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Taxonomic Implications of Leaf Epidermal Anatomy in Some Members of *Genus Quisqualis* L. and *Guiera senegalensis* J.F. GMEL

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ABSTRACT

The genus *Quisqualis* (Combretaceae) is used as an herbal medicine and genus *Guiera* (Combretaceae) distributed widely in Western Africa, and used in traditional medicine. Morphological studies of the leaf epidermis of some members of the genus *Quisqualis* L. and *Guiera senegalensis* J. F GMEL. are presented. Fresh and herbarium species were used. These specimens were fixed, peeled, and epidermal characters were identified and micrographed. The study revealed the leaf epidermal characters such as pattern of epidermal cells, types of stomata, and presence of trichomes which are constant in some species and variable in others, and thus of great significance in understanding the relationships between and within species. The stomata length, width, density, and index also vary in different species. It is concluded that the numerical analysis could be used for understanding the phenetic relationships among various species of *Quisqualis* and *Guiera senegalensis*. The findings contribute to the botanical profiling of the area and provide a valuable reference for future studies in taxonomy, ecology, and conservation.

Keywords: *Quisqualis* (Combretaceae), *Guiera* (Combretaceae), Herbal Medicine, Morphological, Leaf Epidermal, Taxonomy.

1. INTRODUCTION

Genus *Quisqualis* is an exceptionally impressive tropical vine, with a few varieties, distinguishable by its flower color and leaf size. It can reach 21 m in the wild, but generally its length in cultivation ranges between 2 and 9 m. A large, woody and shrubby climber over pergolas, trellises, etc., and yet can be trained as a specimen shrub. Under good growing conditions, are typically seen with lush and fresh green foliage on cascading branches with numerous axillary and terminal drooping racemose inflorescences that is simply spectacular. Leaves with distinct venation, are oblong to elliptic, 7-15cm in length with acuminate tip and rounded base. They are simple and opposite. It non-stop blooms profusely all year around in the tropics

(Sahu et al., 2012). Inflorescences terminal or axillary, simple or sometimes compound spikes. Calyx tube (1.7–)5–9 cm, ± uniformly narrowly tubular except funnel form at apex, deciduous above ovary, hairy or subglabrous; lobes 5, deltoid or triangular-lanceolate, small, apex sometimes cuspidate. Petals 5, white or red, larger (often much more so) than calyx lobes. Stamens 10, not or scarcely exserted from calyx tube. Style partly adnate to inside of calyx tube (in Chinese species) (Flora of China). The fragrant flowers are born in clusters, and each flower has many variations of color, depending on how old the flower is (Alege et al., 2013).

Every plant contains several phytoconstituents in its different parts, showing various pharmacological activities and /or toxicities, likewise, *Quisqualis indica Linn.* also showing many pharmacological activities due to the presence of medicinally active compounds (Ernst and Thompson, 2001). *Quisqualis indica Linn* contains phytoconstituents such as trigonelline (alkaloid), L-proline (α-amino acid), L-asparagine (α-amino acid), quisqualic acid (agonist for both AMPA receptors), rutin (flavonoid) and two forms of the cysteine synthase, isoenzyme A, and isoenzyme B (enzymes). Rutin and pelargonidin-3-glucoside have also been isolated from flowers (Sucher and Carles, 2008). Fruits contain a sugary substance similar to levulose and an organic acid similar to citric acid. Seeds contain a fixed oil, which consists of linoleic, oleic, palmitic, stearic and arachidic acids, a sterol, an alkaloid with anthelimintic properties and a neuroexcitatory amino acid, quisqualic acid (Kessler and Einsenberg, 1999).

2. MATERIALS AND METHODS

2.1. Sample Collection and Plant Identification

Dried herbarium specimens were used for the work, except for *Quisqualis indica,* for which fresh samples from field collection were used. Herbarium studies of *Quisqualis latialata* and *Guiera senegalensis* were carried out at the Herbarium of the Pure and Applied Botany Department, Federal University of Agriculture, Abeokuta, and the Herbarium of the Department of Botany, University of Ibadan, Ibadan. Specimens of *Quisqualis indica* were collected on the right side of the Ogun-Oshun River Basin, off Alabata road, Abeokuta. The geographic coordinates of each of the fresh specimens were recorded using a global positioning system (GPS). A complete list of species including place of collection, GPS coordinates and date of collection are provided in Table 1. The morphological and ecological features of every sample were noted. The plants were properly documented correctly, and well numbered for easy identification. The specimens were processed for the herbarium and voucher specimens were prepared for all collections using standard herbarium procedures and deposited in FUNAAB Herbarium Abeokuta (FHA) for future reference (RBGE, 2017).

Table 1. List of Quisqualis species and Guiera senegalensis from different geographical locations in Nigeria collected for the study

Species	Place of Collection	Date of Collection	GPS Coordinates	
			Latitude	Longitude
Quisqualis indica L.	On the right side of the Ogun-Oshun River Basin, off Alabata road, Abeokuta.	20/10/2022	N07.19684º	E003.43896º
Quisqualis latialata Engl. ex Engl. & Diels) Exell.	Iguelaba Village, Sapoba Forest Reserve community, Orhionmwon LGA, Edo State.	05/07/2017	N06.07568º	E005.81915º
Guiera senegalensis J.F. Gmel.	Open vegetation, off Gwadabawa Kasuwa (Market) Gwadabawa LGA, Sokoto State.	14/12/2017	N13.37729º	E005.22183º

2.2. Micromorphology

2.2.1. Preparation of Epidermal Peels

Portions of the leaf of each species were obtained at standard median portion of the lamina, the specimens were appropriately put in a glass petri-dish and then concentrated 100% trioxonitrate (V) was poured on the arranged leaves to allow for softening and rehydration of the dried leaves. Formation of air-bubbles in the treated leaves indicated that the upper and the lower epidermis have separated from the mesophyll layer and were ready for peeling. The specimens were transferred into new petri-dish containing distilled water for rinsing. The epidermal layers were separated by teasing them apart carefully with a pair of forceps and gently brushing the epidermal layer with soft artist hair brush to remove the residual mesophyll layer, these were then transferred into storage bottles containing 50% ethanol for fixation.

2.3. Preparation of Slides

The leaf epidermal peels were then stained in Safranin 0 for about 3 minutes and rinsed in water to remove excess stain. They were dehydrated in 70% ethanol for about 3 minutes. The epidermal layers were put in xylene for about 3 minutes to remove ethanol and then mounted in 25% glycerin on clean glass slides and covered with coverslips gently, avoiding air bubbles. The edges of the cover slip were then sealed with nail polish to prevent dehydration. Observations and measurements were carried out using a micrometer eyepiece on a pre-calibrated microscope. Measurements were converted by the ocular constant to the power of the objective eyepiece under which they were taken and recorded. Twenty-five different parameters of each character were taken at random from each specimen and the mean and standard deviation calculated. Measurement of the epidermal cell width was taken at the widest point on each cell. Photomicrographs of the specimens were taken using Olympus Cx31 with a digital microscope eyepiece attached and stomata terminologies follow (Dilcher, 1974) and (Stace, 1966). The stomata index (SI) was calculated for each of the representative taxa using the formula of Salisbury as reported by (Ayodele and Olowokudejo, 2006).

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

Where S = Number of stomata per unit area (mm^2)

E = Number of epidermal cells per unit area.

2.4. Numerical taxonomy

2.4.1. Selection of Operation Taxonomy Units (OTUs)

Three OTUs were selected for the numerical taxonomic study. Three (3) OTUs were *Quisqualis* and *Guiera senegalensis* species identified in this study to occur in Nigeria.

The three OTUs are presented below:

1. *Quisqualis indica* .....OTU 1
2. *Quisqualis latialata* .....OTU 2
3. *Guiera senegalensis* .....OTU 3

2.5. Selection of characters

A total of 13 epidermal characters (epidermal cell shape, anticlinal wall pattern, stomata type, trichome type, trichome length, trichome width, number of epidermal cell, epidermal cell width, cell wall thickness, number of stomata, stomata length, width, and stomata index). These characters were scored on a binary system (1= Present and 0 = Absent).

2.6. Data Analyses

A cluster analysis of the morphometric characters was used, and this was performed for all the data matrices. Cladograms were constructed based on Single Linkage Cluster Analysis (SLCA) using the statistical software PAST Version 4.03 package (Hammer et al., 2023). The cluster model was selected from the best suitable algorithm where, Manhattan Distance was used to calculate the similarity measures with the SLCA option.

3. RESULTS AND DISCUSSION

3.1. Leaf Epidermal Anatomy

All the taxa have polygonal epidermal cells with curved to wavy anticlinal walls, except *Guiera senegalensis,* which has irregular cell shape on both adaxial and abaxial surfaces, while *Quisqualis latialata* was amphistomatic; other taxa were hypostomatic with anomocytic stomata (Table 2). Striations are found on the abaxial surface of both *Q. indica* and *Q. latialata,* while in *G. senegalensis*, it was absent on both adaxial and abaxial surfaces (Table 3, Figure 1). Trichomes are glandular (the form of stalked multicellular glands) or non-glandular, uniseriate in members of the genera *Quisqualis* and *Guiera* (Table 2).

The mean number of cells on the adaxial surface ranged from 35 in *G. senegalensis* to 148 in *Q. latialata,* while on the abaxial surface, it ranged from 53 in *Q. indica* to 212 in *G. senegalensis* (Table 3). Epidermal cell width ranged from 40.18 μm in *Q. latialata* to 57.28 μm in *G. senegalensis* on the adaxial surface, and from 46.07 μm in *G. senegalensis* to 48.83 μm in *Q. indica* on the abaxial surface. Cell wall

thickness ranged from 2.03 µm in *Q. indica* to 2.91 µm in *G. senegalensis* on the adaxial surface, and from 1.86 µm in *Q. latialata* to 3.54 µm in *G. senegalensis*. The highest stomata density of 86 was recorded for *G. senegalensis* and the lowest of 58 for *Q. indica* on the adaxial surface, while highest of 126 was recorded for *Q. latialata* and the lowest of 26 for *Q. indica* on the abaxial surface (Table 3). Stomata length ranged from 36.81 µm in *G. senegalensis* to 74.28 µm in *Q. latialata* on the adaxial surface and from 43.62 µm in *Q. latialata* to 51.67 µm in *Q. indica* on the abaxial surface.

The highest mean stomata index of 71.07 % for *G. senegalensis* and the lowest of 28.16 % for *Q. latialata* were recorded on the adaxial surface, and from 48.84 % in the same *Q. latialata* to 32.91 % in *Q. indica* (Table 3). Considerable variation was recorded in the length and width of trichomes (Table 2). The longest trichome was obtained on the adaxial surface in *Q. indica* (1876.3 µm), whereas the shortest trichome was recorded for *Q. latialata* (822.5 µm) on the same surface. On the abaxial surface, the longest trichome (2082.5 µm) was also recorded for *Q. indica*, while the minimum mean length of trichome was obtained in *G. senegalensis* (331.4 µm). The trichome width ranged from 35.12 µm in *Q. latialata* to 58.85 µm in *Q. indica* on the adaxial surface, while on the abaxial surface, it ranged from 20.71 µm in *G. senegalensis* to 56.16 µm in *Q. latialata*.

Table 2: Qualitative leaf epidermal and stomata features of *Quisqualis* species and *Guiera senegalensis*

Species	Leaf Surface	Epidermal cell shape	Anticlinal wall	Stomata type	Striation	Trichome type
Quisqualis indica L.	Adaxial	Polygonal	Curved	Absent	Absent	Stalked glands, Unicellular, non-glandular,
	Abaxial	Irregular	Wavy	Anomocytic	Present	Unicellular, non-glandular
Quisqualis latialata Engl. ex Engl. & Diels) Exell.	Adaxial	Irregular	Undulate	Anomocytic	Present	Capitate glands, Unicellular, non-glandular
	Abaxial	Polygonal	Straight	Anomocytic	Present	Unicellular, non-glandular
Guiera senegalensis J.F. Gmel.	Adaxial	Irregular	Undulate	Anomocytic	Absent	Capitate glands
	Abaxial	Irregular	Straight	Absent	Absent	Capitate glands, Unicellular, non-glandular

Table 3: Quantitative leaf epidermal characters of *Quisqualis* species and *Guiera senegalensis*

Species	Leaf Surface	No of cells/mm²	Epidermal cell width (µm)	Cell wall thickness (µm)	Stomata frequency (mm²)	Stomata length (µm)	Stomata width (µm)	Stomata index (%)	Trichome length (µm)	Trichome width (µm)
Quisqualis indica L.	Adaxial	86±2.59	43.29±1.47	2.03±0.07	Absent	Absent	Absent	Absent	1876.3±21.42	58.85±3.07
	Abaxial	53±2.38	48.83±2.54	2.96±0.10	26±0.57	51.67±1.05	21.40±0.38	32.91	2082.5±46.07	51.76±4.01
Quisqualis latialata Engl. ex Engl. & Diels) Exell.	Adaxial	148±5.23	40.18±2.24	2.25±0.22	58±1.64	74.28±4.57	30.26±2.25	28.16	822.5±29.13	35.12±3.66
	Abaxial	132±3.14	48.65±1.82	1.86±0.14	126±3.58	43.62±2.95	29.60±1.38	48.84	1746.6±15.25	56.16±3.03
Guiera senegalensis J.F. Gmel.	Adaxial	35±2.03	57.28±1.51	2.91±0.12	86±1.74	36.81±2.94	20.96±0.12	71.07	Absent	Absent
	Abaxial	212±8.39	46.07±2.65	3.54±0.06	Absent	Absent	Absent	Absent	331.42±7.63	20.71±0.80

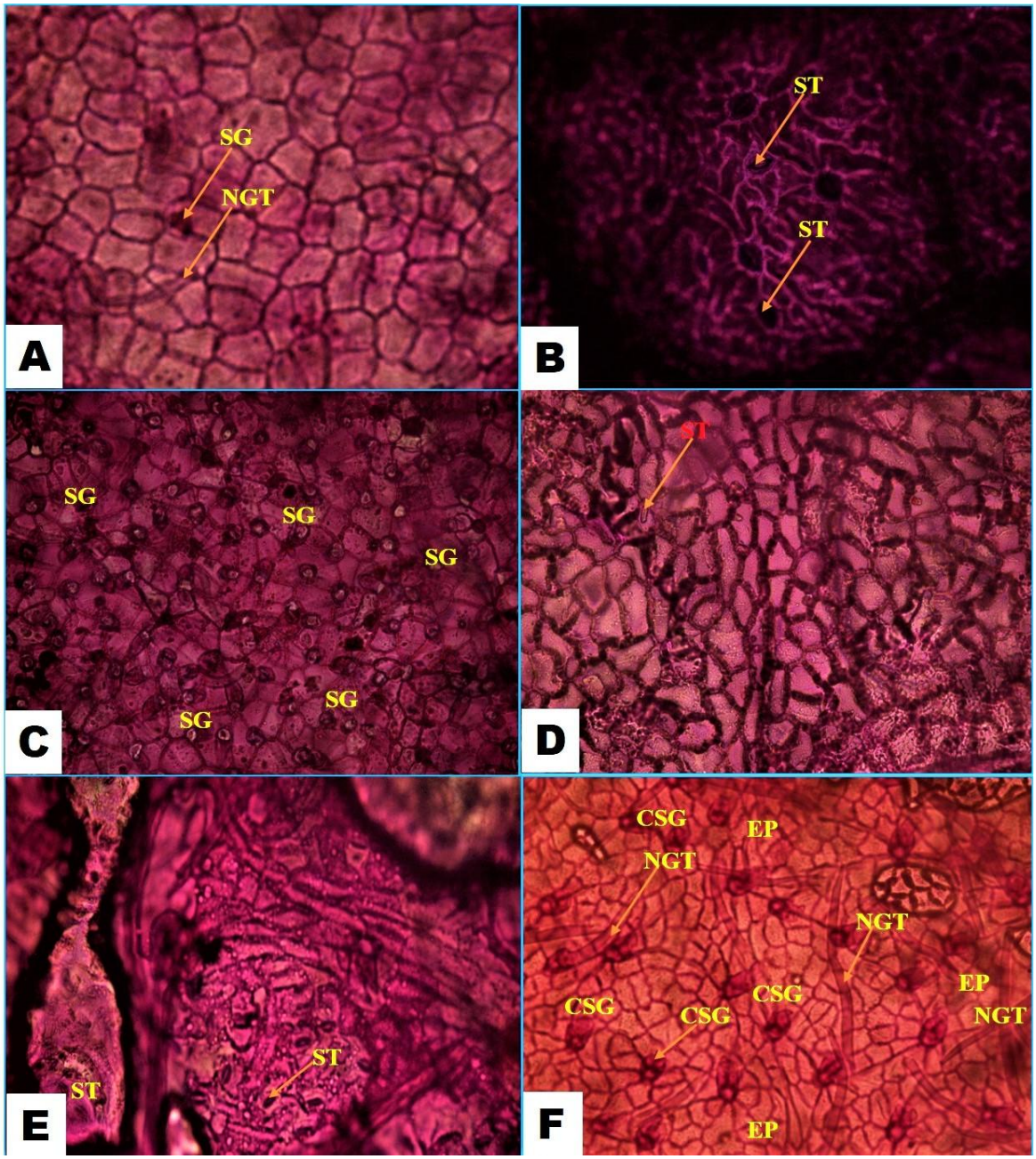

Figure 1. Photomicrographs of Quisqualis species and Guiera senegalensis based on the Leaf Epidermal Characters

A: Adaxial surface of Q. indica showing the stalked glands and polygonal cells with curved anticlinal walls.

B: Abaxial surface of Q. indica showing irregular cells, anomocytic stomata with elliptic guard cells, and striae.

C: Adaxial surface of Q. latialata showing densely distributed multicellular glandular trichomes.

D: Abaxial surface of Q. latialata showing polygonal cells with curved anticlinal outlines.

E: Adaxial surface of G. senegalensis showing anomocytic stomata with narrowly elliptic guard cells.

F: Adaxial surface of G. senegalensis showing capitate stalked glands with dense non-glandular trichomes.

Legend: EP–Epidermal cells; ST-Stomata; SG-Stalked glandular trichome; GT- Unicellular, non- glandular trichome; CSG-Capitate stalked glands.

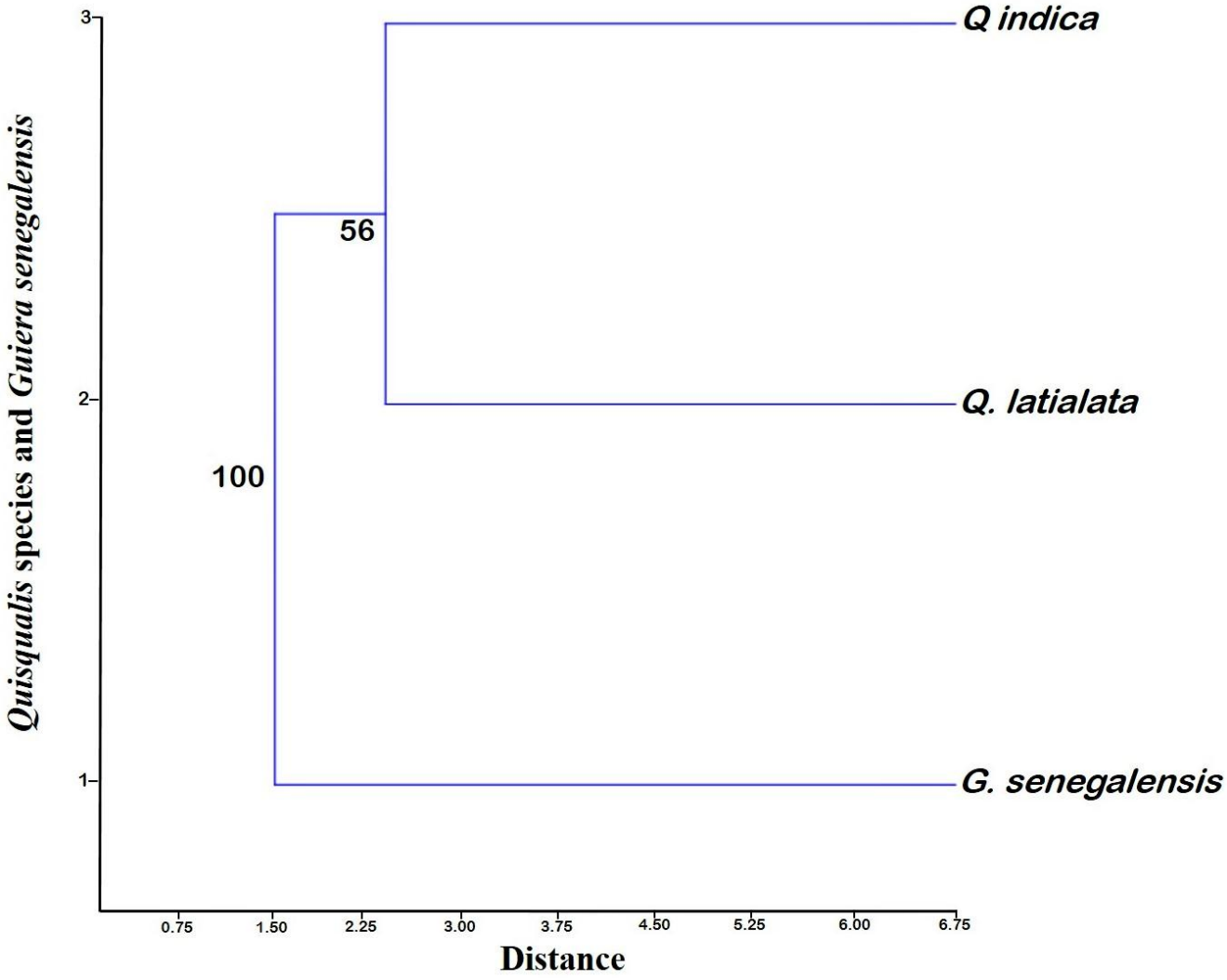

Figure 2. Dendrogram of *Quisqualis* species and *Guiera senegalensis* based on Qualitative and Quantitative Epidermal and Stomata Characters (Note: Numbers under branches indicate bootstrap percentages (%) derived from 1000 replicates).

Leaf size varies considerably in the two genera. The value of epidermal characters in the identification and delimitation of taxa has been stressed by many workers (Ayodele and Olowokudejo, 2006). The upper epidermis differed considerably in size and number among the species studied. According to Salisbury, (1928) and Ayodele and Olowokudejo (2006), such variation may attributed to geographical differences such as light intensity, humidity, and pressure. Stomata are mainly anomocytic while the stomata distribution and sizes in the taxa are important taxonomically because they showed variations. While *Quisqualis latialata* was amphistomatic with anomocytic, *Guiera senegalensis* and *Q. indica* thus distinguish it from the other taxa. Most of the species studied have striation, except *Guiera senegalensis*, with no striae on both adaxial and abaxial surfaces. This distinguishes *Guiera senegalensis* from the rest of the taxa. Observation of trichome bases in Q. indica may be, a result of early loss or degeneration from the plant surface, as noted by (Theobald et al. 1979).

In the first cluster, *Q. indica* is grouped with *Q. latialata* and Unknown, the closeness between *Q. indica* and *Q. latialata* is supported by their stalked multicellular glands and irregular cells with wavy or undulate anticlinal walls. The dendrogram showed (Figure 2) that *Guiera senegalensis* has been found anatomically most distant from all other taxa investigated in this study.

4. CONCLUSION

Therefore, this study has shown how numerical taxonomy justifies the classification of the genus using leaf epidermal characters as supported by several. It is concluded that the numerical analysis could be used for understanding the phenetic relationships among different species of *Quisqualis* and *Guiera senegalensis*.

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Authors' contributions

DTA conceived the main idea, performed the sample and data collection, practical part, writing and submission, MOM supervised and revised the work, IDA analyzed the data, wrote the manuscript and final editing, UOD helped in sample collection and analyzed the data. All authors read and approved the final version of the manuscript.

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Conflict of Interest

The author declares that there are no conflicts of interests.

Informed consent

Not applicable.

Ethical approval & declaration

The ethical guidelines for plants & plant materials are followed in the study for sample collection, identification & experimentation with the help of Dr. O.M. Mudasiru, Federal University of Agriculture, Abeokuta.

Data and materials availability

All data associated with this study are present in the paper.

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