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Effects of Thermal Processing on the Nutritional, Antinutrient, and *In Vitro* Antioxidant Profile of *Monodora myristica* (Gaertn.) Dunal Seeds

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ABSTRACT: Proximate, minerals, and anti-nutrient content of raw *Monodora myristica* seed and *Monodora myristica* seeds boiled and roasted for 10, 20, and 30 min, respectively were assessed. In addition, the effects of boiling and roasting for 10, 20, and 30 min on the antioxidant properties of *Monodora myristica* seed extract were evaluated. Results showed that moisture, ash, fat, and crude protein were significantly reduced by boiling and roasting. Acid and neutral detergent fibres were significantly increased by roasting and boiling, with boiling causing a more significant increase than roasting. Processing resulted in significant decrease in magnesium, potassium, phosphorus, zinc, copper, manganese, and iron while calcium levels were unchanged. Total phenolics levels of raw seeds [(21.94 mg/100 g gallic acid equivalent (GAE)] showed a remarkable decrease (18.64 mg/100 g GAE) when the boiling time was increased to 30 min. Free fraction phenolics increased with thermal processing whereas bound phenolics decreased. Boiling was more effective in reducing anti-nutrients than roasting. Extracts of *Monodora myristica* seeds possess significant 1,1-diphenyl-2-picrylhydrazyl and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) free radical scavenging properties in a concentration-dependent manner, and this was significantly reduced after thermal processing. *Monodora myristica* seed extracts could be pharmaceutically exploited for antioxidant properties, and roasting may be preferred to retain the nutrient composition and antioxidant properties of *Monodora myristica* seeds.

Keywords: *Monodora myristica*, anti-nutrient, antioxidant properties, total phenol, thermal processing

INTRODUCTION

Monodora myristica (Gaertn.) Dunal is a useful but underutilized tropical tree from the *Annonaceae* or custard apple family of flowering plants (1,2). It is variously known as *Iwor* amongst the Itsekiris; *Ikposa* (Benin); *Ehiri* or *Ehuru* (Ibo); *Gujiya dan miya* (Hausa), *Ariwo*, *arigbo*, *Abo lakoshe*, or *eyi naghose* (Yoruba); *Ehinawosin* (Ikale), *Uyengben* (Edo), and *Fausse noix de muscade* (French) (3-5). The most economically important parts are the seeds (3,4). The aromatic seeds are antiemetic, aperient, stimulant, stomachic, and tonic, and they are added to medicines to impart stimulating properties (6-8). The seeds also possess magnesium, calcium, potassium, phosphorus, manganese, iron, sodium, copper, aluminium, and zinc (1,9). It also possesses an impressive range of medicinal and antioxidant properties and has several applications in traditional medicine, which is primarily based on indigenous knowledge systems (IKS) (10).

Monodora myristica seeds are processed locally (in the sub-Sahara African regions) using various IKS-based processing techniques like boiling, roasting, and frying for varying lengths of time. They are then dehulled and crushed into flour for use in local dishes, such as the West African "kunu", "tuwo", and "waina". Some natives simply dehull using stone and crush the raw seed for use in local dishes (10). Different processing techniques utilized often have effects on the levels of nutrients and bioactives (11). These processing treatments are also effective in eliminating the anti-nutritional factors in foods (12). Research on the effects of different processing methods on the chemical composition and organoleptic properties of African Nutmeg (*Monodora myristica*) dealt with a single boiling and roasting time of 20 and 15 min, respectively (13). Also, the antioxidant activity of the flavonoid fraction of the seed extract of *Monodora myr-*

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istica (Gaertn) Dunal was evaluated (3). However, data on the effects of various cooking time on the nutritional, anti-nutrients, and antioxidant properties of *Monodora myristica* seeds are not documented.

There is a need for profiling the indigenous knowledge in the processing of *Monodora myristica* seeds to identify processes that promote nutrient content and bioavailability for improved health and nutrient composition of rural populations whose diets are basically plant based. Understanding the influence of these thermal processing operations is also important in retaining the health benefiting properties of *Monodora myristica* seed flour in processed food products. This study was therefore undertaken to investigate the effect of different cooking methods (boiling and roasting) and cooking times (10, 20, and 30 min) on the nutritional, chemical, and *in vitro* antioxidant properties of *Monodora myristica* seeds with a view to providing information towards effective utilization of the seed in food applications. This information will be used for optimal nutrient retention, thereby improving the nutritional status of consumers. Raw seeds of *Monodora myristica* were used as control.

MATERIALS AND METHODS

Reagents and chemicals

Unless otherwise stated, all the chemicals/reagents used were of analytical grade from Sigma-Aldrich Co., Ltd. (Steinheim, Germany).

Plant materials

Monodora myristica seeds harvested wild at Oke Oro Ekiti were purchased on the 20th May, 2016 from Oja Oba (Kings market) at Ado-Ekiti, Ekiti, Nigeria.

Sample preparation

The seeds (1.5 kg) were cleaned and extraneous materials like dry leaves and stones removed. Samples were divided into seven portions (213 g each) and prepared using the method of Mbah et al. (14) with slight modifications. The first portion was raw and served as the control. The second, third, and fourth portions were boiled (100°C) in a pot of tap water in a ratio of 1:3 (weights of the seeds to volume of water) for varying times: 10, 20, and 30 min. After boiling, the seeds were oven dried at 100°C for 5 h, dehulled and milled into fine flour. The remaining fifth, sixth, and seventh portions were roasted (120°C) for different times (10, 20, and 30 min), dehulled and milled into fine flour. The control seeds were dehulled and milled without any thermal processing. Flour samples (each 210 g) were defatted for 4 h using a Buchi 810 Soxhlet Fat Extractor (BÜCHI Labortechnik AG, Flawil, Switzerland) and packaged in labelled polythene bags (CO, raw flour; B10, B20, and B30, flour from seeds boiled for 10, 20, and 30 min, respectively; R10, R20, and R30, flour samples from seeds roasted for 10, 20, and 30 min, respectively). The packaged flour samples were stored in a cool (4°C) dry place until required for analysis.

Preparation of methanolic extracts

Flour samples were extracted (1:5 w/v) using methanol. Extracts were concentrated to dryness under reduced pressure in a rotary evaporator (40°C). Dried extracts were re-dissolved in methanol for further experiments.

Determination of proximate, macro, and micro mineral elements composition

Standard methods from the Association of Analytical Chemists (AOAC) (15) were used for the analysis of moisture, acid detergent fibre, neutral detergent fibre, ash, and crude fat contents. Nitrogen was determined using the micro-Kjeldahl method. Crude protein content was estimated by multiplying %N by a factor, 6.25. All analyses were carried out in duplicate. Micro and macro mineral contents were determined in duplicate using an atomic absorption spectrophotometer (210 VGP, Buck Scientific, East Norwalk, CT, USA).

Tannin content determination

Quantitative determination of tannins was carried out using the modified vanillin-HCl method as described by Mazahib et al. (16). A 0.2 g of each flour sample was extracted with 10 mL 1% (v/v) HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 mL) was added to the extract (1 mL), and the absorbance was read at 500 nm after 20 min. Oxalates and phytates were determined using the method of AOAC (17).

Total phenol content (TPC) determination

Phenols were determined according to Hertog et al. (18), with slight modifications. Briefly, 0.2 g of *Monodora myristica* flour was mixed with 10 mL methanol [99.8% (v/v)] and vortexed for 30 s. The mixture was left overnight at room temperature to extract the free phenols and subsequently filtered through Whatman® no. 1 filter paper. Bound phenols were released from the remaining flour residue by acid hydrolysis. A 10 mL portion of acidified (2 M hydrochloric acid) 60% (v/v) aqueous methanol was added to each sample, which was then incubated at 90°C for 90 min. Samples were allowed to cool before the supernatant was filtered and analyzed for phenols. Free and bound phenols concentrations were determined by a spectrophotometer at 765 nm. Five milliliters of distilled water, 1 mL Folin-Ciocalteu reagent, 10 mL 7% sodium carbonate, and 8 mL distilled water was added to 1 mL of the extract. The solution was incubated for 3 h in a

dark room and the absorbance read. The calibration curve was plotted by mixing 1 mL aliquots of 50, 100, 150, 200, 250, 300, 350, 400, and 450 μg/mL gallic acid solutions with 5.0 mL of Folin-Ciocalteu reagent (diluted tenfold) and 4.0 mL of sodium carbonate solution (75 g/L). The absorbance was measured after 30 min at 765 nm. The amount of phenols in the seed extract was expressed as gallic acid equivalents (GAE).

Determination of *in vitro* antioxidant activity

***Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH)*:** The free radical scavenging activities (FRSA) of the methanolic extracts against DPPH were evaluated according to the method of Gyamfi et al. (19) with slight modifications. One mL of the extract was mixed with 1 mL of the 0.2 mM methanolic solution of the DPPH radicals. The mixture was vortexed thoroughly and left in the dark for 30 min. The absorbance was measured with a Shimadzu UV 1800 spectrophotometer (Shimadzu, Kyoto, Japan) with wavelength set at 517 nm.

$$\mathrm{DPPHFRSA} = \left(\mathrm{A0} - \frac{\mathrm{A1} - \mathrm{AS}}{\mathrm{A0}}\right) \times 100$$

where A0 is absorbance of the control solution containing only DPPH, A1 is absorbance in the presence of extract in DPPH solution, and AS is the absorbance of the sample extract solution without DPPH.

***2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity*:** To determine ABTS radical scavenging assay, the method of Re et al. (20) was adopted. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was diluted by mixing 1 mL ABTS solution with 60 mL methanol to obtain an absorbance of 0.706±0.001 units at 734 nm using a spectrophotometer. Fresh ABTS solutions were prepared for each assay. Plant extracts (1 mL) were allowed to react with 1 mL of the ABTS solution, and the absorbance was measured at 734 nm after 7 min using the spectrophotometer.

$$\text{ABTS FRSA (\%)} = \frac{\mathrm{A0} - \mathrm{AS}}{\mathrm{A0}} \times 100$$

where A0 is the absorbance of ABTS radical+methanol and AS is the absorbance of ABTS radical+sample extract.

Statistical analysis

All data were expressed as the mean±standard deviation (SD). The Statistical Package for Social Sciences (SPSS, version 20, SPSS Inc., Chicago, IL, USA) was used to obtain mean and standard deviations. Analysis of variance (ANOVA) was done and judged for significance at $P \leq 0.05$. Means were separated using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Effect of cooking methods on nutritional composition of *Monodora myristica* seeds

The proximate analysis of raw and processed *Monodora myristica* seeds are presented in Table 1. Moisture, ash, fat, and crude protein contents were significantly ($P \leq 0.05$) reduced by boiling and roasting. The slight differences in proximate analysis of raw *Monodora myristica* seeds in comparison with proximate compositions reported by Ehirim Fidelis et al. (13) and Ekeanyanwu et al. (9) could be due to variations in environmental and soil conditions (21). However, they are in agreement with the findings of Enwereuzoh et al. (22), Burubai et al. (1), and Zaragozá (23). The fat content of the control (46.4 %) had the highest value when compared to the processed samples. This means that treatment with *Monodora myristica* do not improve the fat content of the seeds. The fat content of the control and processed samples is higher when compared to the fat content of soybean seeds (22.7%) (24), but lower than that of castor seed and sesame seed, respectively (50%) (25). The result indicates that raw and processed *Monodora myristica* seeds are a better source of oil than soybean seed, hence it could be grouped under oil rich plant foods. Its seeds could also be a source of vegetable oil for domestic and industrial purposes. Fat is important in diets because it promotes fat soluble vitamin absorption. It is a high energy nutrient and does not add bulk to the diet (26).

The moisture content of the raw and processed seeds ranged from 6.7~9.0% (Table 1). Processing reduced the moisture content of *Monodora myristica* seeds when compared to that of the raw sample. However, research had shown that low moisture content of food samples is a desirable phenomenon since it reduces microbial activity (27). Low moisture content of these seeds is an indicator that the seeds may not support the growth of microorganisms as high moisture content hastens food spoilage and enhances microbial growth (28,29). Oyedeji et al. (30) reported moisture content of 10.1% for flame of forest (*Delonix regia*) seeds while Akpabio (31) reported moisture content of 25.2% for almond (*Terminalia catappa*) seeds.

The level of crude protein found in raw and processed *Monodora myristica* seeds can qualify it as a good source of protein, if bio-available and easily digestible by the body. Ash content signifies the level of mineral present in the

Table 1. Nutritional composition of raw and processed *Monodora myristica* seeds

Parameters	Raw	R10	R20	R30	B10	B20	B30
Moisture	8.96±0.09^{a}	7.39±0.27^{b}	6.84±0.40^{bc}	7.05±0.12^{bc}	6.95±0.35^{bc}	6.81±0.78^{bc}	6.72±0.11^{c}
Ash	3.46±0.04^{a}	3.29±0.06^{b}	3.12±0.01^{cd}	3.23±0.11^{bc}	3.04±0.09^{de}	2.92±0.00^{e}	2.89±0.01^{e}
Fat	46.36±3.02^{a}	41.15±1.82^{bc}	40.06±0.20^{c}	40.31±1.63^{c}	45.80±1.13^{a}	44.76±0.14^{ab}	35.86±2.14^{d}
ADF	46.14±1.41^{c}	52.03±0.80^{b}	51.54±2.05^{b}	53.73±0.57^{b}	56.78±0.02^{a}	57.20±0.48^{a}	58.30±1.72^{a}
NDF	71.51±3.29^{d}	76.10±0.66^{c}	77.16±1.39^{c}	79.37±0.20^{c}	79.98±2.28^{bc}	83.61±1.27^{ab}	84.69±0.86^{a}
Crude protein	23.32±0.27^{a}	21.23±0.19^{b}	21.11±0.20^{b}	21.40±0.06^{b}	20.42±0.13^{c}	20.44±0.06^{c}	19.92±0.17^{d}
Macro-nutrients (%)							
Ca	0.16±0.00^{a}	0.15±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}	0.12±0.00^{a}	0.14±0.00^{a}	0.13±0.00^{a}
Mg	0.32±0.01^{a}	0.29±0.00^{b}	0.28±0.00^{bc}	0.30±0.00^{b}	0.25±0.00^{e}	0.27±0.00^{cd}	0.26±0.01^{de}
K	1.03±0.01^{a}	0.95±0.00^{b}	0.96±0.01^{b}	0.96±0.02^{b}	0.87±0.00^{c}	0.89±0.01^{c}	0.83±0.03^{d}
Na	0.04±0.01^{ab}	0.00±0.00^{c}	0.04±0.01^{ab}	0.06±0.01^{a}	0.00±0.00^{c}	0.05±0.01^{ab}	0.02±0.03^{bc}
P	0.44±0.00^{a}	0.38±0.00^{bc}	0.41±0.00^{abc}	0.42±0.01^{ab}	0.34±0.00^{d}	0.40±0.00^{abc}	0.37±0.05^{cd}
Micro-nutrients (mg/kg or ppm)							
Zn	28.00±0.741^{a}	20.00±1.41^{cd}	26.00±4.24^{a}	24.00±0.00^{abc}	16.50±0.71^{d}	22.00±1.41^{bc}	16.00±0.00^{d}
Cu	26.50±0.71^{a}	24.00±1.41^{bc}	25.00±0.00^{abc}	26.00±0.00^{ab}	21.50±0.71^{d}	23.00±0.00^{cd}	23.00±1.41^{cd}
Mn	14.00±0.00^{a}	12.00±0.00^{b}	11.50±0.71^{bc}	12.00±0.00^{b}	10.50±0.71^{c}	11.50±0.71^{bc}	11.00±0.00^{bc}
Fe	44.00±0.00^{a}	29.50±0.71^{cd}	31.50±2.12^{bc}	35.00±1.41^{b}	26.00±0.00^{d}	31.50±0.71^{bc}	27.00±4.24^{cd}

Values with different letters (a-e) along the row are significantly different from each other at $P \leq 0.05$.
Values are expressed as mean±SD.
R10~R30, roasted for 10, 20, and 30 min; B10~B30, boiled for 10, 20, and 30 min.
ADF, acid detergent fibre; NDF, neutral detergent fibre.

sample (31). The slightly lower value of ash and crude protein in boiled *Monodora myristica* seeds as compared to the raw and roasted form that recorded in this research might be as a result of leaching of minerals and soluble proteins into the boiling water (43). A similar observation was made by Chukwuma et al. (32) for yellow quality protein maize and Adeparusi (33) for lima beans (*Phaseolus lunatus* L.). Moreover, chick beans (19.4%), lima bean (19.8%) (34), kidney beans (20.9%), and lentils (22.9%) (35) have lower amounts of proteins in comparison with that of *Monodora myristica* seed flour protein. The ash content of the control (3.5%) was lower than the ash content of fluted pumpkin seed and beniseed which were 4.8% and 4.94%, respectively (36,37). However, this level is high when compared to 2.5% recorded for African oil bean seed (38), the value of 1.8% obtained for cashew nut (39).

Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were significantly ($P \leq 0.05$) increased by roasting and boiling from 10 to 30 min with boiling causing a more significant increase than with roasting. The increase in ADF and NDF in boiled samples could be as a result of fibre hydration (40). Herranz et al. (41) also reported that boiling resulted in an increase in NDF, ADF, and cellulose contents for 5 frozen vegetables (raw and boiled). Fibre is important for the physiological role of maintenance of internal distension for a normal peristaltic movement of the intestinal tract (37). Diets with a high content of fibre have a positive effect on health since their consumption has been related to a decreased incidence of several types of diseases (42). The ADF and NDF contents of *Monodora myristica* seeds as shown in Table 1 show that when the seed is incorporated into food, it will help to prevent many metabolic or digestive disorders such as constipation and irritable bowels (37).

Effect of processing on the minerals content of *Monodora myristica* seeds

The results of the mineral analysis of processed and raw *Monodora myristica* seeds as presented in Table 1 show that there was no significant ($P \geq 0.05$) difference in the calcium content of raw and processed samples. Processing resulted in a significant decrease in magnesium, potassium, phosphorus, zinc, copper, manganese, and iron. The decrease in some minerals may be attributed to losses caused by discarding the water used in boiling *Monodora myristica* seed. A similar observation was made by Adeniyan et al. (37) for beniseed. Akinmutimi et al. (43) reported that processing reduces the nutrient composition, and they attributed these reductions to solubilisation of nutrients and leaching as a result of boiling. Results obtained in the current study are in agreement with the results obtained by Ehirim Fidelis et al. (13). Macro and micro elements are necessary for normal physiological function, the deficiency of which causes serious metabolic abnormalities and the increase of which leads to toxicity (44). The most abundant macro-minerals are potassium, phosphorus, and magnesium. However, this is not in agreement with the works of Aremu and Ibrahim (45) who showed that phosphorus, calcium, and magnesium were the most predominant minerals in Nigeria plant foods. The implication of the mineral element content in *Monodora myristica* is that, it could serve as a nutrient supplement and in the formulation of infant's food

Table 2. Chemical/anti-nutrient properties of raw and processed *Monodora myristica* seeds

Sample	Tannins (mg/100 g)	Phytates (mg/100 g)	Oxalates (mg/100 g)	Bound phenol (mg/100 g GAE)	Free phenol (mg/100 g GAE)	Total phenol (mg/100 g GAE)
RAW	0.59±0.00^{a}	4.11±0.00^{a}	1.07±0.04^{a}	20.58±0.16^{a}	1.35±0.01^{c}	21.94±0.10^{a}
R10	0.34±0.01^{c}	3.16±0.01^{b}	1.03±0.00^{a}	20.26±0.21^{b}	1.39±0.00^{c}	21.63±0.04^{b}
R20	0.44±0.00^{b}	2.68±0.10^{c}	0.74±0.00^{b}	19.76±0.10^{c}	1.42±0.04^{bc}	21.16±0.01^{c}
R30	0.18±0.04^{d}	2.15±0.00^{e}	0.60±0.01^{c}	18.82±0.00^{d}	1.79±0.16^{a}	20.64±0.07^{d}
B10	0.46±0.00^{b}	2.52±0.07^{cd}	1.02±0.00^{a}	18.49±0.17^{e}	1.15±0.00^{d}	19.64±0.01^{e}
B20	0.19±0.06^{d}	2.29±0.06^{de}	0.54±0.00^{c}	17.84±0.10^{f}	1.48±0.01^{bc}	19.31±0.09^{f}
B30	0.15±0.00^{d}	1.15±0.26^{f}	0.12±0.06^{d}	17.10±0.03^{g}	1.57±0.03^{b}	18.64±0.11^{g}
Permissible limits	20 mg/g	250～500 mg/g	3～5 mg/kg	NA	NA	NA

Values with different letters (a-g) along the column are significantly different from each other at $P \leq 0.05$.
Values are expressed as mean±SD.
R10～R30, roasted for 10, 20, and 30 min; B10～B30, boiled for 10, 20, and 30 min.
NA, not applicable.

products.

Effect of cooking methods on TPC and antioxidant activities of *Monodora myristica* seeds

The TPC of the methanolic extract of seeds of *Monodora myristica* ranged from 18.64±0.11 to 21.94±0.10 mg/g GAE (Table 2). However, the amount of these plant phytochemicals was higher in the raw extracts, and this value is in agreement with the value of 22.2±0.4 mg/g GAE reported by Ogunmoyole et al. (46). Since phenols are generic names given to a class of compounds with great structural diversities and activities, it is expected that the antioxidant constituents present in *Monodora myristica* may have different antioxidant mechanisms (47). These results strongly suggest that phenolics are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents.

Phenols possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (48,49). The results of this study showed that thermal processing significantly decreased total phenolic contents, and the loss during the boiling treatment was significantly higher than that of roasting methods ($P \leq 0.05$) (Table 2). The same observation was made by Otles and Selek (50) for chestnuts. Khalil and Mansour (51) also stated that cooking treatments significantly decreased the phenolic contents of faba beans. Barros et al. (52) reported that cooking could destroy the structures of phenolics and decrease their contents as some phenolic compounds are unstable and easily become non-antioxidative under heating. Therefore, cooking not only decreased the total phenolic contents, but also changed the type and relative amounts of phenolics (53). On the other hand, Adeniyan et al. (37) reported that the total phenolics levels of raw beniseed (0.2 mg/g) showed a remarkable increase as the boiling time was increased to 30 min with a level of 0.4 mg/g GAE. They reported that aqueous extracts of boiled beniseed contained a higher phenol and flavonoid content than aqueous raw extracts of beniseed. Ju et al. (54) reported that steaming under pressure increased the amounts of soluble phenolic acids of the Chaga mushroom (*Inonotus obliquus*).

Also, from Table 2, after heat treatment, the free fraction of phenolic acids increased whereas the bound fractions decreased. The same observation was made by Xu et al. (55) for citrus peel extracts. Phenolic contents, including the free and bound forms, during processing depend on the type of fruit or vegetable (56). Heat treatment of table beets at 105～125°C for 15～45 min either retained or increased free, bound and total phenolic content, total flavonoids, and total antioxidant activity (57). The same investigators also observed reductions in the antioxidant activity, phenolic contents, and total flavonoids (the majority from free flavonoids) in green beans at similar processing conditions of 100～121°C for 10～40 min.

Antioxidant properties of raw and processed *Monodora myristica* seeds

***DPPH radical scavenging properties*:** Fig. 1 shows the dose-response curve of DPPH radical scavenging property of the methanol extracts of the seeds of *Monodora myristica*. DPPH is a stable free radical, which accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidants induce a reduction of DPPH radicals causing a decrease in the absorbance as a result of a visual discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants since such antioxidant have the ability to readily donate their hydrogen to DPPH (58). The DPPH radical scavenging activity of *Monodora myristica* seeds was concentration dependent.

At a concentration of 50 mg/mL, raw *Monodora myristica* seeds extract scavenged 65.5% DPPH radicals. This result is in agreement with the value reported by Ogunmoyole et al. (46) but higher than the value (41.2%) reported by

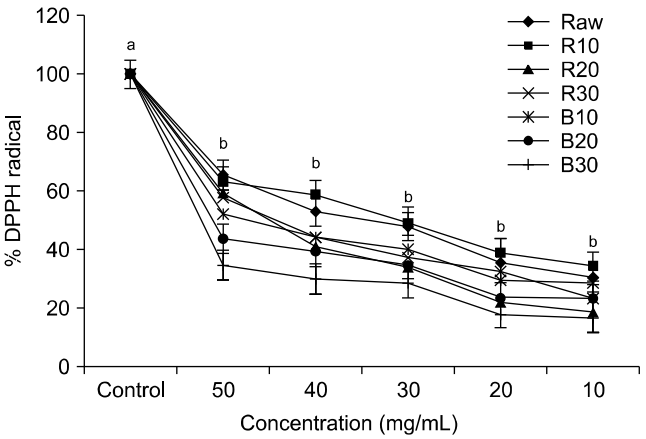

Fig. 1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging ability of raw and processed *Monodora myristica* seed extracts. Values are mean±SD of triplicate determinations. Values with different letters (a,b) indicate significant difference at $P \leq 0.05$. R10~R30, roasted for 10, 20, and 30 min; B10~B30, boiled for 10, 20, and 30 min.

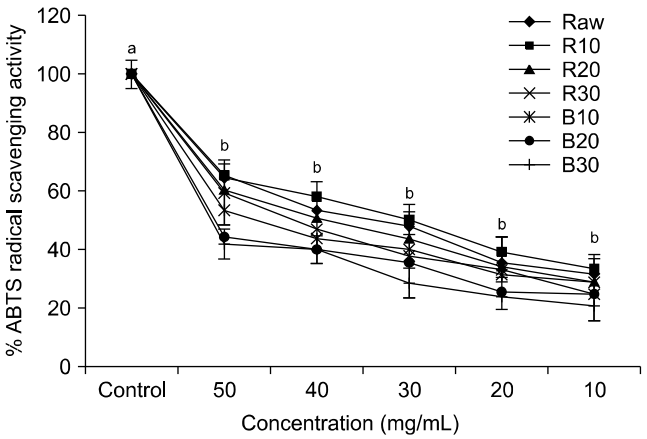

Fig. 2. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging ability of raw and processed *Monodora myristica* seed extracts. Values are mean±SD of triplicate determinations. Values with different letters (a,b) indicate significant difference at $P \leq 0.05$. R10~R30, roasted for 10, 20, and 30 min; B10~B30, boiled for 10, 20, and 30 min.

Feyisayo and Oluokun (3). While the reason for these differences in radical scavenging activity is not completely understood, it could suggest differences in the phytochemical constituents, which are responsible for scavenging free radicals. Logically, extracts with higher phytochemical constituents have higher free radical scavenging effect. Many studies in the literature present positive correlations between the quantity of phenolic compounds and the DPPH free radical scavenging effect (47,53). Furthermore, this can also be attributed to the solubility of the individual phytochemical in different solvents. Most of the phenols which might not be soluble in water may become soluble in ethanol or methanol hence they get extracted leading to increase in the phytochemical content and subsequent increase in radical scavenging activity.

***ABTS radical scavenging activity*:** The methanol extracts of the seeds of *Monodora myristica* seeds were fast and effective scavengers of the ABTS radical (Fig. 2). ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm, which decreases with the scavenging of the proton radicals (59). The scavenging of the ABTS radical by the extracts was found to be much higher than that of DPPH radical. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals (60). Wang et al. (61) found that some compounds, which have ABTS radical scavenging activity, did not show DPPH scavenging activity.

Monodora myristica seed extracts were found to be very effective scavenger of ABTS radical, and the activity increased in a concentration dependent manner (Fig. 2). At 50 mg/mL, the extracts exerted the highest $ABTS^{+}$ scavenging activities ranging from 42.1 to 65.7%. It is apparent that *Monodora myristica* is a good free radical scavenger, and it could effectively act as a primary antioxidant against free radicals and be considered a good source of natural antioxidant in preventing lipid peroxidation and protection from oxidative damage (62).

The DPPH and ABTS radical scavenging activity of *Monodora myristica* seed extract was significantly reduced ($P \leq 0.05$) after thermal processing. This may be due to the instability of strongly antioxidant phenolic compounds at high temperatures (63,64). Reduction in antioxidant activity during roasting could have been a result of decomposition of larger molecular weight phenolic compounds, particularly tannins. High-tannin grains have been reported to exhibit higher antioxidant activity than low-tannin grains (65). Boiling and steaming caused a higher reduction in the antioxidant capacity of red pepper than stir-frying and roasting (66). Siddhuraju and Becker (67) also reported a decrease in % FRSA of dry heated cowpea samples. Also, the antioxidant activities of these samples may be linked to their TPC. For instance, raw *Monodora myristica* seed, which had the highest TPC (Table 2), had a relatively higher DPPH and ABTS FRSA than the other samples. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species (53). Phenolics are commonly known for their antioxidant effects. They react and capture free radicals thereby inhibiting oxidative stress. They are also commonly known to exhibit anti-allergic, anti-inflammatory, antimicrobial, and anticancer activity (68). It is therefore rational to believe that extracts, which contain a higher content of these important phytochemicals, would exhibit a higher free radical scavenging ability. These findings indicate that the processing methods used in the current study would contribute to a decrease in the health-pro-

moting potential of *Monodora myristica* due to reductions in antioxidant activity.

Effect of processing methods on anti-nutrient content of *Monodora myristica* seeds

The presence of anti-nutritional factors in foods hinders the efficient utilization, absorption, or digestion of some nutrients and thus reduces their bioavailability and nutritional quality (69,70). Tannins for instance interact with protein and cause a significant reduction in protein digestibility (71). The results of the current study demonstrated that boiling was more effective in the reduction of anti-nutritional factors than roasting (Table 2). The reason may be attributed to the fact that boiling led to a breakdown of the plant cell wall which permitted the leakage of cell contents including anti-nutrients (72) while roasting is a mere gradual evaporation processes (73). It could also suggest that the anti-nutrients leached into the water (13). The significantly ($P \leq 0.05$) lower levels of oxalate, phytate, and tannins in the flours prepared from the boiled seeds and roasted seeds when compared with their values in the flour from raw seeds are in agreement with the reports by Igbedioh et al. (74), Inyang et al. (73), and Nwosu (75). Reduction in oxalate and phytate contents as a result of boiling and roasting of the seeds may lead to the improvement on the bioavailability of essential minerals like calcium, magnesium, and iron that usually form complexes with these compounds (76). Also, reduction in tannins may lead to the improvement on protein digestibility, better bioavailability, and utilization of amino acid contents in the flour protein (76).

The value of 0.6 mg/100 g of tannin in the raw *Monodora myristica* is slightly higher than the value of 0.5 mg/100 g reported by Ehirim Fidelis et al. (13) but lower than 0.64 mg/100 g reported by Ekeanyanwu et al. (9). Raw *Monodora myristica* seeds had the highest tannin value, which explains that processing methods (boiling and roasting) reduce the tannin content of *Monodora myristica* seeds. The same observation was made by for Mbah et al. (14) for *Moringa oleifera* seeds. However, tannin values obtained for the raw and processed seeds are lower than the critical value of 20 mg/g that could induce tannin toxicity (77). This suggests that the tannin content in the *Monodora myristica* seeds will have no adverse effect on consumers. Antinutritional effects of tannins include interference with the digestive processes either by binding enzymes or by binding to food components like proteins or minerals (78,79). Tannins also have the ability to complex with vitamin B_{12} (80).

Phytate levels of 1.2 to 4.1 mg/100 g were observed. The knowledge of phytate levels in foods is necessary because high levels could cause adverse effects on digestibility (81). Phytate forms stable complexes with copper, zinc, cobalt, manganese, iron, and calcium (9). Phytates consumption should be lowered as much as possible, ideally to 25 mg or less per 100 g or to about 0.03% of the phytate-containing food eaten (82). They are known to reduce bioavailability of minerals, impair protein digestibility caused by formation of phytic-protein complexes, and hinder absorption of nutrients due to damage to the pyloric caeca region of the intestine (83).

Oxalate levels of 0.1 to 1.1 mg/g were observed. These values are lower than the values of 2.5～3.6 mg/100 g reported by Mbah et al. (14) for *Moringa oleifera* seeds. Oxalate has been implicated in the formation of kidney stones and a decrease in calcium absorption (30), but consumption of seeds of low oxalate content such as *Monodora myristica* may not induce any of these.

In conclusion, boiling was more efficient in reducing the anti-nutrients content of *Monodora myristica* seeds implying that the anti-nutrients are more susceptible to moist heat than dry heat. The significant reduction of anti-nutrients during processing suggests that the nutritional components of *Monodora myristica* seeds will be more bioavailable. This research suggests that *Monodora myristica* has tremendous potential in alleviating protein energy malnutrition in Africa since it is nutritionally rich in protein. Methanolic extracts of raw and processed *Monodora myristica* seeds contained large amounts of phenolic acids and exhibited high scavenging activities. These *in vitro* assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress oxidative stress. Further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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