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Research Article

Antioxidant capacities of vegetables consumed in north east India assessed by three different *in vitro* assays

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ABSTRACT

The antioxidant capacity of the vegetables consumed in North East India was estimated by three different *in vitro* methods. A total of 22 (twenty two) vegetables were evaluated using the extracts for their ability to scavenge the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radicals, ability to reduce ferric ions determined by Ferric Reducing Antioxidant Potential (FRAP) assay and Total phenolic content. The antioxidant capacity was expressed as mg Trolox equivalents for DPPH radicals scavenging and FRAP assay while the total phenolic content was expressed as mg Gallic acid equivalent (GAE) per 100 gm of edible portion of the vegetables. The antioxidant capacity of the vegetables estimated by the three methods does not vary markedly though the values of total phenolic content was slightly higher compared to that of the other two methods. The highest antioxidant capacity was observed in stink bean and least in cucumber. Based on the antioxidant capacity, the vegetables were grouped into four categories i.e. extremely high, high, medium and low. Stink bean (*Parkia speciosa*) showed an extremely high antioxidant activity in all the three methods. The vegetables with high antioxidant capacity includes bean leaves, brinjal, mustard leaves, potato and pea while pumpkin leaves, radish, tomato, naga chilli, small chilli and hyacinth bean fall in medium and okra, broccoli, banana flower, cabbage, turnip, common beans, cauliflower, carrot and cucumber in low category.

Keywords: Antioxidants; Vegetables; DPPH; FRAP; Total phenolic content.

INTRODUCTION

Antioxidants are naturally occurring or synthetic chemicals in foods that help to counter the detrimental effects of reactive oxygen species (ROS) and free radicals which causes degenerative diseases such as cancer, heart diseases and cerebrovascular diseases (Wresburger, 2002). They are often reducing agents such as thiols, ascorbic acid or polyphenols which inhibit other oxidation reactions by being oxidized themselves (Sies, 1997). Oxidation reactions are crucial for life, however, they can also be damaging, so plants and animals maintain many types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids (Sies, 1997 and Vertuani *et al*., 2004). It has always been assumed that people who eat fruits and vegetables have a lower risk of heart disease and some neurological diseases (Shenkin, 2006) and there is evidence that some types of vegetables and fruits in general protect against some cancers (Cherubini *et al*., 2005). Antioxidants are found in varying amounts in foods such as vegetables, fruits, grain cereals, eggs, meat, legumes and nuts. Plant based food items contain a wealth of phytochemicals which many have antioxidant properties (Halliwell *et al*., 1995; Hollman & Katan, 1997; Benzie and Strain, 1999; Duthie et al., 2000). The beneficial effects of the fruits and vegetables are hypothesized to owe at least to antioxidants (Halliwell *et al*., 1995; Collins, 1999; Benzie and Strain, 1999). Some antioxidants such as lycopene and ascorbic acid can be destroyed by long-term storage or prolonged cooking (Xianquan *et al.,* 2005 and Rodriguez, 2003) while other antioxidant compounds are more stable, such as the polyphenolic antioxidants in foods such as whole-wheat cereals and tea (Baublis *et al.,* 2000; Rietveld and Wiseman, 2003). Phenolics or polyphenols have received considerable attention because of their physiological functions, including antioxidant, antimutagenic and antitumor activities. They have been reported to be a potential candidate to combat free radicals, which are harmful to our body and food systems (Nagai *et al.,* 2003).

Since there is a diverse group of compounds with different reactivities to different reactive oxygen species, estimation of antioxidants is not a straightforward

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process. In food science, the oxygen radical absorbance capacity (ORAC) has become the current industry standard for assessing antioxidant strength of whole foods, juices and food additives (Cao *et al.,* 1993 and Ou *et al.,* 2001). Other measurement tests include the Folin-Ciocalteau reagent, and the Trolox equivalent antioxidant capacity assay (Prior *et al.,* 2005). In the present study the antioxidant activity of the vegetables as, Ferric Reducing Antioxidant Potential (FRAP), ability to scavenge the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radicals and total phenol content were measured.

MATERIALS AND METHODS

Vegetables and preparation of crude extracts

Vegetables commonly available in North East (Aizawl Market, Mizoram) were bought which includes Stink bean (*Parkia speciosa*), brinjal (*Solanum melangona*), mustard leaves (*Brassica juncea*), potato (*Solanum tuberosum*), pea (*Pisum sativum*), raddish (*Rapnus sativus*), tomato (*Lycopersicon esculentum*), hyacinth bean (*Lablab purpureus*), small chilli also called as African bird's eye (*Capsicum frutescens*), naga chilli (a hybrid of *Capsicum chinense* and *Capsicum frutescens*), okra (*Abelmoschus esculentus*), Broccoli (*Brassica oleracea italica*), cabbage (*Brassica oleracea capitata*), cauliflower (*Brassica oleraacea boytritis*), pumpkin leaves (*Cucurbita maxima*), cucumber (*Cucumis sativus*), bean leaves (*Vigna sesquipedalis*), common beans (*Phaseolus vulgaris*), Bitter gourd (*Momordica charantia*), Carrot (*Daucus carota*), Banana flower (*Musa paradisiaca*) and knol khol (*Brassica caulorapa*).

Figure 1: Stink bean (*Parkia speciosa*)

The vegetables were thoroughly washed under running tap water followed by deionised water. The edible portions of the vegetables were homogenized in a blender and a weighed amount of 1gm of the homogenate was used for the preparation of crude extract. The extracts in both water and methanol were prepared as per the methodology of Pellegrini *et al.* (2003). The extracts obtained were immediately analyzed in triplicate for their antioxidant capacity.

Chemicals and reagents

All the chemicals used were of analytical grade and deionised water was used for entire analysis. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) and 6-hydroxy-2,5,7,8–tetramethyl chromane-2-carboxylic acid(Trolox) were purchased from Sigma Chemicals Co. (St. Louis, USA); Methanol, Ethanol, Sodium acetate trihydrate, ferric chloride hexahydrate ($FeCl_3$. $6H_2O$), Folin-Ciocalteu Phenolic reagent, Sodium carbonate were from Merck (Darmstall, Germany).

Analysis of Antioxidant Activity

DPPH free radical scavenging assay

The DPPH free radical scavenging activity of vegetables was determined using spectroscan 2600 UV/Vis Spectrophotometer (Chemito) according to the method described by Leong and Shui (2001). A 0.1 mM solution of DPPH was prepared in methanol. The initial absorbance of the DPPH was measured at 515 nm. An aliquot (40µl) of the vegetable extracts (with appropriate dilution) was added to 3 ml of DPPH solution. The decrease in absorbance at 515nm was measured at different time intervals until the absorbance remains constant. The antioxidant capacity based on the DPPH free radical scavenging ability of the vegetable extracts was expressed as mg Trolox equivalents per 100 gm of edible portion of the vegetables.

Ferric reducing antioxidant potential (FRAP) assay

The ability to reduce ferric ions was measured using a modified version of the method described by Benzie and Strain (1999). An aliquot (50µl) of the vegetable extracts (with appropriate dilution) was added to 3 ml of FRAP reagents (10 parts of 300 mM solution acetate buffer at pH 3.6, 1 part of TPTZ solution and 1 part of 20 mM $FeCl_3$. $6H_20$ solution) and reaction mixture was incubated at 37^0C for 30 min. The increase in absorbance was measured at 593 nm using spectroscan 2600 UV/Vis Spectrophotometer (Chemito). The antioxidant capacity of the vegetable extracts based on the ability to reduce ferric ions was expressed as mg Trolox equivalents per 100 gm of edible portion of the vegetables.

Total phenolic content determination

The total phenolic content of the vegetable extracts were estimated by the method described by Singleton and Rossi (1965) downscaled to 2 ml final volume. An aliquot (100µl) of the appropriately diluted vegetable extracts was added to 1000µl of 1:10 Folin-Ciocalteau's reagent and incubated at room temperature for 5 min followed by addition of 900 µl saturated (7.5%) sodium carbonate solution. After incubation for 1 hr at room temperature, the absorbance at 640 nm was measured using spectroscan 2600 UV/Vis Spectrophotometer (Chemito). The total phenolic content of the vegetables

Table 1: Antioxidant activity of the vegetables consumed in North East India estimated by DPPH radical scavenging assay, Ferric reducing antioxidant assay (FRAP) and Total phenolic content determination

S. No.	Name of the vegetables	Antioxidant Activity					
		DPPH Assay[1]		FRAP Assay[2]		Total Phenolic content[3]	
		Water extract	Methanolic extract	Water extract	Methanolic extract	Water extract	Methanolic extract
01	Stink beans	7418.28±27	5936.88±19	1617.28±2.5	1897.99	1557.6±0	2464.32±00[d]
02	Bean leaves	71.62±1.35	67.49±3.45	97.29±3.60	72.72±3.54	95.80±0.62	68.23±1.69
03	Brinjal	61.62±1.24	23.74±0.56	57.59±2.99	32.21±1.01	109.81±2.00	28.96±0.31
04	Mustard leaves	59.75±1.84	23.52±2.09	92.78±6.48	48.72±4.44	106.75±2.31	52.06±1.23[c]
05	Potato	55.99±1.96	4.35±0.64	172.27±40.	17.67	114.89±3.08	20.48±0.14
06	Pea	50.65±0.56	64.25±1.80	75.04±0.43	73.37±2.07	56.15±0.19	52.98±0.31
07	Pumpkin leaves	42.78±1.36	59.07±7.035	149.51±2.2	83.19±2.60	248.71±1.97	102.88±3.69
08	Radish	41.56±6.81	8.18±1.24	69.73±3.17	24.35±1.07	62.37±0.77	29.57±0.61
09	Tomato	38.55±2.51	7.66±0.80	60.79±3.17	13.74±1.15	46.36±2.61	13.09±0.15[b]
10	Naga chili	38.35±0.71	93.04±0.34	146.67±2.9	284.99±19.7	156.47±4.00	184.1±5.89
11	Small Chili	31.11±2.02	62.45±0.39	198.74±1.0	223.61±22.0	261.59±11.9	149.09±9.55
12	Hyacinth bean	27.77±2.25	28.18±0.39	37.88±2.60	30.83±1.65	52.01±0.31	31.11±0.92
13	Okra	12.62±2.47	25.85±0.34	51.19±9.70	45.01±0.54	31.00±15.4	25.59±1.69
14	Broccoli	10.37±2.60	19.16±4.98	44.34±0.89	34.25±0.58	99.18±11.1	43.74±2.46
15	Banana flower	8.11±0.22	26.53±1.24	43.92±0.54	47.33±1.00	28.33±3.38	26.36±4.31[a]
16	Cabbage	7.21±0.59	5.48±0.56	21.81±2.93	17.81±1.19	35.88±0.46	18.94±0.15
17	Knol khol	3.23±0.72	5.18±0.81	15.85±1.25	13.89±1.20	39.11±1.23	16.01±0.30
18	Common Beans	2.63±0.56	37.05±3.2	15.34±0.67	42.1±1.15	38.80±3.69	35.73±00
19	Cauliflower	1.95 ±0.68	6.38±0.25	29.05±1.92	25.44±0.33	47.24±0.14	27.72±0.92
20	Bitter gourd	1.73±0.85	3.08±3.08	33.52±3.95	19.27±1.38	51.90±0.77	27.57±1.38
21	Carrot	0	1.95±1.37	15.77±0.98	21.44±0.55	24.79±0.46	15.25±1.07
22	Cucumber	0	1.04±0.90	6.69±2.66	9.81±1.30	24.49±1.08	11.08±2.46

1, 2, 3 Mean of three determinations ± S.D. (standard deviation); a-low, b- Medium, c- High, d- Extremely High

were expressed as Gallic acid equivalents (GAE) mg/100 gm of edible portion of the vegetables.

RESULTS AND DISCUSSION

The interest in the search for new antioxidants has grown over the past years because reactive oxygen species (ROS) production and oxidative stress have been shown to be linked to ageing related diseases (Finkel and Holbrook, 2000). So, measurement of antioxidant capacity of vegetables could be a valuable tool in technology, as the effect of growing conditions, seasonality, storage, processing, preservation techniques, cooking and genetic modification of plant-based foods could be determined (Szeto *et al.,* 2002). The antioxidant activity of the vegetables consumed in North East India were estimated by DPPH radical scavenging assay, Ferric reducing antioxidant potential (FRAP) assay and Total phenolic content determination and the results are given in Table 1.

The vegetables had different antioxidant activities in relation to the method of estimation but Stink bean (*Parkia speciosa*) (Fig.1) showed the highest value in all the three methods. The estimated values are 7418 and 1617 mg Trolox equivalents per 100 gm of edible portion of the vegetables in DPPH and FRAP assay respectively. It also has a total phenolic content of 1557 Gallic acid equivalents (GAE) mg/100 gm of edible portion whereas none of the other vegetables have a value beyond 270 in all the three methods. The antioxidant capacity of the vegetables except of stink bean is shown graphically in Fig. 2, Fig.3 and Fig.4 for DPPH assay, FRAP assay and total phenolic content respectively. Among all the vegetables used in this study cucumber exhibited the least antioxidant activity in both DPPH and FRAP assay. The lower antioxidant capacity of cucumber observed in the two assays when compared to the values of other vegetables analyzed in the study is in agreement with Vinson *et al.* (1998). The

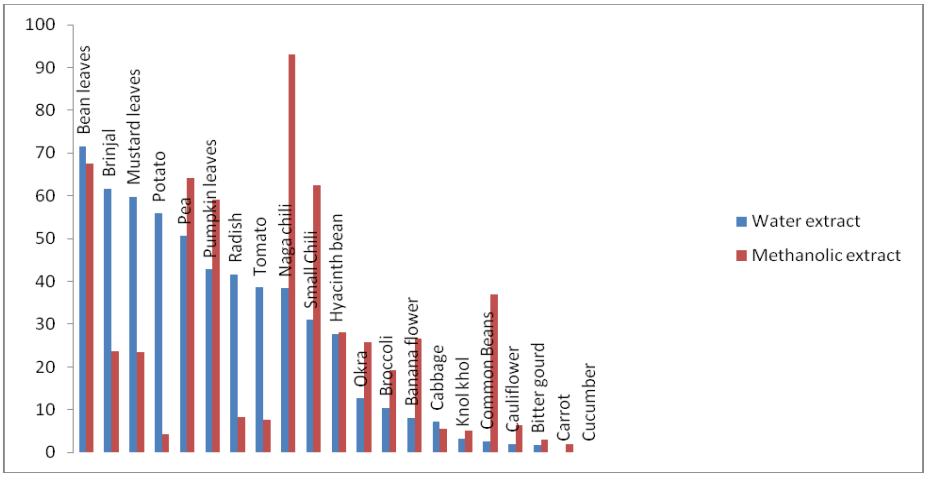

Figure 2: Antioxidant capacity of the vegetables (except for stink bean) in water and methanolic

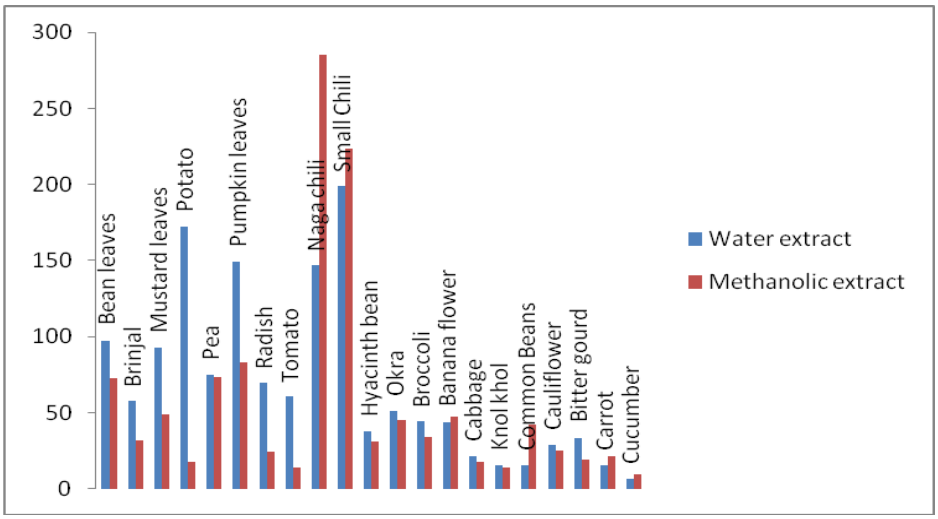

Figure 3: Antioxidant capacity of the vegetables (except for stink bean) in water and methanolic

similar findings of cucumber being the vegetable with least antioxidant activity among other vegetables were also reported by Pelligrini *et al* (2003).

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Brand-Williams *et al.,* 1995). Based on the antioxidant capacity estimated by DPPH free radical scavenging assay, the vegetables were grouped into four categories i.e. extremely high, high, medium and low. Both water and methanolic extract of stink bean (*Parkia speciosa*) showed an extremely high antioxidant activity in all the three methods. The vegetables with high antioxidant capacity includes bean leaves, brinjal, mustard leaves, potato and pea while pumpkin leaves, radish, tomato, naga chilli, small chilli and hyacinth bean fall in medium and okra, broccoli, banana flower, cabbage, turnip, common beans, cauliflower, carrot and cucumber in low category. The same vegetables showed different reactivities in FRAP assay with small chilli, potato, pumpkin leaves, naga chilli, mustard leaves falling into the group of high antioxidant capacity whereas bean leaves, pea, radish, tomato and okra can be grouped into vegetables with medium antioxidant activity.

The vegetables with low antioxidant activity in the FRAP assay includes brinjal, beans, banana flower, cabbage, cauliflower and cucumber. In both FRAP assay and total phenolic content determination, small chilli was found to have highest antioxidant activity next to stink bean with a value of 198.74 mg Trolox equivalents per 100 gm of edible portion and 261.59 Gallic acid equivalents (GAE) mg/100 gm of edible portion respectively. In similar ranking the vegetable with high antioxidant property based on the total phenolic content includes small chilli, pumpkin leaves, naga chilli, potato and brinjal as per the values observed in quantitative estimation given in table 1.

The antioxidant activity of phenolic compounds is mainly due to redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers (Kaur and Kapoor, 2002).

The ranking of the vegetables based on its antioxidant property of the water extract is different from that of

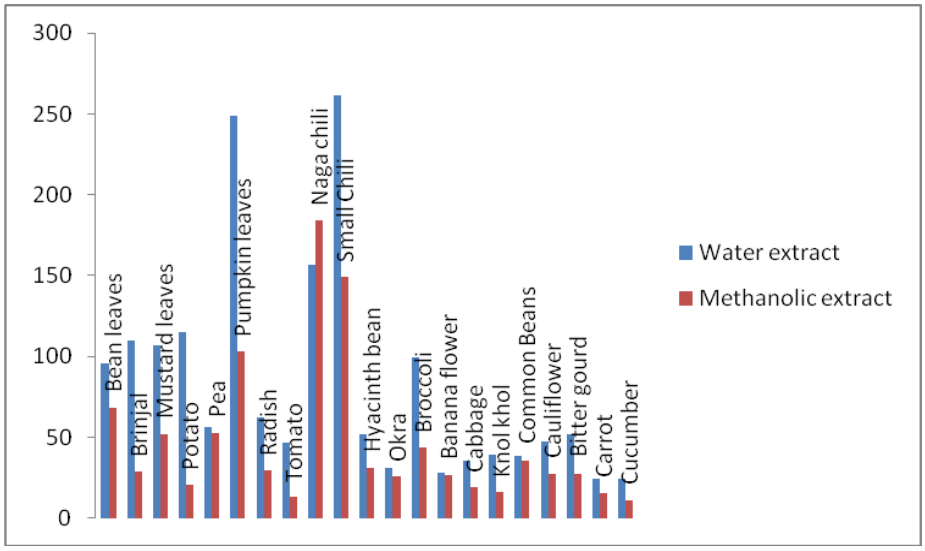

Figure 4: Antioxidant capacity of the vegetables (except for stink bean) in water and methanolic

methanolic extract which indicate the presence of various compounds with different solubility. Several factors might have contributed to the differences in antioxidant capacity of the vegetables of different origin, including variation in cultivars, harvest and post harvest handling and storage conditions, processing techniques during analytical determinations. In addition to these, several physiochemical reactions would have taken place between the harvest time and the open market where the vegetables are sold which may be responsible for the variation in the antioxidant capacities.

In conclusion, the findings of this study has indicated that stink bean (*Parkia speciosa*) is extremely rich in antioxidant property and further work should be carried out on this vegetable which may help in the development of numerous drugs for fighting against various diseases.

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