



Taxonomic Studies on the Genus *Cissus* L. (Vitaceae) Present in Obio/Akpor Local Government Area of Rivers State, Nigeria

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ABSTRACT: This study was aimed at addressing the problematic taxonomy of the genus *Cissus* L. using three species namely; *Cissus aralioides* (Welw. ex Bak.) Planch, *Cissus petiolata* Hook.f and *Cissus lageniflora* Gilg and Brandt found in Obio/Akpor Local Government Area of Rivers State. The investigation was carried out using morphological, anatomical, epidermal and phytochemical lines of evidence. Macromorphology results showed that *Cissus aralioides*, *Cissus lageniflora* and *Cissus petiolata* matured leaf possess digitately compound leaf type, trifoliate compound leaf type and simple leaf type respectively. Micromorphological results showed that all studied species were hypostomatic with anomocytic stomata type present in the abaxial surface of all species. Epidermal cell shape of the adaxial and abaxial surface of all species were pentagonal and irregular to polygonal respectively. Transverse sections of the petiole of studied species shows an arranged and open vascular bundles in all species, variations where however seen in the number of vascular bundles. Methanolic extract of the three *Cissus* species studied revealed the presence of secondary metabolites like; Phenols, Alkaloids, Aglycone Glycosides, Cardiac Glycosides, Steroidal Aglycone Glycosides in the stem and leaf of all species. The results obtained shows that all studied species show diverse characteristic differences but these differences are not adequate and convincing enough to separate them into different genera but it shows that that *Cissus aralioides* and *Cissus lageniflora* are more closely related than they are to *Cissus petiolata*. Moreover, more species and other lines of evidence should be used in future research. A dendrogram to the relatedness of the species and a dichotomous bracketed key to their identification is provided.

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The genus *Cissus* L. belongs to the Family Vitaceae (formerly Ampelidaceae) and comprises of about 350 species distributed all over the world (Airy Shaw, 1985; Egli, 2002). *Cissus* represents the largest of the 14 genera of Vitaceae (Lombardi, 2007; Wen, 2007; Wen *et al.*, 2007). The genus has about 135 species in Africa, 85 species in Asia, 12 species in Australia and 65 species in the Neotropics (Wen, 2007). It is worthy of note that 49 of the 135 species that are present in Africa are found in West Africa with 23 and 15 of these in southern and northern Nigeria respectively, making a total of 38 species present in Nigeria (Hutchinson and Dalziel, 1954; Burkill, 2000). Vitaceae (grape family) *sensu lato* is of great economic importance, but there is little understanding of the relationships between and within the genera, the traditional classifications of grouping some *Cissus* species (e.g. *Cissus artarctica* Vent.) in a clade with *Vitis* species and placing *Cissus* species (e.g. *Cissus quadrangularis* L.) in a monophyletic clade have been countered by recent DNA based phylogenetic studies (Susan *et al.*, 2007).

Members of the genus *Cissus* shows remarkable morphological diversity (Lombardi, 2007), and is generally characterized by well-developed thick and undivided floral disks, four merous flowers, one - seeded fruits and seeds with a long and linear chalaza (Wen, 2007; Chen and Manchester, 2011). It is noteworthy that the members of the genus *Cissus* are climbing shrubs, small tress or herbs from a perennial root stock, with nodes or jointed stem, often with watery juices. It is noteworthy that phylogenetic studies have also moved some other species to another genus, *Cayratia* (Bak.) Suess (Maurizio *et al.*, 2007). Examples of these species are *Cayratia debilis* (Bak.) Suess. Basionym: *Cissus debilis* (Bak.) Planch, *Cayratia gracilis* (Guil and Perr) Suess. Basionym: *Cissus gracilis* Guil and Perr, *Cayratia ibuensis* (Hook.f.) Suess. Basionym: *Cissus ibuensis* Hook.F. (Burkill, 2000). Therefore the objective of this paper is to present detailed characters of the studied species from four taxonomic line of evidence that will be analyzed with a dendrogram to give the relatedness of the species.

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MATERIALS AND METHODS

Collection and identification of plant materials: Fresh and healthy plant samples of the genus *Cissus* L. were collected in separate bags from areas within the University of Port Harcourt Park, Choba Park all in the University of Port Harcourt Campus, Choba in Obio-Akpor Local Government Area of Rivers State. Specimens were also collected from Wokem's estate in Choba community in Obio-Akpor Local Government Area of Rivers State. The plant specimens were identified at the herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State. Voucher specimens were deposited in the herbarium for reference and further studies.

Macromorphological Investigation: Examinations on the macromorphological characters of the *Cissus* species which are of taxonomic interest were performed in matured living plant samples. Both qualitative and quantitative vegetative features were studied. Quantitative features studied were made possible by the aid of meter-rule and measuring tape. Also photographs of relevant morphological features were taken. Plates 1a-1c gives a pictorial representation and view of the species.

Micromorphological Investigation: Foliar materials for epidermal studies were collected fresh from plants growing in the wild. The adaxial and abaxial epidermal surfaces were peeled with blades and in some cases, they were soaked in concentrated Nitric acid or Trioxonitrate (v) acid, rinsed in distilled water and then stained in 1% aqueous safranin solution and mounted in glycerin. Thereafter, the cover slips were placed over the peels and then sealed with nail varnish to prevent dehydration (Agbagwa and Okoli, 2007; Ekeke *et al.*, 2019). The slides were observed using a microscope and pictures were taken with the use of a camera and the leaf epidermal characteristics were determined based on the methods of Metcalfe and Chalk (1979).

Anatomical Studies: Cut sections of petiole and young stems were fixed in FAA (Formaldehyde: Glacial Acetic Acid: Alcohol in the ratio of 1:1:18) for at least 48 hours according to Johansen (1940). Then removed and washed in distilled water after which the specimen was dehydrated in 30%, 50%, 70% and absolute ethanol series for 2 hours each. Thereafter, the specimens were placed in 3:1, 1:1, and 1:3 absolute ethanol: chloroform mixture, for 3 hours each. The specimen was rinsed in pure chloroform. The specimen once again was passed through the ethanol series for 2 hours each from 70%, 50% and then 30% ethanol. The specimen was then sectioned and stained

with 1% aqueous safranin and counter stained with Alcian blue and then rinsed in water and finally mounted on the slide, glycerin was added and then covered with cover slip and sealed with nail polish. The slides were viewed using the microscope and pictures were taken with the aid of a camera.

Phytochemical Investigation: Preparation of solvent extract: Fresh plant parts (stem and leaves) were collected from their natural habitat in separate sterile polythene bags for each of the selected species. The plants were oven dried in the oven of the University of Port Harcourt herbarium for 6-8 days to reduce the moisture content in the samples. The dried samples were collected from the oven and crushed or grounded using the porcelain mortar. It is worthy of note that the stem and leaves of the plants were crushed separately. 10mls of methanol was then added to each of the samples for extraction. The extraction was done at room temperature.

Qualitative analysis of methanolic extract of samples: The following standard protocols as stated by Harborne (1973), Edeoga *et al.* (2005), Sofowora (2008), Nyananyo and Nyingifa (2011), Catherine and Banu (2015) and Rahman *et al.* (2017) were used for qualitative analysis of methanolic extract of samples for the presence of alkaloids, phenols, saponins, cardiac glycosides, aglycone glycosides, steroidal aglycone glycosides, tannins, terpenoids, steroids and flavonoids. Mayer's test for alkaloids, Keller-Kilian test for Cardiac glycosides, Salkowski's test for Steroidal Aglycone glycosides, Liebermann's test for Aglycone glycosides, Follin-denis reagent test for tannin and paper chromatography etc.

Numerical Analysis: Taxonomic characters obtained from the morphological, phytochemical and anatomical investigations of (3) species of the *Cissus* genus were grouped by cluster analysis using the single linkage method based on similarity matrix of Euclidean distances of quantitative and qualitative characters. This statistical analysis was done using the Paleontological statistics (PAST) software.

RESULTS AND DISCUSSIONS

Macromorphological description: Important Macromorphological differences and similarities observed in the three *Cissus* species studied are summarized in table 1. While the pictures of the studied species showing distinguishing features are presented in Plates 1a – 1c. *Micromorphological Investigation:* Abaxial and adaxial surface of the studied species are presented in plate 2a-2f, while table 2 highlighted the important features in all species. *Phytochemical Investigation:* The preliminary

phytochemical analysis of some of the tropical plants investigated in the study area were evaluated and results presented in table 2.

Table 1: Summary of the important macro morphological attributes of the studied species

| Characters | <i>Cissus aralioides</i> | <i>Cissus lageniflora</i> | <i>Cissus petiolata</i> |
|-------------------------------|--------------------------|---------------------------|-------------------------|
| Leaf type | Digitately compound | Trifoliolate compound | Simple |
| Leaf arrangement | Opposite | Opposite | Opposite |
| Leaf shape | Oblong | Lanceolate | Sagitate |
| Leaf apex | Acuminate | Aristate | Aristate |
| Leaf base | Cuneate | Oblique | Cordate |
| Leaf margin | Lobed | Parted | Parted |
| Leaf venation | Pinnately veined | Pinnately veined | Pinnately veined |
| Abaxial leaf pigmentation | Absent | Present | Present |
| Glabrous or pubescent on leaf | Glabrous | Pubescent | Pubescent |
| Leaf length | 11.0-12.6cm | 9.0-10.5cm | 15.5-17.5cm |
| Leaf width | 4.8-5.0cm | 4.0-4.7cm | 12.5-16.5cm |
| Shape of stem | Cylindrical | Circular | Circular |
| Stem pigmentation | Present | Present | Present |
| Stipule | Present | Absent | Present |
| Length of tendril | 23.0-25.0cm | | 24.5-26-5cm |
| Tendril Branching | Absent | Absent | Present |
| Length of petiole | 7.0-8.0cm | 5.5-6.5cm | 11.5-13.5cm |
| Glabrous or pubescent on stem | Glabrous | Glabrous | Pubescent |

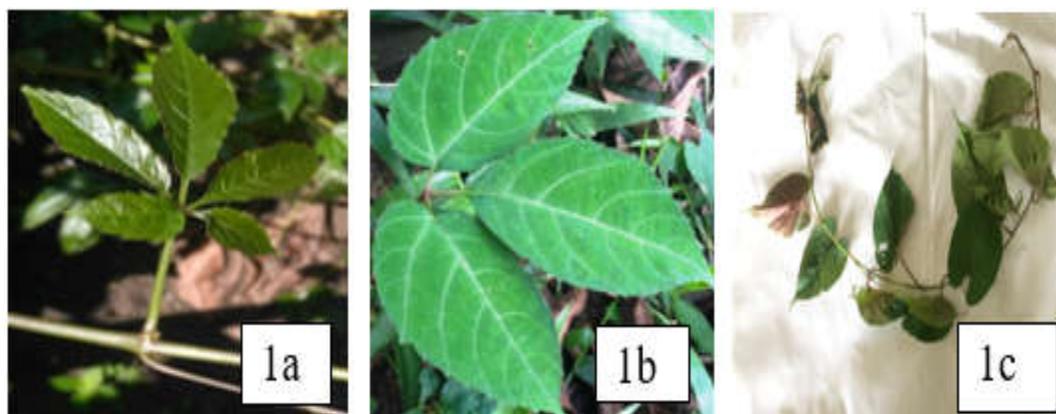


Plate 1a-1c: (1a) *Cissus aralioides* matured leaf showing digitately compound leaf type, oblong leaf shape, acuminate and cuneate leaf apex and base respectively (1b) *Cissus lageniflora* matured leaf showing Trifoliolate compound leaf type, Lanceolate leaf shape, parted leaf margin with aristate and Oblique leaf apex and base respectively (1c) *Cissus petiolata* showing simple leaf type, Sagitate leaf shape, aristate and cordate leaf apex and base respectively with parted leaf margin.

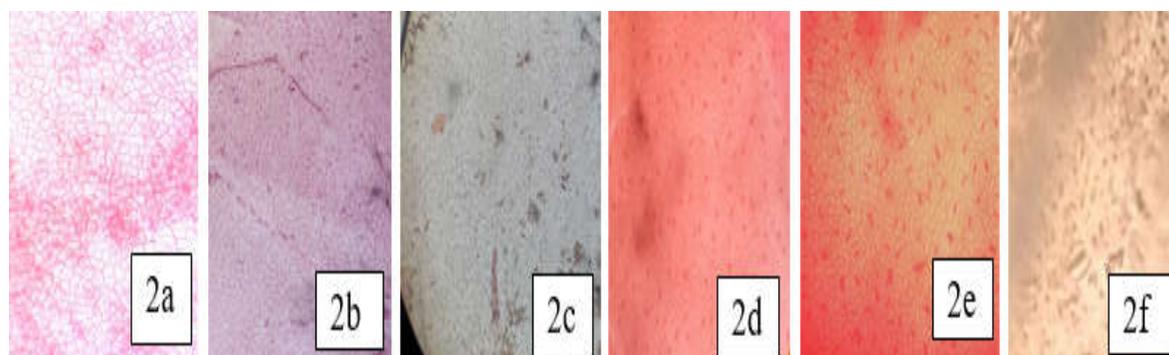


Plate: 2a-2f: All studied species were hypostomatic with anomocytic stomata type present in the abaxial surface of all species. Epidermal cell shape of the adaxial and abaxial surface of all species were pentagonal and irregular to polygonal respectively. Variations were however observed in the presence or absence of trichomes. (2a) Adaxial surface of *Cissus aralioides* (2b) Adaxial surface of *Cissus lageniflora* (2c) Adaxial surface of *Cissus petiolata* with arrow showing presence of trichomes. (2d) Abaxial surface of *Cissus aralioides* (2e) Abaxial surface of *Cissus lageniflora* (2f) Abaxial surface of *Cissus petiolata* showing trichomes.

Anatomical studies (Petiole)

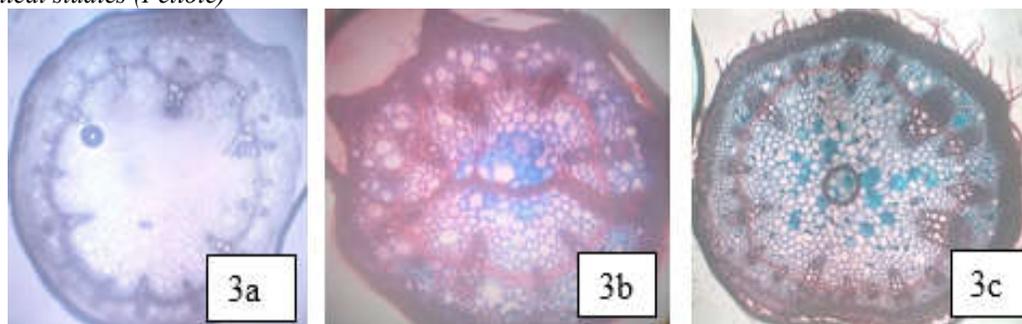


Plate 3a-3c: Transverse sections of the petiole of studied species shows an arranged and open vascular bundles in all species, variations where however seen in the number of vascular bundles as well as in the nature of the Intercellular spaces. **(3a)** Transverse section of the petiole of *Cissus aralioides* showing absence of schlerids, raphid bundles and trichomes. 17 vascular bundles were observed **(3b)** Transverse section of the petiole of *Cissus lageniflora* showing 11 vascular bundles. Schlerids and raphid bundles were observed while trichomes were absent **(3c)** Transverse section of the petiole of *Cissus petiolata* showing 18 vascular bundles. Schlerids and raphid bundles were absents. With arrow showing the presence of trichomes.

STEM

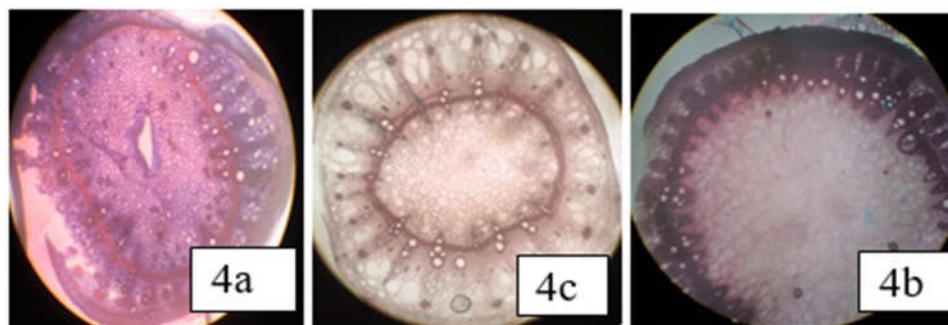


Plate 4a-4f: Transverse sections of the stem of studied species shows an arranged and open vascular bundles in all species, variations where however observed in the nature of the Intercellular spaces as well as in the presence or absence of trichomes **(4a)** TS of the stem of *Cissus aralioides* **(4b)** TS of the stem of *Cissus lageniflora* **(4c)** TS of the stem of *Cissus petiolata* with arrow showing trichomes.

Table 2: Preliminary phytochemical analysis of *Cissus* species studied.

| Phytochemicals | <i>Cissus aralioides</i> | | <i>Cissus lageniflora</i> | | <i>Cissus petiolata</i> | |
|-------------------------------|--------------------------|------|---------------------------|------|-------------------------|------|
| | Leaf | Stem | Leaf | Stem | Leaf | Stem |
| Saponins | - | + | + | ++ | ++ | + |
| Tannins | ++ | - | - | - | +++ | - |
| Phenols | + | + | + | + | + | + |
| Terpenoids | + | +++ | ++ | + | ++ | ++ |
| Steroids | - | - | - | - | ++ | - |
| Alkaloids | +++ | + | ++ | +++ | ++ | ++ |
| Aglycone Glycosides | +++ | ++ | ++ | ++ | ++ | ++ |
| Cardiac Glycosides | ++ | +++ | ++ | ++ | ++ | +++ |
| Steroidal Aglycone Glycosides | ++ | +++ | ++ | +++ | + | +++ |
| Apiginine | + | - | + | - | + | - |
| Kayaflavone | + | - | - | - | + | - |
| Tricin | + | - | + | - | + | - |
| Chrysoeriol | + | - | + | + | + | - |
| Luteolin | + | + | - | - | + | - |
| Gossypetin | - | + | - | - | - | - |
| Quercetin | - | + | - | - | - | - |
| Azelatin | - | - | - | + | - | - |
| Iso-vitexin | - | - | - | - | - | - |
| Kaempferol | - | - | - | - | - | + |
| Iso-orientin | - | - | - | - | - | + |
| Vitexin | - | - | - | + | - | - |
| Orientin | - | + | - | - | - | - |

+++ : highly present, ++ : moderately present, + : Low, - : absent

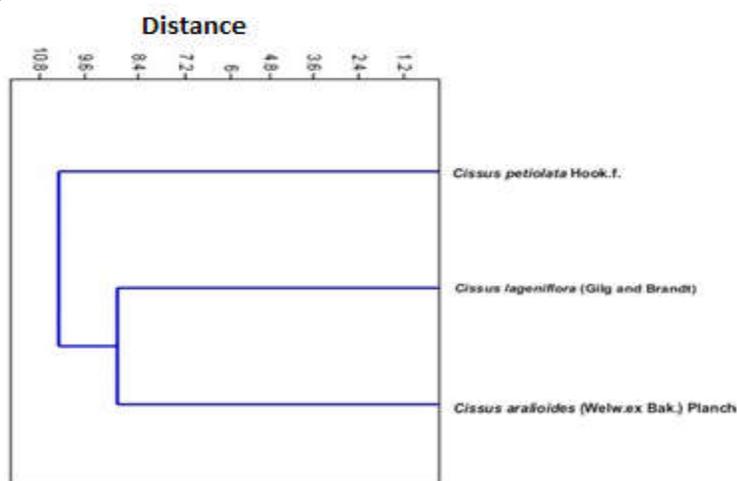
Numerical taxonomy

Fig 2: Dendrogram of the three *Cissus* species studied produced by cluster analysis using Past

Dichotomous bracketed key for identification of the species:

1. Leaf pubescent, apices aristate, margin parted, trifoliolate and simple leaf type present, stem circular, the flavonoid; vitexin present, gossypetin, quercetin, luteolin and orientin absent in stem and saponin present in the leaf.....2

1'. Leaf glabrous, apex acuminate, margin lobed, stem cylindrical, digitately compound, flavonoid; vitexin absent, gossypetin, quercetin, luteolin, orientin, present in stem, saponin absent in leaf*Cissus aralioides*.

2. Trichomes present on stem, petiole and the abaxial and adaxial surfaces of the leaf epidermis, simple leaf present, stem pubescent, stipule present, leaf base cordate, shape sagitate, the flavonoids; kaempferol and iso-vitexin present in stem, luteolin and kavaflavone present in the leaf, chrysoeriol absent in stem and leaf..... *Cissus petiolata*.

2'. Trichomes absent on stem, petiole, and abaxial and adaxial surfaces of the leaf epidermis, trifoliolate compound leaf present, stem glabrous, stipule absent, leaf base oblique, shape lanceolate, the flavonoids; kaempferol and iso-vitexin absent in the stem, luteolin and kavaflavone absent in leaf, chrysoeriol is present in stem and leaf.....*Cissus lageniflora*.

Results obtained from the morphological study of the three *Cissus* species were in conformity with the report of Hutchinson and Dalziel (1954) and Burkill, (1985). *Cissus aralioides* and *Cissus lageniflora* show robust morphological similarity with regards to their stem pigmentation, phyllotaxy and leaf type. Although a good number of differences occur among them and these differences are presented in Table 1 and Plate 2a-

2c above. *Cissus petiolata* nonetheless expresses quite a different form of morphological existence when compared to *Cissus aralioides* and *Cissus lageniflora*.

Methanolic extract of the three *Cissus* species studied revealed the presence of secondary metabolites like; Phenols, Alkaloids, Aglycone Glycosides, Cardiac Glycosides, Steroidal Aglycone Glycosides in the stem and leaf of all species. For *Cissus aralioides*, the compounds tannins and saponins were found in traces and this is in conformity with the study of Omotayo and Borokini (2010) and Nwoguzue *et al.* (2018). For *Cissus lageniflora* It is worthy of note that the secondary metabolites steroids and tannins were absent while terpenoids were found in traces. While the compounds steroids and phenols were found in traces for Methanolic leaf and stem extract of *Cissus petiolata*.

The leaf epidermal studies of the species showed slight variations among the three species as shown in plates 2a-2f. The presence of trichomes (plate 2c) is a unique character to delineate *Cissus petiolata*. It is noteworthy that the species were hypostomatic with anomocytic stomata type found in all, also, the shape of the epidermal cells in both adaxial and abaxial surface were similar.

The anatomical study shows little variation among studied species. For the petiole and stem anatomy of the species, a number of similarities were noticed and some of these are that the vascular bundles are arranged and open as well as the presence of interfascicular cambium ring which indicates that the species undergo secondary growth. The epidermal circumference of the petiole of *Cissus aralioides* (plate 3a) and *Cissus lageniflora* (plate 3b) was angular with

protuberance but was observed to be round without protuberance in *Cissus petiolata* (plate 3c).

The dendrogram produces two groups at point 9.6 along the distance. The first group is made up of *Cissus aralioides* and *Cissus lageniflora* showing that they are more closely related while the second group consists of only *Cissus petiolata*.

Conclusion: The three *Cissus* species in this research work (*Cissus aralioides*, *Cissus lageniflora* and *Cissus petiolata*) although showed some characteristic differences but these differences are not enough to separate them into different genera. It is worthy of note that based on the dendrogram constructed using characters from macromorphology, micromorphology, anatomy and phytochemistry *Cissus aralioides* and *Cissus lageniflora* are more closely related than they are to *Cissus petiolata*. However, further research should be carried out with other lines of evidence with a larger number of species that are members of the genus *Cissus* L. and as well some members of the genus *Cyphostemma* and *Cayratia*

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