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# Extraction, Physicochemical, Phytochemical Analysis And Identification Of Some Important Compounds Of Monodora Myristica (African Nutmeg) Seed Oil

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***Abstract:*** ***The oil from African nutmeg seed (Monodora myristica) also known as ehuru in Igbo or Ariwo in Yoruba was extracted using normal hexane. Physico-chemical analysis, phytochemical analysis and GC-MS was carried out from crude extract. Physico-chemical analysis indicated that Iodine value (4.318 mg), Peroxide value (10.1 meq/ kg), Acid value (0.784 mg KOH /g), Saponification value (246.1 mg KOH g$^{-}$), Refractive index (1.479), Specific gravity (0.968 g/m L) and % yield (36.04%). GC-MS analysis shed n-Hexadecanoic acid, Arachidonic acid, 9- Octadecanoic acid to be a major component of the seed oil. The results suggest that the oil is non drying oil; free from rancidity therefore it is good for cooking.***

## I. INTRODUCTION

*Monodora myristica* Dunal identified as African nutmeg, Calabash nutmeg or Jamaica nutmeg, a tropical tree of a perennial plant that belongs to the class of Annonaceae and it contains aromatic spice that function as a local nutmeg substitute. It is originate from evergreen and deciduous forest of tropical African countries. The plant can grow from 10-35 meters height and 2 meter width with striking and fragrance flow (Edak, Chukwudi and Peter, 2014). It is cultivated in India, Sri lanker and some African countries and also in southern part of Nigeria such as Anambra, Abia, Delta and Enugu state.

The plant is commonly called ehuru ofia in Igbo language, Ariwo in Yoruba. Other names include awerewa, lubushi, Ghana seed or orchid nutmeg. The aromatic seed is crushed into powder and use as food condiments. The seeds are entrenched in a white sweet smelling pulp of a fruit that can be 20cm long by 15cm in diameter (Burubai, Akor, Igoni, and Puyat, 2007).

*Monodora myristica* (African nutmeg) seed has an aromatic flavor and is a popular West African spice. The seed is harvested on maturation from the tree, air dry and store for usage. The leaves are purple in color at an early stage and later change to greenish color at maturity and are physically veined in nature.

*Monodora myristica* (African nutmeg) seed is used as a spice in assorted food such as vegetables, meat and puddings, hot soup with piper guineense (uziza) for newborn mothers to prevent hamorrhage (Onyenibe, Fowoke and Emmanuel, 2015). The addition of these two spices in food facilitate the flow of milk immediately after childbirth. The powdered seed is used for preparation of postpartum tonic after birth (Onyenibe, 2015).

The decayed leaves and branches are used as manure on the farm. The tree is used as firewood and carpentry work. The bark can be used to make necklace and rosaries in the olden

days. The seed is a stimulant which can be used in the treatment of constipation. The bark, when crushed can be used to cure stomach ache. It is added and use as a vapor bath in the treatment of febrile seizure. The seed is used as an insect repellent. The juice pressed from the bark is used in treating itching and wound, also combining it with the bark of Monodora tenuifolia to form lotion which is used in the treatment of various eye diseases. The African nutmeg seed extract can also be used in the treatment of headache, migraine and cold. The seed can be chewed and applied to sores, especially those caused by guinea worms.

*Monodora myristica* (African nutmeg) seed oil contain important compounds such as arachidonic, oleic, pinene and linoleic acid, which can be used industrially for the formulation of perfumes, soap and washing detergents. The oil can also be used as edible cooking oil. The seeds can be chewed and spat on a patient to cure rheumatism. Researchers has also revealed that the extract possesses anti inflammatory and antimicrobial properties against microorganisms and also lowers cholesterol in the human body.

The therapeutic and health promoting properties of the seed are well known since ancient times. The oil is rich in some compounds such as squalene and tocopherol as well as other valuable vitamins A and E. It contains fatty acids such as linoleic and oleic acid, which soothes and protects the skin against microbes. The report stated that the oxygen radicals in a living system are the required compounds in the process of maturation, cellular structures and white blood cells, which release free radicals to fight or destroy the pathogens or microbes as body defense mechanisms (Miguel, 2009). The seed is among the common species in Nigeria that are reported to have medicinal properties which are bactericidal and antibacterial (Irvine, 2000). The biological properties of essential oil from *Monodora myristica* are attributed to the presence of minor compounds like squalene, phytosterols and antioxidant compounds such as phenols and flavonoids (Owen *et al*., 2000). The essential oil from the seed has reported to be used in pharmaceuticals and dental preparation (Njoku, Ibeh, Iwualla, Aboh, Onwuliri, Akah, 1999). Application of the African nutmeg seed (*Monodora myristica*) seed in the food reduces human illness. (Enwereuzoh, Okafor, Uzoukwu, Ukanwoke, Nwakaudu, Uyanwa, 2015). Tocopherols present in *Monodora myristica* seed has nutritional value and antioxidant properties and also protect fat components from autoxidation.

## II. MATERIALS AND METHODS

### MATERIALS

5kg of African nutmeg seed (*Monodora myristica*) was purchased at Agbara market in Ogun State and certified at Herbarium in University of Lagos, Akoka, Nigeria.

### METHODS

#### *PREPARATION OF THE AFRICAN NUTMEG OIL (EXTRACTION)*

5kg of African nutmeg seed (*Monodora myristica*) were air dried at ambient temperature for five weeks. The seeds were crushed using a mechanical grinder. After grinding, it was transported to the laboratory where the oil was extracted with normal hexane as a solvent within 48 hours using soxhlet extraction unit. The oil mixed with the solvent was poured into a schlink flask, set up in a Rotary extractor at 40-50$^{o}$C until the volume was reduced. The oil, concentrated was stored in amber bottles to avoid oxidation. The oil sample obtained was subjected to experimental procedure.

### OIL EXTRACTION PROCEDURE

250 mL of n-hexane was used for the extraction of the oil from African nutmeg seed. 500 g of powdered seed samples was measured and wrapped in a filter paper, tie with rope to avoid blockage and placed in a soxhlet extractor with a boiling point of 40-60$^{o}$C The extraction was done bit by bit within 48 hours. The pure oil was obtained using a rotary evaporator set up at temperatures between 40-50$^{o}$C to remove the excess solvent. The oil was kept in amber bottles wrapped with black nylon to avoid undergoing oxidative rancidity.

#### *DETERMINATION OF PHYSICAL ANALYSIS*

The physical analyses of the seed oil were carried out ranging from colors, specific gravity, and refractive index.

#### *DETERMINATION OF PERCENTAGE (%) YIELD*

The percentage (%) yield of oil recovered after extraction was determined using this equation

$$\text{Percentage (\%) yield of oil} = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100$$

#### *DETERMINATION OF IODINE VALUE*

According to the method of AOAC standard described by (Sabbir, Rownok, Ashrafu,Khatun and Sherif, 2016), Iodine value was determined by Equivalent weight of iodine absorbed by 100 parts by weight of the oil as shown in the equation below:

$$\frac{\text{Eqv; weight of iodine X Volume of } Na_2S_2O_3 \text{ X Normality of } Na_2S_2O_3 \text{ X100 X10}^{-3}}{\text{Weight of the oil.}}$$

#### *DETERMINATION OF PEROXIDE VALUE*

This is done by measuring the weight of oil sample, dissolved in an acetic acid and chloroform. The saturated potassium iodide mixture was added to the sample. The quantity of iodide liberated was determined by titrating 0.1N of sodium thiosulphate using the starch solution as an indicator and blank titration was done as described by (Marinova, seizova, Totoseva, Panayatova, Marekov, Svetlana, 2012). Peroxide value was calculated using Equation 3.5.5 below;

$$\text{Peroxide value (meq/kg)} = \frac{(EPI - BLI) \times TF \times R}{\text{Wt of oil sample}}$$

Equation 3.5.5

Where

EPI = Titre value
BLI = Blank (0.00)
TF = Factor of Reagent
R = Constant (10)
Wt = Weight of sample (2.5g)

DETERMINATION OF ACID VALUE

The acid value was determined according to the Association of Official Analytical Chemist (AOAC) method. 5g of oil were measured into 250ml conical flask. 25ml of ethyl alcohol was measured and added to it, then allowed to boil on water bath. 1-2 drop of phenolphthalein indicator solution was added to the mixture and titrated against the standard potassium hydroxides solution. The pink color appeared and persisted for 30 seconds. The end point was noted. Acid value was calculated using Equation 3.5.6 below:

$$\text{Acid value} = \frac{\text{Volume of KOH x Morality of KOH x Equivalent}}{\text{Weight of oil (g)}}$$

Equation 3.5.6

*SAPONIFICATION VALUE*

Saponification value was determined by weighing 1 g of oil sample into a conical flask, 25 mL of 0.5M alcoholic KOH was added and heated under a condenser for 30-35 mins for the sample to completely dissolve. 2-3 drops of phenolphthalein indicator were added to the mixture after cooling and titrated against 0.2 M HCL until it turns to pink, the end point. A blank solution was prepared by the same procedure.

Saponification Value = Wt. of KOH x M x (Eq.wt of titrate x Volume of KOH in $moldm^3$)

Where
Wt. = Weight of KOH
M = Morality of KOH (56 g/Mol)

*PHYTOCHEMICAL SCREENING*

Phytochemical screening was carried out in Biochemistry laboratory, Covenant University, Ota, Nigeria, to test for the presence of tannin, saponin, flavonoids, alkaloids, betacynin, quinines, glycosides, cardiac glycosides, terpenoids and phenols.

*GC – MS ANALYSIS*

The fatty acid composition of the African nutmeg (*Monodora myristica*) seed oil was determined using GC-MS instrumental analysis. (GC –MS QP2010SE SHIMADZU, JAPAN).

## III. RESULT AND DISCUSSION

The Physio-chemical properties of African nutmeg seed oil (*Monodora myristica*) were represented as data in Table 4.1. The oil extracted from the seed has a pleasant odor and the color is golden yellow with a pH value of 6.48 which indicates that the oil is slightly acidic. The percentage yield of the oil is 36.04% and is higher than other seed oil analyzed as edible oil as reported by the American Oil Chemist Society (AOCS) 2001.

The specific gravity of the oil is 0.968 shows that the oil is less dense than water and it is in conformity with standard report of the Codex Standard for named vegetable Oils (CODEX- STAN 210- 1999). It is within the range of 0.86 - 0.96g/mL oil seed content as reported by Jauro *et al*., (2001). The less specific gravity reduces the mass and density of the oil during frying. The refractive index is found to be 1.479 which is equivalent to the refractive index of edible oil reported by the FAO/WHO (2009) as described by Adegbe *et al*., 2016.

| Physical Parameters | Values for oil |
|---|---|
| Color | Golden yellow |
| Odor | Pleasant Odour |
| % yield of oil | 36.04 % |
| Refractive index | 1.479 |
| Specific gravity | 0.968 g/Ml |

*Table 1: Physical properties of African nutmeg seed oil*

IODINE VALUE

The importance of Iodine value is to determine the amount of unsaturation contained in fatty acids present in the edible oil. The unsaturation shows the structure of the double bond and which react with iodine compound. It also determined the stability of oil to oxidation in other to determine qualitatively the degree of unsaturation. The Iodine value of the seed oil is 4.318 mg. This low value of Iodine value indicates that about 96.1% of fatty acid in the African nutmeg seed oil is saturated and have low C=C double bond. The lower the Iodine values the higher the oxidative storage facilities as described by Marinova *et al.,* (2012). As a result of the low Iodine value, the seed oil is recommended for cooking because of its resistance to Oxidation and polymerization.

PEROXIDES VALUE

Detection of peroxide value shows the evidence of oxidative rancidity in unsaturated fats and oil. It gives the measure of the extent to which an oil sample has undergone primary oxidation. The double bond present in fats and oil plays a fundamental role in autoxidation or oxidative rancidity. And this oxidative rancidity (autoxidation) is a free radical reaction involving oxygen that leads to the deterioration of fats and oil which forms off flavors and odors.

Concentration of peroxide in an oil or fat is useful for investigating the extent spoilage the oil has undergone. Hydro peroxides and aldehydes are responsible for the rancidity in the oil, thereby affecting the nutritional value of the food prepared with the oil. Another common chemical change that influences the flavor of the oil is lipid oxidation. This is as a result of polymerization of secondary oxidation products. It causes the oil to be dark in color, forming a form on the surface of the oil and increases the viscosity of the oil (Kaleem *et al*., 2015). The peroxide value is prepared by measuring the amount of iodine value produced by the reaction of peroxides formed in the oil with iodide ion.

$2I^- + H_20 + ROOH \rightarrow ROH + 2OH^- + I_2$

The iodide produced reacted with excess of acetic acid present. The Iodine liberated is titrated with sodium thiosulphate.

$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^-$

The peroxide value of the seed oil of African nutmeg is 10.1meq/kg, which is within the acceptable value of 10meq/kg approved by FAO/WHO (2009). It was observed that the oil is suitable for cooking.

ACID VALUE

In chemistry, acid value or number is the mass of potassium hydroxide (KOH) in milligrams required to neutralize one (1) gram of free fatty acid in the sample. It is a general parameters in the specification of fats and oil. It determine the amount of free fatty acid (FFA) and the purity of the oil. An increase in the amount of free fatty acids indicates the presence of hydrolysis of triglycerides and glycerol which causes rancidity of the oil. Such reaction occurred by the action of enzymes such as lipase. This is an indicator of inadequate processing and storage conditions such as high temperature and relative humidity. Free fatty acid is responsible for flavor and aroma in a food. The acid value of African nutmeg oil (*Monodora myristica*) is 0.784 mg KOH/g and this value is within the acceptable value of FAO/WHO (2009).

SAPONIFICATION VALUE

Saponification value represents the number of milligrams of potassium hydroxides (KOH) required saponify 1 g of fat or oil. It is also used to measure average molecular weight of all the fatty acids present. It is used to compare the average fatty acid chain. The shortest average chain of fatty acid, leads to high saponification value and low average weight of molecular weight of fatty acid (Ekwu and Nwagu, 2004).

The saponification value of the oil is 246.1 mg KOH $g^-$ which is comparable to the most vegetable oil such as coconut oil (255 mg KOH $g^-$) and palm kernel oil (247mg KOH $g^-$) (Ekeanyawu, Ogu and Nwachukwu, 2010). Therefore the oil obtained from African nutmeg oil contains low fatty acid and can produce a large quantity of KOH. The value obtained for saponification also indicates that the seed oil can be used by cosmetics industries for soap making.

| Chemical Parameter | Value for ANO |
|---|---|
| Iodine value | 4.318 mg |
| Peroxide value | 10.1meq/kg |
| Acid value | 0.784 mg KOH/ g |
| Saponification value | 246.1 mg KOH $g^-$ |

*Where ANO = African nutmeg oil and EO = Edible oil*

*Table 2: Some chemical properties of Monodora myristica (African nutmeg) seed oil*

PHYTOCHEMICAL SCREENING OF AFRICAN NUTMEG (MONODORA MYRISTICA) SEED OIL

The phytochemical screening of the oil extracted from African nutmeg seed is represented in Table 4.6. it shows the presence of quinone, cardiac glycosides, terpenoids, betacyanine and also in flavonoids which is responsible for the unique aroma of the oilseed. The flavonoids are made up of biochemical and pharmacological activities of mammalian systems. The terpenoid indicates that the oil extract of African nutmeg can be used as pest control. The presence of quinone indicates that it can be very effective against the growth of bacteria and metabolism in the human body. The presence of cardiac glycosides indicates that the oil can be used in the treatment of heart failure and irregular heartbeats. Betacynin can be used in place of synthetic food colorant to combat the health effect of synthetic colorant in the food (Henrietta *et al*., 2007)

The screening also revealed that African nutmeg oil has no tannin, saponin, carbohydrates, phenols and alkaloids. The absence of carbohydrates indicates that the oil will be good for diabetes patients and also reduces fat content in the human body.

| Phytochemical | Result |
|---|---|
| Carbohydrate | - |
| Tannin | - |
| Saponin | - |
| Flavonoids | + |
| Alkaloids | - |
| Anthocynin | - |
| Betacynin | + |
| Quinones | + |
| Glycosides | - |
| Terpenoids | + |
| Cardiac glycosides | + |
| Phenols | - |

*Table 3: Phytochemical screening of Monodora myristica (African nutmeg) seed oil*

GC – MS ANALYSIS

The GC-MS analysis carried out on the seed oil indicates the composition of the seed oil present. The chromatogram indicated the peaks plotted against the retention time.

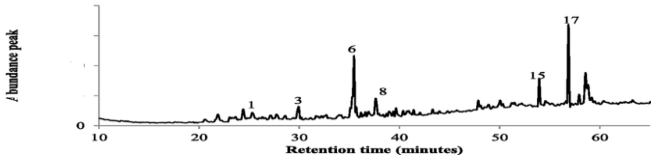


*Figure 1*

Represent the chromatogram of *Monodora myristica* (Africa nutmeg) seed oil.

| PEAK | RETENTION TIME | % AREA | COMPOUNDS |
|---|---|---|---|
| 1 | 18.973 | 6.01 | Cyclohexyl ester |
| 3 | 19.743 | 8.30 | Acetic acid |
| 6 | 20.927 | 13.37 | n- Hexadecanoic acid |
| 8 | 22.341 | 14.22 | Oleic acid |
| 15 | 23.70 | 25.60 | Estrane-3-one |
| 17 | 24.20 | 33.40 | Arachidonic acid |

*Table 4: Compound found in the seed oil*

## IV. CONCLUSION AND RECOMMENDATION

The present study reveals that the African nutmeg seed oil is suitable for cooking and it has satisfied the important food nutrient compare to some seed oil. The chemical analysis indicates that the oil has low fatty acid and saponification value which is economical in cosmetics industries. The phytochemical screening shows that the oil can be used to lower cholesterol and also cure heart disease and irregular heart beat. It can also be used as natural food colorant and as a stimulant in treatment of constipation.

The flavonoids are responsible for the distinct flavour of the oil and pharmacological activities of mammalian system. The terpenoids indicates that the oil extract of African nutmeg can be used as pest control. The presence of quinone indicates that it can be very effective against the growth of bacteria and metabolism in human. The oil can be use as one of the edible oils due to its importance and nutritional value compare to some of the seed oil.

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