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Research Article

Phytochemicals, Proximate Analysis, and Characterizations of Neem (*Azadirachta indica*) Seeds Oil

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The neem plant has long been used to produce industrial items and in traditional medicine to treat various human illnesses. These elements include the plant's leaves, bark, flowers, fruits, seeds, and roots. Among these portions, neem seeds stand out because of their remarkably high oil content compared to other tree parts. Despite its importance and various uses, detailed characterizations and proximate analysis of the seed have not been reported before, as such it is imperative to explore it more. In this work, neem seed oil extraction was done using a solvent extraction method with hexane and methanol as solvents. FTIR and proximate analysis were carried out on the sample before the extraction, the result revealed the presence of various functional groups such as hydroxyl group, amine group, nitriles group, carbonyl group, and aromatics. As well as a moisture content of 20.0%, 5% ash content, 12.4% crude fat, 18.4% crude protein, 42.2 % crude fiber, and carbohydrate content of 1.6%. Phytochemicals screening and UV-Vis analysis were then carried out on the extracted oil and the result revealed the presence of carbohydrates, glycosides, flavonoids, terpenoids, quinines, alkaloids, and other important phytochemicals in both n-hexane and methanol extract. As such, the results obtained from this study revealed that neem seed and its oil could be used in industries for the production of various drugs, paints, pesticides, fertilizers, and other important materials. The oil's potential for use in the creation of numerous medications, including analgesics, antipyretics, antiasthmatics, anti-cancers, antihypertensives, and many more, is indicated by the presence of alkaloids, flavonoids, and glycosides. Similarly, the presence of quinones indicates that the oil can be used to synthesize a wide range of compounds, including oxidizing agents, colors, laxatives, and several chemical intermediates. Similarly, terpenoids are equally essential since, in addition to being used to make cosmetics, flavorings, and insect repellents, they are also utilized in the production of numerous medications that cure cancer and malaria.

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INTRODUCTION

Neem (*Azadirachta indica* A. juss) is a versatile and widely cultivated plant species in most parts of the world like Africa and the Indian subcontinent because of its adaptability to a wide range of habitats. It grows well in tropical and semi-tropical regions (Sulaiman M. et al., 2024; Wasim et al., 2023). The neem plant has long been utilized in indigenous medicine to cure a variety of human ailments as well as in the production of industrial goods. The plant's leaves, bark, flowers, fruits, seeds, and roots are among these components. Neem seeds are notable among these sections due to their unusually high oil content in relation to other tree parts (Nwanekezie et al., 2023). Many uses for this oil include lubricants, pesticides, and pharmaceuticals to treat conditions like diabetes, leprosy, and tuberculosis. Additionally, neem seed oil is a crucial component in the creation of many skincare products, such as face packs, body lotions, and soaps. It is also frequently combined with other organic ingredients (Cesa et al., 2019). In Nigeria, there are more than four million neem trees located in Borno, Kano, Katsina, and Sokoto. Additionally, 90% of the Savanna Zone's forestry is comprised of neem trees as part of the afforestation initiative. Vegetable oil such as neem oil is greatly used in cosmetic, soap, and agricultural industries and the production of pharmaceutical drugs (Goji Usman et al., 2024)

Neem seed oil has garnered significant attention in recent years due to these potential applications (Kumar et al., 2022). The oil is reported to possess antimicrobial, antifungal, and insecticidal properties, making it a promising natural alternative to synthetic pesticides and drugs (Adusei & Azupio, 2022). In addition to being affordable and environmentally beneficial, neem seed extracts may be able to shield certain crop species from fungal infestation, hence boosting crop output and productivity (Campos et al., 2016). *Azadirachta Indica* contains significant levels of azadirachtin, quercetin, and β -sitosterol. Likewise, in addition to its antifungal properties, it may have greater antibacterial and antifungal action than just the bark and leaves (Wylie & Merrell, 2022). Neem seed oil can be extracted from its seeds in several techniques, including mechanical pressing, solvent extraction, and supercritical fluid

extraction. The most used method among them is solvent extraction. In contrast, solvent extraction offers several benefits over supercritical fluid extraction, such as reduced operating costs, enhanced yields, and reduced turbidity compared to mechanical pressing (Sulaiman M. et al., 2024). Over the past 50 years, n-hexane has been the main solvent utilized in industrial oil extraction processes. This is because of its superior oil selectivity, simplicity of evaporation, and relatively low energy costs (Chaudhary et al., 2024).

Despite its potential, limited comprehensive studies have been carried out on the characterization, proximate analysis of the seeds, and phytochemical composition of neem seed oil. However, recently Mustapha *et al* reported various techniques used in the extraction of neem seed oil (Sulaiman M. et al., 2024). Likewise, Nwanekezie *et al* reported the physicochemical and characterization studies of Neem plant extracts without the seed. As such, comprehensive research on the proximate analysis of the seeds, phytochemical composition, and characterization of the oil is therefore necessary. Standardizing the quality of Neem seed oil, guaranteeing its safety and effectiveness for a range of uses, and realizing its full potential as a priceless natural resource all depend on these kinds of studies. Biologically active, naturally occurring chemical compounds called phytochemicals are present in plants. They offer additional health benefits to humans than macronutrients and micronutrients. The process of phytochemical screening is used to find secondary metabolites (Velavan, 2015). Therefore, this study aims to investigate the proximate analysis of the neem seed, phytochemical composition, and characterization of the neem seed oil. Specifically, the study will identify and quantify the bioactive compounds present in the neem seed and its oil. The study also aimed to determine the proximate composition of the seeds and characterize their chemical properties. To the best of our knowledge, a detailed study of this kind has not been reported before. As, such, the results of this study will add to the body of knowledge already available on neem seed and its oil and offer insightful information for potential future uses.

EXPERIMENTAL

The fully matured neem seeds used in this study shown in Figure 1 were found and collected from the Sabon Gari Kanuri ward area of Nguru Yobe State, in northeastern Nigeria. They were authenticated at the Botany section of the Department of Biological Sciences of Yobe State University Damaturu, Nigeria. The seeds were then air-dried and ground to powder using a

mechanical grinder after the removal of the seed's coat. Thereafter the seed powder was subjected to proximate analysis, the FTIR analysis using a BUCK Scientific FTIR spectrometer before the start of the extraction process. The sample was then taken for extraction, after which the oil was subjected to phytochemical studies and UV-Vis analysis using UNICO UV-Vis spectrophotometer.



Fig. 1: Neem seeds before and after drying

Reagents

N-hexane (95%), methanol (96.5%), ethyl acetate (99.8%), petroleum ether (95%), ferric chloride (94%), potassium sulfate (95%), and ethanol (96.5%) were all obtained from BDH Chemicals. Sulfuric acid (99.5%) and DMSO (95.5%) were obtained from Guandong Guanghua Sci-tech Co. Ltd. Sodium hydroxide (97%), magnesium metal (91%), glacial acetic acid (98%), Drangendroff's reagent (99%) and

hydrochloric acid (35.5%) were all from LOBA CHEMIE PVT. LTD. Chloroform (94.6%) was obtained from Sigma-Aldrich.

Determination of moisture content

The moisture content was estimated by maintaining the sample before the extraction at a temperature of 105°C for 2-3 hours and then reweighing after cooling in a desiccator. The moisture content is expressed as a percentage of the dry weight using the following formula

$$\text{Moisture content (\%)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where:

W_1 = weight of empty crucible (g)

W_2 = weight of crucible + Sample before drying (g)

W_3 = weight of crucible + sample after drying (g)

Determination of ash content

2g of the dried sample was weighed into a dry porcelain dish and then heated in a 60 Hz (220V) H-1081 Budapest muffle furnace

(OH85TR) at 6000°C for 6 hours. It was cooled in desiccators and weighed. The percentage ash content was calculated using:

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \times 100$$

Fat content

The fat content was determined using the ultrasonication machine extraction method (AOAC, 1984). 15g of the sample was weighed into the extraction bottle. Cotton wool was used as a plug to avoid loss of sample. The bottle was transferred into the ultrasonicator and sufficient petroleum ether was added until the latter was siphoned into

the receiving flask which had been weighed. After 30min, the ultrasonication machine was switched off and allowed to cool for 10 minutes. The recovered solvent was transferred into an air oven for 1 hour and then cooled in a desiccator and weighed. The amount of oil produced was calculated and expressed as a percentage of the original sample.

$$\% \text{ Crude Fat} = \frac{\text{Weight of extract}}{\text{Weight of the sample}} \times 100$$

Crude fiber:

The crude fiber was determined by treating the defatted sample with 1.25% sulfuric acid and

1.25% sodium hydroxide. The residue was then filtered, washed, dried, and incinerated.

To calculate the crude fiber content from the loss in weight after incineration:

$$\% \text{ Crude Fiber} = \frac{\text{Weight loss on ignition}}{\text{Weight of the sample}} \times 100$$

Crude protein

1g of sample was weighed into a digestion flask. 10g of potassium sulfate, 0.7g mercuric oxide, and 20 ml of concentrated sulphuric acid were added to the sample in the digestion flask. The flask was heated gently at an inclined angle until frothing subsided and boiled until the solution became clear. This was continued for half an hour. When the frothing was in excess, a small amount of paraffin wax was added. On cooling, 90ml of distilled water was added and mixed. A small

piece of pumice was added to prevent bumping. 80 ml of 2M sodium hydroxide solution was added while tilting the flask so that two layers were formed. The condenser unit was rapidly connected and heated while the distilled ammonia was collected in a 50ml boric acid/methyl red indicator. Thereafter, 50 ml of the distillate was collected and titrated against 0.1M hydrochloric acid solution. The percentage nitrogen content percent was calculated thus:

$$\% \text{ Crude protein} = \% \text{ N} \times 6.60$$

The nitrogen content %N of the sample is given by the formula:

$$\% \text{ N} = \frac{T_v \times N_a \times 0.014 \times V_1}{G \times V_2} \times 100$$

Where:

Tv = Titre value of acid (cm³)

Na = Concentration of acid

V1 = Volume of distilled water used for distilling the digest (50 cm³).

V2 = Volume of aliquot used for distillation (10 cm³)

G = Original weight of sample used, g

Total carbohydrate

The total carbohydrate was determined by the differential method. This was achieved by

subtracting the total protein, lipid, moisture, and ash content from 100 thus:

$$\% \text{ carbohydrate} = (100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ fat} + \% \text{ protein} + \% \text{ fibre}))$$

Oil extraction

The n-hexane extract was obtained by complete extraction using an ultrasonicator. 10g of the powdered seeds sample was put into an extraction bottle and placed in an ultrasonication machine (with a boiling point of about 40-60°C) as extracting solvent for 30 mins until the required quantity was obtained. The oil was obtained after filtration and left in a free air condition, to remove the excess solvent from the extracted oil. The oil was kept in the refrigerator without further treatment until further analysis was needed.

Phytochemical screening

Test for alkaloids

0.2g of the extract was dissolved with 1ml of HCl and filtered. A few drops of Mayer's reagent (prepared by dissolving 1.358g of mercury chloride in 60ml of water and poured into a solution of 5g of KI in 10ml of water, made up to 100ml with distilled water) was added to the mixture. The presence of a white turbid precipitate indicates the presence of alkaloids.

Test for cardiac glycosides (Keller-Kelliani's test)

2ml of glacial acetic acid was added to 1ml of each extract (0.1g dissolved in 5ml methanol) in a test tube, followed by a drop of ferric chloride solution. The mixture was carefully overlaid with 1 ml of concentrated sulfuric acid. A brown ring at the interface indicates the presence of deoxysugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may foam.

Test for flavonoids

The extract 1ml was treated with a few drops of sodium hydroxide solution. The formation of an intense yellow color, which becomes colorless with the addition of dilute acid indicates the presence of flavonoids.

Test for phenols

A few drops of aqueous 5% ferric chloride solution were added to the fraction 1ml of extract. The formation of deep blue or black color indicates the presence of phenols.

Test for quinones

A few drops of concentrated HCl were added to the 1ml portion of the extract. The formation of yellow precipitate/coloration indicates the presence of quinones.

Test for saponins

Foam test: 0.2g of the extract was shaken vigorously with 2ml of distilled water for 15 minutes. The formation of foams which persisted for over 10 minutes indicates the presence of saponin.

Test for sterols (Liebermann Burchard test)

A few drops of chloroform, acetic acid, and concentrated sulfuric acid were respectively added to 1ml of the extract. The formation of dark pink or red color indicates the presence of sterols

Test for tannins

A few drops of alcoholic ferric chloride solution were added to 1 ml of the extract. The formation of blue/greenish coloration indicates the presence of tannins.

Test for terpenoids (Salkowski's test), carbohydrates and oxalates

1ml of chloroform was added to 1ml of the extract followed by a few drops of concentrated sulfuric acid, a reddish-brown precipitate produced immediately indicating

the presence of terpenoids. For carbohydrates, 8-10 drops of extract were added to 5 mL of Benedict's reagent, then the mixture was heated for five minutes; the resulting dark red precipitate indicated the presence of carbohydrates. However, for oxalates 2 ml of the extract were treated with a few drops of glacial acetic and a dark green coloration indicated the presence of oxalates.

RESULT AND DISCUSSION

Proximate analysis

Proximate analysis entails examining all the components of a sample, excluding the primary food constituents. It is a type of chemical analysis that gives an estimated and comparative value of moisture content, ash content, crude fiber, and crude protein. The Proximate analysis of the neem seed was successfully conducted and Table 1 reveals the result for the proximate analysis of the neem seed. The result shows that the moisture content is 20%, ash content is 5.0%, crude fat is 12.8%, crude fiber is 42.2%, crude protein is 18.4% and carbohydrate is 1.6%. The moisture content is associated with the storage value, indicating that the seed must be dried before storage to prevent the intrusion of microorganisms that could lead to spoilage. The moisture content value of 20% is lower than the value (60%) reported by (Helen Okoye et al., 2010). The difference in the moisture contents may arise due to the different climatic conditions of the place where the samples were taken or the severity of the dryness of the 2 samples. The sample used in this study was taken in an area with a known average annual temperature of 29-34°C which is a bit high. It was reported that lower precipitations and higher temperatures directly

affect plants by increasing transpiration, photodamage, and lower photochemical efficiency. It may also lead to decreased water use efficiency, decreased productivity, and indirect effects on the abiotic component (soil erosion, nutrient leaching, and more frequent wildfires) (De Dato et al., 2008). The seed's lower moisture content suggested that microorganism activity would decrease and that the neem seed would take longer to germinate or spoil. The ash content is 5% which is low and implies that the sample has lower concentrations of trace elements like copper, lead, chromium, and mercury

The crude fiber is 42% which is much higher than what is mostly reported for plant seeds, this implies that neem seeds are a very good source of fiber. Crude fiber is a portion of plant-derived food that cannot be completely broken down by human digestive enzymes, a high amount of fiber in the body reduces the risk of cardiovascular disease and cancer (Godoy et al., 2025; Ray & Arakera, 2024). Likewise, crude fiber promotes the growth of beneficial gut bacteria and aids in digestion in animals (Yong et al., 2025). The crude fiber content indicates the amount of cell walls in a plant material.

The crude protein is 18.4% which is higher than the 9.63% obtained by Helen Okeye *et al* while the crude fat is 12.8% which is almost the same as crude fats reported for most plant seeds like corn seeds (13.39%), chickpea seeds (10.6%) (Helen Okoye et al., 2010; Saad et al., 2021).

The carbohydrate content is 1.6% which is lower than what was reported for most plant seeds, especially the edible ones (Saad et al., 2021).

Table 1: Proximate analysis of the Neem seed

S/No.	Parameter	Percentage (%)
1.	Moisture content	20.00±1.2
2.	Ash content	5.00±0.5
3.	Crude fat	12.80±0.9
4.	Crude fibre	42.20±2.3
5.	Crude protein	18.40±0.7
6.	Carbohydrate	1.60±0.3

FTIR analysis

Fig. 1 depicts the FTIR spectra of the dried neem seed. The peak observed at 3864 cm⁻¹ and 2754 cm⁻¹ is attributed to O-H stretching

vibration in hydroxyl groups (Ayantola et al., 2024; Smith, 2011). The peak at 3582 cm⁻¹, indicates the presence of N-H stretching vibration in primary and secondary amines

(Yan et al., 2018). The peak at ~ 3176 , ~ 2902 , ~ 2568 , and 1464 cm^{-1} indicates the presence of C-H stretching vibration in alkanes and alkyl groups (Ayantola et al., 2024; Boeva & Sergeyev, 2014). Peaks at ~ 3021 refer to C-H stretching vibration in aromatics (Gu et al., 2012). The vibration peak at $\sim 2289\text{ cm}^{-1}$ is attributed to C-N stretching vibration in nitriles (Tian et al., 2014). The peak at $\sim 1718\text{ cm}^{-1}$ stands for C=O stretching vibration in carbonyl groups (Ayantola et al., 2024; Smith, 2011). The peak at $\sim 1082\text{ cm}^{-1}$ is attributed to C-O stretching vibration while the peak at $\sim 751\text{ cm}^{-1}$ peak usually stands for C-Cl

stretching vibration in chlorinated compounds and the peak at $\sim 627\text{ cm}^{-1}$ refers to C-Br stretching vibration in brominated compounds (Silverstein & Bassler, 1962; Smith, 2011). The presence of various peaks on FTIR spectra attributed to numerous stretching vibrations of compounds is an indicator of the numerous phytochemicals like flavonoids and tannins present in the neem seed as will be seen in the phytochemical analysis result (Pharmawati & Wrasati, 2020). This further revealed the potential of neem seed to be used for various uses and applications.

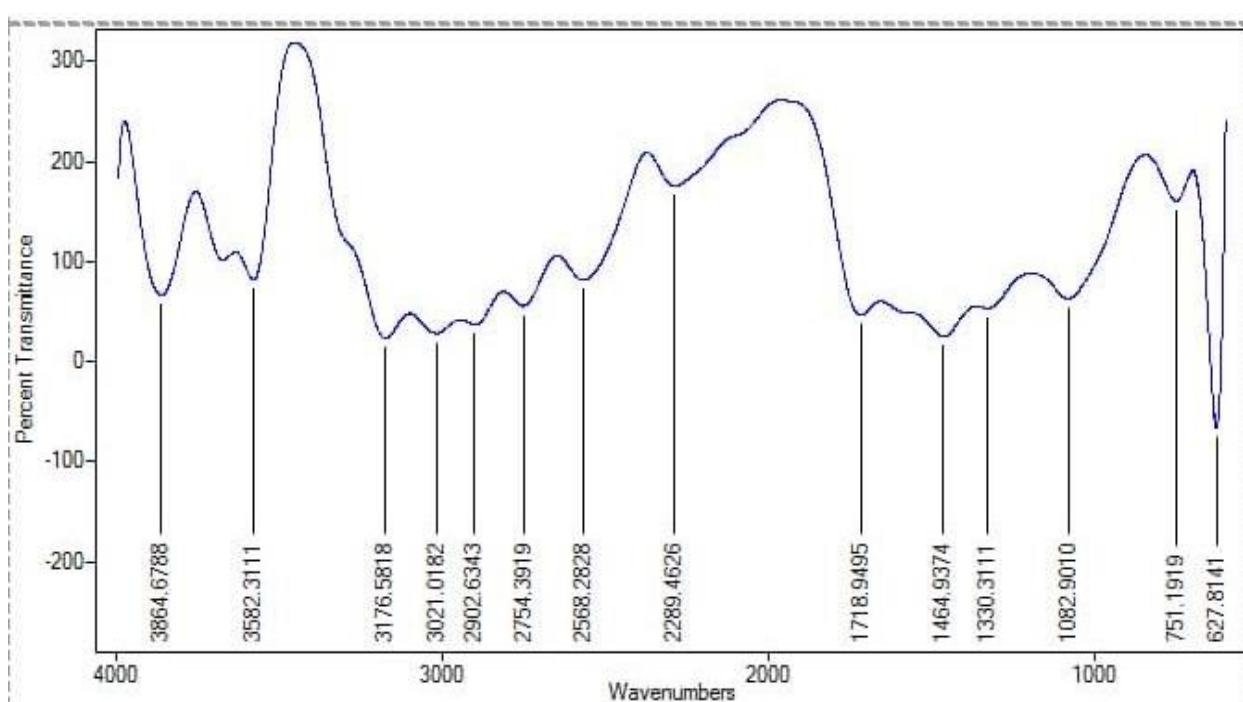


Figure 2: FTIR spectra of the neem seed

Phytochemical analysis

50 g of the powdered sample was weighed into a clean 500 mL beakers and mixed thoroughly with 250 mL of methanol and n-hexane in each case (1:5 w/v). The beakers containing the sample mixture were placed in an ultrasonic bath (Model USC-D, Infitek). The bath was operated at a frequency of 40 kHz and the temperature was maintained at $40\text{ }^{\circ}\text{C}$. The extraction was carried out for 30 minutes. The mixtures were filtered through Whatman no. 1 filter paper after which the filtrates were centrifuged at 5000 rpm for 10 minutes. The filtrates were concentrated using a rotary

evaporator (RV 10, IKA), and the concentrates were stored at $40\text{ }^{\circ}\text{C}$ for further analysis. Phytochemical analysis is usually done to identify bioactive compounds in plants that can be used in developing new therapeutic formulations. The phytoconstituents are well known for their ability to treat a variety of human ailments, including diarrhea, dysentery, malaria, ulcers, and enlarged livers. Numerous phytochemicals with biological activity found in a range of herbs and herbal extracts may have useful medicinal properties. Phytochemicals, which are non-nutrient substances, are responsible for the majority of

the protective benefits of herbal plants (Priyanka Dash et al., 2017).

The result of phytochemical screening of the neem seed oil using n-hexane and methanol as solvents is shown in Table 2. The result revealed the phytochemicals present in the n-hexane extract are; alkaloids, glycosides, quinones, carbohydrates, terpenoids, and flavonoids, while saponins, phenols, tannins, oxalates, and sterols are absent. In methanol extract however, alkaloids, saponins, tannins, glycosides, quinones, carbohydrates, terpenoids, and flavonoids are present, while phenols, oxalates, and sterols are absent.

The presence of alkaloids, flavonoids, and glycosides is an indicator that the oil can be used in the development of various drugs like analgesics, antipyretics, antiasthmatics, anti-cancers, antihypertensives, and many more (Aryal et al., 2021; Lobo et al., 2010; Yattoo et al., 2018). This is because phytochemicals like flavonoids are reported to have an antioxidant effect and serve as protectors against platelet

aggregation. They are also protectors against microorganisms, hepatotoxins, viruses, tumors, ulcers, free radicals, inflammation, and allergies. Similarly, glycoside has been used in the treatment of congestive heart failure due to its direct action which increases the force of myocardial contraction (Ezeonu & Ejikeme, 2016). Likewise, the presence of quinones is an indicator that the oil can be used in the synthesis of various materials like dyes, laxatives, oxidizing agents, and a lot of chemical intermediates (Azevedo et al., 2019; Gonzalez et al., 2020; Zhao et al., 2018). Similarly, the presence of terpenoids is equally important as terpenoids are used in the synthesis of many drugs that treat cancer and malaria apart from their uses in the synthesis of materials like cosmetics, flavoring agents, and insect repellants (Aryal et al., 2021; Azevedo et al., 2019; Cheenpracha et al., 2016; Patnala & Kanfer, 2010; Yattoo et al., 2018).

Table 2: Phytochemical screening of Neem seed oil (using n-hexane and methanol)

S/No.	Phytochemical	Type of test	N-Hexane Extract	Methanol Extract
1.	Alkaloids	Drangendroff	+	+
2.	Saponins	Foaming	-	+
3.	Phenols	Ferric chloride	-	-
4.	Tannins	Braymer's	-	+
5.	Glycosides	Keller-kelliani's	+	+
6.	Quinones	Conc. HCl	+	+
7.	Oxalates	Glacial acetic acid	-	-
8.	Carbohydrates	Molisch's	+	+
9.	Sterols	Liebermann Burchard's	-	-
10.	Terpenoids	Salkowski's	+	+
11.	Flavonoids	Pew's	+	+

UV-Vis Analysis

The optical characteristics of a material are an essential parameter that determines the properties of that material. The absorption spectra of the two extracts are shown in Fig. 3 and 4. It can be seen that in n-hexane extract the higher absorbance is at 420nm and the scope of the wavelength is 300-500nm, while in methanol extract, the wavelength increases with a decrease in absorbance, the higher absorbance is at 1.893 and the wavelength in

such position is 300nm. The difference in the absorption spectra of the 2 samples may arise due to the difference in properties of the two solvents. It is generally known that N-hexane is a non-polar solvent that is less dense than methanol and, as such dissolves non-polar compounds easily unlike methanol which is a polar solvent that can interact with both polar and to some extent non-polar molecules. A solvent's polarity alters the width, location, and strength of the absorption band, which in turn

alters a substance's UV spectrum. This results from interactions known as solvent-solute interactions that occur between the solvent and the material under study (Azizi et al., 2009). Polar solvents are known to provide broader bands and obscure fine spectral details because they change the charge density of the

substance being studied, which alters the electronic distribution. However, non-polar solvents give better resolution and fine spectral details. Due to their small dielectric constant, leading to less solvent effects. These effects are all due to solvent-solute interactions (Nicol, 1974).

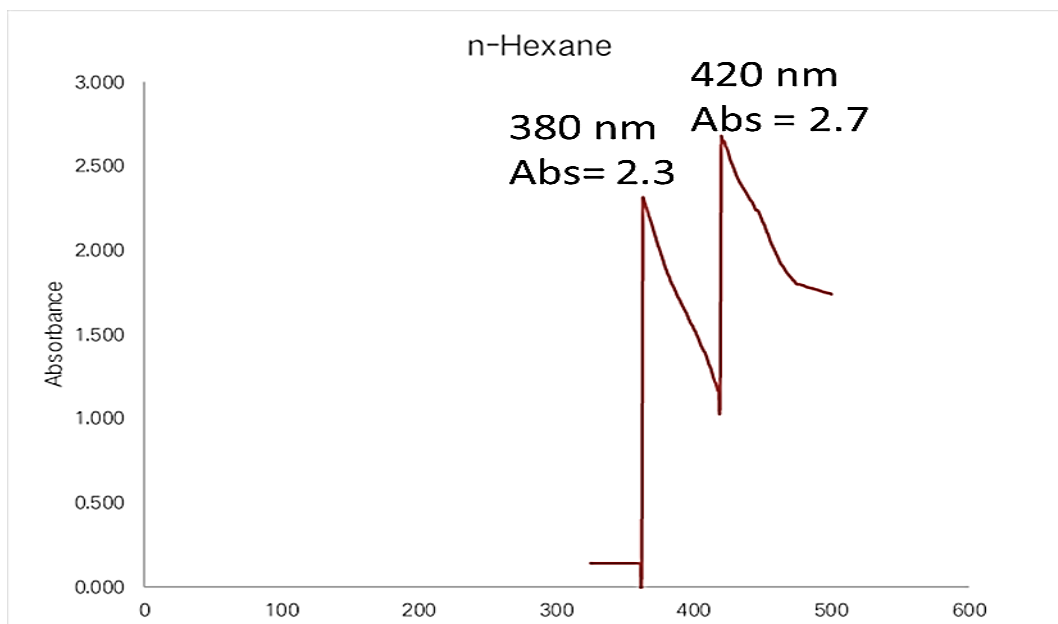


Fig. 3: Absorption spectrum of the n-Hexane extract of the neem seed oil

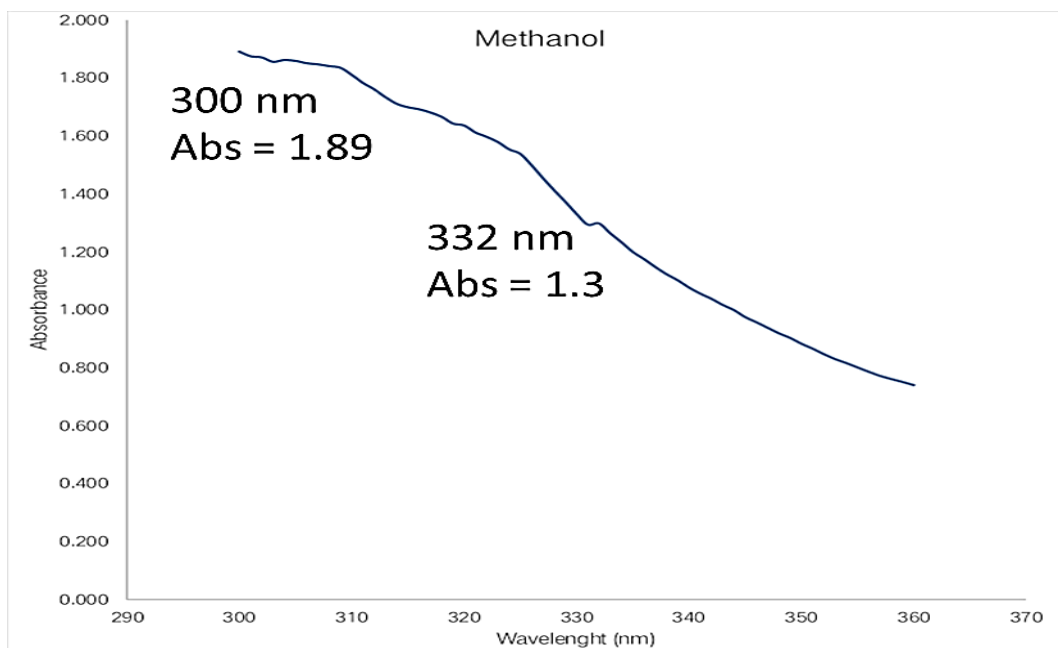


Fig. 4: Absorption spectrum of the methanol extract of the neem seed oil

CONCLUSIONS

In conclusion, hexane and methanol were used as solvents to extract the oil from neem seeds. FTIR and proximate analysis were carried out on the neem seeds before the extraction process. The FTIR result showed the presence of various functional groups such as hydroxyl group, amine group, nitriles group, carbonyl group, and aromatics. The proximate analysis revealed a moisture content of 20.0%, ash content of 5%, crude fat, and crude protein of 12.4% and 18.4% respectively. The crude fiber was however higher (42.2 %) and the carbohydrate content was much lower (1.6%). Phytochemicals screening and UV-Vis analysis were then carried out on the extracted oil and the result revealed the presence of carbohydrates, glycosides, flavonoids, terpenoids, quinines, alkaloids, and other important phytochemicals in both n-hexane and methanol extract. These secondary metabolites (phytochemicals) have been reported to exhibit many pharmacological and physiological effects. The results obtained

from this study revealed that neem seed and its oil could be used in industries for the production of various drugs, paints, pesticides, fertilizers, and other important materials. It is recommended that future research be directed to further characterization of the neem seed oil using Gas chromatography-mass spectrometry (GC-MS) and Nuclear magnetic resonance (NMR) analyses to explore its properties more for other potential industrial applications.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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