Original Research Article

Pharmacognostic Study and Phytochemical profilling of dichloromethane extracts of the leaf and stem of *Vernoniaconferta*Benth. and*Vernoniacolorata* Drake. Asteraceae

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ABSTRACT

Aims: The study determines various taxonomic, pharmacognostic and phytochemical standards helpful to ensure the identity, purity, safety and efficacy of *Vernoniacolorata* and *Vernoniaconferta.*

Study Design: To study these two species of *Vernonia* for their epidermal cell shapes, stomatal types and distribution, trichome types, chemomicroscopic and fluorescence characters, flow properties, ash values, extractive solvents and phytoconstituents of dichlormethane fractions using GC-MS.

Place and Duration: This study was conducted at the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo from June 2023 to December 2023.

Methodology: The pharmacognostic characterization was determined following the guidelines given by the World Health Organization (WHO). Parameters determined included microscopy, powder microscopy, chemomicroscopy, micromeritic properties, ash values, extractive values, fluorescence analysis while phytochemical analysis was done using Gas Chromatography coupled to Mass Spectrometer.

Results: Epidermal cell shapes were both irregular with undulate and sinuous anticlinal wall pattern for both *C. colorata* and *C. conferta* respectively. Stomatal distribution was hypostomatic with paracytic stomata on the abaxial surface for *C. conferta* while amphistomatic with anomocytic stomata in *C. colorata*. Stomatal index was highest in *V. conferta*with 27.31 % and least in *V. colorata*21.52 %.Unicellular trichomes were observed in *V. colorata* and *V. conferta* epidermal surfaces. The chemomicroscopic study revealed the presence of lignin, starch, cellulose, oils, calcium oxalate crystals, mucilage and protein for both leaf and stem respectively. The fluorescence characteristics showed the presence of different colours supporting the presence of various phytoconstituents for both leaf and stem. The quantitative epidermal studies, chemomicroscopic and fluorescence characteristics revealed characteristic features for the drug. The flow properties for both leaf and stem were poor. The total ash values for both leaf and stem ranges between (2.40 – 9.30 and 10.60 – 11.06), acid-insoluble ash values were low, moisture contents were between 8 - 14 %w/w, water and methanol extracted more constituents. In total, 12 chemical constituents each were recorded for the leaves of

*V. colorata*and *V. conferta*, respectively, while 10 and 12 were also recorded for the dichloromethane stem extracts of *V. colorata* and *V. conferta***,** respectively.

Conclusion: This study is useful in pharmacognostic standardization of *Vernoniacolorata* and *Vernoniaconferta*.

Keywords:Vernoniaconferta, Vernoniacolorata, GC-MS, dichloromethane,micromeritic; pharmacogostic.

1. INTRODUCTION

Most research findings on plant source for medicines end up without the researcher reaching a conclusive indication of the implicated chemical name or structure for the cure claimed connecting to the folklore use. Many researcher stops at just authenticating the claimed folklore use of the crude extract of the plant. According to some estimates, almost 80 % of the present-day medicines are directly or indirectly obtained from medicinal plants [1]. Countries are encouraging the use of plant-based medicinal products in their healthcare systems. For example, Natural Health Product Regulations of Canada for the plant-based product in healthcare encourages usage of modern technology and evidence- based scientific support towards promoting medicinal plants and the associated products [2]. However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control. There is the need for documentation of research work carried out on traditional medicines [3]. With this background, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies.
Historically, natural products discovered from plants and their derivatives have provided numerous clinically useful medicines. The value of natural products in new drug delivery will continue to be significant in the years to come, mainly because of intrinsic chemical diversity [4].

The genus *Vernonia* named after William Vernon, an English botanist, who first identified this genus in Maryland is one of the largest genus found predominantly in Africa and South America with about 1000 species of herbs and shrubs in the family, Asteraceae [5]. Some species are edible, for example *V. amygdalina* which has been used for the management of various ailments such as diabetes, stomach aches, dysentery and cancer [40].*Vernonia* is economically valuable and owing to its large nature, some botanists divided this genus into several distinct genera [6].

Vernoniacolorata is endermic to tropical and southern African countries. The leaves have are shown to possess some medicinal uses as it has been used in folk medicine to treat diabetes mellitus, cough, fever, hepatitis, gastritis, stomach pain, gastrointestinal disorders, venereal diseases and skin eruptions [7, 8].

Vernoniaconferta found in Guinea to Southern Nigeria on abandoned farm lands have been also implicated in folkloric medicine as a diuretic, treatment of infections, skin diseases,

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abdominal pains and jaundice amongst many other usefulness [7]. These two species are used in Akwa Ibom State ethnomedicine for both culinary and medicinal uses and also, are usually found to be unduly substituted owing to mistaken identity most especially when collection is undertaken by persons who lack the required knowledge of plants' identification thus creating confusion in their ethno botanical applications. It is on this premise that this study was designed to report the detailed pharmacognostic, physicochemical and phytochemical evaluation of the leaf and stem of *Vernoniacolorata* and *Vernoniaconferta*. These established parameters will be useful in complete authentication and standardization of herbal samples from these species, which can guarantee the quality and purity of the drug and maintain its therapeutic efficacy.

2. MATERIAL AND METHODS

2.1 Collection and Identification of Plants Materials

The fresh samples of *V. colorata* and *V. conferta* were collected in from Uyo and Ikono local governments of Akwa Ibom State, respectively and preserved in FAA (Formalin Acetic Acid). The plants were identified and authenticated by Dr. Imoh I. Johnny in the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, and voucher specimens deposited in the herbarium with the numbers "UUPH $B16_{(1)}$ and UUPH $B17_{(l)}$" for *C. colorata* and *C. conferta*, respectively. The collected leaves and stems were washed under running tap water, rinsed with distilled water, chopped into pieces, dried under shade at room temperature. The dried leaves and stems were pulverized using Sonik SON electric blender, sieved through 350 micron sieve size and stored in airtight bottles to avoid interference from some natural factors such as moisture, microorganisms, humidity etc. before use.

2.2 Microscopic Evaluation of the Leaf

2.2.1 Qualitative microscopy

For anatomical studies, the standard median portion of the well expanded matured leaf was obtained. Epidermal peels of both adaxial and abaxial surfaces were made by placing the leaf on a clean glass slide with the surface to be studied facing down. The specimens were irrigated with water holding it downward from one end and then the epidermis above the desired surface was scrapped off carefully with sharp razor blade. The loose cells were then washed off with water and the epidermis was stained in 1 % aqueous solution of safranin-O for 2-3 minutes and washed again in water to remove excess stain and mounted in 10 % glycerol on a glass slide and covered with a glass cover slip before viewing with an Olympus CX21 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 [9, 41].

2.2.2 Quantitative Microscopy

Quantitative microscopic parameters such as leaf constant studies including stomatal length and width, stomatal pore length and width, guard cell length and width, stomatal number, stomatal index, epidermal cell length and width, epidermal cell number, epidermal cell thickness, vein islet number, vein termination number, areole length and width and trichome length and width were carried out using standard procedures [9, 41]. All measurements were made using a calibrated ocular micrometer and thirty (30) microscopic fields chosen at random were used and data presented as mean ± Standard Error of Mean (SEM).

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The stomata index (S.I) was determined using the formula: Stomatal Index (SI) = S/E +S x 100 Where: S = number of stomata per unit area E = number of epidermal cells in the same area [9].

2.2.3 Evaluation of Powders

Chemomicroscopic studies of the coarse powders of the leaf and stem were carried out to study the microscopical characters as well as their chemomicroscopic[10].

The fluorescent analysis of the studied *Vernonia* dried leaf and stem powders was carried out using the standard method [11]. The physicochemical parameters such as moisture content, ash values (total ash, acid insoluble ash, water soluble ash), soluble extractive values viz. ethanol, ethyl acetate, methanol and water were performed according to the WHO prescribed guidelines on quality control methods for medicinal plant materials [12].The micromeritic characteristics of leaf and stem powder studying bulk density, tap density, angle of repose, Hausner's ratio, Carr's index and pH were determined according to standard methods [33]. Gas Chromatography-Mass Spectroscopy was carried out on the crude dichloromethane extracts according to standard methods [8]. All experiments were repeated at least three (3) times except for the quantitative microscopy where thirty (30) determinations were done. Results were reported as Mean± SEM (Standard Error of Mean).

3. RESULTS AND DISCUSSION

3.1 Qualitative and Quantitative Microscopy

The summary of the leaf qualitative and quantitative micro-morphological characters for the abaxial and adaxial surfaces of the leaves of *V.conferta* and *V.conferta* are shown in Tables 1. While the photomicrographs for the venation, abaxial and adaxial surfaces and trichome were presented in Figure 1. The results of the extractive values for the leaf and stem are shown in Figures 2 and 3. The results of the micromeritic were represented in Table 2. Tables 3 and 4 showed results of the fluorescence properties for the leaf and stem of *V. conferta* and *V. colorata*while moisture contents and ash values of the leaf and stem of *V. conferta* and *V. colorata*are shown in Table 5. Chemomicroscopic result is presented in Table 6. GC-MS analysis of DCM extract for both leaf and stem of *V. colorata* and *V. conferta*are represented in Tables 7 – 10 with their chromatographs.

The quantitative anatomical analysis in Table 1 showed that the stomatal length and width were highest in *V. colorata* with 26.89 ± 0.85µm and least with 16.88 ± 0.41on the abaxial surface. Stomatal pore length was highest in *V. colorata* with 22.39 ± 0.63 µm and least in *V. conferta* with 9.63 ± 0.28 µm. Guard cell length on the abaxial surface was highest in *V. colorata* with 14.57 ± 0.60 ad least on the adaxial surface with 10.65 ± 0.31.Stomata pore was widest in *V. colorata* 7.66 ± 0.25 µm and least with 6.91 ± 0.16 µmon the abaxial and adaxial surfaces respectively. Stomata number was highest in *V. conferta* with 69.1 ± 3.03 µm on the abaxial and least with 9.90 ± 0.78 µm on the abaxial of *V. colorata*. µm respectively. Stomatal index was highest in *V. conferta*with 27.31 % and least in *V. colorata*21.52 %.
Venation studies revealed that the highest areole length was recorded in *V. colorata* with 166.09 ± 1.66 µm adaxial surface and least in *V. conferta* with 45.38 ± 1.59 µm adaxialsuface while on the abaxial surface it was highest in *V. colorata* with 113.69 ± 5.48 µm and least in *V. conferta* with 106 ± 9.53 µm. Areole width on the adaxial surface was highest in *V. colorata* with 144.37 ± 7.99 µm and least in *V. conferta* with 38.12 ± 0.55 µm while on the abaxial surface it was highest in *V. colorata*with 81.60 ± 4.87 µm and least in *V. conferta* with 57.60 ± 8.49 µm. Vein termination number on the adaxial surface was highest

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in *V. colorata* with 10.90 ± 0.43 µm and least in *V. conferta* with 2.90 ± 0.29 µm while the abaxial surface it was highest in *V. conferta* with 5.80 ± 0.34 µm and least in *V. colorata* with 2.70 ± 0.16 µm.
The vein islet number on the adaxial surface was highest in *V. colorata*with 1.90 ± 0.29 µm and least in *V. conferta* with 1.50 ± 0.18 µm while on the abaxial surface it was highest in *V. colorata* with 38.40 ± 2.06 µm and least in *V. conferta*with 2.90 ± 0.25 µm.

Table 1: Quantitative micro-morphological characters for the adaxial and abaxial surface of leaves of *V. conferta*and *V. colorata*

Parameters	Leaf surface	*V. conferta*	*V. colorata*
Stomatal Length (µm)	Adaxial	-	26.55 ±0.35
	Abaxial	25.51 ± 0.46	26.89± 0.85
Stomatal Width (µm)	Adaxial	-	16.88 ±0.41
	Abaxial	12.05 ± 0.51	16.20 ± 0.78
Stomatal Pore Length (µm)	Adaxial	-	19.39 ±1.45
	Abaxial	9.63 ± 0.28	22.39 ± 0.63
Stomatal Pore Width (µm)	Adaxial	-	6.91 ±0.16
	Abaxial	2.16± 0.59	7.66 ± 0.25
Stomatal Number	Adaxial	-	9.9 ±0.78
	Abaxial	69.1 ± 3.03	36.1 ± 2.94
Guard Cell Length (µm)	Adaxial	-	10.65 ±0.31
	Abaxial	12.31 ± 0.43	14.57 ± 0.60
Guard Cell Width (µm)	Adaxial	3.19 ± 0.92	6.94 ±0.18
	Abaxial		12.72 ± 0.20
Stomatal Index (%)	Adaxial	-	-
	Abaxial	27.31	21.52
Length of Trichome (µm)	Adaxial	-	58.22 ±2.06
	Abaxial	84.38± 5.56	207.85 ± 3.09
Width of Trichome (µm)	Adaxial	-	10.72 ±0.47
	Abaxial	9.89± 0.31	19.05 ± 0.13
No of Trichome	Adaxial	-	1.4±0.17
	Abaxial	5.9±0.55	8.5±0.67
Epidermal Cell Number	Adaxial	493.9± 3.17	408.20 ± 2.88
	Abaxial	138.9 ± 1.21	258.6± 10.24
Epidermal Cell Length (µm)	Adaxial	36.12±1.26	36.21± 1.96
	Abaxial	47.34± 2.58	29.76± 1.46
Epidermal Cell Width (µm)	Adaxial	15.39 ± 0.99	22.82± 1.31
	Abaxial	14.4± 1.14	521.66± 1.66
Epidermal Cell Wall Thickness	Adaxial	1.79± 0.64	1.98± 0.24
	Abaxial	3.02 ± 0.09	4.79 ± 0.09

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Areole Length (µm)	Adaxial	45.38 ± 1.59	166.09 ± 3..13
	Abaxial	106.67 ± 9.53	113.69 ± 5.48
Areole Width (µm)	Adaxial	38.12 ± 0.55	144.37 ± 7.99
	Abaxial	57.60 ± 8.49	81.60 ± 4.87
Vein Termination Number	Adaxial	2.9± 0.29	10.90 ± 0.43
	Abaxial	5.8± 0.34	2.7± 016
Vein Islet Number	Adaxial	1.5 ± 0.18	1.9± 0.29
	Abaxial	2.9± 0.25	38.40 ± 2.06

Mean ± SEM of 15 determinations

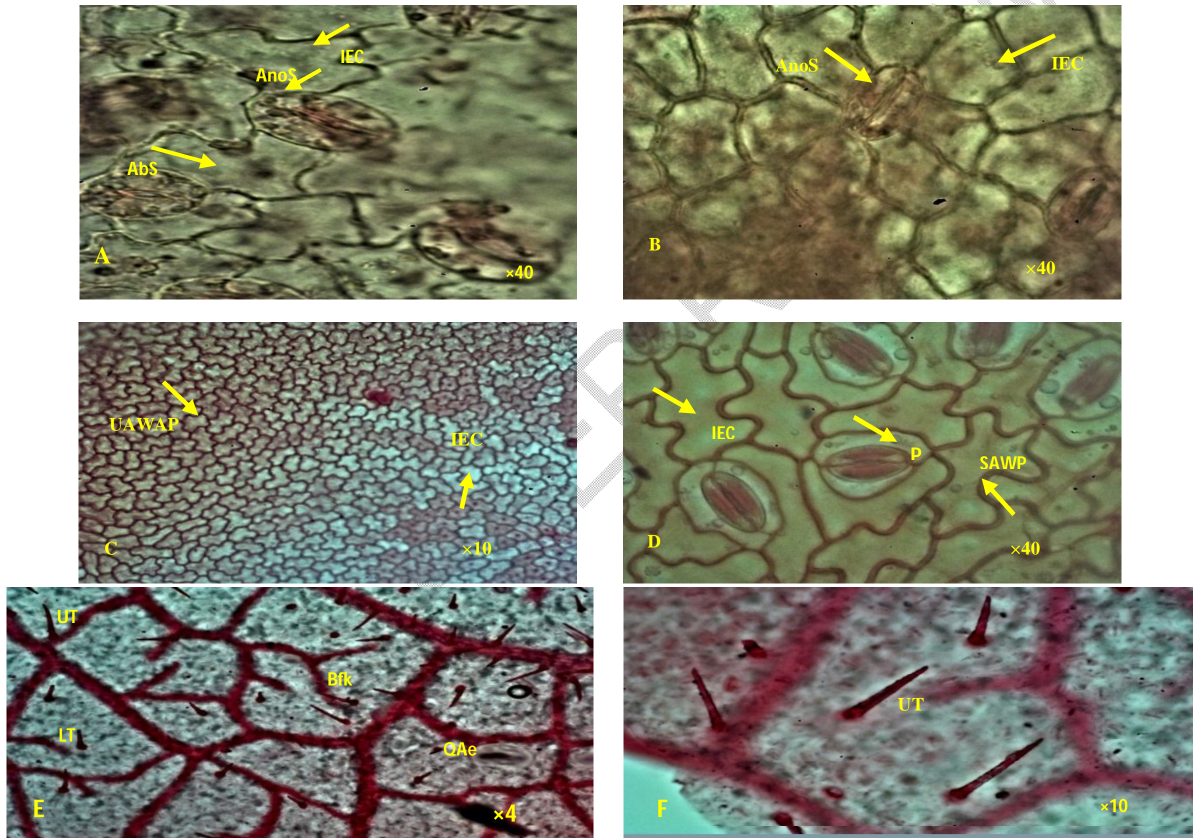

Figure 1. (A): *C. colorata*Adaxial surface ×10 (IEC: Irregular Epidermal Cell: AnoS; Anomocytic stomata; AbS; Abnormal stomata; (B): *C. colorata*Abaxial surface ×10 (IEC: Irregular Epidermal Cell: AnoS; Anomocytic stomata. (C) *C. conferta*Adaxial surface ×4 (UAWP: Undulate anticlinal wall pattern: IEC; Irregular Epidermal Cell. (D) *C. conferta*Abaxial surface ×10 (SAWP: Sinuous Anticlinal Wall Pattern; PS; Parasitic stomata; IEC: Irregular Epidermal Cell. (E) *C. conferta* (UT: Unicellular trichome; Bfk: Biforked termination, QAe; Quadrangular aereolation, LT; Linear vein termination. (F) *C. conferta*Abaxial surface ×10 (UT; Unicelluartrichome

3.2 Evaluation of Powders

The results of the micromeritics, fluorescence analysis,ash values and chemomicroscopy are summarized in Tables 2, 3, 4, 5 and 6, respectively while the extractive values for *V. colorata* and *V. conferta* are presented in Figures 2 and 3, respectively.

3.2.1 Micromeritic Evaluation

The powdered samples of *V. conferta*and *V. colorata* were evaluated for micromeritic features. The results are summarized in Table 2.

Table 2: Micromeritic evaluation of powdered leaf and stem of *V. conferta* and *V. colorata*.

Micromeritic Parameters	Plant Part	*V. conferta*	*V. colorata*
Bulk Volume (mL)	Leaf	38.33 ± 0.41	48.33 ± 0.40
	Stem	52.00 ± 1.22	66.67 ± 0.41
Tapped Volume (mL)	Leaf	27.67 ± 0.41	36.83 ± 0.74
	Stem	34.67± 0.82	46.0 ± 0.71
Bulk Density (g/mL)	Leaf	0.26 ± 0.01	0.2 ± 2.40
	Stem	0.19 ± 0.01	0.14 ± 0.00
Tapped Density (g/mL)	Leaf	0.36 ± 0.01	0.27 ± 0.00
	Stem	0.28± 0.00	0.21 ± 0.00
Hausner Ratio	Leaf	1.38± 0.01	1.33 ± 0.02
	Stem	1.52 ± 0.07	1.49 ± 0.06
Carr's Index (%)	Leaf	28.01 ± 2.73	25.03 ± 1.18
	Stem	32.47 ± 3.39	32.8 ± 2.78
Diameter of Heap (cm)	Leaf	6.88 ± 0.04	7.31 ± 0.04
	Stem	7.3 ± 0.21	8.08 ± 0.08
Height of Heap (cm)	Leaf	2.03 ± 0.11	2.9 ± 0.07
	Stem	3.2 ± 0.14	2.37 ± 0.12
Flow Time (sec)	Leaf	18.67 ± 1.01	33.33 ± 1.78
	Stem	99.6 ± 26.46	33.0 ± 1.41
Flow Rate (g/sec)	Leaf	1.25	0.33
	Stem	3.61	0.29
Angle of Repose (^{0})	Leaf	30.54	38.44
	Stem	41.21	30.28

Mean±SEM (Standard Error of Mean) of Three (3) Replicate

3.2.2Fluorescence Analysis of *V. conferta*and *V. colorata*Leaf and Stem powder

Fluorescence characteristic of powdered leaf and stem *V. conferta*and *V. colorata*were observed for the resolution of doubtful specimen. They were observed in visible light, short and long ultra-violet light (Table 3 – 4).

Table 3: Fluorescence analysis of *V. conferta*leaf and stem powder

Reagent	Sample	Visible light	Under UV light Short Wave length (253.7 nm)	Under UV light Long Wave length (365 nm)
Sample + Ethyl acetate	Leaf	Green	Orange	Red

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	Stem	Colorless	Red	Light red
Sample + Methanol	Leaf	Green	Maroon	Red
	Stem	Light Brown	Light pink	Greenish brown
Sample + Water	Leaf	Light yellow	Yellowish green	Yellow
	Stem	Brown	Light orange	Orange
Sample +n-hexane	Leaf	Brown	Bright yellow	Red
	Stem	Colorless	Yellow	Brown
Sample + Ethanol	Leaf	Green	Red	Red
	Stem	Light brown	Light brown	Greenish brown

Table 4: Fluorescence analysis of *V. colorata*leaf and stem powder

Reagent	Sample	Visible light	Under UV light Short Wave length (253.7 nm)	Under UV light Long Wave length (365 nm)
Sample + Ethyl acetate	Leaf	Green	Red	Orange
	Stem	Colorless	Maroon	Light red
Sample + Methanol	Leaf	Green	Maroon	Pink
	Stem	Ligt green	Light pink	Greenish brown
Sample + Water	Leaf	Light pink	Yellowish green	Orange
	Stem	Brown	Light orange	Orange
Sample +n-hexane	Leaf	Grey	Bright yellow	Red
	Stem	Colorless	Yellow	Brown
Sample + Ethanol	Leaf	Green	Pink	Red
	Stem	Maroon	Light brown	Greenish brown

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3.2.3 Ash values and Moisture Content Evaluation

The moisture content and ash values for the leaf and stem of *V. conferta*and *V. colorata*were evaluated and the results are summarized in Table 5.

Table 5: Moisture Contents and Ash values of the leaf and stem of *V. colorata* and *V. conferta*

Test	*V. colorata*		*C. conferta*	
	Leaf	Stem	Leaf	Stem
Moisture content	12.3	4.45	3.0	15.8
Total ash	2.40	10.60	9.3	11.06

Water-soluble ash	1.19	8.20	3.96	3.87
Acid-insoluble ash	1.45	2.43	0.85	6.55

Value is represented in average % w/w

3.2.4Chemomicroscopic Evaluation

The chemomicroscopic studies for the leaf and stem of *V. conferta*and *V. colorata*were evaluated and the results are summarized in Table 6.

Table 6: Chemomicroscopic evaluation of the leaf and stem of *V. conferta*and *V. colorata*

Test	*V. conferta*		*V. colorata*	
	Leaf	Stem	Leaf	Stem
Lignin	+	+	+	+
Starch	+	+	+	+
Cellulose	+	+	+	+
Oils	+	+	+	+
Calcium oxalate crystals	-	-	-	-
Mucilage	+	+	+	+
Protein	+	+	+	+
Cutin	+	+	+	+

Key: + = Present, - = Absent

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3.2.5 Solvents Extractive values

Figures 2 and 3 are the results of the extractive study on the leaf and stem of *V. colorata* and *V. conferta*

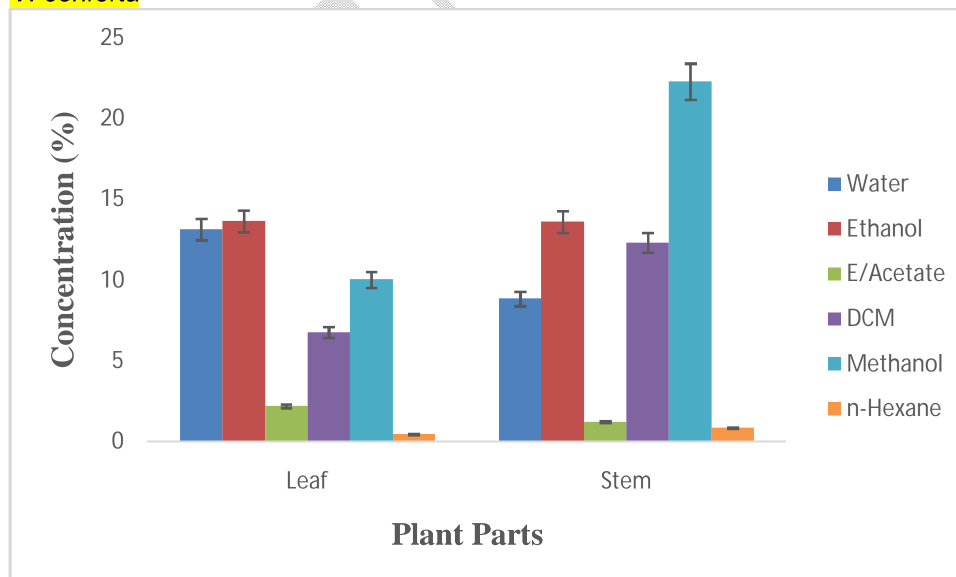

Figure 2: Extractive values for leaf and stem of *V. colorata*

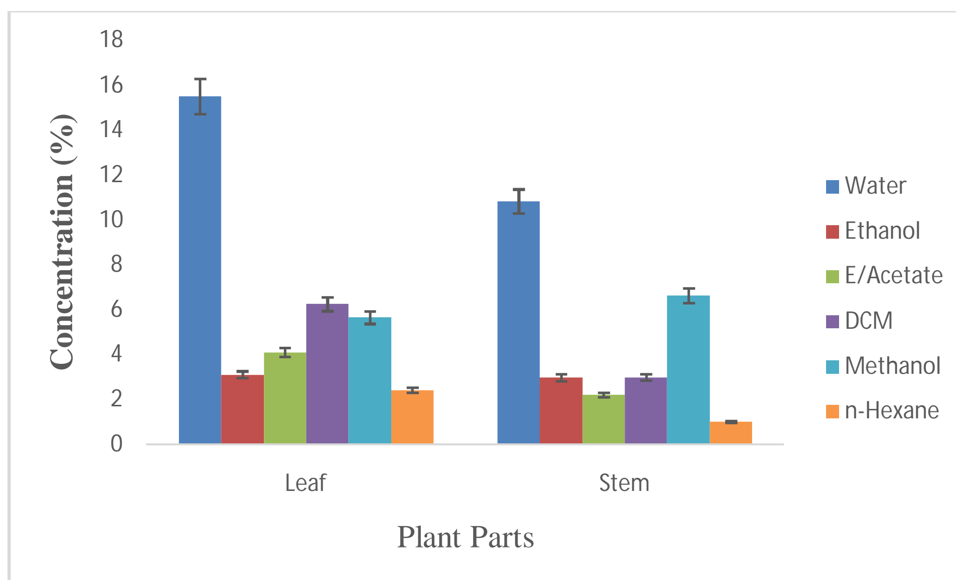

Figure 3: Extractive values for leaf and stem of *V. conferta*

3.2.6 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Dichloromethane Leaf and Stem Extracts of *V. colorata* and *V. conferta*.

The GC-MS analysis of the collected samples of *V. colorata* and *V. conferta* confirmed the presence of various phytochemical constituents in the dichloromethane leaf and stem extracts. The constituents and their retention time (RT), compound name, molecular formular, molecular weight, percentage area (%) and class of constituents in the dichloromethane extracts of the *V. colorata* and *V. conferta* studied are presented in Tables 7 – 10. The results on the GC-MS chromatogram of the ethanol extracts led to identification of various compound on comparison with the National Institute of Standard and Technology (NIST) library.
In total, 12 chemical constituents each were recorded for the leaves of *V. colorata*and *V. conferta* respectively, while 10 and 12 were also recorded for the dichloromethane stem extracts of *V. colorata* and *V. conferta***,** respectively.

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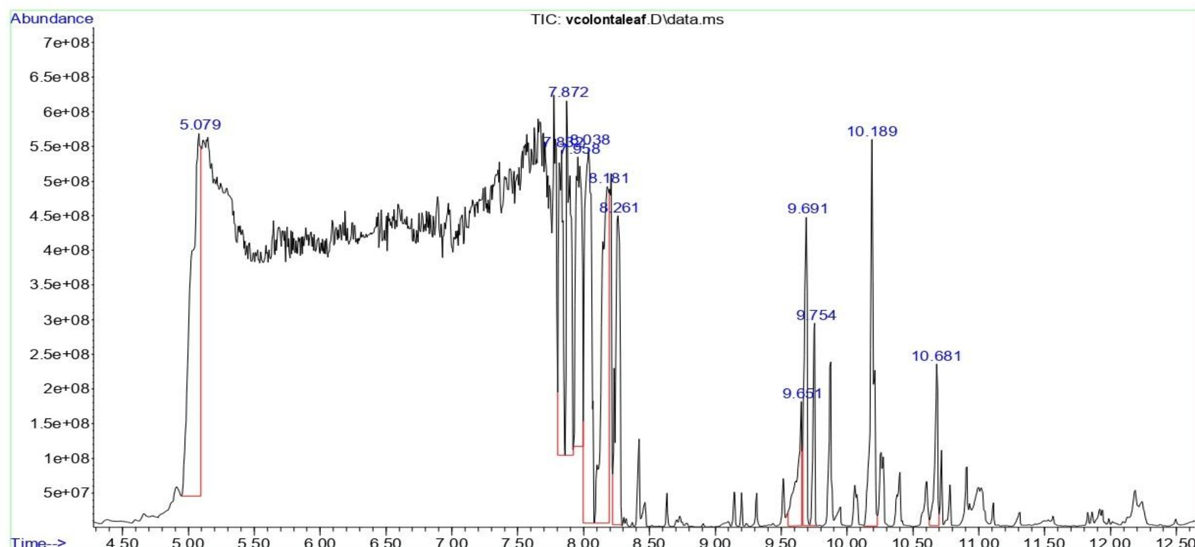

Figure 4: Chromatogram of the dichloromethane leaf extract of *V. colorata*

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Table 7: Phytochemical composition of dichloromethane leaf extract of *V. colorata* by GC-MS analysis.

S/NO	Retention Time	Compound	Molecular Formular	Mol. Weight	Concentration (%)	Chemical Class
1	5.079	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.349	Fatty Acid
2	7.832	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	7.273	Ester
3	7.872	Isopropyl palmitate	$C_{19}H_{38}O_2$	298	8.438	Fatty Acid Ester
4	7.958	Hexadecanoic acid, 1,1-dimethyl-1,2-ethanediyl ester	$C_{36}H_{70}O_4$	566	10.297	Fatty Acid Ester
5	8.038	Hexadecanoic acid, 1-tetradecyl-1,2-ethanediyl ester	$C_{48}H_{94}O_4$	734	14.635	Fatty Acid Ester
6	8.181	Hexadecanoic acid, 1,3-propanediyl ester	$C_{35}H_{68}O_4$	552	14.075	Fatty Acid Ester
7	8.261	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518	7.840	Organosilicon Compound
8	9.651	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	3.417	Organic Compound
9	9.691	13-Docosen-1-ol, (Z)-	$C_{22}H_{44}O$	324	5.145	Alcohol
10	9.754	Z-15-Octadecen-1-ol acetate	$C_{20}H_{38}O_2$	310	2.383	Ester
11	10.189	Hexacosane, 13-dodecyl-	$C_{38}H_{78}$	534	7.406	Hydrocarbon

12	10.681	Tetracosane, 9-octyl-	$C_{32}H_{66}$	450	2.742	Hydrocarbon

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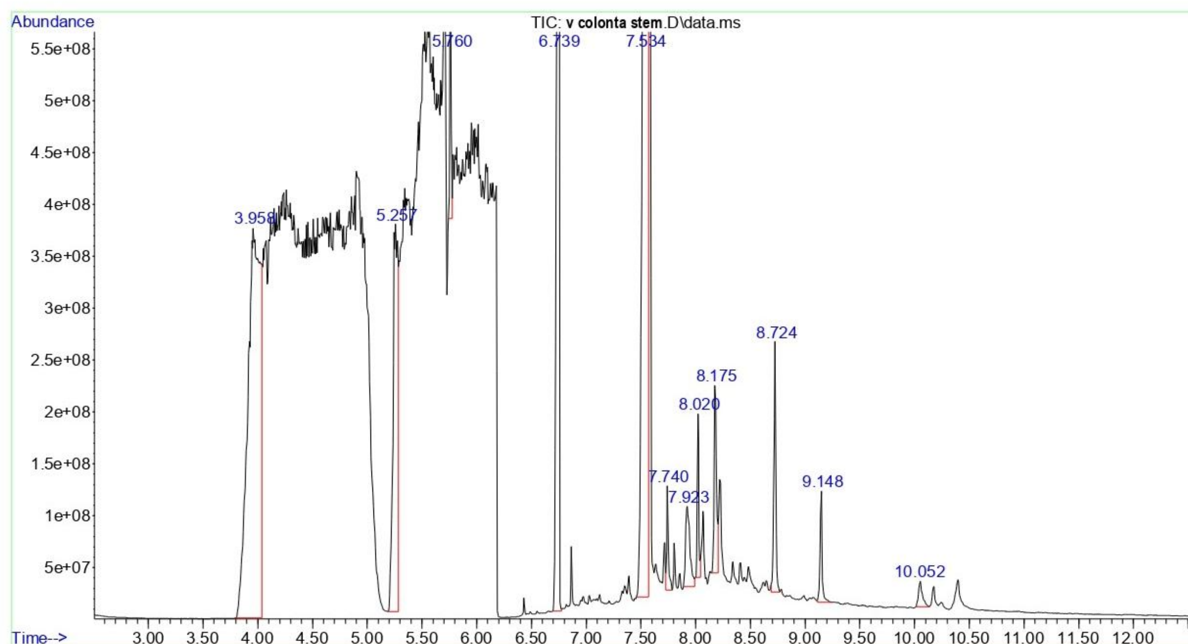

Figure 5: **Chromatogram of the dichloromethane stem extract of *V. colorata***

Table 8: Phytochemical composition of dichloromethane stem extract of *V. colorata* by GC-MS analysis

S/NO	Retention Time	Compound	Molecular Formula	Molecular Weight	Concentration (%)	Chemical Class
1	3.958	Ethanol, 2-(2-butoxyethoxy)-	$C_8H_{18}O_3$	162	24.831	Alcohol
2	5.257	Tricaproin	$C_{21}H_{38}O_6$	386	14.066	Flavonoid
3	5.760	Pentanoic acid, 2-methyl-, anhydride	$C_{12}H_{22}O_3$	214	2.352	Organic Acid Anhydride
4	6.739	Hexanoic acid, 2,4-dimethyl-, methyl ester, (2DL,4L)-	$C_9H_{18}O_2$	158	19.626	Fatty Acid Ester
5	7.534	Octadec-9-en-1-al dimethyl acetal	$C_{20}H_{40}O_2$	312	24.831	Aldehyde
6	7.740	Z-11-Pentadecenal	$C_{15}H_{28}O$	224	1.166	Aldehyde
7	7.923	Docosane, 10-ethyl-10-propyl-	$C_{27}H_{56}$	380	2.478	Hydrocarbon
8	8.020	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	306	1.824	Ester

9	8.175	11-(3-Ethenylcyclopentyl)undec-10-enoic acid, ethyl ester	$C_{20}H_{34}O_2$	306	3.193	Ester
10	8.724	Androsta-5,7-dien-3-ol, 17-(1-methylpent-4-ynyl)-, benzoate	$C_{32}H_{40}O_2$	456	3.351	Steroid
11	9.148	Androst-5-ene-3,17-diol, 17-methyl-, dipropanoate, (3β,17β)-	$C_{26}H_{40}O_4$	416	1.580	Steroid
12	10.052	Androst-5-ene-3,17-diol, 17-methyl-, dipropanoate, (3β,17β)-	$C_{26}H_{40}O_4$	416	0.700	Steroid

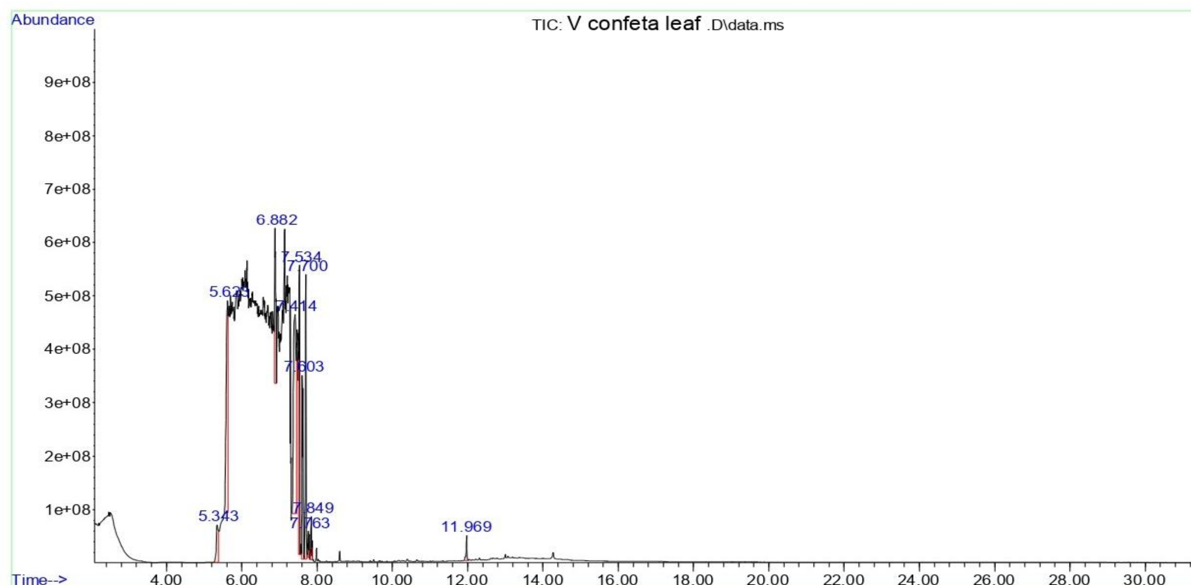

Figure 6: **Chromatogram of the dichloromethane leaf extract of *V. conferta***

Table 9: Phytochemical composition of dichloromethane leaf extract of *V. conferta* by GC-MS analysis

S/N	Retention Time	Compounds	Molecular Formula	Molecular Weight	Concentration (%)	Chemical Class
1	5.343	Benzene, 1,1'-(oxydi-2,1-ethanediyl)bis[3-ethyl-	$C_{20}H_{26}O$	282	4.327	Alkaloid
2	5.623	Benzaldehyde, 2-ethyl-	$C_9H_{10}O$	134	21.504	Alkaloid
3	6.882	1-Piperidinyloxy, 2,2,6,6-tetramethyl-	$C_9H_{18}NO$	156	8.797	Alkaloid

4	7.414	1-Nonene	C_9H_{18}	126	27.600	Alkaloid
5	7.534	Cyclopropane, 1-heptyl-2-methyl-	$C_{11}H_{22}$	154	12.593	Alkaloid
6	7.603	Divinyl sulfide	C_4H_6S	86	9.298	Alkaloid
7	7.700	Benzofuran, 2,3-dihydro-	C_8H_8O	120	11.887	Alkaloid
8	7.763	Indole	C_8H_7N	117	0.972	Alkaloid
9	7.849	Hydrocinnamic acid	$C_9H_{10}O_2$	150	1.917	Flavonoid
10	11.969	2,4-Heptanedione	$C_7H_{12}O_2$	128	1.105	Alkaloid

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Operator : ae
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Instrument : GCMSD
Sample Name:v confetastem
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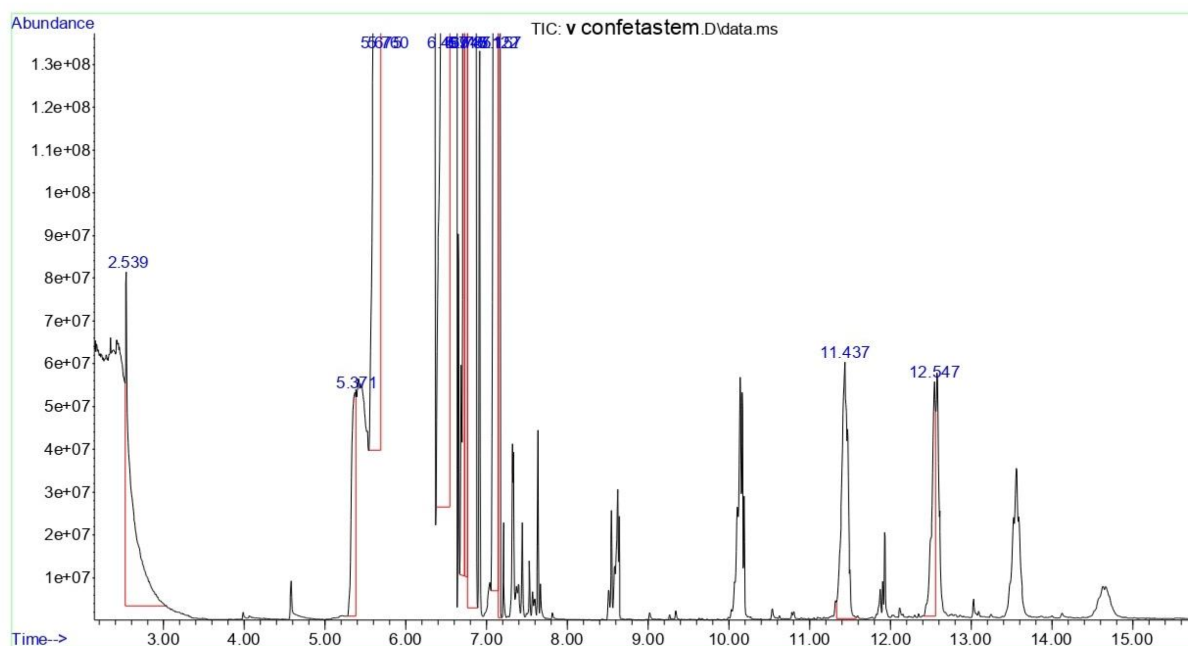
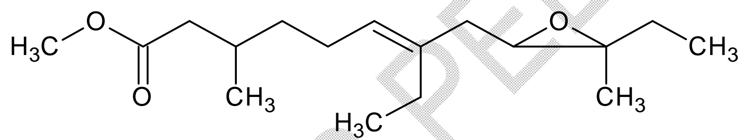


Figure 7: Chromatogram of the dichloromethane stem extract of *V. conferta*

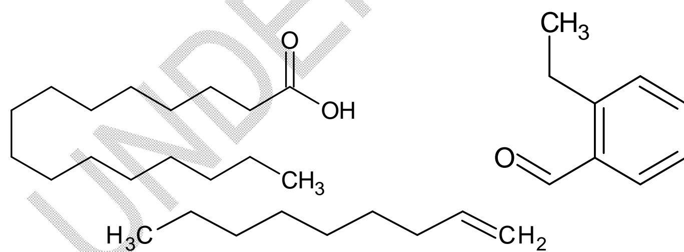
Table 10: Phytochemical composition of dichloromethane stem extract of *V. conferta*by GC-MS analysis

S/N	Retention Time	Compounds	Molecular formula	Molecular weight	Concentration (%)	Chemical Class
1	2.539	Pentane, 3-methyl-	C_6H_{14}	86	3.369	Alkaloid
2	5.371	Hexane, 2,2,4-trimethyl-	C_9H_{20}	128	1.511	Alkaloid
3	5.675	Benzene, 1,1'-(1,1,10,10-tetramethyl-1,10-decanediyl)bis[3,4-dimethyl-	$C_{30}H_{46}$	406	13.277	Alkaloid

4	5.760	Benzoic acid, 4-propyl-, 4-cyanophenyl ester	$C_{17}H_{15}NO_2$	265	9.605	Alkaloid
5	6.499	Divinyl sulphone	$C_4H_6O_2S$	118	17.943	Alkaloid
6	6.716	Benzofuran, 2,3-dihydro-2,2,4,6-tetramethyl-	$C_{12}H_{16}O$	176	3.931	Alkaloid
7	6.745	Butanol, 1-[2,2,3,3-tetramethyl-1-(3-methyl-1-penynyl)cyclopropyl]-	$C_{17}H_{30}O$	250	5.644	Alkaloid
8	6.785	2,6-Nonadienoic acid, 7-ethyl-9-(3-ethyl-3-methyloxiranyl)-3-methyl-, methyl ester, [2R-[2α(2E,6E),3α]]-	$C_{18}H_{30}O_3$	294	17.943	Alkaloid
9	7.122	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-	$C_{25}H_{40}O_6$	436	17.609	Alkaloid
10	7.157	Hydrocinnamic acid	$C_9H_{10}O_2$	150	5.127	flavonoid
11	11.437	Doconexent	$C_{22}H_{32}O_2$	328	2.608	Fatty acid
12	12.547	1H-Pyrrol-2(5H)-one, 4-benzyloxycarbonylmethoxy-3-benzyloxycarbonylmethyl-	$C_{22}H_{21}NO_6$	395	1.433	Alkaloid



2, 6-Nonadienoic acid, 7-ethyl-9-(3-ethyl-3-methyloxiranyl)
-3-methyl-, methyl ester, [2R-[2α(2E,6E),3α]]-



n-Hexadecanoic acid Benzaldehyde,2-ethyl 1-Nonene

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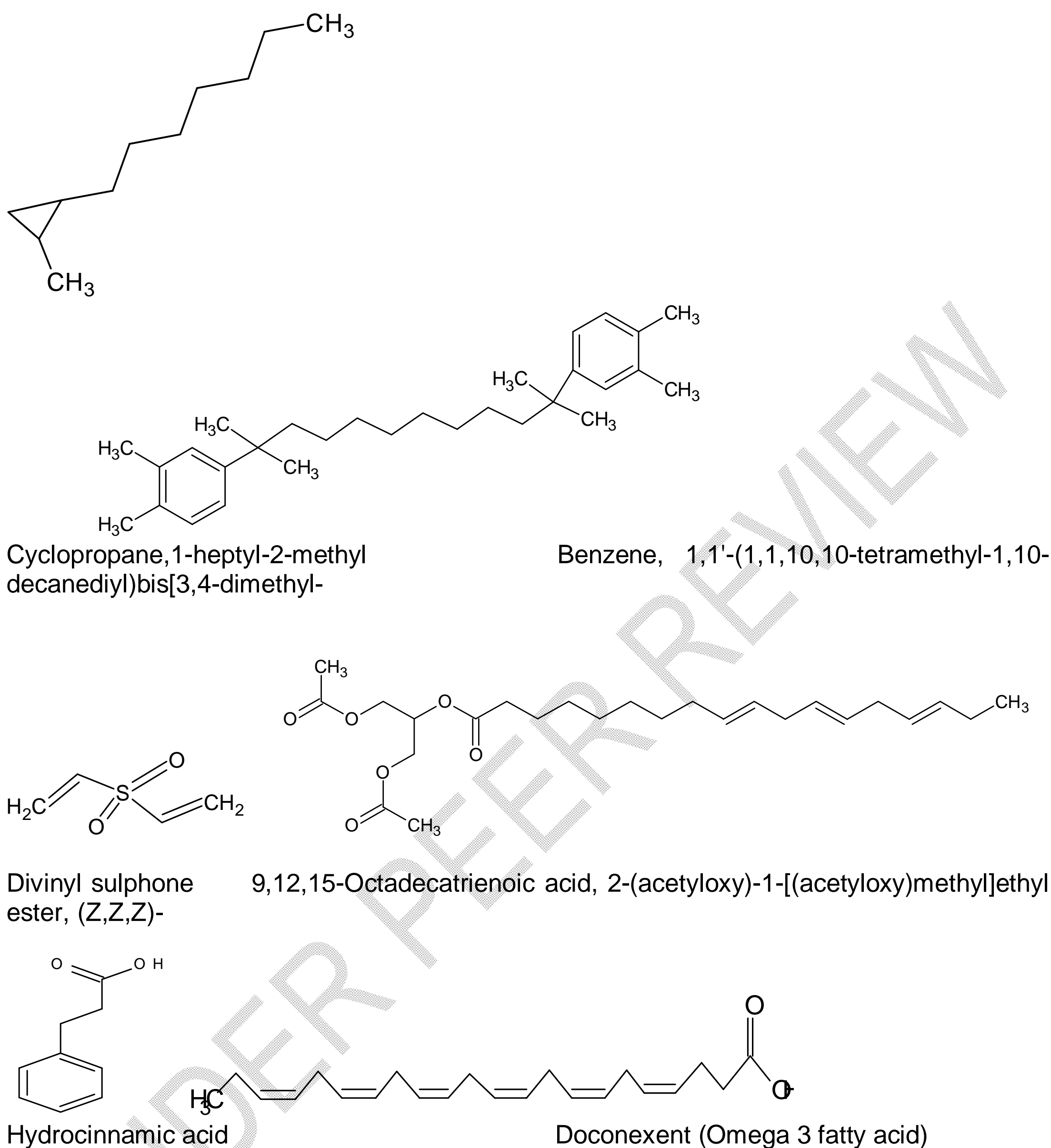

Cyclopropane,1-heptyl-2-methyl

Benzene, 1,1'-(1,1,10,10-tetramethyl-1,10-decanediyl)bis[3,4-dimethyl-

Divinyl sulphone

9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-

Hydrocinnamic acid

Doconexent (Omega 3 fatty acid)

Figure 8:Structures of some abundant phytochemical constituent in the GC-MS analysis

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This study assessed variations of the leaf characters of two species of *Vernonia* using a set of diagnostic characters. Leaf morphometric analysis has been used in order to characterize and elucidate intraspecific variants and morphotypes. The epidermal cell shapes from the abaxial and adaxial surfaces were mostly irregular with undulate to sinuous anticlinal wall pattern. Similar study was reported on 14 *Vernonia* species [13]. Epidermal surfaces of the studied *Vernonia* revealed a number of micromorphological characters and these features showed interesting interspecific variations which were of diagnostic importance for delimitation and identification. The occurrence of undulate walls in the studied *Vernonia* species (*V. conferta* and *V. colorata*) surfaces agreed with the suggestion that undulate (curved) wall is a mesomorphic character and that environmental conditions such as

humidity play significant role in determining the pattern of anticlinal cell walls [14]. Epidermal cell shape was mostly polygonal with a few that were sinuous. Anticlinal wall patterns were mostly straight with a few that were undulate/curved or wavy/undulate. The taxonomic value of the leaf epidermal characters is well documented [15,16,17]. It was earlier noted that the epidermal cells of *V. cinerea* and *V. amygdalina* are slightly irregular to polygonal with wavy or undulating anticlinal walls on the adaxial surface and sinuous anticlinal walls on the abaxial surface [18].

Stomata in the study were hypostomatic for *V. conferta*and amphistomatic for *V. colorata* which is considered a character of unification and distinctive feature for these sets of *Vernonia*. Another study distinguished *Waltheriaindica* from other species using the presence of paracytic stomata which was diagnostic [19]. Stomata was variable in the study on 14 species; *Vernoniaamygdalina*, *Vernoniacalvoana*, *Vernoniacamporum*, *V. glabra* var. *occidentalis* and *Vernoniamigeodii* were amphistomatic whereas four others (*Vernonia glabra*, *Vernoniaglabra* var. *occidentalis*, *Vernoniaguineense* and *Vernoniatenoreana*)were hypostomatic [13]. All other taxa studied possessed stomata at either the adaxial or abaxial surfaces. Parasitic and anomocyticstomatal complex type were reported for *V. conferta* and *V. colorata*, respectively. Also, the presence of parasitic stomata in *V. myriantha* which supports these findings was earlier reported [20]. Also, different shapes of epidermal cells, the type of arrangement of stomata, size and shape as well as trichomes are all vital in systematic botany [20]. Munir *etal*, [17] reported that the foliar epidermis is one of the fundamental taxonomic characters from biosystematics point of view and taxonomic studies of a number of plant families are made on the basis of leaf epidermis anatomy. Leaf epidermal features like the shape of epidermal cells, stomata and trichomes are useful anatomical tools. Unicellular trichome was reported in the abaxial and adaxial surface of *V. conferta*. The presence of multicellular trichome in *V. myriantha* and stomatal distribution of amphistomatic in *V. colorata*. *V. camporun, V. calvoana, V. amygdalina* and *V. migiodii*were also reported [13]. Unicellular trichomes were observed in *V. conferta* and were sparsely distributed. Studies have shown that trichome types can be used as a classifactory tool [21]

Quantitative microscopy was used to study some microscopic characters in the samples. The longest and widest stomata were found in *V. colorata*abaxial surface and *V. colorata*adaxial surface with 16.88 ± 0.41 µm and 16.20 ± 0.78 µm, respectively. The longest stomatal pore was found in *V. colorata*abaxial surface with 22.39 ± 0.63 µm and least in *V. conferta*abaxial surface with 9.63 ± 0.28 µm while the widest stomatal pore was found in *V. colorata*abaxial surface with 7.66 ± 0.25 µm and the least in *V. conferta*abaxial surface with 2.16 ± 0.59 µm and this agrees to earlier study [20]. Study also delimited Althaea and Alcea, the two closely related genera in the family Malvaceae using anatomical sizes of the epidermal cells [22]. *V. conferta* had the highest stomata number (69.10 ± 3.03µm) abaxial surface and the least was found in *V. colorata*adaxial surface (9.90 ± 0.78µm). The occurrence of more stomata on the abaxial surface is an adaptation to water loss as this signifies a coping strategy to survive drought [23].The longest and widest guard cell was recorded in *V. colorata*abaxial as 14.47 ± 0.60 µm and 12.72 ± 0.200 µm respectively while the least was recorded in *V. colorata*adaxial surface and *V. conferta*abaxial surfaces, respectively.
Stomatal index may be used as a diagnostic feature as it was highest in *V. conferta*abaxial surface (27.31 %) and the least in *V. colorata*abaxial surface (21.52 %) as recorded in Table 4. reported that stomatal index and the guard cell provide values that will serve as parameters for comparison among taxa, which can be useful for identification of the studied taxa. A study on three species of *Acalypha* occurring in Nigeria also reported that variation in stomata index and guard cell areas are useful diagnostic tools [25].

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Venation pattern is useful in the number of vein termination number, vein islet number, average areole length and width for delimiting the studied species of *Vernonia*. The vein termination number was highest in *V. colorata*adaxial and lowest in *V. colorata*abaxial surface (2.70 ± 0.16 µm). *V. conferta* had quadrangular areolation. The longest and widest areole was found in *V. colorata*adaxial surface as 166.09 ± 3.13 µm and 144.37 ± 7.99µm, while the least was recorded in *V. conferta*adaxial surface with 45.38 ± 1.59 µm and 38.12 ± 0.55 µm. The appearance and characterictics of the areoles are termed areolation [19]. Foliar venation has been used to provide insight into relationship within the subfamilies and tribes [19].

The moisture contents in the study showed that all fell within the African pharmacopoiea limit of 8 - 14 % w/w for vegetable drug which shows their relatedness except for the stem of *V. conferta* with 15.80 %w/w (Table 5) thus implying that the powdered sample cannot be stored for a long period and would easily be attacked by microbes. High moisture content is uneconomical, and in the presence of suitable temperature could lead to enzymatic activation and hydrolytic reactions as well as proliferation of microbial growth which may ultimately lead to degradation of active constituents [26].

The total ash values for both leaf and stem ranges between (2.40 – 9.30 and 10.60 – 11.06), respectively as shown in Table 5. The European pharmacopoeia limit of total ash value for crude vegetable drugs range should not exceed 14 %w/w[24]. From the result of the study, the total ash value of the leaf and stem were found to be within acceptable limit. Ash value is an indicative of the impurities present in the drug since it is constant for a given drug. Therefore, the values obtained are one of the important diagnostic parameters of the drug as the index of purity which may be used in the delimitation and identification of *V. colorata* and *V. conferta* to avoid adulteration [27].

Acid-insoluble ash values of the studied *Vernonia* were low ranging from 0.85 – 6.55%w/w in Table 5 showed that a minute amount of the inorganic component is insoluble in acid. It indicates that adulteration of raw ingredients by substances, such as silica was less, and a low acid-insoluble ash value may also affect the amount of the component absorbed in the gastrointestinal canal when taken orally in drug after herbal formulation. The results recorded for the acid-insoluble ash shows their relatedness.

The result of the extractive values of water, methanol and ethanol showed high extractives in the leaf and stem powdered samples (Figure 2 and 3) while the least extractive was recorded in n-hexane for both leaf and stem ranging between 0.44 – 1.01 %w/w and this aligns is in agreement with the a previous work [28]. Despite the fact that water as solvent of extraction was better, and is a universal solvent and mostly used as a primarily solvent by traditional healers, alcohol is still given preference in terms of choice of solvent when it comes to medicinal plant researches most especially where preservation is needed. The choice of solvent in research involving plants depend on so many factors among which include the diversity of different phytochemicals to be extracted and also what is intended with the extract. The differences in the extractive values might be caused by the distinct cellular structures of and/or the different dimensions of cells [29].

Angle of repose for the leaf powders of *V. colorata* were 30.54 °- 38.44°while that of the stem powders were 30.28 °- 41.21 °, respectively as seen in Table 2 implies that all had a poor flow rate. The Hausner ratio and Carr’s Index are parameters that are used to determine the powder flow property and powder characteristics. Hausner ratio values less than 1.25 indicate good flow while those greater than 1.25 indicates stand for poor flow [30]. From the study, Hausner ratio and Carr’s index of both the leaf and stem were greater than 1.25 and 23 %, respectively and hence the poor flow property of the powders. This outcome

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may be as a result of moisture contents, temperature, particle size, particle shape (texture) and time of storage at rest that are usually linked to powder's flowability [32].

The angle of repose is considered to be the most classical technique used for characterizing the flow properties of powders. Angle of repose is a characteristic related to interparticulate friction or resistance to movement between particles [30].
Chemomicroscopic studies of the powdered leaves and stems of the studied *Vernonia* were found to have cellulose cell wall, lignin, starch, oil, protein and mucilage except calcium oxalate crystal which was absent in the leaves and stems respectively. The structures, chemical reactions and colour changes are most valuable in the identification of powdered drug as their identification is largely based on the form, the presence or absence of certain cell wall types and cell inclusions. Most of these cell wall materials such as cellulose, lignin and cutin perform the functions of protection, insulation, strengthening and reinforcing vascular plants without which they will topple over [31].

The results of the fluorescence analysis of the powdered leaf and stem of the studied *Vernonia* species when viewed in visible light and under UV light showed different colours ranging from green, light green, brown, red, pink, orange, yellow etc. at both short wavelengths (253 nm) and long wavelength (365 nm). This is due to the presence of different phytochemical constituents in the plant material [32]. Some phytocompounds fluoresce when viewed under the Ultra violet lampat various wavelength. The fluorescence colour is specific for each compound (Tables 3 and 4). The fluorescent method is adequately sensitive and it enables the accurate assessment of the analyte over a satisfactory concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples. The studied *Vernonia* species possessed various phytochemicals which explains the use of the plants for treating different ailments. They have the potential of providing useful drugs for human use.

In recent times, in addition to morphological markers, anatomical, cytological, biochemical and molecular markers, GC-MS maybe a valuable tool using suggested identified phytocompounds to categorize taxa and bring about delimitation [33].
In total, 12 chemical constituents each were recorded for the leaves of *V. colorata*and *V. conferta* respectively, while 10 and 12 were also recorded for the dichloromethane stem extracts of *V. colorata* and *V. conferta*, respectively. Of all the suggested phytochemical constituents identified, the most prevailing constituents were n-Hexadecanoic acid, Hexadecanoic acid, 1-tetradecyl-1,2-ethanediyl ester, Hexadecanoic acid, 1,3-propanediyl ester, Benzaldehyde, 2-ethyl-, 1-Nonene and Cyclopropane, 1-heptyl-2-methyl- for the leaves of *V. colorata* and *V. conferta* respectively.
The major components that delimited the leaves of the species were as follows; In *V. colorata*was n-Hexadecanoic acid (16.349%), Isopropyl palmitate (8.438%), Hexadecanoic acid, 1,1-dimethyl-1,2-ethanediyl ester (10.297 %), Octadec-9-en-1-al dimethyl acetal (24.831 %); in *V.conferta*; Benzaldehyde, 2-ethyl- (21.504 %), 1-Nonene (27.600 %), 4 Benzofuran, 2,3-dihydro- (11.887 %), Benzaldehyde, 2-ethyl- (21.504 %), Hydrocinnamic acid (1.917 %) The major chemical components that delimited the species based on stem were as follows; In *V. colorata,* Ethanol, 2-(2-butoxyethoxy)- (22.4 %), 5-Hydroxymethylfurfural (24.831 %), Tricaproin (14.066 %) and Octadec-9-en-1-al dimethyl acetate (24.831 %), Octadec-9-en-1-al dimethyl acetal (24.831 %) ; in *V. conferta*; Divinylsulphone (17.943 %), 2,6-Nonadienoic acid, 7-ethyl-9-(3-ethyl-3-methyloxiranyl)-3-methyl-, methyl ester, [2R-[2α(2E,6E),3α]]- (17.943 %), 9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)- (17.609 %), 4H Hydrocinnamic acid (5.127 %); These were the most abundant phytoconstituents in the leaves and stems of the studied *Vernonia*.

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The suggested phytochemicals; n-Hexadecanoic acid (fatty acid) was reported as anti-ageing, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukamic, anticancer, vasodilator, anticoronary, antiulcerogenic and antibronchitic in action. Pentanoic acid, 2-methyl-anhydride (Organic Acid Anhydride) as anticonvulsant. Hydrocinnamic acid (flavonoid) was reported as antioxidant, antiturmor, antimicrobial and antidiabetic [34]. Hexadecanoic acid (fatty acid) reported as antimicrobial and chemopreventive [37]. Hexadecanoic acid, 1-tetradecyl-1,2-ethanediyl ester (Fatty acid Ester) reported to possess antioxidant, antimicrobial and anti-inflammatory activities [35]. Tricaporin (flavonoid) also reported to possess anticancer properties [36]. Benzofuran, 2,3-dihydro- (alkaloid) reported to possess several biological properties such as anti-inflammatory, antimicrobial, antifungal, antihyperglycemic, analgesic, antiparasitic and antitumor activities [38, 39].

4. CONCLUSION

The current investigation revealedpharmacognostic features, physicochemical and phytochemical properties of *V. colorata* and *V. conferta*. These parameters could be useful in the preparation of the herbal section of proposed Nigerian Pharmacopoeia. Any crude drug which is claimed to be *V. colorata* and *V. conferta*but whose characters significantly deviate from the accepted standard above would then be rejected as contaminated, adulterated or faked. The high content of polyphenolic secondary metabolites (alkaloids and flavonoids) in the studied *Vernonia* species attest to their uses in ethnomedicine.Further studies on *in vitro* and *in vivo* studies should be carried out on various partition fractions and the isolates obtained from this important medicinal plant may be studied further using clinical trials, to assess the efficacy and safety of the extract for specific diseases.

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

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Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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