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Full Length Research Paper

Antioxidant properties of *Myristica fragrans* (Houtt) and its effect on selected organs of albino rats

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Aqueous extract of the seed of *Myristica fragrans* (nutmeg) was evaluated for its phytochemical constituents, antinutrients and antioxidant properties. Toxicological investigation was also carried out using six groups of Wistar albino rats. The treatment groups were administered varying doses (100-500 mg/kg body weight) of the extract for a period of 28 days. The animals were sacrificed and their livers, kidneys, hearts, spleen and testes harvested for histopathological studies. The results showed that alkaloids, saponins, anthraquinones, cardiac glycosides, flavonoids and phlobatanins were present while tannins were absent in the aqueous extract. The phytate content was 564.11 mg/100 g while the antioxidant indices of 100 mg/100 g, 44% and 0.6 were obtained for the ascorbic acid value, free radical scavenging activity and reducing power, respectively. The results of the histopathological studies showed pathological features of various degrees in the organs with severity corresponding to the concentration of extract. There was lymphoid depletion of the follicles in the spleen, degeneration of the germinal epithelial cells in the testes, bile duct proliferation and congestion of blood vessels in the liver, degeneration, necrosis with desquamation of tubular epithelial cells and congestion of renal blood vessels in the kidney and degeneration of myocardial fibres and myocardial necrosis in the heart in the treatment groups compared with the control. The present results suggest that nutmeg popularly consumed as food and for various medicinal purposes may contain some active principles with antioxidant properties. However, prolonged use at high doses (400-500 mg/kg) could be very toxic to the studied organs.

Key words: *Myristica fragrans*, phytochemical constituents, histopathological studies, antioxidant properties.

INTRODUCTION

Nutmeg (*Myristica fragrans*), whose seed is widely used as a spice, is a tropical, dioeciously evergreen tree native to the Moluccas or Spice Island of Indonesia. Nutmeg has a characteristic pleasant fragrance and is slightly warm taste. It is used to flavour many kinds of baked goods, confections, puddings, meats, sausages, saucers, vegetables, and beverages (Panayotopoulos and Chisholm, 1970). It is also used as components of curry powder, teas and soft drinks, or mixed in milk and alcohol.

Medicinally, it is used as an anti-diarrheal agent for patients with medullar carcinoma of the thyroid. The effectiveness of the treatment may be due to the inhibition of prostaglandin synthesis in the mucosa and sub mucosa of the colon. It is sometimes used as a stomachic, stimulant, carminative as well as for intestinal catarrh and colic, to stimulate appetite, to control flatulence, and has a reputation as an emmenagogue (to promote and regulate menstrual flow) and abortifacient (Green, 1959; Panayotopoulos and Chisholm, 1970). It has also been found useful as tonic for the heart and brain and also in sexual and general debility. Aphrodisiac activity of 50% ethanolic extracts of *M. fragrans* and *Syzygium aromaticum* (L) Merr. and Perry (clove) in male mice has also been reported. The extracts of the nutmeg and clove were found to stimulate the mounting behaviour of male mice, and also to significantly increase their mating performance (Tajuddin et al., 2003). The hypnotic, analgesic and hypotensive activities of *M.*

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fragrans have also been reported (Grover et al., 2002). With the recent gain in popularity of herbal medicine all over the world, it is also possible to abuse the use of *M. fragrans* because of its medicinal properties. It has been reported that the spice can be toxic when ingested in large quantities (1-3 nutmegs) causing convulsions, hallucinations, and possibly death (Forrest and Heacock, 1972).

The medicinal use of nutmeg and its use as a spice suggest that it contains some constituents which are responsible for the reported biological activities. Some of these active principles may at the same time possess some adverse effects. The present work is designed to investigate the phytochemical constituents, antioxidant potentials and *in vivo* toxicity of the aqueous extract of nutmeg.

MATERIALS AND METHODS

Chemicals

Trichloroacetic acid was obtained from Merck Darmstadt, Germany. L-ascorbic acid and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were obtained from Sigma-Aldrich (St. Louis, MO). All other reagents were of analytical grade.

Experimental animals

Adult Wistar albino rats (190-240 g) were obtained from the animal colony of the Department of Biochemistry, University of Ilorin, Nigeria. They were all clinically healthy and maintained under standard environmental conditions of 12 h light/dark cycle and a temperature controlled room. They were fed a standard laboratory diet and water *ad libitum*.

Preparation of extract

Seeds of *M. fragrans* were bought at Oja Oba market, Akure, Ondo State, Nigeria. Authentication was done in the Department of Biology, Federal University of Technology, Akure (FUTA), Nigeria and a voucher specimen was deposited at the herbarium section of Biochemistry laboratory, Medicinal Plants unit, FUTA. Grated nutmeg seed (760g) was soaked in 3 L of hot distilled water and left to stand for 72 h. This was thereafter filtered and the extract obtained. The extract was freeze-dried and kept frozen until used.

Phytochemical screening

This was carried out as previously described (Odebiyi and Sofowora, 1978).

Phytin content determination

The ground sample (4 g) was weighed and dissolved in 100 ml of 2% hydrochloric acid and allowed to stand for 3 h with intermittent shaking. This was then filtered through a two-layer hardened filter paper. The filtrate (25 ml) was precipitated out into a conical flask and then 5 ml of a 0.3% ammonium thiocyanate solution was added as an indicator, and 53.5 ml of distilled water was equally added to obtain the proper acidity (pH 4.5). The solution was then titrated with iron (III) chloride solution containing 0.00195g Fe/ml until there was a brownish yellow colour which persisted for 5 min. Phytin-phosphorus was determined and phytin content calculated by multiplying the value of phytin-phosphorus by 3.55 (Young and Greaves, 1940). Each milligram of iron is equivalent to 1.19 mg of phytin-phosphorus.

Ascorbic acid

The method of Reiss (1993) was used. A blue dye was prepared by dissolving 50 mg of sodium 2, 6-dichlorophenol in 50 ml of distilled water. The mixture was transferred to a 200 ml volume diluted with distilled to the mark filtered and kept in a refrigerator. A standard L-ascorbic acid solution was prepared by dissolving 50 mg of crystalline L-ascorbic in 60 ml of glacial acetic acid and diluted with distilled water to the 100 ml mark. The aqueous nutmeg extract (5 g) was made into solution and then filtered. 30 ml of standard L-ascorbic acid solution was titrated with the blue dye for standardization. The standardized dye was then used to titrate 30 ml of the nutmeg extract. Using the titre value, the ascorbic acid content of the aqueous extract was then calculated using the formula:

Ascorbic acid (mg) / 100 g of nutmeg tissue =

$$\frac{\text{Ascorbic acid (mg) / aliquot} \times \text{total volume of extract (ml)} \times 100}{\text{Volume of aliquot (ml)} \times \text{weight of nutmeg (g)}}$$

Free radical scavenging activity

The free radical scavenging ability of aqueous extract of nutmeg against 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical was evaluated according to the method of Braca et al. (2001). Briefly, 1 ml of the extract was mixed with 1 ml of 0.4 mM methanolic solution containing DPPH radicals. Similarly, a reference solution of methanol and DPPH was prepared in just the same way. Both solutions were kept in a dark chamber for 30 min before measuring the absorbance at 516 nm. Free radical scavenging ability was calculated as follows:

$(A_s - A_o)/A_s \times 100\%$

Where A_s = absorbance of the standard and A_o = absorbance of the sample.

Reducing property

The reducing property of the extract was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution (Jayaprakash et al., 2001). Briefly appropriate dilutions of the extracts (2.5 ml) were mixed with 2.5 ml of 200 mM of sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixture was incubated at 50°C for 20 min after which 2.5 ml of 10% trichloroacetic acid was added. The mixture was then centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with equal volume of deionized water and 1 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power (Jayaprakash et al., 2001).

Toxicological investigation

The animals were randomly divided into six groups with six rats in each group. The control (group 1) received physiological saline

Table 1. Pyhtochemical constituents of aqueous extract of *Myristica fragrans*.

Constituent	Result
Alkaloid	+
Saponins	+
Tannins	-
Anthraquinones	+
Cardiac glycoside	+
Flavonoids	+
Phlobatanins	+

+: present, -: absent

Table 2. Antioxidant Properties of aqueous extract of *Myristica fragrans*.

Antioxidant Properties	value
Ascorbic acid	100 mg/100g
Free radical scavenger ability	44%
Reducing power	0.6
Phytate value	564.11 mg/100 g

Table 3. Effect of varying concentration of aqueous extract of *Myristica fragrans* on the livers of rats.

Concentration (mg/kg bw)	Observation
Control	No observable congested blood vessels and bile duct proliferation congested
100	There was a mild bile duct proliferation.
200	Portal blood vessels were markedly congested. There was mild bile duct proliferation
300	Marked congestion of the portal blood vessels Marked bile duct proliferation.
400	Marked congested blood vessels. Focal areas of moderate periportal lymphocytic infiltration. Moderate bile duct proliferation
500	-marked congestion of the portal blood vessels Focal areas of marked periportal lymphocytic Infiltration.

bw: body weight.

orally throughout the experiment. The remaining groups (2, 3, 4, 5 and 6) received an oral administration of 100, 200, 300, 400 and 500 mg/Kg body weight of aqueous extract of nutmeg, respectively, daily for a period of 28 days. Rats were sacrificed 24 h after the last treatment by cervical dislocation. The spleen and the two testicles were aseptically collected into a mixture of formalin-saline (95:5) for histopathological studies. The organs (spleens and testicles) preserved in formalin-saline were subjected to histopathological studies according to the method of Lamb et al. (1991). A thin section of the organ (thickness being 6 microns) was used for the study.

RESULTS AND DISCUSSION

Aqueous extract of the seed of *M. fragrans*, “a seed of many faces” was studied for its phytochemical constituents, antioxidant potentials and *in vivo* effects on the livers, kidneys, hearts, spleen and the testes of albino rats. Phytochemical screening of extracts revealed the presence of alkaloids, flavonoids and anthraquinones (Table 1). Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents (Chabner and Horwit, 1990; Noble, 1990). Alkaloids also interfere with cell division; hence the presence of alkaloids in *M. fragrans* could account for its use as an antimicrobial agent (Valero and Salmeron, 2003). Flavonoids are a group of chemicals found in varying amounts in foods and medicinal plants which have been shown to exert potent antioxidant activity against the superoxide radical. Epidemiological studies have indicated that the consumption of flavonoids is inversely related to coronary heart disease mortality (Hertog et al., 1983; Knekt et al., 1996). Inhibition of low density lipoprotein (LDL) oxidation has also been attributed to the dietary and supplemental intake of flavonoids and other micronutrients (Knekt et al., 1996). The presence of flavonoids in the plant may be the reason for its therapeutic effects especially in the treatment arteriosclerosis. The antioxidant properties, as presented in Table 2 are likely to be as a result of the presence of flavonoids in the extract. The animals showed some behavioural changes to the extract in the first three days of its administration. The changes include sluggishness, slow response to stimulus and general slowness in body activities. The changes were however absent during subsequent days. The absence is attributable to the adaptation of the animals to the extract over time. Weight gain was also noticed in the animals after the experiment.

The histological results (Tables 3-7) show a progressive increase in degeneration of germinal epithelial cells of the testis across the group with increase in doses. The control recorded no conspicuous lesion. Low sperm counts has been ascribed as the major cause of infertility in men (Austin and Short, 1978) and that low sperm count in itself results from either an impairment in the germinal epithelial cells or in the mature sperm cells during ejaculation (Austin and Short, 1978). The results suggest that large quantity of this extract when consumed can cause low sperm count, although it has been reported to be capable of improving sexual activities (ref). In Table 4, there is degeneration of germinal centers of lymphoid cells. There is gradual increase in pathological changes of the spleen of the treated rats ranging from

Table 4. Effect of varying concentration of aqueous extract of *Myristica fragrans* on the kidneys of rats.

Concentration (mg/kg bw)	Observation
Control	No conspicuous lesion
100	Marked degeneration and necrosis with
200	Desquamation of the tubular epithelial cells. there was marked congestion of renal blood vessels
300	Markedly congested renal blood vessels. Severe diffuse degeneration and necrosis with Desquamation of the tubular epithelial cells.
400	markedly congested renal blood vessels Several diffuse degeneration and necrosis of the tubular epithelial cells.
500	Marked diffuse degeneration and necrosis of the tubular epithelial cells

Table 5. Effect of varying concentration of aqueous extract of *Myristica fragrans* on the hearts of rats.

Concentration (mg/kg bw)	Observation
Control	No conspicuous lesion
100 mg /kg	Diffuse moderate degeneration of the
200 mg/kg	- myocardial fibres
300 mg/kg	No conspicuous lesion
400 mg/kg	No conspicuous lesion
500 mg/kg	Focal areas of myocardial degeneration and necrosis.

bw: body weight.

Table 6. Effect of varying concentration of aqueous extract of *Myristica fragrans* on the testes of rats.

Concentration (mg/kg bw)	Observation
0 (Control, saline)	No conspicuous lesion
100	No conspicuous lesion
200	There is localized area of marked intestinal accumulation of deeply oesoniphilic Oedema fluid.
300	Marked degeneration and necrosis of the germinal epithelial cells were noticed
400	Severe diffuse degeneration and necrosis of the germinal epithelial cell is noticed
500	Severe diffuse degeneration and necrosis of the Germinal epithelial cell is noticed

bw: body weight.

Table 7. Effect of varying concentration of aqueous extract of *Myristica fragrans* on the spleen of rats.

Concentration (mg/kg bw)	Observation
(Control, saline)	No lymphoid depletion
100	Showed slight lymphoid depletion
200	Showed slight lymphoid depletion
300	Moderate lymphoid depletion in the follides and necrosis of the lymphoid cells
400	There was marked lymphoid depletion and necrosis of the lymphoid cells.
500	There was marked lymphoid depletion and necrosis of the lymphoids cells.

bw: body weight.

mild to severe lymphoid depletion in the follicles and necrosis of the lymphoid cells depending on the dosage. Small-sized and degenerated germinal centers of lymphoid cells are indicators of their waning function (Ryabchikova et al., 1996). There was degeneration and necrosis of myocardial focal areas in the hearts and several diffuse degeneration and necrosis of the tubular epithelial cells in the kidneys of the treated animals (400-500 mg/Kg). The control showed little or no pathological features. The possible deduction from these results is

that secondary metabolites, which are largely responsible for therapeutic or pharmacological activities of medicinal plants (Perry, 1980), may also account for their toxicity when the dosage is abused. Caution should therefore be advocated in the intakes of this product.

REFERENCES

Austin CR, Short RV (1978). Reproduction in Mammals. Spermatogenesis. 1st edition, Bartons publishers. USA. pp. 52 – 53.

Braca AT, Nunziatina DB, Lorenzo Di, Pizza C, Politi M, Morelli I (2001). Antioxidant principles from *Bauhinia terapotensis*. J. Nat. Prod. 64: 892-895.

Chabner BA, HortwitzTL(1990). Plant alkaloids. In: Pinedo HM, Chabner BA, Longo DL, eds: Cancer chemotherapy and biological responses.

Grover JK, Khandkar S, Vats V, Dhunnoo Y, Das D (2002). Pharmacological studies on *Myristica fragrans*--antidiarrheal, hypnotic, analgesic and hemodynamic (blood pressure) parameters. Experimental Clinical Pharmacol. 24(10): 675-80

Green RC (1959). Nutmeg Poisoning. J. Jama. 177: 1342-1344.

Haddad LM, Winchester JF Eds. (1983). Clinical Management of Poisoning and drug overdose. Philadelphia, Saunders.

Harbone JB (1984). Phytochemical Methods, A guide to modern Techniques of plant analysis, 2nd edition. Macmillam Publishers London. pp. 401-413.

Hertog MGL, Feskens EJM, Hollman DCH, Katan MB, Kromhout D (1993). Dietary antioxidant flavonoids and risk of coronary heart disease. The Zutphen ederly Study-Lancet 342: 1007 – 1011.

Jayaprakash GK, Singh RP, Sakariah KK (2001). Antioxidant activity of grape seed extracts on peroxidation models in-vitro. J. Agric Food Chem. 55: 1018-1022.

Knekt P, Jarvinen R, Reunanen A, Maatela J (1996). Flavonoid intake and coronary mortality in Finland: a cohort study. Biomedical J. 312: 478-481

Lamb SA, King B, Fisher LA (1991). Introduction to Histopathology. Sectioning. Macmillan publishers. London. pp. 14 - 50

Noble, R.L (1990). The discovery of vinca alkaloids chemotherapeutic agents against cancer. Biochem. cell Biol. 68 (12): 1544-1351

Odebiyi FO, Sofowora A (1993). Heart Puncture Methods, 5th edition. Longman group Ltd. Nigeria. pp. 78 – 101.

Panayotopoulos DJ, Chisholm DD (1970). Hallucenogenic effect of Nutmeg. Brithish medical. J. 1: 754 - 760.

Perry LM (1980). Medicinal plants of East and South East Asia, MIT Press, Cambridge Massachusetts.

Reiss C (1993). Measuring the amount of ascorbic acid in cabbage. In: Tested studies for laboratory teaching vol. 7/8 (Goldman, C.A. and Hauta PL Eds.). Proceeding of the 7th and 8th workshop/conferences of the association of Biology laboratory education (ABLE). pp. 85-96.

Ryobehikora E, Kolesnikora L, Rassadkin (1996). Viral Biotechnol. J. Russian Med. 4: 430-434.

Sofowora EA (1993). Medicinal Plants and traditional medicine in Africa, 2nd edition. John Wiley and sons Ltd. England. pp. 55-62.

Tajuddin, Ahmad S, Latif A, Qasmi IA. (2003) Aphrodisiac activity of 50% ethanolic extracts of *Myristica fragrans* Houtt (nutmeg) and *Syzygium aromaticum* (L) Merr. and Perry. (clove) in male mice: acomparative study. BMC Complement Alternative Med. 20:3(1): 6

Trease EA, Evans B (1983): Medicine and Plants, 3rd edition, Pitman. London. pp. 57 - 90

Valero M, Salmeron MC.(2003) Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. Int. J. Food Microbiol. 15: 85(1-2): 73-81

Weiss G (1966). Hallucinogenic Effects of Powdered Myristica, J. Psychiatry 346: 17- 34

Young SM, Greaves JE (1940). Influence of variety and treatment on phytin content of wheat. Food Res. 5: 103-105.