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Short Communication

Free radical scavenging activity of some Mangroves available in Bangladesh

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SUMMARY

The crude alcoholic extracts of various parts of five different Bangladeshi mangrove plants (*Amoora cucullata, Caesalpinia bonducella, Cerbera odollam, Derris uliginosa* and *Sonneratia caseolaris*) were screened *in-vitro* for anti-oxidant activity using the 1,1-diphenyl-2-picryldydrazyl-hydrate (DPPH) free radical scavenging assay. Of all of them, the ethanolic extracts of the leaves of *Derris uliginosa* and *Sonneratia caseolaris* showed potential antioxidant activity (IC_{50}: 94.406 and 26.30 mg/ml respectively) whereas the ethanolic extracts of aerial parts of *Derris uliginosa*, barks of *Cerbera odollam* and leaves and stems of *Caesalpinia bonducella* showed moderate activity (IC_{50}: 125.89, 211.35 and 301.99 mg/ml respectively). Mild anti-oxidant activity was observed with the methanolic extract of leaves of *Amoora cucullata* and ethanolic extracts of fruits of *Cerbera odollam*.

Key words: *Amoora cucullata*; *Caesalpinia bonducella*; *Cerbera odollam*; *Derris uliginosa*; *Sonneratia caseolaris*; Antioxidant; DPPH radical

INTRODUCTION

Free radicals are metastable chemical species which, after being generated *in vivo* as by products of various biochemical reactions, tend to rob electrons from the molecules in the immediate surrounding in order to replace their own losses. These radicals may be envisaged as molecular sharks, which if not scavenged effectively on time, are capable of damaging crucial bio-molecules including those present in cell membranes, mitochondria, DNA etc. and thus predisposing various pathophysiological states. The role of free radicals, especially of the so called 'reactive oxygen species' (ROS), has been well-established in the pathogenesis of many disease conditions such as rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, some metabolic disorders, neurodegenerative diseases (e.g. Parkinsonism, Alzheimer's disease), gastrointestinal ulcerogenesis, AIDS and even early senescence (Halliwell and Gutteridge, 1985; Halliwell, 1994). ROS is a collective term, which includes not only the oxygen radicals ($O_2^{\cdot-}$, and ·OH) but also some non-radical derivatives of oxygen. These include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3) (Bandhopadhyay *et al*., 1999).

In recent years one of the areas which attracted a great deal of attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated

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with marked oxidative damage. Several plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity (Larson, 1988; Tripathi *et al*., 1996; Sreejayan and Rao, 1997; Vani *et al*., 1997). The objective of the present study was to investigate the antioxidant activity of the crude extracts of different parts of five traditionally used mangroves available in Bangladesh, namely *Amoora cucullata, Caesalpinia bonducella, Cerbera odollam, Derris uliginosa* and *Sonneratia caseolaris*.

MATERIALS AND METHODS

Plant materials

C. odollam, D. uliginosa, and *S. caseolaris* were collected from the Sundarbans' Mangrove Forests, Bangladesh and were taxonomically identified by experts at the Bangladesh National Herbarium (accession no.: *C. odollam*-29788, *D. uliginosa*-29790, and *S. caseolaris*-29787). *A. cucullata* and *C. bonducella* were collected from the Sundarbans' Mangrove Forests and the district of Satkhira respectively, and were taxonomically validated by experts at the Forestry and Wood Technology Discipline, Khulna University, Bangladesh. The botanical and ethnopharmacological features (along with the parts used in extractions) of the plants are listed in Table 1.

Extraction of plant materials

For each plant part, about 400 mg of powdered plant material was taken in a clean, flat bottomed glass container and soaked in 1,300 ml of solvent. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by a filtration through whatmann filter paper and the filtrate thus obtained was concentrated by using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK) to get the crude extract.

Chemicals

Ascorbic acid (Loba, India), DPPH (Aldrich, USA).

Preliminary phytochemical analysis

The crude extracts were subjected to preliminary phytochemical screening for major chemical groups (Evans, 1989). In each test 10% (w/v) solution of the extract in ethanol was used unless otherwise mentioned in individual test.

Screening for antioxidant activity

Antioxidant activity of the extracts was determined on the basis of their scavenging potential of the stable DPPH free radical in both qualitative and quantitative assay.

i) Qualitative assay: A suitably diluted stock solutions were spotted on pre-coated Silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow on purple background) were noted (Sadhu *et al*., 2003).

ii) Quantitative assay: Quantitative assay was performed on the basis of the modified method of Gupta *et al*. (2003). Stock solutions (10 mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 mg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated. The experiment was performed in duplicate and average absorption was noted for each concentration. Ascorbic acid was used as positive control.

Table 1. The botanical and ethnopharmacological features of the selected mangrove plants

Plants	Type	Traditional Use	Chemical Constituents	Reported Bioactivity	Part(s) Used for Extraction (solvent)
Amoora cucullata Roxb.(Meliaceae)	Large tree*	Inflammatory diseases (Kirtikar and Basu, 1987)	Chlorophyll, carotenoids, polyphenols, tannins (Basak *et al.*, 1996)	No report	Leaves (methanol) (Yield: 10%)
Caesalpinia bonducella Flem. (Caesalpinaeceae)	Extensive climber**	Useful in colic, malaria, hydrocele, rheumatism, skin diseases, leprosy, etc. (Kirtikar and Basu, 1987).	Furanoditerpenes, fatty acids, isoflavonoids, lipids, phenolic compounds (Pascoe *et al.*, 1986).	Antitumor and antioxidant (Gupta *et al.*, 2004); hypoglycaemic and hypolipidemic (Sharma *et al.*, 1997; Chakrabarti *et al.*, 2005); antipyretic, antidiuretic, anthelmintic and antibacterial (Neogi and Nayak, 1958); antianaphylactic and antidiarrhoeal (Iyengar and Pendse, 1965); antiviral (Dhar *et al.*, 1968); antiasthmatic (Gayaraja *et al.*, 1978).	Leaves and stems (Ethanol) (Yield: 12%)
Cerbera odollam Gaertn (Apocynaceae)	Small tree*	Emetic and cathartic; emetic, purgative and antirheumatic (Yamauchi *et* al., 1987)	Cardinolide glycosides (Yamauchi *et al.*, 1987a-c; Laphookhieo *et al.*, 2004); lignans (Abe *et al.*, 1988a, b); iridoid glycosides (Abe *et al.*, 1989).	Cytotoxic (isolated cardenolide) (Laphookhieo *et al.*, 2004) and neuropharmacological activities (Hien *et al.*, 1991)	Barks and Fruits (Ethanol) (Yield: 16%)
Derris uliginosa Benth. (Leguminosae)	Large climber***	Used in rheumatism and dysmenorrhoea (Kirtikar and Basu, 1987).	Alkaloids, flavonoids, glycosides, lipids, steroids, triterpenes(Rollet, 1981; Meow-Chan and Choo-Loh, 1987).	No report	Leaves and aerial parts (Ethanol) (Yield: 12%)
Sonneratia caseolaris Linn. (Sonneratiaceae)	Small tree***	Used in sprains and swellings, hemorrhage and in the treatment of piles (Kirtikar and Basu, 1987).	Fatty acids, hydrocarbons, steroids(Rollet, 1981; Hogg and Gillan, 1984)	No report	Leaves (Ethanol) (Yield: 15%)

*Mangrove associates; **Mangrove minors; ***Mangroves

RESULTS

Preliminary phytochemical analysis

All the extracts except that of aerial parts of *D. uliginosa*, gave positive test for flavonoids and tannins (Table 2).

Antioxidant activity study

i) Qualitative assay: The color changes (yellow on purple background) on the TLC plate were observed due to the bleaching of DPPH by the resolved bands.

ii) Quantitative assay: Ethanolic extracts of the leaves of *Derris uliginosa* and *Sonneratia caseolaris* showed potential antioxidant activity where the IC_{50} was 94.406 and 26.30 μg/ml respectively, whereas the ethanolic extracts of aerial parts of *Derris uliginosa,* barks of *Cerbera odollam* and leaves and stems of *Caesalpinia bonducella* showed moderate activity where the IC_{50} was 125.89, 211.35 and 301.99 μg/ml respectively. The methanolic extracts of leaves of *Amoora cucullata* and ethanolic extracts of fruits of *Cerbera odollam* showed mild activity (IC_{50} > 500 μg/ml) against DPPH free radical (Table 3).

DISCUSSION

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study, ethanolic extracts of the leaves of *Derris uliginosa* and *Sonneratia caseolaris* showed potential free-radical scavenging activity. The ethanolic extracts of aerial parts of *Derris uliginosa,* barks of *Cerbera odollam* and leaves and stems of *Caesalpinia bonducella* showed moderate activity. The free radical scavenging property may be one of the mechanisms by which this drug is effective in traditional medicine.

Most of the tannins and flavonoids are phenolic compounds and may be responsible for antioxidant properties of many plants (Larson, 1988). In the present experiment, presence of the tannins and flavonoids in all of the tested plant extracts and absence of flavonoids in fruits of *C. odollam* may be correlated with their antioxidant activity against DPPH free radical. On the other hand moderate antiradical activity was found in the ethanolic extracts aerial parts of *D. uliginosa* where tannins and flavonoids were absent. In this case the activity may be due to lignans or other components (Sadhu *et al*., 2003).

The free radical scavenging property may be one

Table 2. Preliminary screening of the crude extracts for resident phytochemicals

Plant Extract	Alkaloid	Reducing Sugars	Tannins	Gums	Flavonoids	Saponins	Steroids
Me. extract of *A. cucullata* (Leaves)	-	+	+	+	+	+	+
Et. extract of *C. bonducella* (Leaves and stems)	+	-	+	-	+	-	+
Et. extract of *C. odollam* (Bark)	-	+	+	+	+	+	+
Et. extract of *C. odollam* (Fruit)	-	+	+	-	-	+	+
Et. extract of *D. uliginosa* (Aerial parts)	-	+	-	+	-	+	+
Et. extract of *D. uliginosa* (Leaves)	+	-	+	-	+	-	-
Et.. extract of *S. caseolaris* (Leaves)	-	+	+	+	+	+	-

Me.: Methanolic; Et.: Ethanolic

Table 3. Evaluation of antioxidant a ctivity of the plant extracts

Sample	Concentration (μg/ml)	% Inhibition	IC_{50} (μg/ml)
Me. extract of *A. cucullata* (leaves)	1	9.70 ± 0.005	> 500
	5	10.83 ± 0.002	
	10	12.24 ± 0.006	
	50	15.22 ± 0.008	
	100	18.90 ± 0.008	
	500	46.28 ± 0.002	
Et. extract of *C. bonducella* (leaves and stems)	1	18.40 ± 0.003	301.99
	5	21.59 ± 0.006	
	10	32.70 ± 0.005	
	50	34.61 ± 0.004	
	100	37.14 ± 0.003	
	500	55.84 ± 0.005	
Et. extract of *C. odollam* (barks)	1	27.3 ± 0.001	211.35
	5	27.51 ± 0.001	
	10	34.81 ± 0.008	
	50	36.84 ± 0.003	
	100	40.56 ± 0.010	
	500	60.21 ± 0.003	
Et. extract of *C. odollam* (fruits)	1	4.05 ± 0.002	> 500
	5	5.99 ± 0.003	
	10	7.77 ± 0.001	
	50	9.06 ± 0.002	
	100	10.52 ± 0.002	
	500	19.4 ± 0.001	
Et. extract of D. uliginosa (aerial parts)	1	25.96 ± 0.001	125.89
	5	31.93 ± 0.001	
	10	33.26 ± 0.001	
	50	37.19 ± 0.025	
	100	45.47 ± 0.001	
	500	77.96 ± 0.038	
Et. extract of D. uliginosa (leaves)	1	23.60 ± 0.008	94.406
	5	32.12 ± 0.007	
	10	34.08 ± 0.009	
	50	41.20 ± 0.014	
	100	50.42 ± 0.013	
	500	78.21 ± 0.013	
Et. extract of S. caseolaris (leaves)	1	5.55 ± 0.004	26.30
	5	9.41 ± 0.018	
	10	21.53 ± 0.021	
	50	70.14 ± 0.001	
	100	90.58 ± 0.004	
	500	91.06 ± 0.001	

Table 3. Continued

Sample	Concentration (μg/ml)	% Inhibition	IC_{50} (μg/ml)
Ascorbic acid	1	39.78 ± 0.001	3.16
	5	53.82 ± 0.004	
	10	61.40 ± 0.001	
	50	77.47 ± 0.004	
	100	93.96 ± 0.003	
	500	96.84 ± 0.001	

Values are expressed as mean ± S.D; Me.: Methanolic; Et.: Ethanolic

of the mechanisms by which these plants' parts are effective in their ethnopharmacological uses against different ailments. Further studies comprising of thorough phytochemical investigations of the used plants and evaluation for anti-oxidant activity using other models (e.g. various biochemical assays, both *ex vivo* and *in vivo*) are essential to characterize them as biological antioxidants.

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