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Total Phenolic Content, Antioxidant Activity and *in vitro* Bioaccessibility in "Gasnivithi" (*Talinum triangulare)* and "Keren koku" (*Acrostichum aureum*) Available in Sri Lanka

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ABSTRACT

Underutilized wild edible species or neglected crop species are still used at low levels within the local communities, but show a high potential to contribute to the food supply with more health benefits. This study was conducted to assess the nutritional properties of two underutilized wild edible species, namely, *Acrostichum aureum* and *Talinum triangulare* available in Sri Lanka. Plant extracts were analyzed for their ascorbic acid content, total phenolic content, antioxidant activities, and mineral contents (Ca, Cu, Zn, and Fe). Bioaccessibility of minerals was also tested through an *in vitro* digestion model. Ca is the most abundant micronutrient (45.61–102.91 mg/100 g) in both tested species, while the *in vitro* bioaccessibility assay shows a higher fraction of Ca (47.46%) in *A. aureum* and Cu (64.68%) in *T. triangulare*. The highest concentrations of ascorbic acid and total phenolic content were found in *T. triangulare* (AAC: 165 mg/100 g; TPC: GAE,34.8 mg/100 g). Studied species were found to have significant antioxidant activities as evaluated by two different methods, such as DPPH and ABTS radical scavenging activity. In relation to the tested data, *T. triangulare* has shown the greatest antioxidant activity in both methods. Hence, consumption of identified underutilized plant species may serve as good sources of antioxidants and minerals in their natural form.

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INTRODUCTION

The indigenous and traditional diets of Sri Lankan ancestors were rich with greens and seeds of underutilized edible plants (UP), and they consumed those plant species without knowing their nutritional benefits. Even today, some people in rural areas of Sri Lanka still consume UP parts as a normal food source for their diets. However, the present world pays much attention to these species due to their valuable nutritional and functional properties, and it is widely accepted that UP species are important food sources for obtaining a fairly high amount of several nutrients, including micronutrients. People neglected these species as famine food crops or underutilized wild species, ignoring their nutritional and health benefits to the human body. According to Hanelt (2001), nearly 7,000 underutilized plant species are cultivated worldwide to facilitate food needs or medicinal benefits. UP provides a sustainable solution to addressing malnutrition and food and nutrition security, while also playing a critical role in facilitating the economic development of rural farming communities.

Many different forms of UP species are available in Sri Lanka with a poor consumption level, and these plants have not gained sufficient attention while their potential value is also underestimated. “Keren koku” (*Acrostichum aureum*), which belongs to the family Pteridaceae, was identified as one of the UP, which is well-known as a medicinal pteridophyte (Aroloye and Eberechukwu 2021).

This plant can be used to work against parasitic worms (an anthelmintic property) and to prevent excess bleeding in wounds (styptic) (Ningsih, Idroes, and Bachtiar, 2019). Therefore, this plant is used in worm remedy in traditional medicine culture (Thomas, 2012). Moreover, very few studies were carried out to assess the nutrient composition of leaf extracts of *A. aureum* and identify the plant as a healthy food for human consumption (Agra *et al*., 2008; Liao *et al*., 2015).

Talinum triangulare (Portulacaceae) is another wild herb, locally known as "gasnivithi" or "rata nivthi". This is identified as an all-season vegetable and perennial herb with fleshy green leaves and a succulent stem (Agbaire, 2011). Pink color flower was a common authentication part in this plant, which is propagated from both sexual (by seeds) and a sexual (by vegetation parts) parts and is commonly seen in rural village areas (Hughes, 2008). The whole plant has been implicated in the management of a variety of diseases, including hepatic ailments, diabetes, cancer, stroke, obesity, and measles (Liang et al., 2011). A previous study has shown that aqueous extracts of *T. triangulare* possess remarkable levels of antioxidants and flavonoid contents (Andarwulan et al., 2010).

Given the nutritional and medicinal values of *A. aureum* and *T. triangulare*, the plants can possibly serve as an alternate nutritional source in improving the health aspects of humans. Hence, more research is needed to find possible ways to substantially incorporate these UP into food systems. Information on the presence of high levels of fiber, protein, and bioactive compounds in *A. aureum* and *T. triangulare* is available. In addition to knowing the amount of micronutrients present in these plants, it is important to know the bioaccessibility of micronutrients with respect to their absorption in the human body as well. To our knowledge, there is no reported information available on the *in vitro* bioaccessibility of micronutrients of these selected species in Sri Lanka. Thus, the present study was aimed at investigating the total phenol content (TPC), ascorbic acid content (AAC), antioxidant activity, and *in vitro* bioaccessibility of minerals in selected UP collected from the southern province of Sri Lanka.

METHODOLOGY

Collection of plant materials

Selected sample species were collected from the Sothern province, Sri Lanka (Figure 1). The local names, botanical names, and collected locations of the studied UP are presented in Table 1. Specimens were authenticated at the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka.

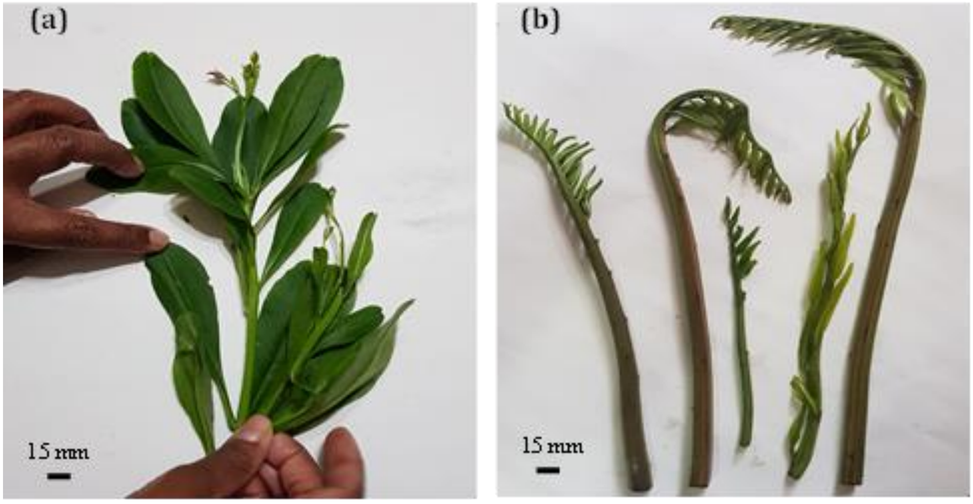

Figure 1. (a) Gasnivithi/ratanivithi (*Talinum triangulare*); (b) Keren koku (*Acrostichum aureum*)

Table 1. Description of the UP used in the study

Local name	Botanical name	Collected location
Keren koku	*Acrostichum aureum*	Ginthota (6.0600° N, 80.1800° E)
Gasnivithi/ratanivithi	*Talinum triangulare*	Mapalagama (6.2236° N, 80.2893° E)

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After authentication, three replicates (yielding 1 kg per plant type) of all identified samples were collected. The collected samples were wrapped in polythene bags and taken to the laboratory, where they were cleaned, the unwanted parts were removed, and the edible parts of the samples were obtained. Prior to extraction, samples were freeze-dried (BK-FD12P, China), powdered, sieved (sieve size: 10), and stored in airtight containers in a refrigerator.

Ascorbic acid content (AAC)

Ascorbic acid content was quantitatively determined using 2,6 dichlorophenol indophenol visual titration method (AOAC. 1984). Ascorbic acid standard was prepared using 100 mg of L-ascorbic acid and 100 mL of 3% metaphosphoric acid (w/v%). The dye solution was prepared by mixing 50 mg of sodium salt of 2.6 dichlorophenol indophenol with 150 ml of hot water containing 42 mg of sodium carbonate. Solution was allowed to cool and it was further diluted with 200 mL distilled water. Freeze dried samples were extracted (2 mg/25 mL) using 3% metaphosphoric acid and extract was filtered using Whatman filter papers (0.45 μm). An aliquot of 3 mL of filtered extract was titrated with standard dye until color of the solution was changed in to a pink end point. The procedure was performed in triplicates and finally, calculation was done according to the following formula;

Total phenolic content (TPC)

As described by Bhandari and Kawabata (2004), TPC was determined using the Folin–Ciocalteu procedure with gallic acid as the standard curve with slight modifications. Freeze dried sample (1 g/10 mL) was extracted by methanol, stirring them overnight in conical flasks. 5 mL of 10% Folin-Ciocalteu reagent were reacted with 1 mL of methanol extract for 5 min under room temperature conditions (28 ˚C). 7.5% sodium carbonate aqueous solution was prepared and added 4 mL of volume to the reacted mixture. The assay was conducted in triplicates while using 100% methanol as the blank. Using a UV-vis spectrophotometer (G10S, USA), samples were measured for their absorbance after 60 min at room temperature at 765 nm. TPC values were calculated using the gallic acid standard curve within the range of 0.02-0.1 mg/mL, and all values were expressed as mg/g of the dry weight sample.

DPPH radical scavenging capacity

2,2-diphenyl-1-picrylhydrazylhydrate (DPPH) was used as a radical to react with a sample extract of selected plant species. The sample extract was taken from the freeze-dried powder of plants after dissolving in ethanol (1 g/10 mL) and was shook for 24 hr, and the supernatant was collected (Bopitya and Madujith, 2012). The sample was tested for initial antioxidant activity, and based on that, a concentration series (0.025-0.1 g/mL) was prepared. Each sample (0.5 mL) was mixed with 2.5 mL of 0.1M DPPH solution and kept under dark conditions to allow the reaction between DPPH radicals and the sample oxidative compounds to take place. It had kept for 60 min at room temperature, and absorbance was measured using a UV visible spectrophotometer (G10S UV VIS, USA) at 517 nm. Using the following equation, DPPH radical capturing activity was evaluated.

$$RAS\ (\%) = [(A^{DPPH} - A^{sample}) / A^{DPPH}] \times 100$$

Where, A^{sample} is the absorbance relative to the final reaction mixture after 60 min and A^{DPPH} is the absorbance relative to the DPPH solution without extraction. A^{sample}, is the absorbance value of the sample after 60 minutes of reacting the methanol extracted sample with DPPH radicals. Final results are expressed in terms of the IC50 value. Here, the IC50 value will be the antioxidant concentration required to reduce the initial DPPH concentration by 50%. Thus, the data analysis is based on the fact that a lower IC50 value indicates a higher antioxidant activity (Phuyal *et al*., 2020).

ABTS radical scavenging capacity

Antioxidant radical scavenging activity was calculated based on $ABTS^{\cdot+}$ radical reaction related to the sample oxidative compounds. Free radicals are generated by a reaction mixture of 2,2'-azobis (2-ethylbenzoline-6-sulfonic acid), diammonium salt (ABTS), and 2.45 mM potassium persulfate. The ethanol extract was taken as previously described in the DPPH assay, and 10 µL of samples were reacted with $ABTS^{\cdot+}$ (1.1 mL of the solution). After a 1-minute reaction period, the absorbance of the samples was measured using a UV visible spectrophotometer (G10S UV-Vis, USA) at 734 nm. The final results are indicated in reference to the Trolox standard curve (range of 100-800 µM) as Trolox equivalent antioxidant capacity (mg TEAC per gram dry weight sample).

Analysis of minerals

Concentrations of minerals such as calcium (Ca), copper (Cu). iron (Fe) and zinc (Zn) were determined using Atomic Absorption Spectrophotometric method (Machado et al., 2017). Sample extraction was conducted using microwave-assisted digestion with the 1 g of the two selected freeze-dried samples. Di-acidic combination including 3 mL of concentrated HNO_3 and 2 mL of H_2O_2 was mixed with sample and digested in a digestion vessel of the microwave digestion oven (MARS 6240/50, USA). Program was optimized as 400 to 1800 W power supply at 180 °C along 15-minute ramp time. Digested samples were volume up to 100 mL with de-ionized water and 0.22 µm nylon membrane filters were used to filter the sample extracts. Atomic Absorption Spectrophotometer (AAS) (iCE 3400 AAS, USA) was used to determine and quantification the minerals in selected samples.

Micronutrient *in vitro* bioaccessibility

Micronutrient bioaccessibility of selected plant leaves was estimated using a simulated *in vitro* gastrointestinal digestion system, adapted from the method previously described by Hedren *et al*., (2002) with slight modifications (Labronbisi *et al*. 2016; Jayathunge *et al*., 2017). Freeze-dried samples (0.5 g) were subjected to gastric digestion stimulation by mixing with 2.5 mL of NaCl/Ascorbic acid solution and 2.5 mL stomach electrolyte in an amber colour glass tubes. The pH of this mixture was adjusted to 4.00 ± 0.05 (with 1M HCl) before the addition of a pepsin solution (0.52% porcine pepsin in stomach electrolyte). Subsequently, the mixture was incubated for 30 min at 37 °C while shaking. Before continuing the incubation in an incubator (IN-010, China) for

30 min, the mixture was acidified to pH 2.00 ± 0.05 (with 1M HCl). The adjustment of the pH in two steps mimicked the gradual drop in gastric pH after a meal.

Then, small intestinal digestion stimulation was done for the partially digested sample. Initially, the pH level was adjusted to 6.90±0.05 with 1 M NaOH. A solution of (1.5 mL) pancreatin, lipase, and bile salt (0.4% porcine pancreatine, 0.2% porcine pancreas lipase, 2.5 % bile extract, 0.5% pyragallol, and 1% α-tocopherol in water) was added to the partially digested sample, followed by incubation in the incubator for 2 hr at 37 °C while shaking. After the incubation period, the mixture was centrifuged (Z 216 M, Germany) at 5000 g for 1 hr, and the supernatant was used for the determination of the bioaccesibility of minerals. The mineral contents in the supernatant of digested samples were measured using an Atomic Absorption Spectrophotometric method (Machado et al., 2017). Amber tubes and vials were used at all steps to protect samples from light. The bioaccessibility of the selected samples was calculated as the ratio (%) of the *in vitro* bioaccessible fraction (BAF) for minerals to the corresponding available mineral content of the samples.

BAF % =

Statistical analysis

SPSS statistical software was used to evaluate the significance of the final data of each analysis conducted for selected plant species in a two-way analysis of variance (ANOVA). Duncan multiple range test was used to assess the difference between treatment means at the level of significance of $P<0.05$.

RESULTS AND DISCUSSION

This study was conducted to quantitatively determine AAC, TPC, minerals and antioxidants quantitatively from *A. aureum* and *T. triangulare* species on a dry weight basis. In addition, in the *in vitro* bioaccessibility of minerals in the plant extract was also investigated. *In vitro* bioaccessibility of minerals has been defined as 'fraction of mineral ingested with plant samples that is released from its matrix into the gastrointestinal tract and has the potential to be absorbed by the intestine during *in vitro* digestion process'. Methanol extracts of selected species were used for sample analysis in the study since methanol is a suitable solvent for the extraction of phenols and many other bioactive compounds from the plants, due to its high polarity of methanol (Fasuyi,2007). Moreover, it has the ability to inhibit the action of polyphenol oxidase, which could affect the antioxidant activity (Lio *et al*., 2015).

Total phenolic content

TPC of methanol extracts of *A. aureum* and *T. triangulare* as determined using the Folin – Ciocalteu method are, presented in Table 2. The obtained results of both UP species were significantly difference and revealed a good recovery of TPC in *A. aureum* and *T. triangulare* as, 279.83 mg of GAE/100 g and 347.45 mg of GAE/100 g, respectively. TPC of *A. aureum* in this study was lower than the reported values in *A. aureum* at *Kuala Lumpur* region, India (Lai *et al*., 2009). Furthermore, the TPC obtained for *T. triangulare* was similar to reported values for *T. triangulare* species from Uganda (Liao *et al*., 2015). Gunathilake *et al*., (2018) screened common leafy vegetables for their nutritional values in Sri Lanka namely, *Centella asiatica* (gotukola), *Gymnema lactiferum* (kuringan), *Sesbania grandiflora* (kathurumurunga) and *Passiflora edulis* (passion fruit). None of the aforementioned plants discussed in that study showed higher TPC values than the species selected in this study. According to Chandrasekhara *et al*., 2020, TPC of methanol extract of *Centella asiatica* ranged between 3.9-12 GAE/100 g. The TPC value related to methanol extract of the selected underutilized cultivars was shown to be higher than that of *Centella asiatica* range, which is one of the leading herbs consumed in Sri Lanka. Therefore, these plants found in Sri Lanka have significant nutritional value. The amount of bioactive compounds such as phenolic compounds and antioxidants contained in plants is determined by the habitat of the plant. It has been discussed by

Zargoosh *et al*., 2019, that factors such as temperature, rainfall and relative humidity have a direct effect on the availability of bioactive compounds in plant species.

Ascorbic acid content

The AAC of both UP species is shown in Table 2, and the highest AAC level was observed at 8100 mg/100 g in *T. triangulare*, followed by *A. aureum* (1650 mg/100 g). These values were consistent with the values reported for several *Acrostichum* and *Talinum* species from Nigeria and Uganda (Nwazue, 2011; Khan et al., 2013; Hanin and Pratiwi, 2017). Moreover, AAC levels in the studied UP were higher than reported values for other common leafy vegetables available in Sri Lanka (Jayawardana et al., 2018). Among them, *Centella asiatica* is one of the prominent leafy vegetables, and the AAC of different *Centella* ranged from 9.73 to 48.5 mg/100 g (Chandrika et al., 2015). It revealed that both selected species contain higher AAC than the identified AAC values in *Centella*. According to Dahanayake and Ekanayake, the amounts of vitamin C related to *Sesbania grandiflora* (Kathurumurunga) and *Alternanthera sessilis* (Mukunuwenna) (according to fresh weight) are 163.4 and 45.8 mg/100g respectively (Dahanayake and Ekanayake, 2020). Accordingly, the methanol extract of the two selected cultivars showed a higher AAC value than both. Thus, it is important to pay attention to these underutilized species, which are more easily available for obtaining the required amount of vitamin C per day.

According to the available data these UP contain important amounts of functional compounds, such as AAC (Vitamin C) and phenols which are beneficial for health. Recently, there has been an increasing interest in biological and health effects of these bioactive compounds, since these molecules are often associated with antioxidant activity. Therefore, these kind of plant extracts are identified as powerful natural antioxidants, leading to a reduction in cardiovascular disease risk factors, a decrease in the incidence of cancer, and protection against a wide range of chronic diseases (Bajpai *et al*., 2005; Leaves and Leaves. 2014).

Antioxidant potential

DPPH and ABTS radical scavenging activities of methanol extracts of UP species are summarized in Table 3. Reported values for DPPH assay were expressed as IC_{50} which is inversely proportional to the antioxidant activity. Table 3 shows that the IC_{50} value of UP extracts ranged from 0.192 mg/mL to 0.173 mg/mL, with *T. triangulare* having the highest antioxidant activity. The IC_{50} of *A. aureum* collected from India was comparable to the data obtained in the present study (Badhsheeba and Vadivel, 2018).

Furthermore, Balasuriya and Dharmaratne (2007) reported that the IC_{50} values of selected prominent leafy vegetables, namely Mukunuwenna, Gotukola, and Kankun range from 185.31 mg/mL to 793 mg/mL.
In the ABTS assay, it measures the relative antioxidant ability of UP species to scavenge the radical-cation $ABTS^{\bullet+}$ released by the oxidation of 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate. The order of extracts in these antioxidant activities in both DPPH and ABTS assays was similar to that of TPC; *T. triangulare* > *A. aureum*. Previous findings indicate that the scavenging and reducing powers of the plant extracts were due to phenolic compounds (Lamien-Meda *et al*., 2008).

Analysis of minerals

Evidence in the literature demonstrates that the UP may be an excellent source of microelements for humans. Underutilized wild greens usually contribute more to the dietary intake of minerals than prominent food crops, and the amount of Ca, Fe, Cu, and Zn present in UP may even reach half of the recommended daily requirement. The results of the analysis show that Ca is the most abundant mineral in both selected UP and higher concentrations of Ca, Zn, and Fe were observed in *T. triangular* species. Similar results with reference to the concentration of Ca, Fe, and Zn were reported in a previous study in Nigeria (Ogbonnaya and Chinedum, 2013). Literature reports were not available for both selected species in Sri Lanka. However, with reference to the high microelement availability in these UP species, a large number of studies have been undertaken worldwide for underutilized or wild edible leaf species, and results were reported for identified elements in this study (Freiberger et al., 1998; Steyn et al., 2001; Lenzi, et al., 2019). Furthermore, the content of Fe in *T. triangular* is higher than that of *Spinacia oleracea*, which was promoted as a dietary source with high Fe concentration (Aygun, 2017). Lack of essential minerals, which are demanded in small quantities by the body for proper growth and development, is identified as a micronutrient deficiency and referred to as 'hidden hunger' (Kennedy et al., 2003). In this context, these identified species can be promoted as low cost, better dietary sources to overcome the prevailing malnutrition in developing countries.

After calculating both digested and undigested mineral contents of two selected cultivars (Table 4), the *in vitro* bioaccessibility of each mineral was calculated. The values obtained for the evaluation of *in vitro* bioaccessibility in relation to the total micronutrient concentrations in undigested samples of *T. triangular* and *A. aureum* are summarized in Figure 2 The *in vitro* bioaccesiibiity of Ca, Cu, Zn, and Fe represented 47.46%, 30.62%, 0.86%, and 0.27% in *A. aureum*, respectively. Moreover, *T. triangular* showed a bioaccessibility fraction of 18.12% for Ca, 64.30% for Cu, 0.25% for Zn, and 0.16% for Fe. The BAF is the portion of the absorbed nutrients that is available for utilization in normal physiological functions and for storage. A number of factors influence the bioaccessibility of nutrients, including their chemical composition, their ability to release from food matrixes, interactions with other nutrients, suppressors and other cofactors, and the formation of stable compounds that are slowly metabolized.

Moreover, this could be due to the specific composition of each sample in terms of, the quantity and quality of proteins and the presence of compounds such as fibers and phenols, which can inhibit the bioaccessibility of minerals, as well as the chemical form of the elements and nutrient interactions (Bertin *et al*., 2016).

Table 2. TPC and AAC of *A. aureum* and *T. triangulare*

Plant species	TPC GAE (mg/100 g)	AAC (mg/100 g)
A. aureum	279.83±5.09[a]	1650.00±0.25[b]
T. triangulare	347.45±1.65[c]	8100.00±0.45[a]

Means with different alphabetic letters in the same column are significantly different at the 5% significance level (P > 0.05).

Table 3. Antioxidant activity in undigested samples of *A. aureum* and *T. triangulare*

Samples	Antioxidant activity	
	DPPH (IC_{50} mg/mL)	ABTS (mg TEAC/ g)
A.aureum	0.192±0.004[a]	342.78±0.01[b]
T.triangulare	0.173±0.001[b]	431.11±0.02[a]

The means with different letter in the same column are significantly different at the 5% significance level (P > 0.05).

Table 4. Mineral contents in undigested and enzyme- digested samples of *A. aureum* and *T. triangulare*

Element		Micronutrient contents in *A.aureum* (mg/100 g)	Micronutrient contents in *T.triangulare* (mg/100 g)
Ca	Undigested	45.61±0.94[b]	102.91±3.63[a]
	Digested	21.60±3.64[a]	18.63±0.13[b]
Cu	Undigested	7.42±1.79[a]	1.45±0.15[a]
	Digested	2.16±0.36[b]	0.93±0.01[b]
Zn	Undigested	5.12±0.15[a]	19.09±0.03[a]
	Digested	0.01±0.001[b]	0.03±0.01[b]
Fe	Undigested	4.67±0.04[a]	17.29±0.20[a]
	Digested	0.04±0.001[b]	0.05±0.04[b]

Values are means ± standard deviation of three replicates. Values within each raw for each element followed by the same letter are not significantly different at $p < 0.05$.

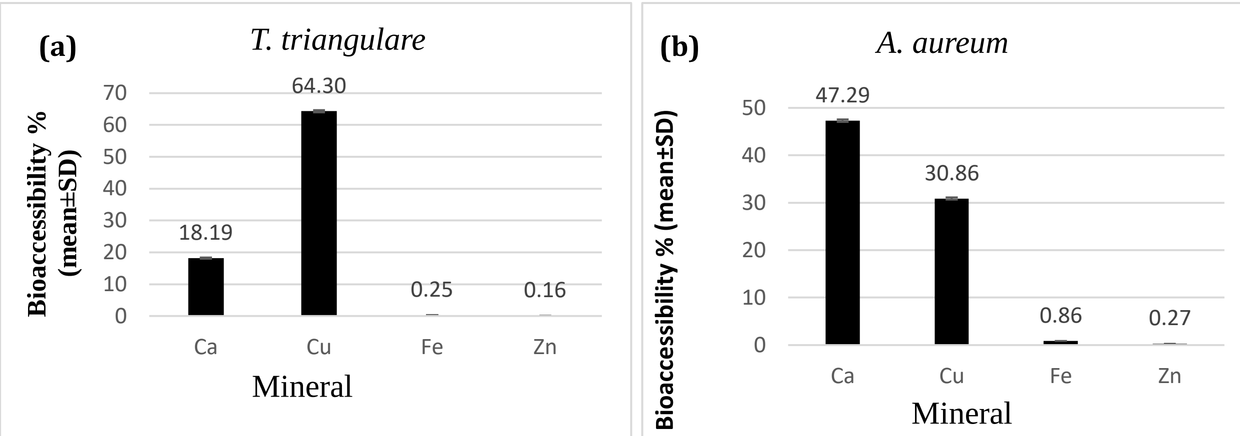

Figure 2. Mean of in vitro accessibility (%) of (a) *T. triangulare* and (b) *A.aureum*. The error bars represent the standard errors of the mean values.

The results of the study indicate the importance of *T. triangulare* and *A. aureum* as potential food sources rich with bioactive compounds. Based on the preliminary trials, different solvent extracts of selected plant species were tested for their bioactive compounds. Among them, methanol was identified as the ideal solvent to extract the maximum amount of bioactive compounds and antioxidants from the selected plants. Overall, all identified bioactive compounds, minerals, and antioxidants were high in *T. triangulare* and *A. aureum* as compared to the majority of highly consumed plant food sources (Jayawardana et al.,2007 2007; Balasuriya et al., 2018). In the work reported here, the lowest antioxidant value (IC50: 0.196 mg/mL) was similar to the leafy vegetables regularly consumed in Sri Lanka (Peduruhewa et al., 2021). However, AAC, antioxidant capacity, and TPC in both species may be affected by cooking or processing during the consumption stage. According to previous findings, the AAC, antioxidant capacity, and TPC of selected fresh vegetables were affected by cooking temperature (Senarathne et al., 2017). These values need to be preserved during the cooking process. Therefore, further studies need to be carried out to improve and identify the best processing methods to gain maximum nutrition values from the selected plants.

Even though the nutritional value of several underutilized or wild edible species consumed by the people in Sri Lanka has been studied, additional knowledge regarding the bioaccassibilty and bioavailability of these nutritional compounds remains to be investigated. Hence, bioavailability and bioaccessibility studies of essential nutritional compounds in UP species are needed to be evaluated in the future. This study provides data to health and food professionals and policymakers in Sri Lanka to encourage the population to consume more UP based products and to increase awareness among people in the country.

CONCLUSION

The selected UP species; *T. triangulare* and *A. aureum*, are potential sources of ascorbic acid (vitamin C) and phenolic compounds. Assessment of the *in vitro* bioaccessibility of minerals (Ca, Cu, Zn, and Fe) in *A. aureum* and *T. triangulare* was performed for the first time using an *in vitro* digestion model. Considering the data from methanol extracts of *A. aureum* and *T. triangulare*, it is concluded that *A. aureum* and *T. triangulare* plants contain bioactive compounds and minerals. These plant species with acceptable antioxidant bioaccessibility percentages will provide an opportunity to obtain quality food at a very minimal cost. However, before these underutilized plants are promoted as a dietary substitute or as a natural antioxidant, it is mandatory to carry out toxicity analysis through *in vitro* or *in vivo* models and performance in edible extracts.

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