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Original Research

Chemical properties of *Monodora myristica* and its protective potentials against free radicals *in vitro*

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Abstract
Distortion of the balance between the production of reactive oxygen species and the body's antioxidant defenses against them produces oxidative stress, which intensifies tissue damage. Spices have been reported to possess antioxidant properties which protect against ROS. *Monodora myristica* is among the common spices in Nigeria with reported medicinal properties. Its proximate composition, phytochemical constituents and antioxidant activities were investigated. The proximate composition ranged from 3.06 to 52.42% with nitrogen free extract having the highest value and ash content, lowest. Phytochemical screening of the spice showed the presence of alkaloids, flavonoids, phenols, tannins and saponins. The 1,1-diphenyl-2 picrylhydrazyl (DPPH) scavenging activity ranged from 16.9 to 71.15 mg gallic acid equivalents (GAE)/g. While the reducing power compared to the standard, gallic acid ranging from 0.028 to 0.126 mg GAE/g. *Monodora myristica* exhibited high antioxidant activities *in vitro*, signifying the protective potential of the spice against free radicals. The phytochemicals, phenolic and flavonoids in particular may be responsible for these effective antioxidant properties.

INTRODUCTION

Recently, plant-based antioxidants are now preferred to the synthetics due to safety reasons [1]. These have led to the extensive screening of plants for possible medicinal and antioxidant properties, the isolation and characterization of diverse phytochemicals and the development and utilization of antioxidants of natural origin [2, 3]. The antioxidant properties of spices are well known [4]. These properties have been reported to be related with their flavonoids, flavones, isoflavones and total phenolic content [5]. Antioxidants protect the body against adverse effects of free radical or reactive oxygen species (ROS) generation [6], which are characterized by their ability in causing oxidative damage to the body. Several studies have shown that ROS are involved in various related physiological processes and diseases such as aging, cancer and atherosclerosis [7]. These antioxidants also play a major role in food quality preservation due to their ability to prevent oxidative deterioration of lipids [3].

Monodora myristica belongs to the Annonaceae family and is one of the most important trees of the evergreen forest of West Africa [8]. It is native to Nigeria, where the seed is called *ehuru* or *ehir* or *abo-lakoshe* among the Yorubas. Its seeds are a popular spice used in cooking to flavour and thicken dishes. Medicinally, the root is chewed to relieve toothaches and arthritis. It is also used in treatment of anemia, hemorrhoids and sexual weakness [9, 10].

This paper aims at investigating the proximate composition, phytochemical constituents of *M.myristica* and its antioxidant activities.

MATERIALS AND METHODS

Plant material

Seeds of *M.myristica* were purchased from a local market in Benin City, Nigeria. They were identified and authenticated at the Botany department, University of Benin, Benin-City, Nigeria. The seeds were dehulled and air-dried at room temperature and grounded to fine powder, using a laboratory mill and stored in air-tight containers for laboratory analysis. All analysis was carried out in triplicates.

Proximate analysis

Proximate analysis was carried out using the standard procedures of the Association of Official Analytical Chemists [11]. Briefly, dry matter content was determined by drying the sample in a vacuum oven at 100ºC and dried to a constant weight. Ash content was determined by incineration of 2 g of the sample in a muffle furnace at 600ºC for 8 h. The percentage residue weight was expressed as ash content. Crude fat was determined by soxhlet extraction method using petroleum ether as solvent. Nitrogen was determined using the Kjeldahl method and crude protein was calculated by multiplying the percentage nitrogen content by the conversion factor 6.25.

Nitrogen Free Extract was determined by following formula:

100 – (%Moisture + %Crude protein + %Crude fat + %Ash)

The total energy was determined by Gallenkamp Ballistic Bomb Calorimeter method.

Extraction

The powdered samples were extracted at room temperature by percolation with ethanol. All extracts were concentrated over a rotary vacuum evaporator.

Phytochemical analysis

The qualitative and quantitative phytochemical properties of the dried powdered sample were determined using standard methods described by Harborne [12]; Boham and Kocipai [13]; and Ebrahimzadeh *et al* [14].

Determination of total phenolic content

The Folin-Ciocalteu reagent assay was used to determine the total phenolic content in the drinks respectively according to the method described by Liu and Yao [15] and expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight. Briefly, 0.5 ml of the diluted extract was dissolved in 100 µl of Folin-Ciocalteau reagent and 6 ml of distilled water. It was vortexed for 1 min, after which 2 ml of 15% Na_2CO_3 was added and the mixture vortexed once again for 30 s. The solution was made up to 10 ml with distilled water. After 1.5 h, the absorbance was read at 750 nm with a UV spectrophotometer.

Total flavonoid assay

Total soluble flavonoid of the spice was determined with colorimetric aluminium chloride methods respectively using rutin as standard as described by Ebrahimzadeh *et al* [14]. 5 ml of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (0.4 mg/ml). Absorption was read at 415 nm using UV spectrophotometer after 10 min against a blank sample consisting of a 5 ml extract solution with 5 ml methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with rutin (0-100 mg/l) as the standard.

Determination of total antioxidant capacity

The total antioxidant capacity was determined according to the method described by Benzie and Strain [16]. Briefly, 2000 µl of freshly prepared FRAP (ferric reducing activity of plasma) reagent was mixed with 30 µl of sample, methanol, or 1.2 M HCl in methanol as appropriate for the reagent blank. The FRAP reagent contained 10 parts of 300 mM acetate buffer (pH 3.6), 1 part of 10 mM tripyridyl triazine (TPTZ), and 1 part of 20 mM ferric chloride. It was incubated at 37ºC for 6 min. The absorbance was read at 593 nm with a UV spectrophotometer.

Determination of reducing property

The reducing power was determined by the method described by Oyaizu [17]. 50-200 µg of the extract was diluted in 1 ml distilled water at different concentration. They were mixed with sodium phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). They were incubated at 50ºC for 20 min. 2.5 ml trichloroacetic acid (10%) was then added to the mixtures, after which they were centrifuged at 3000 rpm for 10 min. The supernatant was dissolved in equal volume of distilled water, and 0.5 ml ferric chloride (0.1%) added. Absorbance was read at 700 nm using a UV spectrophotometer.

Free radical scavenging assay

The free radical scavenging ability of the sample extracts against DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was evaluated as described by Ursini *et al* [18]. Briefly, the extract (50-200 µg) was diluted in 3 ml ethanol and mixed with 3 ml $DPPH^{\cdot}$ solution. The final concentration of DPPH solution was 100 µM. The reaction mixture was shaken, and incubated in the dark. The absorbance of the solution was measured against a blank at 517 nm after 30 min. Percentage inhibition of DPPH was calculated using following equation:

$$\%\text{Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the blank sample and A_1 the absorbance of the tested sample.

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Standard

Different concentrations of gallic acid (25-150 µg/ml) were used as the standard antioxidant.

Statistical Analysis

Statistical significance was established using One-Way analysis of variance (ANOVA) and data were reported as mean ± standard deviation. Statistical analyses were carried out using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, IL, USA).

RESULTS

Proximate composition of the seeds of *M.myristica* is depicted in Fig.1. The proximate composition ranged from 3.06 to 52.42% with nitrogen free extract having the highest value and lowest ash content.

Qualitative screening of the phytochemicals of *M.myristica* (Table 1) revealed the presence of alkaloids, tannins, cardiac glycosides, steroids, terpenoids and saponins. Phlobatannin was observed to be absent. While the quantitative analysis showed a high concentration of saponin, this was followed by tannin, while alkaloids were observed to have the lowest concentration.

The seeds were observed to have a very high total antioxidant activity (82.5 mg/g rutin) as depicted in Fig.2. The total phenol and flavonoid were observed to be 27.6 mg/g GAE and 37 mg/g GAE, respectively.

Fig.3 shows the free radical scavenging activity of the spice against stable DPPH. The scavenging activities of the spice were observed to follow a dose like pattern with increase in concentration, with the highest scavenging activity observed at the highest concentration. The scavenging activity ranged from 16.9 mg GAE/g at 25 µg/ml to 71.15 mg GAE/g at 100 µg/ml. These observed values compared favourably to the standard, gallic acid.

Table 1. Phytochemical properties of *Myristica fragrans* (mean ± SD, n = 3)

Phytochemicals (g/100)	Screening	% Quantity
Tannin	+	1.67 + 0.17
Cardiac glycosides	+	NQ
Steroids	+	NQ
Terpenoids	+	NQ
Saponin	+	4.27 + 0.15
Alkaloids	+	0.51 + 0.25
Phlobatannins	–	–

Key: + = present; – = absent; NQ = not quantified.

The reducing ability of the spice is presented in Fig.4. The spice was observed to have a favorable high reducing power compared to the standard, gallic acid. The reducing ability ranged from 0.028 mg GAE/g at 25 µg/ml to 0.126 mg GAE/g at 100 µg/ml. A dose-like pattern was also observed in the reducing ability of the seeds with increasing concentration.

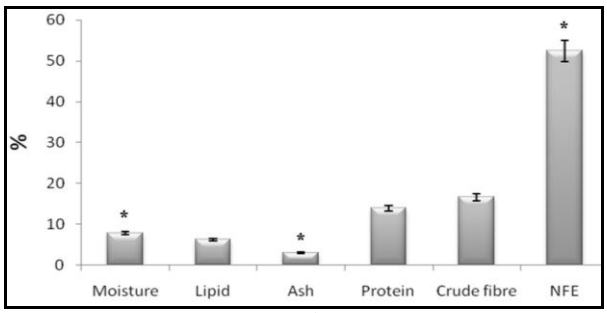

Figure 1. Proximate composition of *Monodora myristica*. Values are mean ± SD (n = 3); *p < 0.05.

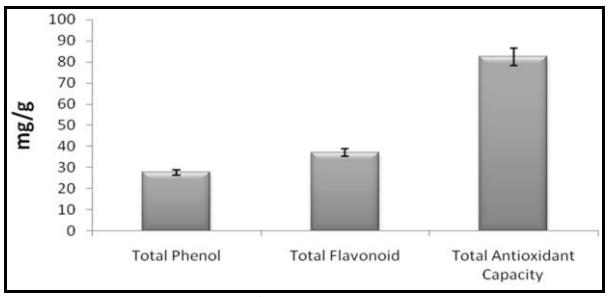

Figure 2. Total phenol, total flavonoid and total antioxidant capacity.

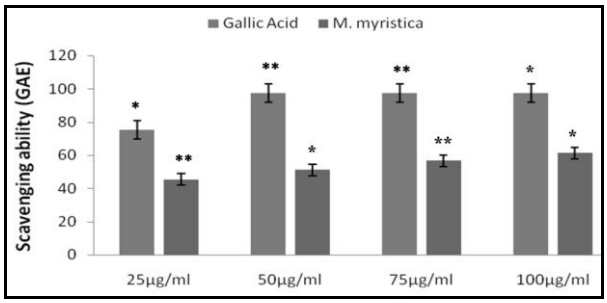

Figure 3. DPPH scavenging actitivities of *Monodora myristica*. Values are mean ± SD (n = 3); *p < 0.05 within group, **p < 0.05 between groups.

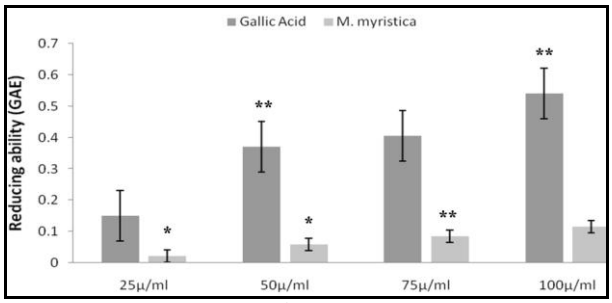

Figure 4. Reducing ability of *Monodora* myristica. Values are mean ± SD (n = 3); *p < 0.05 within group, **p < 0.05 between groups.

DISCUSSION

The protective effects of antioxidants have been shown in different diseases including cardiovascular disease, cancer, aging, and cataracts, their relationship to food have also been extensively conducted [19]. This paper reports the chemical and antioxidant properties of *M.myristica*.

The observed low moisture content of the seeds of *M.myristica* compared favourably to other studied spices like *Myristica fragrans* and *Aframomum sceptrum* [7, 20]. This low moisture content will minimize microbial growth, ensuring longtime storage. This will be of benefits to rural communities which lack access to basic storage facilities and electricity as it will reduce the cost of handling and ensure long term storage [7]. The RDA of dietary fiber for children and adults is reported to be between the ranges of 19-25 and 21-38% [21]. The observed crude fiber was a bit lower than these ranges, but combination with other foodstuffs will meet the RDA. The health benefits of dietary fiber have been reported. It protects against constipation, hemorrhoids, diverticulitis [22]. Recently, the hypoglycemic and hypocholesteremic effect of dietary fiber has been reported [23]. Thus, implying the health benefits of *M.myristica*. The ash content was rather low but compared favorably to results reported by Ekeanyanwu *et al* [24]. They reported an ash content of 3.90% for *M.myristica*. The low ash content signifies a rather low mineral content of the spice. Due to the low level of crude fat in the spice and reported high level of total unsaturated fatty acid of plant oils, its consumption in large amount would be beneficial to individuals suffering from overweight or obesity, and this would constitute a good dietary habit. The crude protein but compared favorably to *Myristica fragrans* (9.96%) and *Aframomum sceptrum* (12.73%) [7, 20]. This signifies the healing properties of *M.myristica* as proteins are essential for the synthesis/repair of body tissues and as enzymes [25].

The pharmacological and biochemical actions of phytochemicals are well known. Phytochemicals screening of *M.myristica* revealed the presence of alkaloids, tannins, cardiac glycosides, steroids, terpenoids and saponins (Table 1). Alkaloids have been reported to be the most efficient therapeutically significant phytochemical [26]. Their analgesic antispasmodic and bacterial properties have been reported and are widely used as cancer chemotherapeutic agents [27]. Dietary tannins have been reported to affect protein digestibility and metal ion availability, but recent studies suggests that free or protein-complex condensed and hydrolysable tannins are more effective than small phenolics [28]. The tannin content of *M.myristica* may therefore contribute to its antioxidant activities. The hemolytic activity and cholesterol binding properties of saponin have been reported. They also serve as natural antibiotics, helping the body to fight infections and microbial invasions [29].

The spice was observed to have appreciable levels of total phenol and flavonoid contents (Fig.3). Over the years, the roles of phenols and flavonoids as powerful antioxidants that protect the human body against free radicals have been widely reported [3]. The best described property of flavonoids is their capacity to act as antioxidants. The flavones and catechins seem to be the most powerful flavonoids for protecting the body against ROS [29]. The antioxidant activity of phenolics is due to their redox properties which allow them to act as reducing agents, metal chelators and free radical quenchers [30].

The DPPH assay has been widely applied in a number of studies to evaluate the radical scavenging ability of antioxidants [31]. The spice was observed to exhibit a dose like pattern in DPPH scavenging activities. This corresponded to several reports that DPPH scavenging activities increased with increasing concentration [20, 32]. However, this contradicts reports by Gong *et al* [33] that the DPPH radical scavenging activity decreased in an order of the ethanol precipitation extract. The DPPH scavenging activity of the spice compared favorably to the standard, gallic acid. The high phenolic and flavonoid contents of the spice could be responsible for its high scavenging activities. This corresponds to reports by Zhou and Yu [34] that total phenolic content of tested vegetable extracts correlated with the DPPH radical scavenging activity, suggesting that total phenolics can play a major role in the antioxidant activity of plant materials. However, increasing evidence suggests high plasma concentration of phenolics could have detrimental pro-oxidant effects [35]. Under certain physiological conditions (high concentrations of phenolic antioxidants, high pH, presence of iron) phenolic antioxidants have been reported to initiate an auto-oxidation process, thus acting like pro-oxidants [36].

The reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [37]. As evidenced in Fig.4, the spice presented a concentration-dependent activity increase. Some phenolic compounds such as flavanoids and phenolic acids have been reported to exhibit antioxidant activity through their reductive capacity in an Fe^{3+}-Fe^{2+} system [38]. The result presented here indicates that the marked reducing power of the spice could be attributed to their antioxidant activity.

In conclusion, the seeds of *Monodora myristica* exhibited high antioxidant activities *in vitro*, signifying the protective potential of the spice against free

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radicals. The phytochemicals, phenolic and flavonoids in particular may be responsible for these effective antioxidant properties. However, there is need of carrying out *in vivo* studies to further authenticate the antioxidant potentials of this spice.

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