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Taxonomic Significance of Leaf Morpho-Anatomical Markers in Identifying *Ficus exasperata* Roxb., *Ficus mucuso* Welw. ex Ficalho and *Ficus thonningii* Blume in Nigeria †

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Abstract: Indigenous Ficus species are well noted for ethnomedicinal uses in Nigeria. Among the available species, *Ficus exasperata*, *Ficus mucuso* and *Ficus thonningii* are very germane to traditional herbal practitioners. Upon this basis, various parts such as leaves, barks and roots are frequently collected for medicinal purposes. Literature has shown that the identification of most of the Ficus species in Nigeria is based on the characterisation results generated by taxonomists several decades ago. Adequate and recent taxonomic evidence is needed for the discrimination and better understanding of the affinities among medicinally important and closely related species. Although macro morphological analysis has hitherto provided clues to differentiating the taxa by a common layman, however, combining it with leaf epidermal characters would produce a better predictive delimitation of the taxa. This study, therefore, investigated the Morpho-Anatomical markers for identifying *Ficus exasperata*, *Ficus mucuso* and *Ficus thonningii*. Leaves samples were collected from the lower canopy portion of the tree species located in the University of Ibadan Campus, Nigeria. Specimens were prepared, analysed for morphological and epidermal characters following standard methods. The result shows that *Ficus exasperata* and *Ficus mucuso* shared appreciable morphological similarities, which are taxonomically different from *Ficus thonningii*. In terms of the anatomical delimitation, *F. mucuso* was differentiated from the other two species by having actinocytic stomata on the abaxial layer of the leaf. Whereas, *F. exasperata* lacks stomata on the adaxial layer. Epidermal cell shape is polygonal for all the species while the trichome present is stellate, foliform and papillary respectively for *F. exasperata, F. mucuso* and *F. thonningii* on the adaxial layer. Based on the identified taxonomic evidence observed in this study, leaf anatomical markers could provide discriminatory information for the taxa even when the plants are in fragmentary forms.

Keywords: Epidermal characters; morphological traits; Ficus species; medicinal plants

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1. Introduction

The genus *Ficus* belongs to the family moraceae. Ficus comprises about 1000 species distributed to the tropics and temperate regions [1]. The genus is economically important most especially because of their medicinal values occasioned by the richness in secondary metabolites such as flavonoids stilbenes, triterpenoids and xanthones [2]. According to [3], the species in the gnus have been found effective for Homoeopathy, Ayurveda, Unani and Siddha traditional system of indigenous medicine. Preparation of the combination of parts such as leaves, barks, latex and fruits proved effective for the treatment of haemorrhoids, diabetes, stomachache, skin diseases, diarrhoea, dysentery and ulcers [3]. In addition, the preparation is equally very useful as anti-cancer agents, carminative, anti-oxidant, anti-inflammatory and astringent. In another sets of studies [4-7], *Ficus* species were

used for the treatment of pile, tuberculosis, ulcer, hypertension, asthma, diarrhoea, diabetes, stomach ache and constipation in southwestern Nigeria.

Specifically, available findings on *Ficus exasperata* Vahl. show that its leaves are a good source of the antipyretic, antiulcer, anti-inflammatory, hypotensive, hypolipidemic and hypoglycemic agent [8-9]. It was also specified that the leaves of the species are very good for pesticidal, antimicrobial, insecticidal and anticandidal activities [10-17]. According to [18], the leaves of *F. thonningii* are used as a remedy for stomach upset in the northern part of Nigeria. It was also mentioned by [19] that the leaves of *F. thonningii* can be utilized for the treatment of liver problems and disease conditions that are linked to jaundice.

Despite the ethnomedicinal importance, there still exists a problem of misidentification of the species, which have many implications on the effective and correct use for medicinal purpose. Although available taxonomic works [16-19] specified that Ficus are easily delimited by the fruit characteristics and that the minute unisexual flowers are usually arranged on shaped receptacles, there is a need for more robust markers for determining their identity. Most especially because flowers and fruits are not always present on the mother trees for identification. Even if the reproductive characters available, sometimes, medicinal samples are obtained from plants that are yet to reach flowering age. It has also been reported that morph-anatomical characters are not very stable for taxonomic purpose, but the molecular markers which ought to be used as an alternative are very expensive to be used by traditional persons that usually utilize the plants for medicinal use. It has been reported recently that epidermal markers are gene dependent. Therefore, this study investigated the possibility of delimiting the selected species of the genus using epidermal and petiole anatomical characters to provide useful information for their taxonomy.

2. Experiments

2.1. Leaf epidermal Characterisation

Leaf samples were collected from the lower canopy portion of the tree species located at the University of Ibadan Campus based on availability. The morpho-anatomical characterization was undertaken at the anatomical Laboratory of the Department of Botany, University of Ibadan. Specimen were obtained from the standard median portion of the matured leaves of each species, mainly the middle way between the apex and the base of the lamina which was done using scissors and transferred into labelled specimen bottles containing 50% ethanol for preservation [20]. The specimens were transferred into a petri dish containing water for rinsing, transferred into NaOH solution and left for about 3 days [21]. The purpose of this is to allow the NaOH solution to penetrate the leaf and separate the adaxial layer from the abaxial layer. After separating the two layers, the sections were stained in Safranin O for three minutes, after which it was rinsed in water to remove excess stain. Each section was mounted on the slide, with a drop of glycerine for preservation and protected with a coverslip. This was then viewed under the microscope for preliminary studies. In other to prevent air from getting to the specimen in the slide, neutral nail varnish was applied to seal the coverslip to the slide and was left for about 20 – 30 minutes to dry before arranging them into the slide box. Each slide was later viewed under a CIWA XSP-35TV biological microscope for studying [20]. Two slides were gotten from each layer of the epidermis. Attention was centred on some structures such as the trichrome, stomatal apparatus, crystals etc. the morphology of the trichome, shape of glands etc. were noted.

2.2. Petiole Analysis

This method was adopted from [22]. Petioles were collected from the lower canopy portion of the tree species. The transverse section of the petiole was carefully cut at 20μm thick using a Rotary microtome. The specimen (i.e. cut sections) were preserved in 50%

ethanol. The cut sections were then stained in 1% aqueous solution of Safranin O for three minutes, rinsed thoroughly in changes of water to remove excess stain. Thereafter, the specimen was mounted on the slide, with a drop of glycerine for preservation, protected with a coverslip. The slides were studied under a CIWA XSP-35TV biological microscope.

3. Results

Table 1 shows the leaf epidermal characteristics of *Ficus* species on the abaxial layer. Stomata were absent in *Ficus thonningii* but present in *Ficus exasperata* and *Ficus mucuso.* Meanwhile, anomocytic and actinocytic type of stomata, which is multidirectional was found on the abaxial layer of *F. exasperata* and *F. mucuso* respectively. However, stomata were more abundant in *F. exasperata* than *F. mucuso.*

Epidermal cell alignment and shape were respectively anticlinal and polygonal. Cell wall type was slightly wavy to wavy in *F. exasperata,* straight to wavy *F. mucuso* and slightly sinuous to sinuous in *Ficus thonningii.* Cristal and trichome were present and not variable in types on the abaxial epidermal layer of the three species.

Table 1. The abaxial layer of the leaf epidermal characteristics of the *Ficus* species.

S/N	Characters	*F. exasperata*	*F. mucuso*	*F. thonningii*
1	Stomata (P/A)	Present	Present	Absent
2	Stomata type	Anomocytic	Actinocytic	Nil
3	Stomata direction	Multidirectional	Multidirectional	Nil
4	Stomata abundance	Many	Few	Nil
5	Cell wall alignment	Anticlinal	Anticlinal	Anticlinal
6	Cell shape	Polygonal	Polygonal	Polygonal
7	Cell wall type	Slightly wavy to wavy	Straight to wavy	Slightly sinuous to sinuous
8	Trichome (P/A)	Present	Present	Present
9	Trichome types	Foliform	Foliform	Foliform
10	Crystal (P/A)	Present	Present	Present
11	Crystal type	Druses	Druses	Druses

The epidermal characteristics of the adaxial (i.e. the upper part of the leaf) for the *Ficus* species are indicated in Table 2. Stomata were absent in *Ficus exasperata* but present in *Ficus mucuso* and *Ficus thonningii.* Similar to the abaxial layer, *F. mucuso* was characterized by actinocytic stomata while *F. thonningii* was having anomocytic stomata. Stomata were multidirectional in both *Ficus mucuso* and *F. thonningii* but few stomata were found in *F. thonningii* and abundant in *mucuso.* Cell wall alignment, cell shape, cell wall and crystal types were not variable for the species on the adaxial layer. Trichome and crystal were present in the three species. Stellate trichome was found in *F. exasperata,* while Foliform and Papillary trichome types were identified in *F. mucuso* and *F. thonningii* respectively.

Table 2. The adaxial layer of the leaf epidermal characteristics of the Ficus species.

S/N	Characters	*F. exasperata*	*F. mucuso*	*F. thonningii*
1	Stomata (P/A)	Absent	Present	Present
2	Stomata type	Nil	Actinocytic	Anomocytic
3	Stomata direction	Nil	Multidirectional	Multidirectional
4	Stomata abundance	Nil	Much	Few
5	Cell wall alignment	Anticlinal	Anticlinal	Anticlinal
6	Cell shape	Polygonal	Polygonal	Polygonal
7	Cell wall type	Slightly wavy to wavy	Slightly wavy to wavy	Slightly wavy to wavy
8	Trichome (P/A)	Present	Present	Present

9	Trichome types	Stellate	Foliform	Papillary
10	Crystal (P/A)	Present	Present	Present
11	Crystal type	Druses	Druses	Druses

The anatomical characteristics of the transverse section of the petiole of the species are shown in Table 3. Petiole shape varied among the *Ficus* species with *Ficus exasperata* having flat to convex shape while *Ficus mucuso* was flat-convex to concave-convex whereas, in *Ficus thonningii,* petiole shape was almost circular. Petiole trace pattern indicates, that there were many free traces in the ring for *F. exasperata* but in *F. mucuso,* it was single free traces in a ring. *F. thonningii* had multitrace in a ring. Papillary and foliform trichome was present on the petiole of *F. exasperata* while in *F. thonningii,* only papillary trichome was discovered but completely absent in *F. mucuso.* Crystals were present in the petiole of all the species, however, they were druses forms in *F. thonningii* and *F. mucuso*.

Table 3. Anatomical characteristics of the transverse section of the petiole of the Ficus species.

S/N	Charcters	*F. exasperata*	*F. mucuso*	*F. thonningii*
1	Petiole shape	Flat to convex	Flat-convex to concave-convex	Almost circular
2	Trace pattern	Many free traces in the ring	Single free traces in a ring	Multitrace in a ring
3	Trichome (P/A)	Present	Absent	Present
4	Trichome type	Papillary and foliform	Nil	Papillary
5	Crystal (P/A)	Present	Present	Present
6	Crystal type	Druses and Prisimatic form	Druses	Druses

4. Discussion

The implication of the differences observed based on the epidermal characteristics is that the markers could provide appreciable taxonomic delimitation among the *Ficus* species. Using the abaxial epidermal characters, *Ficus thonningii* can be discriminated against by the other species by its epistomatic nature. On the other hand, considering the epidermal features on the adaxial layer, *Ficus exasperata* is distanced from *F. muscuso* and *F. thonningii* by having hypostomatic leaves. There exist studies [23-28] in which the positioning of stomata on the epidermal layer was used to delimit the taxa of plants. However, there is presently scarce information according to the literature on the discrimination of *Ficus* species based on the positioning of stomata but there is replete information on other epidermal indices such as stomatal types and epidermal cell shape [29]. The present study aligns with [24], who distinguished some woody species as a result of the hypostomatic nature of the taxa.

The anticlinal cell alignment, polygonal cell shape and the presence of trichome identified in the three *Ficus* species imply that the characters would not be suitable for their description and taxonomic classification. Although, this uniformity in the characteristics on the other hand may be useful for the discrimination of Ficus from other members of moraceae family, which may not exhibit the traits.

The significant analysis of leaf of petiole anatomy of leaves and plant species is imperative since plant anatomy concerns the structure, content and development of cells and tissues [20, 22]. This is because of its importance to all aspects of research in plant sciences such as taxonomy, genetics, morphogenesis, physiology, ecology, evolution and reproduction. The microscopic description of a plant as used in this study is a veritable means towards establishing the identity of the *Ficus* species. Therefore, the petiole anatomical characterization employed may very much applicable, especially when only the morphological approach is not enough to exhibit natural delimitation in the taxa. Hence, the

variableness of petiole shape, trace pattern and existence of trichome vis-à-vis trichome type signifies taxonomic discrimination among the three *Ficus* species.

5. Conclusions

A detailed micro-anatomical study of leaf and petiole structures of the Nigerian Ficus species may provide an invaluable tool for the determination and identification of the three taxa studied, thereby assisting in promoting quality assurance in the genus most importantly in the ethnomedicinal application of the species. The morpho-anatomical characterization employed in this study has provided a reasonable description of the differences and affinities among the selected medicinal Ficus species to some extent. However, to achieve a more robust and natural classification, there is a need for molecular analysis on which the existing morpho-anatomical markers would serve as a baseline.

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