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ANTIOXIDANT POTENTIAL OF COMMON LEAFY VEGETABLES IN BANGLADESH

Sheikh Julfikar Hossain*, M Shahina Sultana, M Iftekharuzzaman, S Amir Hossain and M Abu Taleb

Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh

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Abstract

Amount of polyphenols, flavonoids, anthocyanins in ethanol extracts and antioxidant activity of both ethanol and lipophilic extracts of common leafy vegetables in Bangladesh were compared. Among the 12 leafy vegetables, *Ipomoea aquatica* Forssk. showed the highest content of total polyphenols (38.9 mg gallic acid equivalent (GAE)/g extract) and flavonoids (23.2 mg (+)-catechin equivalent (CE)/g extract). Highest anthocyanins content was found in *Amaranthus gangeticus* L. (1.15 μmol/g extract) followed by *Pisum sativum* L. (0.85 μmol/g extract). Ethanol extracts of *Brassica campestris* L., *Enhydra fluctuans* Lour., *I. aquatica*, *Lagenaria siceraria* (Mol.) Standl. and *P. sativum* exhibited high DPPH free radical scavenging activity with IC_{50} of 104.2, 85.5, 26.9, 125 and 68.5 μg/ml, respectively whereas for the same lipophilic pentane extracts of *Basella alba* L. showed the lowest (68.4 μg/ml) IC_{50} followed by *I. aquatica* (70.4 μg/ml), *E. fluctuans* (75.7 μg/ml), *L. siceraria* (78.3 μg/ml) and *B. campestris* (80.6 μg/ml). *I. aquatica* also showed the highest NO free radical scavenging followed by *B. campestris* and *B. alba*. Highest reducing power (O.D. = 1.7) was observed for *I. aquatica* followed by *P. sativum* (O.D. = 1.12), *B. alba* (O.D. = 0.86) and *L. siceraria* (O.D. = 0.72) at 400 μg/ml extract. They also displayed high total antioxidant capacity. Therefore, the top five potential leafy vegetables consist of both hydrophilic and lipophilic antioxidant(s), the order being *I. aquatica* > *B. campestris* > *B. alba* > *P. sativum* > and *L. siceraria*.

Introduction

Leafy vegetables are widely consumed in many countries because of their nutritional quality and anthocyanin, ascorbic acid, β-carotene, flavonoid, folic acid, polyphenol and alkaloid contents. These components have a wide range of biological functions such as antiallergic, anticancer, antidiabetic, antimicrobial, antioxidant, anticardiovascular diseases (Scalbert *et al.* 2005). Reportedly, various epidemiological studies have suggested that consumption of fruits and vegetables is associated with reduced risk of aging, inflammation, cancer, cardiovascular diseases, Alzheimer's and Parkinson's.

Nowadays, antioxidants are considered as important as vitamins for promotion of health and prevention of various diseases linked to reactive oxygen species (ROS). ROS have been linked to over 100 disorders (Halliwell and Gutteridge 2000). Therefore, for maintaining a healthy biological system, it is critical to have the balance between oxidation and antioxidation. Excess generation of ROS causes oxidative stress that damage DNA, lipids and proteins of cells leading to pathogenesis of various diseases such as cardiovascular disorders, diabetes, cancer, inflammation, aging, brain dysfunction, etc. A variety of phytochemicals have been reported as showing antioxidant potential (Tsao and Akhtar 2005). The majority of antioxidants are hydrophilic and they can easily contribute to keep up physiological health of hydrophilic organs whether lipophilic antioxidants are essential for lipophilic organs such as brain, cell membrane and

*Author for correspondence: <sjhossain_ku@yahoo.com>.

skin. Leafy vegetables are popular in Bangladesh but there is no scientific data available on the content of functional components in it. So the present research was undertaken to study the polyphenol, flavonoid, anthocyanin and antioxidant content of the leafy vegetables of Bangladesh.

Materials and Methods

The leafy vegetables of *Amaranthus gangeticus* L., *Amaranthus viridis* L., *Basella alba* L., *Brassica campestris* L., *Centella asiatica* L., *Chenopodium album* L., *Coriandrum sativum* L., *Enhydra fluctuans* Lour., *Ipomoea aquatica* Forssk., *Lagenaria siceraria* (Mol.) Standl., *Pisum sativum* L. and *Spinacia oleracea* L. were collected from the local markets of Khulna city, Bangladesh. After washing with distilled water the collected vegetables were shed dried. The dried sample was ground to powder by grinding machine and stored in air tight containers. Ethanol and pentane were used as solvents.

Twenty five grams of powder of each sample was taken in 100 ml of ethanol and kept in air tight bottle. After 7 days, the ethanol was filtered by Whatman No. 1 filter paper. The filtrate was air dried and the solid extracts were kept in the refrigerator. For the preparation of lipophilic extract, 10 g powder was shaken vigorously by hand with 200 ml pentane and after filtration the filtrate was air dried to obtain pentane extract. Finally 10 mg of the solid extract was dissolved in 1 ml ethanol or DMSO (10 mg/ml) and used to conduct the experiments.

The total concentration of phenolics (TPH) in ethanol extracts was determined (in triplicate) according to the Folin-Ciocalteu method (Ough and Amerine 1988) with gallic acid (GA) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract (Hossain *et al.* 2008). Total flavonoids and anthocyanins contents were determined according to Zhishen *et al.* (1999) and Padmavati *et al.* (1997), respectively with slight modification (Hossain *et al.* 2013).

The reaction mixture (total volume, 3 ml), consisting of 0.5 ml of 0.5 M acetic acid buffer solution at pH 5.5, 1 ml of 0.2 mM DPPH in ethanol, and 1.5 ml of 50% (v/v) ethanol aqueous solution, was shaken vigorously with the ethanol or pentane extracts (Blois 1958). After incubation at room temperature for 30 min, the amount of DPPH remaining was determined by measuring absorbance at 517 nm. Mean values were obtained from triplicate experiments. The scavenging effect of ethanol extracts on nitric oxide was measured after Marcocci *et al.* (1994), while the reducing power was determined following Oyaizu (1986). Total antioxidant capacity was measured according to Prieto *et al.* (1999).

Results are expressed as mean ± SD for a given number of observations (n = 3 - 5). The level of significance was set at *p* value of 0.05.

Results and Discussion

Table 1 shows the content of bioactive components in ethanol extracts of common leafy vegetables in Bangladesh. Highest amount of polyphenols (38.9 mg GAE (gallic acid equivalent)/g extract) was found in *I. aquatica*. The amount of polyphenols in these leafy vegetables was significantly smaller than that of fruity vegetables (Hossain *et al.* 2014). High content of flavonoids (23.2 mg CE (catechin equivalent)/g extract) was found in *I. aquatica* followed by *P. sativum* (17.6 mg), *L. siceraria* (17.2 mg) and *B. alba* (6.5 mg CE/g extract). In fruity vegetables, it was highest in *Luffa acutangula* (14.46 mg) followed by *Abelmoschus esculentus* (11.9 mg), *Solanum melongena* (11.4 mg) and *Vigna unguiculata* (10.2 mg CE/g extract) (Hossain *et al.* 2014). The highest anthocyanin content was found in *A. gangeticus* (1.15 μmol/g extract) followed by *P. sativum*, *C. album* and *I. aquatica*. However, in fruity vegetables anthocyanin content was highest in *Ficus hispida* (2.22 μmol/g extract) followed by *S. melongena*

Table 1. Total phenolics, flavonoids and anthocyanins contents, DPPH and NO free radical scavenging activities and reducing power of ethanol extracts of common Bangladeshi leafy vegetables.

Sample name	Polyphenols mg GAE/g extract	Flavonoids mg CE/g extract	Anthocyanins µmol/g extract	% DPPH scavenging at 200 µg/ml	% NO scavenging at 800 µg/ml	Reducing power at 400 µg/ml
Amaranthus gangeticus	16.6 ± 0.7	4.3 ± 0.001	1.15 ± 0.05	28.8 ± 2.8	53.3 ± 1.5	0.53 ± 0.02
A. viridis	21.7 ± 0.8	2.8 ± 0.007	0.02 ± 0.1*	34.9 ± 1.1	23.4 ± 2.4	0.30 ± 0.3
Basella alba	21.5 ± 1.0	6.5 ± 0.003	0.33 ± 0.02	71.6 ± 0.6	74.9 ± 0.5	0.86 ± 0.02
Brassica campestris	19.0 ± 0.8	2.8 ± 0.01	0.51 ± 0.03	86.8 ± 0.6	80.1 ± 0.6	0.47 ± 0.01
Centella asiatica	9.6 ± 0.4	1.8 ± 0.004	0.06 ± 0.01*	61.4 ± 1.1	51.3 ± 0.0	0.50 ± 0.04
Chenopodium album	12.9 ± 0.6	4.1 ± 0.01	0.60 ± 0.1	46.2 ± 1.1	25.1 ± 0.0	0.50 ± 0.01
Coriandrum sativum	14.4 ± 0.7	5.7 ± 0.003	0.09 ± 0.1*	68.9 ± 1.6	ND	0.68 ± 0.01
Enhydra fluctuans	25.7 ± 0.9	5.2 ± 0.006	0.03 ± 0.01*	97.8 ± 3.5	28.9 ± 1.2	0.50 ± 0.03
Ipomoea aquatica	38.9 ± 1.7	23.2 ± 0.004	0.51 ± 0.03	100.9 ± 0.6	83.7 ± 0.5	1.70 ± 0.02
Lagenaria siceraria	15.5 ± 0.7	17.2 ± 0.004	0.34 ± 0.03	84.8 ± 1.4	ND	0.72 ± 0.01
Pisum sativum	13.2 ± 0.6	17.6 ± 0.02	0.85 ± 0.01	92.8 ± 0.1	29.6 ± 0.0	1.12 ± 0.01
Spinacia oleracea	21.8 ± 0.8	0.9 ± 0.03*	0.25 ± 0.11	40.2 ± 1.1	39.9 ± 0.9	0.27 ± 0.06

GAE = Gallic acid equivalent; CE = (+)-Catechin equivalent; ND = Not done; *Indicates non-significant.

(1.04 µmol/g extract) (Hossain *et al.* 2014). Anthocyanin limits the development of cancers, cardiovascular diseases, neurodegenerative diseases and diabetes (Scalbert *et al.* 2005). Reportedly, fruits and vegetables are the main sources of antioxidant vitamins such as vitamin E, vitamin C, precursor of vitamin A i.e. β-carotene, which act as antioxidant. Therefore, antioxidant activity of these leafy vegetables might be collective result of their content of polyphenols, flavonoids, anthocyanins and other antioxidant components.

DPPH and NO free radical scavenging and reducing power of ethanol extracts of 12 leafy vegetables are shown in Table 1. Among them *B. campestris*, *E. fluctuans*, *I. aquatica*, *L. siceraria* and *P. sativum* showed more than 80% DPPH free radical scavenging activity at 200 µg/ml of ethanol extract. Fig. 1(A) shows dose-dependent scavenging of DPPH free radical by these potential extracts. The IC_{50} values for DPPH radical scavenging of *B. campestris*, *E. fluctuans*, *I. aquatica*, *L. siceraria* and *P. sativum* were 104.2, 85.5, 26.9, 125 and 68.5 µg/ml, respectively and

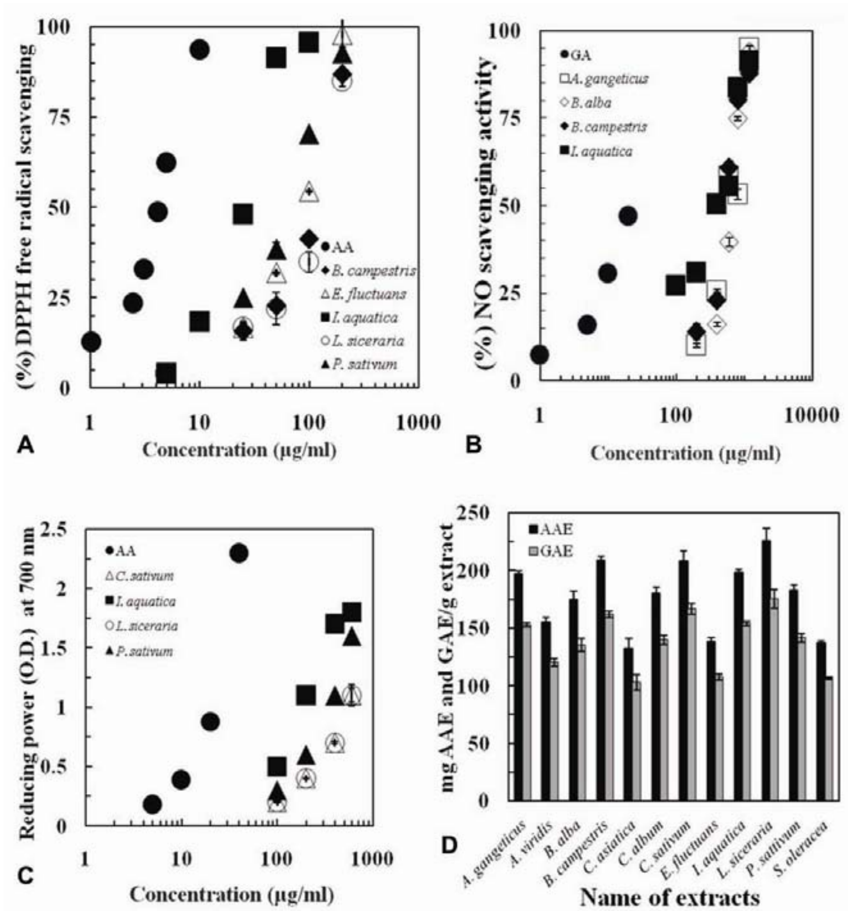

Fig. 1. Antioxidant activity of ethanol extracts of common leafy vegetables in Bangladesh. Dose-dependency of (A): the DPPH and (B) the NO free radical scavenging activities of ethanol extracts (AA: Ascorbic acid; GA: Gallic acid, positive control); (C): Dose-dependent increase of reducing power of the extracts (AA: Ascorbic acid, positive control); (D): Comparison of total antioxidant capacity of the extracts (AAE: Ascorbic acid equivalent; GAE: Gallic acid equivalent). Data were presented as mean ± SD (bar), n = 3-5.

that for ascorbic acid was 4.6. *I. aquatica* showed lowest IC_{50}, which means that among all the extracts tested, it had the strongest DPPH radical scavenging activity. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds, and aromatic amines reduce and decolorize DPPH by their hydrogen donating ability (Blois 1958). Therefore, these extracts possess hydrogen donating capabilities to act as antioxidant. IC_{50} values reported in this investigation for leafy vegetables were found mostly higher than fruits (Hossain *et al.* 2008) and fruity vegetables (Hossain *et al.* 2014). Fig. 2 shows the IC_{50} values for DPPH radical scavenging activity of lipophilic (pentane) extracts of the leafy vegetables. Depending on DPPH free radical scavenging of lipophilic extracts of leafy vegetables, they were graded as *B. alba* > *I. aquatica* > *E. fluctuans* > *L. siceraria* > *B. campestris* > *A. viridis* > *P. sativum* > *C. album*, *A. gangeticus* > *C. sativum* > *C. asiatica* > and *S. oleracea*.

Reportedly, large amounts of NO, peroxynitrite and other reactive nitrogen oxide species are considered to be potentially cytotoxic and capable of injuring the surrounding cells. The dose-dependent inhibition of nitrite production of potential ethanol extracts of *A. gangeticus*, *B. alba*, *B. campestris* and *I. aquatica* has been shown in Fig. 1(B) with IC_{50} values of 694.4, 625, 606.1 and 454.5 µg/ml, respectively. The IC_{50} values for NO scavenging of fruity vegetables, namely *A. esculentus*, *L. siceraria*, *Moringa oleifera* and *S. melongena* were 974.7, 438.6, 684.9 and 450.5 µg/ml, respectively (Hossain *et al.* 2014). Similar reports were also found for lotus seed extract (Yen *et al.* 2006). However, the activity of antioxidants is concomitant with the development of reducing power. Table 1 shows the reducing power of the ethanol extracts as determined by using

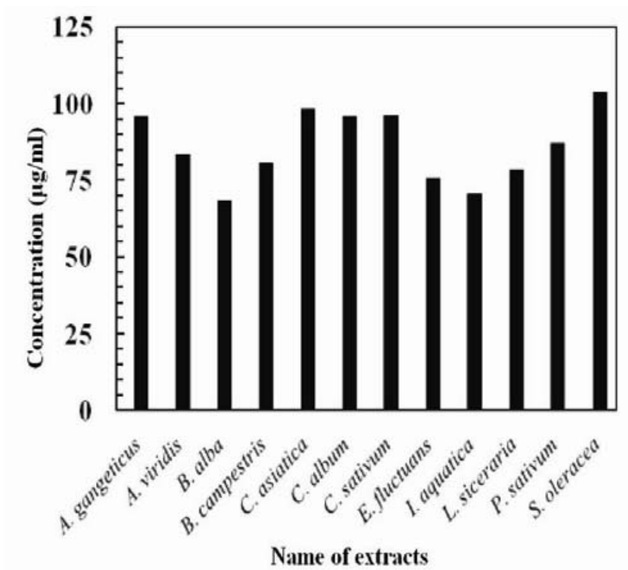

Fig. 2. Comparison of inhibitory concentration 50 (IC_{50}) of lipophilic extracts of common leafy vegetables for scavenging of DPPH free radical.

the potassium ferricyanide reduction method. At a concentration of 400 µg/ml, the extract in phosphate buffer, *B. alba*, *C. sativum*, *I. aquatic*, *L.* siceraria and *P. sativum* had high reducing activity (O.D., optical density) of 0.86, 0.68, 1.7, 0.72 and 1.12, respectively. Since, these extracts displayed high reducing power and their dose-dependent increase of reducing power was shown in Fig. 1(C). Furthermore, total antioxidant capacity that was expressed as the ascorbic acid (AAE), and gallic acid equivalent (GAE) of ethanol extracts, as shown in Fig. 1(D).

Insignificant correlation was observed between total phenolic contents and DPPH free radicals scavenging activity (%) or reducing power of ethanol extracts of leafy vegetables. Reportedly, antioxidant activity generally correlates with total phenolic concentration but the notion does not always hold true. Therefore it is remarkable that unknown components other than polyphenols, in these leafy vegetables, contribute a part to their antioxidant activity. However, among the plant-originated dietary intake of antioxidants, polyphenols are at the top. Attention should be paid when polyphenols are used to prepare functional foods and dietary supplements since some polyphenols perturb the membrane structure (Hossain *et al.* 2002, 2007).

The present observations revealed that among the twelve common leafy vegetables, *I. aquatica* could be considered to be the best source of functional components and antioxidants followed by *B. campestris*, *B. alba*, *P. sativum* and *L. siceraria*. These leafy vegetables should be regarded as potential dietary sources of both hydrophilic and lipophilic antioxidant(s).

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