Effect of Extraction Methods and Storage Time on the Yield and Qualities of Neem Seed (*Azadirachta indica A. Juss*) Oil

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ABSTRACT: The effects of extraction methods and time of storage on the yield and qualities of neem seed oil were investigated. Three extraction methods: cold water, hot water, and n-hexane were used while the extracted oils were stored at room temperature for six months. The yield, chemical properties, qualitative and quantitative phytochemical analysis of the fresh and stored oils were evaluated at every two-month interval using standard methods. The results showed that n-hexane gave the highest oil yield (62 %), followed by hot water (49 %), and cold water (42 %). The chemical properties ranged as follows; peroxide value: (7.02–25.56, 6.30–26.76 and 8.99–24.16 Meq/kg), saponification value: (133.95–245.26, 114.09–288.09 and 141.11–250.12 mg KOH/g oil), iodine value: (51.69–6.98, 56.73–7.88 and 54.87–9.51 mg/wij's) and acid value: (18.01–55.99, 11.34–85.12 and 14.62–56.88 mg KOH/g oil) for cold water, hot water, and n-hexane respectively. The qualitative phytochemical analysis indicated the presence of flavonoids, coumarins, terpenoids, triterpenoid, and steroid contents. Conclusively, the extraction methods and storage time affect the yield and qualities of the extracted neem seed oil, while the chemical and phytochemical results revealed that the extracted oils were good for both medical and industrial applications.

KEYWORDS: Neem seeds, oils, extraction methods, storage time, chemical and phytochemical properties

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I. INTRODUCTION

Oils can be extracted from a wide variety of oilseeds which can be used for foods, skincare products, nutraceuticals, aromatherapies, industrial lubricant productions, and fuels (Adebayo *et al.*, 2012). Oilseeds such as peanut, soybean, cottonseed, sunflower, palm kernel, olive, rapeseeds, and coconut are the largest source of vegetable oils. The oils extracted from these plant seeds served as a relief in human lives to some extent such as in food production, manufacturing of soaps, paints, varnishes, lubricants, and plastics (Adebayo *et al.*, 2012). On the other hand, some of these vegetable oils were not edible like, linseed oils, castor oils, jatropha oils, microalgae oils, karanja oils, rubber seed oils, silk-cotton tree oils, and neem seed oils (Atabani *et al.*, 2013). The availability, renewability, higher heat content, lower sulfur and aromatic content, and biodegradability were reported by Atabani *et al.* (2013) as the main advantages of non-edible oil. However, the multiple usages of some of these oils have created a vacuum that led to their scarcity and higher demand leading to relatively higher prices, which is detrimental to domestic and industrial users.

Neem tree (*Azadirachta indica* A. Juss) is a member of the mahogany family (*Meliaceae)* and endemic to the Indian subcontinent (Liauw *et al.*, 2008). Neem tree proliferates in the tropical and semi-tropic climate countries, including Nigeria where it is known as *Dongoyaro,* with good environmental adaptability. The parts of the neem plant like leaves, barks, flowers, fruits, seeds, and roots were good sources of native medicine for the household treatment of various human illnesses and industrial products (Tesfaye *et al.*, 2018). The neem seed has the highest concentration of oil compared to other parts of the tree and this oil is used as lubricants, insecticides, and drugs for a variety of diseases like; diabetes, leprosy, and tuberculosis (Tesfaye *et al.*, 2018). Neem seed oil is also used in the manufacturing of a large number of skin products such as body lotions, body soaps, and beauty cares facial packs in combination with other natural ingredients.

While the cake that remains after the oil extraction serves as an active ingredient in the manufacturing of mosquito repellent coils (Liauw *et al.*, 2008). Although neem seed oils are not majorly used for cooking purposes because of their offensive odour and bitter taste, similar to the combined odours of garlic and peanut, however, it is used as a preservative agent to prolong the shelf-life of cowpea grains (Ilesanmi and Gungula, 2011). Thus the increase in the production, characterization, and utilization of this seed in vegetable oils to meet global needs.

Recently, many researchers such as Liauw *et al.* (2008); Ilesanmi and Gungula (2011); Jessinta *et al.* (2014); Ikyenge *et al.* (2015); Swapna-sonale *et al.* (2018); Tesfaye *et al.* (2018)

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and Hamadou *et al.* (2020) have worked on the production and characterization of neem seed oil as another possibility to meet the growing demand of non-edible oils. The extractions are basically done by mechanical pressing, supercritical fluid extraction, enzymatic extraction, and solvent extraction techniques. The mechanical technique is the direct application of force on the seeds to press the oil out. It was the most commonly used technique for extracting neem oil; however, the oil obtained with this technique usually has a low price due to its turbid, significant amount of water, and metals contents present (Liauw *et al.*, 2008). The design of the mechanical extractor is another problem associated with the mechanical technique because mechanical extractors are usually designed for a particular seed, thus, the yield will be influenced if that mechanical extractor is used for other seeds (Atabani *et al.*, 2013). Supercritical fluid extraction is an extraction technique that uses a solvent like CO_2 under supercritical conditions to extract oil. The purity of the oil produced by this method is very high (Swapna-sonale *et al.*, 2018), however, the operating and investment costs are very high too (Liauw *et al.*, 2008).

The enzymatic oil extraction technique is a process that used suitable enzymes to extract oil from crushed seeds. This technique's main advantage is that it does not produce volatile organic compounds making it environmentally friendly. However, its longer processing time served has its main disadvantage (Atabani *et al.*, 2013). The solvent extraction technique is the leaching out of the oilseeds' insoluble solid structure with the aid of volatile organic solvents such as acetone, butanol, n-hexane, and isopropanol (Adejumo *et al.*, 2013). These solvents used for the extractions are active as they can extract most of the oil contained in the cells of the oilseed; however, the hazardous nature of the solvents that often lead to fire explosion relatively limit their uses (Efeovbokhan *et al.*, 2015). Also, these solvents have negative environmental impacts due to the wastewater generated, higher emissions of volatile organic compounds, and inflammable chemicals, which are hazardous to the health of the workers and the societies (Atabani *et al.*, 2013). Thus, using the aqueous oil extraction technique will significantly reduce these problems.

The characterization of essential oils through chemical and phytochemical analysis is a mandatory processing step in the production chain. The level of oil deterioration or the extent to which oxidative rancidities have taken place in the oil is usually accessed by the peroxide value (Evbuomwan *et al.*, 2017). The saponification value is an index of the average mass of fatty acids in the oil sample. The higher the oil's saponification value, the higher the use of that oil in the soaps and toiletry manufacturing industries. The iodine value indicated the level of saturation and unsaturation, while the acid value measures the amount of free fatty acid present in the oil sample (Efeovbokhan *et al.*, 2015). The acid value indicates the quality, age, edibility, and suitability of oil for use in industries. Phytochemical screening is also done to identify if the extracted oil has a new source of therapeutically and an industrially valuable compound which have medicinal significance.

Therefore, to retain all these oil qualities, care must be taken when storing them for some time because they readily undergo oxidative deterioration that shortens their shelf-life. Also, the advance in technology nowadays is geared towards using natural products for many purposes. Therefore, there is a need to exploit maximally the potentiality and applicability as well as the agricultural and commercial values of less known oilseeds such as neem seeds as sources of oils that are potentially valuable as medicinal and industrial oil. Hence, the present investigation was carried out to determine the possible effect of extraction methods and storage time on neem seed oils' quality.

II. MATERIALS AND METHODS

A. Procurement and Preparation of Seeds

Neem seeds were collected from Girei in Adamawa State, Nigeria. The seeds were stored at ambient temperature till needed. The reagents and chemicals used were sourced from the analytical laboratories of the Department of Food Science and Technology, Modibbo Adama University of Technology, Yola, and Department of Biochemistry, Bayero University, Kano, Kano State, Nigeria. Neem seeds were manually sorted to remove extraneous materials after that dehulled and winnowed. The seeds collected were then grounded into a paste using an attrition mill. Three extractions; hot water, cold water, and solvent (n-hexane), were used to extract oil from the paste. Completely Randomized Design (CRD) was adopted with 3 replicates.

B. Oil Extraction Processes

The hot and cold water extraction methods described by Ilesanmi and Gungula (2011) was adopted. One thousand and five hundred (1500 g) grams of the neem seed paste was manually kneaded by hand. During kneading, cold water (35±2^{o}C) was occasionally added for the case of the cold water extraction method, while hot water (95±2^{o}C) was added for the case of hot water extraction to aid the extraction process. The extracted oil was thereafter sieved to remove impurities in the oil. The solvent extraction method described by Oladipo and Betiku (2019) with modification was adopted. One thousand and five hundred (1500 g) grams of the neem seed paste was wrapped with a piece of muslin fabric and placed in the conical flask (1500 ml). The flask was filled with 500 ml of n-hexane solvent and firmly secured at the flask's end. The sample was allowed to stay overnight to extract the oil. The experimental setup was thereafter heated with a regulated water bath operated at 70^{o}C. The solvent and oil were decanted, and the solvent was recovered by a distillation process.

C. Measurement of Output Parameters of Extraction Operation

1.) Determination of the percentage oil yield

The parentage oil yield was computed as described by Oladipo and Betiku (2019).

$$\%\ \text{oil yield} = \frac{\text{weight of the oil extracted (g)}}{\text{weight of the paste (g)}} \times 100 \qquad (1)$$

2.) Determination of the chemical properties of neem seed oil

The extracted neem seed oil's chemical properties in terms of peroxide, saponification, iodine, and acid values were determined as described by AOCS (2011) standard method.

D. Qualitative Analysis of the Phytochemical in the Extracted Oils

The extracted oils were analysed for the presence of the following phytochemicals; alkaloids, saponins, flavonoids, tannins, coumarins, terpenoids, triterpenoids, steroids, anthocyanins, quinones, and emodins.

1.) Test for alkaloids

One to two drops of Dragendorff's reagent (potassium bismuth iodide solution) were added to 1 ml of the extracted oil sample inside a test tube. A reddish-brown precipitate shows the existence of alkaloids (Ramadass and Subramanian, 2018).

2.) Test for saponins

About 1.5 g of extracted oil was shaken with water in a test tube. Persistent frothing shows preliminary evidence for the presence of saponins (Ramadass and Subramanian, 2018).

3.) Test for flavonoids

The presence of flavonoids was carried out by putting in 2 to 3 drops of conc. H_2SO_4 into 1 ml of the extracted oil. Observation of orange to crimson red colouration shows the existence of flavonoids.

4.) Test for tannins

The presence of tannin was carried out by adopting the method used by Fasola (2000). Five (5 g) of the extracted oil was introduced into an empty beaker (50 ml) in which 3 drops of ferric chloride was added. A precipitate showed by the solution indicates tannins' presence (Babatunde *et al.*, 2019).

5.) Test for coumarins

Three (3 ml) of 10 % NaOH was added to 2 ml of aqueous extract formation of the extracted oil sample. The appearance of yellow colour in the solution shows the existence of coumarins.

6.) Test for terpenoids

The presence of terpenoid was tested as described by Shanmugavel *et al.* (2018). Five (5 ml) of the extracted oil sample were mixed with 2 ml of chloroform. After that, 3 ml of conc. H_2SO_4 were carefully added to form a layer. The reddish-brown colouration of the inner phase formed shows the existence of terpenoids.

7.) Test for triterpenoids

Ten (10 g) grams of the extracted oil were dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added after the addition of 2 ml of Conc. H_2SO_4. The golden-yellow colour at the bottom of the solution shows the existence of triterpenoids (Ramadass and Subramanian, 2018).

8.) Test for steroids

One to two drops of acetic anhydride were added to 2 ml of the extracted oil samples and boiled. After that, one to two drops of conc. H_2SO_4 from the sides of the test tube were added. The formation of a brown-ring at the point of contact of the two layers. Likewise, the turning of the upper layer to green shows steroids' presence (Ramadass and Subramanian, 2018).

9.) Test for anthocyanin

The presence of anthocyanin was carried out by adding two to three drops of concentrated sulphuric acid into 1 ml of the extracted oil samples. The appearance of a yellowish-orange colour shows the presence of anthocyanin (Babatunde *et al.*, 2019).

10.) Test for quinones

The presence of quinones was carried out by adding two to three drops of concentrated sulphuric acid into 1 ml of the extracted oil samples. The red colour shows the presence of quinones (Babatunde *et al.*, 2019).

E. Quantitative Analyses of Phytochemical in the Extracted Oils

After the qualitative screening of the phytochemical in the extracted oil samples, the quantitative analysis was conducted on these highly present phytochemicals.

1.) Determination of flavonoids

Flavonoids were repeatedly extracted from 10 g of the extracted oil sample with the aid of 100 ml of 80 % aqueous methanol at room temperature. A filter paper was used to filter the mixture into a pre-weighed 250 ml beaker. After that, the filtrate obtained was evaporated to dryness and weighed. The percentage of flavonoid was calculated by difference.

2.) Determination of terpenoids

The method described by Indumathi *et al.* (2014) with little modifications was used to determine terpenoid contents. Ethanol was mixed with ten (10 g) of the extracted oil sample and allowed to stand for 24 hours. The extract (terpenoids) was filtered, and the filtrate was extracted with petroleum ether using a separating funnel. The ether extract was treated as total terpenoids.

F. Shelf-life Study of the Extracted Oil

The oil extracted was kept inside plastic bottles and stored at room temperature for six months. Shelf-life stability of the extracted oil in terms of peroxide, saponification, iodine, and acid values was determined according to AOCS (2011) standard method at every two months interval.

G. Statistical Analysis

The data obtained were analyzed statistically in two ways. Analysis of Variance (ANOVA) and differences among the means were compared with Duncan New Multiple Range Test (DNMRT) at a 5 % ($p < 0.05$) level of significance.

III. RESULTS AND DISCUSSION

A. The Percentage Yield of Neem Seed Oil

Figure 1 shows the percentage of oil yield of neem seed paste samples using three different extraction methods. The extracted neem oil yield was 42, 49, and 62 % for cold water, hot water, and n-hexane extraction methods. It was observed that the cold water extraction method yielded the lowest extraction rate, while the n-hexane extraction method yielded the highest extraction rate. The high non-polarity index of n-hexane, which aided its molecules to penetrate faster through the neem seeds paste was observed to aid the high yield in the n-hexane extraction method (Yang *et al.*, 2014).

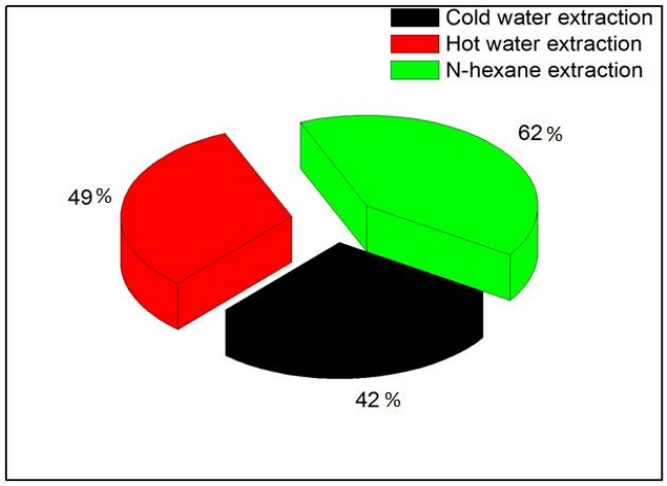

Figure 1: The percentage oil yield from neem.

Also, high yield with n-hexane could be due to the absence of OH group that have been reported to have an undesirable effect on the extraction process of some oilseeds as reported by Oladipo and Betiku (2019). On the other hand, the solubility of cold and hot water as polar solvents could have been affected negatively by the non-polar groups of the fatty acid chains or triglycerides of the oilseeds, which therefore led to lower oil yield when compared with n-hexane, a non-polar solvent. This observation corroborated what was reported by Russin *et al.* (2011) that higher polarity and dipole moment of a polar solvent such as water could have interfered with the solubility of the lipids thus, reduced the oil yield.

The higher oil yield of hot water compared to cold water was observed to be due to high temperature because at elevated temperature, oils become more soluble in hot water, thereby increasing the extraction rate (Tesfaye *et al.*, 2018). Although the yield of cold and hot water extraction methods was not as high as the n-hexane extraction method observed from the results obtained, it offers free solvent recovery, environmentally friendly, and recognized as safe.

B. The Chemical Properties of Neem Seed Oil

The chemical properties of the extracted neem seed oil as affected by the extraction methods and storage time is presented in Table 1. The peroxide value indicates the oxidative rancidity that has taken place in the extracted oil. The peroxide values of the fresh neem seed oil ranged from 6.30 to 8.99 Meq/kg. The samples were significantly different ($p \leq 0.05$), with sample C having the highest value (8.99 Meq/kg) follow by sample A (7.02 Meq/kg), while sample B had the least value (6.30 Meq/kg). These values corroborated 7.82 mg/kg, 8.49 Meq/kg, and ranged of 6.43 – 6.90 Meq/kg obtained for samples A (cold water extraction), B (hot water extraction), and C (n-hexane extraction), respectively as reported by Ikyenge *et al.* (2015), Jessinta *et al.* (2014), and Hamadou *et al.* (2020) for neem seed oils. There was a significant increase oil samples' peroxide values after 2, 4, and 6 months of storage compared to the fresh oil. The peroxide values increased from 10.92 to 13.04 to 25.56 Meq/kg, 7.67 to 17.71 to 26.76 Meq/kg, and 12.97 to 17.21 to 24.16 Meq/kg for Samples A, B, and C after respectively 2, 4, and 6 months storage time. The increase in the peroxide values with storage time could be due to the oxidation of peroxide over time, indicating the presence of oxygen in the oil. A similar increase in the peroxide value over time of storage was reported by Akin-Osanaiye *et al.* (2015) for palm oil.

The increase in the peroxide value over storage time can also be linked to the increase in the number of unsaturated fatty acids in the extracted oil since the rate of oxidation of fats and oils increases with increasing level of unsaturation (Ikyenge *et al.*, 2015). High peroxide value indicates lower stability to oxidative rancidity, while a low peroxide value is an indication of higher strength to oxidative rancidity (Ikyenge *et al.*, 2015). The increase in the peroxide values with storage time showed the oils' poor resistance to peroxidation during storage thereby, reducing the qualities of the oils. Generally, the results showed no significant difference ($p \geq 0.05$) in the extracted oils' peroxide values by the three methods after 6 months of storage. However, the qualities of the oils reduce with an increase in storage time.

Table 1: The chemical properties of neem seed oil.

Sample	Fresh	2 Months	4 Months	6 Months
Peroxide Value (Meq/kg)				
A	7.02 ± 0.35^{bz}	10.92 ± 0.42^{ay}	13.04 ± 0.40^{bx}	$25.56 \pm .91^{aw}$
B	6.30 ± 0.35^{cy}	7.67 ± 0.37^{cy}	17.71 ± 0.71^{ax}	26.76 ± 0.55^{aw}
C	8.99 ± 0.22^{ay}	12.97 ± 0.66^{ax}	17.21 ± 0.41^{ax}	24.16 ± 0.71^{aw}
Saponification Value (mg KOH/g oil)				
A	133.95 ± 0.71^{bz}	177.20 ± 2.04^{ay}	216.17 ± 0.94^{bx}	245.26 ± 0.38^{cw}
B	114.09 ± 0.49^{cz}	158.86 ± 1.37^{by}	241.26 ± 0.58^{ax}	288.09 ± 1.40^{aw}
C	141.11 ± 0.98^{aw}	178.01 ± 1.85^{ay}	218.14 ± 1.04^{bx}	250.12 ± 0.37^{bw}
Iodine Value (mg/wij's)				
A	51.69 ± 0.94^{aw}	43.27 ± 0.63^{bx}	12.34 ± 0.59^{by}	6.98 ± 0.01^{bz}
B	56.73 ± 0.39^{aw}	49.51 ± 0.02^{ax}	17.91 ± 0.23^{by}	7.88 ± 0.03^{bz}
C	54.87 ± 2.03^{aw}	32.19 ± 0.48^{cx}	18.96 ± 0.65^{ay}	9.51 ± 0.01^{az}
Acid Value (mg KOH/g oil)				
A	18.01 ± 0.51^{ax}	20.94 ± 0.31^{cx}	35.13 ± 0.34^{aw}	55.99 ± 0.48^{bw}
B	11.34 ± 0.12^{cz}	29.20 ± 0.53^{ay}	35.11 ± 0.43^{ax}	85.12 ± 0.97^{aw}
C	14.62 ± 0.59^{bz}	24.18 ± 0.51^{by}	32.98 ± 0.46^{bx}	56.88 ± 0.34^{bw}

a-c: Values (mean ± SE) with the same superscript in the same column are not significantly different ($p \leq 0.05$)

w-z: Values (mean ± SE) with the same superscript in the same row are not significantly different ($p \leq 0.05$)

Key: A = Cold water extraction
B = Hot water extraction
C = n-hexane extraction

The saponification values indicate the average molecular weight of triglycerides present in the extracted oils. The saponification values of the fresh neem seed oil ranged from 114.09 to 141.11 mg KOH/g oil. Sample C had the highest value 141.11 mg KOH/g oil follow by sample A 133.95 mg KOH/g oil, while sample B had the least value 114.09 mg KOH/g oil. These values were significantly ($p \leq 0.05$) lower than 213.18 mg KOH/g oil reported by Ikyenge *et al.* (2015), ranges of 194.48 – 202.04 mg KOH/g oil reported by Tesfaye *et al.* (2018), and 199.81 – 200.09 mg KOH/g oil reported by Hamadou *et al.* (2020) for neem seed oils. The variations could be due to varietal differences or cultivation climate and method of extractions. There was a significant increase in the oil samples' saponification values after 2, 4, and 6 months of storage compared to the fresh oil. The saponification values increased from 177.20 to 216.17 to 245.26 mg KOH/g oil, 158.86 to 241.26 to 288.09 mg KOH/g oil, and 178.01 to 218.14 to 250.12 mg KOH/g oil for Samples A, B, and C respectively after 2, 4, and 6 months storage time.

Generally, this increasing trend with a storage time of this oil showed that fatty acids could have been formed, which increased the saponification values, thus increasing the possible utilization of the oil in soaps and cosmetics productions. High saponification value implies the potential tendency to soap formations, and long-stored degraded oils are good for soaps and toiletry product productions (Akin-Osanaiye *et al.*, 2015).

The iodine value indicates the level of saturation and unsaturation of the extracted oil. The iodine values of the fresh neem seed oil ranged from 51.69 to 56.73 mg/wij's were not significantly different ($p \geq 0.05$). However, sample B had the highest value (56.73 mg/wij's) follow by sample C (54.87 mg/wij's) while sample A had the least value (51.69 mg/wij's). These values were significantly ($p \leq 0.05$) lower than 75.26 mg/wij's reported by Ikyenge *et al.* (2015), ranges of 63.81 – 93.09 mg/wij's reported by Jessinta *et al.* (2014), and 73.81 – 74.45 mg/wij's reported by Hamadou *et al.* (2020) for neem seed oils. The variations could be due to varietal differences or cultivation climate and method of extractions. A significant ($p \leq 0.05$) decrease in the samples' iodine value was observed as the storage period progresses compared to the fresh oil. The iodine values decreased from 43.27 to 12.34 to 6.98 mg/wij's, 49.51 to 17.91 to 7.88 mg/wij's, and 32.19 to 18.96 to 9.51 mg/wij's for Samples A, B, and C respectively at 2, 4, and 6 months storage time.

Generally, the decrease in the iodine values with storage time could be a result of the low content of unsaturated fatty acids present in the oil, which could make it suitable for the production of soaps, lighting candles, and lubricating oils that traditionally require fats or saturated oils (Abayeh *et al.*, 1998). These iodine values obtained indicate a high level of unsaturation of fats and oils in the neem seeds oil while the oils are classified as a non-drying oil. Oils with 125 iodine value and above are classified as drying oils, 110 – 140 are semi-drying oil, while less than 110 are non-drying oil (Gore, 2018). Also, because of the oil's non-drying nature, it might not be good in paints and coatings manufacturing industries except that it undergoes dehydration before employ (Abayeh *et al.*, 1998; Adebayo *et al.*, 2012).

The acid value of the extracted oil indicates the amount of free fatty acids present and the extent of oil degradation caused by hydrolysis. The acid values of the fresh neem seed oil ranged from 11.34 to 18.01 mg KOH/g oil. Sample A had the highest value, 18.01 mg KOH/g oil, follow by sample C, 14.62 mg KOH/g oil, while sample B had the least value, 11.34 mg KOH/g oil. These values were in line with 17.40 mg KOH/g oil reported by Ikyenge *et al.* (2015) and ranges of 14.46 – 18.05 mg KOH/g oil reported by Tesfaye *et al.* (2018); however, it was significantly ($p \leq 0.05$) higher than ranges of 8.98 – 9.16 mg KOH/g oil reported by Hamadou *et al.* (2020) for neem seed oils. The high acid values obtained for the fresh neem seed oil indicates that the neem seeds may already have been aged to some extent before their extraction (Efeovbokhan *et al.*, 2015). However, the variations with the other studies could be due to varietal differences or cultivation climate and method of extractions. Atinafu and Bedemo (2011) reported that the fatty acids that are majorly found in a triglyceride form might get hydrolyzed into free fatty acid, thereby increasing the acid value during the extraction process. However, the high acid value implies a decrease in oil quality.

There was a significant increase in the oil samples' acid value after 2, 4, and 6 months of storage compared to the fresh oil. The acid values increased from 20.94 to 35.13 to 55.99 mg KOH/g oil, 29.20 to 35.11 to 85.12 mg KOH/g oil, and 24.18 to 32.98 to 56.88 mg KOH/g oil, for Samples A, B, and C respectively after 2, 4, and 6 months storage time. The increase in the acid value with storage time was observed to be due to the disruption of the unsaturated fatty acids by hydrolysis or oxidation (Akin-Osanaiye *et al.*, 2015). The hydrolysis or oxidation that occurs over storage time could be caused by various agents like, the existence of moisture in the stored oil and lipase enzymes emanating from the source or contaminating microorganisms. This observation agrees with the past work that processing and storage conditions significantly affect the stored oils (Akin-Osanaiye *et al.*, 2015; Saba *et al.*, 2018).

C. Phytochemical Screening of Neem Seed Oils

The phytochemical screening of the extracted neem seed oils was presented in Table 2. The result showed that alkaloids, saponins, tannins, triterpenoids, steroids, anthocyanins, quinines, and emodins were negative (not present) in the extracted oil samples irrespective of the entreated methods. Triterpenoids and steroids presences were low, and coumarins were moderately present, while flavonoids and terpenoids were highly presented in all the extracted neem oil samples. Therefore, quantitative analysis was conducted on these highly present phytochemicals. Similarly, Babatunde *et al.* (2019) stated the presence of flavonoid, anthocyanin, quinones, and terpenoids, with the absence of alkaloid, saponins, glycosides, and phenols in neem seed oil. Also, Abdullahi *et al.* (2020) stated the presence of phenolics, flavonoids, and

anthraquinones, with the absence of alkaloids and saponin in the neem seed oil extracts. The variations in these phytochemicals' occurrence were observed to be due to difference in the varieties or cultivation climate and method of extractions.

Table 2: Phytochemicals in extracted neem seed oils.

Phytochemical	Moringa Hot Extraction	Moringa Cold Extraction	Moringa N-hexane Extraction
Alkaloid	--	--	--
Saponin	--	--	--
Flavonoid	+++	+++	+++
Tannins	--	--	--
Coumarins	++	++	++
Terpenoid	+++	+++	+++
Triterpenoid	+	+	+
Streoid	+	+	+
Anthocyanins	--	--	--
Quinones	--	--	--
Emodins	--	--	--

Key: --: Not Detected
+: Low Present
++: Moderately Present
+++: Highly Present

D. Quantitative Analysis of the Phytochemicals of Neem Seed Oils

Table 3 showed the results of the quantitative analysis of the extracted neem seed oils. The flavonoids of the fresh neem seed oils are 326.83 µg/ml, 272.56 µg/ml, and 165.13 µg/ml for Samples A, B, and C, respectively. A significant ($p \leq 0.05$) increase in the samples' flavonoid content was observed after 2 months of storage compared to that of the fresh oil. Sample A increased from 326.83 to 396.37 µg/ml; sample B rose from 272.56 to 286.47 µg/ml while Sample C increased from 166.13 to 171.31 µg/ml. However, the oil samples' flavonoid contents significantly ($p \leq 0.05$) decrease after 4 months of storage time compared to the result obtained after 2 months of storage time. The flavonoid contents decreased from 396.37 to 382.04 µg/ml, 286.47 to 262.84 µg/ml, and 171.31 to 163.27 µg/ml for samples A, B, and C respectively after 4 months of storage.

The flavonoid contents also indicate a slight increase after 6 months of storage but not significant ($p \geq 0.05$) than that of 4 months of storage time. The values have increased from 382.04 to 384.04 µg/ml, 262.84 to 264.37 µg/ml, and 163.27 to 163.79 µg/ml for samples A, B, and C respectively after 6 months of storage. The fluctuation in the flavonoid contents over storage time may be due to changes in the weather conductions. The presence of flavonoids in the extracted neem seed oil showed that the oil could serve culinary purposes, thereby possessing anti-inflammatory properties (Babatunde *et al.*, 2019).

The terpenoids of the fresh neem seed oils are 89.34 µg/ml, 147.50 µg/ml, and 137.92 µg/ml for Samples A, B, and C, respectively. There was a slight decrease in the oil samples' terpenoid contents at 2 and 4 months of storage compared to the fresh oil. The terpenoid contents decreased to 88.61 µg/ml and 83.07 µg/ml for sample A, 147.14 µg/ml and 145.89 µg/ml for sample B, and 135.88 µg/ml and 129.38 µg/ml for sample C.

Table 3: The phytochemical content of neem seed oil.

	Fresh	2 Months	4 Months	6 Months
Total flavonoids (ug/ml)				
A	326.83 ± 2.02^{ay}	396.37 ± 7.06^{aw}	382.04 ± 0.61^{ax}	384.04 ± 1.57^{ax}
B	272.56 ± 0.17^{bx}	286.47 ± 3.99^{bw}	262.84 ± 2.41^{by}	264.37 ± 1.92^{by}
C	165.13 ± 0.16^{cx}	171.31 ± 2.93^{cw}	163.27 ± 0.09^{cx}	163.79 ± 1.42^{cx}
Total terpenoids (µg/ml)				
A	89.34 ± 0.91^{cw}	88.61 ± 0.94^{cw}	83.07 ± 0.42^{cx}	84.07 ± 0.57^{cx}
B	147.50 ± 0.58^{aw}	147.14 ± 0.09^{aw}	145.89 ± 0.25^{aw}	147.68 ± 0.53^{aw}
C	137.92 ± 0.17^{bw}	135.88 ± 0.54^{bw}	129.38 ± 0.91^{by}	132.01 ± 0.27^{bx}

a-c: Values (mean ± SE) with the same superscript in the same column are not significantly different ($p \leq 0.05$)
z-w: Values (mean ± SE) with the same superscript in the same row are not significantly different ($p \leq 0.05$)

Key: A = Cold water extraction
B = Hot water extraction
C = n-hexane extraction

However, there was a slight increase at 6 months of storage compared to the values obtained at 4 months of storage time. The fluctuation in the flavonoid contents over storage time was also observed to be due to changes in the weather conductions. Based on the extraction methods, hot water extraction gave the maximum terpenoid contents while the cold water extraction method yielded the least amount of terpenoid contents; thus, the hot water extraction method is the adequate method among the methods employed in this study. The presence of terpenoids in the extracted neem seed oil explains why the oil could be used for dietary purposes, like, management of metabolic disorders caused by obesity and the treatment of diabetes in native medicine (Babatunde *et al.*, 2019).

Generally, the results obtained showed that storage time affected the oil sample's phytochemical content in different ways. The flavonoid contents increased, but the terpenoid contents decreased with storage time. However, the presence of these secondary metabolites (flavonoids and terpenoids) explains why neem seed oil is used to treat some illnesses. A similar observation was reported by Abdullahi *et al.* (2020) that the extract of neem seed oil contains some active compounds responsible for therapeutic and pharmacological properties that are known to show curative activity against several ailments in man.

IV. CONCLUSION

This research focused on the effect of extraction methods and storage time on the yield and qualities of neem seed oils. It can be concluded that:

(i.) The n-hexane extraction method gave the highest yield (62 %), next was hot water (49 %), while cold water gave the least yield, 42 %.
(ii.) The neem seed oils extracted might not be appropriate for the manufacturing of paint, varnishes, and surface coatings due to their non-drying attribute.
(iii.) The peroxide values were increasing with storage time, indicating that neem oil has low stability to oxidative

rancidity as storage time increased. The saponification and acid values increased while iodine values decreased with storage time, thus increasing the possible utilization of the neem oil in soaps and cosmetics productions.

(iv.) The presence of the secondary metabolites (flavonoids and terpenoids) showed that the extracted neem seed oils would be useful for treating some illnesses.

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