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GC-MS evaluation of bioactive compounds and antibacterial activity of the oil fraction from the seeds of *Brachystegia eurycoma* (HARMS)

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ABSTRACT

The ethanolic extract of the seeds of Brachystegia eurycoma Harms yielded a dark-brown oil (4.26g). The oil was subjected to GC-MS studies. Twelve phyto-constituents were identified with 9,12-Octadecadienoic acid ethyl ester (25.38%) constituting the bulk of the oil, followed by n-Hexadecanoic acid (15.00%). Other esters, fatty acids and steroid identified include Hexadecanoic acid ethyl ester (10.00%), 9-Octadecenoic acid ethyl ester (13.46%), Octadecanoic acid (13.08%), Eicosanoic acid (4.62%), Docosanoic acid (5.77%), Docosanoic acid ethyl ester (2.31%), 9,12-Octadecadienoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester (1.92%), Tetracosanoic acid (3.85%), Ethyl tetracosanoate (2.69%) and Beter-sitosterol (1.92%). The volatile oil showed antibacterial activity against Escherichia coli, Salmonella typhi and Staphylococcus aureus. These results suggest why Brachystegia eurycoma Harms is used in the treatment of wounds and infections in herbal medicine in Eastern Nigeria.

Keywords: *Brachystegia eurycoma*, GC-MS analysis, Bioactive compounds, Antibacterial activity, Herbal medicine.

INTRODUCTION

There has been a meticulous attempt to harness the bio-potentials of Nigerian vegetation in the area of herbal medicine. That notwithstanding, it is somewhat impossible to explore all that this vegetation can afford even as it battles to survive the onslaughts of deforestation and industrialization. Many naturally occurring chemicals from plants exhibit a broad spectrum of pharmacological profile. These plant chemicals are classified as primary or secondary metabolites[1]. The primary metabolites include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids and chlorophyll[2]. Secondary metabolites are the remaining plant chemicals which are produced from the primary metabolites. These include alkaloids (derived from amino acids), terpenoids (a group of lipids), phenolics (derived from carbohydrate) tannins, steroids and volatile oil[3].

One of such plants with medicinal and food values is *Brachystegia eurycoma* Harms. The plant is native to tropical Africa[4]. It grows mainly along the river banks or swamps in Western and Eastern Nigeria and also in Cameroon[5]. It is a large tree with irregular and twisted spreading branches. The fruit ripens from September to January and is released by explosive mechanism[5]. The exudate is used in fast healing of wounds[6]. The exudate, in right combinations with mucin and honey is used for wound healing, prevention of bacteria infection, scar formation and promotes regeneration of hair follicles[6]. As part of our chemical studies on Nigerian medicinal plants we describe herein the chemical constituents of the volatile oil of *Brachystegia eurycoma* Harms and also evaluate the antibacterial activity of the oil against some pathogenic bacteria for possible development of new drugs for the prevention and treatment of infections.

MATERIALS AND METHODS

Experimental

GC analyses were carried out in SHIMADZU Japan gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25nm x 50m) and the conditions were as follows: Temperature programming from 80 – 200^{o}C held at 80^{o}C for 1 minute, rate 5^{o}C/Min and at 200^{o}C for 20 minutes. FID temperature 300^{o}C, injection temperature 250^{o}C, carrier gas nitrogen at a flow rate of 1ml/min, split ratio 1:75. GC-MS (Gas chromatography Mass spectrometry) analysis was conducted using GCMS-QP 2010 PLUS SHIMADZU JAPAN with injector temperature of 23^{o}C and carrier gas pressure of 100kpa. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. The eluents were automatically passed into a mass spectrophotometer with a dictator voltage set at 1.5kv and sampling rate of 0.2 seconds. The mass spectrum was also equipped with a computer fed mass spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used. Reagents and solvents like ethanol, chloroform, diethyl ether, hexane were all of analytical grade and were procured from Merck, Germany. The nutrient agar was purchased from Scharian Chemical (APHA) Spain.

Plant Materials

Fresh *Brachystegia eurycoma* Harms seeds were bought from Umuahia Ogwumabiri market in Abia State, Nigeria. Clean and wholesome seeds were selected, identified and authenticated by Mr. I. K. Ndukwe of Plant Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Nigeria.

Extraction of Plant Materials

The seeds of *Brachystegia eurycoma* Harms were weighed (1kg) and then decoated by soaking in water for 24 hours. The loosened hull was washed off with several changes of water. The dehulled seeds were air-dried and then ground into powder (820g) using a Thomas Wiley Machine (Model 5 USA). The powdered plant sample (300g) was successively extracted with 2L of benzene (8 hours/3 times/80^{o}C) followed by 2L of ethanol (8 hours/3 times/65^{o}C). The extracts were concentrated under reduced pressure and the supernatant dark oil was decanted (3.2g) after complete removal of the solvent. The oil was centrifuged at 10,000rpm for 20 minutes and the clear supernatant oil was subjected to systematic GC-MS analysis.

Component Identification

Oil components were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature[7].

Bioassay

The *in vitro* antibacterial activity of the oil was carried out for 24h culture of three selected bacteria. The bacteria organisms used were *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. All the test organisms were clinical isolates of human pathogens obtained from stock cultures at the Central Laboratory services Unit f National Root Crops Research Institute, Umudike, Abia State, Nigeria. With the aid of a single hole punch office paper perforator, circular discs of 5 mm diameter were cut from Whatman No 1 filter paper. The paper discs were boiled in distilled water for an hour to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminum foil and sterilized in an autoclave at 121^{o}C for 15 minutes. They were however used within 48 hours of production. The sensitivity of each test microorganism to the oil was determined using the Disc Diffusion Technique[8,9]. A loopful of each test sample organism was aseptically transferred into the surface of a sterile solid medium, appropriate for the test organism. Using a flamed glass hockey, the innoculeum was spread evenly over the surface of the medium, and then with the aid of a flamed pair of forceps, the extract bearing paper discs was carefully place on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 hours in an incubator at 37^{o}C. They were examined daily for growth and for the presence of inhibition zones around the paper discs. The level of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent millimeter rule. The minimum inhibitory concentration (MIC) was determined by comparing the different concentrations of the oil having different zones and selecting the lowest concentration.

RESULTS AND DISCUSSION

The dark-brown oil obtained from the ethanol extract of *Brachystegia eurycoma* Harms seeds showed twelve peaks from the chromatogram of the oil . These peaks indicated the presence of twelve compounds (1 -12) in the oil

(Figure 1). The molecular formula, percentage constituents and molecular masses of the compounds are shown in Table 1. These compounds comprised mainly fatty acids, esters and steroids. The composition of the oil was esters 55.76%, fatty acids 42.32% and steroid 1.92%

Compound **1** was identified as n-Hexadecanoic acid and has molecular formula of $C_{16}H_{32}O_2$ (M/Z 256) with base peak at M/Z 73. The base peak M/Z 73 occurred due to the cleavage of $CO_2C_2H_5$ group from the compound. The compound comprised 15.00% of the volatile oil. Compound **2** was identified as Hexadecanoic acid ethyl ester with molecular formular of $C_{18}H_{36}O_2$ (M/Z 284) and base peak at M/Z 88. The base peak occurred as a result of McLafferty rearrangement leading to the detachment of $CH_2=C(OH)OC_2H_5$ group from the compound. The compound comprised 10.00% of the volatile oil. Compound **3** was named 9,12-Octadecadienoic acid ethyl ester with molecular formular of $C_{20}H_{36}O_2$ (M/Z 308) and base peak at m/z 81. The compound **4** was identified as 9-Octadecenoate acid ethyl ester with molecular formular of $C_{20}H_{38}O_2$ (M/Z 310) and base peak at M/Z 55 which occurred as a result of the cleavage of a butyl group (C_4H_7) from the compound. The compound comprised 3.46% of the volatile oil. Compound **5** was a fatty acid identified as Octadecanoic acid with a molecular formula of $C_8H_{36}O_2$ (M/Z 284). It showed a base peak at M/Z 43 due to the detachment of a propyl group (C_3H_7) from the compound. It comprised 13.08% of the volatile oil. Compound **6** was also a fatty acid known as Eicosanoic acid with the molecular formula $C_{20}H_{40}O_2$ (M/Z 312) and has a base peak at M/Z 43 which was also due to the cleavage of a propyl group (C_3H_7) from the compound. The compound comprised 4.62% of the oil. Compound **7** was another fatty acid which was identified as n-Docosanoic acid with molecular formula of $C_{22}H_{44}O_2$ (M/Z 312) and base peak at M/Z 43. The base peak at M/Z 43 was an indication of another loss of a propyl group (C_3H_7) from the compound. The compound comprised 5.77% of the volatile oil. Compound **8** was an ester identified as Docosanoic acid ethyl ester. It has a molecular formula of $C_{24}H_{48}O_2$ (M/Z 368) and a base peak at M/Z 88 which was as a result of McLafferty rearrangement causing the cleavage of $CH_2=C(OH)OC_2H_5$ group from the compound. The composition of the compound in the oil was 2.31%. Compound 9 was also an ester identified as 9,12-Octadecadienoic acid-2-hydroxy-1-(hydroxyl-methyl) ethyl ester with molecular formula of $C_{21}H_{38}O_4$ and base peak at M/Z 67. The compound comprised 1.92% of the oil. Compound **10** was a fatty acid named Tetracosanoic acid with molecular formula of $C_{24}H_{48}O_2$ (M/Z 368) and a base peak at M/Z 43 which was as a result of propyl group cleavage from the compound. The compound comprised 3.85% of the oil. Compound **11** was identified as ethyl Tetracosanoate with molecular formula $C_{26}H_{52}O_2$ (M/Z 396) and a base peak at M/Z 88 due to McLafferty rearrangement leading to the cleavage of $CH=C(OH)OC_2H_5$ group of the oil. Compound **12** was a steroid identified as beta-sitosterol with molecular formula of $C_{29}H_{50}O$ (M/Z 414). The compound has a base peak at M/Z 43 due to the cleavage of a propyl group (C_3H_7).

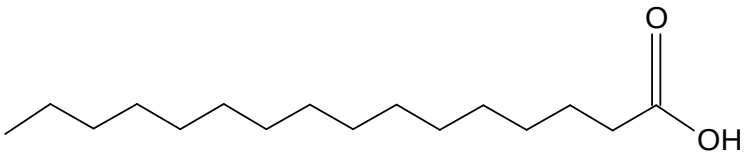
Steroids are abundant in nature; many derivatives of steroids have Physiological activity[10]. Steroid hormones control sexual development and fertility in the human body[11]. As a result of their physiological functions, many steroids are used in medicine in the treatment of cancer, arthritis or allergies and in birth control[10,11]. The detection of a steroid in the seeds of *Brachystegia eurycoma* (HARMS) suggests the use of the seeds in fertility therapy in man where increase in sperm count is important. The seeds may also find use in the management of cancer, arthritis and allergies. Therefore, diets incorporated with the seed flour should be recommended to people with such health problems.

Fatty acids always occur in plants. Fatty acids in plants react with alcohols in an esterification reaction to form esters[12]. The composition of unsaturated fatty acids and fatty acid esters in the seeds of *Brachystegia eurycoma* was found to be 40.76%. The constituent with the highest quantity in the seed was 9,12-Octadecadienoic acid ethyl ester having a composition of 25.38%. Unsaturated fatty acids are important to every cell in the body for normal growth, especially of the blood vessels and nerves and to keep the skin and other tissues youthful and supple trough their lubricating quality[13]. These are nutrients which are invaluable for the production and movement of energy throughout the body, regulation of transportation of oxygen and are vital in maintaining the integrity of cell structure as well as the unique ability to lower cholesterol levels of the blood[13]. The use of *Brachystegia eurycoma* seeds in food possesses no health problems but provides nutritional and medicinal benefits.

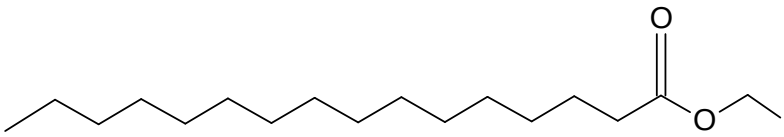
Table 1: GC-MS analysis of Ethanol Fractions from the Seeds of *Brachystegia eurycoma* (HARMS), Showing the Fragment ion Peaks and Retention Time.

Chromatogram peak	Compound name	Molecular formula	Molecular weight	Retention time(min)	Peak height (cm)	Percentage content(%)	Fragment peak(m/z) and % abundance
1	n-Hexadecanonic acid	$C_{16}H_{32}O_2$	256	25.7	3.9	15.00	27(2%), 41(85%), 43(95%), 60(96%), 73(100%), 85(20%), 98(10%), 115(10%), 129(20%), 157(5%), 171(5%), 185 (5%), 213(10%)
2	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284	25.9	2.6	10.00	27(24%), 41(29%), 57(24%), 73(14%), 88(100%), 101(63%), 115(5%), 143(5%), 157(10%), 239 (5%)
3	9,12,-Octadecadienoic acid ethyl ester	$C_{20}H_{36}O_2$	308	28.1	6.6	25.38	41(52%), 55(67%), 67(100%), 81(86%), 95(62%), 109(29%), 123(14%), 136(10%), 150(1%), 164(5%), 178(5%)
4	9-octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	310	28.2	3.5	13.46	27(5%), 41(71%), 55(100%), 69(76%), 83(67%), 88(2%), 101(52%), 123(19%), 137(10%), 152(10%), 180(20%), 22(24%), 246(43%), 266(29%)
5	Octadecanoic acid	$C_{18}H_{36}O_2$	284	28.3	3.4	13.08	27(79%), 41(67%), 43(10%), 60(86%), 73(85%), 85(29%), 98(24%), 155(14%), 129(48%), 143(10%), 171(10%), 185(19%), 199(10%), 277(5%), 241(19%)
6	Eicosanoic acid	$C_{20}H_{40}O_2$	312	30.6	1-2	4.62	27(33%), 41(62%), 43(100%), 7(76%), 73(76%), 85(24%), 98(19%), 115(10%), 129(29%), 171(5%), 185(5%), 213(5%), 254(5%), 269(10%), 298(10%)
7	Docosanoic acid	$C_{22}H_{44}O_2$	312	32.9	1.5	5.77	27(9%), 41(52%), 43(100%), 57(67%), 73(62%), 85(24%), 98(10%), 115(5%), 129(19%), 143(2%), 171(2%), 185(5%), 214(5%), 297(10%)
8	Docosanoic acid	$C_{24}H_{48}O_2$	368	33.1	0.6	2.31	27(14%), 29(36%), 43(83%), 57(71%), 71(29%), 88(100%), 101(67%), 115(5%), 129(2%), 143(5%), 157(10%)
9	9,12-octadecadienoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{38}O_4$	354	33.9	0.5	1.92	27(14%), 41(67%), 55(76%), 67(100%), 81(81%), 95(48%), 109(24%), 121(14%), 135(14%), 149(10%), 163(10%), 185(10%), 234(10%)
10	Tetracosanoic acid	$C_{24}H_{48}O_2$	368	35.0	1.0	3.85	27(7%), 41(48%), 43(100%), 57(81%), 73(76%), 85(33%), 98(29%), 115(14%), 129(48%), 143(5%), 171(10%), 185(14%), 269(5%)
11	Ethyl tetracosanoate	$C_{26}H_{52}O_2$	396	35.2	0.7	2.69	27(5%), 41(19%), 43(43%), 57(29%), 71(14%), 88(1100%), 101(52%), 115(5%), 129)2%), 143(5%), 157(19%), 199(5%), 213(5%), 353(5%)
12	Beta-sitosterol	$C_{29}H_{50}O$	414	44.2	0.5	1.92	4(38%), 43(100%), 57(48%), 81(29%), 95(24%), 107(29%), 119(14%), 133(19%), 145(19%), 161(19%), 173(14%), 213(19%), 231(10%), 255(14%), 273(14%), 303(14%), 315(10%), 329(14%), 382(14%), 396(19%)

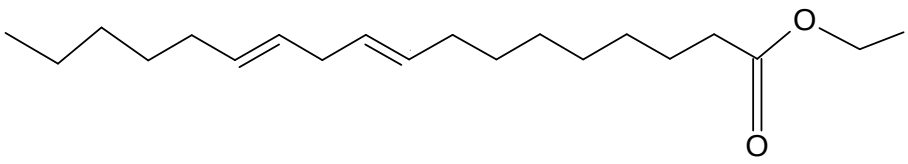
The oil from the seeds of *Brachystegia eurycoma* successfully inhibited *E. coli*, *S. typhi* and *S. aureus* (Table 2). It exhibited highest antibacterial activity against *E. coli*. The minimum inhibitory concentration (MIC) of the oil was 50 – 75%. The microorganisms tested were human commensals and have been incriminated in the infection of wounds[14]. These findings suggest the use of *Brachystegia eurycoma* extracts in the treatment of wounds. The inhibition of the oil against *S. typhi* and *S. aureus* suggests the use of the plant in the treatment of typhoid fever and gonorrhea. The mechanism of inhibitory action may be due to impairment of variety of enzyme systems, including those involved in energy production, interference with the integrity of the cell membrane and structural component synthesis[14]. The oil from the seeds of *Brachystegia eurycoma* possesses phyto-constituents capable of inhibiting the growth of microbial wound contaminants, accelerate wound healing and consequently cause cell proliferation.



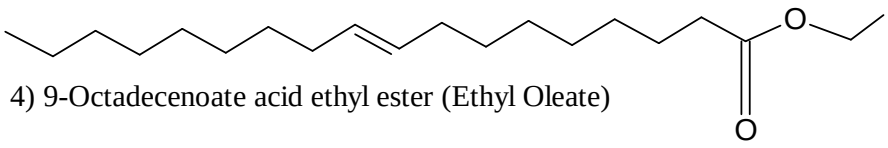
1) n-Hexadecanoic acid (Palmitic acid)



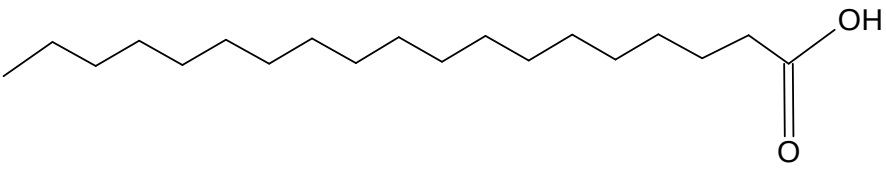
2) Hexadecanoic acid ethyl ester



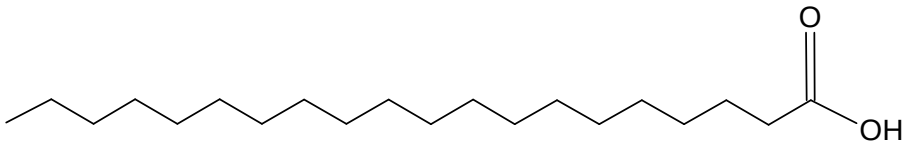
3) 9,12-Octadecadienoic acid ethyl ester (Linoleic acid ethyl ester)



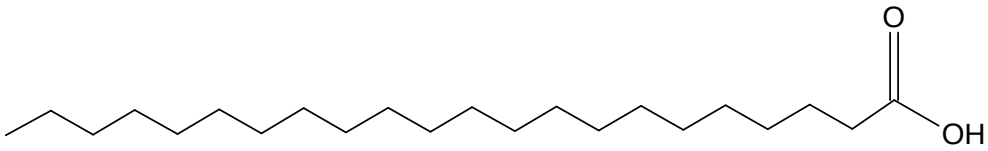
4) 9-Octadecenoate acid ethyl ester (Ethyl Oleate)



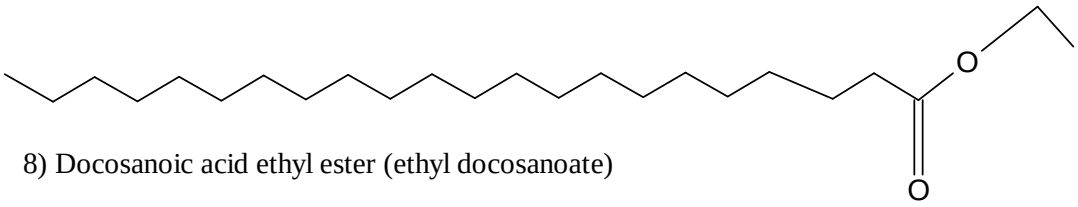
5) Octadecadienoic acid (Stearic acid)



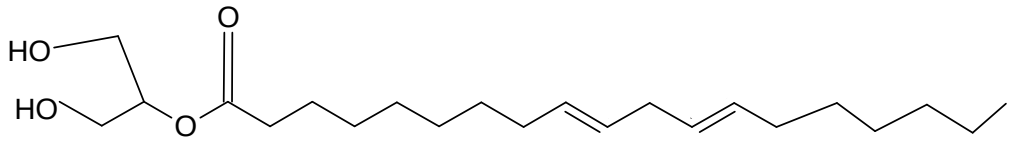
6) Eicosanoic acid



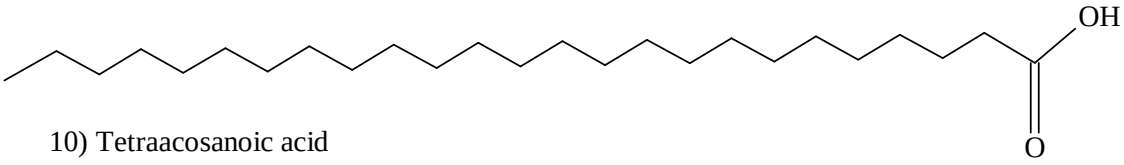
7) n-Docosanoic acid



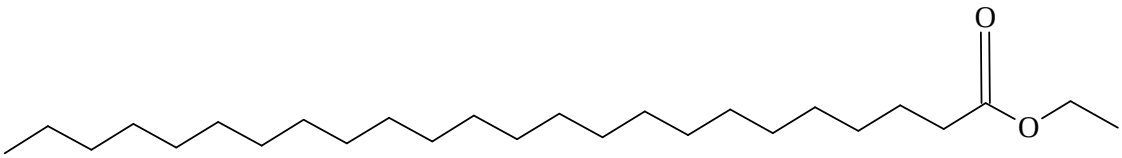
8) Docosanoic acid ethyl ester (ethyl docosanoate)



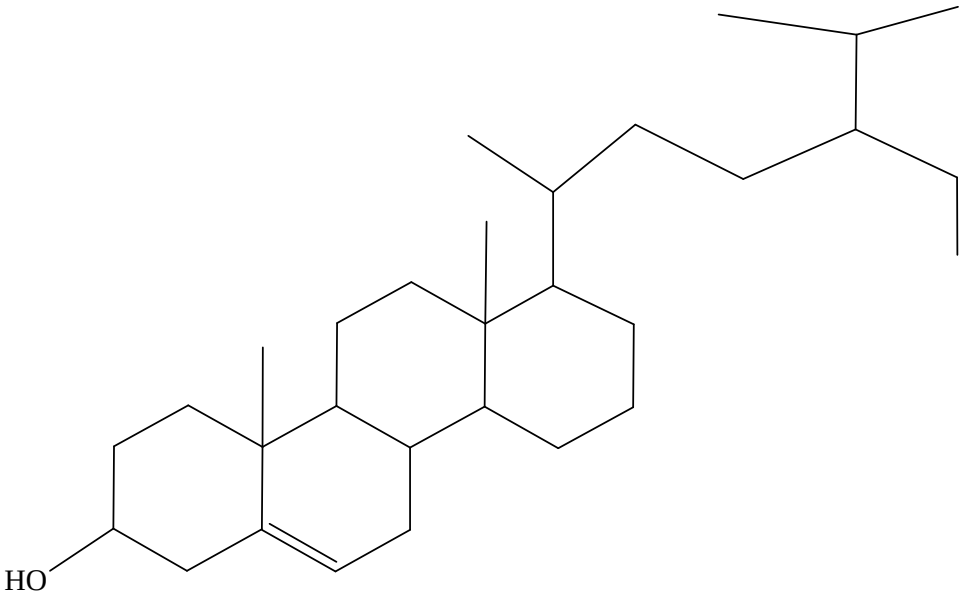
9) 9,12-Octadecadienioc acid-2-hydroxy-1-(hydroxymethyl) ethyl ester



10) Tetraacosanoic acid



11) Ethyl tetracosanoate



12) Beta-sitosterol

Figure 1: Structure of the compounds from GC-MS analysis of the oil from the seeds of *Brachystegia eurycoma.*

Table 2: Inhibitory effects of the oils from the seeds of *Brachystegia eurycoma*

Pathogens	Concentration (%)			MIC (%)
	50	**75**	**100**	
Escherichia coli	9.00	9.67	11.33	50
Salmonella typhi	-	7.67	10.67	75
Staphylococcus aureus	-	5.50	9.67	75

Figures are in mm and include the diameter of the paper disc (5mm). Data are means of triplicate determinations.
MIC = Minimum inhibitory concentration
- = No inhibition

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