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EVALUATION OF THE ANTI-TUMOUR AGENTS IN *ANNONA MURICATA* (LINN.) LEAVES USING COLUMN CHROMATOGRAPHY AND GAS CHROMATOGRAPHY - MASS SPECTROMETRY

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ABSTRACT

Annona muricata which belongs to the family of "Custard-Apple" plants of Annonacae family. has been widely used for its therapeutic benefits. This study was under taken to evaluate the antitumour properties of *Annona muricata* leaves, ethanol extract using column and GC-MS analysis. GC-MS (QP2010SE) analysis of the *Annona muricata* ethanolic leaves extract showed 45 phytocomponents. Some of the prevailing components were 1-Pentadecene (3.21%), 1-Nonadecene (5.63%), Heptacosane (1.06%), 9-Octadecenoic acid (Z)-,phenylmethyl ester (2.17%), 1-Nonadecene (5.52%), 2-Pentadecanone, 6,10,14-trimethyl- (2.67%), Phthalic acid, butyl tetradecyl ester (2.54%), Hexadecanoic acid, methyl ester (2.94%), 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione (1.12%), n-Tetracosanol-1 (3.51%), n-Nonadecanol-1 (2.81%), 1-Heptacosanol (4.41%), Bis (2-ethylhexyl) phthalate (7.63%). The presence of n-Tetracosanol-1, n-Nonadecanol-1 and 1-Heptacosanol suggest the probable use of the plant in ethno-medicine for the treatment of tumour related ailments and other diseases.

KEYWORD: *Annona muricata* leaves, Anti-tumour, ethanol extract, GC-MS.

INTRODUCTION

Annona muricata is a popular tropical fruit with aromatic, sweet, and great tasting fruit that contributes much to the wider economic growth of some of the tropical countries such as tropical America, Australia, African, and Malaysia.[1] It belongs to the family of "Custard-

Apple" plants of Annonacae family. *Annona muricata* is a small, upright tropical evergreen, low-branching, bushy but slender tree, which can reach a height of 7.5-9 m. The large evergreen leaves are smooth and glossy and have a dark green upper surface. The fruits are usually oval or heart-shaped and 10-30 cm long and up to 15 cm in width. The skin of the fruit is leathery and covered with curved, soft, pliable spines. The inside of the fruit is cream-colored and is divided into segments. Closely packed segments are seedless and other segments have a single oval, smooth, hard black seed. One piece of large fruit can contain a dozen to 200 or more seeds.[2] The genus name 'Annona' is from the Latin word 'anon', meaning 'yearly produce', referring to the fruit production habits of the various species in this genus.[3]

Annona muricata leaves are known for its several medicinal uses such as remedy for headaches, insomnia, cystitis, liver problems, diabetes and hypertension and as an anti-inflammatory, antispasmodic and antidysenteric. The decoction of the leaves is also known to have parasiticide, antirheumatic and antineuralgic uses, while the cooked leaves, if applied topically, can alleviate rheumatism and abscesses.[4] The fruit of *Annona muricata* was found to be edible in Yunnan province of China and it is used commercially for the production of juice, candy and sherbets.[5] Although its rind is quite bitter, the fruit's flesh is soft, smooth and sweet and provides carbohydrate as its major nutrient. Soursop also contains abundant vitamin C and several B vitamins such as thiamin, riboflavin and niacin, along with calcium, phosphorus and a small amount of iron.[6]

Intensive chemical investigations of the leaves and seeds of this species have resulted in the isolation of a great number of acetogenins. The isolated compounds display some of the interesting biological or the pharmacological activities, such as antitumoral, cytotoxicity, antiparasitic and pesticidal properties. Roots of these species are used in traditional medicine due to their antiparasitical properties.[7] Various other plants from this family have also been reported for their cytotoxic potential.[8] Traditionally, the bark, leaves, fruit, roots and fruit seeds of *A.muricata* are known for its various medicinal uses. The seeds are crushed and used against internal or external parasites, head lice, and worms. The tea prepared from the leaves in the West Indies and Peruvian Andes are used as a sedative and a soporific (inducer of sleep).[9]

In addition, it is used in medicinal herbal drugs to cure various diseases such as diarrhea, cough, hypertension, rheumatism, tumors, cancer, asthma, childbirth, lactagogue, malaria,

tranquilizer, skin rashes, parasites, worms, liver problems, arthritis etc.[10] It contains a variety of components which attribute to the various biological activities. The roots and bark can be of aid for diabetes, but can also be used as a sedative.[11] These leaves are used to prevent and treat arthritis, asthma, bronchitis biliary disorder, diabetic, heart diseases, hypertension, worm disease, liver disorder, malaria, rheumatism, sedative, tumor and cancer. Also, it is used for the treatment of several types of diseases caused by bacteria such as pneumonia, diarrhea, urinary tract infection and other kinds of skin diseases. This plant has numerous benefits for human life due to high nutrient value. In the food industry soursop can be processed into jam, fruit juice, syrup. Soursop leaves contain flavonoid, tannin, alkaloid, saponin, calcium, phosphor, carbohydrate, vitamin A, B and C, phytosterol, calcium oxalate etc. Plant and plant-derived compounds are alternative sources for treating microbial infections.[5,7,12-14] This study aimed at evaluating the anti-tumour properties of ethanolic extract of *Annona muricata* leaves using column chromatography and GC-MS analysis.

MATERIALS AND METHODS

PLANT MATERIAL

The fresh leaves of *Annona muricata* were collected in July, 2015 from Eziobodo, Owerri west Local Government Area in Imo state, Nigeria. It was identified by Mr. Francis Nwaeze, a senior superintendent of the Forestry and Wildlife Technology Department at Federal University of Technolgy, owerri.

PREPARATION OF PLANT EXTRACT

The leaves were dried in the laboratory at room temperature (25-30^{o}c) and pulverized to powder in a mechanical grinder. 100g of plant sample was weighed, transferred to flask, treated with 500ml of absolute ethanol, incubated for 48 hours and filtered through a Whatman No.1 filter paper. Then, the filtrate is concentrated till dryness at temperature below 60^{o}C using water bath. Afterward, the residue was weighed and further be subjected to purification using column chromatography.

COLUMN CHROMATOGRAPHY

The ethanol extract of *Annona muricata* leaves was separated and purified through silica gel G (60-200 mesh size) column chromatography with various solvent of increasing polarity (n-hexane, ethyl acetate and methanol) in gradient step starting with n-hexane, n-hexane: ethyl acetate, ethyl acetate: metthanol and final elution was performed with 100% methanol. These yielded several fractions (54) which were collected in glass vials. All the fractions

were applied on to the precoated silica gel TLC plates and chromatographed using the solvent system of ethyl acetate: methanol in the ratio of 8:1. Plates were examined under UV and visible light to combine similar fractions. The following samples were combined together due to similarities in colour. 1-5 (100% n-Hexane to 90:10 n-Hexane/ethyl acetate), 6-10 (90:10 n-Hexane/ethyl acetate to 80:20 n-Hexane/ethyl acetate), 11-43 (80:20 n-Hexane/ ethyl acetate to 60:40 n-Hexane/ethyl acetate), 44-46 (60:40 n-Hexane/ethyl acetate to 100% ethyl acetate), 47-54 (80:20 ethyl acetate/ methanol to 100% methanol). Finally fractions 11-43 and 47-54 were combined and concentrated over a water bath at temperature below 40^{o}C. The fractions were subjected to GC-MS analysis to determine the bioactive constituents.[15]

Gas Chromatography - Mass Spectrometry (GC-MS) Analysis

Gas Chromatography - Mass Spectrometry (GC-MS) Analysis GC-MS analysis was carried out on GCMS-QP2010SE Shimadzu, Japan and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30x0.25mm 1Dx1µ df, composed of 100% dimethyl polysiloxane). For GC-MS detention, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 1µl was employed (split ratio of 10:1) injector temperature- 250^{o}C; ion source temperature 280^{o}C. The oven temperature was programmed from110^{o}C (isothermal for 2 min.) with an increase of 10^{o}C/min. to 200^{o}C then 5^{o}C/min. to 280^{o}C/min, ending with a 9 min. isothermal at 280^{o}C. Mass spectra were taken at 70eV; a scan of 0.5s and fragment from 40 to 550Da. Total GC running time was 11.4 minutes. The relative percentage amount of each component was calculated, by comparing its average peak areas and heights to their total respectively, software adopted to handle mass spectra and chromatogram was a turbo-mass. NIST version 1.0 year 2011 library was used for detection.

Identification of Components

Interpretation on mass spectrum of GC-MS was done using the data base of National Institute of Standard and Technology (NIST) having more than 62, 000 patterns. The mass spectrum of the known components were stored in the NIST library. The name, molecular weight and structure of the components of the test materials were determined by applying standard interpretation techniques.

RESULTS AND DISCUSSION

GC-MS analysis of the ethanolic extract and eluents of *Annona muricata* leaves revealed the presence of forty five (45) phyto-compounds in the chromatogram (**Figure 1**). The compounds with their retention time (RT), molecular formula, molecular weight (MW) and percentage composition are presented in **Table 1**.

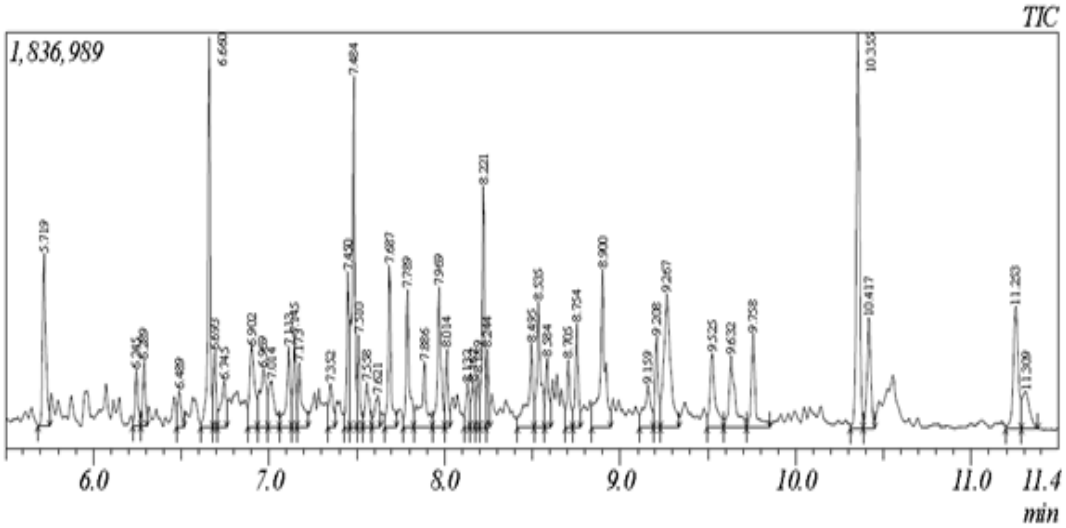

Fig. 1: GC-MS Chromatogram of ethanol extract and combined eluents of *Annona muricata* (Linn.) leaves.

The compounds confirmed in the spectrum profile of GC-MS were 1-Pentadecene (3.21%), Heptacosane (0.94%), Tetradecanal (0.99%), Toluene-4-sulfonic acid, 2,7-dioxatricyclo [4.3.1.0(3,8)]dec-10-yl ester (0.69%),1-Nonadecene (5.63%), Heptacosane (1.06%), 14-Octadecenal (1.33%), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (2.41%), 9-Octadecenoic acid (Z)-, methyl ester (2.17%), 10-Octadecenal (1.48%), Octacosane (1.78%), Hexadecane, 2,6,10,14-tetramethyl- (1.28%), Heptadecanal (1.50%), 2-Dodecen-1-yl(-)succinic anhydride (0.96%), Benzyl Benzoate (2.34%), 1-Nonadecene (5.52%), Hexacosane (1.23%), 2-methyltetracosane (1.04%), Acetic acid, 17-(4-hydroxy-5-methoxy- 1,5 dimethylhexyl)-4,4,10,13,14-pentamethyl- (0.88%), 2-Pentadecanone, 6,10,14-trimethyl- (2.67%), Phthalic acid, butyl tetradecyl ester (2.54%), 2-methylhexacosane (2.09%), Hexadecanoic acid, methyl ester (2.94%),

Table 1: Phyto-compounds detected in the combined eluent fractions from column chromatography of the ethanolic extract of *Annona muricata* Leaves.

Peak#	RT	Name of Compound	Molecular formula	MW	Area%
1	5.719	1-Pentadecene	$C_{15}H_{30}$	210	3.21
2	6.245	Heptacosane	$C_{27}H_{56}$	380	0.94
3	6.289	Tetradecanal	$C_{14}H_{28}O$	212	0.99
4	6.489	Toluene-4-sulfonic acid, 2,7-dioxatricyclo [4.3.1.0(3,8)]dec-10-yl ester	$C_{15}H_{18}O_5S$	310	0.69
5	6.66	1-Nonadecene	$C_{19}H_{38}$	266	5.63
6	6.693	Heptacosane	$C_{27}H_{56}$	380	1.06
7	6.745	14-Octadecenal	$C_{18}H_{34}O$	266	1.33
8	6.902	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	2.41
9	6.969	9-Octadecenoic acid (Z)-, methyl ester	$C_{25}H_{40}O_2$	296	2.17
10	7.014	10-Octadecenal	$C_{18}H_{34}O$	266	1.48
11	7.113	Octacosane	$C_{28}H_{58}$	394	1.78
12	7.145	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282	1.28
13	7.173	Heptadecanal	$C_{17}H_{34}O$	254	1.50
14	7.352	2-Dodecen-1-yl(-)succinic anhydride	$C_{16}H_{26}O_3$	266	0.96
15	7.45	Benzyl Benzoate	$C_{14}H_{12}O_2$	212	2.34
16	7.484	1-Nonadecene	$C_{19}H_{38}$	266	5.52
17	7.51	Hexacosane	$C_{26}H_{54}$	366	1.23
18	7.558	2-methyltetracosane	$C_{25}H_{52}$	352	1.04
19	7.621	Acetic acid, 17-(4-hydroxy-5-methoxy-1,5 dimethylhexyl)-4,4,10,13,14-pentamethyl-	$C_{33}H_{56}O_4$	516	0.88
20	7.687	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	2.67
21	7.789	Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	418	2.54
22	7.886	2-methylhexacosane	$C_{27}H_{56}$	380	2.09
23	7.969	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.94
24	8.014	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	1.12
25	8.132	Phthalic acid, butyl undecyl ester	$C_{23}H_{36}O_4$	376	0.88
26	8.162	2-methyltetracosane	$C_{25}H_{52}$	352	0.88
27	8.189	Acetic acid, 17-(4-hydroxy-5-methoxy1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-	$C_{33}H_{56}O_4$	516	0.84
28	8.221	n-Tetracosanol-1	$C_{24}H_{50}O$	354	3.51
29	8.244	2-methylhexacosane	$C_{27}H_{56}$	380	1.03
30	8.495	4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester	$C_{13}H_{15}NO_3$	233	2.44
31	8.535	n-Nonadecanol-1	$C_{19}H_{40}O$	284	2.81
32	8.584	2-methylhexacosane	$C_{27}H_{56}$	380	1.27
33	8.705	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	592	1.24
34	8.754	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268	1.82
35	8.9	1-Heptacosanol	$C_{27}H_{56}O$	396	4.41
36	9.159	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	502	1.62
37	9.208	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	1.75

38	9.267	Docosanoic acid, docosyl ester	$C_{44}H_{88}O_2$	648	5.20
39	9.525	5-Octadecenal	$C_{18}H_{34}O$	266	2.30
40	9.632	1-Heptacosanol	$C_{27}H_{56}O$	396	2.57
41	9.758	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	2.30
42	10.36	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	7.63
43	10.42	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	2.46
44	11.25	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	3.66
45	11.31	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	1.53
		Total Percentage Composition			100

7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione (1.12%), Phthalic acid, butyl undecyl ester (0.88%), 2-methyltetracosane (0.88%), Acetic acid, 17-(4-hydroxy-5-methoxy1,5- dimethylhexyl)-4,4,10,13,14-pentamethyl- (0.84%), n-Tetracosanol-1 (3.51%), 2-methylhexacosane (1.03%), 4-Oxazolecarboxylic acid, 4,5-dihydro-2- phenyl-, 1-methylethyl ester (2.44%), n-Nonadecanol-1 (2.81%), 2-methylhexacosane (1.27%), Cyclooctasiloxane, hexadecamethyl- (1.24%), Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate (1.82%), 1-Heptacosanol (4.41%), 7,8-Epoxylanostan-11-ol, 3-acetoxy- (1.62%), Cyclononasiloxane, octadecamethyl- (1.75%), Docosanoic acid, docosyl ester (5.20%), 5-Octadecenal (2.30%), 1-Heptacosanol (2.57%), Cyclononasiloxane, octadecamethyl-(2.30%), Bis(2-ethylhexyl) phthalate (7.63%), Cyclononasiloxane, octadecamethyl- (2.46%), Cyclononasiloxane, octadecamethyl- (3.66%), Cyclononasiloxane, octadecamethyl- (1.53%) (**Table 1**).

Yahaya G., et al.[16] carried out GC-MS analysis of bioactive phytochemicals present in ethanolic extracts of leaves of *Annona muricata*. The result obtained from the GC-MS analysis of ethanol leaf extract of *Annona muricata* reveals presence of twenty-five different bioactive compounds. 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%), Oleryl 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.[16]

Some major phyto-compounds and their biological activities obtained through the GC-MS study of the *Annona muricata* leaf extract have been tabulated (**Table 2**). These phytocompounds are responsible for various pharmacological actions of the leaves of the plant.

Table 2: Activity of some phytocomponents identified in the combined eluent fractions from column chromatography of the ethanolic leaf extract of *Annona muricata* using GC-MS.

S/N	Name of Compound	Nature of Compound	Activity
1	1- Nonadecene	Fatty Hydrocarbon	Antibiotic.[13] [17] [18]
2	9-Octadecenoic acid (Z)-,methyl ester	Fatty acid Ester	Antioxidant activity, Anticarcinogenic.[20]
3	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	Ketone	Antimicrobial.[13] [17]
4	n-Tetracosanol-1	Aliphatic alcohol	Anti-bacterial, Anticancer .[17] [18]
5	n-Nonadecanol-1	Aliphatic alcohol	Anti-microbial, Cytotoxic.[17] [19]
6	1-Heptacosanol	Straight chain primary alcohol	Flavor and fragrance agent, cholesterol Lowering, Antimicrobial and Cytotoxicity.[19] Antithrombotic.[13]
7	Heptacosane	Aliphatic	Antioxidant activity.[20]
8	Hexadecanoic acid, methyl ester	Fatty acid Ester	Antifungal, Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavour, haemolytic, 5-Alpha reductase inhibitor, potent antimicrobial activity.[20]
9	Bis(2-ethylhexyl) phthalate	Aromatic Fatty ester	Antimicrobial, Antibacterial.[21] [22]

The structures of some phytocomponents identified in the combined eluent fractions from column chromatography of the ethanolic leaves extract of *Annona muricata* using GC-MS were shown in **Figures 2_{a-i}.**

Fig. 2_a: 1 – Nonadecene.

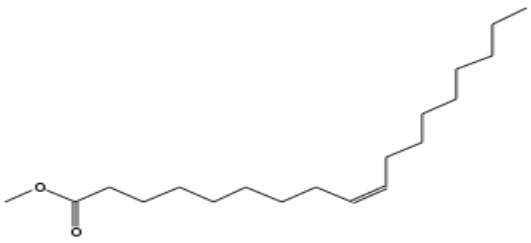

Fig. 2_b: 9-Octadecenoic acid (Z)-, methyl ester.

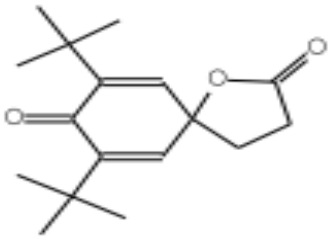

Fig. 2_c: 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione.

Fig. 2_d: n-Tetracosanol-1.

Fig. 2_e: n-Nonadecanol-1.

Fig. 2_f: 1-Heptacosanol.

Fig. 2_g: Heptacosane.

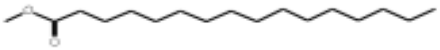

Fig. 2_h: Hexadecanoic acid, methyl ester.

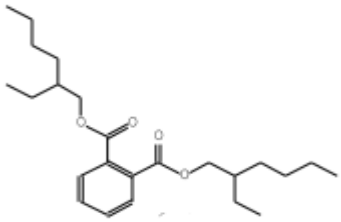

Fig. 2_i: Bis(2-ethylhexyl) phthalate.

1- Nonadecene, a long-chain fatty acid has been reported to be antibiotic.[13][17][18] Ketone 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione has also been reported to possess antimicrobial activity.[13][17] Long chain aliphatic alcohol n-tetracosanol has been found to exhibit anti-bacterial[21] and anticancer activities.[17][18] It has also been reported that long-chain fatty alcohol n-Nonadecanol-1 exhibit anti-microbial and cytotoxic activities.[17][19]

Straight chain primary alcohol 1-Heptacosanol has also been reported to be a flavor and fragrance agent, lower cholesterol and has antimicrobial, cytotoxic,[19] and antithrombotic activities.[13] 9-Octadecenoic acid (Z)-, methyl ester has been reported to be anticarcinogenic and antioxidant Heptacosane also has antioxidant activity (**Table 2**). Hexadecanoic acid**,** methyl ester was reported to have hypocholesterolemic, antifungal, antioxidant, potent antimicrobial, nematicide, pesticide, anti-androgenic flavour, haemolytic, 5-Alpha reductase inhibitory activities.[20] Bis(2-ethylhexyl) phthalate is the compound which has the highest peak height and area from the GC-MS has been reported to exhibit an antimicrobial activity against gram positive bacteria and some parhogenic fungi.[21] It has exhibited a better broad spectrum of antibacterial activity against both Gram positive (*Sta-phylococcus aureus, Bacillus subtilis and Sarcina lutea*) and Gram negative (*Escherchia coli, Shigella sonnei, Shigella shiga and Shigella dysenteriae*) bacteria, with inhibition zones in the range of 07~20 mm.[22]

It has also been reported that free fatty acids including long chain unsaturated fatty acids exhibit antibacterial, anti-inflammatory and antifungal activity.[23] Phthalic acid derivatives were suggested to have been used to cure chronic cardiovascular and cerebrovascular diseases and had anti-tumour, anti-inflammatory, antibacterial functions.[24] Phthalates are reported to have antimicrobial and other pharmacological activities.[25] The anti-microbial activities were believed to be due to phthalic acid derivative.[26] Several authors have shown that natural aromatic compounds possess important biological activities, such as antitumor, antihepatotoxic, antioxidant, anti-inflammatory, estrogenic and antibacterial activities.[17][27]

CONCLUSION

In the present study 45 phytocompounds from the ethanol leaves extract *Annona muricata* (Linn.) were identified by Gas-chromatography– Mass spectrometry (GC-MS) analysis. This study has shown that the leaves extract of *A. muricata* which is extensively rich in bioactive compounds has a high potential pharmacological activities. The presence of n-Tetracosanol-1, n-Nonadecanol-1 and 1-Heptacosanol suggest the probable use of the plant in ethno-medicine for the treatment of tumour related ailments and other diseases. These findings have provided scientific basis for the claimed ethno-medicinal usage of the plant in treating various ailments including tumours. However, further works on isolation of the individual phytochemical constituents, subjecting it to biological activity and toxicity tests will give fruitful results in developing new drugs.

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REFERENCES

1. Moreno-Hernández, C. L., Sáyago-Ayerdi, S. G., García-Galindo, H. S., De Oca, M. M. M, Montalvo- G. Effect of the Application of 1-Methylcyclopropene and Wax Emulsions on Proximate Analysis and Some Antioxidants of Soursop (Annona muricata L.). The Scientific World Journal. 896853, 2014.
2. Adewole, S. O., Ojewole, J. A. O. Protective Effects of Annona muricata Linn.(Annonaceae) Leaf Aqueous Extract on Serum Lipid Profiles and Oxidative Stress in Hepatocytes of Streptozotocin-treated Diabetic Rats. Afr. J. Trad. CAM, 2009; 6(1): 30 – 41.
3. Prasad, P. M., Sajala, P, S., Suresh, K. C. Nutraceuticals: Concept and Regulatory Scenario. International Journal of Pharmacy and Pharmaceutical Science, 2010; 2(2): 14-20.
4. De Sousa, O. V., Vieira, G. D. V., José de Jesus, R. G., De Pinho, J. J. R. G., Yamamoto, C. H. and Alves, M. S. Antinociceptive and Anti-Inflammatory Activities of the Ethanol Extract of Annona muricata L. Leaves in Animal Models. Int. J. Mol. Sci., 2010; 11: 2067-78.
5. Chao-Ming, L., Ning-Hua, T., Hui-Lan, Z., Qing, M., Xiao-Jiang, H., Yi-Neng, H. and Jun, Z. Cyclopeptide from the Seeds of Annona muricata. Phytochemistry, 1998; 48(3): 555-556.
6. Rutkowski, M., Grzegorczyk, K. Modifications of Spectrophotometric Methods for Antioxidative Vitamins Determination Convenient in Analytic Practice. Acta Sci Pol Technol Aliment, 2007; 6: 17-28.
7. Christophe, G., Alain, L., Reynald, H., Olivier L., Laurent S. and Andre C. Acetogenins from Roots of Annona muricata. Phytochemistry, 1997; 44(8): 1541-1545.
8. Paarakh, P. M, Chansouria, J. P. N., Khosa, R. L. Wound Healing Activity of Annona muricata extract. Journal of Pharmacy Research, 2009; 2: 404-406.
9. Technical Data Report for Graviola (Annona muricata). Sage Press, Inc., 2002.
10. Schultes, R. E. and Raffauf. The Healing Forest: Medicinal and Toxic plants of the Northwest Amazonia. Portland: R. F. Dioscorides Press., 1990.

11. Morton, J. F. Caribbean and Latin American Folk Medicine and its Influence in the United States. Q. J. Crude Drug Res., 1980; 18(2): S57-75.
12. Duraipandiyan, V., Ayyanar, M., Ignacimuthu, S. Antimicrobial Activity of Some Ethnomedicinal Plants Used by Paliyar Tribe from Tamil Nadu, India". BMC Complement Altern Med., 2006; 6: 35.
13. Wélé, A., Zhang, Y., Caux, C., Brouard, .J. P., Pousset, J. L. Annomuricatin C, a Novel Cyclohexapeptide from the Seeds of Annona muricata. C R Chim., 2004; 7: 981-988.
14. Jaramillo, M. C, Arango, G. J., Gonzalezb, M. C., Robledo, S. M., Velez, I. D. Cytotoxicity and Antileishmanial Activity of Annona muricata Pericarp. Fitoterapia, 2000; 71: 183-186.
15. Gayathri, G., Vijayalakshmi, K., (Late) Ariamuthu, S. GC-MS and HPTLC Fingerprinting of Bauhinia Variegata Leaves for Anticancer Activity. World Journal of Pharmaceutical Research, SJIF Impact Factor 5.04, 2014; 3(9): 1313-1336. Research Article. ISSN 2277-7105.
16. Yahaya, G., Abou-Elella, F., Fred, W. and El-Sheny, H. A. GC-MS Analysis of Bioactive Phytochemicals Present in Ethanolic Extracts of Leaves of Annona muricata". Pharmacognosy Journal, 2015; 7(5): 302.
17. Ogukwe, C. E., Okhale, S. E., Ezugwu, B. O. Phytochemical and GC-MS Analyses of The Bioactive Components of Securidaca Longepedunculata (Fresen) Roots for Anti-Breast Cancer Activity. World Journal of Pharmaceutical Research, SJIF Impact Factor 5.990, 2015; 4(12): 1503-1518. Research Article ISSN 2277–7105.
18. Kalaivani, M. K., Bhavana J., Sumathy, A. GC-MS Analysis of Chloroform Extract of Croton Bonplandianum. International Journal of Pharma and Bio Sciences, 2013; 4(4): 613-617. ISSN 0975-6299.
19. Isoe, M. E., Alagar, Y. S., Deivamarudachalam, T, P. D. Spectral Analyses of the Bioactive Compounds Present in the Ethanolic Leaf Extract of Strobilanthes kunthiana (Nees) T. Anderson ex. Benth. Advances in Bioresearch (Adv. Biores)., 2015; 6(3): 65-7.
20. Akpuaka, A., Ekwenchi, M. M., Dashak, D.A., Dildar, A. Biological Activities of Characterized Isolates of n-Hexane Extract of Azadirachta indica A. Juss (Neem). New York Science Journal 2013; 6(6): 119-124. (ISSN: 1554-0200). http://www.sciencepub.net/newyork [accessed Dec. 6, 2015].
21. Srinivasan, G. V., Sharanappa, P., Leela, N. K., Sadashiva, C. T. and Vijayan, K. K. Chemical Composition and Antimicrobial Activity of the Essential oil of Leea indica (Burm. f.) Merr. Flowers. Natural product Radiance, 2009; 8(5): 448-493.

22. Habib, M. R. and Karim, M. R. Antimicrobial and Cytotoxic Activity of Di-(2-ethylhexyl) Phthalate and Anhy-drosophoradiol-3-acetate Isolated from Calotropis gigantea (Linn.) Flower. Mycobiology, 2009; 37(1): 31-36.
23. Ogunlesi, M., Okiei, W., Osibote, E. A. Analysis of The Essential Oil from the Leaves of Sesamum Radiatum, a Potential Medication for Male Infertility Factor, by Gas Chromatography - Mass Spectrometry. African Journal of Biotechnology, 2010; 9(7): 1060-1067. Available online at http://www.academicjournals.org/AJB
24. Shengbo, G., Wanxi, P., Dongli, L. Bo, M., Minglong, Z., Daochun, Q. Study on Antibacterial Molecular Drugs in Eucalyptus granlla Wood Extractives by GC-MS. Pak. J. Pharm. Sci., 2015; 28(4): 1445-1448.
25. Saranya, D. K., Sruthy, P. B., Anjana, J. C., Rathinamala, J., Jayashree, S. GC-MS Analysis of Phytocomponents in Resin of Araucaria Columnaris (Cook Pine) and its Medicinal Uses. International Journal of Applied Biology and Pharmaceutical Technology, 2013; 4(3): 272-276. Available online at www.ijabpt.com. [accessed Dec. 6, 2015].
26. Nakalembe, I., Kabasa, J. D. Anti-microbial Activity and Biochemical Constituents of Two Edible and Medicinal Mushrooms of Mid-Western, Uganda. Research Journal of Pharmacological, 2012; 6(1): 4-11. ISSN 1815-9362.
27. Rizvi, S. M. D., Shaikh, S., Sharma, S. K., Shakil, S., Abuzenadah, A. M., Aaqil, H., Sharma, D. C., Khan, S., Manaal Zahera, M., Tiwari, R. K. Combating Multi-Drug Resistance in E. coli and S. aureus with Methanolic Flower Extracts of Spilanthes oleracae and Estimating its Phytochemical Constitutes. World Journal of Pharmaceutical Research, SJIF Impact Factor., 5.990, 2015; 4(8): 1867-1887. Research Article. ISSN 2277-7105.