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GC-MS CHARACTERIZATION OF YEAST DEGRADED SOURSOP LEAF WAX ON ORGANIC SURFACE

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ABSTRACT

The present investigation was carried out to characterize the compounds of yeast degraded soursop leaf wax on organic surface using Gas Chromatography-Mass Spectroscopy (GC-MS). The results of the GC-MS analysis provide different peaks revealing the presence of 28 phytochemical compounds with different therapeutic activities. All the new compounds formed from the yeast degraded soursop wax are useful compounds in pharmaceutical and chemical industries. Thus, *Saccharomyces cerevisiae* degradation of soursop wax has a good techno-economic potential.

Keywords: Degradation; GC-MS Analysis; Organic Surface; *Saccharomyces cerevisiae*; Soursop Wax

INTRODUCTION

Soursop plant also known as *Annona muricata* is a tropical plant that has been used as herbal medicine. It was most abundant in the West Indies, northern South America and in West Africa (Moreno *et al*., 2014). It produces a sweet fruit with lots of health benefits associated with not only the fruit but other parts of the plant such as the seeds, the stem, roots and leaves (Morton and Julia, 1966; Fai *et al*., 2020). The wax covers all the external pat of the plants.

The practical definition of a wax may be "a substance similar in composition or physical properties to bee's wax", irrespective of their source (Kolattukudy *et al*., 1976). Technically wax is nothing but esters of long-chain fatty alcohols with long-chain fatty acids. The plant cuticle covers the epidermis of all aerial parts of plant organs as an uninterrupted extracellular matrix. It is hydrophobic in nature consisting mainly of the complex biopolymer, cutin and cuticular lipids called as waxes collectively (Kolattukudy *et al*., 1976). Plant wax limits the diffusion of water and solutes, permitting a controlled release of volatiles that often deter pests or attract pollinating insects (Prasad *et al*., 2010). The wax layer provides protection from diseases, insects and helps the plants to resist drought (Prasad *et al*., 2010). The wax and other phytochemicals in the plant form part of the herbal medicine.

The use of herbal drugs is getting more attention due to their availability, minimum cost, least side effects and therapeutic potential. About 80% of the world population rely on herbal drugs for their health care need (Sermakkani. and Thangapandian, 2012). For a herb to be considered as a medicinal plant, there is always need to carry out the analysis so as to know the component of the active compounds. The easiest, common and the

best spectroscopic technique use in identifying bioactive constituents is gas chromatography-mass spectrometry (GC-MS). Thus, gas chromatography (GC) and mass spectroscopy (MS) associated with particular detection techniques have become a sophisticated means for identifying various compounds (Vinodh *et al*., 2013).

Soursop leaf wax is an organic substance that can undergo biodegradation. Organic material can be degraded aerobically or anaerobically. Biodegradation can be referred to as chemical or transformation of a substance caused by organisms or their enzymes (Emmanuel *et al*., 2009). Yeasts have a wide range of applications mainly in food industry (wine making, brewing, distilled spirits production, and baking) and in biomass production (single-cell protein) (Zymanczyk-Duda *et al*., 2017; Schisler and Ryder, 2018). Recently, yeast has also been used in the biofuel industry and for the heterologous production of proteins (Branduardi and Salman, 2012). Many yeast strains can function under both anaerobic as well as aerobic conditions of environment, switching their metabolism types easily (Otterstedt *et al*., 2004). Moreover, yeasts as fungi have been exploited by mankind for thousands of years for food and fermentation process. *Saccharomyces cerevisiae (S. cerevisiae*) has been described as mankind's most domesticated organism and still widely exploited yeast species in industry today (Zymanczyk-Duda *et al*., 2017). The number of yeast species described so far is about 1500 and only about a dozen is used at industrial scale (Barnett and Barnett, 2011). Some 70-80 species have been shown to possess potential value for biotechnology (Kurtzman *et al*., 2011). According to modest estimate, known yeast species formed about 5% of the inhabitant of the Earth surface (Hawksworth, 2004). Modern applications of yeasts have been greatly expanded beyond classical applications. Yeasts, especially *S. cerevisiae* and other non-*saccharomyces* yeasts today are increasingly used for the heterologous production of enzymes and pharmaceutical proteins (Çelik and Çalık, 2012). Yeasts have important roles in environmental applications such as bioremediation and removal of heavy metals from waste waters (Tondee *et al*., 2008). They are also used in agriculture as biocontrol agents (Buzzini and Margesin, 2014). Several chemicals can be produced using yeast as a biocatalyst (Kapoor and Gupta, 2012). A detailed literature review on the plant in investigation has shown that so far there are no published reports worldwide related to the yeast degradation of soursop leaf wax on organic surface fractionated into aqueous and organic phases and identification of the new compounds by GC-MS analysis. Thus, the present study was undertaken with aim of degrading soursop leaf wax with *S. cerevisiae* on organic surface in two different phases (aqueous and organic) and characterizing the products by GC-MS.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *A. muricata* were collected from a farm in Malam Inna, Gombe, North Eastern Nigeria, on 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.

Organic Surface

The organic surface was produced from banana leaves that were harvested from Malam Inna quarters in Gombe town. The leaves were rinsed with water and shade-dried for one week and later oven-dried at 40 °C for 4 hours and pulverized using a pestle and mortar, sieved to fine particles using a sieve of 250 microns. A 40g of the pulverized banana leaves were weighed and extracted with n-hexane in a soxhlet extractor for 72 hours, and dried in an oven until a constant weighed and stored in a desiccator as described elsewhere (AOAC, 1984).

Extraction and Degradation of Soursop Leaf Wax by *S. cerevisiae*

The soursop leaf wax was extracted using soxhlet extractor and n-hexane with little modifications as described (AOAC, 1984; Cheung and Leung, 1984).

In other to know if *S. cerevisiae* will digest the organic surface, about 4 g of the processed organic surface and 0.016 g of the yeast (*S. cerevisiae*) were weighed and mixed with 25 ml of distilled water in a 250 ml flat bottom flask and stoppered loosely. The mixture (which serves as a control) was kept for 21 days for fungal degradation to take place. The product of the degraded organic surface by yeast was analyzed with a GC-MS so as to be able to differentiate the degradation products of the wax and any possible degradation products of the organic surface.

In a 100 ml flask containing 25 ml of distilled water, 0.4 g of the soursop leaf wax and 0.016 g of the *S. cerevisiae* were added and mixed thoroughly. The mixture was transferred to a to 250 ml flask containing 3.6 g of the organic surface and allowed to digest for 21 days. More so, 25 ml of water and 30 ml of hexane was added to the digested solution, stirred, filtered and vacuum pump. The hexane layer and the water layer were separated and each of the extracts was transferred to a weighed beaker and dried to a constant weight. The hexane fraction was dried in a fume cupboard while the water fraction was dried in a water bath. GC-MS analysis was carried out to identify the composition of the digestion products.

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 a 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 32.475 psi, the column flow was 3.8379 ml/min the purge flow was 1ml/min. The total flow was 54.838/min. Mass spectra were taken at 74 eV; a scan of 27 mins and fragment from 50 to 550.

RESULTS

The GC-MS analysis of aqueous fraction of the degraded soursop wax revealed the presence of eight (8) different compounds as shown in Figure 1. The compounds with their retention time (RT), molar mass, molecular formula and percentage composition are presented in Table 1. These compounds were not present in the undigested soursop wax as reported in our previous work (Fai *et al*., 2020).

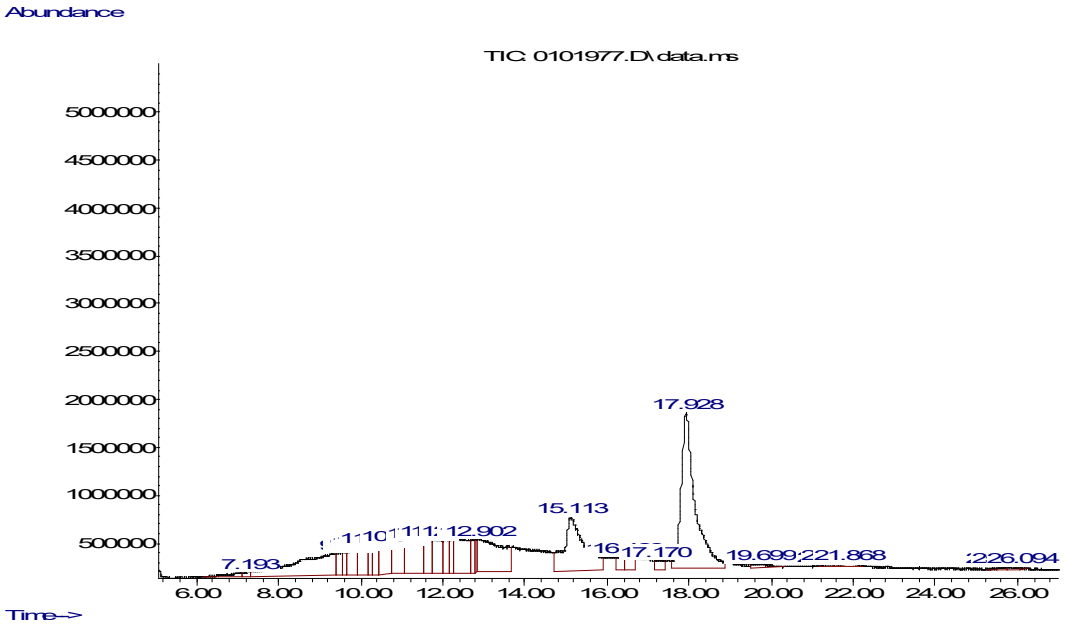

Figure 1: GC-MS chromatogram of aqueous fraction of digested soursop wax.

The compounds confirmed in the chromatogram are: 2-Heptadecenal, 7,11-Hexadecadienal, (Z)- 13-Octadecenal, 2-Methyl-Z,Z-3,13-octadecadienol, (Z)- 14-methyl-8-Hexadecenal, 1-Octadecene, (Z)-14-methyl-8-Hexadecenal, trans-13-Octadecenoic acid, cis-13-Octadecenoic acid, 6-Octadecenoic acid, Oleic Acid, (Z)-13-Octadecenal, (E)- 9-Octadecenoic acid, Hexadecanoic acid, cis-Vaccenic acid, Erucic acid, (Z)- 9-Octadecenoic acid, 9-Octadecenoic acid, Z,E-7,11-Hexadecadien-1-yl acetate, 10-Heneicosene (c,t), 11-Octadecenoic acid, 7-Octadecenoic acid, cis-Inositol-tri-methylboronate.

However, the GC-MS analysis of the organic fraction of degraded soursop wax also revealed the presence of twenty (20) different compounds that are absent in the undigested soursop wax (Figure 2). The compounds with their retention time (RT), molar mass, molecular formulae and percentage composition are presented in Table 2.

Table 1: List of eight (8) different compounds detected in digested soursop wax of aqueous fraction.

Peak#	RT	Name of Compound	Area %	Molecular formulae	Molecular mass ($gmol^{-1}$)
1	7.191	7,11-Hexadecadienal	0.31	$C_{16}H_{28}O$	236.4
2	9.376	(z)- 13-Octadecenal	10.09	$C_{18}H_{34}O$	266.5
3	9.6131	8-Hexadecenal,14-methyl- (Z)-	1.04	$C_{17}H_{32}O$	252.4
4	12.065	6-Octadecenoic acid	2.28	$C_{18}H_{34}O_2$	282.5
5	15.116	Hexadecanoic acid	12.15	$C_{16}H_{32}O_2$	256.42
6	16.471	Erucic acid	1.14	$C_{22}H_{42}O_2$	338.6
7	21.493	Z,E-7,11-Hexadecadien-1-yl acetate	0.23	$C_{18}H_{32}O_2$	280.4
8	21.590	10-Heneicosene	0.08	$C_{21}H_{44}$	296.6

*RT = Retention time

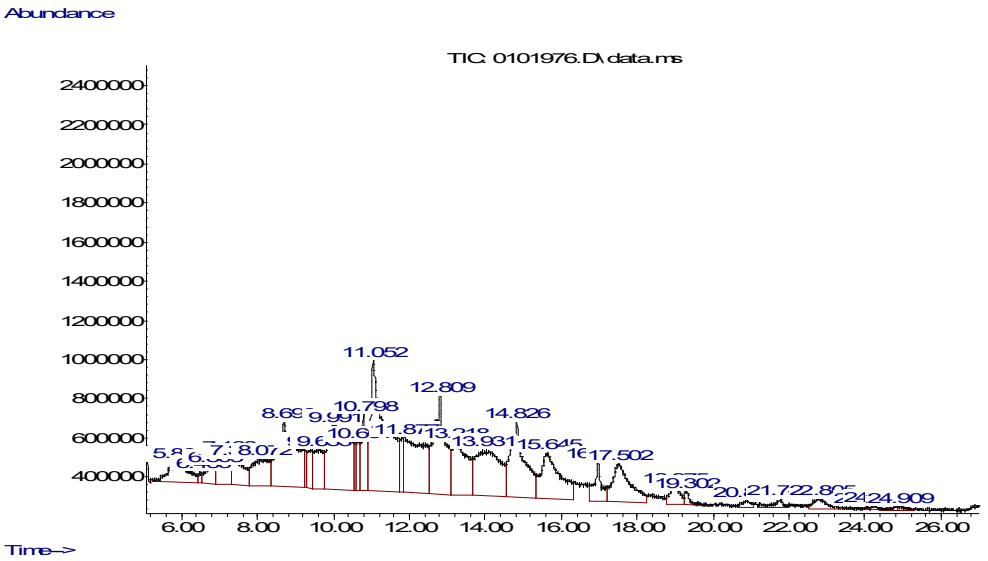

Figure 2: GC-MS chromatogram of organic fraction of digested soursop wax.

The compounds confirmed in the GC-MS chromatogram of the organic fraction of the yeast degraded soursop wax are: 1H-Pyrrolo[2,3-b]pyridine, 2-ethyl, Urea, 1,4-dihydro-1,4-Methanonaphthalene, 1-methyl-Naphthalene, 2-methyl-Naphthalene, Butylated hydroxytoluene, 7,11-Hexadecadienal, (E)-3-Eicosene, 2-Heptadecenal, Cyclohexadecane, Cyclotetradecane, Heptadecanoic acid, *t*-Butyldimethyl(2-styryl[1,3]dithian-2-yl)silane, 3-Thiopheneethanol, Oleic Acid, Z,E-2,13-Octadecadien-1-ol, Octadecane, Cyclohexadecane, 1-Nonadecene, Hexadecanoic acid, 17-Pentatriacontene, Naphtho[2,1-b]furan, (E)- 9-Octadecenoic acid, Octadecane, N-Benzyl-N-ethyl-p-isopropylbenzamide, Fumaric acid, Erucic acid, (Z,Z)- 9,12-Octadecadienoic acid, (E)- 9-Octadecenoic acid, cis-10-Nonadecenoic acid, 2-octyl-Cyclopropaneoctanal (Table 2).

Table 2: List of twenty (20) different compounds detected in degraded soursop wax organic fraction.

Peak#	RT	Name of Compound	Area%	Molecular formulae	Molecular mass ($gmol^{-1}$)
1	5.865	1H-Pyrrolo[2,3-b]pyridine, 2-ethyl	2.55	$C_{10}H_{10}N_2O_2$	190.2
2	6.465	Urea	0.23	NH_2CONH_2	60.1
3	6.806	1,4-dihydro-1,4-Methanonaphthalene	1.01	$C_{11}H_{10}$	142.2
4	7.169	1-methyl-Naphthalene	2.11	$C_{11}H_{10}$	142.2
5	7.339	2-methyl- Naphthalene	2.10	$C_{11}H_{10}$	142.2
6	8.702	Butylated Hydroxytoluene	7.78	$C_{15}H_{24}O$	220.4
7	9.250	7,11-Hexadecadienal	0.68	$C_{16}H_{28}O$	236.4
8	9.991	Cyclohexadecane	8.63	$C_{16}H_{32}$	224.4
9	10.568	Cyclotetradecane	0.69	$C_{14}H_{28}$	196.3
10	10.657	Heptadecanoic acid	1.10	$C_{17}H_{34}O_2$	270.5
11	10.798	*t*-Butyldimethyl(2-styryl[1,3]dithian-2-yl)silane	2.65	$C_{18}H_{28}S_2Si$	336.6
12	11.050	3-Thiopheneethanol	12.61	C_6H_8OS	128.1
13	11.879	Z,E-2,13-Octadecadien-1-ol	6.92	$C_{19}H_{36}O$	280.5
14	12.813	Octadecane	7.34	$C_{18}H_{38}$	254.5
15	14.827	Hexadecanoic acid	7.02	$C_{16}H_{32}O_2$	256.4
16	16.968	Naphtho[2,1-b]furan	1.88	$C_{12}H_8O$	168.2
17	19.301	N-Benzyl-N-ethyl-p-isopropylbenzamide	0.55	$C_{19}H_{23}NO$	281.4
18	21.745	Fumaric acid	0.57	$C_4H_4O_4$	116.1
19	24.056	9,12-Octadecadienoic acid (Z,Z)-	0.04	$C_{18}H_{32}O_2$	280.4
20	24.908	2-octyl- Cyclopropaneoctanal	0.16	$C_{19}H_{36}O$	280.5

*RT = Retention time

1-Nonadecene, a long-chain fatty acid has been reported to be antibiotic as presented in Table 3 (Ogukwe *et al*., 2016). Ketone (7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione) was reported to possess antimicrobial activity (Ogukwe *et al*., 2016). Long chain aliphatic alcohol n-tetracosanol has been found to exhibit anti-bacterial and anticancer activities (Nakalembe and Kabasa, 2012). It has also been reported that long-chain fatty alcohol n-nonadecanol-1 exhibit anti-microbial and cytotoxic activities (Saranya *et al*., 2013).

DISCUSSION

Straight chain primary alcohol 1-Heptacosanol has also been reported to be a flavour and fragrance agent, lower cholesterol and has antimicrobial, cytotoxic and antithrombotic activities (Akpuaka *et al*., 2015). 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 (Ogukwe *et al*., 2016).

Table 3: Medicinal/Industrial activities of some major compounds obtained from digested soursop leaf wax as reported elsewhere (Rizvi *et al*., 2015).

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.

Bis(2-ethylhexyl) phthalate was reported to exhibit an antimicrobial activity against gram positive bacteria and some pathogenic fungi (Akpuaka *et al*., 2015). It has exhibited a better broad spectrum of antibacterial activity against both 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 (Akpuaka *et al*., 2015). It was also reported that free fatty acids including long chain unsaturated fatty acids exhibit antibacterial, anti-inflammatory and antifungal activity (Ogukwe *et al*., 2016).

Phthalic acid derivatives were suggested to have been used to cure chronic cardiovascular and cerebrovascular diseases and had anti-tumour, anti-inflammatory, antibacterial functions (Saranya *et al*., 2013). Phthalates are reported to have antimicrobial and other pharmacological activities (Saranya *et al*., 2013). The antimicrobial activities were believed to be due to phthalic acid derivative. Several authors have shown that natural aromatic compounds possess important biological activities, such as antitumor, antihepatotoxic, antioxidant, anti-inflammatory, estrogenic and antibacterial activities (Ogunlesi *et al*., 2010). Therefore, these compounds with these activities could be obtained from digestion of soursop wax instead of getting from petrochemical industries using petroleum which is not renewable as a raw material.

CONCLUSION

In the present study, soursop wax was degraded by using yeast on organic surface fractionated into an aqueous and organic phase. Twenty-eight (28) different compounds were identified from both the aqueous and organic fractions of the degraded soursop wax by Gas-

chromatography– Mass spectroscopy (GC-MS). This study has shown that the that the products of yeast degraded soursop wax contains some bioactive compounds. Therefore, yeast degraded soursop wax could be a good alternative for many industrial chemicals that are currently source from petroleum which is non-renewable.

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