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Pharmacognostic Studies of *Petiveria alliaceae* L. (Phytolaccaceae)

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ABSTRACT

Background: Petiveria alliacea, a member of the Phytolaccaceae family, is known for its garlic-like odor and medicinal properties, including anti-inflammatory, antimicrobial, immunomodulatory, anticancer, and analgesic activities. This study aimed to establish quality control parameters for the leaves of *P. alliacea*.

Methods: Standard procedures were used for microscopy, micrometry, chemomicroscopy, moisture content, ash values, extractive values, fluorescence, and phytochemical analyses.

Results: The leaf exhibited amphistomatic stomatal distribution with anisocytic and anomocytic types. Stomatal indices were 16.5% (abaxial) and 2.9% (adaxial). Micromeritic analysis showed good flow properties, with a bulk density of 0.25±0.006 and an angle of repose of 30.60°. Chemomicroscopy identified mucilage, lignin, starch, oil, and cellulose, while fluorescence analysis revealed solvent-dependent color variations. Extractive values for water, methanol, and ethanol were 12.7%w/w, 8.3%w/w, and 8.0%w/w, respectively. Moisture content was 18.3%w/w. Ash values (total, acid-insoluble, and water-soluble) were 17.4%w/w, 1.33%w/w, and 5.33%w/w, respectively. GC-MS analysis identified nine phytochemicals, with prominent components including Eicosane, 10-butyl-10-propyl- (23.596%), Isophytol (13.509%), Pentyn-4-one (12.253%) and Hexadecanoic acid (10.178%), known for pharmacological activity.

Conclusion: These findings support the identification and authentication of *P. alliacea*, establishing standards for quality, purity, safety, and efficacy in phytomedicine.

Keywords: Amphistomatic, Micromeritics, *Petiveria alliacea*, Phytolaccaceae and Hausner's ratio

1. INTRODUCTION

Petiveria alliacea, also known as "Guinea Hen Weed" or "Anamu," is a perennial herbaceous plant indigenous to the tropical regions of Central and South America. It is from the family Phytolaccaceae and is recognized by its lance-shaped leaves, greenish-white flowers, and a unique garlic-like smell. The plant contains various bioactive compounds, including alkaloids, flavonoids, tannins and sulfur-containing compounds. The most prominent chemical constituent is dibenzyl trisulfide, which is thought to contribute to its unique odour and therapeutic effects [1]. It is believed to offer potential health benefits for various conditions, including respiratory problems, pain relief, inflammation and as a general health tonic [2]. *Petiveria alliacea* has been used in traditional medicine for the treatment of various central nervous system (CNS) disorders, such as anxiety, pain, memory deficits and seizures, as well as for its anaesthetic and sedative properties [3]. It is used in folk medicine due to its antispasmodic, diuretic, hypoglycemic, abortive, anti-inflammatory and anticancerogenic properties [4]. It further

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possesses antioxidant, analgesics, anti-hypertensive, antidiarrheal and several other activities [1]. Scientific Classification of *Petiveria alliacea* according to Angiosperm Phylogeny Group System (APG, 2016) [5].

Kingdom:	Plantae
Clade:	Tracheophytes
Clade:	Angiosperms
Clade:	Eudicots
Order:	Caryophyllales
Family:	Phytolaccaceae
Genus:	*Petiveriae*
Species:	*P. alliaceae* L.
Common Name:	Guinea hen weed

Figure 1. *Petiveria alliacea* L. (Source: Field data (2023) Faculty of Pharmacy medicinal plant farm)

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

The plant *Petiveria alliacea*

2.1.2 Chemical and reagents

The chemicals and reagents used include; distilled water, glycerol, chloral hydrate, sodium hydroxide, 5% concentrated Hydrochloric acid, ferric chloride, concentrated sulphuric acid, dichloromethane, ethylacetate, methanol, ethanol, n-hexane, dragendorff's reagent, ferric chloride, phloroglucinol, ruthenium red, millon's reagent, N/50 iodine, sosium hypochloride.

2.1.3 Equipment and Apparatus

Materials used include: beaker, electronic weighing balance, test tubes, filter paper, oven, water bath, pen, pencil, funnel, glass stirrer, measuring cylinders, beakers, conical flash, sieves, spatula, marker, masking tape, foil paper, thongs, evaporating dish, silica gel, knife, mortar and pestle, desiccator, oven, furnace, ashless filter paper, Olympus CX21 electronic microscope, microscope slides, cover-slips, full-scape sheets, meter rule, Amscope MD 500.

2.2 Methods

2.2.1 Collection, Identification and Preparation of Plant Material

The leaves of the plant were collected in a location near the Faculty of Pharmacy Herbarium University of Uyo Town Campus, Akwa Ibom State, Nigeria in August 2023. It was identified by Dr Imoh I. Johnny of the

Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo. The voucher specimen number is UUPH 64(C). The fresh leaves of the plant were air-dried, pulverized and packed in a well-labeled dry container.

2.2.2 Microscopic Evaluation of Leaf

The standard median portion of the well-expanded matured leaf was obtained. Microscopical examinations of the transverse section were made, the Epidermis of both adaxial and abaxial surfaces were also made by placing the leaf on a glass slide, scraping gently with a sharp razor blade, irrigated with water until loose cells from the epidermis were washed away and the desired epidermis was reached. The epidermal peels were further cleared with sodium hypochlorite rinsed gently with water and stained with an aqueous solution of safranin-O for (five) 5 minutes and 10% glycerol. The stained samples were mounted on a binocular microscope. Photomicrographs were taken from good preparations using the Olympus CX21 binocular microscope fitted with an MD500 amscope microscope eyepiece camera. Measurements were done at ×10 while ×40 for photomicrographs [6].

2.2.2.1 Quantitative Microscopy of the Leaf

Quantitative microscopy parameters such as leaf constant studies namely stomatal length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, epidermal cell thickness were carried out using standard procedures. All measurements were made using a calibrated ocular micrometer and 10 microscopic fields chosen at random were used and data was presented as mean ± Standard Error of Mean (SEM).

2.2.3 Stomatal Index Determination

The stomatal index (S.I) was determined according to Metcalfe and Chalk [7,8, 9] [X9, X10, X11].
The sample (quantitative microscopy) was placed under the microscope and the stomatal index was determined using the formula;

$$S.I = \frac{S}{E + S} X100$$

Where S = Number of stomata per unit area, E = Number of epidermal cells in the same area

2.2.4 Evaluation of Powders

2.2.4.1 Micromeritic Analysis

The flow property was determined using standard methods [10]. Which constitutes; Bulk Density and Tapped Density. The weight of 10 g of dried powdered leaf was weighed into a 100 ml measuring cylinder and the volume occupied was noted as the bulk volume (Vb). The cylinder was gently tapped repeatedly to obtain a constant volume noted as the tapped volume (Vt). Bulk density was calculated using the formula below;

$$B\rho = M / Vb$$
$$Tv = M / Tv$$

Where Bρ = Bulk density, M=Mass of powder, Bv = Bulk volume of powder, Tρ = Tapped density
Tv = Tapped volume

2.2.4.2 Hausner's Ratio and Carr's index

Hausner's ratio a function of interparticle friction was calculated using the formula.

$$\text{Hausner's ratio} = Tp/Bp$$
$$\text{While } \textit{Carr's index} = Tp - Bp/Tp \times 100$$

Where; Tp = Tapped density, Bp = Bulk density, Angle of repose(θ) = Tan^{-1} (Heap height of powder / Radius of heap base)

2.2.5 Chemomicroscopic Analysis of Leaf Powder

Powdered leaf was examined for its chemomicroscopic properties namely mucilage, lignin, starch, oils, calcium carbonate and calcium oxalate crystals using standard procedures[11].

2.2.5.1 Fluorescence Analysis of Leaf Powders

The fluorescent analysis of dried leaf powder was carried out using the standard method [12].

2.2.5.2 Physico-chemical Evaluation of Leaf Powders

The physicochemical parameters such as moisture content, ash values (total ash, acid-insoluble ash and water-soluble ash values), soluble extractive values such as ethanol, methanol and water-soluble extractive values were performed according to the official method prescribed by the WHO guidelines on quality control methods for medicinal plant materials [7,8,13].

2.3 Statistical Analysis

Data obtained were expressed in Mean ± SEM using (Statistical Package for Social Sciences) SPSS 17.0 and the terminology used in describing epidermal features is that of Metcalfe and Chalk (1979).

3.0 RESULTS

Table 1: Microscopic features of *Petivaria alliacea*

Leaf surface	Abaxial	Adaxial
Stomatal morphology type	Anisocytic and anomocytic	Anisocytic and anomocytic
Stomatal distribution	Amphistomatic	
Stomatal length(µm)	17.04(20.549±2.334)24.70	21.13(23.94±2.574)29.83
Stomatal width(µm)	12.39(13.97±1.523)16.74	16.17(19.40±1.25)20.16
Stomatal pore length(µm)	8.63(10.818±1.142)12.39	13.34(15.695±1.954)20.50
Stomatal pore width(µm)	2.38(3.128±0.537)3.92	1.85(2.605±0.616)3.85
Stomatal number	47(60.9±7.218)70	4(5.5±1.179)7
Stomatal index (%)	16.5	2.9
Guard cell length(µm)	7.54(10.676±1.315)12.69	4.38(6.074±1.205)7.44
Guard cell width(µm)	4.38(6.074±1.205)7.44	5.10(7.615±1.127)8.92
Epidermal cell number	290(308.5±22.510)350	146(195.3±31.65)240
Epidermal cell length(µm)	10.98(46.054±17.047)67.42	37.48(46.49±8.819)65.54
Epidermal cell width(µm)	13.61(17.129±4.225)25.23	20.42(24.52±4.767)33.47
Epidermal cell thickness(µm)	1.24(1.846±0.344)2.63	2.47(2.937±0.355)3.58
Length of trichome(µm)	62.39(104.065±69.013)256.00	
Width of trichome(µm)	10.55(13.047±2.523)18.06	

Values are represented as the mean of (10) replicates ± SEM.

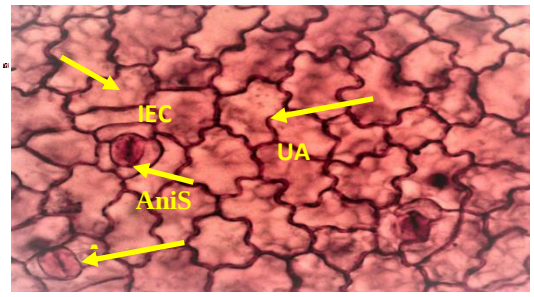

Figure 2: Adaxial surface of the Leaf of *Petiveria alliacea* showing; IEC (Irregular Epidermal Cell); AniS (Anisocytic stomata); AnoS (Anomocytic stomata) and UAWP (Undulate anticlinal wall pattern) Magnification 100

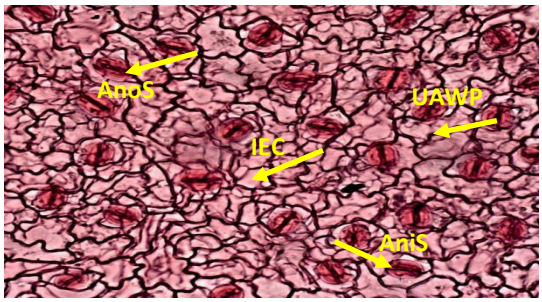

Figure 3: Abaxial surface of Leaf of *Petiveria alliacea* showing; IEC (Irregular Epidermal Cell); AniS (Anisocytic stomata); AnoS (Anomocytic stomata) and UAWP (Undulate anticlinal wall pattern) Magnification 100.

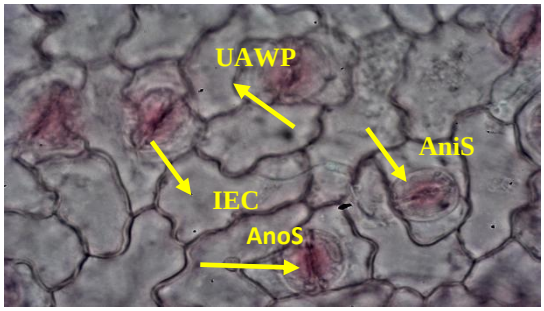

Figure 4: Abaxial surface of Leaf of *Petiveria alliacea* showing; IEC (Irregular Epidermal Cell); AniS (Anisocytic stomata); AnoS (Anomocytic stomata) and UAWP (Undulate anticlinal wall pattern) Magnification 400

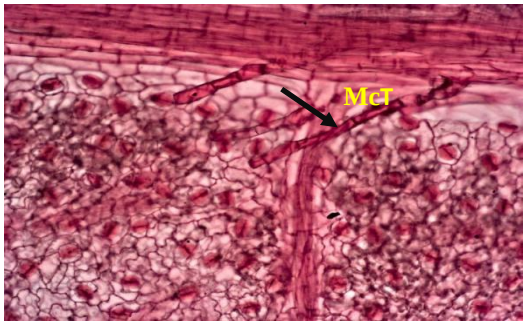

Figure 5: Abaxial surface of Leaf of *Petiveria alliacea* showing; McT (multicellular trichomes) Magnification 40

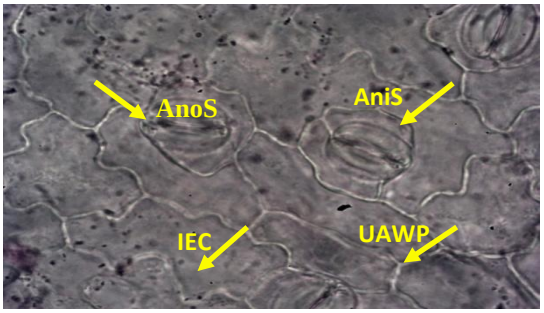

Figure 6: Powder microscopy of the leaf of *Petiveria alliacea* showing; IEC (Irregular Epidermal Cell); AniS (Anisocytic stomata); AnoS (Anomocytic stomata) and UAWP (Undulate anticlinal wall pattern) Magnification 400

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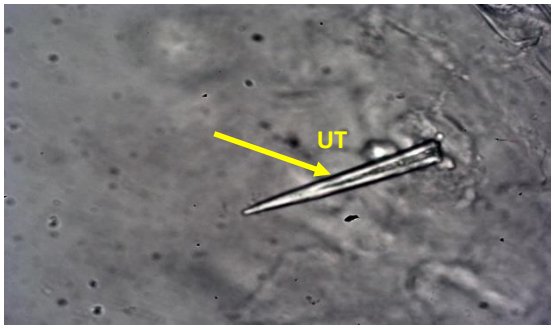

Figure 7: Powder microscopy of the leaf of *Petiveria alliacea* showing; UT (Unicellular Trichome) Magnification 100

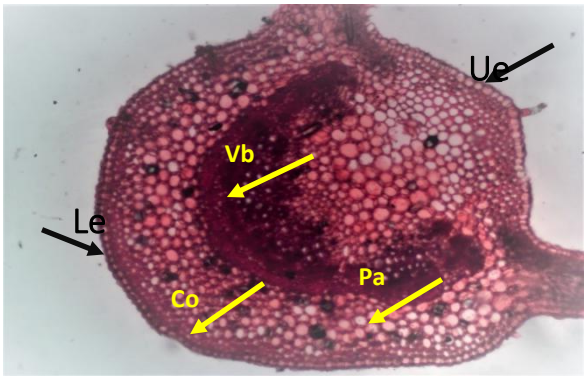

Figure 8: Transverse Section of the Leaf of *Petiveria alliacea* showing; Le(Lower Epidermis); Vb(Vascular bundles); Ue(Upper Epidermis); Co(Collenchyma tissue); Pa(Parenchyma tissue) Magnification 100

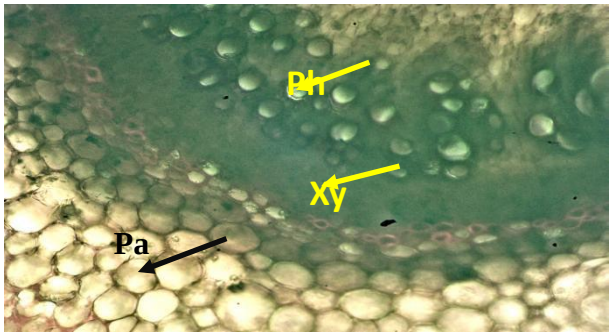

Figure 9: Transverse Section of the Leaf of *Petiveria alliacea* Showing; Pa(Parenchyma tissue); Xy(Xylem tissue); Ph(Phloem tissue) Magnification 400

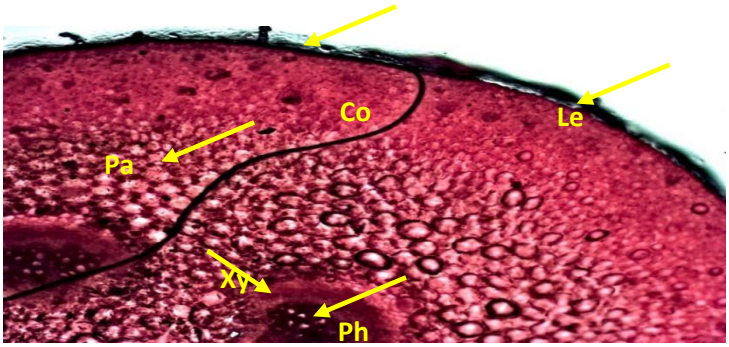

Figure 10 : Leaf Petiole of *Petiveria alliacea* showing; Le(Lower Epidermis); Xy(Xylem tissue); Ph(Phloem tissue) Co(Collenchyma tissue); Pa(Parenchyma tissue) Magnification 400

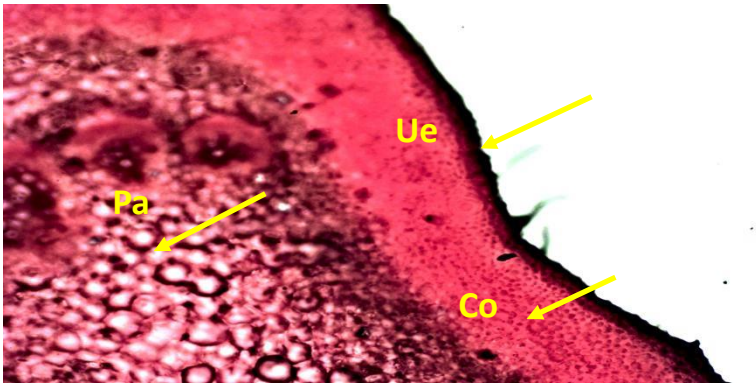

Figure 11: Leaf Petiole of *Petiveria alliacea* showing; Co(Collenchyma tissue); Pa(Parenchyma tissue); Ue (Upper Epidermis) Magnification 40

Table 2: Micromeritic Properties of *Petivaria alliacea* Powdered Leaf

PARAMETERS	RESULTS
Bulk volume (ml)	40.5±0.5
Tapped Volume (ml)	28.33±0.577
Bulk Density (g/ml)	0.247±0.006
Tapped Density (g/ml)	0.353±0.012
Flow Rate (g/s)	23.42±2.147
The angle of Repose is 30.60°	
Hausner's Ratio	0.703±0.006
Carr's Index	30.33±1.155
The diameter of the Heap is 7.1±0	
Height of Heap	2.1±0.1

Values are represented as a mean of three (3) replicates ± SEM

Table 3: Chemomicroscopy of *Petivaria alliacea* Powdered Leaf

Constituents	Qualitative test	Observation	Inference
Mucilage	Sample + Ruthenium red	Sample stains pink	Mucilage present
Lignin	Sample + phloroglucinol + Conc. HCl	Sample stains red	Lignin present
Starch	Sample + N/50 iodine	Blue-black colouration	Starch present
Oils	Sample + Sudan IV	Sample stains pink	Oils present
Calcium oxalate test	The sample was cleared and viewed under a microscope.	No calcium oxalate and crystals seen	Calcium oxalate and crystals absent
Cellulose test	Sample + N/50 iodine + Sulphuric acid	Blue colouration	Cellulose present
Protein	Sample + Picric acid (1%	No yellow stains strand	Protein absent

Table 4: Fluorescence Properties of *Petiveria alliacea.*

Extract	Ordinary Light	UV-365 nm
Water	Ash	Light ash
Methanol	Light Green	Pink
Ethanol	Light Green	Pink
DCM	Light Yellow	Pink
N-hexane	Yellow	Light pink
Ethyl acetate	Light Yellow	Pink

Table 5: Water-soluble Extractive value, Ethanol-soluble Extractive value, Methanol-soluble Extractive value and for Leaf Powders of *P. alliacea*.

Parameters	Weight(g)	Percentage (%$^{w}/_{w}$)
Water	0.1267±0.0058	12.7
Methanol	0.0833±0.0153	8.3
Ethanol	0.08±0	8.0

Values are represented as a mean of three (3) replicates ± SEM

Table 6: Moisture Content, Total Ash Value, Acid-Insoluble Ash Value, Water-Soluble Ash Value for the Leaf of *P. alliacea*.

Parameter	Weight(g)	Percentage (%$^{w}/_{w}$)
Moisture content	0.365±0.0105	18.3
Total ash	0.3483±0.0098	17.4
Acid-insoluble ash value	0.0267±0.0058	1.33
Water-soluble ash value	0.2367±0.0153	5.33

Values are represented as the mean of six (6) replicates ±SEM for moisture content and total ash.
Values are represented as the mean of three (3) replicates SEM for acid-insoluble and water-soluble ash values.

Table 7: GC-MS analysis of DCM extract of *Petiveria alliacea.*

S/N	Retention Time	Compound Name	Molecular Formula	Molecular Weight	Area %
1	12.299	2-Pentyn-4-one	C_5H_6O	82	12.253
2	14.482	Isophytol	$C_{20}H_{40}O$	296	13.509
3	18.975	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.390
4	19.394	Octadecanoic acid	$C_{18}H_{32}O_2$	284	7.062
5	21.338	Hexadecanoic acid,15-[(trimethyl)oxy]-, methyl ester	$C_{20}H_{42}O_3Si$	358	10.178
6	21.856	Eicosa-11,14,17-trienoic acid, picolinyl ester	$C_{26}H_{39}NO_2$	397	7.521
7	23.637	Eicosane, 10-butyl-10-propyl-	$C_{27}H_{56}$	380	23.596
8	23.777	Pentane, 1,1-diethoxy-	$C_9H_{20}O_2$	160	8.137
9	24.015	2-Diethoxymethyl-3-methyl-button-1-ol	$C_{10}H_{22}O_3$	190	9.355

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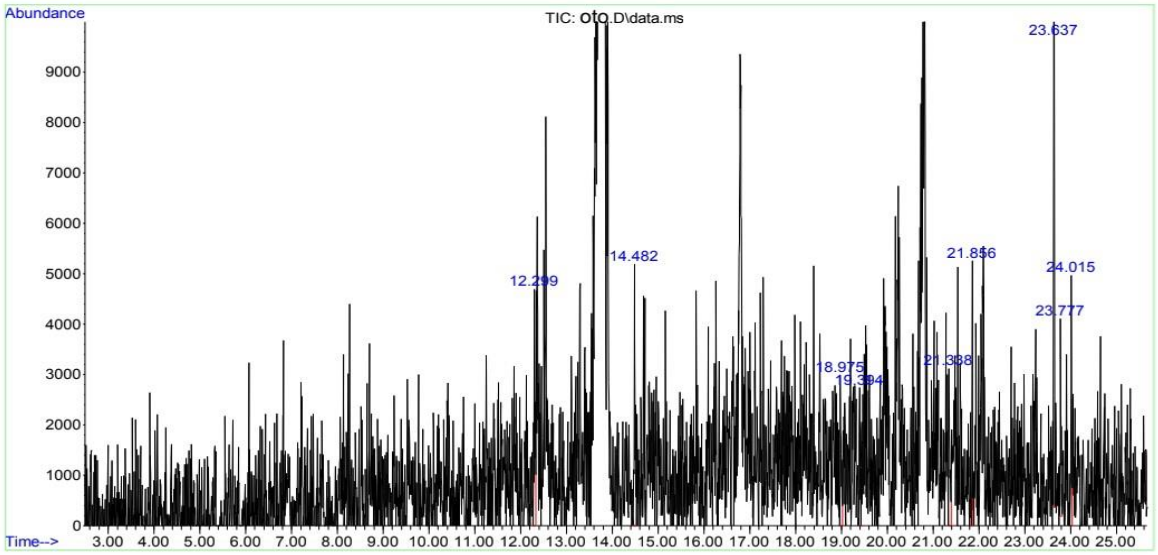
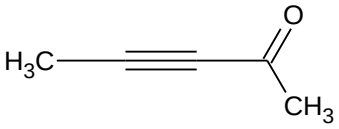
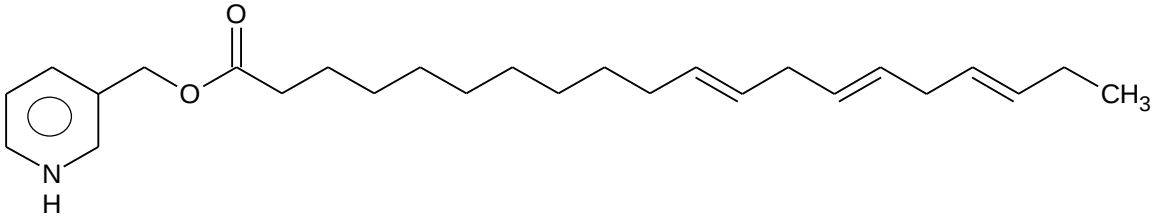


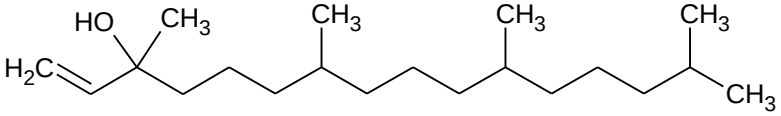
Figure 11: GC-MS Spectrum



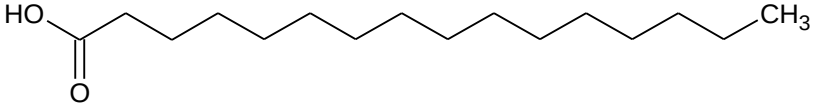
2-pentyn-4-one



Eicosane



Isophytol



Hexadecanoic

Figure 12: Chemical Structures of Compounds

4.0 DISCUSSION

Petiveria alliacea, a member of the Petiveriaceae (pokeweed) family, includes species valued for their ornamental and medicinal properties, as well as their toxicity [14]. According to WHO, all drugs—synthetic or plant-based—must meet safety and efficacy standards. Hence, quality control of herbal drugs is critical for ensuring authenticity and detecting adulteration. Microscopic analysis of *P. alliacea* revealed anisocytic and anomocytic stomata on both adaxial and abaxial surfaces, irregular epidermal cell shapes, and undulated anticlinal walls. Stomatal distribution was amphistomatic, with a stomatal index of 16.5% (abaxial) and 2.9% (adaxial). Stomatal dimensions were 23.94×19.40 µm (adaxial) and 20.55×13.97 µm (abaxial). The transverse section of the midrib revealed vascular bundles (Fig. 8). Micromeritics indicated good flow properties, including an angle of repose (30.60°), Hausner's ratio (0.7033±0.006), and Carr's Index (30.33±1.15) (Table 4.2). Chemomicroscopy confirmed cellulose, mucilage, starch, lignin, and oils (Table 3). Water extractive value was higher than methanol and ethanol aligning with Umoh et al. (2022) [15]. Moisture content exceeded the African Pharmacopoeia limit, potentially causing microbial contamination or degradation of active constituents [8]. Ash values included total (17.4%w/w), acid-insoluble (1.33%w/w), and water-soluble (5.33%w/w), with acid-insoluble ash within European Pharmacopoeia limits [16][17]. GC-MS analysis identified nine phytochemicals, with prominent constituents including Eicosane Isophytol and Hexadecanoic acid known for anti-inflammatory, analgesic, and aromatic properties [18][19][20]. These findings have established *P. alliacea*'s pharmacological potential and importance in quality control.

5. CONCLUSION

The results obtained from the pharmacognostic studies have provided information about the identity, authentication, quality, purity and diagnostic features of the plant *Petiveria alliacea*.

Acknowledgement

The authors would like to acknowledge the entire Laborathaveory staff of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo for their assistance in making the research successful.

Conflict of Interest

The authors have declared that no conflict of interest exists.

Contribution of the Authors

This work was carried out in collaboration with all other authors. RAU and OMU designed the study, performed the experimental procedures, and statistical analysis, and RAU wrote the first draft of the manuscript. Authors RAU and IIJ supervised lab experiments. Authors IIJ, ERI, GEC, AEU, II and NAA organized data, managed the literature searches, and assisted in plant material preparation. All authors read and approved the final manuscript.

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