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MICROSCOPIC EVALUATION OF THE LEAF OF *VERNONIA AMBIGUA* KOTSCHY AND PEYR. (FAMILY: ASTERACEAE/COMPOSITAE) GROWING IN NIGERIA

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ABSTRACT

Vernonia ambigua Kotschy and Peyr. is a plant in the family Asteraceae/Compositae that has been used traditionally for problems related to reproductive health. Substantial amount of work has been done on the phytochemical profile of the leaf. However, there is no report on the microscopiy of the leaf in literature. Result of the microscopic analysis shows wavy-walled epidermal cells on the upper surface while the lower surface has more or less polygonal epidermal cells. Stomata are present on both surfaces of the leaf (amphistomatic) and are of the anomocytic type. The distribution of stomata is more in number on the lower surface. Trichomes are of two types: long multicellular trichomes with broad base and tapering ends (non-glandular) and peltate (glandular) trichomes with short stalk which are more abundant on the lower epidermis. Spiral vessels and fibres with tapering ends are observed. The data obtained from this study will be useful in the identification of the species, in detecting substitution and adulteration of the plant drug and in developing standards for monograph and future reference.

KEYWORDS: Microscopy, Amphistomatic, Asteraceae, Anomocytic stomata, *Vernonia ambigua*, Nigeria.

INTRODUCTION

One of the most important uses of plants known to man is its medicinal value. People have relied on plants to stay healthy and to treat diseases for a long time. The importance of the study of substances obtained from plants and animals, as well as from other substances of natural origin cannot be over-emphasized. Many drugs used in therapy are obtained from plants and more are being discovered (Akerele, 1998). These studies are even more important in third world countries and developing countries like Nigeria where the economic situation limits access to orthodox health care or low financial capability necessitates that a lot of people depend on locally collected and prepared medications (Akerele, 1998). In these countries, the most accessible source of healthcare is the Traditional Medical Practitioner, accounting for 80% of the world's population relying on traditional medicine (Akerele, 1998).

In the African sub-region, there is availability of a vast number of naturally-occurring medicinal plants. The people of this region depend mostly on these plants since they can be accessed quickly and are affordable. The knowledge of these plants and their uses were passed down from one generation to the other and this was done mostly through oral communication (Evans, 2002). In the past, there were no science-oriented studies carried out on these plants and most of the knowledge obtained on their uses was acquired through trial and error. In addition to this, some of the information on these plants were either lost or found to be incorrect after scientific investigations were carried out on them (Evans, 2002). Though herbal medicine use is one of the ways and in some cases the only way of providing healthcare for a majority of the population, especially in developing countries, there still exists the concern and uncertainty about the quality, safety and efficacy of these remedies. The problem of quality arises because of the nature of herbal products which are complex mixtures. The components responsible for the claimed therapeutic effects are frequently unknown or only partly explained and this impedes the level of control which can routinely be achieved with a synthetic drug. The problem of efficacy arises from lack of data on phytochemical constituents and biological studies. There is also the problem of incorrect diagnosis, imprecise dosage and low hygiene standards, secrecy of some healing methods and the absence of written records about patients (De Smet, 2005). There are further concerns over the perceived lack of regulation of herbal medicines in many countries and the encouragement of the sale of unregistered products that are not controlled by regulatory authorities (De Smet, 2005). Despite enormous advances in conventional medicine, there is

the need to look at the needs of people who do not have access to orthodox medicine. Medicinal plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is lower incidence of adverse effects after use. These reasons might account for their worldwide use.

Species of the genus *Vernonia* are found in South America, Africa, Southeast Asia and North America. The genus comprise of about 1000 species. Some species are edible and of economic value and are known for having purple flowers. Species of *Vernonia* include *V. calvoana*, *V. amygdalina*, and *V. colorata* which are eaten as leafy vegetables. Common names for these species include Bitter leaf (English), *Ndole* (Cameroon) and *Onugbu* (Igbo). The leaves have a sweet and bitter taste. They are sold fresh or dried and are typical ingredient in *egusi* soup in Nigeria. *Vernonia amygdalina* is well known as a medicinal plant with several uses including the treatment of diabetes, fever reduction, and recently a non-pharmaceutical remedy for persistent fever, headache, and joint pain associated with AIDS (Elujoba *et al*., 2005). The leaves are exported from several African countries and can be purchased in grocery stores. The root has been used for gingivitis and toothache due to its proven antimicrobial activity (Elujoba *et al*., 2005).
Vernonia amygdalina extracts and its isolated chemical constituents have been studied for their potential pharmacological effects, such as: induction of apoptosis as determined in cell culture and animal studies (Sweeney *et al*., 2005; Song *et al*., 2005). *Vernonia amygdalina* extracts may render cancerous cells to be more sensitive to chemotherapy (Sweeney *et al*., 2005). They also inhibit the growth or growth signals of cancerous cells (Izevbigie *et al*., 2004; Opata and Izevbigie, 2006). *Vernonia amygdalina* may provide anti-oxidant benefits (Erasto *et al*., 2007), Studies conducted using streptozotocin-induced diabetic laboratory animals showed that administration of *V. amygdalina* decreased blood glucose by 50% compared to untreated diabetic animals (Nwanjo, 2005). Extracts of *V. amygdalina* possess *in vitro* anthelminthic anti-parasitic properties (Ademola and Eloff, 2011).

Vernonia galamensis is used as an oilseed in East Africa. It is grown in many parts of Ethiopia, especially around the city of Harare, with an average seed yield of 2 to 2.5 t/ha. It is reported that the Ethiopian strains of *Vernonia* have the highest oil content, up to 41.9% with up to 80% vernolic acid, and is used in paint formulations, and as a reagent for many industrial chemicals (Alamata Pilot Learning Site Diagnosis and Program Design, 2009).

Ethnomedicinal uses of *Vernonia ambigua*

Vernonia ambigua Kotschy and Peyr is widely distributed in upland forests, open grassland, riverine and sometimes savannah region (woodland, dry grass) of Angola, Sudan, Tanzania, Uganda and tropical West Africa. It occurs throughout the drier part of these regions and also widely dispersed in similar parts of tropical Africa (Akobundu and Agyakwa, 1998). Common names include Iron weed, *Orogun* (Yoruba), Tab-taba/Tattaba (Hausa), *Onugbu* (Igbo). The plant is common in West and Central Africa (Akobundu and Agyakwa, 1998). Research on this plant has been gaining momentum in recent years, although much still needs to be done to explore the claimed traditional medicinal uses of the plant (Kunle and Egharevba, 2009). From times past, this plant has been used traditionally for problems related to reproductive health. The leaves are taken orally to treat male and female infertility (Focho *et al*.**,** 2009). A decoction of the plant, which is bitter, is taken as a remedy for cough and fever.

Phytochemical profile and proximate analysis of the leaf have been reported (Kunle and Egharevba, 2009). However, the microscopic profile of the leaf is not available in literature. This information would be useful in developing a monograph for the plant for inclusion in a herbal pharmacopoeia. It will also be useful in detecting substitution or adulteration of the plant drug. The microscopic analysis of the leaf of *Vernonia ambigua* is being reported for the first time in literature.

MATERIALS AND METHODS

Collection of plant

The plant *Vernonia ambigua* was collected in Chaza village, Suleja Local Government Area of Niger State, Nigeria, in October 2014. Identification and authentication were done at the herbarium in the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja-Nigeria. A voucher specimen with number NIPRD/H/6709 was deposited at the herbarium.

Plant processing

The plant was air-dried at room temperature for several days and reduced to moderate coarse powder using electric blender. The powdered sample was sieved using sieve number 40 (420µ) and used for the analysis.

Macroscopic analysis

Various macroscopic examinations were carried out on the fresh leaves. These include the observation of the type of margin, apex, insertion, venation, colour, texture, taste, size, and petioles of the leaves.

Chemo-microscopic analysis

The leaf powder was treated with the appropriate chemical reagents and observed under the microscope at x400 magnification for the presence of starch, oils, tannins, calcium oxalate crystals, proteins and lignin using standard methods in Sofowora (1982).

Microscopic analysis

A quantity of the leaf powder was cleared in chloral hydrate, mounted in dilute glycerol and viewed under the microscope. Leaf epidermal preparations followed the method of Ugbabe and Ayodele, (2008). About $5mm^2$ – $1cm^2$ leaf fragments were obtained from the standard

median portion of the leaf and macerated in concentrated Nitric acid in petri-dish for a period of 24 hrs. The appearance of bubbles on the surface of the leaf fragment indicated their suitability for separation.

The fragments were transferred into water in a petri-dish with a pair of forceps. Both epidermises were carefully separated by teasing them apart and pulling each epidermis back at itself. The leaf epidermises were cleaned with the Carmel hair brush. These were rinsed in distilled water and later transferred into 50% ethanol to harden. They were then stained in Safranin O for 5 minutes and excess stain washed off in water. They were then mounted in glycerin on a slide with the edge of the cover slips ringed with nail vanish to prevent dehydration. The slides were labeled appropriately and examined under the light microscope while photographs were taken using NICON AFX-DX microscope with NICON FX-35DX camera attached at a magnification of x100 and x400. Using a stage micrometer (1 div = 0.01mm), a calibration file was opened using the TUCSEN usb 2.0 H series camera. Photomicrograph of the calibration slide was taken in the same working objectives and resolution. The calibration slide picture was taken with the same lens and microscope settings as the target image taken. Stomata, epidermal cells, vein islets and vein terminations were counted.

Determination of stomata number and stomata index (mag: x100)

Stomata number is the average number of stomata per square millimeter of epidermis. The percentage proportion of the ultimate divisions of the epidermis of a leaf which can be converted into stomata is termed as stomata index. Stomata index can be calculated by using the following equation:

$I = S / E + S \times 100$,

Where, I = stomata index,

S = number of stomata per mm^2

E = number of ordinary epidermal cells per mm^2.

Determination of palisade ratio (mag: x400)

The palisade cells under four epidermal cells (including cells which are more than half and excluding cells which are less than half within the area of epidermal cells) were counted. The determination for five groups of four epidermal cells from fields of view was carried out. The average number of cells under each group of four epidermal cells (palisade ratio) was calculated.

Determination of vein-islet and vein termination numbers (mag: x400)

Vein islet is the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands. Vein termination number is the number of veinlet terminations per mm of leaf surface. The veins within the square (4 mm^2) were counted including those islets which overlap two adjustment sides of the square. The average number of vein islets from five determinations was calculated. The number of veinlet termination present within the square was counted and the average calculated as the vein termination number.

RESULTS

Macroscopic analysis

Results of the organoleptic evaluation of the leaf of *Vernonia ambigua* are shown on Table 1.

Table 1. Organoleptic evaluation of the whole leaf of *Vernonia ambigua*

Characters	Observations
Colour	Green on both sides
Texture	Hairy and rough
Odour	Characteristic
Taste	Bitter
Leaf size	Length (6.2 cm - 11.2 cm) Width (2.2 cm - 3.6 cm)
Apex	Acute
Shape	Oblanceolate
Margin	Entire
Venation	Reticulate
Petiole	Long (2.7 cm - 3.0 cm) Medium (2.0 cm - 2.4 cm) Short (1.7 cm - 2.0 cm)

Chemo-microscopic analysis

The chemo-microscopic evaluation of the leaf of *V. ambigua* showed the presence of starch, protein, oil and tannin (Table 2).

Table 2. Chemo-microscopic Evaluation of *Vernonia ambigua* leaves

METABOLITES	TESTS	OBSERVATION	INFERENCE
Starch	Iodine	Starch grains stained blue-black	Present
Proteins	Millon's reagent	Aleurone grains stained red	Present
Oils	Sudan III	oil globules stained pink	Present
Tannins	Ferric chloride	Blue-black structures observed on unicellular trichomes	Present

Microscopic analysis

The microscopic study revealed the presence of both glandular (peltate) and non-glandular trichomes on the both the lower and upper surfaces of the leaf. The multicellular trichomes are uniseriate with large base and tapering ends (Plates 1 & 2). They consist of about 6-11

segments while the peltate trichomes have short stalks and rounded head. The epidermal cells are wavy-walled on the upper surface and straight-walled on the lower surface. Stomata are found on both the lower and upper epidermis (amphistomatic) and are of the anomocytic type (Plates 1 & 2). Stomata distribution is similar on both surfaces. Fibres are isolated and spindle shaped, tapering at both ends. They are long with narrow lumen and thick edge (Plate 3). Portions of pitted parenchymatous cells are associated with trichomes (Plate 3) and spiral vessels were also present. Vein islets and terminations of the leaf of *V. ambigua* are found on Table 4. Vein-Islet number ranges from 64-87µ while Vein termination number ranges from 62-68µ. Palisade ratio ranges from 8µ and the Stomata index on the abaxial surface s 30.2µ but on the adaxial it is 18.8µ (Table 4).

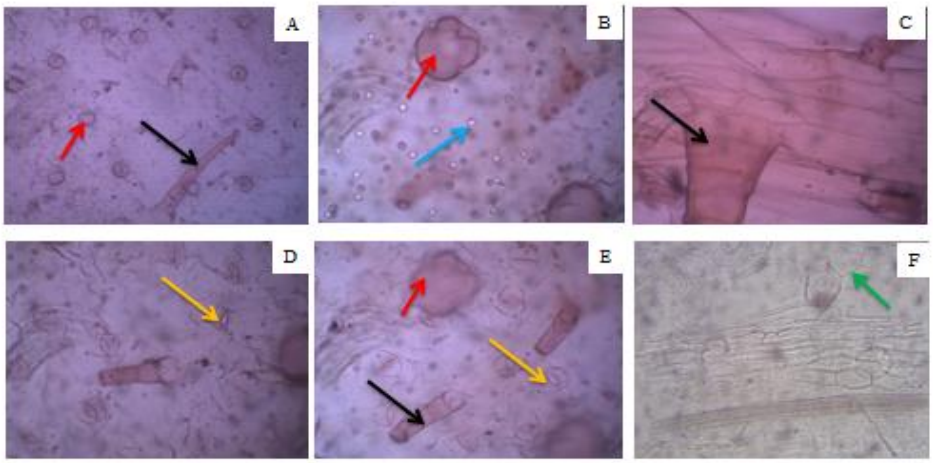

Plate 1. Photo-micrographs of the leaf of *Vernonia ambigua*
A) Lower/Abaxial surface x100 with both glandular (peltate: red arrow) and non-glandular (black arrow) trichomes.
B) Lower/Abaxial surface x400 with glandular trichomes (red arrow) and oil globules (blue arrow)
C) Lower/Abaxial surfacex400 with the stalk of the non-glandular trichome (black arrow)
D) Lower/Abaxial surface x100 with anomocytic stomata (yellow arrow)
E) Lower/Abaxial surface x400 with anomocytic stomata (yellow arrow), glandular trichome (red arrow) and non-glandular trichome (black arrow)
F) Lower/Abaxial surface x400 (unstained) with straight cell walls (green arrow)

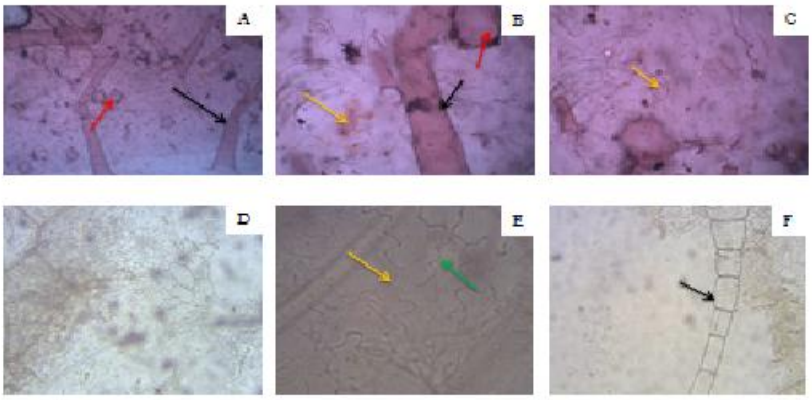

Plate 2. Photo-micrographs of the Leaf of *Vernonia ambigua*
A) Upper/Adaxial surface x100 with both glandular (peltate: red arrow) and non-glandular (black arrow) trichomes.
B) Upper/Adaxial surface x400 with anomocytic stomata (yellow arrow), glandular (red arrow) and non-glandular (black arrow) trichomes.
C) Upper/Adaxial surface x400 with anomocytic stomata (yellow arrow)
D) Upper/Adaxial surface x100 with wavy cellwalls
E) Upper/Adaxial surface x400 with anomocytic stomata (yellow arrow) and wavy cell walls (green arrow)
F) Upper/Adaxial surface x400 with segments of non-gladular trichome (black arrow)

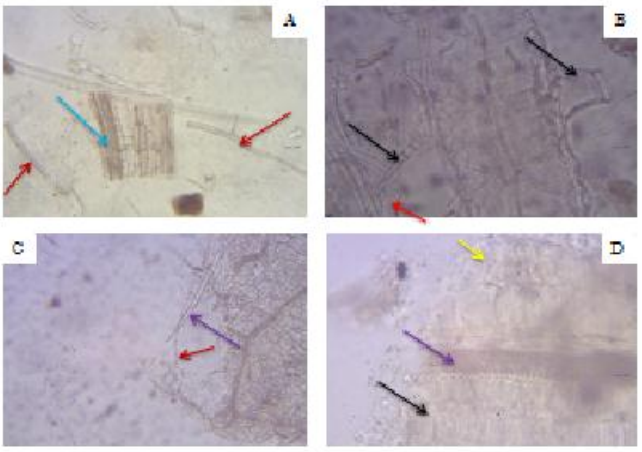

Plate 3. Photo-micrographs of the Leaf powder of *Vernonia ambigua*
A). Spiral vessels (red arrows) and palisade mesophyls (blue arrow)
B). Glandular (red arrow) and non-glandular trichomes (black arrow)
C). Fiber (purple arrow) and spiral vessel (red arrow)
D). Palisade mesophyll (black arrow), Spongy mesophyll (yellow arrow) and vascular bundle (purple arrow)

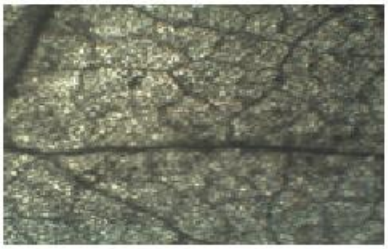

Plate 4. Photo-micrographs of the Vein islets and terminations on the leaf of *Vernonia ambigua* (x40)

Table 3. Quantitative microscopy of *Venonia ambigua* leaf

Stomata number ┼	Abaxial/Lower surface: 126.00 (147.10 ± 3.86) 162.00 Adaxial/Upper surface: 69.00 (79.50 ± 1.87) 87.00
Stomata index ┼	Abaxial/Lower surface: (30.20 ± 0.008) Adaxial/Upper surface: (18.80 ± 0.004)
Palisade ratio*	8.00 (2.68 ± 0.16) 15.00
Vein termination number*	62.00 (63.40 ± 4.20) 68.00
Vein-Islet number*	64.00 (78.40 ± 4.03) 87.00

Key:
Minimum (Mean ± Standard error) Maximum
All measurements in microns
┼ -n = 20; Magnification x 100
*- n = 5; Magnification x 400

DISCUSSION

The organoleptic examination of *V ambigua* showed that the plant had a characteristic odour and bitter taste. The leaves were light green on in colour on both surfaces; the length was 6.2cm-11.2cm and width was 2.2cm-3.6cm. The leaves are hairy and rough.

Chemo-microscopic results showed lignified fibres, proteins and starch. Tannin was found on the trichomes. Microscopic evaluation showed that the leaf has anomocytic stomata on both upper and lower epidermis. Trichomes were of two types: the long multicellular trichomes with broad base and tapering ends (non-glandular) and the peltate (glandular) trichomes with

short stalk which are more abundant on the lower epidermis. The fibres are long with narrow lumen. Parenchymatous cells were also observed.

The presence of numerous stomata on both surfaces of the leaf (Amphistomatic) implied that gaseous exchange takes place on both surfaces for photosynthesis and water loss (opening and closing of stomata). The cell walls are storage organs for carbohydrates and structural support and protection. They also serves as pressure vessels as their major function is preventing over expansion (Turgor pressure) when water enters the cell. Quantitative microscopy of *V. ambigua* showed stomata number on the abaxial surface to be from 126–162 microns while on the adaxial surface it was from 69-87 microns. Palisade ratio ranged from 8–15 microns; vein termination number from 62–68 microns and vein-islet number ranged from 64–87 microns.

CONCLUSION

The microscopic investigation of the leaf of *V. ambigua* is being reported for the first time in literature. The findings will be useful in the identification of the plants, detection of adulterants and monograph of the plant. These parameters can also be used as a standard for future reference.

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