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Full Length Research Paper

Antioxidant activity and total phenolics of some mangroves in Sundarbans

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The antioxidant activity of 23 extracts from different plant parts (leaves, stem bark and root) of 6 mangroves and 4 mangrove associates was examined. The content of total phenolics in the extracts was calculated as gallic acid equivalent (GAE) and anti-radical activity was estimated as IC_{50} values using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Remarkable high phenolic content (GAE > 25 mg/g), strong reducing ability (ascorbic acid equivalent, AAE > 3.5 mg/g) and anti-radical activity (IC_{50} < 2.9 mg/ml) were found in 11 different extracts comprising of 6 mangrove and 4 mangrove associate species. The best results were obtained for *Ceriops decandra* stem bark extract (phenolic content as GAE = 94.4 mg/g, reducing power as AAE = 13.04 mg/g and DPPH radical scavenging ability as IC_{50} = 0.65 mg/ml). A significant correlation was observed between GAE and AAE of respective extracts. The results indicate promising mangrove species for the utilization as significant source of natural antioxidant.

Key words: Mangrove, natural antioxidant, total phenolics, reducing power, radical scavenging ability.

INTRODUCTION

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 (Davies, 2000). These ROS play an important role in the etiology and pathophysiology of human aging (Finkel, 2000) and diseases such as cancer, coronary heart disease, Alzheimer's disease (Ames, 1983; Gey, 1990; Smith et al., 1996) neurodegenerative disorders, atherosclerosis, cataracts and inflammation (Aruoma, 1998). Consequently, search for antioxidant principles from plants has been accelerated and many plants having potential antioxidant activities (Tiwari, 2001) 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 (Lee et al., 2003).

Polyphenols are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers. Indeed, these compounds have been proposed as potential preservatives (Nychas et al., 2003), because consumer pressure on the food industry to avoid chemical preservatives has increased over the past decades. Furthermore, reports on the potential health benefits of polyphenols have increased enormously (Bravo, 1998).

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. (Bandarnayake, 2002). They have been used as traditional medicine in South Asian countries including India. Recently scientists are veering in search of effective remedies from mangroves for diseases such as diabetes, asthma, cancer, ulcer, wounds and AIDS (Premanathan et al., 1999; Babu et al., 2001; Itigowa et al., 2001).

The aim of the present study was to examine the total phenolic content and radical scavenging capacity related to antioxidant potential in different parts of 6 mangroves

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and 4 halophytic mangrove associated plants. The infusions are prepared with regard to efficient extractable phenolics. Antioxidant potential has been determined as the free radical scavenging ability using a stable radical, diphenyl-picryl-hydrazyl (DPPH) and ascertained by measuring reducing power.

MATERIALS AND METHODS

Collection of plant material

The mangroves were collected from Sagar Island, (Longitude 22° 32′ N, Latitude 88° 08′ E), Sundarbans, India, during February – March, 2006. Leaves, stem bark and root of each plant species were sampled from at least five individual tree and segregated for different plant parts. The six mangrove species were *Avicennia alba* Bloch (Fam.- Avicenniaceae), *Aegiceras corniculatum* (L.) Blanco. (Fam.- Myrsinaceae), *Bruguiera gymnorrhiza* (L.) Savigny (Fam.- Rhizophoraceae), *Ceriops decandra* (Perr.) Robinson (Fam.- Rhizophoraceae), *Rhizophora mucronata* Lamk. (Fam.- Rhizophoraceae), *Sonneratia apetala* Buch-Ham. (Fam.- Sonneratiaceae) and four mangrove associates were *Acanthus ilicifolius* L. (Fam.- Acanthaceae), *Ipomoea pescaprae* (Fam. – Convolvulaceae), *Sesuvium portulacastrum* (L.) L. (Fam. – Aizoiaceae), *Sweda maritima* Dumort. (Fam. – Chenopodiaceae) identified by Mr. Aloke Bhattacharya, Botanist, Botanical Survey of India, Indian Botanic Garden, Howrah. The voucher specimens (CU1/039-041, CU1/046, CU1/052-053, CU1/055, CU1/058-060) were preserved in the herbarium of our laboratory. The plant parts were shed-dried, pulverized and stored in airtight containers for further extraction.

Extraction of plant material

In all experiments infusions of plant parts were prepared according to a standard protocol. To 1 g of plant material was added 20 ml of aqueous methanol (20%, v/v) for 18 h at room temperature. The extracts were filtered and diluted to 50 ml and aliquot of that extract were analyzed for their total phenolic content, reducing power and their free radical scavenging capacity.

Determination of total phenolics

The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965). Samples were introduced into test tubes; 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (Ultrospec 2000 UV-visible spectrophotometer, Pharmacia Biotech, USA). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram of dry material.

Measurement of reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Extracts (100 µl) of mangrove plant parts 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. Reducing power is given in ascorbic acid equivalent (AAE) in milligram per gram of dry material.

Free radical scavenging ability by the use of a stable DPPH radical

The free radical scavenging activity of different extracts and butylated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) Blois (1958). Aliquots (20 - 100 µl) of the tested sample were placed in test tubes and 3.9 ml of freshly prepared DPPH solution (25 mg/L) in methanol was added in each test tube and mixed. 30 min later, the absorbance was measured at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = \{(A_c - A_t)/A_c\} \times 100$$

Where A_c is the absorbance of the control reaction and A_t is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.

Statistical analysis

Data were generated for each assay from three separate extracts of each plant material in triplicate. A one-way ANOVA test was performed on the antioxidant activity results to investigate significant differences between the extracts. The method used to discriminate among the means was Duncan's multiple range test. Simple regression analysis was performed to look for relationships between GAE and AAE for different extracts. The computer program employed was SPSS for Windows, version 10.0.

RESULTS

The amount of total phenolics

There was a wide variation in the amount of total phenolics in mangrove plant materials ranging from 4.40 to 94.41 mg GAE/g dry material (Table 1). Among leaves, the highest found in *S. apetala* (47.52 GAE) and lowest in *S. maritima* (4.72 GAE). The amount of total phenolic content of leaves of the plants under investigation can be arranged in descending order *viz. S. apetala* > *A. corniculatum* > *I. pescaprae* > *R. mucronata* > *A. alba* > *S. portulacustrum* > *B. gymnorrhiza* > *A. illicifolius* > *C. decandra* > *S. maritima*. Although tree varieties contain more phenolics than herbaceous plants, *I. pescaprae* is exceptionally good in content of phenolic compounds. Stem bark of mangroves contained considerable amount of phenolics. The highest was found in *C. decandra* (94.41 GAE) and the lowest in the stem bark of *A. alba* (4.40 GAE), the descending order being *C. decandra* > *A. corniculatum* > *S. apetala* > *R. mucronata* > *B. gymnorrhiza* > *A. alba*. Among root materials *C. decandra* was found to contain highest phenolic component (73.60 GAE) and the lowest was found in *A. alba* (4.79 GAE).

Table 1. Total phenolic content, reducing power and antiradical activity of different parts of mangrove plants in Sundarbans.

Name of plant	Part examined	Extractive value (mg/g)	GAE* mg/g of dry material	AAE§ mg/g of dry material	IC_{50} value** (µg dry material)
Avicennia alba (Bloch.)	Leaves	211.90 ± 0.10	11.73 ± 0.69 ^{h,i}	1.59 ± 0.21 ^{g,h}	1331.19 ± 66.87 ^{b}
	Stem bark	50.50 ± 2.46	4.40 ± 0.31 ^{i}	0.83 ± 0.24 ^{g,h,i}	6971.53 ± 304.72 ^{e}
	Root	62.83 ± 10.13	4.79 ± 0.48 ^{i}	0.69 ± 0.01 ^{h,i}	5507.38 ± 309.42 ^{d}
Aegiceras corniculatum (L.)	Leaves	187.83 ± 11.03	40.24 ± 0.97 ^{d,e}	5.31 ± 0.11 ^{e}	129.94 ± 3.29 ^{a}
	Stem bark	147.70 ± 12.26	50.42 ± 4.87 ^{c}	8.18 ± 0.14 ^{c}	96.74 ± 2.52 ^{a}
	Root	115.20 ± 12.38	34.95 ± 2.44 ^{e}	5.03 ± 0.73 ^{e}	233.53 ± 56.25 ^{a}
Bruguiera gymnorrhiza (L.)	Leaves	134.16 ± 3.60	8.25 ± 0.31 ^{h,i}	1.25 ± 0.03 ^{g,h,i}	2052.20 ± 172.01 ^{b,c}
	Stem bark	131.90 ± 8.82	35.86 ± 2.04 ^{e}	2.85 ± 0.09 ^{f}	254.69 ± 21.26 ^{a}
	Root	103.16 ± 3.87	16.37 ± 1.57 ^{g,h}	1.55 ± 0.16 ^{g,h}	1532.71 ± 46.32 ^{b}
Ceriops decandra (Perr.)	Leaves	108.00 ± 2.30	5.14 ± 0.27 ^{i}	0.90 ± 0.66 ^{g,h,i}	5666.86 ± 324.46 ^{d}
	Stem bark	213.33 ± 5.13	94.41 ± 9.63 ^{a}	13.04 ± 0.75 ^{a}	65.55 ± 1.35 ^{a}
	Root	137.70 ± 5.15	73.60 ± 4.30 ^{b}	9.81 ± 0.87 ^{b}	93.65 ± 3.52 ^{a}
Rhizophora mucronata (Lamk.)	Leaves	206.83 ± 8.12	23.81 ± 0.71 ^{f,g}	2.89 ± 0.23 ^{f}	365.37 ± 23.95 ^{a}
	Stem bark	150.33 ± 18.41	40.47 ± 3.18 ^{d,e}	3.62 ± 0.16 ^{f}	193.82 ± 11.14 ^{a}
	Root	82.36 ± 5.94	11.73 ± 0.40 ^{h,i}	1.40 ± 0.00 ^{g,h,i}	1377.45 ± 50.62 ^{b}
Sonneratia apetala (Buch-Ham)	Leaves	174.83 ± 0.60	47.52 ± 2.22 ^{c,d}	5.71 ± 0.24 ^{e}	163.49 ± 6.32 ^{a}
	Stem bark	115.33 ± 5.78	42.68 ± 2.75 ^{c,d,e}	7.06 ± 0.07 ^{d}	193.09 ± 14.35 ^{a}
	Root	97.66 ± 1.45	42.75 ± 1.67 ^{c,d,e}	6.87 ± 0.10 ^{d}	183.04 ± 1.74 ^{a}
Ipomoea pes-caprae (L.)	Aerial part	173.83 ± 6.93	26.32 ± 0.80 ^{f}	3.55 ± 0.54 ^{f}	295.22 ± 27.90 ^{a}
Acanthus illicifolius L.	Leaves	138.33 ± 0.33	6.58 ± 0.25 ^{i}	1.10 ± 0.03 ^{g,h,i}	2501.53 ± 182.62 ^{c}
	Root	150.00 ± 2.00	7.61 ± 0.25 ^{h,i}	1.62 ± 0.03 ^{g,h}	1319.66 ± 150.76 ^{b}
Sesuvium portulacastrum (L.)	Aerial part	214.66 ± 6.56	9.75 ± 0.13 ^{h,i}	1.72 ± 0.02 ^{g}	1452.46 ± 120.06 ^{b}
Suaeda maritima (Dumort)	Aerial part	285.33 ± 8.19	4.72 ± 0.15 ^{i}	0.60 ± 0.24 ^{i}	11923.73 ±1253.30 ^{f}

Data presented as Mean ± SEM, Statistical analysis was done by Duncan's multiple range test.
*GAE means µg Gallic acid equivalent/mg of dry material, r^2 = 0.9967.
§AAE means µg Ascorbic acid equivalent/mg of dry material, r^2 = 0.9980.
**BHT was used as positive control, r^2 = 0.9846.

After examining all plant materials the amount of total phenolics 25 – 50 GAE was found in 3 leaf materials, 4 stem bark and 2 root samples; > 50 GAE was found only in *C. decandra* stem bark and root samples. The phenolic content of different parts of other plants was well below 25 GAE. Considerable amount of phenolics (>40 GAE) were present in the extracts of *A. corniculatum* (leaf, stem), *C. decandra* (stem, root), *R. mucronata* (stem) and *S. apetala* (leaf, stem, root). It was observed that three species of mangrove in Rhizophoraceae family, phenolic contents were exceptionally high in its stem bark than its root/leaves. In *S. apetala* phenolics were not concentrated in a particular part but distributed in leaves, stem and root.

Reducing ability/antioxidant power

The reducing power of mangrove plant materials were evaluated as mg AAE/g dry material as shown in Table 1. A significant linear correlation (Correlation co-efficient '*r*'= 0.950, 95% confidence interval 0.121 - 0.142. Co-efficient of determination (r^2) = 0.9019, p < 0.01) was established between total phenolics (as measured mg GAE/g dry material) and corresponding reducing ability (as measured mg AAE/g dry material) of extracts of mangrove plant parts (Figure 1). According to the reducing power, the mangrove plant materials can be divided into four groups e.g. low <1 AAE/g, n = 4 (2 leaf, 1 stem, 1 root); good 1 – 5 AAE, n =11 (7 leaf, 2 stem, 2 root); very good 5 – 10 AAE, n = 7 (2 leaf, 2 stem, 3 root) and high 10 –15 AAE, n = 1 (1 root) (Table 1). Significant reducing power was observed particularly in some tree variety of mangroves. The reducing ability of the plants in descending order was *C. decandra* (stem)> *C. decandra* (root)>*A. corniculatum* (stem)> *S. apetala* (stem)> *S. apetala* (root)> *S. apetala* (leaf)>*A. corniculatum* (leaf)> *A. corniculatum* (root). The best result was obtained with *C. decandra* stem bark, which is high in phenolic content and showed maximum reducing ability (13.04 AAE).

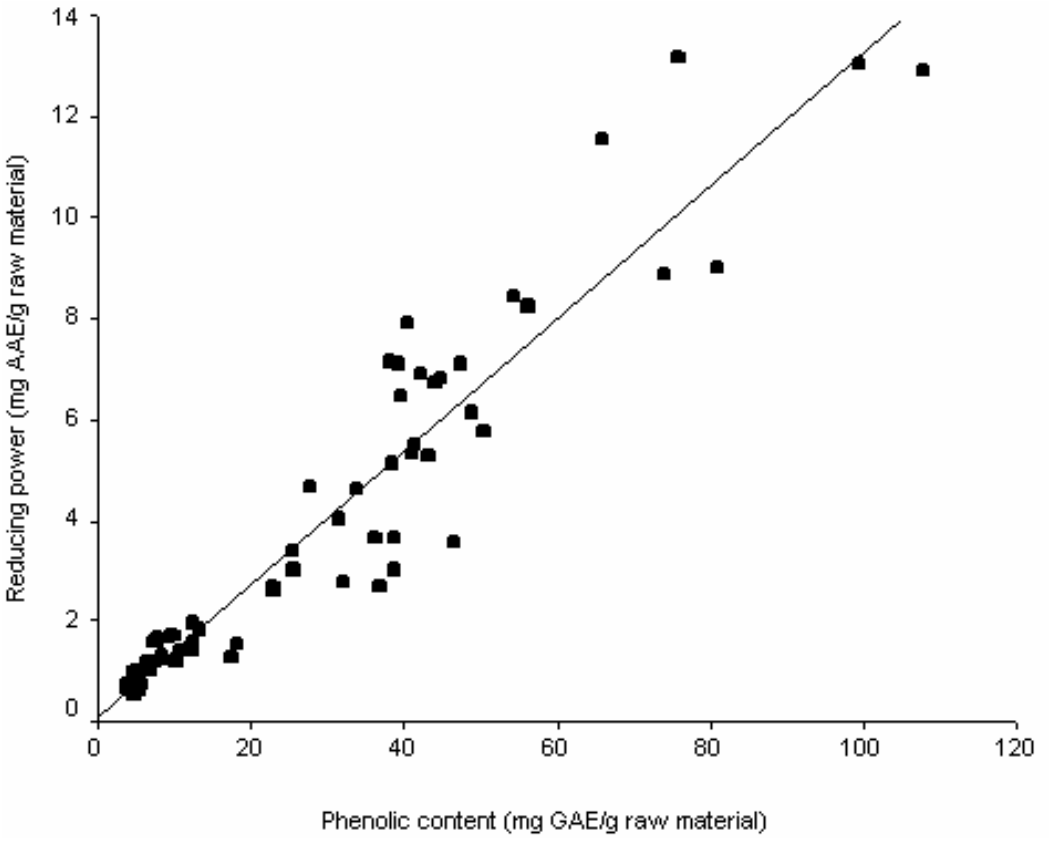

Figure 1. Linear correlation between the amount of total phenolics (GAE) and reducing power (AAE). Correlation co-efficient r = 0.950, 95% confidence interval 0.121 – 0.142, Co-efficient of determination r^2 = 0.9019. The two tailed p value is < 0.01 considered significant.

Free radical scavenging activity

The evaluation of anti-radical properties of mangrove plant materials was performed by DPPH radical scavenging assay. The 50% inhibition of DPPH radical (IC_{50}) by different plant materials was determined (Table 1). *C. decandra* stem bark showed the lowest IC_{50} value (0.65 mg of dry material/ml) whereas *S. maritima* was found to have the highest IC_{50} value (119 mg of dry material/ml). Strong inhibition was observed for *C. decandra* (root, 0.93 mg/ml) and *A. corniculatum* (stem, 0.96 mg/ml).

DISCUSSION

Sundarbans along with its geographical, climatic peculiarrities is very rich in mangrove habitat (Naskar and Bakshi, 1995). Mangroves usually grow in estuarine swamps; have unique adaptations to combat environmental stress conditions e.g. high salinity, high temperature, low nutrient and excessive radiation. An inevitable consequence of this process results in the production of ROS and accordingly the antioxidant enzymes were upregulated due to altered expression of these antioxidant genes (Jitesh et al., 2006). Moreover, mangroves are good source of polyphenols like tannins (Naskar and Bakshi, 1995). Phenolics have been considered classic defence compounds for protecting plants from herbivores, ever since plant secondary metabolites were suggested to have evolved for that reason. In contrast to these concepts, it has been suggested that the main role of many plant phenolics may be to protect leaves from photodamage, not herbivores; they can achieve this by acting as antioxidants; and their levels may vary with environmental conditions in order to counteract this potential photodamage (Close and McArthur, 2002). The phenolics especially flavonoids were shown to protect mangroves from UV radiation (Agati et al., 2007). The different extracts from mangroves were high in phenolic content (Table 1) and reflected greater synthesis since these were grown and survived in stress condition.

In this report we found a linear correlation (r^2 = 0.9500, $p < 0.01$) between the phenolic content (GAE) and ferric reducing capacity (AAE) (Figure 1) of 23 different extracts from 6 mangroves and 4 mangrove associates available in Sagar Island of Sundarbans. In another report, a significant correlation (r^2 = 0.9653, $p < 0.0001$) was ob-

served and authors use ferric reducing power (FRAP) to determine antioxidant power (Katalinic et al., 2006). That was in fair agreement to our observation. Although we have employed a different assay method to determine reducing power, almost similar results are due to identical (Fe(III) / Fe(II) system) mechanism of reaction as in ferric reducing power. In this study we have used this method because it is inexpensive and simple, and the reaction is reproducible. Another group reported no linear response between total phenolics and antioxidant activity, but the authors used different assay methods e.g. oxygen radical absorbance capacity (ORAC), and inhibition of methyl linoleate oxidation to ascertain antioxidant response (Ou et al., 2003; Kahkonen et al., 1999). This fact may add further insight between the chemical nature of phenolic compounds and their antioxidant response.

Many plants have been investigated for the antioxidant activities and the search is gradually increased in recent times since ROS were the salient feature behind many dreadful diseases. Several anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective (Ropetto and Llesuy, 2002; Perry et al., 1999) drugs have recently been shown to have an antioxidant and/or radical scavenging mechanism as part of their activity. Compounds of phenolic nature have been isolated from several species of mangrove and reported to attribute anti-tumerogenic, cytotoxic, anti-inflammatory, wound healing, anti-ulcer activities. But few studies included antioxidant activity to substantiate bioactivity. This report could be useful to delineate mechanism for bioactivity related to antioxidant activity. Naphthoquinones isolated from a gray mangrove *A. marina* of Avicenniaceae family have been shown to exhibit marked inhibitory effect on mouse skin tumor promotion (Itigowa et al., 2001), but authors did not report any antioxidant activity. *A. alba* of the same family were examined for antioxidant potential and leaves extract were shown to be rich in phenolics (11.73 mg GAE/g) and strong reducing power (1.59 mg AAE/g) that corroborated to the presence of flavonoids and naphthoquinones isolated (Ito et al., 2000). A number of polyhydroxy compounds were isolated from *A. corniculatum* and 5-O-hydroxy methylembelin showed cytotoxicity on cell lines (Xu et al., 2004). Here we have reported that *A. corniculatum* plant part extracts were found to contain appreciable amount of phenolics and showed strong antiradical activity in leaves (Table 1).

Triterpenes from *B. gymnorrhiza* showed significant inhibition of the enzyme cyclooxygenase-2 enzyme that played vital role in inflammatory process (Homuhal et al., 2006). The phenolic content in *B. gymnorrhiza* plant parts were found least when compared to other mangroves and consequently the antioxidant potential. The result might be due the extractant solvent used in this experiment where the bioactive terpenes not completely extracted. *C. decandra* is a common habitat in Sundarbans and leaves were known to be antinociceptive (Uddin et al., 2005). The bark of this plant was reported to be a rich source of tannin, which was utilized mainly to stain nets traditionally (Bandarnayake, 2002). The strong reducing power (13.04 mg AAE/g), high phenolic content (94.41 mg GAE/g) and antiradical activity (IC_{50} 0.65 mg/ml) may be correlated to the polyphenolic nature of soluble tannin present in experimental extract. *C. decandra* stem bark extract was shown to be the most potent amongst the ten halophyte species investigated so far. The most studied mangrove in Rhizophoraceae family is *R. mangle*. This plant has hypoglycemic, anti-inflammatory, anti-ulcer, wound healing as well as antioxidant activity (Sanchez et al., 2006). A polysaccharide from *R. mucronata* of the same family was reported to have anti-HIV activity (Premanathan et al., 1999). We also reported for the first time the significant antioxidant potential of different plant parts of *R. mucronata* which is useful to unravel its bioactivity. *S. apetala*, another mangrove species, has been identified to possess high antioxidant activity in all its parts (Table 1), necessitating thorough investigation in immediate future. The most studied mangrove associate is *A. illicifolius*. We found that *A. illicifolius* leaves extract had more antioxidant power than root extract as observed by Babu et al. (2001) although this is not as strong as mangrove extracts presented here. Other halophytic herbs investigated were *I. pescaprae*, *S. portulacastrum* and *S. maritima*. Amongst those *I. pescaprae* was found to be most potent antioxidant halophyte species with high phenolic content (26.32 mg GAE/g), strong reducing power (3.55 mg AAE/g) and significant antiradical activity (IC_{50} 2.95 mg/ml). Phenolic compounds like Quinic acid esters were isolated from this plant and have been shown to inhibit collagenase and impart almost no cytotoxicity (Teramachi et al., 2005).

In conclusion, our screening yielded eleven active extracts comprised of five mangroves and one mangrove associate species, corresponding to 60% to our collection. The active extracts were identified according to their phenolic content between 40 - 95 mg GAE/g and reducing power 4 - 13 mg AAE/g as well as DPPH radical scavenging activity having IC_{50} value 40 - 65 mg/ml of dry material. Further investigation may disclose active compounds responsible for antioxidant activity providing leads for development of new drugs that interfere with the disease processes originated in consequence of reactive oxygen species (ROS).

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