Light Microscope Study of the Stems and Petioles of Some Species of *Cissus* in Nigeria

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Abstract. The internal features in the stems and leaf petioles of *Cissus quadrangularis, C. arguta, C. glaucophylla, C. aralioides* and *C. rufescens* have been studied with the aid of light microscope. Transverse sections of the stems and petioles were prepared into slides following standard procedures. Observations of the slides through the microscope revealed internal features of diagnostic significance in all the *Cissus* species. Features such as outline of stem and petiole epidermis, leaf trace bundles, orientation and number of units of vascular bundles and occurrences of starch grains and raphids where part of the key internal morphological features observed in the species. These internal features were not uniform in their occurrences in the five species as there were instances where some of the features were not found. Consequently, the epidermis of stem of *C. arguta* and *C. quadrangularis* developed six and four loops respectively. These loops gave them characteristic undulating epidermal outline that distinguished them from the rest of the *Cissus* species examined. Each of the loops showed localized collenchymal cells. Furthermore, the occurrences of leaf trace bundles in the stem of *C. glaucophylla* and *C. aralioides* is diagnostic as they were absent in the rest of the species. Raphids were observed in the stem and petiole of *C. quadrangularis* but present only in the leaf petiole of *C. arguta*. In all, only the stem of *C. aralioides* revealed deposits of starch grains in most of the cortical cells. Radial bundles arranged in ring-like pattern were observed in both the stem and leaf petioles of the *Cissus* species. It was asserted that the internal features observed were diagnostic, descriptive, useful and reliable in the identification of the species when they appear in sterile or shriveled forms and also supported the present taxonomic status of the species as separate individuals belonging to the same genus. It was stressed that the internal features of the stems and petioles of *C. arguta, C. glaucophylla, C. aralioides, C. quadrangularis* (petiole) and *C. rufescens* are being reported for the first time as there exist no or little information about their anatomy.

Key words: *Cissus*, stem, petiole, internal features, diagnostic

Introduction

According to Timmons *et al*. (2007) the genus *Cissus* Linn belongs to the family Vitaceae. This family encompasses 14 genera and over 850 species (Mabberley, 1997). In the family, *Cissus* is the largest genus with 200 to over 350 species of tropical distribution (Smith, 1977).

Cissus quadrangularis Linn, one of the species from the monophyletic clade of Rossetto *et al.* (2002a) whose distribution extends along the east coast of Australia (Jackes, 1988), is an example of a species that is used medicinally for the treatment of gout, syphilis and other venereal diseases, piles, leucorrhoea, dysentery and bone fractures (Shirwaikar *et al.*, 2003). This specie has thick, succulent, four-sided, stems that constrict at the nodes. Phyllotaxy is alternate and distichous. Leaves are three-lobed and flanked at the base by a pair of stipules that are attached to and as wide as the elongating shoot. It produces adventitious roots at nodes and is easily propagated asexually by cutting or layering. The simple (unbranched) tendrils are produced after and opposite leaves at each node in a continuous pattern. The Plants climb over substrates using the structural support of firm stems and by the twining of tendrils. Axillary buds, present at every node, are supernumerary with the youngest bud closest to the shoot. Reproductive shoots arise from axillary buds and terminate after initiating three to four

inflorescences. Reproductive shoots follow the same architectural pattern as the vegetative shoots, with the exception that inflorescences occur in place of tendrils and leaves are reduced in size but still contain axillary buds. Inflorescences begin as typical cymes and later produce branches and flowers resulting from bifurcations of the inflorescence meristem. Flowers are perfect and tetramerous, rarely trimerous. Petals are yellow, and reflex at anthesis and basally dehisce within 2 days.

Cissus arguta Hook. f. is an herbaceous climber with somewhat succulent square stems of the forest in Liberia and Southern Nigeria. The stem is widely scandent, somewhat succulent and glaucous. The internodes are short (Hutchinson & Dalziel, 1960). The fruit measures about 1.02cm in diameter. It is ovoid and occasionally eaten. The chromosome number of Nigeria material is recorded as n=10 (Burkill, 2000).

C. aralioides (welw.) Planch. is a strong climber to the top of the forest canopy, stems are green, succulent and woody at the base. It is common in the deciduous forest and fringing jungle across the region of Senegal to North and Southern parts of Nigeria (Burkill, 2000). The whole plant is covered with irritating hairs. The flowers are greenish white, comparatively large in horizontal many-flowered cymes. The fruits measures 2.04cm long, red, ripening blue and making an attractive vine worthy of cultivation. In Congo (Brazzaville), the stem is used for its analgesic and antiseptic attributes to relieve cough and by embrocation for body pain in fever, rheumatism, abdominal and kidney problems. The sap is applied to sores and swollen buboes (Burkill, 2000).

C. rufescens Guill. & Perr. is an herb with a perennial underground root-stock throwing up annual prostrate creeping stems. It is distributed across the Savanna regions of Senegal, Sudan, Uganda and North and Southern Nigeria. The leaf decoction serves as a poison antidote or as a purgative (Burkill, 2000).

C. glaucophylla Hook. f. is a large herbaceous climber with tough stems, pink flowers in bud, cream when open (Hutchinson & Dalziel, 1963).

According to Andreia *et al.* (2012) the anatomy of plants is an important taxonomic parameter for the certification and quality control of medicinal plants, and for the localization of secretion and/or accumulation sites of biologically active compounds. Earlier, Saeed *et al.* (2010), asserted that the physical properties of stem and root are related to their anatomy and there is no way to interpret their role without sufficient knowledge of their structure (Otoide, 2017). To this end, Akinnubi *et al*. (2013) discovered 2-3, bicollateral vascular bundles in petiole anatomy of *Vernonia amygdalina* and 3 oval shaped amphicribal vascular bundles in *Cyanthillium cinereum* (Kemka-Evans *et al.* 2017). Anatomical features are widely used in systematics for identification, placing anomalous groups in a satisfactory position in classification and for indicating patterns of relationship that may have been obscured by superficial convergence in morphological feature. In the recent past, plant species have been removed from their family or genera and classified under another taxon based on anatomical evidence. Characters that are not influenced by environmental factor have proved to be of systematic value (Okoli, 1987). Anatomical characters have been widely used by many workers in taxonomy such as (Stace 1980) who vividly showed that the bundle sheath in *Telfairia occidentalis* Hooker Fil is thin–walled and sclerenchymatous while that of *T. pedata* (Sims) Hooker is thin walled and parenchymatous, thus helping to clarify the taxonomic relationship of these two species. Isawumi (1984) described the shape of leaf trichomes of sixty species of *Vernonia* indicating the presence of T – shaped type of trichomes in *V. amygdalina* and *V.conferta* and uniseriate trichomes in *V. cinerea*. Maryam *et al*. (2013) used the stem epidermis diameter, cortex diameter, palisade diameter, parenchyma diameter, the vessel-to epidermis diameter, stem-to-epidermis diameter, rupture of pith, width of vessels, inner phloem diameter, stem diameter, and stem-to-vessel diameter to evaluate the species relationship in the genus *Anchusa*. Recently, Otoide *et. al*. (2020) studied the epidermis and midribs of four

Vernonia species namely: *V. amygdalina, V. cinerea, V. perrottetii* and *V. adoensis* and further confirm the present taxonomic status of the species as being unbiased and warranted as a result of the anatomical features in the parts examined.

Methods

Matured fresh stems and leaf petioles of *Cissus quadrangularis, Cissus arguta, Cissus aralioides, Cissus rufescens and Cissus glaucophylla* were collected from Ekiti State University environs. Ekiti State University is on geographic coordinates of 7.7141^0N, 5.2601^0E. The specimens were then taken to the Herbarium of Ekiti State University, Ado-Ekiti for authentication.

The transverse sections of the stems and leaf petioles of the four different taxa were made following the methods of Kemka- Evans *et al* (2017). Sections were cut with the aid of a rotary microtome between 15-18 microns and placed on the clean slides smeared with a thin film of Hauptis albumin. The slides were placed on hot plates at 40°C for a few minutes to allow the ribbons to expand and kept in an oven at 30°C for 2 hours. The slides were immersed in pure xylene for 2-5 minutes and in a solution of xylene and absolute alcohol with a 1:1 ratio (v/v) for few minutes. The slides were stained with methylene blue for 30 minutes. The specimens were dehydrated in acid alcohol. (1m1 of concentrated HCl to 99 ml of 70% alcohol). The section were washed in 70% alcohol and left in running water for about 10 minutes. Counter-staining was done with 50% Safranin-O for two minutes, then dehydrated in alcohol, xylene-absolute alcohol solution and pure xylene at intervals of few seconds and mounted in canada balsam.

The prepared slides were observed under the microscope by following conventional procedures of operating the light microscope. Data were collected at X100 and X400 as the case may be and thereafter, photomicrographs were taken.

Results and Discussion

The results obtained in the present study have been summarized in Tables 1-2 and illustrated in Plates 1-10.

The stem epidermis of the five *Cissus* species consist of single layer of cells. However, the layout was undulating in all the species except in *C. glaucophylla* in which the layout was semi-circular (Plate 2). The cortex of the stem of the five species were made of parenchyma cells. As a result of growth and expansion, the cortical cells formed six loops in the stem of *C. arguta*. Each of the loops is permeated with collenchyma cells (Plate1). Leaf trace bundles and numerous air spaces were observed in the cortex of the stem of *C. glaucophylla* (Plate 2). Similarly, the cortex of *C. aralioides* showed leaf trace bundles and starch sheath surrounding the vascular cylinder (Plate 3). The cortex in the stem of *C. quadrangularis* developed into forming four loops oriented opposite one another. Each of the loops was permeated with collenchyma cells. Leaf trace bundles were present in the cortex (Plate 4). Furthermore, the cortex in *C. rufescens* was highly reduced, narrow and developed into forming two opposite loops. Each of the loops were characterized with up to eleven units of metaxylems (Plate 5).

The vascular bundles in the stem of the five species were arranged in a ring-like pattern/ orientation in the vascular cylinder (Plates 1-5). The units of bundles varied in number among the species. Units of 30-35 and 25-30 were observed in the vascular cylinder of *C. arguta* and *C. glaucophylla* respectively. Similarly, 25-28, 35-40 and 30-40 units of vascular bundles were observed in the cylinder of *C. aralioides, C. quadragularis* and *C. rufescens* respectively (Table 1). Radial bundle types were observed in the stem of the five species. However, *C. aralioides* and *C. rufescens* showed well developed metaxylems (Plates 3 & 5).

Ergastic substances such as starch grains and raphids (acicular or needle shaped calcium oxalate crystals) were present in most of the cortical cells of *C. aralioides* and *C. quadragularis*

respectively (Table 1). Conversely, ergastic substance were absent in the stems of *C. arguta, C. glaucophylla* and *C. rufescens.*

On the other hand, undulating layout of epidermis was observe in the petioles of *C. glaucophylla* whereas the layout was entire in *C. arguta* and *C. aralioides* but semi-circular in *C. rufescens.* Conversely, the outline of the epidermis of the petiole of *C. quadrangularis* is pentangonal in shape with short uniseriate epidermal hairs which are more on each of the five angles (Plate 9). In the same vein, although the layout of the epidermis of the petiole of *C. arguta* was entire but at a point, it bifurcated to develop two wings. This could be attributed to the resultant effect of cortical cell activities (Plate 6). The cortex of the petioles of *C. rufescens* and *C. arguta* were large while those of the rest of the species were narrow.

Ring-like arrangement of vascular bundles were observed in the petioles of the five species and each unit of the bundle ranged between 12-14, 13-15 and 16-18 units as in the case of *C. arguta, C. glaucophylla* and *C. aralioides* respectively. Similarly, 13-15 and 10-15 units of vascular bundles were observed in the petioles of *C. quadrangularis* and *C. rufescens* respectively (Table 2). Radial type of bundles were observed in the vascular cylinder of the petioles in the five species.

Uniseriate hairs were observed in the petiole epidermis of *C. quadrangularis* (Plate 9) but absent in the rest of the species. Ergastic substances such as raphids were observed in *C. arguta* and *C. quadrangularis* where they were localized in some of the cells of the cortical parenchyma and vascular cylinder respectively. However, ergastic substances were absent in the rest of the species (Table 2).

Table 1. Anatomical features of the stem of the *cissus* species

Criteria	**Species**				
	C. arguta	***C. glaucophylla***	***C. aralioides***	***C. quadrangularis***	***C. rufescens***
Layout of Epidermis	Single layer of cells and undulating	Single layer of cells and semicircular	Single layer of cells and round	Single layer of cells and undulating	Single layer of cells and undulating
Cortex	Parenchyma cells forming 6 loops. Each loop is permeated with collenchyma cells	Parenchyma cells and numerous air spaces. Leaf trace bundles were present	Parenchyma cells, leaf trace bundles and starch sheath enveloping the cylinder	Parenchyma cells oriented into forming 4 loops which are opposite each other and permeated with collenchyma cells	Parenchymatous, very narrow and developed into two opposite loops each of which are permeated with 10-12 units of metaxylems
Orientation of vascular bundles	Ring-like	Ring-like	Ring-like	Ring-like	Ring-like
Units of bundles	30-35	25-30	25-28	35-40	30-40
Bundle type	Radial	Radial	Radial	Radial	Radial
Ergastic substances	-	-	Starch grains in most of the	Raphids in most of the cortical cells	-

			cortical cells		

Table 2. Anatomical features of the petiole of the *cissus* species

Criteria	**Species**				
	C. arguta	***C. glaucophylla***	***C. aralioides***	***C. quadrangularis***	***C. rufescens***
Layout of epidermis	Single layer of cells and entire	Single layer of cells and undulating	Single layer of cells and entire	Single layer of cells and undulating	Single layer of cells and semicircular
Cortex	Large and bifurcate to develop 2 wings	Very narrow and Parenchymatous	Narrow and Parenchymatous	Very narrow and Parenchymatous	Large and Parenchymatous
Orientation of vascular bundles	Ring-like	Ring-like	Ring-like	Ring-like	Ring-like
Units of bundles	12-14	13-15	16-18	13-15	19-15
Bundle type	Radial	Radial	Radial	Radial	Radial
Ergastic substances	Raphids in some parenchyma cells	-	-	Raphids in some cells in the vascular cylinder	-
Trichomes	-	-	-	Numerous, short and unicellular epidermal hairs	-

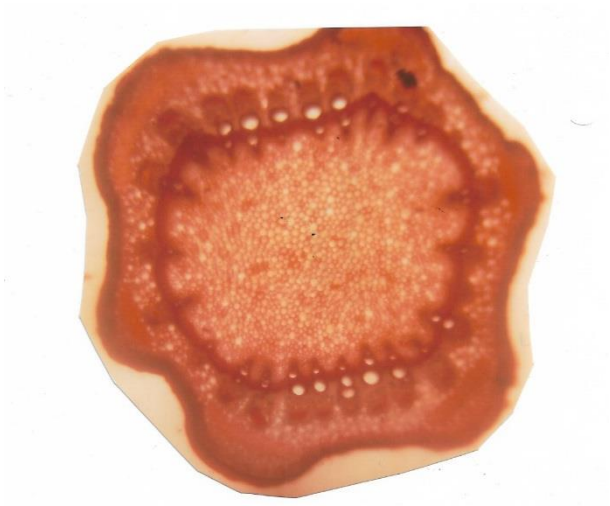

Plate 1: Internal features in stem of *Cissus arguta* in transverse outline. X100

Plate 2: Internal features in stem of *Cissus glaucophylla* in transverse outline. X 100

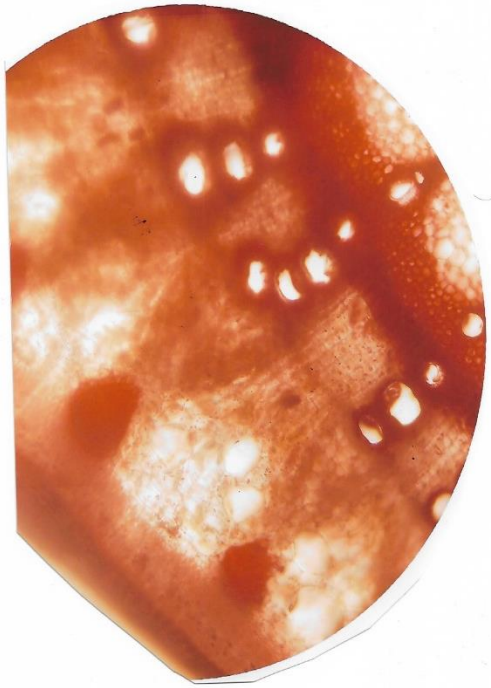

Plate 3: Internal features in stem of *Cissus aralioides* in transverse outline. X 400

Plate 4: Internal features in stem of *Cissus quadrangularis* in transverse outline. X 100

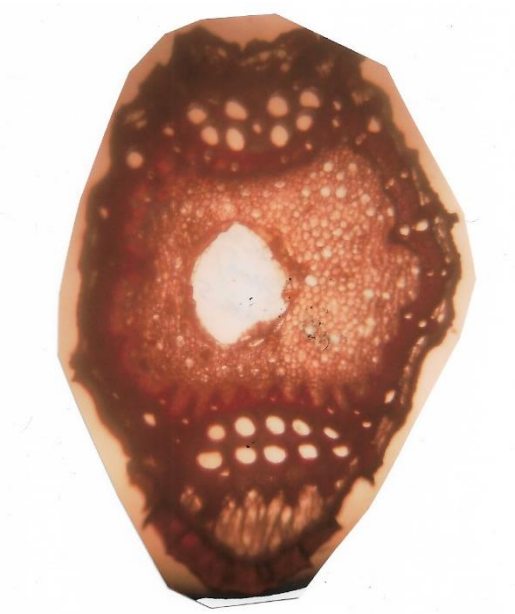

Plate 5: Internal features in stem of *Cissus rufescens* in transverse outline. X 100

Plate 6: Internal features in petiole of *Cissus arguta* in transverse outline. X 100

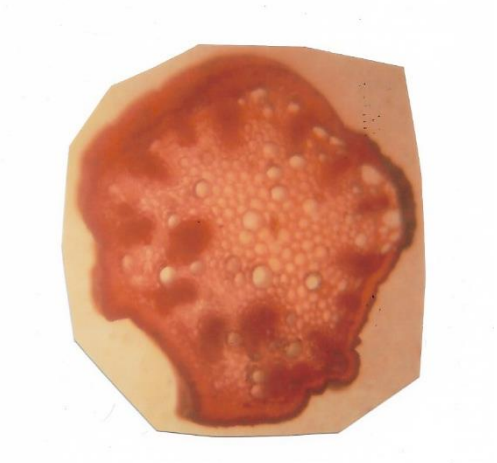

Plate 7: Internal features in petiole of *Cissus glaucophylla* in transverse outline. X 100

Plate 8: Internal features in petiole of *Cissus aralioides* in transverse outline. X100

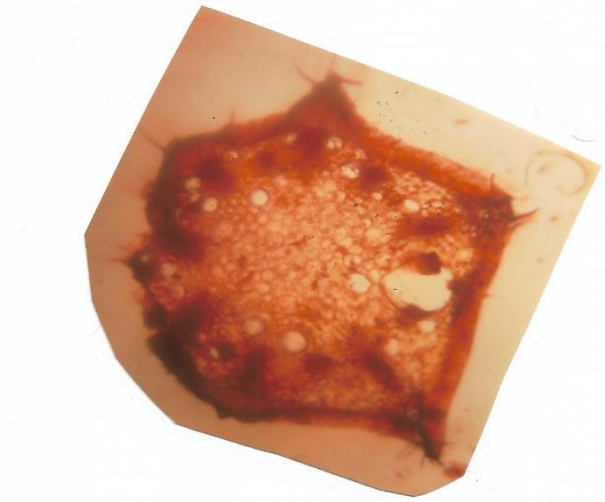

Plate 9: Internal features in petiole of *Cissus quadrangularis* in transverse outline. X 100

Plate 10: Internal features in petiole of *Cissus rufescens* in transverse outline. X 100

The loops in the stem epidermis of *C. arguta* and *C. quadrangularis* is diagnostic as it gave them a characteristic epidermal layout clearly distinguishable from the rest of the species. This observation has lend credence to the earlier report of the development of loops (4) in the stem of *C. quadrangularis* by Anitha and Suji (2012). On this note, it suffices to assert that the characteristic loops would be useful in the identification of these species especially when in sterile or fragmentary forms. The occurrences of leaf trace bundles in the cortex of the stems of *C. arguta, C. aralioides* and *C. quadrangularis* is a useful diagnostic anatomical feature that where not observed in the rest of the species. The occurrences of collenchyma cells in the loops in stems of *C. quadrangularis* comfirms the earlier reports of Anitha and Suji (2012).

The ring like arrangement of the vascular bundles in stems of *C. arguta, C. glaucophylla, C. quadrangularis* and *C. aralioides* is not strange as it is typical of the dicot stem. What is strange however, is the occurrences of two groups of conductive tissues located opposite each other and made up of 11 to 12 metaxylems in *C. rufescens* (Plate 5). The numerous radial-typed vascular bundles common to all the species in the present study is also one of the typical characteristic of the dicot species. In all, *C. aralioides* had the lowest count of bundles by showing 25-28 units while *C. rufescens* had the highest by showing 30-40 units.

The occurrences of leaf trace bundles (which connect the vascular system of the leaf to that of the stem) in the cortex of the stems of *C. glaucophylla* and *C. aralioides* is diagnostic and clearly distinguished them in terms of their internal features from the rest of the *Cissus* species in the present study. This assertion is in line with the one made by Metcalfe and Chalke (1989) that cortical bundles are diagnostically significant because of their taxonomically restricted occurrence.

The observation of raphids in the stem cortex and petiole of *C. quadrangularis* in the present study is not strange as Metcalfe and Chalke (1989) earlier included the vitaceae family among the lists of plant families where raphids are found as they emphasized their diagnostic importance. It also supports the recent reports of Anitha and Suji (2012) on the occurrence of raphids in the stem of *C. quadrangularis.*

The petiole anatomy, on the other hand, displayed useful features that are dependable for their identification. Consequently, the bifurcated layout of the petiole epidermis of *C. arguta* clearly distinguished it from the rest of the species where bifurcation did not exist. Furthermore, occurrences of short and uniseriate epidermal hairs on the five wings of the petiole of *C. quadrangularis* is unique among the species as these were absent in others. Hence, this feature is useful for its identification and quality control when in sterile state being a medicinal plant. Vascular bundles arrangement in the petioles where also in ring-like pattern and the lowest units (i.e. 10-15) occurred in *C. rufescens* while the highest units occurred in *C. aralioides*.

Going by the information available to the researchers, there is little or no comprehensive report on the internal features of the stems and petioles of *C. arguta, C. glaucophylla, C. aralioides, C. quadrangularis* and *C. rufescens* in circulation. Therefore, it is pertinent to point out that the present reports are being comprehensively and adequately documented for the first time. Although, Anitha and Suji (2012) recently studied the internal structure of stem of *C. quadrangularis* in their pharmacognostic evaluation, they however, did not include petiole anatomy in their reports.

From the foregoing, although the internal features reported in the present study are diagnostic and descriptive, they are not sufficient to adjudge or suggest reclassification of the individual species in the genus *Cissus*. The species are easily identifiable and distinguishable by their external morphological features, the results obtained in the present study will be useful and reliable in identifying the species when they appear in sterile or shriveled forms and would also serve as back-up to the already established external morphological features of the species.

Conclusion

The similarities among the species with respect to the anatomical features are overwhelming to support the taxonomic status of individual of the species in the same genus. Therefore, it suffices to affirm the existence of interspecific relationship among the five taxa. Although there is no criticism against the present taxonomic hierarchy and status of these species in the current genus, the present authors wish to suggest the initiation and harnessing of research information from cytology, molecular biology, taximetrics, leaf epidermal anatomy and other sources of taxonomic inferences to support or suggest the regrouping of these species in the genus *Cissus* especially in the cases of *C. glaucophylla* and *C. aralioides* because of the presence of leaf trace bundles in their cortex which is an anomalous feature.

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