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Research Article

Anatomical Studies of the Midrib, Petiole and Epidermal Strip of Some *Vernonia* Species, from Nigeria

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Abstract

Background and Objectives: Leaf epidermal strip, transverse sections of midrib and petiole of five species of *Vernonia* from South-eastern Nigeria namely; *Vernonia ambigua*, *Vernonia amygdalina* (bitter forms), *Vernonia amygdalina* (non-bitter forms), *V. cinerea* and *V. glaberrima* were examined using a calibrated motican microscope to determine leaf epidermal cell and other leaf anatomical features of taxonomic importance. *V. glaberrima* is famed as a very potent anti-malarial plant, though it grows in the wild, it is often spared during bush clearing. *Vernonia ambigua* and *V. cinerea* are both used as medicine though they are regarded as weeds. *Vernonia amygdalina* (bitter and non-bitter) grow in homestead as vegetable (readily available all the year) and as medicine for various ailments. Part of the aim of this study was to examine anatomical differences between these two forms towards contributing to the determination of their varietal or species status. **Materials and Method:** Epidermal preparation of freshly collected leaves together with transverse sections of the midrib and petiole were made. **Results:** Variations were observed among the species in their stomatal index, midrib and petiole outline, number of layers of collenchyma, number and shape of vascular bundles, shape of anticlinal walls in both adaxial and abaxial epidermis and guard cell dimensions. Marked differences were observed in the epidermal strips of the bitter and non-bitter varieties of *V. amygdalina*. It is suggested that DNA test should be carried out to determine if the two should be given species status. **Conclusion:** Anatomical features of the midrib, petiole and epidermal strip provided sufficient data to separate the species of *Vernonia* studied.

Key words: *Vernonia amygdalina* (non-bitter), *V. glaberrima*, midrib petiole, epidermal strips

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anatomical studies of leaf stem and root of a plant is one of the ways employed to study variation and similarities in plant taxonomy. It had been in use before the newer methods of utilization of chemical markers (secondary metabolites) and molecular studies using DNA sequencing¹. It is one of the methods that employ key taxonomically diagnostic characters inherent in plant cells to enhance identification². External morphological features of fruits and flowers are equally useful in plant identification and characterization but are not always available due to the facts that they are seasonal¹. This necessitates the use of other methods earlier mentioned, including anatomy of plant parts^{3,4}.

The genus *Vernonia* has 500-1000 species^{5,6}. The name *Vernonia* was derived after the name of the English botanist William Vernon who first collected and identified the genus⁷ in the late 1600's. Members of the genus *Vernonia* are important as weeds, ornamentals, medicine and are consumed as vegetables⁸. It was observed that of about 25 species of *Vernonia* in Nigeria, <20 species have been reported in literature⁹. *Vernonia ambigua* and *V. cinerea* are regarded as weeds and are often found growing along roadside or farmland but not often cultivated. The bitter and non-bitter varieties of *V. amygdalina* are planted as hedge plant and as a handy medicinal plant for arresting bleeding from minor cuts, as cough antidote and eaten as vegetable after washing in changes of water to remove bitterness. *Vernonia glaberrima*, grows in the wild, but has fame as a very potent anti-malarial herbal. This makes the choice of these species of great interest to study. Some researchers have examined the epidermal strip of some *Vernonia* species in Nigeria especially *V. amygdalina*^{1,10,11}. However, comparative work involving *V. ambigua*, *V. amygdalina* (bitter and non-bitter), *V. cinerea* and *V. glaberrima* has not received any attention in literature.

The root of *V. ambigua* is used in the treatment of cough, fever, urinary tract infection and regulation of body temperature¹². It is also used in the treatment of malaria and various infectious diseases^{13,14}.

Vernonia amygdalina is helpful in facilitating childbirth due to its potential in increasing uterine contraction^{15,16}. It is also used in treatment of constipation, while its antitumor potential and hepatoprotective activity have been reported by Nalule *et al.*¹⁷, Arhoghro *et al.*¹⁸ and Ojiako and Nwanjo¹⁹. *V. amygdalina* have two varieties-the bitter and the non-bitter varieties. The bitter variety has been studied extensively, while the non-bitter variety is yet to be given adequate attention.

Vernonia cinerea was reported as a plant used for the treatment of malaria and skin infections^{20,21}. *Vernonia glaberrima* is used in the treatment of malaria, migraine, dysmenorrhoea, pain, inflammation and microbial infections^{13,22}.

In the present work, an attempt has been made to conduct comparative anatomical studies on leaves, midribs and petioles of four species of *Vernonia* together with the non-bitter species of *V. amygdalina* with the aim of contributing towards the determination of its varietal or species status. Secondly, the present work is aimed at comparing the anatomical features in the species noting their important taxonomic values.

MATERIALS AND METHODS

The five plants studied namely: *Vernonia ambigua* Kotschy and Peyr, *Vernonia amygdalina* Del. (bitter varieties), *Vernonia amygdalina* (non bitter varieties), *V. cinerea* Less. and *V. glaberrima* Wetw ex. Hoffm were collected from Nsukka metropolis in Enugu State, Nigeria. Nsukka is situated between 50°50 and 70°00. Coordinates 6°51'24"N 7°23'45"E²³. The plants were identified by comparison with the specimens at the National Herbarium Ibadan, Nigeria. Voucher specimens were deposited at the Herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The study was conducted between September, 2018 and July, 2019.

The epidermal strips of the species were prepared for studies based on reported method by Kadiri and Olowokudejo⁴. In this, 2-5 cm² portions were cut from standard median parts of the lamina near the midrib. These were soaked in concentrated nitric acid (HNO₃), in capped specimen bottles for about 8-24 h to effect the maceration of the mesophyll. Epidermal layers were separated with forceps, mounting needles and camel brush. They were washed in three changes of distilled water in petri dishes to remove tissue debris. The cells were dehydrated in the normal ethanol series and stained with safranin, mounted on slides and observed under a calibrated microscope.

The stomatal type, epidermal wall patterns, epidermal type and shapes were noted. Quantitative parameters such as epidermal cell wall thickness, length and width of guard cells were randomly measured. The number of stomata per field of view was counted and used to calculate stomatal index with the following equation²⁴:

$$SI = \frac{N}{N+E} \times 100$$

Where:

N = Number of stomata/field of view

E = Number of epidermal cells in the same field of view

The transverse sections of the midrib and petiole of the species were cut using the Reichert sledge microtome. The arrangement of internal tissues were observed and photomicrographed.

Statistical analysis: The data obtained were analyzed using one-way ANOVA.

RESULTS

Midrib of *Vernonia* species: Midrib of *Vernonia* species presented in Fig. 1a-d.

V. ambigua

Outline: The outline of the adaxial surface was convex, invaginating at the median part, the abaxial surface was convex.

Epidermis: Epidermis was uniseriate and below the epidermis was one layer of collenchyma cells (Fig. 1a, Table 1a, b).

Ground tissue: There were 5.50 layers of parenchyma cells at adaxial surface and 4.75 layers at abaxial surface.

Vascular bundle: There were 3 separate bicollateral vascular bundles, the middle bundle consists of 5.50 strands of xylem and each had 5.25 elements.

V. amygdalina (Bitter variety)

Outline: Adaxial surface was flat topped, curving at the left and right hand corners to join the lamina. The abaxial surface was deeply convex with entire margin (Fig. 1b, Table 2a, b).

Epidermis: Single layered with 2.50 layers of collenchymas cells.

Ground tissue: At the median part, there were 13.50 layers of parenchyma adaxially and 6.50 layers at the abaxial surface. At the extreme left and right hand corners, there were 9.50 layers of parenchyma adaxially and 5.50 layers at the abaxial surface.

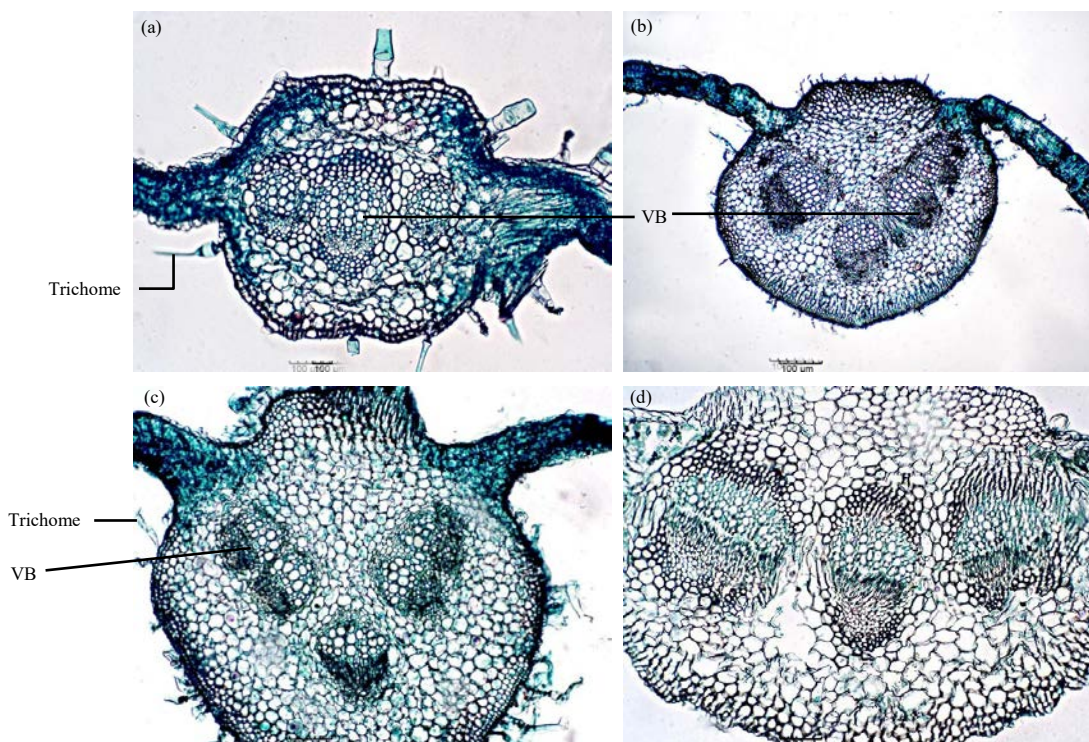


Fig. 1(a-d): Midrib of *Vernonia* species, (a) *V. ambigua* midrib $\times 100$ (b) *V. amygdalina* midrib $\times 100$ (c) *V. amygdalina* (awo) midrib $\times 100$ and (d) *V. glaberrima* midrib $\times 100$
VB: Vascular bundles

Table 1: Midrib of *Vernonia* species (Fig. 1a-d)

Parameters	<i>V. ambigua</i>	<i>V. amygdalina</i>	<i>V. amygdalina</i> (non-bitter)	<i>V. cinerea</i>	<i>V. glaberrima</i>
Outline					
Adaxial	Convex invaginating at median part	Flat topped	Flat topped	Convex	Slightly convex
Abaxial	Convex	Deeply convex with entire margin	Deeply convex with entire margin	Convex with indentation	Convex with indentation
Epidermis	Uniseriate	Uniseriate	Uniseriate	Uniseriate	Uniseriate
Vascular bundle					
Shape	Flattened crescent	V-shaped	V-shaped	Flattened crescent	Flattened crescent
Collenchyma	1.00±0.00 ^c	2.50±0.28 ^b	3.50±0.28 ^a	1.00±0.25 ^e	1.00±0.00 ^c
Ground tissue					
Adaxial	5.50±0.28 ^d	13.50±0.57 ^b	15.00±0.40 ^a	9.00±0.40 ^a	9.50±0.28 ^c
Abaxial	4.75±0.25 ^c	6.50±0.25 ^b	6.25±0.25 ^b	5.75±0.25 ^b	7.75±0.40 ^a
Vascular bundle	3.00±0.00 ^b	5.00±0.00 ^a	5.00±0.00 ^a	2.50±0.28 ^d	3.00±0.40 ^b
Strands of xylem at median bundle	5.50±0.28 ^d	7.50±0.28 ^b	6.50±0.29 ^c	3.00±0.25 ^c	8.75±0.25 ^a
No of xylem elements in a strand	5.25±0.25 ^a	6.00±0.40 ^a	2.50±0.28 ^b	3.00±0.28 ^c	2.75±0.40 ^b

Means with different alphabets along each horizontal array signifies significant differences at $p \leq 0.05$ among the species, while means with similar alphabets along each horizontal array signifies non-significant differences at $p \leq 0.05$ among the species

Vascular bundle: There were 5 separate bicollateral vascular bundles arranged in a V-shape with the middle one located at the lowest point close to the abaxial surface. The median bundle had 7.50 strands with 6.00 elements in a strand.

V. amygdalina (non-bitter variety)

Outline: The adaxial surfaces was flat topped and curved at the left and right sides, while the abaxial surface was deeply convex with entire outline (Fig. 1c, Table 1a, b).

Epidermis: Uniseriate layer of epidermis followed by 3.5 layers of collenchyma cells.

Ground tissue: The parenchyma cells were 15 layers at the adaxial surface and 6.25 layers at the abaxial side of the median bundle.

Vascular bundle: There were 5 vascular bundles that were arranged in a V-shaped crescent with the median vascular bundles being the lowest, while the 2 lateral ones were closer to the adaxial surface. The xylem strands were 6.50 in the median bundle and there were 2.50 elements in a strand.

V. cinerea

Outline: Both adaxial and abaxial surfaces were convex, the abaxial surface had indentations (Table 1a, b).

Epidermis was single layered with a single layer of collenchymas.

Ground tissue: At the median part, there were 9 layers of parenchyma at adaxial surface and 5.75 layers at abaxial surface.

Vascular bundle: There were 2.50 separate bicollateral vascular bundles. The median bundle was big and had phloem sclerenchyma at abaxial surface.

V. glaberrima

Outline: Adaxial surface was slightly convex, while abaxial surface was deeply convex (Fig. 1d, Table 1a, b).

Epidermis: Uniseriate epidermis and 1 layer of collenchyma below it.

Ground tissue: At adaxial surface, there were 9.50 layers of parenchyma and 7.75 layers at abaxial surface.

Vascular bundle: Three separate and bicollateral vascular bundles were present. The median bundle had 8.75 strands of xylem with 3 elements in a strand. The 2 lateral vascular bundles each had 8.75 strands of xylem. Phloem sclerenchyma occurred at adaxial side.

Petioles of *Vernonia* species

V. ambigua

Outline: The adaxial surface was flat topped and the abaxial surface was convex (Fig. 2a, Table 2a, b).

Epidermis: Epidermis was single layered, 3.75 layers of collenchyma cells occurred below the epidermis (Fig. 2a, Table 2a, b).

Ground tissue: Nine layers of parenchyma occurred at the adaxial side and 6.50 layers occurred at the abaxial side.

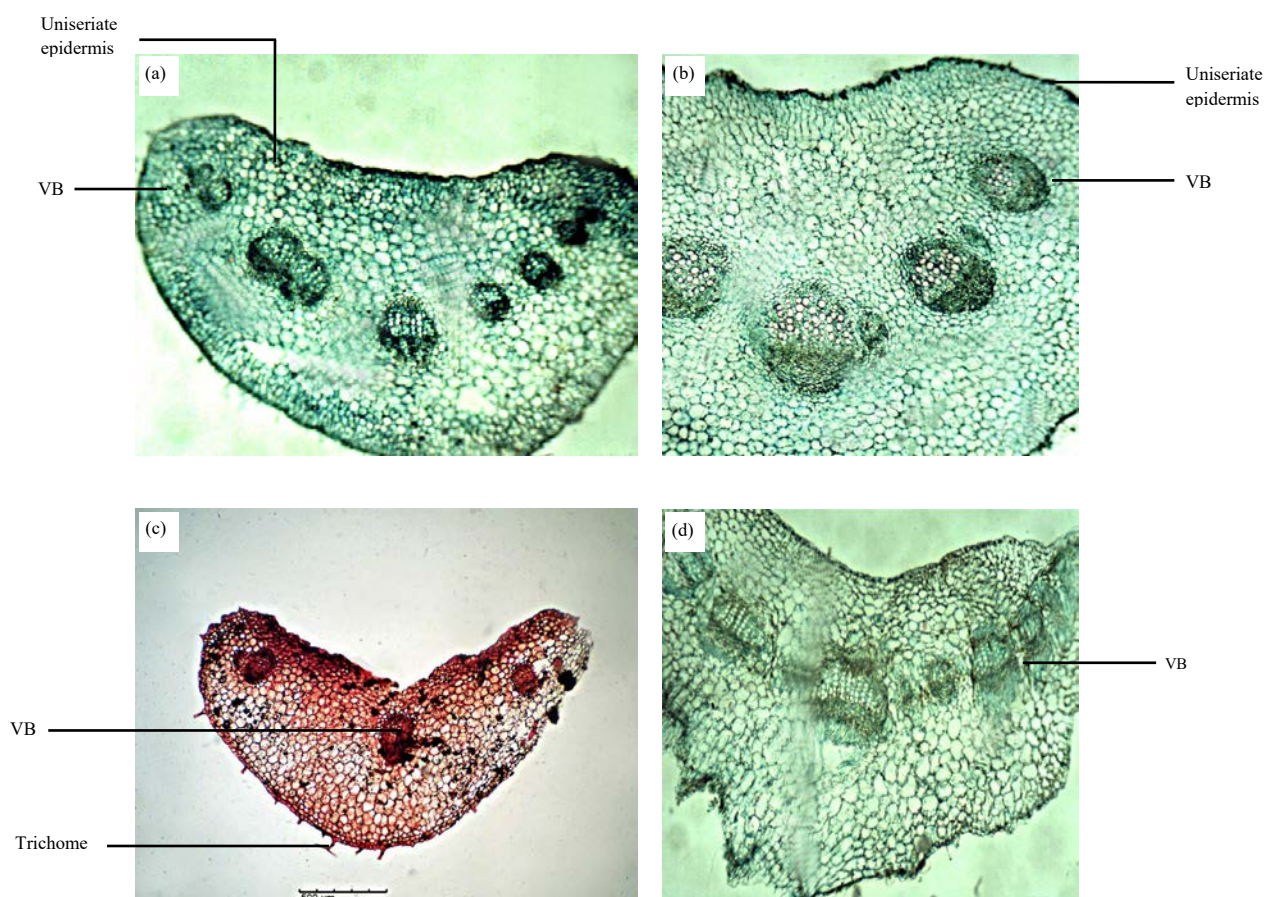


Fig.2(a-d): Petioles of *Vernonia* species, (a) *V. ambigua* petiole $\times 40$, (b) *V. amygdalina* petiole $\times 40$, (c) *V. cinerea* petiole $\times 40$ and (d) *V. glaberrima* petiole $\times 40$

VB: Vascular bundles

Table 2: Petiole of *Vernonia* species (Fig. 2a-d)

Parameters	<i>V. ambigua</i>	<i>V. amygdalina</i> (bitter)	<i>V. amygdalina</i> (non-bitter)	<i>V. cinerea</i>	<i>V. glaberrima</i>
Outline					
Adaxial	Flat topped	Flat topped slightly concave	Flat topped slightly concave	Concave	Concave
Abaxial	Convex	Convex and slightly indented	Convex without indentation	Convex	Deeply convex
Epidermis	Single layered	Single layered	Single layered	Single layered	Single layered
Shape of vascular bundle	Crescent	Crescent	Crescent	Crescent	Flattened crescent
Ground tissue					
Collenchyma	3.75 ± 0.25^a	3.50 ± 0.28^a	2.50 ± 0.28^b	1.50 ± 0.28^c	1.00 ± 0.00^c
Adaxial	9.00 ± 0.40^c	13.00 ± 0.40^a	10.75 ± 0.47^b	5.25 ± 0.25^d	9.00 ± 0.47^c
Abaxial	6.50 ± 0.28^c	12.00 ± 0.00^a	8.75 ± 0.47^b	6.50 ± 0.28^c	8.50 ± 0.28^b
No. of Vascular bundle	9.00 ± 0.00^a	5.00 ± 0.00^d	8.25 ± 0.47^b	3.00 ± 0.00^e	7.00 ± 0.00^c
Strands of xylem at median bundle	6.75 ± 0.25^b	10.75 ± 0.48^a	6.50 ± 0.28^b	5.00 ± 0.00^c	11.75 ± 0.47^a
No. of xylem elements in a strand	2.75 ± 0.47^b	5.25 ± 0.48^a	2.75 ± 0.48^b	4.50 ± 0.28^a	5.25 ± 0.25^a

Means with different alphabets along each horizontal array signifies significant difference at $p \leq 0.05$ among the species, means with similar alphabet along each horizontal array signifies non-significant difference at $p \leq 0.05$ among the species

Vascular bundle: There were nine separate bundles arranged in a crescent shape. The median bundle had 6.75 strands of xylem and 2.75 xylem elements in a strand.

***V. amygdalina* (Bitter variety)**

Outline: The adaxial surface was flat topped and slightly concave, while the abaxial surface was convex and slightly indented (Fig. 2b, Table 2a, b).

Epidermis: Single layered with 3.5 layers of collenchyma cells.

Ground tissue: There were 13 layers of parenchyma at the median part adaxially and 12 layers at the median part abaxially.

Vascular bundle: There were 5 separate vascular bundles that were arranged in form of a crescent. The biggest one was in the middle flanked on either side by two bundles and 10.75 strands of xylem at the median bundle. Xylem elements were 5.25 and of varying sizes in the strands.

***V. amygdalina* (non-bitter variety)**

Outline: The adaxial surface was slightly concave, while the abaxial surface was convex with no indentation (Table 2a, b).

Epidermis: Single layered with 2.50 layers of collenchyma.

Ground tissue: At the median part adaxially, there were 10.75 layers of thin walled parenchyma cells and 8.75 layers at the abaxial surface.

Vascular bundle: There were 8.25 separate vascular bundles arranged in a flattened crescent shape. One vascular bundle in the middle was flanked by 3-4 on the left and on the right hand sides. There were 6.50 strands of xylem in the median bundles and 2.75 elements in a strand.

V. cinerea

Outline: The adaxial surface was concave, while the abaxial surface was convex with entire margin (Fig. 2c, Table 2a, b).

Epidermis: Single layered epidermis was present. 1.50 layers of collenchyma cells occurred at adaxial surface and single layered collenchymas at abaxial surface.

Ground tissue: There were 5.25 layers of parenchyma at adaxial surface of the median vascular bundle and 6.50 layers at abaxial surface.

Vascular bundle: Three separate bicollateral vascular bundles, arranged in a crescent shape were present. The middle was the biggest and had 5 strands of xylem with each having 4.50 xylem elements.

V. glaberrima

Outline: The adaxial surface was concave, while the abaxial surface was deeply convex with some indentations. There were lateral extensions on the left and right hand sides (Fig. 2d, Table 2b).

Epidermis: Single layered epidermis was found, followed by a single layer of collenchyma.

Ground tissue: There were 9.00 layers of parenchyma at the adaxial surface above the median vascular bundle and 8.50 layers below the median bundle (abaxial surface).

Vascular bundle: Seven vascular bundles were present with the biggest at the centre flanked by 3 bundles on either side. In the median bundle, there were 11.75 strands of xylem and 5.25 xylem elements in a strand.

Epidermal strip

V. ambigua

Abaxial surface: Epidermal cells were pentagonal to polygonal and some had irregular shape. The anticlinal walls were straight, there were numerous stomata of anisocytic type (Fig. 3a, Table 3a, b).

Adaxial surface: Epidermal cells were mostly pentagonal with a few that had slightly irregular shape. The anticlinal walls were straight. The numbers of stomata at the adaxial surface were fewer than those at the abaxial surface, they were also of anisocytic type (Fig. 3b).

***V. amygdalina* (bitter)**

Abaxial surface: Epidermal cells were mainly pentagonal to hexagonal, though a few had irregular shape, the anticlinal walls were straight while some were sinuous. The stomata were more numerous at the abaxial surface and stomata were of anisocytic type (Fig. 3c).

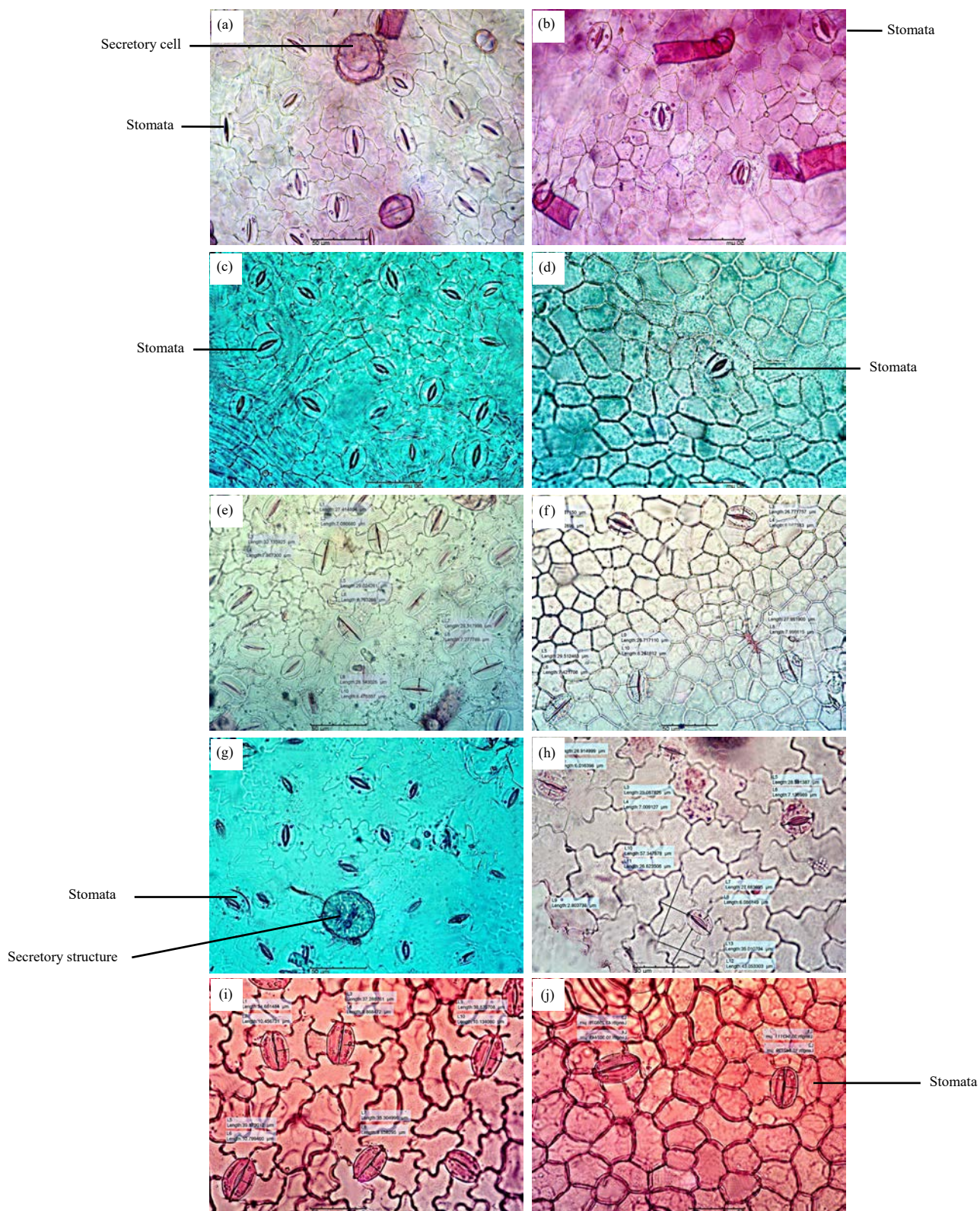


Fig. 3(a-j): Epidermal strip (3), (a) *V. ambigua* AB ×400, (b) *V. ambigua* AD ×400, (c) *V. amaygdalina* AB (bitter) ×400, (d) *V. amaygdalina* AD (bitter) ×400, (e) *V. amaygdalina* (non-bitter) AB ×400, (f) *V. amaygdalina* (non-bitter) AD ×400, (g) *V. cinerea* AB ×400, (h) *V. cinerea* AD ×400, (i) *V. glaberrima* AB ×400 and (j) *V. glaberrima* AD ×400

Table 3: Features of adaxial and abaxial epidermal leaf surfaces of *Vernonia* species (Fig. 3a-j)

Species	Length of guard cell	Width of guard cell	No of stomata/field	No of subsidiary cell/field	Stomatal Index at $\times 400$
<i>V. ambigua</i>	29.26 \pm 0.26 ^b	5.16 \pm 0.12 ^d	4.00 \pm 0.00 ^a	80.16 \pm 0.16 ^b	4.69 \pm 0.00 ^c
<i>V. amygdalina</i> (bitter)	25.10 \pm 0.05 ^d	5.03 \pm 0.33 ^d	4.00 \pm 0.00 ^a	90.00 \pm 0.00 ^b	4.18 \pm 0.01 ^c
<i>V. amygdalina</i> (non-bitter)	29.16 \pm 0.05 ^d	5.40 \pm 0.00 ^c	4.00 \pm 0.00 ^a	171.66 \pm 0.33 ^a	18.00 \pm 0.00 ^a
<i>V. cinerea</i>	27.50 \pm 0.11 ^c	6.60 \pm 0.05 ^b	4.00 \pm 0.00 ^a	40.00 \pm 5.77 ^c	9.00 \pm 0.57 ^b
<i>V. glaberrima</i>	39.50 \pm 0.05 ^a	10.10 \pm 0.05 ^a	2.00 \pm 0.57 ^b	80.00 \pm 5.77 ^b	2.40 \pm 0.05 ^d
<i>V. ambigua</i>	26.53 \pm 0.26 ^b	4.76 \pm 0.03 ^e	15.00 \pm 0.00 ^c	60.00 \pm 0.00 ^d	23.00 \pm 0.00 ^a
<i>V. amygdalina</i> (bitter)	25.50 \pm 0.20 ^c	5.06 \pm 0.03 ^d	16.03 \pm 0.03 ^b	95.66 \pm 0.33 ^b	8.16 \pm 0.16 ^e
<i>V. amygdalina</i> (non-bitter)	24.13 \pm 0.13 ^d	5.40 \pm 0.00 ^c	22.00 \pm 0.00 ^a	115.33 \pm 0.66 ^a	16.00 \pm 0.00 ^c
<i>V. cinerea</i>	25.00 \pm 0.57 ^c	5.56 \pm 0.08 ^b	10.00 \pm 0.57 ^d	40.00 \pm 0.57 ^e	20.00 \pm 0.57 ^b
<i>V. glaberrima</i>	37.00 \pm 0.05 ^a	10.80 \pm 0.05 ^a	7.00 \pm 0.00 ^e	64.00 \pm 0.57 ^c	9.80 \pm 0.05 ^d

Means with different alphabets along each horizontal array signifies significant difference at $p \leq 0.05$ among the species, means with similar alphabets along each horizontal array, signifies non-significant difference at $p \leq 0.05$ among the species

Adaxial surface: Epidermal cells were mostly pentagonal to hexagonal with few that were rectangular. The anticlinal walls were straight and stomata was of animocytic type (Fig. 3d).

V. amygdalina (non-bitter)

Abaxial surface: Epidermal cells were mostly irregularly shaped and anticlinal walls were sinuous. The stomata were numerous and of animocytic type (Fig. 3e).

Adaxial surface: Epidermal cells were pentagonal to hexagonal with straight anticlinal walls. The stomata were few and were of animocytic type (Fig. 3f).

V. cinerea

Abaxial surface: Epidermal cells had irregularly shaped cells. The anticlinal walls were wavy to undulating, the stomata were numerous and animocytic (Fig. 3 g).

Adaxial surface: Epidermal cells were mostly irregular to polygonal in shape. The anticlinal walls were undulating, stomata were few and animocytic (Fig. 3h).

V. glaberrima

Abaxial surface: Epidermal cells were mostly irregularly-shaped and the anticlinal walls were wavy to undulating. The stomata were numerous and of animocytic type (Fig. 3i).

Adaxial surface: The epidermal cells were pentagonal to hexagonal and the anticlinal walls were sinuous. Stomata were few and of animocytic type (Fig. 3j).

Adaxial epidermal strip: The features of epidermal strip at the adaxial surface revealed that the length of guard cells of *V. glaberrima* had the longest dimension while the width had the least dimension. *Vernonia glaberrima* also had the least number of stomata/field of view. The least number of subsidiary cells/field of view was recorded for *V. cinerea*. The

number of subsidiary cells/field of view and the Stomatal Index in *V. amygdalina* (bitter) varied significantly ($p \leq 0.05$) from that of *V. amygdalina* (non-bitter). *Vernonia glaberrima* had the least Stomatal Index value (Table 3a).

Abaxial epidermal strips: The length and width of guard cells in the abaxial surface of *V. glaberrima* had greater value than that of other species examined. The values of number of stomata field of view, number of subsidiary cells/field of view and Stomatal Index of *V. amygdalina* (non-bitter) was significantly ($p \leq 0.05$) higher than that of *V. amygdalina* (bitter) (Table 3b).

The result of Table 3a and b showed that all the *Vernonia* species examined had more stomata at the abaxial than at the adaxial surfaces. Stomata were fewer in number at the adaxial surface for all the species. At the adaxial surface *V. glaberrima* had the least stomatal Index and the least number of stomata with the length and width of guard cells having the greatest dimensions. At the abaxial surface, *V. glaberrima* had the length and width of guard cells with the highest dimensions and the least number of stomata/field of view. The number of stomata/field of view, number of subsidiary cells and Stomatal Index of *V. amygdalina* (non-bitter) was significantly ($p \leq 0.05$) higher than what was observed in *V. amygdalina* (bitter).

DISCUSSION

There are similar features in the midrib of the species of *Vernonia* studied namely; convex shape of abaxial surface, presence of uniseriate epidermis and possession of separate vascular bundles. These similarities buttress the fact that they belong to the same genus. The presence of uniseriate epidermis might be a feature that is common in the family Asteraceae. A study conducted in Turkey showed the presence of uniseriate epidermis in *Tanacetum* (Asteraceae)²⁵. The adaxial surface of midrib of *V. amygdalina*

(bitter and non-bitter) was found to be elevated and flattened. Similar observation was made in a previous study by Ekeke and Mensah²⁶. Bicolateral vascular bundles were observed in all the species in this study. This buttresses the findings of Ekekeh and Mensah²⁶, who observed the same in members of Asteraceae they examined²⁶. The number of parenchyma cells in ground tissue of abaxial and adaxial surfaces varied widely and is useful in identifying each taxon.

Metcalf and Chalk noted that petioles of Asteraceae (compositae) consist of separate bundles which may be arranged in a flattened to deep crescent shape²⁷. They also reported that an arc of phloem may occur at the inner edge of the xylem. In all the *Vernonia* species examined, vascular bundles were separate, crescent shaped and bicollateral thus confirming the earlier report by Metcalfe and Chalk²⁷.

Differences in the outline of petiole, the number of layers of collenchyma and parenchyma cells and the arrangement of vascular bundles exist among members of Asteraceae²⁸. These variations are used in the classification and delimitation of species in the family. In the study of these *Vernonia* species, differences were observed in the outline of adaxial surface, number of layers of collenchyma and parenchyma cells and number of vascular bundles. For example, while *V. ambigua* has 9 separate vascular bundles in the petiole, *V. amygdalina* (bitter variety) has 5, *V. cinerea* has 3 and *V. glaberrima* has 7.

Differences were observed in the midrib of *V. amygdalina* (bitter variety and the non-bitter variety), bitter variety (BV) had 2.50 layers of collenchyma, while the non-bitter (NBV) had 3.50 layers. In the BV, the xylem elements were 6.00, while in the NBV, it was 2.50. The petiole anatomy revealed more differences. There were striking differences in the epidermal strips. The anticlinal walls on the abaxial surface were straight in BV but sinuous in NBV. Other workers also observed the presence of sinuous anticlinal walls on the abaxial surface of *V. amygdalina* (non-bitter)²⁹. The stomatal index at x400 magnification was 4.18% at adaxial surface of BV in this study and is very close to the value of 4.84% reported in an earlier study by Kemka-Evans *et al.*²⁹. Other differences observed in *V. amygdalina* are the shorter life span of NBV, whereas the BV lives for longer years even under adverse conditions. In addition, NBV does not need to be washed in several changes of water to be suitable for consumption unlike the BV that must undergo washing in several changes of water to remove the bitterness. It is suggested that further studies should be done to quantify the number of phytochemicals, chromosome number and molecular DNA analysis to determine if these two are distinct from one another. It is noteworthy that the southeast people of Nigeria (Igbos) gave these 2 plants different names. The bitter variety, they called 'onigbu' and the non-bitter variety, they called 'awo'.

Speaking of indigenous knowledge of an African community (Huri forages) in Central Africa, Cavalli-Sforza stated that these forages are great observers of natural phenomena and their ability as systematists of plants and animals matches those of trained specialists³⁰. This statement can be generalized for many African medicine men including the indigenous people of southeastern Nigeria.

All the species of *Vernonia* examined were amphistomatic (had stomata on both adaxial and abaxial surfaces). The presence of amphistomatic feature in *V. cinerea* and *V. amygdalina* was also reported by Nwakanma *et al.*¹ and Burkill¹². The number of stomata in the adaxial surface was much fewer than the one in the abaxial surface. The stomatal index was lower in the adaxial surface than in abaxial as a consequence. *Vernonia cinerea* had the least no of stomata in the abaxial surface, this could be because the leaf size was also least among the species studied. Length of guard cells in the adaxial surface was longer than the length in the abaxial surface. The wall patterns of the epidermal cells can be used to differentiate the species. The anticlinal walls in abaxial surface of *V. ambigua* and *V. glaberrima* were wavy to undulating but straight at the adaxial surface. In *V. cinerea*, the anticlinal walls were wavy to undulating at both surfaces but straight in *V. amygdalina* (bitter variety) as observed in previous studies by Aworinde *et al.*³¹ and Narayana³².

The length and width of guard cells in *V. glaberrima* was longer than those of other species studied. This could be because they are found in areas where annual fires occur. The number of stomata on both the adaxial and abaxial surfaces is least in *V. glaberrima*. It was reported that climatic factors like rainfall, temperature, humidity and wind can influence the frequency of occurrence and distribution of stomata on leaves³². The report thus corroborates the observed increase in length and width of the guard cells but fewer stomata of *V. glaberrima* in the present study. The features exhibited by the anatomical structure of these species can be used effectively to identify them and to differentiate them from adulterants especially those that grow in the wild like *V. ambigua*, *V. cinerea* and *V. glaberrima*. In the same vein, anatomical features can be used to identify herbarium species that were not preserved with flowers or fruits and also to identify commercial samples of medicinal plants³³.

In conclusion, midrib, petiole and epidermal anatomy of the *Vernonia* species studies, have distinctive features that could be used to accurately identify them and maintain them in the tribe Vernonieae, family Asteraceae. These features of the anatomy can also be used to differentiate the species that are used to treat various ailments from adulterants by the populace.

SIGNIFICANCE STATEMENT

The study noted significant anatomical differences between the bitter and non-bitter forms of *V. amygdalina* that strongly suggest conferring species status on the bitter form. Furthermore, the study has provided a good reason to carry out DNA and phytochemical investigation on these two forms of *V. amygdalina*. The option of domestication of *V. glaberrima* which normally grows in the wild, though it has famed antimalarial potential can be explored for the good of the general populace.

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