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# Chemical Profiling and Industrial Viability of Neem Seed Oil: A Comprehensive Study for Sustainable Biodiesel Production

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A B S T R A C T

The study explores the chemical composition of neem seed oil, highlighting its potential for commercial biodiesel production and various industrial applications. The oil, with a liquid state, pale greenish-yellow color, and pleasant odor, yielded 32.1%, surpassing industrially extracted oils like soybean and cottonseed. Factors influencing its composition include seasonal variations, geographical origin, genetic disparities, growth stages, plant segments, and postharvest processing. Despite these, neem seeds meet the required oil content percentage for large-scale industrial biodiesel production. However, challenges like high free fatty acid content and elevated acid values necessitate acid esterification before alkaline transesterification, potentially increasing production costs. The study also highlights the importance of appropriate seed processing practices to optimize oil yield and quality.

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## INTRODUCTION

The indigenous neem tree, scientifically known as Azadirachta indica, originates from tropical South East Asia. Acknowledged for its rapid growth, resilience to arid conditions and impoverished soils, and persistent foliation, this arboreal species attains an imposing height of up to 30 meters, featuring expansive, leafy branches. Emanating a fragrant aroma reminiscent of honey, numerous white flowers manifest for the first time when the tree attains 2 to 3 years of age, culminating in fruit-bearing after a span of 3 to 5 years. The mature fruit, oval-shaped and measuring approximately 2 centimeters, encapsulate a light-colored seed measuring around 1.5 centimeters in length (Adewoye and Olaosebikan, 2002).

Plate 1: Dried neem seeds with shell

## LITERATURE REVIEW

In the context of nutritional significance, fats and oils represent a vital category among the four major food classifications. Oils find diverse applications, serving prominently in culinary practices, textural enhancement, and industrial realms such as detergent and soap production, oil paint formulation, and cosmetic manufacturing. Within plants, oils predominantly originate in the endosperm alongside carbohydrates, jointly nurturing the embryo (Marchelin et al., 2005). Fatty acids, inherently absent in a free state in nature, commonly exist in combination with glycerol, forming triglycerides (Britannica, 2020). Neem oil emerges as a consequential commercially available organic product with applications in medicine and agriculture (El Mahmood et al., 2010). Azadirachtin, a studied triterpenoid within neem oil, possesses a complex molecular structure featuring secondary and tertiary hydroxyl groups, a tetrahydrofuran ether, and 16 stereogenic centers, including 7 tetrasubstituted centers. The intricacies of its molecular structure present formidable challenges in synthetic organic chemistry methods for its preparation from simple precursors (Veitch et al., 2007). The chemical formula of Azadirachtin is C35H44O16, with a molar mass of 720.721 g·mol-1.

Widely applicable, neem seed oil finds utility in biodiesel production, offering a biodegradable, renewable, and non-toxic alternative through transesterification of seed oils. Its efficacy as a pesticide stems from its interference with the reproductive cycle of target insects (Veitch et al., 2007). The oil's potent aromatic quality serves as an insect repellent (Awasthi and Shikha, 2019). Demonstrating notable efficacy in addressing recalcitrant skin conditions, neem oil proves beneficial in treating psoriasis, eczema, ringworm, and even stubborn warts. Additionally, neem oil serves as a valuable component in cosmetic formulations, contributing to skin clarity, beautification, and rejuvenation (Shujit et al., 2013). Neem's effectiveness extends to the treatment of stomach ulcers, owing to its antihistamine and antibacterial properties.

## METHODOLOGY

Neem seeds were procured from the premises of Usmanu Danfodiyo University Sokoto and subsequently transported to the Herbarium Unit, where they underwent identification by a botanist affiliated with the Department of Biological Sciences within the Faculty of Science at Usmanu Danfodiyo University Sokoto. The execution of this research involved the utilization of conventional laboratory apparatus and reagents, all of which were of analytical reagent grade with a purity level equal to or exceeding 99%.

The neem seeds dehulled using pestle and mortar to obtain the soft seeds which were then cleaned by removing fibers, hay, leaves and other dirt. The dried seeds were then mechanically grinded using pestle and mortar in to powder. The powder was then used to extract oil using soxhlet extraction method.

*Oil Extraction*

Thirty grams (30g) of powdered *Azadirachta indica* seeds was placed in the thimble in soxhlet apparatus and n-hexane 150 cm$^3$ was poured into the round bottom flask. The apparatus was heated at 60$^0$C for 6 h of continuous extraction in the soxhlet apparatus. The experiment was repeated for the same weights of the sample. Remnant solvent in the oil was removed using rotary evaporator. The percentage oil yield was calculated using Equation 1.

$$Oil\ Yield\ (\%) = \frac{Weight\ of\ Extracted\ oil(g)\ x\ 100}{Weight\ of\ sample(g)}$$

*Acid Value of the Oil*

Method described by Adepoju *et al.,* (2013) was used for this determination. Two (2g) of the oil sample (2g) was weighed and transferred into a conical flask to which propan-2-ol (50cm$^3$) was added. The content was titrated against KOH (0.1M) using.
phenolphthalein indicator until it changed to pink colour. Acid value was calculated and recorded using
Equation 2

$$Acid\ Value = \frac{56.1xVxM}{W}$$

V= Volume of KOH used (cm$^3$), M= Molarity of KOH (0.1M), W= Weight of oil sample (g), 56.1= Molecular weight of KOH (g)

*Saponification Value*

The method described by Jagadale and Jugulkar (2012) was used for this determination. The sample (2g) was weighed into a conical flask and ethanolic potassium hydroxide ($25cm^3$, 0.1M) was added. The conical flask containing the mixture was connected to a reflux condenser and heated for five minutes with occasional shaking to ensure complete dissolution of oil. The mixture was allowed to cool and slowly titrated against hydrochloric acid HCl (0.5M) using phenolphthalein indicator until the colour changes from purple to colourless. Saponification value was calculated by using Equation 3.

$$Saponification\ Value\ = \frac{56.1N(V0-V1)}{M}$$

$V_o$ = The volume of HCl used for the blank titration, $V_1$ = The volume of the solution used for the determination, 56.1 = Molar mass of KOH (g), N = molarity of HCl used, M = Mass of the sample

*Iodine Value*

Method described by Jagadale and Jugulkar (2012) was used for this determination. A measured quantity of oil (0.5g) sample was weighed into the conical flask ($250cm^3$), acetic acid ($10cm^3$,) was added and the mixture stirred until the oil sample completely dissolved in the acid. Iodine monochloride ($20cm^3$) was added to the mixture and stirred, then incubated in the dark for (30) minutes. Potassium Iodide solution was later added to the mixture. The mixture was titrated against standardized sodium thiosulphate (0.5M) until pale straw colour was observed. Starch indicator ($2cm^3$) was added to the content, a purple colour was observed. The titration was carried on until the the solution turns colourless, the disappearance of the colour was recorded as end point. The blank sample was treated in a similar manner.

$$Iodine\ Value\ = \frac{56.1N(Vb-Va)x6.2025}{W}$$

$V_a$ = Titre value for $Na_2S_2O_3$ used in the test titration ($cm^3$), $V_b$ = Titre value of $Na_2S_2O_3$ used in the blank titration ($cm^3$), W = Weight of oil (g), 6.2025 = mass of the $Na_2S_2O_3$ in a $250cm^3$ solution.

*Determination of Fatty Acid*

GC-MS analysis was performed on an Agilant Technologies Auto-system GCMS-QP2010 PLUS Shimadzu, Japan, equipped with a split/splitless injector (250 0C) with System operating in the E.I mode at 70 ev,. The transfer line was 280 0C. 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-MSanalysis was performed at Multi-User Science Research Laboratory of the department ofChemistry, Ahmadu Bello University Zaria, Kaduna State Nigeria.

**RESEARCH RESULT**

Table 1. Physicochemical Properties of Neem Seed Oil

| Parameters | Observed Values |
|---|---|
| Percentage Oil Yield | 32.1 ± 0.02 |
| Saponification value (g/KOH) | 150.55 ± 0.03 |
| Iodine value (g/100g) | 60 ± 0.10 |
| Acid value (g/KOH) | 20.54 ± 0.03 |
| Specific gravity | 0.85 ± 0.05 |
| Viscosity ($mm^2$) at $40^0$c | 12.00 ± 0.10 |
| Odour | Unpleasant |
| Colour Yellow | Pale Greenish |
| FFA (%) | 13 ± 0.10 |

Table 2. Trace element composition of Neem seed

| Trace element | Concentration (mg/g) |
|---|---|
| Arsenic | 0.01 |
| Selenium | 0.04 |
| Cadmium | 0.22 |
| Lead | 0.90 |
| Zinc | 0.93 |
| Copper | 1.17 |
| Chromium | 1.93 |
| Mercury | 2.40 |
| Iron | 4.95 |
| Bismuth | 7.20 |
| Sodium | 8.02 |
| Potassium | 15.20 |

Table 3. Fatty acid Determination of Neem (Azadrachta indica) seed oil extract

| Fatty Acid | Systematic Name | Formula | Structure | Area |
|---|---|---|---|---|
| Linoleic acid | 9,12-octadecadienoic | C18H32O2 | 9,12-18:2 | 40 |

| | | | | |
|---|---|---|---|---|
| | acid (Z,Z) | | | |
| Oleic acid | 9-octadecenoic acid (Z) | C18H34O2 | 9-18:1 | 35 |
| Cis-13-octadecenoic acid | 13-octadecenoic acid (Z) | C18H34O2 | 13-18:1 | 8.9 |
| Palmitic acid | Hexadecenoic acid | C16H32O2 | 16:0 | 8.5 |
| Stearic acid | Octadecanoic acid | C18H36O2 | 18:0 | 7.5 |
| Cis-vaccenic acid | 11-octadecenoic acid (Z) | C18H34O2 | 11-18:1 | 0.5 |

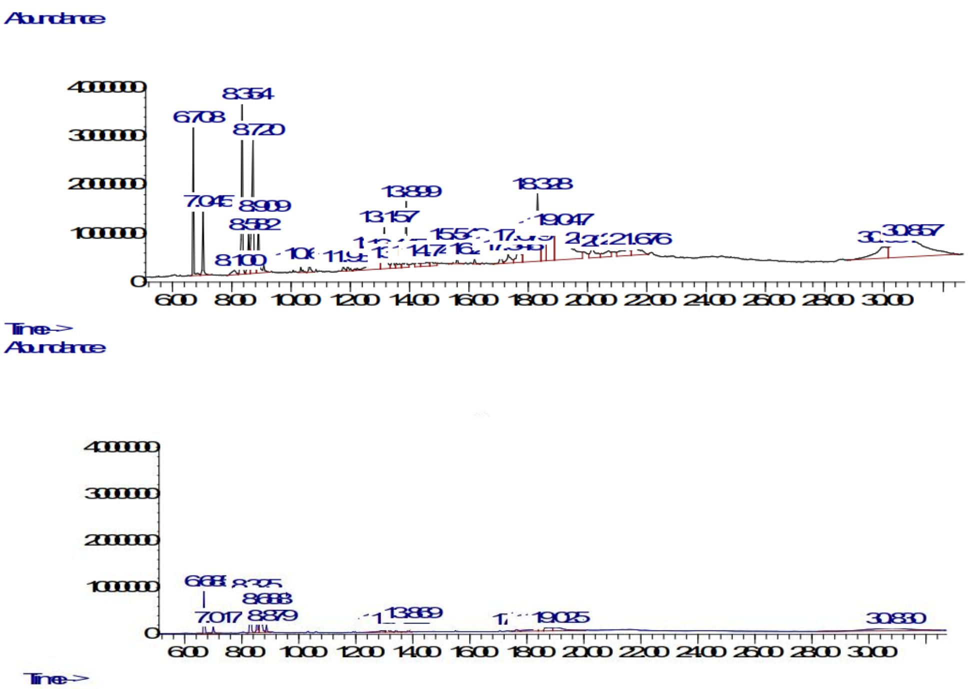


Figure1. Chromatograph showing GC-MS spectrum of Cis-13-octadecenoic acid

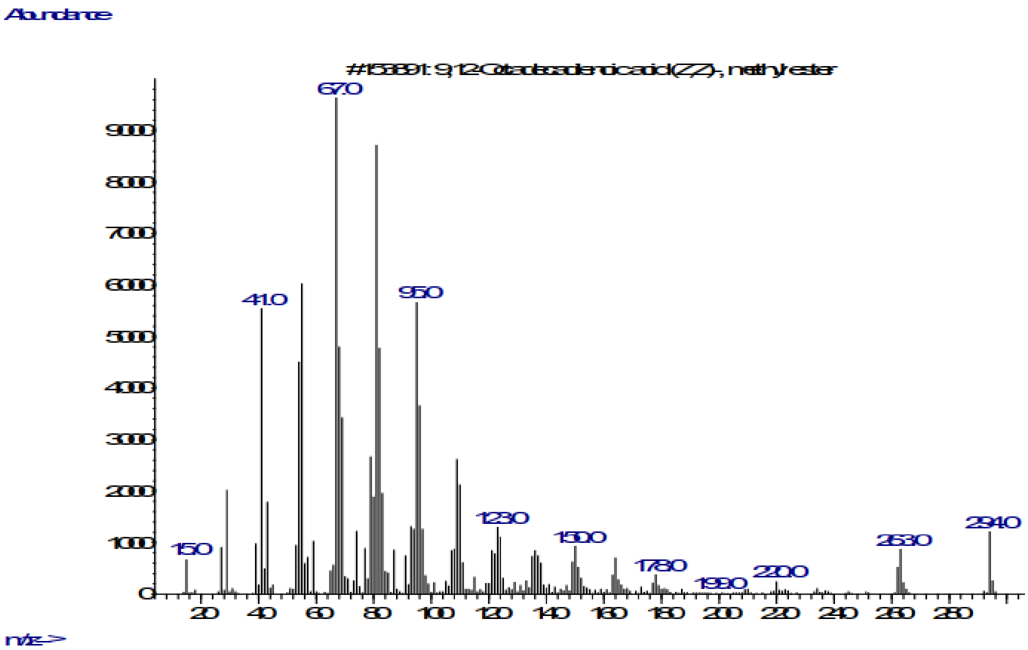


Figure 2. Chromatograph showing GC-MS spectrum of linoleic acid in the extracted oil

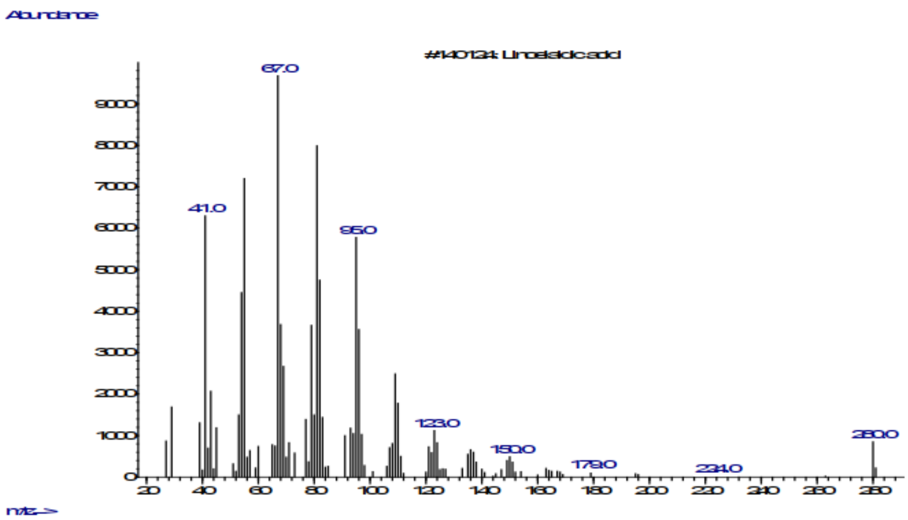


Figure 3. Chromatograph showing GC-MS spectrum of oleic acid in the extracted oil

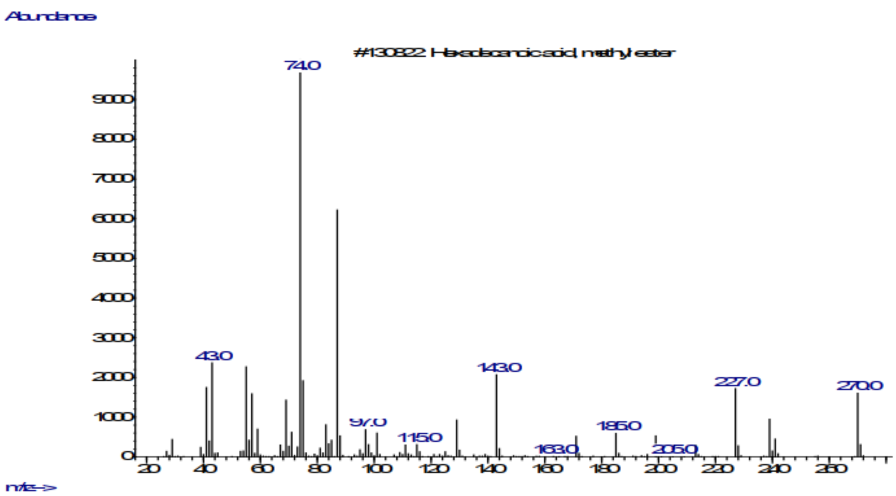


Figure 4. Chromatograph showing GC-MS Spectrum of Palmitic acid in the extracted oil

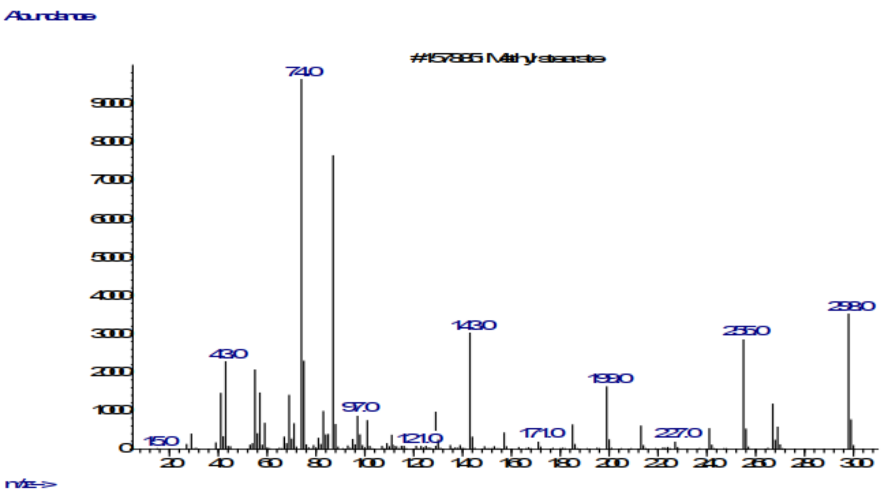


Figure 5. Chromatograph showing GC-MS spectrum of stearic acid acid in the extracted

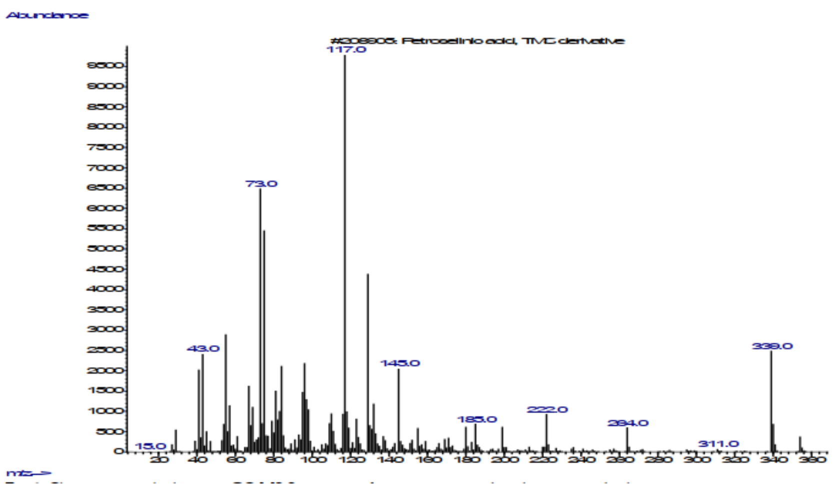


Figure 6. Chromatograph showing GC-MS Spectrum of cis-vaccenic acid in the extracted oil

## DISCUSSION

The physicochemical attributes of neem seed oil, as delineated in Table 1, reveal distinctive characteristics. The oil exhibits a liquid state at ambient temperatures, presenting a pale greenish-yellow hue, coupled with a generally agreeable and non-offensive odor. The compositional and yield determinants of the essential oil are manifold, with interdependencies among various factors that include seasonal and maturity variations, geographical origins, genetic variances, growth stages, plant segments utilized, and postharvest drying and storage conditions (Terblanche, 2000; Marotti et al., 1994). Seasonal and maturity variations are intricately linked, wherein the ontogenic growth stage undergoes dynamic alterations with progressing seasons, consequently affecting the chemical profile of essential oils. Variations in yield are observed seasonally and are influenced by the micro-environment, whether in full sunlight or shade, where the plant is cultivated. Harmonizing with these findings, Sanguanpong (2010) emphasizes the critical impact of harvest timing on both yield and oil composition.

The obtained results indicate a substantial yield of neem seed oil (32.1%), surpassing yields of industrially extracted oils such as soybean, cottonseed, and rubber. This underscores the viability and merit of neem seed oil extraction as a commercially viable venture. Furthermore, this substantiates that neem seeds meet the requisite oil content percentage (30-55%) for large-scale industrial biodiesel production (Belwal et al., 2018). Nevertheless, cautionary notes from Falowo et al. (2019) highlight the potential reduction in oil yield due to incorrect seed processing practices, such as prolonged exposure to direct sunlight. Concurrently, Hasni et al. (2017) postulate that soil type and rainfall quantity can also exert an influence on oil content.

The saponification value of neem seed oil (150 mg KOH/g) is comparatively lower than values reported by Tomic et al. (2020) but falls within the standard range of 175 to 205 mg KOH/g advocated by Elkelawy et al. (2020). This underscores the suitability of neem seed oil as a raw material for biodiesel industries, as oils with higher saponification values find applicability in cosmetic, candle, and soap manufacturing (Asfaram et al., 2017).

The iodine value, indicative of fatty acid unsaturation, aligns closely with values reported in the literature (60 mg iodine/g), although it falls below the maximum value of 105 reported elsewhere (Menkiti et al., 2015). This designates neem seed oil as non-drying and supports its utility as a feedstock for lubricant production, alkyd resin, varnishes, paints, polish, hydraulic brake fluids, and biodiesel. The heightened iodine value suggests increased susceptibility to oxidation and rancidification, as corroborated by Berriors et al. (2007) and Berchman and Hirata (2008).

The free fatty acid content of the neem seed oil sample is notably high (13.77%), necessitating acid esterification before alkaline transesterification for biodiesel production. This adjustment, while enhancing biodiesel yield, potentially escalates production costs. The specific gravity of neem seed oil (0.85 g/cm3) indicates its density relative to water and suggests the absence of heavy elements, aligning with Berriors et al. (2007).

The elevated acid value (20.54 mg KOH/g) surpasses the ASTM D6751 range of 0.4 to 4 mg KOH/g, indicating potential hindrances to biodiesel production. High acid values impede production reactions, resulting in diminished yield and elevated production costs (Berriors et al., 2007). Conversely, low acid values, reflective of neem seed oil's susceptibility to rancidity due to elevated acid content, align with findings from Berchman and Hirata (2008).

The viscosity of neem seed oil at 40°C (12.80 mm2/s) highlights the necessity for transesterification to diminish viscosity, preventing damage to fuel injection systems in engines (Asfaram et al., 2017).

Ash consists of constituents of inorganic substance. This is evident in trac e elements present in the seeds (Table 2).The elemental composition of neem seeds, encompassing zinc, copper, sodium, potassium, chromium, bismuth, argon, iron, calcium, lead, mercury, and selenium, underscores its potential application in animal feed and fertilizer formulations. However, Gawa et al. (2007) caution that animals on raw neem seed diets exhibit lower Packcell volume (PCV), Hemoglobin (Hb), and total protein (TP) values compared to processed diets.

The fatty acid profile reveals cis-vaccenic acid as the least abundant (0.5%). 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. Discrepancies with other studies (Djenontin et al., 2012; Svetlana et al., 2007; Sanderson, 2007; Atabani et al., 2013) are attributed to variances in plant species and extraction methodologies.

## CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study establishes baseline data on the chemical composition of neem seed oil from Sokoto, Nigeria. The oil demonstrates substantial quantity, positioning it as a valuable resource for commercial biodiesel production and various industrial applications. Physicochemical properties align with international standards for biodiesel production. The efficacy of the Soxhlet extraction method with n-hexane affirms its effectiveness in oil extraction.

## ADVANCED RESEARCH

This comprehensive analysis establishes neem seed oil as a promising resource for commercial biodiesel production and diverse industrial applications. Its unique chemical composition, high yield, and alignment with international standards make it a viable alternative to traditional oils. However, challenges such as acid esterification need to be addressed to fully harness its potential. Overall, neem seed oil holds great promise in contributing to sustainable and environmentally friendly industrial practices.

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