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Evaluation of Antioxidant potential of selected Mangrove Plants

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ABSTRACT

Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. A variety of free radical scavenging antioxidants are found in plants. The purpose of this study was to evaluate the antioxidant activity of methanolic extracts of mangrove plants. Our research indicates that India's mangrove plants have the potential in scavenging free radicals and can be a vital source of antioxidant phytochemicals. Our results showed that plant extracts showed significant levels of enzymatic and non-enzymatic antioxidants and also exhibited antioxidant capacity.

Keywords: Mangroves, Radical scavenging properties, enzymatic and non-enzymatic antioxidants.

INTRODUCTION

It has been established that oxidative stress is among the major causative factors induction of many chronic and degenerative diseases and free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, is chemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [1,2] . Considerable scientific evidence suggested that under situations of oxidative stress reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals are generated and the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system [3] .Oxidation process is one of the most important routs for producing free radicals in food, drugs and even living systems. Catalase and hydro peroxidase enzymes convert hydrogen peroxide and hydro peroxides to non radical forms and function as natural antioxidants in human body. Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions.

There is growing interest towards natural antioxidants from herbal [4, 5, 6]. In the longer term, plant species(or their active constituents) identified as having high levels of antioxidant activity in *In vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage. Commercial use of mangroves as source of timber, fuel has long been recognized in tropical coastal zones. Besides, mangroves also provided many non-timber products such as tannin, fish poison, medicine, food, fodder, etc. [7].They have been used as traditional medicine in South Asian countries including India. Search for antioxidant principles from plants has been accelerated and many plants having potential antioxidant activities [8] have been identified. The plants used in traditional medicine are still a large source of natural antioxidants that might serve as leads for the development of novel drugs [9] .

We have very little information on the antioxidant potentials of India's mangroves and halophytes. In the present study, we have evaluated the antioxidant and potential of the methanolic extracts of twenty mangrove plants by determining various enzymatic and non-enzymatic antioxidant capacity assays.

MATERIALS AND METHODS

Plant material:

Avicennia officinalis (Avicenniaceae), *Avicenniaalba* (Avicenniaceae), *Aegicera scorniculatum* (Mysinaceae), *Avicennia marina* (Avicenniaceae), *Bruguiera cylindrica* (Rhizophoraceae), *Bruguiera gymnorrhiza* (Rhizophoraceae), *Ceriops decandra* (Rhizophoraceae), *Excoecaria agallocha* (Euphorbiaceae), *Hibiscus teliaceous* (Malvaceae), *Lumintzera recemosa* (Combretacea), *Myriostachya wigtiana* (Poaceae), *Rhizophora conjugata* (Rhizophoraceae), *Rhizophor amucronata* (Rhizophoraceae), *Salicornia brachiata* (Chemopodiaceae), *Salvodara persica* (Salvodoraceae), *Sonneratia apetala* Sonneratiaceae), *Sesuvium portalucastrum* (Azioaceae), *Thespesia populneoides* (Malvaceae), *Tamarix aphylla* (Tamariscaceae), *Xylocarpus granatum* (Malvaceae) all the plant materials were collected in and around coringa mangrove forest, south coast of Krishna and Godavari delta, Andhra Pradesh, India

Preparation of plant:

Leaves stem bark and flowers of mangrove plant parts were air dried

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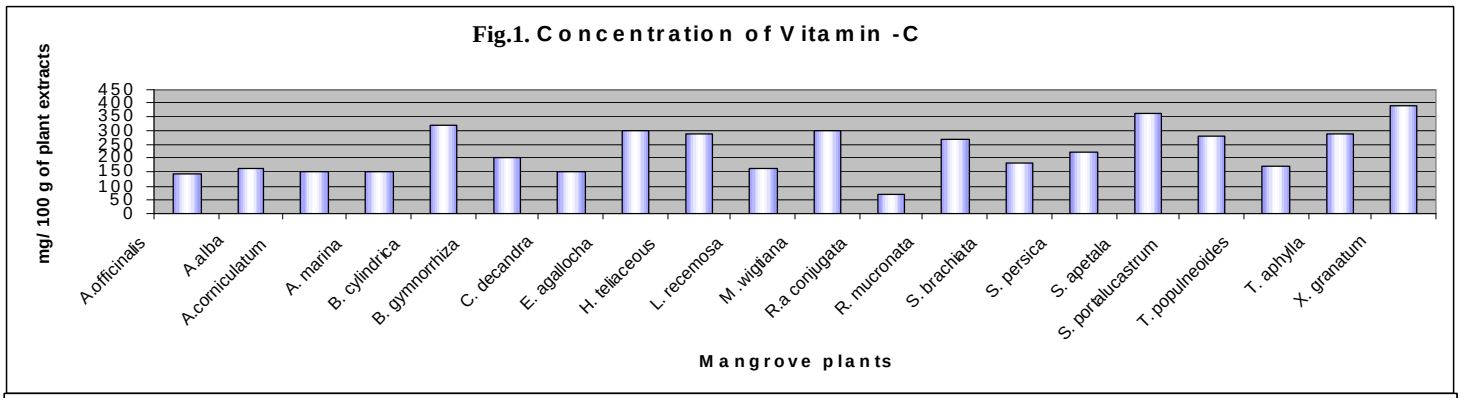
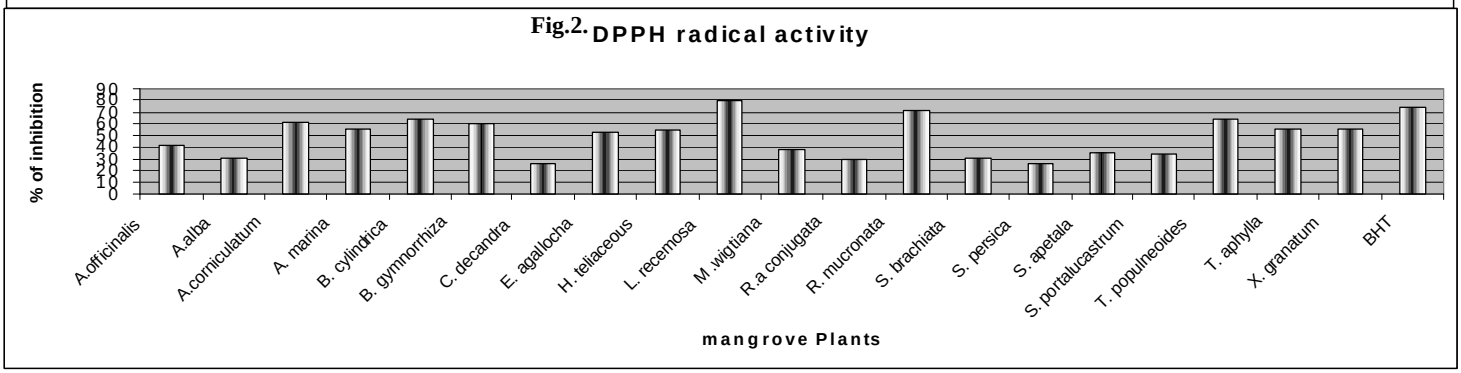
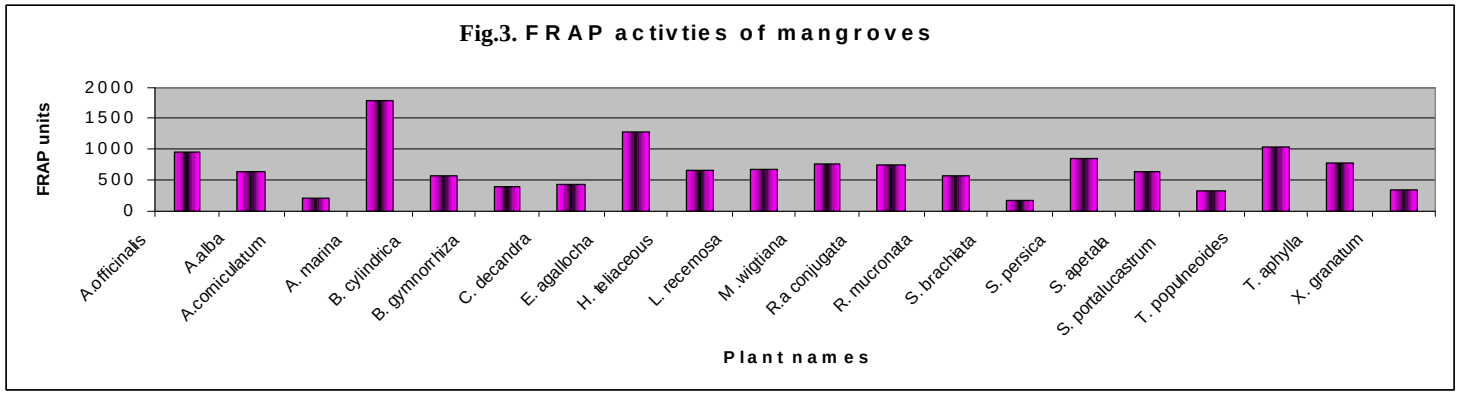
Table: 1 Enzymatic and non - enzymatic antioxidant activities

Name	SOD (U/mg)	Catalase (U/mg)	Ascorbic acid mg / 100 g	DPPH	FRAP units
A.officinalis	1.24 ± 0.05	1.81 ± 0.02	140	42.2	960
A.alba	0.96 ± 0.003	0.98 ± 0.07	160	30.2	625
A.corniculatum	0.67 ± 0.005	0.76 ± 0.018	150	61	186
A. marina	1.44 ± 0.0.17	0.43+0.07	150	56	1772
B. cylindrica	1.27 ± 0.008	0.81± 0.08	320	64	576
B. gymnorrhiza	0.87 ± 0.008	0.60 ± 0.008	200	60	400
C. decandra	1.49 ± 0.036	0.63 ± 0.004	150	26	430
E. agallocha	0.72 ± 0.10	0.55 ± 0.07	300	53	1290
H. teliaceous	0.82 ± 0.05	0.42 ± 0.08	290	55	650
L. recemosa	1.39 ± 0. 09	0.18 ± 0.004	160	79	672
M .wigtiana	1.04 ± 0.024	0.47 ± 0.020	300	38.2	770
R. conjugata	1.31 ± 0.19	0.95 ± 0.06	70	30	730
R. mucronata	0.25 ± 0.27	0.46 ± 0.026	270	71.6	576
S. brachiata	0.92 ± 0.07	0.28 ± 0.16	180	30.2	180
S. persica	0.26 ± 0.09	0.63 ± 0.034	220	25.9	840
S. apetala	1.64 ± 0.025	0.68 ± 0.002	360	35.4	620
S. portalucastrum	0.41 ±0.02	0.27 ± 0.05	280	34.5	320
T. populneoides	1.65 ± 0.16	0.24 ± 0.16	170	64.1	1038
T. aphylla	0.65 ± 0.06	1.6 ± 0.05	290	55.4	790
X. granatum	0.44 ± 0.14	0.24 ± 0.05	390	55.5	350
BHT (1 mg/ ml)	NT	NT	NT	74.5	NT

(For SOD and CAT values presented in average of three determinations and expressed as mean ± S. D), (Each value in an average of triplicate)







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 hrs 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 stock solution.

Assay of enzymatic antioxidants

Assay of super oxide dismutase (SOD)

The assay of super oxide dismutase (SOD) was carried out by the previously described method [10] .In brief, 0.5 ml of plant extract, 1 ml of Na2Co3, 0.4 ml of NBT and 0.2 ml of EDTA were added. The reaction was initiated by adding 0.4 ml of Hydroxyl amine Hydro chloride. Zero time absorbance was taken at 560 nm using Spectrophotometer followed by recording the absorbance after 5 minutes at 25 degrees. The control was simultaneously run without plant extract. Units of SOD were expressed as amount of enzyme required inhibiting the reduction of NBT by 50%. The specific activity was expressed in terms of units per mg protein.

Assay of catalase (CAT)

The catalase (CAT) activity was assayed by the titrimetric method [11]. To 2.5 ml of phosphate buffer, *p*H 7.5 , 2.5 ml of the 0.9% H_2O_2 (v/v) in the same buffer were taken and 0.5 ml of the enzyme extract was added and incubated at room temperature for 30 minutes. The reaction was arrested by adding 0.5 ml of 2 *N* H_2S0_4 and the residual H_2O_2 was titrated with 0.1 *N* KMno4 solutions. A blank was carried out similarly with boiled enzyme extract.Unit of enzyme activity was expressed as ml of 0.1N $KMn0_4$ equivalents of H_2O_2 decomposed per mg protein.

Estimation of non-enzymatic antioxidants

Estimation of Vitamin – C

Ascorbic acid content was determined by the procedure described previously by [12].Pipette out 5.0 ml of the working standard solution in to a 100ml conical flask then 10ml of 4% oxalic acid was added and titrated against the dye. End point was the appearance of pink color, which persists for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid present in the plant extracts. Similar titration was carried out with 5.0 ml plant extracts. Amount expressed as (mg/100 g).

Antioxidant capacity assay

Ferric reducing or antioxidant power assay (FRAP)

The total antioxidant power of the sample was assayed by the method as described earlier by [13]. 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 plants .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 (FeSO4.7H2O) were used for calibration of the FRAP assay and antioxidant The results expressed as ascorbic acid equivalents (µ moles /ml) or FRAP units.

Di*phennyl picrial hydrazyl radical scavengigng assay (DPPH)*

The DPPH (Diphennyl picrial hydrazyl) radical scavengigng assay was carried out as described earlier by [14]. Briefly, 5.0 ml of DPPH solution (0.004 %) in methanol was added to 50 µl of plant extractc. After 30 min 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 sample replaced by positive control. Butly hydroxyl touline (BHT) used as positive control. Inhibition (I) of Diphennyl picrial hydrazyl radical in present was calculated in the following way.

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

RESULTS

Enzymatic antioxidant levels

The results obtained on the enzymatic antioxidants of twenty mangrove plants are presented in **Table 1**. Among all the plant extracts *T. populneoides* (1.65 ± 0.16) (units / mg protein respectively) shown highest SOD activity where as highest CAT were found with *A.officinalis* (1.81 ± 0.02) followed by *T. aphylla* (1.6 ± 0.05) units / mg protein respectively.

Non- enzymatic antioxidant levels

The results obtained on the non-enzymatic antioxidants of twenty mangrove plants are presented in **table 1 and Fig 1**. Significantly high levels of *Vitamin – C* was found in *S. apetala* (360 mg/100 g) or (3.6 mg/g).

Antioxidant capacity

Results presented in **Table 1and Fig 2** of Antioxidant capacity of methanolic extracts of twenty mangrove plants were determined by FRAP method**.** The total antioxidant power (FRAP) was highest in *A. marina* (1772 FRAP units).

Results presented in **Table 1 and Fig 3** the DPPH free radical scavenging of antioxidants is due to their hydrogen donating ability; the plants with higher hydrogen donating capacity have shown higher DPPH free radical scavenging activity [15] . The highest Percentage of inhibition was found in *R. mucronata* (71.6)

The result summarizes the enzymatic and non enzymatic antioxidation activities of mangrove plant extracts are given in **Table 1**. The findings of this study support this view that some medicinal 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 plant materials. Though several mangroves are extensively used in traditional medicine, only some of them were tested for biological activities and a very few were studied for antioxidant activity [16].

DISCUSSION

We found that *T. populneoides, S. apetala, A. marina, R. mucronata* are good sources of natural antioxidants among selected mangrove plants. 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, only some of them were tested for biological activities and a very few were studied for antioxidant activity [15]. Further studies are required to identify the active principles responsible for the significant antioxidant effect.

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