

## EXPLORATION OF ANTIOXIDANT EFFECTS OF CRUDE EXTRACT OF MANGROVE PLANT - *Avicennia marina*

Running Title: Antioxidant activity of *Avicennia marina*

### ABSTRACT:

**Introduction:** Antioxidants are efficient in the prevention of human diseases. Mangroves are high bioactive compounds with good holistic bioactivities including insecticides. *Avicennia marina*, a mangrove plant which has its origin in South Africa included in the family *Acanthaceae*.

**Aim :** To explore the antioxidant potential of **methanolic extract** of mangrove plants, *Avicennia marina*.

**Materials and Method:** The fresh leaves of *Avicennia marina* were collected from Pichavaram mangrove forest area. The leaves were washed and then shade dried for 2-3weeks and turned into a fine powder. Crude methanolic extract of *Avicenna marina* was prepared. Total antioxidant activity, DPPH Assay and scavenging activity of hydrogen peroxide were performed and antioxidant potential was assessed using ascorbic acid as standard.

**Result:** The scavenging activity increased with increase in concentration of the extract and thus antioxidant activity was dose dependent. The total antioxidant activity was more close to the ascorbic acid equivalence.

**Conclusion:** This study concludes that the methanolic extract of *Avicenna marina* is capable of scavenging a wide range of free radicals for which it can be exploited for the treatment of various free radical mediated diseases. It is evident that mangrove species as a collective are rich sources of antioxidants, phenolics and antimicrobial compounds.

**Keywords:** Antioxidant, mangrove plant, innovative technique, ascorbic acid, free radicals.

## INTRODUCTION:

The unique and dynamic environment in mangroves ecosystems owes to its geochemical characteristics and nutrient concentration that is modified by tidal flooding regularly (1). Mangrove is the second most productive marine ecosystem after coral reefs in marine (2). Mangroves are bounded with high bioactive compounds such as steroids, alkaloids, terpenoids, saponins and tannins with good holistic bioactivities including insecticides. Marine natural products possess rich sources of bioactive secondary metabolites with promising potential for biomedical applications (3). Secondary metabolites primarily perform defense mechanisms on parasites and in extreme environmental conditions. Mangroves were able to survive in environments with high salinity, extreme humidity and pH levels as they make secondary metabolites (4).

Mangroves like *Avicennia marina* may possess some endophytic microbes in their tissues which are capable of producing biological or secondary metabolites. It was known that endophytic microbes isolated from a plant are able to produce secondary metabolites similar to those of the original plants (5). *Avicennia marina* belongs to the family **Acanthaceae** (6). The plant is commonly known as the gray or white mangrove (Fig. 1). *Avicennia marina* is the most widely distributed mangrove species in the Indo-Western Pacific area (7). *A. marina* is extremely resistant to environmental stress and can grow under environmental conditions of extreme tides, high salinity, high temperature and anaerobic soil (8). The unique adaptability and history of the plant make us engrossed to investigate the opportunities which can be afforded by the plant for the evolution of new medicines and adequate preparation to combat prevailing health problems and drug resistance diseases. *A. marina* plant has been used traditionally in folk medicine. The leaves of the plant have been potentially used as medical treatment for ulcers, abscess and burns (9). It has been traditionally used to reduce arthritic pain (10) and used worldwide for the treatment of smallpox, snake- bites. Fruits of *A. marina* have been used for digestive disorders such as constipation. The leaves and roots are used to treat wounds (11). The plant is a valuable source of many active constituents that have shown important pharmacological activities and has been reported that it is a rich source of various phytochemicals (12). Generally, many studies exploring anti-inflammatory, antidiabetic, antioxidant, anticancer and cytotoxicity, were reported using plant extract as such or after making nanoparticles (13) (14 – 22). However antioxidants

are essential in the prevention of human diseases. Antioxidants possess the ability to reduce the oxidative damage associated with many diseases and disorders leading to cancer, cataracts, atherosclerosis, diabetes, arthritis and aging (23). Antioxidants work to protect lipids from peroxidation by free radicals (24). Oxidants can damage cells by inducing chain reactions such as lipid peroxidation or by oxidizing DNA or proteins (25). Bioactive compounds derived from the plant have been successfully used to reduce lipid oxidation. It is necessary to understand the ability of natural extracts and preparations to inhibit the metabolizing enzymes that can help the health system for proper diagnosis and treatment of patients, thereby can avoid adverse effects associated with it. Our team has extensive knowledge and research experience that has translated into high quality publications (26 – 36). Thus the aim of the study was to explore its antioxidant potential using the methanolic extract of *Avicennia marina*.

## **MATERIALS AND METHODS:**

**Ethical approval:** Prior to the initiation of the study, clearance was obtained by the scientific review board with approval number [IHEC/SDC/UG-1948/21/92]

### **Collection of plant material and preparation:**

The fresh leaves of *Avicennia marina* were collected from Pichavaram mangrove forest area, Tamil Nadu. The leaves were washed thoroughly with tap water and then shade dried for 2-3 weeks and turned into a fine powder. The study was carried out in the Marine biomedical and environmental research section of Blue lab at Saveetha Dental College, Chennai.

### **Preparation of extraction:**

20g of dried powdered mangrove leaf samples were mixed with 100ml of methanol and allowed to stand at ambient temperature for 24 hours. Then the mixture was passed through Whatman filter paper and the filtrate was centrifuged at 3000 rpm for 10 minutes and further filtered by a 0.45µm syringe micro filter. At last, the solvents are evaporated via vacuum rotary evaporator until samples are obtained in powder form. Then the sample was stored in a shadowy aluminum container at 4°C for further analysis.

### **Total antioxidant activity:**

The 0.3 ml sample was prepared in different concentrations (0.5– 3mg/ml) with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 minutes in a water bath. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity has been expressed as the number of equivalents of ascorbic acid.

#### **DPPH Assay:**

The antioxidant potential of mangrove crude extract was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Different concentrations (0.5-3mg/ml) of samples were mixed with 2.9ml DPPH solution (120µM) in methanol and incubated in darkness at 37°C for 30 minutes. The absorbance was recorded at 517 nm. Inhibition of free radical by DPPH in percentage (I %) was calculated with the following equation:

$$\text{Percentage of Inhibition (I \%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

Where,  $A_{\text{control}}$  is the absorbance of the control and  $A_{\text{sample}}$  is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of the sample. Ascorbic acid was used as positive control.

#### **Scavenging of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>):**

40mM H<sub>2</sub>O<sub>2</sub> was prepared and the concentration was determined spectrophotometrically by measuring the absorption with the extinction coefficient for H<sub>2</sub>O<sub>2</sub> of 81 M<sup>-1</sup> cm<sup>-1</sup>. Mangrove extract and the standard ascorbic acid (0.5-3 mg/ml) were added to 0.6 ml of 40mM H<sub>2</sub>O<sub>2</sub> solution and the absorbance of H<sub>2</sub>O<sub>2</sub> was determined at 230 nm after 10 min incubation against the control, containing phosphate buffer without hydrogen peroxide. The percentage of scavenging of H<sub>2</sub>O<sub>2</sub> was calculated as follows:

$$\text{Percentage of Inhibition (I \%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

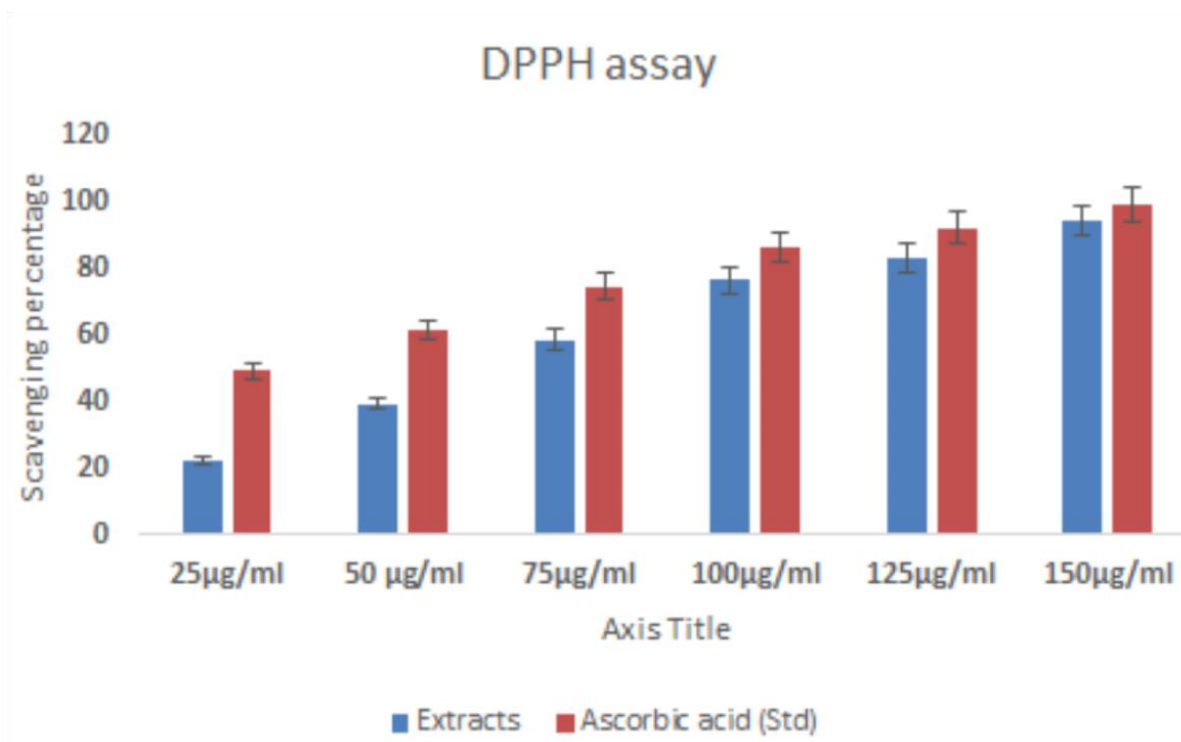


Figure 1: a) *Avicennia marina* b) methanolic extract of *Avicennia marina*

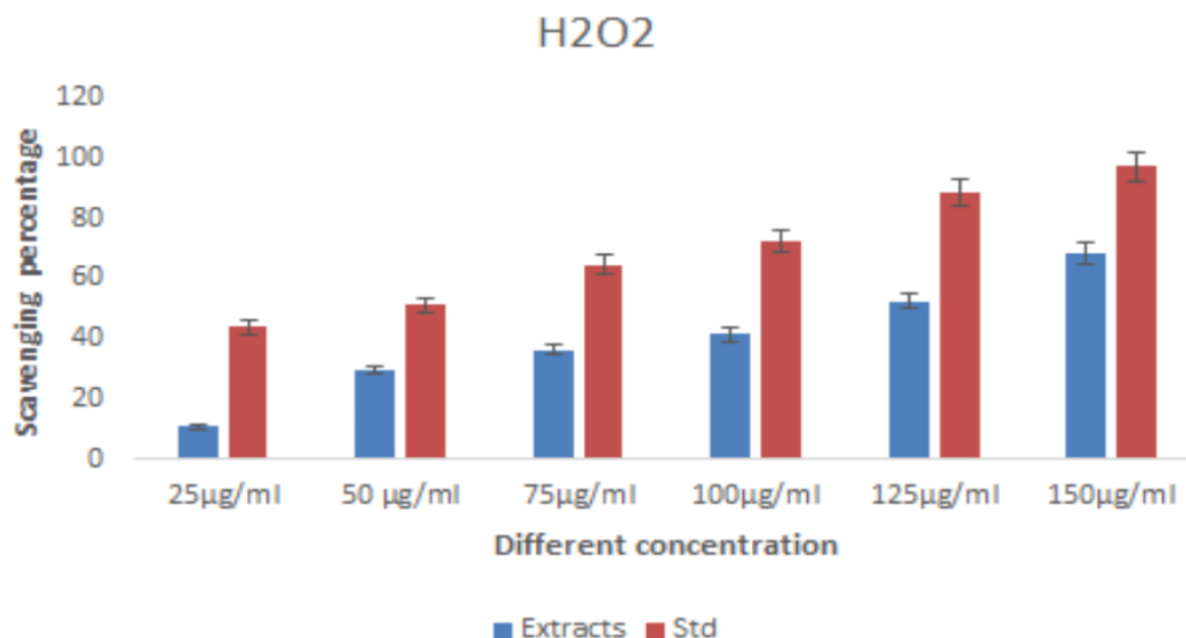
## RESULTS:

Table 1: The shows total antioxidant assay where  $n = 3$  and the values were given as  $\text{mean} \pm \text{SE}$

| Concentration of extract ( $\square$ g/ml) | Ascorbic acid equivalence |
|--------------------------------------------|---------------------------|
| 25                                         | 27.81 $\pm$ 1.21          |
| 50                                         | 49.73 $\pm$ 1.30          |
| 75                                         | 68.29 $\pm$ 0.81          |
| 100                                        | 92.57 $\pm$ 1.22          |
| 125                                        | 112.64 $\pm$ 1.31         |
| 150                                        | 138.49 $\pm$ 1.28         |



**Graph 1:** Bar graph shows the free radical scavenging activity in DPPH assay of the extract in different concentrations against the standard. X -axis represents different concentrations of methanolic extract and ascorbic acid, Y -axis represents scavenging percentage, while blue colour represents the extracts and red represents the standard, ascorbic acid. Values were done in triplicate  $n=3$  with mean  $\pm$ SE . From this, it can be inferred that as concentration of extract increases the scavenging activity increases at highest concentration, the scavenging percentage was almost near to that of the standard.



**Graph 2:** Graph shows the H<sub>2</sub>O<sub>2</sub> free radical scavenging activity of the extract in different concentrations against the standard. X axis represents different concentrations of methanolic extract and ascorbic acid, Y axis represents scavenging percentage while blue colour represents the extracts and red represents the standard, ascorbic acid. Values were done in triplicate n=3 with mean  $\pm$ SE. From this, it can be inferred that as concentration of extract increases, the scavenging activity increases.

The total antioxidant assay involved 25 µg/ml to 150 µg/ml of the extract in which the values observed were more close to ascorbic acid equivalent. At the highest concentration (150 µg/ml) the ascorbic acid equivalent was  $138.49 \pm 1.28$ . However, the lowest concentration (25 µg/ml) of crude extract showed the ascorbic acid equivalence was  $27.81 \pm 1.21$  (Table 1). From the DPPH assay, it can be observed that the scavenging activity of the extract increases with increase in concentration and thus it is a dose dependent activity (graph 1). In the lowest concentration (25 µg/ml) the percentage of scavenging was 22.58 % while the standard, ascorbic acid showed 49.27% of scavenging. But with increase in concentration, the scavenging activity also increased as in the concentration of 100 µg/ml, scavenging percentage was 76.42 and that of standard was 86.37% which is slightly a higher value. At the highest concentration (150 µg/ml), scavenging percentage of the extract was 94.3 and that of standard was 99.2% which is a closer value to the extract. Thus this plant has better potential to act as antioxidant as its scavenging activity remains

more similar to that to ascorbic acid.

On assessing the scavenging activity of extract against the standard in hydroxyl scavenging assay, it was found that with increase in concentration of the extract the scavenging percentage also increases (graph 2). In the lowest concentration (25µg/ml) the percentage of scavenging was only 10.85% while the standard, ascorbic acid showed 43.82% of scavenging. But with increase in concentration, the scavenging activity also increases as at 100µg/ml of concentration, scavenging percentage was 41.38% and that of standard was 72.61%. At the highest concentration (150µg/ml), scavenging percentage of the extract was 68.51% and that of standard was 97.09% and thus the scavenging potential of the extract was comparatively lesser than the standard.

## DISCUSSION:

One previous study suggests that in the presence of an antioxidant, DPPH radical acquires one more electron and the absorbance decreases (37). There are certain methods available to assess antioxidant activity of compounds. DPPH free radical scavenging assay is a rapid and sensitive method for the antioxidant screening of plant extracts. In presence of an antioxidant, DPPH radicals gain one more electron and thus absorbance decreases. Another similar study with concentration ranging from 50-80µg/ml with ascorbic acid as standard, 96.25% and 74.55 % were the percentage of scavenging activity in ethyl acetate and methanolic extract on DPPH respectively (38). This study remains in contrast to the present study. Yet another study established that all the concentrations of *A. marina* extracts showed higher activity than the standard ascorbic acid except petroleum ether and methanolic extracts (39).

Previous study shows that the highest scavenging was seen in 100µg/ml in a DPPH radical scavenging assay (40). In recent years much attention has been devoted to natural antioxidants and their association with health benefits. In a similar study, at 800µg/mL concentration of methanolic extract of *A. marina* pneumatophore showed 69.12% which is a supportive finding of the present study (41). Similar studies on antioxidant, anti-inflammatory potential are in accordance with our study (15,42- 52). This study proved the limitation that the antioxidant property has been assessed through *in vitro* method whereas its effect and potential in living cells

has to be explored. Thus further study will be progressed as an in vivo model to assessment of antioxidant activity of *Avicennia marina*.

## CONCLUSION:

It can be concluded from the results obtained, that methanolic extract of *Avicennia marina* is capable of scavenging a wide range of free radicals. In vitro assay study confirms *Avicennia marina* is a natural antioxidant and can be eminently used for pharmaceutical needs.

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**CONFLICT OF INTEREST:** Nil

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