Antioxidant Activity Evaluation of Methanolic Leaf Extracts of Krishna Estuary Mangroves, Andhra Pradesh., India

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Abstract: *Antioxidants are vital substances that protect the body from damages caused by free radical-induced oxidative stress. A variety of natural antioxidants are found in plants. The present study is aimed at assessment of relative antioxidative potential of methanolic leaf extracts of eight mangrove species. Four different assays have been used to determine the best mangrove with superior antioxidant capacity. B. gymnorrhiza is adjudged as the best species. E. agallocha, A. marina, A. officinalis and A. rotundifolia exhibited moderate activity, where as less antioxidant activity is recorded in A. corniculatum, R. apiculata and B. cylindrica.*

Key words: *Krishna Estuary mangroves, phenolic content, Reducing power, Oxidative stress, Antioxidants.*

1. INTRODUCTION

Free radicals/oxidants are being produced with enhanced rate during oxidative stress in organisms. The family of free radicals generated from the oxygen is known as 'Reactive Oxygen Species (ROS) and responsible for many diseases. Antioxidants are important since they offer protection to organisms from damage caused by free radical ROS. The antioxidants present in plants offer considerable resistance to oxidative damage caused by ROS. Mangrove plants are rich in phytochemicals capable of removing free radicals before they cause damage. Therefore the present study is aimed at evaluation of antioxidant potential of eight mangrove species. Antioxidant potential of mangrove species is assessed through four different assays evaluation of viz., i) DPPH free radical scavenging ii) reducing power iii) total phenolic content and iv) total antioxidant activity.

2. Materials and Methods

Eight mangrove species were collected from Krishna estuary, Nagayalanka, Andhra Pradesh, India which is located between latitude $15^{0}15^{1}$ - $15^{0}55$N and $80^{0}45^{1}$-81^{1}00E longitude. The plant material were identified to their species level with the help of available flora. The plant specimens were preserved in the dept. of Botany & Microbiology, Acharya Nagarjuna University. Methanolic leaf extracts of eight mangrove species constitute the materials for the present study.

Preparation of plant extract

Leaves of eight mangroves were air dried at room temperature to constant weights. The dried plant materials were ground separately to powder. 100 gms 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 by soxhlet apparatus and each extract was resuspended in methanol to make 100 mg/ml stock solution.

DPPH free radical activity

Diphenyl Picryl Hydrozyl radical scavenging assay was determined by Bamionuri *et al*., 2010. Briefly, 5 ml of DPPH solution (0.004%) in methanol was added to 50 µl of plant extract. After 30 minutes 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 were treated in the same way as test sample replaced by positive control. Butyl Hydroxyl Touline (BHT) used as positive control. Inhibition (I) of Diphenyl Picryl Hydroxyl radical was calculated in the following way.

$$\text{Percentage of inhibition (I)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Total Phenolic compounds estimation

The total phenolic contents in the extract, was determined by Bomaniri *et al*., 2010 with minor modifications, including gallic acid as standard and Folincioculteau reagent. To 2.5 ml of 10% Folincioculteau reagent 2 ml of Na_2CO_3 (2%, w/v) was added to 0.1 ml of each sample (3 replicates) of plant extract solution (1 mg/ml). The resulting mixture was incubated at 45^0C with shaking for 15 minutes. The absorbance of the sample was measured at 765 nm using Uv/visible light. Results were expressed as mgs of Gallic acid (20-100 µg/ml) dissolved in water.

Reducing Power assay (RP)

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Extracts (100 ml) of mangrove plant parts were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and 1% Potassium Ferricyanide (2.5 ml). The mixture was incubated at 50^0C for 20 minutes. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured to 700 nm. Reducing power in given in ascorbic acid equivalent (AAE) in milligram per gram of dry material.

Total Antioxidant activity

It is determined by the conjugated diene method of Lingnert *et al* (1979). The extract is mixed with 2 ml of 10 mN linoleie acid emulsion in 0.2 M sodium phosphate buffer (P^H 6.6) and kept in dark at $37^{0C.}$ After 15 hrs of incubation, 0.1 ml from each tube is mixed with 7.0 ml of 80% methanol. The absorbance is read at 234 nm against blank in spectrophotometer. Antioxidant activity (%) = $\frac{AO-A1}{AO} \times 100$ where AO is the absorbance of control-A1 is the absorbance of test. Phenol is the positive control.

3. Results and Discussion

Total phenolic content and total antioxidant activity of methanolic leaf extracts of eight mangrove species are presented in table1. Leaf extract concentrations raging from 100µg to 500 µg were subjected to above assessment. 100 µg methanolic extract of *B. cylindrica* contained less

amount of phenolic substances (34.26 µg) and it was proportionately increased with an increase in extract concentration up to 500 µg in all the species. 500 µg leaf extract of *B. gymnorrhiza* contained the maximum quantity (95.62 µg) of phenolics followed by *E. agalloch* (84 µg), *A. marina* (82.78 µg), *A. rotundifolia* (82.62 µg), *A. officinalis* (79.09 µg), *A. corniculatum* (63.03 µg) *R. apiculata* (62.32 µg) and *B. cylindrica* (52.97 µg).

Total antioxidant activity of 500 µg extract of *B. gymnorrhiza* was found be maximum (93.24%) and it was minimum in *B. cylindrica* extract (52.87%). The other species viz., *E. agallocha, A. rotundifolia* and *A. marina* were rated next to *B. gymnorrhiza* with the range of antioxidant activity between 79.42 to 84.48%.

The DPPH free radical scavenging activity of eight mangroves is presented in table 2. Free radical inhibition percent of mangrove extracts was increased steadly with an increase in extract concentration of mangroves from 100 µg to 500 µg. The free radical scavenging activity of *B. gymnorrhiza* 500 µg extract of excelled other species with 93.15% inhibition *A. corniculatum* extract (500µg) showed the minimum inhibition (62.97%) of DPPH radical while the other six species manifested the DPPH radical scavenging in the range from 72.09% to 75.3%.

Reducing power of the mangroves extracts was presented in the table2. 500µg extract of *A. corniculatum* has the lowest percent (65.82) of reducing power, where as *B. gymnorrhiza* expressed the highest percent (89.71%) of reducing power. The remaining six mangroves exhibited moderate reducing power rangining between 80.41% and 86.48%.

Discussion

Plants serve as a reservoir of effective chemotherapentants. Organisms are equipped with antioxidant system via many natural compounds and enzymes. In addition to the inherent mechanisms, exist in organisms, they must be sometimes supplemented through diet. In this context natural antioxidants from plants appear to be the solution to mitigate the effects of oxidative stress. Hence, there is growing demand for antioxidants from plants. The plant based antioxidants belong to different classes ranging from carotenoids, flavonoids, polyphenols, galic acid derivatives, tannins and catechins.

According to Miles *et al* (1998) mangroves have been considered as great source for chemical compounds of potential value. The flavonoids and polyphenolic compounds are powerful antioxidants due to the presence of C_4-C_7 hydroxyl groups that act as hydrogen donors. The phenolic compounds and flavonoids have positive correlation with free radical scavenging activity (Cushnie &Andrew 2005; Bandini *et al* 2006; Deepanjan *et al* 2008).

The results of present study comprehensively revealed that *B. gymnorrhiza* possess greater antioxidant potential since its extract contain the highest DPPH free radical scavenging activity, reducing power, phenolic content and total antioxidants. Mldadulal Haq *et al* (2011) and Kiran Kumar & Sitaram (2013) also found the highest antioxidant activity in *B. gymnorrhiza.* Moderate antioxidant activity was found in *E. agalloch, A. marina*, *A. officinalis* and *A. rotundifolia*. Relatively less antioxidant activity was recorded in *A. corniculatum*, *R. apiculata* and *B. cylindrica.* In the mangroves of present study Suneeta (2020) reported the presence of flvonoids, terpenoids and hydrocarbons (alkanes and alkenes). Hence, the compounds may be responsible for the antioxidant activity of eight mangrove species in the present study.

Table 1: Total phenolic content and Antioxidant activity of methanolic leaf extracts of eight mangrove species

S.No	Name of the Mangrove species	PHENOLIC CONTENT (µg)					TOTAL ANTIOXIDANT ACTIVITY (%)				
		Extract conc. in µg					Extract conc. in µg				
		100	200	300	400	500	100	200	300	400	500
1	***A.corniculatum***	49.20	54.16	56.92	60.91	63.03	48.09	54.90	59.72	62.27	63.23
2	***A. Marina***	42.76	51.37	68.78	73.23	82.78	33.06	45.21	54.31	76.09	79.42
3	***A. officinalis***	44.02	64.21	65.62	68.78	79.09	23.53	34.06	57.71	59.23	64.34
4	***A. rotundifolia***	51.32	66.42	67.53	73.09	82.62	32.05	49.62	52.23	71.64	82.32
5	***B. cylindrica***	34.26	38.04	49.82	50.20	52.97	33.09	42.32	48.08	51.03	52.87
6	***B. gymnorrhiza***	42.03	73.92	77.62	81.93	95.62	15.87	32.34	50.27	71.14	93.24
7	***E. agallocha***	41.05	57.42	73.92	78.09	84.00	26.52	38.32	59.15	72.62	84.48
8	***R. apiculata***	42.26	54.31	56.42	59.51	62.32	35.57	39.61	42.62	57.43	62.21

Table. 2 : DPPH free radical scavenging and Reducing Power (RP) activity of methanolic leaf extracts of eight mangrove species.

S.No	Name of the Mangrove species	DPPH (%)					REDUCING POWER (%)				
		Extract conc. in µg					Extract conc. in µg				
		100	200	300	400	500	100	200	300	400	500
1	***A. corniculatum***	12.50	21.03	35.01	54.19	62.97	43.23	56.32	58.78	59.04	65.82
2	***A. Marina***	10.90	21.23	34.20	49.52	73.12	42.34	56.28	69.31	74.71	80.41
3	***A. officinalis***	15.25	29.36	46.97	61.06	72.80	42.26	58.29	73.34	78.62	86.48
4	***A. rotundifolia***	13.23	19.31	34.90	47.73	75.30	43.03	50.52	66.25	79.62	82.02
5	***B. cylindrica***	11.08	23.32	35.03	52.13	69.16	46.56	59.23	62.31	71.52	72.09
6	***B. gymnorrhiza***	15.76	32.73	50.35	71.92	93.15	32.98	55.73	62.35	74.82	89.71
7	***E. agallocha***	12.90	26.23	38.20	51.00	75.12	44.06	74.12	74.51	81.32	84.41
8	***R. apiculata***	9.27	20.00	35.23	43.41	72.09	50.62	62.59	74.26	76.31	85.23

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