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Gas chromatography-mass spectroscopy (GC-MS) studies of fixed oil of leaf of *Tetrapleura tetraptera* Taub. (Mimosaceae)

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Abstract
Tetrapleura tetraptera (Schum and Thonn) Taubert (Mimosaceae) also known as Aridan in Yoruba (Nigeria) has been used as an Ethno Pharmacological agent for the treatment of numerous diseases including inflammation, arthritis, hypertension and even as an antioxidant agent. The present study is aimed at investigating the bioactive components of fixed oil of leaf of T. tetraptera. Extraction of plant material was carried out using soxhlet apparatus to obtain fraction of n-hexane, isolation of fixed oil was carried out by open column chromatography and analysis of bioactive components of fixed oil was carried out using gas chromatography-mass spectroscopy (GC-MS). The result of this study showed the presence of thirty one (31) compounds which are basically esters, alkanes, alkenes, terpenoids, fatty acids and vitamins. These compounds have reported pharmacological activities and thus can be used as pharmacological agent.

Keywords: Chromatography-mass, *Tetrapleura tetraptera*, Mimosaceae

Introduction
The use of phytomedicine for the treatment of ailments can be dated back to the history of man. Medicinal plants have been used by different cultures for the management of different diseases [1]. *T. tetraptera* has played significant roles in the management of numerous diseases, different parts of the plant has been used for different purposes. The fruit has been used after birth by breast feeding mothers to prevent postpartum contraction as well as for the treatment of inflammations. Various parts of the plant has been used basically as antihypertensive, cytoprotective, antidiabetic, piscicidal, molluscicidal, antigonadotrope, antimutagenic and antimalarial agents [2], the plant has also been used as an aphrodisiac agent [4]. The various pharmacological properties of the plant have been attributed to its rich phytochemical profile. Different reports have shown that the plant contain various phytoconstituents such as coumarins, alkaloids, flavonoids, fatty acids, amino acids, esters, saponins and glycosides [5-10]
GC-MS analysis is very instrumental in analysing various components of a sample. It provides numerous fractional information at molecular levels; the mass spectrum (MS) provides information on the structure and weight of compounds present in a sample, however, there are some disadvantages of using GC-MS analysis to reveal molecular information for compounds with a boiling point higher than 300 °C because the high boiling point compounds are difficult to be volatilized and detected. Recently, GC-MS (gas chromatography-mass spectrometry) has been proven a fast and reliable method for the investigation of the components in complex mixtures and provides identification of a great deal of compounds in small quantities of plant materials [11].

Materials and Methods
Plant collection and Identification
The leaves of *T. Tetraptera* were collected from the medicinal plant farm of the department of Pharmacognosy and natural medicine, University of Uyo. The plant was identified with the herbarium number UUPHA32 (f) at herbarium of the Department of Pharmacognosy and natural medicine, University.

Preparation of Extract
T. tetraptera leaves were air dried and coarsely powdered with mortar and pestle. About 650 g of the powdered plant material was extracted continuously using soxhlet apparatus with n-hexane, and dried using rotary evaporator to obtain n-hexane fraction.

Chromatographic techniques

Column Chromatographic technique was used in isolation of fixed oil from n-hexane fraction of leaf of *T. tetrapluera.*

Gas chromatography – mass spectroscopy analysis (GC – MS analysis.)

The Gas chromatography–Mass spectroscopy (Gc-Ms QP2010SE Shimazu, Japan Mass Spectrometer) was fitted with electron impact (EI) mode. The Helium was used as the carrier gas at a flow rate of 1mL/min. The temperature was programmed at 70 °C for 5 min then increased to 300 °C at the rate of 15 °C/min. The temperature of injector and EI detector (70eV) were 280 °C and 300 °C, respectively. Fixed oil of 1µL was injected with a Hamilton syringe to the GC-M S manually. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and structure of the components of the test materials were ascertained [12].

Results and Discussions

Nitric oxide assay of fractions of *T. tetraptera* and isolates

Conc (µg/ml)	Ascorbic Acid	n-hexane Fraction	Dichloromethane Fraction	Ethyl acetate Fraction	Methanol Fraction	Fixed oil (NH01)
20	0.054±0.003	0.133±0.169*	0.143±0.246*	0.124±0.136c	0.129±0.054ns	0.136±0.010c
40	0.056±0.002	0.147±0.121*	0.188±0.102ns	0.178±0.003*	0.133±0.002c	0.161±0.001c
60	0.055±0.001	0.200±0.002ns	0.188±0.124ns	0.163±0.104*	0.151±0.001*	0.182±0.012ns
80	0.054±0.002	0.199±0.102c	0.241±0.245*	0.188±0.008c	0.150±0.001*	0.192±0.003ns
100	0.056±0.001	0.221±0.001c	0.283±0.131*	0.204±0.025*	0.157±0.162*	0.178±0.015c

Values represent Mean ± S.E.M

Significance related to control: *p< 0.001, p^c< 0.01

(n=3); ns=not significant

The nitric oxide assay showed an increase in percentage inhibition with an increase in concentration. The Table showed an average percentage inhibition of 50.2% n-hexane fraction, 46.8% dichloromethane fraction, 57.8% ethyl acetate fraction, 64.4% methanol fraction, 65.2% ascorbic acid, 64.8%, and 59.4% NH01, respectively. Ascorbic acid (standard) recorded 65.2%. The NH01 result is slightly more active than the ethyl acetate fraction with a difference of 1.6% percentage inhibition, this is also statistically significant at p <0.01, these results are similar to the report of [13] and support the antioxidant activity of various fractions and isolates of leaf of *T. tetraptera* Taub.

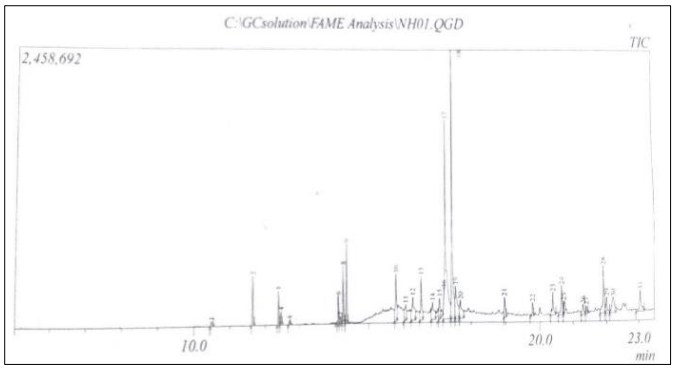

Fig 1: Gc-ms spectra of fixed oil of leaf of *T. tetraptera*

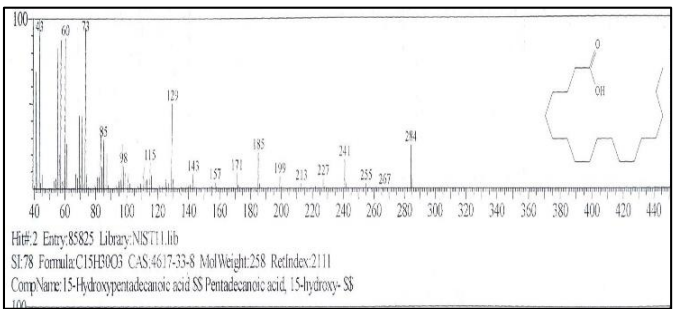

Fig 2: Mass spectrum of Pentadecanoic acid

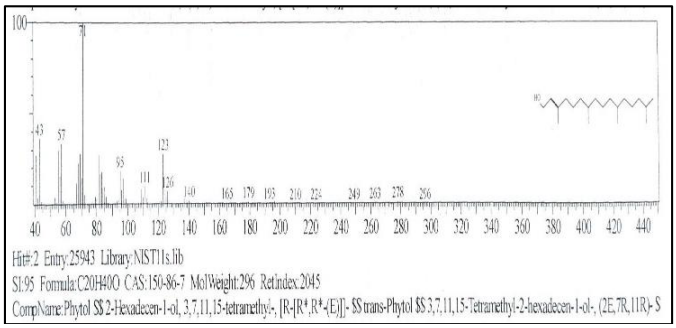

Fig 3: Mass spectrum of Phytol

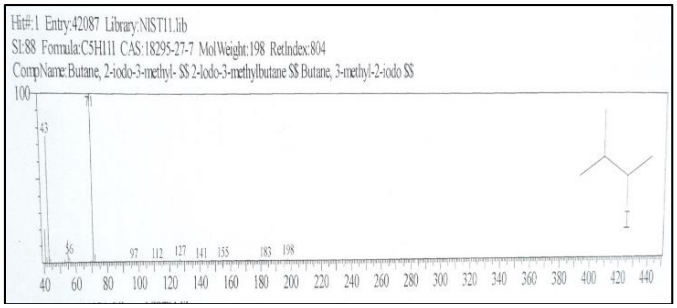

Fig 4: Mass Spectrum of Butane, 2-iodo-3-methyl-

The gas chromatography-mass spectroscopy spectra of fixed oil as shown in figure 1 reveals the presences of thirty one compounds *viz*; 1-Hexene, 3,5-demethyl-, 2-Pentadecanone, 6,10,14-trimethyl-, 5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl, Tridecanoic acid, methyl ester, Butane, 2-iodo-3-methyl-, 6-Octadecanoic acid, methyl ester, (Z)-, Cyclohexanone, 2-butyl-, Phytol, methyl stearate, 15-Hydroxypentadecanoic acid, Oleic acid, Hexanoic acid, trans-Geranylgeraniol, cis-Vaccenic acid, Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)-, 9,12-Octadecanoic acid (Z,Z)-, 13-Octadecanal, (Z)-, 15-Hydroxypentadecanoic acid, Octadecanoic acid, Methyl 16-hydroxy-hexadecanoate, 15-Hydroxypentadecanoic acid, 2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (Z)-, Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pent,

gamma-Tocopherol, beta-Tocopherol, Vitamin E, dl-alpha-Tocopherol, 1-Octacosanol, 2,4,6,8-tetramethyl-, and Octacosanol. These are esters, fatty acids, phenols, terpenoids, vitamins, alcohols, alkenes and alkanes with similar biological activities. About 98% of these volatile oils are pharmacologically active. Table 1 below shows that these volatile oils have played significant roles as antioxidant, antibacterial, antifungal, antimicrobial, anticonvulsive, anticancer, anti-inflammatory, antidepressant, antistress, cytoprotective and ergogenic agents. Figures 2-4 shows the mass spectra of pentadecanoic acid as 258 g, phytol as 296 g and butane, 2-iodo-3-methyl- as 198 g, respectively.

Table 1: NH01 (Fixed oil) Gas Chromatography-Mass Spectroscopy (GC-MS) Spectral Pharmacological Information

S/N	Volatile oil	Biological activity	References
1	1-Hexene, 3,5-dimethyl-	Antibacterial, antimicrobial, antioxidant	[14]
2	2-Pentadecanone, 6,10,14-trimethyl-	Antibacterial, antioxidant, cytotoxic, antimicrobial	[15]
3	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl	Anti-inflammatory, antioxidant, cytotoxic	[16]
4	Tridecanoic acid, methyl ester	Antimicrobial, antioxidant, anti-inflammatory	[17]
5	Butane, 2-iodo-3-methyl-	Antifungal, antibacterial	
6	6-Octadecenoic acid, methyl ester, (Z)-	Antioxidant, antibacterial, anticancer	
7	Cyclohexanone, 2-butyl-	Flavouring agent, antibacterial	[18]
8	Phytol	Anti-inflammatory, anxiolytic, antidepressant	[19]
9	Methyl stearate	Antifungal, antioxidant, anticancer	[20]
10	15-Hydroxypentadecanoic acid	Antioxidant, anticancer, hypotensive	[21]
11	Oleic Acid	Antitumour, antioxidant, hypotensive	
12	Hexanoic acid, 2-tetradecyl ester	Antioxidant, anti-inflammatory, antibacterial	[22]
13	trans-Geranylgeraniol	Insecticide, antibacterial, cytoprotective	[23]
14	cis-Vaccenic acid	Antibacterial	[24]
15	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)	Inhibitors of alpha-glucosidae	
16	9,12-Octadecadienoic acid (Z,Z)-	Antioxidant, anticancer, antidiabetic	
17	13-Octadecenal, (Z)-	Nothing reported	
18	15-Hydroxypentadecanoic acid	Anticancer, antioxidant, hypotensive	[25]
19	Octadecanoic acid	Antibacterial, antioxidant	
20	Methyl 16-hydroxy-hexadecanoate	Nothing reported	
21	15-Hydroxypentadecanoic acid	Anticancer, antioxidant, hypotensive	[26]
22	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-, (Z)-	Nothing reported	
23	15-Hydroxypentadecanoic acid	Anticancer, antioxidant, hypotensive	[27]
24	Oxirane, 2,2-dimethyl-3-(3,7,,16,20-pentamethyl	Nothing reported	
25	Oleic Acid	Anti-inflammatory, antioxidant, anticancer	
26	gamma.-Tocopherol	Anti-inflammatory, antioxidant, anticancer	
27	.beta.-Tocopherol	Anti-inflammatory, antioxidant, anticancer	
28	Vitamin E	Anti-cardiovascular, anti-dementia, Anticonvulsant	
29	dl-.alpha.-Tocopherol	Anti-cardiovascular, anti-dementia, anticonvulsant	
30	1-Octacosanol, 2,4,6,8-tetramethyl-, (all-R)-	Cytoprotective, ergogenic,	
31	Octacosanol	Antioxidant, anti-inflammatory, antiadipose tissue, anticoagulant, antidepressant, antistress	

Table 2: Report Peaks of Gas Chromatography-Mass Spectroscopy (GC-MS) Spectra of NHO1

Peak#	R. Time	Area	Area (%)	Height (%)	Molecular Formula	Name
1	10.484	127892	0.47	0.44	C_8H_{18}	1-Hexene, 3,5-dimethyl-
2	11.642	658023	2.40	4.09	$C_{18}H_{36}$	2-Pentadecanone, 6,10,14- trimethyl-
3	12.376	440967	1.61	2.81	$C_{18}H_{30}$	5,9,13-Pentadecatrien-2-one,6,10,14- trimethyl-, (E,E)-
4	12.449	186614	0.68	1.14	$C_{14}H_{28}$	Tridecanoic acid, methyl ester
5	12.693	81268	0.30	0.46	C_5H_{11}	Butane, 2-iodo-3-methyl-
6	14.104	431631	1.57	2.31	$C_{19}H_{36}$	6-Octadecenoic acid, methyl ester,(Z)-
7	14.158	134073	0.49	0.59	$C_{10}H_{18}$	Cyclohexanone, 2-butyl-
8	14.244	910634	3.32	4.79	$C_{20}H_{40}$	Phytol
9	14.346	1212644	4.42	6.64	$C_{19}H_{38}$	Methyl stearate
10	15.798	1077776	3.93	3.94	$C_{15}H_{30}$	15-Hydroxypentadecanoic acid
11	16.081	487755	1.78 1	1.33	$C_{18}H_{34}$	Oleic Acid
12	16.289	892421	3.25	1.94	$C_{20}H_{40}$	Hexanoic acid, 2-tetradecyl ester
13	16.541	906432	3.30	3.66	$C_{20}H_{34}$	trans-Geranylgeraniol
14	16.867	667819	2.43	1.47	$C_{18}H_{34}$	cis-Vaccenic acid
15	17.074	460842	1.68	1.79	$C_{21}H_{42}$	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
16	17.212	50352	0.18	0.34	$C_{18}H_{32}$	9,12-Octadecadienoic acid (Z,Z)-
17	17.256	5962822	21.74	16.88	$C_{18}H_{34}$	13-Octadecenal, (Z)-
18	17.458	5116758	18.65	22.86	$C_{15}H_{30}$	15-Hydroxypentadecanoic acid
19	17.552	1240982	4.52	2.74	$C_{21}H_{42}$	Octadecanoic acid
20	17.684	782851	2.85	1.52	$C_{17}H_{34}$	Methyl 16-hydroxy-hexadecanoate
21	18.978	410476	1.50	1.66	$C_{15}H_{30}$	15-Hydroxypentadecanoic acid
22	19.794	254494	0.93	1.11	$C_{15}H_{28}$	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-
23	20.404	597892	2.18	1.90	$C_{15}H_{30}$	15-Hydroxypentadecanoic acid
24	20.667	634000	2.31	2.45	$C_{30}H_{50}$	Oxirane, 2,2-dimethyl-3-

25	20.719	200724	0.73	1.15	$C_{18}H_{34}$	Oleic Acid
26	21.299	259502	0.95	0.82	$C_{28}H_{48}$	.gamma.-Tocopherol
27	21.394	195400	0.71	0.68	$C_{28}H_{48}$	.beta.-Tocopherol
28	21.917	1102643	4.02	4.02	$C_{29}H_{50}$	Vitamin E
29	21.999	393493	1.43	1.36	$C_{29}H_{50}$	dl-.alpha.-Tocopherol
30	22.208	794145	2.89	1.32	$C_{32}H_{66}$	1-Octacosanol, 2,4,6,8-tetramethyl-
31	23.043	760019	2.77	1.80	$C_{28}H_{58}$	Octacosanol

Table 2 shows different peaks and areas for each compound as well as their molecular formula. Compounds such as Phytol ($C_{20}H_{40}$) and Hexanoic acid 2, tetradecyl have same molecular formula. This is also characteristic of other compounds such as gamma-Tocopherol and beta-Tocopherol which have the molecular formula $C_{28}H_{48.}$ Vitamin E and dl-alpha-Tocopherol also have the same molecular formula ($C_{29}H_{50}$) as well as 13-Octadecenal, (2)- and Oleic acid ($C_{18}H_{34}$) respectively. The table also shows 13-octadecenal, (Z)- and 15-hydroxypentadecanoic acid to possess the highest area (21.74% and 18.65%) respectively. 9,12-octadecadienoic acid (Z, Z)- and butane, 2-iodo-3-methyl possess the lowest area (0.18% and 0.30%) respectively.

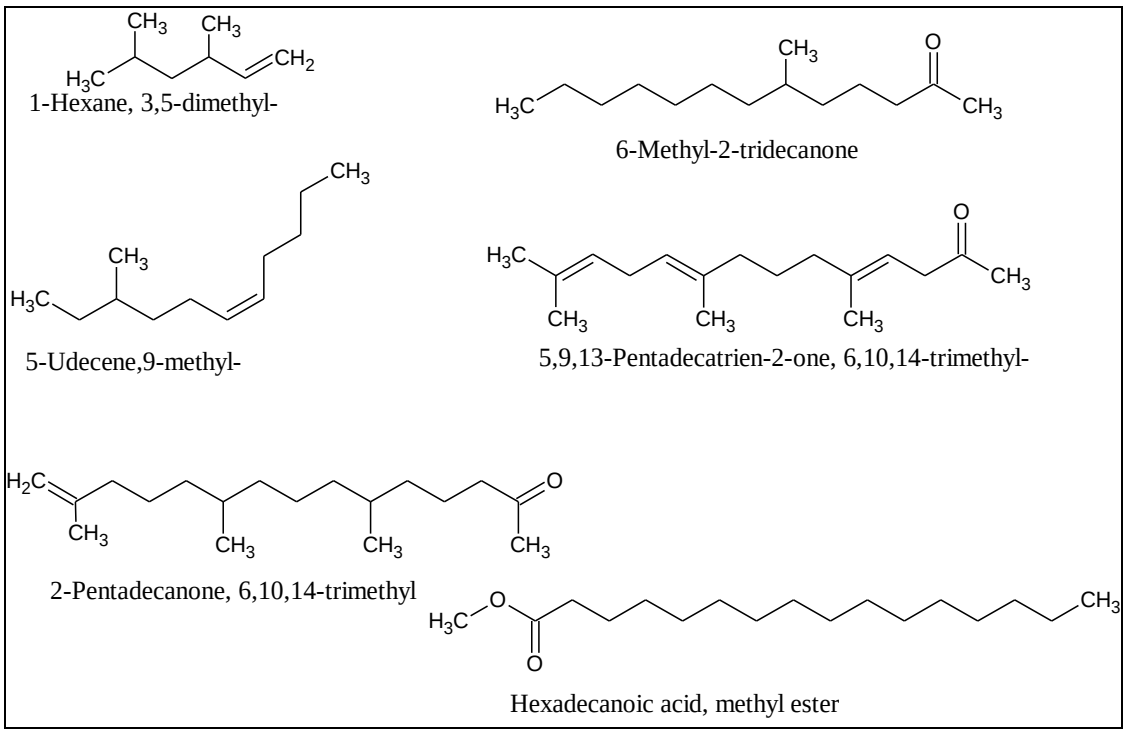

Fig 5: Chemical Structures of Components of leaf volatile oils of *T. tetraptera*

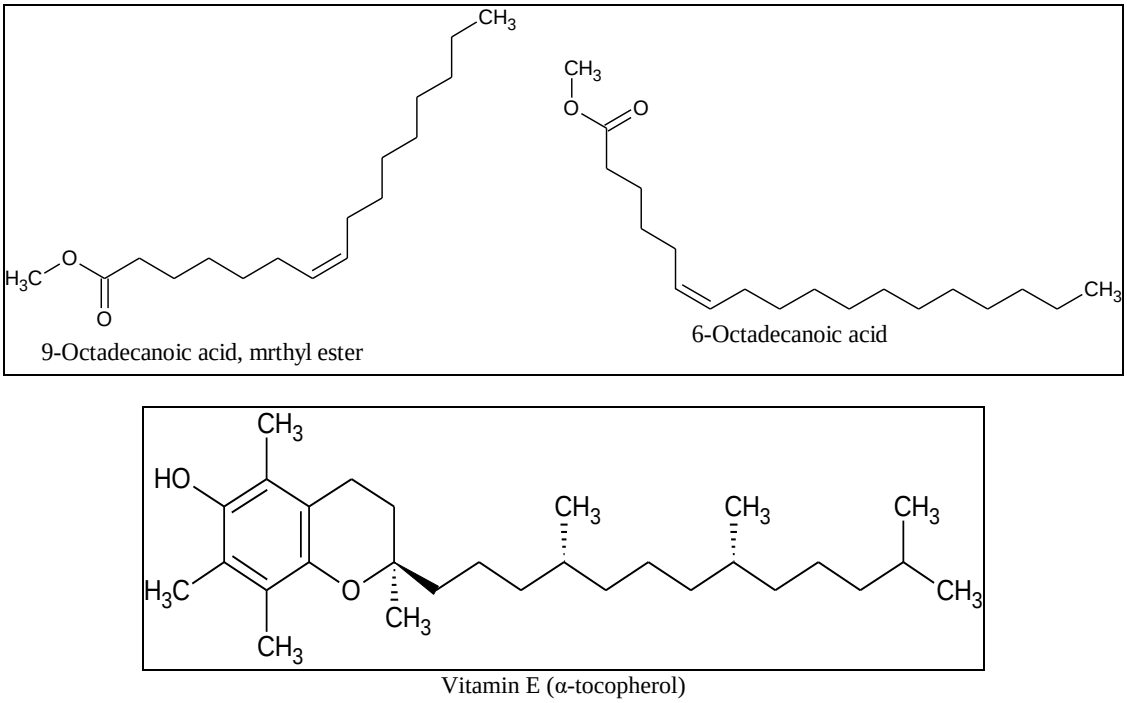

Fig 6: Chemical structures of some identified compounds

Conclusion

T. tetraptera is composed of numerous phytochemicals which are responsible for the numerous bioactivities of different parts of the plant. The presence of saponins such as aridanin is responsible for its molluscicidal properties; the plant has been used as pharmacological agent for the treatment of inflammations, arthritis, and diabetes and even as an analgesic. The Gc-ms analysis of the fixed oil of the leaf reveals the presence of thirty one compounds which are basically esters, fatty acids, alkanes, alkenes and terpenoids as well as vitamins. Compounds such as hydroxypentdecanoic acid, oleic acid, methyl stearate, hexanoix acid, pentadecanone and octadecanoic acid have been reported as antimicrobial and molluscicidal agents.

Conflict of Intrest: None.

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