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Identification of n-Hexane Fraction Constituents of *Archidium ohioense (Schimp. ex Mull)* Extract Using GC-MS Technique

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BAA and OOO designed the study, wrote the protocol and author BAA wrote the first draft of the manuscript. Author AG managed the literature searches, analyses of the study and performed the spectroscopy analysis. Authors OOO, BAA and MAA managed the experimental process and author AMM identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To screen for the presence of bioactive phytoconstituents in hexane fraction of *Archidium ohioense* using the GC-MS technique.
Study Design: Phytochemical screening via GC-MS technique.
Place and Duration of Study: Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, between September 2013 and June 2014.
Methodology: The whole plant material was collected, sundried, pulverized and extracted with 80% (v/v) methanol for 48 hours using cold extraction method. The filtrate was concentrated *in vacuo* on rotary evaporator to yield the crude extract. The crude extract was partitioned with

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saturated n-hexane to afford the hexane fraction. The hexane fraction was analyzed via GC-MS technique. Identification of the constituents was done by comparing the mass spectrum fragmentation pattern of each of the constituents (head to tail) with those stored in the database of National Institute Standard and Technology 11 (NIST11.L) library.

Results: The GC-MS analysis of *A. ohioense* revealed the presence of thirty eight compounds (phytochemical constituents). Three components appeared to be most prominent which constitute 60.03% of the total hexane fraction. The three major compounds are: pentadecanoic acid, 14-methyl-, methyl ester (19.03%), 9, 12- octadecadienoic acid, methyl ester (21.66%), and 9, 12, 15-octadecatrienoic acid, methyl ester, (Z, Z, Z) (28.39%).

Conclusion: GC-MS analysis of the hexane fraction of *A. ohioense* afforded three major constituents identified as: pentadecanoic acid, 14-methyl-, methyl ester, 9, 12- octadecadienoic acid, methyl ester, and 9, 12, 15-octadecatrienoic acid, methyl ester, (Z, Z, Z).These compounds have been reported to exhibit various biological activities, hence hexane fraction of *A. ohioense* could serve as a source for these bioactive compounds.

Keywords: Archidium ohioense; bryophytes, mosses; methanol extract; hexane fraction; GC-MS; biological activities.

1. INTRODUCTION

The use of botanicals as medicine and their methods of application vary from locality to locality [1]. A large number of rural dwellers rely exclusively on the use of indigenous or local medicinal remedies as means of treating various diseases including fever, asthma, constipation, esophageal cancer and hypertension [2]. Although, greater per cent of medicinal plant users come from rural settlements [1,2] today, about 3.4 billion people (representing 88 percent) of the world population rely on herbal medicines to treat ailments. Thus, herbal medicines are speedily overtaking modern medicine because many synthetic drugs are failing to control the spread of infectious diseases such as tuberculosis, typhoid fever, as well as gonorrhea. In addition, some pathogenic bacterial strains are increasingly becoming more resistant to various antibiotics thus increasing the cost of prescription drugs for the maintenance of optimal health [2,3]. Thus, there is need to search for alternative and effective medicines for the treatment of the various diseases; hence this study.

A number of mosses have been identified, classified and reported to express various biological activities such as antitumor [4], anti-fungal [5], anti-feedants, and anti-inflammatory. Some mosses have been reported to produce active inducible substances which enable the plant to resist microbial and fungal attack [58]. Furthermore, some mosses have been reported to possess strong antioxidative machinery which makes them resistant to extreme climates and diseases [9]. Antioxidants constitute an endogenous defensive mechanism against reactive oxygen species (ROS) and hence find extensive application in cosmetic and pharmaceutical industries [10]. For example, the antarctic polar mosses Sanionia uncinata was reported to exhibit antioxidant activity [11] whereas the anti-inflammatory and cytotoxic activities of Sanionia georgico-uncinata had been established [8].

The studied moss, *Archidium ohioense* belongs to the family of *Archidiaceae;* and grows largely on rocky areas. Research on this plant has been neglected probably because it has no economic value and lacks “self-image” [12]. *A. ohioense is a* perennial *plant, short,* tiny and measures about 2-20 mm high. It grows in clusters to form dense and short turfs. The colour ranges from green to yellow-green. *A. ohioense* has simple variable stem and two-leave systems i.e stem and perichaetial leaves [13]. As it grows older, it becomes prostrate, more fragile and often detach.

The aim of this study was to screen the hexane fraction of *A. ohioense* using GC-MS technique with the possibility of discovering compound(s) of therapeutic value.

2. MATERIALS AND METHODS

2.1 The Plant Material

The whole plant material used in this study was collected from Hill II at the Obafemi Awolowo University Campus Ile-Ife, main campus (07°.30`N, 04° 40`E) [14] and authenticated by Dr. Makinde of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. A

voucher specimen (*Archidium ohioense* IFE-17406) was deposited at IFE Herbarium for future reference.

2.2 Preparation of the Extract

The dried powdered plant (500 g) was soaked with 80% (v/v) methanol for 48 hours, then filtered; and the residues were further soaked in freshly prepared 80% (v/v) methanol for another 48 hours. Finally, the filtrates were pooled and concentrated *in vacuo* on the rotary evaporator at 40ºC to obtain the crude extract.

2.3 Partitioning of Crude Extract

The methanol extract was partitioned as previously described [15] based on the principle of differential solubility. The extract (2.0 g) was dissolved in warm distilled water (150 mL) and partitioned with n-hexane (200 mL x 3). The n- hexane fraction obtained was concentrated *in vacuo* on rotary evaporator (Edwards Vacuum Components, Crawley England) at 35°C to afford a viscous and oily substance with amber characteristics. The hexane fraction was used for the GC-MS analyses.

2.4 GC-MS Analysis

The GC-MS analysis was carried out on a Hewlett Packard Agilent Chromatograph GC (Model 7890B series) fitted with flame ionization detector and Hewlett Packard MS 5975 series injector, MS transfer line temperature of 250°C. The GC was fitted with a varian column- Agilent J&W HP-5MS (30 m x 0.320 mm, film thickness 0.25 µm). Sample was dissolved in acetone and 1 µl injected automatically into the column with the injector temperature set at 250°C. GC oven temperature started at 50°C and holding for 5min and it was raised to 280°C at the rate of 5°C/min for 9 min. The injector and detector temperatures were set at 250°C and 280°C respectively. The mass spectrum of compounds in sample was obtained by electron ionization at 70 eV; and its detector was operated in scan mode from 45-450amu (atomic mass units). A scan interval of 0.5 seconds and fragments from 45 to 450 Da was maintained. The total running time was 60 min.

2.5 Identification of the Components

Interpretation of each of the mass spectra from GC-MS analysis was conducted using the database of National Institute Standard and Technology 11 (NIST11.L) library; an online library having more than 62000 patterns. Identification of compounds was done by comparing the mass spectrum fragmentation pattern (head to tail) of each of the constituents of the hexane fraction with those stored in the NIST11.L library.

3. RESULTS AND DISCUSSION

Gas chromatography coupled with mass spectrometry (GC-MS) is an established technique for reliable identification of bioactive compounds existing in medicinal plants including volatile matter, long chain and branched chain hydrocarbons, alcohols, acids, esters [16-19]. For quantitative determination, gas-chromatography with flame ionization detector (GC-FID) and GC-MS are preferred [20,21].

The chromatograms of the GC-MS analysis of the hexane fraction indicating total ion concentration are shown in (Figs. 1.0-1.3) and the compounds identified from each of the mass spectra fragmentation patterns are listed in Table 1. The plant fraction essentially contains hydrocarbons or fatty acid molecules of various carbon lengths. No single aromatic compound was found to be present probably giving the plant its odorless characteristics.

A total of 38 compounds were identified consisting of three prominent compounds (Table 2) and 35 minor constituents. The three prominent compounds constitute 60.03% of the plant hexane fraction. The three major compounds and their percentage abundance are pentadecanoic acid, 14-methyl-, methyl ester (19.03%), 9, 12- octadecadienoic acid, methyl ester (21.66%), and 9, 12, 15-octadecatrienoic acid, methyl ester, (Z,Z,Z) (28.39%). These prominent compounds were represented by peaks 12, 17 and 18 with retention times of 35.031, 38.384 and 38.509 respectively. The test was run in triplicate. Furthermore, the mass spectra of the prominent compounds were analyzed (head to tail) with those stored in the NIST11 library and the results were shown in schemes 1-3.

Table 1. Compounds identified in the hexane fraction of *A. ohioense*

PK no	t_R (min.)	Peak area (%)	Name of compound	NIST matching (%)
1	15.622	3.63	2-Undecenal	62
2	16.880	0.51	3-Chloropropionamide	53
3	17.343	0.74	2, 4-Decadienal, (E, E)-	60
4	17.379	1.00	2, 4-Decadienal, (E, E)-	90
5	27.546	1.82	Cyclohexadecane	96
6	27.730	0.80	Hexadecane	98
7	32.182	2.79	1-Nonadecene	94
8	32.324	1.21	Hexacosane	87
9	33.695	0.67	Dodecylcyclohexane	94
10	33.933	0.65	9-Heptadecanone	99
11	34.781	0.58	9-Hexadecenoic acid, methyl ester, (Z)-	95
12	35.031	19.03	Pentadecanoic acid, 14-methyl-, methyl ester	99
13	35.796	1.65	Dibutyl phthalate	46
14	36.378	3.59	5-Eicosene, (E) -	99
15	36.697	2.60	Eicosane	96
16	37.915	0.69	n-Heptadecylcyclohexane	81
17	38.384	21.66	9, 12-Octadecadienoic acid, methyl ester	99
18	38.509	28.39	9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	99
19	38.598	1.50	9-Octadecenoic acid (Z) -, methyl ester	99
20	38.716	0.76	Bicyclo [3.1.1] heptane, 2, 6, 6,-trimethyl-, (1.alpha., 2. beta., 5. alpha.)	55
21	38.978	1.22	Heptadecanoic acid, 16-methyl-, methyl ester	98
22	39.168	0.89	9, 12-Octadecadienoic acid (Z, Z) -	96
23	39.263	0.99	12-Methyl-E, E-2, 13-octadecadien-1-ol	83
24	40.206	3.59	5-Eicosene, (E) -	96
25	40.307	0.84	Docosane	95
26	41.411	0.68	cis,cis, cis-7, 10, 13-Hexadecatriena	64
27	41.755	0.77	Cyclohexane, decyl-	81
28	42.076	2.24	Cis-11, 14-Eicosadienoic acid, methyl ester	99
29	42.189	0.80	9, 12, 15-Octatrien-1-ol, (Z, Z, Z)-	93
30	43.726	2.06	Cyclotetracosane	99
31	43.815	0.58	Hexadecane	93
32	45.269	0.56	Cyclohexane, nonadecyl-	74
33	46.296	0.49	Bis (2-ethylhexyl) phthalate	64
34	46.978	1.01	1-Heneicosanol	90
35	48.456	0.57	Octadecanal	42
36	48.522	0.68	Cyclohexane, 1, 1'-(1, 3,-propanediyl) bis-	58
37	50.005	0.57	Cyclohexane, 1, 1'- (2-ethyl-1, 3-propanediyl) bis-	38
38	58.807	0.71	Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15, 15-hexadecamethyl-	87

Reported biological activities of some of the phytoconstitutents, especially the prominent compounds, were explored. Interestingly, two of the prominent compounds [9,12-octadecadienoic acid, methyl ester and 9, 12, 15-octadecatrienoic acid, methyl ester, (z, z, z)-] are essential polyunsaturated fatty acid molecules belonging to linoleic (omega-6 fatty acid) and linolenic (omega-3 fatty acid) acid families. Human body cannot make these two important fatty acid

molecules, making it very necessary to obtain them from diets. Thus, the studied plant (*A. ohioense*) could be a rich source of omega-6 fatty acid and omega-3 fatty acid. The 9, 12-octadecadienoic acid, methyl ester (a linoleic acid), had been reported to possess antiinflammatory, nematicide, insectifuge, antiacne, hypocholesterolemic, anticancer, hepatoprotective, antihistaminic, antiarthritic and antieczemic activities [22]. The anticancer, antibacterial, antioxidant, antipyretic, cardioprotective, neural function, antiandrogenic (5-alpha reductase inhibitor) and antiarthritic properties of 9, 12, 15 - octadecatrienoic acid, methyl ester, (z, z, z)- (a linolenic acid) were also documented [23,10]. The pentadecanoic acid, 14- methyl-, methyl ester was reported to possess antifungal and antimicrobial activities [24]. Table 3 showed some of the identified phytoconstituents in n-hexane fraction of *A. ohioense* and their reported biological activities.

Each value represented the mean of three readings. PK = peak number; t_R = retention time; those highlighted in blue colour represent the prominent compounds.

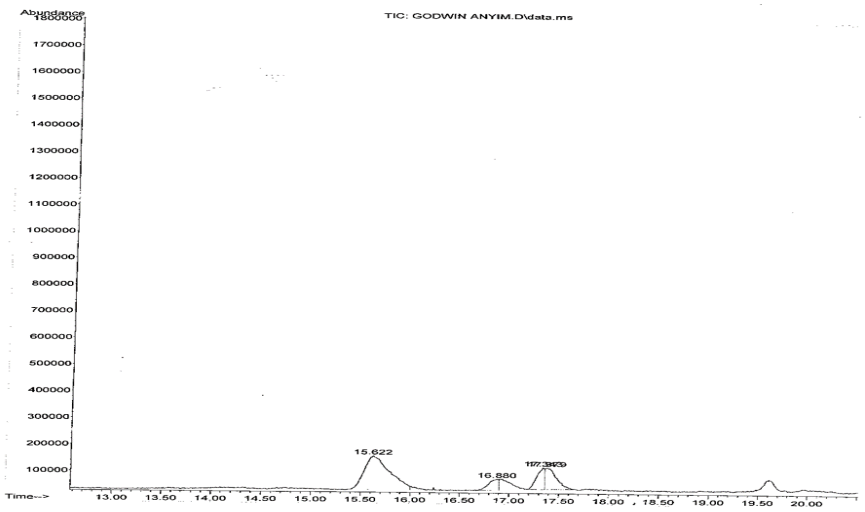


Fig. 1.0. GCMS chromatogram showing t_R between 13.0 and 20.5 min
TIC = total ion concentration

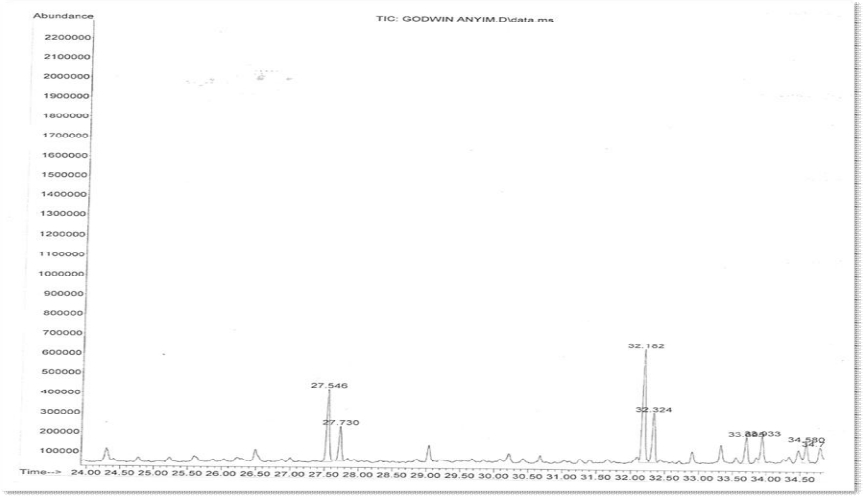


Fig. 1.1. GCMS chromatogram showing t_R between 24.00 and 34.50 min

The mass spectrum fragmentation pattern of each of the constituents of the hexane fraction with those stored in the NIST11.L library was compared (head to tail). In each scheme, the top spectrum represents the spectrum of compound from the hexane fraction; the bottom spectrum containing the molecular structure represents that of the standards from the NIST11 library; and the middle spectrum is the head to tail comparison made.

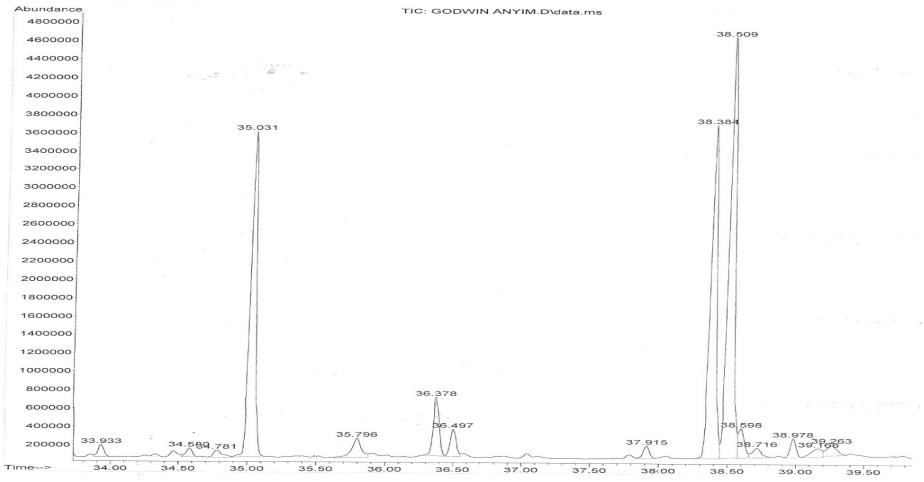


Fig. 1.2. GCMS chromatogram showing t_R between 33.90 and 39.50 min

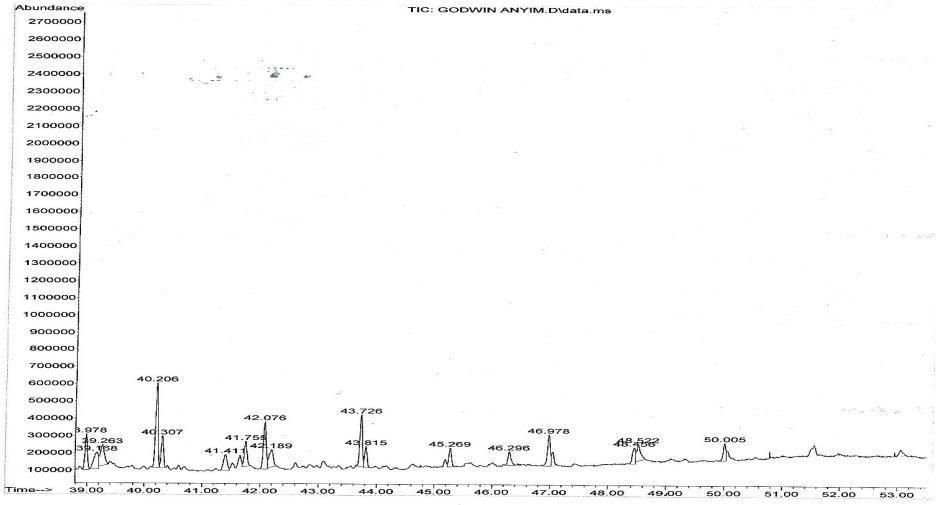


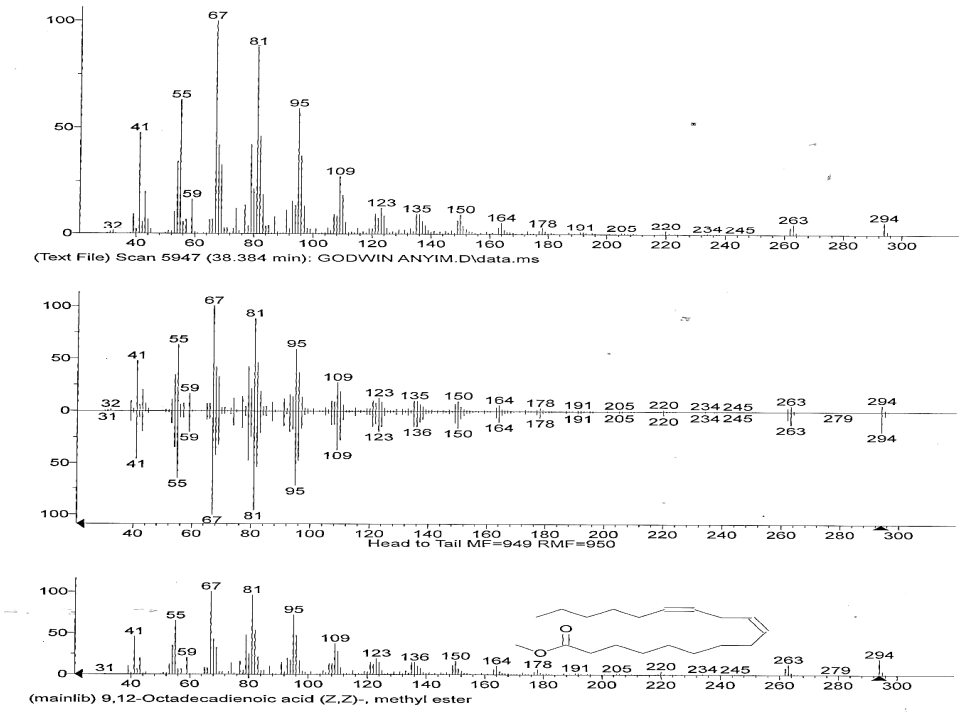
Fig. 1.3. GCMS chromatogram showing t_R between 39.00 and 53.00 min

Table 2. The three (3) prominent compounds and their chemical nature

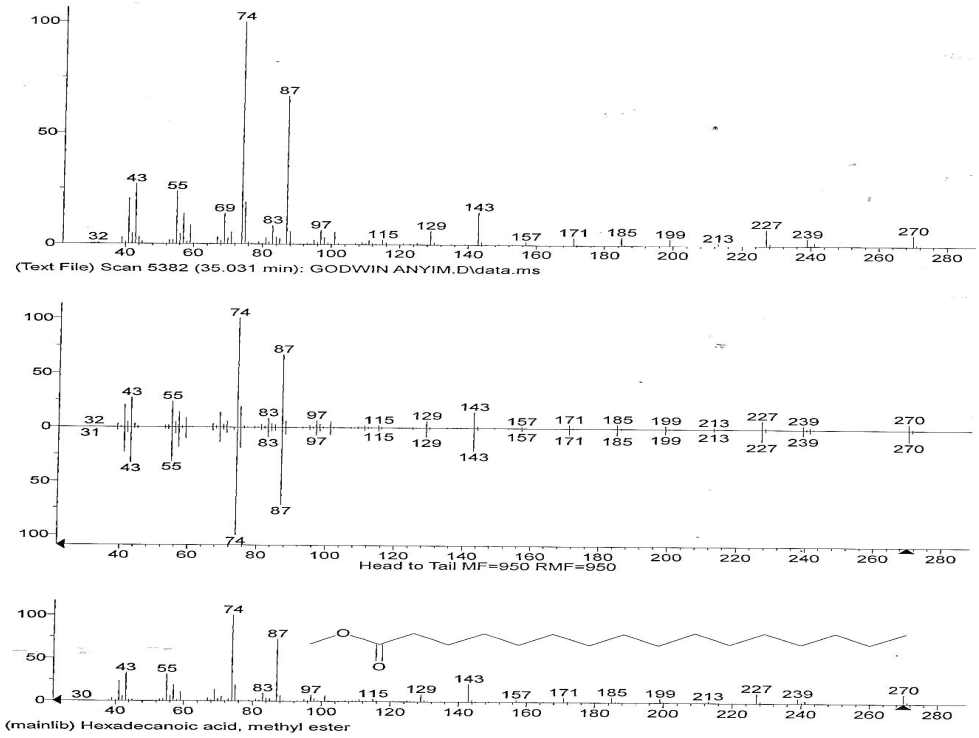
Pk no.	t_R	Compound name	Compound nature
12	35.031	Pentadecanoic acid, 14-methyl-, methyl ester	Pamitic acid
17	38.384	9, 12-Octadecadienoic acid, methyl ester	Linoleic acid (omega-6 fatty acid) (PUFAs)
18	38.509	9, 12, 15-Octadecatrienoic acid, methyl ester, (z, z, z)-	Linolenic acid (omega-3 fatty acid)(PUFAs)

Table 3. Some phytoconstituents identified and their reported biological activities

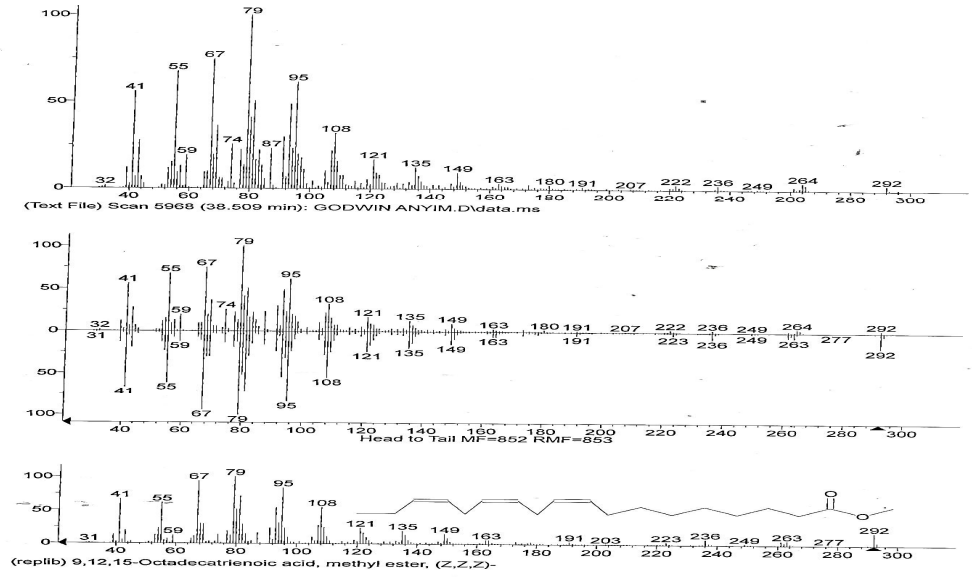
PK no	Compound	Reported activity	References
12	Pentadecanoic acid, 14-methyl-, methyl ester;	Antifungal, Antimicrobial	[24]
17	9, 12-Octadecadienoic acid, methyl ester	Antiinflammatory, Nematicide, Insectifuge, Antiacne, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiarthritic, Antieczemic,	[25,16]
18	9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-.	Anticancer, Antibacterial, Antioxidant, Antipyretic, Cardioprotective, neural function, Antiandrogenic (5-alpha reductase inhibitor), and Antiarthritic properties.	[23,26]
1	2-Undecenal	Anitmicrobial	[27]
2	2, 4-Decadienal, (E, E)-		
13	Dibutyl phthalate	Antifungal, Antibacterial, Antiviral and Antioxidant activities	[23]
15	Eicosane	Antifungal, Antibacterial, Antitumor and Cytotoxic effects	[23]
19	9-Octadecenoic acid (Z)-methyl ester	Antioxidant activity, Anticarcinogenic; Exists in human blood and urine where it serves as endogenous peroxisome proliferator-activated receptor ligand; dermatitigenic flavour	[29,28]
25	Docosane	Antibacterial activity	[23]
31	Hexadecane	Antifungal, Antibacterial and antioxidant activities	[23]



Scheme 1. Comparison of spectra through mass finder



Scheme 2. Comparison of spectra through mass finder



Scheme 3. Comparison of spectra through mass finder

4. CONCLUSION

GC-MS analysis of the hexane fraction of *A. ohioense* afforded three major constituents identified as: pentadecanoic acid, 14-methyl-, methyl ester, 9, 12- octadecadienoic acid, methyl ester, and 9, 12, 15-octadecatrienoic acid, methyl ester, (Z, Z, Z). These compounds have been reported to exhibit various biological activities, hence hexane fraction of *A. ohioense* could

serve as a source for these bioactive compounds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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