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Antioxidant Properties in Leaf and Root Extracts of Some Medicinally Important Mangrove Species of Odisha Coast

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ABSTRACT

The study was aimed to determine antioxidant activity of methanolic extracts of both leaf and root of 12 nos. of therapeutically used mangrove species of Odisha coast using different antioxidant assays i.e., DPPH, FRAP and 'Reducing Power'. Significantly higher DPPH radical scavenging effect was observed in leaf extracts of *Heritierafomes* (IC_{50}=13µg/ml). However, in root extract, the activity was higher in *Rhizophoraapiculata* (IC_{50}=17µg/ml). As far as total antioxidant content is concerned, *Avicennia marina* showed highest content i.e94.16±5.36 mg AEAC/g dry wt.in leaf sample and87.33±0 mg AEAC/g dry wt. in root sample. The highest FRAP value (4.75±0.08 mM AAE/g dry wt.) in leaf samples was found in *Cerberamanghas*. Whereas, highest FRAP value (5.19±0.40 mM AAE/g dry wt.) in root extract was noted in *Cynometrairipa*. As regard to reducing power, *Kandeliacandel* showed highest activity e.g.,3.49±0.08 mg AAE/g dry wt. in leaf extracts among the studied species. In root extract, *Aglaiacucullata* exhibited highest reducing activity (3.35±0.01 mg AAE/g dry wt.). This study revealed that *H. fomes, R. apiculata, A. marina, C. manghas, C. iripa*and *K. candel*were found to be good sources of natural antioxidants for pharmaceutical utilization.

Keywords: Antioxidant, DPPH, FRAP, Mangrove, Medicinal

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INTRODUCTION

Mangroves are specialized group of salt tolerant plants that grow in the intertidal regions of tropic and sub tropic along the coastlines[1]. They are found to have medicinal values and have been used traditionally by local medical practitioners worldwide. They provide innumerable direct and indirect benefits to human beings. Moreover, scientific justifications towards ethno botanical and medicinal use of mangrove plant are yet to be established. Concerning any medicinal properties of plant parts, the most commonly studied factor is their antioxidant effects. Antioxidants play a crucial role in the prevention of chronic ailments such as heart disease, cancer, diabetes, hypertension, stroke and Alzheimer's disease by combating oxidative stress[2]. However, no comprehensive report is available on antioxidant activities in mangrove plants of Odisha coast.

The oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species i.e free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non-radicals like hydrogen peroxide, hypochlorous etc. can be prevented by antioxidants[3,4]. In order to regulate the ROS levels, plant cells are evolved with complex enzymatic and non-enzymatic antioxidant defense mechanisms, which together help in controlling the cellular redox state under changing micro environmental conditions.

Mangroves are to be considered as a valuable source for chemical constituents with potential medicinal and agricultural values[5]. Although the chemical constituents of most mangrove plant parts still have not been studied extensively, investigations are initiated to find out novel compounds with prospective medicinal value for discovery of new chemotherapeutic agents[6]. The plants used in traditional medicine are still a large source of natural antioxidants that might be useful in developing novel drugs[7]. Recently, scientists are searching for effective remedies from mangroves to cure diseases such as diabetes, asthma, cancer, ulcer, wounds and AIDS[8-10]**.** *In vitro* antimalarial activity and cytotoxicity of *Avicennia marina* have also been reported[11]. *A. officinalis* is a folklore medicinal mangrove plant used to cure rheumatism, paralysis, asthma and snake bites, skin disease, ulcer etc. A detection of the plant with sugar candy and cumin is used in dyspepsia with acid eructation[12,13]. *A. marina* have been shown to exhibit marked inhibitory effect on mouse skin tumor promotion[10]. The extracts of some mangrove species indicate significant antioxidant activity[14, 15]**.**

Considering extensive therapeutic uses of leaves and roots of 12 different mangrove species of Odisha coast enlisted in Table-1**,** the present study was undertaken to evaluate antioxidant activities in their leaf and root extracts.

MATERIALS AND METHODS

Collection of plant materials

The leaf and root samples were collected from mangrove forest of the Bhitarkanika and Mahanadi Θ of mangrove of Odisha coast, India. Leaves and roots of each plant species were sampled from at least five individual trees viz. *Aglaiacucullata* (Roxb.) Pellegrin (Meliaceae), *Avicennia marina* (Forssk.) Vierh.(Avicenniaceae), *Bruguieragymnorrhiza*(L.)Savigny (Rhizophoraceae), *Bruguieraparviflora*(Roxb.) Wight &Arn.exGriff. (Rhizophoraceae), *Cerberamanghas* L. (Apocynaceae), *Cynometrairipa*Kostel (Fabaceae), *Excoecariaagallocha* L. (Euphorbiaceae), *Heritierafomes*Buch.-Ham (Sterculiaceae),*Heritieralittoralis*Dryand ex Ait. (Sterculiaceae), *Kandeliacandel (*L.)Druce (Rhizophoraceae), *Rhizophoraapiculata*BI.(Rhizophoraceae), *Xylocarpusgranatum* Koenig (Meliaceae). All the above species were selected because of their therapeutic uses as referred in Table-1.

Table-1: Mangrove plants selected for present study on the basis of their proven medicinal uses

Name of Species	Useful Part	Medicinal uses
Aglaiacucullata	Leaf	Dysentery, Leucoderma, Leprosy, Fever, thirst, tumors and vomiting[30].
	Root	Insecticides[31].
Avicennia marina	Leaf	Antibacterial, anticandidal and antibacteriophage activity[6, 7]**.** (Treatment of rheumatism, smallpox, ulcers[32]. Hepatitis B[22]
	Root	Antimalarial, anticancer activity[11].
Bruguieragymnorrhiza	Leaf	Tumor inhibitors and treatment to burn[33]**.** Treatments of cut and wounds[31,34].
	Root	Constipation, treatment of burns[31]. Antinociceptive and antidiarrhea activity[35].
Bruguieraparviflora	Leaf	Hepatitis[36]
	Root	Hepatitis[31]**.**
Cynometrairipa	Leaf	Leaf decoction against ulcers[36]
	Root	Antibacterial activity[34]
Cerberamanghas	Leaf	Analgesic activity[25]
	Root	Charcoal making, haemorrhage, ulcers, rheumatism, venereal infection[36].
Excoecariaagallocha	Leaf	Treatment of Epilepsy[31, 37]**.** Ulcer[38]. Antifilarial activity[28].
	Root	Leprosy, dermatitis, toothache, Anti-inflammation[34].
Heritierafomes	Leaf	Anticancer activity[39].
	Root	Treatment of gastrointestinal disease, skin disease, hepatic disorders, diabetes[39, **40**]
Heritieralittoralis	Leaf	Menstrual disorder[41]
	Root	Diarrohoea[36]
Kandeliacandel	Leaf	Charcoal, diabetes[36]

	Root	Treatment of diabetes[31]
Rhizophoraapiculata	Leaf	Inhibitory properties of pathogens. Antiseptic for woman after utter, dysentery and stomach disorder[42].
	Root	Astringent for diarrohoea, skin diseases[36]
Xylocarpusgranatum	Leaf	Stomach disorder and fever[42]
	Root	Insect bite[36]

Preparation of samples

The fresh samples were cleaned in running tap water and leaf and root partswere dried in hot air oven (50^0C) for 12 hrs[16]**.** The dried samples were pulverized and stored in freezer in airtight containers forfurther extraction. To 3.0 g of powdered sample, 40 ml of solvent (i.e. absolute methanol) was added in a conical flask and the mixture was stirred using stirrer for 18 h at room temperature. Each extract was filtered using Whatman No.1 filter paper. The filtrate was collected and the residue was re-extracted twice. The two extracts were then pooled out. The solvent (i.e. absolute methanol) in the extract was removed with heating at 40^oC using hot plate till the total volume reached to 10ml. The extracts were filled in the storage vial and stored in air-tight container at 4^oC until further uses.

Radical scavenging activity

The free radical scavenging activities of the samples for the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was measured[17] with some modification[18]. Samples were dissolved in methanol at a concentration of 10-100µl with 2 ml of DPPH (0.06mg/ml methanol) with methanol serving as the blank sample. The mixtures were left for 15 min at room temperature and the absorbance then measured at 517 nm in UV-VIS Spectrophotometer (Secord 2000, Analytik Jena, Germany). Ascorbic acid (1mg/1ml Distilled water) used as standard.

The radical scavenging activity was calculated as follows as: % Inhibition = [(Blank absorbance - Sample absorbance)/Blank absorbance] ×100.

IC_{50} value in DPPH assay

The IC_{50} values of each sample were determined graphically. The IC_{50} was defined as the concentration in µg of dry sample per ml that inhibits the formation of DPPH radicals by 50%.

Antioxidant Content

The antioxidant content was evaluated as described with some modifications[19]. A 50µl of extract was mixed with 2 ml of a 0.06 mg/ml methanol solution of DPPH in methanol. The mixtures were left for 15 min at room temperature and the absorbance then measured at 517 nm. The blank sample consisted of 50µl of sample with 2ml of methanol. The antioxidant content was determined using standard curve for ascorbic acid (1mg/1ml distilled water). The mean of three values were

obtained and the unit was expressed as mg of ascorbic acid equivalent antioxidant content (AEAC) per 1 g of powder sample.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay is used for measuring the total antioxidant capacity[20]. Freshly prepared FRAP reagent (3.0 ml) was mixed with 0.1 ml of test sample and a reagent blank was maintained with methanol. The FRAP reagent was prepared from 300 mmol/L acetate buffer (pH 3.6), 20 mmol/L ferric chloride and 10 mmol/LTPTZ made up in 40 mmol/L hydrochloric acid. All the above three solutions were mixed together in the ratio 25:2.5:2.5. The absorbance of reaction mixture at 593nm was measured spectrophotometrically (Specord 2000 UV-VIS, Germany) after incubation at 25^0C for 10 min. The FRAP values were expressed in mM ascorbic acid equivalent (AAE)/g dry wt. derived from standard curve.

Reducing power activity

The reducing power of the sample was determined using potassium ferricyanide and ferric chloride[21]. Extracts (50µl) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm after allowing the solution to stand for 30 min. A graph of absorbance vs extract concentration was plotted to observe the reducing power. Reducing power is given in ascorbic acid equivalent (AAE) in milligram per gram of dry weight.

Statistical analysis

Mean and standard deviation values of three replicates were calculated. One-way ANOVA was performed to test significant differences among replicates for each species using Graphpad prism software 6.0. The Geisser-Greenhouse's epsilon multiple comparison method was used. Significance was determined at 5% level.

RESULTS AND DISCUSSION

Radical scavenging activity

The radical scavenging activity (IC_{50}) of methanolic extracts of leaves and roots of twelve mangrove species was presented in Table-2. Among leaf extracts, the highest radical scavenging activity was found in *H. fomes* (IC_{50} 13µg/ml) and lowest was in *R. apiculata* (IC_{50} 62µg/ml). In case of root extracts, the highest activity was observed in *R. apiculata* (IC_{50} 17µg/ml) and lowest

obtained in *A. marina* (IC_{50} 41µg/ml) (Figure-1).A low IC_{50} value indicates strong antioxidant activity in a given sample.

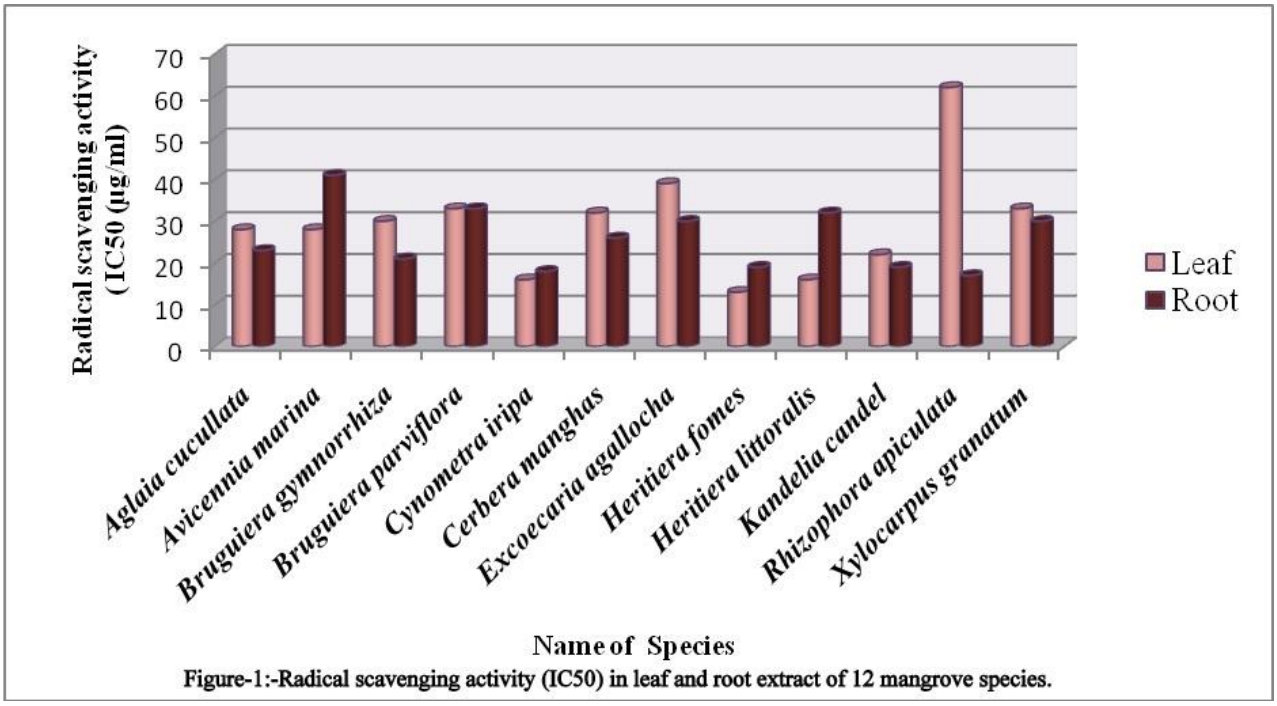

Figure-1:- Radical scavenging activity (IC_{50}) in leaf and root extract of 12 mangrove species.

The radical scavenging activity of the Sunderban mangrove *B. gymnorrhiza* reported to be lower (IC_{50} 2052.20 µg/ml in leaf extract, IC_{50} 1532.71µg/ml in root extract)[14],in comparison to our result. The antioxidant activity in leaf extracts of *A. marina* is higher (IC_{50} 28. µg/ml) when compared to the same species of Pichavaram mangrove forest with IC_{50} 142.05 µg/ml.[22]. According to an earlier report, DPPH radical scavenging activity in leaves of *B. cylindrica* was IC_{50} 175µg/ml[23]. In another study, the antioxidant activity (IC_{50}) of leaf extract of *B. gymnorrhiza* was exhibited 0.038 mg/ml[24]; this value was nearly equal to our observation (IC_{50} 30.0 µg/ml). The leaf extracts of *C. manghas* was having lower antioxidant activity (IC_{50}269.15µg/ml)[25] than the present study (IC_{50} 32.0 µg/ml).

Antioxidant content

Of the twelve mangrove species screened, the results of antioxidant content were shown using standard curves of ascorbic acid. All the studied species exhibited promising antioxidant content of both leaves and roots. The highest antioxidant content was observed in 94.16±5.36 mg AEAC/g dry wt.in *A. marina* followed by *B. gymnorrhiza*, *C. manghas*and *H. fomes*. Values ranged from 27.77±0.76 mg AEAC/g dry wt. in *B. parviflora* to 94.16±5.36 mg AEAC/g dry wt. in *A. marina*.Significance differences between leaf samples among all species were found (P=0.0018) (Table-2) (Figure-2). Antioxidant content of root extracts varied from to *B. parviflora* (21.55±2.34

mg AEAC/g dry wt.) to *A. marina* (87.33±0 mg AEAC/g dry wt.). A significant difference (P=0.0004) was obtained between root sample of all the studied species. It is found in this experiment that *A. marina* exhibited highest antioxidant content in both leaf and root whereas *B. parviflora* obtained lowest antioxidant content. Previous report suggested that mangrove plants have anti-inflammatory and antipyretic activity[26]. In another report, *R. apiculata and E. agallocha* showed 77.58% and 85.61% antioxidant content respectively [27].

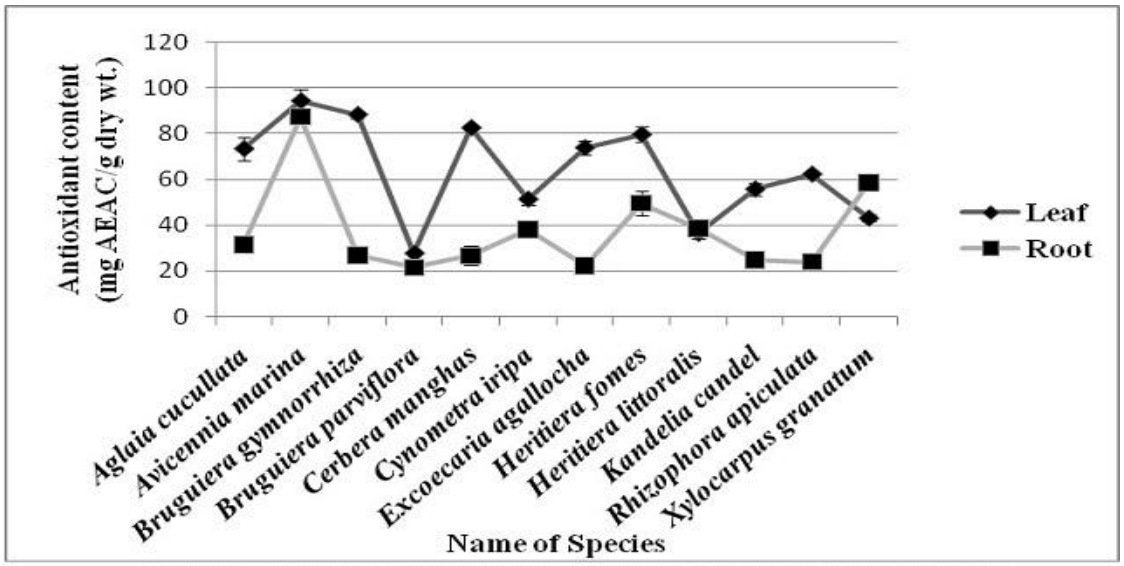

Figure-2:- Antioxidant content (AEAC) in leaf and root extracts of 12 mangrove species.

Figure-2:-Antioxidant content (AEAC) in leaf and root extracts of 12 mangrove species.Ferric reducing antioxidant power (FRAP) of leaves and roots of 12 mangrove species

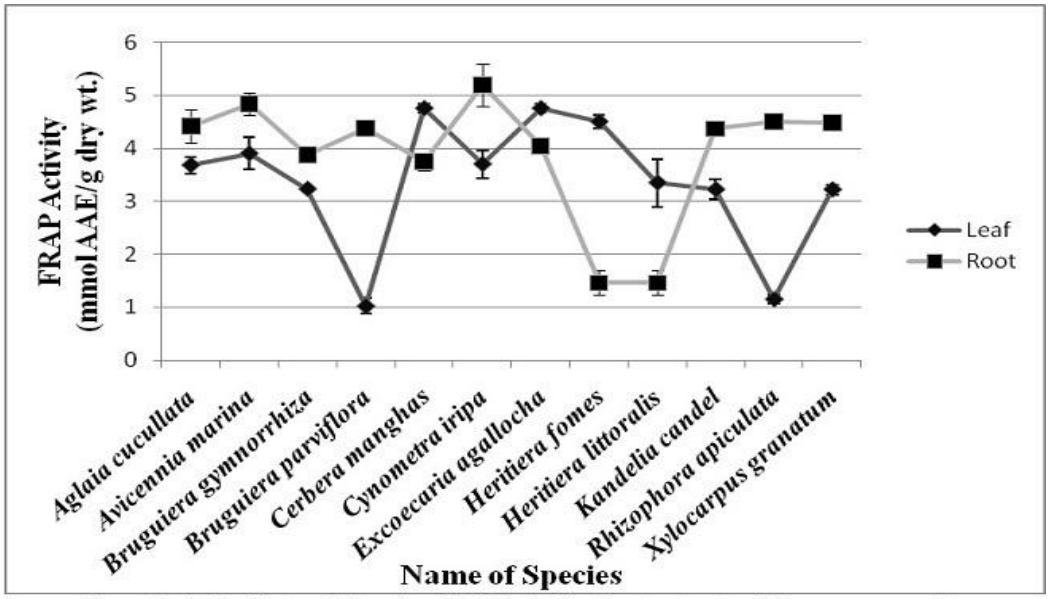

Figure-3:-Antioxidant activity using FRAP in leaf and root extracts of 12 mangrove species.

Figure-3:-Antioxidant activity using FRAPS in leaf and root extracts of 12 mangrove species.

Reducing power assay of leaves and roots of 12 mangrove species

The reducing power of mangrove plant materials were evaluated as mg AAE/g dry sample as shown in Table-2. The best reducing ability of leaf sample was obtained in *K. candel* (3.49±0.08 mg AAE/g dry wt. followed by *A. cucullata (*3.4±0.1 mg AAE/g dry wt.). Statistical significance was found in leaf sample P=0.0003.Among root extracts of all the species, *A. cucullata* showed the highest reducing rate and *B. gymnorrhiza* showed the lowest reducing rate.Statistical significance in root sample among all species P=0.0002(Table-2, Figure-4). Earlier report suggested that higher absorbance corresponds to a higher reducing power[29]. The best reducing activity was obtained in roots of *R. mucronata* (1.40±0.00 mg AAE/g dry wt.) and 2.89±0.23 mg AAE/g dry wt. was observed in leaf[14], when compared to *R. apiculata* in the present study. In case of *B. gymnorrhiza*, the reducing ability was dound higher than the earlier report. The reducing power of *A. marina* wasfound at par with the *A. alba* of the earliers report [14] .

The FRAP value was highest in leaf extracts of both *C. manghas* and *E. agallocha* (4.75±0.08 mM AAE/g dry wt.) and lowest was in *B. parviflora* 1.02±0.15 (mM AAE/g dry wt.). In the present study, *C. manghas* and *E. agallocha* demonstrated significantly (P=0.0006) higher reducing power than other species. The highest FRAP value of root sample was recorded in *C. iripa* (5.19±0.40 mM AAE/g dry wt.) followed by *A. marina* (4.84±0.21 mM AAE/g dry wt.). Sample with lowest FRAP value was observed in both *Heritierafomes* and *Heritieralittoralis* (1.46±0.23 mM AAE/g dry wt.) (Table-2, Figure-3). Statistical significance was found in root samples (P=0.0010). Leaf extracts of *Excoecariaagallocha* possessed a promising antioxidant property and anti filarial activity and it has reducing power activity[28].

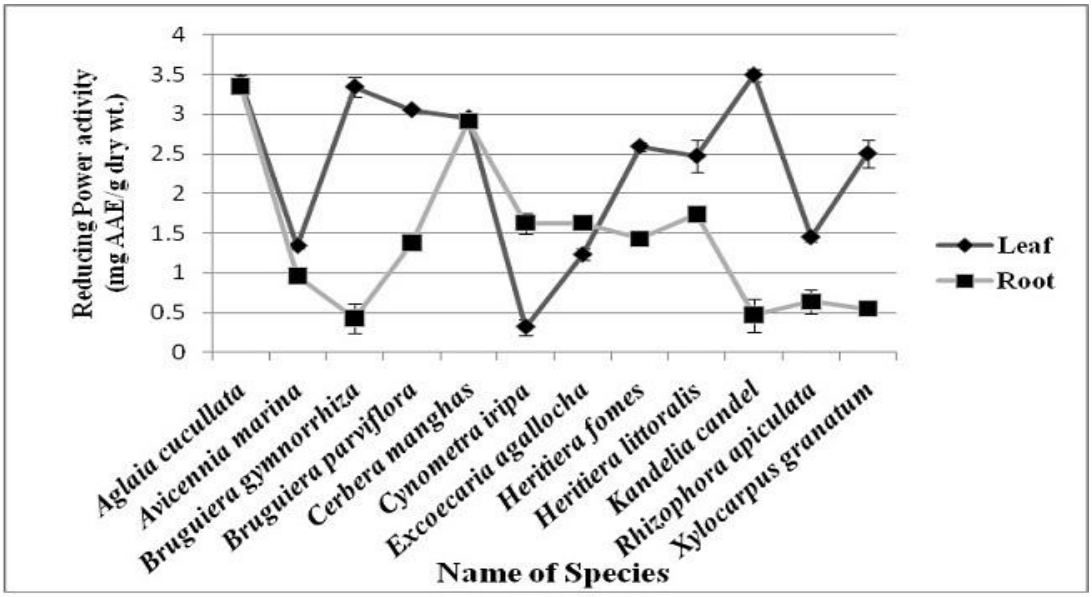

Figure-4:- Reducing power activity in leaf and root extracts of 12 mangrove species.

Figure 4:-Reducing power activity in leaf and root extracts of 12 mangrove species.

Table-2:- Analysis of antioxidant properties in leaf and root extracts of 12 medicinally important mangrove species.

Name of Species	Parts used	Radical scavenging activity (RSA) IC_{50} (µg of dry wt./ml)	Antioxidant content (mg AEAC/g dry wt.)	Reducing power (mg AAE/g dry wt.)	Ferric reducing antioxidant power (mM AAE/g dry wt.)
Aglaiacucullata	Leaf	28	73.33±5.09	3.4±0.1	3.68±0.16
	Root	23	31.55±1.54	3.35±0.01	4.42±0.311
Avicennia marina	Leaf	28	94.16±5.36	1.34±0.034	3.90±0.30
	Root	41	87.33±0	0.96±0.06	4.84±0.21
Bruguieragymnorrhiza	Leaf	30	88.21±1.38	3.34±0.12	3.23±0.05
	Root	21	26.88±0.76	0.43±0.19	3.88±0.04
Bruguieraparviflora	Leaf	33	27.77±0.76	3.05±0.017	1.02±0.15
	Root	33	21.55±2.34	1.38±0.02	4.38±0.06
Cynometrairipa	Leaf	16	51.33±2.30	0.32±0.10	3.70±0.26
	Root	18	38±0	1.63±0.13	5.19±0.40
Cerberamanghas	Leaf	32	82.44±0.76	2.95±0.01	4.75±0.08
	Root	26	26.66±4.05	2.91±0.07	3.75±0.16
Excoecariaagallocha	Leaf	39	73.77±3.00	1.23±0.07	4.75±0.08
	Root	30	22.22±0.38	1.63±0.07	4.04±0.10
Heritierafomes	Leaf	13	79.55±3.35	2.59±0.06	4.50±0.13
	Root	19	49.55±5.59	1.43±0.08	1.46±0.23
Heritieralittoralis	Leaf	16	36.44±2.69	2.47±0.2	3.35±0.45
	Root	32	38.66±0	1.74±0.05	1.46±0.23
Kandeliacandel	Leaf	22	55.77±2.69	3.49±0.08	3.22±0.19
	Root	19	24.66±2.40	0.47±0.21	4.37±0.10
Rhizophoraapiculata	Leaf	62	62.21±1.38	1.45±0.06	1.15±0.08
	Root	17	24±0	0.64±0.15	4.50±0.08
Xylocarpusgranatum	Leaf	33	43.07±1.64	2.5±0.17	3.62±0.10
	Root	30	58.66±2.30	0.55±0.01	4.48±0.10

Values expressed as Mean±Standard deviation.

Abbreviations-: AEAC- Ascorbic acid equivalent antioxidant content

AAE-Ascorbic acid equivalent

IC_{50}- Inhibition coefficient by 50%

CONCLUSION

All the studied species can be considered as good sources of natural antioxidants. Highest radical scavenging activity was found in leaf extract of *Heritierafomes* and root extract of *Rhizophoraapiculata*. *Avicennia marina* showed promising total antioxidant content both in leaf and root extracts. FRAP content was highest in leaf extract of *Cerberamanghas* and root extract of *Cynometrairipa.Kandeliacandel* showed highest reducing power in leaf extract and *Aglaiacucullata*in root extract. The findings of the present study revealed that these plant parts

(roots & leaves) can be exploited in preparation of natural drugs for the treatment of various diseases with appropriate pharmaceutical approaches.

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