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Determination of the Total Phenolic Contents of Essential Oil Obtained from *Cymbopogon Citratus* (Lemongrass) and *Persea Americana Mill (*Avocado Pear Seed) and its Bioactive Component Using Gc-Ms Analysis

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Abstract :-
Background and Purpose: Essential oils are a mixture of volatile lipophilic constituents, most commonly sourced from leaf, bark tissue of higher plants. The study investigated the total phenolic contents of essential oil obtained from *Cymbopogon citratus* (lemongrass) and *Persea Americana mill (*avocado pear seed) and its bioactive component.
Methods: The essential oils of both plants were obtained using the Soxhlet extraction method. Total phenol compositions were assayed using standard colourimetric methods. Gas chromatography-mass spectrometry (GC-MS) analysis was used to ascertain the presence of phytochemicals in the extract.
Results: The total phenolic content of the plants showed a significant ($p < 0.05$) difference ranging from 23.88 to 46.38 mg tannic acid equivalent/g of extract. *Cymbopogon citratus* had the highest value of 46.38 ± 0.26 mg tannic acid equivalent/g compared to *Persea Americana Mill's,* which had a value of 23.88 ± 0.34 mg tannic acid equivalent/g. The chromatograms corresponding to the chromatographic analysis (GC-MS) of *Cymbopogon citratus* and *Persea Americana Mill* allowed the identification of 26 different compounds from *Cymbopogon citratus* 15 different compounds from *Persea Americana Mill*. The peak height of *Cymbopogon citratus* showed a significant ($p < 0.05$) difference ranging from 2781 to 335638. It was observed that Caryophyllene had the highest value compared to that of Myrcenyl acetate, which had the lowest value of 2781. *Persea Americana mill* also showed a significant ($p < 0.05$) difference ranging from 560862 to 13557730, with Tetra decanoic acid having the highest value of 13557730 compared to 1 E-11, Z-13-Octadecatriene with the lowest value of 560862. This difference in peak height could result from the difference in the molecular weight of the volatile compounds.
Conclusions: Overall, the study gave information on the phenolic and volatile compounds of *Cymbopogon citratus* (lemongrass) and *Persea Americana mill (*avocado pear seed) and could be a potential source of natural antioxidants and could be used as a therapeutic agent in preventing or treating degenerative diseases associated with oxidative stress.

Keywords:- *Essential Oils, Cymbopogon citratus, Persea Americana mill, Gas-Chromatography Mass-Spectrophotometry (GC-MS).*

I. INTRODUCTION

There has been an increased search for food substances with health benefits. Such foods contain bioactive components that are advantageous to human health and exhibit properties as inhibitors of the dreaded COVID-19 main protease (Galanakis, 2020). Plants do not just provide food and shelter, oxygen, and beautification to animals but are also sources of phytomedicines. The pharmaceutical industry has identified these bio-active agents in plants and their importance in synthetic medicine and has tested the most commonly used plant species (Nguenang *et al.,* 2005; Khan *et al.,* 2009). Phytomedicines are very beneficial and recently occupied an important place in medicine and plant research (Roger *et al.,* 2015). The medicinal property of plants results from bioactive phytochemicals that they contain (Sheikh *et al.,* 2013). Liu (2004) defined these bioactive non-nutrient plant compounds as phytochemicals.

Cymbopogon citratus (lemongrass) and *Persea Americana mill* (Avacado pear seed) have been shown to contain these bioactive compounds and utilized in food, cosmetics and pharmaceutical industries. Lemongrass is a tall perennial grass generally grown in humid tropical and subtropical regions belonging to *Poaceae* (Olorunnisola *et al.,* 2014). Furthermore, lemongrass contains high amounts of minerals, vitamins and macronutrients. In Nigeria, lemongrass is commonly used as spices and contains phenolic compounds that possess solid antioxidant capacity (Vazquez-Briones *et al.,* 2015). These phenolic compounds contain the benzene ring with a substituted hydroxyl group with a high radical scavenging capacity. They are the most produced

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secondary metabolite in plants, whose distribution is shown throughout the metabolic process. Numerous compounds, including flavonoids, phenolic acids, complex flavonoids, and coloured anthocyanins, are phenolic substances or polyphenols (Babbar *et al.,* 2014). Apart from the defence, which is the function usually associated with these compounds, they also play other vital roles like incorporating attractive substances to accelerate pollination, antibacterial and antifungal activities as well as defence against herbivores (Alasalvar *et al.,* 2001; Acamovic and Brooker, 2005; Edreva *et al.,* 2008).

Like Cymbopogon citratus (lemongrass), studies have shown that *Persea Americana mill*, a dicotyledonous plant from the Lauraceae, is mainly consumed as fresh fruit can be commercialized to give a higher added value product including bioactive compounds (Bruno *et al.,* 2018). These bioactive compounds of avocado, which have beneficial effects on human health, include vitamin C, vitamin E, carotenoids and phenolic compounds. The seeds and peels considered wastes are good sources of antioxidants (Saavedra *et al.,* 2017; Ayala-Zavala *et al.,* 2011) capable of preventing inflammatory diseases. Therefore, the pulp of avocado is an important raw material for the food, cosmetic and pharmaceutical industries (Bruno *et al.,* 2018).

This study aims at the determination of total phenolic contents of essential oil obtained from *Cymbopogon citratus* (lemongrass) and *Persea Americana mill (*avocado pear seed) and its bioactive component using GC-MS analysis.

II. MATERIALS AND METHODS

A. Collection and preparation of plant sample

The fresh *Cymbopogon citrates* (Lemongrass) plant and *Persea Americana Mill* (Avocado Pear) was collected from uncultivated farmland at Amanwo community in Ikwuano Local Government Area of Abia State. The plant was authenticated by a Taxonomist (Dr Ibe K. Ndukwe) from the forestry department, College of Natural Resources and Environment Management (CNREM), Michael Okpara University of Agriculture Umudike (Specimen voucher number = IHF 23142). The fresh leaves were washed thoroughly under running tap water and air-dried for three weeks (21 days) at room temperature (25 ± 2°C). The dried leaves were grounded to a fine powder using a heavy-duty grinding machine (TSK-948 West Point, France) and stored in a tight-lid container pending its use.

B. Extraction of plant material

The extraction was carried out using the Soxhlet apparatus at 40°C with n-hexane as the solvent under reflux for 6 hours. The set-up was allowed to reflux until all the compounds were collected.

The extract was transferred to a sterile clean container and stored at 4°C in a refrigerator until further analysis.

C. Determination of total phenolic content (TPC)

The total phenolic content (TPC) was determined colourimetrically using Folin– Ciocalteu reagent, as described by Paśko et al. (2009). Methanolic solution of the extract in the concentration of 10 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic extract solution, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. After that, a blank solution was made, which contained 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. Both samples and blank were incubated in a thermostat at 45°C for 45 min. The absorbance was determined using a spectrophotometer at λmax = 765 nm. Mean absorbance value was obtained when the samples were prepared in triplicate. The same procedure was repeated for the standard solution of Gallic acid. The concentration of phenolic was read from the calibration line, and then the content in extracts from the measured absorbance was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

D. Gas chromatography-mass spectrometry analysis

An Agilent 6890N gas chromatography equipped with an autosampler connected to an Agilent Mass Spectrophotometric Detector was used. One micro-litre of the sample was injected in the pulsed spitless mode onto a 30m x 0.25mm ID DB 5MS coated fused silica column with a film thickness of 0.15 micrometre. Helium gas was used as a carrier gas, and the column head pressure was maintained at 20psi to give a constant of 1ml/min. Other operating conditions were preset. The column temperature was initially held at 55°C for 0.4min, increased to 200°C at a rate of 25°C/mins, then to 280°C at a rate of 8°C/mins and to a final temperature of 300°C at a rate of 25°C/mins, held for 2mins. The identification time was based on retention time, and components with lower retention time elute first before higher retention time.

III. RESULTS

From our table below, the total phenolic content present in our leaves extract of *Cymbopogon citratus* (lemongrass) plants showed 46.38 ± 0.26(mg TAequ./g) and avocado (*Persea Americana Mill)* pear seed showed 23.88 ± 0.34(mg TAequ./g), both were expressed in terms of tannic acid equivalent and the values obtained for the concentration of total are expressed as mg of Ta/g of the extract.

Table. 1: Total phenolic contents of EOCC *(Cymbopogon citratus) and* EOPAM (*Persea Americana Mill)* leaves

Plants	Total Phenols (mg TAequ. /g)
Lemongrass	46.38 ± 0.26
Avocado pear seed	23.88 ± 0.34

EOS=Essential Oil *Cymbopogon citratus*, **EOPAM**= Essential Oil *Persea Americana Mill* leaves, **TAE** = tannic acid equivalent; **GAE** = gallic acid equivalent

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A. GCMS Screening of The EOCC (Cymbopogon citratus).

The GC-MS total ion content (TIC) chromatograms of the 26 peaks of the compounds detected are shown in Figure 1. The various chemical compounds present in the essential oils obtained from *Cymbopogon citratus* leaves using the Soxhlet extraction method with n-hexane solvent is presented in table 2 below. The mass spectrums of the major compounds are shown in Figures 2 – 6, while the structures are shown in Figures 7 – 31. The structures of the major compounds detected were Myrcenyl acetate (9.703%), Caryophyllene (8.997%), 4-Terpinenyl acetate (7.579%), Citronellal (6.383%) and 4-Androsten-3β,6β-diol-17-one (5.829%).

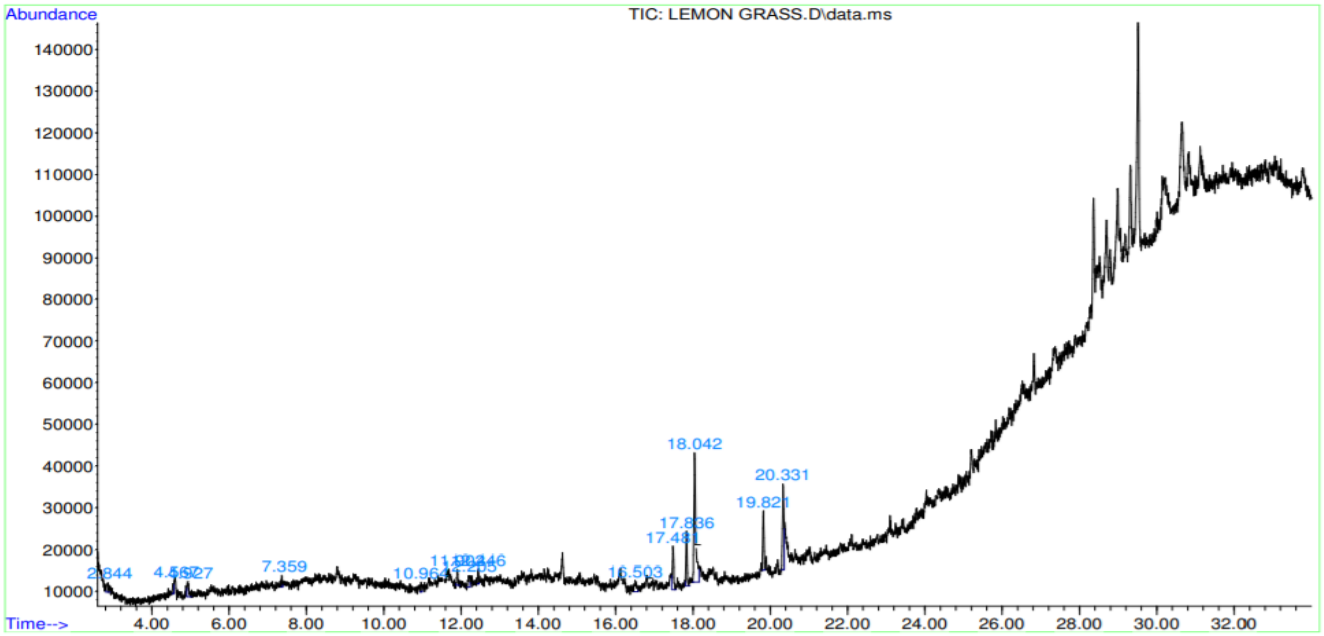

Fig. 1: GC-MS chromatogram of *Cymbopogon citratus* methanol leaves extract

Table 2: Chemical composition (GC-MS) of the n-hexane extract of *Cymbopogon citratus* (lemongrass)

Name	Peak Height (pA)	Retention time (minutes)	Conc (%)	Formular
2,6-Octadienal,3,7-dimethyl-, (E)	4314	4.560	3.720	$C_{10}H_{16}O$
2,6-Octadienal,3,7-dimethyl-, (Z)	3201	4.922	2.522	$C_{10}H_{16}O$
2,6-Octadien-1-ol,3,7-dimethyl-, (Z)	7541	5.550	1.127	$C_{10}H_{18}O$
Geranyl oleate	3815	5.612	0.845	$C_{28}H_{50}O_2$
Citronellal	21685	6.120	6.383	$C_{10}H_{18}O$
Cyclohexene,1-methyl-4-(1-methylethylidene)	21741	6.710	2.577	$C_{10}H_{16}$
Geranyl acetate	32428	6.804	4.943	$C_{12}H_{20}O_2$
Myrcenyl acetate	2781	7.079	9.703	$C_{12}H_{20}O_2$
4-Terpinenyl acetate	4151	7.308	7.579	$C_{12}H_{20}O_2$
2-Nonanone	6364	7.914	1.652	$C_9H_{18}O$
endo-Borneol	6452	8.080	3.662	$C_{10}H_{18}O$
Linalyl acetate	8726	8.257	2.468	$C_{12}H_{20}O_2$
α-Pinene	3010	8.395	0.514	$C_{10}H_{16}$
β-Pinene	7338	11.445	1.142	$C_{10}H_{16}$
Limonene	2887	11.891	0.504	$C_{10}H_{16}$
Linalool	4085	12.154	0.548	$C_{10}H_{18}O$
Caryophyllene	335638	15.364	8.997	$C_{15}H_{24}$
Bicyclo(7.2.0)undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)	2958	16.056	0.584	$C_{15}H_{24}$
Fenitrothion	6078	17.475	1.004	$C_9H_{12}NO_5PS$
Methyl parathion	4064	17.790	0.625	$C_8H_{10}NO_5PS$
Retinol	8319	17.830	1.194	$C_{20}H_{30}O$
6-Beta-hydroperoxy-3alpha,5-cyclo-5alpha-androstan-17-one	3470	17.962	0.659	$C_{19}H_{28}O_3$
4-Androsten-3β,6β-diol-17-one	29747	18.042	5.829	$C_{19}H_{28}O_3$
3β,5α,6β-Trihydroxyandrostan-17-one	7885	19.821	1.260	$C_{19}H_{30}O_4$
δ5-Androsten-3,17-dione	4185	20.331	0.659	$C_{19}H_{26}O_2$

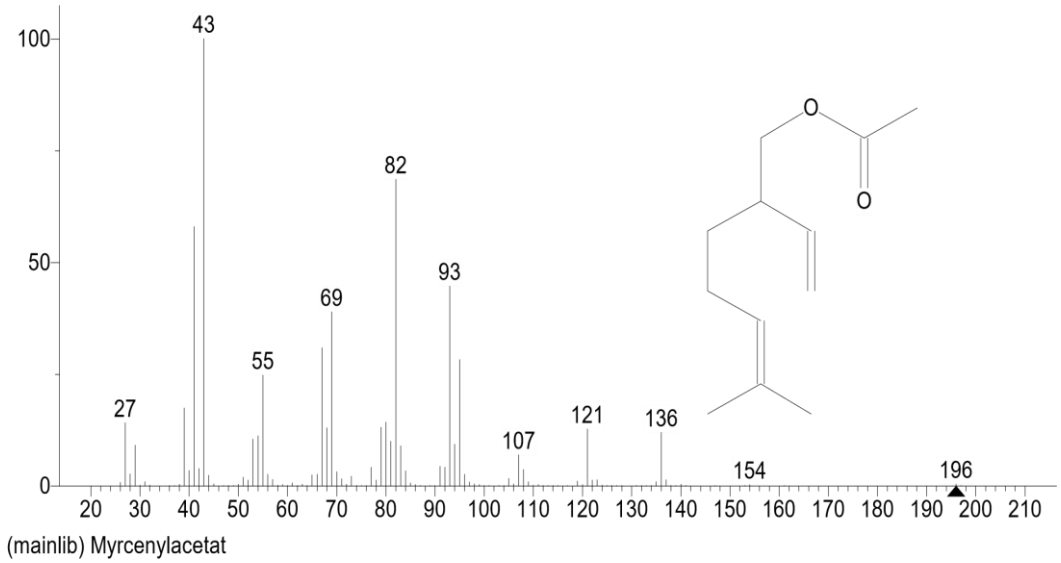

Fig. 2: Myrcenylacetat

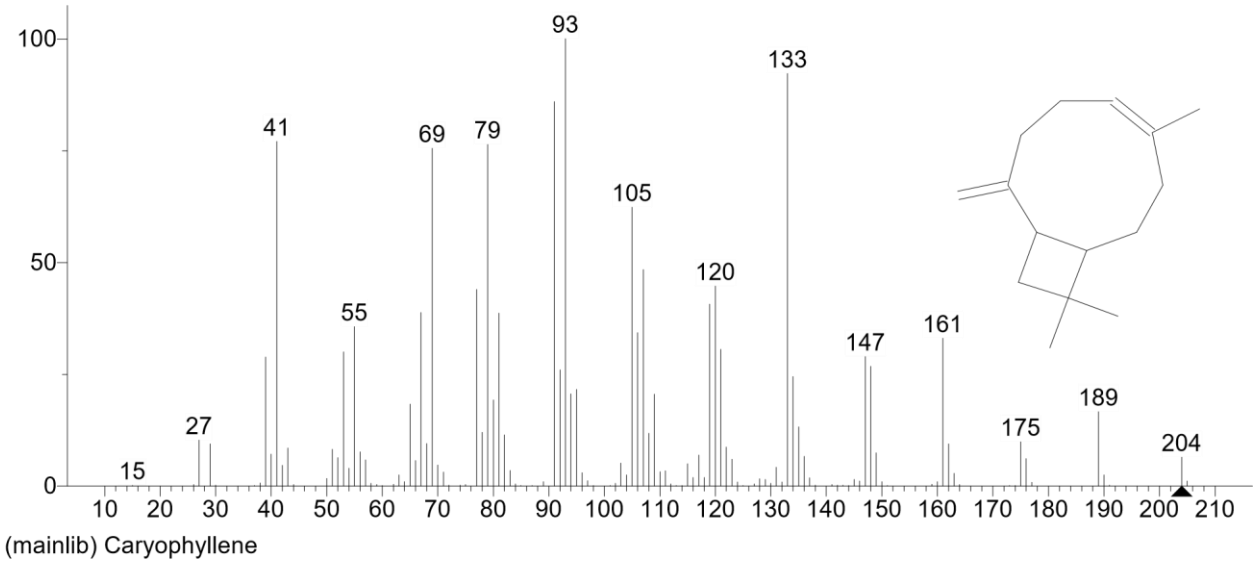

Fig. 3: Caryophyllene

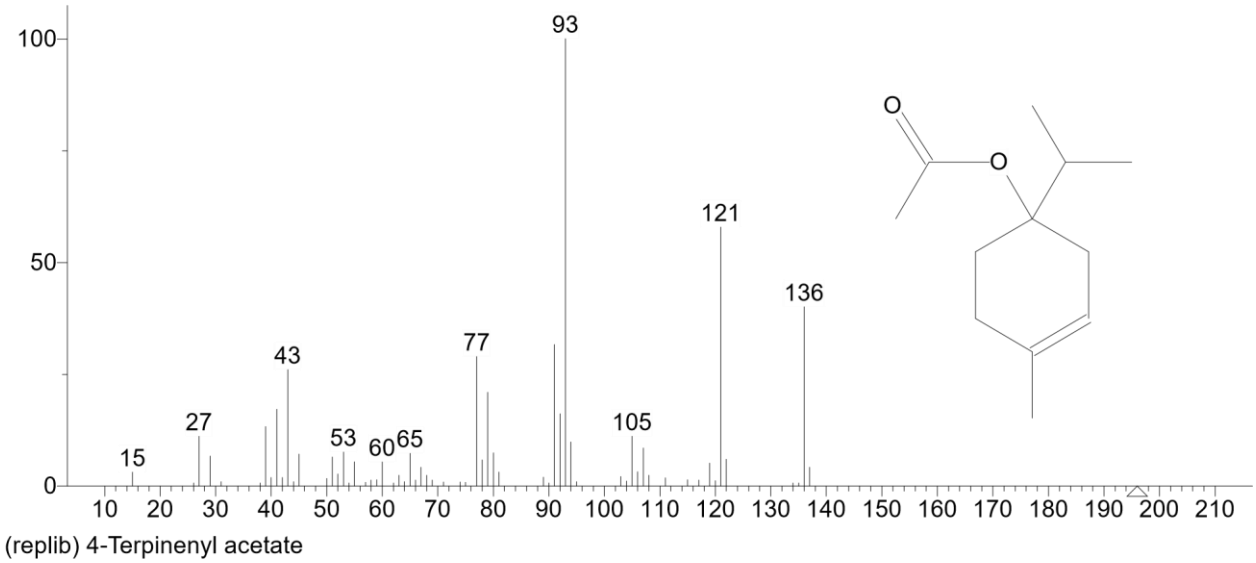

Fig. 4: 4-Terpinenyl acetate

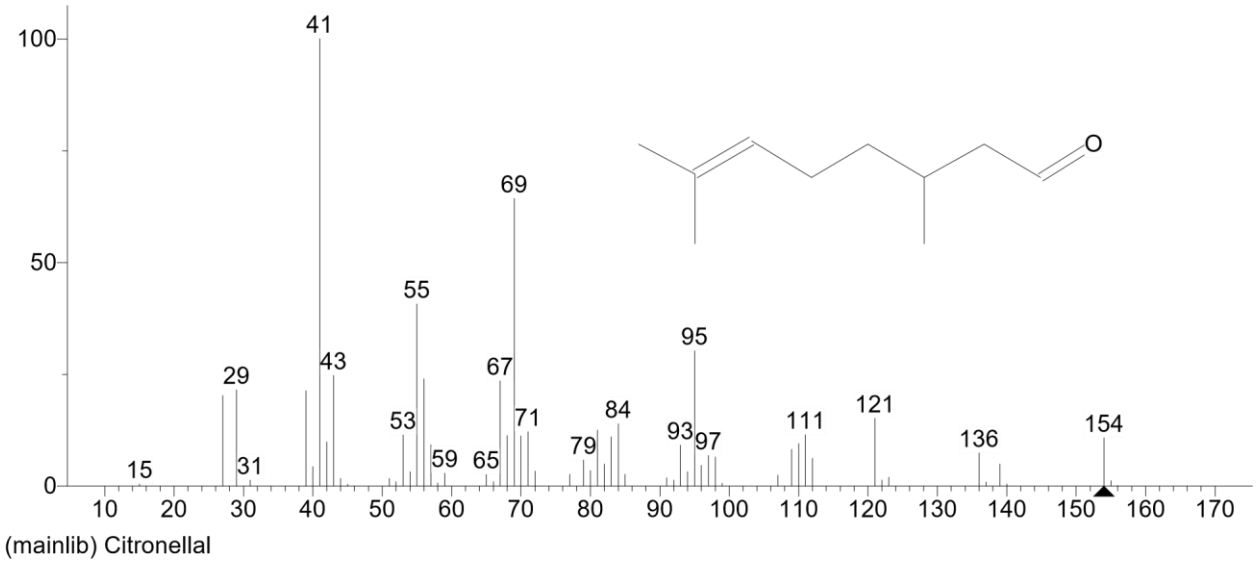

Fig. 5: Citronellal

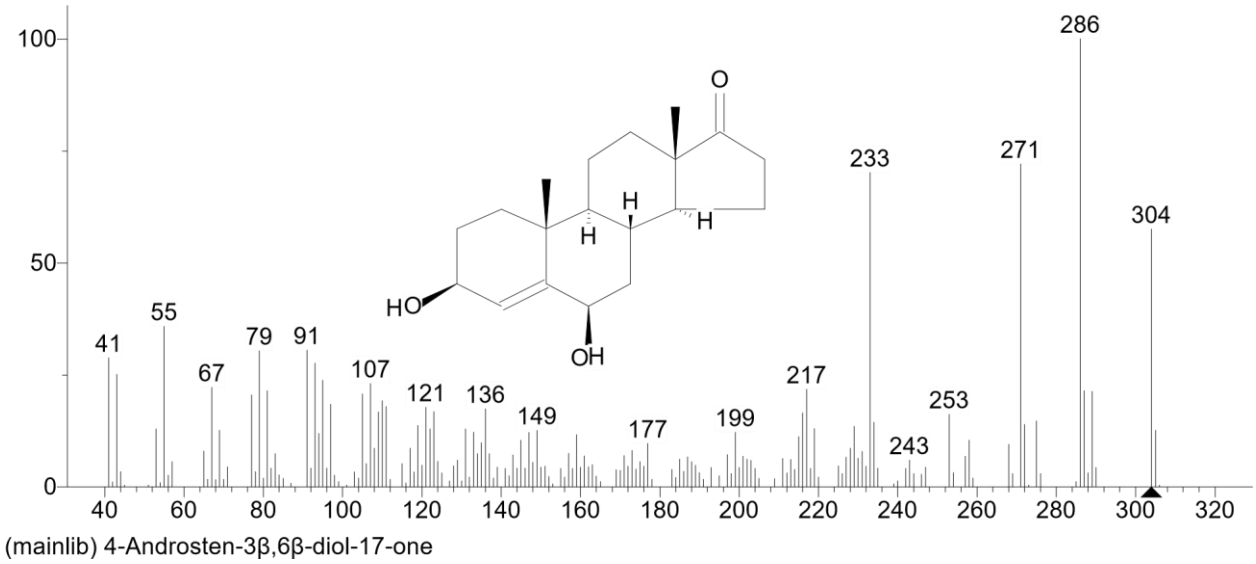

Fig. 6: 4-Androsten-3β,6β-diol-17-one

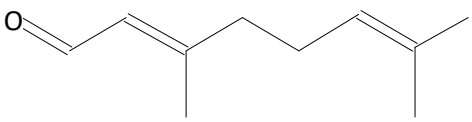

Fig. 7: 2,6-Octadienal, 3,7-dimethyl-, (E)-

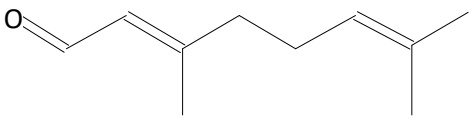

Fig. 8: 2,6-Octadienal, 3,7-dimethyl-, (Z)-

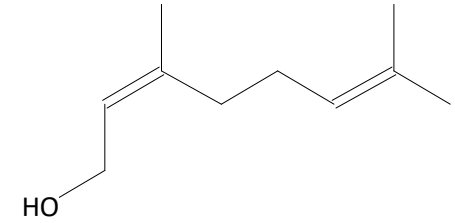

Fig. 9: 2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-

Fig. 10: Geranyl oleate

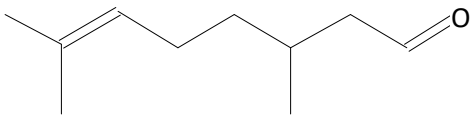

Fig. 11: Citronellal

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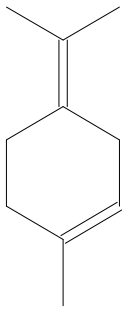

Fig. 12: Cyclohexene, 1-methyl-4-(1-methylethylidene)-

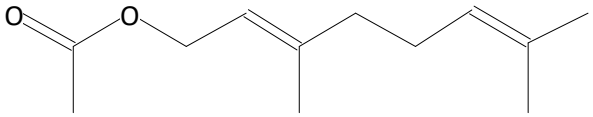

Fig. 13: Geranyl acetate

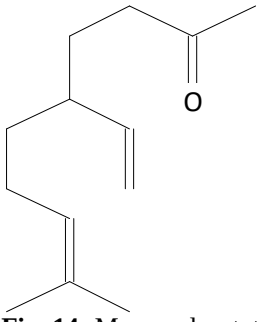

Fig. 14: Myrcenylacetat

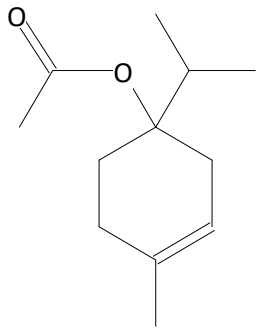

Fig. 15: 4-Terpinenyl acetate

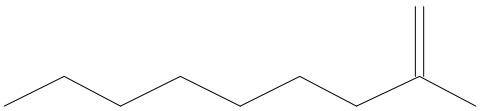

Fig. 16: 2-Nonanone

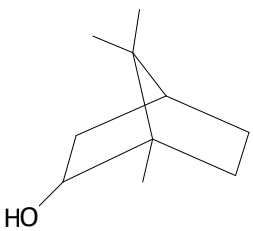

Fig. 17: endo-Borneol

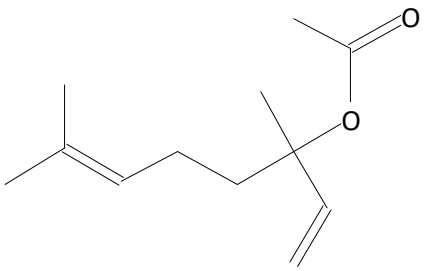

Fig. 18: Linalyl acetate

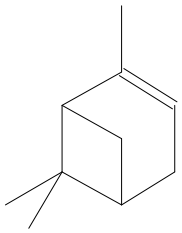

Fig. 19: α-Pinene

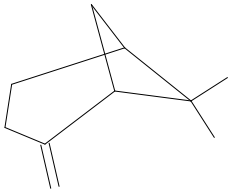

Fig. 20: β-Pinene

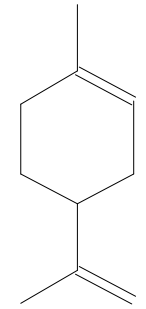

Fig. 21: Limonene

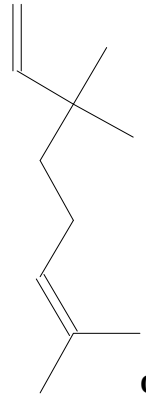

Fig. 22: Linalool

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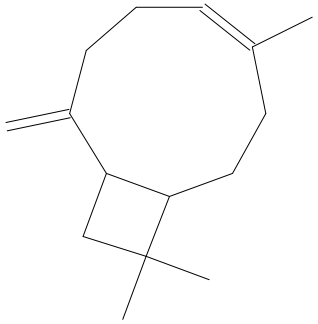

Fig. 23: Caryophyllene

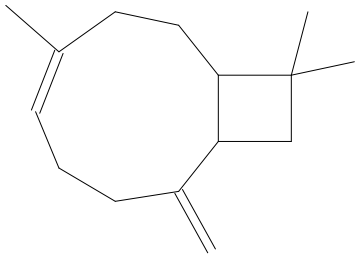

Fig. 24: Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-

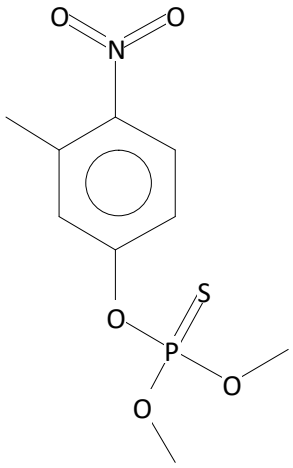

Fig. 25: Fenitrothion

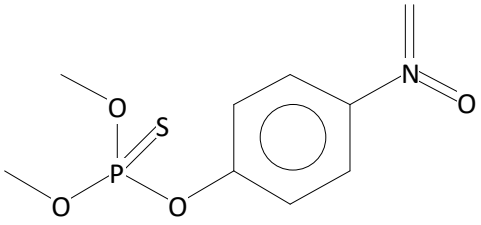

Fig. 26: Methyl parathion

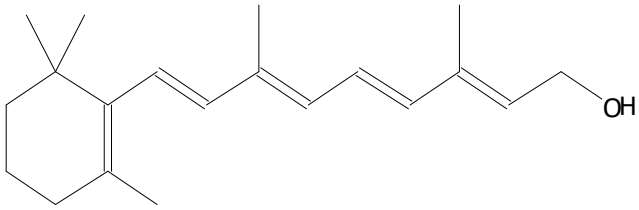

Fig. 27: Retinol

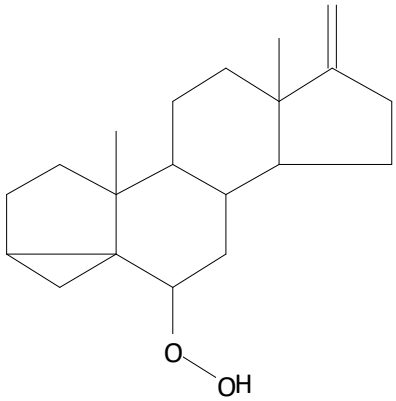

Fig. 28: 6Beta-hydroperoxy-3alpha,5-cyclo-5alpha-androstan-17-one

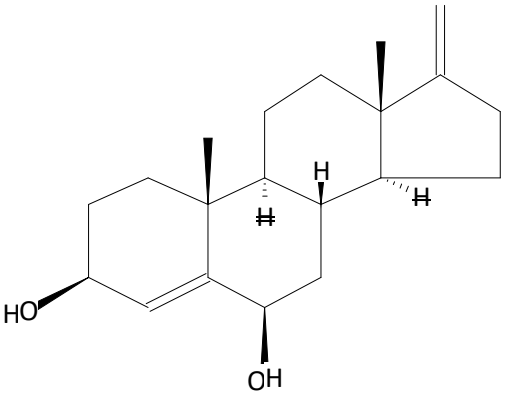

Fig. 29: 4-Androsten-3β,6β-diol-17-one

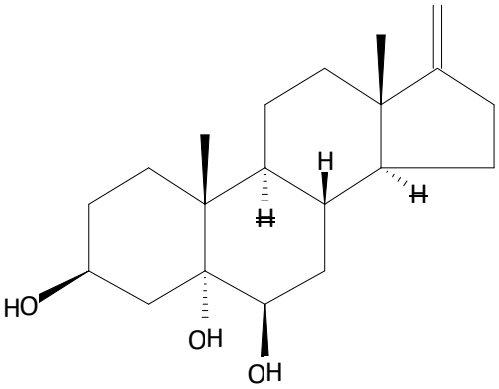

Fig. 30: 3β,5α,6β-Trihydroxyandrostan-17-one

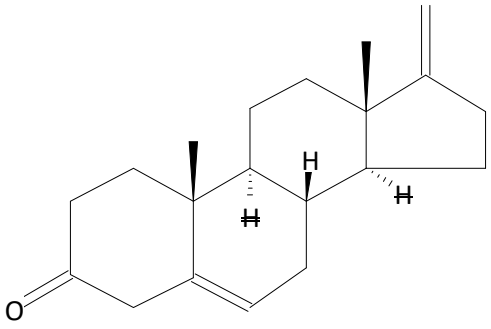

Fig. 31: δ5-Androsten-3,17-dione

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B. ***GCMS Screening of EOPAM*** *(Persea Americana Mill) leaves,*

The GC-MS total ion content (TIC) chromatograms of the 15 peaks of the compounds detected are shown in Figure 32. The various chemical compounds present in the essential oils obtained from *Persea Americana Mill* leaves using the Soxhlet extraction method with n-hexane solvent is presented in table 2 below. The mass spectrums of the major compounds are shown in Figures 33 – 35, while the structures are shown in Figures 36 – 49. The structures of the major compounds detected were Tetradecanoic acid (33.326%), 9,12-Octadecadienoic acid methyl ester (20.122%), and Oleic acid (11.60%).

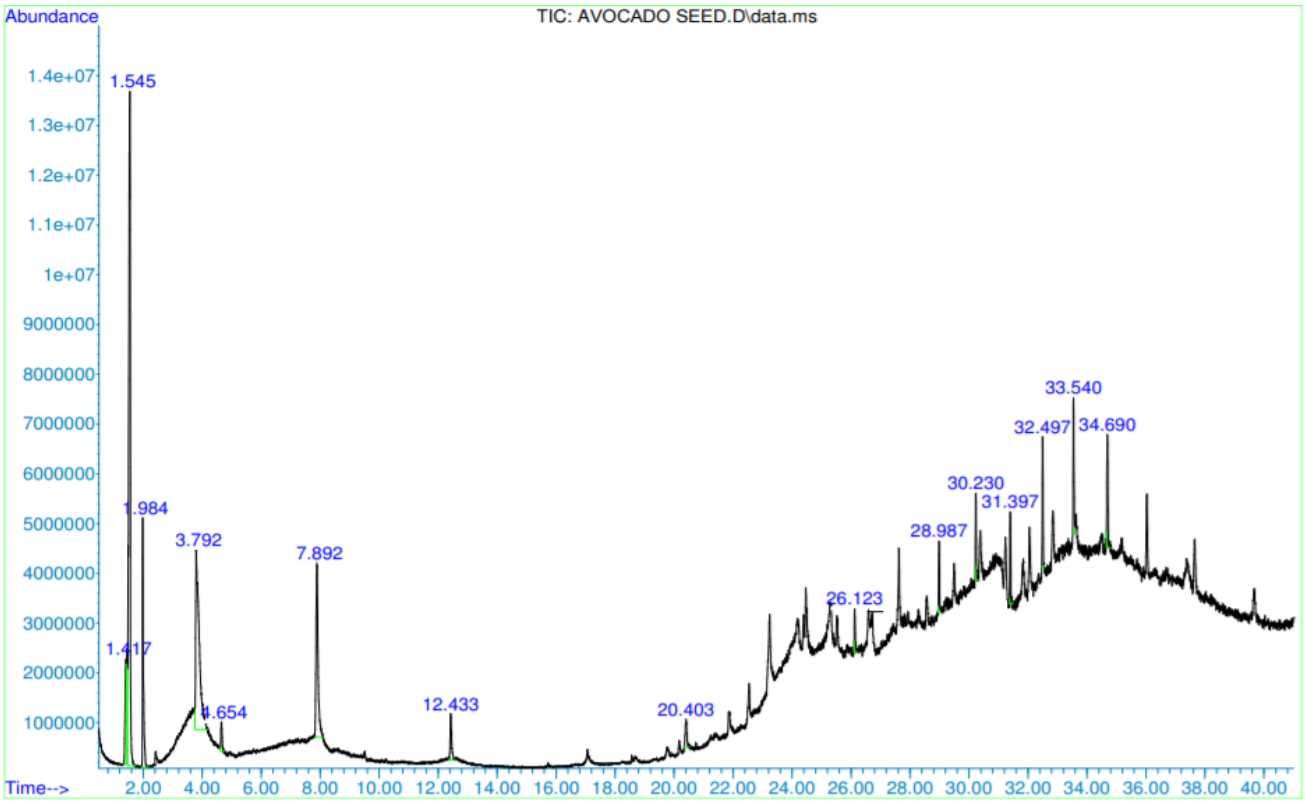

Fig. 32: GC-MS chromatogram of *Persea Americana Mill* methanol leaves extract

Table 3: Chemical composition (GC-MS) of the n-hexane extract of *Persea Americana Mill* (Avocado pear)

Name	Peak-Height (pA)	Retention time (minutes)	Conc (%)	Formular
Dodecanoic acid	2108314	1.417	3.959	$C_{12}H_{24}O_2$
Tetra decanoic acid	13557730	1.545	33.326	$C_{14}H_{28}O_2$
n-Hexadecenoic acid	5008226	1.984	7.065	$C_{14}H_{22}O_2$
9,12-Octadecadienoic acid, methyl ester	3592155	3.792	20.122	$C_{19}H_{34}O_2$
11-Octadecenoic acid, methyl ester	595510	4.654	1.080	$C_{19}H_{36}O_2$
Oleic acid	3497450	7.892	11.600	$C_{18}H_{34}O_2$
9,12-Octadecadien-1-ol, (Z, Z)-	925530	12.430	2.310	$C_{18}H_{34}O$
1, E-11, Z-13-Octadecatriene	560862	20.403	1.400	$C_{18}H_{32}$
E-11(12-Cyclopropyl) dodecen-1-ol	870487	26.124	1.223	$C_{15}H_{28}$
Undecyclenic acid	1444111	28.987	1.922	$C_{11}H_{20}O_2$
1-Dodecanol	1745352	30.230	2.325	$C_{12}H_{26}O$
Palmitaldehyde, disopentyl acetal	1836858	31.397	2.332	$C_{26}H_{54}O_2$
9-Octadecenal	1836858	32.497	3.321	$C_{18}H_{34}O$
(E)-13-Docosenoic acid	2735597	33.540	3.720	$C_{22}H_{42}O_2$
Erucic acid	2276684	34.690	3.246	$C_{22}H_{42}O_2$

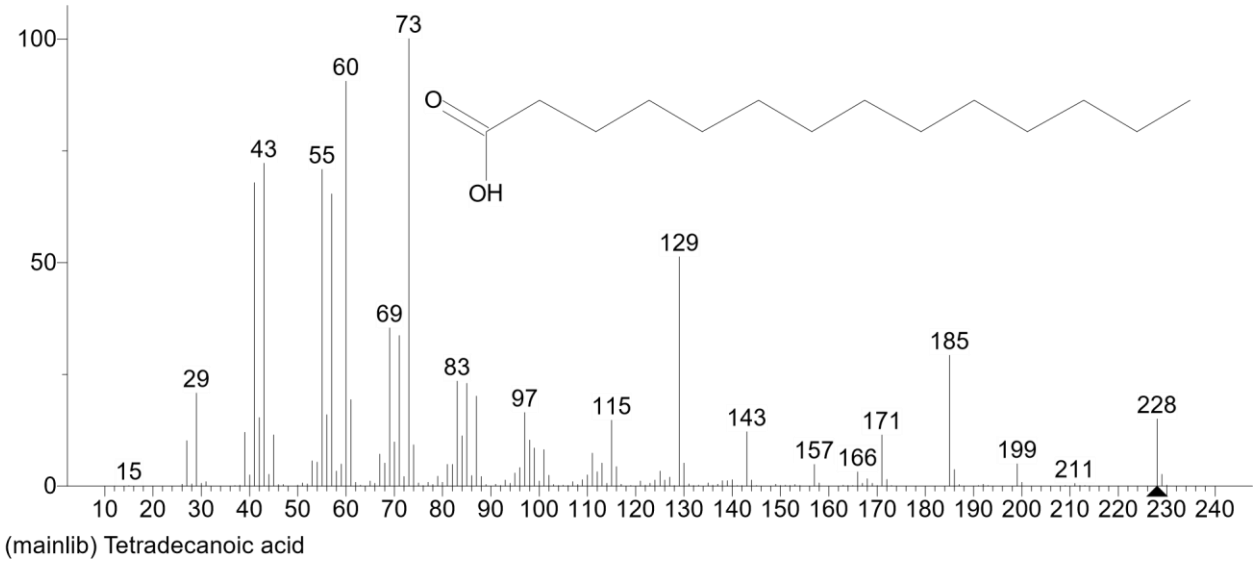

Fig. 33: Tetradecanoic acid

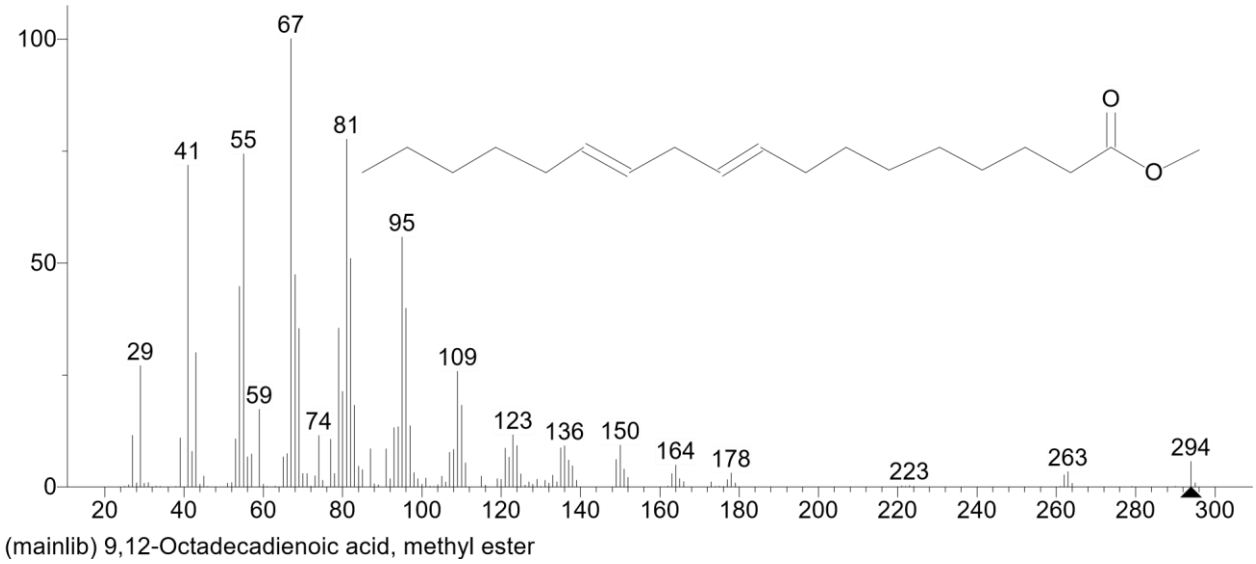

Fig. 34: 9,12-Octadecadienoic acid

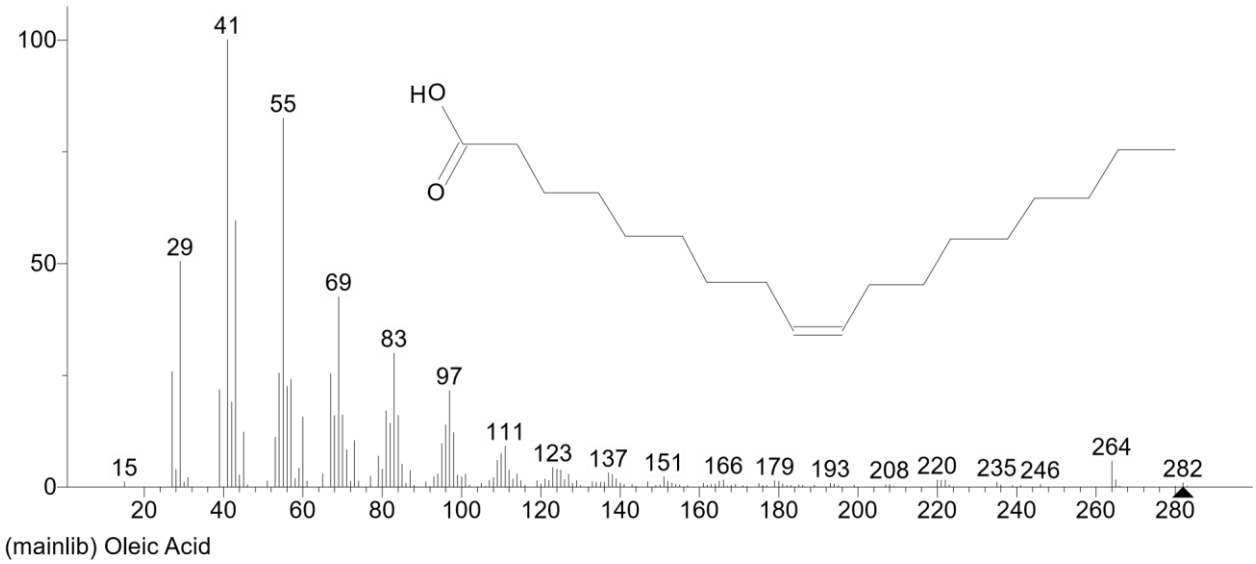

Fig. 35: Oleic Acid

O

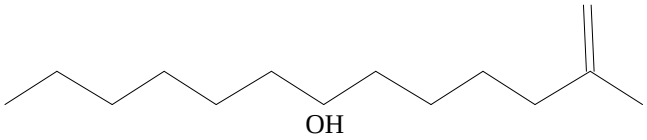

Fig. 36: Dodecanoic acid

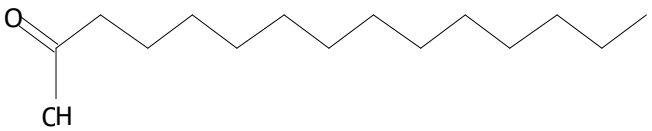

Fig. 37: Tetradecanoic acid

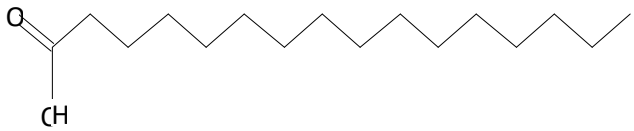

Fig. 38: n-Hexadecanoic acid

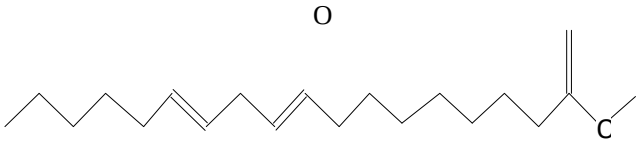

Fig. 39: 9,12-Octadecadienoic acid, methyl ester

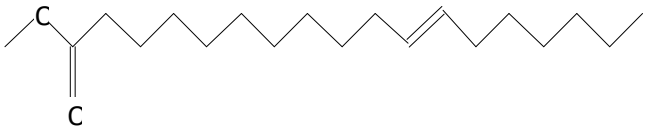

Fig. 40: 11-Octadecenoic acid, methyl ester

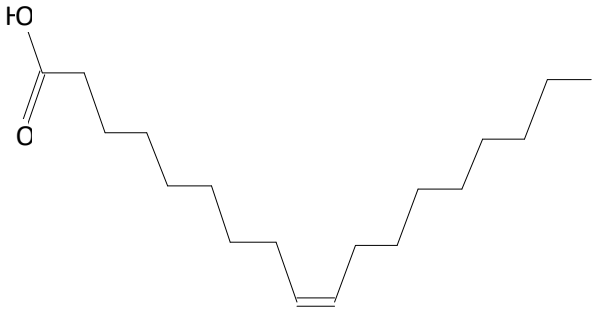

Fig. 41: Oleic Acid

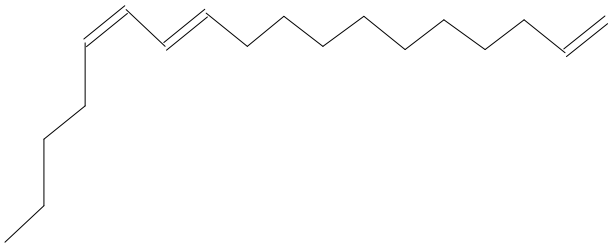

Fig. 42: 1, E-11, Z-13-Octadecatriene

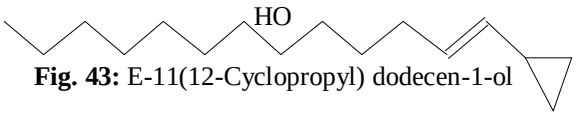

Fig. 43: E-11(12-Cyclopropyl) dodecen-1-ol

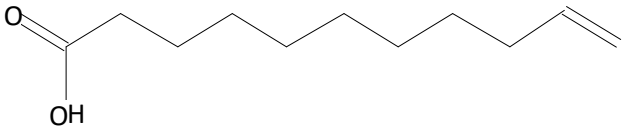

Fig. 44: Undecylenic acid

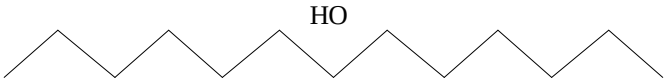

Fig. 45: 1-Dodecanol

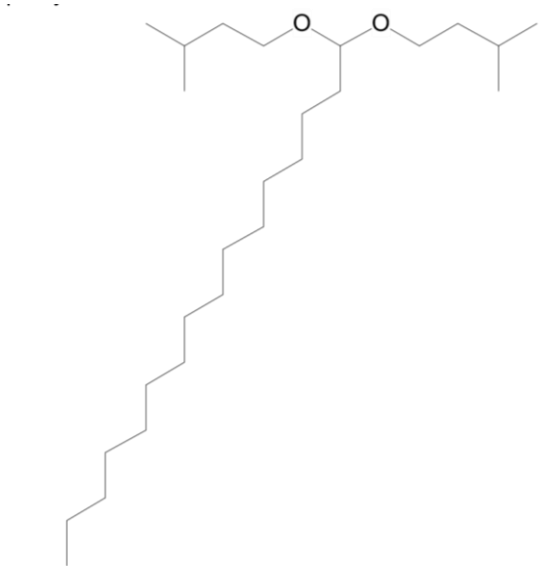

Fig. 46: Palmitaldehyde, diisopentyl acetal

Fig. 47: 9-Octadecenal

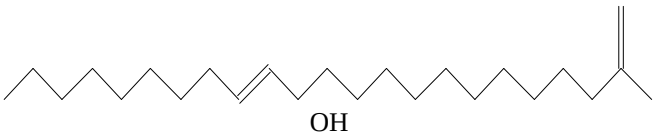

Fig. 48: (E)-13-Docosenoic acid

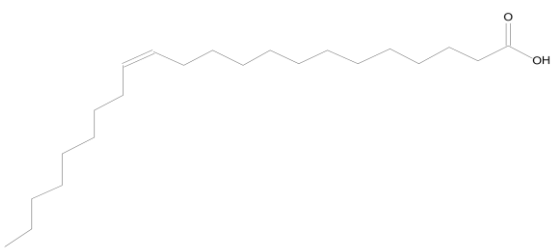

Fig. 49: Erucic acid

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IV. DISCUSSION

Medicinal plants have a recognized medicinal purpose, ranging from plants used in traditional pharmaceutical production to plants used in herbal medicine preparations values (Mohammed *et al.,* 2012). The medicinal effects of plants are due to metabolites, especially secondary compounds produced by plant species. The phenolic compound has been considered the largest category of phytochemicals studied for its antioxidant properties and have a wide range of health benefits, including anti-cancer, anti-inflammatory and anti-diabetic effects (Hamzah *et al.,* 2013). *Cymbopogon citratus* (lemongrass) and *Persea Americana mill* (Avacado pear seed) contain these secondary compounds utilized in foods, cosmetics, and pharmaceutical industries. Hence, this study investigated the total phenolic content in *Cymbopogon citratus, Persea Americana Mill,* and the bioactive compound present in the n-hexane extract using Gas chromatography and Mass spectroscopy.

The total phenolic content of the plants under review showed a significant ($p < 0.05$) difference ranging from 23.88 to 46.38 mg tannic acid equivalent/g of extract. *Cymbopogon citratus* had the highest value of 46.38 ± 0.26 mg tannic acid equivalent/g compared to *Persea Americana Mill's,* which had a value of 23.88 ± 0.34 mg tannic acid equivalent/g. The difference in phenolic content could be due to the type of soil the plants were planted on or climatic conditions. Since phenols are studied for their antioxidant properties against oxidative damage or stress (Mandal *et al.,* 2010) and generally, increased phenolic and flavonoids concentration of plant extract correlates with increased antioxidant activity. Patel et al. (2010) reported that the root of *L. schimperi,* compared to the other tested samples, showed a relatively low number of secondary metabolites as leaves of *R. Abyssinia and H. insignis.* This result is in agreement with the findings of the present study.

The GC-MS analysis of n-hexane extract of *Cymbopogon citratus* and *Persea Americana Mill* revealed that the plant contained a wide range of photo-compounds which may be responsible for its therapeutic potentials. The active principles with their retention times (RT) and total peak area (%) in the chromatogram of each constituent that was identified in the fixed oil obtained from the species depicted twenty-five peaks and fifteen peaks, and this implies the presence of twenty-five (25) phytol-compounds and fifteen (15) phytol-compounds in the extract. The identified phytol-compounds comprise mainly hydrocarbons, fatty acids, alcohols, esters and phenols.

Figure 1 and 32 shows the chromatograms corresponding to the chromatographic analysis (GC-MS) of *Cymbopogon citratus* and *Persea Americana mill,* which allowed the identification of 26 different compounds from *Cymbopogon citratus* and 15 different compounds from the *Persea Americana mill,* of which the major compounds identified were Myrcenyl acetate (9.703%), 4-Terpinenyl acetate (7.579%), Citronellal (6.383%), 4-Androsten-3β,6β-diol-17-one (5.829%), Tetradecanoic acid (33.326%), 9,12-Octadecadienoic acid, methyl ester (20.122%), and Oleic acid (11.600).

The result of the peak height of *Cymbopogon citratus* showed that there was a significant ($p < 0.05$) difference ranging from 2781 to 335638. It was observed that Caryophyllene had the highest value compared to that of Myrcenyl acetate, which had the lowest value of 2781. This difference in peak height could result from the difference in the molecular weight of the volatile compounds. The result of the retention time showed a significant ($p < 0.05$) difference ranging from 4.560 minutes to 20.331minutes. This difference can result from molecular weight, which may also determine the migration capability of the volatile compounds.

The result of the peak height of the volatile compounds found in the *Persea Americana mill* showed that there is a significant ($p < 0.05$) difference ranging from 560862 to 13557730, with Tetra decanoic acid having the highest value of 13557730 compared to that of 1, E-11, Z-13-Octadecatriene with the lowest value of 560862. The retention time result also revealed a significant ($p < 0.05$) difference ranging from 1.417 minutes to 34.690 minutes, with Erucic acid having the highest value and Dodecanoic acid having the lowest value**.** Identifying these phyto-compounds by GC-MS is a valuable technique in suggesting the potential curative properties of plants (McGaw *et al*., 2002).

V. CONCLUSION AND RECOMMENDATION

➢ *Conclusion*

This study showed that *Cymbopogon citratus* and *Persea Americana Mill,* based on their total phenolic presence and the phytochemicals obtained through our GC/MS, could be a potential source of natural antioxidants that could be used as a therapeutic agent in preventing or treatment of degenerative diseases associated with oxidative stress.

➢ *Recommendation*

Already existing literature has shown that the pharmacological effects of plants are a result of the therapeutic activities of their active compounds, of which phenols are vital components. The present study has shown that the essential oil extracted from avocado pear seed and lemon grass contains a significant number of phenols, pointing to the possibility of the efficacy of these oils in exhibiting therapeutic effects. Hence, these essential oils can be used to treat various illnesses that are treatable with phenols. It is suggested that further studies be carried out to ascertain the safety of these oils when used by humans.

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