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STRUCTURAL ELUCIDATION OF PHYTOCHEMICAL CONSTITUENTS OF *ANNONA MURICATA* LEAF USING GAS CHROMATOGRAPHY-MASS SPECTROSCOPY AND FOURIER TRANSFORM INFRARED TECHNIQUES

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Abstract

The phytochemical constituents of the powdered leaf of *Annona muricata* was investigated using Gas Chromatography-Mass Spectroscopy and Fourier Transform Infrared Techniques. The compounds detected in the sample were analyzed using National Institute of Standards and Technology (NIST) library. The GC-MS detected sixteen compounds that were identified as 3, 5-Dihydroxy-6-methyl-2, 3-dihydro-4H-pyran-4-one (3.05%), Iberin nitrile (6.24 %), 7,12-Dihydro-6,7-bis(4-hydroxyphenyl)-6H-[1,2,4]triazolo [1',5':1,2]pyrimido[5,4c]chromen-2-ol (2.11 %), Cyclopentane, 4-cyano-2,2-dimethyl-1-methylene (4.06%), Cyclopentane-4-cyano-2,2-dimethyl-1-methylene (2.19%), Methyl-14-methylpentadecanoate (3.83%), 1-Pentadecanecarboxylic acid (13.62%), Methyl trans, trans-9,12-octadecadienoate (1.67%), 6-Methyl-1-heptanol (1.28%), Methyl-cis-9, 12, 15-octadecatrienoate (1.19%), cis-9-Octadecenoic acid (45.09%), Hystrene T-70 (11.73%), cetane (0.55%), 9-Octadecenal (1.21%), 2-Methyl-Z, Z-3,13-octadecadienol (1.36%) and icosane (0.81%). Similarly, the FTIR spectrum gave seven peaks that corresponded to sulfonic acids, sulfoxides, alcohols, nitroso compounds and halogeno, charged amines, alkanes and primary amides and free NH respectively. The phytochemicals exerts different pharmacological activities individually and synergistically.

Keywords: *phytochemicals, Spectroscopy, structural elucidation, Annona muricata*

Introduction

Diseases have been ravaging humanity since the origin of man. One of such diseases is cancer. Globally, cancer exerts heavy health burden, as researchers are yet to unravel the exert cause and cure of the disease. In Africa, the burden is worst due to inadequacy in health facilities and substandard drugs being supplied. Plants had contributed immensely to the treatment of human diseases [1]. As a result, the use of plant derived medicines become an option to source for cancer cure. *Annona muricata* is one of efficacious plant being used locally in managing cancer case especially prostate cancer. It has been reported to possess anticancer activity [2].

Annona muricata belongs to the family of *Annonaceae* [3]. It is an evergreen plant that is mainly found in the tropical and subtropical regions [2]. Different parts of *A. muricata* have been employed in the preparation of traditional medicine because of their ethnomedicine activities [3]. It is in use as remedy for arthritic pains, treatment of malaria, rheumatism and skin rashes [2]. It also exhibited antibacterial activity [4]; [5]. Other pharmacological activities as reported by researchers include anti-inflammatory, antihelminthic, free radical scavenging activity and antiulcer [3]. These pharmacological properties are functions of the phytochemicals. They are metabolites that exert pharmacological activities on human health [6]. Similarly, Florence *et al*. [7] reported that the substances derived from plants responsible for the protection of human health are phytochemicals. It became pertinent to evaluate the phytochemicals of the leaf of *Annona muricata* using gas chromatography-mass spectroscopy and Fourier transform infrared techniques.

Materials and Methods

The leaf of *Annona muricata* was plucked from its tree at Edem-ani, in Nsukka Local Government Area of Enugu state, Nigeria. The plant was identified by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State. They were washed with distilled water to ensure there is no contamination of the sample with impurities and dried at room temperature. The dried leaf was ground to fine powder using an electric blender. The crude powdered sample was stored for GC-MS/FTIR analysis.

Gas Chromatography-Mass Spectrometry and FTIR analysis

Gas Chromatography-Mass Spectroscopy (GCMS-QP2010 plus Shimadzu, Japan), system is a very efficient technique commonly used for the identification and quantification of compounds. The unknown organic compounds in the complex mixture found in the leaf were matched with the National Institute of Standards and Technology (NIST) library.

The Fourier transform infrared spectroscopy (FTIR-8400S Shimadzu, Japan) was used. The leaf was dried in the oven at 60°C and ground into fine powder, using electric blender. From the prepared sample, two milligrams of the sample was weighed and mixed with 100 mg KBr (FT-IR grade) before compressing it to prepare a salt-disc (3 mm diameter). The disc was immediately transferred to the sample holder and FT-IR spectra were recorded in the absorption range between 500 and 4000 $^{-1}$cm.

Results and Discussion

Gas Chromatography-Mass Spectrometry analysis (GC-MS) has been reported by researchers as one of the best methods used in the identification of bioactive compounds [8]; [9]. The spectra of the study revealed sixteen peaks that corresponded to sixteen compounds from *A .muricata* leaf. The compounds were confirmed by their retention time, percentage area, molecular weight and formulae respectively. From the percentage area calculated, it was observed that 78.51 % of the phytochemicals detected were fatty acids. The major components identified in this leaf were peak 11, which showed the highest composition of 45.09 %, followed by peaks 7 (13.62 %), 12 (11.73 %) and 2 (6.24 %).

The identified phytochemicals present included 3, 5-Dihydroxy-6-methyl-2, 3-dihydro-4H-pyran-4-one (3.05%), Iberin nitrile (6.24 %), 7,12-Dihydro-6,7-bis(4-hydroxyphenyl)-6H-[1,2,4]triazolo [1',5':1,2]pyrimido[5,4c]chromen-2-ol (2.11 %), Cyclopentane, 4-cyano-2,2-dimethyl-1-methylene (4.06%), Cyclopentane-4-cyano-2,2-dimethyl-1-methylene (2.19%), Methyl-14-methylpentadecanoate (3.83%), 1-Pentadecanecarboxylic acid (13.62%), Methyl trans, trans-9,12-octadecadienoate (1.67%), 6-Methyl-1-heptanol (1.28%), Methyl-cis-9, 12, 15-octadecatrienoate (1.19%), cis-9-Octadecenoic acid (45.09%), Hystrene T-70 (11.73%), cetane (0.55%), 9-Octadecenal (1.21%), 2-Methyl-Z, Z-3,13-octadecadienol (1.36%) and icosane (0.81%) as

shown in Table 1. 5-Dihydroxy-6-methyl-2, 3-dihydro-4H-pyran-4-one has been reported to possess antimicrobial and antiinflammatory activities [8]. 9,12-Octadecadienoic acid, ethyl ester is a linoleic acid which has hypocholesterolemic,5-alpha reductase inhibitor and antihistaminic properties and n-Hexadecanoic acid has nematicide, pesticide, anti-androgenic, flavor, hemolytic 5-alpha reductase inhibitor, antioxidant and hypo-cholesterolemic properties [10]. 9,12-octadecadienoic acid (z, z) had been reported by previous researchers to have anti-inflammatory, nematicide, cancer preventive, hypocholesterolemic and hepatoprotective activities [11]. Similarly, 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- had been reported to possess antimicrobial and anti-inflammatory activity [12].

The GC-MS screening of *Xylopia aethiopica* fruit revealed the presence of fifteen compounds including oleic acid and linoleic acid [9]. Similarly, the bioactive components of *Physalis minima* leaves were evaluated using GC-MS as reported by Karpagasundari and Kulothungan [13]. The constituents of *Vitex negundo* leaf analyzed using GC-MS revealed the presence of 9, 12, 15-Octadecatrienoic acid, (Z,Z,Z)-linolenic acid, which possesses anti-inflammatory, cancer preventive and hepatoprotective properties [14]. The GC-MS analysis of methanolic [8] and ethanolic [15] extract of *A. muricata* revealed a common compound (9, 12-octadecanoic acid), also identified in this analysis.

FTIR has been employed by previous researchers as one of the reliable tools for the analysis of bioactive compounds [16]; [9]. Figure 2 showed the various FTIR absorption peaks of the leaf. At 632.67 cm^{-1} there was absorption due to the presence of S=O indicating the presence of sulfonic acids in the leaf. Also, a peak was observed at 1087.89 cm^{-1}, which indicated the presence of S=O (sulfoxides). Subsequently, peaks were observed at 1411.94 cm^{-1}, 1620.26 cm^{-1}, 2353.23 cm^{-1}, 2924.18 cm^{-1}, 3394.83 cm^{-1} corresponding to O-H (Alcohols), N=N (Nitroso compounds and halogeno), $C=NH^{+}$ (charged amines), C-H (Alkanes) and N-H (free amides free NH) (Figure 2, Table 2).

Conclusion

The results of this study revealed the presence of diverse important phytochemicals such as 3, 5-Dihydroxy-6-methyl-2, 3-dihydro-4H-pyran-4-one (3.05%), Iberin nitrile (6.24 %), Cyclopentane, 4-cyano-2,2-dimethyl-1-methylene (4.06%), Methyl-14-methylpentadecanoate (3.83%), 1-Pentadecanecarboxylic acid (13.62%), , cis-9-Octadecenoic acid (45.09%), Hystrene T-70 (11.73%), 2-Methyl-Z, Z-3,13-octadecadienol (1.36%) and icosane (0.81%) in the crude leaf extract of *A. muricata*. Similarly, the FTIR spectrum gave seven peaks that corresponded to sulfonic acids, sulfoxides, alcohols, nitroso compounds and halogeno, charged amines, alkanes and primary amides and free NH respectively which exert the various pharmacological activities.

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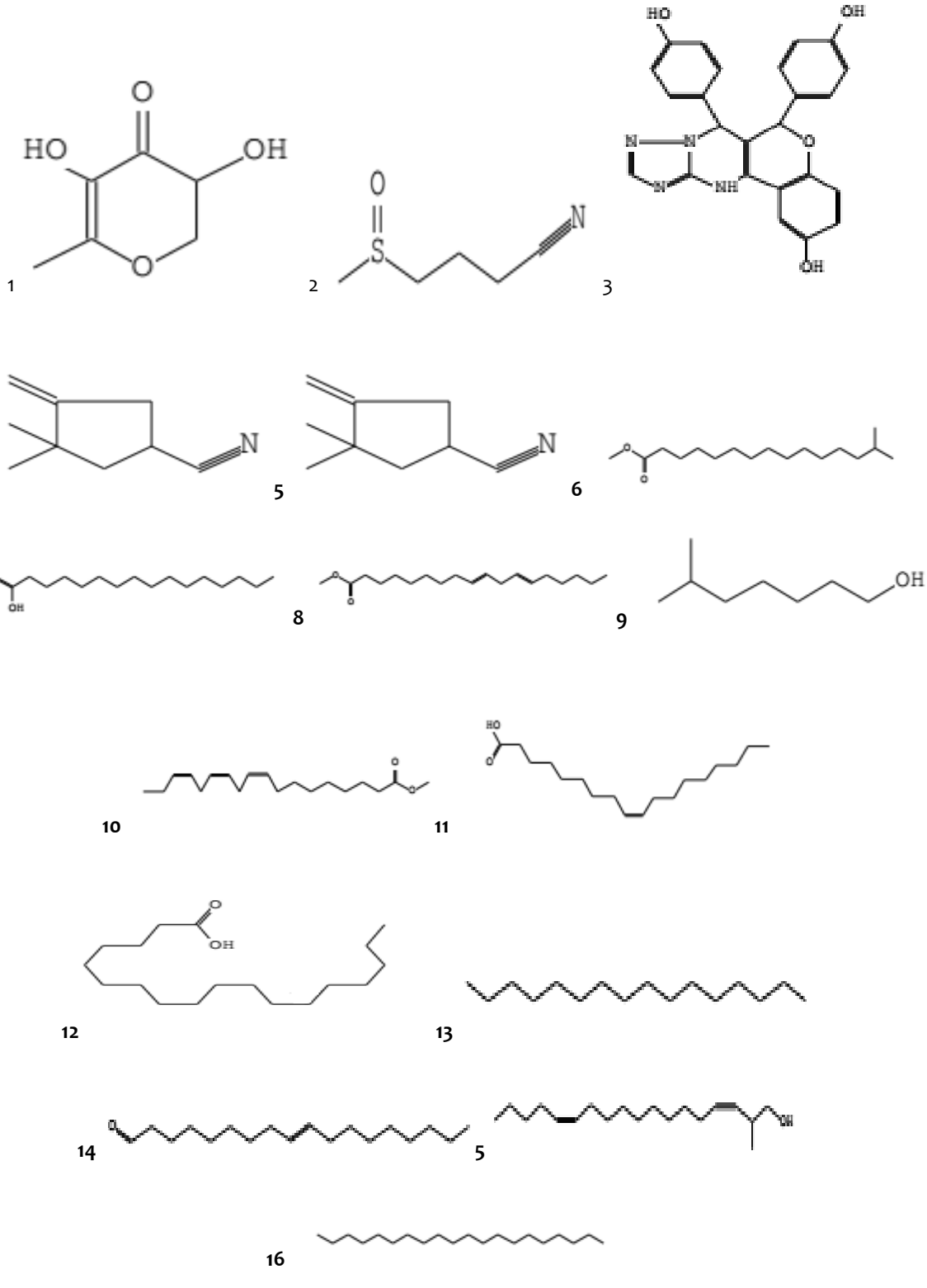
Table 1. Compounds identified from the GC-MS analysis of *A. muricata* leaf

Peak	Compound name	MW	Formular	RT	Area (%)
1	3, 5-Dihydroxy-6-methyl-2, 3-dihydro -4H-pyran-4-one	144	$C_6H_8O_4$	7.144	3.05
2	Iberin nitrile	131	C_5H_9NOS	10.689	6.24
3	7, 12-Dihydro-6, 7-bis (4-hydroxyphenyl) -6H-[1, 2, 4]triazolo [1',5':1,2]pyrimido [5, 4-c] chromen-2-ol	426	$C_{24}H_{18}N_4O_4$	13.189	2.11
4	Cyclopentane, 4-cyano-2, 2-dimethyl -1-methylene	135	$C_9H_{13}N$	14.839	4.06
5	Cyclopentane, 4-cyano-2, 2-dimethyl- 1-methylene-	135	$C_9H_{13}N$	16.402	2.19
6	Methyl 14-methylpentadecanoate	270	$C_{17}H_{34}O_2$	17.167	3.83
7	1-Pentadecanecarboxylic acid	256	$C_{16}H_{32}O_2$	18.663	13.62
8	Methyl trans, trans-9, 12-octadecadienoate	294	$C_{19}H_{34}O_2$	20.160	1.67
9	6-Methyl-1-heptanol	130	$C_8H_{18}O$	20.206	1.28
10	Methyl-cis-9, 12, 15-octadecatrienoate	292	C_4H_6ClN	20.288	1.19
11	cis-9-Octadecenoic acid	282	$C_{18}H_{34}O_2$	21.443	45.09
12	Hystrene T-70	284	$C_{18}H_{36}O_2$	21.618	11.73
13	Cetane	226	$C_{16}H_{34}$	22.526	0.55
14	9-Octadecenal	266	$C_{18}H_{34}O$	24.156	1.21
15	2-Methyl-Z, Z-3, 13-octadecadienol	280	$C_{19}H_{36}O$	24.591	1.36
16	Icosane	282	$C_{20}H_{42}$	26.332	0.81

Table 2: FTIR analysis result of *A. muricata* leaf

Peaks	Functional groups
632.67	**S=O (Sulfonic acids)**
1087.89	**S=O (Sulfoxides)**
1411.94	**O-H (Alcohols)**
1620.26	**N=N (Nitroso compounds and halogeno)**
2353.23	**C=NH$^+$ (Charged amines)**
2924.18	**C-H (Alkanes)**
3394.83	**N-H (Primary amides free NH)**

Chemical structures of the compounds identified.



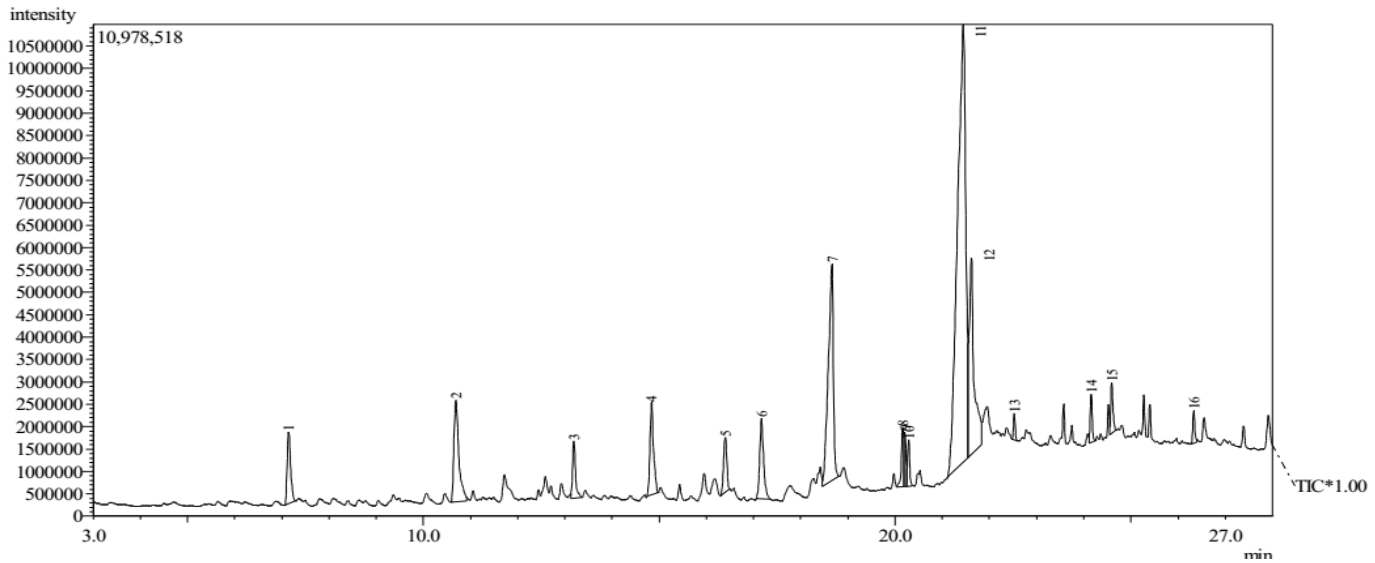


Fig. 1: Chromatogram of *Annona muricata* leaf

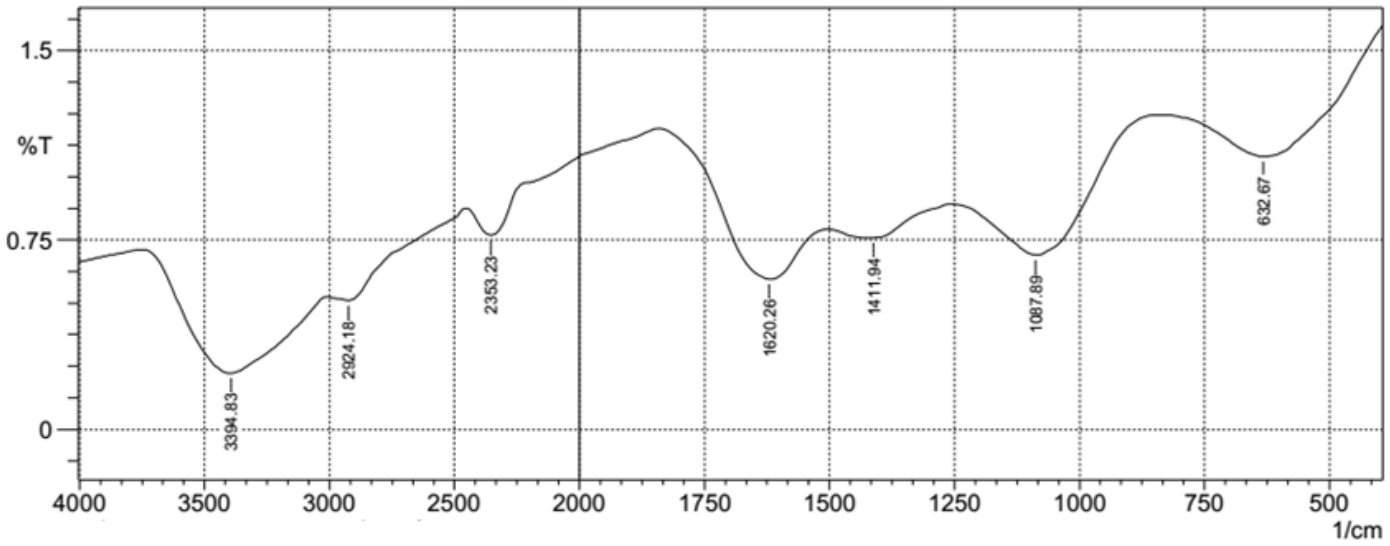


Fig. 2: FTIR spectrum of *A. muricata* leaf