# Antioxidant and Total Phenol Contents of Selected Leafy Vegetables Consumed in Jaffna Peninsula

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**ABSTRACT**

The aim of the study was to determine the antioxidant content and total phenol content of leafy vegetables such as 'Vallarai' (*Centella asiatica*), Leeks (*Allium ampeloprasum*), 'Mulai keerai' (*Amaranthus*), 'Ponnankani' (*Illecebrum sessile*), 'Ilai kova' (*Brassica oleracea*), Moringa (*Moringa olifera*), 'Kurincha' (*Gymnema sylvestre*), 'Thavaci murungai' (*Justica tranquebarensis*), 'Akaththi' (*Sesbania grandiflora*) and Curry leaf (*Murraya koenigii*) available in Jaffna. Total phenolics were measured using the Folin Cio-calteu reagent with gallic acid as standard. The antioxidant contents of leafy vegetables were assayed by both phosphomolybdenum assay and reducing power assay with standards of ascorbic acid and butylated hydroxyl toluene respectively. Based on the phosphomolybdenum assay, highest antioxidant content was observed in leeks [261.51(±0.54) mg/100 g dry samples and the lowest value in 'Ponnankani' [49.69 (±0.96) mg/100 g dry samples. Based on the reducing power assay, highest antioxidant content was found in 'Vallarai' (108.244 (±0.78) mg/100g dry sample], and lowest antioxidant content was detected in'Kurincha' [35.76 (±0.28) mg/100g dry sample]. Highest total phenol content was found in 'Kurincha' [401.88 (±0.16) mg/100 g dry samples and lowest total phenol content was detected in Leeks [53.94 (±0.46) mg/100g Dry Weight] mg/100g dry sample]. From this study, highest antioxidant content and total phenol contents were found in 'Kurincha' while lowest amounts were found in 'Ponnankani'. The present study shows that leafy vegetables contain a lot of antioxidants and total phenols to support human health.

**Key words-** Antioxidant, Ascorbic acid, Butylated hydroxyl toluene, Gallic acid, Total phenol

## 1. INTRODUCTION

Oxidative stress has been defined as a disturbance in the equilibrium status of pro-oxidant/antioxidant systems in intact cells resulting in oxidative damage to lipids, proteins, carbohydrates, and nucleic acids, contributing to pathological dysfunction in the organism [1]. Antioxidants present in the diets can prevent the oxidation of cellular materials. Due to that oxidation of the cellular membrane and other easily oxidisable elements and cellular materials can be damaged and which can cause several illnesses. Consumption of antioxidants can prevent or decrease the diseases such as atherosclerosis, diabetes, neurodegenerative diseases, ageing and cancer [2] [3] [4] [5]. It is always advisable to consume naturally occurring antioxidants rather than going for medication. Food samples such as berries, beans, red cabbage and grapes, etc, are recommended as good sources of antioxidants. Much attention has been focused on the activity of the natural antioxidants present in leafy vegetables, because potentially these components can reduce the level of oxidative stress.

Analysis of antioxidant contents and total phenol contents of leafy vegetables are important for healthy life. The aim of our study is determination of antioxidant and total phenol contents of leafy vegetables which are commonly consumed in Jaffna Peninsula.

## 2. MATERIALS AND METHODS

### Materials

Ten different leafy vegetables such as 'Vallarai' (*Centella asiatica*), Leeks (*Allium ampeloprasum*), 'Mulai keerai' (*Amaranthus*), 'Ponnankani' (*Illecebrum sessile*), 'Ilai kova' (*Brassica oleracea*), Moringa (*Moringa olifera*), 'Kurincha' (*Gymnema sylvestre*), 'Thavaci murungai' (*Justica tranquebarensis*), 'Akaththi' (*Sesbania grandiflora*) and Curry leaf (*Murraya koenigii*) were selected for this study and were purchased from local market and home garden.

All other chemicals used were of analytical grade and were obtained from standard sources.

### Preparation of extracts

Each fresh sample (1g) was weighed and ground with a chilled mortar and pestle with 10 ml of 0.05M Tris HCl, 3mM $MgCl_2.7H_2O$ and 1mM EDTA buffer solution (pH 7.0). The extract was centrifuged at 4°C for 20 min at 3000 rpm. Supernatant was used to determine the antioxidant content (phosphomolybdenum assay method) and total phenol content [6].

**Determination of total phenol content**

To extract (0.2ml), Folin Ciocalteu reagent (1ml) and 0.8ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30min. Absorbance was measured at 765nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per 100gram of extract [7].

**Determination of antioxidant content by Phosphomoly bdenum method**

Extract (0.1ml) was mixed with ammonium molybdate solution (3ml) and mixed well. Tubes were incubated at 95ºC for 90 min. The mixture was cooled to room temperature and absorbance of the solution was measured at 695 nm. The antioxidant content was expressed as Ascorbic Acid Equivalents (AAE) in milligrams per 100gram of the extract [8].

**Determination of antioxidant content by ferric redu cing power assay method**

**Preparation of ethanol extract**

Fresh sample (2g) was weighed and ground with a chilled mortar and pestle with of 99% ethanol (20ml). Finally extract was filtered through filter paper (Whatman No 1).

**Ferric reducing power assay method**

Extract (1ml), 2.5ml of phosphate buffer (pH 6.6) and 1% potassium ferricyanide (2.5ml) were incubated at 50°C for 30min and 10% trichloroacetic acid (2.5ml) was added to the mixture and centrifuged at 3000rpm for 10min. Supernatant (2.5ml) was diluted with distilled water (2.5ml) and shaken with freshly prepared 0.1% ferric chloride (0.5 ml). Absorbance was measured at 700nm. Antioxidant content was expressed as butylated hydroxyl toluene (BHT) equivalents in mg per 100gram sample [9].

## 3. RESULTS AND DISCUSSION

As the phenolic content and antioxidant content are expressed in terms of gram per dry weight, moisture content of the samples was determined. Table 1 shows the moisture content, antioxidant contents of leafy vegetables by phosphomolybdenum assay and reducing power assay and total phenol contents of the leafy vegetables. Moisture content of leafy vegetables ranged from 60.02 to 90.8%. Among the leafy vegetables, highest moisture content was found in leeks [(90.48 (±0.43)%, followed with leafy cabbage [88.01 (±0.21)%], 'Vallarai' [86.58 (±0.21)%], 'Thavaci murungai' [(78.66 (±0.26)%] 'Ponnankani' [78.2 (±0.36)%], 'Mulai keerai' [77.24 (±0.12)%], Moringa [74.07 (±0.34)%], 'Akaththi' [72.93 (±0.08)%] and 'Kurincha' [63.86 (±0.14)%] and lowest moisture content was detected in Curry leaf [60.02 (±0.19)%]. Moisture contents of the leafy vegetable studies are relatively high, ranging from 75.0 to 91.5% [10]. The high moisture content of vegetables makes them to aid the digestion of food. Their shelf life is very short because the high moisture facilitates bacterial action resulting into spoilage.

The phosphomolybdenum method is based on the reduction of molybdenum by the antioxidants and the formation of a green molybdenum (V) complex, which has absorption at 695 nm. The reduction of Mo (VI) to Mo (V) by administration of reference chemicals; ascorbic acid, suggested the presence of effective antioxidants. Therefore the results were correlated with ascorbic acid content of leafy vegetables. Antioxidant contents of leafy vegetables (Phosphomolybdenum assay) significantly ($p< 0.05$) differed from each other. Among the leafy vegetables, highest antioxidant content was observed in leeks [261.51 ($\pm0.54^{a}$) mg/100g Dry Weight], followed with leafy cabbage [201.64 ($\pm0.27^{b}$) mg/100g Dry Weight], Muringa [192.26 ($\pm0.09^{c}$) mg/100g Dry Weight], amaranth [181.54 ($\pm0.65^{d}$) mg/100g Dry Weight), curry leaf [150.27 ($\pm0.13^{e}$) mg/100g Dry Weight), 'Akaththi' [151.7 ($\pm0.43^{f}$) mg/100g Dry Weight], 'Thavasi murungai' [148.65 ($\pm0.45^{g}$) mg/100g Dry Weight] and 'Kurincha' [145.14 ($\pm0.89^{h}$) mg/100g Dry Weight] and 'Vallarai' [138.57 ($\pm0.73^{i}$) mg/100g Dry Weight] and lowest antioxidant content was obtained in 'Ponnankani' [49.69 ($\pm0.96^{j}$) mg/100g Dry Weight].

The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow colour of the test solution changes to green and blue depending on the reducing power of test specimen. Greater absorbance at 700 nm indicated greater reducing power. In this method, antioxidant contents were expressed in BHT equivalent. BHT was used as the standard instead of vitamin E. Therefore the results were correlated with vitamin E content of spices. BHT is the fat soluble antioxidant. Because it is not dissolved in tris buffer extract. Therefore, ethanol was used to prepare the standards and extracts in this method. Antioxidant contents of leafy vegetables based on reducing power assay differed significantly ($p< 0.05$) from each other. In reducing power assay, highest antioxidant content was found in 'Vallarai' ($108.24\pm0.78^{a}$ mg/100g Dry Weight) followed with curry leaf [89.84 ($\pm0.15^{b}$) mg/100g Dry Weight], leafy cabbage ($80.67\pm0.20^{c}$ mg/100g Dry Weight), amaranth ($76.96\pm0.53^{d}$ mg/100g Dry Weight), 'Muringa' [62.34 ($\pm0.37^{e}$) mg/100g Dry Weight], 'Thavasi murungai' [61.41 ($\pm0.51^{f}$) mg/100g Dry Weight], leeks ($52.04\pm0.88^{g}$ mg/100g Dry Weight), 'Ponnankani' ($52.57\pm0.39^{h}$ mg/100g Dry Weight) and 'Akaththi' [45.39 ($\pm0.30^{i}$) mg/100g Dry Weight] and lowest antioxidant content was detected in 'Kurincha' [35.76 ($\pm0.28^{j}$) mg/100g Dry Weight) among leafy vegetables.

The plant phenolic compounds are responsible for effective free radical scavenging and antioxidant activities [11]. Total phenol contents of leafy vegetables significantly ($p < 0.05$) differed from each other. Whereas, highest total phenol content was found in 'Kurincha' (*Gymnema sylvestre*) [401.88 (±0.16$^{a}$) mg/100g Dry Weight] followed with Amaranth [282.65 (±0.37$^{b}$) mg/100g Dry Weight], curry leaf [203.24 (±0.25$^{c}$) mg/100g Dry Weight], 'Muringa' [173.64 (±0.52$^{d}$) mg/100g Dry Weight], leafy cabbage [153.7 (±0.43$^{e}$) mg/100g Dry Weight), 'Akaththi' [133.57 (±0.49$^{f}$) mg/100g Dry Weight], 'Vallarai' [108.06 (±0.80$^{g}$) mg/100g Dry Weight], Thavasi muringai' [86.0 (±0.2$^{h}$) mg/100g Dry Weight] and 'Ponnankani' [66.77 (±0.51$^{i}$) mg/100g Dry Weight] and lowest total phenol content was detected in leeks [53.94 (±0.46$^{j}$) mg/100g Dry Weight] among leafy vegetables.

## 4. CONCLUSION

The present study demonstrated that leafy vegetables showed differences in their antioxidant content and total phenol content. From that observation it can be concluded that 'Kurincha', 'Amaranthus', leafy cabbage and 'Muringa' are good sources of natural antioxidants. Highest amount of antioxidants and total phenols can prevent the health problems in human such as cancer, arthrosclerosis, diabetics, ageing, and neurodegenerative diseases.

## REFERENCES


[1] Sies, H. "Oxidative stress: introductory remarks". In: Sies H, ed. Oxidative stress. Orlando, FL: Academic Press, 1–10, 1985.

[2] Kuo, S.M., "Dietary flavonoid and cancer prevention: evidence and potential mechanism". *Crit. Rev. Oncogenesis.*; 8(1): pp.47–69, 1997.

[3] McCord ,J.M. "The evolution of free radicals and oxidative stress". *Am J. Med.* ; pp. 108:652. 2000.

[4] Sun, J, Chu, Y. F, Wu, X.Z, Liu, R.H., "Antioxidant and antiproliferative activities of common fruits". *J. Agri . Food Chem.* ; Vol 50(25): pp7449–7454 , 2002.

[5] Yang, C.S, Landau, J.M., Huang, M.T., Newmark H.L.,."Inhibition of carcinogenesis by dietary polyphenolic compounds". *An. Rev. Nutr.* ; Vol. 21: pp.381–406, 2001.

[6] Padmaja,M.,Sravanthi,M.and Hemalatha,K.P.J., "Evaluation of antioxidant activity of two Indian medicinal plants". *Journal of phytology*, Vol. 3 (3): pp.86-91, 2011.

[7] Singleton, V.L., Rossi.J.A.. "Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents". *Ame. J. Enol. and Viticult.* 16, pp.144-158, 1965.

[8] Prieto, P., Pineda, M., Aiguel.M. "Spectro photometer Quantization of antioxidant capacity through the formation of Phosphomolybdenum Complex: Specific application to the determination of vitamin E". *Anal. Biochem***.** 269, pp. 337-341, 1999.

[9] Ferreira,C.F.R., Baptista, P., Vilas-Boas, M., Barros,L. "Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity". *Food Chem.* 100, pp. 1511-1516, 2007.

[10] Olaiya, C, Adebisi, J, "Phytoevaluation of the nutritional values of ten green leafy vegetables in South -Western Nigeria". The Internet Journal of Nutrition and Wellness. Volume 9 Number 2, 2009.

[11] Velioglu Y.S., Mazza .G, Gao .L, Oomah .B.D., "Antioxidant activity and total phenolics in selected fruits, vegetables and grain products"**.** *J Agric. Food Chem***.**; 46: 4113- 4117, 1998.


**Table 1**: Moisture, antioxidant and total phenol contents of different leafy vegetables.

| Leafy vegetables | Moisture content (%) | Antioxidant content (mg/100g dry sample) | | Total phenols (mg/100g dry sample) (gallic acid equivalent) |
|---|---|---|---|---|
| | | Phosphomolybdenum Assay (Ascorbic acid equivalent) | Reducing Power Assay (Butylated hydroxyl toluene equivalent) | |
| *Centella asiatica* | 86.58 (±0.21) | 138.57 (±0.73$^{i}$) | 108.244 (±0.78$^{a}$) | 108.06 (±0.80$^{g}$) |
| Leeks | 90.80 (±0.43) | 261.51(±0.54$^{a}$ | 52.04 (±0.88$^{g}$) | 53.94 (±0.46$^{j}$) |
| Amaranth | 77.24 (±0.12) | 181.54 (±0.65$^{d}$ | 76.96 (±0.53$^{d}$) | 282.65 (±0.37$^{b}$) |
| Ponnankani | 78.2 (±0.36) | 49.69 (±0.96$^{j}$) | 52.57 (±0.39$^{h}$) | 66.77 (±0.51$^{i}$) |
| Leafy Cabbage | 88.01 (±0.21) | 201.64 (±0.27$^{b}$ | 80.67 (±0.20$^{c}$) | 153.7 (±0.43$^{e}$) |
| 'Kurincha' | 63.86 (±0.14) | 145.14 (±0.89$^{h}$ | 35.76 (±0.28$^{j}$) | 401.88 (±0.16$^{a}$) |
| Moringa | 74.07 (±0.34) | 192.26 (±0.09$^{c}$) | 62.34 (±0.37$^{e}$) | 173.64 (±0.52$^{d}$) |
| 'Thavaci Murungai' | 78.66 (±0.26) | 148.65 (±0.45$^{g}$) | 61.41 (±0.51$^{f}$) | 86.00 (±0.2$^{h}$) |
| 'Akaththi' | 72.93 (±0.08) | 151.7 (±0.43$^{f}$) | 45.39 (±0.30$^{i}$) | 133.57 (±0.49$^{f}$) |
| Curry leaf | 60.02 (±0.19) | 150.27 (±0.13$^{e}$ | 89.84 (±0.15$^{b}$) | 203.24 (±0.25$^{c}$) |