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Comparative Morpho-Anatomical Investigation of Leaves of *Triumfetta tomentosa* Boj. and *Triumfetta rhomboidea* Jacq.

Robert, Imo U. [a*], Sam, Sunday M. [a] and Okon, Joseph E. [a]

[a] Department of Botany, Faculty of Biological Sciences, Akwa Ibom State University, Ikot Akpaden, Mkpat Enin, Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The plant species *Triumfetta tomentosa* Boj. and *Triumfetta rhomboidea* Jacq using Standard procedures were investigated on morphological characters. The morphological assessment of the species revealed great similarities in their habit, indumentum, nature and type of stem and leaf apices. The differences were seen in their habitat and the number of flowers in the racemose inflorescence. In the foliar anatomy of the species, anisocytic stomata type, the presence of stellate trichomes on both surfaces and the presence of calcium crystals were common in both species. The differences existed in the amphistomatic distribution in *T. rhomboidea* and hypostomatic distribution in *T. tomentosa.* The number, length and width of the stomata, stomata pore and guard cells, the number, length and width of the epidermal cells of and the length of trichomes varied greatly in both species. A combination of morphological and anatomical data alongside others bring about authentication in the delimitation of taxa.

Keywords: Morpho-Anatomical; delimitation; amphistomatic; Triumfetta tomentosa; Triumfetta rhomboidea.

**Corresponding author: E-mail: Imoubom485@gmail.com;*

1. INTRODUCTION

The genus *Triumfetta* L. (Malvaceae: Tilioideae) has a pantropical distribution with nearly 150 described species [1, 2, 3, 4]. The genus is distinguished by the presence of an urceolate, ciliate androgynophore with glands, capsules often globose, indehiscent or bivalvate, with spinules on the whole surface and hyaline apices [4]. *Triumfetta tomentosa is a*n erect shrub, 60 – 80 cm tall. The stem is woody, erect and hairy, 0.4 cm in diameter. The leaf is simple, alternate, petiolate, cuspidate, serrulate, acute, hastate, and hairy on both surfaces and 4- 6 cm long and 2 – 4 cm wide. The flower is pale yellow in colour [5]. *Triumfetta rhomboidea* is an erect shrub which is 25 – 60 cm tall. The stem is woody, erect, branched and hairy with short brown hairs. The leaf is simple, alternate, and variable in size and shape. The upper leaves are ovate, undivided and small, while the lower ones are larger, 2-lobed, 15 cm long and 10 cm wide. They have dentate margin, cuspidate apex, petiolate, hairy on both surfaces. The inflorescence is made up of cluster of flowers at the axils of the leaves. The flower is yellow, 8mm in diameter [5]. The aim of this research is to compare the morphological and anatomical characters of these species in order to provide information for the taxonomic delimitation of these species.

2. MATERIALS AND METHODS

2.1 Study Area

This research was carried out in Akwa Ibom State, Nigeria.

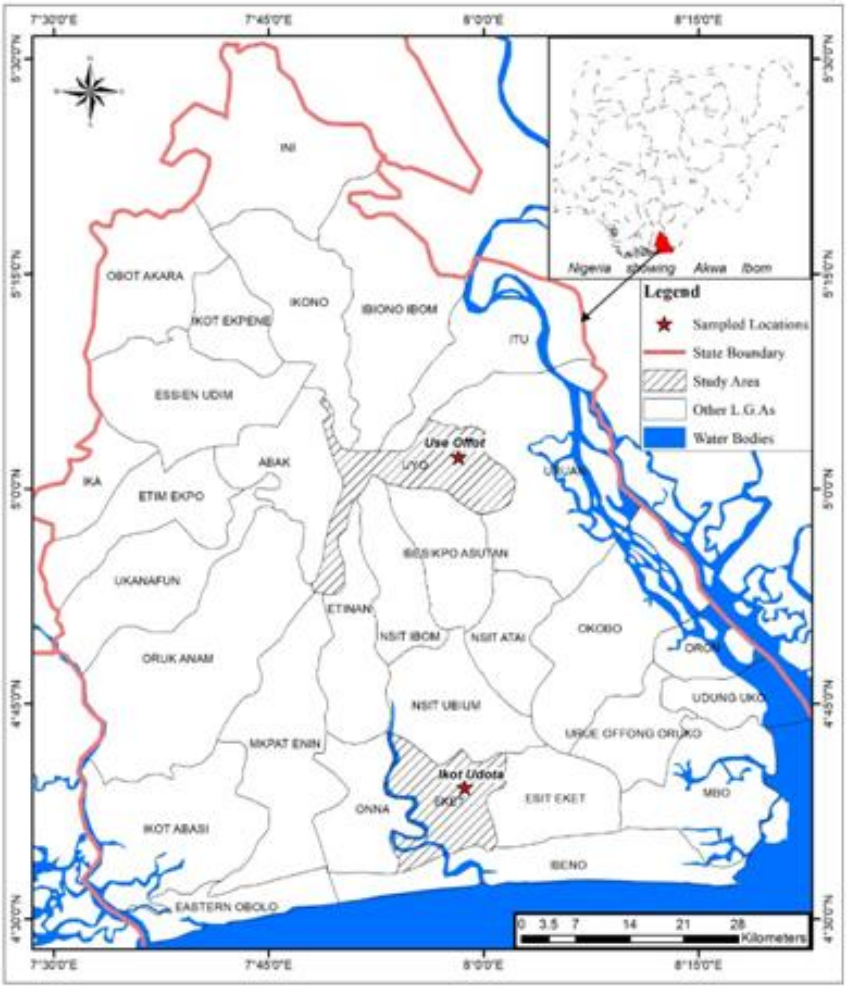

Fig. 1. Map of Akwa Ibom State showing the areas where plant samples were collected

2.2 Collection and Authentication of Plant Materials

This plant samples were collected in different Areas in Ikot Udota in Eket Local Government Area and Use Offot in Uyo Local Government Area of Akwa Ibom State between September and October, 2021 and at their flowering stage.

Fresh plant samples were collected from the field into a sack back with specimen numbers, collection point using global position system (GPS) and date written on the field notebook.

The picture of the collected specimen was taken and drawings made.

The plant materials were identified and authenticated by taxonomist, Mr. Omodot Umoh using the Flora of West Tropical Africa [6].

2.3 Morphological Data

Morphological and related taxonomical observations were made on the stems, leaves (apex, base, margin, texture, hairiness) petioles length and fruits. Measurement of leaflet was taken from the longest median leaflet and the basal leaflet and the characters were described [7].

2.4 Preparation of Surface Specimens of Leaves

The preserved plant samples were rinsed with distilled water. Small portions were obtained from the standard medium part of well expanded mature leaves. The epidermal peels of both abaxial and adaxial surfaces were made by placing the leaf blade in a clean glass slide with the surface to be studied facing down.

The specimens were irrigated with water holding it downward from one end and then the epidermis above the desired surface was scrapped-off carefully with sharp razor blade. The loose cells were washed away from the epidermal peels with the aid of soft camel hair brush. The cleaned portion/epidermis was further cleared in 5% solution of sodium hypochloride (Jik) for about 2-3minutes. The cleared portions of the leaf were finally washed in 3-4 changes of distilled water [8].

The epidermal peels were stained in 1% aqueous solution of safranin-O for 4-8 minutes, carefully washed in water to remove excess stain and mounted in 10% glycerol on a glass slide and covered with a glass cover slip then viewed using an Olympus CX21 binocular microscope. Photomicrographs were taken from good preparation using the Olympus CX21 binocular microscope fitted with an MD500 amscope microscope eyepiece camera.

2.5 Micrometric Evaluation

Measurements of dimensions (length and width) were done of the various diagnostic microscopic characters of the leaf; namely stomatal length, stomatal width, stomatal pore length, stomatal pore width, epidermal cell length, epidermal cell width, guard cell length, guard cell width, length of trichome, width of trichome, areole length and areole width [9].

2.6 Quantitative Leaf Microscopy

Quantitative microscopy was done to determine stomatal number, epidermal cell number, veinislet number, vein termination number and stomatal index. The Stomatal Index was determined according to the method of [7].

$$S.I = \frac{S}{E+S} \times \frac{100}{1}$$

Where S = Number of stomata per unit area
E = Number of epidermal cell

All measurements were made of the widest points using a calibrated ocular micrometer and an Amscope. Thirty (30) microscopic fields chosen at random were used and data presented as Mean ± Standard Error of Mean (±SEM).

3. RESULTS

The plant species *Triumfetta tomentosa* Boj and *Triumfetta rhombiodea* Jacq. were collected in different Areas within Akwa Ibom State as listed in Table 1.

The different co – ordinates are as indicated in Table 1.

3.1 Morphological Descriptions of *Triumfetta rhomboidea.* Jacq and *Triumfetta tomentosa* Boj

3.1.1 *Triumfetta rhomboidea* Jacq

T. rhomboidea is a terrestrial plants, with an erect stem, woody, cylindrical, scabrid. The stem

has a diameter of 0.4cm, the leaf was simple, petiolate. Alternate, acute, serrated at the margin, rounded at base, Ovate to cordate, scabrid on the abaxial and pubescent on the adaxial surface. The leaf is 1-7cm long, and 0.44cm wide. The petiole is 0.5 -1cm long, pubescent and herbaceous. Axillary raceme, cluster of about 3 -5 flowers, 8 – 10 anthers, and a single pistil. The fruit were globose in cluster of 5 -10 fruits, with a diameter of 0.4cm with protrusions of 1mm long all over the fruits making it look like a corona virus (Table 1 and Fig. 2).

3.1.2 *Triumfetta tomentosa* Boj

T. tomentosa is a shrub usually found in swampy forest, with erect stem, woody, scabrid, cylindrical, and it is 0.3cm in diameter.the leaf is simple, petiolate, alternate, acute, with serrated margin, cordate, ovate.younger leaves are cordiately lobed than older leaves.Scabrid adaxial surface and pubescent abaxial surface, the leaf is 7- 11cm long and 3 -9cm wide, the petiole is 1- 4cm long, pubescent and herbaceous. The inflorescence has an axillary to terminal raceme, 21- 25cm long, with many flowers, the fruits were stalked green when fresh, globose in cluster of about 6 – 10 with protrusions of 2mm long all over the fruits, making it look like a corona virus of 0.8 – 1cm long and wide, they were 3 seeds in a fruit minutely ovate in shape (Table 2 and Fig. 3).

Table 1. Samples of *Triumfetta* species and location

S/N	Family	Species name	Locality	Herbarium number	Co-ordinates
1	Malvaceae	*Triumfetta rhombiodea*	Use Offot, Uyo		Latitudes 5.037295^0 Longitudes 7.970847^0
2	Malvaceae	*Triumfetta tomentosa*	Ikot Udota, Eket		Latitude. 4.652855^0 Longitude 7.978002^0

Fig. 2. *Triumfetta rhomboidea* Jacq (A) Fruit (B) Leaf (C) Flower

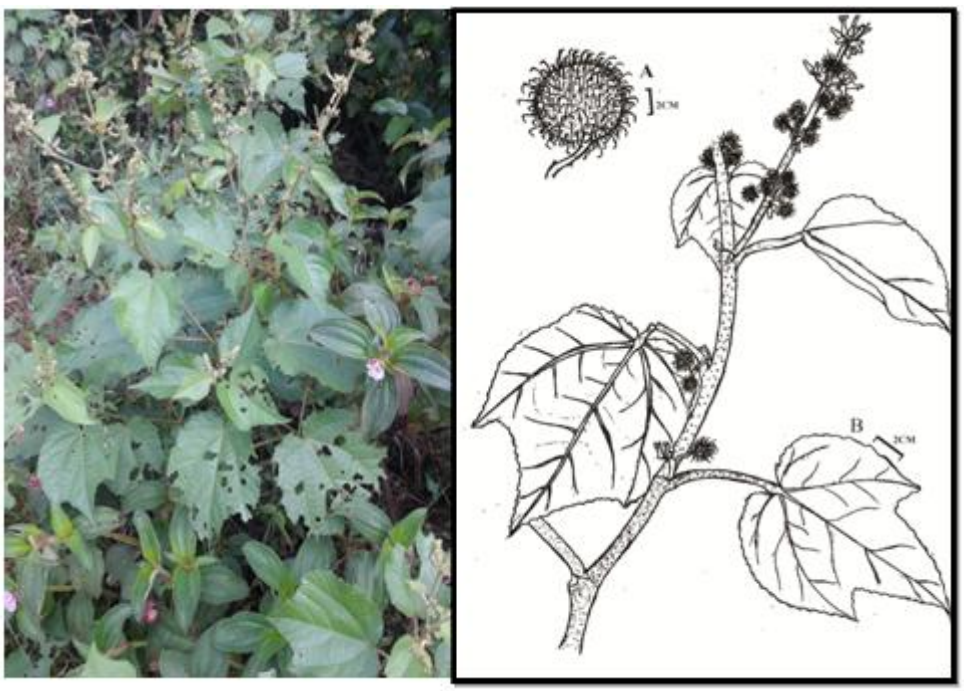

Fig. 3. *Triumfetta tomentosa* Boj (A) Fruit (B) Leaf

Table 2. Morphological characters of *Triumfetta* species

Species/characters	*Triumfetta rhomboidea*	*Triumfetta tomentosa*
Habitat	Terrestrial	Swampy forest
Habit	Shrub	Shrub
Stem type	Erect	Erect
Apex	Acute	Acute
Margin	Serrate	Serrate
Base	Rounded	Cordate
Shape	Ovate	Ovate – cordate
Indumentum	Pubescent	Pubescent
Inflorescence	Axillary raceme with cluster of 3 – 5 flowers	Axillary raceme with cluster of 8 - 10 flowers

3.2 Anatomical Features of *Triumfetta* Species

3.2.1 *Triumfetta rhomboidea.* Jacq

Foliar epidermal cells on the adaxial surface as seen in (Fig. 4, C, D, E. and F), were irregularpolygonal in shape with undulating anticlinal cell wall pattern. Stomata distribution were Amphistomatic (i.e stomata was present at both surfaces). Presence of Anisocytic stomata. presence of calcium oxalate crystals (Druses). Epidermal cell number were 211.7±4.46µm, Length of epidermal cell were 45.33±1.87 µm, Width of epidermal cell were 18.79±1.03µm, Epidermal cell wall thickness were 2.26±0.08µm.Width of guard cells were 4.73±0.34 µm, Length of guard cells were 10.96±0.67 µm, Stomata length were 17.47±0. 44 µm, Stomata width were 2.30±0.77, Stomata Number were 2.6 ±0.23. Length of trichome were 111.32±2.05 µm, Width of trichome were 8.97±0.95 µm, Stomata index were 36.61%, Stomata pore width were 1.69±0.04 µm, Stomata pore length were 3.95±0.17 µm.

On the abaxial surface as seen in (Fig. 4, A,B). The epidermal cells were irregular in shape with sinous anticlinal cell wall pattern, The leaves surface had 3-4 arm of trichome (i.e stellate), presence of Anisocytic stomata were present, Epidermal cell number were 239.7±7.00 µm, Length of epidermal cell were 20.79±0.49 µm, Width of epidermal cell were 10.46±0.36 µm,

Epidermal cell wall thickness were 3.48±0.27 µm, Stomata Number were 3.31±0.11 µm, Length of guard cells were 5.95±0.19 µm, Stomata length were 17.3±0.42 µm, Stomata width were 8.42±0.28 µm, Stomata Number were 6.8±0.34 µm, Length of trichome were 74.6±2.79 µm, Width of trichome were 6.41±0.48 µm, Stomata index were 49.27%, Stomata pore width were 11.55±0.07 µm, , Stomata pore length were 2.44±0.17 µm.

3.2.2 *Triumfetta tomentosa* Boj

Foliar epidermal cells on the adaxial surface were irregular – poloygonal in shape with undulating anticlinal wall pattern. The leaves surface had stellate trichomes, presence of calcium oxalate crystal, stomata distribution was hypostomatic, presence of Anisocytic stomata. Epidermal cell number were 182.5±6.32 µm, Length of epidermal cell was 34.97±2.48 µm Width of epidermal cell were 26.15±2.64 µm. Epidermal cell wall thickness were 2.24±0,09 µm, Length of trichome were 67.355±5.04 µm, Width of trichome was 7.13±0.57 µm (Fig. 5. C,D, E,and F).

On the abaxial surface the epidermal cells were irregular in shape with sinous anticlinal cell wall pattern, the leaves surface had stellate trichomes, stomata distribution were hypostomatic, presence of Anisocytic stomata. Epidermal cell number were 208.06±4.58 µm Length of epidermal cell were 6.82±0.55 µm Width of epidermal cell were 46.69±3.19 µm. Epidermal cell wall thickness were 16.38±1.95 µm Stomata pore length was 11.19 ± 0.23 µm, Stomata pore width were 3.10±0.13 µm Length of trichome were 110.65±4.47 µm Width of trichome were 6.82±0.55 µm (Fig. 5.A,B,).

Stomata length w 21.43±0.70 µm Stomata width were 12.00±0.63 µm. Stomata Number were 45.00±1.28 µm Length of guard cells were width of guard cells were Stomata index were 17.78%.

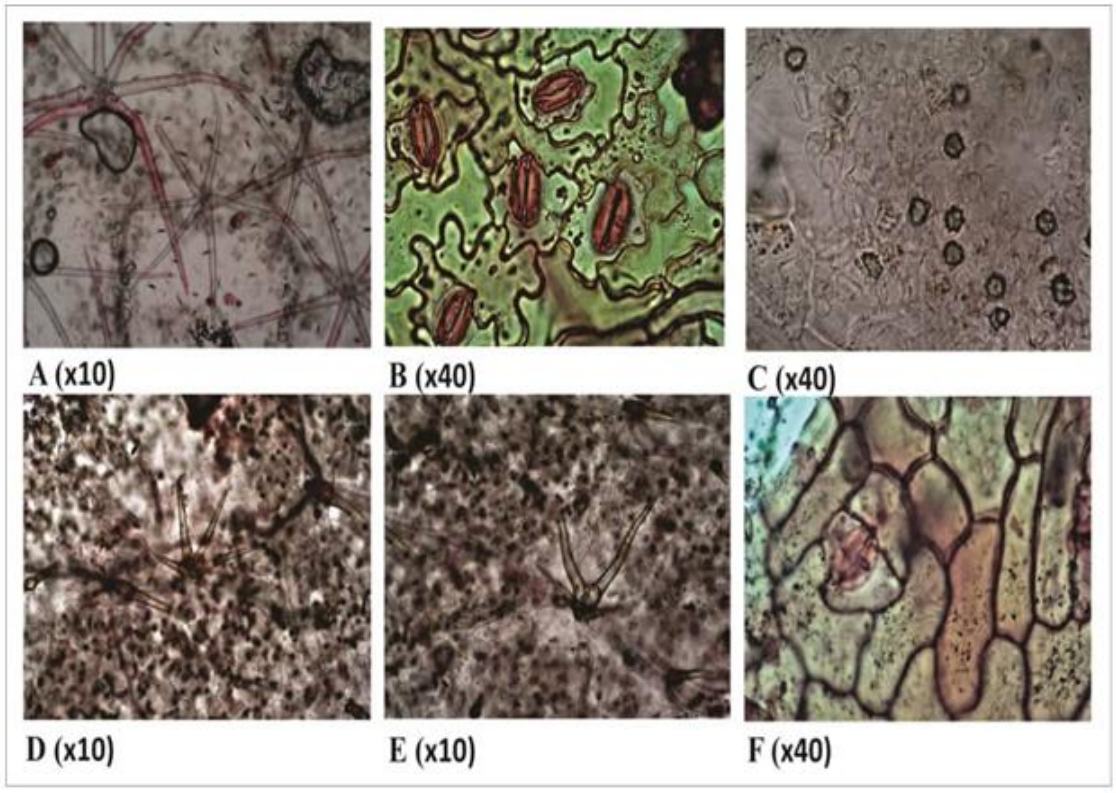

Fig. 4. *T. rhomboidea* Jacq (A). Abaxial surface showing trichome (B). Abaxial surface showing Anisocytic stomata (C). Adaxial surface showing calcium oxalate crystals druse (D).Adaxial surface showing four armed Trichome (E). Adaxial surface showing trichome (F). Adaxial surface showing Anisocytic stomata

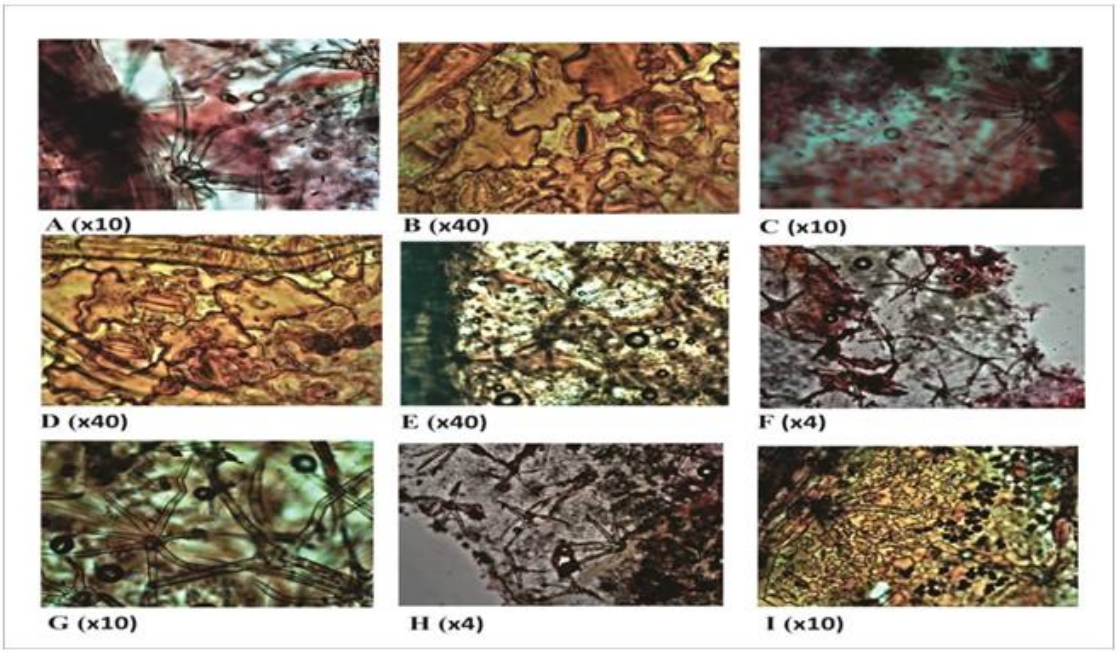

Fig. 5. *Triumfetta tomentosa* Boj (A). Abaxial surface showing trichome (B). Abaxial surface showing Anisocytic stomata (C). Adaxial surface showing calcium oxalate crystals druse (D). Adaxial surface showing Anisocytic stomata (E). Adaxial surface showing trichome (F). Adaxial surface showing four armed trichome (I)

The measurements of anatomical features of the two species of *Triumfetta* were summarised. The values showed that on the adaxial layer of the plants studied, *Triumfetta tomentosa* had the least epidermal cell number (182.5±6.32 µm), while *Triumfetta rhomboidea* had the highest (211.7±4.46). The shortest epidermal cells were found in *T. tomentosa* ((34.97±2.48 µm),while *T. rhomboidea* had the longest (45.33±1.87 µm).The narrowest epidermal cells were found in *T. rhomboidea* (18.79±1.03 µm) while *T. tomentosa (*26.15±2.64 µm) had the widest. The thinnest sepidermal cell wall were found in *T. tomentosa (*2.24±0.09 µm),and thickest in *T. rhomboidea* (2.26±0.08 µm). The shortest trichome length were found in *T. tomentosa (*67.35±5.04 µm) while *T. rhomboidea (*111.32±2.05 µm) had the longest (Table 3).

On the abaxial layer of the two species of plants studied *T. tomentosa* (208.06±4.58 µm) had the least epidermal cells, while *T. rhomboidea (*239.7±7.00 µm) had the highest. The shortest epidermal cells were found in *T. rhomboidea* (20.79±0,49 µm) while *T. tomentosa* (46.69±3.19 µm) had the longest. Epidermal cell walls were narrower in *T. rhomboidea* (10.46±0.36 µm) and widest in *T. tomentosa* (16.38±1.95 µm).The thinnest epidermal cells were found in *T. tomentosa* (1.95±0.07 µm) and thickest in *T. rhomboidea* (3.48±0.27 µm). Stomata Index were least in *T. tomentosa (*17.78%) and highest in *T. rhomboidea* (49.27%). Fewer stomata occurred in *T. rhomboidea* (8.42±0.42 µm) per view, While the highest stomata number occurred in *T. tomentosa (*45±1.27 µm). Stomata width was least in *T .rhomboidea* (8.42±0.28 µm), and highest in *T. tomentosa* (45±1.27 µm). The shortest trichome length occurred in *T. rhomboidea (*74.6±2.79 µm), while *T. tomentosa (*110.65±4.47 µm) had the longest (Table 3).

The narrowest width of trichomes occurred in *T. rhomboidea* (6.41±0.48 µm), while *T. tomentosa (*6.82±0.55 µm) had the widest. The shortest stomata length occurred in *T. rhomboidea* (17.3±0.42 µm), while *T. tomentosa (*21.43±0.70 µm) had the longest. The shortest guard cells were found in *T. rhomboidea (*5.95±0.19 µm) while *T. tomentosa* had the longest (8.76±O.58 µm).The narrowest guard cells were found in *T. rhomboidea* (3.31±0.11 µm), while *T. tomentosa* (3.91±0.06 µm) had the highest. The shortest stomata pore length were found in *T. rhomboidea* (2.44±0.17 µm) and *T. tomentosa* (11.19±0.23 µm) had the longest (Table 3).

Table 3. Measurements of anatomical features of two species of *Triumfetta*

Triumfetta rhomboidea	***Triumfetta rhomboidea***
AD-ECN 211.7±4.46	AD-ECN 182.5±6.32
AB- ECN 239.7±7.00	AB- ECN 208.06±4.58
AD-LEC 45.33±1.87	AD-LEC 34.97±2.48
AB- LEC 20.79±0,49	AB- LEC 46.69±3.19
AD-WEC 18.79±1.03	AD-WEC 26.15±2.64
AB-WEC 10.46±0.36	AB-WEC 16.38±1.95
AD-ECWT 2.26±0.08	AD-ECWT 2.24±0.09
AB-ECWT 3.48±0.27	AB-ECWT 1.95±0.07
AD-SPL 3.95±0.17	AD-LT 67.35±5.04
AB-SPL 2.44±0.17	AB-SPL 11.19±0.23
AD-SPW 1.69±0.04	AD-WT 7.13±0.57
AB-SPW11.55±0.07	AB-SPW 3.10±0.13
AB-LGC 5.95±0.19	AB-LGC 8.76±O.58
AD-WGC 4.73±0.34	AB-WGC 3.91±0.06
AB-WGC 3.31±0.11	AD-LT 111.32±2.05
AD-LT 111.32±2.05	AB-LT 74.6±2.79
AD-WT 8.97±0.95	AB-WT 6.82±0.55
AD-STL 17.47±0.44	AB-STL 21.43±0.70
AD-STW 2.30±0.77	AB-STW 12.00±0.63
AB-STN 8.42±0.42	AB-STN 45±1.27
AD-STI 36.61%	AB-STI 49.27%

Key: AD-ECN = Adaxial Epidermal Cell Number, AD-LEC = Adaxial Length of Epidermal Cell, AD-WEC = Adaxial Width of Epidermal Cell , AD-ECWT = Adaxial Epidermal Cell Wall Thickness, AB-ECN = Abaxial Epidermal Cell Number, AB-LEC = Abaxial Length of Epidermal Cell, AB-WEC = Abaxial Width of Epidermal Cell, AB-ECWT = Abaxial Epidermal Cell Wall Thickness, STN = Stomatal Number, STW = Stomatal width, STL=Stomatal Length, STI=Stomatal Index.AB- LT=Length of trichome,AB-WT= Width of trichome,AD-LT= Length of trichome, AD-WT= Width of trichome,AD-SPL=Stomata pore length,AD-SPW=Stomata pore Width,AB- SPL=Stomata pore length,AB- SPW=Stomata pore Width,AD-LGC=Length of guard cells, AD-WGC=Width of guard cells, AB-LGC=Length of guard cells,AB-WGC= Width of guard cells.

4. DISCUSSION

Based on the relative occurrence and distribution of the *Triumfetta* species in Akwa Ibom State. It was observed that the collected samples were common in the different areas were the samples was collected. The morphology revealed that *Triumfetta rhomboidea* and *Triumfetta tomentosa* both had erect stem, shrub, acute and serrated. Their differences were seen in their inflorescences, the habitat of *T*. *rhomboidea* terrestrial and *T. tomentosa* were swampy while the base for *T*. *rhomboidea* were cordate and *T. tomentosa* were rounded.

Foliar anatomical features of the *Ttriumfetta* species studied showed diagnostic characters that offers clues for identification shape and orientation of stomata, guard cells and subsidiary cells, this is similar to the report of [10], that uses same parameters in the foliar anatomy of some ethnobotanical important species of wild edible fruits. Stating that the epidermis possesses a number of important diagnostic characters that offer valuable clues for identification like size, shape and orientation of stomata, guard cells, and subsidiary cells, structural peculiarities of epidermal cells and stomata frequency. [11], also reported that the leaf of epidermal features have been employed in taxonomy to separate plant genera and species.

The stomata type observed in both species was anisocytic stomata type, [7] stated that stomata type is of taxonomic value Stomata index may be used as a diagnostic feature as it was highest in *T. rhomboidea* (49.27%) , and the least was *T. tomentosa* (17.78%) as recorded in Table 3. [12] used same parameters in the delimitation of some *cola* species.

Essiett et al. [13] also reported that Stomata Index and the guard cell area provided values that served as parameters for comparison among taxa, which can be useful for identification of the studied taxa. Essiet et al. [14] on their study on 3 species of *acalypha* occurring in Nigeria, also reported that variation in stomata index and guard cells areas are useful diagnostic tools. Adedeji et al. [15] also reported that the Stomata

Index is constant for any given species and the value is more uniform on the abaxial surface than the adaxial surface except in an isobilateral leaf. Presence of unique stellate trichomes were present on both surfaces of *T. tomentosa* and *T. rhomboidea.* All of these traits were reported by [16] as a xeromorphic feature for ferns and support the designation of the species as a desiccation tolerant fern reported by [17] based on its environment. The presence of calcium crystals that was observed in the two species of *Triumfetta.* [18] reported that the wide occurrence of calcium oxalate crystals in plants indicates a well-developed system of genetic events.

These events are targeted at provision of variable functions depending on the tissue and organ where they are translocated. Therefore, occurrence of calcium crystals could serve a protective function when they exist in peripheral cells and tissues. For instance, the occurrence of epidermal raphide idioblasts [19] makes banana bracts unpalatable to browsing animals. Where solitary and bundle raphides are numerous, they often give itching property to the species or an organ of it. In this case, the involved plant is protected from herbivores.

5. CONCLUSION

The Morpho-anatomical assessment of *T. tomentosa* and *T. rhomboidea* has revealed a great resemblance in the two species and some differences too. The resemblances in the morphological and anatomical parameters give a clue to justifying their relatedness and the placement in the same genus while the differences justifies their delimitation into species. The differences in the different species from the parameters under consideration show slight modifications in the species which could have occurred in the process of speciation in the species. It is indispensable in modern taxonomy to consider morphological and anatomical parameters in the authentication of delimited plant taxa.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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