



Anatomical Studies on the Stem and Leaf of *Vernonia amygdalina* Del, *Cyanthillium cinereum* (L.) H.Rob and *Brenandendron donianum* (DC.) H.Rob. in Southern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author CIKE designed the study and wrote the first draft of the manuscript. Author CUN performed the statistical analysis. Author OB managed the analysis of the study. Authors CIKE and MI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The stem and leaf anatomical studies of three species namely *Vernonia amygdalina* Del. (Bitter leaf and Non- bitter leaf variety), *Cyanthillium cinereum* (L.) H.Rob and *Brenandendron donianum* (DC.) H.Rob. were undertaken with the aid of a light microscope to ascertain their taxonomic relationship. The anatomy of the stem showed a single layer of epidermis in all the taxa studied, 2-3 layers of collenchyma cells in *V. amygdalina*, 4-5 layers in *C. cinereum* and *B. donianum*. The vascular bundles are arranged in a concentric manner in *V. amygdalina* and *C. cinereum* while it is scattered in *B. donianum*. The scattered vascular bundles and absence of pith in *B. donianum* which is a characteristic feature of the monocotyledonous plant is unique and could be exploited for taxonomic purposes. The occurrence of bicollateral vascular bundles in *B. donianum* and open collateral in the other taxa studied could be of diagnostic importance. The anatomy of the midrib also showed a

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single layer of epidermis usually covered with a cuticle in all the taxa studied. The outline of the midrib is oblong in all the taxa studied. The bundle sheath consists of a cap of 1-3 layers of thick-walled sclerenchyma in *B. donianum* and thin-walled parenchyma in *C. cinereum* and the two different taxa of *V. amygdalina*. The collenchyma cells are 2 – 3 layers in *V. amygdalina*, 4-5 layers in *C. cinereum* and *B. donianum*. The vascular bundle is bicollateral in *B. donianum* and collateral in the other taxa studied. The variation in the type and arrangement of vascular bundles in the midrib is also of taxonomic importance. The similarities in the anatomy of the stem and leaf of *V. amygdalina* (bitter leaf) and *V. amygdalina* (non bitter leaf) suggest that the two taxa are the same species.

Keywords: *Vernonia*; stem; vascular bundles; Southern Nigeria; anatomy.

1. INTRODUCTION

Vernonia amygdalina, *Cyanthillium cinereum* and *Brenandendron donianum* belongs to the Angiosperm family Compositae (Asteraceae). The family Asteraceae is regarded as the largest family of Angiosperms comprising of more than 1,600 genera and 23,000 species of the flowering plants [1] with large number of herbal medicinal plants [2,3]. It is highly advanced, easily recognized and widely distributed dicotyledonous family. Members of this family have a remarkable ecological and economic importance. These include ethno-botanical, phytochemical, antimicrobial and medicinal purposes [4-6]. *Vernonia* occurs in different parts of the world, such as North Africa, Europe, South-East Asia, West Indies and Africa and has attracted the interest of some workers. *Cyanthillium cinereum* is a weed commonly present in tropical and subtropical regions of the world. *Brenandendron donianum* is a tree found in the tropics and other parts of the world. Smith [7] treated the African stengelioid species of *Vernonia*. Adams [8] keyed out the *Vernonia* species in West Africa. Kingharm [9] studied the pollen morphology of African species of *Vernonia* and also grouped the species into sixteen sections. Jones [10] delimited the species of *Vernonia* in Africa and Southeastern Asia into sixteen sections by using pollen morphology and other evidences. Isawumi [11] delimited the *Vernonia* species into five sections based on their foliar trichome studies. Farombi and Owoye [12] reported several health benefits of *Vernonia*. Akinnuubi et al. [13] discovered 2-3, bicollateral vascular bundles in petiole anatomy of *Vernonia amygdalina* and 3 oval shaped amphicribal vascular bundles in *Cyanthillium cinereum* (Synonym: *Vernonia cinerea*). Kemka – Evans and Okoli [14] reported the chromosome number of taxa studied as *Cyanthillium cinereum* (Synonym: *Vernonia cinerea*) $2n = 18$, *Brenandendron donianum* (Synonym: *V. conferta*)

$2n = 20$ and tetraploid number for *V. amygdalina* $2n = 36$. Talukdar [15] showed the taxonomic importance of Cypsela in *Cyanthillium cinereum* (Synonym: *Vernonia cinerea*). *Vernonia amygdalina*, *Cyanthillium cinereum* and *Brenandendron donianum* were formerly placed under the genus *Vernonia*. 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 [16]. Anatomical characters have been widely used by many workers in taxonomy such as [17] 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 [11] 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. [18] 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*. The present study compares the stem and leaf anatomy of *Vernonia amygdalina*, *Cyanthillium cinereum* and *Brenandendron donianum*. The study also reports the stem anatomical characters of *Vernonia amygdalina* (Non- Bitter Leaf) for the first time.

2. MATERIALS AND METHODS

Mature stems and leaves of *Vernonia amygdalina* (Bitter leaf and Non-bitter leaf variety), *Cyanthillium cinereum* and *Brenandendron donianum* were fixed in formalin-acetic acid alcohol mixture (F.AA) for 48 hours (1:1:18, 40% Formaldehyde, acetic acid, 70% alcohol). These materials were later used for anatomical studies according to the method of Johansen [19]. The stems were dehydrated in water alcohol mixtures by steps in the order 30%, 50%, 70%, 95%, absolute alcohol, allowing it for two hours in each solution. They were placed for 3 hours each of the following solutions containing absolute alcohol to pure chloroforms in a volume to volume ratio of 3: 1, 1: 1, 1: 3 and then pure chloroforms. The specimens were placed in an oven at 60°C for 2 days to get rid of all the chloroform. To embed, the contents of the vials were poured into moulds and the specimens kept in place with hot needle. For sectioning, transverse sections were cut on a rotary microtome between 15-18 microns and placed on the clean slides smeared with a thin film of Haptis 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. (1ml 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. Photomicrographs were made from the permanent sides using a

leitzhabolux- 12- microscope fitted with wild maps camera.

3. RESULTS

The anatomical features of the stem of the three species studied namely *Vernonia amygdalina*, *Cyanthillium cinereum* and *Brenandendron donianum* are given in Table 1.

The distribution of tissues in the stem of the taxa studied showed common features of the stem which include single row of epidermal cells which were sometimes covered with thin cuticle. The single row of epidermal cells are flattened tangentially and fitted closely to their radial walls with multicellular hairs. Beneath the epidermis is the collenchyma tissue. The collenchyma cells are 2 to 3 layers in *V. amygdalina* (Bitter-variety and Non-Bitter variety), 4 to 5 layers *C. cinereum* and *B. donianum*. (Figs. 3 and 4). The vascular bundles are arranged in a closed ring in *V. amygdalina* (Bitter-variety and non-bitter variety; Figs. 1 and 2), open ring in *C. cinereum* and scattered in *B. donianum*. The type of vascular bundle is bicollateral in *B. donianum* with interxylary phloem (Fig. 4), while it is open collateral in all the other taxa studied. The bundle sheath consists of 1-3 layers of sclerenchymatous cells in all the taxa. The pith consists of parenchyma tissues in all the taxa studied. The pith is wide in *C. cinereum*. The distribution of tissues in the midrib of the species studied also showed common features of a single row of epidermal cells with thin layer of cuticle usually covering it in all the taxa studied. The outline of the midrib is usually oblong with the abaxial surface being convex and the adaxial concave. Immediately below the epidermis are collenchyma cells, which are thickened at the corners. The collenchyma cells are 2-3 layers in *V. amygdalina* (Bitter-variety and non-bitter variety), 4 to 5 layers in *B. donianum* and

Table 1. Stem Anatomical Features *Vernonia amygdalina*, *Cyanthillium cinereum* and *Brenandendron donianum*

Attribute	<i>V. amygdalina</i> (Bitter-variety)	<i>V. amygdalina</i> (non-bitter variety)	<i>C.</i> <i>cinereum</i>	<i>B.</i> <i>donianum</i>
Epidermal Layer	Single	Single	Single	Single
Epidermal Cell Shape	Rectangular	Rectangular	Rectangular	Rectangular
Collenchyma Layer	2-3 Layers	2-3 Layers	4-5 Layers	4-5 Layers
Collenchyma Type	Angular	Angular	Angular	Angular
Type of Vascular Bundle	Collateral	Collateral	Collateral	Bicollateral
Arrangement of Vascular Bundle	Concentric	Concentric	Concentric	Scattered
Pith	Present	Present	Present	Absent

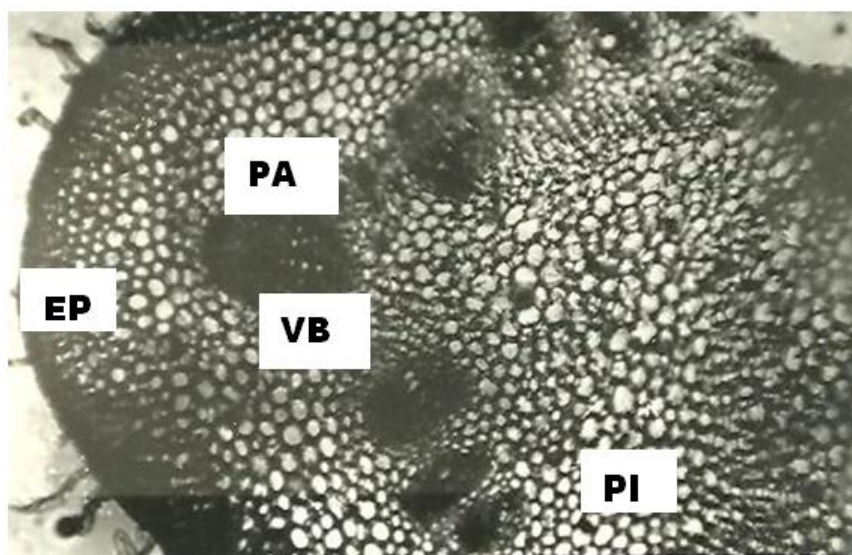


Fig. 1. Showing T.S Stem of *Vernonia amygdalina* (Bitter-variety)

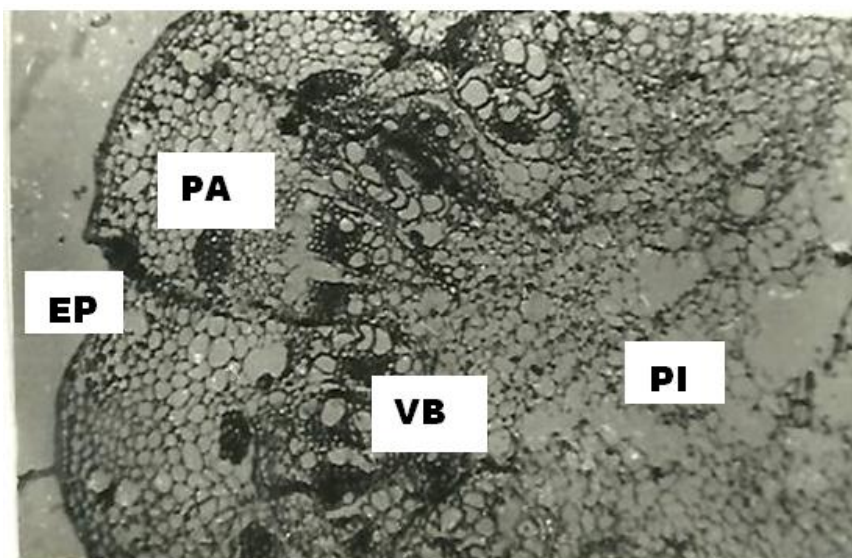


Fig. 2. Showing T.S Stem of *Vernonia amygdalina* (Non-bitter variety). X40

C. cinereum. The vasculature consists of 3-7 bicollateral and separate bundles arranged in a crescent with the small ones in-between the large ones in *B. donianum*, the large bicollateral bundles are arranged in oblong form with vascular bundles occurring at the center (Fig. 8). Bundle sheath consists of a cap of 1-3 layers of thick-walled sclerenchyma in *B. donianum* and thin-walled parenchyma in *C. cinereum* and the two different taxa of *V. amygdalina* (Figs. 5 and 7). The ground tissue of the midrib is parenchymatous in all the taxa studied.

4. DISCUSSION

Anatomical study of the stem and leaf of the species studied revealed that they have highly evolved characters of dicotyledon. The common anatomical features include single layer of epidermis, angular collenchyma and trichomes. The stem anatomy revealed several scattered vascular bundle in *B. donianum*, 18-20 vascular bundles in *V. amygdalina* and 12-13 in *C. cinereum*. *B. donianum* exhibited the bicollateral type of

vascular bundles while the collateral type occurred in *V. amygdalina* and *C. cinereum*. The variation in the number and type of vascular bundles in the stem and leaf the taxa studied could be used as taxonomic characters to delimit these species. The protruding rib of the stem of *C. cinereum* could be a diagnostic character. This work corresponds with the taxonomic update coordinated by the International Compositae Alliance of assigning different genus to the species studied which changed the taxonomic status of *Vernonia cinerea* to

Cyanthillium cinereum and *Vernonia conferta* to *Brenandendron donianum*. The occurrence of scattered vascular bundles in the anatomy of *B. donianum* which is a characteristic of monocotyledon plants is of taxonomic importance. The similarities in both the stem and leaf anatomy of *V. amygdalina* (Bitter-variety) and *V. amygdalina* (Non-Bitter variety) show that it is the same species. The overall anatomical characters revealed in the study are important taxonomic character in the delimitation of the taxa.

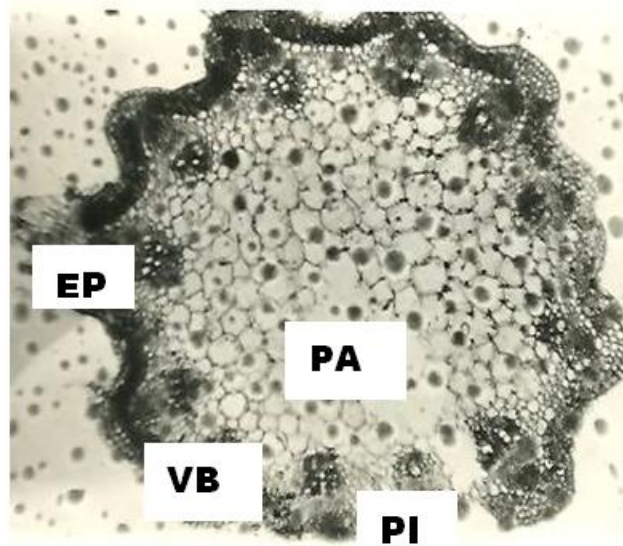


Fig. 3. Showing T.S Stem of *Cyanthillium cinereum*. X 40

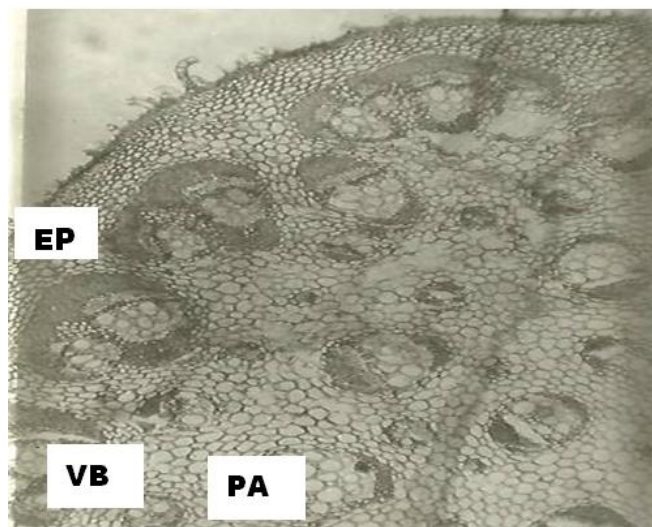


Fig. 4. Showing T.S Stem of *Brenandendron donianum* with scattered and bicollateral vascular bundles. X 40

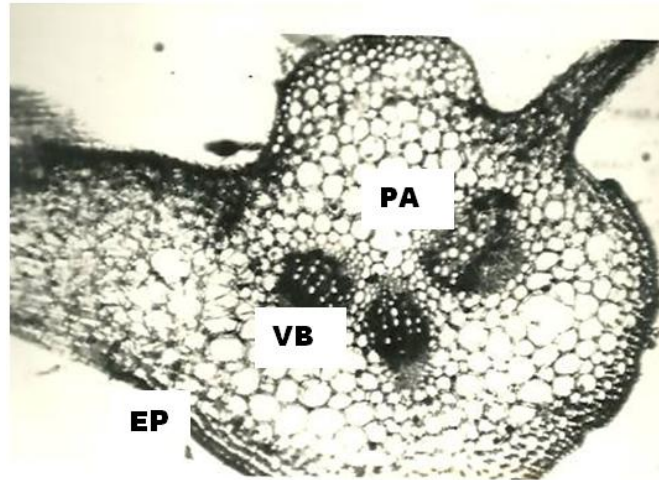


Fig. 5. Showing T.S Lamina of *Vernonia amygdalina* (Bitter-variety) in the region of the midrib. X 40

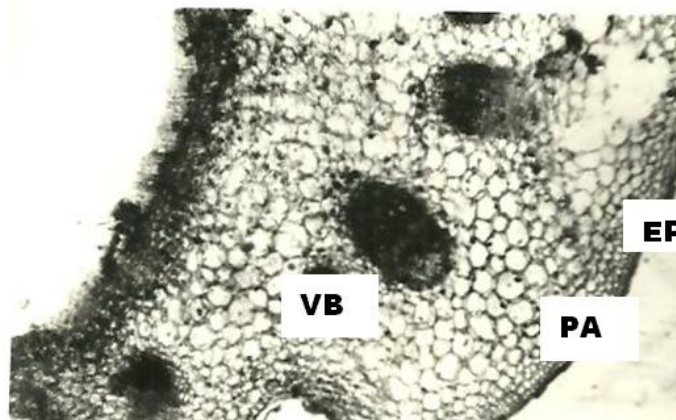


Fig. 6. Showing T.S Lamina of *Vernonia amygdalina* (Non-Bitter variety) in the region of the midrib. X 40

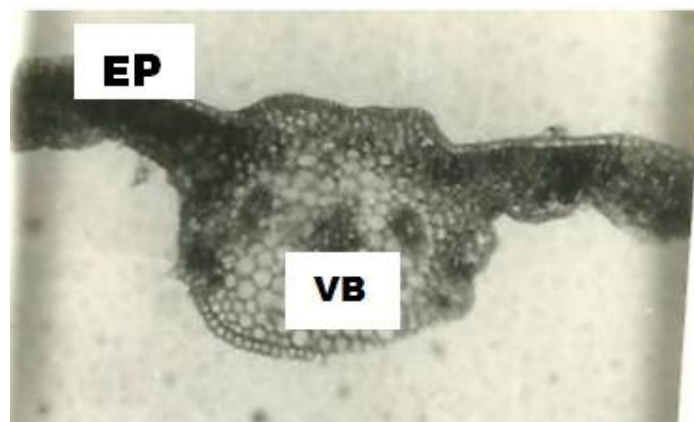


Fig. 7. Showing T.S of Lamina of *Cyathillium cinereum* in the region of the midrib. X 40

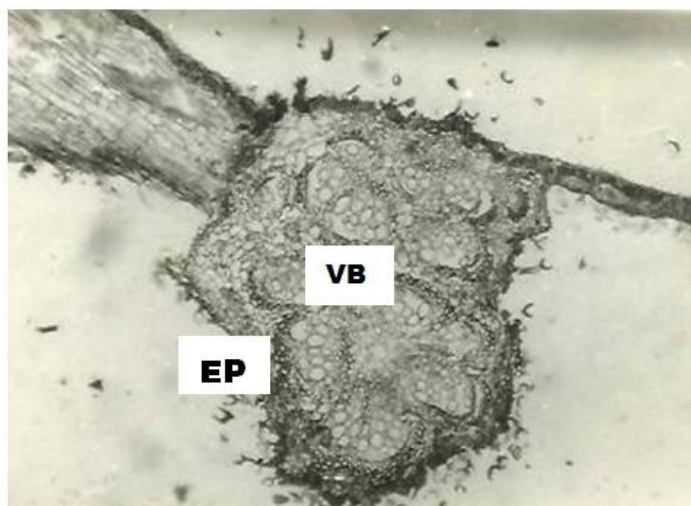


Fig. 8. Showing T.S Lamina of *Brenandendron donianum* in the region of the midrib.

X 40

Key: EP: Epidermis, VB: Vascular bundle, PA: Parenchyma, PI: Pith.

5. CONCLUSION

The overall results from the study show that anatomical characters are of taxonomic importance in the classification and delimitation of the four taxa among the three species studied namely *Vernonia amygdalina* (bitter leaf and non-bitter leaf variety), *Cyanthillium cinereum* and *Brenandendron donianum*. The characters include the variation in the type, number and arrangement of vascular bundles. The similarities in the shape of epidermal cells, type of collenchyma and parenchyma cells show that all the taxa are related.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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