Bulletin of Pure and Applied Sciences.
Vol.39 B (Botany), No.2.
July-December 2020: P.94-105
Original Research Article

Print version ISSN 0970 4612
Online version ISSN 2320 3196
DOI: 10.5958/2320-3196.2020.00015.4
Available online at www.bpasjournals.com

Comparative Taxonomic study on *Crotalaria* L. from some parts of South-Eastern Nigeria

[1]Ekeke C.*, [2]Agogbua J.U.

Author's Affiliation
[1,2]Department of Plant Science and Biotechnology, Faculty of Science University of Port Harcourt, Nigeria

***Corresponding Author:**
Ekeke C.
Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria

E-mail:
ekeke.uche@uniport.edu.ng

Received on 25.06.2020
Accepted on 30.11.2020

Keywords:
Crotalaria,
Petiole,
Anatomy,
Trichome density

Abstract
Morphological and anatomicalcharacteristics of stem, root, petiole, midrib, and lamina of *Crotalaria* L. found in some parts South-Eastern Nigeria were investigated using microtomy and visual observation to provide additional information to delimit them. Anisocytic, paracytic, anomocytic, tetracytic, one-subsidiary cell and contiguous stomata were recorded among the species. anisocytic was the predominant stomata in the three *Crotalaria* species studied occurring on the adaxaial and the abaxial leaf surfaces. Tetracytic stomata were observed on the abaxial surfaces of *C. goreensis* and *C. verrucosa* but not seen on the leaf surface of *C. retusa.* Also, contuigous stomata were observed on the abaxial leaf surface of *C. goreensis* and adaxial surface of *C. retusa.* Furthermore, stomata in groups of 2 to 3 were recorded on the adaxial surfaces of *C. retusa* and *C. goreensis.* On the other hand, stomata with one-subsidiary cell was recorded on the abaxial surface of *C. retusa* and adaxial surface of *C. verrucosa.*The average trichome length include 36.58 µm in *C. retusa*, 56.62 µm in *C. retusa* and 61.15 µm in *C. verrucosa.* The hairiness or trichome density among the species varied *C. verrucosa* (20 – 23 per 100 objective fields of view), *C. goreensis* (40 – 45 OFV), and *C. ratusa* (76 – 100 OFV). A combination of morphological and anatomical of petiole, midrib, stem anatomy, and epidermal characters (stomata types, trichome size, and density) varied among the species studied and are diagnostic especially when combined the morphological data.

INTRODUCTION

The genus *Crotalaria* L. belongs to Fabaceae, subfamily Papilionoideae. Papilionoideaeis the largest group of legumes with about 475 genera, 14 tribes including Crotalarieae,and nearly 14,000 species worldwide (APG, 2012; Duane and Paul, 2012) and about 335 species recorded in Nigeria (Hutchinson and Dalziel, 1954). *Crotalaria* L. is the third largest genus in this subfamily with at least 500 species predominantly found in the tropic and sub-tropic regions of Africa (Nuhu*et al.,* 2000; Samba *et al.,* 2000). Most of the West African species have been morphologically described (Hutchinson and Dalziel, 1954) and are herbs (annuals and perennials), shrubs, vines, and trees

(Heuzé*et al.,*2018), and are common weeds of cultivated crops, bush regrowths, waste areas, and roadsides (Akobundu et al., 2016).

Members of this genus have been known and reported to have many medicinal uses such as the treatment of diabetics (Pullaiah and Chandrasekhar- Naidu, 2003), skin infection, snake bite, and stomach ache prevention (Verdhana, 2008), treating stomach colic, flatulence, cardiac cases, scabies, rashes, leukemia, spasmodity and neo plasticity (Sirhar*et al*., 2007), source of alkaloids, paper pulp and fibers, ornamentals, green manure (Ansari, 2008).They as used for different agricultural and industrial purposes such as preventing liver diseases infarmanimals (Nwude and Ibrahim, 1980), seeds as a source of fibres, silage, and green manure (Cook and White, 1996; Shankar, 2002; Ansari, 2008), and enhance soil fertility, management of weeds and nematodes (Mukurasi, 1986; Keener *et al.,* 2018), and soap production (Akintayo, 1997). *C. retusa*.,*C. lachnosema*, and *C. naragutensis* are the source of feed for animals in Nigeria (Thomas, 2003; Nuhu*et al*., 2009). Though*Crotalaria*species could be toxic due to the accumulation of pyrrolizidine alkaloids which occur at the flowering and seed formation stage (Nuhu*et al.,* 2009), they have immense economic importance (Okeke, *et al.,* 2019).

Anatomical,epidermal and morphological characters of leaves, stems, roots, and petioleplay important role in plant taxonomy, especially of particular groups at generic and specific levels (Hutchinson and Daziel, 1954; Metcalfe and Chalk, 1972; Roeder, and Wiedenfeld, 2009), and have been used to solve taxonomic problems in several plant families (Wollenwebber and Schnesder, 2000). Marianne et al. (2010) have described the fruit morphology and anatomy of *Crotalaria*. Taxonomically members of this subfamily and genus are well investigated (Shah and Gopal, 1969; Sibichen, 2004; Marcelo et al., 2014; Çildir et al., 2017; Massoud& Zahra, 2016; Odewo et al., 2018) but among Nigerian species,only a few of them are studied. Odewo et al. (2018)studiedleaf epidermal characters and pollen of eight species of *Crotalaria* from Nigeria, Okeke et al. (2019) concentrated on the root anatomy of this genus, andOdewo et al. (2015) investigated their ecological distribution. The anatomy of petiole, midrib, and lamina are yet to be studied therefore,our study concentrated on the species found in South-Eastern Nigeria to provide additional information to delimit them.

MATERIALS AND METHODS

Sample collection and study area

Samples of *Crotalaria*species were collected from some parts of South-Eastern Nigeria (Abia and Rivers States). The plants were properly identified, processed, and deposited in the University of Port Harcourt Herbarium (Table 1). The analysis was carried out in the Plant Biosystematics and Taxonomy Research Laboratory, Department of Plant and Biotechnology, Faculty of Science, University of Port-Harcourt between September 2017 and October 2020.

Table 1: **Voucher specimens of *Crotalaria* species studied**

Species name	Locality	Collection date	Name(s) of collector	Collector's number
C. verrucosa L.	University of Port Harcourt Ecological Centre	10 / 10 / 2014	Ekeke, C. & Ogazie, C. A.	014
C. retusa L.	University of Port Harcourt Teaching Hospital	20 / 05 / 2015	Ekeke, C.	132
C. goreensis Guill. & Perr.	Obiga-Asa, along Aba-Port Harcourt Expressway	14 / 01 / 2020	Ekeke, C.	165

Epidermal studies

Fresh foliar materials were collected from plants growing in the wild and the University of Port Harcourt Ecological Center. The adaxial and abaxial epidermal surfaces were peeled, stained with 1%

safranin, rinsed with distilled water to remove excess stain, mounted in a drop of pure glycerine on clean glass slides; coverslips placed over the peels and sealed with nail varnish to prevent dehydration (Ndukwu and Okoli 1992). The good slides were observed using a trinocular research microscope (T340B) fitted with Amcope digital camera. The epidermal features described by Metcalfe and Chalk (1979) and stomata types are described according to Dilcher (1974). Trichome density of the species was determined based on the method from Olowokudejo, 1990 (Table 2).

Table 2: **Scoring of trichome density in the leaf surface of the species studied**

No of trichomes per 100 objective field of view (OFV)	Description
0	glabrous
1 – 10	glabrescent
11 – 29	sparsely hairy
30 – 49	densely hairy
>50	very densely hairy

Source: Olowokudejo (1990)

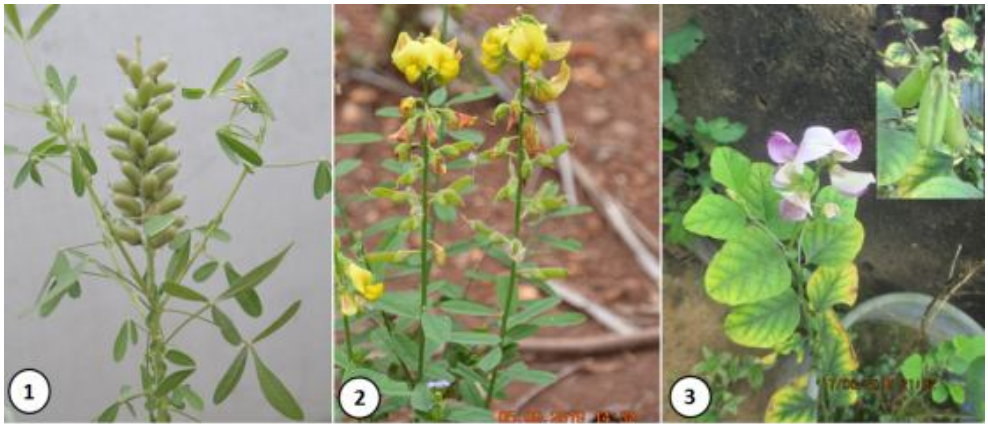
Midrib, petiole, root, and stem anatomy

Two centimeters (2 cm) sections of the petioles, midribs, roots, and young stems were fixed in FAA (formaldehyde: glacial acetic acid: ethanol in the ratio of 1:1:18 parts of 70% ethanol v/v) for at least 48 hours. These materials were washed in several changes of distilled water, dehydrated through alcohol series (30%, 50%, 70%, and 100%) solution for 2 hours in each, and embedded in wax. The embedded specimens were hand-sectionedand thin sections selected, de-waxed and stained with 1% Safranin O and counterstained with Alcian blue, mounted on slides, observed, and micro-photographed with a trinocular research microscope (T340B) fitted with Amcope digital camera.

RESULTS

Macromorphological characteristics

The morphological characteristics of the *Crotalaria* species studied are presented in Figure 1 and Table 2. Noticeable is the difference in flower colour, leaf type, fruit size, and flower size.



Figures 1 - 3: **Epidermal peels of *Crotalaria* species studied. *C. goreensi*, abaxial 1) adaxial 2 and 3); *C. retusa,* abaxial 4), adaxial 5 and 6); *C. verrucossa,* adaxial 7), abaxial 8 and 9); arrow shows contiguous stomata, Tr – trichome base, g – gland, circle – stomata in group.**

Micromorphological characteristics

***C. goreensis*: Epidermis:** Both adaxial and abaxial epidermis are irregular in shape with arced to wavy anticlinal walls. Both surfaces have anisocytic stomata with few anomocytic, tetracytic, contiguous stomata, and average stomatal index of 88.89 on the abaxial surface (Fig. 4), while paracytic stomata including stomata in groups of 2 or 3 on the adaxial surface, and average stomatal index of 75.0 (Fig.

5). Both leaf surfaces have non-glandular trichomes (Fig. 6) measuring 39.14 (56.62) 94.2 µm long, and a trichome density of 40 – 45 per 100 cells on the abaxial surface. The size of stomata on the abaxial surface is 4.68 (5.28) 5.80 × 3.48 (3.96) 4.43 µm, while on the adaxial surface it is 3.63 (4.81) 5.78 × 3.53 (3.78) 4.33 µm (Table 4)**. Lamina:** amphistomatic, abaxial, and abaxial epidermis 1-layer, periclinally elongated. Palisade mesophyll comprised 2 – 3-layers, 17.13 (21.56) 24.91 µm thick, spongy mesophyll 3 – 4-layers 8.23 (10.87) 14.09 µm thick, and vascular bundle interspersed between spongy and palisade mesophylls. Raphides are embedded between the adaxial epidermis and palisade mesophyll or extending to the adaxial surface (Fig. 10, Table 4). **Petiole:** The petiolar cross-section is heart-shaped, V-shaped adaxially, and U-shaped abaxially (Fig. 16). The vascular bundle is U-shaped with 3 – 4-rib traces each on both sides of the arms and surrounded by patches of fiber cells on the abaxial region. **Midrib:** Transverse section shows a V- or U-shaped adaxial and circular abaxial outlines. The vascular bundle formed a semicircular arc surrounded by a layer of parenchyma, and continuous layers of fiber abaxially (Fig. 17, Table 4). The adaxial cortical parenchyma has 3-layers concentrated at the furrow of the midrib, and 3 – 5-layers abaxially. **Stem:** Circular (Fig. 17), 2-layers of epidermis periclinally elongated, the cortex is 4 – 5-layers, with continuous layers of fibers beneath the cortex (Fig. 22, Table 5). **Root:** The root contains lots of starch grains, with patches of sclereids in groups of 2 – 4-cells. The vessels are solitary / partly in radial pairs or multiples of 2 to 3 cells and rays have 2 – 3-cells (Fig. 24).

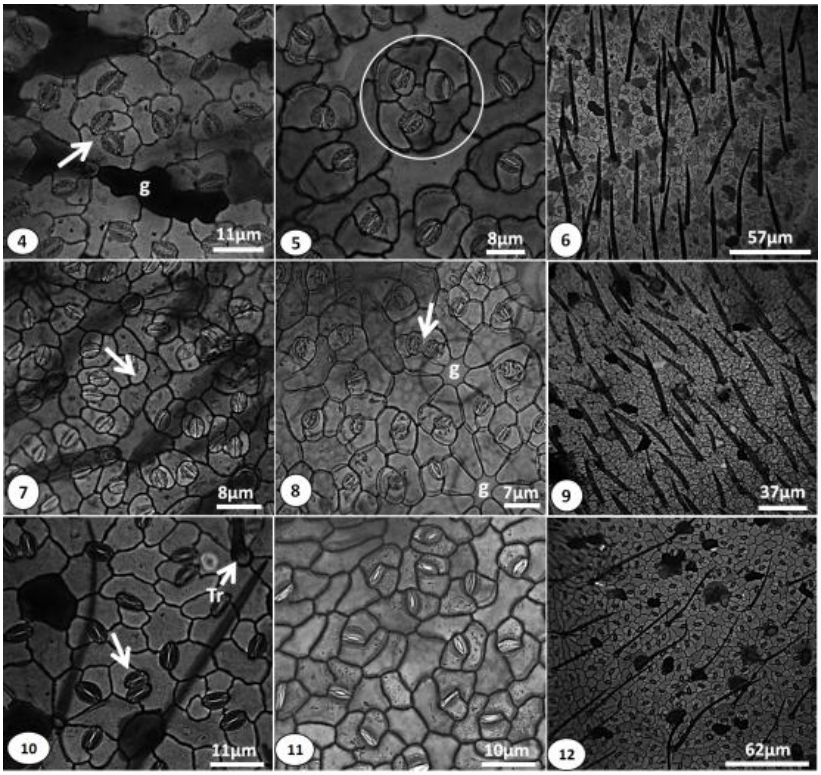
***C. retusa*: Epidermis:** Both adaxial and abaxial epidermis are polygonal in shape with straight to curved anticlinal walls. Both surfaces have mainly anisocytic stomata with few anomocytic or stomata with one-subsidiary cell, and average stomatal index of 82.75 on the abaxial surface (Fig. 7), paracytic stomata including contiguous, stomata in groups of 2 and stomatal index of 72.73 on the adaxial surface (Fig. 8), non-glandular trichomes, (Fig. 9) measuring 33.66 (36.58) 55.02 µm long, and trichome density of 76 – 100 per 100 OFV (Table 4). The size of stomata on the abaxial surface is 3.51 (4.00) 4.87 × 1.99 (2.79) 3.30 µm, while on the adaxial surface it is 2.92 (3.70) 4.06 × 2.40 (2.79) 3.04 µm (Table 3). **Lamina:** Amphistomatic, abaxial, and abaxial epidermis 1-layer, and oval. Palisade mesophyll comprised 3-layers, 32.41 (36.79) 45.16 µm thick with raphides embedded in it. Spongy mesophyll has 2 – 3-layers, 13.41 (15.16) 18.66 µm thick and cylindrical (Table 4). Vascular bundle sheath is embedded between spongy and palisade mesophylls (Fig. 14). **Petiole**: The petiolar cross-section is oval and concave adaxially (Fig. 18). The vascular bundle is arced or semi-circular in shape. Adaxial cortex 11 – 13-layers and abaxial cortex 11 – 12 layers. **Midrib:** Transverse section shows a V-shaped adaxial cuticle. The vascular bundle formed forms an arc surrounded by 2 – 3-layer of parenchymatous cells (Fig. 19). The adaxial cortical parenchyma has 6 – 7-layers concentrated at the furrow of the midrib, and 7 – 8-layers of the abaxial cortex. **Stem:** Has hollow pith with protuberances and stomata on the epidermis, the epidermis has a 2-layer of cells, cortex parenchyma 7 – 11-layers (Figs. 25 and 26, Table 5). **Root:** The root contains patches of sclereids 1 – 4-cells in the phloem and cortex. The vessels are solitary or in tangential pairs or multiples of 2 to 4 cells and rays have 2 – 4-cells (Fig. 27).

Table 2: **Morphological attributes of *Crotalaria* species studied**

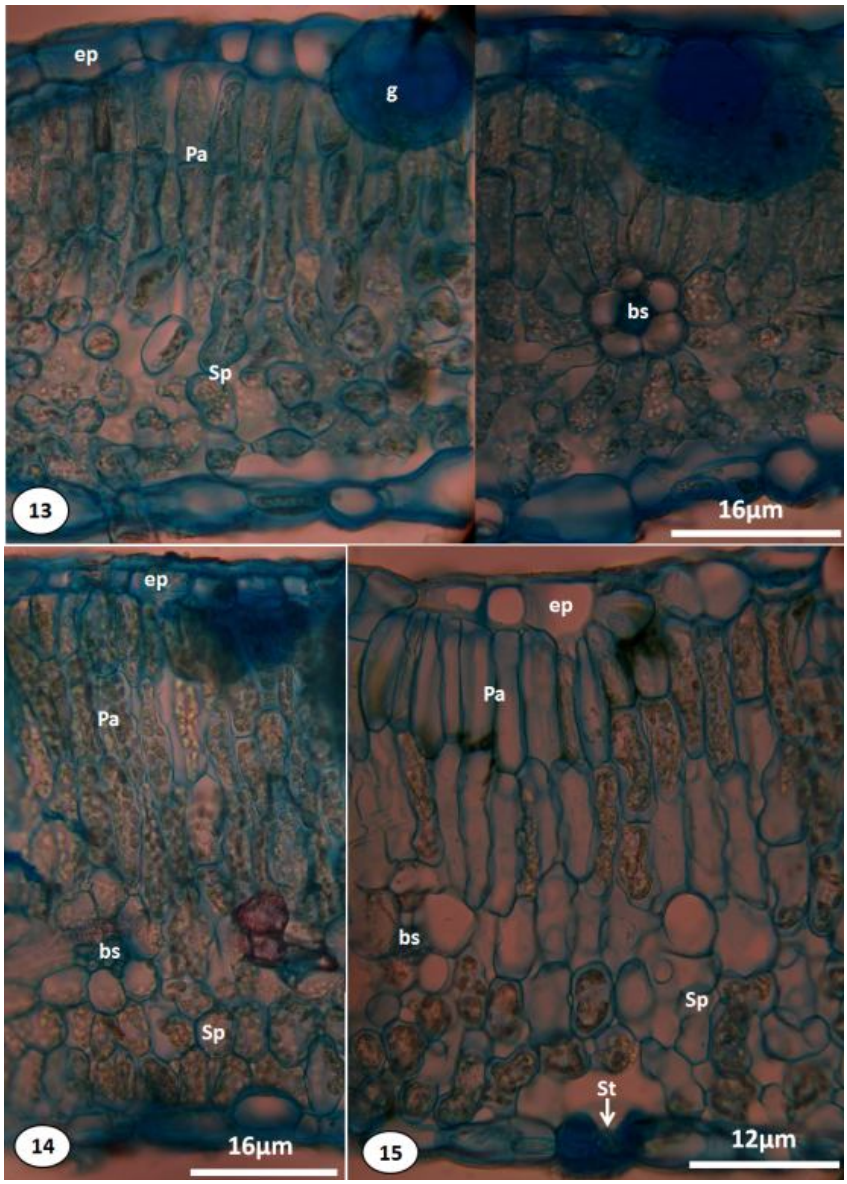
Parameters	**Taxa**		
	C. goreensis	***C. retusa***	***C. verrucosa***
Leaf			
Surface	Hairy	Hairy	Hairy
Shape	Obovate / lanceolate	Obovate / oblanceolate	Ovate / elliptic
Base	Cuncate	Acute	Acuminate
Apex	Rounded / mucronate	Rounded	Rounded
Size (cm)	2.2 – 9.1 × 0.6 – 2.8	3.5 – 8.6 × 1.2 – 2.8	4.8 -12.3 × 3.6 – 7.6
Type	Compound / trifoliate	Simple	Simple
Flower colour	Yellow	Yellow	Blue
Fruit / pod	1.6 – 2.4 cm long, hairy,	2.7 – 5.3 cm long,	3.6 – 6.2 cm long,
Seed size (mm)	2 – 3.5	3.6 – 4.8	3.8 – 5.0
Seed per pod	10 – 18	26 – 28	25 – 30

Petiole length (cm)	2.3 – 6.8		2.0 – 3.5
Stem	4-angled, densely hairy	Glabrous	Hairy
Inflorescence			
Length (cm)	5 – 26 (14.8)	28 – 37 (32.3)	10 – 25 (17.5)
Type	Raceme	Raceme	Raceme

***C. verrucosa*: Epidermis:** Both adaxial and abaxial epidermis are polygonal in shape with straight to curved anticlinal walls. Both surfaces have mainly anisocytic stomata with few anomocytic, and tetracytic on the abaxial surface (Fig. 10), average stomatal index of 86.21; one-celled or paracytic stomata on the adaxial surface (Fig. 11), average stomatal index of 53.84, and non-glandular trichomes (Fig. 12) measuring 43.43 (61.15) 93.81 µm long, and trichome density of 20 -23 per 100 OFV on the of the abaxial surface (Table 4). The size of stomata on the abaxial surface is 5.16 (5.48) 5.92 × 3.17 (3.44) 3.80 µm, while on the adaxial surface it is 4.50 (4.93) 5.46 × 3.86 (4.13) 4.54 µm. **Lamina** amphistomatic, abaxial and abaxial epidermis 1-layer, and oval or anticlinally elongated. Palisade mesophyll cylindrical and comprised 2 – 3-layers, 21.28 (29.38) 47.91 µm thick and spongy mesophyll 3 – 4-layers, 9.07 (11.88) 15.62 µm thick, cylindrical with mucilaginous glands on the adaxial and abaxial epidermis (Fig. 15). The vascular bundle is interspersed between spongy and palisade mesophyll. **Petiole**: The petiolar cross-section is oval or circular, hairy, flat adaxially (Fig. 20). The vascular bundle formed an open arc surrounded by phloem tissues. Adaxial parenchymatous cortex 10 – 12-layers, and abaxial cortical 11 – 12-layers. **Midrib:** Transverse section shows a V- or U-shaped adaxial outline. Adaxial cortex comprised 4 – 6 layer horizontally arranged cells and 5 – 6 layers of vertically arranged cortical cells, abaxial cortex 7 – 10-layers, and vascular bundle formed an open arc (Fig. 21). **Stem:** Hairy, rectangular with 4-projections, the epidermis has a 2-layer of cells, cortex has 6 – 11-layers of but more on the projections, with continuous layers of fibers beneath the cortex (Figs. 28 and 29, Table 5). **Root:** The root contains patches of sclereids in the phloem and cortex. The vessels are in solitary, or radial multiples of 2 to 6 cells, and rays have 2 – 4-cells (Fig. 30).



Figures 4 - 12: **Epidermal peels of *Crotalaria* species studied. *C. goreensi*, abaxial 1) adaxial 2 and 3); *C. retusa,* abaxial 4), adaxial 5 and 6); *C. verrucossa,* adaxial 7), abaxial 8 and 9); arrow shows contiguous stomata, Tr – trichome base, g – gland, circle – stomata in group.**



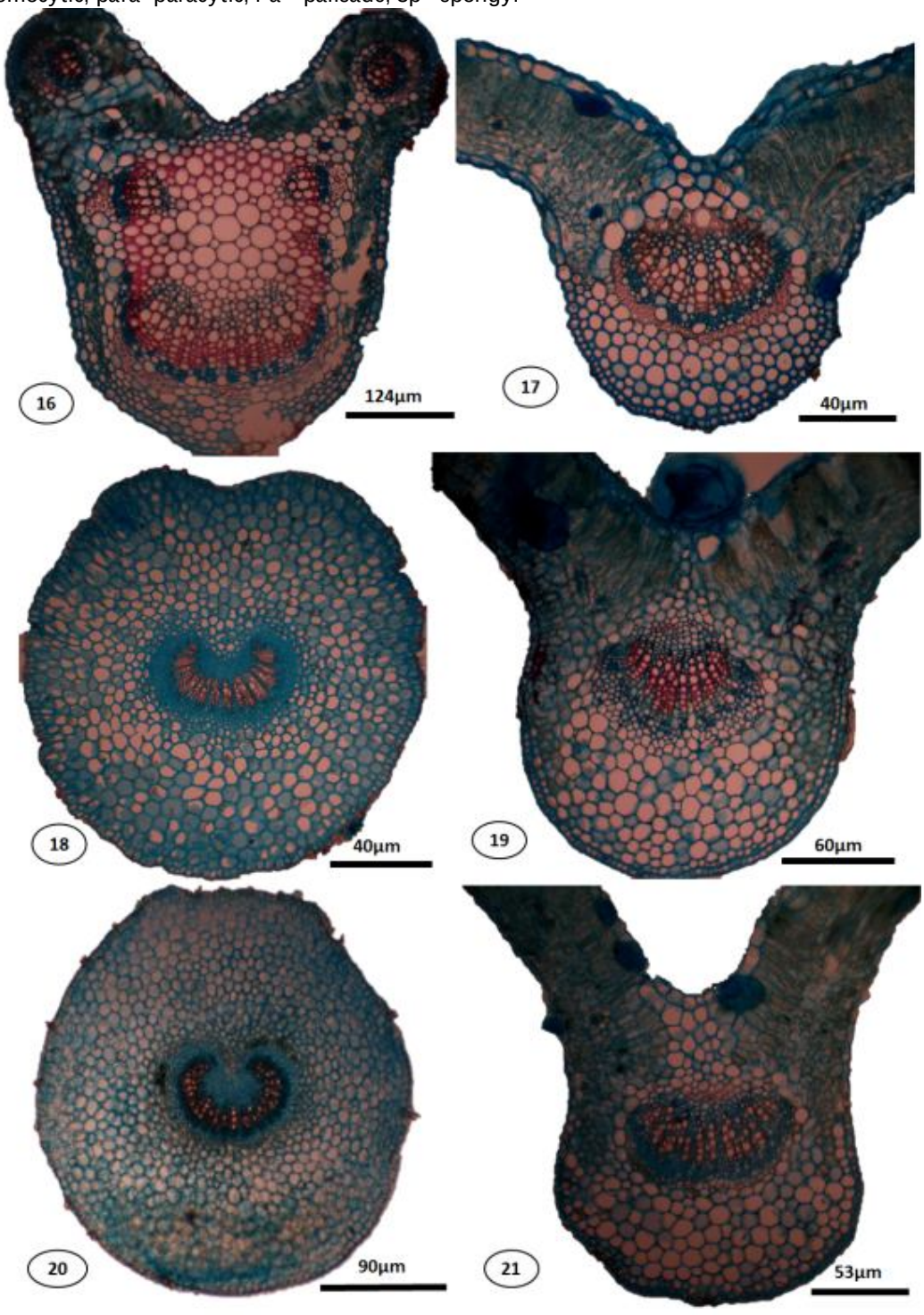
Figures 13 - 15: **Lamina of the *Crotalaria*species studied. *C. goreensis*, 13); *C. retusa,* 14); *C. verricusa,* 15); bs – bundle sheath, Sp – spongy mesophyll, Pa – palisade mesophyll, St – stoma, ep – epidermis, g – mucilage gland.**

Table 4: **Foliar anatomical characteristics of *Crotalaria* species studied**

Parameters	Taxa		
	C. goreensis	***C. retusa***	***C. verrucosa***
Epidermis			
Adaxial	Irregular / wavy anticlinal walls	Polygonal / straight to curved	Polygonal / straight to curved
Abaxial	Irregular / wavy anticlinal walls	Polygonal / straight to curved	Polygonal / straight to curved
Stomata types			
Adaxial	Ani., para and group of 2 or 3	Ani., para, contiguous, and group of 2	Ani.,para. and 1-subsidiary cell stomata
Abaxial	Ani, ano, tetra, contiguous, group of 2	Ani., ano., and 1-subsidiary cell stomata	Ani.,ano., and tetra.
Stomatal size			
Adaxial (µm)	3.63 (4.81) 5.78 × 3.53 (3.78) 4.33	2.92 (3.70) 4.06 × 2.40 (2.79) 3.04	4.50 (4.93) 5.46 × 3.86 (4.13) 4.54
Abaxial (µm)	4.68 (5.28) 5.80 × 3.48 (3.96) 4.43	3.51 (4.00) 4.87 × 1.99 (2.79) 3.30	5.16 (5.48) 5.92 × 3.17 (3.44) 3.80
Stomatal index			
Adaxial	75.0	72.73	53.84
Abaxial	88.89	82.75	86.21
Trichome			
Type	Non-glandular	Non-glandular	Non-glandular
Length (µm)	39.14 (56.62) 94.32	33.66 (36.58) 55.02	43.34 (61.15) 93.81
Density	40 – 45	76 – 100	20 – 23
Lamina			
Epidermis	1-layer, periclinally elongated	1-layer, Oval	1-layer, Partly oval or anticlinally elongated
Palisademasophyll	2 - 3-layers, 17.13 (21.56) 24.91 µm thick	3-layers, 32.41 (36.79) 45.16 µm thick	2 – 3-layers, 21.28 (29.38) 47.91 µm thick
Spongymasophyll	3 – 4-layers, 8.23 (10.87) 14.09 µm thick	2 – 3-layers, 13.41 (15.16) 18.66 µm thick	3 – 4-layers, 9.07 (11.88) 15.62 µm thick
Ratio of Pa / Sp	1.98	2.43	2.47
Mucilage	Present in the adaxial epidermis	In the palisade mesophyll	Present in the adaxial and abaxial epidermis
Petiole			
Shape	Heart-shape	Oval	Oval or circular
Adaxial outline	V-shaped	Concave	Flat
Vascular bundle	U-shaped with 3-4 traces	Open arc	Open arc
Abaxial cortex	4 – 7-layers	11 – 12-layers	10 – 12-layers
Abaxial cortex	2-layers	11 – 13-layers	11 – 12-layers
Midrib			
Adaxial outline	V-shaped	Concave or V-shaped	V- or U-shaped
Vascular bundle	Formed arc surrounded by 1-layer of parenchyma	Formed arc surrounded by 2 – 4-layers of parenchyma	Formed arc surrounded by 2-layers of parenchyma
Adaxial cortex	3-layers	6 – 7-layers	4 – 6-layers
Abaxail cortex	3 – 5-layers	7 – 8-layers	7 – 10-layers

Note: Ani-anisocytic, tetra - tetracytic, ano

- anomocytic, para -paracytic, Pa – palisade, Sp - spongy.



Figures 16 - 21: **Cross-section of petiole and midrib *Crotalaria* species studied. *C. goreensis*, petiole 16) midrib 17); *C. retusa,* petiole 18), midrib 19); *C. verrucosa,* petiole 20), midrib 21)**

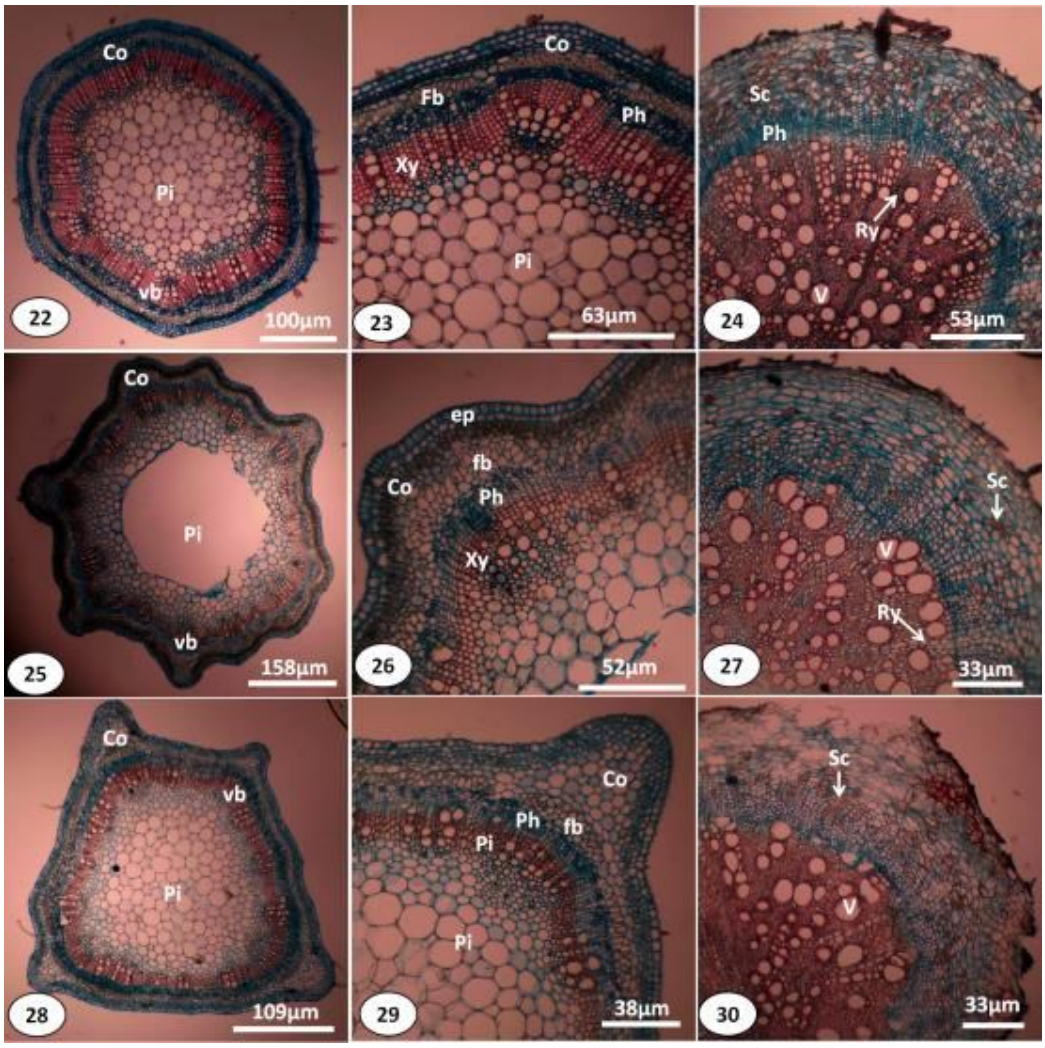


Figure 19 - 27: **Cross-section of Stem and root of*Crotalaria*species studied. *C. goreensis*, stem19-20) root21); *C. retusa,* stem22-23), root 24); *C. verrucossa,* stem 25-26), root27); Co – cortex, vb – vascular bundle, Ph – phloem, Xy – xylem, Sc – scleried,Ry – ray, v – vessel, Pi – pith, fb – fibre, and ep – epidermis.**

DISCUSSION

Morphologically, the major differences among the species studied include the compound/trifoliate leaf in *C. goreensis,* friut sizes, number of seeds per pod or fruit, flower colour and size, and hairiness of the stem. This information conforms with the previous reports by Hutchinson and Dalziel (1954) and Nagar and Albert (2013). Existing reports showed that paracytic stomata are the dominant stomata type in most members of Fabaceae (Massound and Zahra, 2016) and the stomata types in *Crotalaria* L. as paracytic, anisocytic, anomocytic, diacytic, or with one subsidiary cell (Shah and Gopal, 1969; Parveen et al., 2000; Sonje and Bhuktar, 2013). In our study, we observed similar stomata types namely anisocytic, anomocytic, paracytic, or one-subsidiary-celled stomata. Among the *Crotalaria* species investigated by Shah and Gopal (1969), paracytic stomata were dominant followed by anisocytic stomata with diacytic stomata and those with one subsidiary cell being relatively rare. Sonje and Bhuktar (2013) on the other hand reported anisocytic and paracytic measuring 22.5 – 25.0 × 2.5 – 5.0 µm in *C. albida* and average stomatal index of 20.4 and 29.7 on the adaxial and abaxial leaf surfaces respectively. In our work, anisocytic stomata was the predominant stomata in the three *Crotalaria* species studied occurring on the adaxaial and the abaxial leaf surfaces. Other stomata types observed varied slightly in their distribution. For instance, tetracytic stomata

were observed on the abaxial surfaces of *C. goreensis* and *C. verrucosa*but not seen on the leaf surface of *C. retusa.* Also, contuigous stomata were observed on the abaxial leaf surface of *C. goreensis* and adaxial surface of *C. retusa*. Furthermore, stomata in groups of 2 to 3 were recorded on the adaxial surfaces of *C. retusa* and *C. goreensis.* On the other hand, stomata with one-subsidiary cell were recorded on the abaxial surface of *C. retusa* and the adaxial surface of *C. verrucosa.*

Table 5: **Anatomical characteristics of stems of *Crotalaria* species studied**

Parameters	**Taxa**		
	C. goreensis	***C. retusa***	***C. verrucosa***
Shape	Circular	Polygonal with protuberances	Rectangular with protuberances
Pith		Hollow	
Cortex	4 – 5-layers	7 – 11-layers	6 – 11-layers
Vessels	Solitary or in radial multiples or groups of 4 to 6 cells	Radial multiples or groups of 2 to 5 cells	Solitary or in radial multiples or groups of 2 to 4 cells
Fibre	2 – 3-continuous layer of cells	Patched or in clusters	2 – 4 continuous layer of cells or clusters
Rays	1 – 2-tangential cells	1 – 2-tangential cells	1 – 2-tangential cells

Sonje and Bhuktar (2013) reported non-glandular, uniseriate, and unicellular trichomes with an average length of 350 – 1000 µm on the petiole, adaxial, and abaxial leaf surfaces of *C. albida*. Also, Owolabi and Adedeji (2018) observed that *C. retusa* is amphistomatic and contains mucilaginous cells on both leaf surfaces and further reported prevalence of anisocytic stomata including the absence of non-glandulartrichomes on the adaxial leaf surface of this species. Also, Arvinder (1977) reported mucilage in *Crotalaria* and *Tephrosia* species. Our observation is the same as the findings of these authors, however; the trichome size was smaller than the recorded by Sonja and Bhuktar (2013) on *C. albida.* This is evident because among the species studied the average trichome length varied from 36.58 µm in *C. retusa* to 61.15 µm in *C. verrucosa.* The hairiness or trichome density among the species varied *C. verrucosa* (20 – 23 per 100 OFV), *C. goreensis* (40 – 45 per 100 OFV), and *C. ratusa* (76 – 100 per 100 OFV).

Massound and Zahra (2016) separated two varieties of *Trigonellaspruneriana*and*T. disperma* (Fabaceae) base on their leaf epidermal characters (different stomata size and anticlinal cell wall pattern), the shape of cross-section outline (square, circle, and sinuate), presence or absence of crystals, tannins and percentage of anisocytic stomata, number of collenchyma cell-layers and different shape of cross-section (square to circle versus sinuate to elliptic), sclerenchyma fibre layers, and number of vascular bundles in the petiole. Also, Çildir*et al.* (2017) found that anatomical characters such as the number of ray rows in the root, the outline of the stem in the transverse section, the ratio of the wing length to the stem diameter, the number of vascular bundles, the shape of leaf epidermal cells, the pattern of their anticlinal walls, and the presence/absence of trichomes on the leaf of *Lathyrus* L. (Papilionoideae, Fabaceae) from Southwest Asia have taxonomic significance while Parveen et al. (2000), reported that the morphology of epidermal cell walls, nature and number of epidermal cells per unit area, stomatal frequency, and stomatal index is useful in delineating the *Crotalaria* species. Similarly, the combination of anatomical and morphological features such as petiole, midrib, stem anatomy, and epidermal characters (stomata types, trichome size, and density) varied among the species studied and are diagnostic especially when combined with the morphological data.

Acknowledgement

The authors wish to appreciate Mr.ChinedumAlozieOgazie for assisting in plant collection and identification.

Conflict of Interest

The authors have declared no conflict of interest.

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