

PHARMACOGNOSTIC STUDIES OF THE LEAF OF

Ficus benghalensis LINN



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Abstract: Pharmacognostic studies of fresh and powdered leaves of F. benghalensis (Linn.) was carried out to determine the macroscopical features, preliminary phytochemical diagnostic features, physico-chemical properties, quantitative and qualitative microscopical features and chemo-microscopical measures were established. The morphology of the leaves revealed they are simple, alternate, glossy, leathery and glabrous when mature, oval, ovate or elliptical to oblong, base cordate or rounded, thickly coriaceous, obtusely cuspidate, quite entire, basal veins strong. The microscopy reveals the dorsiventral type of leave, with actinocytic stomata, crystal sheath type of calcium oxalate. The quantitative microscopy features such as stomatal number, stomatal index, veinlet termination number, veinislet number and palisade ratio were measured. The physico-chemical properties such as moisture content, total ash value, water soluble ash value, acid soluble ash value, alcohol soluble extractive value, water soluble extractive value were established. The chemo-microscopical analysis revealed the presence of Cellulose, lignin, Suberin/Cutin, aleurone grains, Calcium carbonate, calcium oxalate crystals, Inulin, tannins and the absence of Mucilage and starch. Phytochemical diagnostic features were also established. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant. Keywords: Ficus benghalensis Linn., Moraceae, pharmacognostic studies, preliminary phytochemical screening

Introduction

Ficus benghalensis Linn. commonly known as Indian Banyan or Bengal fig (Gopukumar and Praseetha, 2015) belongs to the family moraceae.Moraceae family is monoecious or dioecious trees, shrubs, lianas, or rarely herbs comprising 40 genera and 1,000 species, nearly all with milky sap. Moraceae or the mulberry family of the rose order (Rosales) are evergreen, distributed mostly in tropical and subtropical regions. Ficus is the largest genus from Moraceae family. It is used in treatment of various diseases such as biliousness, ulcers, erysipelas, vomiting, vaginal complains, fever, inflammations and leprosy (Varanasi, 2007). The latex is used as aphrodisiac, tonic, vulnerary, maturant, lessens inflammations, useful in piles, gonorrhea, neuralgia, rheumatism, lumbago bruises, ulorrhagia, ulitis, odontopathy, hemorrhoids, gonorrhea, cracks of the sole and skin diseases (Ahmad et al., 2011). The leaf buds were act as astringent and leaves infusion is given in diarrhea and dysentery. People prefer F. benghalensis leaves in treatment of various disease such as in ulcers, leprosy, allergic conditions of skin and various types of burning sensations.

Some of the published studies that were conducted on *F. benghalensis* include antihelmintic activity (Aswar *et al.*, 2008), antioxidant activity (Gupta and Sharma, 2010), antidiabetic and ameliorative activity (Mahalingam and Krishnan, 2008) and analgesic/antipyretic activity (Vikas *et al.*, 2010). Most of the studies on this plant were focused on the biological activity; no much attention was given on the establishment of certain pharmacognostic parameters of the leaf hence the need for this work. The aim and objective of this study is to determine some pharmacognostic standards of the leaf of *F. benghalensis*.

Materials and Methods

Plant material

The fresh leaves of the plant *F. benghalensis* were collected from Maigana, Soba Local Government Area of Kaduna State. The plant was identified and confirmed by a Taxonomist at the Herbarium Unit of National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna State, a voucher specimenwas preserved at the Unit Herbarium Library (No. 1027). The leaf was dried under shade, powdered, sieved, weighed and stored in airtight container and subsequently referred to as powdered leaf of *F*. *benghalensis*

Macroscopy

The leaves part was separated from other parts, washed, cleaned and dried for further use. The following macroscopic characters of the fresh leaves were noted: color, odor, taste, size and shape, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina and texture (Evans, 2009).

Microscopy

The free hand thin transverse sections of the fresh leaves through the lamina and the midrib were observed for microscopiccharacteristic. The quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein-islet number and vein let termination number were carried out (Evans, 2009).

Physicochemical investigations

The dried powdered leaf material was used for the determination of moisture content, ash values, water and acid soluble ash values and extractive values. The methanolic leaf extract (extracted using cold maceration) was used for preliminary phytochemical investigation. The chemo-microscopicalexamination of the cleared powdered leaf with chemical reagents were also studied using the method outlined in Evans (2009) and WHO (2011).

Results and Discussion

Macroscopy

The leaves were simple, alternate, spiral; stipules lateral, 2-2.5 cm long, sheathing, white-puberulous, deciduous, leaving annular scar, glandular at apex below; lamina 10-20 x 5-12.5 cm, ovate, base round or subcordate, apex obtuse, margin entire, coriaceous, glabrescent above, minutely pubescent beneath; 3-7-ribbed from base, lateral nerves 4-6 pairs, pinnate, prominent, intercostae reticulate, prominent; odourless and taste is slightly bitter.

Microscopy

Qualitative leaf microscopy

The microscopic examination of the fresh leaf and section of the leaf reveals the following diagnostic features; the calcium oxalate crystals observed were in the form of crystal sheath arranged along the midrib vein (Plate I). Polygonal epidermal

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cells were observed (Plate II). The stomata observed were found at the abaxial layer of the leaf – typical of a dicotyledonous plant. Actinocytic type of stomata was observed (Plate II)

The transverse section through the midrib of the leaf of *F*. *benghalensis* shows the following diagnostic features; the vascular bundle (xylem and phloem) are markedly differentiated which are characteristics features of dicotyledonous plants (Plate IV). Other features include the bundle sheath which is observed surrounding the vascular bundle and between the palisade parenchyma and the spongy parenchyma both of whichare toward the adaxial and abaxial epidermis, respectively (Plate IV).

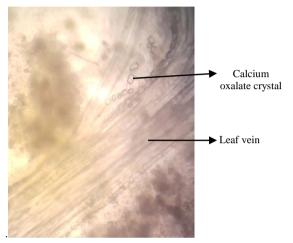


Plate I: Calcium oxalate crystal arranged along the midrib vein (X10).

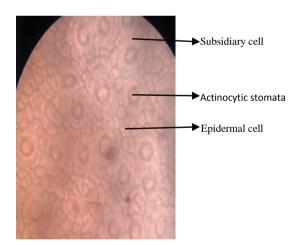


Plate II:Shows stomata, subsidiary cell, epidermal cell and intercellular space (X10)

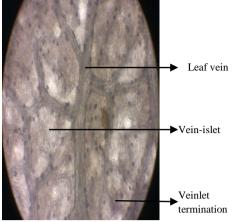


Plate III:Shows the presence of vein, veinlet termination and vein-islet (X10).

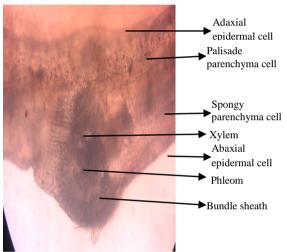


Plate IV: Transverse Section of *F. benghalensis* leaf (X10).

Quantitative leaf microscopy

The result of the average stomatal number, veinlet termination number and veinislet number for upper and lower epidermis were determined under camera lucida (Plate II and III). The results were shown in Table 1;

Ficusbenghalensis is currently being used in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in Herbal Pharmacopoeia, pharmacognostic parameters and standards must be established. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant. The fresh leaf of Ficusbenghalensiswas cleared and evaluated and actinocytic type of stomata was found, at the abaxial epidermal layer whereas no stomata was found at the adaxial epidermal layer. Crystal sheath type of calcium oxalate crystals were found arranged along the midrib veins. The bundle sheath was found between the adaxial and abaxial epidermal layers. The bundle sheath surrounds the vascular bundle (xylem and phloem tissues).

The physical constant reveals a moisture content value of $9.93 \pm 0.07\%$ which is within 8 - 14% general requirements of moisture content in a crude drug (African Pharmacopeia, 1986). Excess moisture content in crude drug may result in the breakdown of important constituents or may lead to the growth of yeast and fungi during storage. The value of moisture content obtained in this study which is $9.93 \pm 0.02\%$

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shows that the chance of this crude drug to be degraded by micro-organism is minimal. In this respect the evaluation of moisture content in setting standard for crude drug is very important (Brain and Tuner, 1975). Total ash value of $11.00 \pm$ 0.06%, water soluble ash value of $14.90 \pm 0.10\%$, acid soluble ash value of $13.60 \pm 0.15\%$, alcohol soluble extractive value of 1.56 ± 0.02 and water soluble extractive value of $2.28 \pm$ 0.09 can all be used to check purity of the crude drug (Table 2). Therefore, the evaluation of physical constants can afford the detection of adulteration of drug.

Numerical standards

The result of the average moisture content, total ash value, water insoluble, acid insoluble, acohol extractive value and water extractive value is presented in Table 2.

Table 1: Quantitative leaf microscopy					
S/N	Parameter	Value (Mean ± SEM)			
1.	Stomatal number	4.8 ± 2.00			
2.	Stomatal index	10.75 ± 4.15			
3.	Veinlet termination number	8.25 ± 4.00			
4.	Vein islet number	11.25 ± 6.00			
5.	Palisade ratio	$10.19{\pm}~7.55$			
SEM	- Standard Error of Mean: Nu	$rac{1}{r}$ mber of count – five (5)			

SEM = Standard Error of Mean; Number of count = five (5).

Table 2: Numerical standards of the powdered leaf of F. benghalensis

Parameter	Value Obtained (%w/w) ± SEM
1. Moisture content	9.93 ± 0.07
2. Total ash value	11.00 ± 0.06
3. Water –insoluble ash value	14.90 ± 0.10
Acid- insoluble ash value	13.60 ± 0.15
5. Alcohol- soluble extractive value	1.56 ± 0.02
6. Water- soluble extractive value	2.28 ± 0.09
SEM - Standard Error of Maan, Num	abor of counts - 5

SEM = Standard Error of Mean: Number of counts = 5

The alcohol soluble extractive value of $1.56 \pm 0.02\%$ shows that the constituents of the plant leaves are slightly soluble in alcohol while water soluble extractive value of 2.28 ± 0.09 is more soluble than that of alcohol soluble extractive value. The alcohol soluble extractive value and water soluble extractive value are meant to detect exhausted and already utilized drug which could be fraudulently used as substitutes and adulterants (Elujoba, 1999).

The total ash value represents the amount of the residual substance not volatilized on continuous heating until moisture - free and completely charred. Total ash value is particularly significant in the evaluation of the purity of the drug and depends largely on the absence of foreign organic matter. Direct contamination such as by sand or earth is immediately detected by the ash value. The total ash of any material, normally consist of inorganic mixture of metallic salt and silica (Danmalam, 2000).

Chemomicroscopical examination

The chemo-microscopical features identified were cellulose cell wall, lignin, cutine/suberine, inulin, aleurone grain, alkaloids and calcium oxalate crystals (cell inclusions), tannins, and calcium carbonate (cell constituents). They are represented in Table 3.

Preliminary phytochemical screening

The result of the preliminary phytochemical screening is shown in the Table 4.

Table 3: Chemo-microscopical analysis of the powdered leaf of F. benghalensis

Constituents tested	Observation	Inference	
Cellulose	Blue stained colour	+	
Lignin	Cherry- red colour	+	
Suberin/ Cutin Orange-red colour		+	
Aleurone grains Yellowish-brown colour		+	
Calcium carbonate	Shinning materials not scattered and deformed	+	
Calcium oxalate crystals	Scattered and deformed shinning materials	+	
Inulin	Brownish-red aggregation of crystals	+	
Mucilage	No change	-	
Starch	No change	-	
Tannins	Bluish- black colouration	+	

+ = presence; - = absence

Table 4: Phytochemical constituents of the powdered leaf of F. benghalensis

Constituents	Test	Observation	Inference
Anthraquinones	Borntrager's test	A bright pink colour	+
	Combined Anthraquinone	A cherry red colour	+
Saponin	Frothing test	Frothing which persist >30 min	+
	Haemolytic Test	Haemolysis of red blood cells	+
Steroid/Triterpenes	Salkowski's test	Cherry-red color	+
•	Lieberman Burchard'stest	Red color ring	+
Tannins	Ferric Chloride test	Greenish-black precipitate	+
	Lead subacetate test	Yellowcoloration	+
Carbohydrate	Molisch test	Reddish colour at interphase	+
-	Fehling test	Brick red precipitate	+
Alkaloids	Wagner's test	No change	-
	Mayer's test	No change	-
	Dragendoff's test	A reddish brown precipitate	+
	Picric acid test	A yellow colouration	+
Cardiac	Keller-kiliani's test	Purple-brown ring at interphase	+
glycosides	Kedde's test	Purple-blue colour	+
Flavonoid	Sodium hydroxide test	Yellow coloration	+
	Ferric Chloride test	Green precipitate	+
	Shinoda's test	A red coloration	+

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The chemo-microscopical evaluation of the leaf of *F*. *benghalensis* revealed the presence of the following; Cellulose, Lignin, Suberin/Cutin, Aleurone grains, Calcium carbonate, Calcium oxalate crystals, Inulin, Tannins while Mucilage and Starch were both absent (Table 3).

The macroscopic, microscopic, physical constants and chemomicroscopic evaluations provide a number of information that can be of importance in preparation of a monograph of the plant. The leaves of *F. benghalensis* have diagnostic potentials in treatment of diseases. *F. benghalensis* contains secondary metabolites which has potentials in treatment of ailments.

Conclusion

The pharmacognostic studies of *Ficusbenghalensis*yielded a set of qualitative and quantitative parameters that are useful in ascertaining the identity of the plant and to determine the quality and purity of the drug materials for future studies. This study has provided additional information that is useful in preparation of the monograph of the plant.

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