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Physiochemical properties, fatty acid composition and characterization of melon seed oil (*Citrullus colocynthis*)

Ikezu UJM and Ugariogu SN

Abstract
Oil from melon has been acclaimed by local populace to be causing high cholesterol in the body therefore it was imperative to study this scientifically. The seed was extracted and characterized using solvent extraction method and standard methods for physiochemical parameters. The results revealed that the saponification value (mg KOH/g), acid value (mg KOH/g), iodine value (mg iodine/100g), peroxide value (mg/peroxide/kg), specific gravity (kg/dm^3) and oil yield (100g/ml) were 220, 2.25, 16.5, 3.6, 0.76 and 15 respectively. Gas chromatography mass spectrophotometer (GCMS) analysis showed that the oil is composed of n-hexadienoic acid, 9,12-octadienoic/Linoleic acid, Oleic acid, Eicosanoic acid, Ricinoleic acid, Cis-vaccenic acid and squalene etc. which have been ascertain to be medicinal and also lower cholesterol. Low acid values obtained for the oil suggest that the oil is edible. High saponification value obtained revealed that the oil has great potential in industrial applications such as soap, cosmetics and medicine. Low iodine value obtained revealed that the oil is a non-drying oil and also suggest that the oil contain unsaturated bonds which are free from cholesterol. While low peroxide value indicated that the oil has low susceptibility to oxidative rancidity and deterioration. The result agreed with the result of some reviewed literatures.

Keywords: Melon seed oil. Physicochemical parameter, GCMS, Characterization, Solvent extraction

Introduction
Attention has not been focused on under-utilized agricultural foods and industrial product and due to increase in human population and industries, the use of plant oil has drastically increased and as a result, oil from plants is less available and costlier, moreover these days there is insufficient scientific information with regard to the chemical constituent of oil from the seeds using GC-MS. Nevertheless, as far as we know, there is no information concerning the various chemical constituent of the oil extracted from egusi seeds grown in Imo state. Hence the research tend to extract and characterized the oil from melon seed using recent methods to identify, revalidate and authenticate the findings of previous researchers on the melon seed. Nuts oils, seed oil and oils of fruit and vegetables are of growing interest due to their high concentration of bioactive lipid components, such as polyunsaturated fatty acids and phytosterols, which have shown various health benefits [1]. Fats and oils, and their several lipid components are extensively used in the food, cosmetics, pharmaceuticals, oleochemicals and other industries. Oils are classified into essential and fixed oils. Essential oils are volatile, and are usually derived from the non-seed parts of the plants while fixed oils are derived from the seeds [2]. Essential oils are used to produce perfumes, flavours, deodorants, antiseptics and pharmaceuticals, while fixed oils are often edible because of their nutritional value [3]. The choice of the melon seeds in this work is due to its high yield of oil compared to other plants. They are edible oils and have various applications in food, medicine and industries [4]. Oils from melon are commonly used in cooking, and are useful as feedstock for biodiesel production, in addition to being used as skin moisturizer [5]. The physicochemical characterization of the oil extracted was based on the Acid value, Saponification value, Iodide value, Peroxide value, specific gravity and fatty acid composition was done using Gas chromatographic mass spectrophotometer (GC-MS). Acid Value is the amount of free fatty acid (FFA) present in oil or fat. The acid value of an oil or fat is the amount of base in milligrams required to neutralize the free organic acid present in 1g of fat or oil [6]. Saponification value of oil or fat is the amount in milligrams of alkali required to neutralize the free fatty acids from complete hydrolysis of the sample. It is a measure of average molecular weights (chain length) of fatty acid present. Peroxide value is a measure of the peroxide contained in the oil. A solution of oil in the mixture of acetic acid and chloroform is titrated with solution of potassium iodide [7].

Specific gravity or relative density refers to the ratio of the weight of sample to the weight of distilled water. It is given as weight of sample/weight of distilled water. It has no unit. The main objective of the study is to extract oil from melon, know the fatty acid composition of the oil and characterize the oil by determining their chemical properties such as saponification value, peroxide value and iodine value in addition to the specific gravity of the extracted oil.

Scientific classification of *Citrullus colocynthis*

Kingdom	:	*Plantae*
Division	:	*Magnoliophyta*
Class	:	*Magnoliopsida*
Order	:	*Cucurbitales*
Family	:	*Cucurbitaceae*
Genus	:	*Citrullus*
Species	:	*colocynthis* [8].

Fig 1: Egusi seed without and with shell

English name – Melon seed, Local name-Egusi, Botanical name- Colocynthis *citrullus*; Family-Cucurbitaceae) is a variety of melon seeds popularly called 'egusi' in West Africa. It is a creeping annual plant and an intercropping plant made use in traditional farming practices, thrives well on rich light soil in the hot climate regions of Africa [8]. It however, has been noted to tolerate low rainfall. In the South-Eastern region of Nigeria, egusi is best cultivated after the first rains of the year [9].

Citrullus colocynthis (L) of the family *Cucurbitaceae* is an annual harbacious monoecious plant with a non-crumbing creeping habit. They cover the soil completely after planting within 3 weeks and flowering starts. The seed is ready for harvest within 9-12 days (3-4 months) after showing. The seeds are obtained either in shelled or unshelled form in West Africa markets and are used greatly in West Africa cooking. The melon seed can be milled and used to prepare a popular egusi soup where it acts as a food thickener. In West Africa where soup are integral to life, it is a common component for daily meals, some are fermented, soaked, boiled and wrapped in leaves and used as condiments to season or flavor soup [10] Melon seeds may also be eaten as snacks either as whole toasted seeds or a fried cake prepared from milled seeds [11]. It is a healthy meal rich in calcium, Iron, Vitamin A and C, sodium, Potassium, proteins and Carbohydrate [12] despite being a significant food stuff even by global standard, it is hardly know to nutritionist outside a few West Africa nations, little nutritional detail on the oil is readily available.

Material and Methods

Melon seed used for the study were purchased from Relief market in Owerri and were identified as *Citrullus colocynthis (L*) by Prof Mbagwu a taxonomist in Imo State University Owerri. The seed was screened to remove the bad ones and pulverized with grinding machine, the ground sample was emptied in an airtight container and stored in desiccators for further analysis. The ground sample was extracted with n-hexane.

Characterization by physicochemical parameter

This was done using the method outlined by Obasi *et al.* [4]

Gas-chromatography mass spectrometry (GCMS)

This was used in determination of the molecular mass of the samples as well as their fragmentation patterns. The instrument used was Agillant 7890a GCMS. The results are shown in appropriate table and appendices.

Fourier Transform Infra-red (FTIR) Spectroscopy

This was done using Shimadzu FTIR-8400S to know the functional groups present.

Result and Discussion

The result of the physicochemical characterization and fatty acid composition were reported in the appropriate tables and discussed below.

Table 1: Result of melon seed oil characterization by physiochemical parameter

Peak	Retention Time in (Min)
Properties	**Values**
Saponification value (mgKOH/g)	220
Acid value (mgKOH/g)	2.25
Iodine value (mg iodine/100g)	16.5
Peroxide value (mg iodine/100g)	3.6
Specific gravity (kg/dm^3)	0.76
Oil yield (100g/ml)	15

The seed oil was an odourless liquid, with a pale yellow colouration.

The tested values serve as important parameter in determining the suitability of the oil in food, cosmetics and medicine. Oils within this saponification values of 220 yields soaps of soft consistency and hence could be used for making shaving creams. Acid value for the oil was (2.25 mgKOH/g). The lower the acid value of oil, the few fatty acid it contains which makes it less exposed to rancidity [4], this means it will not be easier for melon oil to become rancid, the low acid value is also an indication that the oil is edible. The iodine value of melon oil (16.5 mg iodine/100g). The iodine value is a measure of the unsaturation of fats and oils and it is an indicator of double bonds in the molecular structure in terms of classification of fats and oils, this bond implies that the oil is cholesterol free and can protect the heart. Iodine values below 100 confirm that the oil is non-drying [6]. Hence the melon oil is non-drying because their iodine values were lower than 100. The peroxide value of 3.60 (mg peroxide/kg) obtained also served as an index of rancidity. According to Evbuomwan, [2] high oxidative stability is possible when peroxide value is less than 10.00 mg peroxide/kg, hence the oil will be resistant to peroxidation during storage. The specific gravity of 0.78 (kg/dm^3) implies that the oil is less dense than water. Acid value is an important index to determine the quality age, edibility, and suitability of oil for use in industries. Acid value is used to measure the extent to which glycerides in the oil has been decomposed by lipase and other physical factors such as light and heat. Low acid values obtained showed that the oil is edible. Low iodine value obtained revealed that the oil is non-drying oil and also suggest that the oil contain unsaturated bonds which is highly recommended in medicine while low peroxide values indicated that the oil has low susceptibility to oxidative rancidity and deterioration. Hence it confirms that there is presence of high level of antioxidants in the oil. The result of

this research have some level of conformity with that of some previous researchers Obasi *et al.* [4] used Soxhlet and mechanical extraction method to extract and characterized the oil from melon seed, from their result, the mechanical extraction method give high acid value, Free fatty Acids and Saponification Value just like this concluded research. But the iodine value of this present work is low and conforms to that of Oti *et al.*

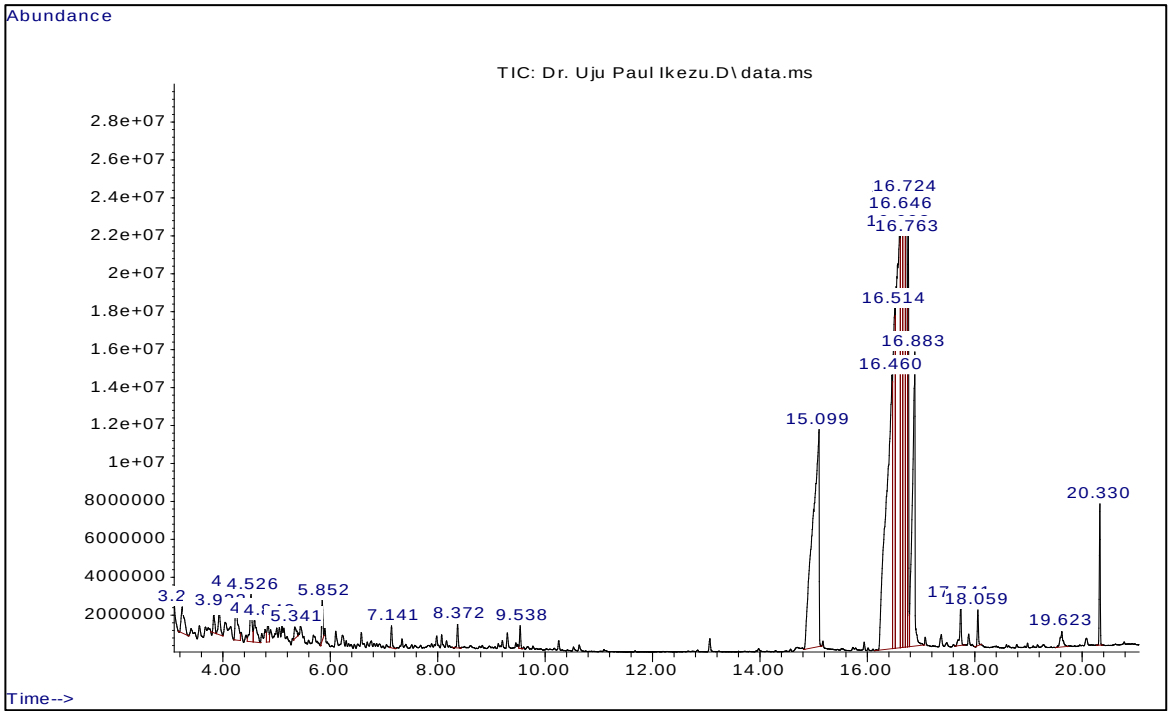

Fig 2: The GCMS result of the melon seed oil

Table 2: GC-MS result of fatty acid composition of melon

Peak	Retention time	Name of Compound	Molar mass
1	3.244	Nonane	C_9H_{20}
2	3.832	1-ethyl-2-methylbenzene	C_9H_{12}
3	3.933	1,2,3-trimethylbenzene	C_9H_{12}
4	4.526	Decane	$C_{10}H_{22}$
5	4.843	4-methyldecane	$C_{11}H_{24}$
6	5.341	4-iodo-1,2 dimethylbenzene	$C_{10}H_{14}I$
7	5.852	Undecane	$C_{11}H_{24}$
8	7.141	Dodecane	$C_{12}H_{26}$
9	8.372	Tridecane	$C_{13}H_{28}$
10	9.538	Tetradecane	$C_{14}H_{30}$
11	15.099	n-Hexadecanoic acid	$C_{16}H_{32}O_2$ 13.772%
12	16.460	9,12-octadecadienoic acid/Linoleic acid	$C_{18}H_{32}O_2$ 15.715%
13	16.763	cis-Vaccenic acid	$C_{18}H_{34}O_2$ 4.514%
14	16.883	Octadecanoic acid/Oleic acid	$C1_8H_{34}O_2$ 7.63%
15	17.740	Ricinoleic acid	$C_{18}H_{34}O_2$ 0.618%
16	18.059	Eicosanoic acid	$C_{18}H_{34}O_2$ 0.385%
17	20.330	Squalene	$C_{30}H_{50}$ 1.273%

The result of the Gas chromatography mass spectroscopy (GCMS) represented above in table (2) showed the presence of seven characteristic fatty acid.

Compound 1: Hexadecanoic Acid with the molecular formular of $C_{16}H_{32}O_2$ has been reported to inhibit phospholipase A (2) in a competitive manner. It was identified from the crystal structure at 2.5A resolution that the position of n-hexadecanoic acid is in the active site of the phospholipase A (2). It was indicated to have potential anti-inflammatory, anti- arthritic and antidiabetic properties [13].

Compound 2: 9,12-octadedienoic acid with molecular formula $C_{18}H_{32}O_2$ also known as Linoleic acid is a polyunsaturated omega-6 fatty acid and is one of two essential fatty acids for humans, who must obtain it through their diet. It is a colorless or white oil that is virtually insoluble in water. 9, 12-octadedienoic acid have been reported to possessed anti-inflammatory and anticancer properties, a diet deficient in linoleate (the salt21orm of the acid) has been shown to cause mild skin scaling, hair loss and poor wound healing [14].

Compound 3: Cis-Vaccenic acid an isomer of oleic acid, is the principal ruminant Trans-fatty acid. Recent data suggested that the consumption of Trans-vaccenic acid may provide health advantages beyond those related to cardiovascular diseases, cancer, immune function and inflammation [15].

Compound 4: Oleic acid or Octadecanoic acid with a molecular formular $C_{18}H_{34}O_2$ has been described to reduce the cardiovascular risk by reducing blood lipid, mainly cholesterol, LDL- cholesterol and triglycerides [16].

Compound 5: Ricinoleic acid which was formally called 12-hydroxy-9-cis-octadecenoic acid is a fatty acid. It is an unsaturated omega-9 fatty acid with molecular formular $C_{18}H_{34}O_2$. Ricinoleic acid increase absorption in the skin and are used in the treatment of various skin conditions including dermatosis, psoriasis and acne [17].

Compound 6: Eisosanoic acid also known as Arachidic acid is a saturated fatty acid with a 20-carbon chain. It is a minor constituent of cupuaçu butter (7%), Perilla oil (0–1%), peanut oil (1.1–1.7%), corn oil (3%). The salts and esters of arachidic

acid are known as arachidates, have been reported to possess anti-inflammation, anti-cancer [18].

Compound 7: Squalene with a molecular formular of $C_{30}H_{50}$ is praised for its anti-cancer and skin protecting effect, lower cholesterol levels and acts as an antioxidant and may even improve the immune response to vaccines. It is a precursor to cholesterol and all steroid hormones. It may be useful in the treatment of skin disorders like psoriasis, dermatitis and acne [19].

The presence of these compounds in the extract of melon seed oil showed that the oil is edible and medicinal.

The result of previous researchers are shown below Mirjana *et al* investigated nutritional quality and characterization of the dried melon seeds (*Citrullus colocynthis L.*) of the family *Cucurbitaceae.* The result revealed that the predominant fatty acid was linoleic (18:2) acid in 62.2%. Other fatty were also presence in the acids range of 10-14% for oleic (18:1), stearic (18:0) and palmitic (16:0) acids, respectively [20]. Giwa *et al* studied melon seed oil as a potential feedstock for biodiesel production. The results showed that melon oil methyl ester (EMOME) yield was 82%. Gas chromatographic analysis showed that it was composed mainly of palmitic, stearic, oleic, linoleic and linolenic esters, which is similar to the profile of sunflower, soybean and safflower oil [21]. Igwenyi *et al.* report that *Citrullus colocynthus* seeds contain about 50% oil, 8.28% potassium, 34.86% protein, 1.49% calcium, 42.29% oil, 3.37ppm copper and 162.76ppm sodium. The result also revealed that it is an excellent source of nutritional minerals and vitamins such as carbohydrate, protein, fat, zinc, vitamin B1 (Thiamine), dietary fibre, sulphur, magnesium, vitamins B2 (Riboflavin), niacin and manganese [22].

Table 3: The TIC result of the GCMS

Peak	Retention Time in (Min)	First Scan	Max Scan	Last Scan	PK TY	Peak Height	Correlation area	Corr elation% max	% of Total
1	3.244	18	33	58	BV 5	1371300	56740133	5.11	0.812
2	3.832	144	157	166	BV	928388	18826819	1.7	0.269
3	3.933	166	178	192	VV	1259131	32948664	2.97	0.471
4	4.246	234	243	260	PV	2545435	75071504	6.76	1.074
5	4.526	287	302	310	VV	2466109	64693872	5.83	0.925
6	4.591	310	316	338	VV 2	1215671	45788051	4.12	0.655
7	4.843	362	369	374	VV	1120322	26162690	2.36	0.374
8	5.341	467	473	486	PV 2	596789	16671549	1.50	0.238
9	5.852	573	581	587	PV 2	2074947	33314974	3.00	0.476
10	7.141	844	851	865	BV	1144681	19012549	1.71	0.272
11	8.372	1091	1110	1121	BV	1269702	20163170	1.82	0.288
12	9.538	1349	1355	1362	VV	1184888	16837884	1.52	0.241
13	15.099	2464	2523	2532	PV	11282933	962878922	86.74	13.772
14	16.460	2741	2808	2810	BV 2	14426336	1098745282	98.97	15.715
15	16.514	2810	2820	2821	VV 3	17855790	517493320	46.62	7.402
16	16.602	2821	2838	2840	VV 2	21932267	1110137955	100.0	15.878
17	16.646	2840	2847	2850	VV 4	22870209	603316631	54.35	8.629
18	16.697	2850	2858	2860	VV 3	23672505	699400559	63.00	10.003
19	16.724	2860	2864	2869	VV 6	23728534	535292355	48.22	7.656
20	16.763	2869	2872	2877	VV 2	21581397	315618307	28.43	4.514
21	16.883	2877	2897	2933	VV	15202235	533936562	48.10	7.637
22	17.741	3056	3077	3090	BB	2214735	43216991	3.89	0.618
23	18.059	3132	3144	3155	BB	1851651	26890884	2.42	0.385
24	19.623	3449	3472	3495	BV 4	821812	29507574	2.66	0.422
25	20.330	3608	3621	3628	BB	7382939	88996151	8.02	1.273

Signal : TIC: data of the GCMS.
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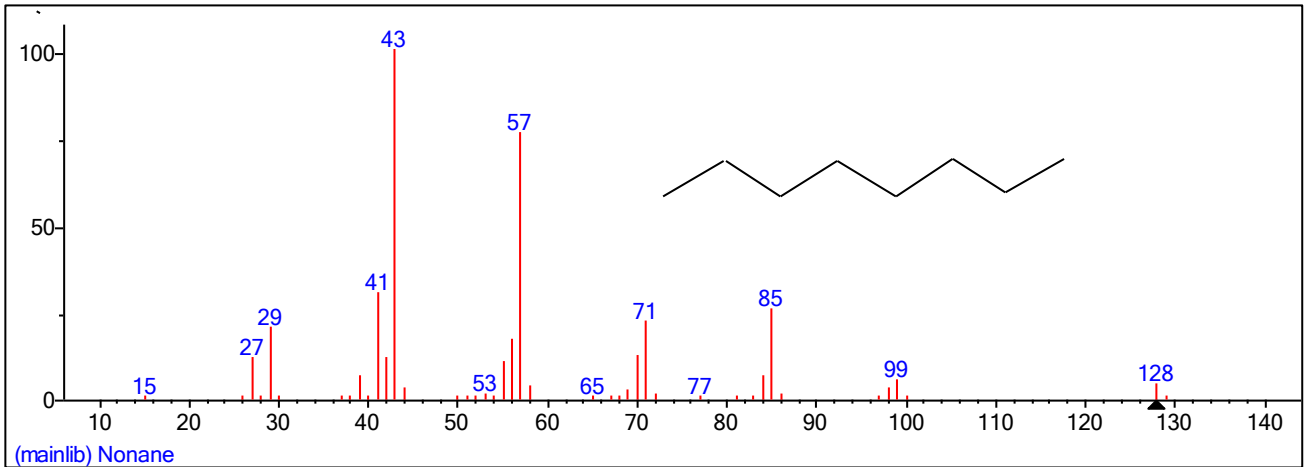
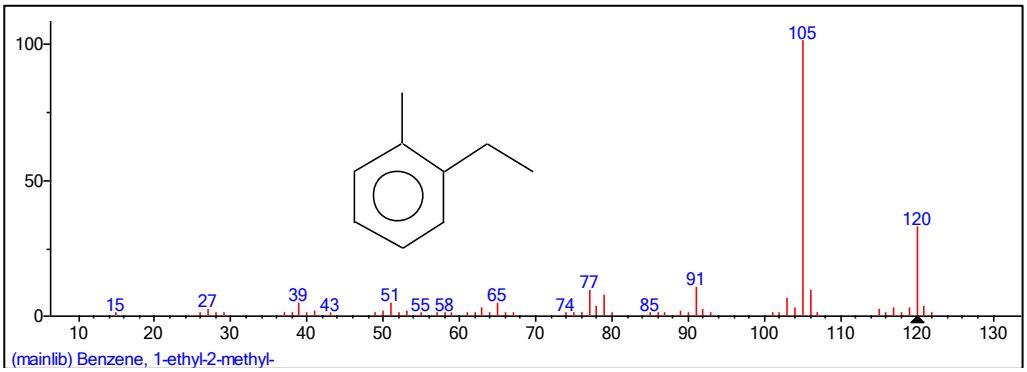
min scan scan scan TY height area% max. total

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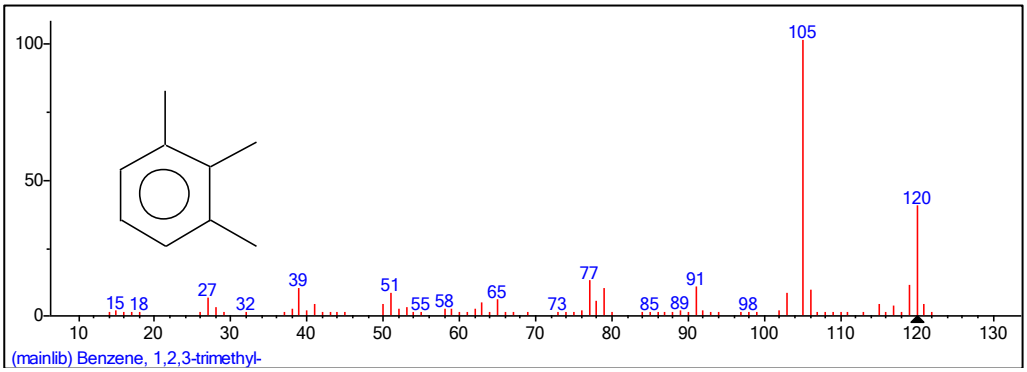
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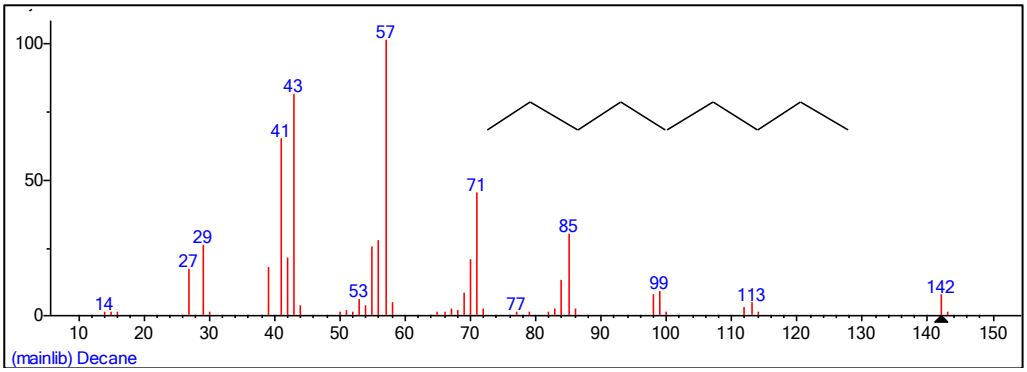
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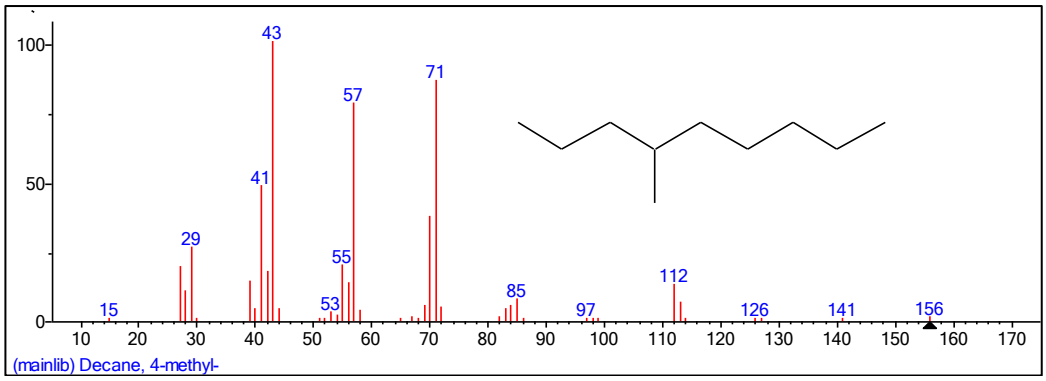
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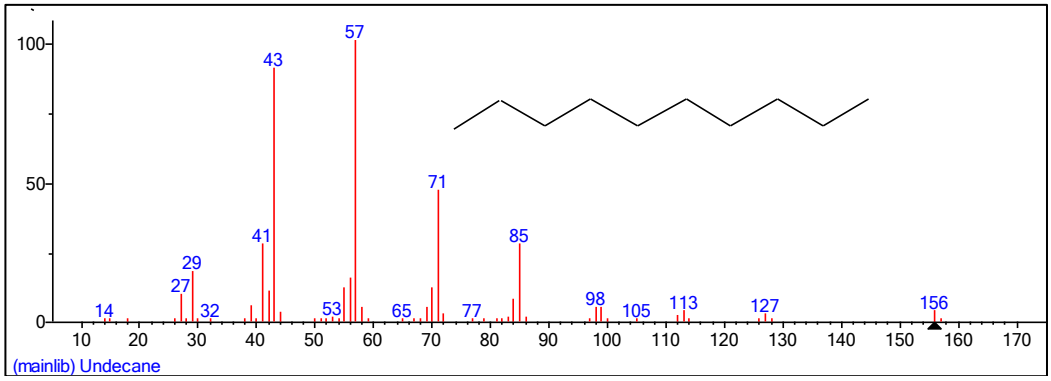
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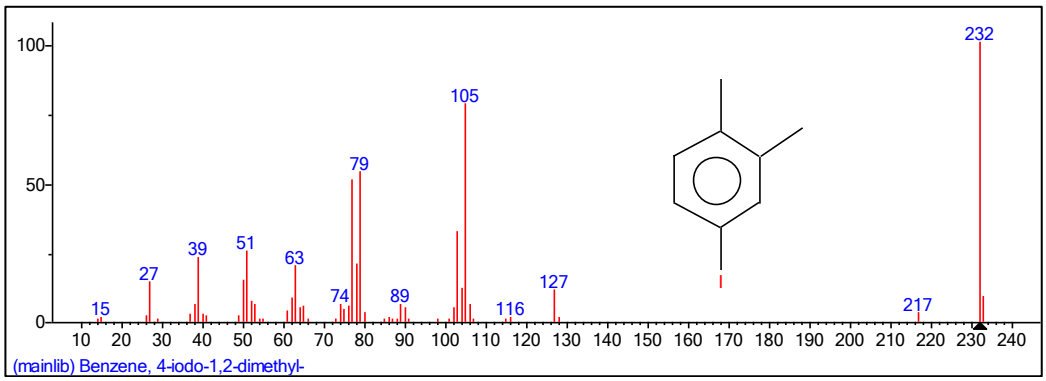
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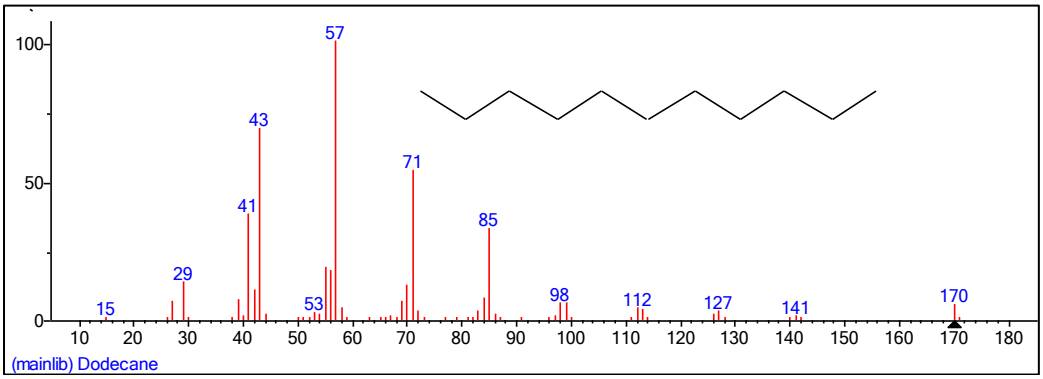
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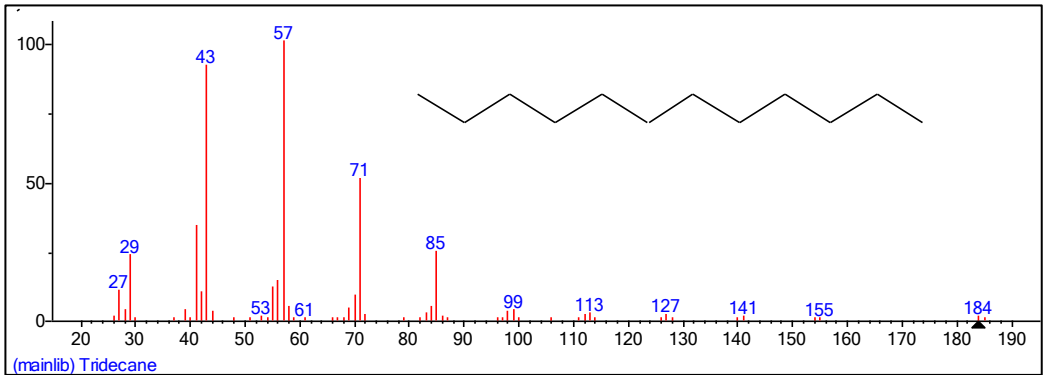
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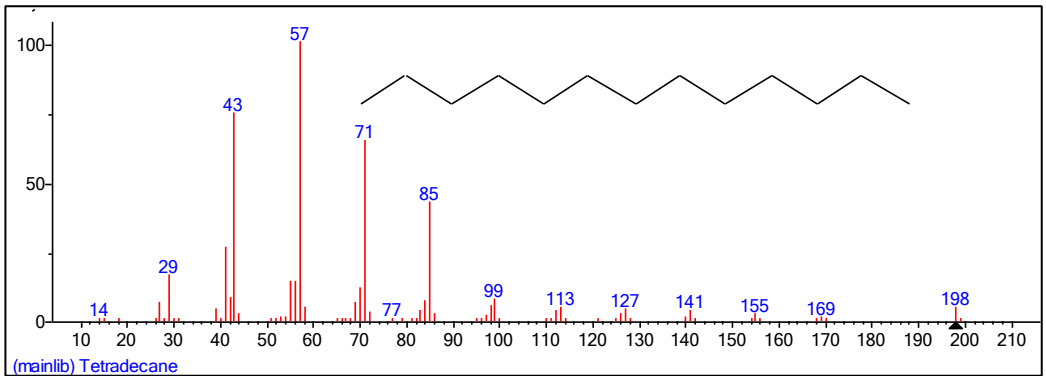
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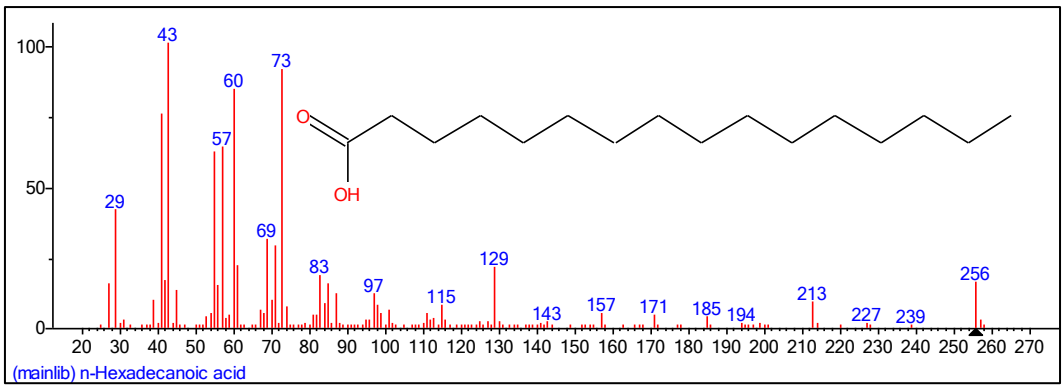
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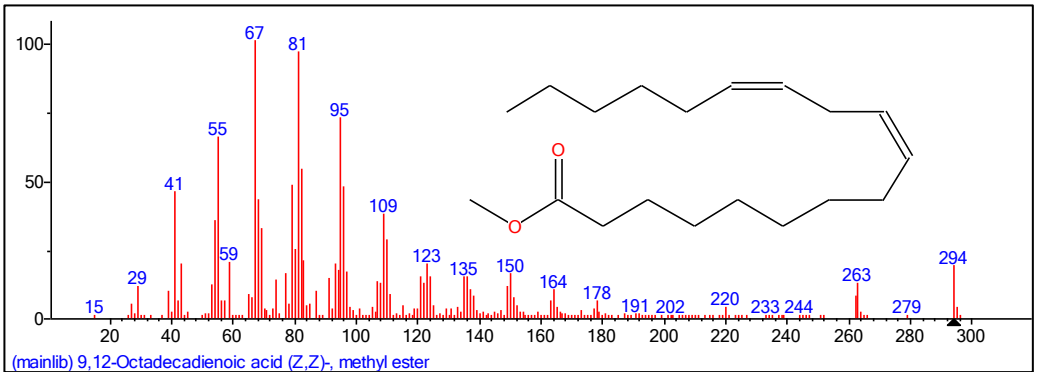
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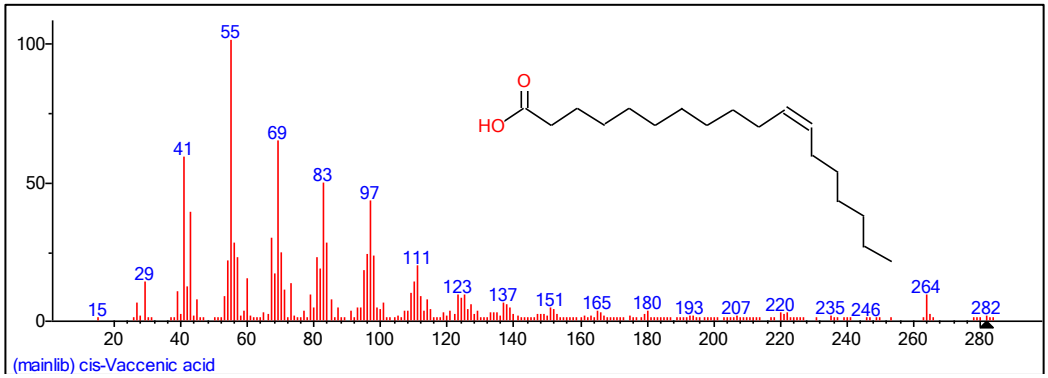
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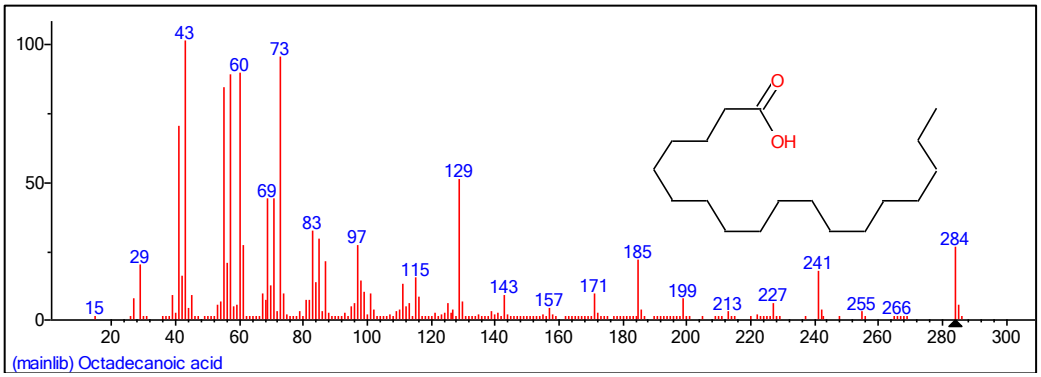
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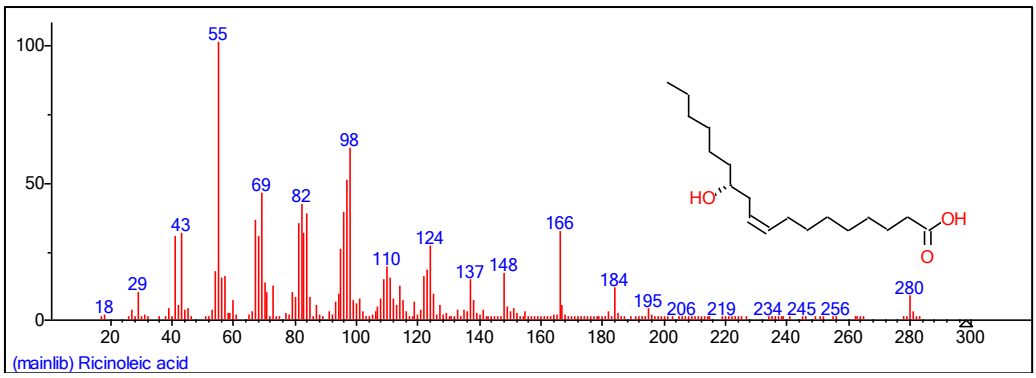
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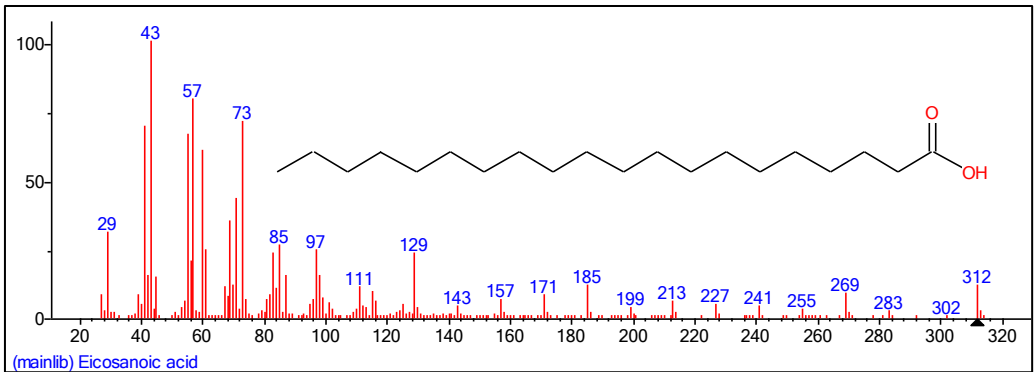
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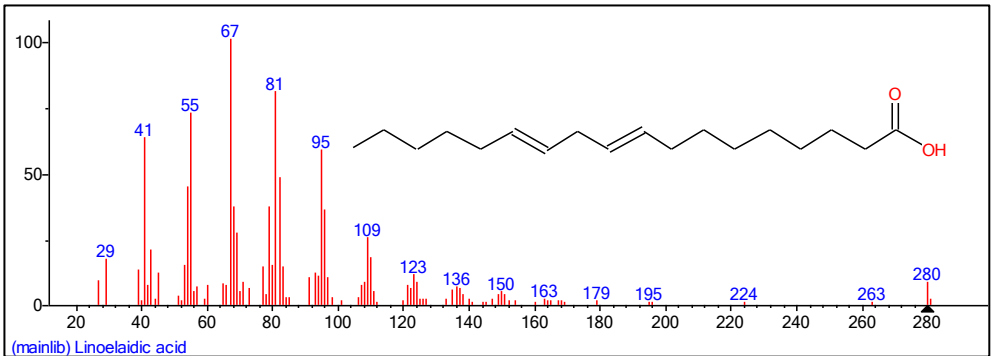

Fig 3: The Spectra result of the compounds.

Table 4: FTIR result of the oil sample

Peak	Functional group	Remark
709.83	-C-H bending	Mono substituted benzene derivative
1033.88	C-O Stretching	Vinyl ester
1165.04	C-O Stretching	Ester
1234.48	C-O Stretching	Alkyl aryl ether
1450.52	C-H bending	Alkane
1728.28	C=O Stretching	Aldehyde
2592.41	O-H Stretching	Carboxylic acid
2924.18	C-H Stretching	Alkane
3610.86	O-H Stretching	Alcohol

The FTIR result of the Oil show the absorbance at 709.83 cm^{-1} was attributed to –C-H bend of mono substituted benzene derivative, 1033.88 cm^{-1} as C-O of stretch of vinyl ester, 1165.04 cm^{-1} as C-O stretching of ester, 1234.48cm^{-1} as C-O stretch of alkyl aryl ether, 1450.52cm^{-1} as C-H bend of alkane. While 1728.28cm^{-1} and 2592.41 cm^{-1} represent the C=O Stretching of aldehyde and O-H stretching of carboxylic acid respectively, peaks at 2924.18 cm-1 and 3610.86 was attributed to C-H and O-H stretching of Alkane and alcohol respectively. These result showed the different functional groups present in the sample and it confirmed the result of GCMS.

Conclusion

It was necessary in the cause of this research work to carry out such test in order to determine the class to which the oil extracted from melon seed belongs. It was found to be classified as non-drying oil, since the iodine value is less than 115. Hence will be edible and very good in cosmetic industries. The project can be considered a profitable venture since it proved that melon is not too oily as people think and that the oil also contain unsaturated bond which help in protecting the heart and not dangerous to health. In the characterization of the oil, some of physicochemical characteristics showed the usefulness of the oil, like in soap production due to its high saponification value and cooking food due its low acid value. Egusi seed oil is very rich in essential fatty acids (linoleic: 9, 12-Octadecadienoic acid and Linoelaidic acid). The result of this research encourages people to continue eating egusi soup because of its medicinal properties due to the fatty acids it contains. Previously many people have sprayed rumours that Egusi soup causes high cholesterol in the body but this scientific research have proved them wrong as egusi was ascertain to contain some good fatty acids which have been reported to possess medicinal properties as reported in the literature review and result discussion above. Government is required to encourage research work on our forest species for their more effective exploitation, consumption of melon seed and oil is highly recommended medically and industrially due to the presence of low acid value, unsaturated bond, low iodine value and high saponification value.

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