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Gas Chromatography-Mass Spectrometry *Vernonia Hymenolepis* Analysis of the Solvent-Solvent Extract of *Vernonia Hymenolepis* Leaves

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Abstract: *The solvent-solvent extraction and gas chromatography-mass spectrometry analysis of the leaves of Vernonia hymenolepis have been carried out. The dried leaves were extracted using n-hexane and partitioned with absolute ethanol. The concentrated ethanol fraction, EVH was analyzed using a Gas Chromatography-Mass Spectrometry, GC-MS instrumentation. The present study has identified thirty-one (31) phytoconstitutions in the volatile extract of Vernonia hymenolepis. 2-Methyl-Z,Z-3,13-octadecadienol (3.16%); cis-verbenol (4.13%); 6-nonynoic acid (2.39%); 1H-pyrrolo[3,4-d]pyrimidine-2,5-di one, 4,6-bis(4-hydroxyphenyl)-1-methyl-3,4,6,7-tetrahydro- (4.91%); myo-Inositol, 4-C-methyl- (9.84%); ethanol, 2-phenoxy-, acetate (3.83%); cyclododecane (2.61%); 2-piperidinone, N-[4-bromo-n-butyl]- (3.52%); tetradecanoic acid (2.05%); hexadecanoic acid methyl ester (5.97%); n-hexadecanoic acid (9.85%); methyl stearate (2.83%); 1-nonadecene (4.97%); octadecanal (2.06%); tetracosane(3.81%); eicosane (2.85%); 2H-indol-2-one, 1,3-dihydro-5-hydroxy- (4.99%) and 1-octen-3-yne (2.07%) were identified as the major constituents.*

Keywords: *Vernonia hymenolepis, solvent-solvent partitioning, GC-MS*

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1.0 Introduction

Vernonia hymenolepsis is an indigenous African plant with over 300 species identified with the family *Asteraceae*. It is locally called a sweet bitter leaf (Isawumi, 1993). It is both a nutritious and medicinal plant with profound local claims of curing pneumonia, bleeding stopping, constipation, malaria, and treatment of jaundice and diarrhoea in babies (Kokwaro, 1993).

Associated with the vegetative and medicinal significance of *Vernonia hymenolepsis* is the phytoconstitutions present in the plant tissues. These phytochemicals in volatile media can be analyzed using Gas chromatography-mass spectrometry (GC-MS) equipment (Sparkman *et al*., 2011; Jones 2019). Reports arising from the evaluation of phytochemicals in *Vernonia hymenolepsis* are scanty. However, several studies have been documented on the phytochemicals in a member of the better leaf family, *Vernonia amydalina* and reports cover the medicinal, pharmaceutical and other applications that are associated with their phytochemicals (Lubus *et al*., 2021; Nowak *et al*., 2022; Odiongenyi *et al*., 2009; Ugbogu *et al*., 2021). To our knowledge, no such documentation is found concerning the leaf extract of *Vernonia hymenolepsis.* Therefore, in line with the need for more research on useful plants especially on their chemical constituents, we are reporting on the phytochemical constituents of the leaf extract of *Vernonia hymenolepsis* obtained from Gas

chromatography-mass spectrophotometer analysis.

2.0 Materials and Methods

2.1 Plant Material

The plant material for the present study was collected from the Otuaba Community, Ogbia Local Government Area of Bayelsa State, Nigeria. The plant material was identified by a botanist with the Department of Biology, Federal University Otuoke, Bayelsa State, Nigeria, to be *Vernonia hymenolepis*. The fresh leaves part of the plant material was dried, ground and subjected to extraction.

2.2 Extraction of Phytoconstituent Using Solvent-solvent Partitioning

A given weight of the ground-dried plant material of *Vernonia hymenolepis* was extracted using 250 ml of n-hexane for 72 hrs. The n-hexane extract was then partitioned using 150 ml of ethanol with the aid of a separating funnel. The ethanol fraction, EVH was collected, concentrated with the aid of a water bath and stored in an air tighten vial. EVH phytochemical constituents were analyzed using gas chromatography-mass spectrometry instrumentation.

2.3 Identification and Quantification of Phytoconstituent

The identification and quantification of phytoconstituents of EVH were done using the method reported by Edet and Onifade, 2020 via the gas-liquid chromatography and mass spectrometry (GC-MS), Agilent Technologies (GC system 7890A coupled with MSD 5975C) equipment with the following specifications: an injection mode 7683B series. Column details: HP5MS, 30 m length, 0.320 mm internal diameter and 0.25 μm thickness. The column temperature was programmed in the range from 37 to 320 ^{o}C at a rate of 18-25 ^{o}C/minute and held for 0.5 and 1.85 mins at 18 and 320 ^{o}C respectively. The MS ion source temperature was maintained at 280 ^{o}C with a full scan and solvent delay of 0-2.30 mins. MS scan range was m/z 35-500 in 0.10 sec. 1μL of the sample was injected per time in Helium carrier gas at split flow of 20 mL/min. Spectra data matching was done using NIST 14. Library.

3.0 Results and Discussion

The EVH fraction has a characteristic smell at room temperature. The result of the GC-MS of the EVH is reported in Table 1.

Thirty-one (31) phyto-compounds have been isolated and identified by the GC-MS instrumental method of analysis. The major constituents include: 2-Methyl-Z,Z-3,13-octadecadienol (3.16%); cis-Verbenol (4.13%); 6-Nonynoic acid (2.39%); 1H-Pyrrolo[3,4-d]pyrimidine-2,5-di one, 4,6-bis(4-hydroxyphenyl)-1-methyl-3,4,6,7-tetrahydro- (4.91%); myo-Inositol, 4-C-methyl- (9.84%); Ethanol, 2-phenoxy-, acetate (3.83%); cyclododecane (2.61%); 2-Piperidinone, N-[4-bromo-n-butyl]- (3.52%); tetradecanoic acid (2.05%); hexadecanoic acid methyl ester (5.97%); n-hexadecanoic acid (9.85%); methyl stearate (2.83%); 1-nonadecene (4.97%); octadecanal (2.06%); tetracosane(3.81%); eicosane (2.85%); 2H-indol-2-one, 1,3-dihydro-5-hydroxy- (4.99%) and 1-octen-3-yne (2.07%).

A total of twenty compounds were reported to have been obtained from the essential oil phyto-content of the leaves extract of *Vernonia* amygdaline (Sonibare *et al*., 2008). The major compounds compared to those of *Vernonia hymenolepsis* are reported in Table 2.

The presence of phytochemicals such as n-hexadecanoic acid and hexadecanoic acid methyl ester are signatures of the bioactivity potentials of *Vernonia hymenolepis*. These phytochemicals are known sources of agents of antioxidant, anticancer, antiandrogenic flavour, alpha-reductase inhibitor, hepatoprotective, antieczemic, hypocholesterolemic, anti-coronary agent and could serve as pesticides (Okagu *et al*., 2018).

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Table 1. The phytoconstituents of the ethanol fraction of *Vernonia hymenolepis*

S/N.	Retention Time (min.)	Concentration (%)	Identified Compounds	Molecular Formula	Molecular Weight
1.	9.064	1.76	1-Octen-3-yne	C_8H_{12}	108.18
2.	9.215	1.57	5-Vinyl-pyrazole	$C_5H_6N_2$	94.12
3.	9.308	3.16	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5
4.	9.417	4.13	cis-Verbenol	$C_{10}H_{16}O$	152.24
5.	11.077	2.39	6-Nonynoic acid	$C_9H_{14}O_2$	154.21
6.	11.908	1.87	2-methyl-1-Hexen-3-yne,	C_7H_{10}	94.15
7.	12.042	4.91	1H-Pyrrolo[3,4-d]pyrimidine-2,5-di one, 4,6-bis(4-hydroxyphenyl)-1-methyl-3,4,6,7-tetrahydro-	$C_{19}H_{17}N_3O_4$	351.36
8.	12.157	9.84	4-C-methyl-myo-inositol	$C_7H_{14}O_6$	194.18
9.	12.193	3.83	Ethanol, 2-phenoxy-, acetate	$C_{10}H_{12}O_3$	180.2
10.	12.338	2.61	Cyclododecane	$C_{12}H_{24}$	168.319
11.	12.572	1.67	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.3975
12.	12.950	2.05	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3709
13.	13.485	1.22	Acetic acid, phenyl ester	$C_8H_8O_2$	136.1
14.	13.149	1.18	1-Phenoxy-2-chloropropane	$C_9\ H_{11}ClO$	170.64
15.	14.652	5.97	hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270
16.	15.000	9.85	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256
17.	15.332	1.63	1-Chlorohexadecane	$C_{16}H_{33}Cl$	260.886
18.	15.555	1.92	9-Octadecene, (E)-	$C_{18}H_{36}$	252.4784
19.	15.934	1.22	2-Norbornaneacetic acid	$C_9H_{14}O_2$	154.206
20.	16.141	1.57	Carbonic acid, isobutyl pentadecyl ester	$C_{19}H_{34}O_2$	294
21.	16.282	1.43	Heneicosane	$C_{21}H_{44}$	296.57
22.	16.551	2.83	Methyl stearate	$C_{19}\ H_{38}O_2$	298.5
23.	16.608	1.79	9,12-Octadecadienoic acid (Z,Z)-	$C_{19}H_{34}O$	294.4721
24.	16.655	4.97	1-Nonadecene	$C_{19}H_{38}$	266.8
25.	16.857	2.06	Octadecanal	$C_{18}H_{36}O$	268.4778
26.	16.930	1.43	(E)-5-Octadecene	$C_{18}H_{36}$	252.4784
27.	17.501	1.31	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O$	282.4614
28.	18.040	3.52	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	234.13344
29.	18.974	3.81	Tetracosane	$C_{24}H_{50}$	338.6538
30.	20.131	2.85	Eicosane	$C_{20}H_{42}$	282.5475
31.	20.863	4.99	2H-Indol-2-one, 1,3-dihydro-5-hydroxy-	$C_8H_7NO_2$	149.147

Myo-Inositol, 4-C-methyl- has been implicated in the reduction of hepatic triglyceride (McCrea and Camilli, 2009), anticancer agent (Nishino *et al*., 1999), antidiabetic substance

(Dona *et al*., 2012), treatment of a bipolar disorder, psoriasis and mood stabilization (Kontoangelos *et al*., 2010).
2-Piperidinone, N-[4-bromo-n-butyl]- (3.52%) is known for its antimicrobial activity (Al-Bahadily *et al*., 2019). Tetracosane has cytotoxic activity against AGS, MDA-MB-231, HT-29 and NIH 3T3 cells (Uddin *et al*., 2012). Eicosane in synergistic constitution with other phytochemicals present in the hexane extract showed antimicrobial and antioxidant activities (Rheto *et al*., 2020). 2H-Indol-2-one, 1,3-dihydro-5-hydroxy- is a derivative of the indole heterocyclic known for its diverse therapeutic applications ranging antimicrobial, antioxidant, antimalarial, antidiabetic, antitubercular, anticholinesterase, antiinflammation to anticancer amongst other activities (Kumar and Ritika, 2020).

Table 2. The major phytoconstitutions of both the leaves of *Vernonia hymenolepsis* and *Vernonia* amygdaline

Vernonia hymenolepsis		***Vernonia* amygdaline (Sonibare *et al*., 2008)**	
Phytoconstitution	**Concentration (%)**	**Phytoconstitution**	**Concentration (%)**
n-hexadecanoic acid	9.85	Thymol	27.0
myo-Inositol, 4-C-methyl-	9.84	(E)-Phytol	15.7
hexadecanoic acid methyl ester	5.97	Ocymene	12.7
1-nonadecene	4.97	β-selinene	8.1
4,6-bis(4-hydroxyphenyl)-1-methyl-3,4,6,7-tetrahydro-	4.91	γ-terpinene	4.4
Verbenol	4.13	β-caryophyllene	3.9
N-[4-bromo-n-butyl]-	3.52	Apiole	3.8

4.0 Conclusions

The present study has identified thirty-one (31) phytoconstitutions in the volatile extract of *Vernonia hymenolepis* using gas chromatography-mass spectrometry (GC-MS). The major constituents include: 2-Methyl-Z,Z-3,13-octadecadienol (3.16%); cis-Verbenol (4.13%); 6-Nonynoic acid (2.39%); 1H-Pyrrolo[3,4-d]pyrimidine-2,5-di one, 4,6-bis(4-hydroxyphenyl)-1-methyl-3,4,6,7-tetrahydro- (4.91%); myo-Inositol, 4-C-methyl- (9.84%); ethanol, 2-phenoxy-, acetate (3.83%); cyclododecane (2.61%); 2-Piperidinone, N-[4-bromo-n-butyl]- (3.52%); tetradecanoic acid (2.05%); hexadecanoic acid methyl ester (5.97%); n-hexadecanoic acid (9.85%); methyl stearate (2.83%); 1-nonadecene (4.97%); octadecanal (2.06%); tetracosane(3.81%); eicosane (2.85%); 2H-indol-2-one, 1,3-dihydro-5-hydroxy- (4.99%) and 1-octen-3-yne (2.07%). The plant has numerous bioactive compounds that have established pharmacological activities such as: antioxidant, anticancer, antimicrobial, pesticide, antiandrogenic flavor, alpha-reductase inhibitor, hepatoprotective, antieczemic, hypocholesterolemic, anticoronary agent, antimalaria, antidiabetic,

antitubercular, anticholinesterase, antiinflammation and anticancer.

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