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Antioxidant Activity of Leaf Extracts of the *Excoecaria Agallocha* and *Agialitis rotundifolia* in Krishna District, Andhra Pradesh., India

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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 methanol, Ethyl Acetate, Chloroform and Acetone extracts of the 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 antioxidants and also exhibited antioxidant capacity.***

Keywords: Mangroves, Antioxidants, *Aegialitis rotundifoliae, Excoearia agallocha*.

1. Introduction

Reactive Oxygen Species (ROS) are various forms of activated oxygen, which include free radicals, e.g., superoxide anions (O_2^-), hydroxyl radicals (OH), non free radical compounds (H_2O_2) and singlet oxygen ($^1O_2$), which can be formed by different mechanisms in living organisms. Oxidative damage of DNA molecules associated with electron transfer reactions is an important phenomenon in living cells, which can lead to mutations and contribute to carcignogenesis and the aging process. ROS species are considered as important causative factors in the development of certain diseases such as diabetes, stroke, arteriosclerosis, cancer and cardiovascular diseases in addition to the aging process. Prior administration of antioxidant provides a close relationship between FRSA and the involvement of endocrinological responses, which help to reverse the effect (Alma *et al*., 2003, Verma *et al*., 2010). Plants are rich sources of phytochemicals such as saponin, tannin, flavonoids, phenolic and alkaloids which possess a variety of biological activities including antioxidant potential. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA stand breaking. Natural antioxidants are in high demand for application as biopharmaceuticals and food additives.

Terrestial plants are considered as potential source of bioactive compounds and pharmacologically active compounds. However, little is known about the therapeutical potential of mangrove plants. Exploration of the chemical constituents of mangrove plant is necessary to find new therapeutic agents and this information is very important to the local community. Important reasons for studying the chemical constituents of mangrove plants are first, mangroves are a type of tropical forest that grows easily and has not as yet been widely utilized. Secondly, the chemical aspects of mangrove plants are very important because of the potential to develop compounds of agrochemical and medical value.

Excoecaria agallocha and *Aegialitis rotundifolia* are the mangrove plants belongs to Euphorbiaceae and Plumbaginaceae. *Excoecaria agallocha* is most widely reported mangrove species. The latex of this plant has been used as a purgative and abortifacient as well as in the treatment of ulcers, rheumatism, leprosy and paralysis.

We have very little information on the antioxidant potentials of India's mangrove. In the present study, we have evaluated the antioxidant and potential of the methanol, Ethyl acetate, Chloroform and Acetone extracts of *Aegialitis rotundifolia* and *Excoecaria agallocha* by determining antioxidant capacity assays.

2. Materials and Methods

Aegialitis rotundifolia (Plumbaginaceae) and *Excoecaria agallocha* (Euphorbiaeceae) leaves were collected in and around Krishna District geographically located between latitude $15^0 15^1$ - $15^0 55$N and $80^0 45^1$-$81^1 00$E longitude mangrove wet land, Nagayalanka, Andhra Pradesh, India.

Preparation of plant extract

Leaves of *Aegialitis rotundifolia* and *Excoecaria agallocha* 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:

Diphenyl Picryl Hydrozyl radical scavenging assay 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 compound estimation

The total phenolic contents in the extract, were 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.

Antioxidant Activity

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*., 1996. The FRAP method of measuring the Ferric reducing power (reduce the TPTZ-Fe(III) complex to TPTZ Fe(II) complex ability) of mangrove plant extracts. In the FRAP assay, an aliquot of the samples (10-40 ml) was mixed with 3 ml of Ferric TPTZ reagent. The change in absorbance was measured at 593 nm after initial mixing and 90 minutes until it reached a plateau. Aqueous solutions of known Fe (II) concentration ($FeSO_4$. $7H_2O$) were used for calibration of the FRAP assay and antioxidant. The results expressed as ascorbic acid equivalents (µ mol/ml) or FRAP units.

3. Results and Discussion

Reactive Oxygen Species (ROS) are various forms of activated oxygen which include free radicals, eg. Superoxide anions (O_2), Hydroxyl radicals (OH_2), non free radical compounds (H_2O_2) and singlet Oxygen ($^1O_2$) which can be formed by different mechanism in living organisms. Oxidative damage of DNA molecules associated with electron transfer reactions is an important phenomenon in living cells which can lead to mutations and contribute to carcinogenesis and aging process. ROS species are considered as important causative factors in the development so many diseases. Plants are rich source of phytochemicals such as saponins, tannins, phenolics, flavonoids and alkaloids which possess a variety of biological activities including antioxidant potential. Antioxidants provide production of ROS and concomitant lipid peroxidation, protein damage and DNA strand breaking.

Determination of DPPH radical scavenging activity:

Excoecaria agallocha leaf extracts of methanol in different concentrations results on the increase of DPPH activity. Gradual raise from 12.90 to 75.12 with the increase from 100 to 500 µg. Higher activity to the extent of 75.12 µg was proved to be better in radical scavenging activity.

The reducing power is also on the increase of 84.41 to higher rate of concentration 500 and in lowest 44.06 at 100 concentration. The reducing power was good enough even in 300 as 74.51 and gradually increased up to 84.41 as the concentration increased up to 500. Phenolic content also had similar effect with the raise in the concentration and minimum of 41.05 recorded at 100 concentration and gradually increased 84.00 at 500 concentration. There is comparison with reference to phenolic content and reducing power as the values are increasing with increase in concentration. With increase in phenolic content, there was increase in reducing power.

Methanol extract at lower concentration 400 and 500 concentrations could bring the increase in the antioxidant content and 84.48 was recorded at 500 where as very low of 26.52 was recorded at low concentration 100. The results are similar to the results of antioxidant (Jayanthaku Patra *et al*., 2009).

The Ethyl acetate extracts of 100 to 500 could not effect the DPPH activity as in 500 concentration the DPPH was said to be 33.21 which was not considerable.

Reducing power also had 72.34 even at higher concentration as 500. The phenolic content in ethyl acetate concentrations was recorded to be 69.26 at 500 concentration which was also not proved good. Antioxidant content against ethyl acetate was also not proved to be better as 500 concentration could produce 69.26 antioxidant content at 500 concentration. Hence methanol extracts of high concentration were proved to be better.

Acetone and chloroform leaf extracts could not produce good effect on bacteria. Although the content is increased as the concentration increased the levels are less than 50% and hence this effect of Acetone and chloroform could not be considerable.

Aegialitis rotundifolia leaf methanol extracts could effect the DPPH maximum of 75.30 recorded at 500 concentration and other concentration were not proved to be better though there is increase with reference to increase in concentration. The reducing power also of higher value at 400 concentration and 500 concentration recorded as 79.62 and 82.02 and the highest at 500. These two were proved to be better.

The phenolic content was proved to be maximum of 82.62 at high concentration 500 and phenolic content increase resulted increase in reducing power as 82.02.

Antioxidants also are of higher range from 71.64 to 82.32 against the concentration of 400 and 500. 500 concentration is better to produce higher values.

Ethyl acetate leaf extracts reducing sugars was shown higher at high concentration 500 as 74.28 and the other values of DPPH, phenolics and antioxidants were not proved to be better even in higher concentration of 500.

The effect of Acetone and chloroform in different concentration could not bring any effect of DPPH, phenolics and Antioxidant whereas Ethyl acetate leaf extract at 500 concentration could be effective in reducing power to an extent of 74.28 is proved to better.

DPPH has been extensively as a free radical to evaluate reducing substances (Cotelle *et al.*, 1996) and is a useful reagent for investigating the free radical scavenging activities of compounds (Duan *et al.*, 2006). Our results show high DPPH scavenging activity with the increase in concentration of methanol and ethyl acetate. Many researchers have reported positive correlation between free radical scavenging activity and total phenolic content, which also matches without finding. The reducing capacity of a compound may serve as indicator of its potential antioxidant capacity (Meir *et al*., 1995). The antioxidant capacity of compounds has been attributed to various factors such as prevention of chain reaction, Chelating metals, reductive capacity and radical scavenging ability etc (Diplock, 1997, Yildirim *et al*., 2001).

Phenolic compounds are commonly found in plants are have been reported to posses several biological activities including a strong antioxidant activity (Duk *et al*., 1999, Chandini *et al*., 2008).

The antioxidant capacity of phenolic extract is attributed to their radical scavenging ability mediated by hydroxyl groups (Hatano *et al.*, 1980). It is suggested that polyphenolic compounds also have inhibitory effect on mutagenins and carcinogenesis in human beings (Gulcin *et al*., 2003)

In our studies the higher amount of phenolic compounds indicating the strong therapeutic potential as antioxidants.

Table 1: Antioxidant activity of leaf extracts of the *Excoecaria Agallocha*

Plant Name	*Concentration*	*DPPH*	*RP*	*Phenolic Content*	*AO*
Methanol	100 µl	12.90	44.06	41.05	26.52
	200 µl	26.23	74.12	57.42	38.32
	300 µl	38.20	74.51	73.92	59.15
	400 µl	51.00	81.32	78.09	72.62
	500 µl	75.12	84.41	84.00	84.48
Ethyl acetate	100 µl	5.02	41.46	31.67	22.67
	200 µl	12.09	55.76	54.03	35.38
	300 µl	20.32	63.12	59.32	57.03
	400 µl	28.59	67.26	62.98	58.98
	500 µl	33.21	72.34	69.26	69.26
Acetone	100 µl	0	14.02	18.21	18.30
	200 µl	0	16.62	23.08	23.62
	300 µl	0	22.73	29.08	28.50
	400 µl	8.35	28.11	30.04	32.05
	500 µl	15.26	32.41	32.01	42.23
Chloroform	100 µl	0	10.12	0	9.52
	200 µl	0	12.18	14.31	12.59
	300 µl	0	16.70	18.11	22.62
	400 µl	6.92	18.61	22.25	28.73
	500 µl	10.24	20.21	26.00	32.01

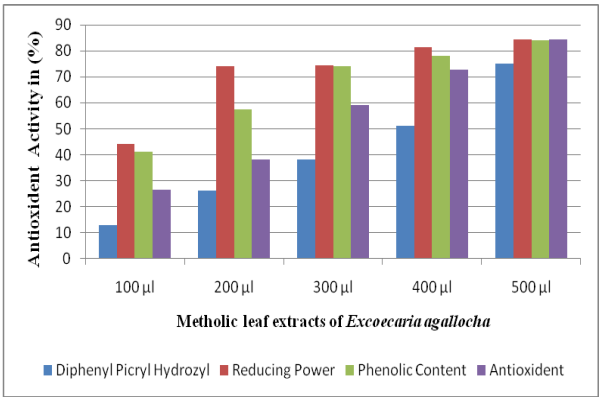
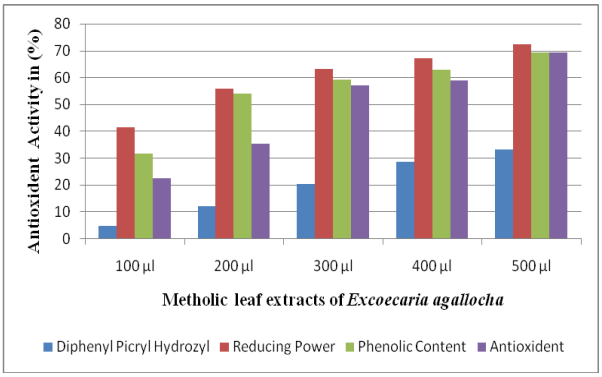


Figure 1



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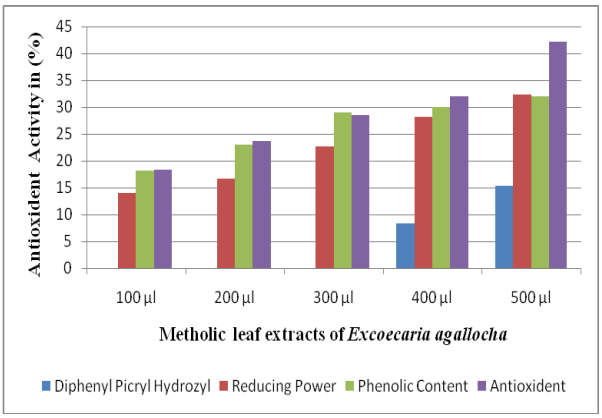

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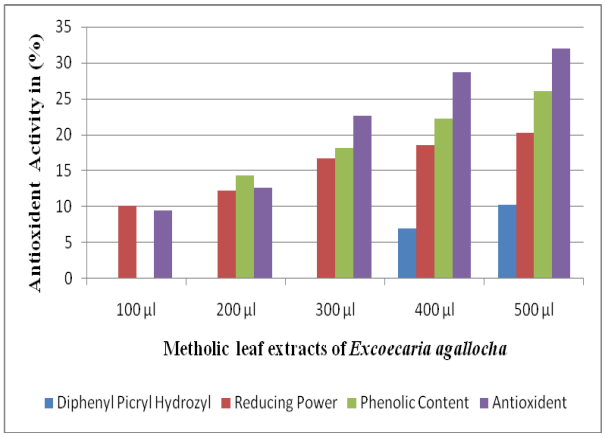
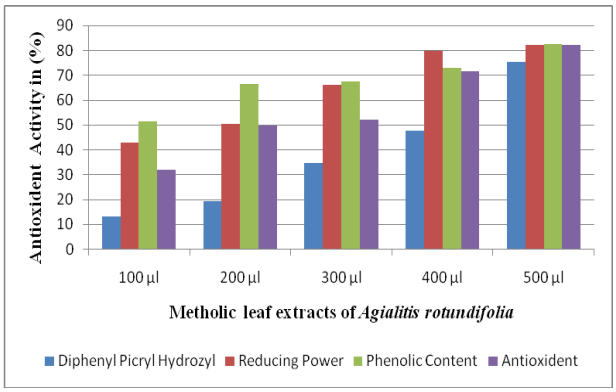


Figure 4

Table 2: Antioxidant activity of leaf extracts of the *Agialitis rotundifolia*

Plant Name	*Concentration*	*DPPH*	*RP*	*Phenolic Content*	*AO*
Methanol	100 µl	13.23	43.03	51.32	32.05
	200 µl	19.31	50.52	66.42	49.62
	300 µl	34.90	66.25	67.53	52.23
	400 µl	47.73	79.62	73.09	71.64
	500 µl	75.30	82.02	82.62	82.32
Ethyl acetate	100 µl	6.14	60.23	35.28	28.28
	200 µl	13.56	62.92	42.20	32.20
	300 µl	21.31	63.09	50.28	40.76
	400 µl	32.09	69.02	59.38	53.03
	500 µl	45.20	74.28	60.06	60.12
Acetone	100 µl	0	4.32	9.51	12.15
	200 µl	0	45.51	22.20	14.30
	300 µl	7.30	56.62	24.00	18.61
	400 µl	16.73	63.60	28.10	22.12
	500 µl	23.21	68.71	35.22	28.14
Chloroform	100 µl	0	20.25	0	8.09
	200 µl	0	24.73	9.00	12.53
	300 µl	0	28.01	18.21	14.61
	400 µl	0	36.65	22.31	19.59
	500 µl	10.62	38.51	36.00	25.62



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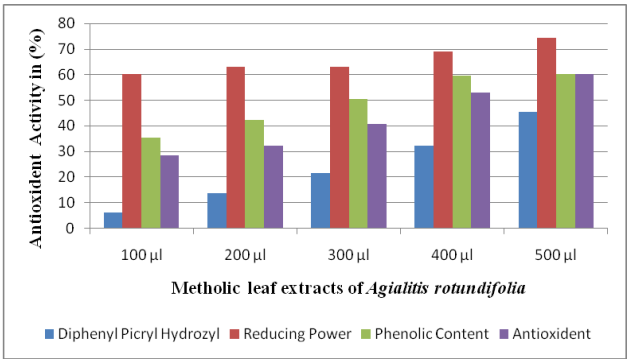

Figure 6

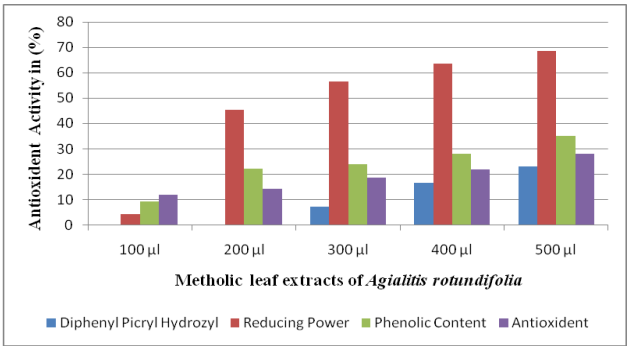

Figure 7

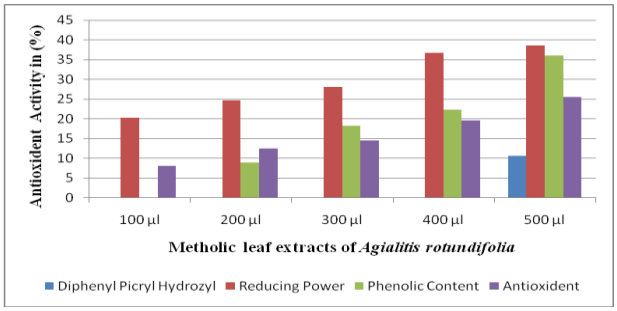

Figure 8

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