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Pharmacognostical & Preliminary phytochemical screening of *Flacourtia inermis* Roxb

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Abstract
Objective: To study detailed pharmacognostic profile and preliminary phytochemical investigation of the leaves of *Flacourtia inermis* (Roxb) belongs to the family Flacourtiaceae. This is an autotrophic plant with an oval shape. The leaves of *F. inermis* (Roxb) used traditionally in Ayurveda in the treatment of various diseases and possessed antioxidant, anti diabetic, antimicrobial activities. The fruits, bark, leaves and roots for some health conditions especially for arthritis, cough, some bacterial infection.
Methods: Leaf of *Flacourtia inermis* (Roxb) was studied by Macroscopical, Microscopical, Quantitative microscopy, Physicochemical, Phytochemical analysis of leaf powder of the plant and other methods for standardization recommended by WHO.
Results: Macroscopically the leaves are simple, alternate, and petiolate with a unicostate reticulate venation. When young, leaves are red and turn green in colour when they mature. The leaves are ovate elliptical in shape, size 5-20 cm long, 3-8 cm width with characteristic odour & bitter taste. The surface is glabrous with serrate margin and acuminate apex. Microscopically, the leaf showed the presence of smooth and uniform thickness lamina. The epidermal layer has thin rectangular walled cells. Beneath the palisade cells are loosely arranged spongy parenchyma cells which are spherical in shape. Calcium oxalate crystals are common in midrib of leaf as druses. The vascular strands are flat. In between xylem lines occur in narrow thin layers of parenchyma cells. There is a thick layer of sclerenchymatous cells forming sclerotic layer. The investigations also included leaf surface data; quantitative leaf microscopy. Physiochemical parameters such as loss on drying, extractive values and ash values were also determined. Preliminary phytochemical screening showed the presence tannins, phenols, flavonoids and triterpenoids, sterols, carbohydrates, protein, alkaloids, cyanogenetic glycosides, tannins, saponins, mucilage, and volatile oils.
Conclusions: The results of the study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigations and applications.

Keywords: *Flacourtia inermis* macroscopy, microscopy physiochemical parameters, preliminary phytochemical screening, fluorescence analysis

Introduction
The importance of medicinal and aromatic crops in the national economy and their potential for the rapid growth of phyto pharmaceuticals, perfumery and allied industries in India has been emphasized from time to time. Medicinal plants belong to the oldest known health care products that have been used by mankind all over the world in the form of folklore medicines or traditional or ethnic medicine[1].The world Health organization (WHO) estimates that about 4 billion people, 80% of the world population presently use herbal medicine for some aspect of primary health care [2] In almost all the traditional medicine, the medicinal plants play a major role and constitute the backbone of the traditional medicine [3]. Indian Materia Medica includes about 2000 drugs of natural origin almost all of which are derived from traditional system and folklore practices. Medicinal plants are inextricably inter-twined with the rich history, cultureand culinary tradition of India. India has a rich and glorious ethno medical heritage [4]. Medicinal plants are also used by the codified systems of medicine such as Ayurveda, Siddha, Unani, Chinese and Tibetian systems of medicine [5]. Medicinal plant research continues to be fruitful approach for the search of new drugs. The endurance of herbal medicine may be explain often without side effects both on the illness and its symptoms. Various latest technological development has lead to increased accuracy in Estimation, Purification, Separation and Determination of principle and therapeutically active constituents in crude drugs. Tribes in India have relied on medicinal benefits of *F. inermis* for centuries. Medicinally we can use (Lovi) the fruits, bark, leaves & roots for some health conditions especially for arthritis, cough, some bacterial infection, indigestion and diarrhoea. It is propagated by seeds.

It is rich in vitamin c. [6-12] Pharmacognostic studies on leaves are not adequate necessitating the present investigation. The current work aims to contribute in solving the problems of controversial drugs prevalent in Ayurveda besides helping in laying down pharmacopoeial standards. Therefore, keeping above view in mind various Macroscopical, Histological and physiochemical and quantitative microscopical studies and Preliminary phytochemical investigation of leaves of *Flacourtia inermis* was carried out in present study.

Materials and Methods

Collection and Authentication

F. inermis leaf was collected, from in Trivandrum district, Kerala, India and authenticated by Mrs. Valsakumari, Curator, Dept of Botany, Kerala University, Trivandrum. The authenticated specimen is deposited in the Department of Botany, a voucher specimen number is KUBH 6036. The fresh leaves were kept for shade drying. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Pharmacognostic Standardization

Organoleptic characters such as shape, size, colour, odour, taste of Leaf was determined. Microscopic studies was carried out by preparing thin hand section of leaf with Chloral hydrate solution, stained with Phloroglucinol-hydrochloric acid (1:1) and mounted in glycerine [13]. And Formalin, Acetic a, Et. OH 70%, tert. Butoh, Tolidineblue, safranin & fast green Histo chemical studies and powder microscopy were carried out to know about the inclusions and detailed anatomical characters of the material [14].

Quantitative microscopy and Physico-chemical Evaluations

The vein islet number, vein terminal number, stomatal number, stomatal index were determined on fresh leaves using standard procedure [15-17]. The parameters were done to evaluate the proceedings of total ash; water soluble ash; acid insoluble ash and sulphated ash were calculated as per Indian Pharmacopoeia [18]. Extracts of the powdered leaf was prepared with different solvents for the study of extractive value. Fluorescence analysis was also carried out for the powder and for extract as per standard procedure [19].

Extraction of plant material

For preliminary phytochemical analysis, extract was prepared by weighing 1kg of the dried powdered leaf was subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether, benzene, chloroform, ethanol and aqueous. The extracts were filtered in each step using What man filters paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-45ºC) and pressure. The presence or absence of the primary and secondary Phytoconstituents was detected by usual prescribed methods [20-21].

Powder analysis

Preliminary analysis of the powder of the leaf powder of *F. inermis* with different chemical reagents was carried out microscopically [22-23].

Results

Macroscopic evaluation

The plant *F. inermis* (Roxb) belongs to the family Flacourtiaceae this is an autotrophic plant with an oval shape. It grows at a height of 9-15 m. the plant is native. It has a smooth bark & hard wood. The leaves are simple, alternate, and petiolate with a unicostate reticulate venation. When young, leaves are red and turn green in colour when they mature. The leaves are ovate elliptical in shape size – 5-20 cm (L), 3-8 cm (b) with characteristic & bitter taste. The surface is glabrous with serrate margin and acuminate apex.

Histological Characters

The detail and systemic pharmacognostical evaluation would give valuable information for the future studies. The Transverse section of the leaf is represented in the given figures. The lamina is smooth and uniform in thickness. The epidermal layer has thin rectangular walled cells. Beneath the palisade cells are loosely arranged spongy parenchyma cells which are spherical in shape. Calcium oxalate crystals are common in midrib of leaf as druses. The vascular strands are flat. In between xylem lines occur in narrow thin layers of parenchyma cells. There is a thick layer of sclerenchymatous cells forming sclerotic layer. Paracytic stomata and covering trichomes are present.

Fig 1: T.s of leaf through mid-rib

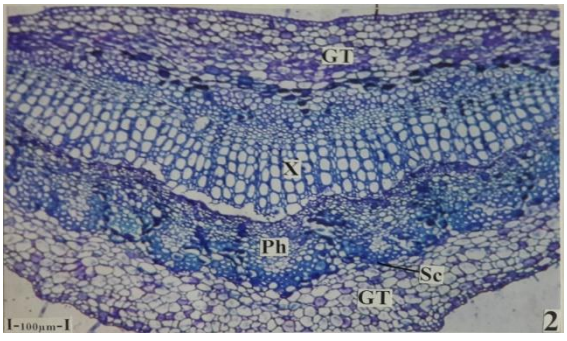

Fig 2: T.S of leaf through Midrib

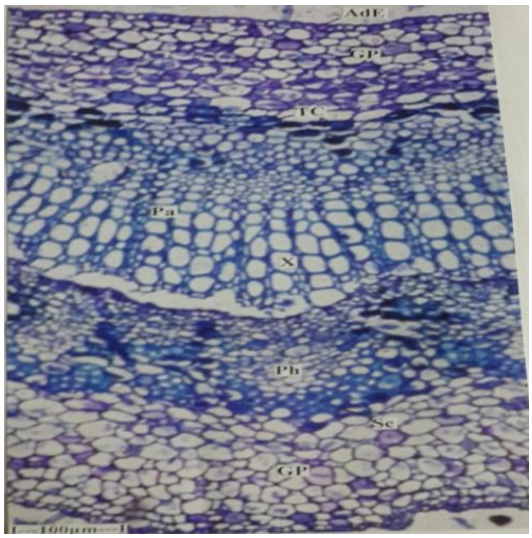

Fig 3: T.S Enlarged

Powder microscopy

Powder characteristics revealed the presence of epidermal cells with starch granules, covering trichomes, lignified fibres, epidermal cells with trichomes, paracytic stomata, vessel elements.

Quantitative microscopy

The quantitative microscopy such as vein- islet number, vein-terminal number, stomatal number and stomatal index were determined and the results were tabulated. (Table 1).

Table 1: Quantitative evaluation of the crude drug of leaf of *F. inermis*

S. No	Plant constants	Values
1	Vein islet no	62
2	Vein termination no	56
3	Stomatal number (upper)	25
4	Stomatal number (lower)	32

Table 2: Dimensions of starch

Diameter	8.68 µm	12.59 µm	µm

Table 3: Dimensions of Fibres

Length	175µm	405 µm	750 µm
Breadth	62.5 µm	98.75 µm	125 µm

Table 4: Dimensions of Trichomes

Length	75 µm	97.5 µm	125 µm

Physico chemical features

The powdered drug was evaluated for its physico-chemical parameters like total ash values, acid insoluble ash, water soluble ash and loss on drying, and the results were tabulated (Table 5).

Table 5: Physico chemical evaluation of the crude drug of leaf of *f. inermis*

S. No	Physical Evaluation	%w/w
1.	Total Ash	9.66
2.	Acid Insoluble Ash	1
3.	Water Soluble Ash	4.12
4.	Sulphated Ash	13.05

Extractive values

The extracts were prepared according to the polarity and they were concentrated and their values were calculated with reference to air dried drug and the results were tabulated (Table 6).

Table 6: Extractive values of leaf of *f. inermis* with different solvents

S. No	Sample	Extractability (%)
1.	Chloroform Extract	16.56
2.	Ethanol Extract	15.12
3.	Aqueous Extract	13.28

Fluorescence analysis of the extracts

The extracts were prepared as per their polarity in hot successive extraction technique, and they were treated with reagents and the colour changes were observed under Ultra Violet light and the results were tabulated (Table 7).

Table 7: Fluorescence analysis of leaf of *F. inermis*

S. No.	Reagents	visible light	Under UV (254nm)	long wave length (365nm)
1	Dry powder	No fluorescence	Light green fluorescence	No fluorescence
2	Powder + 1 NHcl	brown	Brown	Dark brown
3	Powder + 1 N H_2So_4	brown	Brown	Dark brown
4	Powder + 5% $Fecl_3$	green	Greenish brown	Dark green
5	Powder + weak iodine	Yellowish brown	Green fluorescence	Dark brown
6	Powder + dilute $KMnO_4$	Muddy brown	Brown	Black
7	Powder + Acetic acid	brown	Brownish green fluorescence	Brownish black
8	Powder + Acetone	brown	Green fluorescence	Dark brown
9	Powder + Pet. ether	brown	Brownish black	Dark brown
10	Powder + ethyl acetate	Brown	Green	Dark brown
11	Powder + ethanol	Light brown	Light green	Dark brown
12	Powder + picric acid	Yellow	Green fluorescence	Dark brown
13	Powder + NH_3	Bright brown	Dark green fluorescence	Dark brown
14	Powder + Sodium carbonate	brown	Dark green	Dark brown
15	Powder + Con. HNo_3	Light brown	Green fluorescence	Dark brown
16	Powder + Con. H_2SO_4	brown	Dark green fluorescence	Dark brown
17	Powder + Con. HCl	brown	Green	Dark brown

Preliminary phytochemical analysis

The leaf powder and various extracts such as petroleum ether extract, Ethyl acetate extract, chloroform extract, ethanol extract and aqueous extract were subjected to preliminary phytochemical screening for their presence or absence of the constituents and the results were tabulated (Table 8).

Table 8: Preliminary phytochemical tests for drug powder and various extracts of leaf of *f. inermis*

S. No	Test	Drug Powder	Pet Ether extract	Ethyl acetate Extract	Chloroform Extract	methanol Extract	Aqueous Extract
1	Sterols	+	+	-	-	-	-
2	Terpenoids	+	+	-	-	-	-
3	Carbohydrates	+	-	+	+	+	+
4	Flavanoids	+	+	+	+	+	+
5	Proteins	-	-	-	-	-	-
6	Alkaloids		-	-	-	+	-
7	Glycosides	-	-	-	-	-	-
8	Saponins	-	-	-	-	+	-
9	Tannins	+	-	+	+	+	+
10	Mucilages	+	-	-	-	+	+
11	Volatile Oil	+	-	-	-	-	-

+ indicates positive reaction, -indicates negative reaction.

Discussion

Our study has focused on examining Pharmacognostic and Preliminary phytochemical studies of *F. inermis* leaves. Normalization of the macroscopic and microscopic characteristics of the drug *F. inermis* remains essential in other to identify and avoid falsification. Microscopically, the leaf showed, the lamina is smooth and uniform in thickness. The epidermal layer has thin rectangular walled cells. Beneath the palisade cells are loosely arranged spongy parenchyma cells which are spherical in shape. Calcium oxalate crystals are common in midrib of leaf as druses. The vascular strands are flat. In between xylem lines occur in narrow thin layers of parenchyma cells. There is a thick layer of sclerenchymatous cells forming sclerotic layer are the diagnostic features noted from anatomical study. Organoleptic characteristics are important in drugs because they play a role in the detection of adulterated or substituted drugs [24]. The leaves are simple, alternate, and petiolate with a unicostate reticulate venation. When young, leaves are red and turn green in colour when they mature. The leaves are ovate elliptical in shape size – 5-20 cm (L), 3-8 cm (b) with characteristic & bitter taste. The surface is glabrous with serrate margin and acuminate apex. The micrograph performed on the powder has highlighted a number of characteristic elements namely: epidermal cells with starch granules, covering trichomes, lignified fibres, epidermal cells with trichomes, paracytic stomata, vessel elements are diagnostic substances for drugs of plant origin. These diagnostic elements are consistent with botanical standards and WHO guidelines [25-26]. The study of physicochemical parameters such as moisture content and ash values are useful as it determines the physiological and non-physiological state of ash, this will help to determine the possibility of microbial growth and lastly contaminant or impurities. The moisture content of the drug studied had a rate of 7.8 ± 0,1, which is below 10%. This result comply with the standards established by the International Pharmacopoeia, because this water content rate, prevent oxidation reactions, fermentation and give less chance to microbial growth and contamination in drugs [27]. Therefore, for proper conservation of drugs made from the leaves of *F. inermis*, it would be desirable to use those whose water content is less than or equal to 10%.The determination of total ash gave us a rate of 9.66 ± 0.03. This value indicates the level of minerals in drugs. Insoluble ash in hydrochloric acid gave a rate of 1.00 ± 0.02. Indeed, the ash insoluble in hydrochloric acid tells us about the contamination of the drug by siliceous elements [28]. This result is in agreement with Srikanth *et al.* [29] who found rate of 0.97% and 0.5% respectively. The maximum extractive value was found in chloroform (16.56%), followed by Ethanol (15.12%), Aqueous (13.28.%). All the extracts of the drug was subjected to different tests for detecting the presence of various phytoconstituents present in the drug, which revealed the presence of sterols,, flavanoids, alkaloids, saponin, proteins, carbohydrate, and tannins. Preliminary phytochemical analysis indicated a high percentage of quercetine and flavonoids and this may be one of the reasons behind the sedative activity of the plant. These parameters, which are being reported for the first time in this plant, are significant towards establishing the pharmacognostic standards for future identification and authentication of genuine plant material. Though, it is a highly reputed drug used in Ayurveda. Barring the anatomical details and preliminary phytochemical screening, rest of the pharmacognostical parameters, gives us the clue that it can be cashed economically as well to improve the standard of health in the developing countries.

Conclusion

WHO has emphasized the need to ensure quality control of the raw materials used for Ayurvedic medicines by using modern techniques and by applying suitable parameters and standards. In the present study various standardization parameters such as macroscopy, microscopy (histochemical and powder), physicochemical standards, preliminary phytochemical investigation, which are being reported for the first time in this plant and could be helpful in authentication and preparation of a suitable monograph for the proper identification of *F. inermis* for the future.

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