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In vitro Antioxidant Capacity Assay of Few Selected Medicinal and Mangrove Plants

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Antioxidants are micronutrients that have gained importance due to their ability to neutralize free radicals or their actions. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in prevention of the free radical formation by scavenging or promotion of their decomposition and suppression of such disorders. Natural antioxidants have been widely used in different fields of industry and medicine because of their ability to protect against cardiovascular diseases, cancer, deficiencies in immune response, age-related problems, *etc*. In the present study, we have evaluated the antioxidant potential of the methanolic extracts of mangroves and medicinal plants for antioxidant capacity assays.

Key Words: Micronutrients, Free radical, Cardiovascular diseases, Antioxidant potential.

INTRODUCTION

The necessity of compounds with antioxidant activity is increasing as there is a realization that the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been linked in the pathogenesis of several human diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders and certain types of cancer[1,2]. Oxygen free radicals disintegrate DNA, destroy cell membranes and create havoc among cell's basic enzymatic metabolic processes[3]. Radicals which have one or more unpaired electrons are produced in normal or pathological cell metabolism. Free reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. Reactive oxygen species are various forms of activated oxygen, which include free radicals such as superoxide anion radicals (O_2) and hydroxyl radicals (OH), as well as non-free radical species (H_2O_2) and the singled oxygen (O_2). Reactive oxygen species are routinely generated during normal plant metabolic processes. Chloroplasts, mitochondria and peroxisomes are the most important intracellular generators of ROS like O_2^- and H_2O_2. Amongst these, chloroplast-mediated O_2^- and H_2O_2 production remains the most unavoidable consequence of an oxygen-enriched atmosphere. In chloroplasts, O_2^- and H_2O_2 are mainly produced by the electron acceptor of photosystem I, whereas singlet oxygen is generated by the transfer of an electron from an excited chlorophyll molecule to molecular oxygen. ROS are also observed in mitochondrial electron.

Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in prevention of the free radical formation by scavenging or promotion of their decomposition and suppression of such disorders[4]. There is growing interest towards natural antioxidants from herbal sources[5,6]. Search for antioxidant principles from plants and algae has been accelerated and these natural resources having potential antioxidant activities[7] have been identified. Natural antioxidants have been widely used in different fields of industry and medicine because of their ability to protect against cardiovascular diseases, cancer, deficiencies in immune response, age-related problems, *etc*.

In the present study, we have evaluated the antioxidant potential of the methanolic extracts of mangroves, medicinal plants for antioxidant capacity assays.

EXPERIMENTAL

Acanthus ilicifolius, Boerhavia diffusa, Cassia occidentalis, Calotropis procera, Centella asatica, Clitoria ternatea, Cleome viscosa, Datura metel, Dalbergia sissoo, Hildegardia populifolio, Holarrhena antidysenterica, Kyllinga nemoralis, Lantana camara, Lawsonia inermis, Mimosa pudica, Nyctanthes arbortristis, Oxalis corniculata, Pongamia pinnata, Plumeria rubra, Sesuvium portalucastrum, Solanum xanthocarpum, Sida rhombifolia, Sphaeranthus indicus, Terminalia chebula and *Tridax procumbens* collected from

varies places in India including Sundarbans,West Bengal and Kakinada, Godavari-Krishna delta and other areas of Andhra Pradesh.

Preparation of plant extract: The collected plants were properly shade dried and leaves stem bark and flowers of plant parts were air dried at room temperature to constant weights. The dried plant materials were ground separately to powder. One hundred gram (100 g) of each ground plant materials were shaken separately in methanol for 48 h on an orbital shaker. Extracts were filtered using a Whatman No. 1 filter paper. Each filtrate was concentrated and each extract was resuspended in methanol to make 100 mg/mL concentration and kept in dark at -2 ºC until further use.

Solvents and chemicals used: All chemicals were purchased from Merck, Qualigens fine Chemicals and SD Fine Chemicals, Mumbai.

Antioxidant capacity assay

Ferric reducing or antioxidant power assay (FRAP): The total antioxidant activity of the sample was assayed by the method as described earlier by Benzie *et al.*[8]. The FRAP method for measuring the ferric reducing power (reduce the TPTZ-Fe(III) complex to TPTZ Fe(II) complex ability) of plasma (FRAP) or algal extract. In the FRAP assay, an aliquot of the samples (10-40 µL) was mixed with 3 mL of ferric-TPTZ reagent. The change in absorbance was measured at 593 nm after initial mixing and up to 90 min until it reached a plateau. Aqueous solutions of known Fe(II) concentration ($FeSO_4 \cdot 7H_2O$) were used for calibration of the FRAP assay and antioxidant. The results expressed as ascorbic acid equivalents (µ mol/mL) or FRAP units (Fig. 1).

Di phenyl picrial hydrazyl radical scavengigng assay (DPPH): The DPPH (diphennyl picrial hydrazyl) radical scavengigng assay was carried out as described earlier by Cuendet *et al.*[9]. Briefly, 5.0 mL of DPPH solution (0.004 %) in methanol was added to 50 µL of plant extract.

After 0.5 h of incubation period at room temperature, the absorbance was read against a blank containing sample and methanol at 517 nm. Control containing the buffer and reagent was carried out. Similarly positive controls are treated in the same way as test sample replaced by positive control. Butly hydroxyl touline (BHT) used as positive control. Inhibition (I) of diphennyl picrial hydrazylradical in present was calculated in the following way.

Percentage of inhibition (I) = (Absorbance of control – Absorbance of test) /Absorbance of control) × 100

RESULTS AND DISCUSSION

Total antioxidant capacity (TAC) results are summarized in Figs. 1 and 2. The activity of FRAP was highest (900) in *S. xanthocarpum* followed by (825) *C. ternatea* and where as DPPH radical scavenging activity (%) was significant and found 70 % in *N. arbortristis* compared to positive control BHT 74.5 %. Results revealed that methanolic extracts of selected medicinal and mangrove plants antioxidant properties (AOP) or activities (AOA) are significant.

However, the strength of the existing data is not enough to suggest a reasonable mode of action for antioxidant effects. The data of this study may just enrich the existing comprehensive data of antioxidant activity of plant materials. Though several mangroves are extensively used in traditional medicine,

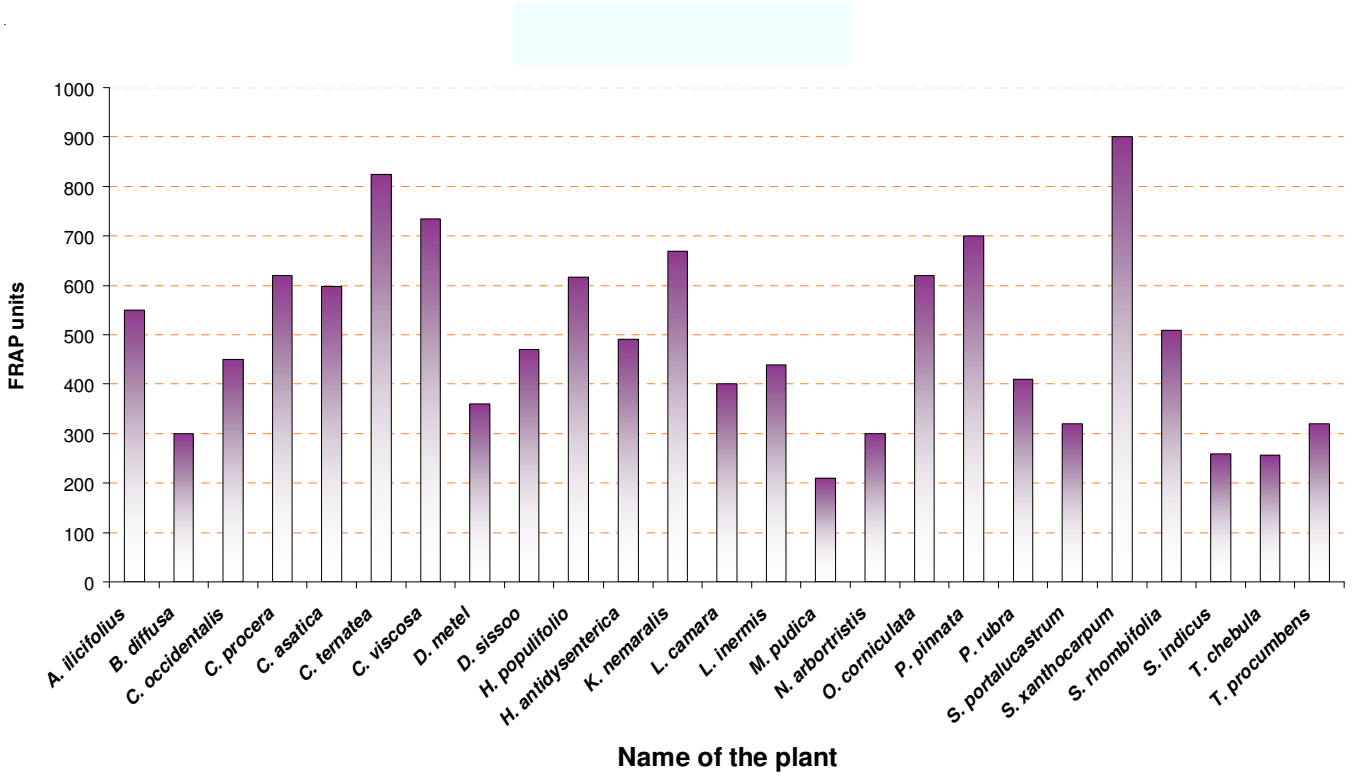

Fig. 1. Antioxidant activity capacity of some medicinal and mangrove plants

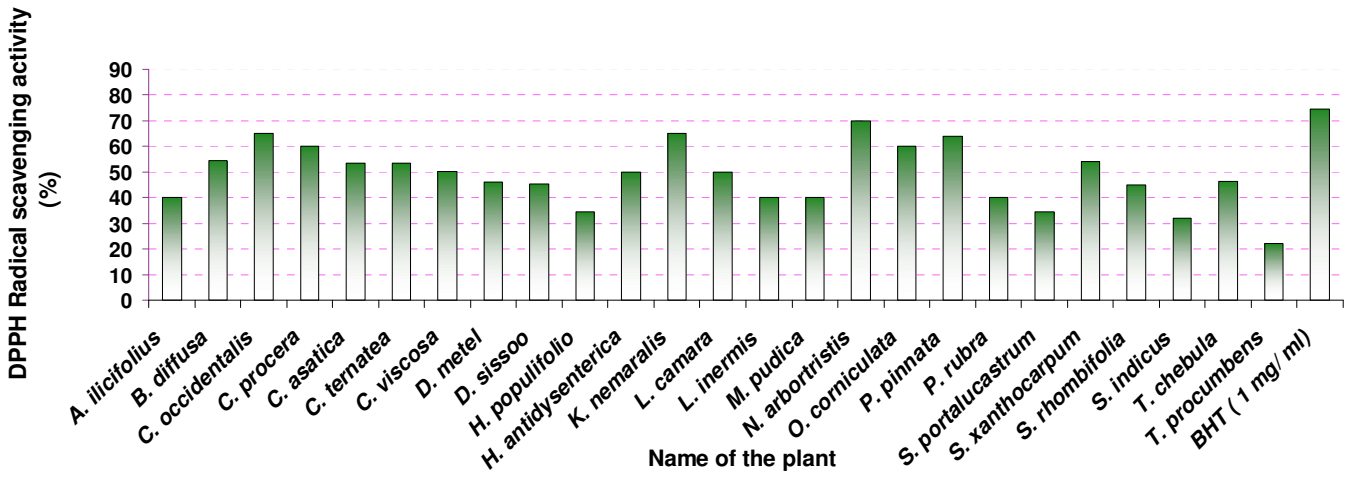

Fig. 2. Antioxidant capacity assay of some medicinal and mangrove plants

only some of them were tested for biological activities and a very few were studied for antioxidant activity. Different reports are found on the literature, whereas some authors found correlation between the total phenolic content and the antioxidant activity, others found no such relationship. Based on these results, it is also suggested that different methods must be applied to determine the antioxidant activity of samples as a single method alone may not be reliable.

Conclusion

Free radicals are often generated as by products of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. Medicinal and mangroves plants are promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of the existing data is not enough to suggest a reasonable mode of action for antioxidant effects. The data of this study may just enrich the existing comprehensive data of antioxidant activity of natural materials. Most of the antioxidant compounds are flavanoids, isoflavones, coumarines, lignans, anthocyanins, flavones and isocatechins. These plants, rich in flavonoids and phenolic acids could be a good source of natural antioxidants. The findings from this work may add to the overall value of the medicinal potential of important plants. The information provided in this studies can be useful to many research groups considering the huge interest in the search for new natural sources of functional ingredients.

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