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PHYSICOCHEMICAL PROPERTIES OF OILS EXTRACTED FROM *Azadirachta indica* SEEDS

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ABSTRACT

The applications of *Azadirachta indica* seed oil are defined by its property through many factors, including methods by which the oil was extracted. The study compared the physicochemical properties of the oils extracted from *Azadirachta indica* seeds with standard values. The *Azadirachta indica* seeds were collected from Gombe State Nigeria and the neem seed oils (NSO) were extracted through the Soxhlet method using *n*- hexane and by mechanical extraction using water as a solvent to get NSO A and NSO B respectively. The percentage yields of both oils were determined and thereafter, their physicochemical properties were carried out. The physical properties of the oils and their physicochemical characteristics had comparable values with the standard. The overall study proved that the extraction methods had impact on the quality of the oil. The medicinal values of NSO could be exploited and put into clinical uses in the production of antiseptic soaps, lubricants, creams, shampoo, insect repellants, etc.

Keywords: *Azadirachta indica* seeds, Physicochemical properties, Sohxlet extraction, Mechanical extraction.

INTRODUCTION

Seed oils are important sources of nutritional oils, industrial raw materials and nutraceuticals. The characteristics of oils from different sources depend mainly on their compositions. No oil from a single source can be suitable for all purposes thus, the study of their constituents is important. Neem oil is a vegetable oil pressed from the fruits and seeds of the neem (*Azadirachta indica*). It is the most important of the commercially available products of neem for organic farming and medicines (Adeeko and Ajibola, 1990, Mongkhol *et al*., 2004). The main reason behind the popularity of the Neem oil is that it is used to treat few of the most common ailments. Neem oil comprises mainly of triglycerides (esters formed from a molecule of glycerol and three molecules of fatty acids), and is very rich in azadirachtin- the key component acting as insect repellent, anti-feedant, anti-fungal and anti-viral, among others, it is perhaps the most important commercial product of neem for organic farming and medicines (Lyons *et al.,* 2008). Neem seed oil is rapidly finding relevance in pharmaceutical and agro-allied industries, Hence the need to research for locally available and economically feasible methods of its extraction (Matt, 1990; Gurulingappa *et al*., 2002).

This study compared the physicochemical properties of the oils extracted from *Azadirachta indica* seeds with standard values. The medicinal values of NSO could be exploited and put into clinical uses in the production of antiseptic soaps, lubricants, creams, shampoo and insect repellants.

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MATERIALS AND METHODS

Collection and Preparation of *Azadirachta indica* Seeds

The ripe neem seeds were plucked from *A. indica* trees from Government Day Secondary School Hashidu located at the north-western edge of the town of Hashidu, Dukku local government, Gombe state, Nigeria. Hashidu lies at 358 m above sea level with an April - October rainy season followed by a dry season.

The plant material was identified, authenticated and assigned a voucher number of "GSUH 30" in the herbarium of the Department of Biological Sciences of Gombe State University, Gombe Nigeria. The ripe neem seeds were sourced in the months of April to early August of 2020 as shown in Figure 1.

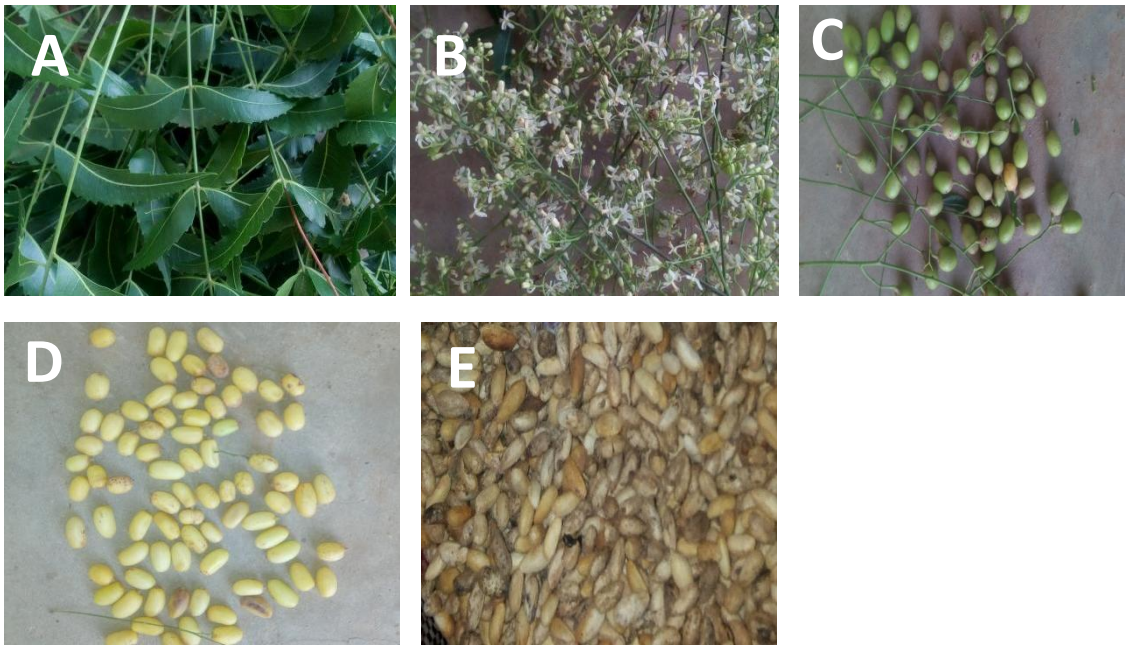

Figure 1: Pictorial representation for obtaining dried *Azadirachta indica* seeds (A- Neem Leaves; B- Neem Flowers to produce fruits; C- Unripe Neem Fruits; D- Ripe Neem Fruit; E- Neem Seeds)

The seeds obtained were thoroughly washed using water to remove dirt and other impurities, cracked to remove the shells and the kernels air-dried in an open space with regular movement for aeration to ensure proper drying, a method also applied by Soetaredjo *et al*. (2008), to reduce the moisture content for proper crushing and to facilitate high oil volume recovery during mechanical extraction. The seeds were stored in a nylon bag and properly kept safely away from the reach of pest organisms and other animals that may contaminate the seeds, and subsequently daily air-dried as previously described with proper monitoring to prevent seed damage as a result of possible moisture fluctuations.

Neem Seed Oil Extraction

Mechanical Extraction Method: The neem seeds were size reduced to finer particles then 500 g was weighed on an electronic balance and transferred into a stainless steel bowl and moisten with some drops of water then pressed in a glass mortar using a pestle until the oil was obtained. The extracted oil was collected, filtered through the filter paper and

the percentage yield of the filtrate (oil) was determined.

Soxhlet Extraction Process: The method of semi continuous extraction method as described by Khittiphoom and Sutasinee, 2011 was adopted. The *A. indica* seeds were powdered and 500 g was weighed extracted using the Soxhlet apparatus for 6 hours with *n*-hexane. The extracted oil was filtered through filter paper and the solvent was evaporated using a rotary evaporator under reduced pressure and temperature and further dried under open air in a dark area. Thereafter the percentage yield was determined.

The mechanical and Soxhlet neem seed oil extraction procedure is summarized in Figure 2.

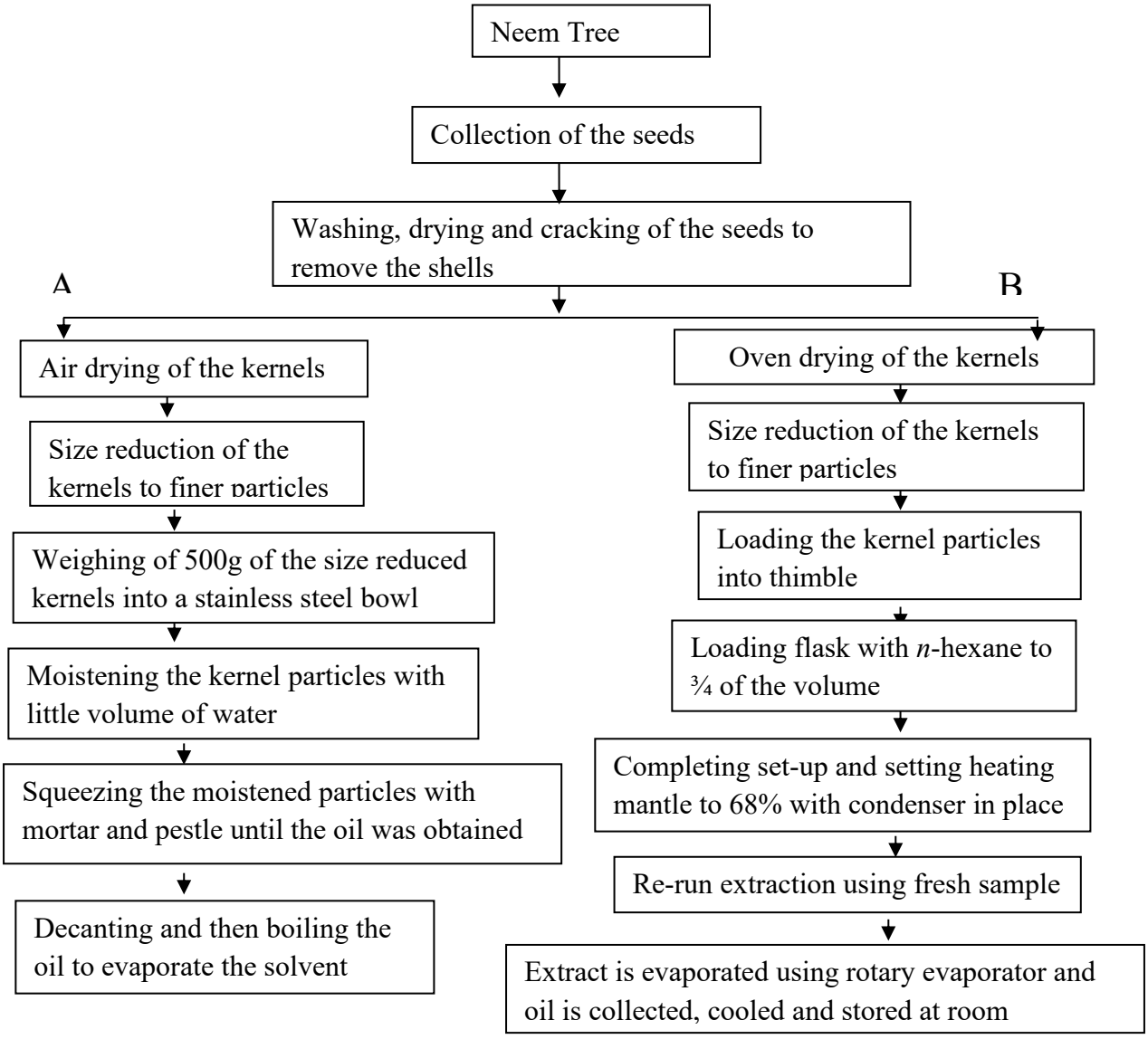

Figure 2: Neem Seed Oil Extraction Processes

Percentage Oil yield determination (%): The oil gotten after the mechanical extraction was transferred into a measuring cylinder which was placed over water bath for 30 min at 70 ^oC to ensure complete evaporation of solvent and volume of the oil was recorded and expressed as oil content percent. Rotary evaporator was used to evaporate the solvent from the oil obtained in sohxlet extraction procedure.

The oils content were calculated as follows:

$$\text{Percentage (\%) yield of oil} = \frac{W1-W2}{W1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where: W_1 = Initial weight of the sample; W_2 = Sample weight after evaporation (Natarajan *et al*., 2003)

Determination of Some Physicochemical Parameters

Determination of moisture content: Moisture content was determined according to Manual of Methods of analysis of Foods: Oils and Fats (2005), using a hot air oven. Five grams of oil was weighed on a previously dried crucible. The crucible was covered with a loose lid and was heated in the oven at 105 ± 1 °C for one hour. The crucible was removed from the oven, cooled in a desiccator and was weighed. The crucible was re-heated for one hour. The cooling and weighing process was repeated until the weight change between two successive observations does not exceed one mg. The following formula was used to calculate the observations;

$$\text{Percentage (\%) of moisture and volatile matter} = \frac{(w1-w2)}{w1} \text{ X } 100 \ldots\ldots\ldots (2)$$

Where; W1 = weight of fresh neem seed oil and W2 = weight of neem seed oil after drying.

Determination of organoleptic properties: The physical properties of colour, odour, and taste were determined for both oils.

pH Determination: The pH of the oils were determined at room temperature (28 °C) using the Oaklon pH meter 1100 series.

Acid Value and Free Fatty Acid: Acid value is the number of milligrams of KOH required to neutralize the free fatty acids present in 1 g of material. Free fatty acids were measured according to IUPAC 2.201 indicator method (Paquot *et al*., 1987). Exactly 2.5 g of neem seed oil was placed in a 250 mL Erlenmeyer flask. About 150 mL of neutralized 1:1 (v/v) ethanol and diethyl ether solution was added to the flask and the mixture was titrated with potassium hydroxide 0.1 N ethanolic solution until the pink color appeared and lasted for 10 seconds. Acid value and free fatty acid value are calculated by the following formulae:

$$\text{Acid value} = \frac{56.1 \, X \, T \, X \, V}{M} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where: T = exact normality of standardize KOH solution; M = Mass of the sample; V = Volume of KOH used

$$\text{Free fatty acid (\%)} = \frac{Acid\ Value}{1.99} \quad \ldots\ldots\ldots\ldots (4)$$

Saponification Value: The saponification value is the number of milligrams of KOH needed to saponify 1 g of fat. It is determined using the colored indicator (phenolphthalein) as described by IUPAC (International Union of Pure and Applied Chemistry). Two grams of the oil sample was added to a flask with 30 mL of ethanolic KOH. A reflux condenser was attached and the flask content refluxed for 30 minutes on a water bath with continues swirling to ensure that the sample is fully dissolved. After sample has cooled 1 ml of phenolphthalein was added and titrated with

0.5 M HCl until a pink colour appeared, indicated the end point.

$$\text{Saponification Value} = \frac{(B-R)X\ 28.05}{M} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

Where: B = the volume of the HCl used for blank test; R = the volume of the HCl used for determination; M = Mass of the sample.

Iodine Value: Iodine value which indicates the degree of unsaturation of the fatty acids in the oil was determined by Hanus method (Association of Analytical Chemist 1990). One gram of oil was placed in 250 mL conical flask followed by 30 ml of Hanus solution, the flask was stopped and the content mixed and placed in a cupboard for 30 minutes,

$$\text{Iodine Value} = \frac{(\text{B}-\text{R})\ \text{X}\ \ 0.14\ \text{X}\ 126.9}{M} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

Where: B = Volume of sodium thiosulphate used for blank; R = Volume of sodium thiosulphate used for determination; M = Mass of the sample.

Peroxide Value: Peroxide value was measured following IUPAC 2.501 (Paquot *et al*., 1987). Exactly 1.0 g of KI and 20 ml of solvent

$$\text{Peroxide Value} = \text{SN}\ \frac{1000}{W} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

Where: S = Average titre volume; N = Normality of $Na_2S_2O_3$ use; W = weight of oil sample used

Statistical Analysis

The mean of three determinations was carried out for each test.

RESULTS AND DISCUSSION

The soxhlet method of extraction using *n*-hexane produced a greater yield (41.10 % w/w) of NSO in comparison with the mechanical method in which water was used as solvent (18.68 % w/w). The color of the NSO samples ranged from greenish-brown to brownish-yellow, as shown in Plates I and II.

All the NSO samples were liquid at room temperature with a bitter taste and a smell that resemble a mixture of garlic and peanut. The physicochemical characteristics such as moisture content, acid value, saponification value, iodine value and peroxide value were determined for both A and B as shown Table 1.

The expression for saponification value (S.V.) is given by:

potassium iodide solution (10 ml of 15 % w/v) added to the flask. This was titrated against 0.14 M $Na_2S_2O_3$, until the solution become light yellow. Starch indicator (2 ml of 10 %) was added and titration continued until the blue color just disappeared. A blank determination was carried out under the same condition. The titre value was recorded and used to calculate Iodine value.

The iodine value (I.V) is given by the expression:

mixture (glacial acetic acid: chloroform, 2:1 v/v) were added to 1.0 g of the oil sample and the mixture was boiled for one minute. The hot solution was poured into a flask containing 20 ml of 5 % KIO3 solution. Few drops of starch solution were added to the mixture and the latter was titrated with 0.002 M $Na_2S_2O_3$ solution.

Plate I: Photograph of Neem Seed oil extracted by mechanical method (A)

Plate II: Photograph of Neem Seed oil extracted by Soxhlet method (B)

Table 1: Physicochemical Analysis of neem seed oil extracted by mechanical and Sohxlet methods compared with Standard

Parameters	Mechanical method (A)	Sohxlet method (B)	Standard
Percentage yield (%)	18.68	41.10	40.0-60.0
Physical state	Liquid	Liquid	Liquid
Color	Brownish Yellow	Greenish brown	Yellow, Brownish-yellow, Greenish-yellow
Odor	Garlic/peanut	Garlic/peanut	Garlic/peanut
Taste	Bitter	Bitter	Acrid, Bitter
Density (g/ml)	0.864	0.971	0.95 – 1.06
Ph	6.30	6.50	5.7 - 6.5
Moisture/Volatile matter content (%)	1.8	0.4	_____
Free fatty acid (%)	8.91	3.38	20.1
Acid value ($mg.g^{-1}$)	17.73	7.63	40
Saponification value ($mg.g^{-1}$)	283.31	272.08	175 – 205
Iodine value ($mg.100g^{-1}$)	46.19	62.80	65 – 80
Peroxide value (mEq $O_2.g^{-1}$)	6.20	3.00	7.82

Standard values: (Erakhrumen, 2011; Workneh, 2011; Koul *et al*, 2012; Zaku *et al*., 2012).

The quality of the oil depends on its composition, which in turn affects its properties. Since neem oil mainly contains fatty acids as one of the active components, it is commonly analyzed for its quality by determining physicochemical parameters (Okonkwo *et al*., 2013; Djibril *et al*., 2015). The difference in the color intensity of the oils that were brownish yellow and greenish brown for A and B oils respectively might be attributed to the presence of various pigments, such as the chlorophyll content. The green color of the immature seeds disappears upon maturation resulting in chlorophyll retention (Ayadi *et al*., 2009). Also, it was reported from the literature that, the presence of moisture content at greater levels affects the color of the oil, whereby the moisture elevates the chlorophyll content and thus contribute in increment of color

intensity (Orhevba *et al*., 2012). The normal and thermal oxidation process of oil contributes towards the deterioration of lipids, and thus it might also influence the color changes of the oil compared to the initial color of the oil (Ayadi *et al*., 2009).

The odor, taste and the pH of both A and B oils were consistent with the typical NSO as was reported in the literature (Dasa Rao and Seshadri, 1941:; Koul *et al*., 2012). The physical state at room temperature was liquid for both A and B, the density was 0.864 g/mL and 0.791 g/mL for A and B respectively, these values deviates slightly from 1.06 g/mL and 0.95 g/mL as reported by Jessinta *et al*., (2014) on analysis of neem seeds from Sudan and Malaysia respectively.

The moisture and volatile matter analysis prove that both oils contain a small amount of moisture and volatile matters, as the presence of water or moisture contributes towards hydrolysis and results in breaking up of triglycerides into glycerol and free fatty acids (Orhevba *et al*., 2012). This process was accelerated by the action of lipase enzymes as catalyst. Therefore, these reactions, both oxidation and hydrolysis reduce the amount of unsaturated free fatty acids and thus contribute towards the reduction of the iodine value and average molecular weight and increases the acid value (Orhevba *et al*., 2012). High moisture content in plant fats and oils usually leads to increase in microbial load as well as lipid oxidation resulting in degradation. Oil extract A has high moisture/volatile matter content with residual un-evaporated solvent (water).

The acid value is the relative measure of rancidity as free fatty acids, which are formed during decomposition or hydrolysis of oil glycerides, due to the action of moisture, temperature and/or lipolytic enzyme lipase. The acid values obtained; 17.73 $mg.g^{-1}$ and 7.63 $mg.g^{-1}$ for A and B respectively were agreeable with the past studies (Erakhrumen, 2011; Zaku *et al*., 2012). Oxidation and hydrolysis processes is also a factor that led towards increment in acid value as the percentage of unsaturated fatty acids increase (Orhevba *et al*., 2012).

The saponification values obtained in this analysis were 283.31 $mg.g^{-1}$ and 272.08 $mg.g^{-1}$ for A and B respectively; these values are higher compared to the standard. High saponification values suggest that the oils have potential to be used in soap and cosmetic industries.

Among various factors of oil classification, the drying quality of the oil is also being considered, whereby it could be drying, semi-drying or non-drying oil through the analysis of the iodine value (Talkit *et al*., 2012). The iodine value for A and B (46.19 g I_2.100 g^{-1} and 62.8 g I_2.100 g^{-1} respectively) suggests that it is a non-drying oil and it is comparable to the standard iodine value of less than 100 g I_2/100 g in accordance with its physical state of being liquid at room temperature of 25 °C. The low iodine value represents the fewer amounts of unsaturated bonds and thus the oil has fewer tendencies to go through oxidative rancidity (Orhevba *et al*., 2012).

The oil obtained through mechanical extraction using water as a solvent had also undergone some chemical decomposition process, whereby the peroxide value (6.2 mEq $O_2.g^{-1}$) is high compared to the oil obtained by soxhlet extraction process using n-hexane as a solvent (3.0 6.2 mEq $O_2.g^{1}$). The peroxide value indicates the rancidity process whereby the higher peroxide value, proves higher oxidation level and the deterioration of lipids (Mohammed and Hamza, 2008). According to literature, oil that displays a high peroxide value is more liable to undergo rancidity that

affects the total stability of the oil (Ibeto *et al*., 2012).

CONCLUSION

The physicochemical characteristics of neem seed oil was determined and showed that soxhlet extraction method using *n*-hexane as a solvent produced a better quality and stable oil with greater percentage yield as compared to water extract using the mechanical method. The medicinal values of NSO could be exploited and put into clinical uses in future with collaborative efforts from National Institute of Pharmaceutical Research and Development and other researchers across the country. The NSO could be used to produce soaps, lubricants, creams, shampoo and insect repellants.

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