



LEAF EPIDERMAL STRUCTURES AND STOMATA ONTOGENY IN SOME MEMBERS OF THE LAMIACEAE FAMILY

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ABSTRACT

The aim of the research was to study the leaf epidermal structures and stomata ontogeny in some members of the family Lamiaceae. The species used in this study were *Vitex doniana*, *Gmelina arborea* and *Tectona grandis*. Leaf epidermal structures, stomata types, sizes, distribution and development were examined and to estimate stomata frequency, stomata index was also evaluated. The epidermal cells were polygonal in *Gmelina arborea*, rectangular and irregular in *Vitex doniana* and isodiametric and some are elongated in *Tectona grandis*. The stomata ontogeny was mesogenous in all the species. Types of stomata observed were anomocytic in *Vitex doniana*, anisocytic and anomocytic in *Gmelina arborea* and paracytic in *Tectona grandis*. For stomata distribution, *Gmelina arborea* was amphistomatic with very scanty stomata on adaxial epidermis, while both *Vitex doniana* and *Tectona grandis* were hypostomatic. The stomata index measured using micrometer and eye piece graticule (μm) were subjected to analysis of variance (ANOVA) at 5% level of significance. The result showed that there were no significant differences in stomata length and pore breadth in *Vitex doniana* and *Gmelina arborea*. However, there was significant difference in the number of epidermal cells and trichomes amongst the three species studied. Therefore, the assertion that three species be placed in the same genus should be rejected and thus recommends the earlier classification which separates the three species into different genera.

Keywords: Epidermal structures, Stomata ontogeny, Lamiaceae, leaf.

INTRODUCTION

Lamiaceae is a family of flowering plants belonging to the order Lamiales which is the assemblage of many families which include Acanthaceae, boraginaceae, bignoniaceae, verbenaceae etc. They are mostly annual or perennial herbs or shrubs with opposite leaves, when crushed; the foliage usually emits various pleasant odors. Stems usually square (Metcalfe, 1950). The Lamiaceae is a relatively commonly encountered family, especially in the temperate regions of the world. It is comprised of about 3500 species distributed among some 200 genera, most of which are herbaceous, less often shrubs, or rarely trees. Many of the species have domesticated and cultivated as ornamentals. Some are highly desirable cooking herbs or flavor producers (e.g. *Mentha*, widely cultivated as a commercial crop plant) (Yuan *et al.*, 2010). The Lamiaceae is one of the most readily recognized families of flowering plants, at least by the layman. Indeed, it can be fairly accurately stated that any herb or shrub having square stems, opposite leaves, and emitting a minty smell when crushed is likely to belong to the Lamiaceae. The family was for many years referred to as the Labiateae, in reference to the strongly bilabiate (2-lipped) flowers exhibited by most of its species. Modern workers tend to use the name Lamiaceae, as

recommended by the International Code of Botanical Nomenclature since consensus has it that each plant family should be based upon a legitimately established genus, in this case the genus *Lamium*. There is no genus "Labia", the latter name merely an ancient common name bestowed upon members of the family by numerous early workers. Many workers including Cantino (1992) believed that the Lamiaceae are closely related to the family Verbenaceae, so much so that elements of the verbenaceae have been transferred into the Lamiaceae, although technically, the Verbenaceae can be said to have entire ovaries, usually rounded stems which, when crushed, lack minty smells. Early workers, because of its 4-lobed ovaries, thought the Lamiaceae might be closely related to the family Boraginaceae, but recent DNA studies (Olmstead *et al.*, 1992), suggest that the latter belongs elsewhere. Heywood (1978) gives a concise and well-illustrated overview of the family. Most species of this family in Texas are attractive roadside flowers, such as *Monarda* and *Salvia*. Correll *et al.* (1970) provided a technical treatment of the family for Texas, recognizing some 120 species in 31 genera, some of these introduced and well established roadside weeds.

Due to the advancement in classification, Lamiaceae is now the sixth largest angiosperm family, containing more than 7,000 species distributed all over the world.

However, although considerable progress has been made in the last two decades, its phylogenetic backbone has never been well resolved. Among the genera, the largest ones are: *Salvia* with 900 species, *Scutellaria* 360 species, *Stachys* with 300 species, *Plectranthus* 300 species, *Hyptis* 280 species, *Teucrium* 250 species, *Vitex* 250 species, *Thymus* 220 species, and *Nepeta* 200 species (Raymond, 2004). *Clerodendrum* was once a genus of over 400 species, but by 2010, it had been narrowed to about 150 (Yuan *et al.*, 2010). This has necessitated the need to conduct research into the family to validate the existing classification and therefore, the present study was conducted to determine the leaf epidermal structures and stomata ontogeny in some members of the Lamiaceae family in order to determine the taxonomic relationship existing among members of the family.

MATERIALS AND METHODS

Sample collection and Sampling method

Leaf samples were collected from Bayero University Kano new and old campus and Ministry of Environment Kano which are located between latitude 11°58'50" and 11°98'18", longitude 8°28'46"E and 8°48'01" and altitude 486.5m. The work was conducted at plant physiology laboratory, Department of Plant Biology, Bayero University Kano.

The epidermal peels were prepared according to the method of (Cutler, 1978). The epidermal peels of both adaxial (upper) and abaxial (lower) surfaces of the leaves were made by placing the leaves on a clean glass slab. The specimens were irrigated with distilled water. The epidermis was carefully scraped off using a sharp razor blade. Loosed cells were washed away using water and soft brush. The epidermal

peels were placed on a clean glass slide and stained with aqueous Safranin for 4-8 minutes, then rinsed with water to remove excess stain. A drop of 50% paraffin was added and then examined under microscope ×10 and ×40 objective magnification.

Stomata measurement

The stomata length, stomata breadth, pore length and pore breadth were measured using an eye piece graticule and stage micrometer, and the values were recorded. This was done according to standard procedure.

Stomata index was calculated using the formula:

$$\text{Stomata index} = \frac{\text{Number of stomata}}{\text{no. of epidermal cells} + \text{no. of stomata}} \times 100$$

RESULTS AND DISCUSSION

Epidermal cell and stomata ontogeny studies

The epidermal cells in *Gmelina arborea* were observed to be polygonal, rectangular and irregular in *Vitex doniana* and isodiametric and elongated in *Tectona grandis*. The stomata development in all the three species were mesogenous as shown in plate (1-4) that is, the guard cells and the subsidiary cells have common origin through asymmetrical division of the guard cell mother cell. The pattern of cell division during stomatal ontogeny in the epidermis as reported by Berger *et al.* (1998) includes an initial longitudinal division, followed by a transverse division. One of the two small cells eventually divides into two guard cells that separate around a stoma. In the mature stomata complexes multiple series of this pattern can reiterate to form larger stomatal complexes.

The findings were also in accordance with those of Daya (2012) who reported single layered epidermis with polygonal cells covered outside with thick walled cuticle, covering trichomes and anomocytic stomata in *Gmelina arborea*.

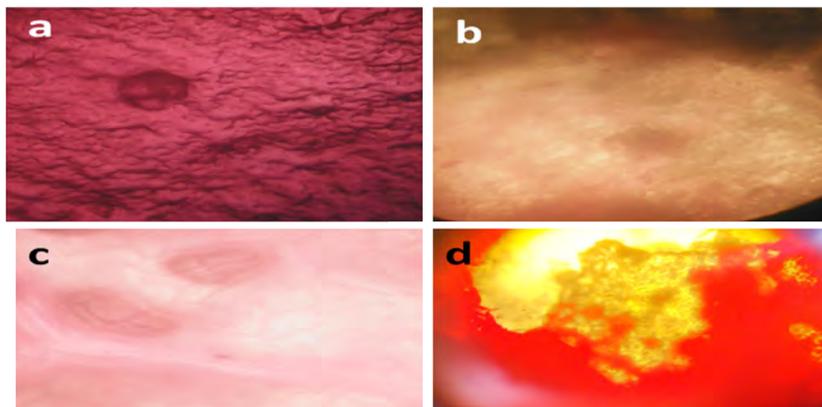


Plate 1: Stomata ontogeny of some members of the family Lamiaceae a. *Gmelina arborea*, adaxial view, b. *Tectona grandis* adaxial view c. *Vitex doniana* adaxial view d. *Gmelina arborea* abaxial view

However, the epidermal cells in *Vitex doniana* and *Tectona grandis* were rectangular or irregular, and isodiametric and or elongated respectively.

Stomata type and distribution

In the abaxial surface of *Gmelina arborea* two types of stomata were observed. Anomocytic type in which there were no subsidiary cells that differ from other epidermal cells. The second type was Anisocytic in which the subsidiary cells were three in number with two larger cells and the other one smaller in size. In *Vitex doniana* only Anomocytic stomata type was observed. In *Tectona grandis* the type of stomata observed was paracytic in which the subsidiary cells were two and are parallel to the long axis of the guard cell wall (Plate 5-8). The result for stomata distribution and types showed that two species (*Vitex doniana* and *Tectona grandis*) were observed to be hypostomatic, that is having stomata only on

the lower epidermis while one of the species (*Gmelina arborea*) was amphistomatic, having stomata on both lower and upper epidermis. At the upper surface no stomata was observed in *Vitex doniana* and *Tectona grandis*. This is in line with findings of Abdulrahman (2013) who also observed *Tectona grandis* to be hypostomatic. In the present study, *Gmelina arborea* was observed to have anisocytic stomata on the upper epidermis, while Abdulrahman (2013) however, reported that *Gmelina arborea* was amphistomatic with paracyticstomatal complex.

At the lower epidermis of all the species there was presence of stomata. *Gmelina arborea* was observed to have anomocytic and anisocytic types of stomata. Daya (2012) also reported anomocytic stomata in *Gmelina arborea*. Paracytic type of stomata was observed in *Tectona grandis* and this corresponds to the findings of Abdulrahman (2013).

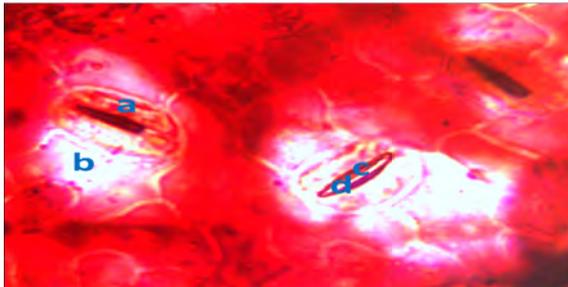


Plate 2: *Gmelina arborea* abaxial (lower) epidermis of leaf showing anomocytic stomata Mg×400. Key : a = guard cell, b = subsidiary cell, c = thick wall of guard cell d = pore

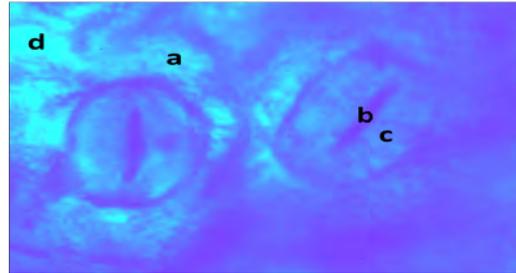


Plate 3: *Gmelina arborea* abaxial(lower) epidermis showing Anisocytic stomata. Mg×400. Key: a = subsidiary cell, b = pore, c = guard cell, d = epidermal cell

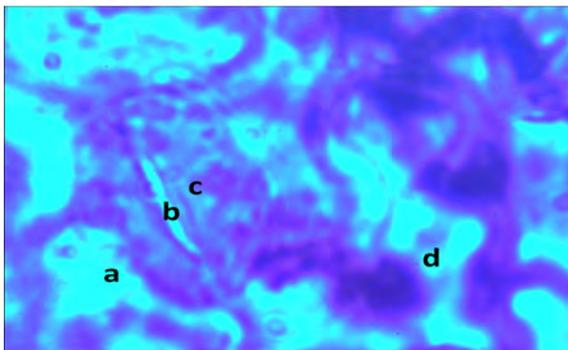


Plate 4: *Vitex doniana* abaxial (lower) epidermis of leaf showing Anomocytic type of stomata. Mg ×400 Key- a = subsidiary cell, b = pore, c = guard cell, d = epidermal cell

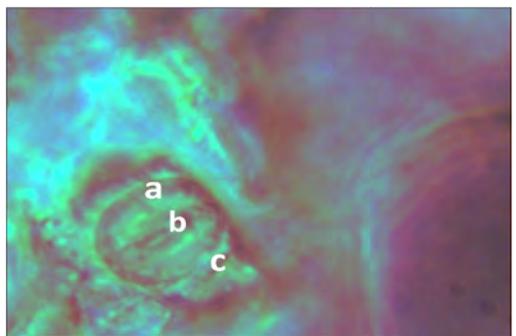


Plate 5: *Tectona grandis* lower epidermis of leaf showing paracytic type of stomata Mg× 400Key - c = subsidiary cell, b = pore, a = guard cell

Stomata measurements on lower epidermis

Table 1 showed the results of the stomata length, breadths and pore length in the leaf of three species of lamiaceae family. The results revealed that stomata lengths, breadths and pore lengths values in *Vitex doniana* and *Gmelina arborea* leaves was significantly higher at $P \leq 0.05$ when compared with what was recorded in *Tectona grandis*. In terms of stomata breadth *Vitex doniana* has significantly recorded highest values and the least values were recorded in *Tectona grandis*. Similar trend of stomata breadths was also observed in Pore lengths. Pore breadth, number of epidermal cells, number of stomata and stomata index were all significant in *Gmelina arborea* and

Vitex doniana at $P \leq 0.05$ when compared with *Tectona grandis* though it has recorded similar results with *Vitex doniana* for number of epidermal cells and stomata index. The report of Tahir and Rajpat (2009) showed that the size and shape of the stomata are taxonomically important characters and the stomata index could be valuable and very reliable in distinguishing some medicinal species (Olowokudejo, 1990). The findings of Ogundipe (2004) revealed that the combination of micro morphological characters such as stomata size epidermal cell size, veinlet termination and other epidermal characters can be used for the delimitation of species.

Table 1: Mean stomata measurements on lower epidermis of leaves in some members of Lamiaceae family measured using a micrometer graticule in μm , magnification $\times 400$.

Species	Stomata length	Stomata breadth	Pore length	Pore breadth	Number of epidermal cells	Number of stomata /field	Stomata index
<i>Gmelina arborea</i>	1.70a	1.19b	0.77b	0.23a	58.00a	9.70a	18.40a
<i>Tectona grandis</i>	1.06b	0.91c	0.66c	0.19b	27.70b	5.30b	15.81b
<i>Vitex doniana</i>	1.73a	1.41a	1.10a	0.23a	41.90ab	11.70a	16.70b
S.E	0.21	0.11	0.108	0.10	20.34	4.36	1.77
C.V	13.5	9.5	12.8	10.4	47.8	48.9	10.4
L.S.D. At 5%	0.19	0.10	0.10	0.07	18.66	4.00	1.63

Result for Trichomes

The type of trichomes in the species was shown in plate 6. *Gmelina arborea* lower epidermis had single uniseriate multicellular non glandular and long trichomes which measured $29.58 \mu\text{m}$ long and $1.45 \mu\text{m}$ wide. In *Tectona grandis* most of the trichomes were uniseriate, non-glandular, short, unicellular and unbranched with long trichomes measured up to $16.53 \mu\text{m}$ long, and short trichomes

measured $4.06 \mu\text{m}$ long and $1.45 \mu\text{m}$ wide while the branched trichomes were measured $20.3 \mu\text{m}$ long and $2.43 \mu\text{m}$ wide. Crystals were also observed on *Tectona grandis*. Hairs and papillae are collectively called trichomes and their occurrence and cellular structure are used extensively by the taxonomist as an aid to identification since there is such a wide range of form (Cutler, 1978).

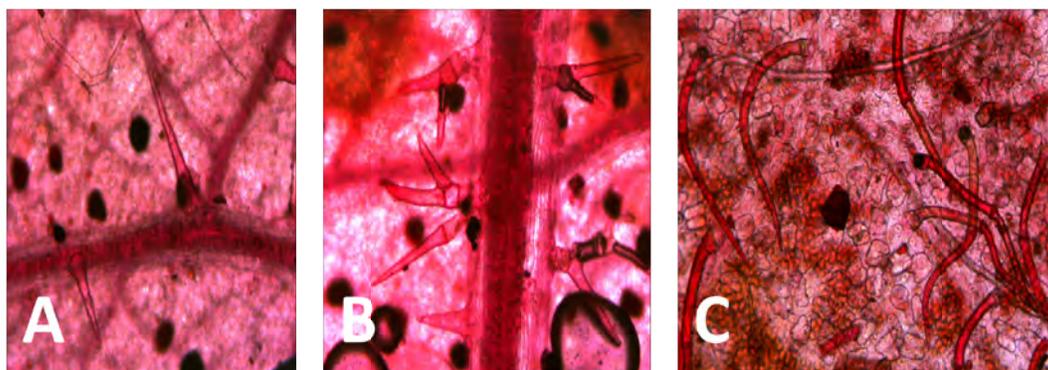


Plate 6: Trichome types in some members of lamiaceae family (a) Simple unicellular trichome *T. grandis* (b) Branched trichome *T. grandis* (c) Simple multicellular trichome on *G. arborea* leaf

CONCLUSION

Based on epidermal cells shapes and arrangement all the species varied. In terms of stomata ontogeny they all have mesogenous development. *Vitex doniana* and *Gmelina arborea* have similar anomocytic stomata, while *Tectona grandis* had paracytic stomata. *Gmelina arborea* was amphistomatic while *Vitex doniana* and *Tectona grandis* were hypostomatic. *Vitex doniana* and *Gmelina arborea* did not differ significantly in terms of stomata length, pore breadth, number of epidermal cells and number of stomata.

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Therefore based on this research, earlier Linnaeus classification that separate the plants into different genera has to be maintained because more differences exist than similarities in the members of the Lamiaceae family studied.

Recommendation

There is need for further research in order to solve classification issues, especially among closely related families. Molecular studies are also recommended so as to have a classification based on the genetic relationships among various plants.