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Nutraceutical and antioxidant evaluation of *Abelmoschus* taxa

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ABSTRACT

The wild species of *Abelmoschus* are not explored for their nutraceutical properties. The study was undertaken to evaluate total phenolic content (TPC), total flavonoid content (TFC), antioxidant activities, proximate composition and mineral profile of wild *Abelmoschus* taxa *A. manihot* (L.) Medik, and *A. ficulneus* (L.) Wight & Arn., and the cultivated taxa *A. esculentus*, and *A. esculentus* cv. Phule Utkarsha. The highest TPC [5.86 ± 0.10 gallic acid equivalent (GAE) mg·g^{-1} DW] and TFC [13.93 ± 0.19 rutin equivalent (RE) mg·g^{-1} DW] were observed in *A. esculentus* cv. Phule Utkarsha. *Abelmoschus ficulneus* had higher 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (10.65 ± 0.06%) and ferric reducing antioxidant property (FRAP) activity [33.91 ± 0.73 ascorbic acid equivalent (AAE) mg·g^{-1} DW]. Taxa affected extraction performance of solvents for phenolics, flavonoids and antioxidant molecules. The Pearson correlation analysis indicated a positive correlation between phenolics, flavonoids and antioxidant activity. Proximate composition was higher for wild *Abelmoschus* over cultivated taxa with higher fat, fiber, protein, carbohydrates content and energy value. The *A. esculentus* cv. Phule Utkarsha had the highest boron, calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc, compositions. Thus, the nutraceutical properties of wild *Abelmoschus* taxa reported may benefit human health and explore the importance of these wild species and their commercial cultivation.

KEYWORDS

Abelmoschus spp; flavonoids; minerals; phenolics; proximate composition

The genus *Abelmoschus* Medik. comprises 11 species categorized as domestic and wild forms (Sutar et al., 2013). Domestic species of okra, *A. esculentus* (L.) Moench., in India is the most popular and cultivated for its fruit which are a source of carbohydrates, protein, fiber, minerals, and vitamins (Jarret et al., 2011). Okra seed is a potential source of edible oil, protein and amino acid (Rao, 1985). Essential oil of *A. moschatus* Medik. seed is used in other industries (Onakpa, 2013).

Okra is used as an antioxidant, anti-inflammatory, antidiabetic, antifungal, analgesic, as a laxative and to treat asthma, diabetes, cardiovascular,

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neurological and skin diseases (Doreddula et al., 2014; Sabitha et al., 2012). Jia et al. (2011) and Huang et al. (2007) isolated several bioactive compounds from *A. esculentus*. Some phenolics and flavonoid compounds isolated from the wild species *A. manihot* (L.) Medik. (Lai et al., 2009), exhibit a neuroprotective effect against cerebral ischemia injury in rats and rabbits (Guo and Chen, 2002; Wen and Chen, 2007). The whole plant is used to treat sprain, bronchitis, and toothache (Anonymous, 1976; Patil and Bhaskar, 2006). *Abelmoschus* taxa have applications in industry (Pandey et al., 2013; Saha et al., 2011). Neglected and underutilized wild *Abelmoschus* species, which are not well known, are being examined for nutritional and phytochemical properties (Anonymous, 2008).

Thus, seeing the importance of *Abelmoschus* species, the current study was undertaken to evaluate phenolic content, flavonoid content, antioxidant activities, proximate composition and mineral profile of both wild (*A. manihot* and *A. ficulneus*) and cultivated (*A. esculentus* and *A. esculentus* cv. Phule Utkarsha) taxa.

Materials and methods

Immature fruits of *A. esculentus, A. manihot, A. ficulneus*, and *A. esculentus* cv. Phule Utkarsha were collected from the Lead Botanical Garden, Shivaji University, Kolhapur. They were authenticated with the help of available literature and discussion with experts and herbarium specimens were deposited at the Herbarium, Department of Botany, Shivaji University, Kolhapur (*A. esculentus*: Voch. No. AVM-105, *A. manihot*: Voch. No. AVM- 106, *A. ficulneus*: Voch. No. AVM-107 and *A. esculentus* cv. Phule Utkarsha: Voch. No. AVM-108). Collected fruits were washed under tap water and dried under shade. Dried materials were powdered using a mixer grinder for solvent extraction.

One-g of dry fruit powder of each accession was extracted with 100 mL of methanol, ethanol or water. All mixes were placed on an orbital shaker (Rivotek, Mumbai, India) at 150 rpm for 24 h at room temperature and then centrifuged at 6,000 × g for 10 min (Remi, Bombay, India). The supernatant was collected and used for estimation of total phenolics, total flavonoids and antioxidant activities. All chemicals and solvents were of analytical grade.

Total phenolic content (TPC) of extracts was analyzed by the Folin-Ciocalteu method developed by Singleton and Rossi (1965) with little modification. Aliquots of plant extract (125 µL) were mixed with 125 µL of Folin-Ciocalteu reagent (10 fold diluted with distilled water) and incubated for 10 min at room temperature. After 10 min, 1.25 mL of 7% Na_2CO_3 was added to the reaction mix. All reaction mixes were allowed to stand for 90 min in the dark at room temperature and absorbance measured at 760 nm

using a UV-visible spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan). Gallic acid was used as positive control and a standard calibration curve was prepared using different concentrations of gallic acid (5–100 $\mu g \cdot mL^{-1}$).

Total flavonoid content (TFC) of extracts was estimated using the aluminium chloride colorimetric method (Chang et al., 2002) with slight modification. Aliquots of extract (1.5 mL) were mixed with 1.5 mL of 2% $AlCl_3$ and kept in the dark for 10 min. Absorbance was measured at 367 nm. Rutin was used as a positive control and a standard calibration curve was prepared using different concentrations of rutin (5–200 $\mu g \cdot mL^{-1}$).

Antioxidant activity of extracts was estimated by DPPH radical scavenging assay (Brand-Williams et al., 1995). The DPPH reagent was prepared by dissolving 2.5 mg DPPH in 100 mL chilled methanol. Aliquots (0.1 mL) of plant extract were mixed with 2.9 mL of DPPH reagent. After shaking the reaction mix was incubated at room temperature in the dark for 30 min. Absorbance was measured at 517 nm.

Detection of antioxidant ability through FRAP assay was determined according to the method described by Benzie and Strain (1996). Aliquots of extract (0.5 mL) were mixed with 2.9 mL FRAP reagent [Acetate buffer (300 mM, pH 3.6): 2,4,6-Tris(2-pyridyl)-s-triazine (10 mM):$FeCl_3$ (20 mM) (10:1:1 v:v:v)]. The reaction mix was kept for 15 min at 37°C. Absorbance was measured at 595 nm. Ascorbic acid was used as the positive control and a standard calibration curve was prepared using 25–600 $\mu g \cdot mL^{-1}$ of ascorbic acid.

Moisture, ash, fat, fiber and crude protein contents were determined according to established methods (Anonymous, 2012). Moisture content was determined by drying 2 g of sample in a pre-weighed crucible at 50°C for 24 h in a forced air oven (Classic Scientific, Mumbai, India). Ash content was estimated by ignition of 2.0 g of sample in a muffle furnace at 600°C for 6 h. Crude protein was determined by multiplying the estimated nitrogen content to nitrogen-to-protein conversion factor 6.25. Crude fat content was analyzed by extracting 2 g of sample with petroleum ether in a Soxhlet apparatus. The extract was evaporated to dry at 50°C in a forced air oven. Crude fiber was estimated using the acid and alkaline digestion method. Carbohydrate content was determined by subtracting percents of ash, crude protein, crude fat and crude fiber on a dry weight basis from 100. The energy value (Kcal) was calculated by multiplying percents crude protein, carbohydrate and crude fat content by factors of 4, 4 or 9, respectively, and energy value (KJ) calculated by multiplying percents of crude protein, carbohydrate and crude fat content by 17, 17 or 37, respectively (Manzi et al., 2004).

Tissues that had not been extracted with solvents were prepared by acid digestion for mineral analysis (Toth et al., 1948). One-g of sample was mixed with 10 mL of concentrated HNO_3 and heated on a hot plate until

all material was completely dissolved. Then 10 mL of perchloric acid was added and the mix heated until the solution became colorless and reduced to 2–3 mL. After cooling, the mix was diluted up to 100 mL with distilled water and used for analyses. Nitrogen was estimated according to the method of Hawk et al. (1948). Sodium and potassium were estimated using a flame photometer (Esico, Parwanoo, India). The remaining inorganic elements: calcium, cobalt, copper, iron, magnesium, manganese potassium and zinc, were analyzed using an atomic absorption spectrophotometer (Systronics, Ahmedabad, India)

All analyses were performed in triplicate. Data were subjected to analysis of variance (2-way ANOVA) using Minitab software (ver. 17.1, SAS Inc., Cary, NC). Proximate and mineral analysis means were tested using one-way ANOVA and separated with Tukey multiple range test.

Results and discussion

Most taxon and solvent interactions affected contents of total phenolics, total flavonoids and antioxidant activity (DPPH and FRAP) because the highest order interaction was significant for all measures it was used to explain results (Table 1).

The estimated TPC and TFC from different taxa of *Abelmoschus* varied (Table 2). Aqueous extract of *A. esculentus* cv. Phule Utkarsha had the highest TPC followed by the methanolic extract of *A. ficulneus*. The least amount of TPC was in the methanolic extract of *A. esculentus*. The results agree with phenolics content recorded from *A. esculentus* (Mohammed et al., 2016; Tiwari et al., 2016). Taxa affected the amount of TFC. The *A. esculentus* cv. Phule Utkarsha had the highest TFC in aqueous extract followed by an ethanolic extract of *A. ficulneus* and *A. manihot*.

Solvent and taxa affected phenolics and flavonoid contents in all solvents (Table 1). The concentration of phenolics and flavonoids was higher in aqueous extract than in ethanol and methanol extracts. Extraction yield of bioactive metabolites largely depends on extrinsic and intrinsic factors (Sun and Ho, 2005; Vadivel et al., 2011).

Table 1. Analysis of variance for TPC[a], TFC, DPPH and FRAP activity.

Source	TPC	TFC	DPPH	FRAP
Taxa (T)	5.3995**	30.4391**	30.232**	665.77**
Solvent (S)	0.4093*	16.8121**	13.092**	312.09**
T × S	1.7483**	9.4127**	9.635**	114.37**

*, ** Significant at $p < 0.05$ and $p < 0.001$.
[a] TPC = Total Phenolic Content; TFC = Total Flavonoid Content; DPPH = 2,2-diphenyl-1-picrylhydrazyl; FRAP = Ferric reducing antioxidant property.

Table 2. Interaction[a] response of taxon and solvent on total phenolic content (TPC), total flavonoid content (TFC), DPPH and FRAP activity of *Abelmoschus* taxa.

Taxon	Solvent	TPC[b]	TFC[c]	DPPH activity[d]	FRAP activity[e]
A. esculentus	Ethanol	3.03^{cd}[f]	8.74^{e}	3.57^{de}	18.74^{c}
	Methanol	2.02^{d}	8.54^{e}	2.60^{e}	18.62^{c}
	Aqueous	3.17^{cd}	6.55^{h}	3.40^{de}	12.24^{d}
A. manihot	Ethanol	4.39^{abc}	12.04^{b}	5.49^{bcd}	29.91^{b}
	Methanol	4.27^{abc}	10.46^{d}	6.80^{bc}	30.57^{b}
	Aqueous	3.40^{bcd}	7.88^{f}	4.47^{cde}	10.74^{de}
A. ficulneus	Ethanol	4.66^{abc}	12.07^{b}	5.55^{bcd}	33.82^{a}
	Methanol	4.94^{ab}	11.93^{b}	6.57^{bc}	33.91^{a}
	Aqueous	4.55^{abc}	7.30^{g}	10.65^{a}	19.45^{c}
A. esculentus cv. Phule Utkarsha	Ethanol	3.88^{bc}	11.94^{b}	3.45^{de}	07.82^{ef}
	Methanol	3.63^{bcd}	11.43^{c}	3.45^{de}	07.45^{f}
	Aqueous	5.86^{a}	13.93^{a}	7.36^{b}	12.66^{d}

[a]data in the interaction analyzed by Least Squares Means and means separated at $p < 0.05$, Tukey's multiple range test.
[b]gallic acid equivalent (GAE) mg·g^{-1} DW.
[c]rutin equivalent (RE) mg·g^{-1} DW.
[d]DPPH = 2,2-diphenyl-1-picrylhydrazyl as % radical scavenging activity.
[e]FRAP = Ferric reducing antioxidant property as ascorbic acid equivalent (AAE) mg·g^{-1} DW.
[f]values in columns (means of 3 replications) followed by the same letter are not statistically different.

Taxa affected antioxidant activity of solvent extracts (Table 2). The aqueous extract of *A. ficulneus* had the highest DPPH activity followed by aqueous of extract *A. esculentus* cv. Phule Utkarsha. The lowest DPPH activity was in the methanolic extract of *A. esculentus*. The DPPH activity in the taxa in this report was similar to that in extract from leaf of *A. manihot* (Sudewi et al., 2017) and *A. esculentus* (Tiwari et al., 2016).

Taxa affected FRAP values (Table 2). The *A. ficulneus* had the highest FRAP activity in methanolic and ethanolic extracts followed by the methanolic extract of *A. manihot*. The least FRAP value was in *A. esculentus* cv. Phule Utkarsha. All solvents produced somewhat comparable results for extraction of antioxidant molecules. The methanol and ethanol extracts produced the highest extraction of antioxidant molecules, which exhibited higher FRAP activity for all taxa except *A. esculentus* cv. Phule Utkarsha. The cultivated taxon *A. esculentus* cv. Phule Utkarsha contained more phenolics and flavonoids. Phenolics and flavonoids content reported in this work might be responsible for antioxidant properties in wild and cultivated *Abelmoschus* taxa (Ignat et al., 2011).

Positive correlations between variables were determined for all tested *Abelmoschus* taxa. The TPC was highly, and positively, correlated to TFC (R-0.666) and DPPH activity (R- 0.724); TPC was positively, correlated to FRAP activity. Correlations between TFC and DPPH and FRAP activity was weak compared to TPC. The results indicated that TPC is the principal antioxidant molecule which participates in the antioxidant activity of the *Abelmoschus* taxa.

Table 3. Proximate composition analysis[a] of *Abelmoschus* taxa.

Taxon	Moisture g/100 g	Crude fat g/100 g	Ash content g/100 g	Fiber content g/ 100 g	Crude protein g/ 100 g	Carbohydrate g/100 g	Energy value (Kcal)/ 100 g	Energy value kJ/100 g
A. esculentus	42.08a[b]	10.25a	29.27a	16.43b	12.16a	31.87c	268.41b	1127.93b
A. manihot	25.10c	10.16a	22.29b	22.90a	05.47b	39.20b	270.17ab	1135.55ab
A. ficulneus	20.40d	09.23b	19.62c	23.49a	05.33b	28.38d	273.67a	1151.71a
A. esculentus cv. Phule Utkarsha	28.05b	02.52c	29.15a	16.20b	03.43c	48.47a	230.35c	975.84c

[a]one-way ANOVA followed by Tukey's multiple range test.

[b]values in columns, mean of 3 replications, followed by the same letter are not statistically different, $p < 0.05$.

The proximate composition of *Abelmoschus* taxa varied (Table 3). Moisture content was highest in *A. esculentus*. Crude fat content from *Abelmoschus* taxa varied. The highest fat content was in *A. esculentus* followed by *A. manihot*. Ash content was affected by taxa with *A. esculentus* being highest followed by *A. esculentus* cv. Phule Utkarsha.

Consumption of dietary fiber reduces risks of cancer, heart diseases and diabetes (Dawczynski et al., 2007). Taxa affected fiber content with *A. ficulneus* being highest followed by *A. manihot*. Proteins are considered essential macronutrients for human nutrition (Reeds et al., 2000). Crude protein content was higher in *A. esculentus* and lowest in *A. esculentus* cv. Phule Utkarsha. Carbohydrate content was highest in *A. esculentus* cv. Phule Utkarsha and lowest in *A. ficulneus*. Carbohydrate and energy values of cultivated and wild taxa were almost comparable to values reported in accessions of *A. esculentus* by Gemede et al. (2015). The proximate composition was found highest in *A. ficulneus* and *A. manihot* than cultivated taxa i.e. *A. esculentus*.

Taxa affected content of mineral elements (Table 4). Minerals are important for the complete physical and mental health and are important components of bones, teeth, muscles, tissues, blood, and nerve cells (Soetan et al., 2010). The highest values of nitrogen and sulphur were in *A. esculentus*; phosphorus and molybdenum were higher in *A. ficulneus*. Values for boron, calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc were higher in *A. esculentus* cv. Phule Utkarsha. Values of mineral elements were lowest in *A. manihot*. Values for copper were not statistically different i.e. *A. esculentus* (0.0026 g/100 g), *A. esculentus* cv. Phule utkarsha (0.0030 g/100 g), *A. ficulneus* (0.0018 g/100 g) and *A. manihot* (0.0021 g/100 g). *Abelmoschus esculentus* cv. Phule Utkarsha had the highest amount of macro- and micro-elements indicating it to be a good source of nutrients

The cultivated and wild species appear to be sources of phenolics, flavonoids, fat, fiber, protein and carbohydrates and dietary energy required for human health. Levels of phytochemicals and nutritional content of wild *Abelmoschus* taxa offer opportunities to develop improved varieties.

Table 4. Mineral analysis[a] of *Abelmoschus* taxa.

Taxon	B	Ca	Fe	Mg	Mn	Mo	N	P	K	Na	S	Zn
A. esculentus	0.0018b[b]	1.22[b]	0.0049[b]	2.74[b]	0.0078[b]	0.0005[b]	1.94[a]	0.08[b]	0.75[b]	1.13[b]	1.09[a]	0.0048[b]
A. manihot	0.0016[b]	1.12[c]	0.0051[b]	2.51[c]	0.0071[bc]	0.0003[b]	0.87[b]	0.07[b]	0.74[b]	1.03[c]	0.55[c]	0.0047[b]
A. ficulneus	0.0012[c]	0.96[d]	0.0043[c]	2.14[d]	0.0061[c]	0.0010[a]	0.85[b]	0.12[a]	0.64[c]	0.86[d]	0.55[c]	0.0040[b]
A. esculentus cv. Phule Utkarsha	0.0021[a]	1.56[a]	0.0071[a]	3.51[a]	0.0100[a]	0.0005[b]	0.55[c]	0.11[a]	1.06[a]	1.45[a]	0.64[b]	0.0066[a]

[a]one-way ANOVA followed by Tukey multiple range test.

[b]values in columns, means of 3 replications (g/100 g); followed by the same letter are not statistically different, $p < 0.05$;

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