Antioxidant activities and phenolic contents of the aqueous extracts of some Indian medicinal plants

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Antioxidants protect the body against oxidative stress by neutralizing free radicals. Plants contain rich amount of polyphenols which are very potent natural antioxidants. The present study was designed to evaluate the relative contribution of different polyphenols such as total phenolics, flavonoids and flavonol contents and their antioxidants activities. For this purpose the total phenolics, flavonoids and flavonol contents of some medicinal plants were determined in the aqueous extracts of leaves of Trichosenthes dioica, fruits of Moringa olifera and Ficus bengalensis as well as seeds of Emblica officinalis. Total antioxidant activity of these extracts was monitored by Free Radical Absorbing Power (FRAP) assay. In this paper, those parts of the plants are used for the analysis of aforesaid parameters which are normally overlooked. The total phenolic content of T. dioica leaves was about two times more than that obtained from the fruits and seeds of M. olifera and E. officinalis, respectively. However, the aerial roots of F. bengalensis registered presence of least phenolic content. The aqueous preparation from E. officinalis exhibited total flavonoid content twice as high as that of the other three plants. The extract from seeds of E. officinalis was found to contain highest antioxidant activity as compared to the preparations from other plants. The high antioxidant activity and flavonoids contents in E. officinalis seeds indicated that it could be exploited as an ingredient in developing a potential antioxidant supplement.

Key words: Antioxidant, flavonoids, flavonols, phenolics, FRAP assay, Ficus bengalensis, Trichosanthes dioica, Emblica officinalis, Moringa oleifera.

INTRODUCTION

Reactive Oxygen Species (ROS) are highly reactive molecules produced as a byproduct of metabolism of oxygen in mitochondrial respiratory chain (Finkel et al., 2000). The term ROS includes super oxide radicals ($O_2^-\circ$), hydroxyl radicals ($^\circ$OH), hydrogen peroxide ($H_2O_2$) and hydroperoxy radicals ($^\circ$HO$_2$) (Oberley, 2002). ROS are fully inactivated by intricate cellular and extracellular Antioxidant Defense Systems (AODS) (Halliwell et al., 1989). An imbalance between the generation and neutralization of ROS due to altered redox homoeostasis within the cell leads to Oxidative Stress (OS) (Oberley, 2002). This imbalance may be either due to an overproduction of ROS or deficiency of the antioxidant system. The OS is highly deleterious to cell as it causes damage to cell membrane via membrane lipid peroxidation and oxidative damage to DNA leading to many chronic and degenerative diseases such as atherosclerosis, diabetes mellitus, immune dysfunctions, Parkinson’s, disease and even cancer (Halliwell, 2000).

Although body’s intrinsic AODS including ROS quencher enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Gluthathione Reductase (GRed), glutathione-S-transferase, monamine oxidase and xanthine oxidase etc. as well as antioxidant molecules such as glutathione, vitamin C, vitamin E, uric acid etc. are able to arrest these ROS (Sies, 1993) but prolonged exposure to xenobiotics and infections overtake the intrinsic AODS
and cause irreversible oxidative damage to body (Tseng et al., 1997). Therefore, under the conditions of pro-
longed OS an exogenous supply of antioxidants (AO) is warranted in order to maintain the redox homeostasis and keep the debilitating diseases in check.

Researches over the years have produced convincing evidence towards application of natural antioxidants in place the synthetic molecules as the later have associated toxicities (Moure et al., 2001; Tseng, 2006). Among the natural antioxidants polyphenolic compounds such as flavonoids, flavonols and terpenoids etc. from plant origin have appeared as favored choice. By virtue of being electron rich these molecules can donate electrons to ROS and neutralize these chemical species (Halliwell, 1996; Gil et al., 1999).

Keeping the aforementioned facts in view we have elaborated our studies taking four Indian medicinal plants such as Moringa oleifera (commonly known as drum stick plant or Sahijan herb), Ficus bengalensis (Banyan tree), E. officinalis (Indian Goose Berry plant) and T. dioica (pointed gourd), which have been extensively explored in our laboratory for their antidiabetic and in vivo antioxidant activities (Rai et al., 2008; Jaiswal et al., 2009; Singh et al., 2009). The parts of the plants used in the present study were fruits of M. Olelra, buttratus root of F. bengalensis, seeds of E. officinalis and leaves of T. dioica, which have not been explored much in traditional Indian medicines. The present study has been conducted in the aqueous extract of the aforementioned parts of the plant. The polyphenolic contents were determined in terms of total phenolics, flavonoids and flavonols as the plant origin have appeared as favored choice. By virtue of being electron rich these molecules can donate electrons to ROS and neutralize these chemical species (Halliwell, 1996; Gil et al., 1999).

Collection of plants and preparation of extract

Trichosanthes dioica

Fresh leaves of T. dioica (7 kg) were collected in the month of June from the local area of Allahabad-U.P. (India) and shade-dried. It was authenticated by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, India. A voucher specimen has been submitted. The dried leaves (2 kg) were mechanically crushed and extracted with distilled water at 70°C using a Soxhlet, up to 54 h. The extract was filtered and concentrated in a rotary evaporator at 35 ± 5°C under reduced pressure, to obtain semisolid material, which was then lyophilized to yield a powder (about 11.3% w/w).

Moringa oleifera

Fresh fruits of M. oleifera (5 kg) were collected from the Botanical garden of University of Allahabad, Allahabad, India. It was identified and authenticated by Prof. Satya Narain in the month of March, 2008. A voucher specimen has been submitted. The fruit were washed thoroughly with distilled water, crushed and extracted twice with distilled water at temperature 60 - 70°C repeatedly, for 48 h. The resulting extract was filtered using Whatman no. 1 filter paper and concentrated in Rotatory evaporator under reduced pressure to give a semisolid residue, which was then lyophilized to get powder (yield: 11.7% w/w) for further exploration.

Emblica officinalis

The fruits of E. officinalis (15 kg) were purchased during the month of November from the local market of Allahabad, India and authen-
ticated by Prof. Satya Narayan. A voucher specimen has been submitted in the University herbarium. The fruits were boiled at 70°C and pulps of fresh fruits were separated to take out the hard seeds. The shade dried seeds (2.5 kg) were mechanically crushed and extracted with distilled water (65°C) using Soxhlet up to 72 h. The extract was filtered and concentrated in rotatory evaporator at 45 - 50°C under reduced pressure, to obtain semisolid material, which was then lyophilized to get a powder (yield 12.3% w/w) (Rai et al., 2008; Jaiswal et al., 2009).

Ficus bengalensis

Fresh aerial roots of F. bengalensis were collected and identified by Prof. Satya Narayan. The roots were dried and cut into small pieces, the pieces were mechanically crushed. Four kg of crushed aerial roots were continuously extracted with distilled water using soxhlet up to 48 h. The extract was filtered and concentrated in rotatory evaporator at 35 - 40°C under reduced pressure to obtain a semisolid material, which was then lyophilized to get a powder (12.32%, w/w).

Determination of total phenolics

Total phenolic contents in the extracts were determined by the modified Folin-Ciocalteu method as described earlier (Wolfe et al., 2003). An aliquot (100 µl) of the extracts was mixed with 5 ml Folin-
Ciocalteu reagent and sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany). All the other chemicals used including the solvents, were of analytical grade.

MATERIALS AND METHODS

Chemicals

2,4,6 tripyridyl -s-triazine (TPTZ), potassium ferricyanide; ascorbic acid, quercetin, AlCl₃ and FeCl₃ were purchased from Sigma Chemical Co. (St. Louis, MO, USA); Folin-Ciocalteu's phenol reagent and sodium carbonate were from Merck Chemical Supplies (Damstadt, Germany). All the other chemicals used including the solvents, were of analytical grade.
Determination of total flavonoids

Total flavonoid contents were determined using the method of Ordon et al. (2006). A volume of 0.5 ml of 2% AlCl$_3$ ethanol solution was added to 0.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content were calculated as quercetin equivalent (mg/g).

Determination of total flavonols

Total flavonols in the plant extracts were estimated using the earlier described method (Kumaran and Kumaran, 2007). 2.0 ml of 2% AlCl$_3$ ethanol and 3.0 ml (50 g/l) sodium acetate solutions were added in 2.0 ml of extract solution. The absorption at 440 nm was read after 2.5 h at 20°C. Sample extract were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content was calculated as quercetin equivalent (mg/g).

Total antioxidant activity (FRAP assay)

A modified method of Benzie and Strain (1996) was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer (3.1 g CH$_3$COONa and 16 ml CH$_3$OOH), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl$_3$·6H$_2$O solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml FeCl$_3$·6H$_2$O. The temperature of the solution was raised to 37°C before using. Plant extracts (150 µl) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were at 593 nm. The standard curve was linear between 200 and 1000 µM FeSO$_4$.

Results are expressed in µM Fe (II)/g dry mass and compared with that of ascorbic acid and quercitin.

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. The data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by using Prism software. The values showing p < 0.05 were regarded as significant.

RESULTS

Results obtained in the present study revealed that the level of these phenolic compounds in T. dioica was found to be 259 mg/g; however in M. oleifera and E. officinalis and F. bengalensis it was recorded to be 125, 120 and 70 mg/g of extract (Figure 1). The data indicates that T. dioica leaves contain very high amount of polyphenolic compounds as compared to the other three compounds. As shown in Figure 2, Flavonoids contents were observed to be highest in the seeds of E. officinalis (12 mg/g extract powder) however flavonoids content of the other three plants was just half of E. officinalis. The Figure 3 represents total flavonol content in the aqueous extract of the plants under observation. Arial root extract
of *F. bengalensis* was recorded to possess highest flavonol content among the four plants; however *T. dioica* and *M. oleifera* were recorded to contain less than half of *F. bengalensis*. *E. officinalis* was found to contain very small concentration of total flavonols.

When these plant extracts were subjected to FRAP assay, *E. officinalis* seed extract exhibited highest antioxidant capacity (85 ± 5.0 μM Fe²⁺/gm) followed by *T. dioica* (65 ± 1.5) and *M. oleifera* (51 ± 2.0) leaf and fruit extracts, respectively. However, *F. bengalensis* was recorded to contain least antioxidant activity (23 ± 1.7 μM Fe²⁺ /gm) as shown in Table 1.

Different ratios of polyphenolic compounds in the plant extracts are shown in Table 2. The data indicated varying levels of flavonoid dependent antioxidant activities in the extracts of different parts of the plants. The trend of presence of flavonoid content in different plant extracts was found to be *E. officinalis > F. bengalensis > M. oleifera > T. dioica*. The extract of *E. officinalis* also exhibited maximum antioxidant activity as shown in Table 1.

### DISCUSSION

Concerns over the safety of synthetic antioxidants have shifted the global interests towards exploration of antioxidant compounds from natural sources (Wanasundara and Shahidi, 1998). A plethora of phenolic compounds extracted from several plant species have been reported to possess strong antioxidant activities (Aqil et al., 2006; Pourmorad et al., 2006; Koleva et al., 2002). Phenolic compounds are ubiquitously present in plants, and when plants are consumed as foods, these phytochemicals contribute to the intake of natural antioxidants in the diets of human as well as animals. Out of all the phenolics, the flavonoids belong to a large family of compounds with a common diphenylpropane structure (C6C3C6) with different degrees of hydroxylation, oxidation and substitution. These compounds commonly occur as glycosides in plants and are reported to be most diverse and efficient as antioxidants (Pietta et al., 1998). The strong correlation observed between antioxidant activity and flavonoid content of different plants suggests a possible use of *E. officinalis* seeds and *T. dioica* leaves in making the active ingredients of antioxidant supplements from these plant tissues.

The flavonoid / total phenolics ratio was highest (0.106) in *E. officinalis*, followed by *F. bengalensis* (0.080). In the other two plants the ratios of flavonoid / total phenolic were very low; the values being 0.04 and 0.021 in *M. oleifera* and *T. dioica*, respectively. The results with *E. officinalis* and *M. oleifera* were found to be concomitant with the total antioxidant activity observed in these plants. However, there were anomalies with the extracts from *F. bengalensis* and *T. dioica* which did not correspond with their flavonoid / total phenolics ratios and the antioxidant activities. The flavonols / total phenolic and flavonol / flavonoids ratios were not consistent with the antioxidant capacities of the different parts of the plants (Table 2). The observed high AO activity in the aqueous extract of *T. dioica* leaves suggests the possible contribution of certain other factors such as vitamin C in its AO activity. These results are supported by our earlier study which had reported the occurrence of high concentrations of Vitamin C in *T. dioica* (Rai et al., 2008). In contrast *F. bengalensis*, despite having high ratios of flavonoid to...
total phenolics and flavonol to total phenolics exhibited very low AO activity. It might due to presence of certain other factors which could impede the AO efficacy of flavonoids in the root extract of *F. bengalensis*.

As antioxidants, flavonoids have been reported to be able to interfere with the biochemical pathways involved in the generation of reactive oxygen species (ROS), quenching free radicals, chelating transition metals and rendering them redox inactive in the Fenton reaction (Heim, 2002). Therefore, the presence of high amount of flavonoids and their multifaceted actions make the aforementioned plant extract a good candidate for exploration of antioxidants. Since, the parts of the plants used in the present study are generally ignored in the traditional system of medicine in India and are thrown away as wastes; the results from the present study indicate that it would be highly economical for the production of potential antioxidant supplement(s).

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**REFERENCES**


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The data presented in this Table have been derived from the values presented in the Figures 1, 2 and 3.

Table 2. Ratio of different polyphenolic compounds in the plant extracts.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Flavonoid/Total Phenolics</th>
<th>Flavonol/ Total Phenolics</th>
<th>Flavonols/Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichosanthes dioica</em> (Leaves)</td>
<td>0.021</td>
<td>0.006</td>
<td>0.270</td>
</tr>
<tr>
<td><em>Moringa oleifera</em> (Fruits)</td>
<td>0.040</td>
<td>0.009</td>
<td>0.220</td>
</tr>
<tr>
<td><em>Emblica officinalis</em> (Seeds)</td>
<td>0.106</td>
<td>0.005</td>
<td>0.051</td>
</tr>
<tr>
<td><em>Ficus bengalensis</em> (Aerial roots)</td>
<td>0.080</td>
<td>0.047</td>
<td>0.589</td>
</tr>
</tbody>
</table>