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Full Length Research Paper

The antioxidant and free radical scavenging effect of *Avicennia officinalis*

P. Thirunavukkarasu, T. Ramanathan*, L. Ramkumar, R. Shanmugapriya and G. Renugadevi

Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai - 608 502, Tamil Nadu, India.

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Avicennia officinalis **is a mangrove plant and is used by coastal village peoples in traditional folk medicine -for a variety of diseases. In general, salt tolerant plants have a more antioxidant profile. In our present study, we examined different types of antioxidant capacity like that of phenolics against DPPH nitroxide, hydroxyl and ABC radicals in two solvent extracts of ethanol and water at different concentrations of 0.1, 0.2, 0.5, 1.0 and 2.0 mg/ml. Our results showed maximum activity in 2.0 mg/ml of leaves extract of ethanol and minimum activity in aqueous extract of 0.1 mg/ml concentration for all antioxidant assays. The results showed highly effective antioxidant activities in two solvent extracts at a different concentrations, therefore, the extracts have good antioxidant properties.**

Key words: *Avicennia officinalis*, mangrove, phenolic, hydroxyl radical, ABTS radical, nitroxide radical.

INTRODUCTION

Avicennia officinalis is a commonly available white mangrove plant in almost all the coastal states of India. It is a folk medicinal plant used mainly against rheumatism, paralysis, asthma, snake-bites, skin disease and ulcer. A decoction of the plant with sugar candy and cumin is used in dyspepsia with acid eructations (Kathiresan and Ramanathan, 1997; Ramanathan, 2000). The fruits are plastered onto tumors in India (Hartwell, 1967). Indian mangrove is a folk remedy for boils and tumors (Duke and Wain, 1981). Unripe seeds are poulticed onto abscess, boils, and smallpox sores. Indochinese uses the bark for skin afflictions, especially scabies. A resinous substance exuded from the bark acts as a contraceptive, and apparently can be taken all year long without ill effects (Perry, 1980). Anti-oxidants are vital substances, which possess the ability to protect the body from damage caused by free radical induced oxidative stress (Ozsoy et al., 2008). There is an increasing interest in natural anti-oxidants, e.g., polyphenols, present in medicinal and dietary plants, which might help prevent oxidative damage (Silva et al., 2005). Polyphenols possess ideal structural chemistry for free radical scavenging activity, and they have been shown to be more effective anti-oxidants *in vitro* than tocopherols and ascorbate. In our present study, antioxidant and free radical scavenging potential of *A. officinalis* was investigated.

*Corresponding author. E-mail: drtrcasmb@gmail.com. Tel: 04144-243223 Extn. 213. Fax: 04144-243555.

MATERIALS AND METHODS

Chemicals

Chemical reagents nitro blue tetrazolium (NBT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid (standard solution), Sodium carbonate and sodium nitroprusside (10 mM) solution and trichloro acetic acid (TCA), hypoxanthine, xanthine oxidase, bovine Cu, Zn superoxide dismutase (Cu, ZnSOD), catalase, nitro blue tetrazolium, diethylenetriaminepentaacetic acid (DTPA), WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)- 2H-5-tetrazolio]-1,3-benzene disulphonate). All other reagents used were of analytical grade and all the chemicals were purchase from -Subra Scientific Co., Pondicherry.

Plant material

The leaves of *Avicennia officinalis* were collected from the

mangrove region of Parangipettai, Cuddalore District Tamilnadu on the Southeast coast of India, during February 2010. The work was carried out at the Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamilnadu,

Preparation of the extract

The air-dried leaves of A. *officinalis* (50 g) were powdered and then extracted with 400 ml of petroleum ether in a soxhlet apparatus, to remove the lipids and other resinous matter from the leaves. Then, the residue obtained after the extraction with petroleum ether was further extracted with 500 ml of chloroform by using soxhlet apparatus. The crude ethanol and aqueous extracts were filtered and evaporated under reduced pressure, which was a viscous dark mass with a percentage yield of 5.20% (w/w). This crude extract was dissolved in ethanol solvent and used in different concentrations of 0.1, 0.2, 0.5, 1.0, 2.0 mg/ ml for the assessment of anti-oxidant activity.

Determination of total phenolic content

Total soluble phenolics in the extracts were determined with Folin–Ciocalteau reagent according to the method using gallic acid as a standard phenolic compound (Slinkard and Singleton, 1977). 1 ml of extract solution containing 1.0 g extract in a volumetric flask was diluted with 45 ml of methanol. 1 ml of Folin–Ciocalteau reagent was added and mixed thoroughly. Three minutes later, 3.0 ml of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract (Kim et al., 2003). All determinations were performed in triplicate. Total content of phenolic compounds in plant extract in gallic acid equivalents (GAE) was calculated by the following formula:

C ¼ c _ V = m

where: C – total content of phenolic compounds, mg/g plant extract, in GAE; c – the concentration of gallic acid established from the calibration curve, mg/ml; V – the volume of extract, ml; m – the weight of pure plant extract.

Determination of DPPH (1-1-diphenyl 2-picryl hydroxyl) radical-scavenging activity

The free radical-scavenging activity of the A. *officinalis* chloroform extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (Blios, 1958). One-tenth mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (0.1 to 5 mg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

Radical scavenging activity (%) = (1- A_{sample}/ $A_{control}$) x 100

where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Determination of hydroxyl radical-scavenging activity

The hydroxyl radical scavenging capacity was measured using modified method as described previously (Halliwell et al., 1987). Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxyribose (10 mM) were prepared in distilled de-ionized water.

The assay was performed by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml of H_2O_2, 0.36 ml of deoxyribose, 1.0 ml of extract (0.1 to 5 mg/ml) each dissolved in methanol, 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 h. 1 ml portion of the incubated mixture was mixed with 1.0 ml of (10%) trichloroacetic acid and 1.0 ml of (0.5%) thiobarbituric acid (in 0.025 M NaOH containing 0.025 M NaOH butyl hydroxyl anisol) to develop the pink chromogen, which was measured at 532 nm. The hydroxyl radical-scavenging activity of the extract was reported as the percentage of inhibition of deoxyribose degradation and was calculated according to the following equation:

Radical scavenging activity (%) = (1- A_{sample}/ $A_{control}$) x 100

where A_c was the absorbance of the control (blank, without extract) and A_s was the absorbance in the presence of the sample of the extract.

All the tests were performed in triplicate and the graph was plotted with the mean values. Ascorbic acid was used as a positive control. Determination of nitric oxide radical-scavenging activity was generated from sodium nitroprusside and measured by the Greiss reaction.

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (Marcocci et al., 1994), which interacts with oxygen to produce nitric ions that can be estimated by using Greiss reagent.

Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate buffer saline (PBS) was mixed with 3.0 ml of different concentrations (0.1 to 5 mg/ml) of the *A. officinalis* extract and incubated at 25°C for 150 min. The samples were added to Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromaphore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance of standard solutions of ascorbic acid treated in the same way with Griess reagent as a positive control.

The percentage of inhibition was measured by the following formula:

Radical scavenging activity (%) = (1- A_{sample}/ $A_{control}$) x 100

where $A_c$was the absorbance of the control (blank, without extract) and A_s was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

ABTS radical scavenging assay

The ABTS assay was employed to measure the anti-oxidant activity

Table 1. Effect of *A. officinalis* aqueous and ethanol leaf extract on DPPH and Hydroxyl radical scavenging activities.

Conc. (mg/ml	DPPH radical-scavenging activity			Hydroxyl radical-scavenging activity		
	AOA	AOE	AA	AOA	AOE	AA
0.1	40.77±3.43	50.55±2.88	65.12±54	38.23±3.84	48.68±7.67	64.35±1.34
0.2	45.78±2.50	65.67±3.41	71.31±2.31	42.55±4.37	62.57±3.57	68.64±0.37
0.5	54.34±2.94.	70.61±5.34	76.15±6.12	51.81±2.47	65.84±4.81	73.45±1.34
1.0	69.85±2.45	80.57±4.81	82.31±3.21	67.22±3.67	75.42±3.27	79.64±2.64
2.0	73.89±4.22.	86.87±5.04	90.32±5.12	71.56±4.84	82.75±2.63	88.54±3.54

AOA: *A. officinalis* aqueous extract; AOE: *A. officinalis* ethanol extract; AA: ascorbica acid; SD, standard deviation. a Values are given as mean of three replicates.

of the leaf extract. ABTS was dissolved in de-ionized water to 7 mM concentration, and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12 to 16 h) in the dark before usage. Half a milliliter of leaf extract was diluted with 0.3 ml ABTS solution and made up to the volume with methanol. Absorbance was measured spectrophotometrically at 745 nm. The assay was performed at least in triplicates. Fresh stocks of ABTS solution were prepared every five days due to self-degradation of the radical. The assay was first carried out on ascorbic acid, which served as a standard. The percentage of inhibition was measured by the following formula:

Radical scavenging activity (%) = $(1- A_{sample}/ A_{control}) \times 100$

where A_c was the absorbance of the control (blank, without extract) and A_s was the absorbance in the presence of the extract.

All the tests were performed in triplicate and the graph was plotted with the mean values.

RESULTS AND DISCUSIÓN

The calculation of total phenolic content of plant extracts was carried out using the standard curve of gallic acid and presented as gallic acid equivalents (GAE) per gram (257 mg/g) of ethanol extract and (293 mg/g) of aqueous extract. The results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. *A. officinalis* extracts contained the highest amount of phenolic compounds and the lowest amount is present in *Ipomea pescapa* extract (Thirunavukkarasu et al., 2010). The same relationship was also observed between phenolics and anti-oxidant activity in roseship extracts (Gao et al., 2000). Phenolic compounds, such as flavonoids, phenolic acid and tannins possess diverse biological activities, such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities. These activities might be related to their antioxidant activity (Chung et al., 1998). Phenols are very important plant constituents because of their scavenging ability owing to their hydroxyl groups (Hatano et al., 1989). Phenolic compounds from plants are known to be good natural anti-oxidants. However, the activity of synthetic anti-oxidants was often observed to be higher than that of natural anti-oxidants (Ningappa et al., 2008).

The DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals was initiated by the lipid autoxidation. In our present study, the result indicated that DPPH radical scavenging activity was higher in the ethanol extract (50.55±2.88) followed by aqueous extract (40.77±3.43) and were compared with ascorbic acid (Table 1). Our results suggest that different concentrations have different activities and maximum activity was observed in 2.0 mg/g concentration (Table 1). Herbal extracts antioxidant principles may act as hydrogen donators preventing microsomal lipid peroxidation and protein thiol group's oxidation (Joyeux et al., 1995; Horie et al., 1999). Similar mechanisms have been proposed for the reaction between DPPH radical and herbal antioxidant principles. The DPPH activity of *A. officinalis* was found to increase in dose dependent manner. The same results were shown in the *in vivo* study of Babu et al. (2001). But in the *in vitro* study of *A. officinalis* at the used concentrations displayed potential effect of DPPH activity as percentage of free radicals inhibition (Table 1). In this study, of the two extracts, maximum activity was seen in ethanol extract and minimum activity in aqueous extract.

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Atallah, 1988). This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an extract is directly related to its anti-oxidant activity (Babu et al., 2001). Our results indicated maximum effect in *A. officinalis* ethanol extract and minimum in aqueous extract, and the concentration of 2.0 mg/ml showed best result compare

Table 2. Effect of *A. officinalis* aqueous and ethanol leaf extract on Nitric oxide and ABTS radical scavenging activities.

Conc. (mg/ml)	Nitric oxide radical-scavenging activity			ABTS radical-scavenging activity		
	AOA	AOE	AA	AOA	AOE	AA
0.1	39.87±4.78	49.78±4.47	62.97±8.64	38.78±9.62	58.39±4.55	61.84±1.33
0.2	40.06±9.12	56.21±3.84	67.64±9.64	39.73±3.89	62.56±5.97	66.87±3.54
0.5	41.44±4.03	61.09±3.88	72.97±5.61	40.37±8.85	67.82±4.92	71.89±9.87
1.0	52.51±8.99	69.45±5.75	77.54±4.64	50.89±5.99	70.33±5.19	75.67±4.44
2.0	70.24±3.99.	80.54±6.63	86.97±5.87	68.67±8.91	81.53±6.29	85.54±6.88

AOA: *A. officinalis* aqueous extract; AOE: *A. officinalis* ethanol extract; AA: ascorbica acid. SD, standard deviation. a Values are given as mean of three replicates.

to others (Table 1). Hagerman et al. (1998) have also explained that high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical-scavenging activity by phenolics than their specific functional groups.

Nitric oxide plays an important role in various types of inflammatory processes in the animal body. In the present study, the crude chloroform extract of the *hj* was investigated for its inhibitory effect on nitric oxide production. Results showed the percentage of inhibition in a dose dependent manner with maximum effect in ethanol extract and minimum effect in aqueous extract (Table 2). The reduction capability of ABTS radical was determined by the decrease in its absorbance at 745 nm, which is induced by anti-oxidants. The extracts of *A. officinalis* leaves showed maximum activity in ethanol and minimum activity in water (Table 2). Our result showed that the concentration of 2.0 mg/ml had the best antioxidant effect.

Conclusion

In this study, using various *in vitro* assay systems, the antioxidant potential of *A. officinalis* extract was evaluated based on DPPH, superoxide, and hydroxyl radical scavenging activities and total phenolic activity. In addition, we further evaluated the inhibition of protein oxidation, as well as reducing power of the extract. The results clearly confirmed the antioxidative and free radical scavenging effect of the *A. officinalis* leaves extracts. Identification of the antioxidative constituents of the plant and evaluation of their probable anti-diabetic, anti-cancer and anti-cardiac vascular disease properties is in progress.

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