Chemical and Antioxidant Properties of Broccoli Growing in Bangladesh

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ABSTRACT: Broccoli (*Brassica oleracea* var. *italica)* a cruciferous vegetable growing in Bangladesh was investigated to determine the fatty acid components, elemental concentrations, ascorbic acid content and antioxidant activity. A total of fifteen compounds were identified from the methylated esters of the fatty acids of flower and stem of broccoli when analyzed by GC-MS. The major compounds were linoleic acid, palmitic acid. linolenic acid, oleic acid, stearic acid and myrestic acid. Elemental analysis was done by XRF method which showed that both the flower and stem contained a significant amount of Na, K, Ca, Mg, Fe, P and Cl. However, heavy metals were not found in any of the broccoli samples. The broccoli flower and stem contained 42.20±0.263 mg and 120±0.254 mg of ascorbic acid per 100g of fresh samples. The methanol extract of broccoli exhibited the highest DPPH radical scavenging activity among methanol, acetone and water extracts. The indegenous broccoli was found to have highest DPPH radical scavenging activity (> 90%) which was comparable to standard ascorbic acid (98.22±1.122%) and BHA (96.01±0.983 %). Methanol extract of flower and stem exhibited highest ferrous ion chelating ability ((91.85±0.951 and 97.38±1.241) respectively whereas the ascorbic acid and BHA hardly demonstrated any ferrous ion chelating ability. These results clearly indicated that Bangladeshi broccoli has a significant potential for uses as nutrients and antioxidant suppliments.

Key words: Broccoli, Fatty acids, Minerals, Vitamin C, Free radical scavenging power, Reducing power, Ferrous ion chelating ability.

INTRODUCTION

Broccoli (*Brassica oleracea* var. *italica)* belongs to the Cruciferous family. It is a biennial vegetable originated from Italy some 2000 years ago.[1,2] Broccoli has been considered as a unique valuable food among Italians.[3] Broccoli grows best when exposed to an average daily temperature between 18-25°C.[4] It is now growing successfully in some areas of Bangladesh. The typical broccoli phytochemicals are sulphur containing compounds, including glucosinolates, dithiolthiones, indoles, glucoraphanin, S-methyl cysteine sulfoxide, isothiocyanates, and indole-3-carbinol etc.[5,6] Sulphoraphane found in this cabbage protects from bacteria, cancer and diabetes.[6-9] Broccoli is a nutritious food and it is a good source of vitamin and minerals.[10,11] It prevents certain types of cancer, diabetes, heart disease, osteoporosis, Alzheimer's disease, joint inflammation and high blood pressure.[12-14] Researchers in different part of the world have indicated that broccoli possessed high antioxidant capacity.[15-18]

So far, no research has been carried out on chemical constituents and antioxidant properties of broccoli from Bangladesh. The present investigation was designed to evaluate the levels of different fatty acid components, nutrients, trace elements, ascorbic

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acid (vitamin C) content and antioxidant activity from flower and stem of broccoli growing in Bangladesh.

MATERIALS AND METHODS

Collection of raw materials. *B. Oleraceae* var. was collected randomly from a garden of Tejgaon, Dhaka, Bangladesh during the year 2006-2007. After harvesting the edible part of broccoli, flower and stem were separated and washed with clean water, cut into small pieces, grounded and freeze-dried. The freeze-dried samples were taken in an airtight container and stored in a cool place.

Determination of the percentage of dry matters. A calculated amount of the fresh samples (stem and flower part) of broccoli were taken and after cleaning it was cut into small pieces. The broccoli samples were then dried under shade followed by oven drying at 40°C and finally it was heated at 60°C until a constant weight was obtained. This experiment was repeated thrice and the mean of the results was taken. Dry matter contents was then calculated and expressed in table I.

Determination of ash content. Accurately weighed powdered vegetable samples (dry weight basis) were burnt according to the methods described in literature.[19] Three sets of experiment were carried out for each of the samples and the mean of the results is expressed in Table 1.

Estimation of elemental concentrations in the ash. The elemental concentrations in the ash of broccoli flower and stem were determined with the help of X-Ray Fluorescence spectrometry (X-RF).

Preparation of tablet for X-RF analysis. The ash of broccoli samples was powdered and sieved through 100 mash screens and dried at 110°C in an electric oven. Dry ash powder (about 4.0 g) in association of 0.4 g stearic acid as binder and 8.0g boric acid as base powder were mixed homogeneously. Then tablet of this mixture was prepared by using pressure machine disc for X-RF analysis. Elemental analyses were carried out by X-RF in which calculation and fixed calculation methods were used in X-ray fluorescence spectrometer (Philips Analytical). Average of three samples is shown in Table 1.

Determination of crude fat and fatty acid components. Crude fat of the broccoli samples were extracted by soxhlet apparatus using n-hexane as solvent.

Soxhlet extraction of fat. About 10 g of dry sample of broccoli was extracted for 16 hours at 70 °C with n-hexane using soxhlet apparatus. The extract was evaporated to dryness and the residue was collected. An average of three experimental results was recorded (Table 2).

Fatty acid analysis by GC/MS spectroscopy. 200 mg of extracted fat was saponified with methanolic sodium hydroxide (10 ml) and the dried saponified material was esterified with BF_3-MeOH (complex). The methylated ester of the fatty acid was isolated by partitioning with n-hexane and water. The n-hexane soluble part was dried and the analysis was done by GC-MS electron impact ionization (E1) method. The compounds were identified by comparing with NIST library data (Table 2).

Estimation of ascorbic acid (Vitamin C) content. Vitamin C contents of stem and flower from broccoli were determined following the method of Ranganan, which is based on the reduction of 2, 6-dichlorophenol-indophenol visual titration method.[20]

Antioxidant activity. The antioxidant activity of broccoli samples (flower and stem) was determined by DPPH radical scavenging activity, reducing power and ferrous Ion Chelating ability test.

Preparation of sample extracts. Freeze dried samples of broccoli stem and flowers (100-1000 mg) were weighed, 50 mL of methanol, acetone and water were then added separately and allowed to stand for 24 hours with occasional stirring. The ratios of sample weight to solvent volume were 2, 4, 8, 12, 16 and 20 mg/mL. The extracts were then vacuum filtered and used as stock solutions for the following tests.

Test for DPPH radical scavenging activity. DPPH radical scavenging activity of broccoli

samples was studied by the method of Shimada *et al.* was followed.[21] During this experiment the test samples methanol, water and acetone extracts of broccoli (5 mL) accompanied with standard compounds Ascorbic acid and BHA methanolic solutions (5mL) were separately mixed with freshly prepared DPPH methanolic solution (1 mM, 1 mL). The reaction mixture was then vortex for few minutes and allowed to stand at dark place at 25°C for 30 min. The absorbance of samples was read against a blank at 517 nm. The percentage of DPPH scavenging activity is expressed by [{1-(test sample absorbance/blank sample absorbance)} x 1000]. An average of three experimental results was recorded and is presented in Figure 1a-b.

Test for ferrous ion chelating ability. The ferrous Ion Chelating ability of broccoli sample was determined following the method established by Decker and Welch.[22] Test samples of broccoli extracts, BHA and Ascorbic acid solutions (each 5mL) were spiked with ferrous chloride (0.1mL, 2 mM) and ferrozine solutions (0.2 mL, 5 mM). The reaction was allowed to stand for 10 minutes and the absorbance of was recorded at 562 nm. The percentage of ferrous ion chelating ability is expressed by [{1-(absorbance of the sample/Absorbance of the blank sample)} x 100]. All the samples were tested for thrice and the mean of the results are expressed in Figure 2a-b).

Test for reducing power. The reducing powers of broccoli samples were determined following the methods developed by Oyaizu.[23] Test samples of broccoli extracts (10 ml) accompanied with ascorbic acid and BHA methanolic solutions (10 mL) were spiked separately with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The mixture was then incubated at 50°C for 20 minutes, then cooled rapidly, again spiked with trichloroacetic acid (2.5 mL, 10%), and centrifuged at 3000 rpm for 10 minutes. The supernatant (5 mL) was then mixed with distilled water (5 mL) and ferric chloride (1 mL, 0.1%). The mixture was then allowed to stand for 10 minutes and the absorbance was recorded at 700 nm. The experiment was repeated thrice and the mean of the results is presented in Figure 3a-b.

RESULTS AND DISCUSSIONS

A comparative study on different edible parts of broccoli from Bangladesh revealed that the flower and stem part contained 12.00 ± 0.088 and 10.50 ± 0.117 g, respectively of dry matter per 100g fresh samples. Crude fat contents were found to be 12.00 ± 0.088 & 10.50 ± 0.117 g and ash content 11.09 ± 0.187 & 11.12 ± 0.148 g in the flower and stem, respectively per 100g of dry samples. 22 elements were estimated in this study. The result of elemental analysis is presented in Table I. Elemental analysis was done by XRF method. Both the flower and stem part were found to be contained a good amount of Na, K, Ca, Mg, Fe, P and Cl (Table 1). Na, K and Cl content was found to be higher in stem part where as the flower part contained higher amount of Ca, Mg, Fe and P than the stem part. Sulfur was found to be present in stem part only (988 ± 0.714 mg/100g dry stem). Cu, Cr, Mn, I, Br were not detected in any of the samples of broccoli. Heavy metals including some radioactive elements Al, Ni, As, Rb, Ba, Ti, U, Zr, & Y was not detected in any of the samples of broccoli during this study except Ti and Y at trace amount in stem part only (Table 1).

Methylated fatty ester of broccoli samples was analyzed by GC-MS. A total of fifteen compounds were identified in the fatty ester of flower and stem part of broccoli. The major components were palmitic, linoleic, stearic, oleic, linoleinic and myrestic acids and the other components were found as ecosanoic, lauric, heneicosanoic, margaric, isostearic, 9-hexadecanoic, nonadecanoic and 7-hexadecanoic acids (Table 2). The relative percentage of the major components in flower and stem parts were palmitic (16.42 ± 0.017 & 15.26 ± 0.002), linoleic (15.41 ± 0.007 & 15.43 ± 0.002), oleic (4.13 ± 0.021 & 5.51 ± 0.004), stearic (7.69 ± 0.027 & 3.50 ± 0.012), Linoleinic (3.26 ± 0.016 & 24.76 ± 0.004) and myrestic acids 3.04 ± 0.006 & 6.52 ± 0.003), respectively.

Our indigenous broccoli was found to possessed 42.20 ± 0.263mg and 120 ± 0.254 mg of ascorbic acid or vitamin C per 100 gram of fresh flower and stem, respectively. The stem contained three times more vitamin C than the flower part.

Table 1. Elemental concentrations in different edible parts of broccoli

No.	Elements	mg/100g flower	mg/100g stem
1	Na	60.99 ± 0.414	69.40 ± 0.843
2	K	3692.97 ± 1.754	4514.72 ± 0.954
3	Ca	1449.46 ± 2.047	589.36 ± 0.548
4	Mg	649.87 ± 0.501	166.80 ± 0.318
5	Fe	26.62 ± 0.041	10.01 ± 0.201
6	Cu	ND	ND
7	S	ND	988.57 ± 0.714
8	P	1366.29 ± 0.243	359.18 ± 0.342
9	Cl	258.40 ± 0.145	930.74 ± 0.543
10	Cr	ND	ND
11	Mn	ND	ND
12	I	ND	ND
13	Rb	ND	5.84 ± 0.216
14	Ni	ND	ND
15	Ba	ND	ND
16	As	ND	ND
17	Ti	ND	<<
18	U	ND	ND
19	Zr	ND	ND
20	Br	ND	ND
21	Y	ND	<<
22	Si	ND	31.13 ± 0.327

Dry matter content: 12.00±0.088 (flower), 10.50±0.117 (Stem);
Ash content: 11.09±0.187 (flower), 11.12±0.148 (Stem).
All the calculations were done on dry wt. basis; <<Indicates for trace amount, ND indicates for not detected. The values are expressed as mean ± standard deviation (n=3).

Antioxidant activity of flower and stem from broccoli was evaluated by using the three complementary test systems; namely DPPH free radical scavenging, reducing power, and ferrous ion chelating power ability tests. The DPPH radical scavenging activity of different solvent extracts of broccoli, ascorbic acid and butylated hydroxyanisole (BHA) methanolic solutions at different concentrations are presented in Figure 1a-b. The result of this study revealed that ascorbic acid and BHA possessed upto 98.22 ± 1.122 and 96.01 ± 0.983 % respectively of DPPH radical scavenging activity at a concentration of 2 mg/mL. The methanol extract of both the flower and stem of broccoli was found to possess highest radical scavenging activity than the water and acetone extracts. The acetone extract exhibited the lowest inhibition 38.34 ± 0.441 and 9.25 ± 0.681 % for flower and stem, respectively. The activity was found to be increased with the increase of concentration but decreased at higher concentrations. The methanolic extract of flower showed maximum inhibition (91.97 ± 0.733) at 4 mg/mL concentration (Figure 1a). The stem extract showed 90.05 ± 0.433% inhibition at 4 mg/mL concentration but exhibited maximum inhibition up to 92.74 ± 0.837 % at 8 mg/mL concentration (Figure 1b). The water extract of flower exhibited maximum inhibition 50.29 ± 0.796% at 12 mg/mL whereas the stem extract showed maximum inhibition of 56.27 ± 0.576 % at 16 mg/ mL (Figure 1a-b). Based on the above results, our indigenous broccoli is considered to have higher DPPH radical scavenging activity than the reported activity of mungbean, soybean[24] and comparable to broccoli of Taiwan.[25]

Table 2. Contents of different Fatty acid components in flower and stem parts of Broccoli (analyzed by GC/MS)

No.	Name of the fatty acids	Relative % in flower part	Relative % In stem part
1	Palmitic acid	16.42 ± 0.017	15.26 ± 0.002
2	Oleic acid	4.13 ± 0.021	5.51 ± 0.004
3	Stearic acid	7.69 ± 0.027	3.50 ± 0.012
4	Linoleic acid	15.41 ± 0.007	15.43 ± 0.002
5	Linoleinic acid	3.26 ± 0.016	24.76 ± 0.004
6	Linolelaidicacid	0.02 ± 0.002	1.23 ± 0.007
7	Myrestic acid	3.04 ± 0.006	6.52 ± 0.003
8	Lauric acid	0.72 ± 0.001	0.03 ± 0.001
9	Ecosanoic acid	2.37 ± 0.013	0.85 ± 0.007
10	Heneicosanoic acid	1.67 ± 0.005	1.29 ± 0.010
11	Margaric acid	0.04 ± 0.002	0.04 ± 0.002
12	Iso stearic acid	1.72±0.012	1.26 ± 0.004
13	9-Hexadeenolic acid	0.03±0.002	1.05 ± 0.005
14	Nonadecanoic acid	0.02±0.001	0.85 ± 0.003
15	7H-exadecanoic acid	0.55±0.011	0.02 ± 0.001

Crude fat content (dry weight basis): 9.91±0.041 (flower), 9.14±0.152 (Stem). All the values are expressed as mean±SD (n=3).

Figure 2a-b demonstrated the ferrous ion chelating ability of the broccoli extracts, ascorbic acid and BHA. Ascorbic acid and BHA exhibited very low chelating power ability. The maximum inhibition was less than 17% in both cases and it was due to the structural properties of these compounds.

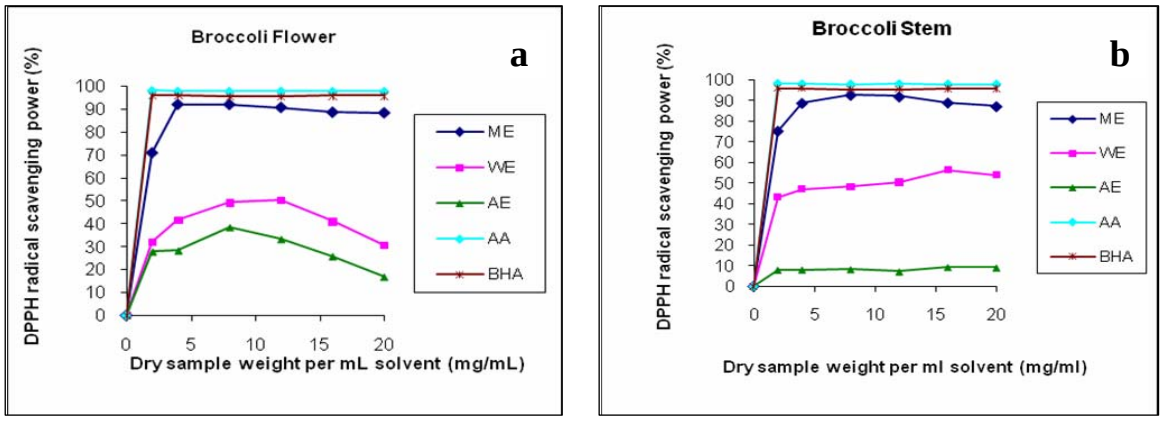

Figure 1a-b: a. DPPH radical scavenging activity of different solvent extracts from flower of *Brassica oleracea* (Broccoli). ME: Methanol extract; WE: Water extract; AE: Acetone extract; BHA: Butylated hydroxy anisole; AA: Ascorbic acid. All the values are expressed as mean ± SD (n=3). b. DPPH scavenging activity of different solvent extracts from stem of *Brassica oleracea* (Broccoli). ME: Methanol extract; WE: Water extract; AE: Acetone extract; BHA: Butylated hydroxy anisole; AA: Ascorbic acid. All the values are expressed as mean ± SD (n=3).

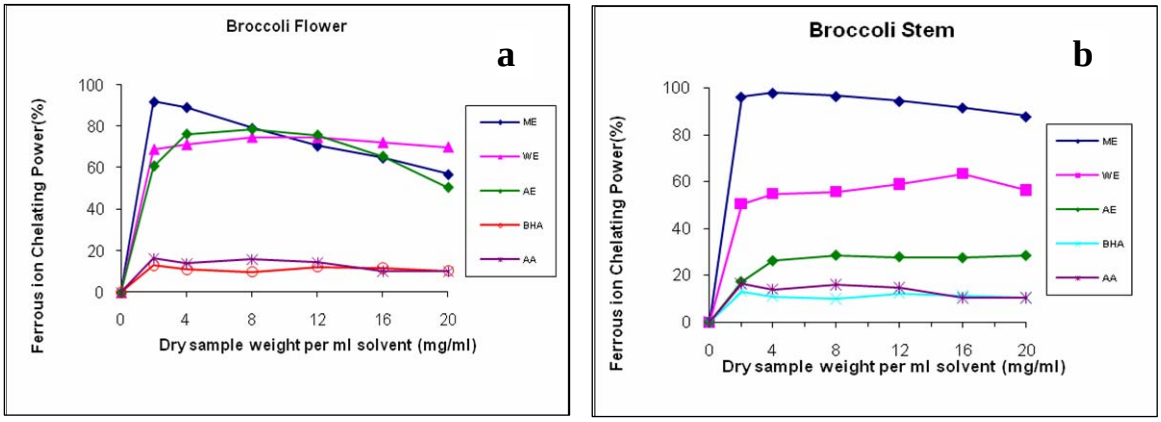

Figure 2a-b: a. Ferrous ion chelating ability of different solvent extracts from flower of *Brassica oleracea* (Broccoli). ME: Methanol extract; WE: Water extract; AE: Acetone extract; BHA: Butylated hydroxy anisole; AA: Ascorbic acid. All the values are expressed as mean ± SD (n=3). b. Ferrous ion chelating ability of different solvent extracts from stem of *Brassica oleracea* (Broccoli). ME: Methanol extract; WE: Water extract; AE: Acetone extract; BHA: Butylated hydroxy anisole; AA: Ascorbic acid. All the values are expressed as mean ± SD (n=3).

All the solvent extracts of broccoli samples except the acetone extracts of the stem exhibited good ferrous ion chelating ability (Figure 2a-b). Among the three different solvent extracts, the methanol extract possessed the highest chelating power ability followed by water and acetone extracts. The methanol extract of flower exhibited the highest ferrous ion chelating ability (91.85 ± 0.951) at 2 mg/mL (Figure 2a). Whereas the methanolic stem extract showed ferrous ion chelating ability by 96.12 ± 1.047 at 2 mg/mL and it gave the best chelating ability up to 97.38 ± 1.241 at 4 mg/mL concentration (Figure 2b). These results revealed that our indegenous broccoli possessed a higher ferrous ion chelating ability than soybean, mungbean and radish sprouts[24] and comparable to broccoli of Taiwan.[25]

Figure 3a-b expressed the reducing power of broccoli extracts, ascorbic acid and BHA. At a concentration of 2 mg/mL, relatively high reducing powers of both ascorbic acid and BHA were observed than the broccoli extracts. The reducing power of all the samples including BHA and ascorbic acid was found to be increased by increasing the sample concentrations. Among the three solvent

extracts methanol and water extracts exhibited higher reducing power than the acetone extract. At concentration higher than 2 mg/mL the water flower extract showed higher reducing power than BHA (Figure 3a). The maximum absorbance of methanol extracts of broccoli flower were up to 1.88 ± 0.0024 & 2.11 ± 0.0019 and that for stem were 2.02 ± 0.0036 & 1.88 ± 0.0038 respectively compared to 3.78 ± 0.0033 and 1.85 ± 0.0024 for ascorbic acid and BHA respectively. At higher concentrations the reducing power of methanol and water extracts of broccoli samples were comparable to BHA. The methanol extract of stem part showed higher reducing power than the flower whereas the water extract of flower possessed higher reducing power than the stem (Figure 2a-b). The results of reducing power of methanol and water extracts from flower and stem of broccoli cultivated in Bangladesh revealed that it possess higher reducing power than several edible plants consumed by Chinese people[26] and comparable to broccoli of Taiwan.[25]

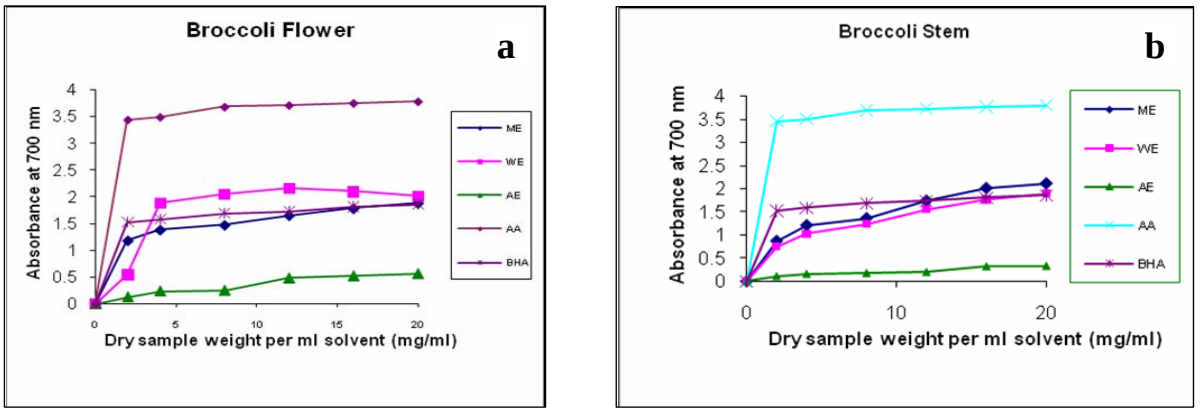

Figure 3a-b: a. Reducing Power assay of different solvent extracts from flower of *Brassica oleracea.* ME= Methanol extract; WE= Water extract; AE= Acetone extract; AA: Ascorbic acid; BHA: Butylated hydroxy anisole. All the values are expressed as mean ± SD (n=3). b. Reducing Power assay of different solvent extracts from stem of *Brassica oleracea.* ME= Methanol extract; WE= Water extract; AE= Acetone extract; AA: Ascorbic acid; BHA: Butylated hydroxy anisole. All the values are expressed as mean ± SD (n=3).

CONCLUSIONS

In the present investigation for evaluation of antioxidant activity the concentration is expressed by the ratio of crude sample per solvent volume instead of extract weight per solvent volume. Based on this concentrations our indegenous broccoli is estimated to have higher DPPH radical scavenging activity and higher ferrous ion chelating ability than the reported activity of mungbean, soybean and radish sprouts[24] and comparable to the broccoli of Taiwan. From the results of reducing power of methanol and water extracts of flower and stem parts it was observed that the indegenous broccoli also possessed higher reducing power than the several edible plants[26] and comparable to broccoli from Taiwan.[25] Our broccoli also contained good amount of vitamin C, different essenential and nutritional elements and fatty acids. These results clearly indicated that broccoli from Bangladesh has a significant potential to be used as nutritional suppliments and as natural antioxidant.

ACKNOWLEDGEMENT

We are grateful to Mr. Akhtaruzzaman, Member Science & Technology, BCSIR & Ex Director BCSIR Labs Dhaka, for his inspirations during this research work. We thank Mr Asaduzzaman, CSO (Ex. In-Charge, Chemical Research Div) and Mr. Shudhangshu Kumar Roy, CSO and In-Charge, Chemical Research Division, BCSIR Labs, Dhaka, for their help and support. We acknowledge the assistance of Mr Shahidur Rahman (Technician) and Mohammed Shajahan (Laboratory Attendant), Chemical Research Division.

REFERENCES

1. Buck, P. A. 2009. Origin and Taxonomy of Broccoli. Department of Food Technology, University of California. http://www.springerlink. com/ content/ ert 85x 3082740212/ fulltext.pdf.Retrived 2009-5-14.
2. Stephens, J. 2009.Broccoli-*Brassica oleracea L.* (Italica group). University of Florida. P.1. http://edis.ifas.ufl.edu/ MV031. *Rtrived* 2009-05-14.
3. Nonneck, I.B.1989. *Vegetable production.* Springer-Verlag New York, LLC.p. 394. ISBN 9780442267216. http://books.google.com/books.
4. Smith, P. June 1999. HGIC 1301 Broccoli. Clemson University. http://www.clemson.edu/extension/hgic/plants/ vegetables/crops/hgic 1301.htm *Retrived* 25 August 2009.
5. Fahey ,J.W., Zalcmann, A.T. and Talalay, P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry.* **56**, 5-51.
6. Talalay, P. and Fahey, J.W. 2001. Phytochemicals from Cruciferous Plants Protect against Cancer by Modulating Carcinogen Metabolism *J. Nutr.* **131** (11 Suppl), 3027S-33S.
7. Conaway, C.C., Getahun, S.M., and Liebes, L,L., 2000. Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. *Nutr Cancer*. **38**, 168-178.
8. Fimognari ,C. and Hrelia ,P. 2007. Sulforaphane as a promising molecule for fighting cancer. *Mutat Res.* **635**, 90-104.
9. Amy, V. G., Ahmed, A.J., Julie, A.S., James, R.B., Paul, F., Clare, A., Moira, A.T. , Christopher, J.H., David, A.B. and Richard F.M. 2005. Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli. *Am J Clin Nutr*. **82**, 1283-91.
10. Moreno, D.A., Carvajal, M., Lopez-Berenguer, C. and Garcia-Viguera, C. 2006. Chemical and Biological Characterization of nutraceutical compounds of broccoli. *J. Pharmaceut. and Biomed. Anal.* **41**, 1508-1522.
11. Kurilich A.C., Tsau G.J., Brown A.,Howard L., Klein B.P., Jeffery E.H., Kushad M., Wallig M.A., and Juvik J.A. 1999. Carotene, tocopherol, and ascorbate contents in subspecies of Brassica oleracea, *J. Agric. Food Chem.*, **47**, 1576-1581.
12. Chiu, B., Houghton, P. 2005. Investigation of Common Vegetables for Cholinesterase Inhibitory Activity, *British Pharmaceut. Conf.* 142nd, 9/26–28/2005, pp.151.
13. Mingzhan, X., Qingwen, Q., Adaikalakoteswari, A., Naila, R., Roya, B.J., and Paul, J. T. 200. Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. *Diabetes* **57**, 2809-2817.
14. Healy, Z., Lee, N., Gao, X., Goldring, M., Talalay, P., Kensler, T. and Konstantopoulos, K. 2005. Divergent responses of chondrocytes and endothelial cells to shear stress: Cross-talk among COX-2, the phase 2 response, and apoptosis. *Proc Nat. Acad. Sci.* **102**, 10410-10415.
15. Plumb, G.W., Price, K.R., Rhodes, M.J.C. and Williamson G. 1997. Antioxidant properties of the major polyphenolic compounds in broccoli, *Free Radical Res.* **27**, 429-435.
16. Kurilich, A.C., Jeffery, E.H., Juvik, J.A., Wallig, M.A. and Klein, B.P. 2002. Antioxidant capacity of different broccoli (*Brassicaoleracea*) genotypes using the oxygen radical absorbance capacity (ORAC) assay, *J. Agric. Food Chem.* **50**, 5053-5057.
17. Sies, H. and Stahl, W. 1995;. Vitamins E and C, B-carotene and other carotenoids as antioxidants. *Chiang Mai J. Sci.* **33**, 1315s -1321s.
18. Zhang, D. and Hamauzu, Y. 2004. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking, *Food Chem.* **88**, 503-509.
19. AOAC (Analysis of Official Analytical Chemist) 1975. *Official method of analysis* (4^{th} edn. Ed. S. Williams) Washington D. C. pp. 152-164.
20. Ranganan, 1991. *Handbook of Quality control for fruit and vegetable productions*. 2^{nd} Ed. Pp. 105-106.
21. Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, *J. Agri. Food Chem.* **40**, 945-948.
22. Decker, E. A. and Welch, B. 1990. Role of ferritin as a lipid oxidation catalyst in muscle food. *J. Agric. Food Chem.* **38**, 674-677.
23. Oyaizu, M. 1986. Studies on products of browning reaction: antioxidative activities of Products of browning reaction prepared from glucosamine. *Jpn. Nutr.* **44**, 307-315.
24. Wong, R.G. and Yen, G. C. 1997. Antioxidant action of mungbean sprouts, soyabean and radish sprouts. *J. Agric. Food Cem.* **40**, 945-948.
25. John-Tzong, G., Hui-Lien, L., Shu-Hsiu, C., Fang-I, L. and Chi-Yue, C. 2001. Antioxidant properties of the extracts from different parts of broccoli in Taiwan. *J. Food Drug Anal.* **9**, 96-101.
26. Liu, B. K., Chang, H. Y. and Yen, G. C. 1999. Antioxidative activity of the methanolic extracts from various traditionally edible plants. *J. Agric. Chem. Soc.* (Taiwan) **37**, 105-116.