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Pharmacological Properties of *Avicennia marina* (Forssk) Vierh

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Abstract

Avicennia marina is the marine mangrove found abundantly along the coastal regions of Thoothukudi District. It is enriched with numerous bioactive compounds having wide range of medicinal properties. In this study, *A.marina* was extracted with five different solvents *viz*. hexane, water, isopropanol, acetone and methanol. Different solvent extracts of *A. marina* was subjected to biochemical assays *viz*. Total antioxidant activity, Protein denaturation inhibition activity, Nitric oxide scavenging activity and Metal chelating ability. Methanolic extract of *A.marina* shown highest activity in all the biochemical assays. Hence, the methanolic extract can be used a source of drug for various diseases.

Keywords

Avicennia marina, Antioxidant, Nitric oxide scavenging and Metal chelating study.

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INTRODUCTION:

Plants are the very important chemical factories of nature. Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicine. Today most of the drugs are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products. Approximately 20 % of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources (Panda, 2000).

Phytochemicals are the natural bioactive compounds found in plants. They are divided into two groups which are primary metabolites and secondary metabolites according to their functions in plant metabolism. Primary metabolites comprise common sugars, amino acids, proteins and chlorophyll while secondary metabolites consist of alkaloids, flavonoids, tannins, saponins, terpenoids and phenolic compounds (Edeoga *et al.,* 2005; Prasad *et al.,* 2012). Most of the novel bioactive principles of plants constituted by secondary metabolites like alkaloids, terpenoids, flavonoids, phenolic compounds, organic acids and lipids (Harborne,1998).

The present study aimed to utilize the mangrove plant which is one of the important sources of mangrove. They are widespread in tropical and subtropical regions, growing in the saline intertidal zones of sheltered coast lines (Chelliah, 2001; Marium Tariq *et al.,* 2007). Mangrove species are woody, seed bearing and highly specialized plants (Duke *et al.,* 1998) found coast lines of estuaries and lagoons (Kamaruzzaman, 2011). Because of their unique adaptations mangroves thrive well in the environment where other plants cannot grow (Shanmugapriya *et al.,* 2012). Mangroves are salt tolerant plants. The specific regions where plants

occur are termed as mangrove ecosystem (Kathiresan *et al.,* 2001; Chelliah, 2001). There are seven species of true mangroves have been identified are also recorded. Mangrove plants also have been also used as traditional medicine in India (Dhayanithi *et al.,* 2012; Bharathi *et al.,* 2011).

However during the last decade screening of mangroves for bioactive compounds (Kokpal *et al.,* 1990), has received high interest as a potential bioresource for novel bioproduct leads (Ishibasi *et al.,* 1993; Miki *et al.,* 1994). Among all the true mangroves of the world *Avicennia marina* (Forsk.) Vierh is a valuable mangrove because of its medicinal values and abundant distribution.

A. marina is commonly called white mangrove belongs to the family Avicenniaceae. It is a small medium sized tree (3-11 meter) with many branches. Extensive underground root system with Pencil root (Pneumatophores or breaking roots) up to 90 mm long. The plant has received some attention in determining its important chemical constituents. Phenolic compounds are secondary plant metabolites and are involved in a wide range of specialized physiological function (Bharathi *et al.,* 2011; Dhayanithi *et al.,* 2012; Borojeni *et al*., 2013). Previous reports suggest that this species was useful in stabilizing banks of estuaries in salty water and that it has tannin rich bark (Bandaranayake, 2002). In this study pharmaceutical properties of *Avicennia marina* was evaluated using certain *in vitro* assays *viz*. Total antioxidant activity, nitric oxide scavenging activity, protein denaturation inhibition activity and chelating ability of metal ions.

MATERIALS AND METHODS:

Collection and Identification of plant *Avicennia marina*

The fresh leaf of mangrove plant, *Avicennia marina* was collected from the estuarine region of Roche Park of Thoothukudi district, South East Coast of Tamil Nadu, India by hand picking method. The leaves were washed thoroughly thrice with tap water and once with sterile distilled water to remove salt and sand. Then they were shade dried for two weeks and were partially powdered using domestic blender and stored in air tight container for further use.

Extraction

Extraction was carried out by using cold percoalation method. An amount (100 gm) of partially crushed and powdered leaves was taken separately into 1000 ml of conical flask with acetone, hexane, methanol, isopropanol and sterilized water individually. This set up was kept on a rotary shaker at 120 rpm for 24 hrs. After shaking, it was filtered through eight layers of muslin cloth, 100 ml of extract was centrifuged at 5000 x g for 15 min. Extraction of solvent was evaporated and dried over Sodium Sulphate in desiccator under vacuum.

Total antioxidant activity of *A. marina* by Phosphomolybdenum assay

Total antioxidant activity was estimated by phosphomolybdenum assay (Prieto *et al*, 1999). *A. marina* extracts of different concentration ranging from 200µg/ml to 1000 µg/ml were taken in individual test tubes and made up to 1 ml using distilled water and 2 ml of Molybdate reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. The tubes were incubated at 95°C for 90 minutes. After incubation, the tubes were cooled to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695nm. Experiments were done in triplicates. Ascorbic acid was used as the positive reference standard.

Protein denaturation inhibition activity of *A. marina* extract

The reaction mixture (0.5ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.1 ml of *A.marina* extract at different concentration (200-1000 µg/ml) was taken in test tubes and incubated at 37ºC for 30 min (Tanford, 1968). After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660nm. 0.5 ml distilled water was used as blank. The percentage inhibition of protein denaturation was calculated by the following formula,

Percent protein denaturation inhibition = $Abs_{Control}$ - Abs_{sample} / $Abs_{Control}$ X 100

Nitric oxide scavenging activity of *A. marina*

Nitric oxide scavenging activity can be estimated by the use of Griess reaction (Garrat, 1964). The compound sodium nitroprusside decomposes in aqueous solution at physiological pH (7.2) producing nitric oxide . Under aerobic conditions, nitric oxide - reacts with oxygen to produce stable products (nitrate and nitrite). The quantities of nitrate and nitrite can be determined using Griess reagent (1% sulfanilamide, 2% H_3P0_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride). Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations (200-1000 µg/ml) of *A.marina* extract and incubated at 30ºC for 2 hours. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthyl ethylenediamine dihydrochloride was

immediately read at 540nm. Inhibition of nitrite formation by the crude extracts and the standard antioxidant ascorbic acid were calculated relative to the control. All experiments were performed in triplicates and the results were expressed as mean ± standard deviation.

Chelating ability of *A. marina* extract on ferrous ions

The ferrous ion chelating potential of the extracts was evaluated by Dinis *et al.* method. The reaction mixture contained 1.0 ml of various concentrations of the extracts (2-10 mg/ml) and 0.05 ml of 2 mM $FeCl_3$. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. The reaction mixture was shaken vigorously and left standing at room temperature for 10 min and the absorbance of the reaction mixture was measured at 562 nm against a reagent blank. A lower absorbance of the reaction mixture indicated a higher ferrous ion chelating ability. The control contained all the reagents except sample. Gallic acid and ascorbic acid was used as standard for comparison. The following formula was used to calculate percent inhibition.

Percent Inhibition = [(Control- Test)/control] × 100

RESULTS AND DISCUSSION:

The total antioxidant activity of *A. marina* extract

The total antioxidant activity is the representation of the compounds ability to neutralize or scavenge free electrons in biological system. Activity of different extracts of *A.marina* was shown in Figure 1. Methanolic extract of *A. marina* shown highest activity followed by acetone, isopropanol, aqueous and hexane. Ascorbic acid was used as control. The results indicated that methanolic extract of *A.marina* has contained highest concentration of bioactive compounds with anticancer activity. Sharma S and and Vig AP, (2013) studied *in vitro* antioxidant properties using methanol and aqueous extracts of *Parkinsonia aculeata* and found that different phytochemicals, present in the leaves, are responsible for the high antioxidant potential. Samydurai et al., 2012 also observed hydroxyl group of the phenolic group donate to superoxide scavenging activity by their electron donor. Kumaran and Karunakaran, 2006 investigated that the antioxidant properties of methanol extract of *Cardiospermum halicacabum* engaging b-carotene-linoleate model system, 1,1-diphenyl-2-picrylhydrazyl (DPPH) superoxide nitric oxide radical scavenging, reducing power, and iron ion chelating activity

Nitric oxide is the important free radical cause severe damage to DNA and proteins. The drug molecules of nitric oxide scavenging potential with less side effects will be the better drug than chemically derived counterparts. The nitric oxide scavenging activity of different extracts of *A.marina* was shown in Figure 2. Methanolic extract of *A. marina* shown highest activity followed by acetone, isopropanol, aqueous and hexane. Ascorbic acid was used as control. Awah and Verla, 2010 studied that Ocimum gratissimum leaf extract possesses high antioxidant and nitric oxide scavenging activity. Pacher et al., 2007 and Sreejayan & Rao, 1997 revealed that toxicity of nitric oxide increases their superoxide to combine oxidant contribute OH group and nitric oxide.

Proteins are the essential biomolecules in human body. The destruction of proteins results in progression of diseases such as alzheimers and Parkinson. The drugs having protein denaturation inhibition activity be used as drugs for protein related diseases (Williams et al., 2008). Many literatures revealed that plant extracts possess protein denaturation (Mitul and Handral, 2013, Mohan et al., 2013, Madduluri et al., 2014, Sarkar, 2015). Protein denaturation inhibition activity of different extracts of *A. marina* was shown in Figure 3. Methanolic extract of *A. marina* shown highest activity followed by acetone, isopropanol, aqueous and hexane.

Metal chelating property is a sign of antioxidant activity; it reduces the concentration of the catalyzing transition (Duh et al., 1999). Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. The reducing ability of a compound generally depends on the presence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom. Metal chelating activity of different extracts of *A. marina* was shown in Figure 4. Methanolic extract of *A.marina* shown highest activity followed by acetone, isopropanol, aqueous and hexane. This hypothesis is also confirmed by numerous reports in the literature showing that cytotoxic herbal extracts and isolated phytochemicals frequently also reveal antioxidant activity (Adam et al., 2018, Duh et al., 1999). Ascorbic acid was used as control.

Figure1. Total Antioxidant activity of *A. marina* extract

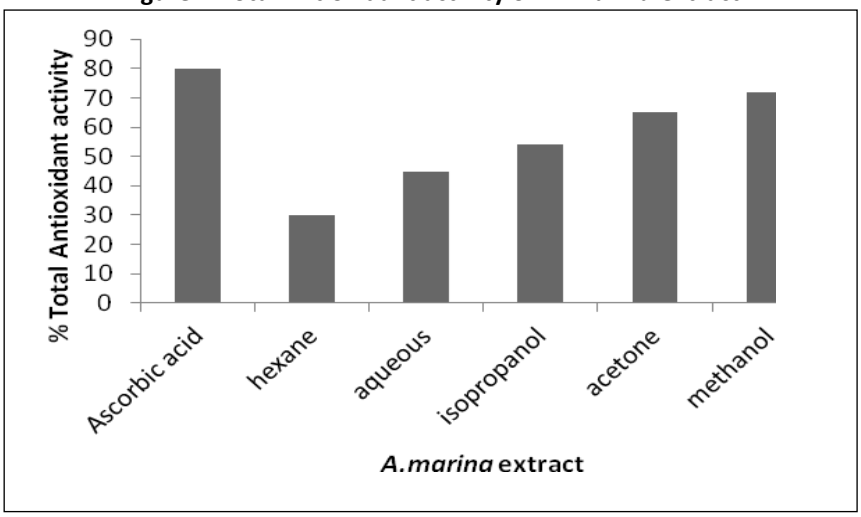


Figure 2. Nitric oxide scavenging activity of *A.marina*

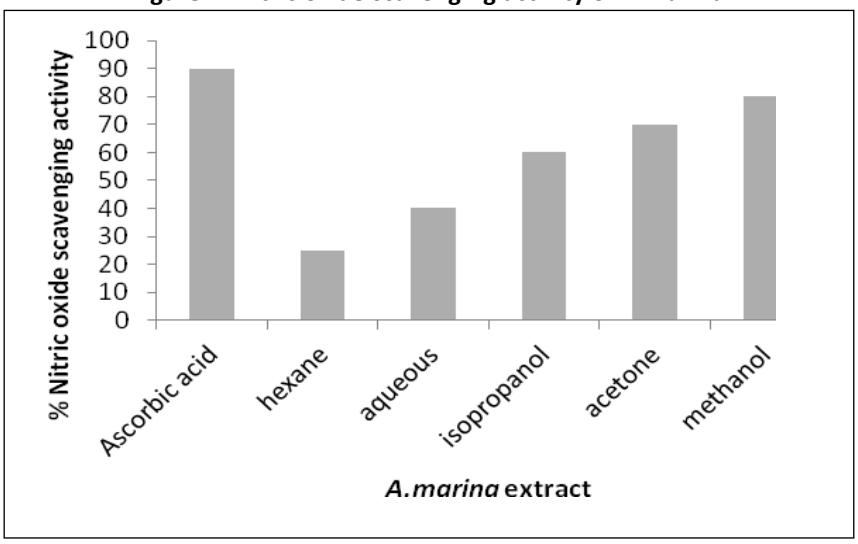


Figure 3. Protein denaturation inhibition activity

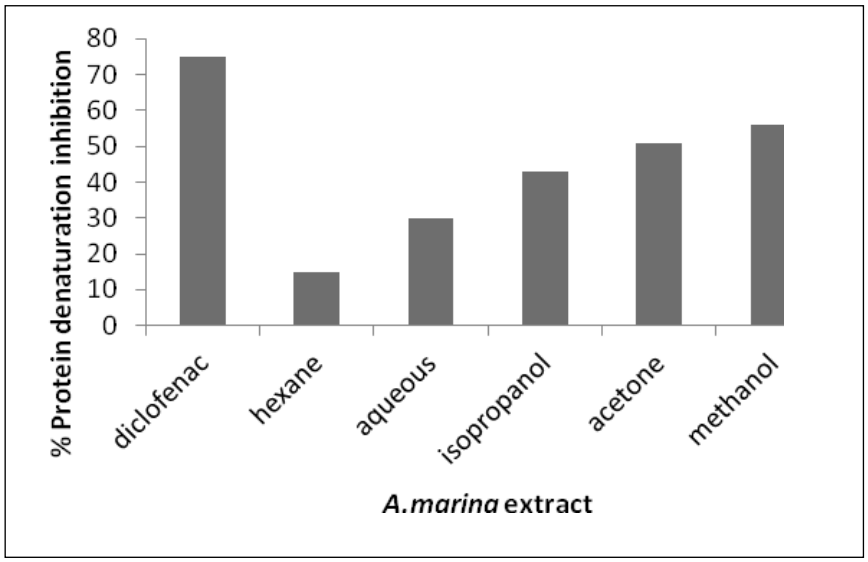
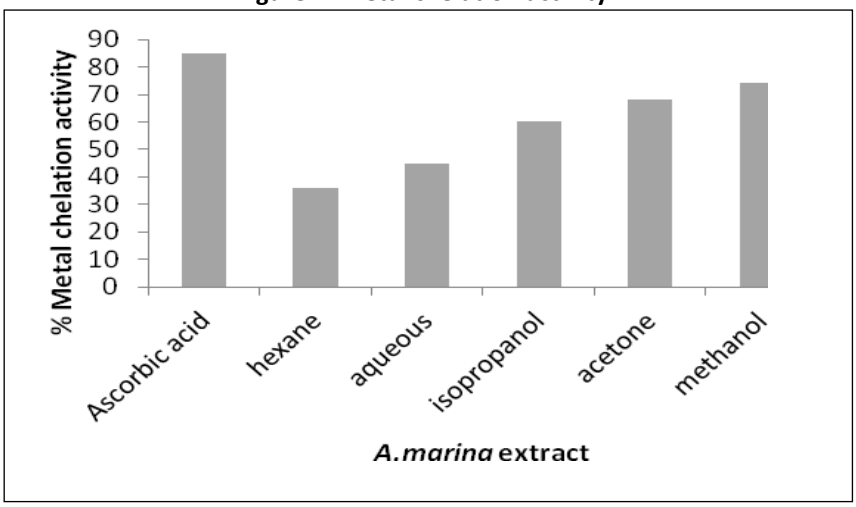


Figure 4. Metal chelation activity



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