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Research Article

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Total Antioxidant Potential of Indigenous Indian Plants

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ABSTRACT

Reactive oxygen species cause much damage to cells by reacting with variety of molecules, whereas antioxidant compounds can delay or inhibit the oxidation processes done by such reactive oxygen species. Present research work was carried out to quantify total flavonoids, total phenolics and total antioxidant potential of ten indigenous plants namely Annona squamosa (Annonaceae), Syzygium cumini (Myrtaceae), Mangifera indica (Anacardiaceae), Musa acuminate (Musaceae), Murraya koenigii (Rutaceae), Combretum indicum (Combretaceae), Vitis vinifera (Vitaceae), Ficus carica (Moraceae), Morus alba (Moraceae), Morus nigra (Moraceae). The results have shown that total antioxidant activity is present in Mangifera indica leaves. It was also observed that Morus nigra exhibited maximum total flavonoid content, while minimum was seen in Combretum indicum. Mangifera indica had also exhibited highest total phenolic content, while minimum level of phenolics was found in Combretum indicum. When statistically analyzed by one way ANOVA it showed significant variation amongst antioxidant potential of studied plants with $p \leq .005$. When correlation was established between total phenolics and total antioxidants, a positive correlation of 0.53 was observed and between total flavonoid and total antioxidants a positive correlation of 0.48 was observed. Our results indicate Flavonoids and phenolics account for major antioxidant activity. Furthermore this antioxidant profile would be advantageous for epidemiological research and providing support for dietary guidelines.

Key words: Antioxidants, Flavonoids, *Magnifera indica*, Phenolics.

INTRODUCTION

Reactive oxygen species (ROS) exists in the form of singlet oxygen, superoxide anion, hydroxyl radical, and hydrogen peroxide (H_2O_2) are usually produced as end products of metabolism or from exogenous factors. These reactive species exert oxidative harm by interacting with almost every cellular component including DNA [1] Excess ROS, if not checked by antioxidants, causes increased quantity of free radicals and lipid peroxides which underlie the initiation of diseases and disorders like atherosclerosis, carcinogenesis, diabetes, cataract, ageing etc. Large number of medicinal plants has been investigated to explore probable sources of antioxidants. Antioxidants obtained from plants either in the form of raw extracts or their chemical constituents, are useful to inhibit any destructive processes caused by oxidative stress [2].

Scientific community is taking antioxidants as a topic of keen interest. Use of synthetic antioxidants in food products is being strictly regulated, due to their associated health risks [3]. Stress-sensitive species display a weak tolerance towards Reactive Oxygen Species under stressed environment and are subsequently exposed to a more severe 'oxidative stress' [4]. Total phenolics, mainly flavonoids and phenolic acids in plants possess the antioxidant

capacity. Flavonoids constitute a class of more than 10,000 structures which are usually secondary metabolites [5]. The biosynthesis of 'antioxidant' flavonoids is rapid in species which are sensitive to stress than in species which are tolerant to stress. Phenolic acids, occurring widely in fruits, vegetables and spices are another major class of phenolic compounds. Though hundreds of herbal extracts have been studied, not each and every compound is characterized and structurally elucidated yet because of being a very diverse and complex group. An aromatic ring along with one or more hydroxyl groups are a characteristic of phenolics. They are mainly synthetized from cinnamic acid [2]. Phenolics are able to scavenge free radicals of oxygen due to their ability to donate electron. Their antioxidant effectiveness in food depends not only on the presence of hydroxyl groups but also on parameters such as physical location, interaction with other food components, and environmental factors (e.g. pH). In several researches, phenolic compounds demonstrated higher antioxidant activity than other free radical scavengers such as carotenoids and vitamins [6].

In the present study ten potential plants, namely *Annona squamosa* (Sugar apple), *Combretum indicum* (Malti), *Ficus carica* (Common fig), *Mangifera indica* (Mango), *Morus alba* (White mulberry), *Morus nigra* (Black mulberry), *Murraya koenigii* (Curry leaves), *Musa acuminate* (Banana), *Syzygium cumini* (Indian blackberry or Jamun) and *Vitis vinifera* (Grapes) which are indigenous to Indian subcontinent are taken. The purpose of the present study is to elucidate the antioxidant properties of aforementioned plants and to measure antioxidant capacity of biomass which may facilitate to formulate the composition of pharmaceutical/ neutraceutical and herbal products for general health and well-being.

EXPERIMENTAL SECTION

Plant Samples

Samples were collected from all above mentioned plants growing in the surrounding areas and departmental garden of Amity University Uttar Pradesh, Lucknow campus. For extraction and analysis only fresh leaves from plants were included in the study. All collected samples were first washed with detergent followed by sterile water. They were blot dried and then used in the study. The plant parts were homogenized and extracted differently for different purposes as mentioned below.

Measurement of Flavonoids:

Total flavonoid was extracted by the method of Kevin et al., (2002) with some modifications[7]. 25gm of tissue was submerged in 100 ml of 80% ethanol overnight. The extract was filtered using Whatman no.1 filter paper. Filtrate was collected and used for estimation of flavonoids. Ethanolic extract was diluted to 1:10 (sample: ethanol) with 80% ethanol to a total of 3 ml. Absorbance was taken at 362 nm using double beam UV-VIS spectrophotometer (Model: UV-1800, Schimadzu, Japan) in triplicate and was calculated using standard curve of Quercetin (QE) as standard.

Measurement of Total Phenolics

Total phenolic content was determined by the modified protocol of Fatma et al., (2013) with some modifications [8]. 25 gm of tissue was homogenized with 100 ml of 70% ethanol. The extract was filtered through Whatman no. 1 filter paper. The total phenolic content was determined by using Folin-Ciocalteu reagent. A volume of 0.5 ml of the plant extract was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and then neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated for 30 min. The absorbance of the resulting color was measured at 765 nm. All the sets were prepared in triplicate. The total phenolic content was determined using standard curve of gallic acid (G.A) as standard.

Total Antioxidant Activity

Total antioxidant activity was estimated by the protocol of Cacig et al., (2005) with some modifications [9]. One gm of tissue was homogenized in 4 ml of double distilled water and incubated for 24 hour at 4°C. It was then filtered twice with Whatman no. 1 filter paper and the collected filtrate was stored at 4°C. 100µl of sample was taken in a 3 ml glass cuvette containing the oxidative mixture of 0.18 ml potassium permanganate (0.01 M); 0.42 ml sulfuric acid (2 M) and 2.3 ml distilled water. The decrease in absorbance was measured at 535nm, with standard using ascorbic acid.

In order to quantitatively compare the antioxidant activities, the following formula was used:

$$A50 = t\ standard \times \frac{C\ standard}{t\ plant\ sample} \times \frac{V\ standard}{m\ plant} \times \frac{V\ extract}{V\ plant\ sample}$$

where:
*A*50 — antioxidant activity articulated, reflected in the time until the sample induces a decrease of the oxidizing agent [$KMnO_4$] concentration up to one half, compared against a standard [ascorbic acid] (mmol equivalent / g plant tissue)

t plant sample — the time until the sample induces a decrease of the permanganate concentration up to one half (min)

t standard — the time until the standard (ascorbic acid) persuade a decline of the permanganate concentration up to one half (min) [0.66 minutes as seen in standard curve]
Cstandard — standard (ascorbic acid) concentration [0.01 mmol/ml]
m plant — weight (g) of the plant sample subjected for extraction [1g]
Vplant sample — volume of the plant extract subjected for the analysis [0.1 ml]
Vstandard — volume of the standard subjected for the analysis [1 ml]
Vextract — volume (ml) of the obtained extracts [4 ml]

Statistical Analysis
The data analysis was performed using Microsoft Excel 2010 software [10]. One way ANOVA (without replication) was done for analysis of variance to establish variation in the antioxidants among respective plants and their different parts.

RESULT AND DISCUSSION

A total of ten plants were tested for their antioxidant potential. Leaves were studied for flavonoids and phenolic content. The total antioxidant activity was also calculated in all of them. The results are represented in Table 1. Total phenolics were estimated spectrophotometrically using Folin-Ciocalteu method. It was observed that *Mangifera indica* exhibited highest total phenolics content; while minimum was observed in *Combretum indicum*. Significant variability was observed when one way ANOVA was applied with ($P< 0.05$). A previous comparative study of total phenolic content in some fruits also revealed that *Mangifera indica* has highest phenolic contents; while significant variation amongst different fruits in polyphenolic contents was established [11]. Further it was reported by Naseer et al., (2014) that *Azadirachta indica* possesses more phenolic content than those reported in *Mangifera indica*, and therefore *Azadirachta indica* is a better source for polyphenolics [12]. Comparative study by Srivastava et al., (2013) for total phenolic content in fruits also revealed that *Mangifera indica* is having good phenolic activity and the maximum flavonoid/phenolic ratio (F/P ratio) was found in *Lactuca sativa* which is a leaf vegetable [13]. In our study when total flavonoids were estimated spectrophotometrically using Quercetin (QE) as standard, *Morus nigra* exhibited highest total flavonoid content; whereas *Combretum indicum* had the lowest levels. The study by Iqbal et al., (2012) also supports that the flavonoid content is higher in *Morus nigra* as compared to other species [14]. Additionally, for flavonoids significant variability was observed when one way ANOVA was applied with ($p \leq .005$). In the present study, F/P ratio was found to be highest in *Morus alba*, whereas Sulaiman et al., (2012), having studied twenty medicinal plants, recorded the highest F/P ratio in *Cassia fistula*[15].

Total antioxidants were estimated spectrophotometrically and as evident from Table 1, *Mangifera indica* had the highest antioxidant content; while minimum was observed in *Morus alba*. The Pearson correlation was also calculated as a measure of strength for relationship between two variables. When correlation was established between total phenolics and total antioxidants, a positive correlation of 0.53 was observed; whereas correlation between total flavonoid and total antioxidants gave a value of 0.48. It was reported by Oruma et al., (2007) also that *Mangifera indica* exhibited high level of antioxidant activity even when stored for three months; however decrease in activity was noted [16]. In another study, antioxidant activities were evaluated in terms of total phenolics content, total antioxidant activity, and reducing power in *Kalanchoe pinnata* extract. All these antioxidant activities increased with increasing concentrations in a dose dependent manner and were found to be significant and valuable [17].

Table 1: Phenolics, flavonoids and total antioxidant values in studied plants

Plants	Phenolics (mg equivalent GA /gm of tissue) ±S.D.)	Flavonoids (mg equivalent QE /gm of tissue) ±S.D)	Flavonoids / Phenolics ratio (F/P ratio)	Total Antioxidnts (mM equivalent ascorbic acid/g tissue) ±S.D.)
Annona squamosa	2.36±0.04	1.61±0.17	0.68	0.23±0.01
Combretum indicum	1.25±0.01	1.26±0.15	1.01	0.20±0.08
Ficus carica	1.89±0.60	1.53±0.25	0.80	0.16±0.02
Mangifera indica	2.89±0.58	2.03±0.32	0.70	0.31±0.08
Morus alba	1.28±0.01	2.03±0.32	1.57	0.14±0.01
Morus nigra	1.48±0.57	2.16±0.30	1.45	0.30±0.07
Murraya koenigii	1.91±0.57	2.08±0.40	1.09	0.28±0.06
Musa acuminate	1.50±0.57	1.85±0.21	1.23	0.28±0.06
Syzygium cumini	2.69±0.57	1.53±0.40	0.56	0.29±0.05
Vitis vinifera	1.36±0.01	1.43±0.15	1.05	0.14±0.03

*N=3±SD**

**Each experiment was repeated thrice and Mean±SD wa s used in the study.*

CONCLUSION

The total antioxidant potential is a relevant tool for investigating the relationship between dietary antioxidants and pathologies induced by the oxidative stress [18]. Natural antioxidants have shown their roles in cancer, cardiovascular diseases and age related degeneration of cell components [19,20]. Flavonoids are particularly beneficial, acting as anti-oxidants and giving protection in cardiovascular disorders, certain cancer and age related degeneration of cell components. The biflavonoids have the ability to suppress the release of histamines and the adhesion of blood platelets [21]. The present work is a preliminary study to check the antioxidant potential of some indigenous plants of the subcontinent, where in the highest antioxidant activity was observed in *Mangifera indica* followed by *Morus nigra*, *Syzygium cumini*, *Musa acuminate* and *Murraya koenigii*. In addition, the flavonoid and phenolic content of leaves correlated closely with their total antioxidant activity. Moreover the F/P ratio was highest in *Morus alba*. The results indicate that total phenolics and flavonoids are the major contributors to the antioxidant activity in plants. The findings of the present study also support the recommendations of many national dietary guidelines which suggest eating a variety of foods every day. Efforts to encourage their utilization should be continued for overall health benefits. This antioxidant profile would be advantageous for epidemiological research and providing support for dietary guidelines.

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