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Research Article
Mineral and Vitamin Contents of *Monodora myristica* (African Nutmeg) Seeds from Nsukka, Enugu State, Nigeria

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Abstract
Background and Objective: *Monodora myristica* is a perennial, edible plant, widely used in many parts of the world as a spice for local delicacies and as a part of polyherbal formulations for disease management. To our knowledge, there is paucity of data on the nutritional content of *M. myristica* seeds from Nsukka, an urban town in Enugu State, Nigeria, with wide availability of the plant. This study was undertaken to determine the mineral and vitamin contents of *M. myristica* (African nutmeg) seeds from Nsukka, Enugu State, Nigeria. **Materials and Methods:** Seeds of *M. myristica* used were deshelled, oven-dried and ground into fine powder. The powdered sample was subjected to mineral analysis using atomic absorption spectrometry and the vitamin content was determined using spectrophotometry. **Results:** The mineral analyses showed that the seed had the highest calcium content (333.33±1.4 mg/100 g). Other minerals found were magnesium (182.99±0.03 mg/100 g), manganese (10.35±0.13 mg/100 g), sodium (5.47±0.31 mg/100 g), lead (0.00 mg/100 g), nickel (1.43 ±0.25mg/100 g), iron (90.0±0.01 mg/100 g), zinc (3.73±0.2 mg/100 g) and chromium (9.26±0.55 mg/100 g). Vitamin C had the highest vitamin concentration at 9.88±1.72 mg/100 g; the other concentrations were as follows: vitamin B_{12} (0.17±0.03 mg/100 g), vitamin B_3(0.68±0.14 mg/100 g), vitamin B_9 (0.22±0.04 mg/100 g), vitamin B_6 (0.54±0.07 mg/100 g), vitamin B_1 (5.63±0.92 mg/100 g), vitamin B_2 (0.82±0.65 mg/100 g), vitamin A (0.92±0.07 mg/100 g) and vitamin E (5.50±1.41 mg/100 g). **Conclusion:** The results showed that the seed is rich in minerals and vitamins and the levels vary compared to those found in the literature, which could be attributed to the different soil types and the nutritive state of the soil where the plant is cultivated.

Key words: African nutmeg, atomic absorption spectrophotometry, minerals, *Monodora myristica*, vitamins

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Monodora myristica is a perennial edible plant of the Annonaceae family that grows wild in the evergreen forest of West Africa[1] and is widely distributed from Africa to Asia, Central and South America[2]. *Monodora myristica* has the common names of African nutmeg and calabash nutmeg and in Nigeria, it is called “Ehuru” in Igbo, “Abolakoshe” in Yoruba and “Ebenoyoba” in Benin[3]. In addition, the bark, seeds and leaves are used in treating various ailments in African traditional medicine[4]. However, the most economically important parts are the plant is the seeds, which are embedded in the white, sweet-smelling pulp of the subspherical fruit[5]. The seeds have nutritive and calorific values that make them necessary in diets[6]. The kernel obtained from the seed has an aromatic fragrance, which makes it suitable as a spicing agent in both African and continental cuisines in Nigeria[7]. In eastern countries, the kernel is used as a drug more than as a condiment. *Monodora myristica* has a bitter pungent taste and is therefore used for bronchitis and to improve appetite. Following roasting and grinding, the seeds are rubbed on the skin for skin diseases[8], thus suggesting that the seeds of the *M. myristica* plant could be germicidal or antiseptic[9]. When ground to powder, the seed may be taken as a stimulant to relieve constipation and to control passive uterine hemorrhage in women immediately after child birth[10]. The essential oil from the seed is used in pharmaceutical and dental preparations[11]. The stem bark of *M. myristica* is used in the treatment of hemorrhoids, stomach aches, fever pains and eye diseases[12]. The nutrient contents of the seed from different locations have been reported[1,7,13-16]. To our knowledge, there have not been any reports on the seed from Nsukka and we hypothesize that the environmental conditions and the type and nutritive status of soil have an impact on the mineral and vitamin contents of the plant seeds. This study was therefore aimed at determining the mineral and vitamin contents of *Monodora myristica* seeds from Nsukka, Enugu State, Nigeria, to compare the availability and levels with those obtained from other parts of the world, as reported in the literature.

MATERIALS AND METHODS

Plant material: Seeds of *M. myristica* used for this study were procured from a habitat at Ibagwa in Nsukka, Enugu State, Nigeria and were identified by Mr. Alfred Ozioko, a certified taxonomist at the Bioresources Development and Conservation Programme, (BDCP), Nsukka, Enugu State, Nigeria.

Chemicals and reagents: All chemicals used in this study were of analytical grade and were products of May and Baker (England). Reagents used for the analysis were all Sigma products (USA).

Determination of mineral and vitamin contents of *Monodora myristica* seeds: The fresh, matured seed (500 g) were washed, deshelled, oven-dried at 40°C and ground into fine powder, which was subjected to mineral and vitamin analyses. The mineral contents of the sample were determined using an atomic absorption spectrometer (AAS) (Model AA-7000, Shimadzu, Japan) as follows.

Procedure for determining mineral content: The powder of *M. myristica* seeds was digested (wet digestion method). The powdered sample (1 g) was weighed in a digestion flask and 10 mL of HNO_3/H_2SO_4 mixture was added and digested in a fume cupboard at 150°C until a clear solution was obtained. The solution was cooled and filtered. The filtrate volume was brought to the 50 mL mark in a standard volumetric flask with deionized water. The remaining residue was dissolved in hydrochloric acid. The AAS was set up to measure the absorption from the hollow cathode lamp of each of the cations using an acetylene/air flame. The appropriate wavelength for each mineral corresponding to the lamps was set. The working standard solution of each of the cations being measured was nebulized and the controls were adjusted until steady zero and suitable maximum readings were obtained. The intermediate standards were nebulized and a graph relating galvanometer reading to $\mu g\ mL^{-1}$ of cation in all the standard solutions was constructed. The concentration of each cation was read from the standard graph equivalent to the galvanometer readings of solutions and blank solutions. The vitamin contents of the powdered *M. myristica* seeds were determined using modified methods[17] and the analyses were performed in triplicate (n = 3).

Determination of vitamin A concentration: Koche[18] method was used. *Monodora myristica* seed powder (1 g) was macerated with 200 mL of petroleum ether for 10 min and allowed to stand for 1 h with intermittent shaking every 1 min. The mixture was centrifuged for 5 min and 3 mL of the supernatant was transferred into triplicate test tubes. Each supernatant in the test tube was evaporated to dryness and the residue was redissolved in 0.2 mL of acetic anhydride/chloroform (1:1) and 2 mL of 50% trichloroacetic acid (TCA) in chloroform. The absorbance of the resulting solution was then taken at a wavelength of 620 nm at 15 and 30 sec against the corresponding blank. The vitamin concentration of the samples was calculated as follows:

$$\text{Vitamin A content (Koche}^{18}) = \frac{\text{Absorbance of test} \times \text{Concentration of standard}}{\text{Absorbance of standard weight of sample}}$$

Determination of vitamin C (ascorbic acid) concentration: The AOAC[17] method was used. *Monodora myristica* seed powder (1 g) was macerated with 20 mL of 0.4% oxalic acid for 10 min and centrifuged for 5 min. The supernatant (1 mL) was transferred into triplicate test tubes to which 9 mL of 2,6-dichlorophenol indophenol (12 mg L^{-1}) had been previously added and then mixed thoroughly by shaking. The absorbance of the resulting solution was taken at 520 nm at 15 and 30 sec against the corresponding blank. The vitamin concentration of the samples was calculated thus;

$$\text{Concentration of vitamin C (AOAC}^{17}) = \frac{\text{Abs} \times \text{Df} \times \text{volume of curvette}}{\text{E}}$$

Where
Abs = Absorbance
Df = Dilution factor
E = Extinction factor

Determination of vitamin E (α-tocopherol) concentration: The AOAC[17] method was used. *Monodora myristica* seed powder (1 g) was macerated with 20 mL of petroleum ether for 10 min and allowed to stand for 1 h with intermittent shaking every 1 min and centrifuged for 5 min. Supernatant (3 mL) was transferred into triplicate test tubes, evaporated to dryness and the residue redissolved in 2 mL ethanol and shaken. A known volume, 1 mL of 0.2% ferric chloride in ethanol and 1 mL of 0.5% α-dipyridyl in ethanol, was added to the resulting solution and then made up to 5 mL with ethanol. The mixture was thoroughly shaken and the absorbance of resulting solution was taken at a wavelength of 520 nm against the corresponding blank. The vitamin concentration of the samples was calculated as follows:

$$\text{Concentration of vitamin E (AOAC}^{17}) = \frac{\text{Abs} \times \text{Df} \times \text{volume of curvette}}{\text{E}}$$

Where
Abs = Absorbance
Df = Dilution factor
E = Extinction coefficient

Determination of vitamin B_1 (thiamine) concentration: Koche[18] method was used. *Monodora myristica* seed powder (1 g) was homogenized with 50 mL of ethanolic sodium hydroxide solution and filtered into a 100 mL flask. Filtrate (10 mL) was pipetted into a beaker and 10 mL potassium dichromate was added for color development. A blank sample was prepared and the absorbance was taken at 560 nm. The concentration of each sample was extrapolated from a standard curve.

$$\text{Vitamin } B_1 \text{ content (Koche}^{18}) = \frac{\text{Absorbance of sample} \times \text{Concentration of standard}}{\text{Absorbance of standard weight of sample}}$$

Determination of vitamin B_2 (riboflavin) concentration: Koche[18] method was used. *Monodora myristica* seed powder (1 g) was extracted with 100 mL of 50% hydrogen peroxide and allowed to stand for 30 min. Thereafter, 2 mL of 40% sodium sulfate was added to bring the solution volume to the 50 mL mark. The absorbance at a wavelength of 510 nm was read in a spectrophotometer. The vitamin concentration was calculated as follows:

$$\text{Vitamin } B_2 \text{ content (Koche}^{18}) = \frac{\text{Absorbance of sample} \times \text{Concentration of standard}}{\text{Absorbance of standard weight of sample}}$$

Determination of vitamin B_3 (niacin) concentration: Koche[18] method was used. *Monodora myristica* seed powder (1 g) was added to 50 mL of sulfuric acid and shaken for 30 min. Thereafter, 3 drops of ammonia solution was added to the mixture and filtered. Potassium cyanide (5 mL) was added to 10 ml of the filtrate in a 50 mL volumetric flask and the mixture was acidified with 0.02 M H_2SO_4. The absorbance was read at a wavelength of 470 nm in a spectrophotometer. The vitamin concentration was calculated as follows:

$$\text{Vitamin } B_3 \text{ content (Koche}^{18}) = \frac{\text{Absorbance of sample} \times \text{Concentration of standard}}{\text{Absorbance of standard weight of sample}}$$

Determination of vitamin B_6 (pyridoxine) concentration: The AOAC[17] method was used. *Monodora myristica* seed powder (1 g) was extracted with 500 mL of distilled water for 1 h and filtered. Then 2 mL of distilled water, 0.4 mL of 50% sodium acetate, 0.1 mL of diazotized reagent and 0.2 mL of 5.5% sodium carbonate were added to 1 mL of the filtrate and mixed thoroughly. The absorbance of the solution was read at a wavelength of 540 nm. The vitamin concentration was calculated as follows:

$$\text{Concentration of vitamin } B_6 \text{ (AOAC}^{17}) = \frac{\text{Abs} \times \text{Df} \times \text{volume of curvette}}{\text{E}}$$

Where

Abs = Absorbance
Df = Dilution factor
E = Extinction coefficient

Determination of vitamin B_9 (folic acid) concentration: The AOAC[17] method was used. *Monodora myristica* seed powder (1 g) was weighed into a beaker and extracted with 100 mL of distilled water with slight heat. The mixture was shaken thoroughly and filtered after cooling. The absorbance of the filtrate was read spectrophotometrically at a wavelength of 325 nm. The vitamin concentration was calculated as follows:

$$\text{Vitamin } B_9 \text{ content (AOAC}^{17}) = \frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample}}$$

Determination of vitamin B_{12} (cobalamin) concentration: Koche[18] method was used. *Monodora myristica* seed powder (1 g) was macerated with 50 mL of distilled water for 5 min and filtered. The filtrate (2 mL) was taken and the pH was adjusted between 9.5-10 using 10% NaOH. Sodium cyanide (0.1 g) was added to the solution and then allowed to stand for 5 h. Sodium sulphate (1 g) was added and the pH of the mixture was adjusted between 11 and 11.5. A volume of 2 mL of benzyl alcohol extract was added and the aqueous layer was discarded. To the benzyl alcohol layer, 3 mL of chloroform was added and centrifuged for 5 min. Then, 10 mL of distilled water was added to the organic layer. A volume of 5 mL of the solution was taken, 1 mL of 10% sodium cyanide was added and the pH was adjusted between 5-6 using a 12.5% potassium dihydrogen phosphate solution. The absorbance was read at 582 nm.

$$\text{Vitamin } B_{12} \text{ content (Koche}^{18}) = \frac{\text{Absorbance of test} \times \text{Dilution factor}}{\text{Absorbance of standard volume of sample use}}$$

Statistical analysis: Data obtained from the laboratory were analyzed using one-way analysis of variance (ANOVA) in IBM Statistical Product and Service Solutions (SPSS), version 18. The results were expressed as the Mean±standard deviation (SD) and are presented in tables.

RESULTS AND DISCUSSION

The present study evaluated the mineral and vitamin contents of *M. myristica* seeds, which are a highly consumed spice in Africa. The results obtained from this study, as shown in Table 1, revealed that calcium is the most abundant mineral in the seed, with a concentration of 333.3 mg/100 g. This finding is comparable to the observations of Bouba *et al.*[16], who reported a calcium content of 375 mg/100 g and Uhegbu *et al.*[14], who reported a calcium content of 297 mg/100 g in the seed. Meanwhile, Ekeanyanwu *et al.*[13] and Enabulele *et al.*[19] reported calcium contents of 416.0 mg/100 g and 421.84 mg/100 g, respectively, in the seed. Other studies found lower calcium contents in the seed: 184.46 and 15.92 mg/100 g were reported by Okonkwo and Ogu[15] and Ameh *et al.*[20], respectively. These differences in calcium concentration might be due to the stage of maturity of the seed used, the type and nature of the soil in which the plant was grown and the mode of seed processing. Calcium is needed for regulating most internal organs, including the heart and liver and it is needed for the integrity of most physiological function including normal functioning of the heart and, skeletal system and of cell membranes, blood clotting, nerve-signal transmission and regulation of enzymes and hormones[21]. Calcium is required in the diet in an amount of 100 mg or more per day, which implies that less than 100 g of this seed is needed to provide the recommended daily intake. Calcium deficiency in the body leads to malfunctioning of organs and to conditions such as osteoporosis, osteopenia and calcium deficiency diseases (hypocalcemia).

Table 1: Mineral contents of powdered *Monodora myristica* seeds

Mineral	Concentration (mg/100 g)
Manganese (Mn)	10.35±0.13
Magnesium (Mg)	182.99±0.03
Sodium (Na)	5.47±0.31
Lead (Pb)	0.00
Nickel (Ni)	1.43±0.25
Iron (Fe)	90.00±0.01
Chromium (Cr)	9.20±0.55
Calcium (Ca)	333.33±1.4
Zinc	3.73±0.2
Potassium (K)	89.17±0.1

Values are the Mean±SD (n=3)

The magnesium content of the seeds obtained in this study (182 mg/100 g) is comparable with the previously obtained result (132 mg/100 g)[16]. However, the magnesium content of *M. myristica* seeds obtained in this study is twice as high as that reported by Ameh *et al.*[20] (64 mg/100 g), Ekeanyanwu *et al.*[13] (56.32 mg/100 g) and Okonkwo and Ogu[15] (64.52 mg/100 g). The variations in magnesium content reported in these studies might be linked to the differences in the method of analysis and environmental factors associated with the plant source. Magnesium is required in the plasma and extracellular fluid where it helps maintain osmotic equilibrium. Additionally, magnesium is required in many enzyme-catalyzed reactions and prevents some heart disorders and low blood pressure. Magnesium and potassium support human biochemical processes and activate enzymes necessary for carbohydrate metabolism[21]. A lack of magnesium is associated with abnormal irritability of muscles.

Another major mineral reported in this study is potassium, with a concentration of 89.17 mg/100 g, as shown in Table 1. This finding is comparable with the previously obtained result (79.7 mg/100 g)[14]. However, Enabulele *et al.*[19] found a very high potassium content (800.2 mg/100 g) in the seeds and Ameh *et al.*[20] reported a lower potassium content (50.22 mg/100 g) than that in the present study. The differences in mineral composition might be due to differences in soil characteristics and climatic conditions in the areas where the seeds were cultivated. The analysis revealed that *M. myristica* seeds contain sodium (5.47 mg/100 g) which agrees with the levels of 9.1 mg/100 g[16] and 9.41 mg/100 g[20] previously reported. Other reports had varied reports regarding sodium contents, such as 0.13 mg/100 g[22] and 42 mg/100 g[14]. Sodium and potassium regulate water balance, muscle contraction and nerve-signal conduction. The Na/K ratio result of the present study is 0.061. The ratio of Na/K in the body is of great concern for the prevention of high blood pressure. For proper functioning of the body, the ratio should be less than 1 (< 1). This value is recommended to regulate normal body pH for muscle movement and nerve stimulation. A balanced Na/K ratio controls glucose absorption and enhances the normal retention of protein during growth; it also influences glucose and lipid metabolism. However, extremely high sodium intake has been associated with fluid retention leading to hypertension, heart failure and instant death[21].

The iron content of the seeds as reported in this study was 90 mg/100 g. This content is higher than those reported by other studies: (31.83 mg/100 g)[22], (21.71 mg/100 g)[13], (36.7 mg/100 g)[14], (3.16 mg/100 g)[15] and (1.90 mg/100 g)[20]. These deviations in results might be the effect of the edaphic factor where nutrients determined are not exclusively those taken up by the plants. Iron is necessary for oxygen transport and plays an important role in human metabolism and it facilitates the oxidation of carbohydrates, proteins and fats to control body metabolic rate, which is a very important factor in diabetes. Iron deficiency leads to anemia and such conditions can present severe complications and adverse consequences for expectant mothers. The zinc content observed in this study was 3.73 mg/100 g, which is in line with reports on ginger (4.99 mg/100 g)[23], *Xylopia aethiopica* (3.8 mg/100 g)[16] and *Mondia whitei* (3.7 mg/100 g)[16]. This finding shows that the zinc contained in most spices is relatively small in quantity; hence, the body contains only a small quantity of a biologically active pool of zinc. Therefore, a dietary supply of zinc is continually needed[24]. Zinc is involved in ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) synthesis needed for cell division and cell growth. A lack of zinc in the body causes rapid suppuration on the surface of wounds and may delay quick healing[21]. The Manganese content obtained in this study was 10.35 mg/100 g, which is in accordance with previous reports (11.57 mg/100 g)[22] and (10.90 mg/100 g)[16]. Manganese helps the body form connective tissue, bones, blood clotting factors and sex hormones[25].

Lead was not detected in the seeds of *M. myristica,* which is in accordance with previous studies where lead was not detected[13] or was present at a very low concentration (0.001 mg/100 g)[14]. Recently, lead and cadmium were listed by the Agency for Toxic Substances and Disease Registry[26] as the second and seventh priorities of toxic substances, respectively and the accumulation of these important hazardous metals causes cell damages[27]. The absence of these metal elements in these seeds makes them relatively safe for consumption.

In the vitamin analysis, the concentration of vitamin C (9.88 mg/100 g) was the highest, as reported in Table 2. Another study obtained a higher vitamin C content (243.43 mg/100 g)[15]. The low concentration of vitamin C content recorded in the present study could be attributed to the effect of heat during seed processing. Sun-drying has been reported to cause a marked decrease in the vitamin content of food material[28]. Vitamin C is mainly used for the synthesis of collagen, a major protein for building connective tissues[29] and is a general antioxidant that enhances iron absorption and is needed for synthesizing some hormones and neurotransmitters[21]. Vitamin C maintains blood vessel flexibility and improves circulation in the arteries of smokers; it also acts as an antioxidant in the body system where it scavenges oxygen free radicals, which are by-products of many of the normal metabolic processes in the body. Vitamin C deficiency results in scurvy, which is evidenced by poor wound healing, pinpoint hemorrhage in the skin and bleeding of the gums[30]. Vitamin B_2 content of the seeds, as

Table 2: The vitamin contents of *Monodora myristica* seed powder

Vitamins	Vitamin content (%)
Vitamin A	0.92±0.07
Vitamin B_1	5.63±0.92
Vitamin B_2	0.82±0.65
Vitamin B_3	0.68±0.14
Vitamin B_6	0.54±0.07
Vitamin B_9	0.22±0.04
Vitamin B_{12}	0.17±0.03
Vitamin C	9.88±1.72
Vitamin E	5.50±1.41

Values are the Mean±SD (n = 3)

reported in this study (0.82 mg/100 g), is not in line with the previous studies, which reported (0.06 mg/100 g)[22] and (0.034 mg/100 g)[15]. Additionally, the vitamin B_3 content reported in this study (0.68 mg/100 g) is comparable with that from one previous study (0.75 mg/100 g)[25] but not in close agreement with other findings, (2.95 mg/100 g)[22] and (0.013 mg/100 g)[15]. Thiamine, riboflavin and niacin play key roles as coenzymes in energy-yielding metabolism. The recommended dietary allowance (RDA) is 1.1-1.2 mg for thiamin, 1.1-1.3 mg for riboflavin and 14-16 mg for niacin. A deficiency of the three vitamins may result in brain damage, poor nervous coordination and disorder in the gastrointestinal track of affected persons[24, 31].

CONCLUSION

The *M. myristica* seed is very rich in minerals and vitamins. The appreciable antioxidant vitamin and mineral contents suggest potential benefits to health. These findings also showed that the nutritional contents of *M. myristica* seeds vary based on environmental conditions, the nutrient status of the soil where the plant is grown and analytical procedures.

SIGNIFICANCE STATEMENT

This study discovers that *M. myristica* (African nutmeg) seeds from Nsukka, Enugu State, Nigeria, contain high mineral content, non-detectable amount of heavy metals and an appreciable amount of vitamins. This study will help researchers in area of micronutrient deficiency by informing them that the seed can supply minerals needed to make supplements which can minimize the prevalence of disorders related to micronutrient in our rural settings. It also supported the view that nutritional content of plant materials depend on the soil where it is obtained.

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