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GC-MS analysis, pharmaceutical and industrial significance of phytochemicals present in *Annona muricata* from Eziobodo, Imo State, Nigeria

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Abstract

Background: The conduct of screenings for therapeutic alternatives derived from medicinal plants has emerged as a useful tool for drug discovery and valuable information for further phytotherapeutic studies.

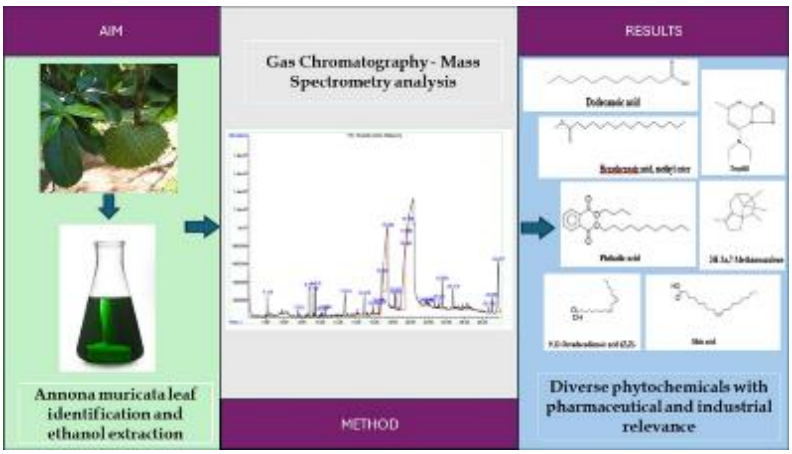
Objective: To carry out chemical characterization of ethanol leaf extract of *Annona muricata* using gas chromatography mass spectroscopy (GC-MS).

Materials and method: Apparently healthy leaf samples were taxonomically identified, air-dried and subjected to maceration to obtain ethanol extract stock. GC-MS analysis was utilized to investigate phytochemical composition.

Results: The GC-MS analysis spectra was characterized by a total of twenty-six constituents at different retention times which were all identified. Dominant components present included n-Hexadecanoic acid, Oleic Acid and γ-Sitosterol with peak areas 32.60%, 24.35% and 9.10% respectively. Additional constituents were Dodecanoic acid (peak area 4.31%), 13 Octadecenal, (Z) (peak area 2.83%), Bis (2-ethylhexyl) phthalate (peak area 2.31%), Tetradecanoic acid (peak area 2.34%), Trapidil (peak area 2.10%) while others occupied less than 2% peak area.

Conclusion: This study suggests that the ethanol leaf extract of *A. muricata* could possess vital compounds of pharmaceutical and industrial relevance.

Graphical Abstract



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Keywords: GCMS; *Annona muricata*; Ethanol extract; Phytochemicals

1. Introduction

Medicinal plants have historically played a fundamental role in traditional medicine throughout a wide range of civilizations, serving as the primary foundation for the treatment of numerous maladies [1, 2, 3, 4]. Nigeria possesses a diverse array of plant species that have significant therapeutic properties and are utilized as ethnobotanical treatment strategies [5, 6]. Recently, phytochemicals have been intensively studied, as the search for agents linked with optimum health upkeep as well as a likely decline on the risks of diseases such as diabetes, cancer, cardiovascular diseases, stroke, metabolic syndrome, hypertension and other degenerative diseases has drawn global attention [7, 8, 9, 10, 11]. The conventional approach to the development of novel pharmacological entities necessitates a comprehensive scientific assessment including potency, effectiveness, bioavailability, adverse effects on non-target locations, safety, as well as preclinical and clinical trials [12, 13]. However, numerous artificial drugs that are not derived from natural sources can result in significant adverse reactions as these drugs were previously only considered as a last resort for treating incurable illnesses like cancer. Alternatively, the byproducts found in medicinal plants may offer a way to avoid the side effects associated with synthetic drugs, as they tend to build up within living cells [14].

Annona muricata, a member of the Annonaceae family, has garnered significant attention in the academic community owing to its considerable therapeutic prospects including hypoglycemic, antispasmodic, sedative, hypotensive and smooth muscle relaxant effects [15, 16, 17]. It is distributed in the tropical regions of Central and South America, Western Africa and Southeast Asia [18]. The immature fruit, seeds, leaves, and roots of the plant are employed as biopesticides and insect repellents [19]. Phytochemicals including alkaloids, flavonoids, phenolic compounds, glycosides, saponins, tannins, terpenoids, and phytosterols have been identified as various components of *A. muricata* [20, 21]. The leaves of *A. muricata* are reportedly extensively employed for various ethnomedicinal purposes, with a particular emphasis on their notable anticancer, anti-inflammatory, immunomodulating, and anti-proliferative attributes [22, 23, 24]. A comparative analysis to assess the antioxidant and anti-inflammatory properties of various extracts of *A. muricata* identified the ethanol extract with the most exhibited level of activity than the n-hexane extract, ethyl acetate extract [25]. Thus, this study aims to identify using GCMS, the industrial and pharmaceutical applications of the chemical components of ethanol leaf extract of *A. muricata* found in Eziobodo, Imo State, Nigeria.

2. Material and methods

2.1. Plant material

Healthy leaves of *A. muricata* were obtained from agricultural farms located in Eziobodo, Owerri West Local Government Area of Imo State, Nigeria. The plant specimen was taxonomically recognized at the Department of Forestry and Wildlife Technology, Federal University of Technology, Owerri, Nigeria, with the corresponding voucher number FUTO/FWT/ERB/2022/71.

2.2. Sample extraction

The plant samples were air-dried at room temperature until a stable weight was achieved. Dried leaves of *A. muricata* were weighed, homogenized into a fine powder, and stored in an air-tight container for future utilization. The maceration extraction method, which entails the process of soaking, filtration, and subsequent evaporation, was employed. A total of 1300 mg of *A. muricata* plant material was subjected to extraction using 70% v/v ethanol (BDH, England) for a duration of 48 hours, with continuous agitation. The finished sample was thereafter poured and separated through the use of Whatman No. 1 filter paper. The filtrate was subjected to evaporation using a water bath (Memmert WNB10 Germany) to obtain a molten extract with a consistent weight. The stock extract was kept at a cool temperature throughout the duration of the investigation.

2.3. GC-MS analysis

The ethanol leaf extract of *A. muricata* was analyzed quantitatively for phytochemical content via chemical characterization using Gas Chromatography Mass spectrometry (GC-MS). 2ul of the Sample Extract was injected into the GC column for analysis. The GC (Agilent 7890N) and MS (5975B MSD) is equipped with DB-5ms capillary column (30 m×0.25 mm; film thickness 0.25 µm). The initial temperature was set at 40°C which increased to 150°C at the rate of 10°C/min. The temperature again increased to 230°C at the rate of 5°C/min. The process continued till the temperature reached 280°C at the rate of 20°C/min which was held for 8 minutes. The injector port temperature remained constant at 280°C and detector temperature was 250°C then. Helium was used as the carrier gas with a flow

rate of 1 mL/min. Split ratio and ionization voltage were 110:1 and 70 eV respectively. To identify the unknown components, present in the extract, their individual mass spectral peak value was compared with the database of National Institute of Science and Technology 2014.

3. Results and discussion

3.1. GC-MS analysis results of ethanol leaf extract of *A. muricata*

The GC-MS Chromatogram revealed a total of 28 peaks with different retention times. Though the peak number is 28, the identified compounds were 26 due to the reiteration of some compounds.

Table 1 Compounds identified in the ethanol leaf extract of *A. muricata*

Peak No.	R.T min	Name of compound	% A	Molecular formular	MM (g/mol)
1	4.128	2-Heptenal, (E)	1.74	$C_7H_{12}O$	112.17
2	7.575	Naphthalene	0.38	$C_{10}H_8$	128.17
3	8.757	2-Decenal (E)	1.55	$C_{10}H_{18}O$	154.25
4	9.198	1-Ethynylcyclopentanol	1.39	$C_7H_{10}O$	110.15
5	9.510	2-Octyne	1.61	C_8H_{14}	110.20
6	10.257	n-Decanoic acid	0.42	$C_{10}H_{20}O_2$	172.26
7	10.598	3H-3a,7-Methanoazulene	0.38	$C_{15}H_{24}$	204.35
8	12.816	Dodecanoic acid	4.31	$C_{12}H2_4O_2$	200.32
9	14.915	Tetradecanoic acid	2.34	$C_{14}H_{28}O_2$	228.37
10	15.915	Phthalic acid	0.49	$C_8H_6O_4$	166.13
11	16.486	Hexadecanoic acid, methyl ester	0.77	$C_{17}H_{34}O_2$	270.50
12	16.709	Palmitoleic acid	1.20	$C_{16}H_{30}O_2$	254.41
13	16.862	Trapidil	2.10	$C_{10}H_{15}N_5$	205.26
14	17.486	n-Hexadecanoic acid	32.60	$C_{16}H_{32}O_2$	256.42
15	18.327	9, 12 – Octadecadienoic acid (Z), methyl ester	1.23	$C_{19}H_{34}O_2$	294.50
16	18.409	9-Octadecenoic acid	1.20	$C_{18}H_{34}O_2$	282.50
17	19.456	Oleic Acid	24.35	$C_{18}H_{34}O_2$	282.50
18	19.586	cis 7, cis-11-Hexadecadien-1-yl acetate	1.46	$C_{18}H_{32}O_2$	280.40
19	19.768	Cyclodecanol	1.82	$C_{10}H_{20}O$	156.26
20	21.703	9,12-Octadecadienoic acid (Z,Z)-	0.42	$C_{18}H_{32}O_2$	280.40
21	21.968	9,12-Octadecadienoic acid (Z,Z)-	0.50	$C_{18}H_{32}O_2$	280.40
22	22.138	9,12-Octadecadienoic acid (Z, Z)	0.39	$C_{18}H_{32}O_2$	280.40
23	23.221	15-Hydroxypentadecanoic acid	0.71	$C_{15}H_{30}O_3$	258.40
24	23.550	Bis (2-ethylhexyl) phthalate	2.31	$C_{24}H_{38}O_4$	390.60
25	24.744	13-Octadecenal, (Z)	2.83	$C_{18}H_{34}O$	266.50
26	28.720	Campesterol	0.69	$C_{28}H_{48}O$	400.70
27	29.056	Stigmasterol	1.71	$C_{29}H_{48}O$	412.70
28	29.767	γ-Sitosterol	9.10	$C_{29}H_{50}O$	414.70

RT= retention time, A = Abundance; MM = Molecular mass

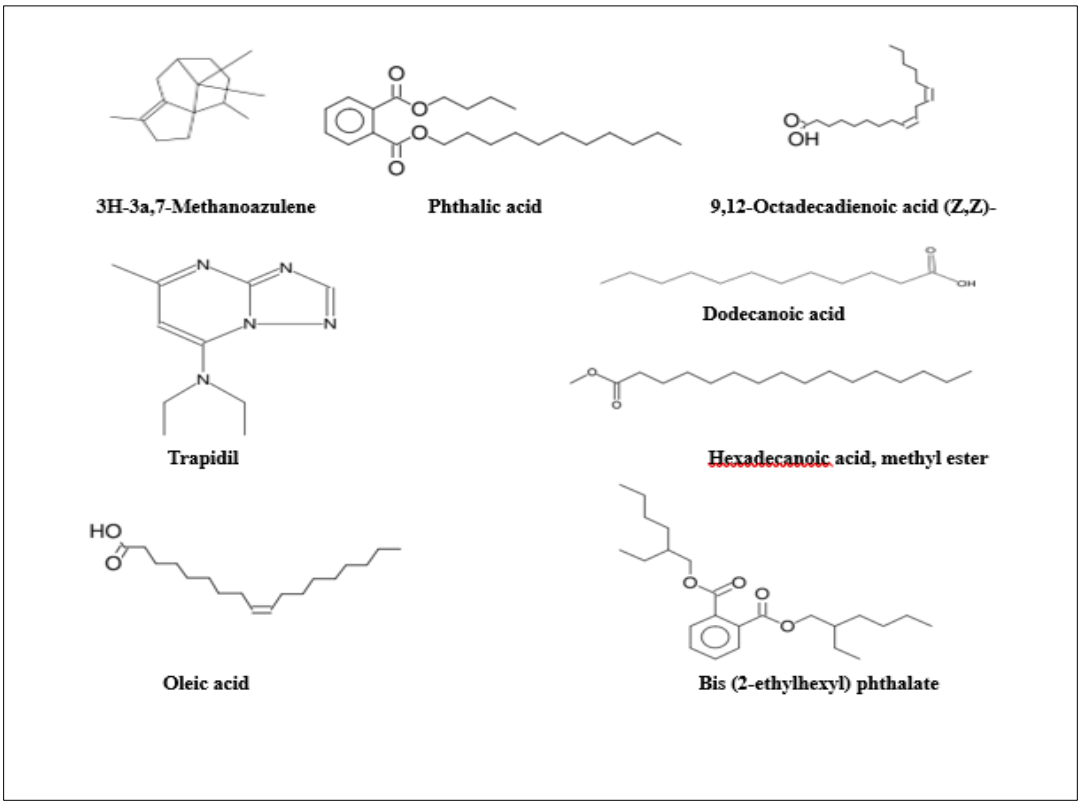

Figure 1 Chemical structure of some compounds identified via GC-MS in A. muricata ethanol leaf extract

In this study, the ethanol leaf extract of *A. muricata* was analyzed with GC-MS to determine its chemical composition and the results are as shown (Table 1). The most abundant compound present in the sample was n-Hexadecanoic acid (32.60%), also known as palmitic acid. Gavamukulya *et al.*[14] reported that palmitic acid possessed antioxidant, anti-inflammatory, hypocholesterolemic and hemolytic 5-alpha reductase inhibitory attributes. Previous investigations of natural products implicated palmitic acid as a potent bioactive molecule with antiviral inhibition against HIV-1 infection via direct binding mechanisms to CD4 receptors [26, 27]. In addition, the selective apoptotic activity of palmitic acid on U266 multiple myeloma cells has been reported [28]. Palmitic acid is employed industrially as a flavouring agent [29].

Oleic, a monounsaturated fatty acid, was the second most abundant compound present at 24.35%. Recent studies indicate that oleic acid exhibited a strong DPPH radical scavenging activity and reduced the level of superoxide radical O^{2-} of cadmium-induced lipid peroxidation [30]. According to Ismail *et al.* [31], oleic acid in combination with aromatase inhibitors enhanced the release of cytochrome c and subsequent apoptosis induction in estrogen receptor-positive human breast cancer (MCF-7) cells. In another study, oleic acid reportedly induces minimal occurrence of inflammatory conditions through functional modulations on the activities of immune system players [32]. Furthermore, oleic acid has been observed to possess an efficacious modulation of endoplasmic reticulum stress autophagy as a mechanism of hepatic lipotoxicity protection [33]. Industrially, oleic acid is used as a food additive, emulsifying agent, skin penetrant, herbicide, insecticide, and fungicide [29].

The third most ample compound from the analysis (9.10%) was γ-Sitosterol, a phytosterol present in many plants. It has the capacity of exhibiting strong antioxidant, anticholesterolemic, anti-diabetic, anticancer, anti-inflammatory, antifungal, antibacterial and anti-angiogenic activity [34]. It is a major component in cosmetics as an emulsion stabilizing agent, skin conditioner and humectant [29].

Further identified phytocompounds present in the sample (Table 1) include Dodecanoic acid (4.31%), 13 Octadecenal, (Z) (2.83%), Bis (2-ethylhexyl) phthalate (2.31%), Tetradecanoic acid (2.34%) and Trapidil (2.10%). Dodecanoic acid, commonly known as lauric acid was recently described to play a role in reversing deoxynivalenol-induced damage on intestinal epithelium through a mechanism of accelerated intestinal stem cell regeneration, thus serving as a potent therapeutic agent in jejunal diseases [35]. Tetradecanoic acid, known as myristic acid, is a flavouring agent and used to process sugar beets and yeasts [29]. Chen *et al.* [36], demonstrated supported evidence on the use of myristic acid as a natural antibacterial agent. Trapidil, a member of the triazolopyrimidines, is recognized for its role as a platelet-derived

growth factor antagonist, phosphodiesterase inhibitor, vasodilator, and anti-platelet agent in its current use for the treatment of patients with ishemic coronary heart, kidney, and liver conditions [37]. Wang *et al.* [38] suggested that trapidil employed a mechanism of blocking endoplasmic reticulum stress in right heart failure therapy. In addition, trapidil contributed to the right ventricle restoration in a model of pulmonary arterial hypertension condition by ameliorating redox balance indices [39].

In this study, some of the phytocompounds that had less than 2% peak area were identified to possess noteworthy characteristics. Stigmasterol has been associated with the capacity to confer neuroprotection by downregulating signalling pathways involved in glutamate-induced toxicity in HT-22 cell culture [40]. In a study by AmeliMojarad *et al.* [41], stigmasterol demonstrated its potential to induce apoptosis in breast tumour cells by downregulating Bcl-XL and Bcl-2 gene expressions. 9,12-Octadecadienoic acid (Z,Z), also known as linoleic acid, is found in many vegetable oils (cottonseed, soybean, peanut, etc.), and industrially used in the manufacture of emulsifiers, margarine, vitamins, food additive, dietary supplements as well as paints, soaps and coatings [29, 42]. Hexadecanoic acid methyl ester, commonly known as methyl palmitate, is suggested to have anti-inflammatory, antioxidant, and anti-proliferative potentials [43]. Another study reported that methyl palmitate exerted anti-inflammatory and antifibrotic effect via NF-kB inhibition [44]. 3H-3a,7-Methanoazulene, known as cyperene, is a sesquiterpenoid identified as a significant constituent of essential oils that demonstrated antioxidant, antibacterial and protective effect against DNA damage [46]. 2-Decenal (E) and 2-Heptanal are both utilized as flavouring constituent of many foods [29]. In addition, Campesterol is added to food as a source of phytosterols owing to its anti-hypercholesterolemic property [42].

4. Conclusion

It is evident that the rich diversity of phytocompounds present in *A. muricata* could serve as an economically viable resource with pharmaceutical and industrial significance in Nigeria. Further studies could support commitment towards conservatory practices of this specie for long term sustainability goals.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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