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Antioxidant activity of some non-conventional green leafy vegetables of North-East India

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Abstract. Methanolic extracts of 15 traditionally consumed non-conventional green leafy vegetables were examined for *in vitro* antioxidant activity using three different assays. IC_{50} values measured by DPPH assay ranged from 8.98 to14.97 mg/L. In ABTS assay, trolox equivalent antioxidant capacity (TEAC) value varied from 18.3 to71.8 μM trolox/g of dry weight (dw). Ferric reducing antioxidant power (FRAP) values ranged from 107.7 to 275.6 μM Fe(II) per g dw). Total phenolic content (4.62 to 14.74 mg GAE/g dry dw), flavonoid content (0.65 to 7.72 mg QE/g of dw) and Vitamin C contents (35.79 to 106.7 mg/100 g dw) were evaluated by colorimetric methods. There was a positive linear correlation between the total phenolic content and antioxidant activities measured by three different methods.

Keywords: Green leafy vegetables, antioxidant activity, phenolic content, flavonoid content

1. Introduction

Reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), and singlet oxygen ($^1O_2$) are generated in the body either as a by-product of normal cellular aerobic respiration or exposure to environmental factors such as pollution, radiation, cigarette smoke and herbicides [1, 2]. In healthy individuals, production of ROS is controlled by an antioxidant defense system [3]. A serious imbalance between the production of ROS and antioxidant defense system is responsible for oxidative stress where excessive build-up of ROS results in damage to nucleic acids, proteins, enzymes and other biological molecules containing a lipid component of polyunsaturated fatty acids through oxidation [4, 5]. Oxidative damage and lipid peroxidation caused by the action of ROS may initiate and promote the progression of many diseases including cancer, liver disease, Alzheimer's disease, ageing, inflammation, rheumatic disorder, diabetes, Parkinson's disease, atherosclerosis and AIDS [6–8]. Dietary antioxidant nutrients, which include vitamin E, vitamin C, carotenoids and polyphenols/flavonoids, are believed to be effective in prevention of these oxidative stress related diseases [9, 10].

Antioxidants are mainly of two types, *viz*. synthetic and natural [11]. Synthetic antioxidants are synthesized in the laboratory. Some common synthetic antioxidants are phenolic compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and propyl gallate (PG) which are most commonly used for food and pharmacological applications [12]. But synthetic antioxidants are suspected of being responsible for some severe toxic effects. Synthetic antioxidants are believed to play a role as promoter of carcinogenesis and liver swelling [8, 13]. Hence there is a rising interest amongst researchers to explore the potential natural antioxidants and

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establishing their association with health benefits. Natural antioxidants are found in almost all plants, microorganisms, fungi and even in animal tissues [12]. The major natural dietary antioxidants are vitamin C and E, carotenoids, and phenolic compounds especially flavonoids. Vegetables and fruits are the least expensive sources of such natural antioxidants. Epidemiological data have clearly shown an inverse relationship between consumption of plant-based foods, such as fruits, vegetables and legumes and chronic ailments such as cardiovascular diseases, cataract and macular degeneration and cancer [14]. Therefore there has been a considerable increase in interest to find natural antioxidants from medicinal plants, vegetables and fruits to replace synthetic antioxidants due to their presumed safety, nutritional and therapeutic values [15].

North-eastern region (NER) of India is rich in biodiversity. NER consists of eight states of India *viz*. Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim. It has the richest reservoir of plant diversity in India and is one of the 'biodiversity hotspots' of the world supporting about 50% of India's biodiversity [16]. Ethno-botanical studies have reported that 200 plant species from Arunachal Pradesh, 286 plant species from Assam, 526 plant species from Nagaland and 194 plant species from Tripura are used for treatment of different diseases and ailments in these states [17]. In this study an effort has been made to study and report the antioxidant activities and total phenolic contents of some non-conventional vegetables of north east India. The relationship between phenolic content and antioxidant activity has also been investigated.

2. Materials and methods

2.1. Plant materials

The 15 leafy vegetables (Table 1) have been identified by a taxonomist (Dr. Dinesh Ch. Deka). Fresh vegetables are cleaned and external moisture blotted dry and non edible portions separated and discarded. The edible portions are cut into small pieces. 100 g of each cleaned fresh plant material is air dried in a well-ventilated room at room temperature under shade and ground into a fine powder by laboratory mill and samples kept at 20–25ºC in dark until analysis.

2.2. Preparation of extracts

The methanolic extract has been prepared as described by Proestos et al. [18] with some modification. Earlier 62.5% methanol was used but better results are achieved when the methanol concentration has been increased up to

Table 1
List of vegetables

Scientific name	Family	Local name (Assamese name)	Edible part used
Achasma nigra (Gaertn) Buru	Zingiberaceae	Tora	Stem, leaf
Alternanthera sesilis (L.) R. Br. ex DC.	Amaranthaceae	Matikanduri	Twigs
Amorphophallus paeoniifolius (Dennst.) Nicolson	Araceae	Olkasu	Whole plant
Ardisia colorata Roxb.	Primulaceae	Noltenga	Leaf
Centella asiatica (L.) Urban	Apiaceae	Bar manimoni	Whole plant
Enhydra fluctuans Lour.	Asteraceae	Helachi	Twigs
Houttuynia cordata Thunb	Saururaceae	Masandari	Leaf
Hydrocotyle sibthorpioides Lamk	Apiaceae	Sarumaninoni	Whole plant
Ipomoea aquatica Forssk	Convolvolaceae	Kalmou	Twigs
Lasia spinosa (L) Thw	Araceae	Sengmora	Whole plant
Oxalis corniculata L	Oxalidaceae	Sarutengesi	Whole plant
Oxalis debilis var. corymbosa (DC) Lour	Oxalidaceae	Bartengesi	Whole plant
Paederia scandens (Lour) Merr.	Rubiaceae	Vadailata	Leaf
Polygonum microcephalum D. Don	Polygonaceae	Madhusaleng	Twigs
Talinumtria ngulare (Jacq.) Willd.	Portulacaceae	Piralipaleng	Leaf

80%. A 0.5 g of homogenized plant material has been mixed with 50 ml methanol (80% v/v) containing 0.1% HCl in a round bottom flask. The mixture is stirred for 30 minutes and then sonicated for 15 minutes. After sonication the mixture is bubbled with nitrogen and refluxed in a water bath at 90ºC for 2 hours. After cooling it is concentrated under reduced pressure in a rotary evaporator (Buchi, R-200) and then lyophilized and dried. Dried material has been dissolved in the same solvent. The extract is purged with nitrogen and kept in a deep freezer (–20°C) until analysis.

2.3. DPPH free radical scavenging assay

The radical scavenging activity for the DPPH assay is calculated by the previously described method [19]. Briefly, 3 ml of DPPH solution (10^{-4} M) has been added to 1 ml of sample and the mixture is then shaken and kept in dark at room temperature. The absorbance is measured after 5, 20 and 30 min against a blank (80% v/v methanol) by UV/Vis Spectrophotometer (Shimadzu, UV-1800).

A mixture of 1 ml methanol (80%) and 3 ml DPPH solution is used as control. The radical activity is calculated by the following formula

$$\% \text{ Inhibition} = [(A_B - A_A)/A_B]$$

where, A_A and A_B are the Absorbance (Abs) of test sample and control respectively.

IC_{50} values of the samples have been calculated from graph by plotting % inhibition against extract concentration (200, 100, 50, 25, 10 and 5 mg/L).

2.4. ABTS cation scavenging assay

The ABTS assay described by Re et al. [20] is used to determine antioxidant capacity of the plant extracts. A calibration curve has been constructed using trolox as standard and the antioxidant capacities are expressed as μM trolox per g of dry weight (dw).

2.5. Ferric reducing antioxidant power (FRAP) assay

Total antioxidant capacity has also been determined using FRAP assay by Benzine and Strain method [21]. A calibration curve is constructed using $FeSO_4.7H_2O$ solution and results are expressed as μM Fe(II) per g dry weight (dw).

2.6. Determination of total phenolic content

Total phenolic content has been estimated using Folin-Ciocalteu colorimetric method [19]. Results are expressed as mg of Gallic acid equivalent (GAE) per 100 g dry weight (dw).

2.7. Determination of total flavonoid

Total flavonoid content has been estimated by colorimetric method described previously using quercetin [3]. Aliquot of 1 ml of dilute extract (1:10 v/v) is added to 5 ml of distilled water followed immediately by 0.3 ml of $NaNO_2$ (5%) and after 6 min 0.6 ml of 10% $AlCl_3$ in ethanol is added followed by addition of 1M NaOH solution, and the total volume is made up to 10 ml. Absorbance of the resulting pink coloured solution is measured at 510 nm against a blank prepared from the same solvents. The flavonoid content has been expressed as mg of quercetin equivalent (QE) per g of dw.

2.8. Determination of Vitamin C content

Vitamin C content of the vegetables has been determined by the method proposed by Al Duas et al. [22]. Comparing the absorbance of experimental samples with calibration curve, vitamin C content is estimated and the results are expressed as mg ascorbic acid per 100 g of sample.

2.9. Statistical analysis

All the experiments have been done in triplicates ($n = 3$), and the results are expressed as mean ± SD (standard deviation). SPSS (16.0) and Excel 2003 software have been used to perform statistical analyses.

3. Results and discussion

3.1. Antioxidant activity of the vegetables

The results of the three assays for antioxidant capacity are presented in Table 2. In the DPPH assay antioxidant capacity has been expressed as IC_{50} value, which is defined as the concentration of antioxidant required for 50% scavenging of DPPH radical in a specific time period [3]; a smaller IC_{50} value corresponds to a higher antioxidant activity of the plant extract. The antioxidant capacity measured by DPPH assay and expressed as IC_{50} values ranges from 89.8 to149.7 mg/L in the samples under study. The IC_{50} value of *Houttuynia cordata* is the lowest (89.8 mg/L) and hence it has the highest antiradical activity among all studied vegetables, followed by *Achasma nigra* (91.8 mg/L) and *Lasia spinosa* (99.8 mg/L) (Table 2). Similar results have been reported by other researchers for some traditional medicinal plants and for some Indian leafy vegetables [10, 23]. In ABTS assay, antioxidant capacity is expressed as trolox equivalent antioxidant capacity (TEAC) simply as μM trolox/g of dw. Table 2 shows TEAC value varying from 18.3 to 71.8 μM trolox/g of dw. *Achasma nigra* (71.8 μM trolox/g of dw) has the highest TEAC value followed by *Houttuynia cordata* (57.6 μM trolox/g of dw) and *Amorphophallus paeoniifolius* (54.9 μM trolox/g of dw). As indicated in Table 2, the FRAP values range from 107.7 to 275.6 μM Fe(II) per g dw. *Houttuynia cordata* (275.6 μM Fe(II)/g dw) has the highest FRAP values followed by *Amorphophallus paeoniifolius* (217.7 μM Fe(II)/g dw) and *Achasma nigra* (192 μM Fe(II)/g dw) whereas *Polygonum microcephalum* (107.7 μM Fe(II)/g dw) has the lowest FRAP value. These results are supported with the findings of many research groups who have reported similar data for antioxidant activities of other common vegetable samples measured by ABTS and FRAP assay [24, 25].

3.2. Total phenolic, total flavonoid and vitamin C content in the vegetables

There is a variation in the total phenolic content of the vegetables investigated; the values range from 4.62 to 14.74 mg GAE/g dw of raw sample (Table 3). *Achasma nigra* (14.74 mg GAE/g of dw) has the highest phenolic content, followed by *Houttuynia cordata* (14.30 mg GAE/g of dw) and *Amorphophallus paeoniifolius* (13.24 mg

Table 2
Total antioxidant capacity of vegetables. All data are the means ± SD of triplicate experiment ($n = 3$)

Samples	DPPH (IC_{50} value (mg/L)	ABTS (μM trolox/g dw)	FRAP(μM Fe(II)/g dw)
Achasma nigra	91.8 ± 1.5	71.8 ± 1.28	192.0 ± 0.57
Alternanthera sesilis	108.5 ± 1.3	43.8 ± 0.40	165.3 ± 0.49
Amorphophallus paeoniifolius	106.1 ± 1.3	54.9 ± 1.36	217.1 ± 1.63
Ardisia colorata	113.9 ± 6.1	31.4 ± 0.62	132.6 ± 0.40
Centella asiatica	105.3 ± 1.0	47.2 ± 0.89	170.1 ± 0.87
Enhydra fluctuans	139.0 ± 2.1	23.2 ± 0.32	116.9 ± 0.57
Houttuynia cordata	89.8 ± 1.5	57.6 ± 0.49	275.6 ± 2.14
Hydrocotyle sibthorpioides	101.8 ± 1.2	47.6 ± 0.44	171.5 ± 1.20
Ipomoea aquatica	132.2 ± 1.5	34.0 ± 0.54	116.2 ± 1.21
Lasia spinosa	99.2 ± 1.2	41.4 ± 0.46	164.2 ± 1.02
Oxalis corniculata	116.4 ± 1.8	28.1 ± 0.61	150.8 ± 0.57
Oxalis debilis	117.4 ± 2.8	28.6 ± 0.53	151.1 ± 1.00
Paederia scandens	116.2 ± 3.2	32.9 ± 0.61	141.1 ± 0.74
Polygonum microcephalum	149.7 ± 1.2	23.4 ± 0.24	147.7 ± 0.53
Talinum triangulare	142.6 ± 5.4	18.39 ± 0.24	117.5 ± 0.47

Table 3
Total phenolic content, total flavonoid content and the ascorbic acid content of the raw vegetables. All data are the means ± SD of triplicate experiment

Samples	Total phenolic content (mg GAE/g dw)	Total flavonoid content (mg QE/g dw)	Vitamin C (mg/100 g dw)
Achasma nigra	14.74 ± 0.17	5.32 ± 0.15	35.79 ± 0.76
Alternanthera sesilis	8.28 ± 0.54	2.17 ± 0.14	35.16 ± 1.69
Amorphophallus paeoniifolius	13.24 ± 0.48	6.23 ± 0.04	48.88 ± 0.87
Ardisia colorata	8.78 ± 0.19	3.74 ± 0.02	89.71 ± 4.21
Centella asiatica	11.14 ± 0.21	4.24 ± 0.12	75.72 ± 3.21
Enhydra fluctuans	5.11 ± 0.07	0.81 ± 0.04	52.16 ± 2.10
Houttuynia cordata	14.30 ± 0.45	7.72 ± 0.23	57.45 ± 2.78
Hydrocotyle sibthorpioides	11.90 ± 0.60	4.65 ± 0.29	50.54 ± 3.33
Ipomoea aquatica	5.20 ± 0.30	1.44 ± 0.16	67.97 ± 2.51
Lasia spinosa	7.64 ± 0.20	3.20 ± 0.04	49.57 ± 1.78
Oxalis corniculata	5.60 ± 0.28	1.12 ± 0.06	47.14 ± 2.11
Oxalis debilis	5.71 ± 0.79	2.63 ± 0.01	83.71 ± 3.45
Paederia scandens	6.33 ± 0.05	1.40 ± 0.14	106.7 ± 4.21
Polygonum microcephalum	6.21 ± 0.08	1.22 ± 0.06	104.1 ± 3.76
Talinum triangulare	4.62 ± 0.58	0.65 ± 0.02	46.71 ± 2.78

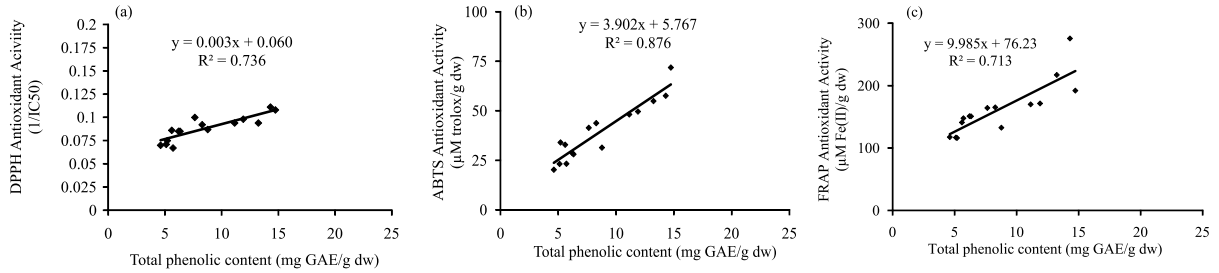

Fig. 1. Correlation between total phenolic content antioxidant activity. (a) Total phenolic content and DPPH antioxidant activity ($1/IC_{50}$). Correlation coefficient R = 0.80 and coefficient of determination $R^2 = 0.73$ ($p < 0.05$). (b) Total phenolic content and ABTS antioxidant activity (TEAC). Correlation coefficient R = 0.92 and coefficient of determination $R^2 = 0.87$ ($p < 0.05$). (c) Total phenolic content and FRAP antioxidant activity. Correlation coefficient R = 0.84 and coefficient of determination $R^2 = 0.71$ ($p < 0.05$).

GAE/g of dw). Similar results for total phenolic content have been reported by Sreeramulu and Raghunath [15] for some root tuber and vegetables consumed in India. The amount of flavonoid content is found in the range of 0.65 to 7.72 mg QE/g of dw of raw sample (Table 3). *Houttuynia cordata* (7.72 mg QE/g of dw) containes the highest flavonoid followed by *Amorphophallus paeoniifolius* (6.23 mg QE/ g of dw) and *Achasma nigra* (5.32 mg QE/g of dw). Similar findings for flavonoids contents of some Thai indigenous plants have been reported by Maisuthisakul et al. [26]. The ascorbic acid (vitamin C) content of the vegetables is found to be high, ranging from 35.79 to 106.7 mg/100 g dw (Table 3). *Oxalis corniculata* contains the highest vitamin C (106.7 mg/100 dw) followed by *Oxalis debilis* (104.1 mg/100 g dw) and *Ardisia colorata* (89.7 mg/100 g dw). Vitamin C is a water soluble antioxidant which is in a unique position to scavenge aqueous peroxy radicals before they damage the lipids.

3.3. Correlations between phenolic content and antioxidant capacity

A positive correlation is observed between total phenolic content and antioxidant activity of the vegetables measured by all the three assays (Fig. 1). Highest correlation between total phenolic content and antioxidant activity ($R^2 = 0.87$,

$p < 0.05$) is observed in ABTS (TEAC) followed by DPPH assay ($R^2 = 0.73$, $p < 0.05$) and FRAP assay ($R^2 = 0.71$, $p < 0.05$). Therefore phenolic compounds contribute significantly to the antioxidant capacity of the investigated vegetables. These results are in agreement with the findings of many research groups who have reported such positive correlations between antioxidant activity and total phenolic content [1, 27].

4. Conclusion

The antioxidant activity of investigated green vegetables is well correlated with total phenolic content. The phenolic compounds may contribute directly to antioxidative the major phenolic action. The linear positive correlation between total phenolic content and total flavonoid content indicates that flavonoids are compounds. Vitamin C content is also found to be high. Finally it can be concluded that the investigated non-conventional leafy vegetables are potential sources of natural antioxidants.

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