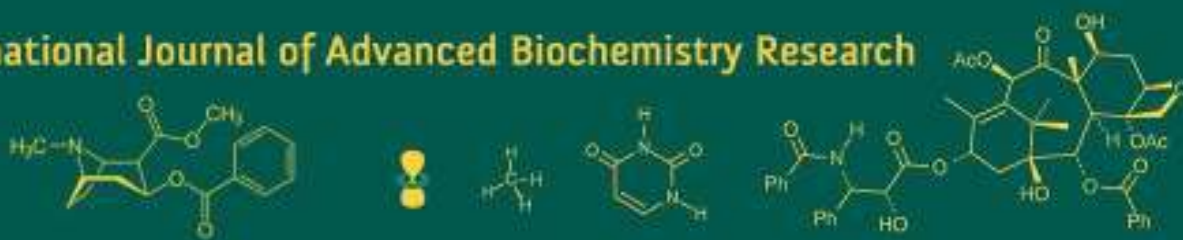


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 IJABR 2024; SP-8(10): 1245-1254
www.biochemjournal.com
 Received: 20-08-2024
 Accepted: 21-09-2024

BR Jana
 ICAR-RCER, Research Centre
 for Makhana, Darbhanga,
 Bihar, India

Anuradha Srivastava
 ICAR-Directorate of
 Mushroom Research, Solan,
 Himachal Pradesh, India

BP Bhatt
 ICAR-RCER, ICAR Parishar,
 Patna, Bihar, India

Nutritional, pharmacological and antioxidant properties of water chestnut (*Trapa* spp.) as affected by genotypes

BR Jana, Anuradha Srivastava and BP Bhatt

DOI: <https://doi.org/10.33545/26174693.2024.v8.i10So.2704>

Abstract

Background and Objective: For potential detoxifying capacity of *Trapa* spp., increasing attention has been paid to the nutritional and pharmacological values for clinical purposes.

Materials and Methods: Nutritive components and minerals of fresh water chestnut kernel were estimated by standard methods whereas the total phenolic and flavonoids by spectro-photometrically. The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method and the ferric reducing antioxidant power (FRAP) assay.

Results: In fresh seed kernel of eight genotypes protein, carbohydrates, fat, fiber and minerals content varied from 2.79 ± 0.13 - $3.90 \pm 0.19\%$, $22.79 \pm 0.94\%$ - $25.63 \pm 0.77\%$, 0.38 ± 0.02 - $0.50 \pm 0.04\%$, 1.76 ± 0.05 - $2.02 \pm 0.09\%$ and 1.42 ± 0.25 - $2.08 \pm 0.06\%$, respectively. The total phenolic and flavonoid contents ranged from 340.81 ± 10.16 to 651.56 ± 18.44 mg GAE/100 g FW and 76.64 ± 1.43 to 147.68 ± 2.85 mg QE/100 g FW respectively. The commercial cultivars, Bihar Large Red had the highest DPPH and FRAP values 389.34 ± 6.92 mg AEAC/100 g FW and 248.22 ± 8.2 mg AEAC/100 g FW, respectively. However, the DPPH activities of Green Spineless (387.70 ± 11.93 mg AEAC/100 g FW) was at par with Bihar Large Red and FRAP values of former was 211.80 ± 6.5 mg AEAC/100 g FW and acquired 2nd rank. Higher phenolic content was associated with higher DPPH scavenging activity in fresh water chestnut kernel. Furthermore, high K/Na ratio and total of cations ($\text{Ca}^{2+} + \text{Mg}^{2+}$) content of fresh kernel were promising for controlling B.P. and obesity.

Conclusions: For having more plentiful nutritional, phyto-chemicals and antioxidant components water chestnut may be considered as nutrient dense high value medicinal super food.

Keywords: Water chestnut, protein, fat, fiber and anti-oxidant

Introduction

Water chestnut, a rooted and beautiful floating aquatic herb, belongs to the family Lythraceae or Trapaceae has three different species like *T. bispinosa*, *T. natans* and *T. quadrispinosa* or *T. bicornis*. Nutritional and medicinal qualities of *Trapa natans* have been recognized in India, China, Hong Kong, Malaya, Thailand, and Russia [1]. Fresh kernel is well known for its high water content (80.0%) [2], protein (1.87%) and TSS (7.0-8.0%) [3]. According to Adkar *et al.*, [4] and Alfassane *et al.*, [5] protein and carbohydrate content of fresh water chestnut are 4.40% and 22.3%, respectively. Water chestnuts are excellent source of crude fiber [green genotype 2.13% and red genotype 2.27%] [6]. It is also a fair source of Vitamin B along with Ca, K, Fe and Zn [7-8]. Water chestnut kernel flour is generally consumed as a nutritious diet on the fasting days, in Indian traditional festival "Navratri" [9]. Water chestnut fruits are antibacterial [10] and anti-inflammatory [11] and anti-diabetic [12-13]. Water chestnuts are cholesterol and gluten free and have cooling and detoxifying effects. Besides carbohydrates and protein, water chestnut kernel contained high amount of flavonoids and antioxidants [14]. Antioxidant potential in plants is mainly due to the redox properties of phenolic compounds, which act as hydrogen donors, singlet oxygen quenchers and reducing agents [15]. According to Bogucka-Kocka *et al.*, [16] antioxidant activity of poly-phenols may act as scavenging and reducing agent, cofactors of enzymes catalyzing oxidative reactions, terminate radical chain reactions, inhibit enzyme oxidizes, and stabilize free radicals in cell. Antioxidants such as polyphenols are important for the maintenance of health and effective against cardiovascular diseases and cancer by preventing oxidative stress [17].

Corresponding Author:
BR Jana
 ICAR-RCER, Research Centre
 for Makhana, Darbhanga,
 Bihar, India

Water chestnut (*Trapanatans*) possess strong antioxidant, antimicrobial and anticancer activities, which have been attributed to their bioactive components, such as polyphenols, flavonoids and alkaloids [18-19]. Of late, there has been a lot of attention to polyphenols, owing to their antioxidant capacity, and free radical scavenging, and beneficial effects on human health [20]. According to Wollgast *et al.*, and Scalbert *et al.*, [21-22] poly-phenols are very complex group of molecules present in plants. Several compounds can be identified under the term “polyphenols”; these include mainly phenolic acids and flavonoids [23]. Based on some clinical and epidemiological report, an international interest has been raised on dietary poly-phenols for their considerable role in the prevention of various degenerative diseases [21]. Water chestnut extracts are also reported to have high inhibitory activity against glycolytic enzymes such as amylase that inhibits blood glucose elevation and a unique feature of reducing insulin secretion and hence can be effective food additives in managing type 2-diabetes [24]. Therefore, the present study was conducted with a view to know the exact nutritive values and anti-oxidant potential of water chestnut for human health, the possible role as detoxifying agent clinically and to augment awareness regarding water chestnut consumption as a medicinal food among health conscious people.

Materials and Methods

Plant materials

The experiment was conducted in ICAR-RCER, Research Centre for Makhana, Darbhanga, during 2018-19. The genotypes under study were Balia Red (G-1), Bihar Large Red (G-2), Bihar Small Red (G-3), Green Spine (G-4), Green Spineless (G-5), Lucknow Green (G-6), Orange Spineless (G-7) and Red Spineless (G-8). Three samples of each genotype of water chestnut collected from farm field and washed, peeled and fresh water chestnut kernels were analyzed in following characters.

Proximate composition

Nutritive properties including moisture, ash, crude fat, and crude protein contents were determined by AOAC methods [25]. Crude protein was analyzed using Kelplus Elite Ex Micro Kjeldahl method using conversion factor 6.25. Carbohydrates (by difference) % weight was determined as followed by the method of Gopalan *et al.*, [8]. Total dietary fiber (TDF) were determined by digesting the sample with α -amylase (AOAC method 991.43). All the biochemical analyses were carried out in three replications using fresh water chestnut kernel.

Minerals estimation

Minerals content (N, P, K, calcium, magnesium, sulphur, sodium, iron, zinc, copper and manganese), Samples of fresh water chestnut kernels were washed initially using tap-water followed by dilute hydrochloric acid (0.05 N) and finally with double-distilled water. The samples were then dried in an air oven at a temperature of 65 ± 5 °C till the weight remained constant for three consecutive days. The dried samples were then ground and passed through a 80-mesh sieve (180 μ m) to be used for analysis of minerals. All above cited minerals were estimated by [25] with an atomic absorption spectrophotometer (Analyst 100, Perkin Elmer, and Norwalk, CT, USA) and expressed as mg mineral/100 g

FW for individual elements. All mineral values were added to get total minerals content and expressed as per cent.

Estimation of total poly-phenolic

In fresh water chestnut kernel, levels of phenolic content are varied with different genotypes and were expressed in terms of Gallic acid equivalent (GAE). Total phenolic content in water chestnut kernel was measured by following Folin-Ciocalteu method [26] with slight modification [27]. A 0.25 mL aliquot of the extract obtained above was mixed with 0.25 mL of the Folin-Ciocalteu reagent and 2 mL of distilled water. After 3 minutes at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added and the mixture placed at 37 °C in a water bath for 30 minutes. Absorbance of the mixture at 750.0 nm was then recorded. A calibration curve was established from a serial dilution of Gallic acid for estimating the total phenolic content in the samples. Concentration of total phenolic compounds was expressed as mg of Gallic acid per 100 g of fresh matter of water chestnut kernel (mg GAE/100 g FW).

Total flavonoid

Total flavonoid content in water chestnut kernel was determined by aluminum chloride colorimetric assay [28]. The absorbance of the final product was measured at 517.0 nm and the standard curve was prepared from different concentrations of quercetin and expressed as mg QE/100g FW.

Antioxidant activity

Antioxidant activity of the water chestnut fresh kernel samples was determined using two assays, viz. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric reducing antioxidant potential (FRAP). Both assays are highly reproducible, accurate, and simple and can be rapidly performed [29-30].

Extraction

Sample extraction for both DPPH and FRAP assays were carried out using 80% methanol. One gram of fresh water chestnut kernel sample was homogenized with 20 ml of methanol (80%) in a pestle and mortar, and centrifuged at 4000 rpm. Supernatant was collected which was to be used for measuring the antioxidant activity. The pellet was again homogenized with 10 ml of methanol (80%). The procedures were repeated for 2-3 times to make volume up to 50 ml.

DPPH radical scavenging activity assay

Anti oxidant activity of was measured by DPPH radical scavenging ability [31]. The assay was based on the reduction of DPPH radicals in methanol by anti-oxidants, which cause a reduction of absorbance at 517.0 nm. The decrease in absorbance with sample addition was used for calculation of anti oxidant activity of the sample (fresh water chestnut kernel).

Ferric reducing antioxidant power (FARP)

This is based on the reduction of colorless ferric complex (Fe^{3+} -tripyridyl-triazine) to blue color ferrous complex (Fe^{2+} -tripyridyl-triazine) by the action of electron donating antioxidant to low pH [32]. Anti-oxidant activity was calculated by measuring the change in absorbance at 593.0 nm. A standard curve using different concentration of

ascorbic acid 20-100 mg was also developed and results were expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC/100 g FW) for both the assay.

Statistical Analysis

The Analysis of Variance (ANOVA) was employed to compare means between genotypes through the RBD test, with a significance level of 0.05. Results were expressed as mean of triplicate measurements (replications) $\pm$ SD. According to SAAS^[33] significant value was measured with in column for each characters and mean separation for the genotypes through CD value.

Results

Nutritive properties

Data pertaining to Table-1 reflected that the weight of water chestnut kernel (WCK) varied from genotype to genotype. Green Spineless genotype had the highest kernel weight of 52.33 ± 0.36 g/100g fruit. This genotype is popular for fresh as well as dry nut production in India. The effect of different cultivars on proximate composition of WCK is shown in Table-2. A close perusal of the Table-2 also revealed that the genotype Green Spineless had the highest protein content of $3.90 \pm 0.19\%$ and the moderate carbohydrates of $25.23 \pm 0.44\%$ on fresh weight basis. According to the results, the highest fat content of $0.50 \pm 0.04\%$ was observed in the genotype Balia Red, which was very small fruit indeed and not commercially cultivated or exploited in India. The Green Spineless genotype also possessed the maximum fiber content of $2.02 \pm 0.09\%$. The minerals content of different water chestnut were varied from $1.42 \pm 0.25\%$ in Balia Red to $2.08 \pm 0.06\%$ in Green Spineless which was at par with Bihar Large Red ($1.97 \pm 0.10\%$). The prolific bearer genotype Green Spineless had moderate fat ($0.42 \pm 0.03\%$) and the highest ash content of $1.43 \pm 0.05\%$. The moisture content of water chestnut kernel ranged from $63.65 \pm 1.97\%$ in Green Spineless to $66.59 \pm 3.01\%$ in Orange Spineless, which was a mutant genotype of water chestnut at RCM, Darbhanga, India.

Total phenolic and flavonoid

Free radical scavenging of phenolic compounds is an important property underlying their various biological and pharmacological activities. In our present study, data of Fig-1 elaborated that the highest total phenolic content was found in genotype Bihar Large Red (651.56 ± 20.18 mg, GAE/100 g FW) followed by Green Spineless (615.50 ± 18.44 mg GAE/100 g FW). Furthermore, the highest flavonoid was observed in fresh water chestnut kernel (147.68 ± 2.85 mg QE/100 g FW) of Green Spineless genotype and data were more consistent than that of Bihar Large Red (147.68 ± 10.79 QE/100g FW).

Antioxidant properties

A close perusal of the Fig-2 depicted that all DPPH values were greater than the FRAP values. Regarding quantification of antioxidant, the commercial cultivar Bihar Large Red had the highest DPPH and FRAP values of 389.34 ± 6.92 mg AEAC/100 g FW, and 248.22 ± 8.20 mg AEAC/100 g FW, respectively. The Green Spineless genotype had statistically similar value of DPPH (387.7 ± 11.93 mg AEAC/100 g FW) to Bihar Large Red but FRAP values were not at par and acquired second rank (211.8 ± 6.50 mg AEAC/100g FW). Green Spineless

showing FRAP value of (211.8 ± 6.50 mg AEAC/100g FW), which was at par with Red Spineless cultivar (210.80 ± 6.47 mg AEAC/100g FW). The DPPH activity and FRAP values were showed highly significant ($p < 0.05$) through mean separation technique. Moreover, the results of this study revealed that the DPPH radical scavenging activity expressed the lowest in Orange Spineless among the other (rest of the) genotypes. In DPPH radical scavenging assay, hydroxyl benzoic acid acts as a weak scavenger of DPPH radical. The highest FRAP value was found in Bihar Large Red (248.22 ± 8.20 mg AEAC/100 g FW). The another important variety Red Spineless exhibited moderate FRAP value (210.8 ± 6.47 mg AEAC/100g FW,) which was at par with Green Spineless cultivar (211.80 ± 6.50 mg AEAC/100g FW) and acquired second rank.

Important nutrient elements

It was evident from table-3 that the mineral contents Na, K, Ca^{2+} and Mg^{2+} in fresh flesh/seed ranged between 17.92 ± 2.77 – 22.72 ± 7.08 mg/100 g, 109.89 ± 10.19 – 207.06 ± 14.59 mg/100 g, 77.69 ± 0.89 – $187.98.75 \pm 14.93$ mg/100g and 32.24 ± 7.85 – 46.96 ± 1.89 mg/100 g, respectively. Low level of sodium was observed in Green Spineless (17.92 ± 2.77 mg /100 g) as compared to spine once. High level of K (207.06 ± 14.59 mg/100g) was observed in Green Spineless followed by Red Spineless (161.99 ± 8.31 mg/100 g) and Orange spineless (144.51 ± 12.11 mg/100 g). The highest Ca^{2+} (187.98 ± 14.93 mg/100 g) and Mg^{2+} (46.96 ± 1.89 mg/100 g) content were found in Green Spineless genotypes.

Discussion

Mineral content

The nutrient status of different water chestnut was investigated in the fresh water chestnut kernel as it is consumed in India as fresh. Data from our investigation supports the assumption that the level of different nutritional component like protein and carbohydrates and fat etc. changes according to different cultivars and their growing practices. Significantly higher amount of protein and moderate carbohydrates were found in Green Spineless $3.90 \pm 0.19\%$ and $25.23 \pm 0.44\%$, respectively, on fresh weight basis. The findings were corroborated by Gopalan *et al.*,^[8] when they estimated nutritive values of different nuts. The water chest cultivar Balia Red showed the highest fat content of $0.50 \pm 0.04\%$. The similar finding was also obtained by Faruk *et al.*,^[6] Crude fiber of the fresh water chestnut kernel was found to be 0.72% slightly more than reported in Chinese water chestnut as reported by Khare^[34]. The findings were corroborated by the findings of Puste^[12] and Faruk *et al.*,^[6] Our findings regarding fiber and mineral and ash content of Green Spineless cultivar of water chestnut, as indicated by its size and less water content supported its hardness and solid kernel structure. Despite of sufficient moisture content, the fresh kernel of water chestnut Bihar Large Red also had appreciable amount of protein, carbohydrates, fat, fiber and minerals.

Total phenolic and flavonoid

In our present study, The Folin–Ciocalteu method was used to determine the total polyphenol content of different water chest nut cultivar. The results are shown in Figure 1. The cultivar Bihar Large Red showed the highest content of poly-phenols (651.56 ± 20.18 mg, GAE/100 g FW) followed

by Green Spineless (615.5 ± 18.44 GAE/100 g FW) and finally Orange Spineless (340.81 ± 10.16 GAE/100 g FW). Our investigation showed a significantly higher amount of total phenolic content in Bihar Large Red cultivar which might be due to its presence of spine in seed coat which could accumulate higher amount of alkaloids for defense mechanism. Green Spineless cultivar had relatively lower total phenolic content. Phenolic content of other edible nuts were also observed by Abe *et al.*,^[35] in cashew nut (raw), peanut raw and pecan nut raw were 381 ± 6 mg GAE/100 gFW, 597 ± 6.0 mg GAE/100 gFW and 703 ± 44 mg GAE/100g FW, respectively. Water chestnut contained a great quantity of antioxidants, such as flavonoids, flavones, and total phenol contents [36]. Phenolic extracts have been reported to retard lipid oxidation in oils and fatty foods [37] and decrease the risk of heart diseases by inhibiting the oxidation of low-density lipoproteins. During early growing stage, phenolic and flavonoids content of water chestnut seed were more, and then gradually decreased up to the harvesting. In the light of these above research results, it can be said that flavonoid in fresh water chestnut kernel were valuable and varied from Orange Spineless (76.64 ± 1.43 mg QE/100 g FW) to Green Spineless (147.68 ± 2.85 mg QE/100 g FW) at harvesting stage. This result was inconformity with the findings of Shafi *et al.*,^[38] and they reported that water chestnut dry flour had higher flavonoid contents of 1.92 g QE/1000 g or 192 mg/100 DW. Our results showing asimilarly high flavonoid content in cvs. Green Spineless and Bihar Large Red. According to Chiang *et al.*, and Elhamirad *et al.*,^[39-40], one of the major flavonoids present in water chestnut flour was quercetin and they also reported that quercetin to have higher values of standard coefficient (Qs). As a stable flavonoid, a measure of antioxidant activity even at higher temperatures ($140-180$ °C) was constant (unchanged). An improved retention of antioxidant activity in water chestnut even at higher temperatures can be due to hydroxylation of quercetin (B-ring).

Anti-oxidant

Antioxidants are mainly defined as polyphenolic compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals (ROS) and diminishing oxidative stress of the cell^[41]. The reducing capacity of a compound may serve as indicator of its potential antioxidant capacity^[42]. In our studied materials of different water chestnut fresh kernels, we found higher amount of phenolic compounds, free radical acceptors and chain breakers which indicated their strong therapeutic potential as an antioxidant substance. Hence, water chestnut has potential application to reduce oxidative stress with consequent health benefits through inhibition of lipid oxidation and oxidation of other molecules by rapid donation of hydrogen atom to free radicals^[41]. DPPH radical scavenging activity is associated with high phenol content. Furthermore, the DPPH free radical, can receive easily an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule^[43]. In DPPH radical scavenging assay, hydroxyl benzoic acid acts as a

weak scavenger of DPPH radical^[44]. We observed that water chestnut cv. Bihar Large Red had the highest DPPH and FRAP values; this might be due to reduce ferric ion and the high chlorogenic acid content. Indeed, a previous study supported a strong relationship between ferric reducing power activity and chlorogenic acid content^[45]. We found that cv. Green Spineless cultivar had significantly similar value of DPPH as that of Bihar Large Red. Cultivar Green Spineless had also higher mineral content which might involve in different enzymatic reaction and could facilitate/accelerate toscavenge ROS (Reactive Oxygen Species) through DPPH activity in cell. In lotus seed, Kim *et al.*,^[46] reported that the HANN exhibited strong free radical scavenging activity as evidenced by the low IC (50) value in DPPH (1,1-diphenyl-2-picryl hydrazyl) was 612.0 ± 0.41 mg /100g DM. The presence of anti-oxidant in water chestnut was also noticed by Kachre *et al.*,^[34] and Karmakar *et al.*,^[47]. Antioxidant present in water chestnut inhibited increased lipid peroxidation and restored glutathione peroxidase and catalase activities in cerebral cortexas compared to ageing accelerated control group^[48]. DPPH-radical scavenging activity and tyrosinase inhibition activity were also estimated by Leong *et al.*,^[49] in lotus nut. DPPH-radical scavenging activity were also noticed by Haleema *et al.*,^[50] in makhana pop, Lee *et al.*,^[51] in makhana seed and Al Doghathier *et al.*,^[52] in brewed coffee. The ferric reducing antioxidant power confirmed that the Orange Spineless showed the lowest antioxidant activity and we found the difference between Bihar Large Red and Green Spineless /Red Spineless being the antioxidant activity highest in Bihar Large Red (Fig-1).

Important nutrient elements

In human nutrition, K/Na ratio and total of Ca^{2+} and Mg^{2+} content have important role regarding control of B.P and obesity. Table-3 shows that the mineral content of different cultivars presented the highest values for Na, K, Ca^{2+} and Mg^{2+} . The eight genotypes assessed showed similar concentration of K, Ca and Mg^{2+} presented the highest value on Green Spineless. The most abundant macro-element was K in agreement with previous reports^[8]. The mineral contents varied between 17.92 ± 2.77 – 22.74 ± 7.08 mg/100g, 109.89 ± 10.19 – 207.06 ± 14.59 mg/100 g, 77.69 ± 0.89 – 187.98 ± 14.93 mg/100 g and $32.24.61 \pm 7.85$ – 46.96 ± 1.89 mg/100 g for Na, K, Ca^{2+} and Mg^{2+} respectively. Generally, Spineless cultivar contained the highest concentration of Ca^{2+} and K and lowest in Na content. Green Spineless cultivar had the highest Mg^{2+} content, followed by both the spineless and spine genotypes. In our study, the concentration of these minerals was a little bit higher according to the results showed in Table 3 due to better adaptation of Green Spineless and Bihar Large Red at Darbhanga, Bihar condition, and we only found difference on Na content being this value higher in Lucknow Green (22.74 ± 7.08 mg/100 g). Our results are similar to the results obtained by Adkar *et al.*^[4] but less than the content published by Faruk *et al.*,^[6].

Table 1: Physical characteristics of different water chestnut genotypes at RCM, Darbhanga.

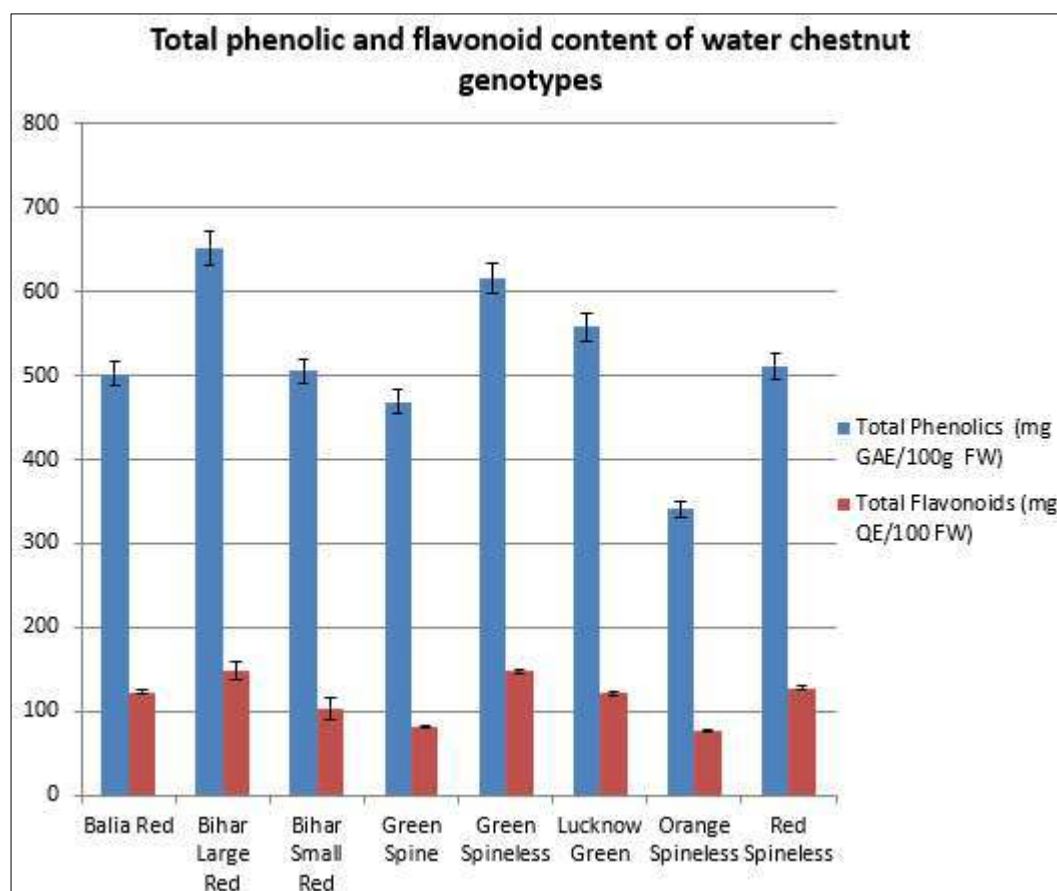
Genotypes	Kernel weight / 100 g fruit	Skin weight/ 100 g fruit	Appearance of kernel
Balia Red	49.32±0.39 ^f	50.68±0.39 ^c	Triangular, Soft
Bihar Large Red	51.83±0.30 ^b	48.16±0.30 ^f	Triangular, Semi-hard
Bihar Small Red	47.31±0.54 ^g	52.79±0.55 ^b	Triangular, Soft
Green Spine	42.55±0.31 ^h	57.45±0.55 ^a	Triangular, Hard
Green Spineless	52.33±0.36 ^a	46.50±0.30 ^h	Oval, Semi-hard
Lucknow Green	50.35±0.30 ^c	47.67±0.37 ^g	Triangular, Semi-hard
Orange Spineless	50.79±0.94 ^e	49.42±0.19 ^d	Oval, Soft
Red Spineless	51.31±0.32 ^d	48.60±0.32 ^c	Oval, Soft
CD (5%)	0.249	0.242	...

*Results were expressed as mean of triplicate measurements± SD. Mean in the same column with common letters are not significantly different ($p < 0.05$)

Table 2: Nutritional quality of different water chestnut fresh kernel.

Genotypes	Carbohydrate (%)	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Minerals (%)	Moisture (%)
Balia Red	25.43±0.76 ^b	3.22±.15 ^e	0.50±0.04 ^a	1.10±0.02 ^g	1.76±0.05 ^c	1.42±0.25 ^d	64.95±2.74 ^{bc}
Bihar Large Red	25.63±0.77 ^a	3.27±0.16 ^d	0.46±0.04 ^{bc}	1.32±0.03 ^c	1.85±0.07 ^c	1.97±0.10 ^a	64.20±2.16 ^{cd}
Bihar Small Red	24.03±0.72 ^d	2.79±0.13 ^h	0.43±0.02 ^{bc}	1.24±0.03 ^d	1.80±0.06 ^d	1.67±0.08 ^c	64.44±2.63 ^{cd}
Green Spine	23.23±0.69 ^f	2.90±0.14 ^f	0.43±0.03 ^{bc}	1.20±0.03 ^e	1.92±0.08 ^b	1.50±0.06 ^d	65.65±2.16 ^{ab}
Green Spineless	25.23±0.44 ^c	3.90±0.19 ^a	0.42±0.03 ^b	1.43±0.05 ^a	2.02±0.09 ^a	2.08±0.06 ^a	63.65±1.97 ^d
Lucknow Green	24.10±0.73 ^d	2.82±0.14 ^g	0.44±0.03 ^{bc}	1.23±0.04 ^d	1.78±0.06 ^d	1.73±0.08 ^{bc}	64.44±1.85 ^{cd}
Orange Spineless	22.79±0.94 ^g	3.33±0.16 ^c	0.44±0.05 ^{bc}	1.16±0.03 ^f	1.91±0.08 ^b	1.77±0.08 ^{bc}	66.59±3.01 ^a
Red Spineless	23.43±0.70 ^e	3.48±0.17 ^b	0.38±0.02 ^c	1.35±0.05 ^b	1.80±0.06 ^d	1.83±0.09 ^b	65.40±2.62 ^b
CD(5%)	0.183	0.034	0.076	0.020	0.024	0.112	0.941

*Results were expressed as mean of triplicate measurements± SD. Mean in the same column with common letters are not significantly different ($p < 0.05$).

**Fig 1:** Total phenolic and flavonoid content of water chestnut genotypes grown in RCM Darbhanga.

*Results were expressed as mean of triplicate measurements± SD. Means are significant at $P < 0.05$ following RBD.

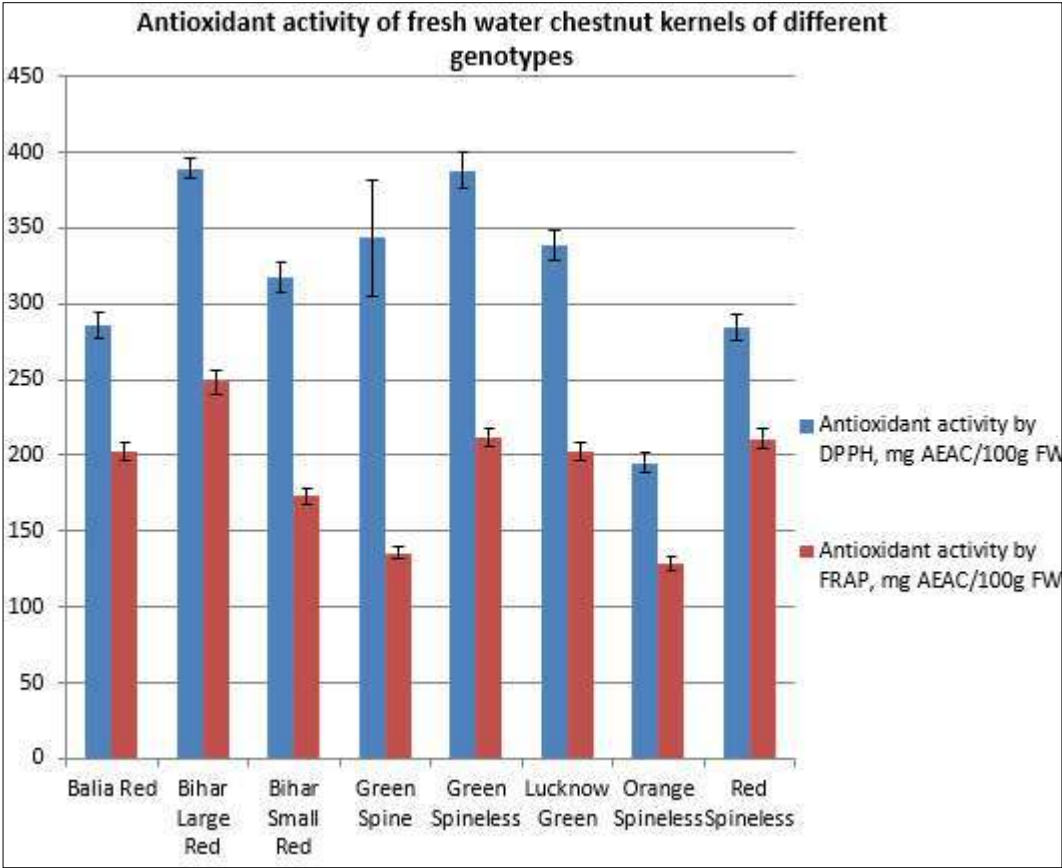
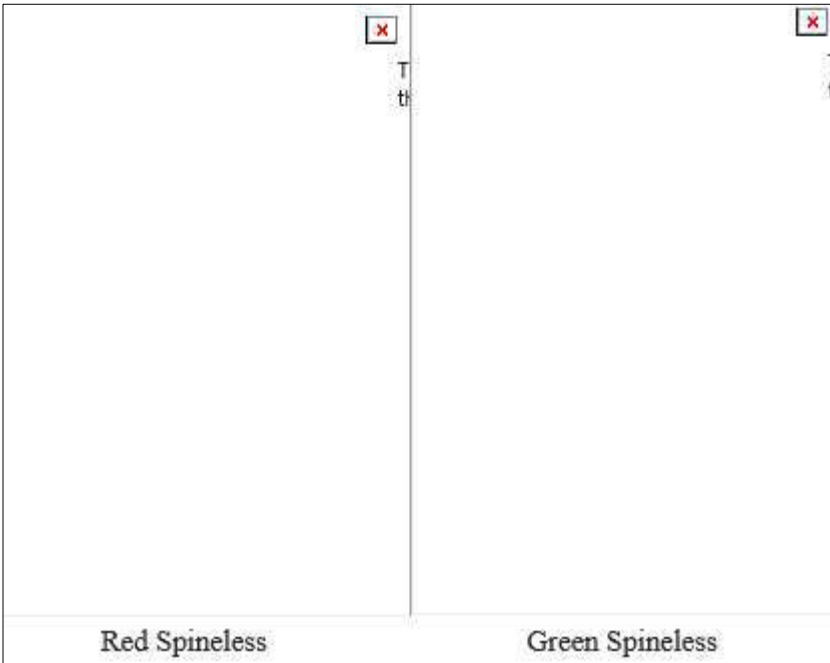


Fig 2: Antioxidant activity of fresh water chestnut kernels of different genotypes.
*Results were expressed as mean of triplicate measurements± SD. Means are significant at $p<0.05$ following RBD.



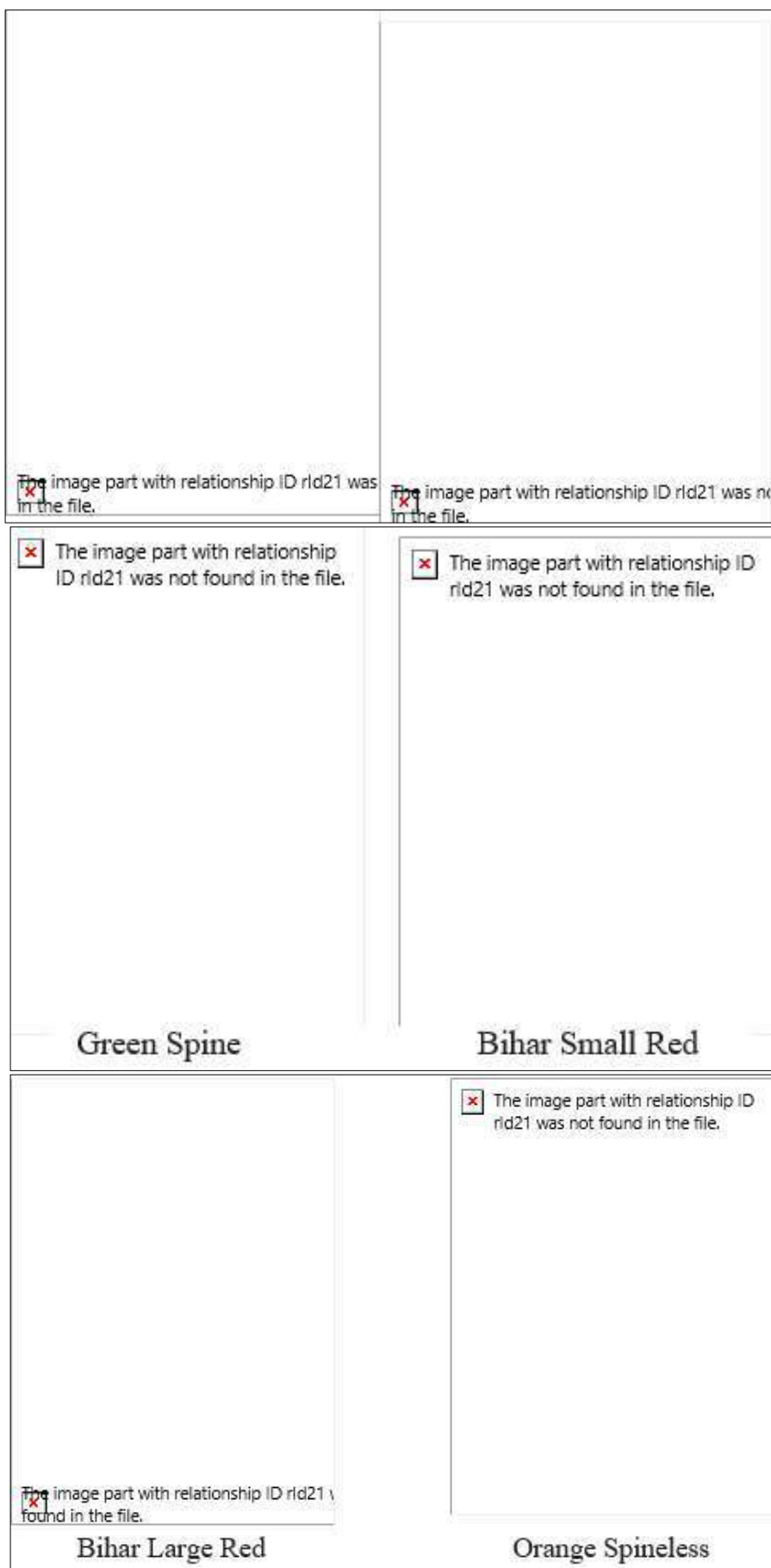


Table 3: Important nutrient elements in water chestnut genotypes

Genotypes	Na (mg/100 g)	K(mg/100 g)	Ca(mg/100 g)	Mg(mg/100 g)
Balia Red	21.11±2.86 ^a	129.81±12.80 ^c	116.05±5.30 ^c	32.24±7.85 ^c
Bihar Large Red	20.98±4.92 ^a	135.79±11.98 ^d	117.82±11.93 ^c	33.35±4.26 ^c
Bihar Small Red	22.55±2.39 ^a	114.36±10.21 ^s	105.53±15.32 ^d	40.55±6.31 ^b
Green Spine	21.72±3.04 ^a	109.89±10.19 ^b	77.69±0.89 ^e	40.02±4.07 ^b
Green Spineless	17.92±2.77 ^b	207.06±14.59 ^a	187.98±14.93 ^a	46.97±1.89 ^b
Lucknow Green	22.74±7.08 ^a	119.20±15.31 ^f	114.84±7.17 ^c	42.74±4.03 ^a
Orange Spineless	18.59±0.65 ^b	144.51±12.11 ^c	131.86±1.76 ^b	38.63±8.86 ^{ab}
Red Spineless	18.95±1.92 ^b	161.91±8.31 ^b	138.78±9.13 ^b	40.38±9.05 ^b
CD	1.86	3.51	8.50	5.18

*Results were expressed as mean of triplicate measurements± SD. Mean in the same column with common letters are not significantly different ($p < 0.05$).

Conclusion

In this study, the effect of cultivars on nutritional and phenolic composition as well as antioxidant activity was described. Water chestnut fresh kernels were very rich in carbohydrates, protein, fat and fiber content. Bihar Large Red and Spineless Green and Spineless Red genotypes of water chestnut had valuable anti-oxidant content measured as AEAC equivalent. DPPH radical scavenging activity was associated with high phenol content. Therefore, water chestnut kernel powder could be used as potential detoxifying agent clinically for human health and may be recognized as a medicinal food among health-conscious people due to having low fat and gluten with high minerals and antioxidant content.

Significant Statement

The fresh kernel of water chestnut (*Trapa natans* L.) possessed various nutritional, phyto-chemicals and antioxidant components with major divalent cations viz; Mg^{2+} and Ca^{2+} and significant monovalent nutrient element 'K'. Therefore, it may be considered as nutrient dense high value food and mainly used as detoxifying agent clinically all over world.

Funding

This work was supported by Institute research project and grant was obtained from ICAR-RCER, Patna.

Acknowledgement

Authors are thankful to Director ICAR Research Complex for Eastern Region, Patna, and Bihar for funding and technical guidance for smooth running of experiment.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contribution

Dr. B.R. Jana conceptualized and defined the research idea and developed the research design. Dr (Mrs.) Anuradha Srivastava searched the literature and performed the lab analysis. Dr Jana analyzed the data statistically and prepared the first draft of the manuscript. The Director, Dr. B. P. Bhatt wrote and edited the second draft of the manuscript.

References

- Hummel M, Kiviat E. Review of world literature on water chestnut with implications for management in North America. *J Aquat Plant Manage.* 2004;42:17–27.
- Puste AM. Agronomic management of wetland crops. Kalyani Publishers; c2004. India.
- Singh GD, Singh S, Jindal N, Saxena DC. Physico-chemical characteristics and sensory quality of singhara (*Trapa natans* L.): An Indian water chestnut under commercial and industrial storage conditions. *Afr J Food Sci.* 2010;4(11):693–702.
- Adkar P, Dongare A, Ambavade S, Bhaskar VH. *Trapa bispinosa* Roxb.: A review on nutritional and pharmacological aspects; c2014. Available from: <https://www.hindawi.com/journals/aps/2014/959830/>
- Alfasane Md, Almujaaddade M, Khonder M, Rahman M. Biochemical composition of the fruits of water chestnut (*Trapa bispinosa* Roxb.). *Dhaka Univ J Biol Sci.* 2011;20(1):95–98. Available from: <https://www.banglajol.info/index.php/DUJBS/article/view/8879/6590>
- Faruk MO, Amin MZ, Sana NK, Saha RK, Biswas RK. Biochemical analysis of two varieties of water chestnuts (*Trapa* spp). *Pak J Biol Sci.* 2012;15(21):1019–26.
- Singh GD, Bawa AS, Singh S, Saxena DC. Physicochemical, pasting, thermal and morphological characteristics of Indian water chestnut (*Trapa natans*) starch. *Starch.* 2009;61:35–42.
- Gopalan C, Ramasastri BV, Balasubramanian SC, Rao BSN, Deosthale YG, Pant KC. Nutritive value of Indian foods. National Institute of Nutrition, ICMR; 2004. Hyderabad, India.
- Majee C, Mazumder R, Chakraborty GS. A review on potential of plants under *Trapa* species. *Int J Res Pharm Chem.* 2013;3:502–8.
- Singh G, Singh S, Jindal N. Environment friendly antibacterial activity of water chestnut fruits. *J Bio Env Sci.* 2011;1:26–34.
- Patel AS, Patel NC, Shah MH, Shah VN. Evaluation of anti-inflammatory activity of fruits of *Trapa natans* L. *Int J Pharm Res Dev.* 2010;3(6):97–102.
- Das PK, Bhattacharya S, Pandey JN, Biswas M. Anti-diabetic activity of *Trapa natans* fruit peel extract against streptozotocin induced diabetic rats. *Global Pharmacol.* 2010;5:186–90.
- Shalabh B, Jain A, Choudhary J. *Trapa natans* (Water Chestnut): An overview. *Int Res J Pharma.* 2012;3(6):31–3.
- Mann S, Gupta D, Gupta V, Gupta R. Evaluation of nutritional, phytochemical, and antioxidant potential of *Trapa bispinosa* Roxb. fruit. 2014. Available from: <http://www.ijppsjournal.com/Vol-4Issue-1/3050.pdf>
- Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its

- relevance to therapeutic applications. *Int J Biol Sci*. 2015;11:982–91. DOI:10.7150/ijbs.12096.
16. Bogucka-Kocka A, Zidorn C, Kasprzycka M, Szymczak G, Szewczyk K. Phenolic acid content, antioxidant and cytotoxic activities of four *Kalanchoë* species. *Saudi J Biol Sci*. 2016;23:678–85. DOI:10.1016/j.sjbs.2016.01.037.
 17. Karakaya S, El SN, Tas A. Antioxidant activity of some foods containing phenolic compounds. *Int J Food Sci Nutr*. 2001;52:501–8.
 18. Yu L, Nanguet *al*, Beta T. Comparison of antioxidant properties of refined and whole wheat flour and bread. *Antioxidants*. 2013;2:370–83.
 19. Chiang PY, Ciou JY. Effect of pulverization on the antioxidant activity of water caltrop (*Trapa taiwanensis* Nakai) pericarps. *LWT Food Sci Technol*. 2010;43:361–5.
 20. Wollgast J, Anklam E. Polyphenols in chocolate: Is there a contribution to human health? *Food Res Int*. 2000;33:449–59. DOI:10.1016/S0963-9969(00)00069-7.
 21. Scalbert A, Morand C, Manach C, Rémésy C. Absorption and metabolism of polyphenols in the gut and impact on health. *Biomed Pharmacother*. 2002;56:276–82. DOI:10.1016/S0753-3322(02)00205-6.
 22. Yardim Y. Electrochemical behavior of chlorogenic acid at a boron-doped diamond electrode and estimation of the antioxidant capacity in coffee samples based on its oxidation peak. *J Food Sci*. 2012;77:408–13. DOI:10.1111/j.1750-3841.2011.02609.x.
 23. Samanidou V. Determination of polyphenols and major purine alkaloids in coffee: an overview. In: Preedy VR, editor. *Coffee in Health and Disease Prevention*. Amsterdam: Elsevier; 2014.
 24. Yasuda M, Yasutake K, Hino M, Ohwatari H, Ohmagari N, Takedomi K, *et al*. Inhibitory effects of polyphenols from water chestnut (*Trapa japonica*) husk on glycolytic enzymes and postprandial blood glucose elevation in mice. *Food Chem*. 2014;165:42–9.
 25. AOAC. Official methods of analysis. 20th ed. Washington, DC: Association of Official Analytical Chemists; 2016.
 26. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*. 1999;299:152–79.
 27. Genovese MI, *et al*. Determinação do conteúdo de fenólicos totais em frutas. *Rev Bras Cienc Farm*. 2003;39(3):167–9.
 28. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J Agric Food Chem*. 2002;50:3010–4. DOI:10.1021/jf0115589.
 29. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem*. 2006;94:550–7.
 30. Thaipong K, Boonprakob K, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J Food Compos Anal*. 2006;19:669–75.
 31. Kang HM, Saltveit ME. Antioxidant capacity of lettuce leaf tissue increases after wounding. *J Agric Food Chem*. 2002;50:7536–41.
 32. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Anal Chem*. 1996;239:70–6.
 33. SAAS. SAAS/STAT. Guide for personal computers. Version 6.1. Cary, NC: Statistical Analytical System Inc.; c1990.
 34. Khare CP. Indian medicinal plants: An illustrated dictionary. Berlin: Springer; c2007.
 35. Abe LT, Lajola FM, Genovese M. Comparison of phenol content and antioxidant capacity of nuts. *Food Sci Technol*. 2010, 30(1). DOI:10.1590/50101-20612010000500038.
 36. Kachare S, Tiwari S, Tripathi N. Water chestnut: biotechnological and pharmacological advances. *Prog Res*. 2016;11:5149–56.
 37. Rumbaoa RGO, Cornago DF, Geronimo IM. Phenolic content and antioxidant capacity of Philippine potato (*Solanum tuberosum*) tubers. *J Food Compos Anal*. 2009;22:546–50.
 38. Shafi MWN, Baba F, Masoodi FA. Wheat-water chestnut flour blends: effect of baking on antioxidant properties of cookies. *J Food Sci Technol*. 2016;53:3540–6. DOI:10.1007/s13197-016-2423-5.
 39. Chiang PY, Ciou J, Hsieh L. Antioxidant activity of phenolic compounds extracted from fresh and dried water caltrop pulp (*Trapa taiwanensis* Nakai). *J Food Drug Anal*. 2008;16:66–73.
 40. Elhamirad AH, Zamanipoor MH. Thermal stability of some flavonoids and phenolic acids in sheep tallow olein. *Eur J Lipid Sci Technol*. 2012;114:602–6.
 41. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*. 2010;15:7313–52.
 42. Meir S, Kanner J, Akiri B, Philosoph-Hadas S. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *J Agric Food Chem*. 1995;43:1813–5.
 43. Aksoy L, Kolay E, Ağilönü Y, Aslan Z, Kargiöglu M. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic *Thermopsis turcica*. *Saudi J Biol Sci*. 2013;20:235–9. DOI:10.1016/j.sjbs.2013.02.003.
 44. Somporn C, Kamtuo A, Theerakulpisut P, Siriamornpun S. Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of arabica coffee beans (*Coffea arabica* L. cv. Catimor). *Int J Food Sci Technol*. 2011;46:2287–96. DOI:10.1111/j.1365-2621.2011.02748.x.
 45. Moreira DP, Monteiro MC, Ribeiro-Alves M, Donangelo CM, Trugo LC. Contribution of chlorogenic acids to the iron-reducing activity of coffee beverages. *J Agric Food Chem*. 2005;53:1399–402. DOI:10.1021/jf0485436.
 46. Kim J, Shin WK, Kim Y. Effect of lotus seed on viscosity and antioxidant activity of soy-based porridge. *Cereal Chem*. 2018;95:276–82. DOI:10.1002/cche.10108.
 47. Karmakar UK, Rahman KS, Biswas NN. Anti-diarrheal, analgesic and antioxidant activities of *Trapa bispinosa* Roxb. fruits. *Res J Pharm Tech*. 2011;2:111–5.

48. Ambikar DB, Harle UN, Khandare RA, Bore VV, Vyawahare NS. Neuro-protective effect of hydro alcoholic extract of dried fruits of *Trapa bispinosa* Roxb on lipo-fuscinogenesis and fluorescence product in brain of d-galactose induced ageing accelerated mice. Indian J Expt Biol. 2010;48:378–82.
49. Leong ES, Tan S, Chang YP. Antioxidant properties and heavy metal content of lotus plant (*Nelumbo nucifera* Gaertn) grown in ex-tin mining pond near Kampar, Malaysia. Food Sci Technol Res. 2012;18(3):461–5.
50. Haleema K, Srigiripura CV, Urooj A. Antioxidant content and *in vitro* antioxidant activities of extracts from popped Euryale ferox seeds. Agric Sci Res J. 2016;6(4):86–92.
51. Lee SE, Ju EM, Kim JH. Antioxidant activity of extracts from Euryale ferox seed. Exp Mol Med. 2002;34(2):100–6.
52. Al Doghaither HA, Almowalad AM, Shorbaji AM, Al-Ghafari AB, Omar UM. Assessment of the antioxidant properties of the most common coffee brews available in the local market of western region of Saudi Arabia. J Expt Bio Agril Sci. 2017;5(1):70–6.
DOI:10.18006/2017.5(1).070.076.