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COAST
JOURNAL OF THE SCHOOL OF SCIENCE
Volume 6, Issue 1. May, 2024 ISSN: 2714-3716
Coast, J. Sch. Sci. 6 (1): 954 - 962

COMPARATIVE WOOD ANATOMY OF FOUR *Vernonia delile* SPECIES (ASTERACEAE) IN SOUTHWESTERN NIGERIA.

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https://doi.org/10.61281/coastjss.v6i1.2

Abstract

Wood anatomy of four *Vernonia* Delile species in Southwestern States of Nigeria was investigated with a view to identifying the anatomical features of the genus that could be used to delimit the taxa. Plant samples were collected from Oyo, Ekiti, Osun and Ondo States. Transverse, tangential and radial longitudinal sections were made from the wood of the species of *Vernonia* using Reichert sliding microtome at 10µm thickness. The woods were also macerated with Schultze's fluid to separate the tissues and cells. The macerates and cut sections were stained with Safranine O reagent to reveal the differences in tissue arrangements, shapes and cell inclusions. The four species showed common anatomical features: pore shape at transverse plane are oval, round, rectangular circular to cylindrical, simple pitting of vessel elements and presence of - uniseriate, biseriate and multiseriate rays. The wood characteristics that can be used to distinguish the taxa are absent and presence of : tylose, fibre length and percentage of solitary vessel, axial parenchyma type and its absence. These features conferred significant taxonomic difference in the species and members were categorized into three major groups. These findings serve as baseline for further research such as molecular studies.

Keywords:- ***Anatomy, axial parenchyma, comparative, rays, seriate, wood***

Introduction

The genus name *Vernonia* was derived from the name of the English Botanist, William Vernon who first collected and identified species of the genus in the late 1600's (Toyang and Verpoote, 2013). The genus *Vernonia* belongs to the family Asteraceae. This family is regarded as the largest family of angiosperm comprising of 950 genera and 23,000 species, with large number of herbal medicinal plants (Gills, 1988). The genus *Vernonia* has 500-1000 species (Bremar and Anderberg 1994; Keeley and Jansey 1994). Members of this family have ecological and economic importance; such as ethno-botanical, phytochemical, anti-microbial and medicinal purposes. Most Asteraceae species are herbaceous although some could be shrubs or trees. Members of the family Asteraceae are economically important as weeds, ornamentals, herbs and green vegetables with alternate or opposite leaf arrangement (Olorode, 1984).

Twenty-five (-25) species of *Vernonia* were recorded to occur in Nigeria, out of which *Vernonia cinerea* (Linn), *Vernonia*

amygdalina Delile, *V*ernonia *conferta* Benth and *Vernonia galamensis* Delile form interesting groups to study because they have similar anatomical features. *V. cinerea* is an herbaceous weed, *V. galamensis* is a shrub while *V. conferta* is a small tree and *V. amygdalina* is usually treated as a shrub with two varieties which are bitter leaf and non- bitter varieties. Kemka-Evans *et al.* (2014) studied the leaf epidermis of three species of *Vernonia* and reported the occurrence of contiguous stomata and sinuous anticlinal walls on the abaxial leaf surface of non-bitter variety of *V. amygdalina* and on both leaf surfaces of *V. cinerea*. Similarly, Aworinde *et al.* (2013) compared the leaf architecture of some *Vernonia* species in Southwestern Nigeria. They reported the occurrence of wavy anticlinal wall with polygonal cell shape in *V. ambigua* and straight anticlinal wall with irregular cell shape in *V. amygdalina* and *V. cinerea*.

The Nigerian species of *Vernonia* have been described (Hutchinson and Dalziel, 1963; Burkill, 1985; Isawumi, 2008), however there is yet to be sufficient information especially on micro-morphological studies of members of the genus in Nigeria. Anatomical studies especially on woods are scare and the anatomical relationships between and among the species is not clear - and this had hindered proper species identification. This work investigates wood anatomical features of the four *Vernonia* species that can be used for comparism. These new set of information will be used as diagnostic tools for proper species identification, better conservation approaches as well as encourage optimal utilization of *Vernonia* species in Nigeria.

The aims of this study is to provide information on the wood anatomy of *V. amygdalina*, *V. cinerea*, *V. galamensis*, and *V. colorata*. The specific objective of the study is therefore to examine the variations that exist among the four species with a view of identifying wood anatomical features/characters that could be employed in delimiting the taxa in the genus.

Materials and Methods

Fresh mature stems of the four *Vernonia* species under study were collected from straight matured branches, within Southwestern Nigeria and authenticated at Obafemi Awolowo University (OAU), Ile-Ife, Herbarium (IFE), Osun State, Nigeria. The transverse section, (T.S), tangential longitudinal section, (T.L.S) and radial longitudinal section (R.L.S) of the matured stems were made at 10µm using sliding microtome. Sections were preserved in 50% ethanol prior to staining (Wikremasinghe and Herat, 2006). Mature wood of each of the *Vernonia* species was macerated using Schulze's fluid. The macerated wood samples were washed in five changes of water. Wood macerates were preserved in 50% ethanol prior to staining (Oladipo and Oyeniran, 2013). Wood sections were stained for 5 minutes with Safranine O and rinsed in water, then counter stained with Alcian blue and rinsed again, dehydrated and differentiated in series of grades of ethanol (50%, 70%, 80%, 90%, and 100%). The stained sections were mounted in DPX mountant on clean slides and appropriately labeled (Oladipo and Oyeniran, 2013). Microscopical observation of the prepared slides of the wood sections, macerates were made at 10×, 40×, 100× objectives using LEICA DM500 binocular light microscope. Tissues, cells and cell inclusion identification and description were done following the terms described by International Association of Wood Anatomist (IAWA, 1989). Wood characters studied were xylem vessels, axial parenchyma, fibres, rays, tylose, secretive duct/canal and crystals.

The following quantitative parameters were

measured: vessel pore diameter at the transverse section, length and width of the vessels, ray height and width at the tangential longitudinal section using calibrated microscope, fibre length and width, lumen and wall thickness of the macerates. Quantitative wood data were subjected to One Way Analysis of Variance with means separated by Duncan Multiple Range Test. The data were subjected to Principal Component Analysis and Cluster Analysis using Paleontology Statistics (PAST). Photomicrographs of the slides showing anatomical features of the wood sections were taken using Accu-Scope trinocular microscope (ACCU-Scope 33001 LED Trinocular Microscope with 3.2MP CMOS digital camera.

Results

Quantitative studies

Table 1 showed the results of the quantitative anatomical parameters measured. Mean of vessel diameter of both *Vernonia amygdalina* and *Vernonia colorata* are not significantly different from one another while *Vernonia galamensis* is significantly different from both except *Vernonia cinerea* which is between the mean vessel diameters of the three species. Mean of vessel length of both *Vernonia amygdalina* and *Vernonia galamensis* are not significantly different while *Vernonia cinerea* and *Vernonia colorata* are both significantly different. The means of fibre length and fibre diameter of the four species are not significantly different from one another. Means of width thickness of both *Vernonia cinerea* and *Vernonia colorata* are not significantly different but significantly different from the mean of width thickness of *Vernonia amygdalina* and *Vernonia galamensis.*

Table 1: Means of Wood anatomical features of *Vernonia* species.

Vernonia species	Mean of VD ± S. E.	Mean of VL ± S. E.	Mean of FL ± S. E.	Mean of F D ± S. E.	Mean of FWT ± S. E.
V. amygdalina	12.84 ±0.82 ^{a}	77.36 ± 3.02 ^{ab}	33.20 ± 2.44 ^{a}	1.00 ± 0.00 ^{a}	3.12 ± 0.09 ^{a}
V. cinerea	11.62 ± 0.93 ^{ab}	73.96 ± 3.15 ^{b}	35.31 ± 2.79 ^{a}	1.00 ± 0.00 ^{a}	2.69 ± 0.17 ^{b}
V. colorata	12.44 ± 0.70 ^{a}	84.44 ± 2.27 ^{a}	30.16 ± 1.65 ^{a}	1.00 ± 0.00 ^{a}	2.36 ± 0.11 ^{b}
V. galamensis	9.92 ± 0.74 ^{b}	81.08 ± 3.00 ^{ab}	30.96 ± 2.32 ^{a}	1.00 ± 0.00 ^{a}	1.80 ± 0.15 ^{c}

Legend: Mean value ± SE with the same letter along columns are not significantly different from one another at P≤ 0.05. S.E: Standard error; VD: Vessel Diameter, VL: Vessel length, FL: Fibre length, FD: Fibre Diameter, FWT: Fibre Width Thickness.

Quantitative anatomical characters of the four species of *Vernonia* studied showed that the fibre lumen of *Vernonia amygdalina* and *Vernonia cinerea* are not significantly different from one another while both *Vernonia colorata* and *Vernonia galamensis* are significantly different (Table 2). The ray width of *Vernonia amygdalina*, *Vernonia cinerea* and *Vernonia colorata* are not significantly different while *Vernonia galamensis* has significantly different ray width. Number of pores in *Vernonia amygdalina* and *Vernonia colorata* are both not significantly different from one another and the number of pores present in both *Vernonia cinerea* and *Vernonia amygdalina* are not significantly different from one another. The mean of pore diameter of both *Vernonia colorata* and *Vernonia galamensis* are not significantly different from one another (Table 2).

Table 2: Means of anatomical parameters of *Vernonia* species.

Vernonia species	Mean of FLU ± SE	Mean of RH ± SE	Mean of PD ± SE	Mean of RW ± SE	Mean of NP ± SE
V. amygdalina	8.04 ± 0.64 b	74.84 ± 9.98 cb	5.64 ± 0.81 ab	15.36 ± 1.90 a	55.04 ± 4.51 a
V. cinerea	7.62 ± 0.34 b	115.58 ± 6.89 a	6.92 ± 0.35 a	14.96 ± 1.68 a	32.81 ± 1.54 b
V. colorata	9.28 ± 0.34 a	56.24 ± 5.00 c	4.36 ± 0.43 b	13.88 ± 1.22 a	48.84 ± 2.22 a
V. galamensis	5.92 ± 0.40 c	84.60 ± 5.09 b	4.60 ± 0.38 b	4.64 ± 0.46 b	27.04 ± 1.77 b

Legend: Mean value ± SE with the same letter along columns are not significantly different from one another at P≤ 0.05. S.E: Standard error; FLU: Fibre Lumen; RH: Ray Height, PD: Pore Diameter, RW: Ray Width, NP: Numbers of Pores.

Wood anatomical features/characters

***Vernonia amygdalina* (Plates 1A and 2A)**

Porosity is diffuse, vessel pore at transverse section ranges from circular, oval, short cylindrical, short rectangular, arc to polygonal. Pore cluster 2-9, pore multiple 2-10. Pore cluster and pore multiple predominant with scanty solitary vessel. Perforation plate simple, inclination transverse to oblique with tail at one end, at both ends and no tail at all. Pitting is simple and alternate. Tylose is present in few vessels (scanty). Secretive duct is present. Axial parenchyma has scanty unilateral paratracheal. Rays are uniseriate, multiseriatre and compound

***Vernonia cinerea* (Plates 1B and 2B)**

Porosity is diffuse, vessel pore shape at transverse plane ranges from circular, oval, short cylindrical, short rectangular, arc to polygonal. Solitary vessel is predominant with few pores that are in multiples of 2. Pore cluster is absent. Perforation plate simple, inclination oblique with tail at one end, both ends and no tail at all. Pitting is scarlariform and reticulate. Tylose is absent, Secretive duct is present on vessel. Crystal is present among fibres and vessel element. Ray is uniseriate, biseriate, multi-seriate, compound ray, non-storied and heterogeneous. The multiseriate rays tapered at their ends to form uniseriate rays (the uniseriate rays do not occur singly but as taperings of the multiseriate rays). This feature is unique to this taxon. There is also the presence of flask shaped compound ray that is diagnostic for *V. cinerea.*

***Vernonia colorata* (Plates 1C and 2C)**

Porosity is diffuse, vessel pore shape at transverse plane ranges from circular, oval, short rectangular, short cylindrical, arc to polygonal. Pore multiple 2-5, pore cluster 2-7. Solitary vessel are about equal number with pore multiple and cluster. Perforation plate simple, inclination is transverse to oblique with tail at one end, both end and no tail at all. Tylose is absent, Crystal housed in the parenchyma cell of the pit. Ray is uniseriate, biseriate, multiseriate, heterogeneous (procumbent and upright) and non-storied.

Vernonia galamensis

Porosity is diffuse, vessel is partly woody, the woody part form a circle around large pit which is occupied by various shape parenchyma cells whose shape ranges from circular, oval, short cylindrical, arc to polygonal. Pore cluster absent. Perforation plate simple, inclination is transverse to oblique with tail at one end, at both ends and no tail at all. Pitting is scalariform and alternate. Tylose is absent. Axial parenchyma is absent. Crystal housed in the parenchyma cells of the pits. Rays are uniseriate to multi-seriate, homogeneous and non-storied.

Figure 1 is the dendogram of the quantitative anatomical parameters measured. It showed

that the four species studied are related, however there is a closer relationship between *Vernonia colorata* and *Vernonia amygdalina* and this suggests that one of the two species is likely to be a variant of the other. *V. cinerea* is distinct from the other three species though they belong to the same genus. *Vernonia colorata* and *Vernonia galamensis* are also closely related. *Vernonia colorata*, *Vernonia amygdalina* and *Vernonia galamensis* are all related to *Vernonia cinerea,* with these findings, it shows that the four species can be grouped together within the same genus.

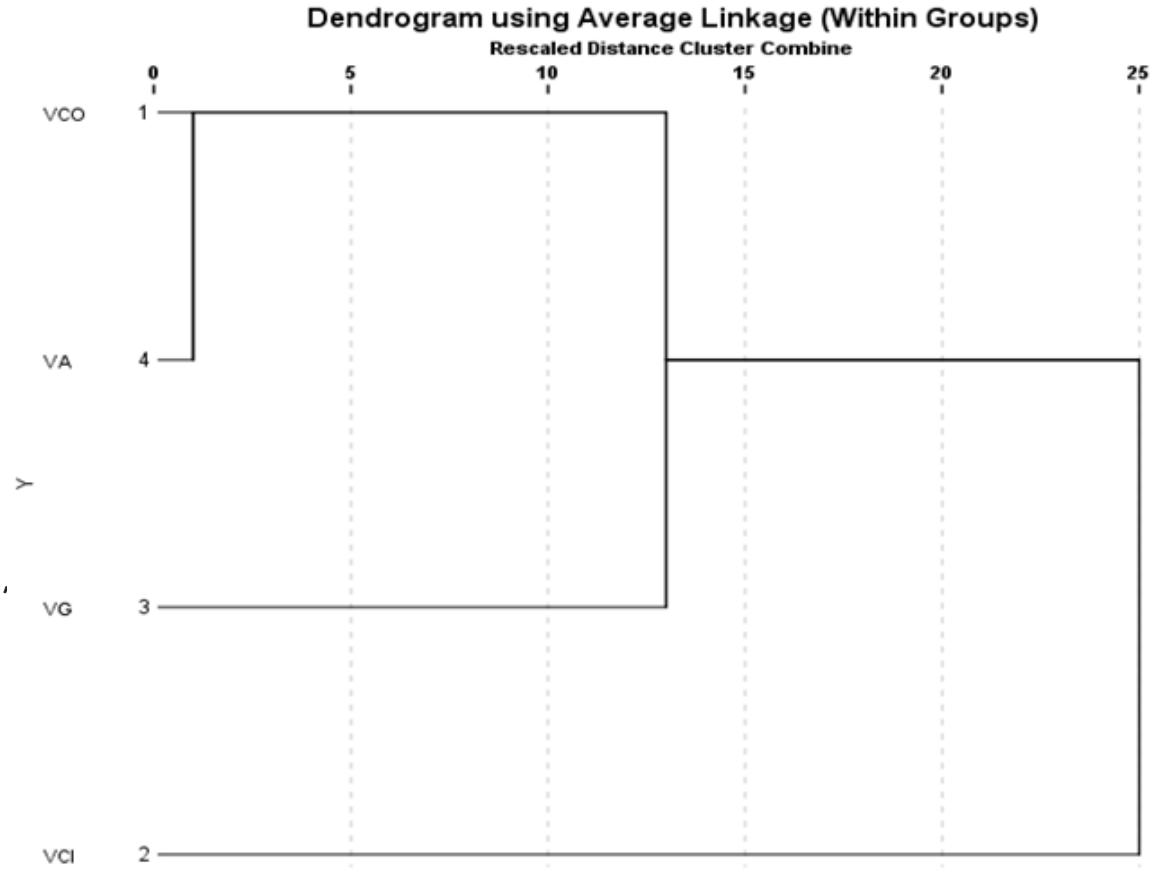

Figure1: Dendrogram Showing the Relationship among Four Species of *Vernonia* studied.

LEGEND: VCO: *Vernonia colorata*, VCI*: Vernonia cinerea*, VG*: Vernonia galamensis*, VA: *Vernonia amygdalina*

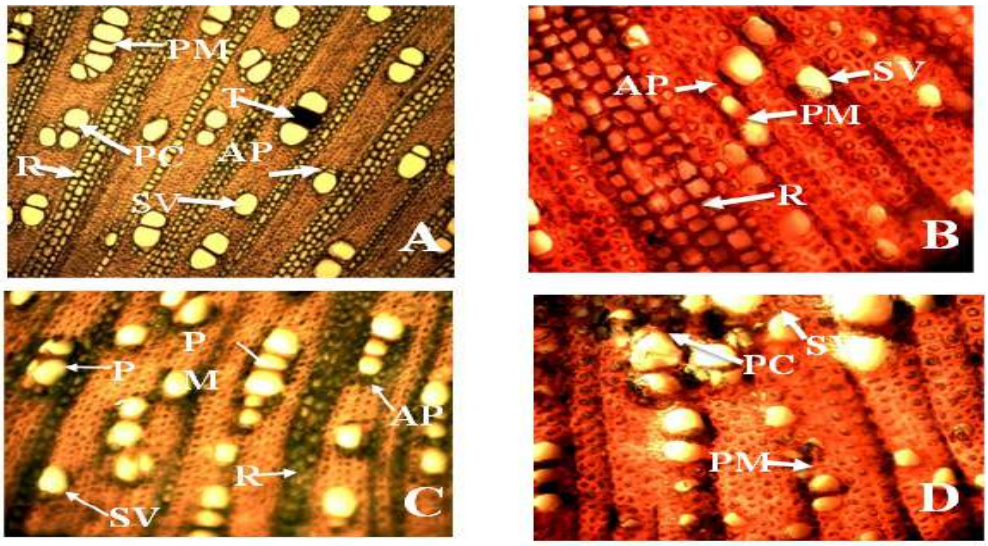

Plate 1: Transverse Sections (T/S) of wood of the four *Vernonia* species

LEGEND:
A: Transverse Section of wood for *Vernonia amygdalina*
B: Transverse Section of wood for *Vernonia cinerea*
C: Transverse Section of wood for *Vernonia colorata*
D: Transverse Section of wood for *Vernonia galamensis*

Key:
AP: Axial parenchyma
SV: Solitary vessel
PC: Pore cluster, R: Ray,
PM: Pore multiple, T: Tylose,

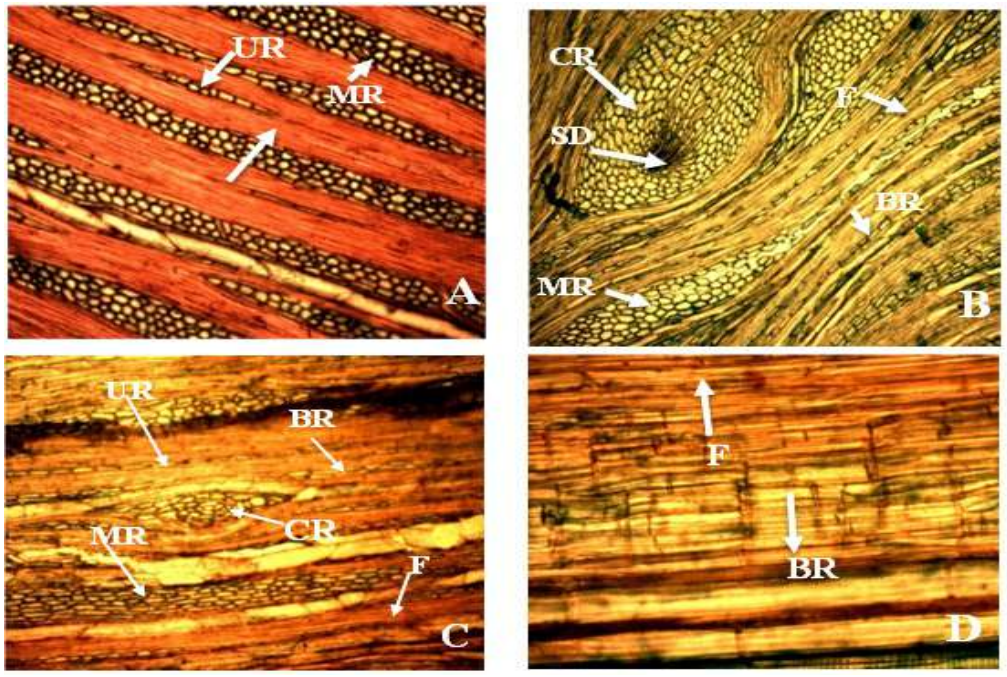

Plate 2: Tangential Longitudinal Sections (TLS) of wood of the four *Vernonia* species

LEGEND:
A: TLS of Wood for *Vernonia amygdalina*
B: TLS of Wood for *Vernonia cinerea*
C: TLS of Wood for *Vernonia colorata*
D: TLS of Wood for *Vernonia galamensis*

Key:
UR: Uniseriate ray, CD: Crystal druses
MR: Multi-seriate ray
F: Fibre, BR: Biseriate ray
SD: Secretive duct, CR: Compound ray
FSRWNN: Flask shaped ray with narrow neck

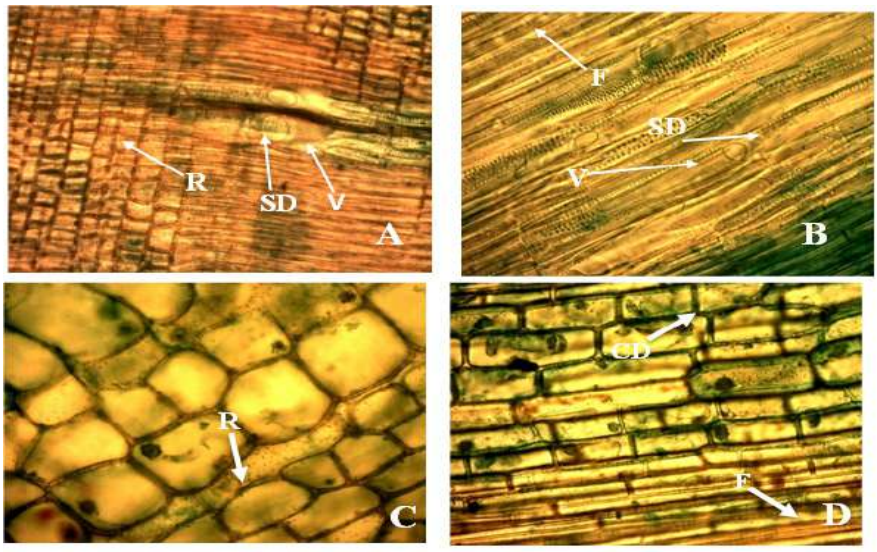

Plate 3: Radial Longitudinal Sections (RLS) of wood of the four *Vernonia* species

LEGEND:
A: RLS of Wood for *Vernonia amygdalina*
B: RLS of Wood for *Vernonia cinerea*
C: RLS of Wood for *Vernonia colorata*
D: RLS of Wood for *Vernonia galamensis* SD: Secretive duct

Key:
V: Vessel, R: Ray, F: Fibre
BR: Biseriate ray,
CD: Crystal druses

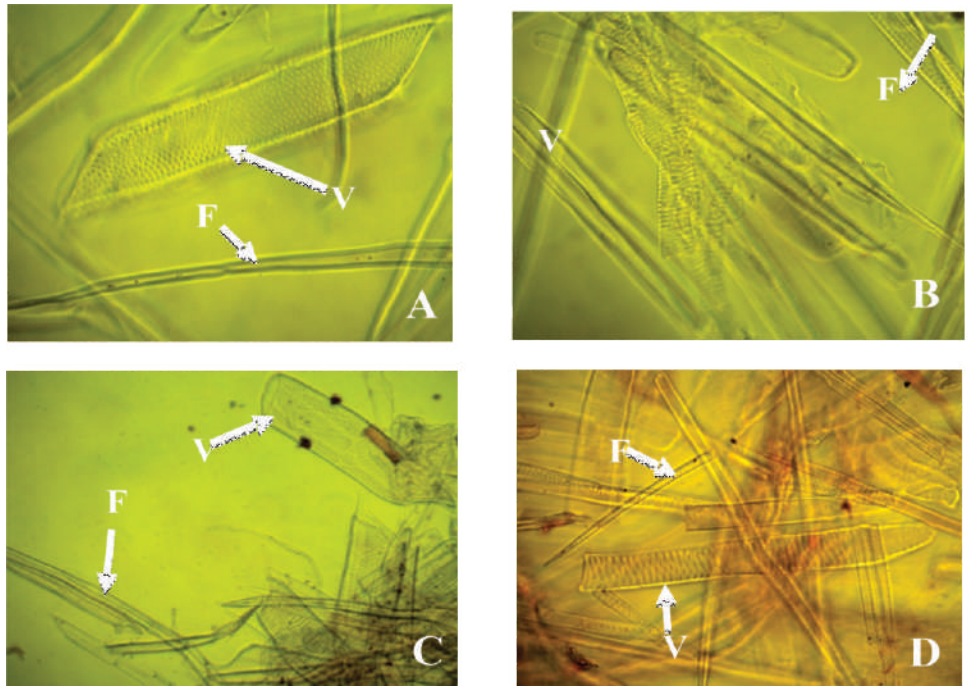

Plate 4: Macerate of the four *Vernonia* species

LEGEND:
A: Macerate Wood of *Vernonia amygdalina*
B: Macerate Wood of *Vernonia cinerea*
C: Macerate Wood of *Vernonia colorata*
D: Macerate Wood of *Vernonia galamensis*

Key:
V: Vessel
F: Fibre

Discussion

All the taxa have diffused porous wood, solitary vessels, pore clusters and pore multiple and absence of growth rings. The perforations of the vessel element are simple and the orientation is oblique to transverse with tail at one end, both ends and without tail at all (Plates 3 and 4). Pitting is simple alternate. Pore shape at transverse plane ranges from circular, oval, arc, saucer, rectangular to cylindrical. A number of quantitative anatomical parameters are not significantly different from each other among the species studied. These are fibre length and diameter, number of pores and pore diameter, ray width are not significantly different in *V. amygdalina*, *V. cinerea* and *V. colorata.*

Rays in all the species are predominantly uniseriate, multiseriate and compound; non-storied and heterogeneous in all the four species as showed in Plate 2. However, in *V. cinerea* the multiseriate rays tapered at their ends to form uniseriate rays (the uniseriate rays do not occur singly but as taperings of the multiseriate rays) as well as the presence of flask shaped compound ray that are diagnostic for *V. cinerea*. These features are unique to this taxon.

Fibres are non-storied, no pitting, narrow wall and lumen (Plate 4). Axial parenchyma is apotracheal in *V. cinerea* and *V. colorata* but para-tracheal in *V. amygdalina* but absent in *V. galamensis.*

The members of the genus *Vernonia* studied have anatomical features that generic affinity among the four species studied. These features include presence of solitary pores, simple perforation plates, and great overlap in most of the quantitative anatomical features (an indication that quantitative anatomical characters may not be useful in distinguishing the four members studied). Ray width, ray height and fibre lumen could also be useful in delimiting the studied species. The four species can however be categorized into three groups.

1. Those with paratracheal axial parenchyma, tylose and upright rays, druses in the vessels.....*V. amygdalina*
2. Tylose absent, and procumbent rays absent of druses in the vessels.....*V. cinerea, V. colorata, V. galamensis*
    a. Those with apotracheal axial parenchyma, flask shaped compound rays present .....*V. cinerea.*
    b. Those with apotracheal axial parenchyma, flask shaped compound rays absent.....*V. colorata*
3. Apotracheal axial parenchyma absent..... *V. galamensis*

The presence of pore cluster confers on *V. colorata* closer affinity to *V. amygdalina* and therefore account for the relationship between the two species as indicated by the dendogram.

This study has greatly increased our understanding of the genus *Vernonia* and opened up knowledge gaps that need to be filled by researchers.

According to Oladipo and Oyeniran (2013), dominance of solitary vessels is an indication of evolutionary primitiveness. In this study, solitary vessels are dominant in all except *V. cinerea* and *V. amygdalina*. Since the presence of tyloses and dominance of solitary vessel can be an indication of evolutionary primitiveness, all the species studied are primitive because all of them have one or both of these features. According to Aworinde *et al* (2007) solitary vessel elements could be linked to coping strategy for water conservation. Also according to Baas (1982) less specialized plant taxa have longer vessels

than the specialized form. In this study, similar observations were made.

In conclusion, wood of Southwestern *Vernonia* species are very similar micro-morphologically. Further research should be directed on DNA sequence of South-western Nigeria *Vernonia* species to provide more information on the relationship within and among the species.

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