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## Nutritional activity, antioxidant and anti arthritic activity of selected green leafy vegetables

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### Abstract

**Introduction:** Nutrient density and phyto chemical richness of green leafy vegetables make them an important component of the diet. Therefore, the present study aimed to estimate the nutrient content, antioxidant activity and anti-arthritic activity in 10 commonly consumed green leafy vegetables.

**Methodology:** A quasi-experimental design using purposive sampling technique was used. Ten green leafy vegetables namely, Sirukeerai, Araikeerai, Mulaikerai, Mint, Manathakali, Agathi, Ponnagani, Drumstick leaves, Fenugreek leaves and Spinach were analyzed for antioxidant activity using DPPH scavenging method and anti-arthritic activity was estimated using sodium diclofenac tablets as standard. The green leafy vegetables were also analyzed for nutrient content namely moisture, carbohydrate (Anthrone method), protein (Lowry's Method) and crude fiber (Maynard analysis).

**Results:** Drumstick leaves contained appreciable amounts of carbohydrate ((9.3 g/100g), protein (6.9 g/100g) and crude fibre (13.3 g/100g) when compared with the other green leafy vegetables used in the present study. On the basis of antioxidant activity, Sirukeerai (98.42%), Mint (96.85%) and Spinach (96.06%) was found to have highest scavenging activity and Manathakali (11.1 mg/ml) and Mint (10.2 mg/ml) showed highest anti arthritic activity.

**Conclusion:** Green leafy vegetables are excellent sources of antioxidants and potential sources of anti-arthritic activity.

**Keywords:** Green leafy vegetables, antioxidant activity, protein, carbohydrate, crude fibre, and anti-arthritic activity

### Introduction

Green leafy vegetables contain immense variety of bioactive non-nutritive health promoting compounds such as antioxidants and phytochemicals, which provide health benefits beyond basic nutrition. The leafy vegetables contain major components namely flavanoids, coumarins, tannins and other phenolic compounds which play a vital role in health management especially in lowering the risk of chronic human ailments such as cancer, cardiovascular disease and other degenerative disease.

Green leafy vegetables are good source of macronutrients namely carbohydrate, protein and fibre content. Leafy vegetables also act as anti-diabetic, anti-histaminic, anti-carcinogenic and anti-inflammatory agent due to their potential richness in antioxidants and phytochemical content. They are also rich source of nutrients which make them natural anti-ageing wonders. Therefore, the present study was conducted with the aim of investigating the nutrient value, antioxidant activity and anti-arthritic activity of ten selected green leafy vegetables namely Agathi (*Sesbania Grandiflora*), Araikeerai (*Amaranth Tristis*), Manathakali (*Solanum Nigrum*), Mulaikerai (*Amaranth Spinousus*), Drumstick leaves (*Moringa Oleifera*), Spinach (*Spinacia Oleraceae*), Ponnagani (*Alternathera Sessilis*), Mint (*Mentha Spicata*), Sirukeerai (*Amaranthus Polygonoides*) and Fenugreek leaves (*Trigonella Foenum Graecum*) that are commonly consumed by the local population.

### Material and Methods

#### Materials

Green leafy vegetables were purchased from five different markets in the city. Hundred grams of each leafy vegetable were purchased on the day of the analysis from five different outlets namely Koyambedu market, Pazhamudhir Cholai, Vegetable vendor, footpath greens vendor

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and Reliance super market. The sample from different markets were pooled together and made into a bundle to ensure that a representative sample of green leafy vegetables purchased locally was used for analysis. All chemicals used for the study were of analytical grade (AR) and were obtained from Sigma Aldrich India Ltd. De-ionized distilled water was used for the preparation of reagents used in the entire analysis.

### Preparation of the Samples

Green leafy vegetables were cleaned and washed with distilled water followed by washing with de-ionized distilled water, and allowed to air dry. Two gram portion of the dry sample were weighed and made into paste using pestle and motor. The juice was extracted and made upto 5ml with de-ionized distilled water and poured into a centrifuge tube and after the process of centrifuging the aqueous extract collected was packed in polythene pouches and stored in the refrigerator. Fresh leaves were also used for certain biochemical analysis namely crude fibre and moisture content whereas aqueous extract were used for carbohydrate, protein, and antioxidant activity and *in vitro* anti arthritic activity. All glass ware used for the analysis were washed with detergent and rinsed with water and soaked overnight in 1N HCL and rinsed again with distilled water and acetone before drying. Care was taken to prevent contamination at every stage.

### Biochemical Analysis

#### Moisture Content

One gram portion of leafy vegetable was subjected to drying at 60 °C in hot air oven for 24 hours. Each dried sample was weighed to calculate the difference between the fresh and dry weight that gives the amount of moisture content present in the leaves.

#### Carbohydrate Content

Carbohydrates were determined using Anthrone method using anthrone reagent and glucose solution. Anthrone reagent were added to the 2ml of test sample and made upto 5ml with deionized distilled water. The solution was heated in water bath for 5 minutes and incubated for 20 minutes. The readings were noted colorimetrically at 670nm.

#### Protein Content

Protein content of enzyme extract was determined by Lowry's method using Folin-Ciocalteu reagent. To two ml of test sample, reagents were added and incubated for 30 minutes in dark condition. Five standard solutions and a blank solution were prepared. The sample were estimated colorimetrically at 660nm.

### Crude Fibre Content

The crude fibre content in substrate was estimated by the method by Maynard. The greens undergo acid and alkaline treatment. Two gram sample were taken and boiled for 30 minutes with sulphuric acid for acid treatment and washed with boiled deionized distilled water. Then the greens are treated for alkali treatment, in which the sample were heated for 30 minutes along with sodium hydroxide and again washed with deionized distilled water. The sample was then kept in hot air oven and made into dry ash under 600 degrees centigrade and kept for cooling. After 24 hours, the dry ash weights were noted.

### *In vitro* anti-Arthritic Activity

To 2ml test solution phosphate buffer saline and bovine serum albumin solution were added along with distilled water. The solution was incubated at 37 degree centigrade for 15 minutes and then heated for five minutes in a water bath. After cooling, readings were noted at an optical density of 660nm (Procedure given in Appendix XI). The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ Inhibition of Protein Denaturation} = 100 \times [V_c - V_t / V_c]$$

$V_t$  = absorbance of test sample,

$V_c$  = absorbance of control.

### Antioxidant Assay

Antioxidant assay of leafy vegetables were estimated with its free radical scavenging method by using DPPH (2, 2-diphenyl-1-picrylhydrazyl). DPPH is a stable free radical with purple colour absorbed at 517nm and compared with known synthetic standard 0.16% of Butylated hydroxy toluene (BHT). A screening method was done before the quantitative assay to know which sample were antioxidant positive and was then subjected to further analysis. In this screening method the observation for the sample were found by discolouration from purple to yellow and pale pink which was considered as strong and weak positive respectively. After that the quantitative assay was carried out with 2 ml of acetone aqueous extract of leafy vegetables with 0.1% of methanolic DPPH which was added over the sample and incubated for 30 minutes in dark condition. Discolourations were observed in the sample after 30 minutes from purple to yellow and pale pink colour. If free radicals have been scavenged, DPPH will degenerate to yellow colour. Yellow colour was considered to be strong positive and pale pink colour to weak positive respectively.

### Results and Discussion

**Table 1:** Nutrient Content of Green Leafy Vegetables

| S.No | Sample           | Carbohydrate (g) | Protein (g) | Moisture (g) | Crude Fibre (g) |
|------|------------------|------------------|-------------|--------------|-----------------|
| 1.   | Sirukeerai       | 3.3              | 5.9         | 81.2         | 5.2             |
| 2.   | Araikeerai       | 3.3              | 1.9         | 87.0         | 1.2             |
| 3.   | Mulaikeerai      | 2.6              | 2.9         | 84.0         | 5.5             |
| 4.   | Mint             | 4.0              | 4.9         | 86.0         | 5.8             |
| 5.   | Agathi           | 6.2              | 4.9         | 79.0         | 12.6            |
| 6.   | Drumstick leaves | 9.3              | 6.9         | 75.9         | 13.3            |
| 7.   | Fenugreek leaves | 3.3              | 4.3         | 87.6         | 1.0             |
| 8.   | Ponnagani        | 10.1             | 5.3         | 85.5         | 6.0             |
| 9.   | Manathakali      | 4.6              | 7.9         | 83.0         | 1.2             |
| 10.  | Spinach          | 3.3              | 1.9         | 94.0         | 3.2             |

\*Values expressed are means of triplicates

The carbohydrate content of most green leafy vegetables namely Sirukeerai, Araikeerai, Mint, Manathakali, Agathi and Spinach ranged between 3g/100g - 7 g/100g. The lowest carbohydrate content was found in Mulaikeerai (2.6g/100g) whereas Ponnagani (10.1g/100g) had the highest amount of carbohydrate followed by Drumstick leaves (9.3g-/100g). However, the carbohydrate values for green leafy vegetables obtained in the present study are found to be much lower than the values for carbohydrate quoted by Onwordi, Ogungbade and Wusu (2009) [14] for locally available Nigerian green leafy vegetables which ranged between 28-32g/100g depicting geographical differences in nutrient composition of green leafy vegetables.

With regard to protein content of green leafy vegetables it is observed that Manathakali has the highest content of protein (7.9g/100g) followed by Drumstick leaves (6.9g/100g) but Spinach and Araikeerai are found to have lowest protein content at (1.9g/100g). Moreover these values for protein in green leafy vegetables found in the present study are in concordance with the range of protein 2-10 g/100g obtained for green leafy vegetables by Saha, Biswal and Deka (2015) [21].

Moisture content of the green leafy vegetables is found to range between 75g/100g-90g/100g wet basis. The highest value for moisture content is seen in Spinach at 94.0g/100g followed by Fenugreek leaves (87.6g/100g) and lowest value is found in Drumstick leaves (75.9g/100g). High moisture content provides greater activity of water soluble enzymes and co-enzymes which is needed for metabolic activity of leaves (Badau, 2013) [5]. Further the values for moisture content of green leafy vegetables in the present study compare well with the values for moisture content that ranged between 70-92 g/100g for African green leafy vegetables (Patricia, Zoue, Megnanou, Doue & Nimake 2014) [15].

On examining the results for crude fibre content of green leafy vegetables in table 1, it is evident that Drumstick leaves showed the highest value for crude fibre (13.3g/100g) followed by Agathi (12.6g/100g) whereas the lowest value was found in Fenugreek leaves (1.0g/100g), Manthakali (1.2g/100g) and Araikeerai (1.2g/100g). The other green leafy vegetables had crude fibre content ranging between 3-6g/100g. According to Ramulu and Rao (1997) [18] components of plant fibre lowers blood cholesterol and works as an excellent cleanser to the body. The values obtained in the present study are also found to be similar to the crude fibre value reported by Saha, Biswal and Deka (2015) [21] which ranged between 1-8 g/100g for green leafy vegetables.

From the results of nutrient analysis of green leafy vegetables it is thus observed that Drumstick leaves, Ponnagani, Manathakali, Mint and Sirukeerai are found to have appreciable amounts of carbohydrate, protein and crude fibre.

**Table 2:** Antioxidant Activity of Selected Green Leafy Vegetables

| Sample           | Scavenging Activity (%) |
|------------------|-------------------------|
| Sirukeerai       | 98.42                   |
| Araikeerai       | 88.97                   |
| Mulaikeerai      | 88.18                   |
| Mint             | 96.85                   |
| Agathi           | 84.25                   |
| Drumstick leaves | 89.76                   |
| Fenugreek leaves | 87.40                   |
| Ponnagani        | 88.97                   |
| Manathakali      | 88.18                   |
| Spinach          | 96.06                   |

\*Values expressed are means of triplicates

Antioxidant activity ranging between 80-95% is observed in all the green leafy vegetables chosen in the study. Highest antioxidant activity is found in Sirukeerai (98.42%) and Spinach (96.06%). All the other green leafy vegetables had an antioxidant activity that ranged between 84-89.76%. The range of antioxidant activity for green leafy vegetables obtained in the present study (80-95%) is found to be similar to the range cited by other researchers (Sahu, Routray & Kar 2013; Raghavendra, Reddy, Yadav, Raju & Kumar 2013) [19, 16, 25] at 80-85% for green leafy vegetables. These findings conform to the observation that amaranth varieties contain high antioxidant activity and higher amount of pigments and total polyphenols. (Ali, Khanadaker & Oba, 2010) [4]. Furthermore, green leafy vegetables are known to contain immense variety of bioactive non-nutritive health promoting compounds such as antioxidants and phytochemicals that provide health beyond basic nutrition (Aletor, Oshodi & Ipinmoroti, 2002) [3].

**Table 3:** Anti Arthritic Activity of Selected Green Leafy Vegetables

| Sample           | 50% Inhibition Values (mg/ml) |
|------------------|-------------------------------|
| Sirukeerai       | 4.5                           |
| Araikeerai       | 4.0                           |
| Mulaikeerai      | 5.5                           |
| Mint             | 10.2                          |
| Agathi           | 9.6                           |
| Drumstick leaves | 2.6                           |
| Fenugreek leaves | 3.7                           |
| Ponnagani        | 9.7                           |
| Manathakali      | 11.1                          |
| Spinach          | 1.4                           |

\*Values expressed are means of triplicates

Spinach (1.4 mg/ml), Drumstick leaves (2.6 mg/ml) and Fenugreek leaves (3.7 mg/ml) are found to have the lowest anti arthritic activity whereas moderate anti arthritic activity is seen in Sirukeerai (4.5 mg/ml) and Araikeerai (4 mg/ml), Manathakali (11.1mg/ml) and Mint (10.2mg/ml) showed the highest anti arthritic activity that reduces inflammation upto 50%. Ibrahim, N (2008) [10] point out that high concentration of anti-arthritic activity is directly proportional to phyto chemical content. Antioxidant potential is also stated to play an important role in anti-inflammatory activity. Reddy and Vinodini (1993) [20] found that neutralization of free radicals by antioxidants and radical scavengers also eased inflammation. Thus, it is understood that high antioxidant activity seen in green leafy vegetables could have added to the anti-arthritic activity observed.

## Conclusion

From the study, it is observed that appreciable amounts of carbohydrate, protein and crude fibre is found in Drumstick leaves among the selected green leafy vegetables. On the other hand, adequate antioxidant activity is found in all the green leafy vegetables studied. Highest antioxidant activity is found in Sirukeerai (98.42%), Mint (96.85%) and Spinach (96.06%). Lowest amount of antioxidant activity is seen in Agathi (84.25%). And in the case of anti-arthritic activity, Manathakali (11.1 mg/ml) and Mint (10.2 mg/ml) showed the highest anti arthritic activity whereas the lowest anti arthritic activity are found in Spinach (1.4 mg/ml), Drumstick leaves (2.6 mg/ml) and Fenugreek leaves (3.7 mg/ml). Thus, it is evident that green leafy vegetables are excellent source of antioxidants and potential source of anti-arthritic activity.

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