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# NUTRITIONAL AND SAFETY ASSESSMENT OF AQUEOUS EXTRACT OF NUTMEG SEEDS, *MYRISTICA FRAGRANS*

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## ABSTRACT

Nutmeg, *Myristica fragrans* is used as a spice or medicinal plant to manage ailments. This study evaluated the nutritional and safety assessment of an aqueous extract of Myristica fragrans in Wistar rats. Thirty male Wistar rats weighing 180 - 240 g were randomly distributed into five groups: Control received distilled water, while 100, 200, 300, and 400 mg/kg body weight represented the treatment groups, respectively. The extract was orally administered to the animals for 28 days and then euthanized under anesthesia. Blood, serum, and liver homogenates from rats were used for biochemical analysis. The phytate and tannin contents were 0.38 mg/100 g and 0.15 mg/100 g, respectively. The calculated phytic acid to zinc ratio was 0.12. The respective percentage proximate components of the extract include moisture, ash, fat, protein, and carbohydrates: 10.61%, 45.42%, 5.95%, 1.81%, 13.78%, and 22.43%, respectively. Sodium, potassium, calcium, iron, magnesium, zinc, manganese, and lead were present in the extract. There was a significant increase ($p<0.05$) in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and acid phosphatase (ALP) activity in the groups treated with the extract compared to the control groups. Similarly, there was a significant increase ($p<0.05$) in serum total bilirubin and albumin concentrations in the extract treatment groups compared to control groups. On the contrary, there was a significant decrease ($p<0.05$) in the liver AST and ALP activity in the treatment groups compared to the control. The liver total protein and albumin concentrations showed a significant decrease ($p<0.05$) in the extract treatment groups compared to the control. There was a significant decrease ($p<0.05$) in the pack cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), Mean cell volume (MCV), and platelet (PLT) levels in the animals in the extract treatment groups compared to the control group. Myristica fragrans is abundant in essential nutrients crucial for maintaining optimal physiological functions within the body, however, it is important to avoid excessive dosage or prolonged consumption which might be toxic to liver cells.

**Key words:** *Myristica*, animals, tannins, phytic acid, chemical and drug Induced liver injury, minerals

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## INTRODUCTION

Nutmeg is the dried kernel of the broadly ovoid seed of *Myristica fragrans* (Family: *Myristicaceae*). The genus comprises about 100 species throughout the tropics, especially in the Malayan region. Of these, *Myristica fragrans* alone contains enough aromatic essential oils to make it valuable for cultivation. Nutmeg is used in food and beverage preparations. It is used in baking desserts, sauces, pasta, meat and vegetable soups. It is also utilized as a preservative. The isopropymyristate component is found in cosmetic and topical preparations [1, 2, 3]. *Myristica fragrans* has been mentioned in ethnomedical literature as an aphrodisiac, stomachic, carminative, expectorant, sialagogue, emmenagogue, tonic, and nerve stimulant [4]. It is beneficial in the management of paralysis and increases blood circulation. Nutmeg is used as an anti-diarrhoea, hypnotic, analgesic, aphrodisiac, stomachic, carminative, tonic, nervous stimulant, and also for nausea, arthritis, constipation, fatigue, muscle aches, neuralgia, poor circulation, rheumatism, and slow digestion [5-9]. The reported medicinal potential of *Myristica fragrans* are believed to be due to some secondary metabolites which are equally potent toxicants at certain dose levels. Hence, this present study evaluated the nutritional and safety assessment of the aqueous extract of *Myristica fragrans* seed.

## MATERIALS AND METHODS

### Reagents

Ammonium thiocyanate and ferric chloride were obtained from Sigma-Aldrich (St. Louis, MO), while assay kits were all products of Randox Laboratories (Antrim, UK). All other reagents were of analytical grade.

### Study location, sample collection and preparation

*Myristica fragrans* seeds were bought at Oba Market, Akure, Ondo State, Nigeria. Authentication was done in the Department of Biology, Federal University of Technology, Akure (FUTA), Nigeria. A voucher specimen was deposited in the herbarium section of the Biochemistry Laboratory, Phytomedicine Unit, FUTA. Grated *Myristica fragrans* seeds (700 g) were soaked in 3 L of hot distilled water and left to stand for 72 h with periodic stirring. This was then filtered and an extract was obtained. The extract was freeze-dried and kept frozen until used.

### Phytochemical Screening

The aqueous extract was screened for the presence of the following: alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, flavonoids, and phlobatannins according to the methods described by Sofowora [10].

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## Antinutrients Determination

Phytate and tannin contents in the extracts were determined according to the method of Wheeler and Ferrel [11] and Makkar and Blümmel [12], respectively.

## Proximate analysis

The proximate analysis was carried out according to the method of Horwitz [13].

## Experimental animals

Adult Wistar albino rats weighing between 180-240 g were obtained from the animal colony of the Department of Biochemistry, FUTA, Nigeria. Ethical approval was obtained from the Department's ethics committee. Rats were all clinically healthy and maintained under standard environmental conditions of a 12 h light/dark cycle and a temperature-controlled room. They were fed a standard laboratory diet and water ad libitum.

## Animal grouping and treatment

The animals were randomly distributed across five groups with five rats in each group. Group 1 (control) received distilled water orally, throughout the experiment. Groups 2 to 5 received oral administration of 100, 200, 300, and 400 mg/kg body weight of aqueous *Myristica fragrans* extract, respectively, daily for 28 days. Rats were euthanized under anaesthesia 24 hours after treatment ended. Blood samples were collected into plain serum tubes by cardiac puncture and centrifuged at 3500 rpm for 10 min to obtain sera. In addition, portions of the whole blood were collected for haematological analyses. The liver was quickly removed, rinsed in an ice-cold 1.15% potassium chloride solution, blotted with filter paper, weighed, and homogenized for the evaluation of liver function markers.

## Biochemical estimation

Enzyme activity (AST, ALT, and ALP) and concentrations of total bilirubin, albumin, and total protein in liver homogenates, and serum were estimated using assay kits from Randox Laboratories Ltd. (Antrim, UK) according to the manufacturer's instructions.

## Hematological studies

Hematological analysis was performed using an automated hematological analyzer (Sysmex KX21, Tokyo, Japan). This includes; red blood cells (RBC), hemoglobin (Hb) level and packed cell volume (PCV) and platelets. Mean cell volume (MCV) was calculated from the PVC and RBC [14, 15].

## Statistical analysis

The results were expressed as mean ± SD. One Way Analysis of Variance was used followed by Duncan post hoc multiple comparisons for estimation of

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significant difference between means at $p<0.05$. The statistical analysis was performed with SPSS 16.0 software.

## RESULTS AND DISCUSSION

The results showed that alkaloids, phlobatannins, anthraquinones, cardiac glycosides and flavonoids were present (Table 1). Proximate analysis of the extract revealed that *Myristica fragrans* seeds have a higher fat content (45.42%) compared with other components proximate constituents (Table 2). Results in Table 3 revealed that *Myristica fragrans* seeds are rich in essential elements needed by the body, with magnesium being the most abundant mineral present (403.6 ppm), and lead appeared to be the least abundant in the aqueous extract of *Myristica fragrans* seeds (95.3 ppm). The phytate and tannin contents were 0.38 mg/100 g and 0.15 mg/100 g, respectively while the calculated phytic acid to zinc ratio was 0.12 as shown in Table 4.

The antioxidant and medicinal potential of the seed can be linked to the presence of alkaloids, phlorotannins, anthraquinones, cardiac glycosides, and flavonoids. The anthraquinones form a natural group of purgative drugs, and being a laxative, they may be responsible for the use of *Myristica fragrans* against constipation, slow digestion, nervous stimulant to the gastro-intestinal tract [5] and also its use in pile treatment [16, 17]. Flavonoids are known for their antimicrobial activities, as they are a member of the phytoalexins; this may explain using of *Myristica fragrans* against skin diseases like eczema and scabies and removing blotches from the face[18]. It also could account for *Myristica fragrans* seeds' use as a treatment for tuberculosis and against colds, fever, and general respiratory ailments. Cardiac glycosides are characterized by their specific action on heart muscles, increasing the excitability and contractility of the heart. They are, for this reason, valuable in treating congestive heart failure. This may account for the importance of *Myristica fragrans* in cases of paralysis, increased blood circulation, fatigue, muscle ache, or neuralgia [5, 7, 16]. In addition, the high-fat content is also an effective source of some essential oils accounting for various therapeutic functions of *Myristica fragrans* seeds. For instance, the hepatoprotective activity was suggested to be due to the inhibition of TNF-alpha release from macrophages by myristicin, as myristicin suppressed lipopolysacharide/D-Gal N-induced enhancement of serum TFN-alpha concentration markedly [7, 19]. Fats also act as lubricants in the intestines, which may account for their use as a stomach and gastrointestinal stimulant [2, 17]. *Myristica fragrans* was also found to be rich in protein (10.61%), which implies that it contributes to growth and is a component of critical biological substances like hormones and enzymes[20]. The crude fibre content (5.95%) could also account for its use in constipation and as a carminative [5, 17] since fibre are essential in normal digestion because they promote peristalsis, absorption of

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nutrients, and elimination of undigested food. Minerals are critical to the human system since they are used by the body in a variety of ways [21]. The study shows that *Myristica fragrans* contains minerals needed by the body. These minerals are either part of the body's rigid structure or are a constituent of body fluids. For example, iron could account for its use as a tonic; zinc is also very significant in promoting growth. Also, although Lead was detected, the levels were far below the permissible limits of 50-300 ppm [22]. This study also found *Myristica fragrans* seeds to contain phytate and tannin as total phenol. These anti-nutrients reduce the bioavailability of some nutrients in the body. Tannins affect foods' nutritive value by forming a complex with proteins, inhibiting digestion and absorption [23]. Phytates also interfere with Ca, Fe, Mg, and Zn absorption due to their ability to chelate divalent cationic minerals [24]. However, since the tannin content is far below the harmful level of 0.76 – 0.90 mg/100 g, it could be considered safe. Furthermore, the calculated [phytate]/[Zinc] ratio of *Myristica fragrans* was 0.124, which is far below the ratio of 10:1 considered to cause reduced growth and decreased plasma zinc [25]. Values of about 5.7 or higher were found to significantly lower zinc absorption in humans [26]. This implies that, *Myristica fragrans'* phytate level is still safe for human consumption. Furthermore, the *in-vivo* evaluation of the safety limit of *Myristica fragrans* aqueous extract showed that there was a significant decrease ($p<0.05$) in the liver AST and ALP activity in the groups administered aqueous extract of *Myristica fragrans* (200-400) mg/kg weight compared to the control. On the contrary, the group given 100 mg/kg body weight of the extract showed no significant change in AST and ALP activity compared to the control. The activity of liver ALT showed no significant change across the treatment groups compared to the control (table 5). The liver total protein concentration showed a significant decrease ($p<0.05$) in groups given the extract (200 – 400) mg/kg body weight compared to the control, while the group administered 100 mg/kg body weight showed no significant change (table 5). There was no significant change in liver total bilirubin concentrations compared across the treatment groups compared to the control (table 5). The liver albumin concentration showed a significant decrease ($p<0.05$) in the treatment groups administered, the extract at 300 and 400 mg/kg body weight compared to the control, while at 100 and 200 mg/kg body weight there was no significant change (table 5). There was a significant increase ($p<0.05$) in serum AST, ALT, and ALP activity in the groups treated with the extract in comparison to the control groups (table 6). Similarly, there was a significant increase ($p<0.05$) in serum total bilirubin concentration in the groups administered at 400 mg/kg body weight, compared to the control groups (table 6). There was a significant increase ($p<0.05$) in serum albumin concentrations in the treated groups, compared to the control groups (table 6). There was a significant decrease ($p<0.05$) in the PCV, Hb, RBC, MCV,

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and PLT levels in the animals in the treatment groups compared to control group (table 7). The intake of *Myristica fragrans* at a high enough dosage could lead to liver damage or hepatic disorders. The reduced concentrations of albumin and total protein in this study could indicate a reduction in the production of albumin and other proteins produced in the liver. This is usually associated with many chronic liver disorders that affect the liver's ability to make proteins. Decreased albumin synthesis results in hypoalbuminemia [27, 28]. The increased serum albumin level in the rat administered the extract despite a decrease in the liver albumin level may be due to the long half-life of most proteins, including albumin, which needs a more extended period of the liver's incapacity to produce before it reflects in the serum as having lower levels [29]. The elevated serum activity of ALT and AST may indicate hepatocellular necrosis as damaged liver cells, which results in increased serum activities of ALT and AST due to leakage from the cells [30]. These two enzymes are also biomarkers of chronic hepatitis. Alkaline phosphatase is a membrane-bound enzyme, therefore, can leak into the serum due to compromise of membrane integrity [31]. In addition, the increased activity of serum ALP may indicate obstruction of bile flow and liver injury [32]. Elevated serum bilirubin may also indicate obstruction of bile flow due to liver injury [33]. The decrease in the hematological parameters observed by this current study may be due to adverse effects on blood parameters. This finding contradicts a previous study by Ram and Lauria [34] which reported the absence of any adverse effects on various hematological parameters in toxicity studies involving Myristica fragrans. The low PCV in this study can be associated with the bioaccumulation of lead from the seed. Lead is known to cause anemia by obstructing heme biosynthesis [35].

## CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The antioxidant and medicinal potential exhibited by *Myristica fragrans* seed can be directly linked with plant bioactive compounds present in the seed. Similarly, high fat and carbohydrate constituents of seed will be useful for an optimal growth and energy production in human. On the contrary, prolonged consumption of the seed could result in the accumulation of heavy metals such as lead in the liver. Therefore, this accumulation might result in the death of the cell. The use of *Myristica fragrans* seeds should be done with extreme caution to avoid bioaccumulation. Therefore, more studies should be undertaken to discover a food processing method to remove the antinutrients and lead content of the seeds.

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**Table 1: Qualitative phytochemical composition of *Myristica fragrans***

| Constituent | Inference |
|---|---|
| Alkaloids | + |
| Saponin | - |
| Tannin | + |
| Anthraquinones | + |
| Phlobatannin | + |
| Cardiac glycosides | + |
| i. Legal Test | + |
| ii. Leiberman's | - |
| iii. Salkowski | + |
| Flavonoids | + |

+ = Present, - = Absent

**Table 2: Proximate composition of aqueous extract of *Myristica fragrans* in dry weight**

| Nutrient | Percentage (%) |
|---|---|
| Protein | 10.61 ± 2.10 |
| Crude fat | 45.42 ± 1.15 |
| Crude fibre | 5.95 ± 0.19 |
| Ash | 1.81 ± 0.04 |
| Moisture | 13.78 ± 0.13 |
| Carbohydrate | 22.43 ± 6.49 |

Values are expressed as Mean ± S.D (n=3 replicates)


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**Table 3: Mineral composition of aqueous extract of *Myristica fragrans***

| Mineral | Concentration (ppm) |
|---|---|
| Sodium | 141.82 ± 1.07 |
| Potassium | 194.80 ± 3.63 |
| Calcium | 161.55 ± 2.79 |
| Iron | 106.12 ± 0.95 |
| Magnesium | 403.63 ± 12.50 |
| Zinc | 167.32 ± 5.81 |
| Manganese | 32.33 ± 0.67 |
| Lead | 5.31 ± 0.07 |

Values are expressed as Mean ± S.D (n=3 replicates)

**Table 4: Antinutritional factors of aqueous extract of *Myristica fragrans***

| | Phytate (mg/100g) | Tannin Content (mg/100g) | Calculated Ph/Zn ratio |
|---|---|---|---|
| Extract | 0.38 ± 0.01 | 0.15 ± 0.00 | 0.12 ± 0.00 |

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**Table 5: Effects of an aqueous extract of *Myristica fragrans* on some biochemical indices in the liver of the Wistar rat**

| Group | TB (mg/dL) | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | TP (mg/dL) | ALB (mg/dL) |
|---|---|---|---|---|---|---|
| Control | 1.40±0.11$^{a}$ | 7.31±0.15$^{b}$ | 0.39±0.03$^{a}$ | 15.00±1.15$^{c}$ | 14.00±0.00$^{c}$ | 4.00±0.00$^{b}$ |
| 100 mg/kg | 1.4±0.11$^{a}$ | 7.40±14$^{b}$ | 0.40±0.05$^{a}$ | 15.24±1.16$^{a}$ | 14.00±0.01$^{a}$ | 4.00±00$^{b}$ |
| 200 mg/kg | 1.32±0.17$^{a}$ | 7.25±0.17$^{b}$ | 0.40±0.15$^{a}$ | 10.50±1.29$^{b}$ | 10.50±1.29$^{b}$ | 4.00±0.81$^{b}$ |
| 300 mg/kg | 1.35±0.13$^{a}$ | 4.30±1.26$^{a}$ | 0.39±0.03$^{a}$ | 8.25±0.95$^{a}$ | 8.15±0.57$^{a}$ | 3.75±0.95$^{a}$ |
| 400 mg/kg | 1.45±0.3$^{a}$ | 4.20±0.18$^{a}$ | 0.40±0.08$^{a}$ | 8.00±0.81$^{a}$ | 8.00±0.17$^{a}$ | 2.25±0.95$^{a}$ |

Values with same superscript letters down the column are not statistically significantly different from each other ($p<0.05$). TB: Total Bilirubin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; ALB: Albumin; TP = Total Protein

**Table 6: Effects of an aqueous extract of *Myristica fragrans* on some biochemical indices in serum of Wistar rat**

| Group | TB (mg/dL) | AST (IU/L) | ALT (IU/L) | ALP (IU/L) | ALB (mg/dL) |
|---|---|---|---|---|---|
| Control | 1.38±0.38$^{a}$ | 37.0±0.83$^{a}$ | 25.0±0.58$^{a}$ | 34.0±2.30$^{b}$ | 24.50±3.11$^{a}$ |
| 100 mg/kg | 1.15±0.37$^{a}$ | 50.50±8.69$^{b}$ | 28.25±0.50$^{a,b}$ | 35.25±3.95$^{b}$ | 26.75±4.03$^{a}$ |
| 200 mg/kg | 1.00±0.12$^{a}$ | 103.50±20.30$^{c}$ | 30.5±1.73$^{a,b}$ | 45.0±4.24$^{b}$ | 26.5±1.00$^{a}$ |
| 300 mg/kg | 1.33±0.15$^{a}$ | 149.25±4.19$^{c,d}$ | 34.50±1.00$^{a}$ | 97.25±22.07$^{c}$ | 25.25±2.50$^{a}$ |
| 400 mg/kg | 2.43±0.20$^{b}$ | 147.00±24.26$^{c,d}$ | 33.00±4.08$^{c}$ | ND | 29.0±2.94$^{a}$ |

Values with same superscript letters down the column are not statistically significantly different from each other ($p<0.05$). TB: Total Bilirubin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; ALB: Albumin; ND: Not determined

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**Table 7: Hematological variables of rat treated with aqueous extract of *Myristica fragrans***

| Group | PCV (%) | Hb (g/100 ml) | RBC ($1^{-1}$) | MCV (ft) | PLT ($1^{-1}$) |
|---|---|---|---|---|---|
| Control | 56.25±4.26$^{b}$ | 18.95±1.90$^{a,b}$ | 10.10±0.58$^{a}$ | 56.22±0.90$^{a}$ | 187.50±9.57$^{b}$ |
| 100 mg/kg | 52.75±2.06$^{a,b}$ | 16.33±2.63$^{a,b}$ | 8.78±2.06$^{a}$ | 60.24±14.92$^{a}$ | 156.00±3.65$^{a}$ |
| 200 mg/kg | 53.50±4.12$^{a,b}$ | 17.53±1.28$^{a,b}$ | 10.10±0.47$^{a}$ | 52.92±1.81$^{a}$ | 165.50±12.37$^{a,b}$ |
| 300 mg/kg | 48.25±2.36$^{a}$ | 15.80±0.77$^{a}$ | 8.00±1.97$^{a}$ | 62.62±12.84$^{a}$ | 184.75±25.97$^{b}$ |
| 400 mg/kg | 52.50±7.05$^{a,b}$ | 17.33±2.29$^{a,b}$ | 9.30±2.27$^{a}$ | 58.06±9.08$^{a}$ | 184.75±22.62$^{b}$ |

Values with same superscript letters down the column are not statistically significantly different from each other ($p<0.05$). PCV: Packed cell volume; Hb: Haemoglobin; RBC: Red blood cells; MCV: Mean cell volume; PLT: Platelets; ND: Not determined

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