

**INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY,
BIOLOGY AND CHEMISTRY****Research Article****Comparative Pharmacognostic Study of *Ficus abutilifolia*
Miq. (Moraceae) Plant Leaf, Stem bark, and Root****Cletus Anes UKWUBILE**Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences,
Ahmadu Bello University Zaria, Nigeria.**ABSTRACT**

Comparative microscopical features were carried out on the leaves, stem barks and roots of *Ficus abutilifolia* with a view to determining some parameters that would help in the identification of the plant. Results obtained showed that the plant has covering trichome of unicellular and uniseriate stalks, acute apex and swollen base on both surfaces of the leaf. Diacytic stomata were in the range of 22-23.3-25 mm² and 31-34.1-38mm² upper and lower surfaces respectively. Stomata index were 12.5-13.24-14.2% and 22.4-23.80-26% on the upper and lower epidermis respectively. Vein islet and vein termination numbers were 12-14-16 mm² and 20-21-22 mm² respectively. Palisade ratios were found to be 8-9.4-12 mm² and 4.2-7.5-10 mm² upper and lower epidermis respectively at n = 10. Cellulose and hemicelluloses were observed in the stem barks and roots powders, cutin and suberin, lignin and starch were noticed in all the parts at different locations. Calcium oxalate crystals were twin prisms of 4um small and 18um large occurring in the brachy sclereids of the stem barks and parenchymatous cell of the leaf. Calcium carbonates were observed with effervescence in the upper epidermal cells in all the parts. Single and crossed fibres with acute apex, lumen and lignified walls were observed in the stem bark powder as well as cork cells. The xylem vessel showed both spiral and annular thickenings in the stem barks and roots powders. The study showed that using the above features, the plant can easily be identified and differentiated from closely related species.

Keywords: Comparative, Pharmacognostic, Trichome, Stomata, Calcium oxalate, powders.

INTRODUCTION

Ficus abutilifolia belongs to one of the largest families of the angiosperms. Collectively they are regarded as figs, and produced sap, leaves and flower buds (Ijeh and Ukwani, 2007). Most of them are found growing very well on rocks, bush veldts, swamps and on hard surfaces (Abbiw, 1990). They are mainly trees of range 21-50m high (Aluka, 2008). Flowers are produced between August and February of each year, and these flowers are borne inside the plant, which is unique among the fig (Bouquet, 1969), and propagated by seeds. The plant is distributed in South Africa, Mozambique, Zimbabwe, Botswana, Nigeria, and Sudan (Berg, 1992). Chemically, the plant contains mainly glycosides such as saponins, flavonoids, anthraquinones as well as alkaloids and tannins (Ukwubile and Nuhu, 2010). Antibacterial activities of ethanol extract of leaves had been reported against *Salmonella typhi*, *Shigella dysenteriae*, and *Staphylococcus aureus* (Ukwubile and Nuhu, 2010). Traditionally, the plant had been used to treat various diseases in Nigeria such as typhoid fever, dysentery, food poison and STIs.

This study was aimed at comparing the various microscopical features of the morphological parts with a view to help in the identification of the plant from closely related species.

MATERIALS AND METHODS**Collection and Preparation of Plant Materials**

Three morphological parts of the plant were collected- leaves, stem barks and roots, and identified by Mr. U.S Gallah of the Department of Biological Sciences, Ahmadu Bello University Zaria, where a voucher number of 900742 was deposited for the plant. Freshly collected leaves were kept for determining leaf surface data while others were air-dried alongside other parts. They were grinded using mechanical blender into fine powders, and then stored for further usage.

Chemo microscopy of Powdered Drugs

Chemo microscopy of the powdered drugs were carried out in order to determine the presence of the following cell wall materials like cellulose, hemicelluloses, suberin, cutin, gums and mucilage, lignin as well as cell inclusions/features like starch, calcium oxalate crystals, calcium carbonate, fats and oils, and tannins, were determined using appropriate reagents.

Determination of cell wall materials**Cellulose and hemicelluloses**

0.5 g of the powdered leaves, stem barks and roots were each stained in N/50 iodine solution and observed; A blue colour confirmed the presence of hemicelluloses, and when treated with 80 % (w/w) H₂SO₄ blue colour confirmed cellulose. The powdered drugs were also treated with ammoniacal solution of copper II oxide and dilute H₂SO₄, cellulose is precipitated (Evans, 2006).

Suberin and cutin

0.5 g of the powdered drugs was each treated with chloral-zinc-iodine. A yellow to brown colour indicates suberin or cutin. On staining the powdered drugs with potash, suberin and cutin are stained yellow, but warming suberin with a 20 % solution of potash, yellowish droplets exude; cutin is more resistant (Evans, 2006).

Gums and Mucilage

0.5 g of powdered drugs were each stained with ruthenium red and treated with few drops of lead acetate solution. Mucilage was stained pink.

Lignin

0.5 g of the powdered drugs was mount in 1 % phloroglucinol in 90 % ethanol with HCl. Presence of red colour was taken to indicate the presence of lignin substance.

Determination of cell inclusions**Starch**

0.5g of the powdered drugs was each stained in solution of N/50 iodine, a blue –black colour indicate the presence of starch.

Calcium oxalate crystals

0.5 g of the powdered drugs was each mount in glycerine solution and viewed in a polarised light. Presence of crystals indicates calcium oxalate.

Calcium carbonates (CaCO₃)

0.5g of the powdered drugs were mount in HCl solution, the presence of effervescence indicates CaCO₃

Fats/oils

0.5 g of the powdered drugs was each mount in Sudan IV solution and heat lightly. The presence of red colour was taken for the presence of fats/oils.

Tannins

Two drops of ferric chloride solution were added to 0.5 g of the powdered drugs on white tie and mixed with glass rod and then viewed. Green colour confirmed tannins.

Examination of epidermal characters of leaves

The fresh leaf of the plant was nicked with razor blade followed by peeling of the epidermis with finger on both upper and lower surfaces. The material was mounted in one drop alcohol and one drop dilute glycerol and observed starting with the scanning objective. Features observed were recorded as well as drawn in laboratory note book, with the aid of camera Lucida and pencil.

Quantitative microscopy of *F. abutilifolia*.

The quantitative microscopy of the plant was done using freshly prepared leaves in order to determine the following leaf surface data, and using the methods adopted from Kokate (1993) as well as Evans (2006): stomatal number, stomatal index, Vein-islet and vein let termination numbers and palisade ratio. A total of ten readings were taken and values recorded as means of the original values.

Determination of stomatal number and stomatal index

Stomatal number is defined as the average number of stomata per sq mm of epidermis of the leaf while stomatal index is the percentage which the number of stomata form to the total number of epidermal cells, each stoma being counted as one cell. Briefly,

a fragment of leaf from the middle lamina was cleared by boiling in chloral hydrate solution. The upper and lower epidermis of the leaf were peeled using forceps, and mount separately in glycerine water. A square 2 x 2 mm was drawn using stage micrometer and camera Lucida on drawing paper, and the stage micrometer was replaced by the cleared leaf preparation and focused. The number of the stomata and epidermal cells were counted (two guard cells and ostiole being counted as one) within the square. The stomatal index was calculated using the formula:

$$S.I = \frac{S}{E + S} \times 100$$

Where S.I = stomatal index, E = epidermal cells, and S = number of stomata.

Determination of vein-islet and vein let termination numbers

Vein-islet number is defined as the number of vein islets per sq mm of the leaf surface midway between the midrib and the margin. A vein termination on the other hand is the ultimate free termination of vein let. Briefly, a leaf sample was boiled in chloral hydrate solution in a test tube placed in boiling water bath until it was cleared. It was then mounted in glycerine water. A camera Lucida was set up to divide the paper into 2 sq mm by means of the stage micrometer. The stage micrometer was then replaced by the cleared leaf preparation, and the veins were traced in a square of 2mm x 2mm, following the superimposition of image of the leaf portion on paper. The numbers of vein – islets and vein lets termination present within the square were counted and the total numbers of each were divided by four to get the value in square mm. A total of ten sets of counts were made.

Determination of palisade ratio

Palisade ratio is defined as the average number of palisade cells beneath each epidermal cell. Scrapings from each of the leaf surface were cleared by boiling with chloral hydrate solution and mounted in glycerine water and then focused under high power (x40) magnification. A camera Lucida was attached and the outlines of four continuous epidermal cells were traced and marked using pencil. Palisade cells within each group were counted including those that were more than half covered by the epidermal cells. The values obtained from the counts were divided by four to obtain the palisade ratio.

RESULTS**Chemo microscopy and Quantitative Microscopy**

Cell wall indicates the presence of the following materials: cellulose, hemicelluloses; at the cell wall layers, cutin; at the cuticles of the three parts and cork cells of the stem barks and roots powders, suberin; at the cuticle of the parts. The plant leaf shows some macroscopic features (Table 1). Gums and mucilage as well as lignin were noticed in the leaves and stem barks as well the root powders at various locations (Tables 1 and 2). The powdered parts of the plant also revealed the presence of starch in all the parts with 25 µm large and 6 µm small (6-25 µm diameter), with hilum at the eccentric position. Starches are simple grains located in the parenchymatous cells. They are spherical in shape and many in numbers. Calcium oxalate crystals are in the form of twin prisms with sizes 18 µm large and 4 µm small, and are rare in occurrence, and also occurring with calcium carbonates found in upper epidermal cells and outgrowths. They are located in the parenchyma of leaves and stem bark powders as well as in the sclereids of the stem bark (Table 4).

Table 1: Macroscopical examination of *F. abutilifolia*

Morphology	Features	Observed
Leaves :	Petiole:	3 – 5cm long
	Lamina:	
	Composition:	Simple
	Venation:	Reticulate
	Margin:	Entire
	Apex:	Acute
	Base:	Cordate
	Surface:	Smooth
	Outline:	Ovate
	Surface:	Pubescent
	Texture:	Brittle (dry), papery (fresh)
Size:	12cm – 16cm length 10cm – 13cm width	

	Odour:	Slight unpleasant
	Taste:	Bitter
Stem bark :	Shape:	Curved
	Outer surface:	Lichens, cork and whitish
	Inner surface:	Brownish
	Fracture:	Short (outer), fibrous (inner)
	Transverse surface:	LE – MR – CK
	Odour:	Slightly unpleasant
	Taste:	Neutral
Roots :	Kind:	Tap
	Size:	-
	Shape:	Cylindrical:
	Surface:	Cracks:
	Fracture:	Short
	Transverse section:	No pith, contains fibres
LE = Lignified elements, MR = Medullary rays, CK = Cork, - = not determined		

Table 2: Cell wall materials of *F. abutilifolia*

Cell wall materials	Test	Observation	Conclusion/location
<i>Cellulose/Hemicelluloses</i>	N/50 iodine	Blue colour	Cell wall layer of
	N/50 iodine + 80% w/w H ₂ SO ₄	Blue colour	(R,S)
	Ammoniacal CuO	Cellulose ppt.	Cellulose is stained(L,R,S)
<i>Cutin</i>	Chlor-zinc-iodine	Yellow to Brown colour	Cuticle (L,R,S), cork cell (SR)
<i>Suberin</i>	Chlor-zinc-iodine	Yellow to Brown	Cuticle (L,R,S)
<i>Gums/Mucilage</i>	Ruthenium red	Pink colour	Below cuticle (L,SR,)pith
<i>Lignin</i>	Pgnl + HCl	Red colour	Endodermal cells (L,S,R)

L (Leaf), S (Stem) and R (Root), Pgnl (Phloroglucinol), CuO (Copper II oxide)

Table 3: Examination of cell inclusions of *F. abutilifolia* powdered parts

Description	Starch	COX	CaCO ₃	Fats/oils	Tannins
Shape :	Spherical	Twin prisms	-	-	-
Size :	25 µm (Large)	18 µm (Large)	-	-	-
	6 µm (small)	4 µm (Small)	-	-	-
Hilium :	Eccentric	-	-	-	-
Striation :	None	-	-	-	-
Frequency:	Numerous	Rare	-	-	-
Aggregation:	Simple grains	-	-	-	-
Location :	Cortex, Parenchyma	Parenchyma (LS) ,	-	-	Vacuole
	(LRS)	Sclereids (S)	ue	-	-

- = not applicable or not detected, L (Leaves), S (Stem barks) and R (Roots), COX (Calcium oxalate), CaCO₃ (Calcium carbonates); present in the upper epidermal cells (ue).

In the quantitative microscopical features (Table 4) of the leaves, the diacytic stomata were found to be more in number on the abaxial than the adaxial surface with means of 34.1 ± 0.52 and 23.3 ± 0.16 respectively, and were attached with covering trichomes 25 µm width and 40 µm long (Figure 1; Table 5) with apex acute and swollen base (Figure 2). However, the palisade ratio of the adaxial surface was more with a mean of 9.4 ± 0.30 as

against 7.5 ± 0.15 of the abaxial surface (Table 4). Calcium oxalate crystals are in the parenchymatous cells, and calcium carbonates in upper epidermal cells of the plant (Table 3). The stem barks have medullary rays crossed with fibres (Figure 4 a₁ and a₂) as well as Para tracheal parenchyma below the xylem vessels (Figure 4 c and d). Figure iv a, b, and e are sclereids, cork cells, as well as annular and spiral thickenings of the xylem vessel in the stem barks respectively, as well as pericyclic fibres while plate i is the brachy sclereids from the root powder. Some fibres are also single while others are crossed in the stem barks powders.

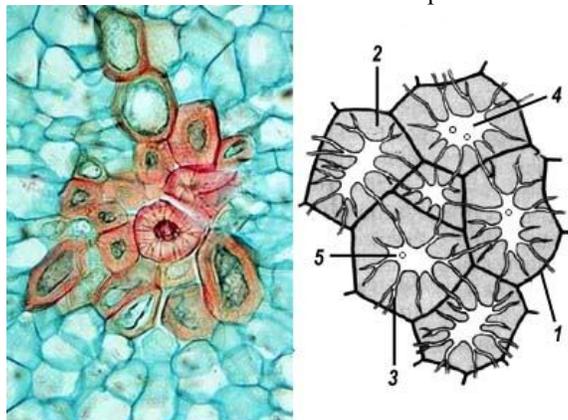


Plate 1: Brachy sclereids of the powdered roots of *F. abutilifolia*; 1; primary wall, 2; secondary wall, 3; ramified pit, 4; cell lumen, 5; cross-sectioned pit, x 400.

Table 4: Quantitative microscopical features of the Leaf of *Ficus abutilifolia* Miq.

Parameter (mm ²)	Upper Surface	Lower Surface
	Range	Range
SN	22-23.3-25	31-34.1-38
SI	12.5 - 13.24 - 14.2 %	22.4- 23.80 -26 %
VIN	12- 14 -16	-
VTN	20 - 21 -22	-
PR	8 - 9.4 -12	4.2- 7.5 -10

Key: SN (stomatal number), SI (stomatal index), VIN (vein islet number), VTN (vein termination number), PR (palisade ratio), numbers in bold are means of original values at n = 10, - = not applicable.

Table 5: Epidermal characters of *F. abutilifolia* fresh leaf

Description	Characters observed		
	Cells	Stomata	Trichome
Shape :	Elongated	-	-
Anticline walls:	slightly wavy (U,L)	-	-
Thickenings :	Beaded	-	-
Papillae :	Absent	-	-
Cuticle :	Striated	-	-
Type :	-	Diacytic	Covering
Frequency:	-	Rare (U) Numerous(L)	Numerous (U)
Position :	-	U, L	U
Form:	-	-	Uniseriate
Surface :	-	-	Smooth
Apex:	-	-	Acute
Base :	-	-	Swollen

U = Upper epidermis, L = Lower epidermis, - = not applicable.

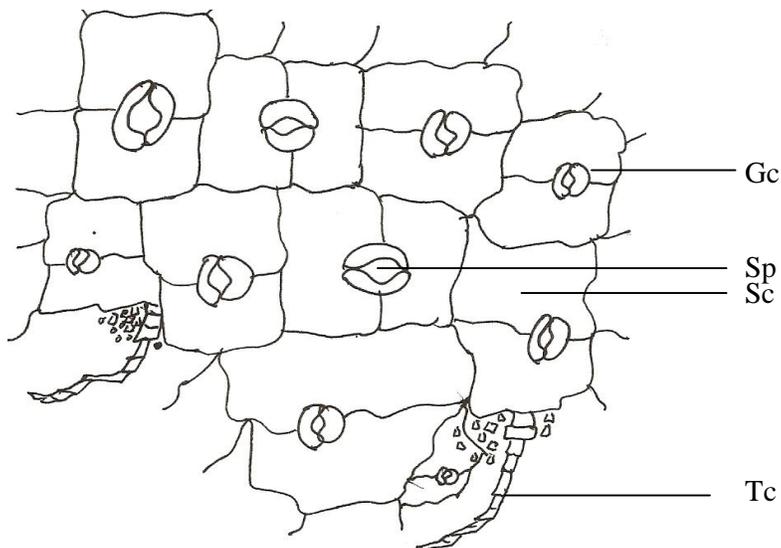


Figure 1: Diacytic stomata of *F. abutilifolia* x 400, Gc (guard cell), Sp (stomata pore), Sc (subsidiary cell), Tc (trichome).

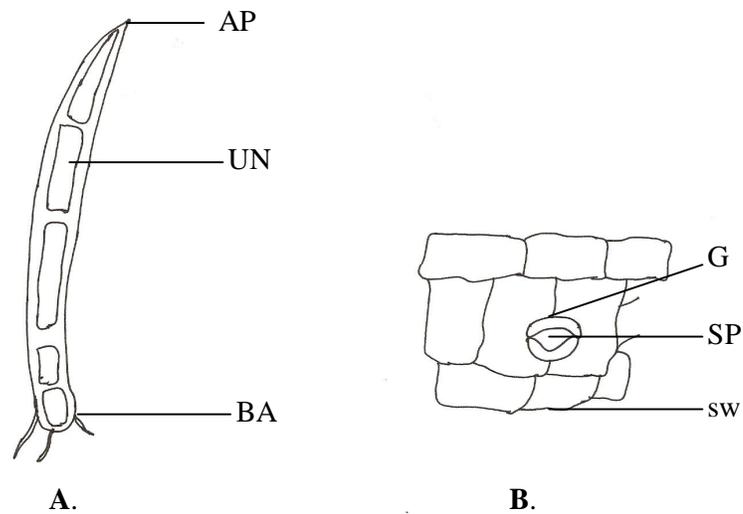


Figure 2: A; covering trichome, AP (apex), UN (uniseriate stalk), BA (swollen base), B; epidermal cell surface view, Gc (guard cell), SP (stomatal pore), sw (slightly wavy wall), X400.

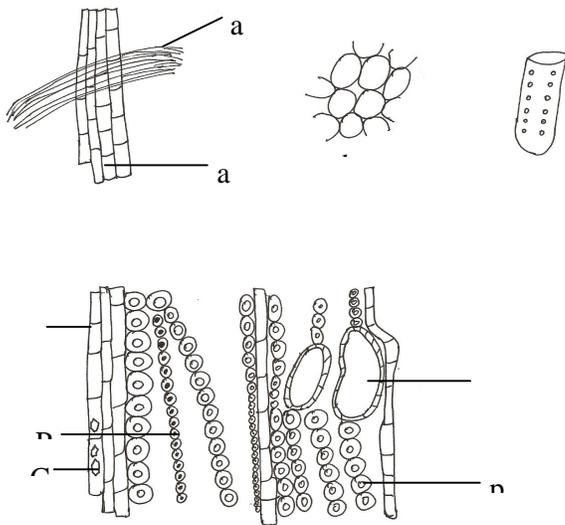


Figure 3: a; medullary rays and fibre (a₁; medullary rays, a₂; fibre), b; parenchymatous cells, c; vessel, d; TS powdered stem bark x 400, mr (medullary rays), pf (pericyclic fibre), Cox (calcium oxalate crystal), ve (vessel), pp (Para tracheal parenchyma).

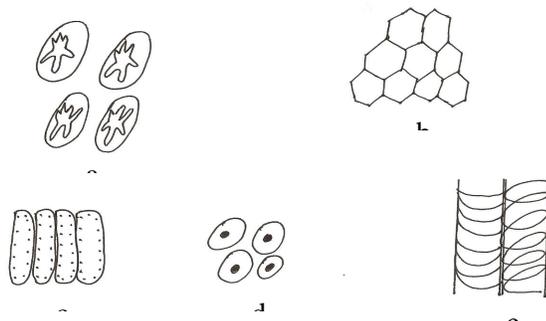


Fig. 4: Powder characteristics of the plant's parts a; sclereids, b; cork, c; palisade cells, d; pericyclic fibres, e; xylem vessels annular and spiral types.

The xylem vessel undergoes both spiral and annular thickenings to offer strength to the plant, the cork cells are hexagonal in shape and joined to each other, the palisade cells have barrel or cylindrical shapes, on both sides.

DISCUSSION

The chemo microscopy of the powdered drug revealed the absence of fats/oils in *Ficus abutilifolia* Miq and this support the work by Kumar and Singh (1984) when they reported that “the absence of fats/oils in this plant accounts for the widely use of the plant in traditional medicine”, since fats/oils intake in large quantities had been reported to offer some degree of resistance to drugs by pathogens (Ojalla *et al.*, 1999).

The study on the leaf revealed some important diagnostic features that would help in the identification of the plant, since there are close resemblances between *F. abutilifolia* and *F. capreifolia* (Potgieter and Vanwyk, 1999). The presence of starch in the powdered plant materials also offer important diagnostic tool in the identification of the plant. The starch contains helium at eccentric position and does not have striations. The walls of the granules are thick and isodiametric in all or part of the plant. By visual comparisons, they were more in number in the stem and the root than the leaves probably because of the time of collection of the plant. Because the green parts of plant contain small granules of transitional starch which are moved to storage organs during darkness (Evans, 2006). The position and form of helium and the presence or absence of well-defined striations are important in the characterization of starches (Evans, 2006). This attribute will help to differentiate the plant from other genera.

Suberin and cutin are water proof in the organ where they occur, and it is possible that these materials prevent the loss of water to the surrounding, ensuring that plant survive adverse conditions. The anticline walls are slightly wavy and not polygonal as was reported by Jayeola (1998), Juniper and Jeffree (1983), (Figure 1 b). This is an adaptation to reduce water lose from the stomata. The palisade cells are single layer that are found in the upper and lower surface (isobilateral), spongy parenchyma are five to six (5-6) layer cells, collenchymas are 4-6 layer cells occurring in both surfaces (Fig.3), and these are tendencies to survive water shortages in its environment (Shanahan *et al.*, 2001). The presence of fibres in the plant is of significant in providing strength to the plant against forces of wind since it is mainly a savannah plant, and it indicates that the cell is ageing, hence the xylem cells of the plant are spirally thickened at this stage accompanied by various depositions such as calcium oxalate crystals, corks cells, and sclereids as were seen in the powdered parts of the plant (Fig. 4). A cork cambium or phellogen usually arises which by its activity, produces new protective tissues, collectively called periderm, which replace the epidermis and part or the entire primary cortex (Fig. 4 b).

The cell wall is composed of inner and outer cellulose layers and median suberin lamella which may be lignified as in the case of acacia bark. Mature cork cell is dead, impermeable to water and often filled with tannins (Evans, 2006). The presence of cork cells in the powdered drugs may show adulteration or low quality or improperly peeled drug; with cinnamon, ginger and liquorice as typical examples. The formation of cork puts out the action of the stomatal apparatus leading to the formation of lenticels (Ashutosh, 2007). The cork cells protect the plant especially against mechanical injuries and water loses.

Sclereids or stone cells are sclerenchymatous cells approximately isodiametrical in shape with lignified thick walls, well-marked stratification and traversed by pit-canals. This will help in microscopical identification of the stem bark as well as the root from other related species (Fig.4 a, plate 1). These brachy sclereids also give strength to the plant in its habitat. Calcium oxalate is usually present in about 1 % in plants but may exceed 20 % in rhubarb (Polygonaceae) rhizome in dry weight. Single and twin prisms had been reported in henbane (Evans, 2006) and they often occur in cells which differ in size, form or content (idioblasts) from those surrounding them (Evans, 2006). The presence of twin prisms form of calcium oxalate in this plant is of great diagnostic feature for the plant.

Calcium carbonates may be found embedded in or incrustated in the cell walls. Concretions of calcium carbonates formed on the outgrowths of the cell wall such as trichomes are called cystoliths; occurring in the families Moraceae, Urticaceae, Cannabinaceae and Acanthaceae and some Combretaceae and Boraginaceae (Evans, 2006). This may explain the presence of calcium carbonates in *Ficus abutilifolia*. Thus, the plant can serve as an indicator of the presence of this mineral (limestone) in the soil where they grow. The diacytic stomata of *F. abutilifolia* are deeply sunken (Fig. 1) and this is an adaptation to survive drought. These stomata were distributed more in numbers on the lower surface of the leaf epidermis. These help the plant to minimize the rate of transpiration through the leaves.

The taxonomic value of trichomes in angiosperms (Fig. 2 a) is well documented in botanical literature (Theobald *et al.*, 1979; Batterman and Lammers, 2004). In this present study, their presence or absence on the epidermal surfaces of *Ficus* species were found to be less informative taxonomically. The nature of the trichomes in the genus seems to be more reliable than their mere presence or absence. Three basic types of trichomes had been identified among the figs; uniseriate (stalked with flat plate), covering and glandular (Sonibare *et al.*, 2005). The leaf epidermis of *F. abutilifolia* showed that it contains, covering type of trichome which are both uniseriate and unicellular and it is possible that the plant respond to its environment in specific ways by modifying the basic plan of certain features to improve its adaptation. Trichomes serve various functions in plants such as chemical and physical protection of plants, maintenance of still air on leaf surfaces thus reducing water loss, and secretory of substances which contain pharmaceutical necessity like narcotic

resins in some genera. The function of trichome in this plant *F. abutilifolia* could be for the reduction of water loss from leaf surfaces as the plant grows on hard surfaces with only little water.

CONCLUSION

The study showed that these parameters provide in-depth microscopical features which also provide pharmacopoeia standards for easy identification of the plant hence, differentiating it from closely related species.

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