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Antioxidant and Free Radical Scavenging Effect of *Acanthus ilicifolius*

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Abstract: *Acanthus ilicifolius* is a mangrove plant and it used coastal village peoples for traditional folk medicine method for varies disease. In general salt tolerance plants have a more antioxidant constitute. In the present study researchers have examine different type of anti oxidant capacity like that total phenolic, DPPH radical, nitraxcite radical, hydroxyl radical and ABC radical in two solvent extract of ethanol and aqueous for different concentration like 0.1, 0.2, 0.5, 1.0 and 2.0. In the result showed that maximum in 2.0 mg mL^{-1} of leaves extract of ethanol and minimum in aqueous extract in 0.1 concentration for all antioxidant assay. In this result showed that highly effect in antioxidant levels in two solvent extract and varies concentration so there would be a good antioxidant property.

Key words: *Acanthus ilicifolius*, phenolic, hydroxyl radical, ABTS radical, nitraxcite radical, India

INTRODUCTION

India is a rich source of medicinal plants and a number of plant extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Sidha. Only few of them were scientifically explored. *Acanthus ilicifolius* Linn. (Acanthaceae) is a folklore medicinal plant used against rheumatism, paralysis, asthma and snake bite (Kathiresan and Ramanathan, 1997; Ramanathan, 2000). The whole plant extract has been reported to possess analgesic and anti-inflammatory activities (Agshikar *et al*., 1979) while no such report has been published in reference to the leaves of *Acanthus ilicifolius*. Methanolic extract of the leaves has been reported to exhibit hepatoprotective (Babu *et al*., 2001) and tumor reducing activities (Babu *et al*., 2002). Leishmanicidal activity of 2-benzoxazolinone, isolated from the leaves of this plant has also been documented (Kapil *et al*., 1994). Phytochemical studies with the plant revealed the presence of lignans (Kanchapoom *et al*., 2001) and megastigmane glycosides (Wu *et al*., 2003). According to various medical literatures, several adverse.

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 radicalscavenging activity and they have been shown to be more effective anti-oxidants *in vitro* than tocopherols and ascorbate. In the present study to carried out antioxidant and free radical scavenging potential of *A. ilicifolius*.

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 gradeand all the chemical purchase from (Subra Scientific Co., Pondicherry).

Plant material: The leaves of *A. ilicifolius* were collected from the sand mash region of Poondiyankuppam, Cuddalore district Tamil Nadu on the Northeast coast of India during November 2009. The research out of centre of advanced study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu.

Preparation of the extract: The air-dried leaves of *A. ilicifolius* (50 g) were powdered and then extracted with

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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 extract was 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 concentration like that 0.1, 0.2, 0.5, 1.0, 2.0 mg mL^{-1} 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), 1.0 mL of extract solution containing 1.0 g extract in a volumetric flask was diluted with 45 mL of methanol. The 1.0 mL of Folin-ciocalteau reagent was added and mixed thoroughly. About 3 min 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^{-1} 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^{-1} plant extract in (GAE)
- c = The concentration of gallic acid established from the calibration curve (mg mL^{-1})
- V = The volume of extract (mL)
- m = The weight of pure plant extract

Determination of DPPH (1-1-diphenyl2-picryl hydroxyl) radical-scavenging activity: The free radical-scavenging activity of the *A. ilicifolius* chloroform extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (Blois, 1958). The 0.1 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 (0.1-5 mg mL^{-1}). The 30 min 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 Eq. 1:

Inhibition (%) ¼ ðA0_AtÞ = A0_100 (1)

Where:

- A0 = The absorbance of the control (blank, without extract)
- At = 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 (0.1-5 mg mL^{-1}) 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. The 1.0 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 MNaOH 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 from Eq. 1. Ascorbic acid was used as a positive control. 2.7. Determination of nitric oxide radical-scavenging activity nitric oxide 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 reduce production of nitric oxide. Sodium nitroprusside (5 mM) in Phosphate Buffer Saline (PBS) was mixed with 3.0 mL of different concentrations (0.1-5 mg mL^{-1}) of the *A. ilicifolius* 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 napthylethylenediamme 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 Eq. 1.

ABTS radical scavenging assay: The ABTS assay was employed to measure the anti-oxidant activity 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 (12-16 h) in the dark before usage. The 0.5 mL 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 5 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 Eq. 1.

RESULTS AND DISCUSION

The calculation of total phenolic content of plant extracts was carried using the standard curve of gallic acid and presented as Gallic Acid Equivalents (GAE) per gram 257 mg g^{-1} of ethanol extract and 293 mg g^{-1} of aqueous extract. These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. In the result remain that the calculation of total phenolic content of plant extracts was carried using the standard curve of gallic acid and presented as Gallic Acid Equivalents (GAE) per gram *A. ilicifolius* extracat 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 the present study the result indicated that DPPH radical scavenging activity higher the ethanol extract (50.55±2.88) followed by aqueous extract (40.77±3.43) and their compare to ascorbic acid (Table 1). In the result suggested that in different constration like 0.1, 0.2, 0.5, 1.0, 2.0 among the constration maximum observed in 2.0 mg g^{-1} and followed by 0.1, 0.2, 0.5, 1.0 mg g^{-1} (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. ilicifolius* was found to increase in dose dependent manner. The same result indicated that *in vivo* study of (Babu *et al*., 2001). But in the *in vitro* study the *A. ilicifolius* at the used concentrations displayed potential effect of DPPH activity as percentage of free radicals inhibition (Table 1). In this study two extract of among the two extract maximum in ethanol extract and minimum in aqueous.

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 Attalah, 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). In the result indicated that maximum effect in *A. ilicifolius* ethanol extract and minimum in aqueous extract and in the result 2.0 mg mL^{-1} is best result compare to others (Tabel 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 *A. ilicifolius* was checked for its inhibitory effect on nitric oxide production. Results showed the percentage of inhibition in a dose dependent manne 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 ethanol extract of *A. ilicifolius* leaves maximum in ethanol extract and minimum in aqueous extract (Table 2). In the result showed that 2.0 mg mL^{-1} is the good anti oxidant effect followed by 1.0, 0.5, 0.2 and 0.1.

Table 1: Effect of *Acanthus ilicifolius* aqueous and ethanolic leaf extract on DPPH and hydroxyk R radical scavenging activities

Con.	DPPH radical-scavenging activity			Hydroxyl radical-scavenging activity		
(mg mL^{-1})	AIA	AIE	AA	AIA	AIE	AA
0.1	40.77±3.43	50.55±2.88	65.12±54.0	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

AIA: *Acanthus ilicifolius* Aqueous extract, AIE: *Acanthus ilicifolius* Ethanol extract; AA: Ascorbica Acid; SD: Standard Deviation; a values are given as mean of three replicates

Table 2: Effect of *Acanthus ilicifolius* aqueous and ethanolic leaf extract on nitric oxide and ABTS radical scavenging activities

	Nitric oxide radical-scavenging activity			ABTS radical-scavenging activity		
Con. (mg mL^{-1})	AIA	AIE	AA	SM.A	SME	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

AIA: *Acanthus ilicifolius* Aqueous extract, AIE: *Acanthus ilicifolius* Ethanol extract, AA: Ascorbica Acid; SD: Standard Deviation. a values are given as mean of three replicates

CONCLUSION

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

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