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Full Length Research Paper

Phytochemical and Pharmacognostic Analysis of *Ficus thonningii* Blume Leaves for Monograph Development

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Abstract

The plant Ficus thonningii Blume is used in ethnomedicine for the treatment of ailments like diarrhoea, dysentery, diabetes mellitus, gonorrhoea, etc. For proper identification and authentication of the plant leaves mostly used in herbal medicine, a phytochemical and pharmacognostic study was undertaken. Macroscopic examination of the leaves showed they are simple, glossy, dark green, thin and papery or slightly leathery. The leaves are elliptic or obovate, sometimes rather elongated or slightly oblanceolate. Chemo-microscopy revealed the presence of lignin, tannin, protein, and oval and round shape starch grains. Oils glands were observed on parenchymatous cells. Microscopic examination of the leaf powder revealed straight-walled epidermal cells with thick cuticle. Palisade cells and group of fibers and spiral vessels were seen. A few unicellular trichomes were also seen. Prisms of calcium oxalate crystals were observed. The phytochemical screening revealed the presence of carbohydrate, saponin, tannin, and flavonoid. Proximate analysis showed a total ash value of 10.57 %, acid-insoluble ash value of 0.80%, water-soluble ash value of 6.34%, water-extractive value of 1.37% and alcohol-extractive value was 1.72 %. The findings of this study would be useful in developing a monograph for the plant.

Key words: *Chemo-microscopy, Ficus thonningii Blume, Microscopy, Phytochemistry.*

Introduction

The World Health Organisation (WHO) has estimated that about 80% of the world's population rely mostly on traditional medicine (Akerele, 1988). The importance of the study of substances obtained from plants and animals, as well as from other substances of natural origin cannot be overemphasized. Many orthodox drugs used in therapy are obtained or conceptualized from plants sources (Evans, 2002). The WHO's goal of "Health for all" would not be met without the contribution of herbal medicine. These studies are even more important in third world countries like Nigeria where the economic condition necessitates that a lot of the people depend on locally collected and prepared medications due to limited access to orthodox healthcare or low financial capability.In the African sub-region, there is availability of a vast number of naturally occurring medicinal plants. The people of this region depend mostly on these plants since they can be accessed quickly and are affordable.

Ficus thonningii Blume, synonyms *F. acrocarpa* (Miq.) *F. dekdekena* (Miq.) A. Rich, *F. eriocarpa* Warb, *F. hochstetteri* (Miq.) A. Rich, *F. natalensis* Hochst, being found abundantly in Africa, is used in traditional medicine to treat a lot of ailments. *F. thonningii* Blume, (Figure 1) is an evergreen tree 6-21 m tall, with a rounded to spreading and dense crown. In English it is known as *bark-cloth fig* or *common wild fig* or *strangler fig* while in Hausa it is called *chediya* and in Yoruba it is called *odan*. It belongs to the family *Moraceae* of the order Rosales. The generic name is the classical Latin name for the cultivated fig derived from the Persian word *'fica'*. The species is widely distributed in upland forest, open grassland, riverine and rocky areas and sometimes in the savannah. It occurs naturally from the Democratic Republic of Congo and Tanzania in the north to the Eastern Cape in South Africa. Trees are relatively drought resistant. Flowers are unisexual, pollinated by small wasps, which develop in some of the flowers and live symbiotically inside the syconium. Its durability is low, and it is easily attacked by termites. A considerable amount of useful latex is produced by the tree.The leaves are used in combination with other herbs in the treatment of malaria. They are also believed to have analgesic and anti-inflammatory properties (Otimenyin et al., 2004).The bark is used in treating colds, sore throat, dysentery, wounds, constipation, nose-bleeding and to stimulate lactation. Latex is used as an antipyretic while an infusion of the root and fibre is taken orally to help prevent abortion. Powdered root is taken in porridge to stop nosebleed, the milky latex is dropped into the eye to treat cataracts.The plant is also used to treat epilepsy, diarrhea, gonorrhea and diabetes mellitus (Adebayo and Osman, 2012; Coker et al., 2009).

Although the use of herbal remedies in general and *F. thonningii* in particular is on the rise, there still exits the concern and uncertainty about the quality, safety and efficacy of these remedies. There is also the problem of incorrect diagnosis, imprecise dosage, low hygiene standards, the secrecy of some healing methods and the absence of records about patients (Kunle et al., 2012). There are also concerns over the lack of regulation of herbal medicines in many countries and the ineffective monitoring and control of the sale of unregistered products (De Smet, 1995). The problem of efficacy may arise from dearth of data on

chemical constituents and biological activities, which may vary with species, seasonal changes and geographical differences (Kunle and Egharevba, 2012). The macro-economic study of plants is targeted at achieving best practices in the cultivation, harvesting, preservation and processing. But pharmacognostic studies which involve macroscopic and microscopic morphological studies, and proximate analysis such as total-ash value, water and alcohol extractive values, pesticides and herbicide levels, etc., are necessary for proper identification and authentication of the plant for safe use, consumption and the establishment of standards for quality control of the plant or plant product and development of monographs (Kunle et al., 2012).

Unfortunately, not much is found in literature on the pharmacognosy and chemistryof *F. thonningii* Blume. The pharmacognostic profiling of the plant could be of great advantage in herbal medicine development. This study is aimed at determining the pharmacognostic and phytochemical parameters with a view of generating standards for future reference and monograph.

Fig 1: *Ficus thonningii* Blume

Materials and Method

Materials

All reagent used were of Analar grade and procured from Zayo-Sigma Limited, Nigeria.

Plant's collection and preparation

Ficus thonningii Blume leaves were collected in Nasarawa-Gwom, Jos North Local Government Area of Plateau state, Nigeria, and identified in the Department of Horticulture and Landscape Technology, Federal School of Forestry, Jos, Plateau State, Nigeria. A voucher specimen was deposited at the Herbarium, Department of Pharmacognosy, University of Jos. The fresh leaves were air-dried at room temperature for two weeks and reduced to moderately coarse powder using an electric blender. The powdered material was stored in sample bottles until required.

Phytochemical Screening

The phytochemicalscreening was carried out according to the methods outlined in Evans (2002), Sofowora (1993), Trease and Evans (1989) and Sofowora and Adebiyi (1978). Briefly, the procedures include:

Chemo-Microscopic Examination

The powdered leaf was treated with the appropriate chemical reagents and observed under the microscope for the presence of substances such as cellulose, lignin, mucilage, tannins, oils, starch and calcium oxalate crystals.

Test for Lignin

The powdered leaf was mounted in a little quantity of phloroglucinol, allowed to dry and concentrated hydrochloric acid added. The slide was viewed under the microscope. Pinkish/red stained structures indicate the presence of lignin.

Test for Tannins

The powdered leaf was mounted in a little quantity of ferric chloride solution and viewed under the microscope. A bluish-black or green colour indicates the presence of tannins.

Test for Starch

The powdered leaf was mounted in small quantity of N/50 iodine and viewed under the microscope. Blue-black stained structures indicate the presence of starch.

Test for Calcium Oxalate Crystals

The powdered leaf was mounted in chloral hydrate solution and observed for bright structures of calcium oxalate crystals. After this, a few drops of dilute sulphuric acid were added, observed for effervescence and viewed under the microscope. The disappearance (dissolution) of calcium oxalate crystals, without effervescence, confirms their presence.

Test for Fats and Oils

A small quantity of the powder was mounted with Sudan III and viewed under the microscope. Pink stained structures indicate the presence of oils. A small quantity of the powder was put on a filter paper and the paper squeezed to observe for translucent stains indicating the presence of oil.

Test for Proteins

A small quantity of the leaf powder was mounted with Million's reagent, warmed and viewed under the microscope. Pink stained structures indicate the presence of protein

Proximate Analyses

Determination of Total Ash Value

Six porcelain crucibles were heated at 105°C to a constant weight and their weights were noted after cooling in a desiccator. 2 g of the leaf powder were accurately weighed and poured into each crucible. Each crucible and its contents were then heated in a muffle furnace at a temperature of 405° C until it was moisture free and completely charred. The muffle furnace temperature was increased to 600° C for 2 hrs until most of the carbon was combusted and volatilized.The ash was cooled, weighed and the weight noted. The heating and cooling was continued until the weight of the ash was constant. The weight of the ash was calculated for each crucible by subtracting the weight of the crucibles from the final weights of crucibles and ash. The total ash value was calculated as a percentage of the weight of the ground leaf powder used.

Determination of Acid-Insoluble Ash Value

The ash obtained from the experiment above was transferred into a 100 ml beaker containing 25 ml of dilute hydrochloric acid. The beaker and its content were boiled for 5 minutes on a water bath and filtered through an ashless filter paper. The beaker was washed with distilled water and the washing was passed through the same ashless filter paper. The washing was repeated three times and on each occasion, the washings were passed through the ashless filter paper. The weight of a clean and heated porcelain crucible was accurately determined using a sensitive balance. The filter paper with its residue was folded into a small cone and transferred into the weighed crucible. The crucible was gently heated until the filter paper was completely ashed, then heated strongly for a few minutes in the muffle furnace. The crucible was cooled and weighed and the final weight was noted. The weight of the residual ash was then calculated. This was done by subtracting the constant weight of the crucible from the constant weight of the crucible and ash. The acid-insoluble ash value, with reference to the initial weight of the leaf powder, was calculated and expressed in percentage. The weight of the ash divided by the initial weight of the sample multiplied by100 was taken as the acid-insoluble ash value. (British Pharmacopoeia, 1980).

Determination of Water-Soluble Ash Value

The procedure described above in acid-insoluble ash value was repeated for the leaf powder but using 25 ml of distilled water instead of dilute hydrochloric acid. The weight of the water-soluble ash was determined by subtracting the weight of the residual ash from the total ash obtained in the experiment above that was initially dissolved in water. The water-soluble ash was then calculated andexpressed as a percentage.

Determination of Alcohol-Soluble Extractive Value

5 g of the powder were weighed into 250 ml stoppered conical flask. 100 ml of 90% ethanol were added into the conical flask and the stopper was replaced firmly. Theflask was shaken on a mechanical shaker for 6 hours and then allowed to stand for 18 hours. The extract was filtered by suction filtration and poured into the weighed evaporating dish and evaporated to dryness. The dry residue obtained was weighed andthe alcohol extractive value was calculated with reference tothe initial weight of the powdered leaf and expressed as a percentage (British Pharmacopoeia, 1980).

Determination of Water-Soluble Extractive Value

The above procedure was repeated using 0.25% chloroform water instead of 90% ethanol as the extracting solvent. The water-soluble extractive value was similarly calculated as in the above procedure (British Pharmacopoeia, 1980).

Qualitative macroscopic and microscopic investigation

The macroscopic description of matured fresh leaves was done according to terms outlined by Wallis (1995) to observe margin, apex, insertion, veination and other important characteristics of the leaves. The epidermal strips, anatomical sections and powdered samples of the leaves were used for microscopic investigation as outlined in African Pharmacopoeia (1986). The chemo-microscopy was conducted on anatomical sections and powdered samples of the leaves. A small quantity of the powder was cleared in dilute choral hydrate, mounted in dilute glycerol and viewed under the microscope.

Results

The results of phytochemical screening, proximate analysis, and qualitative microscopic investigation are shown in Tables 1, 2 and 3 respectively. The results of chemo-microscopy are shown in Table 4 and that of microscopic investigation are schematically represented in Figures 2a-8.

Table 1: Results of Phytochemical screening

Test	Method	Inference
Carbohydrates	Molisch's test	+
	Barfoed's test	_
	Fehling's test for reducing sugars	_
	Fehling test for combined reducing sugars	_
	Resorcinol test	_
	Phoroglucinol test	+
	Iodine test	+
Saponins	Froth test	+

Tannins	Ferric chloride test	+
Cardiac Glycoside	Keler-killiani test	_
	Kedde's test	_
Cyanogenic Glycoside	Picrate paper	_
Anthraquinones	Bontragger's test	_
Alkaloids	Mayer's	_
	Hager's	_
	Dragendorff's	_
	Wagner's what of	_
	10% tannic acid	_
Steroids	Burchard's test	_
	Salkowski's test	_
Flavonoids	Lead sub-acetate	+
	Sodium hydroxide test	+
	Shinoda's test	+

Key: - absent, + present

Table 2: Results of Proximate analysis

Parameter	Value
Total ash value	10.57 ± 0.24
Acid-insoluble ash value	0.80 ± 0.18
Water-soluble ash value	4.48 ± 0.47
Alcohol-soluble extractive value	1.72 ± 0.04
Water-soluble extractive value	1.37 ± 0.09

Table 3: Qualitative macroscopic and microscopic investigation of leaf

Investigation	Character	Observation
Macroscopic	Apex	Acuminate
	Margin	Entire
	Leaf base	Symmetrical
	Outline of lamina	Lanceolate
	Veination	Reticulate
	Petiole	Long (3.5 cm), glabrous, cylindrical and green
	Insertion	Alternate (simple)
	Upper and lower surface	green, glabrous, dotted (only upper surface)
	Texture	Slightly leathery and smooth
Organoleptic	Colour	Green
	Taste	Tasteless
	Odour	characteristic
	Texture	Slightly leathery and smooth
Microscopic Studies	Epidemis	A layer of which is straight walled with thick cuticle and palisade cells (Figure 2a).
	Parenchymatous cells	Large parenchymatous cells are seen showing attached oil glands and calcium oxalate crystals (Figures 3a & 4). Parenchyma with seriated borders were also observed (Figure 2b).
	fibres and spiral vessels	Group of fibres and spiral vessels were also seen (Figures 2a, 5a, 5b).
	Trichomes	A few unicellular trichomes were seen (Figure 6)
	Starch grains	Starch grains were present with most being oval and round in shape.
	prism sheaths	There were prism sheaths containing lots of calcium oxalate crystals (Figures 3a & 4).
	Stomata	Upper epidermal layer showed straight walls and no stomata (Figure 7), while lower epidermal layer showed straight wall and numerous stomata what type of stomata (Figure 8)

Table 4: Results of Chemo-microscopy

Test	Observation	Inference
Lignin	Pinkish/reddish stained structures in fibres and conducting vessels	Present
Tannins	Bluish black stained structures Seen on virtually all the structures	Present
Protein	Yellowish stained structures on fibres	Present
Starch	Few grains of bluish-black stained structures	Present
Calcium Oxalate Crystal	Bright Crystal structures mostly on fibres	Present
Oils	Pink in any structure Seen all over especially in parenchymatous cells	Present

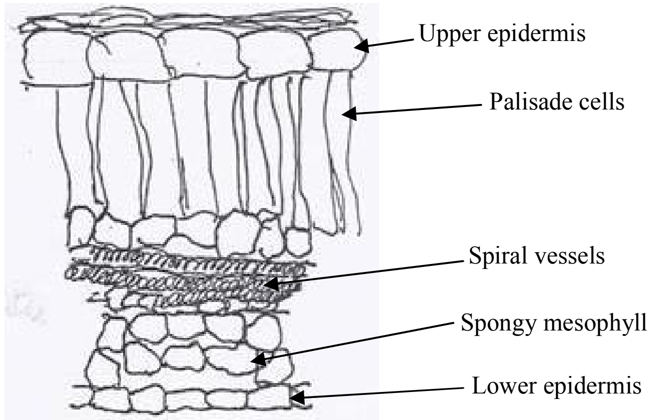

Fig 2a: A schematic diagram of the microscopic view of the leaf of *F. thonningii* showing upper epidermis, Palisade cells, spiral vessels,

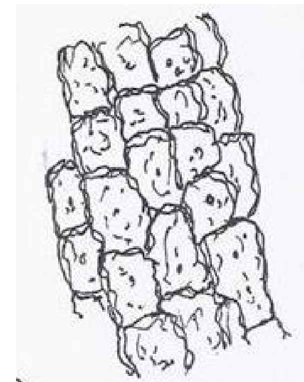

Fig 2b: Parenchyma with seriated borders

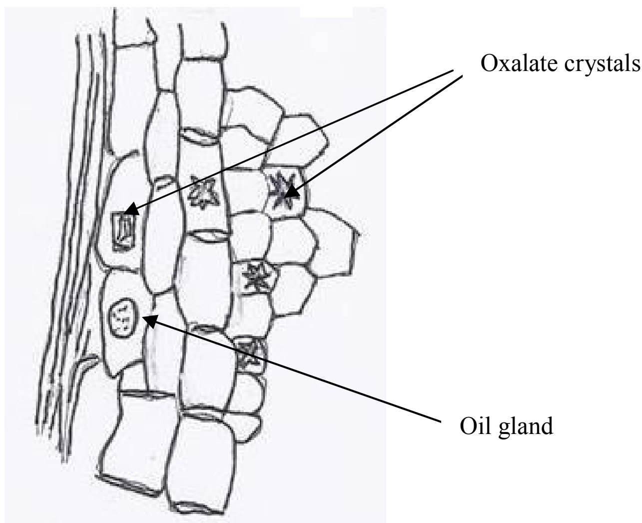

Fig 3a: Large parenchymatous cells with calcium oxalate crystals and oil glands

Fig 3b: Starch granules

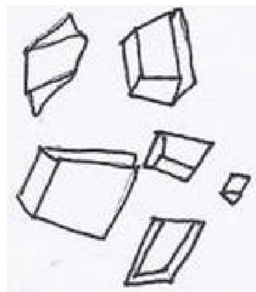
Fig 4: Oxalate prisms

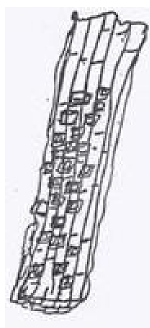
Fig 5a: Fibre

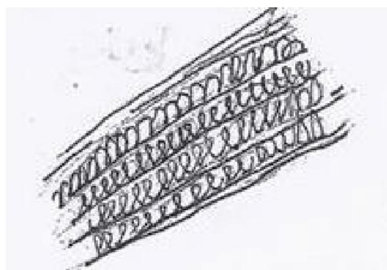
Fig 5b: Spiral bundles

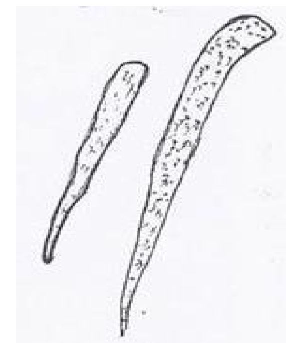
Fig 6: Unicellular trichomes

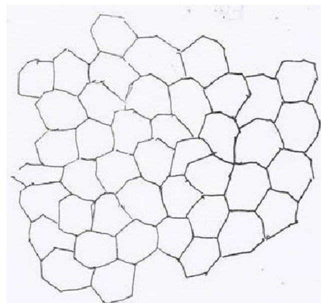
Fig 7: Upper epidermis with no stomata

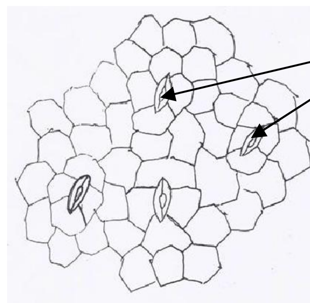

Fig 8: Lower epidermis with numerous stomata

Discussion

The results of the phytochemical screening (Table 1) revealed that carbohydrates, saponins, tannins, and flavonoids were presence, while alkaloids, cyanogenetic glycosides, cardiac glycosides, anthraquinones and steroids were absent. The plant may be good as an astringent since tannin and saponins containing drugs have been used for its styptic property. Tannin and saponin possess astringent and antibiotic properties and are known to exert inhibitory effects on many enzymes (Huzaifa *et al*., 2014; Evans, 2002).*F. thonningii* Blume may also possess anti-inflammatory, anti-allergic, anti-diabetic and vaso-protective activity as the plant is rich in flavonoids and flavonoids are known for their anti-inflammatory, anti-allergic, antidiabetic and vaso-protective properties. Many flavonoid-containing plants are diuretic or antispasmodic. Some have anti-tumour and antibacterial activities (Evans, 2002). Saponins are also astrigents and antiseptics and therefore the plant may have good anti-bacterial activity (Huzaifa *et al*., 2014). The presence of carbohydrate may explain why the leaves are readily being eaten by animals for energy. The presence of some of these metabolites may be responsible for some of its ethnomedicinal use.

Chemo-microscopy revealed the presence of lignin which was seen on fibres and the conducting vessels and confirmed the presence of tannin which was widely distributed all over the different structures. Proteins occurred mostly on the fibres. The presence of a few starch grains whichwere seen mostly as oval and round shape also confirmed the presence of carbohydrate in the phytochemical screening. Calcium oxalate crystals were seen mostly on fibres. Oils were observed on parachymatous cells.

The total ash value was determined as 10.57 ± 0.24 % the acid insoluble ash value was determined as 0.80 ± 0.18 % while the water soluble ash value was determined as 4.48 ± 0.47 %. These ash values can serve as standards for *F. thonningii* Blume and could be useful in determining contaminated products. The low acid insoluble ash value is suggestive of the high absorbability/bioavailability of the mineral salts in the plant if eaten by animals.The water extractive value was determined to be 1.37 ± 0.09 % and this was lower than that of alcohol extractive value that was 1.72 ± 0.04 %. This indicated that alcohol is a more suitable solvent for extraction of the leaf powder. These values can also be used to detect adulterant in a product. (British Pharmacopoeia, 1980).

Conclusion

The findings of this study suggest the plant holds great potential for use as herbal drug since it contains very important secondary metabolites like flavonoids and saponins. These metabolites may be responsible for the observed activities and use in ethnomedicine. Hence they could be source of new drug lead/hit. The results would also serve towards generating standard for the plant which would be useful in developing a monograph for the plant.

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