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Proximate Analysis of *Monodora myristica* (Gaertn.) Dunal (African Nutmeg) in Ogun State, Nigerian

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Abstract

The study examines the proximate analysis of *Monodora myristica* in Ogun State of Nigeria. The analysis showed that the oil content is 25% which is considered fairly high and economical for extraction purposes; moisture content (10%), Ash (3.33%), protein (12%), crude fibre (8.33%) and carbohydrate (39.67%). The chemical characteristics of the oil are: Saponification value (418.95 mg/KOH/g), free fatty acid (34.55%), and iodine value (98.96%). The oil is not very good for human consumption. The proximate analysis of the seed coat showed the following values: Total ash (5%), Calcium (53.33%), Magnesium (15.6%). The seed coat can be used as livestock feed complement.

Key words: Saponification value, proximate analysis , Monodora mysristica, oil, and seed coat.

INTRODUCTION

FAO (1991) defined non-wood forest products as subsistence goods and series for human or industrial consumption derived from renewable forest resources and biomes bearing promise for augmenting real rural household incomes and employment. The products include the use of plants for food, frage, fuel, medicine, fibre, biochemical's as well as animals, birds, reptile and fishes for food, fur and feathers. Forest has been of innumerable values to man since pre-historic times. Apart from the major products got from the forest, such as timber, pole and firewood, other non-timber forest products include nut resins, gums and vegetables (Okeke *et al,* 2006). According to Burkill (1985), *Monodora mysristica* is a tree of the evergreen and deciduous forest to 35m high by 2m in girth, distributed from Liberia to W Cameroon and on to Uganda and Angola.The flowers are conspicuous attractive and scented. The bole is usually clear. The wood is white or greyish, hard, somewhat tough and does not split well. It is easy to work and is suitable for carpentry and turnery and walking sticks. The bark is used in the Ivory Coast to treat heamorrhoids, stomach-ache and febrile pains, and mixed with that of *M. tenuifolic* a collyrium is prepared for use in various eye-troubles. The preparation of a collyrium for treating filarial in the eye is recorded from Congo where the bark is also used in a vapour-bath as a defatigant and to relieve febrile lumbago, and the juice expressed from it to paint over itch. The fruit, subspherical, may attain 20cm length by 15cm diameter, and lends its pot shape to the English and French names, calabash nutmeg. The seeds are embedded in a white sweet-smelling pulp and are the most economically important part of the tree. These, as are the seeds of the other *Monodora spp*, are sold all over the West African region. They are aromatic and are used after grinding to a powder as a condition in food providing a flavour resembling that of nutmeg.

They are used as an aromatic and stimulating addition to medicines and to snuff. Ground to a powder they may be taken as a stimulant or stomachic or to relieve constipation, the powder may be sprinkled on sores, especially those caused by the guinea worm, or the powder fried and made up into oily pomade may be applied. Dusting or application of the pomade is used to disinfest from fleas and lice. The seeds chewed up are applied to the forehead for headache and for migraine in Gabon and ground up for headaches, rhino-pharygitis, or loss of voice, to apply on sores, or eaten as an anti-emetic aperative and tonic in Congo. Burkill (1985). The study therefore examines the proximate analysis of *Monodora myristica* in Ogun State, Nigeria.

Materials And Method

The Study Area

The study area is Ogun State. It was divided into four sub ethnic group viz: Egba, Yewa, Remo and Ijebu. Two major markes were selected from each sub-ethnic group: Egba (itoku and lafenwa) Yewa (Tube, Agosasa); Ijebu area was excluded for logistic reasons.

The vegetation of these areas is that of rainforest with uniform distribution of rainfall throughout the year with two peaks. The temperature ranges between 23^{0}C and averaging 12^{0}C. The people are Yorubas and they are mainly farmers and traders.

Phytochemical Screening:

The *Monodora myristica* seeds were bought from Itoku market, Abeokuta, Ogun State. The seeds were sun-dried and weighed. The seeds were then dehulled and the coat collected. The seeds (embryo) were ground to a powdery form to increase the surface area in order to enhance total extraction of the oil present in the seeds.

Oil Extraction

Material: ***Monodora myristica*** ground seeds:

Apparatus: Soxhlet extractor, water bath, distiller, conical flask, beaker and clamp.

Solvent: Hexane

Procedure: 60.0gm of powdered *Monodora myristica* seeds were weighed into a filter paper and the wrapped filter paper was placed inside the inner part of the soxhlet extractor. The apparatus was then fitted to a round bottom flask, which contained 200cm3 of hexane solvent. It was then attached to a reflux condenser. The set-up was clamped and heated on s water bath. After the extraction has been certified completed by the extracting solution being clear, the solvent was distilled off in the distillation set. The oil was then poured into a bottle and left for 5 days for the remaining solvent to evaporate. The oil was then weighed and the percentage oil content determined.

$$\% \text{ Oil yield} = \frac{\text{Weight of oil} \times 100}{\text{Weight of sample}} \%$$

% Residue = (100-oil yield) %

CHEMICAL ANALYSIS OF THE OIL

Saponification value determination reagents

Chloroform – analytical reagent grade 0.5m alcoholic potasium hydroxide prepared by dissolving 2.8g of KOH in absolute ethanol and made up to 100.0 cm^3 mark. 0.5m Hydrochloric acid – 10.75cm^3 of concentrated HCl was diluted with distilled water and made up to 250 cm^3 mark.

Procedure

1.0g of the oil was weighed into flask and 3.0cm^3 of chloroform added. Then 25.0cm^3 of the 0.5M ethanolic KOH was added and the solution then refluxed for 30 minutes with frequently shaking at an interval of 10 mins. 25.0cm^3 of the resulting solution while still hot titrated with the 0.5M HCl using phenolphthalein as indicator, until a colourless end point was reached. Another 25.0cm^3 of fresh ethanolic KOH was titrated against the 0.5M KCl using phenolphthalein as indicator. This served as blank. The difference between the blank and the sample reading gives the amount in cm^3 of 0.5m KOH required to saponify the 1.0 of oil.

$$\text{Saponification value} = \frac{(B-S) \times 28.50}{\text{Weight of oil}}$$

Where B = Blank titre value (cm^3)

S = Sample titre value (cm^3)

Free Fatty Acids Determination Reagents

Phenolphthalein indicator

0.1m NaOH - 0.4g of sodium hydroxide pellets were weighed and dissolved in 100.0cm^3 standard flask with little quantity of distilled water and made up to mark Absolute Ethanol.

Neutralized ethanol- About 150.0cm^3 of absolute ethanol was boiled for few minutes and 2 drops of phenolphthalein indicator added. Aqueous NaOH was added until the pink colour just discharged.

Procedure

1.0g of the soil was measured into a conical flask 50.0cm^3 of hot neutralized ethanol was added and the mixture was boiled in a water bath. While hot, the mixture was titrated with 0.1m NaOH until the pink colour appeared.

$$\text{Free fatty acids value} = \frac{\text{Volume of 0.1m NaOH X Factor X 2}}{\text{Weight of oil}}$$

Where = Factor of NaOH used.

Iodine Value Determination Reagents

Chloroform

10% potasium iodine solution – 10g of potasium iodine pellet was weighed into 100m volumetric flask and starch indicator-1.00g of starch was dissolved in 5.0cm^3 of distilled water in 100 cm^3 volumetric flask and made up to mark dissolved with some quantity of distilled water before making it up to the mark.

Darn's Reagent – 8.2 ml of pyridine was dissolved in 6cm^3 concentrated tetraoxosulphate (vi) acid in cooled 2cm^3 glacial acetic acid. The mixture was added to solution of 2.6ml Bromine in 20cm^3 acetic acid and was kept in the dark because of the poisonous gas produced from the preparation. 0.0125m sodium thiosulphate pellets was weighed into a volumetric flask and then dissolved with 200m^3 of distilled water up to the mark.

Procedure

0.5g of the oil sample was weighed and 5 cm^3 of chloroform solution was added. 5cm^3 of Darn's reagent was added and the mixture was kept in the dark cupboard for ten minutes, after which 5cm^3 of 10% potassium iodine solution and 20cm^3 of distilled water were added. The mixture was swirled several times and later titrated to a colourless end point with the 0.0125m $Na_2S_2O_5$ using 1% starch solution as indicator, added towards the end point of the titration. A blank titration was carried out containing all other things except the oil.

$$\text{Iodine value} = \frac{\text{(B-S) X 0.003175 X 40 X 100}}{\text{Weight of oil}}$$

0.003175g = 1cm^3 of 0.0125m $Na_2S_2O_3$

B = Blank titre value (cm^3)

S = Sample titre value (cm^3).

Free Fatty Acids Determination Reagents

Phenolphthalein indicator

0.1mNaOH – 0.4g of sodium hydroxide pellets were weighed and dissolved in 100.0cm^3 standard flask with little quantity of distilled water and made up to mark.

Absolute Ethanol

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Procedure

0.5g of the oil sample was weighed and 5 cm^3 of chloroform solution was added. 5cm^3 of Darn's reagent was added and the mixture was kept in the dark cupboard for ten minutes, after which 5cm^3 of 10% potassium iodine and 20cm^3 of distilled water were added. The mixture was swirled several times and later titrated to a colourless end point with the 0.0125m $Na_2S_2O_2$ using 1% starch solution as indicator, added towards the end point of the titration. A blank titration was carried out containing all other things except the oil.

$$\text{Iodine value} = \frac{(B-S) \times 0.003175 \times 40 \times 100}{\text{Weight of oil}}$$

0.003175g = 1cm^3 of 0.0125m $Na_2S_2O_2$
B = Blank titre value (cn^3)
S = Sample titre value (cm^3).

Analysis of the seed nutritional factors
Moisture Content Determination
Material: *Monodora myristica* seeds
Apparatus: Crucible, dessicator, oven and weighing balance.

Procedure
2.0g of the ground seeds were weighed into pre-weighed crucible. The crucible and the content were weighed again. This was then put in the oven at 150^{0}C for 5 hours after which it was removed, cooled and weighed until a constant weight was obtained.

$$\% \text{ Moisture content} = \frac{\text{Weight loss} \times 100}{\text{Weight of sample}}$$

Ash Content Determination
Material: *Monodora myristica* residue
Apparatus: Crucible, muffle furnace, Bunsen burner and dessicator.

Procedure:
2.0g of the residue was weighed into a pre-weighed crucible and burnt over a Bunsen burner flame until there was no more smoke.
The sample was then placed in the muffle furnace at 600^{0}C until it turned grey white. This was cooled in a dessicator and weighed to a constant weight.

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

Crude Fibre Determination

Material*: Monodora myristica*
Reagents: Tricholoacetic acid (TCA), methylated spirit
Apparatus: Funnel, reflux flask, filter paper heating mantle crucible, dessicator, muffle furnace, weighing balance.

Procedure:
2.0g of the residue were weighed into the reflux flask, 50.0cm^3 of TCA was added and the flask as heated to boil for 40 minutes on a heating mantle. It was then filtered. The residue obtained called the filter cake was washed with hot water six times followed by methylated spirit once. The filter cake was transferred into a pre-weighed crucible. The crucible was kept in the oven at 105^{0}C for 5 hours after which it was put in the dessicator to cool. The weight was determine, then it was put in the muffle furnace at 6000C for 4 hours to ash. The ash (grey white in colour) was coded in the dessicator and weighed to a constant weight.

$$\% \text{ Crude fibre} = \frac{\text{Weight of dry sample} \times 100}{\text{Weight of wet sample}}$$

Qualitative Analysis of the seed coat

Ash Determination

Material: *Monodora myristica* seed coat
Apparatus: Crucible, bunsen burner, muffle furnace, dessicator and weighing
balance.

Procedure:
3.0g of the seed coat was weighed into a pre-weighed crucible and ignited over the Bunsen burner flame. The crushed sample was placed in the muffle furnace set at 600^{0}C until it ashed. The sample was then cooked in a dessicator and weighed to a constant weight.

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

Calcium Content Determination
Material: Ashed sample content
Preparation of the sample: The ashed sample was dissolved in 1cm^3 concentrated HCI in a 250.0cm^3 volumetric flask and made up to mark with distilled water. This made the ash extract.

Reagents:
Ammonium chloride Buffer – prepared by adding 65.0cm^3 of 13.5m ammonia solution to 6.75g of Ammonium chloride crystal and diluted to 100.0cm with distilled water (pH 10).
0.2m Magnesium sulphate (MgSO4) solution prepared by dissolving 4.936g of MgSO4 with distilled water in a 100.0cm^3 flask and made up to mark with distilled water.
0.2M EDTA – 6.7242g of EDTA crystal was weighed into a 100.0cm^3 volumetric flask and made up to mark with distilled water.
0.2MgSO4 – EDTA – Equal volume of 0.2m MgSO4 and 0.2m EDTA were measured into a conical flask and mixed together. It was neutralized with 0.1m NaOH solution to a pH between 8 and 9.
Eriochrome Black Tn – 0.5(g) of Eriochtrome Black T crystal was dissolved in 100.0cm^3 ethanol in a volumetric flask.

Procedure:
5.0cm^3 of the ash extract was measured into a 250.0cm^3 conical flask, 2.0cm^3 of the 0.2M Mg-EDTA complex solution was added along with 2.0cm^3 of the NH4cl buffer solution and about 3 drops of Erichrome Black T indicator was added. The mixture was then titrated against the 0.2M EDTA solution. The procedure was repeated again.

Magnesium Content Determination
Material: Ashed sample extract
Reagents:
Ammonium chloride buffer
Erichrome Black indicator
0/2m EDTA solution

Procedure:
5.0cm^3 of the ash extract was measured into 250cm conical flask. 2.0cm^3 NH4cl buffer solution was added followed by about 3 drops of Erichrome Blacn T indicator. This was titrated with the 0.2m EDTA solution. The titration was repeated again.

Protein Content Determination
Material: Oil from *Monodora myristica*
Reagents:
Sodium Hydroxide solution – prepared by dissolving 20.0g of NaOH pellet in distilled water and made up to 1000cm mark with distilled water. Copper Sulphate solution: Prepared by dissolving 0.5g of CuSO4 pellet (salt) in distilled water and made up of 100cm^3 mark.
Sodium carbonate solution: prepared by dissolving 10g of $Na2CO_3$ salt in distilled water and made up to the 100cm^3 mark.

Potassium Sodium Tartrate – prepared by dissolving 1.0g of potassium sodium tartrate salt in distilled water and made up to the 100cm^3 mark.

Lowry's reagent: prepared by adding 50ml of $Na2CO_3$ and NaOH solution in ratio 1:1 to 5ml CuSO4 and potassium sodium tartrate solution in ratio 1:1.
Preparation of the sample – 1ml of the oil from *Monodora myristica* seeds was dissolved in 10ml of Hexane. The mixture was then filtered.

Preparation of the standard – these sets of standard solution were produced using different grams of the standard tables 0.1, 0.4 and 0.8g of the standard tablets were each placed in different test tube and dissolved with 10ml of distilled water by warming in water bath.

Procedure:
5ml of the Lowry's reagent was added to 5ml of the sample solution and the absorbance was measured at 750nm in the spectrophometer. The same way 5ml of the Lowry's reagent was added to 5ml of each of the standard solutions and the absorbance was also measured at 750nm. Note that the solutions should be left to stand for 10 minutes at room temperature before determining the absorbance.
A standard curve relating absorbance at 750nm to protein content should be prepared within the range of the protein concentration in the neutral sample (10-100Ng).

Carbohydrate Content Determination
This was determined by difference in percentage.

(% Carbohydrate = 100 - total weight of other nutritional factors) %.

RESULT

RESULT OF PHYTO-CHEMICAL ANALYSIS OF *MONORORA MYRISTICA* SEEDS
The result of the chemical analysis of the nutritional factors are summarised on table below;

Table 1: proximate Analysis of Seed Meal.

NUTRIENT	CONTENTS (%)
Oil	25.0
Moisture	10.0
Ash	5.0
Protein	12.0
Crude Fibre	8.33
Carbohydrate	39.67

CHEMICAL ANALYSIS OF *MONODORA MYRISTICA* SEED OIL

Table 2 gives the result of the various test carried on the oil

Table 2: Chemical Characteristics of *Monodora myristica* Seed Oil

TEST	VALUE
Saponification (mg/KoH/g)	418.95
Free fatty acid (%)	34.55
Iodine (%)	98.96

QUALITATIVE ANALYSIS OF THE SEED COAT

Table 3: Shows the result of qualitative analysis of *Monodora myristica*

PARAMETER	CONTENT (%)
Total Ash	3.33
Magnesium	15.6
Calcium	53.33

DISCUSSION

Proximate Analysis of *Monodora myristica*

The oil yield is low compared to that of *Jatropha curcas*, which is 36%, cocoa butter (45-56%), sheabutter (34-44%), (Godwin and Spensley, 1971). The oil yield is the same as that of Soyabean (25%) but is higher than that of *Brachestagia eurycoma* (3.33%) (Amiolemen, 1999). Therefore the production of oil from *Monodora myristica* will not be too expensive and hence economical. The moisture content obtained was 10%. This is low signifying that the seeds will dry and store well. This means that the dry matter is 90%. The ash content of 5.0% is very low though slightly lower than that of *Brachetagia eurycoma* (8.35%). This indicates that the mineral content is very low. The crude protein content was 12%. This is comparable to that of maize (12%). This is good value when compared with that of *Brachetagia eurycoma* (14%) and Cassava seed 18.81%. However, this is low when compared with soyabean meal which is 30-40% (Akinlawon, 1998).

The crude fibre content of 8.33% is average in quantity as revealed from the analysis. This implies that when this seed is incorporated into food, it will help to prevent many metabolic or digestive disorders such as constipation, irritable bowels etc, as this is the work of fibre in the body (Akinlawon, 1998). The percentage carbohydrate content is very high 39.67%, much higher than that of cassava seed (16.81%) (Akinlawon, 1998). Hence the seed is a good source of energy for animals when put in their feed and also for human beings if incorporated into diet. The saponification value of *Monodora myristica* is 418.95. Saponification value is the number of milligramme of alkali required to produce one gramme of fat. The larger the number, the smaller the chain length. This value is rather high indicating short chain fatty acid. Because of the high values, it is not economical for soap manufacture, as it will require a large quantity of KOH. In addition, the oil will not congeal. The free fatty acid is 34.55%. This is the amount of fatty acids that are free and not triglycerides. It is very high when compared with that of cassava seeds oil (5.8%) (Godwin and Spensley, 1971). Free fatty acid value is an important variable in considering the quantity of the oil, because the lower the free fatty acid values, the better the quality of the oil. The iodine value obtained for the oil was 98.96%. Iodine value measures the degree of unsaturation. It is the number of gram of iodine absorbed by 100g fat. The larger the number, the more the degree of unsaturation. It has medicinal value. The health improving effect of the unsaturated fatty acids has become widely known since about 1950 (FAO 1998). The percentage ash content obtained for *Monodora myristica* is 3.33%. This is low when compared with that of mango seed (13.3%) and cassava seed (11%). This indicates that the seed coat is not a good compliment of animal feed to provide calcium and magnesium for bones and teeth.

CONCLUSION

This work provides a basis for bio prospecting for drugs and chemicals. In addition, the *monodora myristica* seed contain oil in appreciable quantity, which can be used industrially.

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