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Gas chromatography-mass spectrometry phytochemical studies of ethanol leaf extract of *Annona muricata*

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Abstract

Folk medicine has taken an important place especially in developing countries where limited health services are available. However, the absence of scientific evaluation of medicinal plants may cause serious adverse effects. The phytochemical composition of the ethanolic extracts of leaves of *Annona muricata* was analyzed using gas chromatography -mass spectroscopy (GC-MS). The GC-MS analysis revealed thirty (30) constituents of which all were matched and identified. The major constituents were two compounds with percentage peak areas of 17.21% and 17.10%. Of the identified compounds, the outstanding in composition were 1,2-Benzisothiazol-3-amine, TBDMS Derivative, Propanamide, N-(4-methoxyphenyl)-2,2-dimethyl-1H-Indole with (peak area 17.21%), 6-Octadecenoic acid, (Z)-9-Octadecenoic acid, (E)-6-Octadecenoic acid (peak area 17.10), 9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester, Oleic Acid, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester (peak area 15.54%). The current study suggests that ethanolic extracts of leaves of *Annona muricata* are a potent therapeutic agent and paves the way for the development of several treatment regimens based on compounds from this extract.

Keywords: *Annona muricata*; Ethanolic Leaf Extracts; GC-MS Analysis; Phytochemicals

1. Introduction

Plant use in treatment of diseases is as old as civilization (1,2) and complementary medicine is still a major part of habitual treatments of different maladies (2,3). Generally, complementary medicine has a long history of serving people all over the world (4,5). In recent times and due to historical, cultural, and other reasons, folk medicine has taken an important place especially in developing countries where limited health services are available. However, the absence of scientific evaluation of medicinal plants may cause serious adverse effects (3,6,7).

Natural products are extremely an important source of medicinal agents. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer based molecular modeling design, none of them can replace the importance of natural products in drug discovery and development (8). Advancement in analytical technique has been extensively utilized for identification, structural determination and separation of bioactive compounds in a mixture by utilizing GCMS (9).

Annona genus belongs to Annonaceae family classified under flowering plant. Species is muricata, common name: Soursop.

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Many non-natural, synthetic drugs cause severe side effects that were not acceptable except as treatments of last resort for terminal diseases such as cancer and that the metabolites discovered in medicinal plants may avoid the side effect of synthetic drugs, because they must accumulate within living cells (5).

Annona muricata L commonly known as Soursop belongs to the family of Annonaceae and is the most tropical semi deciduous tree with the largest fruits of the *Annona* genus. It is a typical tropical tree with heart shaped edible fruits and widely distributed and native to Sub- Saharan Africa countries that lie within the tropics including Nigeria (7). The leaves are lanceolate with glossy and dark green in color and had been traditionally used to treat headaches, hypertension, cough, asthma and used as antispasmodic, sedative and nervine for heart condition, (10,11) as well as cancer. It is widely used for complementary treatment in many countries such as Amazonia, Barbados, Borneo, Brazil, Cook Islands, Curacao, Dominica, Guatemala, Guam, Guyana, Haiti, Jamaica, Madagascar, Malaysia, Peru, Suriname, Togo and West Indies, (12,13,14) as well as in Nigeria (7). It is hoped that traditional medicine will in future provide the cure to many tropical diseases that have defied orthodox prescriptions.

Annona muricata leaves possess lots of secondary metabolite, vitamins and mineral with variable valuable biological activities. Hence, this informs the decision to carryout phytochemical screening of the leaf ethanol extract of *Annona muricata* using GCMS (9).

2. Materials and methods

2.1. Sample collection and authentication

Fresh leaves of *Annona muricata L*. were collected from Ogidi town of Anambra State in Nigeria during the month of February 2025. The plant was identified and authenticated by Mr. Francis Iwunze from the Department of Forestry and Wildlife, School of Agricultural Technology, Federal University of Technology Owerri, Nigeria. A voucher specimen was deposited in the herbarium under the collection number FUTO/FWT/ERB/2025/71.

2.2. Samples preparation and Extraction

The leaves of *Annona muricata* were washed with water and cut into small pieces, drying was done at room temperature under the shade for about thirty days, and the dried leaves were powdered by using a blender. 100 g of powdered leaves were extracted using 700 ml ethanol for three days by the plant tissue homogenization method as previously described (15). The extract was then concentrated using a rotary evaporator and stored in the refrigerator at 4^{o}C until used.

2.3. Chemicals and reagents

All chemicals and reagents were procured from certified suppliers and were of the highest analytical standard.

2.3.1. Gas Chromatography Mass Spectroscopy

Gas chromatography mass spectroscopy (GC-MS), a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose was used. The unknown organic compounds in the complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (16).

2.3.2. Preparation of extract

The ethanolic extract of the leaves was analyzed using Gas Chromatography Mass Spectroscopy for the identification of the phytochemical compounds present. A solvent blank analysis was first conducted using 1 µl of absolute ethanol. Then 1 µl of the reconstituted ethanolic extract solution was employed for GC-MS analysis as previously described with modifications (16,17)

2.3.3. Analysis

GC-MS analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument; Schimadzu GCMS-QP2010, employing the following conditions: Column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) as carrier gas at a constant flow of 1ml/ minute and a sample injection volume of 1 µl which was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minute, to 200°C, then 5°C/minute to 280°C, ending with a 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of

0.5 s and fragments from 40 to 550 Da. Total run time was 30 min. The compounds were then identified from the GC-MS peaks, using library data of the corresponding compounds. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library using NISP Search. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software (17).

3. Results

The results obtained from this study represented an important step towards the effective characterization of the secondary class metabolite compounds from this plant using GC-MS analysis. Preliminary qualitative phytochemical analysis of extracts revealed it to be rich in secondary class metabolite compounds of alkaloids, saponins, terpenoids, flavonoids, coumarins and lactones, anthraquinones, tannins, Cardiac glycosides, phenols and phytosterols.

Each row in table 1 lists a unique bioactive component identified within the extract, along with its retention time during the analysis and the percentage it constitutes of the total extract. The results indicated that thirty (30) phytochemical constituents were identified from the leaf ethanol extract of *Annona muricata* (Table 1).

Table 1 Phyto-components generated in the ethanolic leaves extract of *Annona muricata* by GC-MS analysis

Peak #	Retention time/ minutes	% Composition by Area	Bioactive Components	Comment
1	10.193	0.43	Hexadecane Hexadecane 2,6,10-Trimethyltridecane	Matched
2	13.024	0.10	9-Eicosene, 3-Eicosene, E-14-Hexadecenal	Matched
3	13.171	0.34	1-Octadecanesulphonyl chloride 10-Methylnonadecane Tritetracontane	Matched
4	14.710	0.54	Dibutyl phthalate Dibutyl phthalate 1,2-Benzenedicarboxylic acid, buty –l- octyl ester	Matched
5	15.771	0.45	1-Octadecene 1-Docosene 1-Nonadecene	Matched
6	15.898	0.43	Sulfurous acid, butyl tetradecylester, Tetratetracontane Methoxyacetic acid, 2-tetradecyl ester	Matched
7	18.289	0.34	1-Docosene 1-Hexadecanol, 2-methyl-9-Nonadecene	Matched
8	18.402	0.26	Octadecanesulphonyl chloride, Carbonic acid, Tridecyl vinyl ester, Tetratetracontane	Matched

9	20.609	1.54	1-Docosene 1-Docosene Cyclotetracosane	Matched
10	20.707	0.14	Docosane Sulfurous acid, butyl heptadecyl ester, Carbonic acid, eicosyl vinyl ester	Matched
11	21.516	0.34	2-Piperidinone, N-[4-bromo-n-butyl,]- Oxirane, tridecyl- Cyclohexane, 1,1'-(1-methyl-1,2- ethanediyl) bis-	Matched
12	21.783	3.34	Bis(2-ethylhexyl) phthalate Bis(2-ethylhexyl) phthalate Bis(3-methylbutan-2-yl) phthalate	Matched
13	22.164	0.29	2-Piperidinone, N- [4-bromo-n-butyl, 1-Heptadecene, Hexadecane,	Matched
14	22.756	1.54	17-Pentatriacontene Octacosyl, trifluoroacetate Pentadecafluorooctanoic acid, octa decyl ester	Matched
15	22.841	0.84	Docosane, 9-octyl- Carbonic acid, eicosyl vinyl ester, Tritetracontane	Matched
16	24.857	0.43	Squalene Supraene, Squalene	Matched
17	30.006	0.23	4-Cholesten-3-one semicarbazone, Hydrazinecarboxylic acid, 1,1-dimethylethyl ester, N-Acetoacetyl-deacetylcolchicine	Matched
18	30.860	0.10	1,2-Benzisothiazol-3-amine, TBDMS derivative, Trichloroacetic acid, 2-tetradecyl ester 3-Trifluoroacetoxypentadecane	Matched
19	30.934	0.43	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)- 1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)- 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	Matched
20	31.034	0.11	Erucic acid, Heptadecanolide, Cyclopentadecanone	Matched
21	31.052	0.03	Pentatriacontane, 13-docosenylidene-2-Pentadecanol, 2-Trifluoroacetoxypentadecane	Matched
22	31.295	2.84	Propanamide, 2,2-dimethyl-N-(4-methylphenyl)- Propanamide, 2,2-dimethyl-N-(3-methylphenyl)- 2-(2-Butoxyethoxy) ethyl 2,2,3,3,3-pentafluoropropanoate	Matched
23	31.335	2.45	Pyrrolidine, 1-methyl-3,2'-spiro-benzo-1,3-dioxolane-2,6-Di-tert-butyl-4-methylphenol, O-heptafluorobutyryl, Pregn-16-en-20-one, 3-hydroxy-,.beta.,5.beta.)-	Matched

24	33.372	17.21	1,2-Benzisothiazol-3-amine, TBDMS Derivative, Propanamide, N-(4-methoxyphenyl)-2,2-dimethyl-1H-Indole,	Matched
25	33.448	3.54	1,2-Benzisothiazol-3-amine, TBDMS derivative, Benzo[h]quinoline, 2,4-dimethyl-Cyclotrisiloxane, hexamethyl	Matched
26	33.473	9.26	Acetamide, N-(4-fluorophenyl)-2,2, 2-trifluoro-1,2-Benzisothiazol-3-amine, TBDMS derivative, 9-Octadecene, 1-[3-(octadecyloxy)propoxy]-(z)	Matched
27	33.553	14.10	1,2-Benzisothiazol-3-amine, TBDMS derivative, 2-Ethylacridine, 9(10H)-anthracenone, 10,10-dimethyl	Matched
28	34.901	2.83	Cyclotrisiloxane, hexamethyl-9-Borabicyclo [3.3.1] nonane, 9-[3-(dimethylamino)propyl]- Benzo[h]quinoline, 2,4-dimethyl	Matched
29	35.384	17.10	6-Octadecenoic acid, (Z)- 9-Octadecenoic acid, (E)- 6-Octadecenoic acid	Matched
30	36.755	15.54	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester, Oleic Acid, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	Matched

The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the GC-MS NISP library. Phytochemical analysis by GC-MS analysis of the ethanolic extract of leaves of *Annona muricata* revealed the presence of different fatty acids, heterocyclic compounds, esters among others. Thirty peaks were generated.

The detailed tabulations of GC-MS analysis of the extracts are given in Table 1. From the analysis, thirty compounds were elucidated in this study on *Annona muricata*, of which all compounds were effectively matched and identified. The leaf ethanolic extract of the plant generated thirty constituents, the major constituents were at peaks 24 (peak area 17.21%), peak 29 (peak area 17.10%), 6-Octadecenoic acid, (Z)-9-Octadecenoic acid, (E)-6-Octadecenoic acid, peak 30 (peak area 15.54%), 9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester, Oleic Acid, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, Peak 27 (peak area 14.10%), 1,2-Benzisothiazol-3-amine, TBDMS derivative, 2-Ethylacridine, 9(10H)-anthracenone, 10,10-dimethyl, peak 26 (peak area 9.26%) Acetamide, N-(4-fluorophenyl)-2,2, 2-trifluoro-1,2-Benzisothiazol-3-amine, TBDMS derivative, 9-Octadecene, 1-[3-(octadecyloxy)propoxy]-(z), peak 25 (peak area 3.54%) 1,2-Benzisothiazol-3-amine, TBDMS derivative, Benzo[h]quinoline, 2,4-dimethyl-Cyclotrisiloxane, hexamethyl, peak 12 (peak area 3.34%) Bis(2-ethylhexyl) phthalate, Bis(2-ethylhexyl) phthalate, Bis(3-methylbutan-2-yl) phthalate, peak 22 (peak area 2.84%) Propanamide, 2,2-dimethyl-N-(4-methylphenyl)-Propanamide, 2,2-dimethyl-N-(3-methylphenyl)- 2-(2-Butoxyethoxy)ethyl 2,2,3,3,3-pentafluoropropanoate, peak 23 (peak area 2.45 %) Pyrrolidine, 1-methyl-3,2'-spiro-benzo-1,3-dioxolane-2,6-Di-tert-butyl-4-methylphenol, O-heptafluorobutyryl,Pregn-16-en-20-one, while the rest had less than 2% composition by peak area.

4. Discussion

The presence of various secondary class metabolites identified puts these results in line with earlier studies that were carried out on the ethanolic seeds extract of *Annona muricata*, and the phytochemical tests showed that ethanol soursop seeds extract contains secondary metabolites compounds group of saponins, alkaloids and triterpenoids, flavonoids, anthraquinones, tannins, and cardiac glycosides, which they noted that they are defense chemical compounds of plants produced in the plant tissue (17,18). The plant could thus be used for the management of various healthy conditions associated with the metabolites screened.

Using GC-MS Analysis, 30 compounds have been elucidated for the first time in our study on *Annona muricata*, of which all the compounds were effectively matched and identified.

1,2-Benzenedicarboxylic acid, butyl octyl ester is a plasticizer compound with antimicrobial, antifouling, antioxidant and hypo-cholesterolemic activities. (19) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol is recorded to have anti-tuberculosis, insecticidal, anti-inflammatory, antioxidant and antimicrobial activities. n-Hexadecanoic acid on the other

hand which is commonly known as Palmitic acid has nematicide, pesticide, lubricant, anti-androgenic, flavor, hemolytic 5-alpha reductase inhibitor, antioxidant and hypo-cholesterolemic properties. (17).

Hexadecanoic acid, ethyl ester is a fatty acid ester with nematicide, pesticide, lubricant, anti-androgenic, flavor, and has hemolytic 5-alpha reductase inhibitor properties. (20,21)

Phytol and phthalate are diterpenes with antimicrobial, anticancer, anti-inflammatory, anti-diuretic, immune-stimulatory and anti-diabetic properties. 9,12-Octadecadienoic acid, ethyl ester is a linoleic acid which has hypo-cholesterolemic, 5-alpha reductase inhibitor, antihistaminic, insectifuge, anti-eczemic, and anti-acne properties. Finally, 1,2-Benzenedicarboxylic acid, diisooctyl ester is a plasticizer compound with antimicrobial and antifouling properties. (16,20,21)

It is worth noting that of the major constituents identified in our extract are the compounds with the highest composition at peaks 24 (peak area 17.21%) and Peak 29 (peak area 17.10**%)**. These two could be very novel compounds that need to be analyzed further in order to elucidate their nature. The same applies to the remaining 28 compounds which have lower concentrations.

5. Conclusion

GC-MS analysis of the ethanolic extract of leaves of *Annona muricata* revealed the presence of different fatty acids, heterocyclic compounds, esters among others. This confirms the results on presence of the various secondary metabolite compounds detected by the qualitative procedures. These mass spectra are fingerprint of the compound which can be identified from the data library. Hence, the identified phyto-components using GC-MS can be used as a pharmacognostical tool for the identification of adulterants. The current pioneering study suggests that ethanolic extract is a potent therapeutic agent. It paves the way for the development of several treatment regimens based on this extract. In addition, further research is necessary to identify and purify the active compounds responsible for therapeutic activity, as well as the unidentified compounds.

Compliance with ethical standards

Ethical standards were complied with throughout the course of this research.

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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