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Antioxidant, anticancerous and cytotoxic effects of *Avicennia marina*

Sheela Devi A*, Joseph J, Bhuvaneshwari V

Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Chinna Kolambakkam-603308, Tamil Nadu, India

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ABSTRAC

Mangrove plants have been used in folklore medicines. Mangroves are woody trees and shrubs that grow in the intertidal zones of tropical and sub-tropical regions. *Avicennia marina* is an important mangrove species with a wide geographical and climatic distribution which suggests that a large amount of genetic diversity are available for conservation and breeding programs. Leaves of this plant are mainly used in the clinical approach. Antioxidant, anticancerous and cytotoxic effects of methanol extract of *A.marina* (MEAM) leaves have been tested in this study. The various antioxidant model systems viz., 1, 1-diphenyl-2picryl-hydroxyl, (DPPH) superoxide dismutase (SOD), lipid peroxidation by thiobarbituric acid reactive substances (TBARS) and iron chelating activity by ferric ion reducing antioxidant power (FRAP) assay were performed. Followed by the anticancerous activity of the extract on cervical cancer and cytotoxic effect on peripheral blood mononuclear cell were also studied. The result of DPPH scavenging capacity of both ascorbic acid and MEAM was increased when the concentration increases. The 50% inhibitory concentrations (IC_{50}) values in all antioxidant models viz, DPPH, SOD, TBARS and FRAP radical scavenging activity of MEAM were found to be 154, 187, 179 and 245 µg/ml respectively. The IC_{50} value of MEAM on Hela cell lines and the cytotoxic concentration (CC_{50}) of MEAM on PBMC was found to be 107 and 1370 µg/ml respectively. Finally, it has been concluded that the MEAM leaves showed significant antioxidant and anticancerous activity.

* Corresponding Author

Name: A. Sheela Devi
Phone: 044-27565485
Email: sheeladevi.kvcet@gmail.com

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INTRODUCTION

Mangrove plants have been used in folklore medicines and extracts from mangrove species have proven activity against human, animal and plant pathogens. This group of plants provide many resources for utilization in the forestry, fisheries, food, agricultural and medicinal industries. Secondary metabolites like alkaloids, phenolics, steroids, terpenoids have been characterized from mangroves and have toxicological, pharmacological and ecological importance (Howard Miles *et al.*,1999). Several latest studies mentioned the therapeutic value of mangroves and associated plants persist to provide invaluable treatment modalities, both in modern and traditional systems of medicine. Mangrove plants are used for the treatment of rheumatism, painful arthritis, inflammation, asthma antioxidant, free radical scavenging, anti- inflammatory, antinociceptive, diabetes and hepatoprotective actions (Roome *et al.*, 2008). Antioxidants are important in the prevention of human diseases. Naturally occurring antioxidants in leafy vegetables and seeds, such as ascorbic acid, vitamin E and phenolic compounds, possess the ability to reduce the oxidative damage associated with many diseases, inducing aging, atherosclerosis, arthritis, cancer, cardiovascular disease, cataracts, diabetes and immune deficiency

diseases (Basniwal *et al*., 2009). These antioxidants are capable of inhibiting the oxidation of biomolecules by removing free radical intermediates and inhibiting other oxidation reactions. Antioxidants might interrupt peroxidation by donating hydrogen to a lipid radical, forming a new radical, more stable than the initial one oxidative stress occurs when there are low levels of antioxidants or inhibition of the antioxidant enzymes resulting in cell damage or cell death (Awah *et al.,* 2012). Thus, it is vital to study antioxidant activity of the plants used in the herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on the antioxidant activity of these herbal plants (Molan *et al.,* 2012).

Cancer is one of the most prominent diseases in humans. At present, there is considerable interest in performing the detection of new anticancer agents from natural products (Kinghorn *et al*., 2003). A chemical compound such as chloroquine act as an autophagy inhibitor in cancer treatment and also the various safety issues concerning with the same (Sydha *et al.,* 2018). Hence, in the present study, an effort was to evaluate the antioxidant and anticancer activity.

MATERIALS AND METHODS

Sample collection and authentication

Healthy and fresh leaves of *A.marina* were collected from Pichavaram mangrove forest, Tamil Nadu, India. They were authenticated at Arignar Anna Siddha Central Research Institute, Arumbakkam, Chennai, Tamil Nadu, India. After the authentication of the leaf samples, it was washing with distilled water, the leaves were shade dried, powdered and used for crude extract preparation.

Preparation of methanol extract of *A. marina* (MEAM)

The MEAM was prepared and lyophilized to remove the solvents completely. Then the extract was stored at 4°C in airtight bottles and used for further studies (Harbone 1973). The percentage yield for extract such as methanol and was calculated as 8.7%.

Determination of antioxidant activity of *A. marina*

Their ability studied the antioxidant activity of *A. marina* extract: to scavenge superoxide radicals by DPPH radical scavenging assay, SOD activity assay and, to inhibit lipid peroxidation (LPO) by thiobarbituric acid assay, followed by FRAP assay. Each test was performed in triplicates and mean values calculated. The stock solution of *A. marina* extract was prepared by taking 10 mg of crude MEAM dissolved in 1ml of dimethyl sulfoxide.

DPPH radical scavenging assay

The antioxidant activity of extracts was determined by the scavenging activity of the stable DPPH free radical by the method (Braca *et al.,* 2001). The antioxidant activity was compared with that of the natural antioxidant, L-ascorbic acid (Positive control).

SOD activity Assay

The SOD activity assay (Beauchamp & Fridovich 1971) was carried out by adding different concentrations of the extract and the activity was compared with that of L-Ascorbic acid.

Determination of Lipid peroxidation inhibitory activity by assay of TBARS

The ability of the extract to inhibit malondialdehyde (MDA) formation, it is one of the final product of lipid peroxidation, and therefore, lipid peroxidation was determined colorimetrically by using a modified TBARS assay (Niehius & Samuelson 1968).

FRAP (iron chelating activity) assay

The chelation of ferrous ions by the test sample was estimated by FRAP assay (Decker & Welch 1990; Dinis *et al*., 1994). The activity was compared with that of EDTA (Positive control).

Determination of cytotoxicity of MEAM

Cytotoxicity of MEAM was performed by 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay (Mosmann 1983). It was carried out by using PBMC.

Determination of the anticancerous activity of MEAM

The anticancerous activity of MEAM was performed by the MTT assay (Mosmann 1983). It was carried out by using a hela cell lines. The cell line was derived from cervical cancer cells.

RESULTS AND DISCUSSION

Antioxidant activity of MEAM

The percentage of DPPH radical scavenging activity at a concentration of 100, 200 and 500 µg/ml of ascorbic acid (positive control) and MEAM was found to be 43.37±1.63, 60.33±1.07 and 81.30±1.13; and 32.37±0.55, 43.47±1.24 and 58.60±1.25 respectively. It was shown in Fig. 1. The result of this study showed that the DPPH scavenging capacity of both ascorbic acid and

MEAM was increased when the concentration increases. The 50% inhibitory concentrations (IC_{50}) value of ascorbic acid and MEAM was recorded as 115µg/ml and 154µg/ml respectively. The above results are comparable to the results obtained by Bangaru Chandrasekaran *et al.*, (2013). They have reported that the scavenging ability of a methanolic extract of *Salicornia brachiata* on DPPH was 23% and 85% at a concentration of 200 and 1000 µg/ml respectively.

The percentage of superoxide radical scavenging activity at a concentration of 100, 200 and 500 µg/ml of ascorbic acid (positive control) and MEAM was found to be 48.77±2.60, 74.23±4.65 and 80.53±3.27; and 22.73±2.54, 53.43±3.97 and 69.53±3.19 respectively. It was shown in Fig. 2. The result of this study showed that the superoxide radical scavenging activity of both ascorbic acid and MEAM was increased when the concentration increases. The IC_{50} value of ascorbic acid and MEAM was recorded as 103µg/ml and 187µg/ml respectively.

The percentage of lipid peroxidation inhibitory activity at a concentration of 100, 200 and 500 µg/ml of alpha-tocopherol (positive control) and MEAM was found to be 38.90±0.70, 59.90±1.44 and 80.37±0.50; and 35.53±0.93, 55.83±0.31 and 74.50±0.50 respectively. It was shown in Fig. 3. The result of this study showed that the lipid peroxidation inhibitory activity of both alpha- tocopherol and MEAM was increased when the concentration increases. The IC_{50} value of alpha- tocopherol and MEAM was recorded as 170µg/ml and 179µg/ml respectively.

The percentage of iron chelating activity at a concentration of 100, 200 and 500 µg/ml of ethylenediaminetetraacetic acid (EDTA) (positive control) was found to be 43.10±0.57, 61.20±0.95 and 82.97±0.58; and 20.40±1.05, 36.10±0.95 and 67.53±0.67 respectively. It was shown in Fig. 4. The result of this study showed that the iron chelating activity of both EDTA and MEAM was increased when the concentration increases. The IC_{50} value of EDTA and MEAM was recorded as 116µg/ml and 245µg/ml respectively. Sudha & Vinayagam (2011) stated that phenols are important plants constituent with many biological functions including antioxidant activity because of their radical scavenging capability due to their hydroxyl groups. The presence of flavonoids might be responsible for the antioxidant activity of the plants. Soobrattee *et al.*, (2005) documented that phenols and flavonoids serve as potent antioxidants because of high redox properties. Mathew & Abraham (2006) reported that phenols and flavonoids from plants have been reported to be potent free radical scavengers. As observed in previous studies, the MEAM have potentials as antioxidants, owing to the presence of phenols and flavonoids.

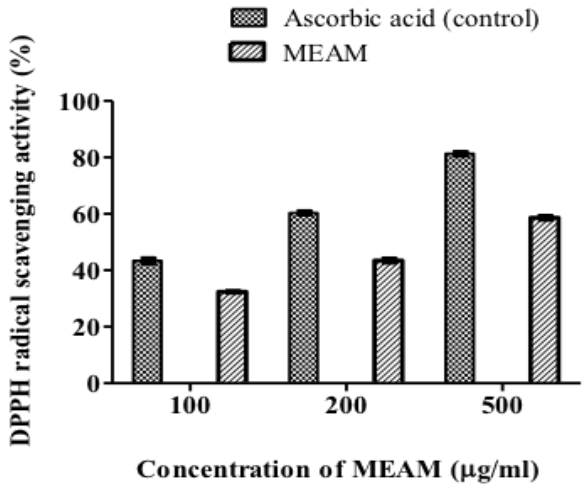

Figure 1: DPPH radical scavenging activity (%) of ascorbic acid and MEAM. The values were reported as mean ± S.D (n = 3).

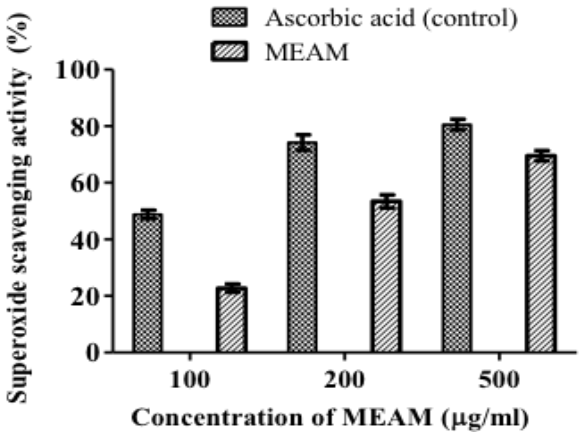

Figure 2: Superoxide radical scavenging activity (%) of ascorbic acid and MEAM. The values were reported as mean ± S.D (n = 3).

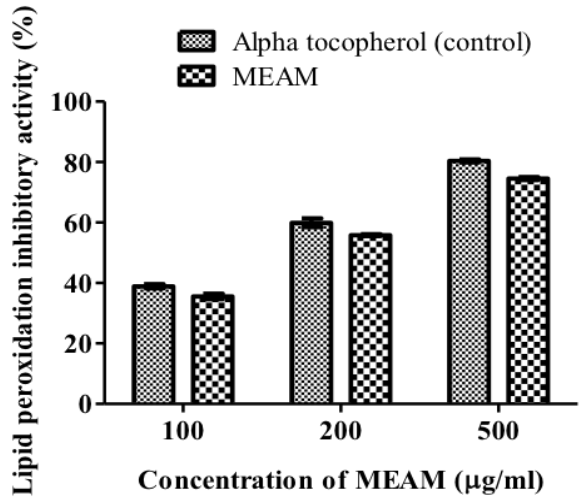

Figure 3: Lipid peroxidation inhibitory activity (%) of alpha-tocopherol and MEAM. The values were reported as mean ± S.D (n = 3).

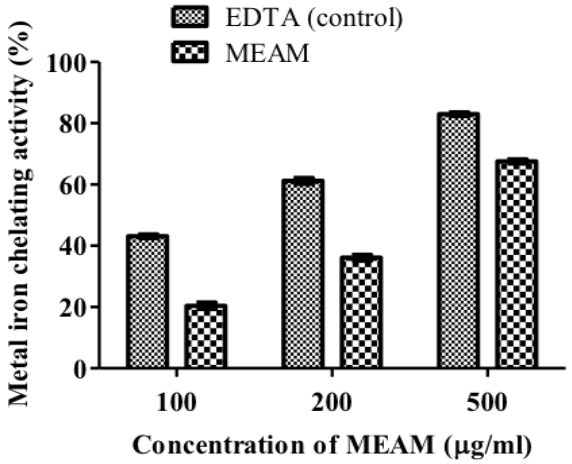

Figure 4: Metal chelating activity (%) of EDTA and MEAM. The values were reported as mean ± S.D (n = 3).

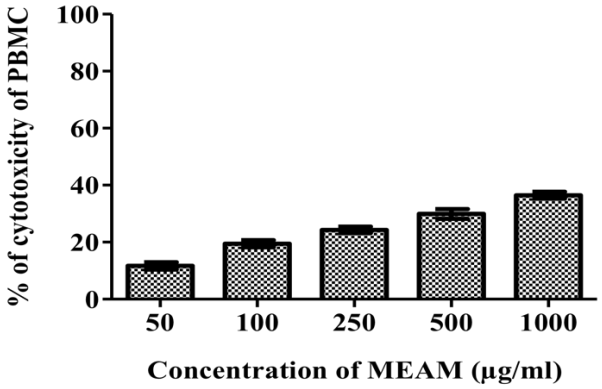

Figure 5: Cytotoxic effect (%) of MEAM on the peripheral blood mononuclear cell (PBMC). The values represented mean ± S.D (n = 3).

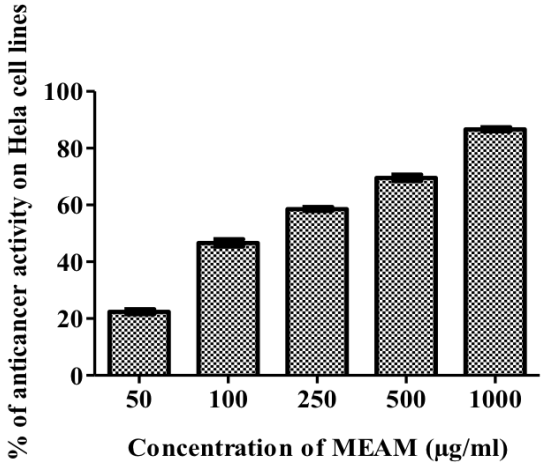

Figure 6: Anticancerous activity of MEAM on HeLa cell lines. The values represented mean ± S.D (n = 3).

Cytotoxic effect of MEAM

The percentage of the cytotoxic effect of MEAM at a concentration of 50, 100, 250, 500 and 1000 µg/ml on the PBMC was found to be 11.67±1.26, 19.47±1.26, 24.33±1.11, 29.92±1.68 and 36.49±1.24 respectively. It was shown in Fig. 5. The result of this study showed that the cytotoxic activity of the extract is increased when the concentration increases. The CC_{50} of MEAM on PBMC was found to be 1370 µg/ml. This was in agreement with other studies by Hojjat Hojjat Sadeghi-aliabadi *et al*., (2013). They mentioned that the cytotoxic activity of *A.marina* on human breast cancer cell line (MDA-MB 231) was more than human embryonic kidney (HEK) normal cell line. Therefore, consistent with previously published data, it is notable that the extract showed more cytotoxic activity on cancer cell line than normal cell line.

Anticancer activity of MEAM

The percentage of the anticancerous activity of MEAM at a concentration of 50, 100, 250, 500 and 1000 µg/ml on Hela cell lines (which were derived from human cervical cancer) was found to be 22.40±0.90, 46.67±0.30, 58.55±0.71, 69.57±1.14 and 86.67±0.71 respectively. It was shown in Fig. 6. The result of this study showed that the anticancerous activity of the extract is increased when the concentration increases. From the result, the IC_{50} value of MEAM was found to be 107µg/ml. This was comparable with the result reported earlier by Luke Esau *et al*., (2015). They reported that the ethyl acetate extract of leaves of *A.marina* inhibited breast cancer cell (breast adenocarcinoma - MCF-7) growth in a concentration-dependent manner. Saeed Irian *et al*., (2012) had also reported that the ethanol extract of leaves of *A.marina* exhibited anticancerous activity on human leukemic cell line (HL-60) with IC_{50} values of 600, 400, and 280 µg/ml after 24, 48, and 72 h, respectively, in a concentration and time-dependent manner.

CONCLUSION

The current project revealed that the methanol extract of *A. marina* might contain bioactive anticancerous compounds that kill human cervical cancer cells (Hela cell line), which was analyzed by MTT assay. Also, the extract showed the anti- oxidant effect, which was evaluated by using different free radicals (Superoxide, Hydroxyl, metal iron and DPPH) and compared with some standards like ascorbic acid, alpha-tocopherol and EDTA. Further, the cytotoxic effect of the extract on PBMC was studied. These activities of the mangrove mentioned above plant are due to the presence of phytoconstituents. Thus the results of the study suggest that it can serve as a candidate for the development of anti-oxidant and anti- cancer herbal agents against cervical cancer (Hela cells).

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