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Bioactive phytochemicals in *Annona muricata* fruit juice ethanol extract

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Abstract

Phytochemical contents of *Annona muricata* fruit juice ethanol extract was determined using gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). Lyophilized fruit juices were extracted in 10 % v/v aqueous solution of ethanol at 60 0C in a water bath. The GC-MS screening of the extract revealed the concentrations (mg/ml) of thirty-three (33) phytochemicals which included glycerine (9.12), levoglucosenone (5.18), 1,3-propanediol-2-(hydroxymethyl)-2-nitrobenzene (11.83) and D- allose (7.28), benzofuran-2,3-diydro-(1.87), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.52), Cis-11,14-Eicosadienoic acid, methyl ester (1.53), 9-Octadecenoic acid, methyl ester (1.68), 2-Methoxy-4-vinylphenol (1.69), which are reported to exhibit various biological activities. The HPLC analyses of the extract revealed various polyphenolic compounds (mg/ml) such as naringenin (12.97), proanthocyanin (0.23), tannin (44.17), coumaric acid (4.12), catechin (39.20), resveratrol (42.27) and kaempferol (29.86). which also possess various health benefits like antibacterial, antifungal, anti-inflammatory, anticancer, antimutagenic, antioxidant, antitumor, and others. It can be concluded that the *Annona muricata* fruit juice ethanol extract contained bioactive phytochemicals that exhibit promising health-promoting activities.

Keywords: *Annona muricata;* Bioactive phytochemicals; Ethanol extract; GC-MS; HPLC; Molecular docking; ADME.

1. Introduction

Medicinal plants are a source of natural ingredients that are widely used as medicines. They constitute the oldest type of medicine, which have been used as traditional medicines for thousands of years across many nations. Over centuries, human communities have shared empirical knowledge about their beneficial effects [1]. Natural products are an important source of drug compounds, and many contemporary drugs used in modern pharmacotherapy are derived from traditional herbal medicine [2]. Despite these, there is still hope for medicinal plants because more research is needed to fully understand the phytochemical makeup and potential health benefits of many species [3]. Medicinal plants are used to treat specific conditions, maintain health, or both, in traditional medicine or modern medicine [4, 5]. Due to their safety and effectiveness, natural products that have been discovered thus far have been essential in enhancing human health and have been the preferred medications even in the face of fierce competition from compounds originating from computational and combinatorial chemistry. The structural diversity of natural products, which is still largely unexplored, is what stands out most in relation to their enduring significance in drug discovery.

The majority of the world's population still depends heavily on the traditional herbal medicine system for their principal health needs [6, 7]. The demand for herbal products has resurfaced due to increased knowledge of the negative effects of synthetic drugs and the minimal side effects of plant-based medicines; this demand is predicted to increase steadily by 2050 [8, 9].

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The phytochemical components of these plants, which affect human physiology in various ways, are linked to their medicinal benefits [10]. The most prevalent of these components are phenolic compounds, tannins, alkaloids, and flavonoids. There have been reports of antioxidant activity in a number of herbs, and polyphenols are a significant potential source of antioxidant [11]. Among the lot, *Annona muricata* is one of the most extensively used traditional plants.

Annona muricata Lin. also known as soursop, belongs to the *Annonaceae* family, which includes over 2300 species and over 130 genera. It contains a variety of pharmacologically active substances. The plant is widely grown in tropical and subtropical regions like Southeast Asia, South America, Nigeria and the African rainforests. The plant produces edible fruit all year round in addition to being widely used as a traditional medicine for bacterial infections, fever, skin conditions, respiratory conditions, diabetes, insomnia, cystitis, arthritis, hypertension, and cancer [12,13,14].

An important step in the investigation of the bioactive compounds derived from plant sources is the extraction process. Modern extraction techniques, like ultrasound-assisted and supercritical fluid extraction methods, are currently being used in addition to more conventional methods [15]. Furthermore, phytochemical research was greatly enhanced by the creation of sophisticated instruments for the qualitative and quantitative evaluation of phytochemicals, such as high-performance liquid chromatography (HPLC) and gas-chromatography mass spectrometry (GC/MS) [16]. Thus, this study aimed at using GC-MS and HPLC to evaluate the biologically active phytochemicals of *Annona muricata* fruit juice ethanol extract.

2. Materials and Methods

2.1. Plant collection and processing

The *Annona muricata* was purchased at a local market (Eke-Onuwa), in Owerri, Imo State, Nigeria. The fruit was identified by Dr. C.M. Duru, a taxonomist in the Department of Biology, School of Biological Sciences (SOBS), Federal University of Technology, Owerri, and assigned a voucher number: FUTO/FWT/ERB/2022/71.

The fruit was washed, peeled and the seeds removed. The white soft flesh was extracted, blended and the juice was squeezed out with a clean Muslin cloth. The yield from *A. muricata* fruit was 10 % (g/g). The extract was put in a bottle in preparation for the extraction process.

2.2. Extraction

The freshly squeezed juice was filtered into a bottle using Whatman No. 4 filter paper and was then stirred until the residues were all left on the filter paper. The extract was kept at 4 °C in a refrigerated until it was lyophilized. Then, 0.5 g of sample was transferred into a test tube and 15 ml of 10 % ethanol was added. The test tube was allowed to stand in a water bath at 60 °C for 60 min. The content of the test tube was transferred into a separating funnel and washed successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3 ml hexane, then three times with 10 ml of 10 % (v/v) ethanol aqueous solution. The solution as dried with anhydrous sodium sulphate and the solvent was concentrated at 40 °C [17]. The sample was solubilized in 1000 µl of methanol of which 200 µl was transferred to a vial for analyses.

2.3. GC-MS analyses of the phytochemicals

Shimadzu GC-MS QP 2010Ultra with MS detector equipped with RTx 5MS column 60 m x 0.25 mm ID x 0,25 u film was used to carry out the GC-MS analysis. The following were the temperature ramp used injector temperature of 250 °C, oven initial temperature of 60 °C held for 1 min and heated to 180 °C held for 2 min at 15 °C/min, then heated to 300 °C held for 7 min at 13 °C/min. The characterization of the plant extract was completed in the SCAN mode with m/z range varied from 40 to 650. The flow rate of the helium gas was 1.96 ml/min, injection volume was 1 ul in a spitless mode. The ion source temperature was 230 °C while the interface temperature was at 250 °C. The detector voltage was relative to the tuning file [18].

2.4. Identification of chemical constituents

Bioactive compounds detected from the extract were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Mainlab data of GC-MS systems).

2.5. Procedure for HPLC analysis

The analysis was performed using an Agilent Technologies 1200 High Performance Liquid Chromatography (HPLC) system, which was outfitted with a SUPELCOSIL LC-18 column. The column measured 250 mm in length, 4.6 mm in diameter, and 5 mm in particle size. The temperature in the column was adjusted to 20 °C. Two components made up the mobile phase composition: component "B" was acetonitrile, and component "A" was an aqueous acetic acid solution (0.5 percent v/v). The elution process began with the administration of 100% solvent A for two minutes. This was followed by a 58-minute interval during which a mixture of 40% solvent A and 60% solvent B was used. The injection volume was 25 µL, and a flow rate of 1 mL/min was established. A UV detector with a 280 nm wavelength calibration was used to find polyphenols. The study evaluated the retention periods of the detected polyphenolic compounds using a single standard solution of 100 mg/L. [19]

2.6. Identification of bioactive compounds

Bioactive compounds were determined by the ratio between the area and mass of internal standard and the area of the identified. The concentration of the different bioactive compound was expressed in µg/g.

3. Results

The *Annona muricata* GC-MS analysis of the extract revealed various distinct compounds. The compounds are listed in Table 1 together with their molecular formulae, compositions, retention times, and molecular weights. Thirty-three (33) compounds were isolated from the *Annona muricata* fruit juice ethanol extract; with glycerine (9.12 mg/ml), levoglucosenone (5.18 mg/ml), 1,3-propanediol-2-(hydroxymethyl)-2-nitrobenzene (11.83 mg/ml) and D- allose (7.28 mg/ml) being the most prevalent phytochemicals while benzofuran-2,3-diydro-(1.87 mg/ml), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.52 mg/ml), Cis-11,14-eicosadienoic acid, methyl ester (1.53 mg/ml), 9-octadecenoic acid, methyl ester, (E) (1.68 mg/ml) and 2-Methoxy-4-vinylphenol (1.69 mg/ml) being the least common.

Phenolic compounds were identified in the ethanolic extracts of *A. muricata* fruit ethanol extract using HPLC showed the presence of twenty (20) polyphenolic compounds. Adhumulone (32.9 mg/ml), cohumulone (34.6 mg/ml), catechin (39.2 mg/ml), resveratrol (42.2 mg/ml), tannin (44.1 mg/ml), and humulone (36.7 mg/ml) were the most common compounds while proanthocyanin (0.15 mg/ml) and naringin (2.3 mg/ml) were the least common.

Table 1 Phytochemicals present in *Annona muricata* fruit juice extract using GC-MS analyses

PK	RT	Area %	Height %	Phytochemical	Molecular formula	Molecular weight(g/mol)	Concentration (mg/ml)
1	5.847	1.30	0.63	Glycerin	$C_3H_8O_3$	92.09	9.12
2	6.385	0.23	0.29	2-methyl-7-oxabicyclo [2.2.1] heptane	$C_7H_{12}O$	112.17	3.49
3	6.780	0.32	0.36	2,5-dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_4$	144.12	3.97
4	6.978	0.67	0.87	Cyclohexanamin, N-3-butenyl-N-methyl-	$C_{11}H_{21}N$	167.29	3.41
5	7.070	0.56	0.51	Methyl-2-furoate	$C_6H_6O_3$	126.11	3.89
6	7.245	0.92	0.79	Levoglucosenone	$C_6H_6O_3$	126.11	5.18
7	7.743	0.57	1.10	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-	$C_6H_8O_4$	144.12	2.30
8	8.031	0.48	0.76	1,2-Epoxy-3-propyl acetate	$C_5H_8O_3$	116.11	2.79
9	8.259	2.48	4.99	Catechol	$C_6H_6O_2$	110.11	2.21
10	8.359	3.56	6.74	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	2.34
11	8.454	0.37	0.87	Benzofuran-2,3-dihydro-	C_8H_8O	120.15	1.87
12	8.870	0.24	0.51	Hydroquinone	$C_6H_6O_2$	110.11	2.06

13	9.469	0.17	0.45	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	1.69
14	9.967	2.56	4.52	1,2,3-Benzenetriol	$C_6H_6O_3$	126.11	2.51
15	10.570	2.45	0.92	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro	$C_4H_9NO_5$	151.12	11.83
16	10.932	0.69	0.42	D-Allose	$C_6H_{12}O_6$	180.16	7.28
17	111.893	0.18	0.41	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,-	$C_8H_{10}O_2$	138.16	2.00
18	12.065	0.27	0.34	Benzenepropanal, 4-hydroxy-alpha-methyl-,	$C_{10}H_{12}O_2$	164.2	3.44
19	12.264	0.53	0.69	Quinic acid	$C_7H_{12}O_6$	192.17	3.41
20	14.986	11.89	1.96	Gamma-sitosterol	$C_{29}H_{50}O$	414.71	26.89
21	15.503	0.23	0.66	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	1.52
22	16.080	0.35	0.30	9-octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.49	5.28
23	16.169	0.70	1.67	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	1.85
24	16.255	0.84	1.81	Cis-9-hexadecenoic acid	$C_{16}H_{30}O_2$	254.41	2.06
25	16.472	7.35	13.18	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	256.42	2.47
26	17.598	0.23	0.67	Cis-11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322.5	1.53
27	17.666	1.89	4.98	9-Octadecenoic acid, methyl ester, (E) -	$C_{18}H_{34}O_2$	282.5	1.68
28	17.819	0.52	1.25	Phytol	$C_{20}H_{40}O$	296.5	1.85
29	17.880	0.46	1.09	Methyl Stearate	$C_{19}H_{38}O_2$	298.5	1.87
30	18.026	56.01	44.46	Oleic acid	$C_{18}H_{34}O_2$	282.5	5.59
31	20.418	0.35	0.81	Hexadecanoic acid, 2-hydroxy-1-(hydromethyl) ethyl ester	$C_{19}H_{38}O_4$	330.5	1.88
32	25.897	0.63	0.98	Vitaine E	$C_{29}H_{50}O_2$	430.7	2.84
33	8.17	0.63	12.09	Allicin	$C_6H_{10}OS_p$	162.3	5.76

Table 2 Compounds identified in *Annona muricata* fruit juice ethanol extract using HPLC analyses

PK	RT	Area	Height	Compound	Molecular formular	Molecular weight (g/mol)
1	44.170	10566	175.989	Tannin	$C_{76}H_{52}O_{46}$	1701.2
2	0.230	2673	77.285	Proanthocyanin	$C_{31}H_{28}O_{12}$	592.5
3	2.390	12463	207.166	Naringin	$C_{27}H_{32}O_{14}$	580.5
4	4.120	6453	109.082	Coumaric acid	$C_9H_8O_3$	164.16
5	6.016	18473	303.874	Flavan-3-ol	$C_{15}H_{14}O_2$	226.27
6	7.466	8209	141.562	Anthocyanin	$C_{47}H_{54}O_{27}$	207.24
7	10.366	19406	325.524	Ribalinidine	$C_{15}H_{17}NO_4$	275.3

8	12.970	6015	102.717	Naringenin	$C_{15}H_{12}O_5$	272.25
9	15.460	4747	81.456	Spartein	$C_{15}H_{26}N_2$	234.38
10	17.966	10838	185.757	Sapogenin	$C_{58}H_{94}O_{27}$	1223.3
11	20.313	12126	207.446	Phenol	C_6H_6O	94.11
12	22.730	9256	157.532	Flavonones	$C_{15}H_{12}O_2$	224.25
13	25.650	9899	166.836	Steroids	$C_{19}H_{28}O_2$	288.4
14	29.860	5193	89.413	Kaempferol	$C_{15}H_{10}O_6$	286.24
15	32.996	14260	237.682	Adhumulone	$C_{22}H_{32}O_5$	375.5
16	34.600	5663	98.627	Cohumulone	$C_{20}H_{28}O_5$	348.4
17	36.876	6483	112.946	Humulone	$C_{21}H_{30}O_5$	362.5
18	39.200	9912	168.507	Catechin	$C_{15}H_{14}O_6$	290.27
19	42.276	3472	58.519	Resveratrol	$C_{14}H_{12}O_3$	228.24

4. Discussion

The exploration of phytochemical compounds within *Annona muricata* fruit juice ethanol extract using GC-MS revealed a wealth of potential health benefits. These phytochemicals included phenolic compounds like flavonoids, phenolic acids, and tannins which exhibit diverse biological activities crucial for human well-being [20]. Their significance extends to mitigating the risk of anti-inflammation, anti-carcinogenicity, anti-atherosclerosis, and degenerative diseases through the reduction of oxidative stress and inhibition of macro-molecular oxidation [21, 22]. Notably, 9-octadecenoic acid (Z)-methyl ester demonstrates anticarcinogenic and antioxidant properties, while hexadecanoic acid methyl ester presents a variety of bioactivities such as antifungal, antioxidant, strong antimicrobial, nematicide, pesticide, anti-androgenic, hemolytic, and 5-alpha reductase inhibitory effects [23, 24, 25]. The 5-Hydroxymethylfurfural exhibits antimicrobial, anti-inflammatory, anti-diabetic, and anti-allergenic properties, and the ketone 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl is known to possess antimicrobial and anti-inflammatory attributes. Oleic acid, a monounsaturated fatty acid, documented by Albrathy, displays a variety of properties including antimicrobial, antifungal, antioxidant, anti-inflammatory, antibacterial, anticancer, antiulcer, antiandrogenic, antihepatotoxic, and dermatogenic effects [26, 27, 28]. Furthermore, 6-octadecanoic acid methyl ester manifests strong antimicrobial, analgesic, anti-inflammatory, and antipyretic properties [29]. Strong antibacterial efficacy against *Escherichia coli* is demonstrated by 9-hexadecanoic acid from *Tribolium castaneum* methanol extract [30]. Additionally, 11,14-eicosadienoic acid exhibits anti-inflammatory, antioxidant, anti-arthritic, and anti-coronary properties [31]. Methyl stearate also functions as a γ-aminobutyric acid (GABA) aminotransferase inhibitor, showcases anti-inflammatory, antihelmintic, antinociceptive, and intestinal lipid metabolism regulating properties [32, 33].

The HPLC analysis performed with the extract of *A. muricata* revealed the presence of various polyphenolic compounds such as quercetin, anthocyanin and kaempferol which possess various health benefits. Prominently, flavonoid like isorhamnetin exhibits potent anti-proliferative effect against human breast cancer [8]. Kaempferol, another prominent constituent, showcases remarkable anti-proliferative and apoptosis-inducing activities in various human carcinoma cells, including osteosarcoma, breast (MCF-7), stomach (SGC-7901), and lung (A549) [34]. Quercetin emerges as a multifaceted player, inducing cell cycle arrest and growth inhibition across diverse malignant tumour cell lines, spanning leukemia, colon, breast, and ovarian cancer cells [35]. The catechins, including catechin, epicatechin, and epigallocatechin gallate, along with the flavonol quercetin, contribute to an oxidative burst, raising reactive oxygen species (ROS) production and subsequently enhancing membrane permeability causing damage [36]. Beyond their anti-proliferative effects, flavonoids such as naringenin, kaempferol, quercetin, and genistein showcase interference with biofilm formation, while quercetin, and myricetin, exhibit inhibitory effects on bacterial DNA replication [37, 38].

5. Conclusion

The phytochemical compounds identified in *Annona muricata* fruit juice ethanol extract, as analysed through GC-MS and HPLC, presented a diverse array of biochemical properties due to their various classes of functional groups. The extract also contained many secondary metabolites dominated by polyphenolic compounds. Thus, the *A. muricata* fruit juice ethanol extract has many compounds of biological importance.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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