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EXTRACTION AND CHARACTERIZATION OF SOKOTO NEEM TREE (*Azadirachta Indica)* SEED OIL

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ABSTRACT

Neem (Azadirachta indica) is a medicinal plant known for its rich phytochemical and therapeutic potential, particularly its seed oil. This study focuses on the extraction and characterization of neem seed oil obtained through Soxhlet and traditional methods, with a focus on physicochemical properties, phytochemical content, antioxidant vitamins, and fatty acid composition. The Soxhlet method yielded significantly more oil (47.08%) than the traditional method (17.16%) ($p < 0.05$). Soxhlet-extracted oil exhibited significantly higher saponification (103.78 mg KOH/g) and iodine values (9.05 g I_2/100 g), suggesting better suitability for industrial applications such as soap and biodiesel production. However, it showed significantly higher acid (145.11 mg KOH/g) and peroxide values (408.33 meq/kg), indicating increased oxidation. Phytochemical screening revealed the presence of alkaloids, phenols, flavonoids, steroids, and saponins in both extracts, with alkaloids and phenols being more prominent. Antioxidant vitamins A and E were significantly ($p < 0.05$) higher in traditional method extracted oil, while both extracts had low vitamin C content. Thin-layer chromatography identified predominantly present fatty acids as stearic, oleic, linoleic, and palmitic acids, consistent across both extraction methods. The results suggest that neem seed oil especially the Soxhlet extracted has high yield and may be of use in industrial application such as soap making, however, both oils require extensive processing to make them better for use in industry and.as edible oils. Further optimization of extraction techniques could enhance the yield and functional quality of neem seed oil from local neem trees.

Keywords: *Azadirachta Indica*, Neem seed oil, Soxhlet extraction, Phytochemicals, Antioxidant vitamins

INTRODUCTION

Natural products derived from medicinal plants, whether as pure compounds or standardized extracts, continue to be a vital source of new drug discovery due to their extensive structural diversity and biological activity (Newman & Cragg, 2020). These natural medicines are not only essential for meeting the primary healthcare needs of over 80% of the global population, especially in developing countries, but they are also gaining significant popularity in developed nations because of their cost-effectiveness and minimal side effects (World Health Organization [WHO], 2023).

Plants are an abundant source of bioactive phytochemicals such as alkaloids, flavonoids, tannins, glycosides, phenols, and essential oils, all of which contribute to their medicinal value (Salehi et al., 2022). Among these plant-derived products, plant oils stand out as versatile, sustainable, and health-promoting alternatives to animal fats and petroleum-based compounds. Historically, they have been used for culinary, medicinal, cosmetic, and industrial purposes. In particular, Neem (*Azadirachta indica*), a tropical tree belonging to the Meliaceae family, has become one of the most economically and therapeutically important plants in South Asia and sub-Saharan Africa (Kumar et al., 2021). The neem seed, rich in oil and bioactive components, is considered the most valuable part of the tree. Neem oil, primarily extracted from the seed kernel, contains compounds like azadirachtin, nimbin, and salannin, which are responsible for its insecticidal, antimicrobial, antifungal, and contraceptive properties (Isman, 2020). It has proven effective as a botanical pesticide, insect repellent, and antifertility agent, especially in traditional Indian medicine and modern integrated pest management systems (Ogbanje et al., 2023).

Beyond agriculture, neem oil is widely used in soap manufacturing, cosmetic formulations, candle and lamp oil, textile protection, and even biodiesel production due to its high triglyceride content and biodegradability (Ghosh et al., 2021). In Nigeria, neem is locally known as Dogonyaro, and is maily cultivated in the northern regions, where it thrives due to its tolerance to arid conditions. Despite its abundance, neem seed oil remains underutilized industrially, especially in applications requiring detailed profiling of its physicochemical and phytochemical properties. This study, therefore, aims to explore the extraction and characterization of neem seed oil to evaluate its potential for pharmaceutical, agricultural, and industrial uses. The research provides insights into the composition and functional qualities of neem oil, helping to develop value-added products from indigenous bioresources.

MATERIALS AND METHODS

Solvents and Reagents: n-Hexane (C_6H_{14}), ethanol (CH_3CH_2OH), chloroform ($CHCl_3$), diethyl ether $(C_2H_5)_2O$, glacial acetic acid (CH_3COOH), hydrochloric acid (HCl), potassium hydroxide (KOH), phenolphthalein ($C_{20}H_{14}O_4$), Hanus iodine solution, sodium thiosulfate ($Na_2S_2O_3$), potassium iodide (KI), starch solution, iodine crystals (I_2), cholesterol, oleic acid ($C_{18}H_{34}O_2$), linoleic acid ($C_{18}H_{32}O_2$), palmitic acid ($C_{16}H_{32}O_2$), stearic acid ($C_{18}H_{36}O_2$), ferric chloride ($FeCl_3$), and distilled water.

Apparatus and Equipment

Beakers, conical flasks, burettes, round-bottom flasks, measuring cylinders, volumetric flasks, Soxhlet extractor, TLC plates, TLC chamber, refractometer, heaters, water baths, ovens, spatulas, retort stands, cotton wool, Pasteur pipettes, micro-pipettes, weighing balances, filter paper (Whatman No. 1), mortar and pestle, grinding stones, rulers, pencils, sample bottles, reagent bottles, and glass funnels.

Sample Collection, Identification, and Pre-treatment

Fresh neem (*Azadirachta Indica*) seeds were collected within the premises of Usmanu Danfodiyo University, Sokoto, Nigeria. The plant was identified and authenticated at the Department of Plant and Biological Sciences, Usmanu Danfodiyo University, Sokoto, where a voucher specimen (UDUH/ANS/0755) was deposited. The seeds were thoroughly rinsed with clean tap water, followed by distilled water to remove dirt and surface contaminants. The seeds were then air-dried under sunlight, followed by oven-drying at 40 °C to reduce moisture content. The dried seeds were shelled manually using a grinding stone to obtain clean kernels, which were further dried and ground into a fine powder using an electric blender. The powder was sieved using a muslin cloth and stored in an airtight container until further use

Oil Extraction

Two methods were used for oil extraction: Soxhlet extraction and the traditional aqueous method.

Soxhlet Extraction Method

Fifty grams (50 g) of neem seed powder was weighed, wrapped in filter paper, and placed in the Soxhlet extractor. Two hundred and fifty millilitres (250 mL) of *n*-hexane was used as the solvent. The extraction was carried out for 4 hours. Afterward, the solvent was recovered by distillation, leaving the oil residue in the round-bottom flask. The extracted oil was then weighed, recorded, and stored for further analysis.

Traditional Extraction Method

The powdered neem seed was stirred with a pestle in a mortar while gradually adding hot water to form a thick dough. The dough was manually kneaded and pressed on a grinding stone until oil separated. The oil was then decanted and collected into clean containers. The oil was heated to remove moisture by evaporation, indicated by the cessation of crackling sounds, following the traditional method described by Oluwole et al. (2012).

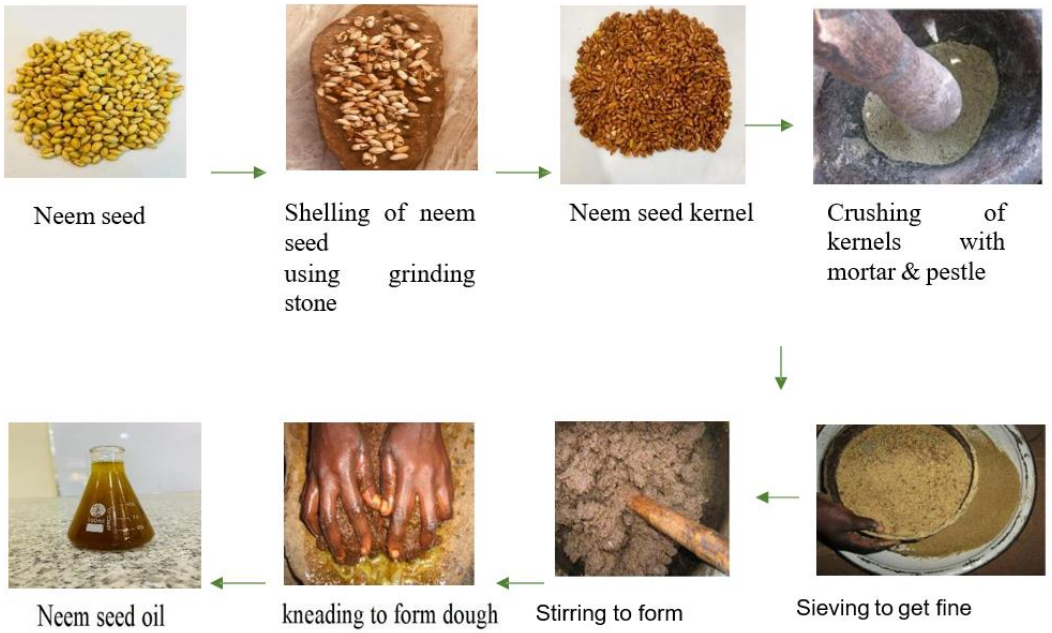

Figure 1: Procedures of traditional extraction of neem seed oil

Physicochemical Analysis

The following physicochemical analysis was carried out for the extracted oil. Vital properties of the oils were characterized in line with the Association of Official Analytical Chemists (AOAC) (Table 1).

Table 1: Physicochemical Analysis of Neem Seed Oil

S/N	Parameter	Principle/Procedure	Indicator of Presence	Formula
1	Acid Value (AV)	Titration with KOH using phenolphthalein indicator	Pink color persistence	AV (mgKOH/g) = ((Vs − Vb) × N × 56.1) / W
2	Peroxide Value (PV)	Iodometric titration with KI and starch (AOAC method)	Disappearance of blue color	PV (meqO_2/kg) = ((B − A) × N × 1000) / W
3	Iodine Value (IV)	Hanus iodine titration with starch	Disappearance of blue color	IV (g I_2/100g) = ((B − S) × N × 12.69) / W
4	Saponification Value	Reflux with alcoholic KOH and titration with HCl	Disappearance of pink color	SV (mgKOH/g) = 28.05 × (Vb − Vs) / W
5	Specific Gravity	The weight ratio of equal volumes of oil and water	Numerical density ratio	SG = Weight of oil / Weight of equal volume of water
6	Refractive Index	Measured with refractometer at 25 ± 1°C	Boundary between blue and white field	Direct reading
7	Moisture Content	Oven drying and mass loss calculation	Mass difference before and after drying	Moisture (%) = ((M1 − M2) / M1) × 100
8	pH	Direct pH meter reading	Digital pH display	—

Vs = volume of titrant for sample; Vb = volume of titrant for blank; N = normality of titrant; W = sample weight (g); M1 = initial mass; M2 = final mass

Qualitative Phytochemical Screening

Neem seed oil was screened for the presence of some secondary metabolites as described for alkaloids (Harborne, 1998), steroids (Trease and Evans, 1989), saponins (Wall *et al.,* 1954), phenolics and flavonoids (Awe and Sodipo, 2001), tannins (Odebiyi and Sofowora, 1978).

Table 2: Qualitative Phytochemical Screening of Neem Seed Oil

S/N	Phytochemical	Test Method	Reagent Used	Indicator of Presence
1	Flavonoids	3.0 mL Neem extract mixed with 4.0 mL 1% KOH and observe for color change	1% Potassium hydroxide (KOH)	Dark yellow color
2	Tannins	1.0 mL of Ethanolic KOH added to 1.0 mL of extract, then observe.	10% ethanolic KOH	White precipitate
3	Saponins	2.0 mL of extract boiled in 10.0 mL water, filtrate was mixed with 5.0 mL of distilled water, and shaken for 5 minutes	Distilled water	Persistent froth
4	Alkaloids	1 mL extract treated with 5 mL HCl, filtered, and tested with Dragendorff's reagent	Respective alkaloid reagents	Characteristic red precipitates
5	Steroids	Salkowski test using H_2SO_4 (5 drops of concentrated H_2SO_4 was added to 1.0 mL of the extract) and observed color change	Concentrated sulfuric acid	Red coloration
6	Phenolics	2 drops of 5% w/v of $FeCl_3$ was added to 1.0 mL of the plant extract	5% Ferric chloride ($FeCl_3$)	Greenish precipitate

Determination of Antioxidant Vitamins

The levels of antioxidant vitamins A, C, and E in neem seed oil were determined using spectrophotometric methods as described by Rutkowski *et al.* (2006). Each vitamin was identified and quantified based on specific reactions and absorbance wavelengths, with the procedures and corresponding calculation formulas summarized in the table below.

Table 3: Determination of Antioxidant Vitamins in Neem Seed Oil

S/N	Vitamin (Rutkowski *et al.*, 2006)	Principle/Procedure	Wavelength (nm)	Formula
1	Vitamin A	1g each of the samples and standard 5 ml of ethanol shaken vigorously and then extracted with petroleum ether by allowing to stand for 1 hour. The mixture was centrifuged at 2000 rpm for 5 minutes, and 1 ml of supernatant was taken, and its absorbance was taken using spectrophotometer (6300, Jenway)	450	$\frac{Abs\ Sample}{Abs\ Std}$ x Conc Std (mg/dl)
2	Vitamin C	1 ml of dissolved samples, distilled water, and standard ware added in separate centrifuge test tubes. + 1 ml phosphotungstic acid was added, thoroughly mixed and left to stand at room temperature for 30 minutes. Then centrifuged at 3,500xg for 10 minutes, 1 ml from each of the test tubes was	700	$\frac{Abs\ Sample}{Abs\ Std}$ x Conc Std (mg/dl)

3	Vitamin E	aspirated, transferred into cuvette and measure spectrophotometrically 0.5 ml of diluted samples + 0.5 ml of anhydrous ethanol shaken vigorously for 1 minute + 3 ml of xylene shaken vigorously for 1 minute centrifuged at 1500xg, 10 min to separate the extract supernatant + 0.25ml $FeCl_3$ and 0.25 ml of H_3PO_4 measured spectrophotometrically	539	$\frac{Abs\ Sample}{Abs\ Std}$ x Conc Std (mg/dl)

Abs = absorbance, conc. of std= concentration of standards

Fatty Acid Composition of Neem Seed Oil

The fatty acid composition of neem seed oil was determined using Thin Layer Chromatography (TLC), a technique employed to separate and identify non-volatile mixtures based on differences in their affinity between a stationary phase and a mobile phase. Silica gel-coated glass plates were used as the stationary phase. Samples were spotted onto the plates using a micro-pipette and developed in a solvent system composed of *n*-hexane, diethyl ether, and glacial acetic acid (80:20:1 v/v/v). The mobile phase ascended the plate by capillary action, facilitating the separation of components. Visualization of the separated fatty acids was achieved using iodine vapor, and identification was performed based on their retention and refractive indices.

Data Analysis

All data obtained were expressed as mean ± standard error of the mean (SEM). Statistical significance was evaluated using Student's t-test in Microsoft Excel, with $p<0.05$ considered significant.

RESULTS AND DISCUSSION

Physicochemical Properties of Neem Seed Oil

Table 4 shows the physicochemical properties of neem seed oil extracted using traditional and Soxhlet methods. The Soxhlet method produced significantly more oil (47.08%) compared to the traditional method (17.16%). It also resulted in oil with higher saponification and iodine values, indicating a greater presence of short-chain and unsaturated fatty acids, respectively. However, the Soxhlet-extracted oil had higher acid (145.11 mgKOH/g) and peroxide values (408.33 meq/Kg), which may suggest increased oxidation or degradation. The traditional extract had a slightly higher specific gravity (1.02), lower pH (4.54), and lower moisture content (0.43.%) compared to the Soxhlet extract (0.98, 4.70, and 0.48%, respectively). Notably, the moisture contents for both extracts are higher maximum limit of 0.2% for edible oils, and thus poses the risk of easy rancidity. The refractive indices of both extracts (1.345 and 1.350) showed no significant difference, with measurements taken at room temperature.

Table 4: Physicochemical Properties of Neem Seed Oil

Parameters	Traditional extract	Soxhlet extract
Percentage yield (%)	17.16^b	47.08^a
Saponification value (mgKOH/g)	13.37 ± 0.33^b	103.78 ± 0.99^a
Iodine value (gl_2/100g)	1.10 ± 0.12^b	9.05 ± 0.24^a
Acid value (mgKOH/g)	132.58 ± 0.69^b	145.11 ± 0.26^a
Peroxide value (meq/Kg)	385.00 ± 8.6^b	408.33 ± 6.24^a
Specific gravity	1.02 ± 0.002^a	0.98 ± 0.00^b
pH	4.54 ± 0.03^b	4.70 ± 0.02^a
Moisture content	0.43 ± 0.003^b	0.48 ± 0.001^a
Refractive index	1.345^a	1.350^a

Values are presented as mean ± SD of replicates. Values having the same superscript across the column are not significantly different $p < 0.05$.

Free Fatty Acid Composition of Neem Seed Oil

The fatty acid profile of the neem seed oil was determined using Thin Layer Chromatography (TLC), which allowed for the qualitative identification of key fatty acids. Both the traditional and Soxhlet extracts revealed similar banding patterns, indicating relatively high (+++) presence of stearic acid, oleic acid, and linoleic acid in, along with moderate (++) presence of palmitic acid and cholesterol.

Table 5: Free Fatty Acid Composition of Neem Seed Oil

Parameters	Traditional Extract	Soxhlet Extract
Stearic acid	+++	+++
Linoleic acid	+++	+++
Oleic acid	+++	+++
Cholesterol	++	++
Palmitic acid	++	++

Key: +++ indicates presence in higher amount, ++ indicates presence in average amount

Qualitative Phytochemical Screening of Neem Seed Oil

The qualitative phytochemical analysis revealed the presence of important secondary metabolites in both oil extracts. Alkaloids and phenols were notably of higher (+++) presence, while steroids, flavonoids, and saponins were observed to be of moderate (++) presence.

Table 6: Qualitative Phytochemical Screening of Neem Seed Oil

Parameter	Soxhlet Extract	Traditional extract
Steroid	++	++
Alkaloid	+++	+++
Flavonoid	++	++
Saponin	++	++
Phenol	+++	+++

Keys: +++ indicates presence in higher amount, ++ indicates presence in average amount

Anti-oxidant Vitamin Level

The result of the vitamin composition of traditional and Soxhlet extract of neem seed oil in the chart below reveals that oil obtained through the traditional method contains a higher concentration of vitamin A, along with elevated levels of vitamin E. However, both extraction methods yield oils with a low level of vitamin C.

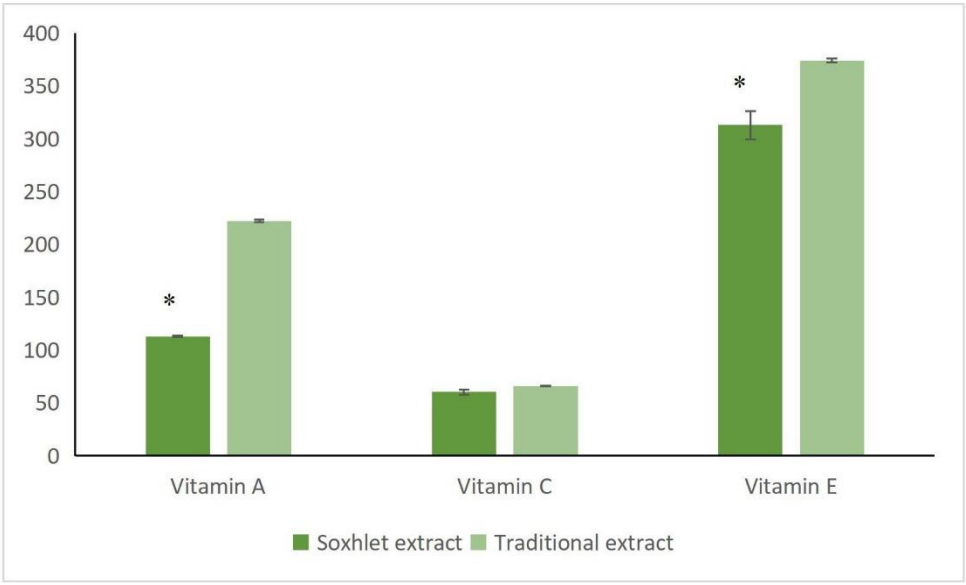

Figure 2: Vitamin Composition of Traditional and Soxhlet Extract of Neem Seed Oil

Data are presented as mean± standard deviation of triplicate samples. Points marked with an asterisk (*) indicate statistically difference ($p < 0.05$, t-test).

Discussion

The extraction and characterization of neem seed oil provided valuable insights into its composition, quality indicators, and potential uses. Among the two extraction techniques studied, the Soxhlet method produced a significantly higher oil yield (47.08%) compared to the traditional method (17.16%), consistent with findings by Adejumo et al. (2013), who credited higher oil recovery to better solvent penetration and extraction efficiency. The traditional method, though simpler and more environmentally friendly, resulted in a lower yield, aligning with reports by Olufunmilayo et al. (2020).

Analysis of physicochemical properties showed that the Soxhlet-extracted oil had higher saponification (103.78 mgKOH/g) and iodine values (9.05 gI_2/g) than the traditionally extracted oil (13.37 mgKOH/g and 1.10 gI_2/g, respectively). These suggest a likely higher concentration of short-chain and unsaturated fatty acids in the Soxhlet extract, which could benefit applications like soap making and biodiesel production (Farooq et al., 2018; Ogbomida et al., 2017). However, acid (145.11 mgKOH/g) and peroxide values (408.33 meqKOH/g) were also higher in the Soxhlet oil, indicating more oxidative degradation, likely from thermal exposure during extraction, supporting the observations of Uzoh et al. (2021). A slight increase in pH was noted in the Soxhlet oil (4.70 vs. 4.54), but both oils remained acidic. The moisture contents, 0.48% for Soxhlet and 0.43% for oil are higher than the maximum allowable limit of 0.2% for edible oils (Federation, 2011) and raise a concern of possible easy rancidity of the oils. Hence, this could significantly reduce oils' stability, making them unsuitable for most industrial, cosmetic, or pharmaceutical uses, as moisture promotes hydrolysis and microbial growth, which decrease stability (Abdulkadir & Jimoh, 2014). However, the moisture content was lower than that reported by Kabo and Ogbesejana (2020) in desert date oil.

Fatty acids profiling based on qualitative indicators showed strong presence (+++) of stearic, oleic, and linoleic acids in both oils, with moderate (++) presence of palmitic acid and cholesterol. While this fits with known properties of neem oil (Ahmed et al., 2019; Okolie et al., 2018), the lack of quantitative data limits the strength of these conclusions. Similarly, phytochemical screening revealed high (+++) presence of alkaloids and phenols, and moderate (++) presence of flavonoids, steroids, and saponins, supporting their antioxidant and antimicrobial properties, as reported previously (Benisheikh et al., 2019). However, without quantitative values, precise comparisons of bioactivity are limited.

The vitamin content analysis showed that oil obtained via the traditional method retained more vitamin A, likely due to less heat and solvent exposure, which matches the findings of Das et al. (2014). Conversely, Soxhlet extraction resulted in higher vitamin E levels, probably because of better solubility in the organic solvent used (Adewale et al., 2020). Both methods

produced oils with low vitamin C content, indicating their inherent instability or limited presence in neem oil (Yusuf et al., 2016). However, it not unusual to have low or even absent vitamin C in oils, since vitamin C is water soluble, henece difficult to exist in lipid medium. While the Soxhlet method was more efficient at extracting oil and unsaturated components, it also caused increased oxidative degradation and possibly excessive moisture, which could threaten product stability. The traditional method, though less efficient, appears to preserved heat-sensitive compounds better. These findings highlight the importance of improving post-extraction handling and conducting more detailed quantitative analysis to support application-specific recommendations.

CONCLUSION

The study evaluated neem seed oil extracted using both traditional and Soxhlet methods. While Soxhlet extraction yielded a higher oil output with increased saponification and iodine values, it also resulted in elevated acid, peroxide, and moisture contents, which may reduce the oil's stability and limit its industrial suitability without additional processing. The traditional method, employing milder conditions, better preserved heat-sensitive vitamins such as vitamin A, whereas the higher vitamin E content in Soxhlet-extracted oil likely resulted from solvent-enhanced extraction. Both methods revealed similar types of bioactive compounds; however, differences in antioxidant levels suggest variations in composition. These findings indicate potential applications of neem seed oil in medicinal, cosmetic, and industrial contexts, although further research is needed to optimize extraction methods for improved quality tailored to specific uses.

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