Research Article

ANTIOXIDANT ACTIVITIES OF DIFFERENT PARTS OF TREE TOMATO FRUIT

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ABSTRACT

Cyphomandra betacea (Cav.) Sendtn. (Solanaceae) is commonly known as 'Tree Tomato'. It is a common shrub, which widely grows in the Darjeeling Himalaya. The fruits are good sources of provitamin A, vitamin C, B_6, E and iron. This study was conducted to determine the antioxidant activities of methanol extracts from five different parts (placenta, endocarp, epicarp, seed and mesocarp) of this fruit. Antioxidant activities were evaluated by DPPH (2,2-diphenyl-1-picryllhydrazyl) radical, nitric oxide (NO), hydroxyl radical (OH), superoxide scavenging activity, metal chelating, reducing power (RP) and anti-lipid peroxidation. Placenta and endocarp showed significantly higher superoxide scavenging and metal chelating activity whereas inhibition of lipid peroxidation was much better in mesocarp. Therefore, different parts of *C. betacea* fruit mainly placenta, endocarp and epicarp are potential functional food ingredient and their incorporation into human diets might provide protection and help to reduce oxidative damage in different vital organs.

Keywords: *Cyphomandra betacea*, Antioxidant, Free-Radical, Phytochemicals.

INTRODUCTION

Cyphomandra betacea (Cav.) Sendtn. is domesticated plant in the hills of Darjeeling and commonly known as 'Tree Tomato'. The fruits are eaten fresh, cooked in stews and sauces, prepared as chutney, pickles as well as directly consumed with salads. *C. betacea* mature fruit juice is traditionally used in Ecuador for the treatment of tonsillitis, high cholesterol and stomach pain.[1] This fruit, naturally acidic, is recommended for its nutritional qualities, as a good source of provitamin A, vitamin E, C, B_6 and iron.[2-5] The fruit has significant amount of bioactive phytochemicals like carotenoids, anthocyanins and phenolic compounds.[6] Fruits and vegetables may protect against numerous chronic diseases including carcinoma, cerebro- and cardiovascular stroke, ocular and neurological disorders[7,8] due the presence of such type of antioxidant constituents, like vitamin C, R-tocopherol, carotenoids, glutathione, flavonoids, and phenolic acids.[9] The potential of the antioxidant constituents of plant materials for the maintenance of health and protection from chronic diseases has also raised interest among scientists and food manufacturers as consumers move towards functional foods with specific health effects.[10] A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems.[11] The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defense mechanisms. It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, *diabetes mellitus*, cancer, immunosuppression, neurodegenerative diseases and others.[12] Several widely consumed vegetables are rich in various phenolic compounds and vitamins which are the main source of natural antioxidant. Among vegetables, tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), chilli pepper (*Capsicum annuum* L.), and potato (*Solanum tuberosum* L.), which belong to the solanaceae family, are important for their richness with healthy components, due to which they are also widely consumed.[13] But *C. betacea* is a plant which belongs to this family and its use is mainly restricted only in the hilly region as vegetables.

The aim of the present study was to investigate the different parts of the fruits of *C. betacea* from Darjeeling hills as a potential functional food and antioxidant source, as an alternative to synthetic compounds. In this work we have determined the radical scavenging efficacy of different parts of this fruit as well as the phytonutrients like total carotene, lycopene, anthocyanin, total phenolics and flavonol.

MATERIALS AND METHODS

Plant materials

Ripe and fresh *C. betacea* fruits were collected from Sorang Basti, Darjeeling, West Bengal, India. Different parts of this fruits like placenta, endocarp, epicarp, seed and mesocarp were surgically separated. The plant material was authenticated from Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal. The material was deposited in the 'NBU Herbarium' and recorded against the accession no 9579 dated 04-03-09.

Animal material

Goat liver, which was used for anti-lipid peroxidation assay, was collected from slaughter house immediately

after slay. Experiment was conducted within one hour after collection.

Chemicals

Methanol (M), 2.2-diphenyl-1-picryl hydrazyl (DPPH), nitroblue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide sodium salt monohydrate (NADH), phenazine methosulphate (PMS), sulfanilamide, glacial acetic acid, napthylethylenediamine dihydrochloride, ferrozine, ferrous chloride, trichloroacetic acid (TCA), thiobarbituric acid (TBA), $FeSO_4.7H_2O$, KOH, KH_2PO_4, ethylene-diamine tetra acetic acid (EDTA), ascorbic acid, vitamin-E, 2-deoxyribose, potassium ferricyanide, ferric chloride ($FeCl_3$), hydrogen peroxide (H_2O_2), sodium nitroprusside, gallic acid, Folin-Ciocaltue reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), acetone, petroleum ether, sodium sulphate, and sodium hydroxide (NaOH) were either purchased from Sigma Chemicals (USA), or of Merck analytical grade.

Extraction and determination of methanol extractive values

Fresh parts of tree tomato were extracted by standard solvent extraction method.[14,15] The plant materials were ground into fine paste and separately extracted with methanol under soxhlet extractor for eight hours. The solvents were completely removed by vacuum rotary evaporator at 50°C. These crude extracts were used for further investigation. The extractive value of the plant materials were calculated on dry weight basis from the formula given below:

$$\text{Percent extractive value (yield \%)} = \frac{\text{Weight of dry extract}}{\text{Weight taken for extraction}} \times 100$$

Antioxidant activity assay

DPPH scavenging activity assay

The free radical scavenging capacity of different parts of tree tomato was determined by using DPPH (16). 0.1 mM solution of DPPH in methanol was prepared. 1.8 ml of this solution was added to 0.2 ml of test solutions at different concentrations (40 mg/ml-200 mg/ml FWT) in different test tubes. Thirty minutes later, the absorbance was measured at 517 nm. Methanol was used as a blank. The percentage of free radical scavenging activity was calculated as follows:

$$\text{Percent inhibition of DPPH radical} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Determination of Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined by using the 2-deoxyribose oxidation assay.[17] A solution (0.2 ml) of 10 mM $FeSO_4$, $7H_2O$ and 10 mM ethylenediamine tetraacetic acid was prepared in a screw capped test tube. Then, 0.2 ml of 10 mM 2-deoxyribose solution, 0.5 ml of each sample (different concentration) and 0.1M sodium phosphate buffer (pH 7.4) were added to give a total volume of 1.8 ml. Finally, 200 µl of 10 mM H_2O_2 solution were added and incubated at 37°C for 120 min. After incubation, 1 ml each of 2.8% trichoroacetic acid and 1.0% thiobarbituric acid were added to the reaction mixture. The mixture was boiled at 100°C for 10 min, cooled in ice, and then its absorbance was measured with a spectrophotometer at 515 nm. The IC_{50} value was calculated by the same process mentioned above.

Determination of superoxide anions scavenging activity

The superoxide anions generated by phenazine methosulphate (PMN) and nicotinamide-adanine dinucleotide-phosphate, reduced form (NADPH), were detected by the reaction with 2,2´-di-*p*-nitrophenyl-5,5´-diphenyl-(3,3´-dimethoxy-4,4´-diphenylene) di-tetrazolium chloride (nitro blue tetrazolium-NBT).[18] The reaction mixture contained 1 ml of different samples (different concentration), 1 ml of NBT solution (312 µM prepared in phosphate buffer, pH-7.4) and 1ml of NADH solution (936 µM prepared in phosphate buffer, pH-7.4). Finally, the reaction was accelerated by adding 100 µL PMS solution (120 µM prepared in phosphate buffer, pH -7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min and absorbance at 560 nm was measured against methanol as control. Percentage inhibition and IC_{50} value was calculated using the same formula mentioned above.

Determination of reducing power

One milliliter of fruit extract, 2.5 ml sodium phosphate buffer (0.2 M, pH 6.6), and 2.5 ml potassium ferricyanide (1% w/v) were incubated at 50°C for 20 minutes. The tube was cooled on ice and 2.5 ml 10% trichloroacetic acid was added. The mixture was centrifuged at 3000 rpm for 10 minutes to collect the upper layer of solution (2.5 ml) and mixed with distilled water (2.5 ml) and 0.25 ml of $FeCl_3$ (0.1% w/v). Finally, the absorbance was measured at 700 nm against blank sample.[19]

Metal chelating assay

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al.* with slight modification.[20] To 0.4 ml of methanol extract diluted with 1.6 ml of methanol, 0.04 ml of $FeCl_2$ (2 mM) was mixed. After 30 s, 0.8 mL ferrozine (5 mM) was added. Then after an interval of 10 min at room temperature, the absorbance of the Fe^{2+}–Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated by the same procedure mentioned above.

Anti-lipid peroxidation (ALP) assay

Lipid peroxidation was measured *in vitro* with the extracts of liver homogenate of male goat in terms of formation of thioburbituric acid reactive substances by the method of Dhalwal *et al.*[21] 0.1 ml of the plant samples were

individually added to 2.8 ml of 10% liver homogenate and 0.1 ml of 50 mM $FeSO_4$. The reaction mixture was incubated at 37°C for 30 min. 1 ml of reaction mixture was mixed with 10% TCA-0.67% TBA (2 ml) in acetic acid. Then the combination was boiled for one hour at 100°C and centrifuged for 5 minutes at 10000 rpm. Supernatant was used for measuring absorbance at 535 nm. Blank contained all reagents except liver homogenate and extract. Control was prepared by mixing all reagents without extract and $FeSO_4$ and iron induced sample was organized by mixing all reagents devoid of extract. ALP percentage was calculated by using the following formula:

$$\text{ALP \%} = \frac{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{Abs. of sample}}{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{Abs. of control}} \times 100$$

Determination of Nitric oxide activity

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction.[22] Briefly, 320 µL methanol extract, 360 µL (5mM) sodium nitroprusside-PBS solution and 216 µL Greiss reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) was mixed and incubated at 25°C for one hour. 2 ml water was added and absorbance was taken at 546 nm. The percent inhibition was calculated by the same procedure mentioned above.

Phytochemical Estimation

Total phenol estimation

Total phenolic compounds of fruit extracts were determined by Folin-Ciocalteu method.[22] For the preparation of the calibration curve, 1 ml aliquot of 0.025, 0.05, 0.075, 0.1, 0.2 and 0.3 mg/ml methanolic gallic acid solution was mixed with 5 ml of Folin-Ciocaltue reagent (10 times diluted) and 4 ml sodium carbonate (75 g/L). The absorbance at 765 nm was measured after 1 hr. at 20°C and the calibration curve was drawn. To the similar reagent, 1 ml methanolic fruit extracts (10 mg/ml) was mixed as described above and after 1 hr. the absorbance was measured.

Total flavonoids estimation

Aluminum chloride spectrophotometric method was used for flavonoids estimation.[23] Each fruit extracts (0.5 ml of 200mg/ml FWT) were separately diluted with 4 ml double distilled water. Diluted extracts of fruits were mixed with 5% (0.3 ml) $NaNO_2$. 10% aluminum chloride was then added with reaction mixture. After 6 minute 2ml (1.0 M) NaOH and 2.4 ml distilled water was added and mixed well. Thereafter, absorbance was measured at 510 nm in spectrophotometer. For preparation of standard curve, quercetin (0-500 mg L^{-1}) was used.

Determination of total anthocyanin content

Total anthocyanins content was measured using a spectrophotometric pH differential protocol described by Giusti and Wrolstad, and Wolfe *et al*. with slight modifications.[24,25] Briefly, 0.5 ml of the extract was mixed thoroughly with 3.5 ml of 0.025 M potassium chloride buffer pH 1. The mixture was mixed with vortexing and allowed to stand for 15 min. The absorbance was then measured at 515 and 700 nm against distilled water blank. The extract was then combined similarly with 0.025 M sodium acetate buffer pH 4.5, and the absorbance was measured at the same wavelength after being allowed to stand for 15 min. The total anthocyanin content was calculated by using the following equation:

Total anthocyanin content (mg/100 g of dried sample) = A x MW x DF x1000/(e x C), where A is absorbance = (A515 – A700)pH 1.0 – (A515–A700)pH 4.5; MW is a molecular weight for cyanidin-3-glucoside = 449.2; DF is the dilution factor of the samples, e is the molar absorbtivity of cyanidin-3-glucoside = 26900; and C is the concentration of the buffer in mg/ml. Results were expressed as mg of cyanidin-3-glucoside equivalents in 100 g of fresh sample (mg c-3-g eq./100 g FWT).

Estimation of lycopene content

Lycopene content was measured spectrophotometrically at one of its absorption maxima.[26] 5 ml of methanolic fruit extract was dissolved with 15 ml of acetone. The extract was transferred to a separating funnel containing about 20 ml of petroleum ether and mixed adequately. To it, about 20 ml of 5% Na_2SO_4 solution was added and gently shaken. 20 ml of petroleum ether was added again to the funnel for clear separation of the two layers. The upper layer was mostly coloured. The two phases were separated and the lower aqueous phase was re-extracted with 20 ml of petroleum ether until the aqueous phase become colourless. The petroleum ether extract was pooled and washed once with a little distilled water. The petroleum ether extracts enriched with lycopene was transferred into a brown bottle containing about 10 gm anhydrous Na_2SO_4 and kept aside for 30 minutes. Petroleum ether extract was decanted into a 100 ml volumetric flask through a funnel containing cotton wool. The Na_2SO_4 slurry was washed with petroleum ether until it became colourless and was transferred to the volumetric flask. Finally the volume was made up and the absorbance was measured in a spectrophotometer at 503 nm using petroleum ether as blank. The lycopene content was calculated by the formula:-

$$\text{Lycopene Content} = \frac{3.1206 \times \text{final volume} \times \text{Mean value}}{\text{Initial weight of the sample}}$$

Where, 1 O.D = 3.1206 µg/gm

Estimation of the total carotene content

Total carotenoids were estimated spectrophotometrically through solubility based solvent partitioning followed by hydrolysis of bound carotenoid esters with concentrated KOH solution.[26] 1 ml of methanolic fruit extract was mixed with 2 ml of petroleum ether in separating funnel. Two layers of aqueous phase were observed. The upper phase was collected whereas the lower phase was decanted, this procedure was repeated thrice. The collected upper phase containing carotenoids was

evaporated at 37°C and the residue was dissolved in 2 ml of ethanol. 2 ml of aqueous 60% KOH was added to the residue mixture and boiled for 5 to 10 min. 2 ml of water was then added and partitioned thrice with petroleum ether and evaporated. To the reaction mixture 2.5 ml of ethanol was added and the absorbance was taken at 450 nm. The carotene content was calculated by the formula:-

$$\text{Carotene content} = \frac{D \times V \times F \times 10}{2500} \text{ mg}$$

Where, D = Absorbance value.

V = Volume of original extract in ml.

F = Dilution factor.

2500 = Average extinction coefficient of the pigment.

Statistical analysis

The data were pooled in triplicate and subjected to analysis of correlation co-efficient matrix using SPSS (version 12.00) for drawing the relation between phytochemical properties and antioxidant attributes and MS Excel of Microsoft Office, 2007 was used for comparing the antioxidant attributes of different fruit parts. Smith's Statistical Package (version 2.5) was used for determining the IC_{50} values of antioxidants and their standard error of estimates (SEE). In order to examine and visualize relationships between different phytochemicals and antioxidant traits, a principal component analysis (PCA) based on the correlation matrix was calculated using Multivariate Statistical Package (MVSP 3.1).

RESULTS AND DISCUSSION

The extractive yields of five different parts of tree tomato fruits were presented in Figure 1. Relatively higher extraction yields were obtained from mesocarp and epicarp than other parts like endocarp, seeds and placenta. The highest extraction yield was found in the methanol extract from the mesocarp with 8.95%, while the seed part (3.25%) had the lowest extraction yield. These results showed that the extraction yield varied with methanol, indicating that each part of this fruit consists of different components. Several studies reported the increase of extraction yield by the action of pectinases, cellulases and hemicellulases.[27,28] Reduction of particle size increases the polyphenols extraction rate and the extraction yield. The fruits like tomato have superior level of phenolic compounds (flavonoids, flavones, cinnamic acid derivatives), phytoalexins, protease inhibitors, glycoalkaloids and carotenoids, but especially enriched with lycopene and β-carotene.[29-31] Similar results were obtained in case of tree tomato fruits, which are rich in total phenolics. Figure 2 shows that the total phenolic contents were highest in the placenta (4.11 mg/g) of this fruit. Simple phenolic derivatives and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources and they have been shown to possess significant antioxidant activities.[32] Total flavonoid contents were also determined to be accumulated in higher amount (0.199 mg/g) in placenta than the other fruit parts of tree tomato (Figure 3).

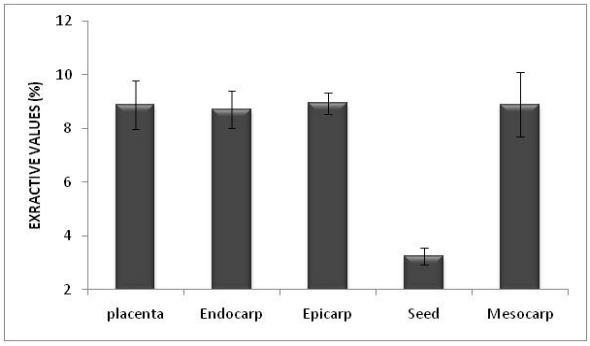

Figure 1: Determination of extractive values of different parts of *C. betacea* fruits.

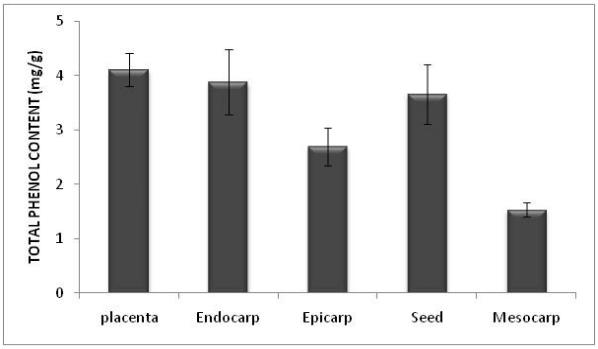

Figure 2: Total phenol content (mg/g FWT) of different parts of *C. betacea* fruits.

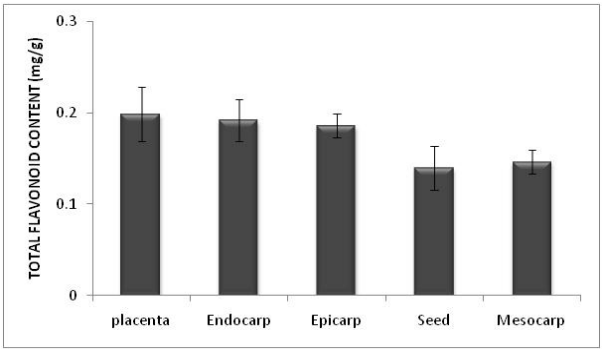

Figure 3: Total flavonoid content (mg/g FWT) of different parts of *C. betacea* fruits.

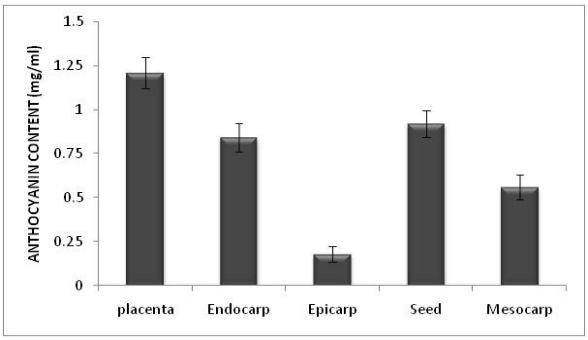

Figure 4: Anthocyanin content (mg/g FWT) of different parts of *C. betacea* fruits.

According to Vasco *et al*. (2009) both purple-red and golden-yellow varieties of *C. betacea* have high absorbance at 520 nm due to their anthocyanin content.[33] Parallel results were found when anthocyanin contents of

tree tomato were measured in our research. Figure 4 showed the anthocyanin content of each extract at concentration of 25 mg/ml fresh weight tissue. Extracts from the placenta of tree tomato fruits demonstrated the presence of significant amount of total anthocyanin (1.21 mg/100g) than the extracts from other fruit parts. It is well known that along with phenolics, carotenoids are responsible for bright colours and these chemical class acts as antioxidant, with functions that include protection of membranes against damage by free radicals and retardation of ageing processes.[34] Figure 5 exhibited the spectrophotometric measurement of total carotenoids based on absorbance at 470 nm after petroleum ether partitioning from aqueous alkaline phase of extracts. The results revealed that the placenta (0.601 mg/100g), endocarp (0.588 mg/100g) and epicarp (0.498 mg/100g) of the fruit of tree tomato are affluent with this component. The seed extracts presented the lowest value. These carotenoids are yellow, orange, and red pigments present in all plant tissues. Lycopene is the main carotene, accumulated significantly in ripe tomato fruits.[35] This pigment is a powerful natural antioxidant that acts as most efficient singlet oxygen quencher *in vitro* among common carotenoids[36] and plays a determinant factor in reducing the mortality from several cancers.[37-38] Like carotene, similar results were also found in case of lycopene content. In Figure 6, it is clear that significant amount of lycopene was present in placenta (0.512 µg/g), epicarp (0.554 µg/g) and endocarp (0.435µg/g), when compared with other two parts of tree tomato fruit. During oxidative stress, large quantities of reactive oxygen species like hydrogen peroxide, superoxide, hydroxyl radical, singlet oxygen and nitrogen species are generated and it is now recognized that there is no single test to evaluate antioxidant activities of the compounds with wide spectra of structures, modes of action, and physico-chemical properties; thus different assays were employed as a part of our investigation. DPPH assay has been largely used as a quick, reliable, and reproducible parameter to search the *in vitro* general antioxidant activity of pure compounds as well as plant extracts.[39,40] The potential antioxidant activities of different parts of tree tomato fruit extracts were evaluated by this method and the results were shown on Figure 7. The ability of free radical scavenges were determined by change of stable purple to yellow colour. As shown in the figure, extracts from placenta and endocarp parts had relatively strong DPPH scavenging activity (low IC_{50} value), exhibiting high antioxidant capacity compared to extracts from epicarp, seed and mesocarp. The IC_{50} values for hydroxyl radical (Figure 8), superoxide scavenging ability (Figure 9), and reducing power capability (Figure 10), showed a similar trend with those for DPPH. It was observed that purple red variety of tree tomato showed higher hydroxyl radical scavenging activity than golden yellow variety due to the presence of greater amount of anthocyanin pigment.[41] The potentially reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins; the effect of fruit extracts on the inhibition of free radical mediated deoxyribose damage was assessed by means of iron (II)-dependent DNA damage assay, which showed significant results.[42] On the other hand superoxide radical anions are potential precursors of hydroxyl radical and play an important role in the formation of reactive oxygen species such as hydrogen peroxide, hydroxyl radical, and singlet oxygen.[43]

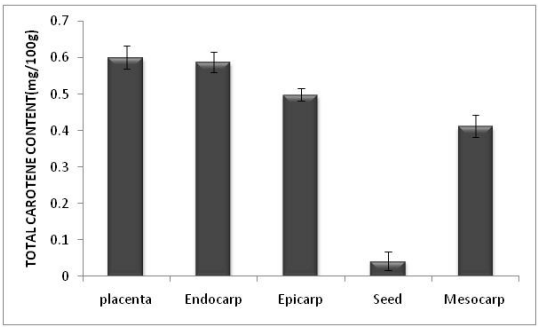

Figure 5: Total carotene content (mg/100g FWT) of different parts of *C. betacea* fruits

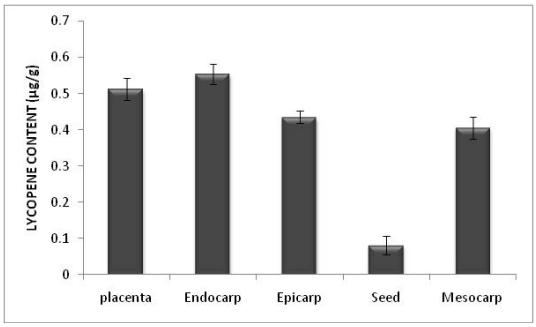

Figure 6: Lycopene content (µg/g FWT) of different parts of *C. betacea* fruits

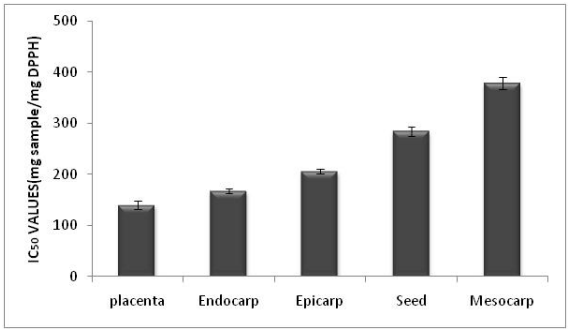

Figure 7: DPPH radical scavenging (IC_{50}) activity of different parts of *C. betacea* fruits

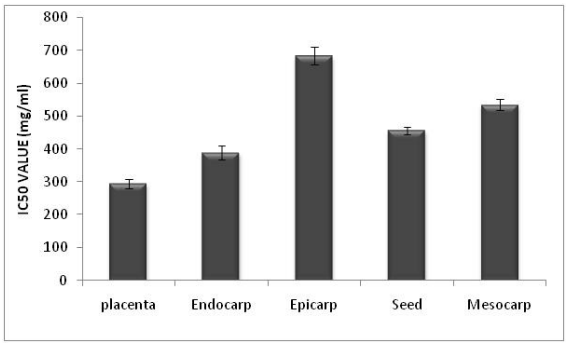

Figure 8: Hydroxyl scavenging (IC_{50}) activity of different parts of *C. betacea* fruits

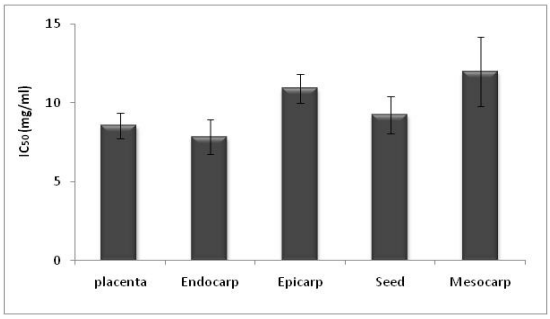

Figure 9: Superoxide scavenging (IC_{50}) activity of different parts of *C. betacea* fruits

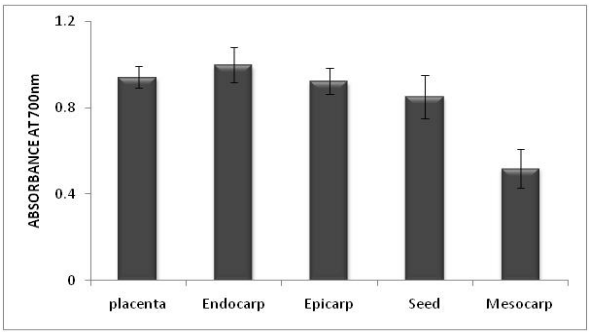

Figure 10: Reducing Power of different parts of *C. betacea* fruits

Results of superoxide anion scavenging activities of different parts of tree tomato fruit, measured by the PMS-NADH superoxide generating system are shown in Figure 9. Figure 10 showed the reducing potential of different parts of fruit extracts at concentration of 100 mg/ml (FWT) and the capacity of reducing power of these extracts increased in a concentration dependent manner (data not shown). The values of the absorbance for the tested extracts at a concentration of 100 mg/ml was determined in this assay and the absorbance is ranged from 0.521 to 0.999 and followed the order of effectiveness as: endocarp (0.999) > placenta (0.943) > epicarp (0.923) > seed (0.852) > mesocarp (0.521). In general, the extracts of the tested fruit materials, exhibiting greater total phenol content, also depicted good reducing power in the present analysis. The reducing potential of antioxidant components is very much associated with their total phenol content. The fruit or seed extracts with higher level of total phenolics also exhibit greater reducing power in monocot species like wheat under different bio-physical stresses.[44] In addition, some phenolic compounds exhibit antioxidant activity through the chelation of metal ions.[45] Chelating agents may inhibit radical generations by stabilizing transition metals, consequently reducing free radical damage. Phenolic compounds may be permitting that bond to metal ions due to their chemical structures. The observation of the present study has shown the chelating activity and ferrous ion capturing before ferrozine formation. The formation of ferrous and ferrozine complex has helped in inhibition of lipid peroxidation. In the presence of chelating agents, red colour of the complex was decreased due to the disruption in the complex formation. From Figure 11 it was revealed that all the fruit parts impeded with the formation ferrous-ferrozine complex, suggesting that the fruit has high chelating activity and captured ferrous ion before ferrozine. It was found that mesocarp of tree tomato fruit possessed high anti-lipid peroxidation with IC_{50} of 19.5mg/ml (Figure 12). Phenolic compounds are very important plant constituents because they exhibit antioxidant activity by inactivating lipid free radicals, or by preventing the decomposition of hydroperoxides into free radicals.[46] Table 1 also confirmed that point through the high correlation between phenolic content with anti-lipid peroxidation activity against goat liver. Like anti-lipid peroxidation, the scavenging activity against nitric oxide was optimal in the mesocarp portion (IC_{50} of 9.65 mg/ml) of tree tomato (Figure 13).

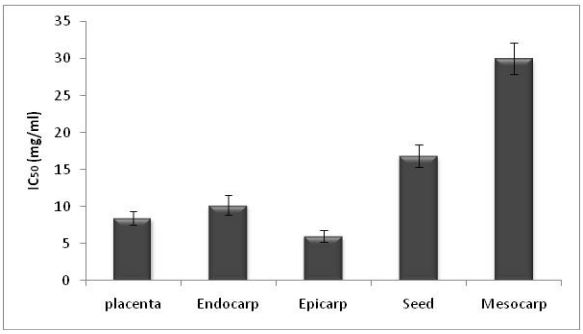

Figure 11: Metal chelating (IC_{50}) activity of different parts of *C. betacea* fruits.

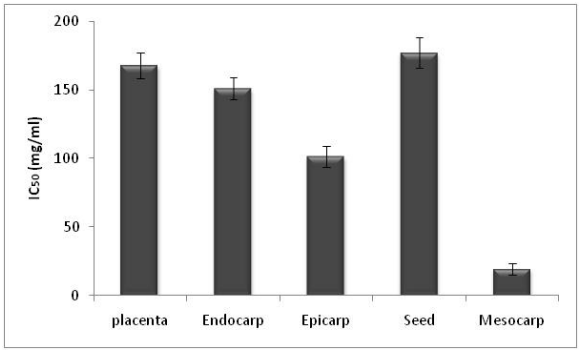

Figure 12: Anti-lipid peroxidation (IC_{50}) activity of different parts of *C. betacea* fruits.

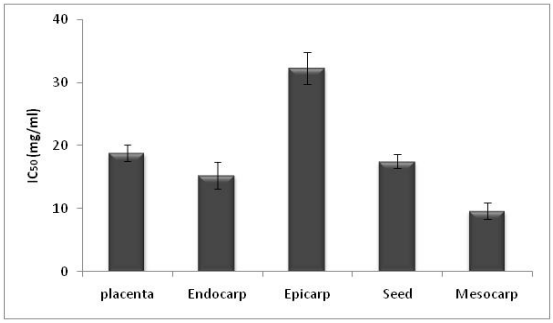

Figure 13: Nitric oxide scavenging (IC_{50}) activity of different parts of *C. betacea* fruits.

Other authors also suggested that the crude extracts of certain plants under the family solanaceae like *Solanum melongena* L., *Solanum tuberosum* L. *Lycopersicon esculeutum* Mill. potentially scavenge nitroprusside induced nitric oxide generation *in vitro*.[47] The extracts

inhibit nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide which is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities.[22]

Table 1: Correlation matrix

	DPPH	SO	MC	NO	ALP	OH	RP	EV	TPC	TFC	CARO	LYCO
SO	0.763											
MC	**0.922(*)**	0.589										
NO	-0.472	0.046	-0.758									
ALP	-0.707	**-0.880(*)**	-0.672	0.225								
OH	0.433	0.758	0.099	0.524	-0.578							
RP	**-0.926(*)**	-0.806	**-0.943(*)**	0.546	0.829	-0.305						
EV	-0.270	0.179	-0.143	0.098	-0.474	0.077	-0.027					
TPC	-0.814	**-0.953(*)**	-0.705	0.156	**0.970(**)**	-0.692	0.864	-0.272				
TFC	**-0.899(*)**	-0.502	-0.789	0.428	0.330	-0.284	0.709	0.664	0.502			
CARO	-0.570	-0.172	-0.394	0.136	-0.141	-0.180	0.285	**0.936(*)**	0.082	0.866		
LYCO	-0.511	-0.166	-0.318	0.047	-0.190	-0.182	0.235	**0.939(*)**	0.040	0.819	**0.992(**)**	
ANTHO	-0.349	-0.711	-0.058	-0.496	0.635	**-0.972(**)**	0.261	-0.271	0.697	0.131	-0.024	-0.034

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).
DPPH: 2,2-diphenyl-1-picryl hydrazyl; SO: Superoxide radical; MC: Metal chelating; NO: Nitric oxide; ALP: Anti-lipid peroxidation; OH: Hydroxyl radical; RP: Reducing power; EV: Extractive value; TPC: Total phenol content; TFC: Total flavonol content; CARO: Total carotene content; LYCO: Lycopene content; ANTHO: Anthocyanin content; FWT: Fresh weight tissue; c-3-g: Cyanidin-3-glucoside; eq.: Equivalent.

Several previous studies have evaluated the relationship between free-radical scavenging capacity and bioactive secondary metabolites.[48,49] The results achieved with Pearson's correlation have shown that DPPH IC_{50} values were more associated with total flavonol content than accessory pigments like total carotene, lycopene and anthocyanin (Table 1). As represented in Table 1, the strong negative correlation between IC_{50} values of specific free radical scavengers and different bioactive polyphenols deserves detailed attention. Basically antioxidants are reducing agents and are capable of donating a single electron or hydrogen atom for detoxifying free radicals.[50] In this study, the cohesiveness of superoxide scavenging activity with total phenol content and hydroxyl radical scavenging with anthocyanin indicated that free hydroxyl groups present in phenolics and anthocyanin-based phytochemicals play important role as reducing agent. The ferric reducing power of different parts of *C. betacea* fruits were also highly correlated with DPPH radical scavenging and metal chelating capacity. Arnous *et al*. reported a strong correlation between DPPH free radical scavenging ability and ferric ion reducing capacity of wines.[51] Pulido *et al*. also suggested that ferric ion reducing capacity correlates with the results from different methods used to estimate antioxidant activity.[52] In order to clarify which group of compounds mainly contributes to the antioxidant activity, principal component analysis (PCA) were performed and analyzed. The data matrix was standardized by an auto-scaling procedure to furnish same importance to all parameters. When PCA was performed with all phytochemical and antioxidant parameters across all the different parts of *C. betacea* fruit, PCA identified two uncorrelated linear combination of measurements that account for 80.17% (Figure 14); with first principal component (PC1) and the second principal component (PC2) account for 50.68 and 29.48% of the total variability respectively.

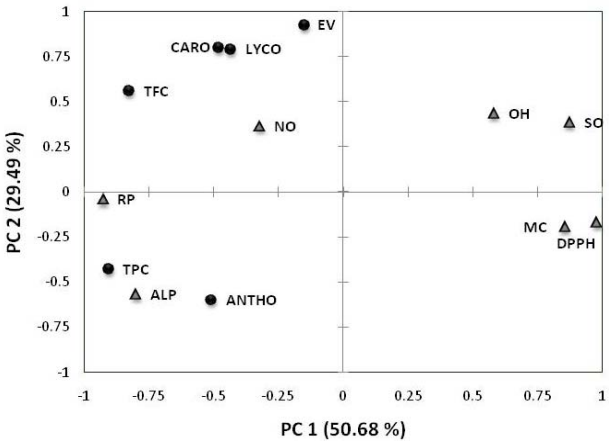

Figure 14: Principal component analysis of DPPH, SO, MC, NO, ALP, OH, RP, EV, TPC, TFC, CARO, LYCO and ANTHO.

From the distribution profile of loading factors of different phytochemical groups, it is apparent that hydrophilic antioxidants are regulated by PC1, whereas PC2 mainly contributes to the antioxidant capacity through hydrophobic interaction. Among them, DPPH, MC and SO were found to be similarly loaded on PC1, which indicated that the three properties are closely associated with primary antioxidant activity. Inverse relationship between the groups DPPH, MC and SO verses TFC, TPC and RP indicated that the polar metabolites like free phenolics and flavonoids and the reducing ability of the same metabolites are responsible for lowering the

IC_{50} values of specific free-radical scavengers. PC2, on the other hand, loads highly on the plant extracts' ability to inhibit ALP on negative domain and hydrophobic antioxidants like carotene and lycopene on positive domain. So, anti-lipid peroxidation activity is controlled principally by hydrophobic carotenoids group. Moderate values of nitric oxide scavenging and anthocyanin on both components pointed out that nitric oxide scavenging potential is not so significant and the presence of anthocyanin does not necessarily translate into primary antioxidant property in *C. betacea* fruit.

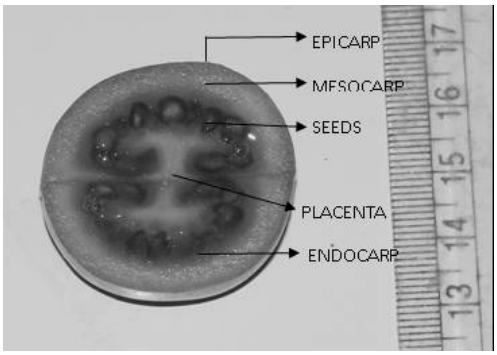

Figure 15: T.S. of the *C. betacea* fruit

CONCLUSION

The study indicates that the methanol extract obtained from different parts of edible fruit of *C. betacea* contain high amount of phenolics and flavonoids. It also exhibited significant antioxidant activity mainly in placenta and endocarp. The high radical scavenging activity may be due to hydroxyl groups existing in phenolic compounds that can able to reduce free radicals by donating additional electron. In the longer term the constituents of the fruits of *C. betacea*, mainly from placenta and endocarp, as having high antioxidant activity, can be used as functional food ingredients and may unravel novel treatment strategies for various disorders associated with oxidative stress induced damage in different vital organs of human being.

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REFERENCES

1. Tene V, Malangon O, Vita Finzi P, Vidari G, Armijos CH, Zaragoza T, An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe Ecuador, Jounal of Ethnopharmacology, 111, 2007, 63-81.
2. Popenoe H, King S, Leon J, Kalinowski L, Vietmeyer N, Dafforn M, Mortino M, Lost Crops of the Incas: Little-known Plant of the Andes with Promise for Worldwide Cultivation, National Academy Press, Washington, DC, USA, 1989, 306-316.
3. Romero-Rodriguez MA, Vazquez-Oderiz ML, Lopez-Hernandez J, Simal-Lozano J, Composition of babaco, feijoa, passionfruit and tamarillo produced in Galicia (north-west Spain), Food Chemistry, 49, 1994, 23-27.
4. Vera de Rosso V, Mercadante AZ, HPLC-PDA-MS/MS of anthocyanins and carotenoids from dovyalis and tamarillo fruits, Journal of Agricultural and Food Chemistry, 55, 2007, 9135-9141.
5. Wills RBH, Lim JSK, Greenfield H, Composition of Australian foods: 31 Tropical and subtropical fruit, Food Technology Association of Australia, 38, 1986, 118-123.
6. Schmeda-Hirschmann G, Feresin G, Tapia A, Hilgert N, Theoduloz C, Proximate composition and free radical scavenging activity of edible fruits from the *Argentinian yungas*, Journal of the Science of Food and Agriculture, 85, 2005, 1357–1364.
7. Bloc G, Patterson B, Subhar A, Fruit, vegetables and cancer prevention: a review of epidemiological evidence, Nutrition and Cancer, 18, 1992, 1–29.
8. Youdim KA, Joseph JA, A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: multiplicity of effects, Free Radical Biology and Medicine, 30, 2001, 583–594.
9. Sies H, Stahl W, Vitamins E and C, β-carotene and carotenoids as antioxidant, American Journal of Clinical Nutrition, 62, 1995, 1315–1321.
10. Loliger J, Use of antioxidants in food,Taylor and Francis, London, 1991, 121-150.
11. Halliwell B, Superoxide Dependent formation of hydroxyl free radicals in the presence of iron chelates, FEBS Letters, 92, 1978, 321.
12. Young IS, Woodside JV, Antioxidants in health and disease, Journal of Clinical Pathology, 54, 2001, 176-186.
13. Helmja K, Vaher M, Gorbatsova J, Kaljurand M, Characterization of bioactive compounds contained in vegetables of the Solanaceae family by capillary electrophoresis, Proceedings of the Estonian Academy of Sciences Chemistry, 56, 2007, 172–186.
14. Hennebelle T, Sahpaz S, Joseph H, Bailleul F, Phenolics and iridoids of *Lippia alba,* Natural Product Communications, 1, 2006, 727-730.
15. Saha MN, Alam MA, Aktar R, Jahangir R, *In vitro* free radical scavenging activity of *Ixora coccinea* L., Bangladesh Journal Pharmacology, 3, 2008, 90-96.
16. Blois MS, Antioxidant determinations by the use of a stable free radical, Nature, 181, 1958, 181: 199-1200.
17. Jung MJ, Heo SI, Wang MH, Free radical scavenging and total phenolic contents from methanolic extracts of *Ulmus davidiana,* Food Chemistry, 108, 2008, 482-487.
18. Nishikimi M, Rao NA, Yagi K, The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen, Biochemical and Biophysical Research Communications, 46, 1972, 849-854.
19. Aiyegoro OA, Okoh AI, Phytochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of *Helichrysum*, International Journal of Molecular Sciences, 10, 2009, 4990-5001.
20. Dinis TCP, Madeira VMC, Almeidam LM, Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavenging, Archives of Biochemistry and Biophysics, 315, 1994, 161.
21. Dhalwal K, Deshpandey YS, Purohit AP, Kadam SS, Evaluation of antioxidant activity of *Sida cordifolia,* J Pharmaceutical Biology, 43, 2005, 754-761.
22. Marcocci L, Packer L, Droy-Lefaix MT, Sekaki A, Gardes-Albert M, Antioxidant action of *Ginkgo biloba* extract EGb 761, Methods in Enzymology, 234, 1994, 462-475.
23. Sultana B, Anwar F, Ashraf M, Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts, Molecules, 14, 2009, 2167-2180.
24. Guisti MM, Wrolstad RE, Characterization and measurement of anthocyanins by UV-visible spectroscopy, John Wiley & Sons, New York, 2001, F1.2.1-F1.2.13.
25. Wolfe K, Wu X, Liu RH, Antioxidant activity of apple peels, Journal of Agricultural and Food Chemistry, 51, 2003, 609–614.
26. Thimmaiah SK, Standard Methods of Biochemical Analysis, Kalyani Publishers, New Delhi, 2004, 304-307.

27. Furhman B, Volkova N, Suraski A, Aviram M, White wine with red wine-like properties: Increased extraction of grape skin polyphenols improves the antioxidant capacity of the derived white wine, Journal of Food Science, 49, 2001, 3164-3166.
28. Landbo AK, Meyer AS, Enzyme-extraction of antioxidative phenols from black currant juice press residues (*Ribes nigrum*), Journal of Agricultural and Food Chemistry, 49, 2001, 3169- 3177.
29. Gahler S, Otto K, Bohm V, Alteration of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products, Journal of Agricultural and Food Chemistry, 51, 2003, 7962–7973.
30. Gomez-Romero M, Arraez-Roman D, Segura-Carretero A, Fernandez-Gutierrez A, Analytical determination of antioxidants in tomato: Typical components of the Mediterranean diet Separation, Science and Technology, 30, 2007, 452–461.
31. Naczk M, Shahidi F, Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis, Journal of Pharmaceutical and Biomedical Analysis, 41, 2006, 1523-1542.
32. Van Acker SA, Van Den Berg DJ, Tromp MN, Griffioen DH,Van Bennekom WP, Vander Vijgh WJ, Bast A, Structural aspects of antioxidant activity of flavonoids, Free Radical Biology and Medicine, 20, 1996, 331-342.
33. Vasco C, Avila J, Ruales J, Svanberg U, Kamal-eldin A, Physical and chemical characteristics of golden-yellow and purple-red varieties of tamarillo fruit (*Solanum betaceum* Cav.), International Journal of Food Sciences & Nutrition, 60, 2009, 278-288.
34. Bulda O, Rassadina V, Alekseichuk H, Laman N, Spectrophotometric measurement of carotenes, xanthophylls, and chlorophylls in extracts from plant seeds, Russian Journal of Plant Physiology, 55(4), 2008, 544-551.
35. Bartley GE, Coomber SA, Bartholomew DM, Scolnik PA, Genes and enzymes for carotenoid biosynthesis,Academic Press, New York, 1991, 331-364.
36. Di Mascio P, Kaiser S, Sies H, Lycopene as the most efficient biological carotenoid single oxygen quencher, Archives of Biochemistry and Biophysics, 274, 1989, 532–538.
37. Gerster H, The potential role of lycopene for human health, Journal of the American College of Nutrition, 16, 1997, 109–126.
38. Zhang S, Tang G, Russell RM, Mayzel KA, Stampfer MJ, Willet WC, Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast cancer cases and control subjects, American Journal of Clinical Nutrition, 66, 1997, 626–632.
39. Goncalves C, Dinis T, Batista MT, Antioxidant properties of proanthocyanidins of *Uncaria tomentosa* bark decoction: a mechanism for anti-inflammatory activity, Phytochemistry, 66, 2005, 89-98.
40. Koleva II, Van Beek T, Linssen JPH, De Groot A, Evstatieva LN, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods, Phytochemical Analysis, 13, 2002, 8-17.
41. Gordon A, Papagiannopoulos M, Marx F, Antioxidant Capacity of Tamarillo Fruit (*Cyphomandra betacea*), Journal of Agricultural and Food Chemistry, 53, 2005, 103-110.
42. Jornot L, Petersen H, Junod AF, Hydrogen peroxide induced DNA damage is independent of nuclear calcium but dependent on redox active ions, Biochemical Journal, 335, 1998, 854-855.
43. Halliwell B, Superoxide Dependent formation of hydroxyl free radicals in the presence of iron chelates, FEBS Letters, 92, 1978, 321.
44. Cheng Z, Su L, Moore J, Zhou K, Luther M,Yin JJ, Yu LL, Effect of postharvest treatment and heat stress on availability of wheat antioxidants, Journal of Agricultural and Food Chemistry, 54, 2006, 5623-5629.
45. Zhao HF, Fan W, Dong JJ, Lu J, Chen J, Shan LJ, Lin Y, Kong WB, Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties, Food Chemistry, 107, 2008, 296–304.
46. Maisuthisakul P, Pongsawatmanit R, Gordon MH, Characterization of the phytochemicals and antioxidant properties of extracts from Teaw (*Cratoxylum formosum Dyer*), Food Chemistry, 100, 2007, 1620-1629.
47. Bor JY, Chen HY, Yen GC, Evaluation of Antioxidant Activity and Inhibitory Effect on Nitric Oxide Production of Some Common Vegetables, Journal of Agricultural and Food Chemistry, 54, 2006, 1680-1686.
48. Aires A, Fernandes C, Carvalho R, Bennett RN, Saavedra MJ, Rosa EA, Seasonal effects on bioactive compounds and antioxidant capacity of six economically important brassica vegetables, Molecules, 16, 2011, 6816-6832.
49. Ninfali P, Mea G, Giorgini S, Rocchi M, Bacchioca M, Antioxidant capacity of vegetables, spices and dressing relevant to nutrition, British Journal of Nutrition, 93, 2005, 257-266.
50. Wong SP, Leongm LP, Koh JHW, Antioxidant activities of aqueous extracts of selected plants, Food Chemistry, 99, 2006, 775-783.
51. Arnous A, Makris DP, Kefalas P, Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece, Journal of Food Composition and Analysis, 15, 2002, 655–665.
52. Pulido R, Bravo L, Saura-Calixto F, Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing antioxidant power assay, Journal of Agricultural and Food Chemistry, 48, 2000, 3396–3402.

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