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Foliar epidermal studies of the genus *Crotalaria* in Nigeria

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Abstract
Foliar epidermal studies of the genus *Crotalaria* in Nigeria were conducted in Forestry Research Institute of Nigera and Botany Department in University of Ibadan. Eight species of the genus *Crotalaria* namely; *Crotalaria retusa, C. bongensis, C. ononoidea, C. lachnosema, C. comosa, C. naragutensis, C. goreensis* and *C. mucronata* were studied on the basis of micro morphological features of the leaf and pollen with a view to obtaining reliable taxonomic characters for easy identification and delimitation of the species even when they are in fragmentary conditions. Methods follow conventional practice as reported by previous authors of related studies. The mean stomata length in abaxial surfaces of the species studied ranged from 1.2 µm in *C. bongensis* to 25.3 µm in *C. ononoidea* while in adaxial surfaces, the mean stomata length range from 1.1 µm in *C. bongensis* to 20.5 µm in *C. ononoidea*. The distribution of stomata in *C. lachnosema* and *C. comosa* was hypostomatic and amphistomatic respectively delimit the species from others. Anisocytic stomata type was prominent in the studied taxa and this characterized the species in the genus. Additional stomatal type such as Anomotetracytic was also observed in *C. lachnosema, C. comosa, and C. naragutensis.* The shape of epidermal cells in *C. retusa, C. naragutensis* and *C. goreensis* were polygonal on both surfaces while it was irregular in other species (*C. comosa, C. lachnosema, C. mucronata, C. ononoidea.* and *C. bongensis*). Anticlinal walls were straight to curved in all species of the studied except in *C. ononoidea* (Undulate). Trichome observed were simple, unicellular and unbranched on the abaxial surfaces while on adaxial surfaces, trichomes were absent. Pollen grains were tricolporate in the species studied.

Keywords: Epidermal, *Crotalaria*, micro morphological, features, stomata, trichome

Introduction
The genus *Crotalaria* L. belongs to subfamily Papilionaceae, family Leguminosae. It is the third largest genus in the family. According to Polhil (1982) [9], it consists of 600 species worldwide. The genus is mostly found in the tropic and subtropic region and at least 500 species occur in Africa (Nuhu *et al.*, 2000) [6]. They are commonly known as rattlepod, shake-shake or devil-bean. *Crotalaria* species are erect herbaceous, variably hairy plants and may be annual or perennial. The leaves are simple or one to three foliate, alternate, lanceolate to obovate, with a finely hairy under surface. The flowers are yellow on long terminal or axillary clusters, each 5-parted and pea-like, with the leguminous calyx longer than the corolla. The fruit is a leguminous pod, in fliated, hairless, becoming black with maturity and contain 10 to20 glossy black, heart-shaped seeds, which often detach and rattle with many seeds (Alice *et al*, 1997) [2].

The genus is generally adapted to a tropical climate (Samba *et al.*, 2000) [11]. The majority of species have a high requirement for growth; consequently, they usually absent from forest interior but are relatively common in clearings and forest margins (Polhil, 1982) [9]. In Nigeria, this genus has played important role in vertinary Pharmacy. According to Nwude and Ibrahim (1980) [7], it acts as preventive measures in liver diseases of domestic animals. Nuhu *et al.*, (2000) [6] reported the traditional uses of some *Crotalaria* species in Zaria, Nigeria, among which are *C, retusa* L., *C. lachnosema* Stapf, *C. naragutensis* Hutch and many others for feeding of sheep and goat. The genus is also good in traditional uses for treating stomach colic, flatulence, cardiac cases, scabies, rashes, leukemia, spasmodity and neo plasticity (Shirhar *et al.*, 2007) [12]. Most of the species of *Crotalaria* assist in weed and nematodes control (Mukurasi, 1986) [5]. They are very useful in the management of soil fertility. Thomas (2003) [16] recorded that most of the species in the genus are used as food source by some of the larvae of Lepidoptera species such as *Utetheisa ornatris, Etiella zinckenella* and *Endoclita sericeus.*

Anatomical and micro morphological characteristics of leaves have played an important role in plant taxonomy, especially of particular groups at generic and specific levels. Anatomical traits integrated with morphological features help to understand relationship between species (Roeder, and Wiedenfeld, 2009) [10]. Moreover, the use of anatomical features from leaf has been evaluated in solving different kind of taxonomic problems in several plant families (Wollenwebber and Schnesder, 2000) [12]. Although many studies have been conducted in this area for the purpose of correct identification of the genus but little is known about the anatomy and micro morphology of the species under the study. Therefore, the study aimed at determines the anatomical features that would aid the identification and distinguish the species from one another.

Materials and Methods

Plant collection

Fresh specimens of *Crotalaria comosa, C. retusa, C. naragutensis, C. lachsennoma, C. mucronata, C. bongensis* and *C. ononoidea* were collected in Forestry Research Institute of Nigeria, Ibadan and Eruwa-Ibadan road, Oyo state, Nigeria. The specimens were identified and authenticated at the Forest Herbarium, Ibadan (FHI), Nigeria.

Leaf epidermal preparation

Epidermal preparations were obtained using the technique of Olowokudejo and Ayodele (2006). Fresh plant specimens were used for this species. Each sample was macerated in concentrated Trioxonitrate (V) acid for 2-4 hours. The sample was transferred to water in Petri-dish while adaxial and abaxial epidermises were carefully separated using forceps and dissecting needle. The inner parts (mesophyll tissue) of leaves were carefully cleared with camel hair brush and the isolated epidermal layers were washed in several changes of water before transferring in 50% alcohol for 2 minutes to harden them. The tissue was then transferred to a clear glass microscopic slide and stained after draining off excess water, with safranin for less than 4 minutes and excess stain was washed off using a dropping pipette to add and remove water from the tissue. They were later mounted in glycerin on a slide with edge of the cover slip ringed with nail varnish to prevent dehydration and to seal the cover slips on the slides. The slides were labeled appropriately and examined under the light microscope while photomicrographs were obtained at magnification of X400 using Olympus Biological microscope model ex31, fitted with Olympus E-330 digital SLR camera through E330- ADU 1.2 microscope adapter.

Pollen morphology

Pollen morphology was studied using acetolysis procedure by (Sowumi, 1973). Dried buds of each species were used for the study. The flower buds were crushed with a glass rod in centrifuge tube. About 3ml of the prepared acetolysis mixture (9:1 of acetic anhydride and concentrated tetraoxosulphate (VI) acid) were added to the content in the tubes and heated in water-bath at 75°C of boiling point, shaking the tubes occasionally. The tubes were left in water bath for about five minutes. The supernatant was then be decanted into specially labeled bottles (acetolysis waste bottles) leaving the sediment in the tube. Water was added to the sediments in the tubes and shaken vigorously until it is formed. Drops of methylated spirit were added to remove the foam, and the suspension was centrifuged and supernatant decanted. It was then washed with water and centrifuging was repeated four times. Fifty percent aqueous glycerin was added to the sediment and left for two hours. The tubes were shaken vigorously and centrifuged for ten minutes at 4000 r.p.m., which were then decanted and inverted over filter paper for draining. The tubes were left in this position for three hours after which 100% glycerin was added to each tube and shaken vigorously. Each tube was transferred into appropriately labeled storage vials. Slides of the pollen grains were prepared by placing a drop of each content on the slide and cover with cover slip. The cover slip on the slides was sealed using paraffin wax. Photomicrographs of the slides of the pollen grains were taken using Olympus Biological microscope with camera attachment.

Results

Table 1: Stomata characters of eight selected *Crotalaria* species in Nigeria

Species name	Stomata length (µm)		Stomata width(µm)		Number of stomata per view X400	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
C. bongensis	13.5-16.2 (1.2±0.3)	13.5-16.2 (1.1±0.3)	10.8-14.8 (1.3±0.3)	10.8-13.5 (0.9±0.2)	68-88 (7.0±1.7)	57-70 (3.5±0.9)
C. comosa	25.7-31.1 (1.3±o.4)	16.2-37.9 (5.3±1.4)	13.5-22.9 (2.0±0.6)	13.5-24.3 (2.9±0.8)	27-39 (2.5±0.7)	3-17 (2.5±0.7)
C. lachnosema	13.5-18.9 1.6±0.4)	AB	16.2-24.3 (2.3±0.6)	AB	24-89 (12.5±3.7)	AB
C. retusa	21.6-31.0 (1.7±0.5)	21.6-25.7 (1.4±0.3)	16.2-21.6 (1.2±0.3)	13.5-20.3 (1.4±0.4)	33-54 (4.9±1.4)	28-39 (3.3±0.9)
C. naragutensis	16.2-29.7 (2.6±0.7)	16.2-21.6 (1.2±0.4)	13.5-24.3 (3.3±0.8)	13.5-18.9 1.3±0.4	35-71 (11.0±2.8)	27-40 (3.4±0.8)
C. mucronata	16.2-27.0 (1.7±0.5)	16.2-28.4 (2.7±0.7)	21.6-25.7 (1.6±0.4)	10.8-21.6 2.4±0.7	36-68 (6.8±2.0)	17-36 (3.9±1.2)
C. ononoidea	18.9-29.7 (25.3±0.5)	16.2-22.9 (20.5±0.4)	16.2-23.0 (19.6±0.4)	13.5-18.9 (16.7±0.4)	13-21 (16.8±0.7)	13-18 (15.7±0.7)
C. goreensis	17.0-29.1 (3.0±0.9)	16.2-28.4 (2.7±0.7)	13.5-24.8 (3.3±0.9)	13.5-18.9 (1.3±0.4)	36-72 (12.3±2.8)	28-42 (3.6±1.0)

*AB - Absent

Table 2: Qualitative micro characters of eight selected *Crotalaria* species in Nigeria

Species	Shape of epidermal cell		Anticlinal cell wall	
	Adaxial	Abaxial	Adaxial	Abaxial
C. retusa	Polygonal	Polygonal	Straight wall	Straight wall
C. ononoidea	Irregular	Irregular	Undulate	Undulate
C. lachnosema	Irregular	Irregular	Straight undulate curved	Straight undulate curved
C. comosa	Polygonal	Polygonal	Straight to curved	Straight to curved
C. mucronata	Polygonal	Irregular	Straight to curved	Straight to curved
C. bongensis	Polygonal	Polygonal	Straight to curved	Straight to curved
C. goreensis	Polygonal	Polygonal	Straight to curved	Undulate
C. naragutensis	Polygonal	Polygonal	Straight to curved	Straight to curved

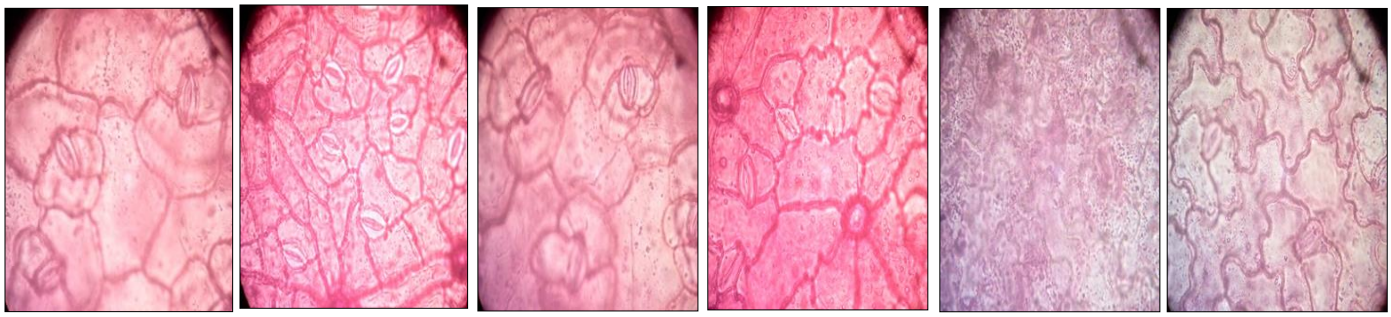
Table 3: Stomata and Trichome features of eight *Crotalaria* species in Nigeria

Species	Stomatal type		Trichomes	
	Adaxial	Abaxial	Adaxial	Abaxial
C. retusa	Anisocytic	Anisocytic	AB.	Trichome base modified
C. ononoidea	Anisocytic	Anisocytic	AB.	AB.
C. lachnosema	AB.	Anisocytic/Anomotetracytic	AB.	Simple, long, unicellular
C. comosa	Anisocytic/ Anomotetracytic	Anisocytic/ Anomotetracytic	AB.	Simple, long, unicellular Scanty
C. mucronata	Anisocytic	Anisocytic	AB.	Simple, long, unicellular
C. bongensis	Anisocytic	Anisocytic	AB.	AB.
C. goreensis	Anisocytic	Anisocytic	AB.	Trichome base modified
C. naragutensis	Anisocytic/Amphianisocytic	Anisocytic/Anomotetracytic	AB.	Simple, long, unicellular

*AB - Absent

Table 4: Pollen features of selected *Crotalaria* species in Nigeria

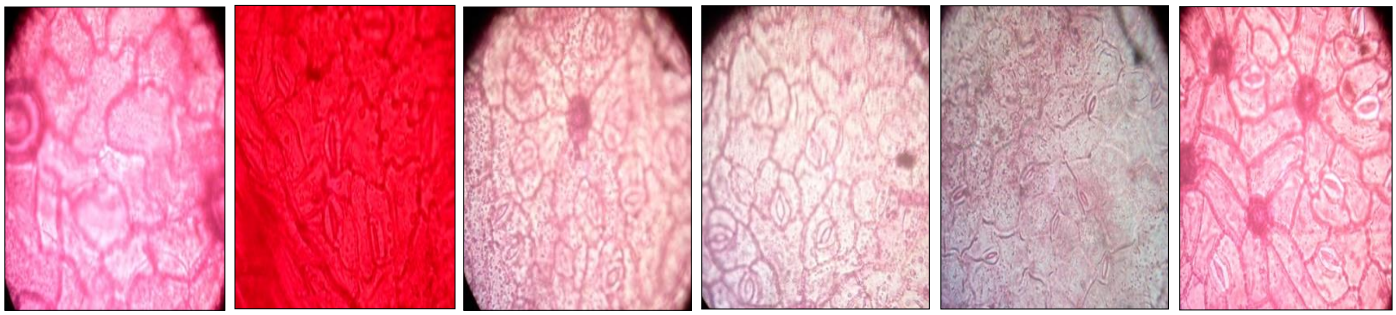
Species	Polar axis (µm)	Equatorial axis (µm)	Shape class
C. comosa	15.5-22.5 (19.3±0.6)	13.8-20.0 (16.0±0.4)	Tricolporate
C. bongensis	15-27.5 (21.8±1.0)	13.8-26.3 (19.0±1.2)	Tricolporate
C. mucronata	15.0-30.0 (23±0.8)	12.5-22.5 (17.3±0.6)	Tricolporate
C. retusa	20.0-35.0 (24.1±1.4)	17.5-30.0 (7.0±1.0)	Tricolporate
C. naragutensis	12.5-20.0 (17.1±0.40)	12.5-17.5 (14.3±0.4)	Tricolporate
C. lachnosema	15.0-21.3 (17.3±1.2)	13.0-17.5 (15.7±2.6)	Tricolporate
C. ononoidea	20.0-31.25 (28.4±0.7)	17.5-2.5 (23.3±0.7)	Tricolporate
C. goreensis	12.5-22.5 (18.3±0.7)	10.0-22.5 (16.8±0.6)	Tricolporate



A B C D E F

A. Adaxial surface of *C. naragutensis* showing polygonal cells, straight to curve anticlinal walls, anisocytic and amphianisocytic stomata.
B. Abaxial surface of *C. naragutensis* showing polygonal cells, straight to curve anticlinal walls, anisocytic and anomotetracytic stomata.
C. Adaxial surface of *C. comosa* showing polygonal cells, straight to curve anticlinal walls, anisocytic and anomotetracytic stomata.
D. Abaxial surface of *C. comosa* showing polygonal cells, straight to curve anticlinal walls, anisocytic and anomotetracytic stomata
E. Adaxial surface of *C. ononoidea* showing irregular cell shape, undulate anticlinal walls, and anisocytic stomata.
F. Abaxial surface of *C. ononoidea* showing irregular cell shape, undulate anticlinal walls, and anisocytic stomata.

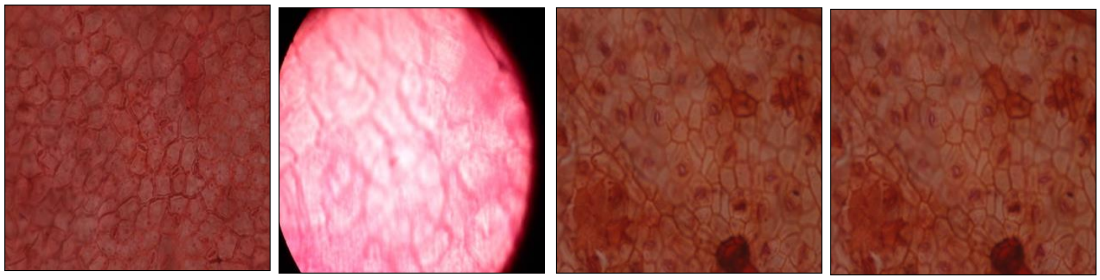
Plate 1: Photomicrographs of leaf surface of *Crotalaria species* x 400



G H I J K L

G. Adaxial surface of *C. lachnosema* showing irregular cell shape, straight, curved and undulate anticlinal walls.
H. Abaxial surface of *C. lachnosema* showing irregular cell shape, straight, curved and undulate anticlinal walls, anisocytic and anomotetracytic stomata
I. Adaxial surface of *C. mucronata* showing polygonal cell shape, straight to curve anticlinal walls, and anisocytic stomata
J. Abaxial surface of *C. mucronata* showing irregular cell shape, straight to curve anticlinal walls. and anisocytic stomata.
K. Adaxial surface of *C. bongensis* showing polygonal cell shape, straight to curve anticlinal walls, and anisocytic stomata
L. Abaxial surface of *C. bongensis* showing polygonal cell shape, straight to curve anticlinal walls, and -anisocytic stomata.

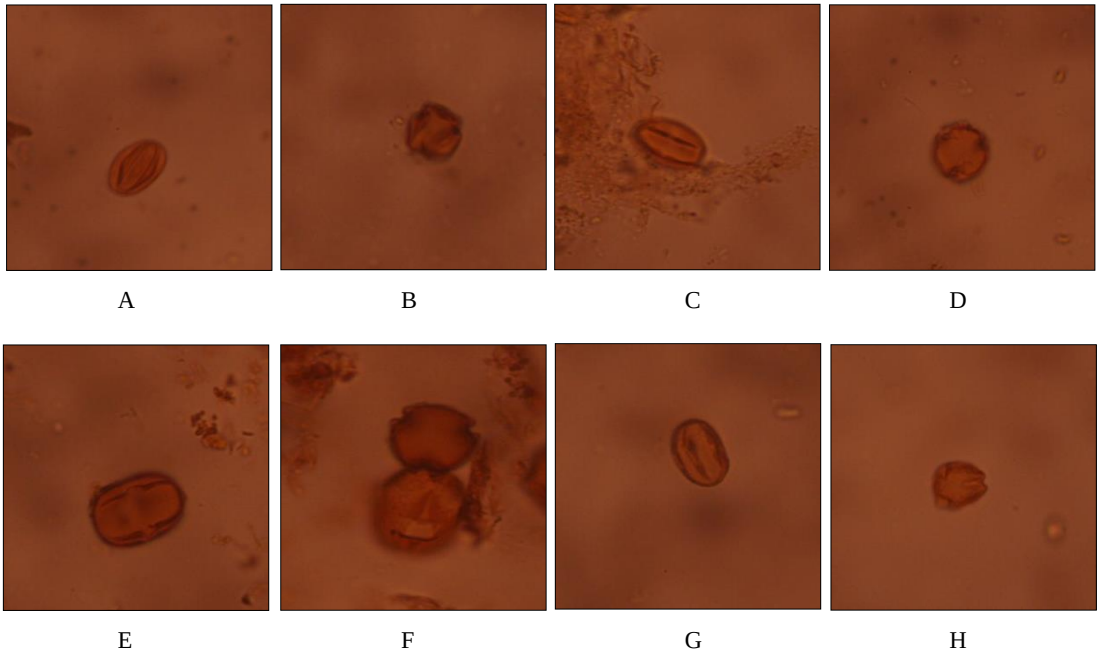
Plate 2: Photomicrographs of leaf surface of *Crotalaria* species x 400



M N O P

Plate 3: Photomicrographs of leaf surface of *Crotalaria* x 400

M. Adaxial surface of *C. retusa* showing polygonal cell shape, straight, undulate anticlinal walls and anisocytic stomata.
N. Abaxial surface of *C. retusa* showsing polygonal cell shape, straight anticlinal walls, and anisocytic stomata.
O. Adaxial surface of *C. goreensis* showing polygonal cell shape, straight to curve anticlinal walls, and anisocytic stomata.
P. Abaxial surface of *C. goreensis* showing polygonal cell shape, straight to curve anticlinal walls, anisocytic stomata.



A B C D

E F G H

A: Equatorial view of the pollen of *C. goreensis*
B: Polar view of the pollen of *C. goreensis*
C: Equatorial view of the pollen of *C. comosa*
D: Polar view of the pollen of *C. comosa*
E: Equatorial view of the pollen of *C. retusa*
F: Polar view of the pollen of C. *retusa*
G: Equatorial view of the pollen of C. *ononoidea*
H: Polar view of the pollen of C. *ononoidea*

Plate 4: Pollen grains of *Crotalaria* species

Discussion

Table 1 shows the stomata characters of the eight selected *Crotalaria* species. The mean stomata length in abaxial surfaces of the species studied ranged from 1.2 µm in *C. bongensis* to 25.3 µm in *C. ononoidea* while in adaxial surfaces, the mean stomatal length range from 1.1 µm in *C. bongensis* to 20.5 µm in *C. ononoidea*. The mean stomata width in abaxial surfaces of the species also range from 1.2 µm in *C. retusa* to 19.6 µm in *C. ononoidea* while in adaxial surfaces, the mean stomata width range from 0.9 µm in *C. bongensis* to 16.7 µm in *C. ononoidea*. This suggests that both the mean stomata in adaxial and abaxial width and length of *C. ononoidea* is higher than other selected species in the study. Ahmed (1979) [1] and Stace (1965) [14] reported that other characters with useful variation in epidermal cells include size, distribution and frequency of stomata are significant parameters in taxonomy and phylogeny. The mean number of stomata per view in both adaxial and abaxial surfaces of the species studied range from 2.5 in *C. comosa* to 16.7 in *C. ononoidea*. This is supported by Ohewandamilo and Akinriulade (1991) that the systematic value of epidermal characters vary from group of plants to another. Stomata are absent in adaxial surfaces of *C. lachnosema* (Plate 2) while the number of stomata in abaxial surfaces of other species are more than their adaxial surfaces (Plate 1, 2, 3). This implies that stomata distribution in *C. lachnosema* and C. comosa is hypostomatic and amphistomatic respectively; however, other species of study are hypo amphistomatic. Epidermal cells are polygonal in *C. retusa*, *C. comosa*, *C. bongensis*, *C. naragutensis* and *C. goreensis* (Plate 1 ,2, 3) on both surfaces except in *C. ononoidea* and *C. lachnosema* (irregular surfaces) (Table 2, Plate 1 and 2) while it is different in *C. mucronata* which contains polygonal and irregular on adaxial and abaxial surfaces respectively (Plate 2). The anticlinal

walls in adaxial surfaces of all the species studied are straight to curve except in *C. retusa* and *C. lachnosema* which are straight to undulate (Plate 2 and Plate 3) while the adaxial surfaces of *C. ononoidea* are completely undulate, thus delimit the species from other species of study (Table 2, Plate 1). Ayodele and Olowokudejo (1997) [3] reported that straight or curve walls are characteristic of species growing in drier conditions while undulate walls are found mostly in species growing in areas of high humidity. Presence of anisocytic stomata (Table 3) characterized all the species of studied while other stomata type such as Anomotetracytic (*C. comosa, and C. lachnosema*) and Amphianisocytic (*C. naragutensis*) delimit the species from other species of study (Plate 1 and 2). This is in line with the study of other Leguminosae family by Maria and Rodriigo (2003) [4] who reported five species of *Bauhinia* with anisocytic stomata type as prominent one in all the species studied. Generally, trichomes are simple, unicellular and unbranched in all abaxial surfaces except in *C. ononoidea* and *C. bongensis* that are absent distinguished those from other species of study (Table 3, Plate 1 and 2).

The pollen characters of all taxa studied are shown in Table 4. The mean polar axis of the species studied ranges from 17.1µm in *C. naragutensis* to 28.4 µm in *C. ononoidea* while the mean equatorial axis ranges from 7.0 µm in *C. retusa* to 23.3 µm in *C. ononoidea*. The shape class of the species studied is tricolporate which characterized all the species (Plate 4). This indicates that the pollen can help in suggesting relationship at specific level or to determine variation within a species or even below the species level.

Conclusion

The characters observed in the genus Crotalaria are diagnostic enough to distinguish and separate the species even when they are sterile or fragmentary. In this study, micro morphological characters such as presence of Anisocytic stomata, straight, curve and undulate pattern of anticlinal walls in both surfaces (Adaxial and abaxial), tricolporate shape of pollen, affinity in term of sizes equatorial diameter and polar diameter are consistent and uniform in all species of study. Hence, these features can be considered for identification of the species. However, the selected species differ in their anatomical characters like absence of stomata in adaxial surfaces (*C. lachnosema)*; hypostomatic and amphistomatic stomata distribution (*C. lachnosema* and *C. comosa*); polygonal and irregular surfaces of epidermal shape; Anomotetracytic (*C. comosa, and C. lachnosema*) and Amphianisocytic (*C. naragutensis*) stomata type and absence of Trichome (*C. ononoidea* and *C. bongensis*) and hence delimit and distinguish the taxa.

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