Morpho-anatomical and histological characters in the systematics of the *Croton* species (*Euphorbiaceae: Crotonoideae*) in Southern Nigeria

Cornelius Onyedikachi Nichodemus* & Chimezie Ekeke

Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria. P.M.B 5323. *email: cornel4nic@gmail.com (corresponding author)

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Abstract. Three *Croton* species, namely *C. hirtus*, C. *lobatus*. and *C. zambesicus* have been examined with a view of using the morphological, anatomical and histological characters in the systematics of the *Croton* species. *Croton hirtus* and *C. lobatus* are herbs with simple and lobed leaves, respectively, while *C. zambesicus* is a shrubby plant, with simple leaves. Variations in the trichome morphology and stomata orientation clearly distinguished the species. Localization, abundance and morphology of tannins and calcium oxalates have differed across the species. The three lines of study have proved useful in highlighting the basic diagnostic characters of the species.

Key words: anatomy, calcium oxalates, *Croton*, morphology, stomata, tannins, trichome

Introduction

Crotonoideae is a subfamily in *Euphorbiaceae*, along with *Acalyphoideae* and *Euphorbioideae* (APG 2009). Taxonomic classification of this species-rich family includes *ca.* 37 tribes, 334 genera and 8000 species (Sá-Haiad & al. 2009). Like *Apocynaceae* and some Monocotyledons and Dicotyledons, *Euphorbiaceae* are characterized by a milky latex (Rudall 1987; Feio & al. 2016), which is innocuous in *Crotonoideae* but poisonous in *Euphorbioideae* (Ramos & al. 2019). The milky latex is secreted from some specialized cells or from a row of parenchymal cells called laticifers (Ramos & al. 2019). *Euphorbiaceae* is one of the largest and complex plant families due to its species richness, diversity in gross morphology and overlapping geographical distribution, which has created ample taxonomic concern within the family and only few taxonomists have general knowledge about the entire family (Punt 1962). Thus, several taxonomists have attempted to resolve the taxonomic problems within the family *Euphorbiaceae*.

Within the family *Euphorbiaceae*, systematic palynological studies have been carried out for the purpose of its classification (Punt 1962; Saad & El-Ghazaly 1988), molecular phylogenetic analysis by plastid sequences (Wurdack & al. 2005; van Ee & Berry 2009), morphological descriptions, and phylogeny of *Croton* (Hutchinson & Dalziel 1958; van Ee & Berry 2009). With ample information on characterization of the member-species of *Euphorbiaceae*, there still exist some levels of complexity within this family. With a view of understanding the complexity within *Eurphorbiaceae*, a taxonomic review of the *Croton* species was carried out (van Ee & Berry 2009; van Ee 2011).

The focus of this work is on *Croton* L., the type genus within the subfamily *Crotonoideae* and tribe Crotoneae (APG 2009), with over 1200 species (Govaerts & al. 2000). This flowering angiosperm genus is pantropical, with few species occurring in the temperate areas and has great diversity of habit, ranging from herbs and shrubs to trees. By way of distribution, *ca.* 150 species are native to Madagascar (Schatz 2001) and 13 species to West Africa, of which eight are endemic to Nigeria (Hutchinson & Dalziel 1958). The economic importance of these species includes ecological aspects and ornamental and medicinal potentials (Burkill 1985). In ethnomedicine, these species help in the treatment of skin diseases, infections, epilepsy, diabetes, malaria, convulsions, have anti-inflammatory and pain-killing effect, etc. due to the presence of glycosides, saponins, steroids, alkaloids, phenolics, and flavonoids (Burkill 1985; Schmelzer 2007; Salatino & al. 2007; Okokon & Nwafor 2009; Soladoye & al. 2012). Although *C. zambesicus* is usually cultivated for ornamental purposes, it is believed to have cultural, religious and superstitious magic impact (Burkill 1985).

Studies on the taxonomic characters of plants with respect to morphology, anatomy and histology are well documented (Franceschi & Nakata 2005; Omoigui & Aromose 2012; Ekeke & Agbagwa 2014; Feio & al. 2016; Ekeke & al. 2020). The importance of such information in understanding the phylogenetic relationships and delimitation of species are highly significant in plant taxonomy (Hutchinson & Dalziel 1958; Metcalfe & Chalk 1979; van Ee & Berry 2010; Vitarelli & al. 2015; Feio & al. 2018). The existing reports on *Croton* mainly concern the non-Nigerian species and are based on species from India, Spain, and Brazil (Bhavana & al. 2020; Ana & al. 2018). In Nigeria, ample information is available on the medicinal potential of the *Croton* species as stated above, and scanty information on their taxonomic characters. Hence, there has been an urgent need to document the basic taxonomic information on plants with a great ethnomedicinal value as keys to understanding their taxonomy. In this light, this research work is aimed at providing more diagnostic characters necessary for the delineation and delimitation of three *Croton* species using morphological, anatomical and histological components.

Material and methods

Plant material

The plant material for this study was collected in the field, pressed, authenticated and deposited in the University of Port Harcourt Herbarium (UPH), Nigeria (Table 1).

Morphological studies

Observations and measurements of the qualitative characters and morphometric (quantitative) characters of the species were carried out. The morphological (vegetative and floral) characters of the species were assessed based on 12 samples from each mature species. Diagrammatically, the species are presented in sketch form.

Epidermal studies

The epidermal layers (adaxial and abaxial) of the species were peeled, scrapped and stored in 70 % ethanol. They were stained with Alcian blue, mounted and viewed under the microscope for clear photomicrographs (Okoli & Ndukwu 1992) using a trinocular research microscope (T340B) fitted with Amscope digital camera. Several fields were observed for the stomata studies (type, index and complex) and trichome morphology. The adopted stomata and trichome classification models were based on Prabhakar (2004) and Webster & al. (1996), respectively.

Anatomical studies

The plant parts were fixed in FAA (formaldehyde: glacial acetic acid: ethanol, in the ratio of 1:1:18) for at least 48 hours, subjected to alcohol series (30 %, 50 %, 70 % and 100 %) for two hours each and stored in 70 % ethanol. Free-hand sections of the stem, leaf midrib and inflorescence were made. Thin sections were stained with 1 % Safranin O, counter stained with Alcian blue, rinsed with distilled water, mounted on slides and viewed under the microscope (Cutler 1978) with modifications. Photomicrographs were taken from clear sections by a trinocular research microscope (T340B) fitted with Amscope digital camera.

Histological studies

The stem, leaf midrib, petiole, and fruit stalk were fixed in FAA (formaldehyde: glacial acetic acid: eth-

Table 1. Checklist of the studied *Croton* species.

S N	Species	Locality, date of collection	Collector(s)	Herbarium number
1.	*C. hirtus* L'Hèrit.	University of Port Harcourt Teaching Hospital; 29/05/2020	Ekeke, C. & Nichodemus, C.O.	UPH/V/1465
2.	*C. lobatus* Linn.	University of Port Harcourt Teaching Hospital; 29/05/2020	Ekeke, C. & Nichodemus, C.O.	UPH/V/1466
3.	*C. zambesicus* Müll. Arg.	Alakahia in Rivers State; 29/05/2020	Nichodemus, C.O. & Ekeke, C.	UPH/V/1467

anol, in the ratio of 1:1:18) for 48 hours, subjected to alcohol series (30 %, 50 %, 70 %, and 100 %) for two hours each and stored in 70 % ethanol. The samples were free-hand sectioned and stained as follows:

Test for calcium oxalates: Selected thin sections were stained with silver nitrate and hydrogen peroxide ($AgNO_3$ + H_2O_2). Records of the morphology, distribution and localization of calcium oxalates were made.

Test for tannins: Thin sections were stained with a mixture of iron II sulphate and formalin ($FeSO_4$ + formalin). Areas stained dark brown to black indicated the presence of tannins (Okoli 1988).

The stained sections were rinsed, mounted, viewed, and photomicrographs were taken by a trinocular research microscope (T340B) fitted with Amscope digital camera.

Results

Plant morphology

A summary of the morphological characters of the species is presented in Table 2. The results are based on 12 samples of each species, with assessed qualitative and morphometric characters. The three species are erect, branching and perennial. Figs 1–3 show the morphological features of the species. The fruits are three-chambered, with non-glandular trichomes.

Epidermal characteristics

Generally, the epidermal results revealed dependable characters across the species. These characters ranged between stomata type and density, epidermal cell shape and anticlinal wall pattern. The stomata orientation revealed that *C. hirtus* and *C. lobatus* were amphistomatic, while *C. zambesicus* was hypostomatic (Figs 4–6 and Table 3). The epidermal cell shapes varied from rectangular and polygonal, to irregular, while the anticlinal cell wall was straight and wavy (Figs 4–6). The present common stomata types were anisocytic (three subsidiary cells), anomocytic (four or more subsidiary cells), tetracytic (four subsidiary cells arranged opposite each other, with uniform shapes), and paracytic (two subsidiary cells parallel to each other), and are presented in Figs 4–6.

C. hirtus

Both adaxial and abaxial epidermis surfaces contained anisocytic, anomocytic and paracytic stomata types (Fig. 4a–b), but differed in the presence of tetracytic stomata on the abaxial surface (Fig. 4b). The epider-

Table 2. Summary of the morphological characters of the studied *Croton* species.

Characters	Plant species/section		
	C. hirtus Geiseleria	***C. lobatus*** Astraea	***C. zambesicus*** Argyrocroton
Plant height	0.9–1.3 m	1.2–1.5 m	12–16 m
Plant habit	Herb	Herb	Shrub or small tree
Leaf shape; apex; base; margin	Ovate; acute; acute; aerrate to dentate	Lobed; acuminate; acute; dentate	Elliptic; acuminate; acute; entire
Leaf type and phyllotaxy	Simple and opposite but seldom alternate	Lobed (3–5) and opposite	Simple and alternate
Leaf venation	Ternate	Palmate	Pinnate
Leaf length by width	2.5–4.6 × 1.4–3.2 cm	3.6–5.1 × 4.8–7.2 cm	3.6–9.8 × 1.6–2.8 cm
Petiole length and hairiness	0.8–1.8 cm; tomentose	1.6–4.4 cm; pubescent	1.1–2.9 cm; tomentose
Vestiture on leaf (adaxial/abaxial)	Pubescent/tomentose	Pubescent on both sides	Glabrous/tomentose
Hairiness of stem	Tomentose (simple to stellate)	Glabrous to pubescent (simple)	Tomentose (lepidote)
Stem width	2–5 mm	3–6 mm	10–15 cm
Pigmentation	Green	Red	Gray
Inflorescence type	Raceme	Raceme	Raceme
Inflorescence length	2.4–4.7 cm	4.7–9.6 cm	2.2–5.4 cm
Hairiness	Pubescent	Glabrous	Tomentose
Number of fruits	6–12	3–5	1–8
Fruit length by width, hairiness	3.6–4.8 × 3.6–3.9 mm; pubescent; persistent styles	5.3–6.2 × 4.8–5.0 mm; pubescent; persistent styles	6–9 × 10–12 mm; tomentose
Styles on fruit	Long style	Branched style	Absent
Flower	White, calyx 5 sepals (4–5 mm) and petals	White, calyx 5 sepals (5–6 mm) and petals	Calyx 5 sepals (3–4 mm), petals

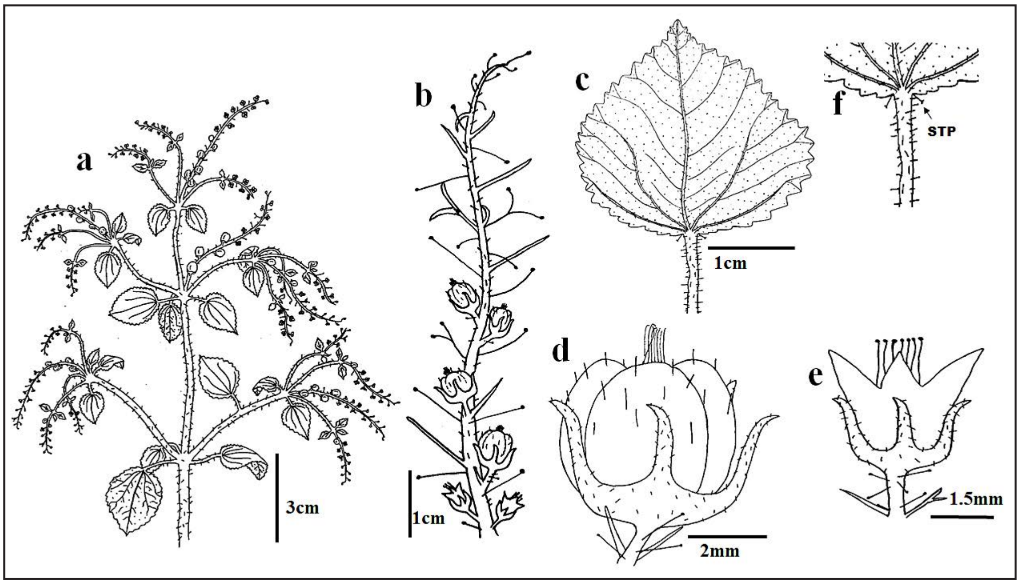


Fig. 1. *C. hirtus* (**a**) Plant habit (**b**) Inflorescence (**c**) Leaf-abaxial surface (**d**) Fruit and sepals with persistent styles (**e**) Flower (**f**) STP- Stipitate gland.

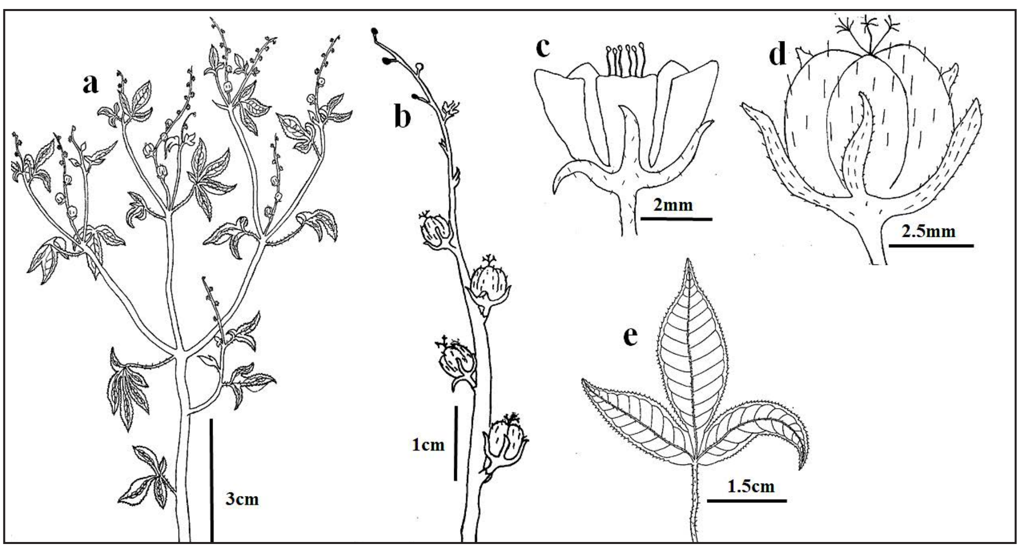


Fig. 2. *C. lobatus* (**a**) Plant habit (**b**) Inflorescence (**c**) Flower (**d**) Fruit and sepals with persistent styles (**e**) Leaf-abaxial surface.

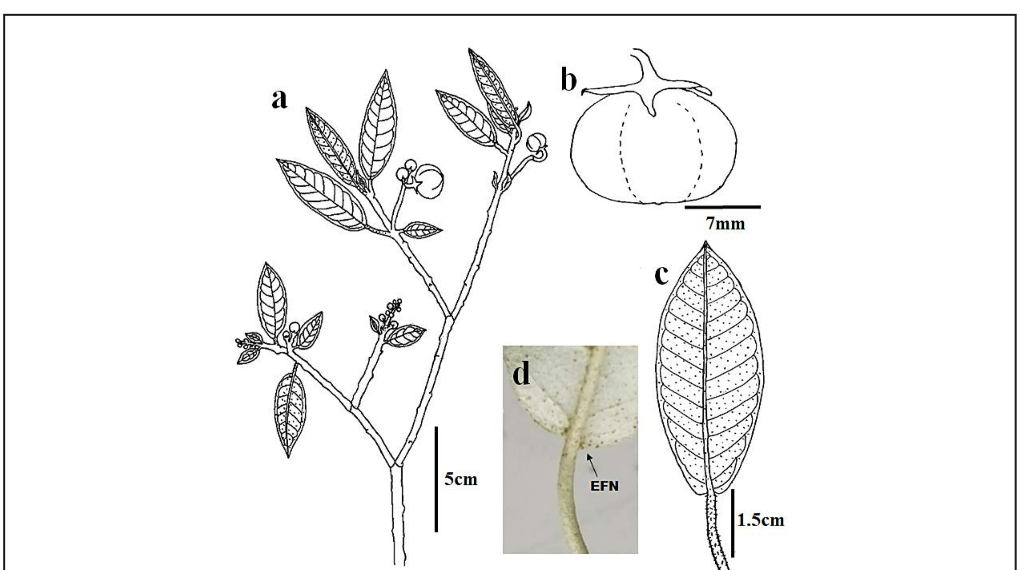


Fig. 3. *C. zambesicus* (**a**) Plant habit (**b**) Fruit with sepals (**c**) Leaf abaxial surface (**d**) EFN- Extrafloral nectaries.

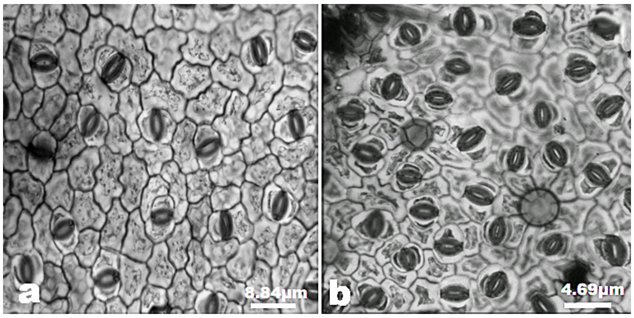


Fig. 4. Epidermal layers of *Croton hirtus* (**a**- adaxial layer; **b**- abaxial layer)

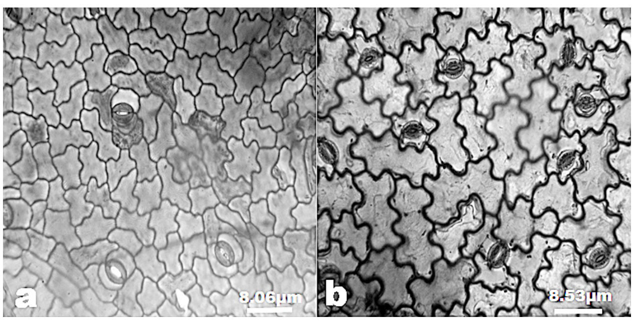


Fig. 5. Epidermal layers of *C. lobatus* (**a**- adaxial layer; **b**- abaxial layer).

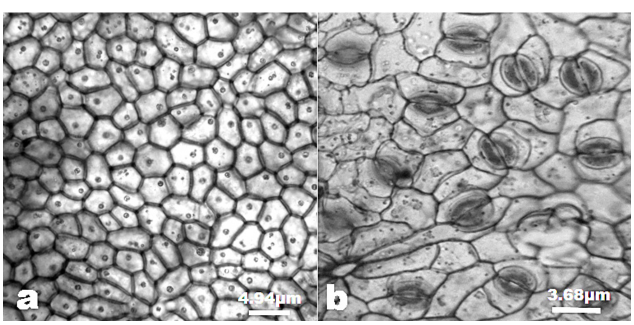


Fig. 6. Epidermal layers of *C. zambesicus* (**a**- adaxial layer; **b**- abaxial layer).

mal cell shape was irregular and the anticlinal wall pattern was slightly wavy on both epidermal surfaces (Fig. 4b).

C. lobatus

Both adaxial and abaxial surfaces had tetracytic and paracytic stomata (Fig. 5a–b), but differed in the presence of anisocytic stomata on the abaxial surface (Fig. 5b). Irregular epidermal cell shapes and a wavy anticlinal wall pattern were observed on both surfaces (Fig. 5a–b).

C. zambesicus

It was hypostomatic, since no stoma was observed on the adaxial surface. The epidermal cell shapes were rectangular and polygonal, with straight cell walls (Fig. 6a). On the abaxial layer, paracytic and tetracytic stomata types were present, with a straight to slightly curved anticlinal wall pattern and polygonal shape of the epidermal cells (Fig. 6b).

Trichome morphology

A variety of trichome types was observed in the studied *Croton* species. These trichomes varied in size, length, shape, distribution, and abundance (Fig. 7 and Table 3). The trichomes were glandular (simple) and non-glandular (simple to multicellular). Morphologically, they ranged from simple, multiradiate or stellate, to lepidote trichomes (Fig. 7a–l). In *C. hirtus*, the adaxial surface had simple and multiradiate or stellate trichomes (Fig. 7a–d), while the abaxial surface had multiradiate or stellate trichomes

Table 3. Summary of the epidermal characters in the studied *Croton* species.

Characters		***C. hirtus***	***C. lobatus***	***C. zambesicus***
Stomatal orientation		Amphistomatic	Amphistomatic	Hypostomatic
Stomatal index	Adaxial	19.6	6.6	–
	Abaxial	45.6	26.9	40.4
Stomatal complex (µm)	Adaxial	8.47–16.51	5.43–14.15	–
	Abaxial	3.43–9.1	6.34–18.34	3.4–6.12
Trichome density/ abundance	Adaxial	++	+	–
	Abaxial	+++	++	+++
Number of radiates per trichome		1–12	1–6	44–62
Trichome orientation	Adaxial	Simple-stellate	Simple	Glabrous
	Abaxial	stellate	simple-stellate	lepidote

Key: – =Absent; + = 1–10 trichomes/83521 μm^2; ++ = 11–20 trichomes/83521 μm^2; +++ = above 21 trichomes/83521 μm^2. Trichome counts were taken at 10× magnification.

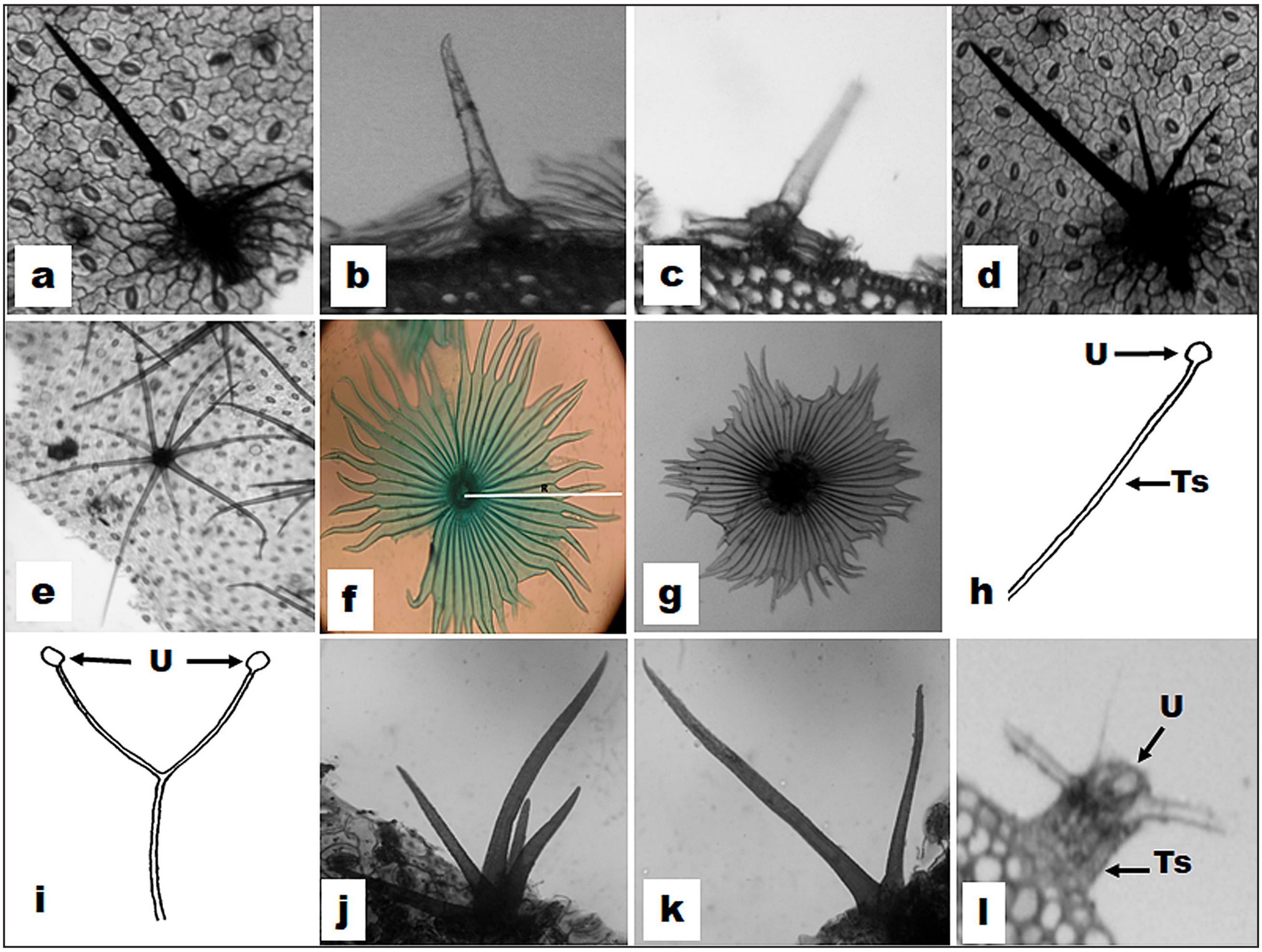


Fig. 7. Variations in trichome morphology observed across the *Croton* species (**a-b**) simple unicellular non-glandular trichome; (**c-e**) non-glandular multiradiate or stellate trichomes; (**f-g**) lepidote trichome; (**h-i**) simple glandular trichome (**j-k**) non-glandular multiradiate or stellate trichome; (**l**) multicellular glandular trichome. **Ts**- Trichome stalk, **U**- Umbo, **R**- Radius.

(Fig. 7a–e), with 6–8 basal cells. The fruit stalk or peduncle of *C. hirtus* had branched and unbranched glandular (Fig. 7i–h), multicellular glandular (Fig. 7l) and non-glandular stellate trichomes (Fig. 7j). *Croton lobatus* showed both simple and multiradiate or stellate trichomes (Fig. 7a, d, e, j and k) on both surfaces, with the abaxial surface containing more trichomes than the adaxial surface. In *C. zambesicus*, the adaxial surface was glabrous, while the abaxial surface was covered with lepidote trichomes (Fig. 7f–g), with 7–9 basal cells. The trichome length ranged from 32.62–200.35 μm. Mention deserves the fact that these trichomes were also found on the stem, petiole, fruit, and inflorescence of the studied species.

Plant anatomy

Summary of an anatomical study of transverse sections is presented in Tables 4–6 and Figs 8–16. The transverse sections of the stem, midrib and fruit stalk showed basic internal structures, such as layers of parenchyma, fiber cells, epidermis, pith, vascular bundles, and trichome morphology.

Distribution and localization of calcium oxalates and tannins

The above-listed ergastic substances were found in the studied species but varied in their abundance, morphology, distribution, and localization across the species (Tables 7a, 7b and Figs 17–20). Tannins and calcium oxalates were localized around the phloem and xylem tissues, parenchyma and collenchyma cells of the stem, at midrib, petiole, and fruit stalk (Figs 17–19; Table 7b). These calcium oxalates were mainly druses and prismatic crystals (Fig. 20). Druse crystals were more abundant in *C. hirtus* and *C. zambesicus*. Tannins were more abundant in the stem of *C. hirtus* and *C. zambesicus*. Density of the druse crystals and tannins differed across the species and is presented in Table 7a. For druse crystals, the ratio for *C. hirtus*, *C. lobatus* and *C. zambesicus* in the stem was 3:2:3, for midrib 3:1:3, petiole 3:1:3, and fruit stalk 2:0:1. For tannins, it was 3:2:3 for the stem, for midrib 2:1:2, petiole 2:2:2, and fruit stalk 2:1:3.

Table 4. Transverse section of the midrib, illustration and description (Ph- Phloem, Xy- Xylem, T- Trichome).

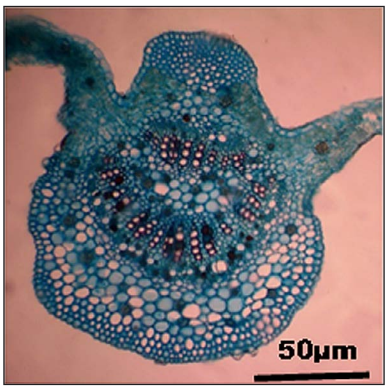
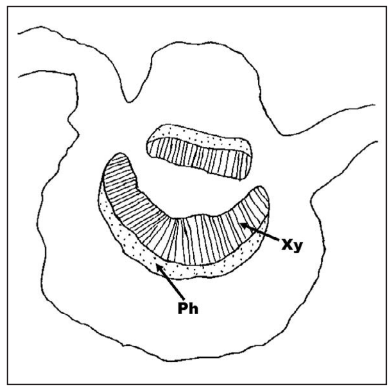
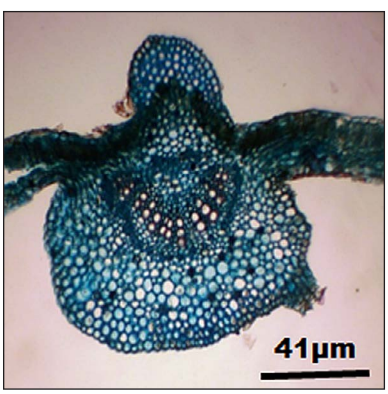
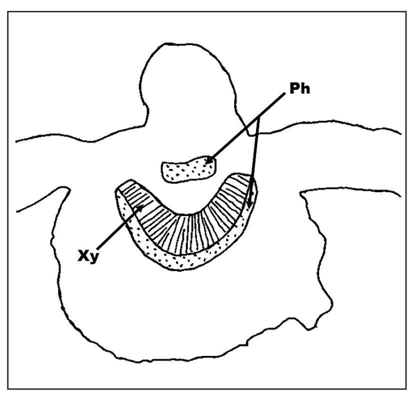
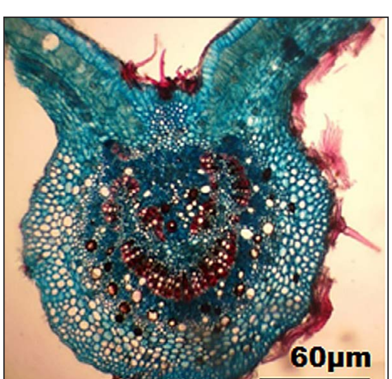
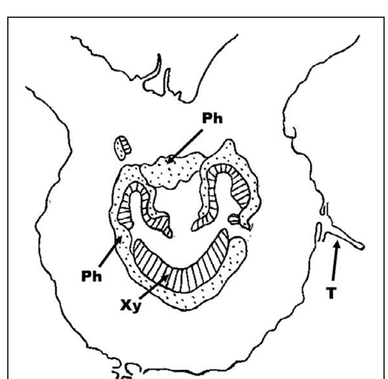
Midrib	Illustration	Description
 *C. hirtus*		Semicircular abaxial surface; convex adaxial surface; 1 epidermal layer; 8–10 layers of parenchymatous cortex; curved vascular bundle with dorsal plate on the adaxial surface; slightly packed palisade and spongy mesophyll; presence of papillae;
 *C. lobatus*		semicircular abaxial surface Convex adaxial surface; 1 epidermal layer; 8–9 layers of parenchymatous cortex; curved vascular bundle with phloem plate on the adaxial surface; palisade mesophyll cuts across the midrib; closely packed palisade mesophyll and loose spongy mesophyll
 *C. zambesicus*		Semi-circle abaxial surface; slightly flat adaxial surface; 1 epidermal layer; 7–9 layers of parenchymatous cortex; curved vascular bundle with slight invaginated ends; closely packed palisade and spongy mesophyll; stellate and lepidote trichomes on the abaxial surface

Table 5. Transverse section of the stem, illustration and description (Ph- Phloem, Xy- Xylem, Fb- Fibre cell, T- Trichome).

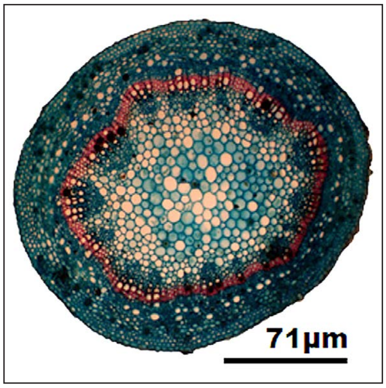
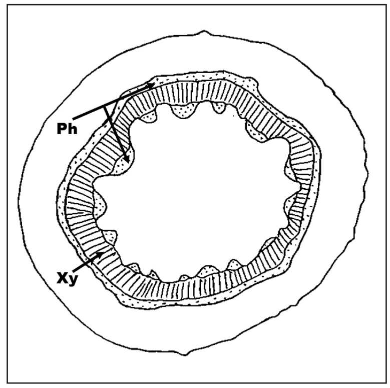
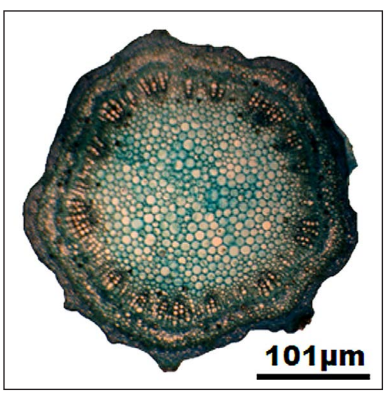
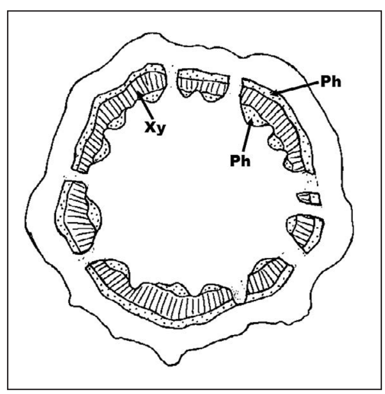
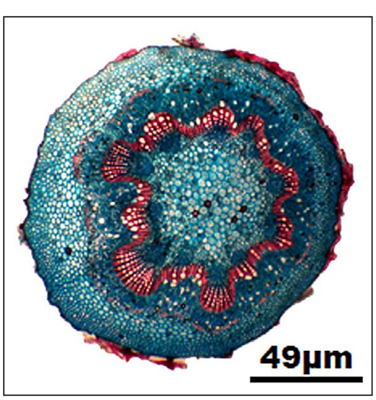
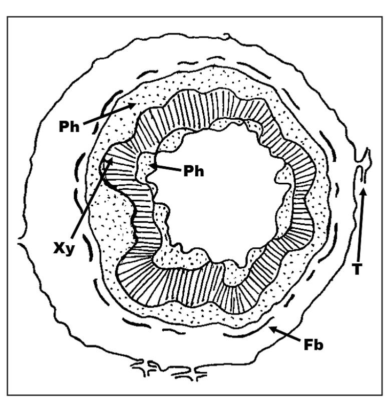
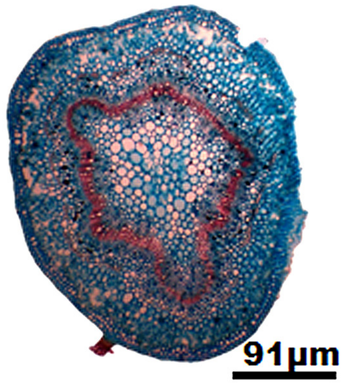
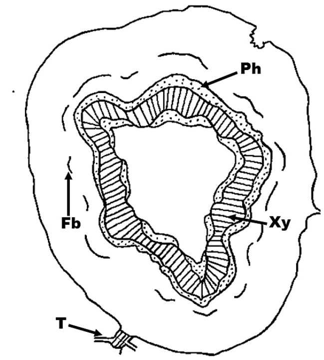
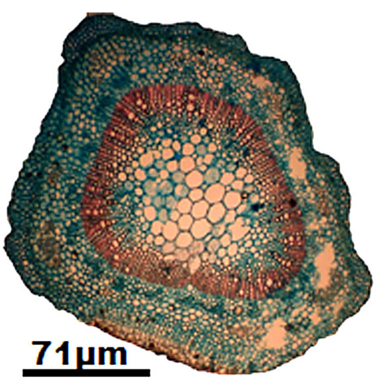
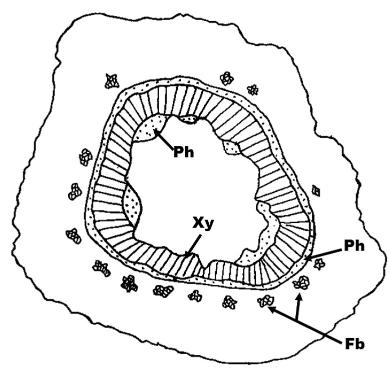
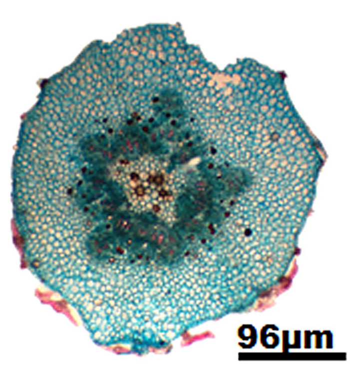
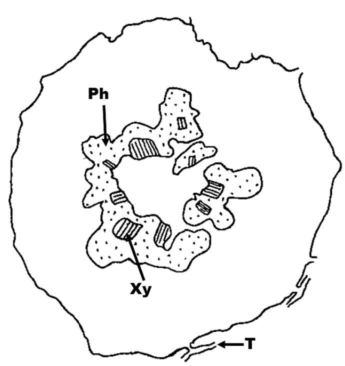
Stem	Illustration	Description
 *C. hirtus*		1 epidermal layer; 2–3 layers of hypodermis; 5–6 layers of endodermis; continuous ring of bicollateral vascular bundle; radial vessel elements; pith; simple and stellate trichomes
 *C. lobatus*		1 epidermal layer; 4–5 layers of hypodermis; 4–6 layers of endodermis; discontinuous ring of bicollateral vascular bundle; radial vessel elements; pith; simple trichomes
 *C. zambesicus*		1 epidermal layer; 4–5 layers of hypodermis; 6–7 layers of endodermis; patches of fiber cells; continuous ring of bicollateral vascular bundle; radial vessel elements; pith; lepidote and stellate trichomes

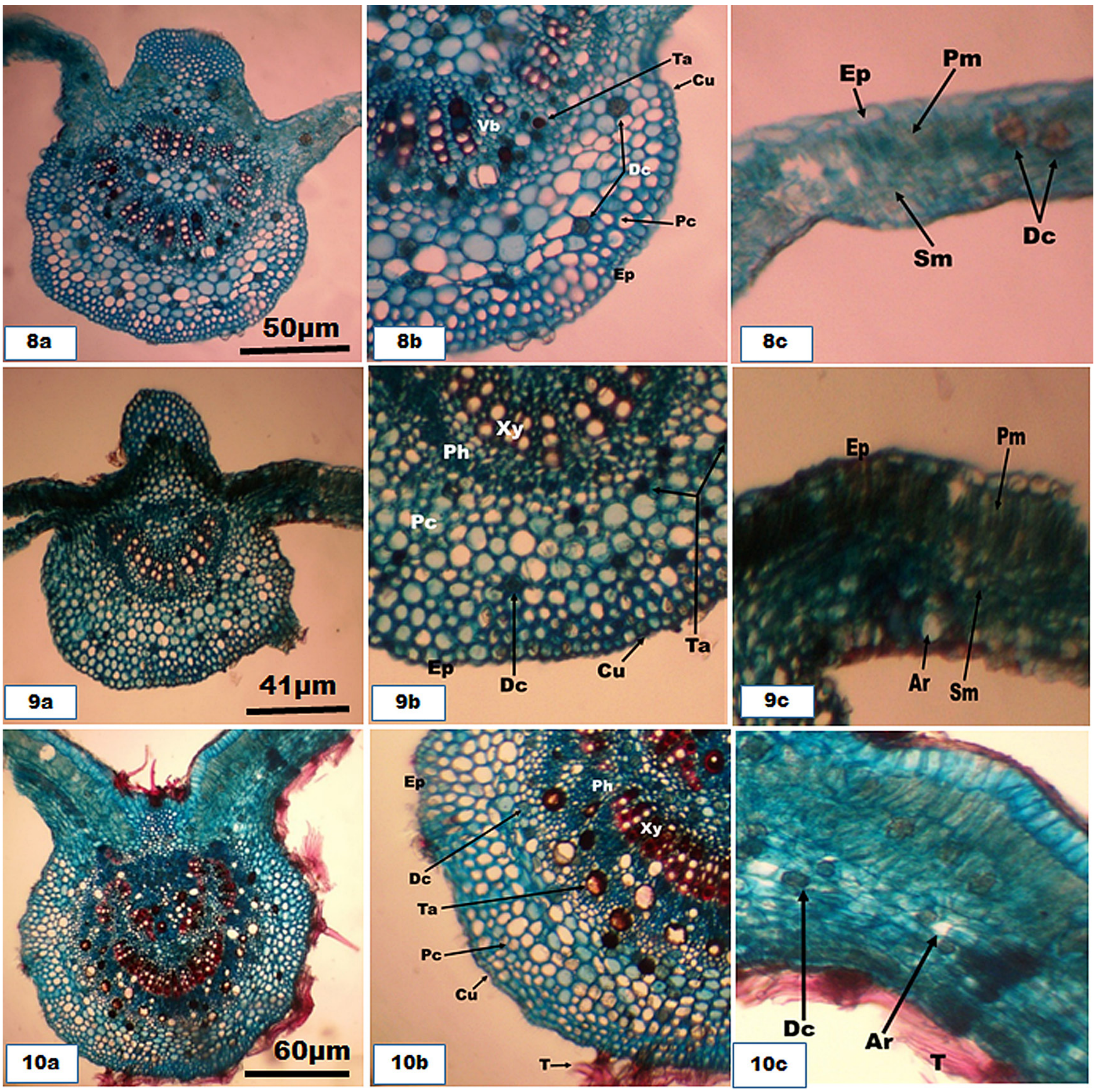
Table 6. Transverse section of the fruit stalk, illustration and description (Ph- Phloem, Xy- Xylem, Fb- Fibre cell, T- Trichome).

<table>
<tr><th>Fruit stalk</th><th>Illustration</th><th>Description</th></tr>
<tr><td>

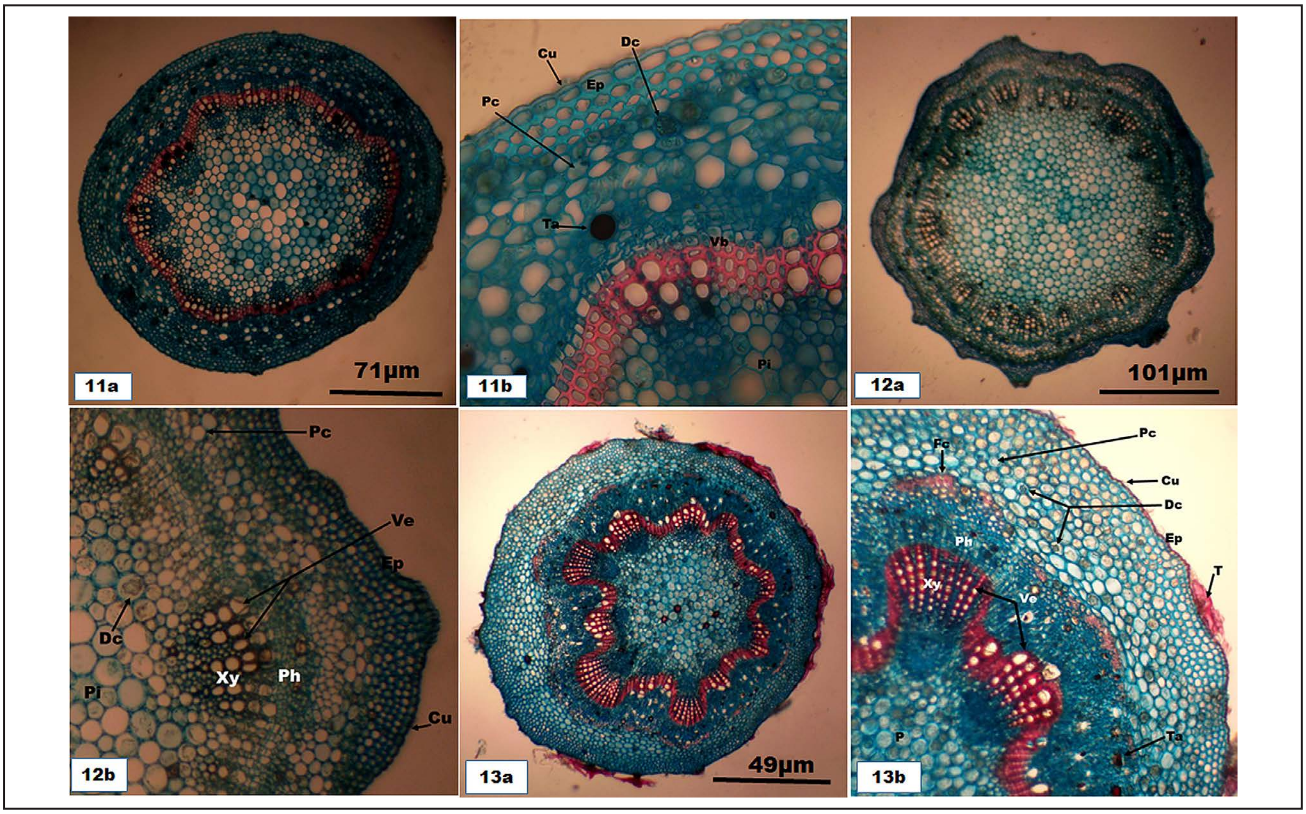
C. hirtus</td><td>
</td><td>1 epidermal layer;
8–11 layers of parenchymatous cortex;
linear fibre cells;
continuous ring of bicollateral vascular bundle;
multicellular glandular trichome;
pith</td></tr>
<tr><td>

C. lobatus</td><td>
</td><td>1 epidermal layer;
10–12 layers of parenchymatous cortex;
closely packed fibre cells;
continuous ring of bicollateral vascular bundle;
pith</td></tr>
<tr><td>

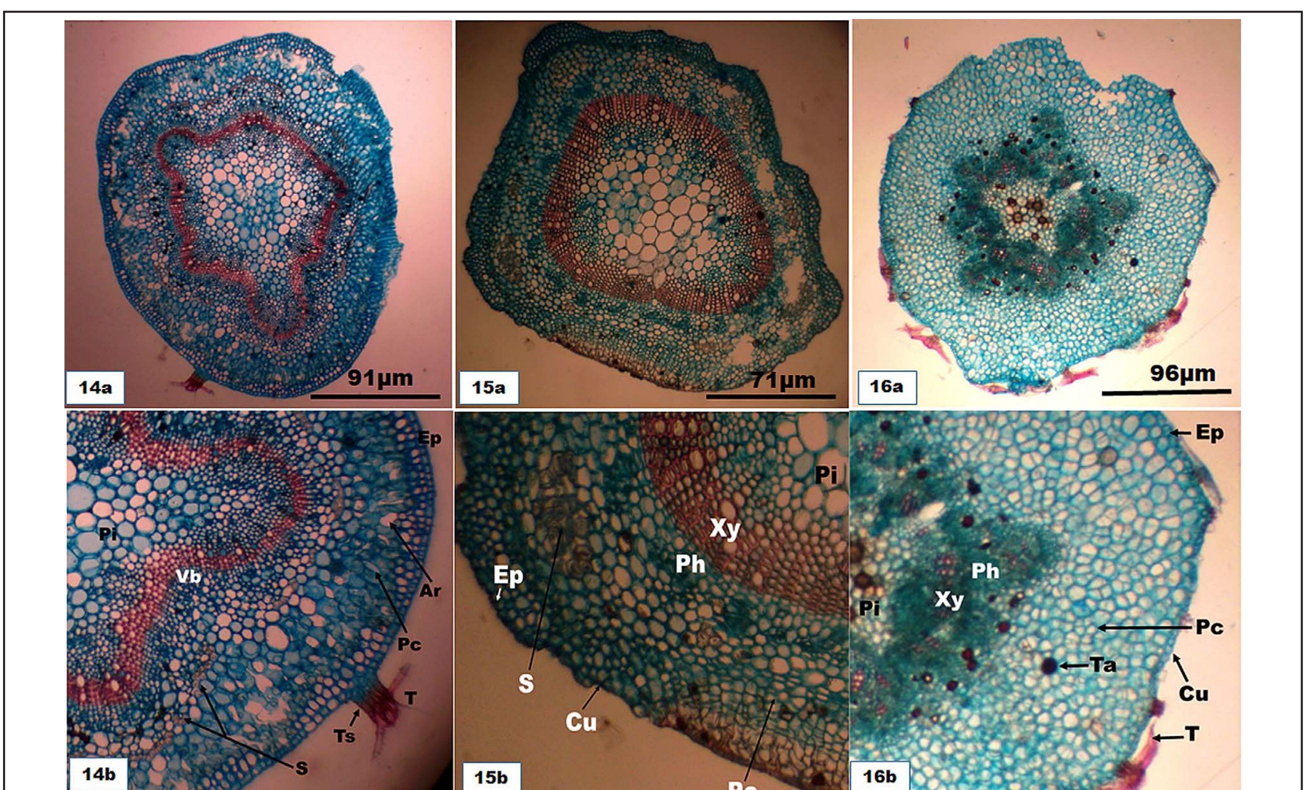
C. zambesicus</td><td>
</td><td>1 epidermal layer;
11–15 layers of parenchymatous cortex;
discontinuous vascular bundle in ring form;
pith;
non-glandular trichomes</td></tr>
</table>



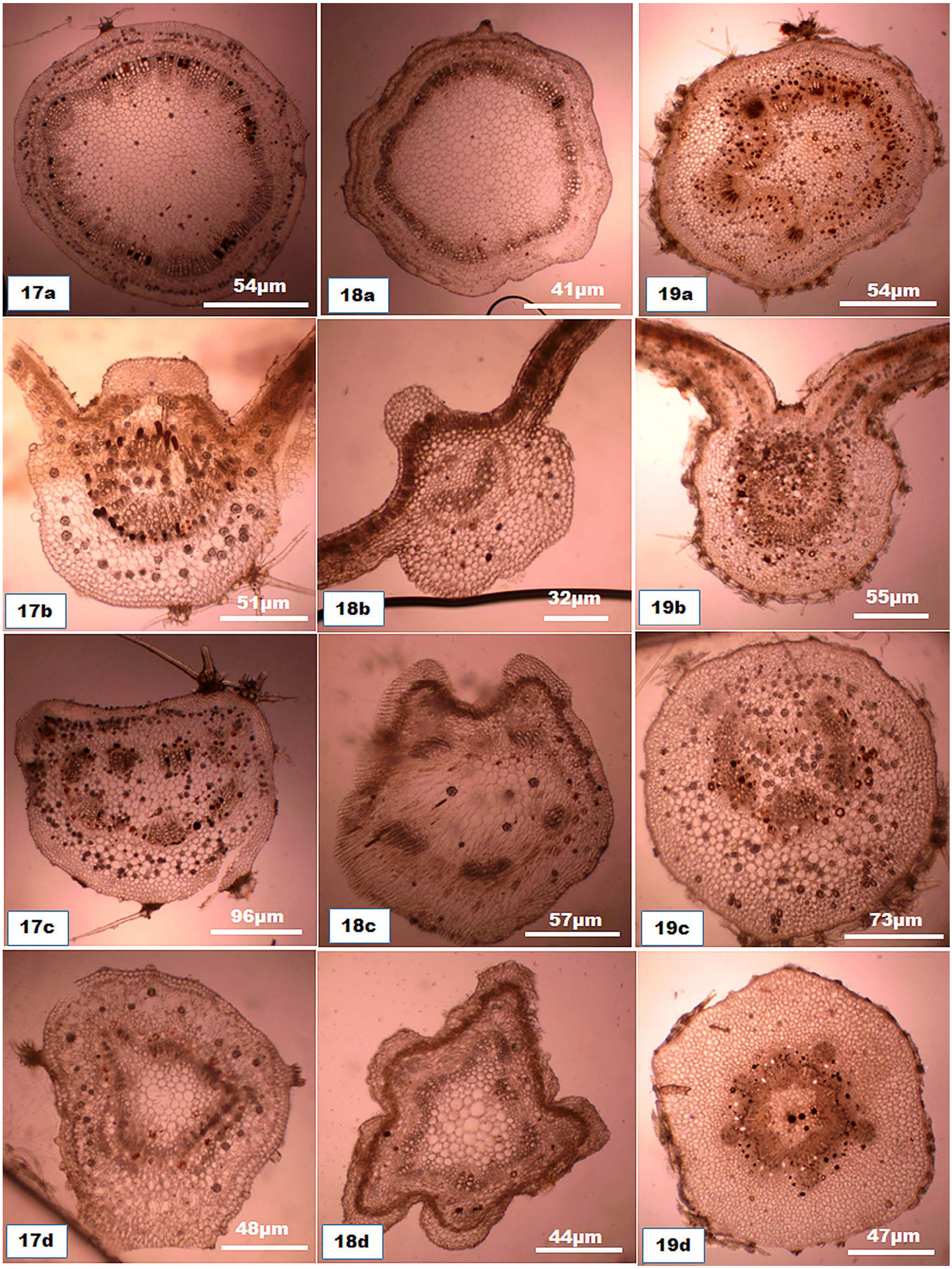
Figs. 8-10. Midrib anatomy. (8) *C. hirtus* (9) *C. lobatus* (10) *C. zambesicus* (**Vb**- Vascular bundle, **Pc**- Parenchymatous cortex, **Cu**- Cuticle, **Ep**- Epidermis, **Ar**- Air space, **T**- Trichomes, **Dc**- Druse crystals, **Ta**- Tannins, **Pm**- Palisade mesophyll, **Sm**- Sponge mesophyll).



Figs. 11-13. Stem anatomy. (11) *C. hirtus* (12) *C. lobatus* (13) *C. zambesicus* (**Vb**- Vascular bundle, **Ph**- Phloem, **Xy**- Xylem, **Pc**- Parenchymatous cortex, **Cu**- Cuticle, **Ep**- Epidermis, **Fc**- Fiber cells, **Ve**- Vessel elements, **T**- Trichome, **Dc**- Druse crystals, **Ta**- Tannins, **Pi**- Pith).



Figs. 14-16. Fruit stalk anatomy. (14) *C. hirtus* (15) *C. lobatus* (16) *C. zambesicus* (**Vb**- Vascular bundle, **Ph**- Phloem, **Xy**- Xylem, **Pc**- Parenchymatous cortex, **Cu**- Cuticle, **Ep**- Epidermis, **S**- Sclereids or fibre cells, **T**- Trichome, **Ts**- Trichome stalk, **Ta**- Tannins, **Pi**- Pith).



Figs. 17-19. Abundance and localization of druse crystals and tannins in the *Croton* species. (17) *C. hirtus* (18) *C. lobatus* (19) *C. zambesicus* **a**- stem; **b**- midrib; **c**- petiole; **d**- fruit stalk.

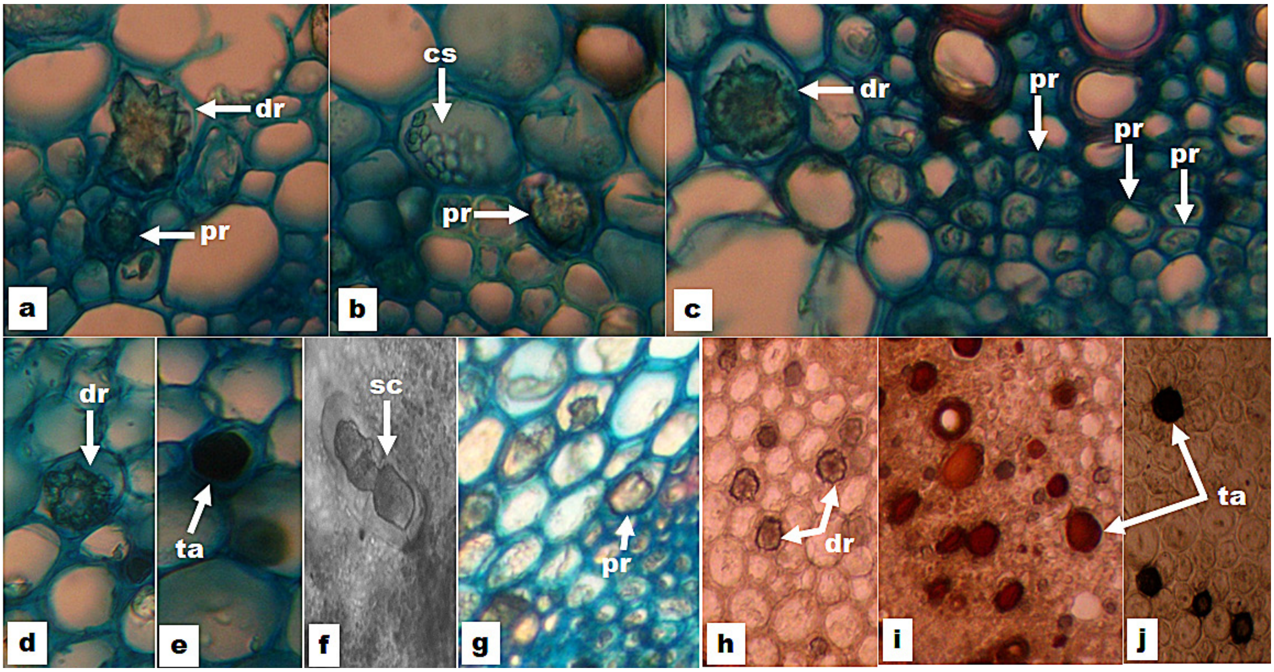


Fig. 20. Morphology of calcium oxalates (a-d, g-h), tannins (e, i-j) and secretory glands (f) in the studied species (**dr**- druse crystals, **cs**- crystal sand, **pr**- prismatic crystals, **ta**- tannins, **sc**- secretory gland).

Discussion

The value of plant taxonomy in description, collection of data and provision of a classification system has been widely acknowledged over the years (Stace 1980). In support of that, we have examined three *Croton species*: two herbaceous plants (*C. hirtus* and *C. lobatus*) and a shrubby one (*C. zambesicus*) in the following order.

Morphology

Hutchinson & Dalziel (1958) provided a unique characterization of the *Croton* member- species found in West Africa based on their morphological features. Among the studied species, a number of diagnostic characters were further observed ranging from the level of pubescence or vestiture, habit and height, pigmentation, branching, and leaf shape (Table 2). A triple branching pattern arising from the node was peculiar to *C. hirtus* and *C. lobatus,* while *C. zambesicus* had a single branching pattern. Similarly, an inflorescence type known as raceme was observed in the species, with alternate fruit arrangement. The inflorescence of *C. hirtus* was characterized by both glandular (2–3 mm) and non-glandular trichomes (1–2 mm).

Generally, the fruits were three-chambered, with trichomes, capsule and dry dehiscence. The considered morphological features confirmed further the reports of Hutchinson & Dalziel (1958). Vestiture (cover of plant hairs) of the *Croton* species was an important feature in the systematics, physiology and ecology of the genus *Croton* (Vitarelli & al. 2016). The trichomes of *C. hirtus* were stronger and spine-like in nature. In general, the trichome length ranged from 32.62 to 200.35 µm. Five types of secretory elements existed within the genus *Croton,* namely, laticifers, colleters, extrafloral nectaries, secretory idioblasts, and secretory trichomes (Machado & al. 2015; Vitarelli & al. 2015; Feio & al. 2016, 2018). The authors have further observed presence of a pair of long stipitate glands at the leaf base in *C. hirtus* and extrafloral nectaries at the leaf base of *C. zambesicus,* which were not spotted in *C. lobatus*. These observation have further confirmed the results of Hutchinson & Dalziel (1958) and Sá-Haiad & al. (2009), which mentioned presence and position of the stipitate glands and extrafloral nectaries on the margin, base and leaf blade of some *Croton* species.

Leaf architecture is an essential tool used in both field and herbarium taxonomy (Dilcher 1974; Stace 1980). Information gathered on the basis of leaf morphology of species clearly shows the distinction of species. Both *C. hirtus* and *C. zambesicus* have simple leaves, while *C. lobatus* has lobed leaves (3–5 lobes). The leaf margin of

C. zambesicus is entire, while *C. hirtus* and *C. lobatus* have dentate margins. Three net venation patterns were observed: ternate-netted in *C. hirtus*, palmate-netted in *C. lobatus* and pinnate-netted in *C. zambesicus*. Leaf morphology of *C. lobatus* has shown some variations in the number of lobes, within the 3–5 range.

Leaf epidermis

Stomata orientation of the species showed that both *C. hirtus* and *C. lobatus* were amphistomatic, while *C. zambesicus* was hypostomatic. Four major stomata types were observed (anisocytic, anomocytic, paracytic and tetracytic), with paracytic stomata as the most dominant. Dominance of paracytic stomata among these species was in conjunction with the findings of Sá-Haiad & al. (2009) of some closely related species in the tribe Crotoneae. Therefore, this implies that the *Croton* species could be characterized by the dominance of paracytic stomata at epidermal layer. The epidermal structures showed that the epidermal cell and anticlinal wall pattern were polygonal to irregular, and straight to wavy, respectively. The *Croton* species were typically characterized by variations of foliar trichomes among its member-species (Berry & al. 2005). Webster & al. (1996) reported seven different trichome types, namely: stellate, fasciculate, multiradiate-rosulate, dendritic, lepidote, papillate, and glandular. Within the framework of this study, the authors have noted three distinct non-glandular trichome shapes, namely: simple, multi-radiate or stellate, and lepidote and simple glandular trichomes. These foliar trichomes presented an integral taxonomic tool for the species differentiation based on their morphology, distribution and density. Simple and stellate non-glandular trichomes were found in *C. hirtus* and *C. lobatus,* while *C. zambesicus* had lepidote and stellate trichomes. Simple glandular trichomes were found on the inflorescence of *C. hirtus*. The presence of such trichomes in these species agreed with the report of Webster & al. (1996) and Vitarelli & al. (2016).

Stem, midrib and fruit stalk anatomy

The anatomical features of the studied *Croton* species are similar to those observed in other species within the same genus (Metcalfe & Chalk 1979, 1983; Hayden & Hayden 1994; Riina & al. 2015; Naidoo 2018). In the present study, the authors have observed variations in the vascular bundle shapes, number of parenchyma layers, vessel elements, and wood fibre cells in the stem, midrib and fruit stalk. In the stem anatomy, fibre cells were found only in *C. zambesicus*. All species had a bicollateral vascular bundle system. A continuous vascular arrangement was found in *C. hirtus* and *C. zambesicus,* and a discontinuous one in *C. lobatus*. Vascular bundle arrangement in midrib anatomy provided a highly diagnostic feature: curved vascular bundle with a dorsal plate (*C. hirtus*), curved vascular bundle with a phloem plate on the adaxial surface (*C. lobatus*), and curved vascular bundle with invaginated ends (*C. zambesicus*). In the lamina, only the palisade mesophyll in *C. lobatus* cut across the midrib. This feature was absent in other species. Fibre cells were found in the fruit stalk of *C. hirtus* and *C. lobatus* around the vascular bundle, but were absent in *C. zambesicus*. The arrangement of fibre cells in *C. hirtus* was linear and in *C. lobatus* was clustered. Only *C. zambesicus* had a discontinuous vascular bundle arrangement. The bicollateral vascular bundle system observed in the stem anatomy of these species was similar to the findings of Hayden & Hayden (1994) and Naidoo (2018) about the vascular system of *C. glandulosus* and *C. gratissimus,* respectively. Therefore, it could be concluded that the *Croton* species have a bicollateral vascular system. Mention deserves the fact that the internal phloem is larger or more extensive than the external phloem.

Histology

Localization, distribution and morphology of ergastic substances in plants are significant in the species taxonomy (Edeoga & Ugbo 1997; Franceschi & Nakata 2005; Idu & Onyibe 2011; Ekeke & Agbagwa 2014). The studied species have revealed diagnostic characters based on the distribution and localization of the ergastic substances (calcium oxalates and tannins). Morphologically, calcium oxalates observed among the species are prismatic crystals, druse crystals and crystal sands. Morphology of the present prismatic crystals is rectangular, rhombus and rod-like. Different variations of the prismatic crystals and large-sized druses have been observed in *C. zambesicus,* which are of taxonomic value. Druse crystals of varying sizes (medium to large) have predominated in the stem, midrib and petiole of *C. hirtus* and *C. zambesicus*. Morphology of the druse crystals has been similar to the findings of Ekeke & Agbagwa (2014) in the genus *Combretum*. Calcium oxalates and tannins were more abundant in *C. zambesicus* and *C. hirtus*, and scanty in *C. lobatus*, which indicates differences in the density of druse crystals and tannins across the transverse section of stem, midrib, petiole, and fruit stalk of the species. Druses have been also found in the lamina of *C. hirtus* and *C. zambesicus*. No calcium oxalate has been found in the fruit stalk of *C. lobatus*. The tannins in these species have been localized around the phloem tissues of the vascular bundles and parenchymatous cells of the stem, midrib, petiole, and fruit stalk. Abundance of tannins (tanniferious tissues) has differed between species and could be of diagnostic importance. This contributes further information on the presence of tanniferious tissues in the *Croton* species reported by Feio & al. (2016) in the parenchyma cells of *C. echinocarpus* and *C. urucurana*.

Mention deserves the fact that the taxonomic implications of the three lines of evidence (morphology, anatomy and histology) employed in this study have generated dependable diagnostic characters that could aid identification and delimitation of the species. Morphologically, *Croton hirtus* and *C. zambesicus* have simple leaves, while *C. lobatus* have lobed leaves. These leaves are important in ethnomedicine and are used in the treatment of various ailments, in addition to the roots of *C. zambesicus*. Furthermore, a combination of druses and tannins, along with the other characters, could have notable taxonomic implications. Investigations of the anatomy and histology of the studied *Croton* species in Nigeria are presented for the first time.

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