RESEARCH ARTICLE

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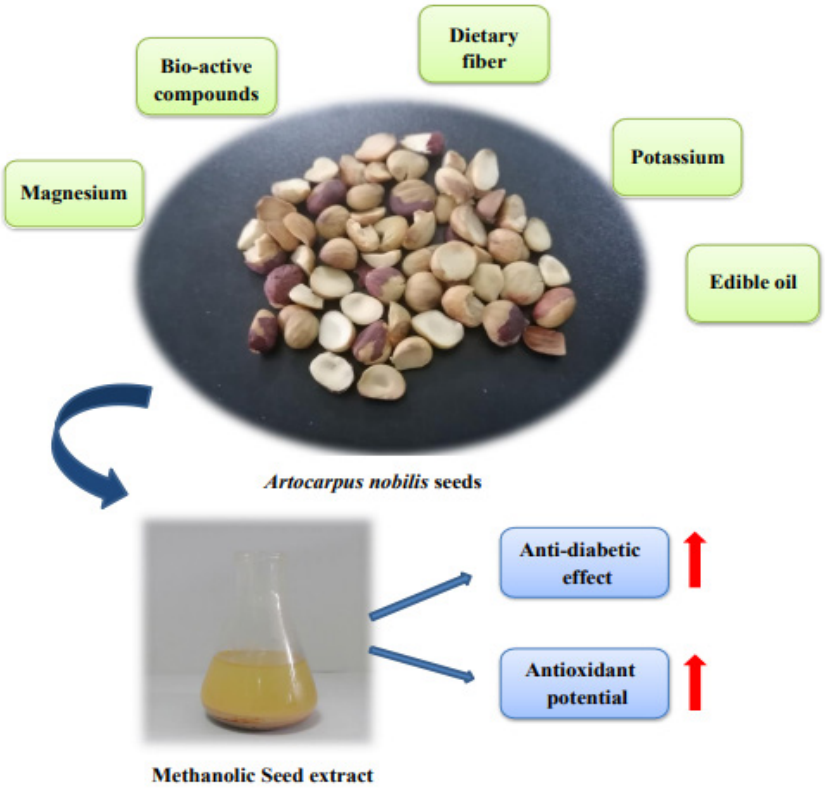

Highlights

- Underutilized *Artocarpus nobilis* seeds can be categorized as nutrient-dense plant food.
- Methanolic seed extract exhibited significantly higher antioxidant potential and α-amylase enzyme inhibitory activity than pistachio, almond, and cashew nut.
- Can be used as an inexpensive source of natural antioxidant.
- Roasting and microwaving improved the nutritional value of *Artocarpus nobilis* seeds.

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Nutritional properties, antioxidant potential and antidiabetic effect of raw and processed *Artocarpus nobilis* (Ceylon breadfruit) seeds

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Abstract: *Artocarpus nobilis* Thwaites (Ceylon breadfruit) is an under-utilized, native plant in Sri Lanka that produces seeds with a unique taste. This study investigated the nutritional properties, antioxidant potential, and anti-diabetic effect of raw and processed (roasted, boiled, and microwaved) *A. nobilis* seeds in comparison with some popular nuts. According to the analysis, both raw and processed *A. nobilis* seeds were rich in proteins (11.73-12.94 %), fat (26.45-28.02 %), total dietary fiber (27.72-30.08 %), along with a higher level of potassium (5188.5-5838.2 µg/g), magnesium (1077.0-1176.1 µg/g), and contained a considerable amount of iron (21.30- 24.51 µg/g) and zinc (15.30-19.23 µg/g). *A. nobilis* seeds had significantly higher total phenolic content, flavonoid content, and antioxidant activity than commonly consumed pistachio, almond, and cashew nuts. Moreover, methanol extracts of roasted *A. nobilis* seeds strongly inhibited α-amylase enzyme (IC_{50} = 48.72 µg/ml) and exhibited higher anti-diabetic properties. Processing significantly modulated the proximate composition and antioxidant activity in tested samples. Pan roasting and microwaving are preferable processing methods to improve their nutritional value and antioxidant potential. The current study concluded that *A. nobilis* seeds are rich in macro- and micro-nutrients and antioxidants, and could be considered as a potential source in mitigating undernutrition and oxidative stress.

Keywords: *Artocarpus nobilis;* processing; nuts; nutrients; antioxidants.

INTRODUCTION

The role of bioactive compounds in preventing diet-related non-communicable diseases has received considerable research interest lately (Gul *et al*., 2016; Teodoro, 2019). These compounds are known to modulate the metabolic process and exert several physiological, immunological, and pharmacological effects (Dillard & German, 2000). Studies have already revealed that tree nuts and seeds are rich sources of phytochemicals and excellent sources of antioxidants ranking third behind spices and fruits (Wu *et al*., 2004; Pérez-Jiménez *et al*., 2010). Tree nuts and seeds like almond, walnut, pistachio, pecan, hazelnut, pumpkin, sesame, sunflower seeds have been subjected to extensive investigations for their health benefits (Nyam *et al*., 2009; Rusu *et al*., 2018). According to the statistics of the International Nut and Dried Fruit Council (INC, 2020), 95% of the world trade in tree nuts is based on just five nut species. Nevertheless, there are many indigenous species of edible nuts with a great potential to address food insecurity and oxidative stress-related health problems in developing countries. Similarly, the seed of *Artocarpus nobilis* Thwaites (Ceylon breadfruit/ Waldel/ Badi del) is a native underutilized tree nut in Sri Lanka that is yet to be explored for its nutritional and functional properties.

Artocarpus nobilis belonging to the family Moraceae is endemic to Sri Lanka and is mainly distributed in the southern wet zone of the country. Plants in the genus *Artocarpus* are enriched with bioactive compounds and have the potential for use in ethnopharmacological purposes (Jagtap and Bapat, 2010). Several previous studies have screened the bioactivities and chemical constituents of the bark, leaves, and the whole fruit of *A. nobilis.* Cycloartane-type triterpenoids, flavonoids, benzofurans, and stilbene derivatives were isolated from the extract of the bark (Zahid *et al*., 2007) while geranyl chalcone derivatives were found in the extracts of the leaves (Jayasinghe e*t al*., 2004a). Interestingly, these phytochemicals exhibited significant antifungal, antioxidant activity (Jayasinghe *et al*., 2004b; Jayasinghe *et al*., 2008; Iverson *et al*., 2010), antibacterial, moderate acetylcholinesterase (AChE) inhibitory activity (Zahid *et al*., 2007), and glutathione S-transferase (GST) enzyme inhibitory activity (Iverson *et al.*, 2010). According to Jayasinghe *et al*. (2006), the whole fruit of *A. nobilis* also resulted in high antioxidant activity without showing significant antifungal activity against *Cladosporium cladosporioides.* However, none of the studies has reported the nutritional composition and bioactive properties of *A. nobilis* seed. According to Soong and Barlow (2004), fruit

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seeds of jackfruit (*Artocarpus heterophyllus*), which are from the same family were found to contain significantly higher phenolic content and antioxidant activity than their edible portions. Therefore, there is a high probability of *A. nobilis* seeds having promising bioactive properties. There is evidence that these seeds had been eaten well before 30000 years according to the evidence from archaeological excavations done at Kitulgala, Beli-Lena, Sri Lanka (Wedage *et al*., 2020). In Sri Lanka, these seeds are consumed mainly after pan-roasting and boiling and there is a flavor enhancement after pan-roasting. Hence, the present study evaluated the proximate composition, mineral composition, phenolic and flavonoid contents, antioxidant activity, and *in vitro* anti-diabetic properties of both raw and processed *A. nobilis* seeds.

MATERIALS AND METHODS

Sample collection and preparation

Mature seeds of *A. nobilis* (Badi del or Wal del in Sinhala) (Figure 1A) were collected from eight locations in Sri Lanka. Seeds were authenticated by the National Herbarium of the Peradeniya Botanical Garden, Sri Lanka, and a voucher specimen was deposited at the National Institute of Fundamental Studies, Kandy. Seeds were hand-sorted to remove splits and foreign matters. Approximately, 250 g of seeds were randomly selected from each location, and a composite sample was prepared. A pool of raw *A. nobilis* seeds was divided into four groups and subjected to three different processing methods: pan roasting, boiling, and microwaving. In pan-roasting, seeds were roasted using a nonstick pan and electric hot plate at 200 °C for 20 min. The optimum roasting condition for the nut was decided on the basis of sensory attributes such as roasted oily aroma, brown color, and crunchy texture. Samples were microwaved at a medium-high temperature for 3 min. The boiled sample was prepared as follows; raw seeds were boiled for 20 min by adding distilled water in a 1:3 (w/v) ratio until seeds were soft. The inedible outer cover was peeled off manually prior to grinding and processed samples (Figure 1B) were stored at -20 °C.

Phytochemicals analysis, antioxidant activities, and anti-diabetic effect of raw and processed seeds of *A. nobilis* were compared with those of the three commercial nut types (almond, pistachio, and cashew). Unprocessed almonds (*Prunus dulcis*), cashew (*Anacardium occidentale*), and pistachio (*Pistacia vera*) were purchased from the local market, Sri Lanka. Almond, cashew, and pistachio were roasted according to the optimum time and temperature combination reported by Lin *et al*. (2016), Chandrasekara and Shahidi (2011), and Hojjati *et al*. (2013), respectively. Cashew, pistachio, and almond were roasted at 130 ℃ for 33 min, 135 ℃ for 20 min, and at 200 ℃ for 20 min, respectively.

Determination of proximate composition

Moisture, lipid, crude protein, ash, dietary fiber, and carbohydrate contents were analyzed according to the relevant standard procedures (AOAC 2000). Crude protein and crude fat contents were determined using Kjeldahl and Soxhlet extraction methods respectively. Dietary fiber content was analyzed using an enzymatic kit (TDF 100A-1KT, Sigma-Aldrich) following the Prosky-AOAC method (AOAC 2000). The Digestible carbohydrate (CHO) content on dry basis was calculated using the following formula: Carbohydrate (%) = 100% - (crude protein% + crude fat% + ash%+ total dietary fiber%). The energy values of the seeds were estimated in kilojoules by multiplying the protein, fat, and carbohydrate percentages by the food energy conversion factors 16.7, 37.7, and 16.7, respectively as mentioned by Eknayake *et al*. (1999).

Determination of micro-mineral composition

Dried samples (0.25g) were digested according to the method previously described by Naozuka *et al*. (2011) using a microwave assisted closed vessel digestion system (MARS, CEM Corporation, North Carolina). The digestion procedure was done in triplicate for each sample. Samples obtained from the acid digestion were filtered using a cellulose acetate filter (0.45 µm) and analyzed for Ca, Mg, K, Na, Zn, Fe, Zn, Mn, Co, Cu, and Ni by Inductively coupled plasma-optical emission (ICP-OES) (iCPA 7000

(A) (B)

Figure 1: (A) Mature seeds of *Artocarpus nobilis* (B) de-hulled processed *Artocarpus nobilis* seed.

series, Thermo Scientific).

Determination of anti-nutritional compounds and their effect on mineral bioavailability

The phytic acid content of *A. nobilis* seeds was determined by the method described by Singh *et al.* (2015) and the oxalate content was determined according to the procedure reported by Hassan *et al*. (2011). The molar ratios for Phytate:Ca, Phytate:Fe, Phytate:Zn, Oxalate:Ca (mol/kg) were calculated according to Gemede *et al*. (2016) and the effect of oxalate and phytate level on the bioavailability of minerals was evaluated.

Evaluation of phenolic content and total antioxidant capacity

Preparation of crude extracts

Raw and processed nut samples were defatted with n-hexane. The defatted powder was extracted into methanol according to the method described by Tomaino *et al*. (2010) with a slight modification. Briefly, defatted seed powder (5 g) was mixed with methanol/ water (2:1) solution in the ratio of 1:5 (W/V), homogenized for 10 min, and ultra-sonicated at 30 kHz, in-room temperature for 15 min. The homogenate was centrifuged at 4500 rpm for 15 min, and the supernatant was separately collected. The residue was again subjected to the same extraction procedure. Combined supernatant was concentrated with the aid of a rotary evaporator and lyophilized for 48 hr. The weight of the methanol extract of each sample was recorded and stored at -20 °C until use.

Determination of total phenolic content

Total phenolic content (TPC) in methanol extracts was determined according to the method described by Pajal *et al*. (2014) with some minor modifications. Briefly, 65 µL of sample extract was mixed with 105 µL of 10% Follin-Ciocalteu reagent and 80 µL of 7.5% Na_2CO_3 in a 96-well micro-plate. After incubating the samples for 30 min (25 ± 2º C), the absorbance was measured at 760 nm. TPC in samples was calculated using a Gallic acid standard curve and expressed as milligrams of Gallic acid equivalent per gram of defatted sample using a microplate reader (Fluostar Omega, BMG Labtech, Germany).

Determination of total flavonoid content (TFC)

Flavonoid content in samples was determined according to the method described by Singh et al. (2015) with some minor modifications. Briefly, 20 µL of 5% $NaNO_2$, 20 µL of 10% $AlCl_3$, and 200 µL of 4 % NaOH were mixed with 50 µL of sample extract. The total flavonoid content in each extract was determined using a catechin standard curve, which was prepared using a 0.2 mg/mL catechin stock solution. Sample analysis was done in triplicate, and the absorbance was recorded at a wavelength of 510 nm after 15 min incubation period.

DPPH radical scavenging capacity

The capacity to scavenge DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical was monitored by the method described by Brand-Williams *et al*. (1995) with slight modifications. Briefly, 0.12 mg/mL DPPH solution in absolute methanol was freshly prepared to obtain an absorbance between 0.8 and 0.9 at 517 nm. Then, 100 µL of DPPH solutions and 150 µL of sample extract with varying concentrations were added into a 96-well microplate. Triplicates were carried out for each sample concentration, and the sample blank was prepared by adding methanol extract without DPPH. As the control blank, instead of the sample, 150 µL of absolute methanol was mixed with 100 µL of DPPH. Finally, the mixture was left to incubate for 30 min, and the absorbance of reaction mixtures was measured at 517 nm. The inhibition percentage of DPPH• was calculated using the following equation: $[(A_o - A_e)/ A_o]$ X 100, where A_o is the absorbance of the blank and A_e is the absorbance of the tested sample.

The inhibition percentage was separately calculated and the concentration vs. inhibition percentage was plotted. From each graph, the sample concentration required to obtain 50% radical inhibition (IC_{50} value) was calculated.

Trolox equivalent antioxidant capacity (TEAC) by ABTS

Total antioxidant activity of sample extracts was determined using TEAC by ABTS according to the method described by Gouveia & Castilho (2011) with some modifications. Each well of the microplate consisted of 50 µL of a sample or Trolox and 150 µL of ABTS solutions. Sample blank was carried out using 50 µL of sample and 150 µL of distilled water. The ABTS•+ bleaching was measured at 734 nm after 6 min incubation. Antioxidant activities were expressed as µmol of Trolox equivalent per gram of sample dry weight.

FRAP Assay

The FRAP Assay was carried out as described by Benzie & Strain (1996), with minor modifications for assay on a 96-well microplate. Briefly, 50 µL of sample extract was reacted with 200 µL of working FRAP solution and incubated for 4 min. The analysis was in triplicate for each sample, and the absorbance was measured at 593 nm. The FRAP absorbance data were calculated against a series of dilutions of ferrous sulfate.

Oxygen Radical Absorbance Capacity (ORAC) Assay

Reagents and standards for the $ORAC_{FL}$ were prepared according to the procedure mentioned in Huang *et al*. (2002). The assay was carried out using a FLUOstar Omega plate reader with an inbuilt incubator, and auto-injector, with fluorescein as the probe. Briefly, a sample with known concentration or Trolox (25 µL; 12.5-200 µm) was added to the black microplate and then fluorescein (150 µL; 10 nm of concentration) was quickly added to each well using a multichannel pipette and incubated at 37 °C for 30 min. Afterward, AAPH (25 µL; 240 mM) was injected using the auto-injector. The program of the plate reader was as follows: excitation filter, 485 nm; emission, 520 nm; cycle time, 90 S; number of cycles, 80; shaking mode, orbital shaking for 4S after injection. The final ORAC value was calculated using MARS data analysis software.

Alpha-amylase inhibitory assay

Alpha-amylase inhibitory activity was performed according to the Visvanathan *et al*. (2019) and the following reagents were added to each well: 40 µL of PBS (0.02 M, pH 6.9), acarbose or plant extract, and α-amylase enzyme (15 Unit/mL). Then the mixture was incubated for 10 min at 37 °C and 40 µL of soluble potato starch (3g/L) was added immediately. Finally, 100 µL of glucose oxidase/peroxidase (GOD/POD) reagent was added using the onboard injector of the microplate reader. The absorbance was measured at 505 nm after 15 min incubation using Fluostar Omega, BMG Labtech, Germany.

Statistical analysis

Results were calculated and expressed as mean ± standard deviation and statistically analyzed by one-way analysis of variance (ANOVA) using SAS software (SAS 9.3.1). Mean differences were compared using Duncan multiple range test at a P-value of ≤0.05. The correlation between phenolic content and antioxidant activity of the extracts was determined using Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of raw and processed *A. nobilis* seeds is presented in Table 1. The raw seed contained 11.73% of protein, 26.45% of lipid and 59.48% of carbohydrate on a dry matter basis (DM). Calculated available carbohydrate content and estimated gross energy (GE) in raw seeds were around 29.41% and 16 842 KJ/kg DM, respectively. Although Jackfruit (*A. heterophyllus*) and Ceylon breadfruit (*A. nobilis*) belong to the same genus, the proximate compositions of their seeds showed considerable differences. The main compositional difference between the seeds of *A. heterophyllus* and *A. nobilis* was the lipid content. Interestingly, *A. nobilis* seeds had approximately 30-fold higher lipid content than *A. heterophyllus* seeds. However, protein and ash contents were roughly similar between the two species, and the carbohydrate content in *A. nobilis* seed was lower than that in *A. heterophyllus* seed (Eke-Ejiofor *et al*., 2014).

According to the data presented in Table 1, the processing modulated the mean moisture content of the *A. nobilis* seed. The moisture content in both the roasted and microwaved seeds was significantly ($p<0.05$) lower than that of the raw seeds. However, after boiling, the moisture content of seeds has become doubled. There was no significant difference ($p>0.05$) in mean moisture content between microwaved and roasted samples. Low moisture content decreases microbial growth, unwanted fermentation, and undesirable enzyme activities. Therefore, the roasted and microwaved seeds could be stored for a more extended period than the boiled seeds (Tenyang *et al*., 2016).

The processing method may alter the lipid content in different ways. According to the data presented in Table 1, pan roasting and microwaving increased ($p<0.05$) the lipid content of the raw *A. nobilis* seeds. Moreover, processing had an impact on the protein content. Significantly higher protein content was observed in roasted, microwaved, and boiled samples than the raw seeds. Results were in accordance with the observation of Mariod *et al*. (2012) and Karuna *et al*. (2016) that boiling and roasting resulted in significantly higher lipid and protein content than that of raw safflower and sesame seeds, respectively. The observed result may be due to the moisture loss during heat treatment, which apparently increased lipid (Tonfack Djikeng *et al*., 2018) and protein concentration (Sanchiz *et al*., 2019). The mean ash content of the raw and processed *A. nobilis* seed was in the range of 1.77 to 2.61%. The highest ash content was observed in the roasted sample (2.46±0.15), while the lowest ash content was in the boiled sample (1.86±0.06). However, the thermal processing did not make any significant ($p>0.05$) difference in ash content except in the boiled sample.

The total dietary fiber (TDF) content of *A. nobilis* seed

Table 1: Proximate composition of raw and processed *Artocarpus nobilis* seeds.

Analyses	Raw	Composition (%) Roasted	Microwaved	Boiled
Moisture (fresh weight)	12.92±1.08^{b}	10.06±0.94^{c}	8.05±0.26^{c}	24.77±1.76^{a}
Protein	11.73±0.25^{c}	12.24±0.26^{b}	12.36±0.25^{b}	12.94±0.07^{a}
Fat	26.45±0.86^{b}	28.02±0.93^{a}	27.98±0.19^{a}	27.15±0.69^{ab}
Ash	2.33±0.01^{a}	2.46±0.15^{a}	2.41±0.09^{a}	1.86±0.06^{b}
Total dietary fiber	30.08±0.28^{a}	28.36±0.18^{b}	28.16±0.42^{b}	27.72±0.22^{b}
Insoluble	27.58±0.80^{a}	25.23±0.69^{b}	25.54±0.36^{b}	26.02±0.49^{ab}
Soluble	2.50±0.51^{ab}	3.12±0.51^{a}	2.62±0.05^{ab}	1.71±0.27^{b}
Carbohydrate (by difference)	29.41	28.92	29.09	30.33
Energy (KJ/kg dry matter)	16,842.20	17,437.26	17,470.61	17,461.64

Protein, lipid, carbohydrate, ash, and dietary fiber composition are expressed on dry matter basis (DM). Values (mean ± standard deviation, n=3) bearing different superscript letters within the same row are significantly different ($P < 0.05$).

Table 2: Elemental concentrations of *Artocarpus nobilis* seeds. .

Element	Raw	Roasted	Boiled	Microwaved
Na	30.15±0.93^{a}	24.43±2.96^{b}	28.08±0.91^{ab}	25.64±2.49^{ab}
K	5838.18±138.20^{a}	5337.78±225^{b}	5188.52±39.30^{b}	5239.78±199.95^{b}
Mg	1176.12±67.83^{a}	1171.40±39.50^{a}	1109.78±69.09^{a}	1076.99±78.08^{a}
Ca	567.29±21.55^{a}	660.40±32.40^{a}	570.44±91.28^{a}	563.27±57.77^{a}
Fe	24.51±0.97^{a}	21.30±2.50^{a}	22.66±0.98^{a}	21.81±0.64^{a}
Zn	16.35±0.70^{ab}	19.23±0.85^{a}	15.91±2.27^{b}	15.30±0.92^{ab}
Mn	6.21±0.17^{a}	5.36±0.24^{bc}	5.63±0.22^{b}	5.05±0.16^{c}
Co	0.015±0.004^{c}	0.046±0.007^{b}	0.063±0.006^{a}	0.034±0.003^{b}
Cu	4.33±0.10^{b}	5.22±0.03^{a}	5.17±0.11^{a}	5.15±0.19^{a}
Ni	0.26±0.08^{b}	0.41±0.03^{a}	0.37±0.03^{ab}	0.32±0.02^{ab}

Significant differences are denoted using different superscript letters.
Values are expressed in µg/g of sample. Values (mean ± standard deviation, n=3)

ranged from 27.72- 30.08% and could be considered a good source of fiber (Dhingra *et al*., 2012). The raw seed had significantly higher TDF content than the processed sample. Nevertheless, there was no significant difference in TDF content among roasted, boiled, and microwaved samples. Insoluble dietary fiber contents of the raw and processed samples were in the range of 25.23-27.58 % DM where SDF was recorded in less than 3.2 %. As the energy density of raw *A. nobilis* seed was lying between 4-9 kcal/g (16 736- 37 656 KJ/kg), this seed can be classified under the group of 'high energy density food" (Rolls, 2017). Moreover, roasted and microwaved samples had higher energy value than raw seed which agrees with the observation of Brufau *et al*., (2006) that reported higher energy value in the roasted almond, cashew, hazelnut, pecan, and pistachio than their raw seeds.

Mineral composition

As shown in Table 2, potassium, calcium and magnesium were the most abundant minerals in *A. nobilis* seed. Reported K content in raw *A. nobilis* ranged from 5699.98-5976.18µg/g, where corresponding Mg and Ca values were 1108.29-1243.95 µg/g, and 545.74-588.84 µg/g. Similar elemental distribution was observed in other common nuts which are well known as rich sources of healthy minerals (Ros, 2010). The consumption of foodstuff that is rich in K, Ca and Mg is associated with the protection against insulin resistance, arterial hypertension and alleviates overall cardiovascular risk (Ros, 2010). When considering the micro-mineral distribution, Fe was the most abundant mineral (23.54-25.48 µg/g) followed by Zn (15.65-17.05µg/g) and Mn (6.04-6.38 µg/g) while Co, Ni were found in very low concentrations. The processing method slightly affected the mineral composition of the raw sample. The K, and Ca concentration in *A. nobilis* was considerably lower than the reported value of cashew, and brazil nuts but higher than that of tropical nuts: babassu and capuassu seeds (Naozuka *et al*., 2011).

Anti-nutritional compounds

Phytic acid and oxalate are two main anti-nutritional compounds that decrease the bioavailability of several minerals including Ca, Fe and Zn (Hassan *et al*., 2011). As indicated in Table 3, raw *A. nobilis* seed had 5.59-6.75 mg/100g of phytate content where total oxalate content ranged 246.9-286.6 mg/100g. Generally, legumes, oilseeds, and nuts contain comparatively higher phytate content than other plant sources (Schlemmer *et al*., 2009). The phytate content of commercial nuts could vary from 0.17-9.42 mg/100g (Schlemmer *et al*., 2009), and total oxalate content varied 42-469 mg/100g (Chai & Liebman, 2005). Considering the impact of processing, boiling reduced the phytate and oxalate content in raw seed, but not at a significant level. Roasting and microwaving resulted in higher phytate and oxalate content than its raw state. The changes observed upon processing agreed with results reported previously (Ayemhenre *et al*., 2015).

The calculated molar ratios (MRs) between antinutrients and minerals have been proposed as an indicator for predicting the bioavailability of dietary minerals. Bhandari & Kawabata, (2004) has mentioned that the MRs of [Phy]/[Ca] > 0.24 is associated to reduce the bioavailability of Ca. The obtained ratio for [Phy]/[Ca] in raw and processed *A. nobilis* was lower than the critical value (0.24). Therefore, phytate content in *A. nobilis* seeds may not significantly affect the Ca bioavailability. However, the reported MR for [Oxa]/[Ca] was above the critical limit (>1) (Gemede *et al*., 2016), indicating that the bioavailability of calcium in *A. nobilis* may adversely be affected by the oxalate content. The critical value for the [Phy]/[Fe] and [Phy]/[Zn] has been established as 1 and 15, respectively (Gemede *et al*., 2016). As the calculated MRs for [Phy]/[Fe] and [Phy]/[Zn] were below than their critical limit, phytate content in *A. nobilis* seed may not significantly affect the bioavailability

Table 3: Phytate and oxalate content of *Artocarpus nobilis* seed and calculated molar ratios for Phy:Ca, Phy:Fe, Phy:Zn and Oxa:Ca.

	Raw	Roasted	Microwaved	Boiled
Phytate	6.17±0.58^{b}	6.38±0.29^{ab}	7.61±0.29^{a}	5.35±0.58^{b}
Total oxalate	294.90±11.92^{ab}	320.02±7.94^{a}	311.68±11.91^{a}	266.75±19.85^{b}
Phy:Ca	0.0066±0.0006^{b}	0.0058±0.0004^{b}	0.0082±0.0006^{a}	0.0058±0.0007^{b}
Phy:Fe	0.2131±0.0107^{c}	0.2564±0.0379^{b}	0.2953±0.0031^{a}	0.1996±0.0106^{c}
Phy: Zn	0.3739±0.0141^{b}	0.3292±0.0173^{b}	0.4940±0.0329^{a}	0.3359±0.0308^{b}
Oxa: Ca	2.368±0.069^{a}	2.211±0.1324^{a}	2.540±0.387^{a}	2.175±0.445^{a}

Oxalate and Phytate contents are expressed in mg/100g of sample. Molar ratios are expressed in mol/kg. Values (mean ± standard deviation, n=3). Same superscript letters within a row are not significantly different ($p>0.05$). Phy: Phytate, Oxa: Oxalate

Table 4: Total phenolic, flavonoid content and antioxidant activity of nuts.

Sample	Total Phenolic Content (GAE mg/g DW)	Total flavonoid content (CE mg/g DW)	$ORAC_{FL}$ (mM of TE/g)	DPPH activity (IC_{50} mg/ml)
Artocarpus nobilis				
Raw	20.85 ± 0.98^{c}	14.78 ± 0.15^{c}	193.46 ± 1.74^{a}	0.059 ± 0.002^{e}
Roasted	25.28 ± 0.29^{a}	20.08 ± 0.42^{a}	164.64 ± 6.00^{b}	0.036 ± 0.004^{e}
Microwaved	22.49 ± 0.65^{b}	16.61 ± 0.25^{b}	205.30 ± 20.3^{a}	0. 039 ± 0.003^{c}
Boiled	6.57 ± 0.22^{d}	1.47 ± 0.04^{d}	77.54 ± 15.44^{c}	0.150 ± 0.001^{e}
Almond Raw	1.32 ± 0.03^{e}	0.47 ± 0.02^{f}	16.62 ± 2.16^{d}	1.906 ± 0.019^{b}
Almond Roasted	1.51 ± 0.09^{e}	0.57 ± 0.02^{ef}	18.77 ± 2.07^{d}	1.357 ± 0.025^{c}
Pistachio Raw	5.59 ± 0.11^{d}	0.99 ± 0.03^{e}	26.31 ± 0.44^{d}	0.678± 0.041^{d}
Pistachio Roasted	6.52 ± 0.12^{d}	0.90 ± 0.02^{ef}	27.11 ± 3.58^{d}	0.778±0.019^{d}
Cashew Raw	2.02 ± 0.04^{e}	0.53 ± 0.05^{ef}	19.95 ± 1.60^{d}	1.941±0.108^{b}
Cashew Roasted	2.27 ± 0.06^{e}	0.61 ±0.02^{ef}	22.85 ± 1.53^{d}	2.550±0.100^{a}

Results are presented as mean ± SD (n=3) on dry basis. Significant differences are denoted using different superscript letters ($p>0.05$). GAE: Gallic acid equivalent; CE: Catechin equivalent, DM: Dry matter basis, TE: Trolox equivalent, ORAC: Oxygen radical absorption capacity

of Fe and Zn. Considering the processing methods, boiled samples showed the lowest MRs for anti-nutrients and minerals, but the reduction was not significant compared to the raw samples. Nevertheless, there was significant difference in molar ratios between microwaved and boiled samples.

Total phenol (TPC) and flavonoid content (TFC)

As shown in Table 3, total phenolic content (TPC), total flavonoid content (TFC), and the antioxidant activity of the raw and processed *A. nobilis* seeds were compared with raw and roasted almonds, pistachio, and cashew nut. Almond, pistachio, and cashew were selected as controls for the experiment mainly due to several reasons. According to the US Department of Agriculture (USDA) and Phenol-Explorer databases, pistachio, almonds, and cashew nuts are positioned in the top, middle, and bottom, in the "total phenolic content" ranking, respectively (Bolling *et al*., 2011) and almond and pistachio are the most studied nut types for their phenolic composition and antioxidant activity (Chang *et al*., 2016). Therefore, a comparison may clarify the phytochemical value of *A. nobilis*.

According to the results, roasted *A. nobilis* had the highest TPC and TFC, while raw-almond seed had the lowest TPC and TFC. Interestingly, *A. nobilis* seeds had significantly higher ($p<0.05$) TPC and TFC than pistachio, cashew, and almond. As a comparison, raw *A. nobilis* seed showed 15.7fold higher TPC than almond seed extract and 3.7fold higher TPC than raw pistachio. Observed TPC for raw almond and pistachio seeds in the present experiment was within the value range reported by Kornsteiner *et al*. (2006).

According to the present research findings, pan roasting increased the phenolic content and flavonoid content of the raw *A. nobilis* seed by 1.21fold and 1.35fold, respectively. Microwaving has significantly increased both phenolic and flavonoid contents of raw *A. nobilis* by 1.07fold and 1.12fold, respectively. Nevertheless, a significant reduction in phenolic and flavonoid content was observed in the boiled sample. Further, processing improved ($p>0.05$) the TPC in almond, pistachio, and cashew nuts. However,

there was a reduction in TFC in cashew upon roasting. In the available literature, the effect of processing on the phenolic content and antioxidant activities of nuts and their byproducts are inconsistent. For instance, significant enhancement of phenolic content was reported in cashew (Chandrasekara and Shahidi, 2011), almond (Lin *et al.*, 2016) pistachio upon roasting, whereas a significant loss of phenol was reported in hazelnut *(*Pelvan *et al*., 2012), macadamia, and walnut (Schlörmann *et al*., 2015). Higher phenolic and flavonoid content in the processed samples might be ascribed to several reasons. As reported in previous studies, it may occur due to the liberation of bound phenolic compounds, breakdown of complex molecules, and the formation of Millard Reaction Products (MRPs) (Chandrasekara & Shahidi, 2011; Lin *et al*., 2016).

The significant loss of phenolics and antioxidant activity of the nuts may be due to the removal of skin after processing (Yang *et al*., 2015; Chang *et al*., 2016). When considering the *A. nobilis* seed structure, there is an inedible outer cover that protects the skin of the nut. Because of that, it avoids the loss of skin due to thermal processing. That may be the probable reason for the higher TPC and antioxidant activity of the roasted *A. nobilis* seed. A significant reduction in the phenolic and flavonoid content of *A. nobilis* seeds was observed upon the boiling process. The result was in accordance with Sanchiz *et al*. (2019), who reported a significant reduction of phenols, flavonoids, and tartaric esters in boiled pistachio and cashew compared to unprocessed control.

Antioxidant activity and reducing power

Three different *in vitro* chemical assays were performed to acquire a better idea about antioxidant capacity.

Oxygen radical absorbance capacity: As shown in Table 4, ORAC values for the methanolic extract of raw and processed seeds ranged from 16.62 - 205.30 mM of Trolox equivalent (TE)/g of the defatted meal. Microwaved *A. nobilis* seed had the highest ORAC value whereas the raw almond seed had the lowest. Although microwaved *A. nobilis* had a higher ($p>0.05$) ORAC value than raw seeds, pan-roasted seeds had a significantly lower ORAC value than that of raw seed. Compared to the raw sample, roasted almond cashew, pistachio had a higher ($p>0.05$) ORAC value. Obtained results for the cashew in this study were higher than the reported value in Chandrasekara & Shahidi, (2011) for cashew kernel. Similarly, the ORAC value for almonds (18.18 ±1.89 mM TE/g) was higher than the reported literature value (Monagas *et al*., 2009).

Trolox equivalent antioxidant activity: ABTS·+ radical scavenging activity expressed in micromole of Trolox equivalent (TE) per gram of defatted meal is presented in Fig. 2A, Roasted *A. nobilis* seed had the highest Trolox equivalent antioxidant activity (TEAC) value (267.04±6.41 µmol of TE/g of the defatted sample), while raw almond seed extract showed the lowest value (12.74±0.49 µmol of TE/g). Raw, roasted, and microwaved *A. nobilis* seeds showed significantly higher ($p>0.05$) TEAC than almond, pistachio, and cashew. As a comparison, raw *A. nobilis* seeds showed nearly 100fold higher TEAC than almond seed extract and 7fold higher TEAC than raw pistachio. The ABTS radical scavenging activity of the *A. nobilis* seed was significantly increased ($p>0.05$) with thermal processing.

Reducing power: Ferric reducing antioxidant power (FRAP) given by the different seed extracts are represented in Fig. 2B. Raw *A. nobilis* seed extract yielded the highest ferric ion reducing power (161156.7 ±11741 mM Fe^{2+}/g),

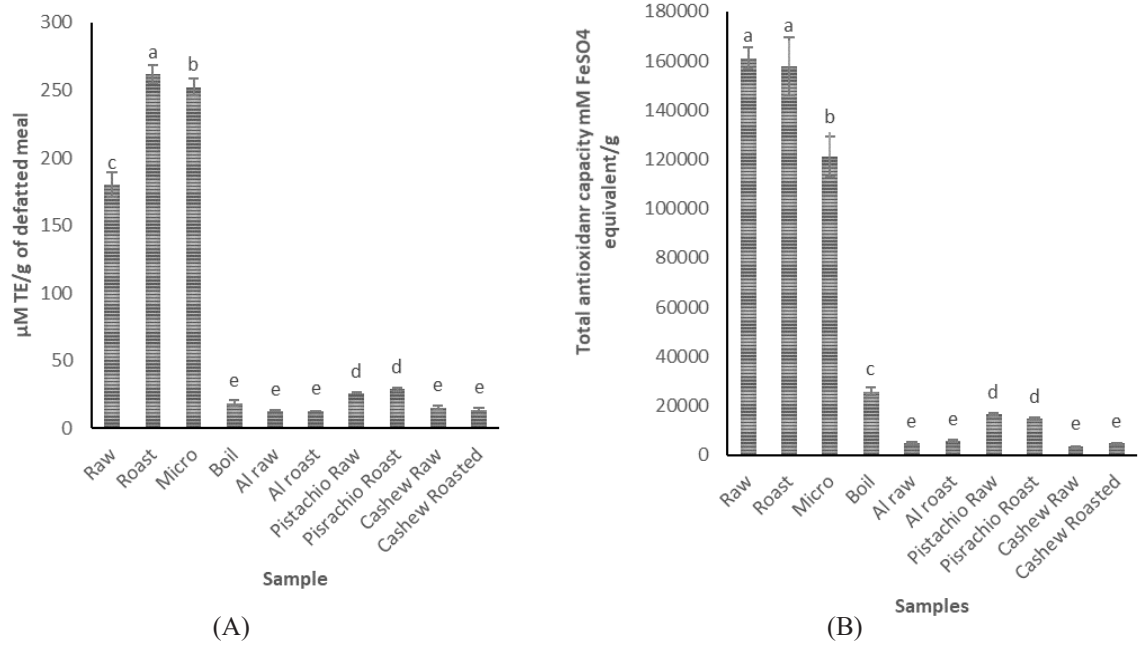

Figure 2: (A) Trolox equivalent antioxidant activity by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and (B) reducing power capacity by Ferric Reducing Antioxidant Power (FRAP) assay from methanolic extract of defatted raw, roasted, microwaved, boiled *Artocarpus nobilis* seed and raw and roasted almond, pistachio, and cashew. Data are expressed as mean ± SD (n=3). Mean ± SD followed by the same letter on the bar are not significantly different ($p>0.05$). TE: Trolox equivalent.

whereas raw cashew had the lowest value (3643.9±79.5 mM Fe^{2+}/g). The processing method didn't modulate the ferric ion reducing power of *A. nobilis*, almond, pistachio, and cashew. However, almond, pistachio, cashew seeds showed significantly lower reducing power than the *A. nobilis* seed.

Radical scavenging activity: DPPH assay was performed to determine the sample concentration required obtaining 50% radical inhibition (IC_{50}), and the results are presented in Table 1. Roasted *A. nobilis* seed had the highest DPPH radical scavenging activity bearing the lowest IC_{50} value of 36.53± 0.4 µg/ml followed by microwaved sample (39.26±0.36 µg/ml). Almond, pistachio, and cashew showed a significantly lower antioxidant activity than *A. nobilis* seed extract. The roasting and microwaving process didn't significantly modulate the antioxidant activity measured by DPPH assay in *A. nobilis* seeds. Nevertheless, the radical scavenging activity of almond, pistachio, and cashew was significantly affected by the roasting process. Pistachio and cashew showed a significant reduction in their antioxidant activity upon the roasting process, whereas roasted almonds had a significantly higher DPPH radical scavenging activity than its raw sample.

Correlation analysis

The ABTS radical scavenging activity of defatted nut extracts highly correlated with TPC (r^2=0.979; p<0.0001) and TFC (r^2=0.993; p<0.0001). Furthermore, a strong positive association was observed between ferric ion reducing power and TPC (r^2=0.979; p<0.0001) and TFC (r2 =0.974; p<0.0001) in this study. Overall, it was observed a positive association given by the ORAC, ABTS, FRAP into the TPC and TFC. These results suggested a strong involvement in phenolic and flavonoid to antioxidant activity of studied nut samples. However, IC_{50} value resulted by DPPH was negatively correlated with TPC (r = 0.78, p<0.0001), and TFC (r = 0.684, p<0.0001).

Alpha-amylase inhibitory activity

In this study, the inhibition of α-amylase enzyme with the presence of natural seed extract was assessed. Table 5 shows the α-amylase inhibitory activity of methanol extract of raw and processed nuts. According to the results, the IC_{50} value of the nut extracts ranged from 48.72-10711.64 µg/mL. Roasted *A. nobilis* showed the highest α-amylase inhibition while the least activity was observed in almond seed extract. Roasted and microwave seed extract showed potent α-amylase inhibitory activity giving evidence as a nut with high antidiabetic properties. However, none of the seed extracts was effective as acarbose which resulted in an IC_{50} value of 13.96 ± 10.04 µg/ml at the same experimental condition.

CONCLUSIONS

A. nobilis seed can be characterized as an energy-dense food with a considerable amount of protein, oil, and essential minerals. In addition, methanolic seed extract was rich in bioactive compounds, resulting in significantly higher total phenolic content and flavonoid content than the almond, pistachio, and cashew. Roasted and microwaved *A. nobilis* seed extracts showed remarkable antioxidant potential and α-amylase inhibitory activity. Considering the processing method, Pan roasting and microwaving are preferable to improve their nutritional value and antioxidant potential. This study highlighted the potential of *A. nobilis,* which is currently underutilized as a healthy nut in mitigating undernutrition and oxidative stress.

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Table 5: α-amylase inhibitory activity of the methanolic extract.

Sample	amylase inhibitory activity IC_{50} (µg/mL)
Artocarpus nobilis	
Raw	160.49 ± 17.26^{f}
Roasted	48.72 ± 3.58^{f}
Microwaved	70.54 ± 7.96^{f}
Boiled	1834.40 ± 42.14^{e}
Almond Raw	10711.64 ± 493.43^{a}
Almond Roasted	9217.95 ± 145.81^{b}
Pistachio Raw	4192.88 ± 114.34^{d}
Pistachio Roasted	4059.37 ± 86.61^{d}
Cashew Raw	5392.58 ± 258.35^{c}
Cashew Roasted	5022.42 ± 328.35^{c}
Acarbose	13.96 ± 1.044

Data are presented as mean standard deviation (n=3).

Different letters within a column indicate significant differences (p<0.05)

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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