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Identification of Active Compounds of *Annona muricata* (Soursop) Leaf Wax Extract Using GC-MS

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Authors' contributions

This work was carried out in collaboration among all authors. Authors FFY, BM and WLD designed, supervised and reviewed all the drafts of the manuscript. Author AMA carried out the research and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Soursop plant (*Annona muricata L.*), is widely used for both industrial and medicinal applications. In view of this, the present study aims at extraction and characterization of soursop leaf wax using gas chromatography-mass spectrometry (GC-MS). The results of the GC-MS analysis of soursop wax show the presence of 26 different compounds. From the results, it could be concluded that *A. muricata* contains various compounds that could be useful in different industries. Thus, soursop wax could be a good alternative source for many industrial chemicals that are currently sourced from petroleum which is non-renewable.

Keywords: Annona muricata; characterization; GC-MS analysis; soursop wax.

1. INTRODUCTION

Soursop (*Annona muricata L.*) also known as graviola, belongs to the family *Annonaceae* and is spread throughout the tropics. It is the largest species in the genus *Annona* [1]. The leaves, roots, bark, and fruits of the graviola tree are known for various medicinal uses. The fruit is used to combat parasites, which lower fevers, and also increases lactation after childbirth [2].

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The tea prepared from the leaves are used as a sedative and a soporific (inducer of sleep). This infusion is also used to relief pain or for antispasmodic purposes [2]. It is mostly used in medicine as a remedy for diseases such as indigestion, overweight, hypertension and heart diseases [1]. The practical definition of a wax may be "a substance similar in composition or physical properties to "bee's wax", irrespective of their source [3]. Technically wax is nothing but esters of long-chain fatty alcohols and fatty acids. The plant cuticle covers the epidermis of all aerial parts of the plant organs as an uninterrupted extracellular matrix. It is hydrophobic in nature consisting mainly of the complex biopolymer, cutin and cuticular lipids called waxes collectively [3]. Plant wax limits the diffusion of water and solutes, permitting a controlled release of volatiles that often deter pests or attract pollinating insects [4]. The wax layer provides protection from diseases, insects and helps the plants to resist drought [4]. Over the last few decades, the use of herbal drugs has been emphasized due to their easy availability, therapeutic potential, least side effects and minimum cost. At present about 80% of the world population rely on plant based drugs for their health care need [5]. Gas chromatography-mass spectrometry is the best technique to identify the active constituents of alcohols, acids, esters, alkaloids, steroids, long chain hydrocarbon, amino and nitro compounds etc. Thus, gas chromatography (GC) and mass spectroscopy (MS) associated with particular detection techniques have become a sophisticated means for identifying various compounds [6].

To date, no available work published on line worldwide on extraction and identification of constituents of *A. muricata* wax. So, the present study is aimed to investigate the possible chemical compositions of soursop prepared from the hexanolic leaf extract, separation and identification of the compounds by GC-MS analysis.

2. MATERIALS AND METHODS

2.1 Plant Material

The fresh leaves of *A. muricata* were collected from a farm in Malam Inna, Gombe, North-Eastern Nigeria, in August, 2019 using polyethene bag. The leaves were identified in Gombe State University. The leaves were shade-dried for one week and ground using a pestle and mortar, and then sieved to fine particles.

2.2 Extraction of Soursop Leaf Wax

The soursop leaf wax was extracted using soxhlet extractor and n-hexane with little modifications as described by AOAC [7] and Cheung and Leung [8]. A 300 ml of *n*-hexane was poured in to a round bottom flask. A 20 g of the grounded soursop leaf was placed in a thimble and inserted in the extractor. The soxhlet was heated at 60-70°C and the vapour rose through the vertical tube in to the condenser at the top. The condensate dripped in to the filter paper thimble in the centre which contained the solid sample to be extracted. The extract seeped through the pores of the thimble and filled the siphon tube, where it flowed back down in to the round bottom flask. This was allowed to continue for 36 hours. It was then removed from the tube, dried in the oven, cooled in the desiccators and re-weighed to determine the amount of wax extracted.

2.3 GC-MS Analysis

The GC-MS analysis was carried out using GC-MS-7890A, Agilent Technologist at the American University of Nigeria, Adamawa. The investigation of the hexanolic extract was performed on an Inert MSD-597CM with the following conditions: Column agilent-1 fused silica capillary column (30 m x 250 µm x 0.25 µm, composed of 5% Phenyl Methyl Silox). For GC-MS detention, an electron ionization system with ionization energy of 74 eV was used. Helium gas was used as the carrier gas at constant flow rate of 3.8379 ml/min and an injection volume of 1 µL was employed with split less injection mode, injector temperature 270°C; ion source temperature 250°C. The oven temperature was programmed initially at 80°C for 0 min then decreased to 10°C for 1 min then finally increased to 300°C for 5 mins. The flow control mode was at an average velocity of 72.418 cm/sec, pressure 2.239 Bar, the column flow was 3.8379 ml/min the purge flow was 1 ml/min. The total flow was 54.838/min. Mass spectra were taken at 74 eV; a scan of 27 min and fragment from 50 to 550.

3. RESULTS AND DISCUSSION

The GC-MS analysis of soursop leaf wax revealed the presence of twenty-six (26) compounds. The compounds with their retention time (RT), molar mass, molecular formula and percentage composition are presented in Table 1.

1-nonadecene, a long-chain fatty acid has been reported to be antibiotic [9] as presented in Table 2. Ketone 7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione have also been reported to possess antimicrobial activity [9]. Long chain aliphatic alcohol n-tetracosanol has been found to exhibit anti-bacterial and anticancer activities [10]. It has also been reported that long-chain fatty alcohol n-nonadecanol-1 exhibit anti-microbial and cytotoxic activities [11].

Straight chain primary alcohol 1-heptacosanol has also been reported to be a flavor and fragrance agent, lower cholesterol and has antimicrobial, cytotoxic and antithrombotic activities [12]. 9-octadecenoic acid (*Z*)-methyl ester has been reported to be anticarcinogenic and antioxidant heptacosane also has antioxidant activity. Hexadecanoic acid, methyl ester was reported to have hypocholesterolemic, antifungal, antioxidant, potent antimicrobial, nematicide, pesticide, anti-androgenic flavour, haemolytic, 5-alpha reductase inhibitory activities [9]. Bis(2-ethylhexyl) phthalate has been reported to exhibit an antimicrobial activity against gram positive bacteria and some pathogenic fungi [12]. It has exhibited a better broad spectrum of antibacterial activity against gram-positive (*Staphylococcus 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 [12].

It has also been reported that free fatty acids including long chain unsaturated fatty acids exhibit antibacterial, anti-inflammatory and antifungal activity [9]. Phthalic acid derivatives were suggested to have been used to cure chronic cardiovascular and cerebrovascular diseases and had anti-tumour, anti-inflammatory, antibacterial functions [11]. Phthalates are reported to have antimicrobial and other pharmacological activities [11]. The antimicrobial activities were believed to be due to phthalic acid derivative. Several authors have shown

Table 1. List of 26 different compounds detected in soursop leaves wax

Peak no.	RT (min)	Name of compound	Area%	Molecular formula	Molecular mass (g/mol)
1	6.250	4-methyl-2H-benzopyrane	3.51	$C_{10}H_{10}O$	146.19
2	7.280	2-methyl-naphthalene	2.01	$C_{11}H_{10}$	142.2
3	7.524	Cycloheptatriene	5.43	C_7H_8	92.14
5	8.406	Oleic acid	1.24	$C_{18}H_{34}O_2$	282.5
6	9.080	(*E*)-3-octadecene	4.40	$C_{18}H_{36}$	252.5
7	9.339	*Cis*-vaccenic acid	1.86	$C_{18}H_{34}O_2$	282.5
8	9.443	(*Z*)-14-methyl-8-hexadecenal	1.25	$C_{17}H_{32}O$	252.4
9	9.591	(*E*)-3-eicosene	1.81	$C_{20}H_{40}$	280.5
10	9.828	(*Z*)-9-tetradecenal	3.93	$C_{14}H_{26}O$	210.4
11	11.020	Oxirane	5.62	C_2H_4O	44.05
12	11.220	(*Z*)-9,17-octadecadienal	1.85	$C_{18}H_{22}O$	264.4
13	11.339	2-methyl-*Z*,*Z*-3,13-octadecadienol	1.57	$C_{19}H_{36}O$	280.5
14	11.561	Cyclohexane	4.42	C_6H_{12}	84.16
15	11.746	*Cis*-13-octadecenoic acid	7.43	$C_{18}H_{34}O_2$	282.5
16	12.361	*Trans*-13-octadecenoic acid	7.31	$C_{18}H_{34}O_2$	282.5
17	11.183	(*E*)-9-eicosene	8.17	$C_{20}H_{40}$	280.5
18	13.738	(*Z*)-3-eicosene	5.23	$C_{20}H_{40}$	280.5
19	14.398	2-octadecyl-propane-1,3-diol	0.68	$C_{21}H_{44}O_2$	328.6
20	14.457	(*Z*)-9-octadecenal	4.31	$C_{18}H_{34}O$	266.5
21	15.235	Hexadecanoic acid methyl ester	5.65	$C_{17}H_{34}O_2$	270.6
22	16.168	1-octadecene	3.10	$C_{18}H_{36}$	252.5
23	21.012	*Cis*-10-nonadecenoic acid	0.06	$C_{19}H_{36}O_2$	296.5
24	21.108	17-pentatriacontene	0.22	$C_{35}H_{70}$	490.9
25	23.937	1-nonadecene	0.37	$C_{19}H_{38}$	266.5
26	25.330	1-octadecanethiol	0.12	$C_{18}H_{38}S$	286.6

**RT = Retention time*

Table 2. Medicinal/Industrial activities of some major compounds obtained from soursop leaf wax [13]

S/N	Name of compound	Nature of compound	Activity
1	1-nonadecene	Fatty hydrocarbon	Antibiotic
2	(*Z*)-13-octadecenoic acid,methyl ester	Fatty acid ester	Antioxidant activity, Anticarcinogenic
3	2-octadecyl-propane-1,3-diol	Aliphatic alcohol	Anti-bacterial, Anticancer
4	2-methyl-*Z*,*Z*-3,13-octadecadienol	Aliphatic alcohol	Anti-microbial, Cytotoxic
5	Hexadecanoic acid methyl ester	Fatty acid ester	Antifungal, Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavour haemolytic, 5-Alpha reductase inhibitor, potent antimicrobial activity
6	Octadecane	Aliphatic	Antioxidant activity
7	1,4-dihydro-1,4-methanonaphthalene	Aromatic fatty ester	Antimicrobial, Antibacterial
8	Urea		Manufacture of fertilizer

that natural aromatic compounds possess important biological activities, such as antitumor, antihepatotoxic, antioxidant, anti-inflammatory, estrogenic and antibacterial activities [14].

4. CONCLUSION

The yield crude wax from soursop leaves was 0.90% (w/w). The GC-MS results of this study revealed the presence of twenty-six (26) different compounds of many classes of functional groups like alkane, ester, alcohol, fatty acids etc. Fatty acids have many beneficial effects in human nutrition whereas; alkane octadecane has good antioxidant, cytotoxic, antimicrobial and anti-fungal effects. Apart from medicinal and nutritional applications, wax can be useful in different industries. Thus, soursop leaf wax has many compounds of biological and industrial importance hence; it could be a good alternative source for many industrial chemicals that are currently sourced from petroleum which is non-renewable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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