Pharmacognostic studies and thin layer chromatography profile of the aerial parts of Vernonia cinerealess. (Asteraceae)

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Abstract

Vernonia cinerea(VC) Less is synonymously known as *Cyanthillium cinereum* and *Vernonia abbreviate* belongs to Asteraceae family. Different parts of the plant are extensively used in traditional medicine for treatment of various disease conditions. However, there was no scientific evaluation of the plant's pharmacognosy and thin layer chromatography (TLC), which necessitate these studies, to bring out characters that will facilitate its proper identification, standardization and, nature of secondary metabolites in aerial parts of the plant. To carry out detailed pharmacognostic studies and TLC evaluation on the aerial parts of *Vernonia cinerea*. Aerial plant parts of *V. cinerea* were studied for their organoleptic, macroscopic, microscopic, physicochemical features and bitterness properties, their elemental and nutritional values were evaluated using standard procedures. In the TLC, reagents were used to evaluate presence of various bioactive constituents in the parts studied. Pharmacognostic studies of aerial parts of VC highlighted features that are diagnostic from microscopic characters and their dimensions, different ergastic substances in the chemomicrocopical study. The elemental analysis showed the presence of macro, micro and trace elements. Nutritionally, there are presence of macro molecules such as carbohydrates, protein and fats. All these will serve as criteria to differentiate it from other species in the same and other families. The TLC profile showed spots of different colour shades after spraying with general and specific reagents and, their R_f values determined. Detailed pharmacognostic studies on the aerial parts of VC showed characters which might be useful to provide information with regard to its identification, standardization and types of phytochemicals present.

Keywords: Chromatography, Diagnostic, Pharmacognosy, Nutritional, *Vernonia abbreviate*

Introduction

Plants are vital for the survival and wellbeing of other organisms (Abubakar *et al*., 2014), hence, they are used as medicine since time immemorial (Jothi *et al*., 2018). The major sources of herbal drugs are from wild plants and most of those available in markets are in powdered form and prone to adulteration (Nivedithadevi and Somasundaram,2012). For safety and efficacy of these herbal products,

accurate knowledge and standardization evaluations have become indispensable (Chanda, 2014). Importance of pharmacognosy has been widely felt through the studies of standardization parameters; namely organoleptic, macroscopic, microscopic, micrometry, physicochemical, chemomicroscopy, these helps to provide a unique identification of the plant even if it is in dry powdered form, because, once the plant is converted into dry powder, it loses its morphological identity and easily prone to adulteration. Hence, these studies will help in authentication of the plant and ensures reproducible quality herbal products (Jothi *et al*., 2018).

Vernonia cinerea belongs to the Asteraceae family (Toyang and Verpoorte, 2013). The plant is commonly known as little ironweed and purple flea bane in English (Singh *et al*., 2014). Local names the plant is identified with are Bojure in Yoruba, Oriwo in Edo (Sonibare *et al*., 2016) and Ba gashi in Hausa languages respectively. It is an annual herb, erect and highly branched, it can grow up to a height of 80 cm, it is found mostly at the roadside, open waste places and dry grassy sites, among crop plantations (Ahsanul *et al*., 2012; Josh, 2014), and fallowed farm land. This genus is predominantly found in Africa, Asia and South America (Antonio *et al*., 2015). *V. cinerea* has many therapeutic applications in the practice of traditional medicine with every part of the plant used medicinally for treating ailments such as fevers, helminths, arthritis, diuresis, cancer, abortion, eye infection and various gastro-intestinal disorders (Antonio *et al*., 2015; Verma, 2018). The plant is also used for smoking cessation, wound healing and eruptive boils (Sonibare *et al*., 2016) as well as quicker healing of accidental wounds (Rajendra and Abirami, 2012). Juice of this plant is given to children to treat bed- wetting (Varsha *et al*., 2016). The present study aimed at evaluating pharmacognostic, elemental, nutritional and bitterness characters as well as TLC on the aerial parts of *Vernonia cinerea*.

Materials and Methods

Collection, Identification and Preparation of Plant Material

The aerial parts of *Vernonia cinerea* were first identified in the field and sample was taken to the Herbarium Section, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, where it was compared with the existing specimen and authenticated by Mal. Sunusi Namadi, and voucher specimen number ABU 0289 was collected for future references. On November 7^{th,} 2018, sufficient quantity of aerial plant parts was collected from Bogari village in Zaria Local Government Area, Kaduna, Nigeria, washed, air-dried at room temperature under shade to a constant weight, powdered using a wooden mortar and pestle and then stored in a clean air tight container before usage.

Chemicals, Reagents, and Solvents

All chemicals, reagents, and solvents used for the study were of analytical grade.

Pharmacognostic Study of Aerial Parts of *Vernonia cinerea*

The aerial parts of *V. cinerea* were evaluated by examining the organoleptic, macroscopical, microscopical, micrometry, quantitative leaf microscopy, chemomicroscopic characteristics and physicochemical parameters using procedures mentioned in literature (Evans, 2009; WHO, 2011).

Elemental Analysis of Aerial Parts of *Vernonia cinerea*

X-Supreme 8000 device was used, it is made up of trays, membrane key board, integrated high performance compact industrial PC with embedded windows software for easy communication and flexibility for qualitative and quantitative analysis (AMC, 2006; Oxford Instrumentation Manual, 2015).

Determination of Bitterness Value of Aerial Parts of *Vernonia cinerea*

Dilution of Quinine Hydrochloride

Exactly 0.1 g of quinine hydrochloride was dissolved in sufficient safe drinking-water to produce 100 mL. Subsequently, 5 mL of this solution was diluted to 500 mL with safe drinking-water to give the stock standard solution of quinine hydrochloride labeled Sq, and contained 0.01 mg of the quinine standard /mL. Nine test-tubes labeled 1 to 9 were set up to contain 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6 and 5.8 mL of Sq respectively. And into these tubes 1 to 9 were then added 5.8, 5.6, 5.4, 5.2, 5.0, 4.8, 4.6, 4.4 and 4.2 mL of drinking-water, respectively. By this, tubes 1 to 9 contained 0.042, 0.044, 0.046, 0.048, 0.050, 0.052, 0.054, 0.056 and 0.058 mg of quinine hydrochloride, respectively.

Preparation and dilutions of *Vernonia cinerea* stock solution

Exactly 20 g of powdered aerial parts of *V. cinerea* was extracted with safe drinking-water to produce 1000 ml of aqueous extract. Subsequently, 5 mL of the extract was diluted to 500 mL with drinking water. This solution was labelled as the stock test extract (St). It contained 20 mg of the herb /mL. Ten tubes labeled 1 to 10 were set up to contain 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mL of St respectively. Into tubes 1 to 10 was added 9, 8, 7, 6, 5, 4, 3, 2, 1 and 0 mL of safe drinking-water, respectively.

Procedure for the test

First, the participants made up of 5 adults (male and female) each rinsed their mouths with good drinking water, and then taste 10 mL of the most dilute solution by swirling it in the mouth for 30 seconds, noting whether or not the solution tasted bitter. The solution was held in the mouth by the participants for another 30 seconds, it was noted whether or not there is a loss of bitterness. Subsequently, the solution was spit out, and the mouths rinsed with the same drinking water. The participants waited for 10 minutes before the next higher concentration was tasted. These procedures were repeated until the dilution with threshold bitter concentration (that is the lowest concentration at which a solution continues to taste bitter after 30 seconds) attained by the participants. After the first series of tasting (either with the quinine solution or the herbal extract), the mouth was rinsed thoroughly with the drinking water until no bitter sensation remained. A waiting time of 10 minutes must elapse before carrying out the second series of tasting. Bitterness value was computed using this equation;

$$\frac{2000\ X\ c}{a\ x\ b},$$

where a = the concentration of the test stock solution (St) (mg/mL);

b = the volume of St (in mL) in the tube with the threshold bitter concentration;

c = the quantity of quinine hydrochloride (Sq in mg) in the tube with the threshold bitter concentration (Amehe*t al*., 2010; WHO, 2011).

Nutritional Analysis of Aerial Parts of *Vernonia cinerea*

Crude fiber, fat and protein of shade dried powder of aerial parts of *V. cinerea* were determined following the methods described by the Association of Official Analytical Chemists (AOAC, 1980) and crude carbohydrate was determined following the procedure of Pearson (1976).

Thin Layer Chromatographic Profile of Methanol Extract and Fractions of Aerial Parts of *Vernonia cinerea*

The methanolic extract and its fractions were subjected to thin layer chromatography using dimensional ascending method in silica gel plate. Pre-coated silica gel plate 20 x 20 cm (Merck), thickness 0.25 mm) were cut into desired sizes and used appropriately. Plate markings were made with ruler and soft pencil. Glass capillary tubes were used to spot the samples, air dried and developed. (Evans, 2009; Rajendra and Estari, 2013; Isa *et al*., 2017). Pictures were taken immediately and documented. The chromatograms were dried and sprayed with general reagent (*p*-Anisaldehyde). More chromatograms from best solvent systems were developed and used for specific reagents spraying such as ferric chloride for phenolic compounds, aluminum chloride for flavonoids, Lieberman- Burchard for steroids/triterpenes and Dragendorf's for alkaloids.

The solvent systems used were; Hexane: Ethyl acetate (9:1), Hexane: Ethyl acetate (8:2), Hexane: Ethyl acetate (7:3), Hexane: Ethyl acetate (1:1), Hexane (100 %), Ethyl acetate: Methanol (9:1), Chloroform (100 %), Hexane: Chloroform (1:1), Ethyl acetate (100 %), Butanol: Acetic acid: Water (6:1:1), Butanol: Acetic acid: Water (8:1:1), Butanol: Acetic acid: Water (10:1:1), Butanol: Acetic acid: Water (10:2:1) and Butanol: Acetic acid: Water (20:1:1).

Statistical Analysis

Results were presented as figures and tables, data generated were expressed as mean ± Standard Error of Mean (± SEM).

Results and Discussions

Pharmacognostic Evaluation of Aerial Parts of *Vernonia cinerea*

Organoleptic and macroscopic examination of aerial parts of V. cinerea

Organoleptically, the colour of *Vernonia cinerea* leaf, stem, bract and seed are green while flower head is purple to whitish purple. Taste and odour of the entire aerial parts of plant is bitter. Texture of the leaf is smooth and soft. The stem of this plant is smooth, solid and strong and seed case and bract is rough. Macromorphology of aerial parts of the plant showed alternately arranged leaves which are elliptic to lanceolate in shape, short petioles, margin is dentate, apex is acute, leaf base is symmetrical, and vein arrangement is reticulate. The stem is round in shape and the flower heads are grouped together in clusters with arrays of numerous purplish disc florets and, seeds are almost flat with shallow grooves on both sides (Plates I, II and Table 1).



Plate I: Aerial parts of *Vernoniacinerea*



a b c

Plate II: Macromorphological Features of Aerial parts of *Vernonia cinerea*; Showing branch with Seed Case (a), Seeds (b) and Matured Leaf (c)

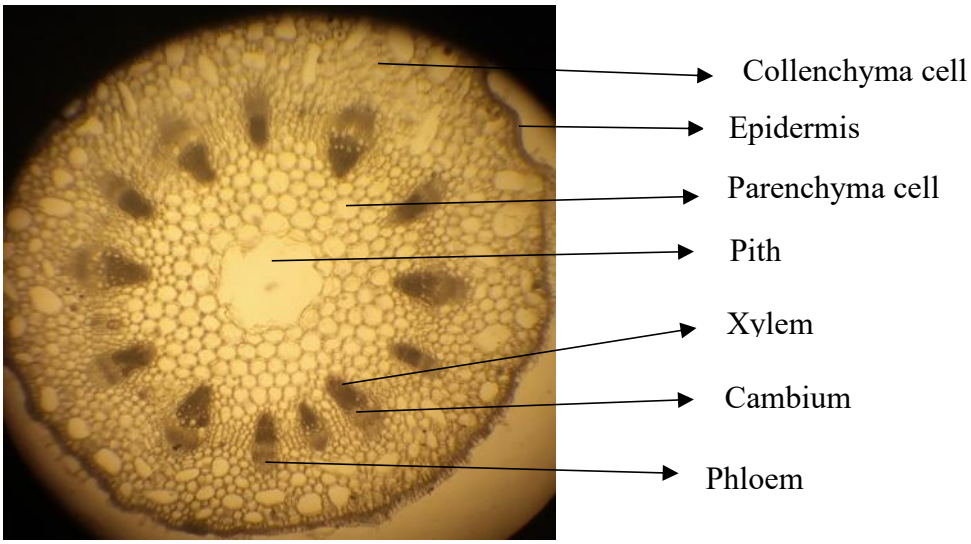
Table 1: Morphological Features for the Aerial Parts of*Vernonia cinerea*

Plant Features	Observation
Leaf	
Type	Simple and Single
Size	Length: 14.57 ± 0.55 cm, Breadth: 4.33 ± 0.17 cm
Shape	Elliptic to lanceolate
Apex	Acute
Margin	Dentate
Venation	Reticulate
Base	Symmetrical
Petiole	Petiolate
Surface texture	Smooth and soft
Phyllotaxy	Alternate
Stem	
Shape	Round and highly branched

Texture	Smooth and strong
Flower	
Head	Clustered
Seed	
Shape	Almost flat, broader top, tapered end with two grooves

Microscopic examination of the aerial parts of Vernonia cinerea

The transverse section of *V. cinerea* stem revealed diagnostic features including epidermis, collenchyma and parenchyma cells, phloem, cambium, xylem pith. Epidermis is the outermost part of the stem, the cortex contains small collenchyma cells with thin cell walls that are circular, and bigger parenchyma cells with thick cell walls that are also circular. There are conjoint vascular bundles of the type open collateral where cambium is between xylem and phloem. The



pith is the inner most part of this stem and it is centrally positioned (Plate III).

Plate III: Micrograph of the Transverse Section *Vernonia cinerea* Stem (Mag.: X 100)

The transverse section of *V. cinerea* leaf was observed to be dorsiventral in character with some prominent features such as epidermal hairs, epidermis, parenchyma cells and conjoined vascular bundles that extend into the leaf margin (Plate IV).

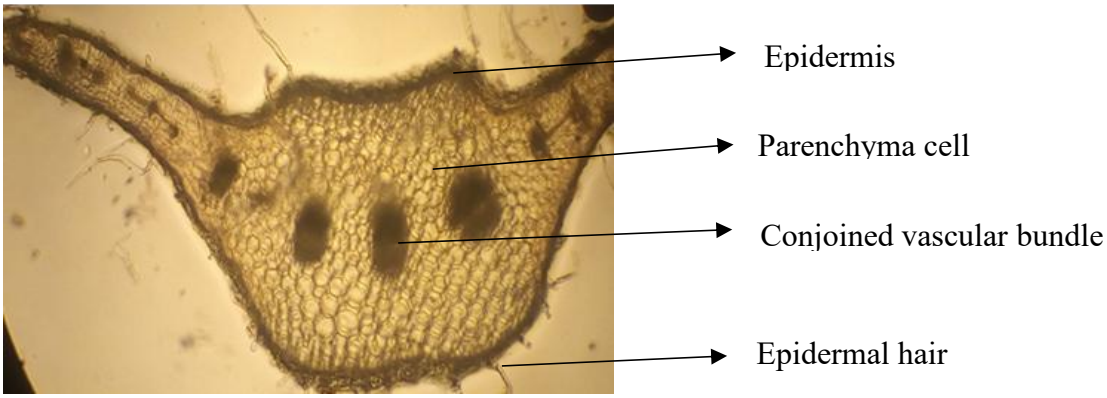


Plate IV: Micrograph of the Transverse Section *Vernonia cinerea* Leaf (Mag.: X 100)

Longitudinal section (LS) of upper and lower surfaces (Plates V and VI) showed anomocytic type of stomata, this type of stomatal arrangement is known asamphistomatic, this characteristic was also in agreement with the research work of Abraham, (2015). Upper epidermal cells were observed to be polygonal in shape with slightly straight anticlinal walls and lower epidermal cells are irregular in shape outline, the epidermal cells on the upper surface is slightly smaller than those in the lower surface in appearances (Table 2). The occurrence of the above-mentioned features was observed among some members of the vernonia as presented in the work of Kemka-Evans *et al.,* (2014).

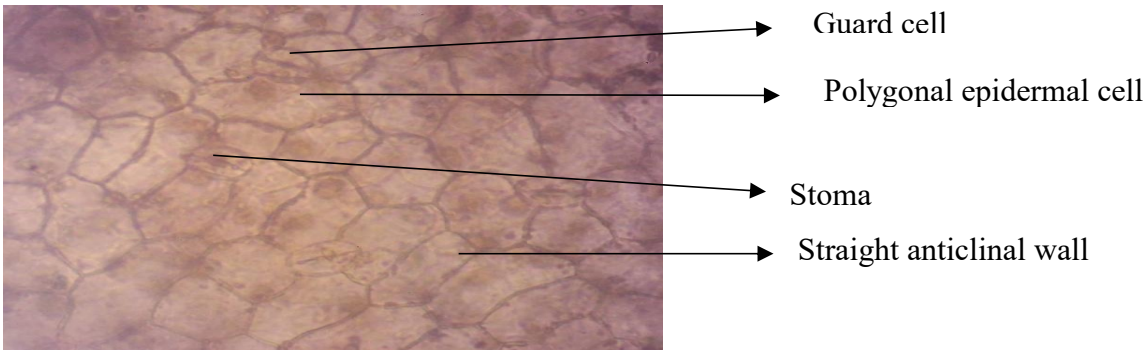


Plate V: Micrograph of Upper Epidermal Layer of *Vernonia cinerea* Leaf (Mag.: X 400)

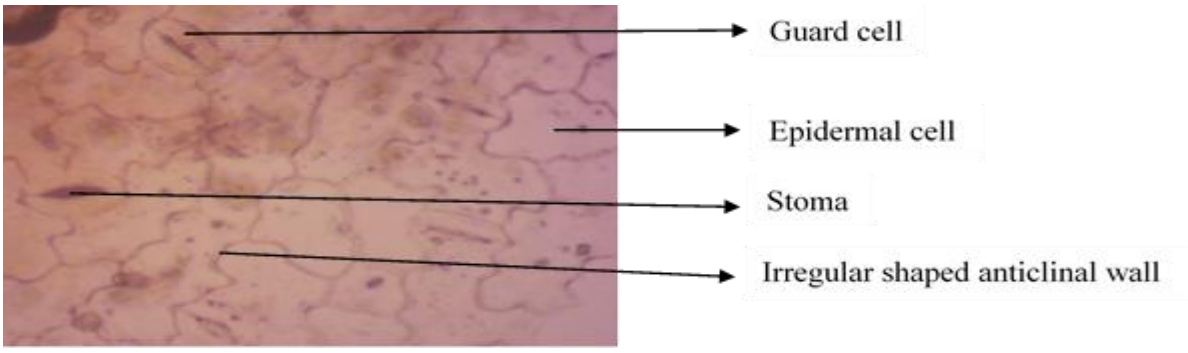


Plate VI: Micrograph of Lower Epidermal Layer of *Vernonia cinerea* Leaf (Mag.: X 400)

Table 2: Microscopical Characters on the Upper and Lower Surfaces of the Leaf of *Vernonia cinerea*

Microscopical Characters	Observation
Adaxial surface	
Epidermal Cells	Polygonal in shapes
Anticlinal walls	Slightly straight
Stomata	Present
Types	Anomocytic
Frequency	Evenly distributed
Abaxial surface	
Epidermal Cells	Irregular in shapes
Anticlinal walls	Thin
Stomata	Present
Types	Anomocytic
Frequency	Evenly distributed

Quantitative microscopic standards of the leaf of Vernonia cinerea

Plate VII is a photomicrograph of surface preparation showing vein termination and vein islet (a small photosynthetic area encircled by a conducting strand). Quantitative microscopy is a technique that is used to study microscopic characters not easily characterized by general microscopy; vein islet and veinlet termination numbers, palisade ratio, stomatal number and index have all been investigated and reported for the leaf of this plant.

The veinlet termination number (3.75) and vein islet number (2.75) of the plant were of diagnostic importance, the vein islet and termination numbers may appear to vary according to the preliminary treatment the leaf has received (Evans, 2009), the palisade ratios for upper and lower epidermis were found to be 4.69 and 4.06 respectively, this important parameter can be determined even on quite fine powders unlike the veinlet termination and vein islet numbers which require fresh and large portion of leaf and are preferably determined on a particular part of a leaf and for these reasons; it is an important parameter that is used primarily for evaluating intact leaves as well as the powder (WHO, 2011). The stomatal number was found to be 31.25 (upper surface) and 27.50 (lower surface) while the stomatal index of the upper surface was 76.63 % and lower surface was found to be 83.72 % (Table 3), the stomatal index is a more useful value and supportive evidence which, taken together with other factors, can make useful identification possible and it is less subjected to variations with external conditions (Brain and Turner, 1975).

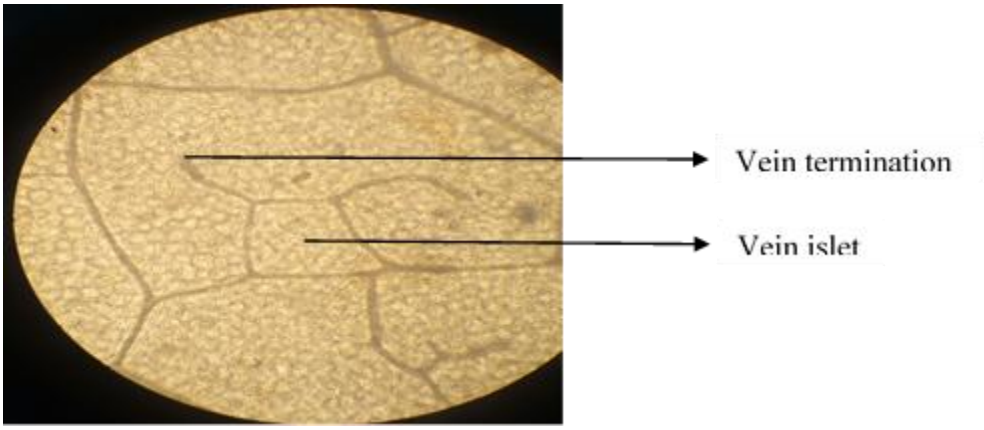


Plate VII: Micrograph of Surface Preparation of *Vernonia cinerea* Leaf (Mag.: X 100)

Table 3: Quantitative Microscopy of Physical Leaf Standards of the Leaf of *Vernonia cinerea*

Parameter	Mean ± SEM	Lower Range	Upper Range
Vein termination number / mm^2	3.75 ± 0.48	3.19	4.31
Vein islet number / mm^2	2.75 ± 0.25	2.34	3.16
Palisade ratio (upper epidermis) / mm^2	4.69 ± 0.21	3.99	5.39
(lower epidermis) / mm^2	4.06 ± 0.25	3.45	4.67
Stomatal number (upper epidermis) / mm^2	31.25 ± 0.85	26.56	35.94
(lower epidermis) / mm^2	27.50 ± 0.65	23.37	31.63
Stomatal index (upper epidermis) %	76.63 ± 0.48	65.11	88.15
(lower epidermis) %	83.72 ± 3.44	71.16	96.28

Note: Four determinations were made in each case and the average taken.

Micrometry evaluation of microscopic features on the leaf of Vernonia cinerea

The micrometry evaluation revealed the dimensions of upper and lower epidermal cells as well as those of the stomata as follows; mean for the length and breadth were 31.50 mμ x 22.50 mμ (lower epidermis), 19.25 mμ x 11.75 mμ (stomata from lower epidermis), 40.25 mμ x 30.75mμ (upper epidermis) and 17.25 mμ x 12.00 mμ (stomata from upper epidermis) respectively (Table 4). These findings were in agreement with the work of Asuzu, (2020) where some Vernonia species were found to have distinct different upper and lower epidermal cells and their respective stomata.

Table 4: Dimensions of Microscopical Characters on the Adaxial and Abaxial Surfaces of the Leaf of *Vernonia cinerea*

Parameter	**Mean ± SEM**	**Lower Range**	**Upper Range**
Upper epidermal cells			
Length (μm)	40.25 ± 2.16	34.21	46.29
Breadth (μm)	30.75 ± 1.29	26.14	35.36

Stomata (upper epidermis)			
Length (µm)	17.25 ± 1.02	14.66	19.84
Breadth (µm)	12.00 ± 1.11	10.20	13.80
Lower epidermal cells			
Length (µm)	31.50 ± 0.82	26.77	36.23
Breadth (µm)	22.50 ± 0.95	19.12	25.88
Stomata (lower epidermis)			
Length (µm)	19.25 ± 1.06	16.36	22.14

Chemomicroscopical evaluation of the powdered aerial parts of Vernonia cinerea Chemomicroscopical examination of the powdered aerial parts of *Vernonia cinerea* revealed the presence of cellulose cell wall, suberin and lignified cell wall as cell wall materials while starch, tanninsand aleurone grains are ergastic substances (Table 5) then, features such as fibres, trichome and parenchyma cells (Plates VIII and IX).

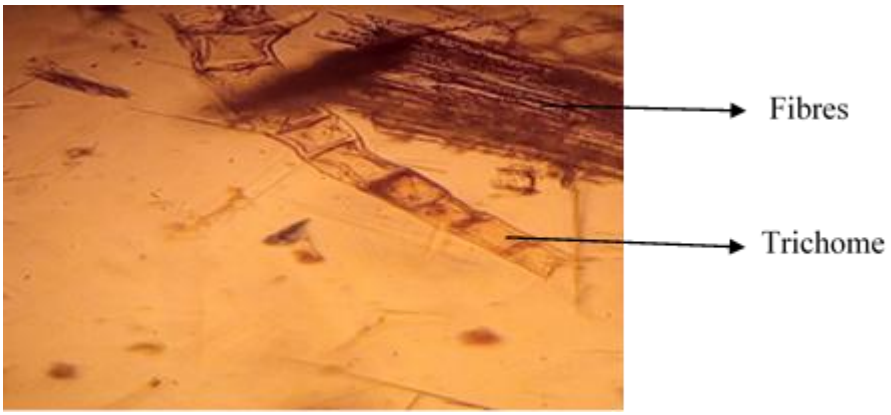


Plate VIII: Micrograph of Powdered Aerial Parts of *Vernonia cinerea* showing Trichome and Fibres (Mag.: X 400)

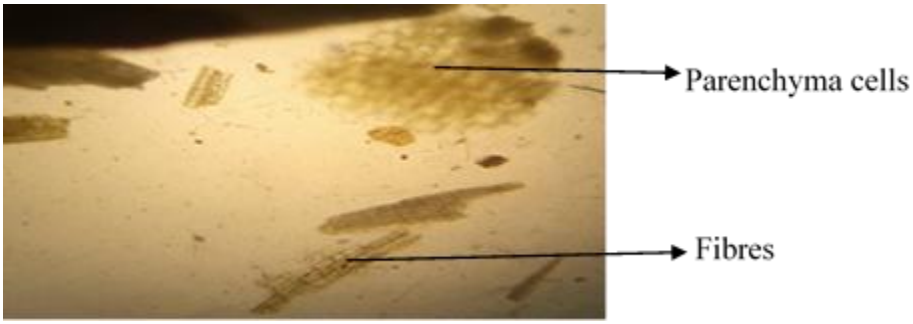


Plate IX: Micrograph of Powdered Aerial Parts of *Vernonia cinerea* showing Parenchyma cells and Fibres (Mag.: X 400)

Cell wall materials

(i) Test for Cellulose: Bluish-black colour was observed on the walls of the epidermal cells after addition of two drops of iodinated zinc chloride followed by one drop of

H_2SO_4,which indicated the presence of cellulose.

(ii) Test for Suberins: Slight reddish colour was observed on the cell wall of the plant when two drops of Sudan red were added followed by gentle heating, which is an indication for the presence of suberin.

(iii)Test for Inulin: On addition of a drop of 1- naphtol and H_2SO_4 each and viewed under the microscope, there were no spherical aggregation of crystals that later dissolves to indicate the absence of inulin.

(iv) Test for Lignin: Red stain was observed on the walls of some lignified cells in the plant after two drops of phloroglucinol were added to the cleared sample and allowed to stand until almost dry, and a drop of H_2SO_4 was added which indication for the presence of lignin.

(v) Test for Gums and Mucilage: When a drop of ruthenium red was added to the sample on the slide and viewed under the microscope, there was no pink colour observed in the epidermis and vascular tissues of the plant which is an indication for absence of gums and mucilage.

(vi) Test for Anthraquinone: When a drop of KOH solution was added to the sample, no appearance of red stain to indicate the presence of anthraquinone.

3.1.5.2 Cell Contents/Cell inclusions /Ergastic substances

(i) Test for Calcium Carbonates: On addition of concentrated HCL to the powdered sample on slide, there was no appearance of effervescence to indicate the presence of calcium carbonates.

(ii) Test for Calcium Oxalate Crystals: Two drops of concentrated HCL were applied on the sample and observed under the microscope, there were no dissolution of shining crystals on the anatomical sections of the materials, an indication of absence of calcium oxalates.

(iii) Test Aleurone grains: After addition of few drops of iodine in ethanol to the powdered sample and observed under the microscope, a yellowish brown colour was observed which indicated the presence of aleurone grains.

(iv) Test for Tannins: When a single drop of ferric chloride was added to the cleared samples and observed under the microscope, observation of greenish black colour in some parenchyma cells indicated the presence of tannins.

(v) Test for Starch: After addition of two drops of N/50 iodine to the cleared sample and viewed under the microscope, bluish black colour was seen within cells of the plant which indicated the presence of starch.

Table 5: Summary of the Chemomicroscopical Analysis of the Powdered Aerial Parts *Vernonia cinerea*

Parametersanalysed	Detecting reagents	Conclusions
Cell wall Materials		
Cellulose	Chlor - Zinc – Iodine	Present
Suberin	Sudan red	Present
Lignin	Phloroglucinol	Present
Inulin	1-Naphtol and H_2SO_4	Absent
Gums and Mucilage	Ruthenium red	Absent
Anthraquinones	KOH	Absent
Cell Contents/ Cell inclusions/ Ergastic substances		
Calcium carbonates	HCl	Absent

Calcium oxalates	HCl	Absent
Aleurone grains	Iodine in ethanol	Present
Tannins	5 % $FeCl_3$	Present
Starch	N/50 Iodine	Present

The chemomicroscopical features of powdered aerial parts of VC revealed the presence of cellulose, suberin, and ligninas cell wall materials while starch, tannins and aleurone grains are cell inclusions. However, it was absent for gums and mucilages, calcium carbonate, and calcium oxalate crystals (Table 5). When medicinal plants are evaluated chemomicroscopically, the type of compounds and their accumulation in the plant tissues are revealed, and based on this, one can choose the organ or tissue where the required compounds are located (Udodiong *et al*., 2014). The cell contents of interest in pharmacognosy are non-living (ergastic) substances which include either food-storage products or by-products of metabolism such as carbohydrates, proteins, fats, tannins, calcium oxalate, calcium carbonate and silica (Evans, 2009). To assess these substances, there is a need to apply chemomicroscopy (microchemical analysis), which is a valuable technique in the evaluation of medicinal plants due to its ability to identify plant components, authenticate and control the quality of plant materials, determine therapeutic potential, aid in standardization and formulation, and support research and development efforts in the field of herbal medicine (Ghokale*et al*., 2012).The chemomicroscopical features of the powdered aerial parts of *V. cinerea* revealed the presence of cellulose, suberin, and ligninas cell wall materials while starch, tannins and aleurone grains are cell inclusions. However, it was absent for gums and mucilages, anthraquinone, calcium carbonate, and calcium oxalate crystals (Table 4.8). When medicinal plants are evaluated chemomicroscopically, it provides us with ideas on type of compounds and their accumulation in the plant tissues and based on this, one can choose the organ or tissue where the required compounds are located (Odoh *et al*., 2011). Cellulose as an important part of plant cell walls provides structural support and rigidity to plant cells so they can be able to maintain their shape and withstand mechanical stress. They act against pathogens and pests as a physical defense mechanism (Wang *et al*., 2016). In addition, cellulose regulates water transportation within the cell walls and acts as barrier to prevent excessive water loss (Cosgrove, 2014). Suberin on the other hand is essentially found in the epidermis and periderm of plant tissues as a waterproofing agent and formed a barrier against transpiration, protecting plants from desiccation. It is known to act as a physical and chemical defense against pathogens to inhibit spread of microbial infections (Zeier, 2013). Lignin is a complex phenolic polymer that provides strength and rigidity to plant tissues, and forms a major component of secondary cell walls in many plant species. It is also a cell wall material that protection plants against microbial invasion (Vanholme *et al*., 2010). Starch is a complex carbohydrate synthesized in the chloroplasts of green plants during photosynthesis during the day time (transitory starch), this type of starch is degraded at the night to sustain metabolism, energy production and biosynthesis in the absence of photosynthesis. There is also storage starch found in tissues such as seeds, tubers, and roots which are readily available sources of glucose for energy production during periods of growth, germination, or when energy demands are high (Zeeman and Pfister, 2016). Tannins are

found in different parts of plants where they protect individual plant species from being infected by disease causing organisms such as bacteria or fungi. Most of these tannins produced antioxidant effects that lower total cholesterol, lower blood pressure and stimulate the immune system. Tannins promote rapid healing and the formation of new tissues on wounds and inflamed mucosa, hence, are used in the treatment of ulcers, hemorrhoids, minor burns, frostbite, as well as inflammation of gums. Internally tannins are administered in cases of diarrhoea, intestinal catarrh, and in cases of heavy metal poisoning as an antidote (Tong *et al*., 2021). Aleurone grains are specialized organelles found in the outermost layer of the endosperm in seeds. They are rich in proteins, lipids, minerals, and enzymes. In plants, aleurone grains function to provide nutrients and enzymes required for the germination and early growth of the plant embryo. Aleurone grains in whole grains and wheat bran may help to reduce the risk of cancer (Brouns *et al*., 2012).

Physicochemical features of powdered aerial parts of Vernonia cinerea

The physicochemical characters determined for the powdered aerial parts of the plant include: water extractives value (16.33 %), alcohol extractives value (14.67 %), moisture content (5.33 %), total ash value (8.00 %), acid insoluble ash (2.33 %), water soluble ash (4.00 %) and swelling index (1.14 %) (Table 6). These values are useful as criteria to judge the identity and purity of crude drugs (WH0, 2011). It also indicates the presence of various impurities like carbonate, oxalate and silicate in plant materials (Kaneria and Chanda, 2011). Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The compositions of these phytoconstituents depend on the nature of the drug and the solvent used. It also gives an indication whether the crude drug is exhausted or not (Tatiya *et al.,* 2012; Khan *et al*., 2013). It was observed that there was a reduction in the extractive values with decrease in extractive solvent polarity. This study indicated that water had high extractive value of 16.33 % w/w compared to alcohol which had an extractive value of 14.67 % w/w. Despite the higher extractive value of water over alcohol, methanol was chosen as the solvent of extraction in this study (Isa *et al*., 2023), this is due to the higher activities of extract from this solvent as compared to aqueous extract (Tiwari *et al*., 2011). Moreover, water promotes the occurrence of microorganisms as compared to methanol (Lapornik, *et al*., 2005). Water is an important component of biological materials, the physical and chemical properties of these materials especially medicinal plants are determined by their moisture content (Rocha *et al*., 2011). Drying is the most common and basic method for preservation of medicinal plants after harvest, it allows quick conservation of the medicinal qualities (Özgüven *et al*., 2019), this is achieved through process of increasing the shelf life by slowing or stopping the growth of microorganisms and prevention of certain biochemical reactions that might alter the organoleptic characteristics (Sahar *et al*., 2016). Plants usually contain maximum level of moisture and microorganisms (Din *et al*., 2016), these vegetable drugs themselves served as essential food requirements for mould, insects and mites, hence, deterioration can be very rapid once infestation has taken place (Ganesan*et al*., 2019), therefore immediate drying is the most important process. According to Din *et al*., (2016), natural way of drying herbal medicinal products is drying under the shade drying. For the plant sample under study, moisture

content was found to be 5.33 % (Table 6). The general requirement of moisture content in crude drugs is that, it should not be more than 14% (B. P, 1990) and the value obtained in this work is within the accepted range. The purpose of dehydration is to enhance the keeping quality of plants especially those with medicinal values and ensure year round availability of raw materials that are comparatively cheaper than fresh products, and once in dried form, they are long lasting and require less care as well as being ecologically friendly and biodegradable (Janakiram *et al*., 2016). Total ash value (8.00 %) (Table 6) represents both the physiological and non-physiological ash from the plant. The non-physiological ash is an indication of inorganic residues after the plant drug is incinerated. The study of ash content is very important to the extent that it provides an insight into the nutritionally important inorganic mineral elements present in a sample (Bhattacharjee *et al*., 2013). The acid insoluble ash values (2.33 %) obtained in this study indicated that the plant was in good physiological condition and it contained little extraneous matter such as sand, silica and soil. Water soluble ash in this study was found to be 4.00% (Table 6), all these are used as criteria to judge the identity and purity of crude drugs especially those of plant origin (WHO, 1996). The physico chemical results were found to be almost in agreement with those found in the research work of Abraham, (2015). In comparison with the research work of Usunobun and Okolie, (2016), where some physicochemical compositions of *Vernonia amygdalina* was found to contain moisture and ash as high as 9.32, 16.65 %, looking at this result, it is quite different from what was obtained for *Vernonia cinerea*, this can serve as criteria to differentiate them apart during the processes of identification and standardization.

Table 6: Physicochemical Parameters of the Aerial Parts of *Vernonia cinerea*

Parameters	**Values (%w/w) ± SEM***
Water soluble extractives	16.33 ± 0.88
Alcohol soluble extractives	14.67 ± 0.88
Moisture content	5.33 ± 0.19
Total ash	8.00 ± 1.04
Acid insoluble ash	2.33 ± 0.44
Water soluble ash	4.00 ± 0.50
Swelling index	1.14 ± 0.22

Note: Three counts were determined and average taken

Elemental Analysis of the Aerial Parts of *Vernonia cinerea*

X-ray fluorescence (XRF) is a very useful and nondestructive analytical technique for qualitative and quantitative determination of the elemental composition of all kinds of materials (Biswajit and Bhabesh, 2016), including medicinal plants. In the elemental studies of medicinal plants, the XRF technique was used, being well suited for multi elemental evaluation of samples, is a sensitive, rapid and simple analytical technique (Hüsniye *et al*., 2013). Elements are naturally occurring inorganic solid substances in the biosphere including all types of foods, they are found following the degradation of plant and animal tissues, and function in the regulation of metabolic pathways in living body (Solayman *et al*., 2016). These elements are classified into major, trace and ultra-trace

elements on the basis of body requirements. Plants take up both essential and non-essential elements from the soil and environment during growth and serve as an important link in the transfer of elements from soil to humans, deficiency or excessive levels of these in medicinal plants could lead to toxicity when ingested by humans (Hüsniye *et al*., 2013). In medicinal plants, these elements participate in reactions that lead to the formation of phytochemical compounds with pharmacological activities (Yamashita *et al*., 2006; Khan *et al*., 2013), hence, knowledge of contents of these elements are important in determining the quality of herbal products. Elemental evaluation of aerial parts of *V. cinerea* was carried out and the concentrations of mineral contents in parts per million (ppm) indicated the presence of Mg, Al, Si, P, S, Cl, Ca, Ti, Mn, Fe, Zn and Sn (Table 7) Based on the above classification, calcium (Ca), magnesium (Mg), phosphorus (P), sulfur (S) and chlorine (Cl) are the major elements while iron (Fe), zinc (Zn) and manganese (Mn) are the trace elements and the ultra-trace elements include aluminum (Al), silicon (Si), tin (Sn) and titanium (Ti). The mineral contents showed that calcium (Ca) concentration (1952.21 ppm) was higher in the elements analyzed followed by chlorine (Cl) (1874.61 ppm). Calcium is essential in many enzyme-mediated processes, blood clotting and keeping the bones strong and reducing osteoporosis (Maigari*et al*., 2016), especially in old age. The concentration of magnesium in powdered aerial parts of *Vernonia cinerea* was 715.83 ppm.According to Maigari *et al*., 2016 in FAO/WHO (1984), this is very high compared to the permissible level of 200 ppm. Mg is an essential mineral in biological systems especially in humans, it is essential for the excitability of muscles and nerves, protection against diabetes, reduce blood pressure and maintenance of bones and teeth integrity in the presence of calcium and phosphorus (Hüsniye *et al*., 2013). Iron (Fe) in human functions is an essential component of hemoglobin (HB), it facilitates the oxidation of carbohydrates, protein and fat to control body weight, it is responsible for oxygen transport, maintains a healthy immune system, prevention of anaemia and being a constituent of several enzymes, is responsible for energy production. It is also an active site for several enzymes (Abdelhafeez *et al*., 2013; Anal and Chase, 2016). The permissible limit of Fe set by FAO/WHO (1984) in edible plants was 20 ppm, and comparing it with the studied medicinal plant, it was found to be 250.90 ppm, however, plants are known to accumulate Fe above this limit. Phosphorous (P) function in the maintenance of blood sugar levels; and normal heart contraction, it is also important for normal cell growth and repair, bone growth and kidney function. It plays an important role in maintaining the body's acid-alkaline balance (Maigari*et al*., 2016), concentration was 57.96 ppm. Manganese (Mn) is an important electrolyte responsible for proper bones and liver function, it also works as a co-factor in more than 300 metabolic reactions (Patil *et al*., 2013), it is involved in the synthesis of fatty acids and cholesterol (Gbekele-Oluwa, 2013). The concentration of manganese was 36.49 ppm while the permissible limit set by FAO/WHO (1984) in edible plants was 2 ppm. Comparison showed value obtained in the medicinal plant studied is higher, however, FAO/WHO (1984) proposed that all plants accumulate Mn above this limit. Zinc (Zn) is an essential mineral element of importance, necessary for plants, animals and microorganisms, it is a component of more than 270 enzymes (Abdelhafeez *et al*., 2013), it is essential for growth and development (Gbekele-Oluwa, 2013) especially sperm manufacturation, fetus development and proper function of immune response (Abdelhafeez *et al*., 2013). It is used in the prevention and treatment of diarrhoea, pneumonia, cold, respiratory infections as

well as malaria (Gbekele-Oluwa, 2013). The permissible limit of zinc set by FAO/WHO (1984) in edible plants was 27.4 ppm andits ontent in thiswork is 21.99 ppm showing that concentration of zinc is within acceptable limit. Silicon in silica form stimulates the formation of collagen, a protein that give bones their strength, joint cartilage its cushioning ability (Bhattacharjee *et al*., 2013), concentration was found to be 224.56 ppm. The remaining elements are sulphur, tin, aluminum and titanium, and their contents were 411.89, 767.04, 174.97 and 88.35 ppm respectively. It is worth mentioning that limit is not set by FOA/WHO for some of the mineral elements detected in the plant of study. Important elements such as calcium, magnesium, iron, zinc and manganese were known to be present in Vernonia species as seen in the work (Aliyu *et al*., 2015) where three species of Vernonia were evaluated. Lead, mercury and cadmium are some of the heavy metals which are absent in *Vernonia cinerea* aerial parts, there absence is good because they are elements of toxicity particularly when consumed in powdered form, suggesting that the plant is safe for consumption.

Table 7: Concentrations of Elemental Content (ppm) of the Aerial Parts of *Vernonia cinerea*

Elements	Concentration (ppm)
Magnesium (Mg)	715.83
Aluminum (Al)	174.97
Silicon (Si)	224.56
Phosphorus (P)	57.96
Sulphur (S)	411.89
Chlorine (Cl)	1874.61
Calcium (Ca)	1952.21
Titanium (Ti)	88.35
Manganese (Mn)	36.49
Iron (Fe)	250.90
Zinc (Zn)	21.99
Tin (Sn)	767.04

3.3 Nutritional Evaluation of Aerial Parts of *Vernonia cinerea*

The nutritional analysis showed important macromolecules such as carbohydrates, proteins and fats as well as crude fibres (Table 8). Carbohydrate contents was found to be 43.19 %. The importance of carbohydrates cannot be over emphasized as they play significant roles in human health, apart from the supply of energy, they are also needed in numerous biochemical reactions not directly concerned with energy metabolism, these carbohydrates may serve as substrates for the production of aromatic amino acids and phenolic compounds through the Shikimic acid pathway and this may confer high phenolic and antioxidant potentials on the plant (Bhattacharjee *et al*., 2013), thus the carbohydrate levels of the studied sample suggest its usefulness as alternative source of glucose. The level of protein was found to be (6.80 %). This can contribute to the formation of hormones which controls a variety of body functions such as growth, repair and maintenance of body (Bhattacharjee *et al*., 2013). Crude fiber is being recognized as a useful tool for the control of oxidative processes in food products and as functional food ingredient, its presence in the diet is

necessary for digestion and elimination of wastes as it stimulates the contraction of muscular walls of the digestive tract thereby counteracting constipation, it also decreases the absorption of cholesterol from the gut, above all, it functions in the protection against cardiovascular disease, colorectal cancer and obesity (Bhattacharjee *et al*., 2013). The percentage fiber content was (24.10 %). The consumption of excess amounts of fats has been recognized as the most important dietary factor aiding increased levels of cholesterol and high content of this cholesterol causes obesity which is a factor in the causation of some diseases (Bhattacharjee *et al*., 2013). Crude fat content of VC was 5.35 %, from this evaluation, the content of fat is small when compared to the fibre content, hence it can decrease the absorption of this fat especially if this fat is cholesterolic in nature.In the research work conducted by Ramaswamy and Mani, (2016), *V. cineria* was evaluated for phytonutrients among others, it was found to contain high contents of proteins and fats, and a very low content of carbohydrates, this could be as a result of the geographical location and the soil content where the plants are grown. Usunobun and Okolie, (2016) showed in their research work that the nutritional composition of *Vernonia amygdalina* to contain crude protein, fiber, fat and carbohydrate as 22.81, 18.17, 4.34 and 38.03 % respectively, this results can also be used as criteria to differentiate the macromolecular content of *V. cinerea* from its sister species above, as clear difference can be observed between the two species.

Table 8: Nutritional value of the aerial parts of *Vernonia cinerea*

Nutritional value (%)	**Mean ± SEM of Nutritional Composition**
Lipid	5.35 ± 0.09
Protein	6.80 ± 0.20
Fibre	24.10 ± 0.28
Carbohydrate	43.19 ± 1.14

Note: Three counts were determined and average taken

Bitterness Value of Aerial Parts of*Vernonia cinerea*

Results from the determination of bitterness value of *Vernonia cinerea* showed that females are more sensitive than males subjects to taste bitterness at a lower concentration The mean bitterness values of 0.80 and 0.66 respectively (Table 9).

Table 9: Bitterness Value of the Aerial Parts of *Vernonia cinerea*

Volunteer's code	**a (mg/ml)**	**b (ml)**	**c (mg)**	**BV**	**Mean ± SEM**
A (m)	20	9	0.058	0.64	
B (m)	20	8	0.056	0.70	
C (m)	20	9	0.058	0.64	
D (m)	20	9	0.058	0.64	**0.66 ± 0.02**
E (f)	20	7	0.054	0.77	
F (f)	20	7	0.054	0.77	
G (f)	20	6	0.052	0.87	
H (f)	20	7	0.054	0.77	**0.80 ± 0.03**

Note: m= male volunteers and f= female volunteers

Medicinal plants with bitter characteristics are therapeutically employed as appetizing agents, the bitterness from these plants stimulate secretions of gastric juice in the digestive tract (Ameh *et al*., 2010). This study showed bitterness values of 0.66 for male subjects and 0.80 for female subjects (Table 9). Male subjects revealed lower bitterness value when compared with the female subjects, this implies that female subjects can recognize bitterness at a lower concentration than the male subjects.

Thin Layer Chromatography (TLC) Profile of Methanol Extract and Fractions of Aerial Parts of *Vernonia cinerea*

3.5.1 Using general detecting reagent

After several solvent systems used for the development of the TLC plates, analysis revealed the following solvent systems to be the most suitable for the extract and fractions; Hexane: ethyl acetate (H:E = 9:1) for HF, hexane: ethyl acetate (H:E = 8:2) for EAF and butanol: acetic acid: water (B:A:W = 10:1:1) for ME, BF and AF; Plates X, XI and XII below.

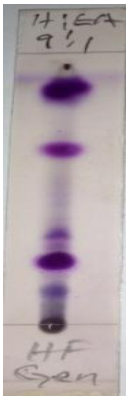


Plate X



Plate XI



Plate XII

Plate X: Chromatogram of HF on precoated silica gel plate developed in H: E (9:1) sprayed with *p*-Anisaldehyde. revealed 5 spots

Plate XI: Chromatogram of EAF on precoated silica gel plate developed in H: E (8:2) sprayed with *p*-Anisaldehyde revealed 4 spots

Plate XII: Chromatogram of ME, BF and AF on precoated silica gel plate developed in B:A:W(10:1:1) sprayed with *p*-Anisaldehyde.reveaved6, 5 and 4 spots for ME, BF and AF respectively, colours and R_f values are shown in table 10

3.5.2 Using specific detecting reagents

More chromatograms were developed for specific sprays as follows:



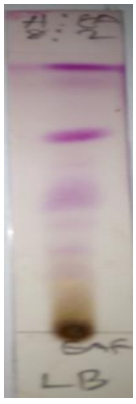




Plate XIII Plate XIV Plate XV

Plate XIII: Chromatogram of HF on precoated silica gel plate developed in H: E (9:1) sprayed with Libermann-Burchard. revealed 5 spots

Plate XIV: Chromatogram of EAF on precoated silica gel plate developed in H: E (8:2) sprayed with Libermann-Burchard revealed 4 spots

Plate XV: Chromatogram of ME on precoated silica gel plate developed in B:A:W(10:1:1) sprayed with Libermann-Burchard revealed 2 spots. Colours and R_f values are shown in Table 11







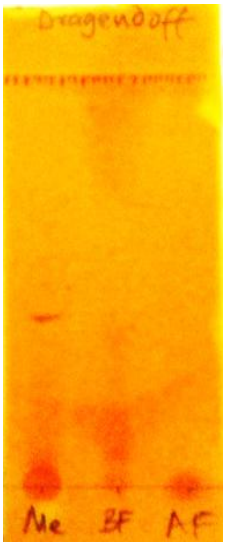


Plate XVI Plate XVII Plate XVIII Plate XIX

Plate XVIII: Chromatogram of ME, BF and AF on precoated silica gel plate developed in B:A:W(10:1:1) sprayed with Aluminum chloride revealed 7 spots (ME), 2 spots (BF) and 1 spot (AF)

Plate XIX: Chromatogram of EAF on precoated silica gel plate developed in H:E (8:2) sprayed with Aluminum revealed one spot

Plate XX: Chromatogram of ME, BF and AF on precoated silica gel plate developed in B:A:W(10:1:1) sprayed with Ferric chloride revealed5 spots (ME) and 3 spots (BF)

Plate XXI: Chromatogram of ME, BF and AF on precoated silica gel plate developed in B:A:W(10:1:1) sprayed with Dradgendorff revealed 2 spots (ME) and 1 (BF). Colours and R_f values are shown in table 11

Table 10: Summary of TLC Profile of Methanol Extract and Fractions of Aerial Parts of *Vernonia cinerea* sprayed with *p*-Anisaldehyde

Extract/Fraction	Solvent	No. of spot	Colour	of R_f values

	system		spots	
Hexane Fraction	H:E (9:1)	5	Purple	0.16
			Purple	0.28
			Purple	0.35
			Purple	0.57
			Purple	0.81
Ethyl Acetate Fraction	H:E (8:2)	4	Purple	0.41
			Purple	0.65
			Purple	0.93
			Purple	0.97
Methanol Extract	B:A:W(10:1:1)	6	Grey	0.07
			Grey	0.28
			Grey	0.32
			Grey	0.47
			Grey	0.67
			Grey	0.85
Butanol Fraction	B:A:W(10:1:1)	5	Grey	0.04
			Grey	0.25
			Grey	0.35
			Grey	0.56
			Grey	0.68
Aqueous Fraction	B:A:W(10:1:1)	4	Grey	0.05
			Grey	0.11
			Grey	0.35
			Grey	0.44

Table 11: Summary of TLC Profile of Methanol Extract and Fractions of Aerial Parts of *Vernonia cinerea* Sprayed with Specific Reagents

Extract/Fraction	Solvent system	Specific Detecting Reagents	No. of spot	Colour of spots	R_f values
Hexane Fraction	H:E (9:1)	Libermann-Burchard	5	Pink	0.16

				Pink	0.28
				Pink	0.35
				Pink	0.57
				Pink	0.81
Ethyl Acetate Fraction	H:E (8:2)	Libermann-Burchard	4	Pink	0.41
				Pink	0.65
				Pink	0.93
				Pink	0.97
Methanol Extract	B:A:W(10 :1:1)	Libermann-Burchard	2	Light pink	0.48
				Light pink	0.59
Methanol Extract	B:A:W(10 :1:1)	Aluminum chloride	3	Yellow	0.04
				Yellow	0.23
				Yellow	0.47
			4	Light blue	0.07
				Light blue	0.13
				Light blue	0.20
				Light blue	0.36
Butanol Fraction	B:A:W(10 :1:1)	Aluminum chloride	2	Light blue	0.05
				Yellow	0.33
			1	Light blue	0.40
Aqueous Fraction	B:A:W(10 :1:1)	Aluminum chloride	1	Yellow	0.04
Ethyl Acettate Fraction	H:E (8:2)	Aluminum chloride	1	Yellow	0.05
Methanol Extract	B:A:W(10	Ferric chloride	5	Bluish	0.60

	:1:1)			black	
				Bluish black	0.65
				Bluish black	0.87
				Bluish black	0.89
				Bluish black	0.95
Butanol Fraction	B:A:W(10 :1:1)	Ferric chloride	3	Bluish black	0.41
				Bluish black	0.49
				Bluish black	0.57
Methanol Extract	B:A:W(10 :1:1)	Dradgendorff	2	Orange	0.07
				Orange	0.39
Butanol Fraction	B:A:W(10 :1:1)	Dradgendorff	1	Orange	0.08

Chromatography as a technique is based on the interaction of the mixture components with the mobile and stationary phases of the chromatographic system which results in division of the mixture into various components between the two phases (Tomczyk *et al*., 2019), this separation process is important due to interaction of the mobile and stationary phases (Monteiro *et al*., 2016).

TLC profiling of the extract and fractions showed impressive results directing toward the presence of phytochemicals already determined qualitatively (Isa *et al*., 2023). Various phytochemicals give different R_f values in different solvent system. This variation in R_f values of the phytochemicals provide a very important clue in understanding their polarity and also helps in the selection of appropriate solvent systems for separation of pure compounds by column chromatography (Udoidong *et al*., 2014).

TLC profiling of ME and fractions of aerial parts *V. cinerea* in different solvent systems at different ratios gave various degrees of separation after spraying with general reagent (*p*- anisaldehyde). The chromatograms of ME, BF and AF were developed in butanol: acetate acid: water (B:A:W) in ratio (10:1:1), this revealed 6 spots for ME, 5 spots for BF and 3 spots for AF, the colour observed was grey. The chromatogram of HF was developed in hexane: ethyl acetate (9:1), it revealed 5 spots that are purple in colour. EAF was developed in hexane: acetic acid (8:2), 4 spots were seen and the colour observed was purple. More

chromatograms were developed for specific spraying, HF after spraying with Libermann-Burchard reagent revealed a clear separation with 5 spots which are pink in colours, the positions of these spots correspond to those sprayed with specific reagent. EAF after spraying with Libermann-Burchard reagent revealed a clear separation with 4 spots which are pink in colour, also the positions of these spots correspond to those sprayed with general reagent. ME sprayed with Libermann-Burchard reagent revealed 2 spots. ME sprayed with aluminum chloride revealed 8 spots that are yellow, orange and light blue in colours. BF gave 2 spots, AF and EAF also gave 1 spots each with aluminum chloride. ME and BF showed 5 and 3 spots respectively that are light grey in colour after spraying with ferric chloride reagent. ME give 2 spots while BF and AF give 1 spot each that are orange in colour after spraying with dradgendorff reagent (Plates X – XIX and Tables 10 and 11). TLC profiling is an indispensable technique which play significant roles analysis of these therapeutically important compounds (Soni *et al*., 2018). The healing properties of medicinal plants are usually linked with the presence of phytochemicals in the medicinal plants and they differ in type and concentrations from one plant to another, this account in part for the difference in pharmacological effects of these plants (Ugwoke *et al*., 2017). The findings of this work showed pharmacognostic features, elemental, primary metabolites present and bitterness values, and the TLC which showed presence of secondary metabolites in the aerial parts of *V. cinerea* were determined, and could serve as authentication source and standardization information for preparation of a monograph on the plant.

Conflict of interest statement

No conflict of interest to declare by authors.

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