



## ANTIOXIDANT PROPERTIES OF SOME SEASONAL LEAFY VEGETABLES FROM RATNAGIRI DISTRICT OF MAHARASHTRA

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### ABSTRACT

Analysis of antioxidant potential of seasonal vegetables occurring in Ratnagiri district and which are a part of regular diet and also possess medicinal properties was undertaken. Methanolic, ethanolic and aqueous extracts of five wild vegetables namely *Colocasia esculenta* (L.), *Clerodendrum serratum* (L.), *Cassia tora* (L.), *Celosia argentea* (L.) and *Amorphophallus paeoniifolius* (Dennst.) were prepared for estimating various antioxidant properties such as DPPH, FRAP, FICA and reducing power assay. Total phenolic content of the vegetable extracts was also determined in order to understand the correlation with antioxidant properties. Results revealed that all the vegetables possess a substantial antioxidant potential and can add to the nutritional supplement when consumed in the daily diet. DPPH, FRAP and FIC activities were maximum in the methanol and ethanol extracted samples whereas reducing ability was greater in the aqueous extracts of vegetables.

**KEYWORDS:** DPPH, FIC activity, FRAP, Leafy vegetables, Reducing power, Total phenols



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## INTRODUCTION

Antioxidants found in large quantities in the crude extracts of fruits, herbs, vegetables, cereals and other plant materials act as reducing agent and maintain health by acting against stress related disorders such as infections, diabetes, cancer and coronary heart diseases<sup>1</sup>. The most common antioxidants present in fruits and vegetables are ascorbic acid, phenols, flavonoids etc. which prevent accumulation of reactive oxygen species<sup>2</sup> and help to protect human body from chronic illness<sup>3</sup>. A large numbers of plants have been extensively studied in search of novel antioxidants and other important nutrients<sup>4</sup>. A variety of vegetables are used in rural areas which are nutritionally rich but are less known due to their limited availability and lack of awareness. According to Khanzadi<sup>5</sup> the knowledge of these ignored indigenous vegetables needs urgent scientific analysis. Many wild leafy vegetables are consumed by local peoples in the coastal districts of Maharashtra like Sindhudurg and Ratnagiri. Their nutritional and medicinal properties are less known or ignored as they are not popularly used. In the present task antioxidant activities of a few wild vegetables from Ratnagiri district on the west coast of Maharashtra are evaluated. Methanolic, ethanolic and aqueous extracts of some such vegetables namely *Clerodendrum serratum* (L), *Cassia tora* (L), *Amorphophallus paeoniifolius* (Dennst.), *Celosia argentea* (L) and *Colocasia esculenta* (L) are prepared and used for the purpose.

## MATERIALS AND METHODS

### **Collection and Extraction of Plant Samples**

Vegetable samples were collected from Ratnagiri district during monsoon (June to August, 2012). The leaves and young shoots were washed to remove any soil debris and dust, blotted to dry and dried in hot air oven at 45°C. Dried material was powdered in a grinder and then stored in air tight containers for further use. Two grams of powdered samples were

extracted in 20 ml solvent (Methanol/Ethanol/distilled water) for 24h using a mechanical shaker. The extracts were filtered through Whatman No. 1 filter paper and stored in air tight glass vials in refrigerator.

### **Ferric Reducing Antioxidant Property**

FRAP assay was performed as described by Benzie and Strain<sup>6</sup>. FRAP reagent (2.7ml) was mixed with 40µl of sample. The reaction mixture was allowed to stand for 30 min. at room temperature before reading the absorbance at 593nm.

### **Ferrous Ion Chelating Activity**

Ferrous Ion Chelating Activity of the samples was measured by the method given by Decker and Welch<sup>7</sup>. 0.5 ml of individual extract was mixed with 0.1 ml FeCl<sub>2</sub> (2mM) and 0.2 ml Ferrozine (5mM) solutions and reaction mixture was incubated for 10 min. at room temperature. Absorbance was measured at 562nm on a spectrophotometer. FIC activity is expressed as the percent inhibition of Fe<sup>+2</sup> to ferrozine complex.

### **Reducing Power Assay**

Reducing power of the extracts was evaluated as per Yen and Chen<sup>8</sup>. One ml extract was mixed with 0.2ml phosphate buffer (0.2M, pH 6.6), 1ml potassium ferricyanide (1%) and incubated at 50°C for 20 min. After cooling 1ml trichloroacetic acid (10%) was added and mixed well. 1.5 ml of this mixture were transferred to other test tube containing distilled water (1.5ml) and FeCl<sub>3</sub>.6H<sub>2</sub>O (0.5 ml, 0.1%). The contents were centrifuged and kept at room temperature for 10 min. before reading the absorbance at 700 nm on a spectrophotometer. The antioxidant activity is expressed as ascorbic acid equivalent

### **DPPH Scavenging Activity**

The ability of plant extract to scavenge DPPH (1, 1 diphenyl, 2-picryl hydrazine) was assessed by using the method of Wang et al<sup>9</sup>. 0.5 ml of extract was mixed with 3 ml of 2.5mM DPPH

solution prepared in methanol. After 30 minutes incubation in dark at room temperature, absorbance was measured at 517 nm against a blank of methanol without DPPH. Methanol with DPPH solution was used as the control. DPPH activity is expressed as percent inhibition of the DPPH.

### Total Phenolic Content

Total Phenolic Content of vegetable samples was determined according to Folin-Ciocalteu

method<sup>10</sup>. 0.1 ml extract was diluted to 3 ml by adding distilled water followed by 0.5 ml Folin-Ciocalteu reagent. After 3 min. two ml of 20% Na<sub>2</sub>CO<sub>3</sub> were added and the contents were boiled for one min. on a water bath. The absorbance was measured at 650 nm after cooling to room temperature. Amount of phenols was calculated using catechol as the standard and expressed as mg/g dry weight.

**Table 1**  
**Antioxidant properties of some wild vegetables from Ratnagiri District**

Vegetables					
Antioxidant Activities & Solvents	<i>Colocasia esculenta</i>	<i>Clerodendrum serratum</i>	<i>Cassia tora</i>	<i>Celosia argentea</i>	<i>Amorphophallus paeoniifolius</i>
<b>Methanol</b>					
FRAP*	1.74	5.72	4.35	2.41	1.06
FICA#	57.47	56.10	57.89	76.03	68.17
Reducing Power*	0.62	0.74	0.66	0.62	0.71
DPPH#	78.21	85.83	83.53	83.73	87.47
<b>Ethanol</b>					
FRAP*	2.34	5.81	2.92	0.77	1.65
FICA#	59.39	69.82	68.21	79.40	62.73
Reducing Power*	1.47	1.25	1.08	0.57	0.95
DPPH#	67.62	75.03	85.06	31.47	77.08
<b>Aqueous</b>					
FRAP*	2.42	3.47	5.93	4.54	2.70
FICA #	10.93	65.78	76.24	51.04	57.40
Reducing Power*	0.91	1.18	1.02	0.96	0.90
DPPH #	49.96	89.19	15.53	3.25	38.88

\* values are expressed as % inhibition values are expressed in mg/g

**Table 2**  
**Phenolic content (mg/g dry wt) of some wild vegetables from Ratnagiri District**

Vegetable	M	E	Aq
<i>Colocasia esculenta</i>	2.94	4.39	10.33
<i>Clerodendrum serratum</i>	8.95	10.89	39.35
<i>Cassia tora</i>	8.77	4.32	28.00
<i>Celosia argentea</i>	3.76	0.98	8.42
<i>Amorphophallus paeoniifolius</i>	3.43	3.62	14.16

M – Methanol Extract, E – Ethanol Extract, Aq – Aqueous Extract

## RESULTS AND DISCUSSION

Results of antioxidant activities observed in different vegetables are presented in Table 1.

### FRAP Assay

FRAP is a measure of reductive ability and is evaluated by the transformation of Fe<sup>+3</sup> to Fe<sup>+2</sup> in the presence of sample extract. The reducing capacity of a common compound may serve as

a significant indicator of its potential antioxidant activity<sup>11</sup>. In the present work FRAP activity ranged from 0.77 to 5.9 mg/g in different extracts and ethanolic and methanolic extracts of *Clerodendrum serratum* it was maximum (5.81mg/g and 5.72mg/g, respectively) and in the remaining samples FRAP activity was less than 3.0 mg/g. In aqueous extracts, *Cassia tora* showed the highest (5.9mg/g) activity. Chanda et al<sup>12</sup> reported that acetone extract of

*Colocasia esculenta* leaves did not show any activity, while the methanol extract showed good activity. *Diplazium esculentum* is a green vegetable of North India showing maximum antioxidant power in aqueous extract (7.6 mM/dry wt. of extract) but methanol and acetone extracts were having less activities (4.16, 3.8 mM/dry wt. of extract)<sup>13</sup>. Katerere et al<sup>14</sup> have reported the lowest and the highest FRAP activities in *Vigna unguiculata* and *Corchorus olitarius* (0.58 and 5.04mmol/100g respectively).

### **Ferrous Ion Chelating Activity**

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However it is an extremely reactive metal and catalyses oxidative changes in lipids, proteins and other cellular components<sup>15</sup>. All the vegetable extracts in the present analysis exhibited more than 50% FICA the highest being in the ethanolic and methanolic extracts of *Celosia argentea*. In aqueous extracts, *Cassia tora* gave maximum activity. Arya and Yadav<sup>16</sup> have reported maximum chelating capacity in methanolic extract of *Cassia tora*, commonly consumed in Haryana. According to Gacche et al<sup>17</sup> FICA activity was higher in the leaves of *Spinacia oleracea* (43.9%) than that in *Trigonella foenum-graecum* (16.8%).

### **Reducing Power**

Reducing Power is often used to evaluate the ability of natural antioxidants to donate electrons<sup>18</sup>. In the present study, the ability of vegetable extract to reduce Iron (III) to Iron (II) was determined. All the extracts showed some degree of electron donating capacity in which ethanolic and aqueous extracts exhibited more or less similar results. The reducing power in methanolic extract of all vegetables was less. Reducing power of *Clerodendrum inerme* leaves has been reported to be maximum followed by *Clerodendrum serratum* leaves<sup>19</sup>. Reducing power in the wild leafy vegetable *Tricyrtis pilosa* was found the highest (256.62mg/g) whereas in *Allium porum* it was the lowest (12.07mg/g)<sup>20</sup>. According to Lobo et

al<sup>21</sup> *Cassia tora* in Thane district showed lower reducing power in aqueous and ethanolic extracts at lower concentration and it was increased with increasing concentration.

### **DPPH**

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. In the presence of hydrogen donor, DPPH is reduced. All vegetables in methanolic and ethanolic extracts showed appreciable free radical scavenging activity in the present study. The aqueous extract of *Clerodendrum serratum* showed the highest value (89.19%) while that of *Celosia argentea* had the lowest activity (3.25%). According to Jacob and Shenbagaraman<sup>22</sup> in *Hibiscus subdariffa* which is a leafy vegetable of Chennai, Tamilnadu, 98% free radical inhibition occurred at 250µg/ml concentration followed by *Centella asiatica* which exhibited a 100% inhibition at 400µg/ml concentration in ethanolic extract. Amongst the aqueous extracts, only *Moringa oleifera* showed a strong free radical inhibition capacity of 100% at 400µg/ml concentration. Gupta and Prakash<sup>23</sup> have reported a stronger radical scavenging activity (83.44%) in the leaves of *Murraya koenigii* than that in other green leafy vegetables. In *Amaranthus species* the scavenging activity recorded by them was minimum (38.18%).

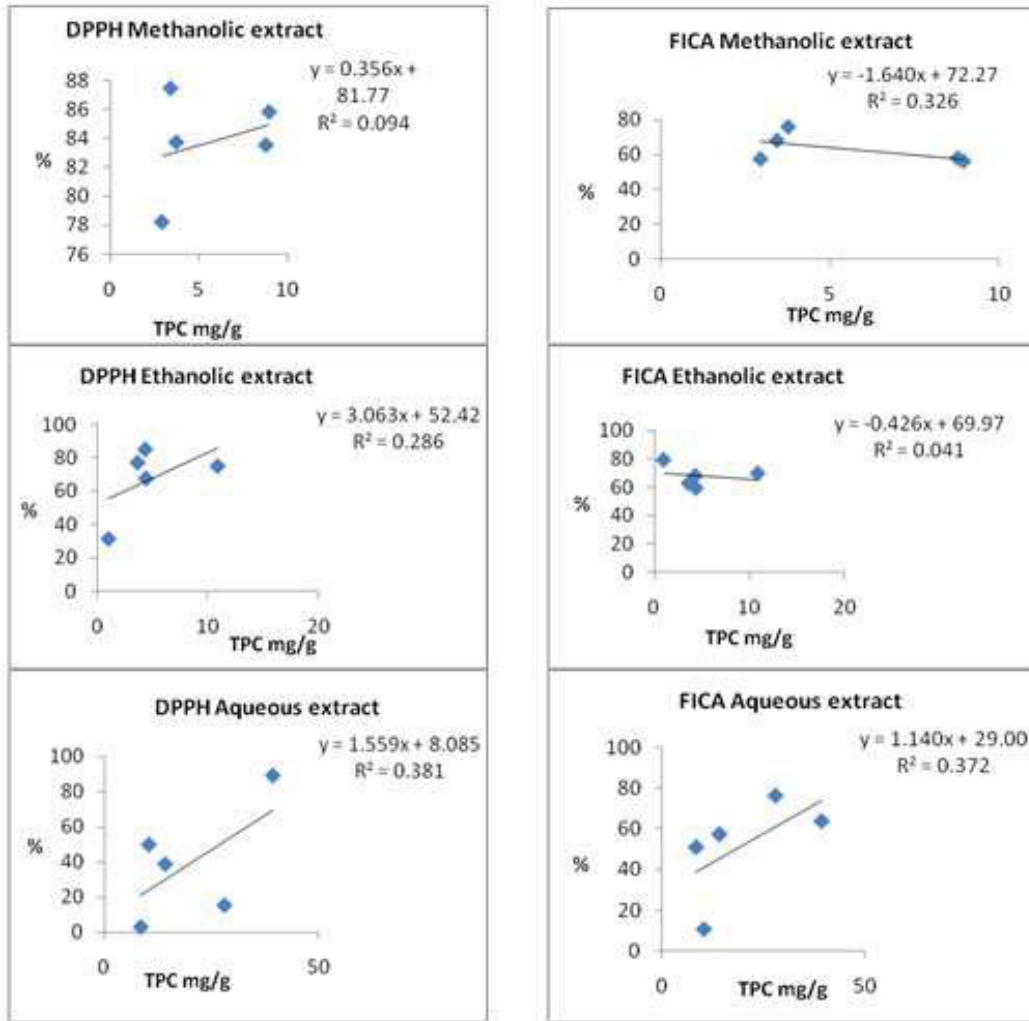
### **Total Phenolic Content**

It is well known that plant phenolics function as highly effective free radical scavengers and antioxidants. Phenol content of different vegetables from Ratnagiri District was higher in the aqueous extracts (Table 2). A high phenolic content has been reported in some vegetables grown in Turkey<sup>24</sup>. Khanzadi et al<sup>5</sup> claimed that in the leaves of *Cicer arietinum* the highest phenolic content (221.0mg/g) was present followed by *Brassica juncea* (189.3mg/g) and *Portulaca oleracea* (111.7 mg/g). The phenolic content of vegetables from Indonesia ranged from 0.33 to 1.52 mg/g fresh weight<sup>25</sup>.

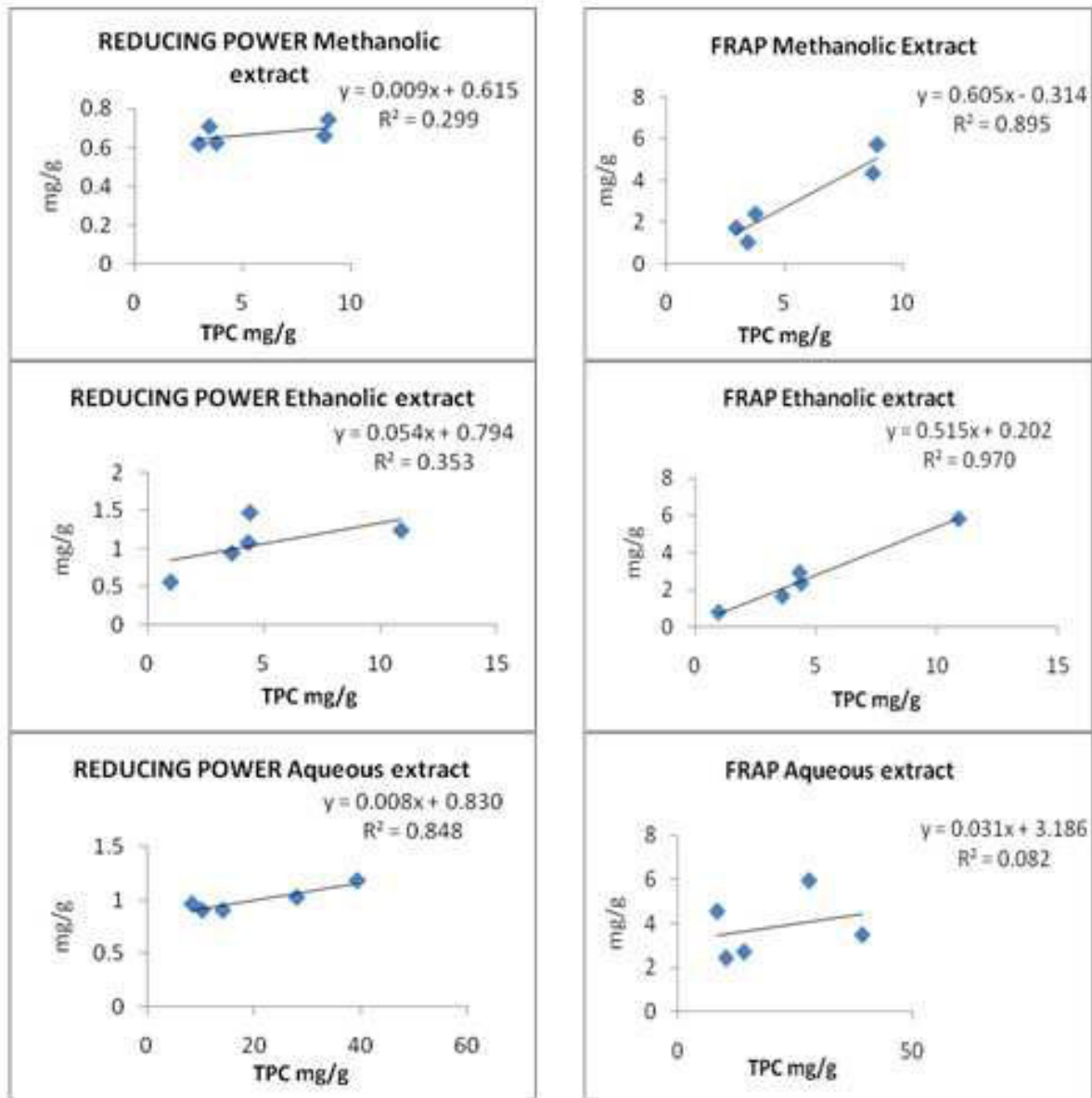
**Statistical Analysis**

The scavenging activity of phenolic compounds is due to the presence of hydroxyl groups in their structure. A correlation between the phenol content and antioxidant activities has been widely studied in different plants or foodstuffs.

From the present study, it is found that the FRAP activity in methanolic and ethanolic extracts and Reducing power scored in aqueous extracts correlate significantly with the total phenolic content (Figures 1 and 2).



**Figure 1**  
*Correlation between the total phenolic content (TPC) and DPPH and FICA in leafy vegetables*



**Figure 2**  
**Correlation between the total phenolic content (TPC) and FRAP and Reducing Power in leafy vegetables**

## CONCLUSION

A remarkable level of antioxidant potential was noticed in the seasonal vegetable samples collected from Ratnagiri District. These vegetables definitely add to the nutritive value and enrich the regular diet of coastal inhabitants.

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