose of adulteration. Ash value is useful tool for detecting low grade products or exhausted products or excess of sandy or any earthy substance with drug. Acid insoluble ash denotes the presence of only earthy matter i.e., sand or silica in the drug whereas Water soluble ash detects the material exhausted with water, if admixed with exhausted drug will show much greater reduction in water soluble ash than total ash. So, it's an important indicator when exhausted material is substituted for the genuine drug. In the present study Total ash, Acid insoluble ash and Water soluble ash were found to be 5.42, 2.67 and 1.36 % w/w respectively. The results of Physicochemical parameters were depicted in Table 3.

Table 3: Physicochemical parameters of *Lawsonia inermis* flowers.

S. No	Constants	Yield (N=3)
1.	Foreign matter	0
2.	Moisture content(Loss on drying) %	20.97 %
3.	Swelling Index ml	2.5
4.	Moisture Sorption Capacity /g	0.36
5.	Total ash (% w/w)	5.42
6.	Acid insoluble ash (% w/w)	2.67
7.	Water soluble ash (% w/w)	1.36

The consistency and extractive values of different extracts of *Lawsonia inermis* flowers

An extractive value signifies the amount of constituents in the given amount of plant material extracted with solvents. It provides an indication of the extent of polar, medium polar and non-polar constituents present in crude drug. In the present study water soluble extractive value and alcohol soluble extractive values were found to be higher when compared to pet ether, chloroform and ether soluble extractive values, which denotes that *Lawsonia inermis* flowers contains more quantity of polar elements. The extractive values of different extracts of *Lawsonia inermis* flowers and their behavior analysis were shown in Table 4 and Table 5.

Table 4: The consistency and extractive values of different extracts of *Lawsonia inermis* flowers.

S. No	Treatment	Consistency	Extractive values (%)
1.	Water	Sticky	32.33
2.	Ethanol	Sticky	33.8
3.	Pet Ether	Sticky	3.6
4.	Chloroform	Sticky	5.4
5.	Ethyl acetate	Powder	7.2

Table 5: Behavior and Fluorescence analysis of extracts of *Lawsonia inermis* flowers

S. No	Treatment	Day Light	UV Light (254nm)	UV Light (365nm)
1.	Aqueous	Rust	Bright green	Dark green
2.	Alcohol	Saddle brown	Olive	Dark brown
3.	Pet Ether	Olive	Olive drap	Saddle brown
4.	Chloroform	Raw umber	Cinnamon	Bister
5.	Ethylacetate	Burnt orange	Yellow green	Sepia

Micrometric parameters of *Lawsonia inermis* flowers

Bulk density, Tapped Density, Angle of repose, Hausner's ratio and Carr's index was determined as a part of micrometric analysis. The Carr's compressibility index & Hausner's ratio are reflective of the difference in the bulk and tapped densities. While the Carr's index shows strength and the ability of a material to reduce in volume, the Hausner's ratio reveals inter particulate friction. As the values these indices decrease, the flow property of the powder increases. The angle of repose is a traditional characterization method for determining powder flow property. The result showed that the powder has fair flow ability as angle of repose of powder was found to be 37.56 ^{0}. The values of micrometric parameters were depicted in Table 6.

Table 6: Micrometric parameters of *Lawsonia inermis* flowers

S. No	Constants	Yield
1.	Bulk density	0.2 g/ml
2.	Tapped Density	0.28 g/ml
3.	Angle of repose	37.56 ^{0}
4.	Hausner's ratio	1.4
5.	Carr's index	30 %

Preliminary phytochemical screening of different extracts of *Lawsonia inermis* flowers

Phytochemical screening showed that maximum presence of phytoconstituents in ethanolic and aqueous extract extracts. The results of Preliminary phytochemical screening of different extracts of *Lawsonia inermis* flowers were shown in Table 7.

Table 7: Preliminary phytochemical screening of different extracts of *Lawsonia inermis* flowers

S. No	Plant constituent	Aqueous Extract	Alcohol	Pet Ether	Chloroform	Ethyl acetate
	Alkaloids	+	+	-	+	-
	Glycosides	-	+	-	+	+
	Carbohydrates	+	+	+	+	+
	Flavonoids	+	+	-	+	+
	Tannins	+	+	-	-	+
	Proteins	+	+	-	-	+
	Amino acids	+	+	-	-	+
	Fixed oils	-	+	-	-	+
	Sterols	+	+	+	-	+
	Starch	-	-	-	-	-
	Cardiac glycosides	-	+	-	-	-

Conclusion

It was concluded from current investigation of *Lawsonia inermis* flowers that the pharmacognostical data will provide the standards for its identification and authentication. The other parameters which are useful in the establishment of its quality control parameters are ash value, extractive values, moisture content, swelling and foaming index, leaf constants, fluorescence analysis and phytochemical parameters. The findings of the current research will help in the evaluation, identification and authentification of the plant.

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