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The Extraction of Oil from *Moringa oleifera* for Pharmaceutical Utilization

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ABSTRACT

Research work was conducted on *Moringa oleifera* for oil extraction with the aid of n-hexane as basis for solvent extraction and water for steam extraction. The result obtained reveals that n-hexane solvent extraction yielded optimal volume when compared with the steam extraction. The product obtained from steam extraction has less fatty acid than the n-hexane solvent extraction, but the processing period of n-hexane solvent extraction is faster compared to steam extraction. The products recovered from both process was analyzed using GC and result obtained revealed that both products are found useful in the pharmaceutical industries.

Key words: Extraction, oil *Moringa oleifera*, pharmaceutical, utilization, product

1. INTRODUCTION

Many works have seen recorded on the medicinal values and therapeutic properties of *Moringa oleifera*, this has prompted the need for a research work on the moringa seed to be carried out to investigate the lipid profile of the *Moringa* seeds [1-8]. As per the description of issues to be addressed during this research work, we are to be doing an intensive work on the extraction of the main component (oil) which is done by reaction kinetics [9]. One good story of this work is due to dismissing resources of fats and oils, there is need for the search of new sources *Moringa oleifera*, a very rapid growing tree found in varying range of climate is a promising tree and has the potential to become a new source of oil for Nigeria striking revelation about the *Moringa oleifera* is this, 3000kg of seeds could obtained from 1 hectre equivalent to 900kg oil/hectre, (30% oil yield) [10-14]. As compound of soya bean which a source of oil but is also with only 20% oil yield/ the process used in the extraction of this oil from *Moringa oleifera* depends of the botanical material used [15-21]. The different methods include mechanical, tradition and solvent extraction [22-27]. But in this research we will employ the mechanism of low energy consumption, large production capacity, fast action, and easy contentious operation and ease of automation [28-33].

The aim of this research work is to examine the methods that will enhance the energy of consumption of production in terms of quality and yields. The following objectives were considered in this research work, such as to;

Analyze the chemical composition of the *moringa oleifera* seed mesh, determine the yield volume from both extraction methods, analyze the products obtained from the different extraction methods and comparison of the product in terms of quality.

This present work on *Moringa oleifera* describes a quantitative study of the oil from the aforementioned seed with the goal of exploring the very significant medicinal properties of this oil and benefits to the pharmaceutical industry.

This research work covers analytical and experimental techniques. Therefore, the scope covers the following activities; determination of the percentage/amount of oil extracted by the tow extraction process [Solvent (n-hexane) and steam extraction, ascertain the better extraction process for *Moringa* oil between solvent extraction and steam extraction], to determine the proximate analysis of the oil extracted from the *Moringa oleifera* in both processes and to determine some physiochemical parameters of the oil extracted from the *Moringa oleifera*.

2. MATERIALS AND METHOD

Materials

The following materials are required for successful implementation of the experimental techniques or methods. The agricultural feed for extraction process is the *Moringa* seed (*Moringa oleifera*). Essentially, they need to be collected and prepared into samples. The collections and preparation of samples are stated below;

The *Moringa oleifera* falls from its tree, which normally grows 12 to 18 meters (40 to 60 feet's) high. They were allowed to ferment or decay for 4-6 days. The fruit head soften for easy removal of the seeds. Some were sliced for quick fermentation. The seeds were manually extracted from the freshly harvested and partially fermented fruit heads. The mucilaginous layer of the freshly extracted seeds were removed using graded concentration (1-5%) of trona (complex salt) and wood ash as hydrolyzing acid for 5-25 minutes. The freshly extracted seeds were cleaned with fine sand and washed with water. The fine sand and water served as the control. The seed samples are parboiled for 7-10 minutes to soften the endocarp for easy removal. The seeds later extracted from the endocarp by manual process. With sand filled and well corked, was rolled on the seeds on a hard flat surface to dehull the endocarp. The seed samples were then separated from endocarp. They were dried for two days on a hot sun to reduce the moisture content, and then be reduced in a milling machine to powdered form.

Methods

The method adopted for this research is by the application of experimental techniques throughout the process. Wherever necessary, analytical models relating to stoichiometric balance was stated for purposes of calculations and clarity. This section focuses on the chemical extraction and parameter analytical steps.

Experimental Techniques (Oil Extraction Process)

During the extraction process the following experimental materials were utilize. Class thimble extractor, 500ml flat-bottomed flask, reflux condenser,, heating mantle, retort sand and the different media of extraction: Steam (Water) and Solvent (Hexane). The *Moringa oleifera* serves as material for extraction of the oil.

Procedure

The oil was extracted from the *Moringa oleifera* by solvent extraction method using hexane and as well, steam extraction using water respectively. The oil concentration was carried out using 20g of the grinded *Moringa* seed. The seed sample was weighed and fed into the porous glass thimble. The thimble was fixed into the extractor. Then the extractor was fixed into a 250ml capacity flat-bottomed flask.

The flat-bottomed flask contains some quantity of the extraction medium (hexane/water). The mesh was subjected to heating and process help to promote regular ebullition during the heating of the hexane/water. The hexane/water was coupled to a double surface reflux condenser. The set up was placed on a heating mantle to secure firmly in position by clamping to a retort stand.

The flask is heated gently over the heating mantle to start up extraction. The solvent evaporates and moves up into the condenser. The vaporized solvent condensed into the thimble and the oil is extracted, in the extraction chamber, when the solvent surrounding the sample exceed a certain, level. It overflows and trickles back down or refluxed back into the flask. This process continued until the extraction was completed as observed by a colour change of the oil solvent mixture in the extractor. The mixture became more yellow. The extraction mechanism was in the same way with both solvents (hexane) and steam (water).

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Figure 1: Apparatus and Materials during the Extraction Process

Analysis of the Extracted Oils

Materials and Method for Gas Chromatography (G.C/MS)

- Agilent 6890N Gas Chromatograph with Agilent 5975Mass Selective Detector

Materials

- Auto sampler vials, 150µL vial inserts, and crimp seals
- Vial crimper and decrimper
- 2.5mL airtight syringe or 3mL disposable hypodermic syringe
- 10 micro-liter autosampler syringe
- Supelco capillary column (hp-innowax, Agilent, 100 m×0.25 mm, i.d. 0.20 µm)
- centrifuged

Reagents

- Petroleum ether –Optima Grade
- Air–Zero grade
- Nitrogen gas –UHP grade
- n-hexane
- Methanol
- Potassium hydroxide
- sulfuric acid,
- The FAME standards(internal standard)

Sample Preparation for G.C Analysis

The soxhlet method was introduced using 5g and 80ml of petroleum ether at temperature of 60-90℃ for a period of 8h and it was observed that the solvent evaporated and remaining sample stored at temperature of 4℃.

Procedure for G.C Analysis

Lipids obtained after the extraction of the samples were converted to the corresponding FAMEs. In this procedure, 40µL of the was placed into 10mL centrifuge tubes to which 0.7mL of potassium hydroxide (10M) solution and 5.3mL of methanol were added. The reaction was performed at 55°C for 1.5h with mixing for 5s every 20 min. The solution was allowed to cool to room temperature and then 0.58m of H_2SO_4 (10m) was then added and the obtained solution was further subjected into heating at temperature of 55℃ for a

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period of 15h with stiring. Then the obtained solution was allowed to cool to room temperature and 3ml of n-hexane was introduced with stirring for period of 5mins. Subsequently, the tubes were centrifuged for 5min and the extracts were removed for GC analysis.

GC–MS analysis was carried out using an Agilent 6890 gas chromatograph with a 5973MS detector equipped with 60 m×0.2 mm, i.d. 0.25μm/MS DB-WAX capillary column (Agilent). The following temperature ramp was used: injector at 250 °C, oven initially at 200°C, held for 1 min and heated to 230°C (1.5°C min-1, then held for 10 min). The characterization and identification of FAMEs from the sample was completed in the SCAN mode with them and range varied from 35 to 450. The flow rate of the nitrogen as carrier gas was 1mL min-1; manual injection; the injection volume was 1μL. The composition of fatty acid of the FAMEs from the sample was determined using an Agilent 6820 gas chromatograph equipped with a Supercar capillary column (hp-inn wax, Agilent, 100 m×0.25mm, i.d. 0.20μm), injection port. The initial oven temperature was 200 °C, which was held for 1 min, subsequently increased to 230 °C at 1.5 °C $min^{-1}$and then held for 1 min. The injector was set at 250 °C, and the detector at 280 °C. Nitrogen was used as the carrier gas at a flow rate of 1 mL min-1. The split ratio was 50:1, and the sample size was 1 μL

3. RESULTS AND DISCUSSION

Gas Chromatography – Mass Spectrometric Analysis (GC-MS)

Gas Chromatography – Mass Spectrometric Analysis technique was used to identify the phytocomponents present in the extract. The gas chromatograph was interfaced to a Mass Spectrometer equipped with Elite-1 fused silica capillary column of Length: 30.0m, Diameter: 0.25mm, Film thickness: 0.25 and composed of 100% Dimethyl polysiloxane. The column oven temperature was maintained at 70°C and injector temperature at 240°C. The oven temperature was programmed as follows: 70°C for 2 minutes raised to 300°C for 7 minutes at the rate of 10°C/mm.

Steam Extraction

Thirty compounds were identified in the steam extract of *Moringa oleifera* (seeds). The peak report of the total ion current chromatogram obtained with details of retention time, molecular weight and composition. The best matched hit with the target was molecular formula, molecular weight and structure could be followed. The best matched hit results for the prevailing compounds were represented in Figure 2. The same was adopted for the identification of other compounds too.

The following results are the outcome of the project work presented in tables and figures below;

Table 1: Fatty Acid Calibration for Oil Obtained from Steam Extraction

	ARAL Laboratory Quantitation Report (Not Reviewed)
Data Path	C:\msdchem\1\data\060217k\
Data File	A1.D
Operator	ARAL
Acquired	12 April. 2021 09:53:27 AM using AcqMethod PAH_TEST2.M
Instrument	GCMS
Sample Name	Steam Extraction Sample
Misc Info	PROJECT
VialNumber	001 Sample Multiplier:2
Quant Method	C: \MSDCHEM\1\METHODS\PAH_TEST2.M
Quant Title	FATTY ACID CALIBRATION
Qlast Update	Thur. 07 Jan., 2021 09:53:28 AM
Response Via	Initial Calibration

R.T (min)	Compound	Name of Compound	Molecular Formula	M.W (g/mol)	Composition (%)
08.15	C8:0	Caprylic acid	CH_3-[CH_2]-COOH	144.21	1.76
08.78	C9:0	Nonanoic acid	$C_9H_{18}O_2$	158.24	2.39
12.08	C10:0	Decanoic acid	$C_{10}H_{20}O_2$	172.27	2.52
12.27	C11:0	Undecanoic acid	$C_{11}H_{22}O_2$	186.30	1.49

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12.75	C11:1	Decanoic acid	$CH_3[CH_2]_8COOH$	184.30	0.31
16.48	C12:0	Dodecanoic acid	$C_{12}H_{24}O_2$	200.35	6.54
16.56	C13:0	Tridecanoic acid	$CH_3[CH_2]_{11}COOH$	214.35	1.93
17.05	C13:1	Tridecanoic acid	$C_{12}H_{26}O_2$	212.43	2.51
17.29	C14:0	Tetradecanoic acid	$CH_3[CH_2]_{12}COOH$	228.38	3.64
18.17	C14:1	Tetradecanoic acid	$C_{14}H_{26}O_2$	226.38	1.72
18.84	C15:0	Pentadecanoic acid	$CH_3[CH_2]_{12}COOH$	242.41	13.25
19.36	C16:0	Hexadecanoic acid	$CH_3[CH_2]_{14}COOH$	256.43	3.91
20.45	C16:1	Palmitoleic acid	$CH_3[CH_2]_5CH$	254.43	7.52
20.71	C17:0	Heptadecanoic	$CH_3[CH_2]_{15}COOH$	270.48	4.20
20.80	C17:1	Ginkgolic acid	$CH_{17}H_{34}O_2$	268.48	1.24
21.22	C18:0	Octadecanoic acid	$CH_{18}H_{36}O$	284.48	5.27
21.81	C18:1	Elaidic acid	$CH_{18}H_{34}O_2$	282.48	1.56
22.56	C19:0	Nonadecanoic acid	$CH_{19}H_{38}O_2$	298.51	2.82
23.34	C20:0	Icosanic acid	$CH_{20}H_{40}O_2$	312.54	3.63
23.88	C20:1	Eicosenoic acid	$CH_{20}H_{32}O_2$	310.54	2.51
24.71	C20:2	Arachidonic acid	$CH_{20}H_{32}O_2$	308.53	0.86
24.98	C20:4	Icosanic acid	$CH_{20}H_{40}O_2$	304.52	1.72
25.51	C22:0	Docosanoic acid	$CH_{22}H_{44}O$	340.59	1.52
26.12	C22:1	Cetoleic acid	$CH_{22}H_{42}O_2$	338.59	2.36
26.46	C22:4	Docosanic acid	$CH_{22}H_{44}O_2$	332.57	0.54
26.62	C22:5	Docosanic acid	$CH_{22}H_{44}O_2$	330.57	2.67
27.40	C22:6	Docosahexaenoic acid	$CH_{22}H_{32}O_2$	328.57	1.85
27.87	C23:0	Tricosanic acid	$CH_{23}H_{46}O_2$	354.61	1.52
28.24	C24:0	Tetracosanic acid	$CH_{24}H_{48}O_2$	366.63	0.16
28.76	C24:1	Tetracosanic acid	$CH_{24}H_{48}O_2$	366.63	2.74

(#) = qualifier out of range (m) = manual integration (+) = signals summed
ESS(+) – Limonene_TEST2.M Thur. 07 Jan. 2021 09:53:28 AM

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Operator        : ARAL
Acquired        : 12 April. 2021  09:53:27 AM  using AcqMethod PAH_TEST2.M
Instrument      : GCMS
Sample Name     : Steam Extraction Sample
Misc Info       : PROJECT
VialNumber      : 001
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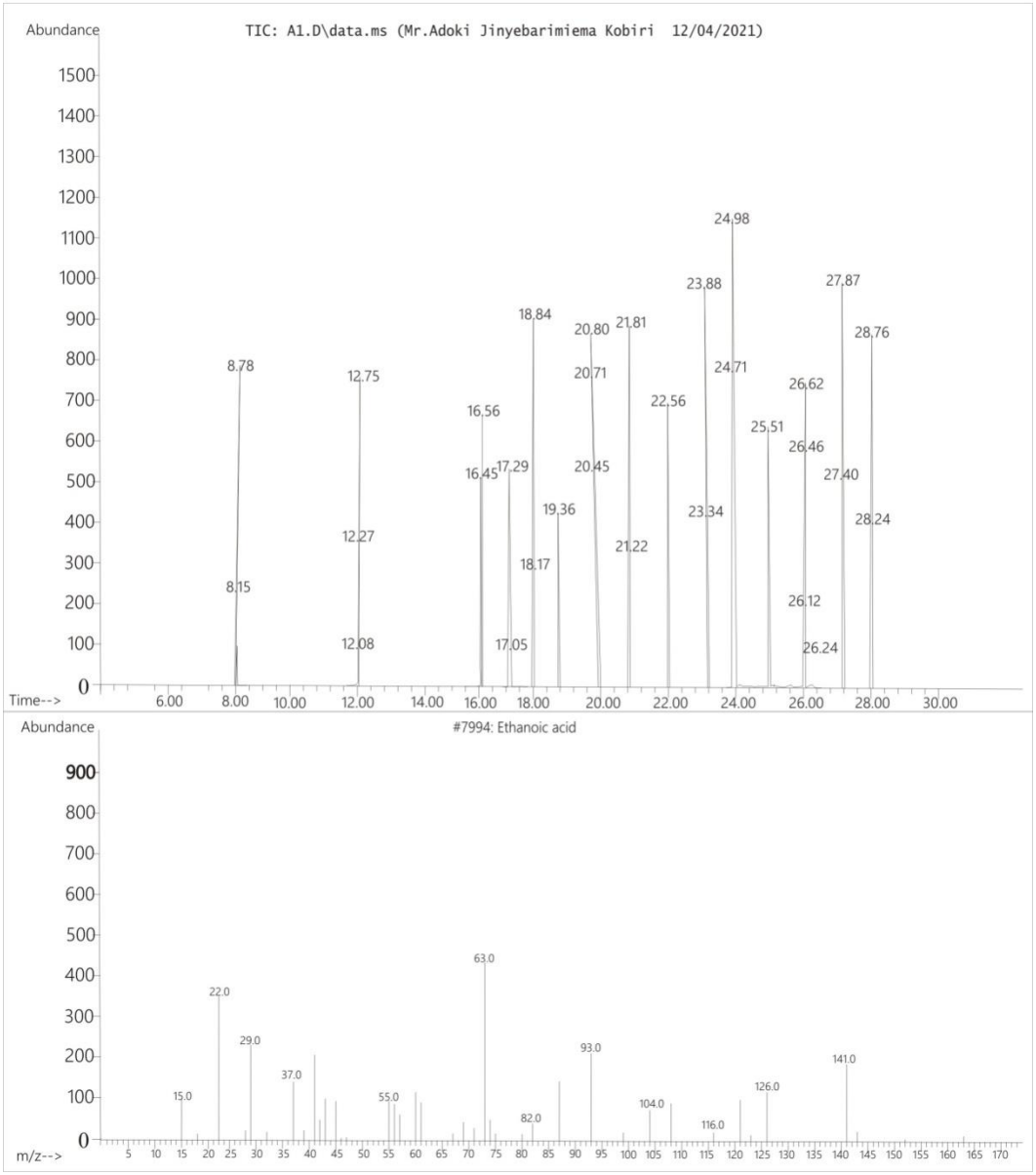

Figure 2: Chart Showing Fatty Acid Calibration for Oil Obtained from Steam Extraction

Thirty compounds were identified in the hexane of *Moringa oleifera* seed as presented in Table 2. From Figure 3 it is seen that the chromatogram with the peaks of the test compounds with respect to retention time. Each compound vary in their respective retention time. The mass spectrum was obtained with mass/charge ratio on x-axis and abundance on y-axis.

Table 2: Fatty Acid Calibration for the Oil Obtained from Solvent Extraction

	ARAL Laboratory Quantitation Report (Not Reviewed)
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Data File	A1.D
Operator	ARAL
Acquired	12 April. 2021 10:28:47 AM using AcqMethod PAH_TEST2.M

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Instrument	GCMS
Sample Name	Solvent Extraction Sample
Misc Info	PROJECT
VialNumber	002 Sample Multiplier:2
Quant Method	C: \MSDCHEM\1\METHODS\PAH_TEST2.M
Quant Title	FATTY ACID CALIBRATION
Qlast Update	Thur. 07 Jan., 2021 09:53:28 AM
Response Via	Initial Calibration

R.T (min)	Compound	Name of Compound	Molecular Formula	M.W (g/mol)	Composition (%)
8.46	C8:0	Caprylic acid	CH_3-[CH_2]-COOH	144.21	3.38
8.72	C9:0	Nonanoic acid	$C_9H_{18}O_2$	158.24	4.61
12.13	C10:0	Decanoic acid	$C_{10}H_{20}O_2$	172.27	2.78
12.29	C11:0	Undecanoic acid	$C_{11}H_{22}O_2$	186.30	4.63
12.72	C11:1	Decanoic acid	$CH_3[CH_2]_8COOH$	184.30	2.71
16.28	C12:0	Dodecanoic acid	$C_{12}H_{24}O_2$	200.35	7.99
16.63	C13:0	Tridecanoic acid	$CH_3[CH_2]_{11}COOH$	214.35	3.54
17.17	C13:1	Tridecanoic acid	$C_{12}H_{26}O_2$	212.43	4.21
17.54	C14:0	Tetradecanoic acid	$CH_3[CH_2]_{12}COOH$	228.38	2.31
18.38	C14:1	Tetradecanoic acid	$C_{14}H_{26}O_2$	226.38	15.96
18.38	C15:0	Pentadecanoic acid	$CH_3[CH_2]_{12}COOH$	242.41	2.27
19.31	C16:0	Hexadecanoic acid	$CH_3[CH_2]_{14}COOH$	256.43	8.52
20.12	C16:1	Palmitoleic acid	$CH_3[CH_2]_5CH$	254.43	6.37
20.28	C17:0	Heptadecanoic	$CH_3[CH_2]_{15}COOH$	270.48	3.86
20.70	C17:1	Ginkgolic acid	$CH_{17}H_{34}O_2$	268.48	6.91
21.63	C18:0	Octadecanoic acid	$CH_{18}H_{36}O$	284.48	3.27
21.84	C18:1	Elaidic acid	$CH_{18}H_{34}O_2$	282.48	3.68
22.51	C19:0	Nonadecanoic acid	$CH_{19}H_{38}O_2$	298.51	4.21
23.42	C20:0	Icosanic acid	$CH_{20}H_{40}O_2$	312.54	6.30
23.77	C20:1	Eicosenoic acid	$CH_{20}H_{32}O_2$	310.54	2.89
24.52	C20:2	Arachidonic acid	$CH_{20}H_{32}O_2$	308.53	2.25
24.86	C20:4	Icosanic acid	$CH_{20}H_{40}O_2$	304.52	4.30
25.46	C22:0	Docosanoic acid	$CH_{22}H_{44}O$	340.59	3.87
26.28	C22:1	Cetoleic acid	$CH_{22}H_{42}O_2$	338.59	3.25
26.67	C22:4	Docosanic acid	$CH_{22}H_{44}O_2$	332.57	1.27
26.72	C22:5	Docosanic acid	$CH_{22}H_{44}O_2$	330.57	2.90
27.24	C22:6	Docosahexaenoic acid	$CH_{22}H_{32}O_2$	328.57	2.25
27.53	C23:0	Tricosanic acid	$CH_{23}H_{46}O_2$	354.61	3.93
28.17	C24:0	Tetracosanic acid	$CH_{24}H_{48}O_2$	366.64	1.75
28.40	C24:1	Tetracosanic acid	$CH_{24}H_{48}O_2$	366.63	1.52

(#) = qualifier out of range (m) = manual integration (+) = signals summed

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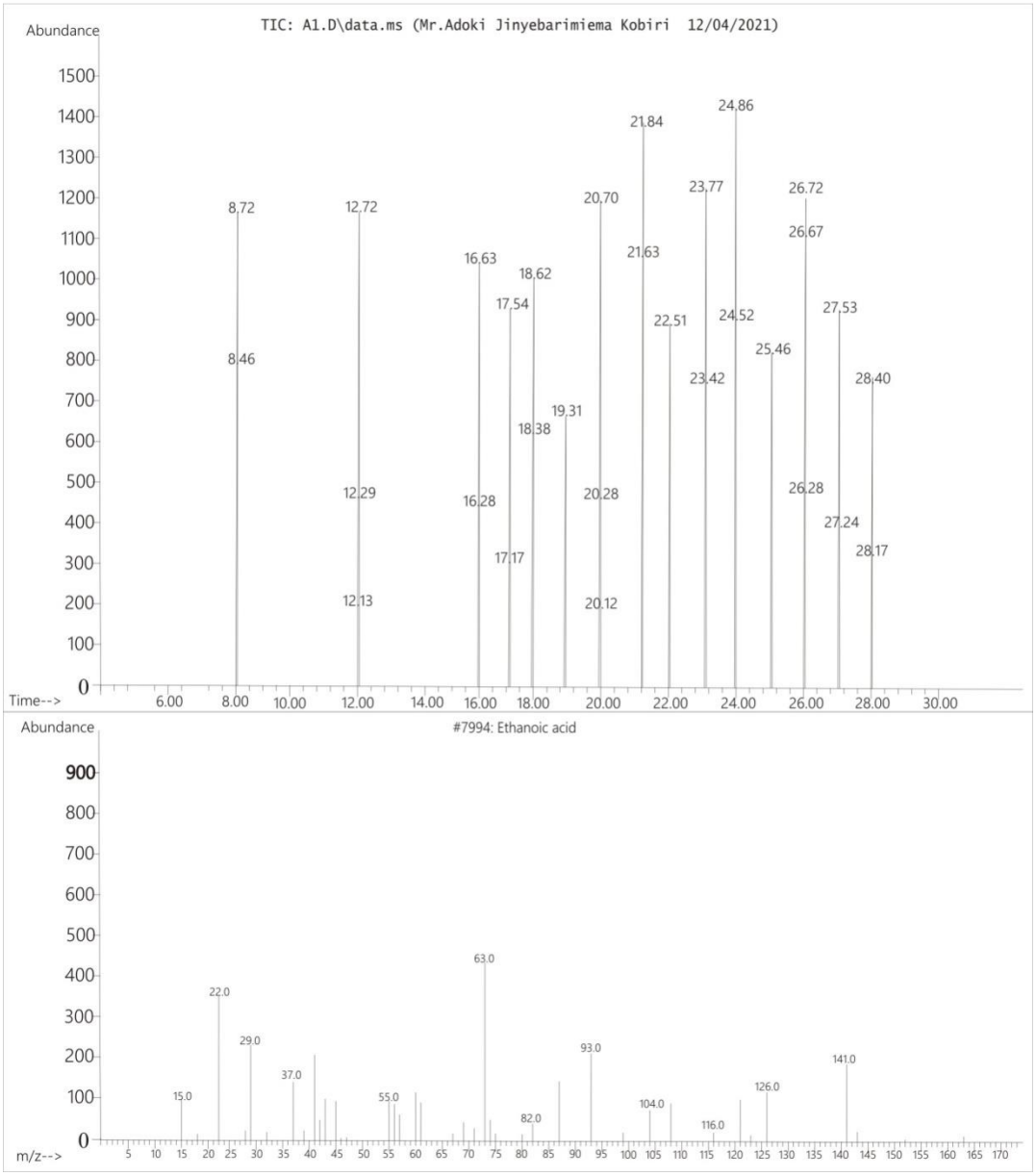

Figure 3: Chart Showing the Fatty Acid Calibration of the Oil from Solvent Extraction

3. CONCLUSION

The following conclusions are drawn from the research carried out on extraction, characterization and proximate analysis of Moringga oleifera. Oil concentration of *Moringa oleifera* is very high when compared with other oil seed so processing the oil is considered economical. For commercial purposes steam extraction is the more appropriate method to extract the oil. Solvent extraction is the more effective extraction method among other types of extraction method. From the analysis carried out on the *Moringa oleifera*, It shows that it has a high nutritive value of 15.01% protein and the carbonhydrate content is 37.08%. The acid value of *Moringa oleifera* is 2mgkoH/g showing that it is of low value. This low value is desirous because when the value of free acid is above 2.0% there is increase in the level of serum cholesterol, which may lead to coronary heart problem. The project reveals that due to the iodine and saporification value *Moringa oleifera* is not to be stored for a long time as oxidation may take place thereby

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increasing the level of free fatty acid and rancidity. The saponification value of the oil shows that it could be used in liquid soap and shampoo. This project revealed that the location of extraction plant does not affect oil yield.

Funding:
This study has not received any external funding.

Ethical approval
The ethical guidelines for plants & plant materials are followed in the study for experimentation.

Conflict of Interest:
The authors declare that there are no conflicts of interests.

Data and materials availability:
All data associated with this study are present in the paper.

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