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The Mineral, Amino Acid and Fatty Acid Evaluations of *Myristica fragrans* Seeds Extracts †

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Abstract: The mineral elements, amino acid constituents and fatty acids present in *Myristica fragrans* seeds extracts were identified and quantified using atomic absorption spectrometry and flame photometry, Technicon sequential multi-sample amino acid analyzer (TSM) and Shimadzu GC-MS machine respectively. Six hundred grams of powdered seeds were extracted in methanol and n-hexane (2:1 *v*/*v*) using a rotary evaporator. Calcium, magnesium, and iron were the most abundant in methanol extract with concentrations of 3.21 ± 0.05 part per million (ppm), 3.00 ± 0.05 ppm, and 1.00 ± 0.02 ppm respectively. The major essential amino acids in the seed were leucine 6.24 g/100 g, valine 3.72 g/100 g, and threonine 3.50 g/100 g while the non-essential amino acids were glutamate 10.6 g/100 g, aspartate 7.60 g/100 g, and arginine 5.50 g/100 g. The major biological compounds in the methanol extract as revealed by the GC-MS analysis were 9,12-Octadecadienoic acid methyl ester (RT: 20.768, 27.13%), Cyclododecyne (RT: 26.458, 19.33%) and octadecanoic acid (RT: 14.360, 12.24%) while the hexane extract constituents were margarinic acid (RT: 14.746, 27.04%), oleic acid (RT: 20.947, 18.96%) and 9,12-octadecadien-1-ol (RT: 26.523%, 15.10%). These compounds have shown nutritional, pharmacological, and medicinal importance in different studies.

Keywords: mineral elements; calcium; *Myristica fragrans*; amino acids; fatty acids

1. Introduction

Myristica fragrans (nutmeg) are known to be native of the Banda Islands (formerly known as the Spice Islands) tiny archipelagos in Eastern Indonesia (Moluccas). Malaysia and Grenada have been known to be producers of the seed. It is also known to be cultivated in China in Guangdong, Guangxi, and Yunman provinces. Grenada nutmeg is also exported to other countries but mainly to the USA [1].

The seed of the plant also known as "nutmeg" and is used for flavouring food [1]. Nutmeg is oval and weighing about 8 g dried, it is usually used in powdered form.

Myristica fragrans has been reported to be useful in paralysis and increases blood circulation and has been demonstrated to have antioxidant properties [2]. It also possesses aphrodisiac, anti-fungal, nervous stimulant, anti-dysenteric, and anti-inflammatory properties [3]. Studies have shown that it lowers blood pressure [4,5] and relieves stomach ache as well as stops diarrhoea and (in low doses) helps in detoxification in the body [6]. The medicinal properties of the seed are overwhelming and it is used in spicing of foods and in baking industries, there is a need to harness the nutritional constituents in the seed.

This work was carried out to analyze the nutritional and biological active constituents present in *Myristica fragrans* seed extracts using atomic absorption spectrometry and flame photometry, amino acid analyzer, and gas chromatography/mass spectrophotometer (GC-MS) to give more insight into its medicinal properties.

2. Materials and Methods

2.1. Materials

The following chemicals and apparatus were used in this research: n-hexane, methanol, evaporator (Model 349/2, Corning Ltd., England), 1000 mL capacity separating funnels, GC-MS (GCMS-QP2010 PLUS SHIMADZU, Japan), among others.

2.2. Methods

2.2.1. Collection of Plant Material

Myristica fragrans seeds were sourced from Ogige main market Nsukka and authenticated by a plant taxonomist, Mr. Alfred Ozioko of the Bioresources Diversity and Conservation Programme, Nsukka.

Preparation of Plant Material

The seeds of *M. fragrans* were ground into powder form using an electronic blender (Model MGB 1212, Japan). The ground plant material was stored in an airtight container for further use.

2.2.2. Extraction of Plant Material

Six hundred grams of powdered *M. fragrans* seed was macerated in 3.5 L of methanol and n-hexane (2:1 *v*/*v*). The solution was filtered with Whatman No. 4 filter paper; it was then poured into 1000 mL of separating funnel to give two layers which were then decanted separately. Finally, the filtrates were concentrated to a semi-solid mass using a rotary evaporator at a temperature of 40 °C.

2.2.3. Mineral Element Analysis

The extracts were digested using a concentrated sulphuric acid. The mineral constituents in the sample were analyzed using atomic absorption spectrophotometer (AAS) (Hitachi model 170–10). Sodium (Na) and potassium (K) analyses were carried out using a flame photometer.

2.2.4. Amino Acid Analysis in the Seed

The sample was dried, defatted, and evaporated in a rotary evaporator and loaded into the Technicon sequential multi-sample amino acid analyzer (TSM) (Model 6890N, USA). The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The amino acid present was calculated from the chromatogram peak [7].

The nor-leucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula [8].

$$NE = \frac{Area\ of\ Nor-leucine\ Peak}{Area\ of each\ amino\ acid}$$

Finally, the content of the amino acids was calculated in g/100 g protein via the following formula:

$$\text{Concentration (g/100 g protein)} = NH \times W@NH/2 \times S_{std} \times C$$

where

$$C = \frac{Dilution \times 16 \div NH \times w\ (nle\mu)}{Sample\ Wt(g) \times N\% \times 10 \times vol.\ loaded}$$

NH = Net height; W = Width at half-height; nleμ = Norleucine.

2.2.5. GC-MS Analysis

Methanol and hexane extracts were dissolved in methanol and n-hexane respectively and injected separately in a QP2010 GC system by an author injection at National Research Institute for

Chemical Technology, Zaria, Kaduna State, Nigeria. The operating conditions and instructions of the GC-MS set for the analysis were followed. The sample component was carried at 70 eV ionization rate while the total running time was 28.00 min [9,10].

NIST 14 library (2019) was then used to compare the structures of the compounds with that of the NIST database. Retention times and mass spectra were used to identify similar compounds in the NIST library [9].

3. Results and Discussion

The atomic absorption spectrometry and flame photometry analysis of *M. fragrans* seed methanol and hexane extracts and the concentrations of the identified minerals are shown in Table 1. Calcium was higher in methanol extract (3.21 ± 0.05) ppm compared to the hexane extract (0.35 ± 0.10) ppm and other mineral contents in the extracts. The methanol extract had the highest yield in all the mineral constituents analyzed. Potassium and sodium were not detected in both extracts.

These minerals play vital roles in disease management and general wellbeing in humans [11]. Iron is an essential component of haemoglobin and it carries oxygen from the lungs to the tissues. Iron deficiency in humans mostly caused by insufficient supply in diets and menstrual flow which can lead to anaemia [12]. Calcium was the highest constituent in methanol extract and is vital for strong bone formation and homeostasis regulations [13]. Magnesium was the second-highest content; this plays several functions that are not limited only to maintaining osmotic equilibrium and cofactor in many enzyme-catalyzed reactions. It can prevent heart disorder and lower blood pressure. Lack of Mg causes irritability of muscle and convulsion while its excess depresses the central nervous system [11].

Trace amounts of Zinc, manganese, and copper were found in both extracts. These mineral elements are important micro-nutrient cofactors in antioxidant glutathione peroxidase, catalase, and superoxide dismutase catalytic activity. Inadequate supply of these trace minerals in diets may shut down these antioxidant defense mechanisms [13].

Table 1. Mineral compositions of methanol and hexane seed extracts of *Myristica fragrans.*

Minerals	Hexane Extract (ppm)	Methanol Extract (ppm)
Fe	0.49 ± 0.15	1.00 ± 0.02
Ca	0.35 ± 0.10	3.21 ± 0.05
Mg	0.32 ± 0.00	3.00 ± 0.05
Zn	0.11 ± 0.13	0.17 ± 0.13
Mn	0.04 ± 0.00	0.38 ± 0.18
Cu	0.02 ± 0.20	0.02 ± 0.00

Each value represents the mean ± SD (n = 3).

Technicon sequential multi-sample amino acid analyzer (TSM) result is shown in Table 2.

Glutamic acid and aspartic acid were more abundant non-essential amino acids, while the essential amino acid had leucine as the most predominant. Other essential amino acids are also contained in the varying amounts in the sample. Methionine (0.80 g/100 g) which is a sulphur-containing amino acid was present in very little quantity. The total amino acid value in this study was 67.05 g/100 g which was lower as reported for *Monodora myristica* (90.9 ± 0.63 g/100 g) [14]. Amino acids initiate hormone synthesis and production of nitrogenous substances which are vital body homeostasis [15]. This result showed that *M. fragrans* are rich in most essential amino acids which can serve as an adjuvant for food deficient in essential amino acids.

Table 2. Amino acids constituent of *Myristica fragrans* seeds.

Essential Amino Acids (g/100 g)		Non-Essential Amino Acids (g/100 g)	
Leucine	6.24	Proline	3.24
Lysine	3.16	Tyrosine	2.58
Isoleucine	2.62	Arginine	5.50
Phenylalanine	3.72	Cysteine	0.73
Threonine	3.50	Alanine	4.10
Valine	3.39	Glutamic acid	10.60
Methionine	0.80	Glycine	3.99
Histidine	2.17	Serine	3.11
		Aspartic acid	7.60

Gas chromatography-mass spectrometry (GC-MS) analysis of *Myristica fragrans* seed extract of methanol and hexane revealed the presence of twenty-three and twenty-seven bioactive compounds respectively as shown in Tables 3 and 4.

The relative abundant bioactive compounds in hexane extract was in the order margarinic acid (27.04%) > oleic acid (18.96%) > 9,12-octadecadien-1-ol (15.10%) > palmitic acid (6.79%) > Glycerol-1,2-dipalmitate (6.08%). The least fatty acid present the extract was 2,6-octadien-1-ol, 3,7-dimethylacetate (0.24%) (Table 3) while methanol extract of *Myristica fragrans* had 9,12-octadecenoic acid methyl ester (27.13%) as the most abundant followed by cyclododecyne (19.33%) and octadecanoic acid (12.24%). The least fatty acid constituent was 2-cyclohexen-1-ol (0.22%) as shown in Table 4. Literature has reported that these compounds play crucial roles in general metabolism and homeostasis of the human body. Monoterpenoid rich extracts such as alpha-terpineol and 4-allyl-2, 6-dimethoxyphenol has antioxidant activity [16] while terpinen-4-ol suppresses cancer cell growth [17] and eugenol exhibit apoptosis-inducing effects [18].

Palmitic acid, undecane, alpha-phellandrene, eugenol, and oleic acid prevent insect pests that are resistant to insecticides [19,20].

Alcohols (4-isopropenyl-1-methyl cyclohexanol, 2-cyclohexen-1-ol and 2,6-dimethyl-5,7-otadien-5, 7-octadien 2-ol) act as antifungal agents and prevent food spoilage [21].

Reports have shown that 10-octadecenoic acid methyl ester and its likes are rich in antioxidants and attenuate cancer regeneration [22,23].

Table 3. Bioactive components and fatty acid profile of hexane seed extract of *Myristica fragrans.*

	Retention Time (min)	Compound Name	Formular	Area %
1	3.455	Alpha phellandrene	$C_{10}H_{16}$	0.46
2	3.993	Bicyclo[3.1.1]heptanes, 6,6-dimethylene	$C_{10}H_{16}$	0.45
3	5.049	Alpha pinene	$C_{10}H_{16}$	0.25
4	5.224	7-Decen-2-one	$C_{10}H_{18}O$	0.93
5	5.573	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethyl)	$C_{10}H_{18}O$	0.69
6	5.660	4-Isopropenyl-1-methyl cyclohexanol	$C_{10}H_{18}O$	1.31
7	5.899	4,7,7-Trimethylbicyclo[4.1.0]heptan-3-ol	$C_{10}H_{18}O$	0.39
8	6.783	1-Terpinen-4-ol	$C_{10}H_{18}O$	3.07
9	6.960	2,6-Dimethyl-5,7-octadien-5,7-octadien-2-ol	$C_{10}H_{18}O$	0.76
10	8.273	1,3-Benzodioxole, 5-(1-propenyl)	$C_{10}H_{10}O_2$	0.69
11	9.505	2,6-Octadien-1-ol, 3,7-dimethylacetate	$C_{12}H_{20}O_2$	0.24
12	9.730	7-(2,4-Dinitrophenoxy)-2,2-dimethyl-2,3-dihydro-1-benzofuran	$C_{16}H_{14}N_2O_6$	1.81
13	10.458	2-Methoxy-4-(1-propenyl)phenol	$C_{10}H_{12}O_2$	0.85
14	10.964	Eugenyl methyl ether	$C_{11}H_{14}O_2$	1.29
15	11.405	2-phenyltetrazole-5-carboxylic acid	$C_8H_6N_4O_2$	4.17
16	11.624	2,5-Dimethoxycinnamic acid	$C_{11}H_{12}O_4$	1.15
17	11.913	Myristic acid	$C_{14}H_{28}O_2$	1.73
18	12.282	1(4,5-Dimethyl-2-nitrophenyl)-1H-tetraazole	$C_9H_9N_5O_2$	1.45
19	13.551	Myristic acid methyl ester	$C_{15}H_{30}O_2$	0.38

20	14.746	Margarinic acid	$C_{17}H_{34}O_2$	27.04
21	18.079	Palmitic acid	$C_{16}H_{32}O_2$	6.79
22	19.861	Methyl linolelaidate	$C_{19}H_{34}O_2$	0.42
23	20.947	Oleic acid	$C_{18}H_{34}O_2$	18.96
24	22.619	Glycerol-1-palmitate	$C_{19}H_{38}O_4$	1.15
25	24.225	Linoleic acid chloride	$C_{18}H_{31}ClO$	2.38
26	24.673	Glycerol-1,2-dipalmitate	$C_{35}H_{68}O_5$	6.08
27	26.523	9,12-octadecadien-1-ol	$C_{18}H_{34}O$	15.10

Table 4. Bioactive compound and fatty acid profile of methanol seed extract of *Myristica fragrans.*

	Retention Time (min)	Compound Name	Formular	Area %
1	4.067	2-Decenal	$C_{10}H_{18}O$	0.24
2	5.211	7-Decen-2-one	$C_{10}H_{18}O$	0.37
3	5.563	2-Cyclohexen-1-ol	$C_{10}H_{18}O$	0.42
4	5.645	Terpineol	$C_{10}H_{18}O$	0.50
5	5.890	2-Cyclohexen-1-ol	$C_{10}H_{18}O$	0.22
6	6.759	4-Terpineol	$C_{10}H_{18}O$	1.19
7	9.699	3,5,7-Cycloheptatriene-1,3-dimethanol	$C_9H_{12}O_2$	1.12
8	11.315	Oxalic acid	$C_{18}H_{19}N_3O_2$	3.36
9	11.587	Benzene 1,2,3-trimethoxy-5-(2-propenyl)	$C_{12}H_{16}O_3$	0.76
10	11.820	Myristic acid	$C_{14}H_{28}O_2$	0.57
11	12.257	2,8-Decadiyne	$C_{10}H_{14}$	0.78
12	14.360	Octadecanoic acid	$C_{18}H_{36}O_2$	12.24
13	16.809	Palmitic acid	$C_{16}H_{34}O_2$	0.72
14	17.892	1-Pentadecanecarboxylic acid	$C_{16}H_{32}O_2$	6.36
15	19.850	11,14-Eicosadienoic acid	$C_{21}H_{38}O_2$	2.06
16	19.932	10-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	1.15
17	20.768	9,12-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	27.13
18	21.016	Octadecadienoic acid, 2-(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$	3.33
19	22.414	1-[(6-Methylheptyl)oxy]ethylene	$C_{10}H_{20}O$	1.21
20	24.210	9,12-Octadecadienoyl chloride	$C_{18}H_{31}ClO$	5.03
21	24.647	Hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl)ethyl ester	$C_{16}H_{32}O_2$	4.48
22	26.458	Cyclododecyne	$C_{12}H_2O$	19.33
23	26.641	2,3-Dihydroxypropyl palmitate	$C_{19}H_{38}O_4$	7.68

4. Conclusions

The methanol extract of *Myristica fragrans* had the most abundant elements (calcium and magnesium) which are essential elements necessary for animal life. The extracts contain a large amount of non-essential amino acids and an appreciable amount of other essential amino acids. These amino acids play vital roles in cell metabolism. The major biological compounds in the extracts of *Myristica fragrans* were 9,12-octadecenoic acid methyl ester and margarinic acid. All the bioactive compounds identified have been reported to be medicinally active and exhibit anticancer activities.

The study provides empirical evidence of the nutritional and medicinal credibility of *Myristica fragrans* seed which therefore serves as a reservoir of nutrient and therapeutic compounds for the wellbeing and development of humans and animals.

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