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# Determination of Fatty Acids and Physicochemical Properties of Neem (*Azadrachta Indica* L) Seed Oil Extracts

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## Abstract
*Neem tree is a folklore plant mostly used in medicinal preparations. Therefore, neem seeds were investigated with the aim of determining its fatty acid composition and physicochemical properties of the oil extract. The oil was extracted from the powdered seed using n-hexane with the help of Soxhlet which yielded 29.71% oil. Results revealed that the oil was liquid at room temperature and physically stable at varying temperatures (0, 50 and 100°C). It appeared to be pale greenish yellow, garlic-like odour, had a little bitter taste, viscosity of 12.2Pas and pH value of 6.78 ± 0.0135. The chemical parameters were identified to be 1.22 ± 0.029%, 2.36 ± 0.054 mg NaOH/g oil, 172.84 ± 0.559 mgNaOH/g oil and 1.88 ± 0.059 meq/kg oil for free fatty acids, acid value, saponification value and peroxide value respectively. The GC-MS analysis showed that the oil extract contained six different fatty acids with total composition of 63.07% oil. The compound with the highest composition was linoleic acid (40%) followed by oleic (35%), cis-13-octadecenoic acid (8.9%), palmitic acid (8.5%), stearic acid (7.5%) while the least compound was cis-vaccenic acid (0.5%). However, contrary to previous work where it was reported that oleic acid or linoleic acid was the dominant fatty acid found in neem oil. Linoleic acid was found to be dominant in this current research work. It is however recommended that under-utilized neem seeds should be explored the more with a view to producing viable products.*

**Keywords**: Fatty acid composition, Neem tree, oil, Physicochemical properties, seeds,

## INTRODUCTION
Neem tree is known as *Azadirachta indica* belonging to the mahogany family called Meliaceae (Jessinta *et al*., 2014), It is one of the two species in the genus Azadirachta that is native to the Indian subcontinent. It is typically grown in tropical and semi-tropical regions (Dua *et al.,* 2009). This plant has its origin in India but it is currently and widely distributed in the tropics, subtropics, semi-arid and wet-tropical regions of the world (Dua *et al*., 2009). Interestingly, the barks, leaves, seeds, roots, stems, flowers and fruits of *A. indica* possess biologically active substances ranging from alkaloids, tannins, peptides, phenols, sterols, glycosides and

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flavonoids which enhanced in combating against some bacterial infections. Among all the parts of *A. indica*, the seeds are documented as one of the most major sources of pharmaceuticals in antibacterial property as the oil has broad spectrum against antibacterial infections (El-Mahmood *et al*., 2010). However, the seeds are mostly available in Nigeria, plenty of the seeds generated by the neem tree are presently unattended to and massively underutilized (Erakhrumen, 2011).

Fats and oils are nutritionally vital as they are classified as one of the four major categories of foods. Oils are employed in different ways. Some are mostly utilized for food baking, texturing and also used industrially in the production of detergent, soaps, oil paints and cosmetics. In plants, oils are mostly produced in the endosperm along with the carbohydrates where they jointly nourish the embryo (Oyeyiola, 1993). Fatty acids are not found in a free state in nature but exist commonly in combination with glycerol (an alcohol) in the form of triglyceride (Britannica, 2020).

The 16- and 18-carbon fatty acids, equally known as stearic acid and palmitic acid are amongst most extensively disseminated fatty acids in nature. It has been established that both palmitic and stearic acids are found in the lipids of the majority of organisms. For instance, the body fat of animals is made up of 30% palmitic acid. Fatty acids equally contribute to 5 to 50% lipids found in vegetable fats and most abundantly found in palm oil. Stearic acid is copious in some vegetable oils like cocoa butter and shea butter thereby making up for a comparatively high percentage of the lipids found in ruminant tallow (Britannica, 2020). Commercially, fatty acids have got an extensive range of applications. For instance, they are used not only in the manufacture of plentiful food merchandises but equally in the production of soaps, cosmetics and detergents. Soaps are known as the sodium and potassium salts derivable from fatty acids. According to (Britannica, 2020), some products meant for skin-care have fatty acids which can ultimately help retain healthy skin look and function. Fatty acids, principally omega-3 fatty acids are also usually sold as dietary supplements (Britannica, 2020),

The oil generated is a vegetable oil obtained from the seed kernels, which is an evergreen of the tropics and sub-tropics. It is deep yellow in colour with garlic–like odor. It is one of the important commercially available products used for organic medicines and farming (El-Mahmood *et al*., 2010). One of the studied triterpenoid in neem oil is Azadirachtin. However, nimbin is another triterpenoid with some antimicrobial properties (Kraus, 1995). The complex molecular structure of Azadirachtin possesses both secondary and tertiary hydroxyl groups and a tetrahydrofuran ether in its molecular structure coupled with 16 stereo genic has a, 7 of which are tetrasubstituted. These characteristics can be attributed to the serious difficulty that is normally met in its preparation from simple precursors through the employment of synthetic organic chemistry methods (Veitch *et al*., 2007). The chemical formula of Azadirachti is $C_{35}H_{44}O_{16}$ with molar mass of 720.721 g·mol$^{-1}$. Neem seed oil has wide applications ranging from biodiesel, which is biodegradable, a clean, renewable biofuel and non-toxic, can be produced by transesterification of seed oils. It is used in the manufacture of pesticides as it works by interfering with the reproductive cycle of target insects (Veitch *et al*., 2007).

Neem oil equally have a powerful aromatic odour that chases insects away (Awasthi and Shikha, 2019). Neem has excellent effect on chronic skin conditions that often fail to respond to medical drugs, psoriasis, eczema, and ringworm, even stubborn warts are among the conditions that can clear up easily. In addition, neem oil can be used as an excellent component of cosmetics to help clear, beautify and rejuvenate the skin (Shujit *et al*., 2013). Neem has proven successful in treating stomach ulcers. Its antihistamine and antibacterial

compounds can reduce inflammation and destroy ulcer causing bacteria (Shujit *et al*., 2013). Neem has been proven effective for its ability to reduce blood clots, blood pressure, heart irregularities and cholesterol levels. Since the antihistamine properties of nimbidin found in neem leaves have been found to cause blood vessel to dilate, it confirmed the reason of its capability to reduce high blood pressure. A recent research showed that neem lowered high cholesterol values in only one month (Shujit *et al*., 2013).

Previous work have been done on the neem seed- determination of the fatty acid composition, the physicochemical properties has also been reported in literature. The objectives of this research was to validate and compared the determination of some of the physicochemical properties and fatty acids composition of neem seed oils using gas chromatography-mass spectrometer.

## METHODS AND MATERIALS

The study area is in Ringim Local Government Area, Ringim emirate Jigawa State Nigeria. Which is located between Latitudes 110 38' 31''N and 11o46' 16''N and longitudes 9018' 33''E and 9024' 24''E. with estimated population of 192,024 as at 2006 census (NPC, 2006). The climate is tropical wet dry climate (Koppen AW) classification and the temperature is warm to hot throughout the year, even though there is slightly cold period around November through February. The mean annual temperature is $26^0$C but, mean monthly value ranges between $21^0$C in the coldest months (February) and $31^0$C in the hottest months (April/May) (Aminu, 2015)

### Collection of Samples and Identification

The neem seeds used in this research project were collected from a farm in Ringim Local Government Area, Jigawa State, Nigeria in January 2021and transported to the Herbarium Unit where it was identified by a botanist, Department of Biological Sciences, Faculty of Science, Federal University Dutse, Jigawa State for identification

### Preparation of the Neem Seed

The neem seeds (hulled) used in this research project were collected from a farm in Ringim Local Government Area, Jigawa State, Nigeria in January 2021. Prior to use, the neem seeds (hulled) were washed three times to remove the dirt and solid impurities. It was then dried under the absence of light at room temperature for about one month until it reached constant moisture content (Plates 1 showing neem tree and 2 pulp neem seeds).

Plate 1NEEM TREE

Plate 2. Washed and dried pulped neem seeds

The heating mantle was used to heat the solvent (n-hexane) above room temperature, the reflux condenser attached to the Soxhlet extractor used to condense the n-hexane back to the chamber (with a constant flow of cold water throughout the condenser). The oil obtained was separated from the solvent using a rotary evaporator at a reduced pressure which was used to recover the solvent. The oil was further distilled to ensure that all the solvent was totally removed. The percentage oil content of the neem seeds was calculated using;

$$\frac{Mass\ of\ oil}{Mass\ of\ sample} \times 100\%$$

Manually, the seeds were crushed by removing the simple hulls where the almonds were obtained. It was then grounded to reduce the size, so as to enable smooth extraction process (Plates 3 and 4). The grounded powdered sample was added into a thimble which was then inserted into the main chamber of Soxhlet extractor and the solvent (n-hexane) was poured into the round bottomed flask.

*Plate 3.Size reduction of the seeds*

*Plate 4. Introduction of sample into thimble*

Standard methods were employed to determine the physical properties of the processed neem seed oils. The extract was observed carefully by naked eyes. The instrument label NDJ-5S viscometer was used to determine the viscosity of the extract. L-4 spindle was used (dipped into the extract) and ran for 10 minutes. The electrode of pH meter was cleansed using distilled water and dried. The meter was switched on and the electrode was dipped into the buffer solution (a solution whose pH is known to be 7.0 and then prepared by adding a weak acid to its salt). Adjustments were made till the pH value for the buffer solution was at exactly 7.0, the electrode was then removed and dipped into a portion of the extract in a beaker. The reading was allowed to pulsate till it maintain a consistent value. This was taken as the pH

value. The physical stability of the oil was determine by exposing a sample of the oil to varying temperature (100, 50 and 0°C). This was done to see whether the oil could still retain its physical properties under different ranges of temperature. The oil was also subjected to vibration and compression so as to determining its stability.

Fifty (50) mL of ethanol (99%) was added to 10 g sample oil. The resulting solution was heated using magnetic heated stirrer until the oil was dissolved in the ethanol. While the solution was still hot, a few drops of phenolphthalein indicator was added and titrated against 0.1N NaOH. The titration was stopped when the colour of the solution changed to pink. The replicate of the same procedure was done and recorded. The acid value was calculated using:

$$\text{AV} = \frac{56.1VN}{Ws}$$

Av = acid value

V = vol. in ml of standard NaOH, N = normality of NaOH solution, Ws = weight of sample oil.

The same procedure used for determination of acid value was adopted for the determination of free fatty acid except 0.1N KOH was used instead of 0.1N NaOH (AOAC, 2009). The free fatty acid (FFA) was calculated as follows;

$$\text{FFA} = \frac{28.2VN}{Ws}$$

V = vol. in ml of standard KOH, N = normality of KOH solution, Ws = weight of sample oil

An approximately 5 g of sample oil was weighed and transferred in to a round bottomed flask labeled with 'sample' and 50 mL of 4% ethanolic KOH was added to the sample flask. Equivalent volume of 4% ethanolic KOH was added to a separate container labeled 'Blank' (no oil content in it). The sample mixture was refluxed using a heating mantle and reflux condenser for about 30 minutes with a constant flow of cold water. The heating was stopped when there was no separate layer in the solution which indicated a complete saponification. The solution was then allowed to cool down. The same procedure was carried out on the Blank sample solution. The solution was titrated against 0.5N HCl using a few drops of phenolphthalein indicator with a vigorous agitation of the mixture until the pink colour formed disappeared from the solution. The cold 4% ethanolic KOH was also titrated using the same procedures and the burette reading was noted in all cases (AOAC, 2009). The saponification value (SV) was calculated using;

$$\text{SV} = \frac{56.1N\ (Vb - Vs)}{Ws}$$

Vs = Vol. in ml of 0.5N HCl required for the sample

Vb = Vol. in ml of 0.5N HCl required for the blank, N = normality of KOH solution

Ws = weight of sample oil.

The weight of sample oil was weighed between 10-12 g into a conical flask and 30 mL mixture of acetic acid and chloroform was added to the sample oil and shaken so as to mix it thoroughly. One (1) mL of saturated potassium iodide (KI) was added to the mixture followed by 30 mL of distilled water. One (1) mL of starch solution was used as an indicator. The solution was titrated against 0.01N $Na_2S_2O_3$ until the colour changed from blue to colourless. The peroxide value was calculated using;

$$\text{PV} = \frac{VN \times 1000}{Ws}$$

V = vol. in ml of standard $Na_2S_2O_3$, N = normality of $Na_2S_2O_3$ solution, Ws = weight of sample oil

GC-MS analysis was performed on an Agilant Technologies Auto-system GCMS-QP2010 PLUS Shimadzu, Japan, equipped with a split/splitless injector (250 $^0$C) with System operating in the E.I mode at 70 ev,. The transfer line was 280 $^0$C. Helium was used as carrier gas (1.3ml min$^{-1}$) and the capillary columns used were on Hp 5Ms (30 x 0.25 mm with Film thickness 0.25 mm and an Hp innovvax (30 x 0.32 mm, Film thickness 0.50 mm). The GC-MS analysis was performed at Multi-User Science Research Laboratory of the department of Chemistry, Ahmadu Bello University Zaria, Kaduna State Nigeria.

**Results:**

Physicochemical properties are those properties that define both the physical and chemical behaviour of a substance. Among those characteristics observed in this study are colour appearance, odour, test, conductivity and resistivity, viscosity, pH value, acid value, refractive index, iodine value, physical stability, free fatty acids, peroxide value and saponification value. The results of the extraction, determination of fatty acids composition and physicochemical properties of the oil are presented in Table 1.0 and 2.0 respectively. The seed oil analyzed in this study revealed a conductivity value (0 mScm$^{-1}$) which implied that the oil will be stable under conditions of proper storage, handling and processing. The value of viscosity (12.2 Pa.s) recorded from the processed neem oil showed that a spreader is necessary to make a formulation that can be burnt or sprayed easily (Table.1.0). A pH value of 6.78 ± 0.0135 was equally detected (Table 1.0). The fatty acids determination resulted in the detection of six (6) fatty acids, whereby the dominant compound was linoleic acid (40%) followed by oleic acid (35%), cis-13-octadecenoic acid (8.9%) palmitic acid (8.5%) and stearic acid (7.5%) while the least compound detected was cis-vaccenic acid (0.5%) (Table 2.0) and was compared with report by (Atabani *et al*., 2013).

Table I.0: Physicochemical properties of neem (Azadrachta indica) seed oil extract

| Parameter | Value |
|---|---|
| Oil Content (%) | 29.71 |
| Colour | Pale greenish yellow |
| Odour | Garlic-like |
| Taste | A little bitter |
| Physical stability (at 100, 50 and 0 °C) | Physically stable |
| Conductivity (mScm$^{-1}$) | 0 |
| Viscosity (Pa.s) | 12.2 |
| pH value | 6.78 ± 0.0135 |
| Acid value (mgNaOH/g oil) | 2.36 ± 0.054 |
| Free fatty acids (%) | 1.22 ± 0.029 |
| Saponification value (mgNaOH/g oil) | 172.84 ± 0.559 |
| Peroxide value (meq/kg oil) | 1.88 ± 0.059 |

Data are mean value ± standard deviation of four replicates

Table 2.0: Fatty acid determination of neem (Azadrachta indica) seed oil extract

| Fatty Acid | Systematic Name | Formula | Structure | Area (%) |
|---|---|---|---|---|
| Linoleic acid | 9,12-octadecadienoic acid (Z,Z) | $C_{18}H_{32}O_2$ | 9,12-18:2 | 40 |
| Oleic acid | 9-octadecenoic acid (Z) | $C_{18}H_{34}O_2$ | 9-18:1 | 35 |
| Cis-13-octadecenoic acid | 13-octadecenoic acid (Z) | $C_{18}H_{34}O_2$ | 13-18:1 | 8.9 |
| Palmitic acid | Hexadecenoic acid | $C_{16}H_{32}O_2$ | 16:0 | 8.5 |
| Stearic acid | Octadecanoic acid | $C_{18}H_{36}O_2$ | 18:0 | 7.5 |
| Cis-vaccenic acid | 11-octadecenoic acid (Z) | $C_{18}H_{34}O_2$ | 11-18:1 | 0.5 |

In the systematic names the Z and E represent Zasumen for the hydrogen atoms at the same side of the double bond. The structure of fatty acids represented by e.g. 9,12-18:2; where the number (s) before the hyphen (-) represents the position (s) of the double bond, 18 and 2 represent the number of carbon atoms and double bonds per molecule respectively.

The chromatographs obtained from the GC-MS analysis of the extracted neem oil are depicted in Figs 1-6 respectively. It can be observed that the five (5) major organic acids were detected in the analyzed oil.

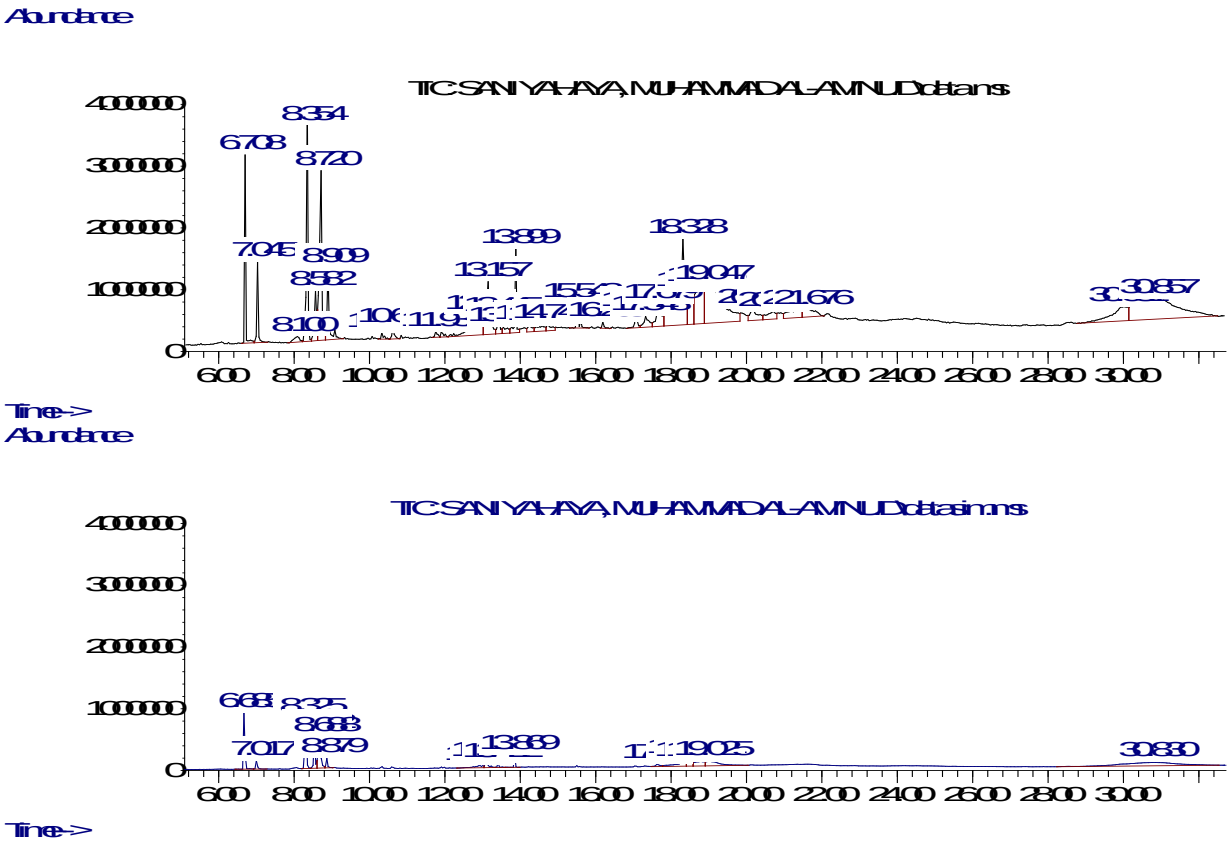


Fig 1: Chromatograph showing GC-MS spectrum of Cis-13-octadecenoic acid

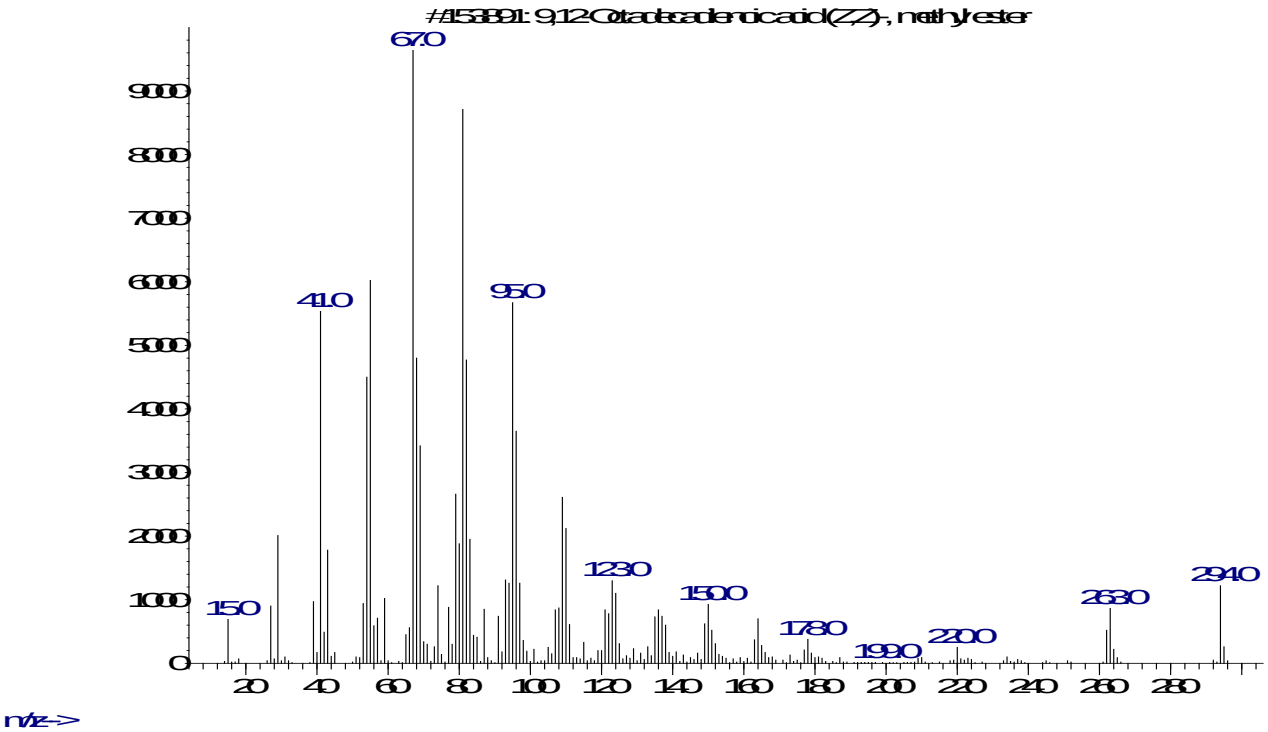


Fig 2: Chromatograph showing GC-MS spectrum of linoleic acid in the extracted oil

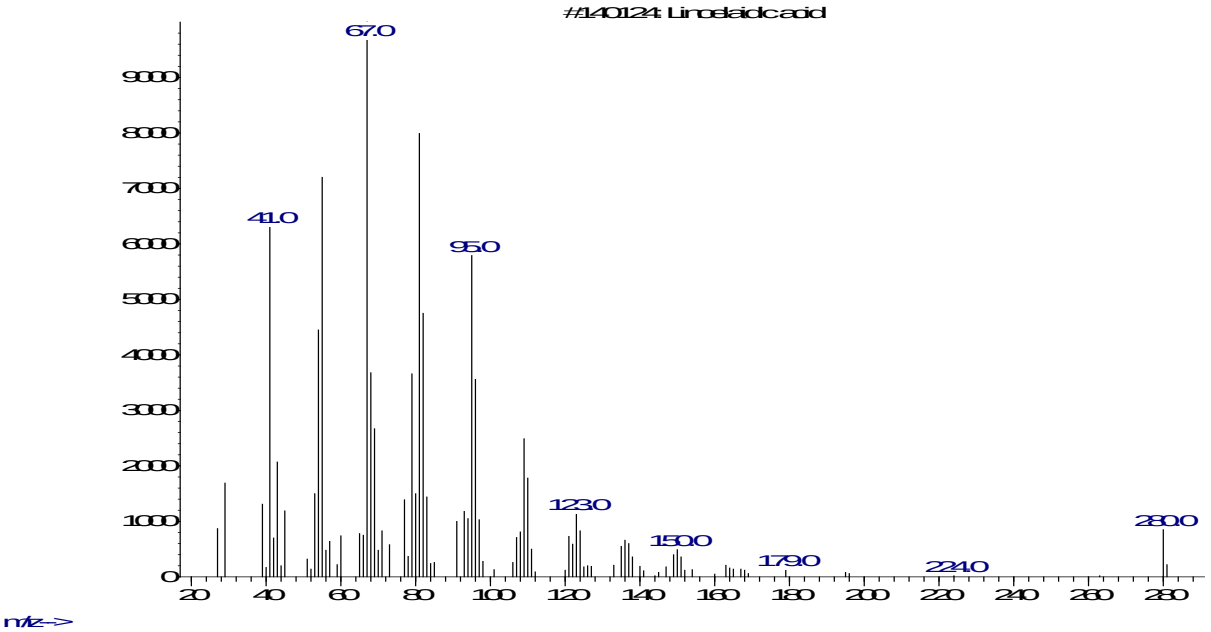


Fig 3 Chromatograph showing GC-MS spectrum of oleic acid in the extracted oil

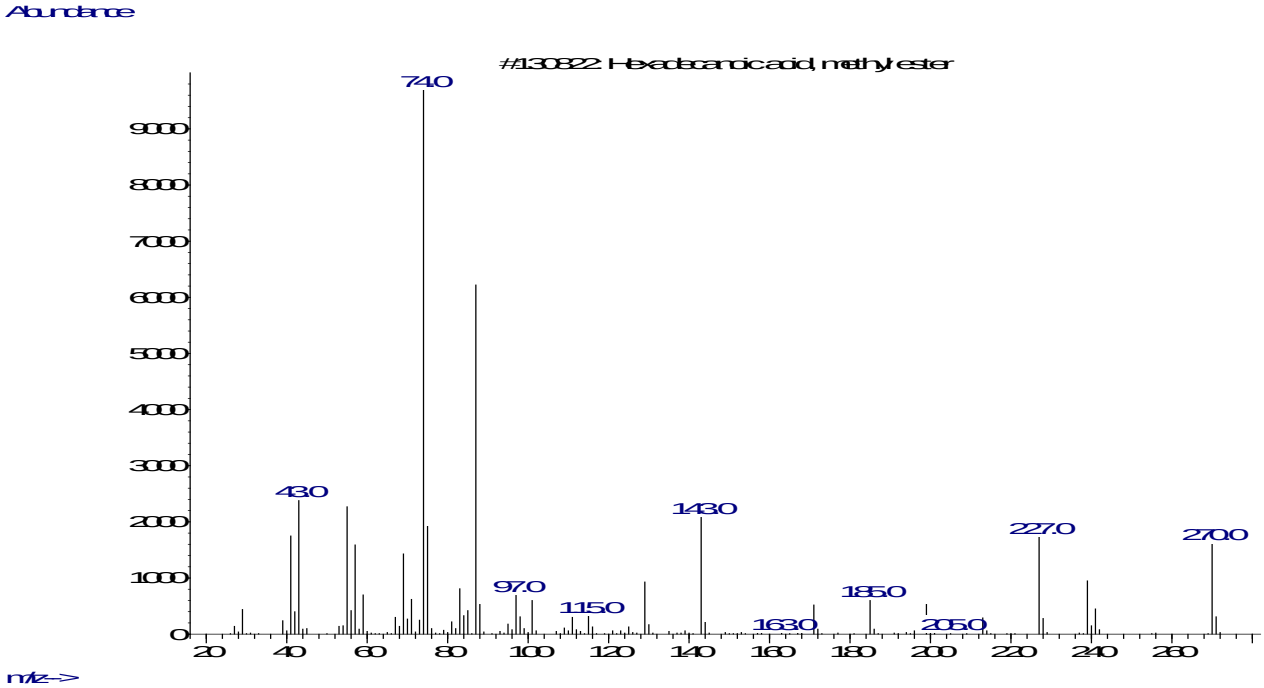


Fig 4: Chromatograph showing GC-MS Spectrum of Palmitic acid in the extracted oil

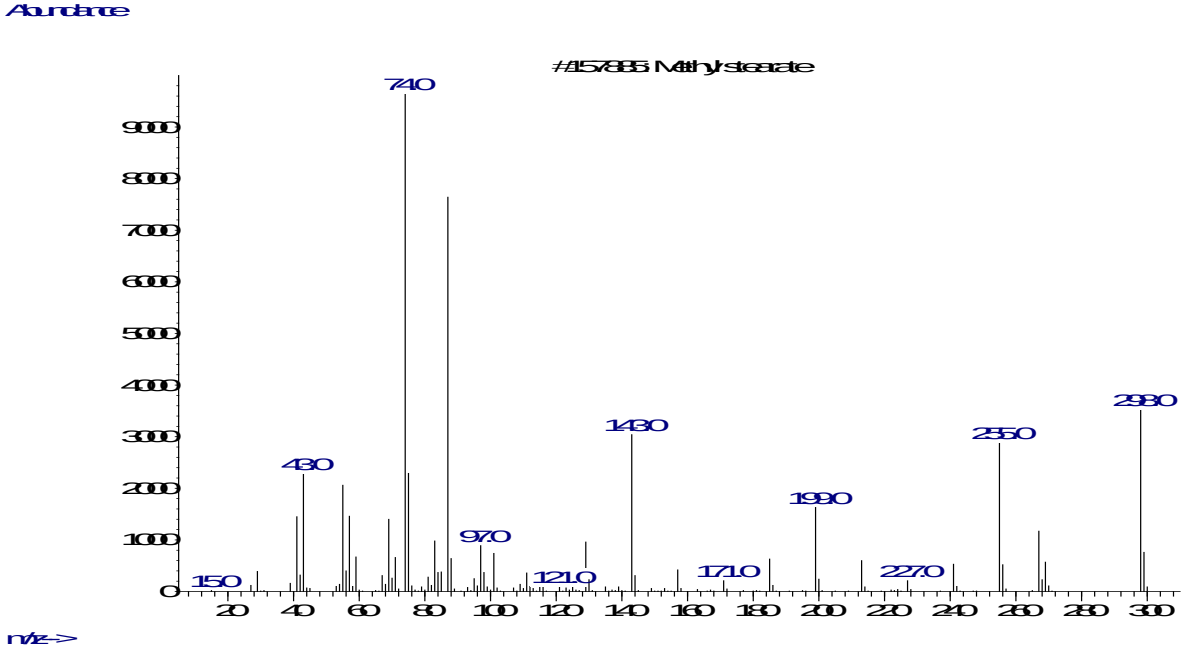


Fig 5: Chromatograph showing GC-MS spectrum of stearic acid acid in the extracted

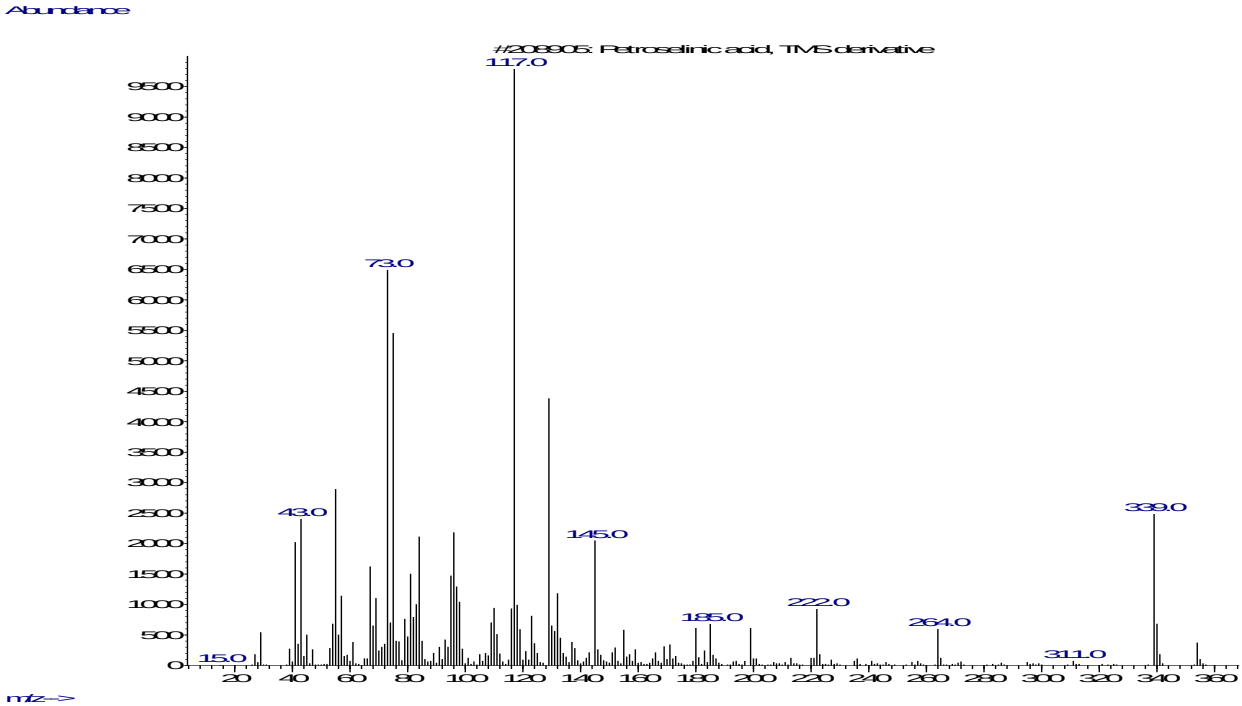


Fig 6: Chromatograph showing GC-MS Spectrum of cis-vaccenic acid in the extracted oil

## DISCUSSION

The percentage yield (% w/w) of oil obtain from the seeds was 29.71%. The extracted oil had pale greenish yellow colour with a pungent smell. It was compared with other work which yielded as 28.4% of oil by (El-Mahmoud *et al*., 2010) and 37% (Sandanasamy *et al*., 2013). While acid value was 2.36 ± 0.054 mg NaOH/g, and compared to report conducted by Sampson (2018) revealed that neem seed oil contained acid value of 3.56 mgKOH/g.

The free fatty acids in this present work was 1.22 ± 0.029% lower when compared (5.39 %) as reported by(El-Mahmoud *et al*., 2010). While saponification value obtained was 172.84 ± 0.559 mgNaOH/g oil and compared favourably with previous work showing no significant difference as reported by El-Mahmoud *et al*., 2010.was 198.26 mgKOH/g oil.

The peroxide value in this present study gave 1.88 ± 0.059 meq/kg oil which was very low compared to (8.7 meq/kg) as reported by (El-Mahmoud *et al*., 2010). Some of the documented results are compared well with the present work which are in agreement with the present study. However, the difference in percentage yield and physicochemical properties might be due to the origin of the plant, time of harvesting of the seeds, maturity of the seeds and the drying process employed.

Previous research have reported that the major fatty acid content of neem seed fixed oil is oleic acid whereby the percentage ranged between 25-61.9% (Kaushik and Vir, 2000; Ahmad *et al*., 2011; Atabani *et al*., 2013; Bakari *et al*., 2020). Nevertheless, the results were supported by other reported claiming that the major content was linoleic acid at 38.26% and 34.69% (Muthu *et al*., 2010) followed by oleic acid at 34.09% and then 20.46% (Sandanasamy *et al*., 2013).

In this present study, the lowest content of fatty acid was represented by cis-veccenic acid (0.5%) which is a geometrical isomer of linoleic acid. According to Djenontin *et al.* (2012), neem seed fixed oil's palmitic-, oleic-, stearic-, arachidic- and behenic acid ranged between 17.3-34.3, 6.6-24, 25.4-57.9, 1.24- 1.3, and 0.23-1.73%, respectively. The difference may be attributed to the plant species and methods of extraction adopted. Similar finding has been reported by Svetlana *et al*. (2007); Sanderson (2007); Atabani *et al.* (2013).

The conductivity of the neem oil obtained in this study implied that it was stable under normal conditions of proper handling, processing and storage. The viscosity recorded from the processed neem oil showed that a little spreader is necessary to make a formulation that can be burnt or sprayed easily. The oil content (29.71%) detected in this study showed that the analyzed seed kernel contained an appreciable quantity of oil and hence can be utilized for large-scale industrial production. The physical properties of the oil implied that it possessed the basic characteristics of good insect pest repellent (Awasthia and Deepti, 2019). The pH value of the oil, implied that it is acidic therefore cannot cause skin burns when applied on the skin, as such the neem oil can be used in the production of soap and vaseline for protection against mosquito bites (Awasthia and Deepti, 2019).

## CONCLUSION

The oil obtained from the neem seeds had an appreciable quantity compared with published information on extraction of oil from neem seeds. The neem oil extracted was stable under processing and storage. It equally contained essential components of fatty acids and considerable saponification value. Owing to the results obtained in this study coupled with the abundance of neem seeds in study area, production of viable oil from neem seeds is hereby recommended with a view to making the underutilized neem seeds a sustainable source of edible oil production.

## REFERENCES

Association of Official Analytical Chemists (AOAC) 2000. *Official Method 965.33 Peroxide Value of oils and fats*. (17th ed).Pp.73-85

Association Of Official Analytical Chemists (AOAC).(2009).Official Method 920.160 Saponification number of oils and fats. (17th ed).Pp.111-134

Aminu S. Z (2015), land use, land cover changes in Dutse 1986-2014, Jigawa state, Nigeria. (Master's thesis). Department of Geography, Faculty of Science Ahmadu Bello University, Zaria Nigeria.

Ahmad, M., Khan, M. A., Zafar, M. and Sultana, S. (2011). *Biodiesel from Non-Edible Oil Seeds: a Renewable Source of Bioenergy,* Chap. 13, Economic Effects of Biofuel Production, Marco Aurelio DosSantos Bernardes. Pp. 101-121

Awasthia, R. and Deepti S., (2019). Solvent Extraction of Neem Oil from Neem Seed for Development of Ecofriendly Pesticides, Published in *International Journal of Trend in Scientific Research and Development (IJTSRD),* **3(3);** 119-122.

Atabani, A. E., SillitongaI, A. S., Ong, H. C., Mahlia, T. M., Masjuki, H. H., Irfan A. B. and Fayaz, H. (2013)**.** Non-edible vegetable oils: A critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production. *Renew. Sustain. Energy Rev.,* **18**: 211-245.

Bakari, H., Djomdi, R. Z., Delattire, C., Pilerre, G., Dubessay, P., and MilchaudI, P., (2020). Influence of Physicochemical Characteristics of Neem Seeds (*Azadirachta indica A*. Juss) on Biodiesel Production. *Biomolecules* **6(2**); 341-350.

Britannica (2020). Fatty acid. *Encyclopedia Britannica,* 6th edition, pp 765-860. Retrieved from https://www.britan nica.com/science/fatty-acid.

Djenontin, T. S., Wotto, V. D. Avless, F. Lozano, P. Sohounhloue, D. K. C. and Pioch, D.(2012). Composition of *Azadirachta indica and Carapaprocera (Meliaceae*) seed oils and cakes obtained after oil extraction. *Ind. Crops Prod.* **38**: 39-45.

Dua, V. K., Pandey, A. C., Raghavendra, K., Gupta, A., Sharma, T. and Dash, A. P. (2009). Larvicidal Activity of neem oil *(Azadirachta indica)* formulation against mosquitoes. *Malar. J.,* **8,** 124.

EL-Mahood, A. M., Ogbonna, O. B. and Raji, M. (2010). The Antibacterial Activity of *Azadirachta indica* (neem seeds extracts against bacterial pathogens associated with eye and ear infections. *J. Medi. Plants Res.,* **4**: 1414-1421.

Erakhrumen, A. (2011). Selected Physical and Chemical Properties of Mechanically Extracted Neem Seed Oil Sourced as a Preservative for Lignocellulose in South Western Nigeria. *Forestry Studies in China*, **13 (4**): 263 – 269.

Jessinta, S H. N. Azhari, N. T. Saiful, S.H and Abdurahman, H. N. (2014). Impact of Geographic Variation on Physicochemical Properties of Neem (*Azadirachta indica*) Seed Oil. *International Journal of Pharmaceutical Sciences and Research.* **5 (10**): 4406-4413.

Kaushik, N. and Vir, S. (2000). Variations in fatty acid composition of neem seeds collected from the Rajasthan state of India. *Bio. Chem. Soc. Trans*, **28**: 880-882.

National Population Commission (NPC) (2006). Census Report

Oyeyiola, J.A. (1993). Nigerian Foods and Feeding Stuff *IUP Ibadan*: pp 50-52.

Sanderson, K. (2007). Chemists synthesize a natural-born killer. Nature 448 (7154): 630–1. Bib code: 2007Natur.448Q.630S. doi:10.1038/448630a. PMID 17687288.

Sampson I. E. (2018). Physico-Chemical Properties of Neem Seed Kernel Extract. *International Journal of Advanced Academic Research Sciences, Technology & Engineering*, **4(10**), 87-112.

Sandanasamy, A. H.N., Saifulnizam, B.T. and Abduranhamid, N., (2013) Fatty Acid Composition and Antibacterial Activity of Neem (*Azadirachta indica*) Seed oil. *The Open Conference Proceedings Journal,* **4**: 234-276.

Shujit, C.P., Muhammed, Y. M. and Shovon, B.S., (2013). Neem Oil From Neem Seed And Applications, *Lampard Academic Publishing*, pp 76-97.

Svetlana, M., Daniela, A., Iloko, M., Liliana, K., Boryana, N. D., and Gulab, J.(2007). Fatty Acids, Triacylglycerol, and Sterols in Neem Oil (*Azadirachta indica* A. Juss) as Determined by a Combination of Chromatographic and Spectral Techniques, *Journal of Liquid Chromatography & Related Technologies,* **30** (1): 11-25.

Veitch, G. E., Beckmann, E., Burke, B. J., Boyer, A., Maslen, S. L. and Ley, S. V. (2007). Synthesis of Azadirachtin: A long but successful journey. Angew. *Chem. Int. Ed. Engl.* **46** (40): 7629–7632.

Kraus, W. (1995). Biologically active ingredients-Azadirachtin and other triterpenoids. In: H. Schutterer (Ed.). *The Neem Tree Azadirachta indica A. Juss and Other Meliaceous Plants,* Weinheim, New York, pp 35-88.