Extraction And Characterization Of Oil From Neem Seeds, Leaves And Barks

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ABSTRACT

The extraction and characterization of oil from Neem seeds, leaves and bark was studied. The results obtained showed that the moisture content of Neem seeds, leaves, and bark are 41.0%, 57.6% and 34.2% respectively, which indicates that Neem leaves possesses the highest amount of oil. Furthermore, the results from the characterization of the oils showed that Neem leaf oil is denser than those obtained from Neem seeds and bark, and so is the best for soap making. The results also indicated that the oil extract from Neem bark is the most acidic.

Key Words: Neem Seed, Neem Oil, Neem bark, Neem Leaves, Extraction, Characterization

1. INTRODUCTION

Plant oils are oils derived from plant sources, as opposed to animal fats or petroleum. Oils derived from plants have been used for thousands of years. Plant oils have been a healthy alternative to animal derived oils since their discovery. Plant oils are composed of compounds called *triglycerides*; which may exist in a highly unsaturated form or less**{1}** . Saturated compounds are compounds "saturated" with hydrogen; all available places where hydrogen atoms could be bonded to carbon atoms are occupied. Unsaturated compounds have double bonds (C=C) between carbon atoms, reducing the number of places where hydrogen atoms can bond to carbon atoms. Saturated compounds have single bonds (C-C) between the carbon atoms, and the other bond is bound to hydrogen atoms.

The three fatty acids in the first set of equations are usually different, but many kinds of triglycerides are known.

There are broadly three types of triglycerides viz: *Saturated*, *Monounsaturated* and *Polyunsaturated* triglycerides**{2}.**

In saturated triglycerides, most of the fatty acids are saturated.

In monounsaturated triglycerides, most of the fatty acids are monounsaturated; one pair of hydrogen atoms in the middle of the molecule is missing.

A polyunsaturated triglyceride has most of its fatty acids polyunsaturated. Polyunsaturated fatty acids are two or more pairs of hydrogen atoms short of saturation; two examples are omega-3 and omega-6 fatty acids (American Heart Association., 2010). However, unsaturated triglycerides are the main constituents of plant oils.

There are broadly two classes of plant oils viz: *Essential Oils* and *Fixed Oils.*

Essential oils are volatile, and are usually derived from the non-seed parts of the plants. Most fixed oils are the so-called "fatty oils", and a majority of the fatty oils are derived from the seeds – hence the term oilseeds, meaning oil-bearing seeds. Some of the fixed oils are derived from vegetables & nuts. Essential oils have been used for centuries. They have been used extensively in ancient Rome, Greece, Egypt & the Middle East – as perfumes, flavours, deodorants, antiseptics & pharmaceuticals. As a result of new processing technologies, they can today be used for more functions as well. Essential oils refer to the subtle, aromatic and volatile liquids extracted from the flowers, seeds, leaves, stems, bark and roots of herbs, bushes, shrubs and trees through distillation**{3}**. They are quite different from oils produced from oilseeds – that is soybeans, sunflower etc. and glands or hairs originating from epidermal cells. Essential oils are concentrated liquids containing volatile aromatic compounds. Essential oils are responsible for the aroma and flavor associated with herbs, spices, and perfumes. Also called volatile oils because they easily diffuse into the air where they are then detectable by our olfactory senses, essential oils are usually terpenoids, another large class of "secondary" chemicals.

Fixed oils are oils obtained from plants that are fatty, dense and non-volatile, such as olive and sweet almond oil. This is in contrast to essential oils which are volatile in nature. Some of the prominent fixed oils are almond oil, castor oil, coconut oil etc.

Materials and methods

2.2.1 *Apparatus*

2.2.1 *Pre-treatment of Samples*

Pre-treatment was carried out on samples prior to the extraction process to ensure maximum yield and purity of the extracted oil.

Mature Neem seeds, leaves and barks were gotten from the Abuja campus of the University of Port Harcourt. They were washed and sundried for 3 days and subsequently crushed mechanically via the use of a manual grinder to particulate sizes of 2mm to obtain a larger surface area. Finally, resulting samples were artificially dried using a tray drier at a temperature of 50°C for two hours.

2.2.2 *Extraction Procedure*

40g, 50g and 61.4g of neem seeds, leaves and barks respectively, were each weighed and put into the thimble of the Soxhlet extractor. 300ml of the solvent (ethanol) was measured with a measuring cylinder and poured into the still pot of the Soxhlet extractor, the apparatus was then coupled and the condenser unit was connected to an overhead water tank to cool rising solvent vapour. The heat source was a Bunsen burner operating at a temperature of 68°C. The solvent evaporated through the distillation path, thimble and the expansion adapter after which it condensed at the condenser unit of the Soxhlet extractor. At this point the condensed vapour returned to the thimble as liquid droplets and got in contact with the sample therein. It

then broke the sample membranes to release the oil content which accumulated with the solvent at the siphon (or reflux arm) of the Soxhlet extractor. When the solvent in the thimble rose to the level of the siphon top, the entire content of the thimble and siphon was emptied back into the still pot of the Soxhlet extractor (Fig. 2(e)). The process was repeated severally for about nine refluxes in 3 hours after which the extraction process was completed. Temperature was regulated using a thermometer.

2.2.3 ***Recovery of Extracted Oil***

After extraction, the resulting liquid was a mixture of the solvent used for extraction and the oil extract. The liquid was discharged into a Liebig condenser to separate the solvent from the oil extract. The mixture was distilled at a temperature of 68°C until the oil extract was completely free of the solvent. Diethyl ether was then used to purify the oil extract after which it was exposed to the atmosphere for a while to ensure elimination of the solvent odour.

2.2.4 ***Determination of Moisture Content***

The samples were weighed before and after drying in the tray drier oven and their initial and final masses were noted in each case respectively. The difference in mass gave the individual moisture contents by employing the formula:

$$Percentage\ Moisture\ Content = \frac{M_1 - M_2}{M_2} \times 100$$

Where, M_1 = Initial mass;
M_2 = Final mass

2.2.5 ***Determination of Percentage Yield***

The initial masses of individual samples were weighed and recorded before extraction (after oven drying) and after the extraction process. The mass of oil extract obtained was also weighed and the percentage yield calculated using the equation below:

$$Percentage\ Yield = \frac{Mass\ of\ oil\ extracted}{Initial\ mass\ of\ sample\ used} \times 100$$

2.2.6 ***Determination of Characterization Parameters***

Briefly highlighted in this sub-chapter are the procedures employed in characterizing the oil extract.

(i) Acid Value

- 5g of sample was accurately weighed and placed in a 250ml flask.
- 50ml of a mixture of equal volumes of ethanol and ether, which had been neutralized by 0.5N of potassium hydroxide, was added.
- The resulting mixture was heated for 10 minutes to allow for complete dissolution of the sample and then cooled.
- 1ml of phenolphthalein was added as indicator while shaking the contents vigorously.
- The mixture was titrated with 0.5N potassium hydroxide until a pink colour, which persisted for 15 seconds, was obtained.
- The entire procedure was repeated without the sample (blank).
- The acid value was calculated using the formula:

$$Acid\ Value = \frac{TD \times N \times 56.1}{M}$$

Where, TD = Titre Difference = $B - S$

B = Titre value blank; S = Titre value with sample

N = Normality of titrating solution (KOH used herein)

M = Mass of sample (g)

(ii) Peroxide Value

- 5g of sample was accurately weighed and placed in a 250ml flask.
- 30ml of glacial acetic acid-chloroform solution was added swirling the flask and carefully warming the mixture with an electric heating mantle until the sample was completely dissolved.
- 0.5ml of saturated potassium iodide solution was added and the contents swirled for exactly 1 minute.
- 30ml of distilled water was then added and the contents shaken vigorously to liberate iodine from the chloroform layer.
- 1ml of starch solution was added as indicator.
- The resulting mixture was titrated with 0.1N sodium thiosulphate until the blue-gray colour disappeared in the aqueous layer.
- The entire procedure was repeated without the sample (blank).
- The peroxide value was calculated using the formula:

$$Peroxide\ Value = \frac{TD \times N \times 1000}{M}$$

Where, TD, N, and M are as defined in (i) above.

(iii) Saponification Value

- 2g of sample was accurately weighed and placed in a 250ml flask.
- 25ml of a mixture of equal volumes of ethanol and potassium hydroxide was added.
- The mixture was heated in a water bath (coupled to a reflux condenser from the soxhlet extractor) for 30 minutes while being stirred continuously.
- 1ml of phenolphthalein was added as indicator.
- The resulting mixture was titrated with 0.5N hydrochloric acid.
- The entire procedure was repeated without the sample (blank).
- The saponification value was calculated using the formula as given in (i) above.

(iv) Iodine Value

- 0.2g of sample was accurately weighed and placed in a 250ml flask.
- 20ml of chloroform was added to the sample.
- 25ml of Wijs reagent was added with the aid of a pipette.
- The resulting mixture was stirred and stored in a dark place at 25°C for 30 minutes.
- 10ml of 30% potassium iodide was then added to the mixture as well as 100ml of distilled water.
- The mixture was titrated with 0.1N sodium thiosulphate until the yellow colour almost disappeared.
- 1ml of starch solution was then added and the mixture was titrated further until the blue starch-iodine colour disappeared.
- The entire procedure was repeated without the sample (blank).
- The Iodine value was calculated using the formula below:

$$Iodine\ Value = \frac{TD \times 1.269}{M}$$

Where, TD and M are as defined in (i) above.

(v) Free Fatty Acid (FFA)

The free fatty acid value is usually regarded as half the acid value of the oil extract and was obtained as such.

(vi) Specific Gravity

The specific gravity bottle was oven dried to remove existing moisture after which its mass (empty) was measured and recorded. It was then filled with 10ml of water and its mass measured and recorded. The specific gravity bottle was then filled individually with equal volume of each oil extract while its mass was measured and recorded in each case. The specific gravity of individual oil extracts were calculated using the formula below:

$$Specific\ gravity = \frac{W_1 - W_2}{W_3 - W_2}$$

Where, W_1 = Mass of specific gravity bottle + Oil extract

W_2 = Mass of empty specific gravity bottle

W_3 = Mass of specific gravity bottle + Water

(vii) Density

The density was obtained using the formula below:

$$\text{Density} = \text{Specific gravity} \times 1000\ \text{kg/m}^3$$

3. RESULTS AND DISCUSION

3.1 Comparison of Percentage Oil Yield by One-Way ANOVA

Oil Yield (%)

Sample / Run	Neem seed	Neem leaf	Neem bark
1st	35.2	39.0	26.7
2nd	34.5	36.0	27.8
3rd	____	38.2	27.7
Mean	**34.85**	**37.73**	**27.70**

In computing the value of CM (correction for the mean), we have:

$$\text{CM} = \frac{(\text{Total of all observations})^2}{(\text{Total number of observations})}$$

$$= \frac{(35.2 + 35.4 + 39.0 + 36.0 + 38.2 + 26.7 + 27.8 + 28.6)^2}{8} = 8844.5$$

In computing the value for SS(Total) (Total Sum of Squares), we have:

$$\text{SS(Total)} = (\text{Uncorrected Sum of Squares}) - \text{CM}$$

Uncorrected Sum of Squares

$$= (35.2)^2 + (34.5)^2 + (39.0)^2 + (36.0)^2 + (38.2)^2 + (26.7)^2 + (27.8)^2 + (28.6)^2$$

$$= 9009.22$$

Therefore;

$$\text{SS(Total)} = 9009.22 - 8844.5 = 164.72$$

In computing the value of SST (Treatment Sum of Squares), we have:

$$T_1 = 35.2 + 34.5 = 69.7$$

$$T_2 = 39.0 + 36.0 + 38.2 = 113.2$$

$$T_3 = 26.7 + 27.8 + 28.6 = 83.1$$

$$\text{Total Sum for each Treatments} = \frac{(69.7)^2}{2} + \frac{(113.2)^2}{3} + \frac{(83.1)^2}{3} = 9002.328$$

$$\text{SST} = 9002.328 - \text{CM} = 9002.328 - 8844.5 = 157.828$$

In computing the value for SSE (Error Sum of Squares), we have:

$$\text{SSE} = \text{SS(Total)} - \text{SST} = 164.72 - 157.828 = 6.892$$

Finally, in computing the values for MST (Mean Square of Treatments), MSE (Mean Square of Error) and their ratio, F (or ANOVAs Coefficient), we have:

$$\text{MST} = \frac{\text{SST}}{\text{k} - 1} = \frac{157.828}{2} = 78.914$$

$$\text{MSE} = \frac{\text{SSE}}{\text{N} - \text{k}} = \frac{6.892}{5} = 1.3784$$

$$\text{ANOVAs Coefficient F} = \frac{\text{MST}}{\text{MSE}} = \frac{78.914}{1.3784} = 57.25$$

Where, k = Number of Treatments = 3

N = Total number of observations = 8

The resulting ANOVA table is given as,

3.1.2 Results from ANOVA analysis

Source	SS	DF	MS	F
Treatment	157.828	2	78.914	57.25
Error	6.892	5	1.3784	
Total (corrected)	164.72	7		
Correction Factor	8844.5	1		

3.2 DISCUSION

Neem leaves possesses the highest oil yield with the mean percentage yield of 37.73% while that of Neem bark and seed are 27.70% and 34.85% respectively. This also corresponds with their moisture contents which showed that Neem leaf possesses the highest moisture content of 57.6% while that of Neem bark and seeds are respectively 34.2% and 41.0%. The oil extracts from Neem seeds, leaves and bark was also characterized. The densities of Neem oil from leaves, bark and seeds are 1108kg/m^3, 1029kg/m^3, and 1089kg/m^3, respectively. This indicates that the oil extract from Neem seeds is the best for soap making**{4,5}.** The acidic values of Neem seeds, bark and leaves were found to be 12.34, 13.46, and 10.56 respectively. The oil extract from Neem seeds has the highest iodine value of 7.61 which indicates that it is the most unsaturated**{6}.**

The coefficient obtained from ANOVA statistical analysis(57.25) is much larger than the critical value(5.786) as given by the F distribution table. This indicates that there is a significant difference among the means of the percentage oil yields.

CONCLUSION

The oil extracts obtained from various parts of the neem tree (seeds, leaves and barks) serve a variety of useful functions in several fields like medicine, agriculture and industry. The oil extracts obtained form a basis for the harnessing of the neem's vast potentials. By-products of the extraction process may also find applications in other areas (e.g. bio-fuel production), improving waste reduction and energy conservation efforts considerably.

The extraction process is solvent selective as certain solvents failed to extract the required solute while other solvents had greater affinity for some samples than others, hence the choice of ethanol as the optimal solvent.

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