

Antioxidant Activity of Selected Organic Green Leafy Vegetables

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Abstract: The present study was conducted with the objectives such as ascertaining the presence of phytochemicals in the selected green leafy vegetables, determining the total antioxidant activity by using DPPH radical scavenging assay and determining the total phenol. The experimental procedures were used to find out the antioxidant activity. Standard procedures were used to assess the alkaloids and tannin content, total antioxidant activity and total phenol content. Twelve different leafy vegetables collected from Payyanad Village, Malappuram District, Kerala were used for the analysis. Alkaloid and tannin content assessed in the selected green leafy vegetables revealed that drumstick leaves, curry leaves, chekkurmanis, green amaranth and amara leaves showed a positive result to alkaloids test. The presence of tannins was found only three selected green leafy vegetables which are pumpkin leaves, chekkurmanis and curry leaves. Pumpkin leaves, sambar cheera and bottle gourd leaves shows the total antioxidant activity. With regard the total phenol content curry leaves, drumstick leaves, chekkurmanis, red amaranth, green amaranth and amara leaves have high content of phenol. Hence, it is summarised that green leafy vegetables have antioxidant activity and this will help to reduce several chronic and degenerative diseases including atherosclerosis, heart diseases, diabetes mellitus and cancer, etc.

Keywords: Antioxidant, Phytochemicals, organic, free radicals, green leafy vegetables

INTRODUCTION

Green leafy vegetables are used since ancient periods as source of food as they contain many nutrients and minerals which are helpful in maintaining human health. Plant foods are sources of energy and micronutrients essential to health, in addition to phytochemicals with further health benefits including glycemic control, immune-stimulation or antioxidant

activity. Traditional vegetables are valuable sources of nutrition in poor rural areas. Green leafy vegetables hold an important place in well-balanced diets (Gacch et al., 2010).

Plants are considered to be biosynthetic innovative, which produce primary and secondary metabolites. Many primary metabolites like carbohydrates, proteins and lipids and secondary metabolites like glycosides, alkaloids, tannins, volatile oils etc., which have therapeutic effects in human beings. Green leaves are found to be an excellent source of minerals like iron, selenium, copper, zinc and also of antioxidants like carotene and ascorbic acid. Since green leafy vegetables supply not only iron but also vitamin C and folic acid, hence their increased consumption can play a role in control of anemia. Green leafy vegetables are offering a cheap but rich source of a number of micronutrients and other phytochemicals having antioxidant properties (Tarwadi et al., 2003).

In a country like India, where most of the people are engulfed in poverty and cannot afford expensive food products, there is an immediate need to identify cheap and easily available sources rich in Iron, Vitamin A and other micronutrients and green leafy vegetables can be one of them (Joshi et al., 2010).

The present study entitled 'Antioxidant activity of selected organic green leafy vegetables' was undertaken with the aim of evaluating the antioxidant activity of selected organic green leafy vegetables. Antioxidants in green leafy vegetables provide a multitude of health benefits.

The specific objectives of the study are to ascertain the presence of phytochemicals in selected green leafy vegetables, to find out the total antioxidant activity by using DPPH radical scavenging assay and to

determine total phenol content present in the selected green leafy vegetables

MATERIALS AND METHODS

Selection of green leafy vegetables

Twelve different green leafy vegetables were selected for the present study. The samples that are selected for the present study were further used for the phytochemical screening, total antioxidant activity and total phenolic content.

The selected samples were as follows:

Table 1: Selected Green Leafy Vegetables

Sl. No	Scientific Name	Common Name	Malayalam Name
1	<i>Cucurbita maxima</i>	Pumpkin leaves	Mathayila
2	<i>Moringa oleifera</i>	Drumstick leaves	Muringayila
3	<i>Vigna radiata</i>	String bean leaves	Payarila
4	<i>Sauropus androgynous</i>	Chekkurmanis	Velicheera
5	<i>Cephalandra indica</i>	Ivy gourd leaves	Kovai ila
6	<i>Benincasa hispida</i>	Ash gourd leaves	Cumbalanga ila
7	<i>Murraya koenigii</i>	Curry leaves	Curryvepila
8	<i>Thalassia asiaticam</i>	Sambar cheera	Sambar cheera
9	<i>Amaranthus viridis</i>	Amaranth Green	Pacha cheera
10	<i>Lagenariasiceraria</i>	Bottle gourd leaves	Churakka ila
11	<i>Phaseolus lunulatus</i>	Lima bean leaves	Amarakka ila
12	<i>Amaranthustricolor</i>	Amaranth red	Chuvanna cheera

The plants were cultivated by the investigator in her own home at Payyanad village, Malappuram District, Kerala. The soil in which the plants were grown had not been applied with any prohibited substances (pesticides and chemical fertilizers) any time earlier. USDA (2012) recommends a non-applicative period of three years for a produce to be certified as 'organic'. Therefore, the home-grown leaves used for the present study was termed as 'organic'.

The leaves were washed and used for analysis. Double distilled water was used for the preparation of reagents used in the entire analysis. All chemicals used for the study were of analytical grade.

Phytochemical screening of the selected green leafy vegetables

The study of antioxidant activity of the samples was done in the Biochemistry laboratory of St. Teresa's College, Ernakulum. Standard procedures were used to assess the alkaloid and tannin content, total antioxidant activity and total phenol content.

Preparation of the Extract for Phytochemical Analysis
Methanolic extracts were used for the determination of alkaloid and tannin content, total antioxidant activity and total phenol content. Fresh leaves of the plants were collected, washed and homogenized using methanol for the preparation of aqueous and

methanolic extracts. Samples were ground in a mortar and pestle and 2gm of grounded samples were homogenized by using 20 ml of methanol. These extracts were then centrifuged at 2000 rpm for 10 minutes and the supernatant was collected in 50ml volumetric flask and that extract was used for further study.

Phytochemical screening of the extract

Phytochemical screening was performed using standard procedures. All the chemicals used in the study were analytical grade.

a) Test for Alkaloids:

Two tests were performed using Meyer's reagent and Wagner's reagent respectively.

b) Test for Tannins

The test for tannins was performed using lead acetate.

Total antioxidant activity of leafy vegetables

The antioxidant activity of methanolic extracts of samples was measured using DPPH radical scavenging assay (Ayoola, 2008). DPPH radical scavenging assays are electron transfer-based assays. Vitamin C was used as standard in DPPH assay for comparing the antioxidant activity of green leafy vegetables.

The radical-scavenging activity was determined using Diphenyl Picryl Hydrazyl (DPPH) radical. This

provides information on the reactivity of the test compounds with a stable free radical and gives a strong absorption band at 517nm in the visible region. The absorbance of the mixture was measured spectrophotometrically at 517nm using vitamin C as reference.

Total phenol content of selected green leafy vegetables
The total phenol content was estimated by Folin Ciocalteu method. Phenolic contents were estimated as per the procedure described by Sridevi et al., (2014). Folin Ciocalteu rely on the reducing ability of phenols to quantify the amount of total phenolics in the sample. Absorbance of the extract was measured spectrophotometrically at 650nm.

RESULT AND DISCUSSION

Twelve different locally available green leafy vegetables in Kerala were identified for the present study. Nine of these green leafy vegetables were underutilized, less familiar green leafy vegetables. Three of the selected green leafy vegetables i.e., curry leaves, green cheera and red cheera were included as those were the most commonly used green leafy vegetables. Many of these selected green leafy vegetables are creepers and some are short herbaceous plants. Pumpkin leaves, ivy gourd leaves, string bean

leaves, ash gourd leaves, bottle gourd leaves and Amara leaves are creepers. Chekkurmanis, green amaranth, red amaranth and sambar cheera are short lived herbaceous plants. Woody plants like curry leaves and drumstick leaves whose leaves can also be used were included as green leafy vegetables.

Phytochemical screening of selected green leafy vegetables

Qualitative analysis was carried out to ascertain the presence of phytochemicals. The phytochemical screening of methanolic extracts of the plant samples were evaluated by different standard procedures. The study revealed the presence of two types of phytochemicals which included alkaloids and tannins.

Presence of Alkaloids

Most alkaloids are precipitated from neutral or slightly acid solution by Mayer's reagent (potassiomeric iodide solution), by Wagner's reagent (solution of iodine in potassium iodide), by Hager's reagent (a saturated solution of picric acid), or by Dragendorff's reagent (solution of potassium bismuth iodide).

Phytochemical investigations of the samples were done to test the presence of alkaloids in twelve selected green leafy vegetables. Results are given in table 2.

Table 2: Presence of alkaloids in selected GLVs

Sl No	Common Name of the sample	Presence of alkaloids
1	Pumpkin leaves	Absent
2	Drumstick leaves	Present
3	String bean leaves	Absent
4	Chekkurmanis	Present
5	Ivy gourd leaves	Absent
6	Ash gourd leaves	Absent
7	Curry leaves	Present
8	Sambar cheera	Absent
9	Green amaranth	Present
10	Bottle gourd leaves	Absent
11	Lima bean leaves	Present
12	Red amaranth	Absent

Drumstick leaves, curry leaves, Chekkurmanis, green amaranth and Amara leaves showed a positive result to alkaloids test in the selected green leafy vegetables. Thus, it was found that only five out of twelve GLVs had the presence of alkaloids.

Preliminary phytochemical screening of *Murraya koenigii* by Darvekar et al. (2011) has also indicated the presence of alkaloids.

Presence of Tannins

Tannins are widely distributed in plant flora. Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. They form complexes with proteins, carbohydrates, gelatin and alkaloids.

A phytochemical investigation for the tannins was conducted in 12 different green leafy vegetables. The test was performed using lead acetate. Table 3 shows the results.

Table 3: Presence of tannins in selected GLVs

Sl No	Common Name of the Sample	Presence of Tannins
1	Pumpkin leaves	Present
2	Drumstick leaves	Absent
3	String bean leaves	Absent
4	Chekkurmanis	Present
5	Ivy gourd leaves	Absent
6	Ash gourd leaves	Absent
7	Curry leaves	Present
8	Sambar cheera	Absent
9	Green amaranth	Absent
10	Bottle gourd leaves	Absent
11	Lima bean leaves	Absent
12	Red amaranth	Absent

The presence of tannins was found only three selected green leafy vegetables. Pumpkin leaves, Chekkurmanis and curry leaves showed the positive results for the presence of tannins. Determination of Total Antioxidant Activity of selected green leafy vegetables

Total antioxidant capacity is the cumulative capacity of food components to scavenge free radicals. The total antioxidant content of fruits and vegetables may contribute to the protection they offer from disease. Total antioxidant activity of *Cucurbita maxima*, *Lagenaria siceraria* and *Thalinum asiaticum* were analyzed by DPPH assay.

Table 4: Free radical scavenging activity of selected GLVs in methanolic extracts

Sl. No	Concentration of the sample Mg/ml	Free radical scavenging activity (% Inhibition)			
		Ascorbic Acid	Pumpkin leaves (<i>Cucurbita maxima</i>)	Bottle gourd leaves (<i>Lagenaria siceraria</i>)	Sambar cheera (<i>Thalinum asiaticum</i>)
1.	0.1	29.26	26.609	76.76	2.01
2.	0.2	39.02	30.901	80.46	4.29
3.	0.3	46.34	51.716	80.84	5.15
4.	0.4	51.21	52.145	81.45	7.9

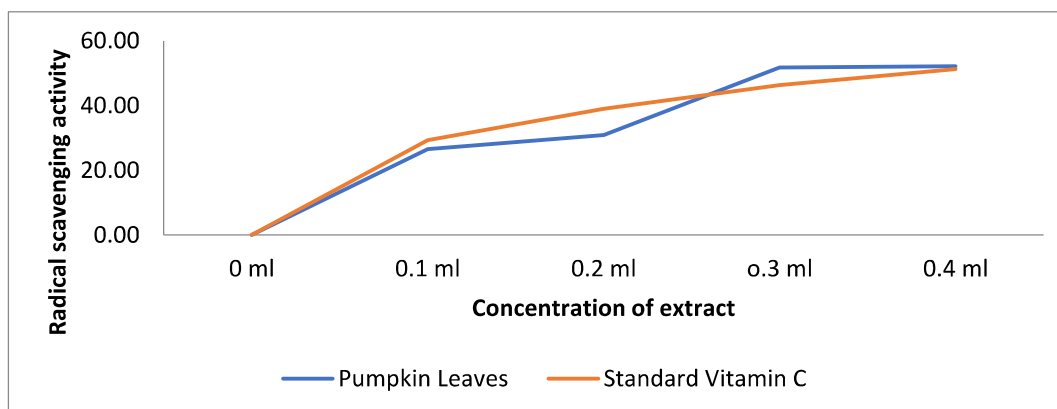
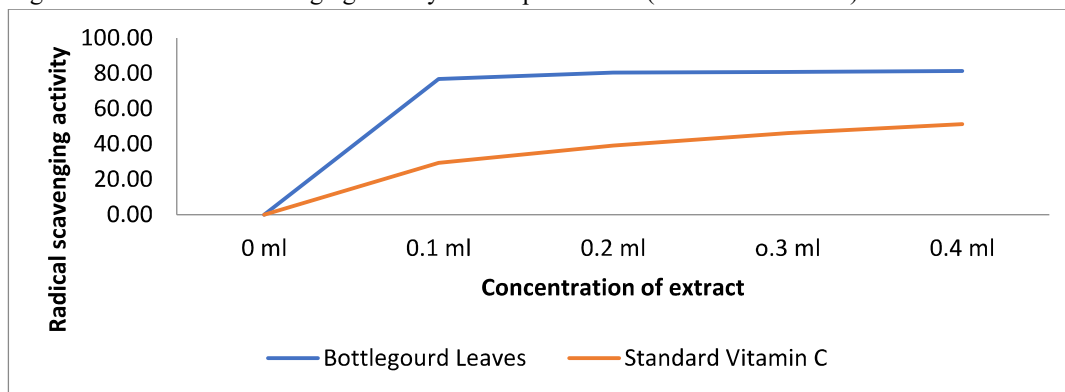
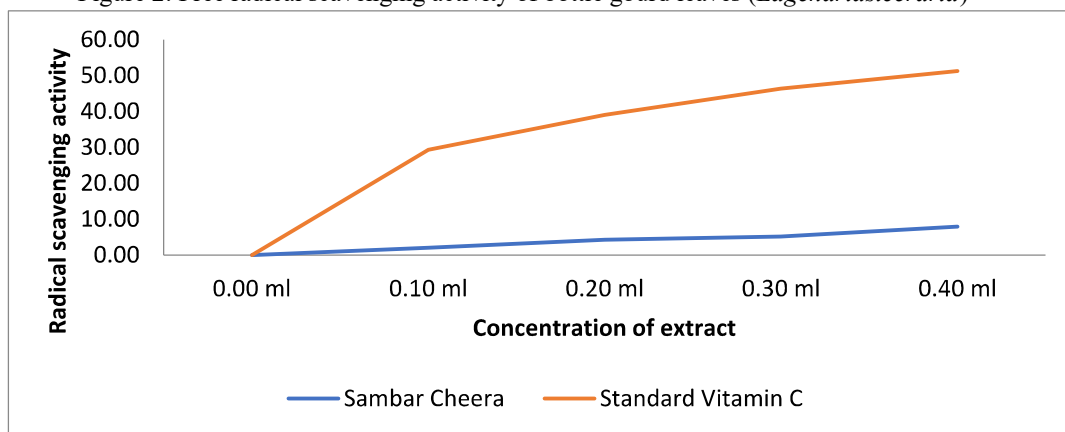


Figure 1: Free radical scavenging activity of Pumpkin leaves (*Cucurbita maxima*)Figure 2: Free radical scavenging activity of bottle gourd leaves (*Lagenariasiceraria*)Figure 3: Free radical scavenging activity of sambar cheera (*Thalinum asiaticam*)

Radical scavenging activity of standard Vitamin C increased in proportion with concentration of extract. Pumpkin leaf, Bottle gourd leaf and Sambar cheera leaf extracts had comparable antioxidant activity as standard vitamin C.

Total Phenol Content of selected green leafy vegetables

Total phenol content in the extract was determined by using Folin Ciocalteu method. Absorbance of the solution was measured at 650nm. Total phenol content was expressed as mg/g gallic acid equivalent.

Table 5: Total Phenol Content of selected green leafy vegetables

Sl No	Name of the sample	Absorbance	Total Phenol (mg/GAE)
1	Pumpkin leaves	0.342	2.814
2	String bean leaves	0.511	4.205
3	Sambar cheera	0.171	1.407
4	Kovai leaves	0.770	6.338
5	Ash gourd leaves	0.541	4.452
6	Bottle gourd leaves	0.932	7.670
7	Chekkurmanis	0.878	7.225
8	Lima bean leaves	0.997	8.204
9	Drumstick leaves	1.079	8.879
10	Curry leaves	1.323	10.887
11	Green Amaranth	0.867	7.134
12	Red Amaranth	0.786	6.468

*GAE Gallic Acid Equivalent

The commonly used leafy vegetable like curry leaves, green amaranth, red amaranth, drumstick leaves, Chekkurmanis had high content of phenol. Sambar cheera, string bean leaves, ash gourd leaves had less phenols when compared to another leaves.

CONCLUSION

Alkaloids were found to be present in only five selected green leafy vegetables. They were drumstick leaves, curry leaves, Chekkurmanis, green amaranth and Amara leaves. The presence of tannins was found only in three selected green leafy vegetables- Pumpkin leaves, Chekkurmanis and curry leaves. Pumpkin leaf, Bottle gourd leaf and Sambar cheera leaf extracts had comparable antioxidant activity as standard vitamin C. The commonly used leafy vegetable like curry leaves, green amaranth, red amaranth, drumstick leaves, Chekkurmanis had high content of phenol. Sambar cheera, string bean leaves, ash gourd leaves had less phenols when compared to another leaves. Incorporating the above green leafy vegetables in daily diets has to be popularized for its many health benefits.

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