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Characterization of the Volatile Bioactive Compounds in Ethylacetate Leaf Extract of *Annona muricata* Linn.

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Abstract: *Annona muricata* Linn (*A. muricata*) leaf has long history of folkloric use for the management of cancer, infections and arthritis. The hexane, ethyl acetate, methanol successive and straight run methanol extracts were screened against standard strains of *Salmonella paratyphi* (ATCC 9150), *Candida albicans* (ATCC 22015) and clinical isolates of *Pseudomonas aeroginosa* ATCC 27853, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsiella pneumoniae* from NIPRD clinic using agar dilution method. The ethyl acetate extract exhibited the strongest antimicrobial activity against *Salmonella paratyphi* (ATCC 9150), *Pseudomonas aeruginosa*, *Candida albicans* (ATCC 2876), *Klebsiella pneumonia* ATCC 13883 and *Escherichia coli* (clinical isolate) at 2000µg/ml, while the hexane, methanol successive and straight run methanol extracts showed no activity against all the test micro-organisms. Amoxicillin, which was used as standard, inhibited the growth of all the test micro-organisms at the same concentration. The ethyl acetate extract was subjected to gas chromatography-mass spectrometry (GC-MS) characterization to identify volatile bioactive compounds. The GC-MS analysis revealed 22 different volatile bioactive compounds including 2,8-dimethylundecane (8.79%), n-hexadecane (8.17%), phytol (8.11%), n-tetradecane (7.76%), 3,7-dimethyl-1,6-octadiene (7.59%), (Z,Z)-α-farnesene (6.98%), 1,11-dodecadiene (6.95%), palmitic acid (6.31%) and α-tridecene (5.05%). These compounds maybe responsible for the observed antimicrobial activities of the ethyl acetate extract.[Samuel Ehiabhi Okhale[1]* and Chinyere Imoisi. **Characterization of the Volatile Bioactive Compounds in Ethylacetate Leaf Extract of *Annona muricata* Linn.** *Life Sci J* 2022;19(11):57-62]. ISSN 1097-8135(print);ISSN2372-613X (online). http://www.lifesciencesite.com. 08.doi:10.7537/marslsj191122.08.

Keywords: *Annona muricata*, GC-MS, Ethylacetate, Antimicrobial, Bioactive, Leaf

1. Introduction

The use of medicinal and aromatic plants in the treatment of various diseases has increased rapidly in both developed and developing countries, attributable to affordability, safety and efficacy. Medicinal plants contain phytochemical substances with pharmacological properties. *Annona muricata* is one of such medicinal plants used for treating diseases including cancer, infections and arthritis (Hamid *et al*., 2012). *A. muricata* commonly known as "soursop" or "graviola", is a tropical tree with aromatic, sweet, and great tasting fruit. *A. muricata* belongs to the Annonaceae family, with about 2500 species and more than 130 genera, it belongs to the genus Annona and the family is concentrated in the tropics, with few species found in temperate regions (Tai *et al*., 2014). *A. muricata* is a slender, evergreen tree, 5-10 m in height and 15 cm in diameter, with an open, roundish canopy and is usually found in tropical countries around the world (Torres *et al*., 2014). The fruit of *Annona muricata* is edible and is used commercially for the production of juice, candy and sherbets (Gajalakshmi *et al*., 2012).

Different parts of the *A. muricata* have been shown to contain a variety of phytoconstituents. Some of the isolated constituents are alkaloids, tannins, sugars, saponins, free fatty acids, steroids, terpenoids, flavonoids, and anthraquinones, also, volatile oils including mono- and sesquiterpenes, triterpenes like α-amyrin, β-amyrin and squalene and esters of aliphatic acids; alkaloids such as anonaine, isolaureline and norisolaureline, annonamine, murisolin, annoreticuin-9-one, annoreticuin and sabadelin; sterols such as β-sitosterol and stigmasterol, β-sitosterone, β-sitosteryl fatty acid ester; megastigmanes like annoionols A and B and annoionoside and several phenolic and antioxidant principles (George *et al*., 2015). *A. muricata* also contained megastigmanes, cyclopeptides as well as minerals, such as K, Ca, Na, Cu, Fe and Mg (Aidy *et al*., 2018).

Most importantly, like other members of the Annonaceae family, *A. muricata* has been shown to contain a special group of compounds known as acetogenins including annoreticuin-9-one, annoreticuin, sabadelin, muricins J, K and L, muricoreacin, murihexocin C, annomuricin E,

annocatacin A, annocatacin B, neoannonin, desacetyluvaricin, bullatacin, asimicin, annoglaucin, squamocin, rollimusin, epomuricenins-A and-B, epomusenins-A and B, epomurinins-A and B, muricatenol, 2,4-cisgigantetrocinone, 2,4-transgigantetrocinone, 2,4-transIsoannonacin-10-one, 2,4-transisoannonacin, gigantetrocins-A and B, annomontacin, gigantetronenin, annonacin, isoannonacin, goniothalamicin and these are responsible for a good number of their biological effects (George *et al*., 2015).

A. muricata has been shown to have quite a number of biological and pharmacological activities such as antidiabetic, anticancer via several mechanisms such as regulation of apoptosis, antiproliferative activity, antinociceptive and anti-inflammatory, larvicidal, antioxidant, antiplasmodial and antiulcer (Moghadamtousi *et al*., 2014). The aim of this study was to identify the volatile bioactive compounds in ethyl acetate extract of *Annona muricata* leaf using gas chromatography-mass spectrometry (GC-MS).

2. Materials and Methods

Reagents

Hexane, ethyl acetate and methanol were of analytical grade purchased from Sigma-Aldrich (Germany).

Plant material and processing

The leaf was collected from the National Institute for Pharmaceutical Research and Development (NIPRD) garden Idu, Abuja, Nigeria. The plant was identified and authenticated by a taxonomist at the herbarium of the Department of Medicinal Plant Research and Traditional Medicine, NIPRD, Abuja, Nigeria. Sample of the plant was deposited in the herbarium for reference purpose with voucher number NIPRD/H/6965. Initial weight of the pulverized plant sample (46g) was macerated with 500ml of hexane, (45g) of marc was macerated with 500ml of ethyl acetate, (40g) of marc was macerated with 400ml of methanol (successive), (25g) of the plant sample was extracted with 70% of methanol. Weight of hexane extract, ethyl acetate extract, methanol extract and 70 % of methanol extract were 0.8421g, 1.1538g, 1.9431g and 1.4758g respectively. Percentage yield of the extracts corresponded to 1.8 %, 2.6%, 4.9%, and 5.9% respectively.

Gas Chromatography-Mass Spectral analysis

The extract was analyzed by GC-MS using Shimadzu QP- 2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy = 70eV), scan range = 45-400amu, and scan rate = 3.99 scans/sec], and Shimadzu GCMS solution data system. The GC column was HP-5MS fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, length 30 m, internal diameter 0.25mm and film thickness 0.25µm. The carrier gas was helium with flow rate of 1.61ml/min. The program used for GC oven temperature was isothermal at 60^0C, followed by 60-180^0C at a rate of 10^0C/min, then held at 180 ^{0}C for 2 minutes; followed by 180-280^0C at a rate of 15^0C/min, then again held at 280^0C for 4 minutes. The injection port temperature was 250^0C. The ionization of sample components was performed in the E.I. mode (70eV). Injector temperature was 250^0C while detector temperature was 280^0C. Helium was used as carrier gas at a flow rate of 1.61ml/min. 1.0µl of diluted sample (1/100 in ethyl acetate, v/v) was injected using auto sampler and in the split mode. Split ratio was 10:90 (Okhale *et al*., 2018).

Antimicrobial activities

Preparation of the test organisms

According to the method of Okhale *et al*. (2014), the test micro-organisms were taken aseptically from their respective slants, sub-cultured into freshly prepared nutrient agar and placed in the incubator for 24 hours at 37ºC. Pure, discrete colonies of the culture were inoculated into normal saline to match 0.5 McFarland standards (approximately 1.25 x 10^6 – 1.25 x 10^7 colony forming units).

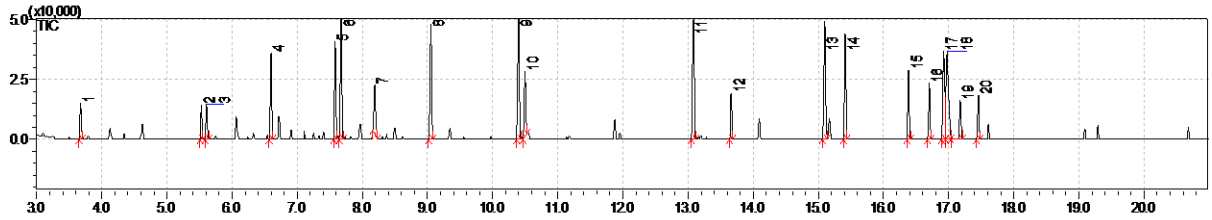
Screening against micro-organisms

According to the method of Okhale *et al*. (2014), the extracts were screened for anti-microbial activity against typed strains of *Salmonella paratyphi* (ATCC 9150) and *Candida albicans* (ATCC 2876) and clinical isolates of *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Escherichia coli*, *Bacillus subtilis* ATCC 21332and *Klebsiella pneumoniae* ATCC 13883 using agar dilution method. Five hundred microliters of dimethylsulphoxide (DMSO) was used to dissolve the crude extracts and made up to 1ml with sterile water. One milliliter each of the dissolved crude extracts (containing 40mg) was introduced into 19 ml of molten nutrient agar placed in water at 45ºC. These were mixed properly and poured into sterile Petri-dishes to give final concentration of 2000µg/ml. The dishes which were prepared in duplicates and then allowed to gel and thereafter, the test microorganisms were inoculated by surface streaking onto the nutrient agar using a wire loop. Control dishes were also set containing only agar and test organisms (organism viability control), dishes containing agar and DMSO and plates containing agar and sterile water, also served as controls. The Petri-dishes were incubated over night at 37ºC for 24hr after which they were observed for microbial growth inhibition. All procedures were done aseptically in the biosafety cabinet to avoid the introduction of unwanted micro-organisms from the environment.

3. Results and Discussion

Table 1: Extraction yields for different extracts of *A. muricata* leaf.

Material weight (g)	Extraction method/ Solvent	Weight of extract (g)	% Yield w/w
46	Maceration, hexane extract	0.9	2.0
45	Maceration, ethyl acetate successive extract	1.2	2.7
40	Maceration, methanol successive extract	2.0	5.0
25	Maceration, 70% of methanol extract	1.5	6.0



Peak#	Name	Kovats Index	Retention	% Composition
1	1,1,3-Trimethylcyclopentane	725		1.65
2	3,7-Dimethyl-1,6-octadiene	949		7.59
3	2,2-Dimethyl-3-hexanone	1179		1.88
4	1,11-Dodecadiene	1179		6.95
5	4,7-Dimethylundecane	1212		4.48
6	2,8-Dimethylundecane	1223		8.79
7	α-Tridecene	1291		5.05
8	Capric acid	1370		2.17
9	n-Tetradecane	1400		7.76
10	(Z,E)-α-Farnesene	1493		4.11
11	(Z,Z)-α-Farnesene	1497		6.98

Figure 1: GC-MS Spectra of *Annona muricata* leaf ethyl acetate extract

12	n-Hexadecane	1600	8.17
13	E-14-Hexadecenal	1825	4.22
14	n-Hexadecanol	1880	2.91
15	1-Nonadecene	1896	3.94
16	Hex-4-enoic acid	1923	1.94
17	Palmitic acid	1975	6.31
18	E-15-Heptadecenal	2085	2.54
19	Phytol	2123	8.11
20	11-Tricosene	2289	2.5
Total % Composition:			98.05

Table 2: GC-MS result of volatile bioactive compounds in *Annona muricata* leaf ethyl acetate extract

Antimicrobial activity

Table 3: Antimicrobial activity of *Annona muricata* leaf extracts

Extracts	**Activity at 2000µg/ml**						
	Sa	**St**	**Bs**	**Ps**	**Ca**	**Kp**	**Ec**
Hexane extract	NA	NA	NA	NA	NA	NA	NA
Ethyl acetate successive	NA	Active	NA	Active	Active	Active	Active
Methanol extract successive	NA	NA	NA	NA	NA	NA	NA
70% aqueous methanol extract	NA	NA	NA	NA	NA	NA	NA

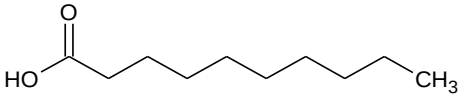
Key: NA = No activity at 2000µg/ml; Sa = *Staphylococcusaureus* (ATCC 25923), St = *Salmonella paratyphi* (ATCC 9150), Bs = *Bacillus subtilis* (ATCC 21332), Ps = *Pseudomonas aeruginosa* (ATCC 27853), Ca = *Candida albicans* (ATCC 2876), Kp = *Klebsiella pneumonia* (ATCC 13883), Ec = *Escherichia coli* (Clinical isolate NIPRD Clinic)

4. Discussion

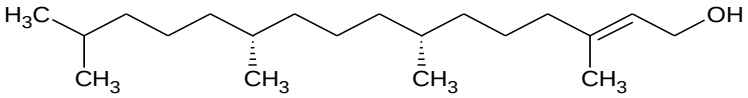
Weight of hexane extract, ethyl acetate extract, methanol extract and 70% of methanol extract obtained were 0.9g, 1.2g, 2.0g and 1.5g respectively. Percentage yield of the extracts corresponded to 2.0%, 2.7%, 5.0%, and 6.0% respectively. The four extracts hexane, ethyl acetate, methanol and 70% aqueous methanol (CEMEST) were screened against seven microorganisms at 2000µg/ml concentration. A single concentration of 2000µg/ml was used for all the extracts as shown in Table 3. Antimicrobial studies of the four extracts revealed that only the ethyl acetate extract was active against five microorganisms namely, *Salmonella paratyphi, Pseudomonas aeroginosa, Candida albicans, Klebsiella pneumonia and Escherichia coli.*

The antimicrobial activity could be linked to the presence of the bioactive components (Li *et al*., 2001). There were 20 different volatile bioactive compounds found from the GC-MS analysis of the ethyl acetate leaf extract of *Annona muricata*. Fig.1 and Table 2 showed the volatile bioactive compounds identified from the ethyl acetate leaf extract of *Annona muricata* through GC-MS analysis. Among them were 2,8-dimethylundecane (8.79%), n-hexadecane (8.17%), phytol (8.11%), n-tetradecane (7.76%), 3,7-dimethyl-1,6-octadiene (7.59%), (Z,Z)-α-arnesene (6.98%), 1,11-dodecadiene (6.95%), palmitic acid (6.31%) and α-tridecane (5.05%), identified as the major bioactive compounds that have different biological properties such as antimicrobial, anti-tumor, anti-fungal, and anti-inflammatory activities (Liu *et al*., 2011).

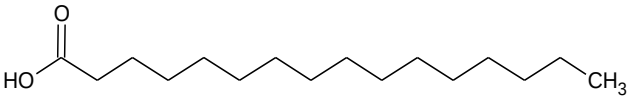
Phytol was also found to be effective at different stages of arthritis (Liu *et al*., 2011). Palmitic acid showed selective cytotoxicity to human leukemic cells, but no cytotoxicity to normal HDF cells. Furthermore, it induces apoptosis in the human leukemic cell. It also shows in vivo antitumor activity in mice. One molecular target of palmitic acid in tumor cells is DNA topoisomerase I, however, interestingly, it does not affect DNA topoisomerase II, suggesting that palmitic acid may be a lead compound of anticancer drugs (Harada *et al*., 2002).



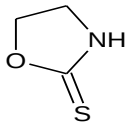
capric acid



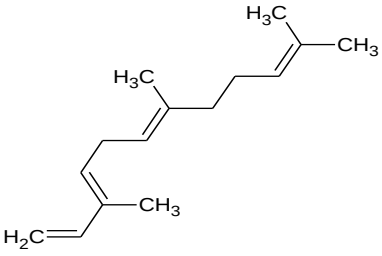
Phytol



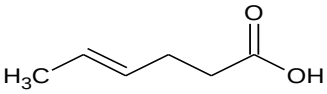
palmitic acid



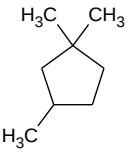
2-thiooxazolidone



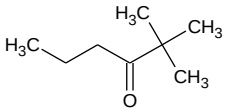
(E,E)-a-farnesene



Hex-4-enoic acid



1,1,3-Trimethylcyclopentane



2,2-dimethyl-3-hexanone

Figure 2: Chemical structures of the volatile constituents of *Annona muricata* leaf extract

The pharmacological and biological benefits of the chemical constituents of *Annona muricata* have been well documented. With respect to the leaf extracts, the pharmacological effects include Anti-inflammatory, anti-arthritic and analgesic. *Annona muricata* have long been used to treat arthritis pain (Hamid *et al.*, 2012). This has been further substantiated by positive results from investigation of the ethanolic leaf extract in rats. Hamid *et al.* (2012) demonstrates the use of the leaves for acute and chronic inflammation; Ishola *et al.* (2014) demonstrates anti-inflammatory and analgesic activity through suppression of inflammatory mediators and interaction with opioid pathway. The ethylacetate leaf extract of *Annona muricata* induced apoptosis on colon and lung cancer cells, besides inhibiting the migration and invasion of colon cancer cells (Moghadamtousi *et al.*, 2014 (a); Moghadamtousi *et al.*, 2014 (b). The ethylacetate leaf extract also induced apoptosis in myelogenous leukemic K562 cells as confirmed by TUNEL assay (Ezirim *et al.*, 2013). Interestingly, a case study of a 66-year old woman with a metastatic breast cancer showed that consumption of *Annona muricata* leaves boiled in water and *Xeloda* resulted in the stabilization of the disease. This anticancer activity has led to tablet formulation of the ethylacetate-soluble fraction of the leaves, which can be used as a cancer adjuvant therapy (Elisya *et al.*, 2014).

Hamid *et al.* (2012) reported that oral administration of the ethylacetate extract of *A. muricata* showed significant anti-ulcer potential. This was due to the protective effects against gastric wall mucosal damage.Some findings suggested the use of *Annona muricata* against jaundice due to potential hepatoprotective activity. In a study, the aqueous extract of *Annona muricata* was screened on phenylhydrazine-induced jaundice in adult rats; results showed significant reduction to hyperbilirubinemia, which almost neared normal levels (Arthur *et al.*, 2012). Little wonder the plant is traditionally used to treat jaundice in Ghana. DRSA, FRAP and HRSA tests on leaf extract of *Annona muricata* showed marked antioxidant property; DNA protective effect against hydrogen peroxide-induced toxicity was also observed (George *et al.*, 2015). This suggests that *Annona muricata* could be a natural source of antioxidants.

The decoction of *Annona muricata* leaves is traditionally used in many African countries to control fever and convulsive seizures (N'gouemo *et al.*, 1997). According to Isela *et al.* (2008), the MIC of ethanolic extract of *Annona muricata*, screened against Herpes Simplex Virus-1 (HSV-1), was found to be 1 mg/ml. this indicates that it could be used as potential antiherpetic drug. The leaf extracts of *Annona muricata* is used in the treatment of various bacterial infectious diseases such as pneumonia, diarrhea, urinary tract infection and even some skin diseases. Its spectrum of activity covers a wide range of bacteria responsible for the most common bacterial diseases (Pathak *et al.*, 2010). Other pharmacological activities documented on the leaf extracts of *Annona muricata* included wound healing, antimalarial and antitrypanosomal (anti-parasitic), antihypertensive, antispasmodic, antidiabetic and hypolipidemic activity and anxiolytic and anti-stress activity (Moghadamtousi *et al.*, 2015).

Conclusion

Twenty (20) different volatile bioactive compounds were found from the GC-MS analysis of ethyl acetate leaf extract of *Annona muricata*. These volatile bioactive compounds identified from ethyl acetate leaf extract of *Annona muricata* maybe responsible for its observed biological and pharmacological properties such as antimicrobial, anti-tumor, anti-fungal, and anti-inflammatory activities, wound healing, antimalarial and antitrypanosomal (anti-parasitic), antihypertensive, antispasmodic, antidiabetic and hypolipidemic activity, anxiolytic and anti-stress activity.

Conflict of interests

The author declared no conflict of interests.

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