

GC-MS Analysis of Bioactive Phytochemicals Present in Ethanolic Extracts of Leaves of *Annona muricata*: A Further Evidence for Its Medicinal Diversity

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ABSTRACT

Background: 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. **Objective:** To analyze the phytochemical composition of the ethanolic extracts of leaves of *Annona muricata* using gas chromatography mass spectroscopy (GC-MS). **Materials and methods:** GC-MS Analysis was used. **Results:** The GC-MS Analysis revealed 25 constituents of which 12 of the compounds were identified. The major constituents were two unidentified compounds with percentage peak areas of 23.51% and 16.8%. Of the identified compounds, the outstanding in composition were 7-Tetradecenal, (Z) (peak area 9.39%), n-Hexadecanoic acid (peak area 7.12%), Oleyl Alcohol (peak area 6.15%), Phytol (peak area 5.61%), cis, cis, cis-7,10,13-Hexadecatrienal (peak area 4.26%), 2-Pentadecanol (peak area 3.93%), 9,12-Octadecadienoic acid, ethyl ester (peak area 3.21%), 1,2-Benzenedicarboxylic acid, butyl octyl ester (peak area 2.67%), and 1,E-11,Z-13-Octadecatriene (peak area 2.15%), while the rest had less than 2% composition by peak area. **Conclusion:** 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.

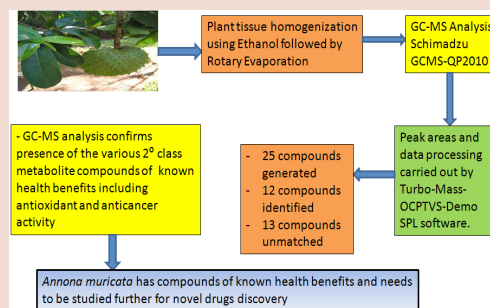
Key words: *Annona muricata*, Ethanolic extracts, GC-MS, Medicinal diversity, Phytochemicals.

SUMMARY

- *Annona muricata* is widely used to treat various diseases.
- **Objective:** To conduct a GC-MS Analysis on ethanolic leaves extracts 25 compounds were generated, 12 compounds were identified, 13 were not.
- The major constituents were two unidentified compounds with percentage peak areas of 23.51% and 16.8%.
- Identified compounds included: 7-Tetradecenal, (Z) (peak area 9.39%), n-Hexadecanoic acid (peak area 7.12%), Oleyl Alcohol (peak area 6.15%), Phytol (peak area 5.61%) cis, cis, cis-7,10,13-Hexadecatrienal (peak area 4.26%),

2-Pentadecanol (peak area 3.93%), 9,12-Octadecadienoic acid, ethyl ester (peak area 3.21%), 1,2-Benzenedicarboxylic acid, butyl octyl ester (peak area 2.67%), 1,E-11,Z-13-Octadecatriene (peak area 2.15%). The rest had less than 2% composition by peak area.

- All identified compounds of known health benefits. Further studies needed on the plant's therapeutic value.



PICTORIAL ABSTRACT

Abbreviations used: GC-MS: Gas Chromatography Mass Spectroscopy, TIC: Total Ion Chromatogram.

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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 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 Graviola or 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 Uganda.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,^{9,10} 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,^{11,12,13} as well as in Uganda.⁷ It is hoped that traditional medicine will in future provide the cure to many tropical diseases that have defied orthodox prescriptions. The objective of this study was therefore to undertake phytochemical composition analysis using GC-MS on the ethanolic extracts of leaves of *Annona muricata*.

MATERIALS AND METHODS

Sample collection and authentication

Fresh leaves of *Annona muricata* L. were collected from the wild in Eastern Uganda in the district of Kaliro during the month of August 2013. The plant was identified and authenticated in the Makerere University Botanical Herbarium (MHU) by Ms. Olivia Wanyana Mangeni. A voucher specimen was deposited in the herbarium under the collection number GY 021- 10/13- MB 300-0007/12-001.

Samples preparation and Extraction

The leaves of *Annona muricata* were washed with water and cut into small pieces, drying was done at room temperature, and the dried leaves were powdered. 150 g of powdered leaves were extracted using 500 ml ethanol for three days by the plant tissue homogenization method as previously described.¹⁴ The extract was then concentrated using a rotary evaporator and kept at 4°C until used.

Chemicals and reagents

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

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.

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 μ l of absolute ethanol. Then 1 μ l of the reconstituted ethanolic extract solution was employed for GC-MS analysis as previously described with modifications.^{15,16}

Analysis

GC-MS analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument; Shimadzu GCMS-QP2010, employing the following conditions: Column Elite-1 fused silica capillary column (30 \times 0.25 mm ID \times 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 μ l 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.

RESULTS

Our previous study,⁷ reported that ethanolic leaves extracts of *Annona muricata* L showed anticancer and antioxidant activities. 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 as shown in the Figure 1.

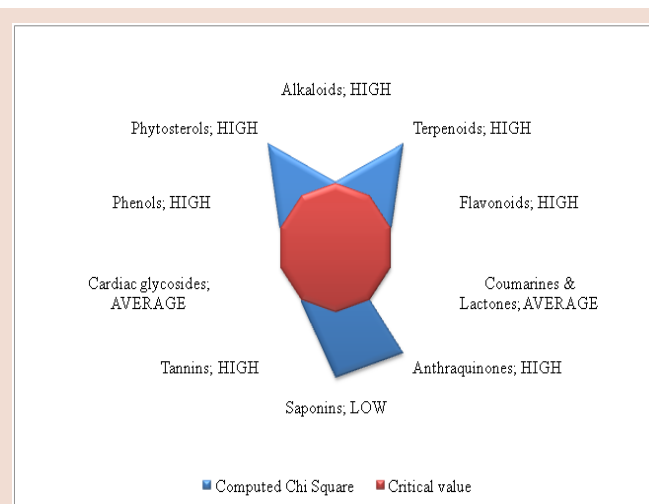


Figure 1: Phytochemicals present in Ethanolic leaves extracts of *Annona muricata* with relative abundance computed from the χ^2 Test.⁷

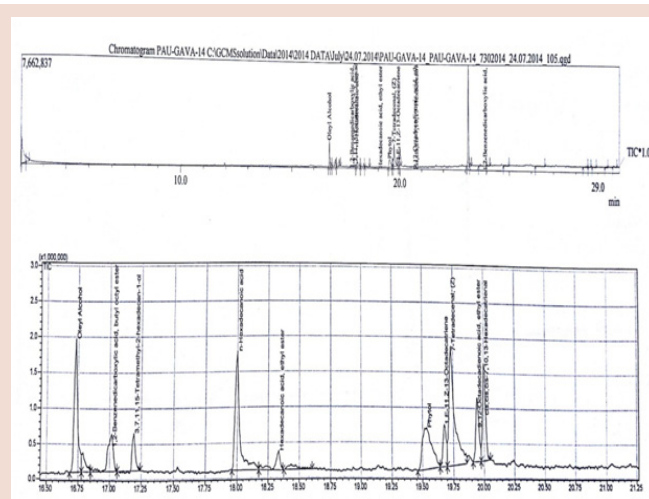


Figure 2: Total Ion Chromatogram (TIC) of ethanolic extract of leaves of *Annona muricata*

Table 1: Phyto-components generated in the ethanolic leaves extract of *Annona muricata* by GC-MS Peak Report TIC

Peak #	Retention time/ minutes	% Composition by Area	Matched compound IUPAC Name	Chemical Formula	Comment
1	3.034	16.8	-	-	Unidentified
2	3.342	3.93	2-Pentadecanol	C ₁₅ H ₃₂ O	Matched
3	16.732	6.15	Oleyl Alcohol	C ₁₈ H ₃₆ O	Matched
4	16.792	1.04	-	-	Unidentified
5	17.015	2.67	1,2-Benzenedicarboxylic acid, butyl octyl ester	C ₂₀ H ₃₀ O ₄	Matched
6	17.19	1.37	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	Matched
7	17.998	7.12	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	Matched
8	18.331	1.29	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	Matched
9	18.431	0.73	-	-	Unidentified
10	19.531	5.61	Phytol	C ₂₀ H ₄₀ O	Matched
11	19.678	2.15	1,E-11,Z-13-Octadecatriene	C ₁₈ H ₃₂	Matched
12	19.733	9.39	7-Tetradecenal, (Z)	C ₁₄ H ₂₆ O	Matched
13	19.946	3.21	9,12-Octadecadienoic acid, ethyl ester	C ₂₀ H ₃₆ O ₂	Matched
14	20.003	4.26	cis, cis, cis-7,10,13-Hexadecatrienal	C ₁₆ H ₂₆ O	Matched
15	23.143	23.51	-	-	Unidentified
16	23.225	1.08	-	-	Unidentified
17	23.695	1.74	-	-	Unidentified
18	24.025	0.75	1,2-Benzenedicarboxylic acid, diisooctyl ester	C ₂₄ H ₃₈ O ₄	Matched
19	24.75	1.25	-	-	Unidentified
20	26.402	1.54	-	-	Unidentified
21	28.35	1.12	-	-	Unidentified
22	28.567	0.74	-	-	Unidentified
23	28.875	0.85	-	-	Unidentified
24	29.331	0.74	-	-	Unidentified
25	29.704	0.95	-	-	Unidentified

The total ion chromatogram (TIC) of the ethanolic extract, showing the GC-MS profile of the compounds identified is given in Figure 2. 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. 25 peaks were generated.

The detailed tabulations of GC-MS analysis of the extracts are given in Table 1. From the analysis, 25 compounds have been elucidated for the first time in this study on *Annona muricata*, of which 12 compounds were effectively matched and identified. The ethanolic extract of the plant generated 25 constituents, the major constituents were at peaks 15 (peak area 23.51%), Peak 1 (peak area 16.8%), 7-Tetradecenal, (Z) (peak area 9.39%), n-Hexadecanoic acid (peak area 7.12%), Oleyl Alcohol (peak area 6.15%), Phytol (peak area 5.61%), cis, cis, cis-7,10,13-Hexadecatrienal (peak area 4.26%), 2-Pentadecanol (peak area 3.93%), 9,12-Octadecadienoic acid, ethyl ester (peak area 3.21%), 1,2-Ben-

zenedicarboxylic acid, butyl octyl ester (peak area 2.67%), and 1,E-11,Z-13-Octadecatriene (peak area 2.15%), while the rest had less than 2% composition by peak area.

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.^{16,17} The plant could thus be used for the management of various healthy conditions associated with the metabolites screened.

Using GC-MS Analysis, 25 compounds have been elucidated for the first time in our study on *Annona muricata*, of which 12 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.¹⁸ 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 nematocide, pesticide, lubricant, anti-androgenic, flavor, hemolytic 5-alpha reductase inhibitor, antioxidant and hypo-cholesterolemic properties.¹⁶

Hexadecanoic acid, ethyl ester is a fatty acid ester with nematocide, pesticide, lubricant, anti-androgenic, flavor, and has hemolytic 5-alpha reductase inhibitor properties.^{19,20}

Phytol is a diterpene 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, insecticide, anti-eczemic, and anti-acne properties. Finally, 1,2-Benzenedicarboxylic acid, diisooctyl ester is a plasticizer compound with antimicrobial and antifouling properties.^{15,19,20}

It is worth noting that of the major constituents identified in our extract, the compounds with the highest composition at peaks 15 (peak area 23.51%) and Peak 1 (peak area 16.8%) have not been matched in the library. 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 11 compounds which have not been identified irrespective of their percentage composition being less than 2%.

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.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR'S CONTRIBUTIONS

All authors contributed immensely towards the research work and development of the manuscript. YG–designed experiment, collected samples, conducted laboratory analysis, analyzed data, participated in write-up of manuscript. FA–participated in the design, analysis, write up and reviewed drafts of the manuscript. FW–participated in the design, coordination, analysis, write up and reviewed drafts of the manuscript. EHA -participated in the design and coordination of the study, data interpretation and write-up of manuscript. All authors read and approved the final manuscript.

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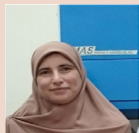
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