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Effect of Processing on Nutritional Quality and Antioxidant Potentials of Leafy Vegetables

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Abstract

Blanching is a primary step in processing of Green Leafy Vegetables. Despite of its preserving advantage, it leads to partial destruction of some nutrients like vitamin C which is heat liable and sensitive to light, oxygen and oxidizing agents. The study was carried out to identify a suitable blanching temperature, time and chemical media for the green leafy vegetables namely, *Alternanthera sessilis*, *Cardiospermum helicacabum* and *Celosia argentea* that ensures enzyme inactivation and maximum nutrient retention. The leaves were processed by the following methods (i) Blanched at 80°C, 90°C and 100°C for 5 min in distilled water (ii) Blanched in water containing chemical media (potassium metabisulphite (KMS), sodium bicarbonate and sodium chloride) at 80°C for the 1 min, 2 min and 4 min respectively. Blanching time and temperature increased, there is a reduction in the retention of Vitamin C in all the greens. The statistical analysis (P≤0.05) showed significant retention of vitamin C on blanching the leaves at 80°C for 1 min in potassium metabisulphite. Reduction in the moisture content, fiber, Iron was also found to be statistically significant. Blanching at 80°C for 1 min in potassium metabisulphite was sufficient to inactivate peroxidase in leafy vegetables. The antioxidant properties of the fresh and blanched green leafy vegetables were subsequently determined. The study revealed the presence of phenolics (gallic acid equivalents 3.89-8.55 mg/g), flavonoids (quercetin equivalents 9.47-37.66 mg/g) and tannins (tannic acid equivalents 10.47-13.58 mg/g). Three of the samples were exhibited remarkable DPPH radical scavenging activities (>70%) with significant IC_{50} values of 653.10 and 760.34 µg/ml respectively. The result showed that blanching at 80°C for 1 min with potassium metabisulphite as a chemical media had a good retention of Vitamin C and least effect on other nutritional content. Hence it is found that blanching is the best method of preserving GLV without compromising its nutritional quality and antioxidant potentials.

Keywords: Green leafy vegetables; Blanching; Antioxidant activity; Vitamin C; Nutritional quality

Introduction

Green Leafy Vegetables (GLVs) are the most nutritious agricultural products and are widely and preferably consumed in fresh. They are rich sources of calcium, iron, β-carotene, Vitamin C, dietary fiber and many trace elements. India is blessed with an array of leafy vegetables of which some are cultivated many are gathered [1]. Fruits and vegetables contain different antioxidant compounds such as Vitamin C, vitamin E and carotenoids, whose activities have been established in recent years. Flavonoids, tannins and other phenolic constituents present in food of plant origin are also potential antioxidants [2,3]. The potentially cancer inducing oxidative damage might be prevented or limited by dietary antioxidants found in fruits and vegetables. Studies to date have demonstrated that phytochemicals in common fruits and vegetables can have complementary and overlapping mechanisms of action, including scavenging of oxidative agents, stimulation of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism, antibacterial and antiviral effects [4]. Imbalance of oxidizing agents and natural antioxidants in the body results in oxidative stress and triggers pathogenic advancement of some severe diseases such as, atherosclerosis, liver cirrhosis, cataracts, cardiovascular, cancer, neurodegenerative disorders and diabetes [5].

Green leafy vegetables are generally consumed in the cooked form apart from the salads. Therefore, there is a need to assess the changes that occur in the antioxidant activity on cooking. Few works have reported the effect of cooking on antioxidant activity of vegetables. Blanching the Nigerian GLV reduces their antioxidant properties drastically [6]. The uncooked GLV had significantly higher values of TBARS when compared to the cooked vegetables [7]. Some evidences showed that Generation of oxygen radicals, such as superoxide radical (O_2^{-1}), hydroxyl radical (OH^-) and non-free radical species, such as H_2O_2 and singlet oxygen (O_2^{-1}) are associated with cellular and metabolic injury and accelerating aging, cancer, cardiovascular diseases, neurodegenerative diseases and inflammation [8,9]. Natural antioxidants like α-tocopherol and synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene, tert-butyl hydroquinone and propyl gallate are widely used as food preservatives. But the use of these synthetic antioxidants is increasingly getting limited because of their toxicity and detrimental effects on health [10]. It is the largest producer of fruits and vegetables in world. It is estimated that India processes less than 1% of production and about 30% to 35% production cannot be utilized due to lack of adequate technology for processing, handling, storage and processing infrastructure [11].

Blanching is a primary step in processing of vegetables. It stops the enzyme action, sets the colour and shortens the drying and dehydration time. This technique is used by indigenous people to reduce or eliminate the bitterness of the vegetables and acid components that are common on leaves [12]. More than 90% of the vitamin C in human diets are supplied by fruits and vegetables. But vitamin C is most difficult of the vitamin to preserve during blanching and dehydration [13]. Controlled blanching can contribute to retention of vitamins and nutrients in

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processed foods [14,15]. Peroxidase is one of the most heat stable enzymes and is often used as an index of blanching [16]. Peroxidase inactivation is faster at higher temperature [17].

The present study aims at drawing a comparative sketch of some green leafy vegetables and the effect of blanching on nutritional value and antioxidant potentials of selected widely consumed in Tamil Nadu and other parts of India.

Materials and Methods

Plant materials

The leafy vegetables were procured from local vegetable market such as *Alternanthera sessilis* (L) Family: Amaranthaceae (local name: Ponnagani), *Celosia argentea*(L) Family: Amaranthaceae (local name: Pannai Keerai), *Cardiospermum helicacabum*(L) Family: Sapindaceae (local name: modikkottan) at Chidambaram, Tamil Nadu. Plants were identified and the voucher specimens and plants were kept preserved in the herbarium collections of the Department of Botany, Annamalai University and Food Processing Laboratory, Department of Chemical Engineering, Annamalai University. The leaves were separated in edible portions and washed under running water to remove the adhering mud particles and drained completely.

Blanching

Washed leaves were processed by the following methods: (i) Blanched at 80°C, 90°C and 98°C for 5 min in water, (ii) Blanched in a) water containing potassium metabisulphite (KMS) (5 g/L), b) water containing sodium bicarbonate (1 g/L) and c) water containing Sodium chloride (20 g/L) at 80°C for 1 min, 2 min and 4 min respectively. The time required for blanching was judged by residual peroxidase activity.

Analysis

The fresh green leafy vegetables and blanched green leafy vegetables were analyzed for the following components to study the effect of blanching. Moisture content was determined by standard method of AOAC [18]. Protein was estimated by Lowry et al. [19] and Fiber was determined by AOAC method [20]. The blanched samples are tested for peroxidase inactivation by Guiacol peroxide [21]. The Vitamin C content was determined using DNPH (Di Nitro Phenyl Hydrazine) method [22]. Iron content of the fresh and blanched leaves was determined using Atomic Absorption Spectrophotometer by Vogel method.

Determination of phenolics, flavonoids and tannin contents

Sample preparation for phenolic and flavonoids content. 0.5 g of each of the powdered tissues was weighed and extracted with 50 ml 80% aqueous methanol for 2.5 h at room temperature with intermittent shaking. The extract was then centrifuged at 14,000 rpm for 5 min and the supernatant obtained was used for the assays.

Total phenolics determination

Total phenolics content was determined by the Folin-Ciocalteu colorimetric method [23] using gallic acid as a standard phenolic compound. 450 µl of distilled water was added with 50 µl of the extract and treated with 2.5 ml of 0.3 N Folin-Ciocalteu reagent. After 5 min, 2 ml of 7.5% Na_2CO_3 solution was added and the mixture was incubated at 30°C for 1.5 h with intermittent shaking. The absorbance of the resulting blue colored solution was measured at 765 nm against blank. The evaluation was carried out, based on the six-point standard calibration curve of gallic acid (20, 100, 200, 300, 400, 500 mg/L) in 80% aqueous methanol. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry material.

Total flavonoids determination

Total flavonoids content was measured by the aluminum chloride colorimetric assay [24] using quercetin as a standard flavonoid. 1 ml of the extract was added to 4 ml of distilled water and 0.3 ml of 5% $NaNO_2$ and the mixture was incubated at room temperature for 5 min. After incubation, the mixture was treated with 0.3 ml 10% $AlCl_3$ solution. After 1 min, 2 ml 1 M NaOH was added and 2.4 ml distilled water was added to the whole solution. The solution was mixed well and the absorbance was measured at 510 nm against blank. The assay was performed, based on the 6 point standard calibration curve of quercetin (40, 80, 120, 160, 200, 300 mg/L) in 80% aqueous methanol. The total flavonoid content was expressed as quercetin equivalents (QE) in milligrams per gram of dry material.

Tannin determination

Tannin determination was carried out according to the method described by Van and Robinson [25] with minor modifications as described by Kaur and Arora [26] using tannic acid as standard. 50 ml of distilled water was added to 250 mg of powdered sample and shaken for 1 h using a mechanical shaker. It was filtered into a 50-ml volumetric flask and the final volume was adjusted with distilled water. Then 1 ml of the filtrate was added to 4 ml of distilled water and treated with 2 ml (10 fold diluted) of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The solution was mixed well and the absorbance was measured at 605 nm within 10 min against blank. The evaluation was performed based on the 5 points standard calibration curve of tannic acid (20, 40, 60, 80, 100, 140, 200 mg/L) in distilled water. The tannin content was expressed as tannic acid equivalents (TAE) in milligrams per gram of dry material.

Antioxidant activity determination by DPPH assay

The free radical scavenging activity of methanolic extract was determined according to Liu et al. [27]. Briefly, the sample stock solutions (1 mg/ml) were diluted with methanol to final concentrations of 5, 10, 20, 40, 60, 80, 100 and 200 µg/ml. 2 ml of each of the sample solution at different concentrations was mixed with 1 ml of 0.25 mM DPPH solution in methanol and allowed to react at room temperature in dark for 30 min. The change in color was observed in terms of absorbance using a spectrophotometer at 517 nm. And 1 ml of methanol served as control. Quercetin was used as the standard antioxidant. The percentage of scavenging activity was calculated using the formula,

DPPH radical scavenging activity (%)=((A_0 of control-A_1 of sample)/A_0 of control) × 100.

Where,

A_0: Absorbance of the control

A_1: Absorbance of the sample extract/ standard

Lower IC_{50} value indicated higher free radical scavenging activity.

Determination of reducing power

The reducing power of the extracts was determined according to the method of Oyaizu [28]. Different amounts of methanolic extracts (100, 200, 300, 400 and 500 µg) in 1 ml of methanol was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After 2.5 ml of 10% TCA were added, the mixture was centrifuged at 650

rpm for 10 min. The upper layer (2.5 ml) solution was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% $FeCl_3$, and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis

All the analyses were carried out in triplicate and expressed as mean standard deviation (SD). The calculation of means, SD, IC_{50} values and analysis of variance (ANOVA) were carried out using Microsoft Office Excel 2007 and Duncan's multiple range tests (DMRT) were performed to study the differences at 5% level of significance among the phytochemical contents and antioxidant activities of the experimental leafy vegetables.

Results and Discussion

Effect of blanching temperature on retention of vitamin C

The effects of blanching temperature on retention of vitamin C are shown in Table 1. Vitamin C content of all greens blanched at 80°C showed a reduction but the extent of loss varied between the vegetables. Retention was comparatively higher in *Alternanthera sessilis* (>50%), moderate in *Cardiospermum helicacabum* (20%) and lower in *Celosia argentea* (Table 1). The loss of Vitamin C in the leafy vegetables treated under identical temperature could be attributed to the fact that vitamin C is not stable at high temperature. With increase in temperature by another 10°C, showed a further reduction of (1%-10%) in all the three samples. When the temperature of blanching in water was raised to 100°C, *Alternanthera sessilis* and *Cardiospermum helicacabum*, which exhibited a higher retention of vitamin C at 80°C and 90°C, reduced by another 3%-5% while in *Celosia argentea* it was marginal. Vitamin C is a highly soluble substance and thermal processing results in maximum losses [29]. Increase in temperature though reduced the Vitamin C content, the extent of difference between the samples treated with other temperature was not significant (P>0.05). These findings are in accordance to those reported by Gupta and Prakash [30] wherein they have shown that there is an increase in the loss of ascorbic acid in *Amaranthus tricolor* with every 10°C rise in the temperature of blanching media.

Effect of blanching time and media on retention of Vitamin C

When the greens blanched at 80°C for 1 min in distilled water, *Celosia argentea* showed least retention (5%-10%) while *Alternanthera sessilis* and *Cardiospermum helicacabum* showed a moderate retention (20%-30%) of Vitamin C as shown in Table 2. With increase in blanching time for another 1 min, *Alternanthera sessilis* and *Celosia argentea* showed a further loss of 5%-10% while in *Cardiospermum helicacabum* it was marginal. When the blanching time increased to 4 min, *Celosia argentea* and *Cardiospermum helicacabum* showed a reduction of 10% and 20%, respectively in comparison with the samples blanched for 2 min. Analysis of variance revealed that increasing the blanching time by 1-2 min at same temperature is not significantly affect the losses of nutrients (P>0.05).

Leafy vegetables	Vitamin C			
	mg/100 g	% retention		
		Blanched for 4 minutes in water		
		80°C	90°C	100°C
A. sessilis	22.3	26.8	16.9	10.5
C. helicacabum	44.6	22.7	14.0	11.9
C.argentea	75.2	8.4	8.0	7.2
Statistical analysis				
F- ratio	-	1.73^{ns}		
ns: Not significant				

Table 1: Effect of blanching temperature on the retention of Vitamin C (%).

Leafy Vegetables	Blanched at 80ºC											
	Time in minutes											
	KMS			NaCl			$NaHCO_3$			Water		
	1	2	4	1	2	4	1	2	4	1	2	4
A. sessilis	22.9	15.9	9.2	17.9	15.3	12.2	26.9	23.4	13.5	26.8	10.5	8.7
C. helicacabum	32.9	14.5	11.6	40.9	34.6	17.3	45.3	32.8	15.3	22.7	11.7	7.0
C. Argentea	15.8	11.7	8.2	22.3	21.3	9.8	23.4	17.4	12.9	8.4	7.4	5.4
Statistical analysis												
Time variant	KMS			NaCl		$NaHCo_3$		Water				
F-ratio	5.84^{*}			1.82^{ns}		3.66^{ns}		3.67^{ns}				
Time vs Media												
Time in min.				F-ratio								
1				0.73^{ns}								
2				3.76^{*}								
4				5.50^{*}								
ns: Not Significant; *: Significant at 5% level												

Table 2: Effect of blanching media and time on the retention of Vitamin C (%).

The blanched leafy vegetables in KMS solution at 80°C for 1 min showed better retention of vitamin C where in other media, salt and sodium carbonate showed a least retention. The results shown in Table 2 with increase in blanching time to 4 min, *Alternanthera sessilis* and *Cardiospermum helicacabum* showed a further loss of 10% while in *Celosia argentea* the losses were remarkable. No significant losses were encountered with increase in the blanching time by 1-2 min at the same temperature in potassium metabisulphite. The GLV of blanched at 80°C for 1 min in KMS had 10%-15% higher retention of ascorbic acid compared to other media. Even with increase in blanching time by 1-2 min, potassium metabisulphite blanched greens showed statistically significantly (P<0.05) retention over other blanching media. Similar observations were reported for fenugreek leaves and other vegetables [31]. These findings infer that blanching media offer a protection against oxidation of vitamin C and thereby a higher retention of the water-soluble vitamin was observed.

Effect of blanching on nutritional content of green leafy vegetables

The values obtained for nutrient content of green leafy vegetables were higher than the values reported by Narasingha et al. [32]. Moisture content of blanched leaves is 70% for *Alternanthera sessilis,* 82.7% for *Cardiospermum helicacabum* and 83.4% for *Celosia argentea* (Table 3). Reduction in the moisture content on blanching was found to be statistically significant (P<0.05). Nkafamiya et al. [33] reported that the moisture content was lower in blanched sample of some vegetable**.** However, the high moisture content indicates short shelf life and high vulnerability to microbial attack.

The protein content of fresh and blanched GLV was found to be similar. The slight variations that observed were found to be statistically insignificant (P>0.05). This agrees with the findings of Sobowale et al. [34]. In fresh samples the fiber was found to be in the range of 0.2-2.7 g (fresh weight basis). Blanching caused significantly higher (P<0.01) reductions in the fiber content. According to Punna and Rao [35] no

significant effect of processing /cooking on insoluble dietary fiber content of green leafy vegetables. Among the fresh GLV, *Celosia argentea* had the maximum amount of iron, whereas *Alternanthera sessilis* and *Cardiospermum helicacabum* had the least. Blanched *Celosia argentea* had 22.5 mg/100 g whereas *Alternanthera sessilis* and *Cardiospermum helicacabum* had 1.96 mg/100 g and 1.88 mg/100 g. The results (Table 3) indicate that blanching caused significantly higher (P<0.01) reductions in the Iron content of Green Leafy Vegetables. Oladunmoye et al. [36] were observed similarly that reduction in iron content of blanched and cooked tender and mustard cassava leaves.

Phenolics, flavonoids and tannin content determination

Phenolics are aromatic secondary plant metabolites widely spread throughout the plant kingdom. 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 [37]. The phenolic compounds evolved to protect the plants from herbivores. It has been reported that by acting as antioxidants the phenolics counteract the photo damage and their level varies with the extent of exposure [38]. The correlation between the phenolic content and the observed antioxidant activity of a particular plant species are evident from a number of publications [39,40]. Shahidi et al. [41] reported different classes of phenolics possessing varying antioxidative strengths and the synergistic effect of polyphenolics (flavonoids, condensed tannins and gallotannins) in the observed antioxidant activity. Flavonoids constitute majority of the polyphenolic compounds and include anthocyanins, proanthocyanins, flavonols and catechins [42]. Flavonoids act by scavenging or chelating [43]. All these compounds found in plants therefore have great beneficial effects on human health. This study was initiated to investigate these potential sources of natural antioxidants in leafy vegetables that are consumed in many parts of India. The total phenol content of GLV is presented in Table 4. The GLV were found to have varying levels of phenol, ranging *C. argentea* 3.89 to 8.55 for *A. sessilis*. Kaur and Kapoor [44] reported the total phenolic content of *Trigonella foenumgraecum* to be 217.5 mg of catechol/100 g of fresh vegetable. Salvatore et al. [37] in their study on antioxidant characterization of some Sicilian edible greens stated that the frequently consumed greens in the Mediterranean areas were very rich in antioxidants such as flavonoids and carotenoids. The total polyphenol content of some common Indian leafy vegetables was found to be in the range of 5 mg-69.5 mg of tannic acid/g of extract [45]. Therefore, it can be said that the total polyphenol content of vegetables varies widely depending on the variety of vegetable and a comparison is difficult, as different standard compounds have been used for their analysis. Our results were revealed that highly significant differences in the total phenol content of the GLV analyzed.

GLV/ Nutrient	*A.sessilis*	*C.helicacabum*	*C. argentea*	F-ratio
Moisture (%)				
Fresh	75	84	85	17.12*
Blanched	70.2	82.7	83	
Protein (g)				
Fresh	5.6	4.2	3.8	0.68^{ns}
Blanched	3.5	3.9	3.2	
Fiber (g)				
Fresh	2.7	0.2	2.4	54.5*
Blanched	2.4	-	1.9	
Iron (mg/100 g)				
Fresh	1.93	2.03	24.6	422.4*
Blanched	1.88	1.96	22.5	
ns: Not Significant; *: Significant at 5% level				

Table 3: Effect of blanching on nutritional content of green leafy vegetables.

DPPH radical scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The methodology involves reaction of specific compounds or extracts with DPPH in methanol solution. In the presence of hydrogen donors, DPPH is reduced and a free radical is formed from the scavenger. The reaction of DPPH is monitored by the decrease of the absorbance of its radical at 517 nm, but upon reduction by an antioxidant, the absorption disappears [46]. Exponential increase of antioxidant activity due to the development of the reducing power signifies that the antioxidant properties are associated with the development of reducing power [46,47]. DPPH radical scavenging activity of the GLV extracts at varying concentrations (6.37-18.42 mg/ml) were measured and the results are depicted in Table 5. All the GLV studied showed appreciable free radical scavenging activities. Of the three leafy vegetable extracts tested, extracts of *A. sessilis* had the strongest radical scavenging activity compared to the other GLV (83.44%). *A. sessilis* has been found to exhibit the most potent DPPH scavenging activity followed by *C. helicacabum*. The IC_{50} value of *C. argentea, A. sessilis* and *C. helicacabum* is 760.34, 653.10 and 590.54 µg/ml, respectively. The strong DPPH radical scavenging activity of *E. fluctuans* is in line with earlier studies by Dasgupta and De [48]. The IC_{50} value of the reference compound quercetin has been found to be 3.65 µg/ml and has exhibited higher activity than all the leafy vegetables extracts tested. ANOVA analysis has shown the presence of highly significant differences in the free radical scavenging activity of the different species of leafy vegetables studied (P=0.000). Use of purified antioxidative compounds of phenolic origin from the plant species with potent antioxidant activities might exhibit better activity than observed with crude extracts. Phenolic compounds isolated from the genus *Corchorus* have been reported to possess significant antioxidant activity in the linoleic acid peroxidation system [49].

Reducing power determination

The antioxidant effect exponentially increases as a function of the development of the reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power [28]. Reducing power of tannins from medicinal plants prevents liver injury by inhibiting formation of lipid peroxides [50]. The antioxidant and reducing capacity of spices is closely related to the presence of chemical constituents with antioxidant activity, mainly to the phenolic

Leafy Vegetables	Total phenolics (mg GAE/g dry weight)	Total flavonoids (mg QE/g dry weight)	Total tannin (mg TAE/g dry weight)
A.sessilis	8.55 ± 0.08	9.47 ± 1.03	13.58 ± 0.75
C. helicacabum	7.89 ± 0.29	37.66 ± 2.04	10.47 ± 0.15
C. argentea	3.89± 0.13	17.54 ± 2.74	12.14 ± 0.78
Each value is the mean ± standard deviation from three replicates			

Table 4: Quantitative determination of phytochemicals.

DPPH radical scavenging activity (%)[a]									
Leafy Vegetables	**5 µg/mL**	**10 µg/mL**	**20 µg/mL**	**40 µg/mL**	**60 µg/mL**	**80 µg/mL**	**100 µg/mL**	**200 µg/mL**	**IC_{50} µg/mL**
A. sessilis	6.57 ± 1.15	8.35 ± 0.24	8.64 ± 0.13	9.39 ± 0.16	10.90 ± 0.78	12.22 ± 1.16	12.74 ± 0.68	18.42 ± 1.10	653.10
C. helicacabum	6.75 ± 1.46	7.56 ± 1.62	8.38 ± 2.36	9.63 ± 1.51	10.67 ± 0.08	11.80 ± 0.46	13.72 ± 0.41	22.15 ± 0.76	590.54
C. argentea	6.37 ± 0.97	6.45 ± 0.86	6.53 ± 0.69	7.60 ± 0.40	8.65 ± 1.00	9.55 ± 1.06	11.46 ± 1.22	15.21 ± 1.26	760.34
-	0.5 µg/mL	1 µg/mL	2 µg/mL	3 µg/mL	4 µg/mL	5 µg/mL	6 µg/mL	7 µg/mL	
Quercetin	8.68 ±1.40	14.21 ± 1.48	23.50 ± 1.38	38.47 ± 1.23	51.44 ± 1.22	83.41 ± 1.00	67.65 ± 0.68	86.40 ± 0.21	3.65
[a]Each value is the mean ±standard deviation from three replicates									

Table 5: *In vitro* free radical scavenging activity by DPPH method.

Reducing power assay (absorbance)[a]					
Plants	**100 µg/mL**	**200 µg/mL**	**300 µg/mL**	**400 µg/mL**	**500 µg/mL**
A. sessilis	0.013 ± 0.001	0.037 ± 0.004	0.070 ± 0.016	0.085 ± 0.019	0.096 ± 0.003
C. helicacabum	0.040 ± 0.005	0.089 ± 0.006	0.121 ± 0.002	0.149 ± 0.005	0.190 ± 0.004
C. argentea	0.018 ± 0.002	0.055 ± 0.009	0.069 ± 0.002	0.087 ± 0.001	0.106 ± 0.002
-	5 µg/mL	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
Quercetin	0.078 ± 0.020	0.288 ± 0.016	0.514 ± 0.029	0.724 ± 0.039	0.908 ± 0.028
[a]Each value is the mean ± standard deviation from three replicates					

Table 6: *In vitro* antioxidative activity by reducing power determination.

compounds [51-54]. The antioxidant capacities were in the order of *A.sessilis*>*C.helicacabum*>*C.argentea* (Table 6). There are very few studies on the antioxidant capacity of vegetable measured by the phosphomolybdenum method. Dasgupta and De [48] studied the antioxidant capacity of three varieties of Piper betle leaves and reported that the Kauri variety had higher total antioxidant capacity (equivalents of gallic acid) in comparison with tea. The activity of the reference compound quercetin is higher than the experimental leafy vegetables. ANOVA analysis (P=0.000) has suggested the presence of significant differences in the reducing power of these leafy vegetables.

Conclusion

In the present study, all of the three leafy vegetables *Alternanthera sessilis*, *Cardiospermum helicacabum* and *Celosia argentea* have been found Vitamin C was sensitive to heat and oxidation during blanching. Among the plants selected, *Alternanthera sessilis* had a better retention of Vitamin C followed by *Cardiospermum helicacabum* and *Celosia argentea*. Potassium metabisulphite showed better retention of vitamin C followed by sodium bicarbonate and sodium chloride. Blanching at 80°C for 1 min ensured peroxidase inactivation in all greens. The losses of nutritional content protein, moisture, fiber and iron were marginal. But Vitamin C which is a major antioxidant known for proper functioning of the human body had a better retention during blanching**.** Thus, it can be suggested that blanching at 80°C for 1 min in Potassium metabisulphite are the most ideal for blanching greens. These GLVs have been found to possess minor to major DPPH radical scavenging activities. Of all the leafy vegetables tested, *A. sessilis*, *C. helicacabum* and *C. argentea* possess the maximum activity with regard to the antioxidative potential. Pertaining to the stability of antioxidative activity of leafy vegetables at higher temperatures suggests that the consumption of leafy vegetables in daily diet either in raw or in cooked form can be a good source of natural antioxidants, beneficial for health. Therefore, the leafy vegetables can be potential sources of natural antioxidants for domestic consumption as well as possible substitutes for synthetic antioxidants for industrial purpose. These above study results may serve as a guide to select blanching temperature, time and media for processing the vegetables. In conclusion, these findings may be useful for the selection of spices for further application in different food formulations to support their function as antioxidants. Statistical package was found to be an effective tool for analysis of variance.

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