Gas chromatography-mass spectrometry analysis of ethanol and ethyl acetate fractions of *Annona muricata* (L) seed oil

Alamu, O.[1*], Ofuya, T.I.,[1] Oni, M.O.[1] and Idoko, J.E.[1]

[1]Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria.
[2]Department of Extension and Linkages, National Centre for Genetic Resources and Biotechnology (NACGRAB) PMB 5382 Moor Plantation, Ibadan, Nigeria.
*Corresponding author: *bisialamu@gmail.com*

Abstract

This present study characterized ethanol and ethyl acetate fractions of oil extract obtained from *Annona muricata* seed of which 18 and 22 compounds were separated and identified respectively using Gas-chromatography-mass spectrometry(GC-MS).The predominant compounds were 9, 12-Octadecadienoic acid (Z,Z)- (66.52%,), Hexadecanoic acid ethyl ester (13.98%),9-Octadecenoic acid (Z)- 2-hydroxy-1-(hydroxymethyl)ethyl ester (8.87%),n-Hexadecanoic acid (7.07%) and Squalene (1.66%) ; 2-Methyl-Z,Z-3,13-octadecadienol (21.70%) ,Tetradecanoic acid ethyl ester(12.77%), n-Hexadecanoic acid (9.85%) , 1,8,11-Heptadecatriene, (Z,Z)-(9.59%) and Methyl p-coumarate, TMS derivative (1.08%). Some of these compounds can be exploited in future as insecticide or pesticide in the management of pest of agricultural importance while conscious efforts should be made for ex-situ conservation of the plant.

Keywords: *Annona muricata*, GC-MS, medicinal plants, plant oil, insecticide

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Introduction

Plants are pillars of life on earth and essential sources of food, medicine, shelter, fuel, and forage, and provides wide range of additional ecosystem and cultural services to humanity (Alamu *et al*., 2020). Higher plants are rich sources of novel natural substances that can be used to develop environmental safe methods for insect control and other purposes (Jbilou *et al*., 2006). There are several medicinal plants around the world of which about fifteen percent (15%) have been screened for their therapeutic values (Hatta and Shafei, (2013).

The use of traditional medicine and medicinal plants in some developing countries, as a basis for the maintenance of good health, has been observed by UNESCO (1996). Nigeria, a country in West Africa has a rich diversity of plants some of which are used medicinally and needs to be further explored (NACGRAB, 2008). *Annona muricata* (L.), commonly known as soursop or graviola belongs to the Annonacea family which consists of 135 genera and approximately 2500 species (Chatrou *et al*., 2004). It's closely related relatives are *Annona squamosa* and *Annona reticulata*. The active ingredients are in the unripe fruit, the seeds, leaves and in the roots. There has been unconfirmed report of soursop in alternative cancer treatment **(**Mishra *et al*., 2013).

One of the reliable techniques of compounds separation and characterization is Gas chromatography- mass spectrometry (GC-MS) which has been acknowledged for its reliability, sensitivity and accuracy in time and space (Jie, *et al.,* 1988). However, for the rational development and utilisation of botanicals that originated from plants in the management of pest of agricultural importance, there is the need to evaluate the intrinsic chemical compounds and validate them accordingly. On this backdrop, this present study thereby seeks to investigate the differential in ethyl acetate and ethanol fractions of oil obtained from *Annona muricata* seed using GC-MS technique towards efficiency in product development.

Materials and Methods

Collection of plant materials

Mature fruits of sousop, *Annona muricata* were collected from the Field Genebank of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan (07º 23 11 3"N) latitude and (003º 50 25 0"E) longitude, Nigeria. The mature fruits were cut longitudinally and the black seed removed with the aid of a sharp knife. The mucilage covering of the seeds were removed manually and air dried under a shade for 4-5 days before pulverized into a fine power. The powder obtained was stored in air tight containers and used for further studies.

Extraction of plant oil

Sample weighing 500g each of the powdered material was macerated three times at room temperature (27±2ºC) and suspended in 500ml of absolute ethanol (99.9 %, ANALAR ®grade) and ethyl acetate separately for 48hrs with occasional stirring using a magnetic stirrer and then filtered with Whatman filter paper (9mm size). The slurry was placed in a thimble of glass (Soxlet apparatus) in order to extract the active ingredients. The filtrate was later concentrated using a rotary evaporator (Resona Technics ®) at 78º C to separate the organic solvent and concentrate the oil. The oil extract was exposed to air so that traces of the volatile solvent evaporate, kept in reagent bottles and stored in the freezer at temperature of 4ºC for further studies.

GC-MS procedures

Gas chromatography-mass spectrometry (GC-MS) analysis of the samples was performed using Hewlett Packard 7820A gas chromatograph coupled to a 5975C inert mass spectrometer (triple axis detector) with electron-impact source (Agilent, Pablo Alto, CA, USA) as described by Adams (1995).The stationary phase of the separated compounds in HP-5 capillary column was

coated with 5% Phenyl Methyl Siloxane (30m length x 0.32mm diameter x 0.25µm film thickness (Agilent Technologies). Helium was used as the carrier gas at constant flow of 1.49 ml/min at an initial nominal pressure of 1.49 psi and average velocity of 44.22 cm/sec. 1µl of the aliquot samples were injected in splitless mode at an injection temperature of 300 °C. Purge flow was 15 mL/min at 0.75 min with a total flow of 16.67 mL/min; gas saver mode was switched on. Oven was initially programmed at 40 °C (1 min) then ramped at 12 °C/min to 300 °C (10 min). Run time was 32.67 min with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization (EI) mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150°C and transfer line temperature of 300°C. Scanning of possible phytochemical compounds was from 45 to 550 m/z at 2.00s/scan scan rate and identified by the corresponding peaks, retention time and comparing measured mass spectral data with those in National Institute for Standards and Technology Mass Spectral Library and literatures (NIST, 2005).

Result and Discussion

GC-MS analysis of the aliquot of the *A.muricata* seed oil yielded several chemical compounds of economical and industrial importance. These compounds were identified based on their respective peak area, retention time, molecular formula, molecular mass, and calculated fragments. The total ion chromatogram of the ethanolic and ethyl acetate fractions of *A. muricata* showing 18 and 22 separated compounds with their peak areas and retention times are shown in Figure 1 and 2 respectively. The first five compounds in ethanol fractions with highest percentage were 9,12-Octadecadienoic acid (Z,Z)-, Hexadecanoic acid ethyl ester, 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, n-Hexadecanoic acid and

Squalene in proportion of 66.52%, 13.98%, 8.87%, 7.07% and 1.66% respectively (Table 1). Whereas, in the ethyl acetate fractions the first five compounds with highest percentage were 2-Methyl-Z, Z-3,13-octadecadienol,Tetradecanoic acid ethyl ester, n-Hexadecanoic acid, 1,8,11-Heptadecatriene, (Z,Z)- and Methyl p-coumarate, TMS derivative in proportion of 21.70%, 12.77%, 9.85%, 9.59% and 1.08% (Table 2). The molecular structures of these compounds were presented in figures 3-7 and 8- 11 respectively. Slightly more compounds were elucidated in the ethyl acetate (non- polar) than ethanol (polar) fractions of the oil extracts, which was in contrast to the observation of Bobadilla *et al.*, (2005) concerning the rational management of natural products.

Table 1: List of five most abundant compounds in ethanol fraction of *A.muricata* oil

S/N	Compound Name/Hit Name	Percentage Total of all compound (% Total)	Chemical formula	Molecular weight
1.	9,12-Octadecadienoic acid (Z,Z)-	66.524	$C_{19}H_{34}O$	294.4721
2.	Hexadecanoic acid, ethyl ester	13.976	$C_{18}H_{36}O_2$	284.4772
3.	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	8.872	$C_{21}H_{38}O_4$	354.5240
4.	n-Hexadecanoic acid	7.077	$C_{16}H_{32}O_2$	256.4241
5.	Squalene	1.663	$C_{30}H_{15}$	410.7180

Table 2: List of five most abundant compounds in ethyl acetate fraction of *A.muricata* oil

S/N	Compound Name/Hit Name	Percentage Total of all compound (% Total)	Chemical formula	Molecular weight
1.	2-Methyl-Z,Z-3,13-octadecadienol	21.698	$C_{19}H_{36}O$	280.5
2.	Tetradecanoic acid, ethyl ester	12.770	$C_{16}H_{32}O_2$	256.42
3.	n-Hexadecanoic acid	9.845	$C_{16}H_{32}O_2$	256.4241
4.	1,8,11-Heptadecatriene, (Z,Z)-	9.585	$C_{17}H_{30}$	234.4201
5.	Methyl p-coumarate, TMS derivative	1.081	$C_{10}H_{10}O_3$	178.1846

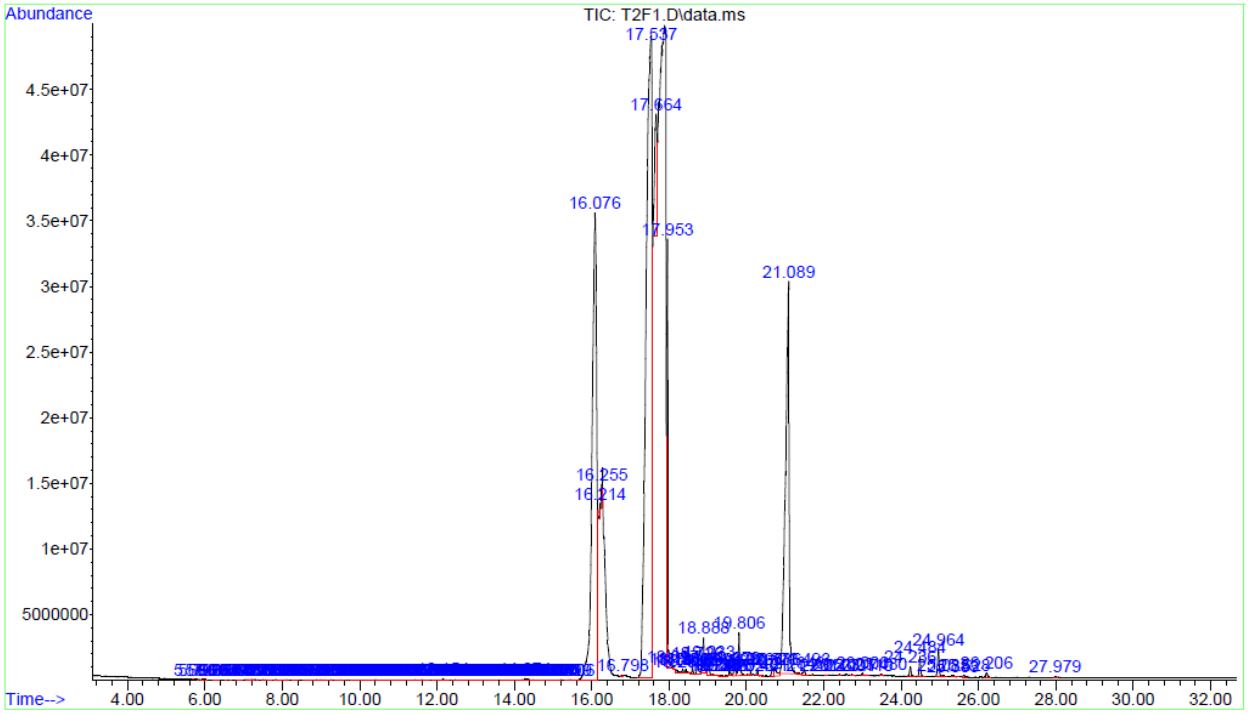

Fig 1: Total ion chromatogram of the of ethanol active fractions of *A. muricate.*

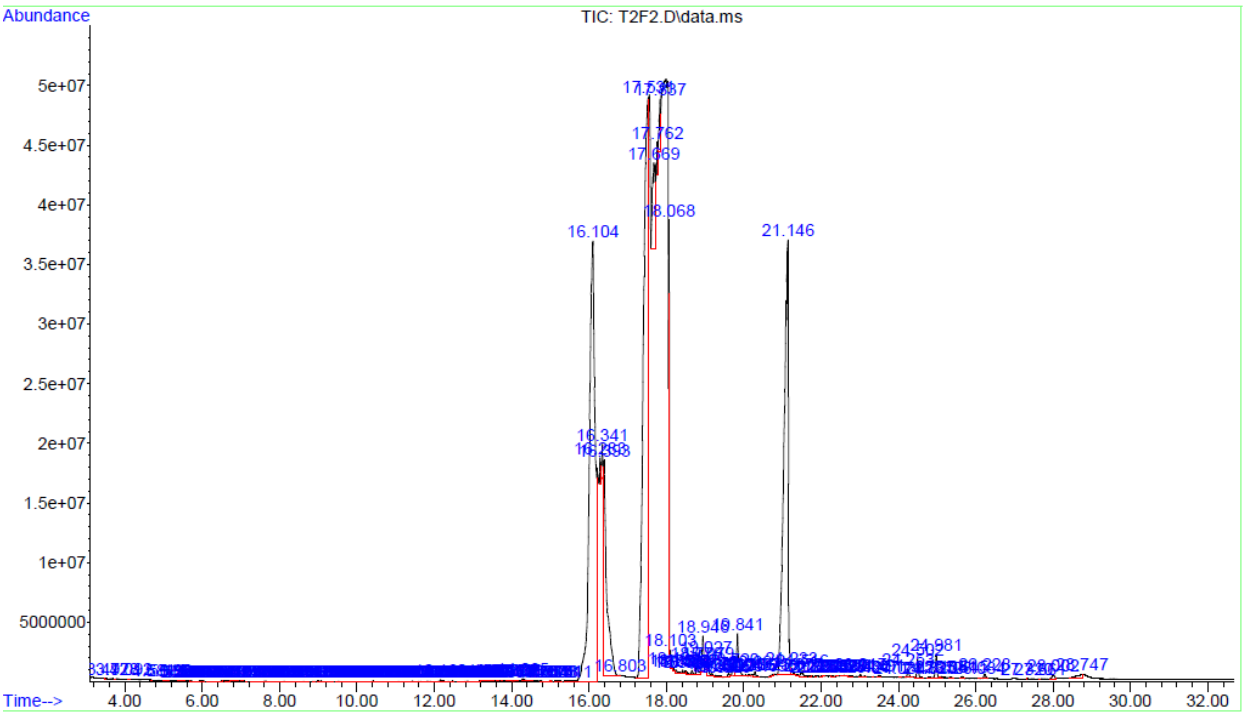

Fig.2: Total ion chromatogram of the of ethyl acetate active fractions of *A. muricate.*

Furthermore, n-Hexanoic acid was yielded by the two fractions in varying concentrations (7.077% and 9.585%) respectively. These compounds present different forms of bioactive activities against living organisms and major constituents of other plants. For instance, N-hexadecanoic acid was reported as one of the main constituents in essential oil of *Acalypha hispida* with toxicity (LC_{50}) values of 122.28µg/ml against brine shrimps larvae, *Artemia salina* (Aboaba and Omotoso (2012). Methanolic leaf extract of *Blighia sapida* was reported by Edewor and Kazeem (2018) to yield n- hexadecanoic acid (12.214%). Squalane, a derivative of squalene, obtained from ethanol fractions of *A.muricata* was noted to have huge potentials in nutraceutical and pharmaceutical industries, and reported to have anticancer, antioxidant and skin hydrating properties (Kim and Karadeniz , 2012; Salini and Shankar, 2014). Likewise, Oni *et al.,* (2019) reported squalene as a constituents of n-hexane leaf extract of *Acalypha godseffiana* which offered some form of protection to wheat grain against *Rhizoperca dominica*. Okonkwo and Onyeji, (2018) reflected the insecticidal activities of essential oils obtained from the leaves of *Phyllanthus amarus* and *Stachytarpheta cayennensis* against American cockroach, *Periplaneta americana* , American grasshopper *Schistocerca americana* and African malaria mosquito *Anopheles gambiae* and observed Hexadecanoic acid ethyl ester as one of the major constituents of these plant species. In addition, coumarin, a derivatives of Methyl- p coumarate have been shown to exhibit mortality effect of 30-80% with doses between 10 and 100 µg/ml against common fruit fly, *Drosophila melanogaster* larvae (Vargas –Soto *et al*., 2017). Other notable compounds of interest elucidated from the assay were 9,12-Octadecadienoic acid (Z,Z)-, 1,8,11-Heptadecatriene, (Z,Z)- and 2-Methyl-Z,Z-3,13-octadecadienol.

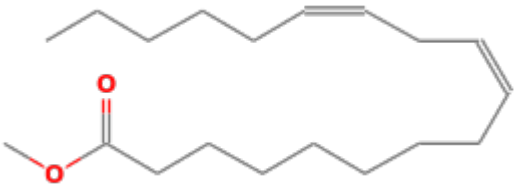

Fig. 3: 9, 12-Octadecadienoic acid (Z, Z) -.

Fig. 4: Hexadecanoic acid, ethyl ester.

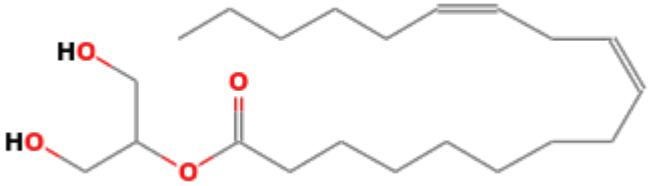

Fig. 5: 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester.

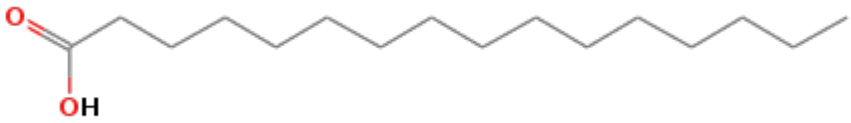

Fig. 6: N-Hexadecanoic acid.

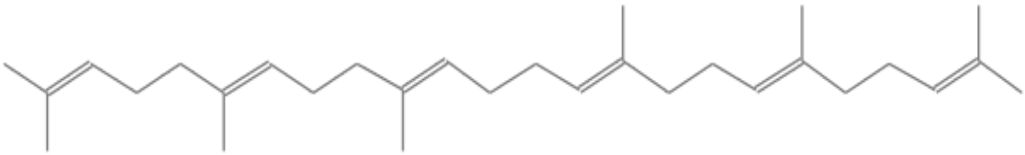

Fig. 7: Squalene.

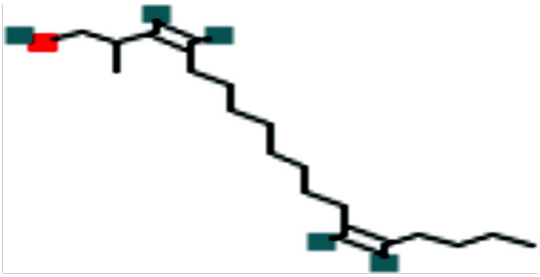

Fig. 8: 2-Methyl-Z, Z-3, 13-octadecadienol.

Fig. 9: Tetradecanoic acid, ethyl ester.

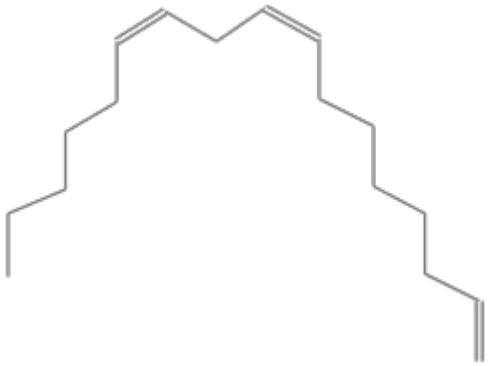

Fig. 10: 1, 8, 11-Heptadecatriene, (Z,Z)-.

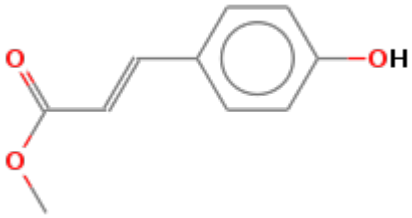

Fig. 11: Methyl p-coumarate.

Conclusion

Medicinal plants has been used virtually in all cultures of the world as a source of medicine both traditional and modern, and perform diverse ecosystem functions. Findings from this study unravel the importance of plants oil, especially from *Annona muricata* as rich source of novel phytochemicals and secondary metabolites that can be used in either chemical,

pharmaceutical and agricultural industries. Bioactive compounds are naturally occurring secondary metabolites in plants, and are potential source of new materials for commercial and biomedical purposes. In some instances, some of these compounds have been noted to have pesticidal properties. Conscious effort should be made to develop protocol for vegetative propagation and conservation of this plant to ensure availability of the plant oil all year round. Further development of identified compounds through crystallization and research on their toxicity, mode and mechanisms of action and fate in the environment should be explored**.**

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