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Research Article

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Antibacterial and antioxidant activity of *Avicennia marina* Leaf

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ABSTRACT

Leaf extracts of Avicennia marina L. were prepared in ethyl acetate, acetone, methanol and ethanol. The crude extracts were screened for antioxidant and antibacterial activity. The antioxidant activity of all these extracts was done by ABTS method. Methanol infusions demonstrated the highest antioxidant activity. The antibacterial activity of all the extracts were evaluated against Enterobacter cloaca, Proteus vulgaris, Bacillus cereus and Enterococcus faecalis by agar well diffusion method and compared with the standard antibiotic gentamicin. The extent of inhibition by the leaf extracts diverge from one solvent system to the other. Among them methanol and ethanol extracts exhibited higher level of inhibition against the gram positive test cultures compared to gram negative test cultures employed. The present study reveals the potential of leaf extracts of Avicennia marina L as antioxidant and antibacterial agent.

Keywords: *Avicennia marina* L., Leaf extracts, Antioxidant activity, Antibacterial activity.

INTRODUCTION

The plant kingdom is endowed with various biologically active compounds such as alkaloids, flavonoids, lignins, phenols, sterols, saponins, tannins and terpenes. These metabolites are the major sources of antibacterial, antioxidant and anticancer agents [1,2]. The plants used in traditional medicine are a large source of natural antioxidants. Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease [3]. Plants serve as a reservoir of effective chemotheraputants and provide valuable sources of natural products in control of several bacterial and fungal diseases [4,5.] Antibiotics are the most important weapons in fighting against bacterial infections. Many pathogenic organisms are developing plasmid-mediated resistance to popular drugs [6]. Hence, there is a need for the isolation of novel compounds either from microorganisms or from plants. The potential of mangrove plants as a source of new principles is still unexplored. The present study is paying attention on the exploitation of leaves of *Avicennia marina* L for extraction in different organic solvents to screen for antioxidant activity by ABTS method and antibacterial activity by agar well diffusion method.

EXPERIMENTAL SECTION

Collection of plant material

The leaves of *Avicennia marina* L. were collected from East Godavari mangroves at Corangi Reserved Forest, (Geographically located between 16^{o} 39' N longitude – 17^{o} N longitude and 82^{o} 14' E latitude -82^{o} 23'E lattitude) Kakinada, Andhra Pradesh, India. The leaves were collected in new polythene bags and surface sterilized with 1% mercuric chloride solution. The leaves were chopped separately into small pieces and shade dried at room temperature for seven days.

Extraction

The extraction of leaves was carried out with different solvents in their increasing order of polarity viz., ethyl acetate, acetone, methanol and ethanol by soaking the leaves in the respective solvents overnight at room temperature one after the other [7] . Chopped fruit material (250g) was initially soaked in 1000ml of the respective solvent in a round bottom flask at room temperature for 24h . The contents of each flask were subjected to reflux below the boiling point of the respective solvents viz., ethyl acetate (77^{o}C), acetone (55^{o}C), methanol (65^{o}C) and ethanol (78^{o}C) for 6-8h in order to solubulize the active compounds into the solvent. The extracts were filtered through whattman No.1 filter paper and the residual material was re-extracted with fresh solvent. After 24h the process was repeated. Pooled extracts were individually concentrated by removing the solvent under reduced temperatures using vacuum rotator evaporator. These extracts were further concentrated by solvent evaporation using thin film method. Dried leaf extract of 100mg each was dissolved in 10ml of 1:10 diluted DMSO in sterile distilled water so as to obtain the final concentration of 10mg/ml [8]. All the extracts thus prepared were stored in a refrigerator at 4^{o}C.

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Determination of antioxidant activity by ABTS method

Total antioxidant capacity of each extract was measured using 2, 2´-azinobis [3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) assay. ABTS and potassium per-sulfate were separately dissolved in deionized distilled water to a final concentration of 7mM and 2.45mM respectively. The two solutions were mixed and allowed to stand in dark at room temperature for 16h before use in order to produce ABTS radical (ABTS•+). The resultant intensely-coloured ABTS•+ radical cation was diluted with 0.01M PBS (phosphate buffered saline), pH 7.4, to give an absorbance value of ~0.70 at 734 nm. The test compound was diluted 100X with the ABTS solution to a total volume of 1ml. Absorbance was measured spectrophotometrically at time intervals of 3min after addition of each extract. The assay was performed at least in triplicate. Controls were run using PBS in place of the extract. The assay relies on the antioxidant capability of the samples to inhibit the oxidation of ABTS to ABTS•+ radical cation. Percent inhibition was calculated using the following formula and it was compared with ascorbic acid. [9].

$$\% \text{ inhibition of oxidation of ABTS to ABTS•+} = \frac{\text{Initial absorbance - Final absorbance X 100}}{\text{Initial absorbance}}$$

Determination of antibacterial activity

The antibacterial activity of the crude extracts was screened with six gram negative and six gram positive cultures viz., *Enterobacter cloaca, Proteus vulgaris, Bacillus cereus* and *Enterococcus faecalis* by agar well diffusion method [10]. About 20 ml of melted Mueller Hinton agar was mixed with 1 ml of bacterial suspension homogeneously and allowed to solidify in petri dishes (143 mm diameter). Wells (8mm diameter.) were made using a sterile cork borer on the solidified medium and are filled with 100 µl of the extract (10mg/100µl). The inoculated plates were incubated at 34-38^{o}C. The diameter of the inhibition zones were measured in mm. Each experiment was performed in triplicate and the average value of inhibition and standard deviation was calculated. Gentamicin (30µg) discs are used as positive control and DMSO with solvents (1:10 dilution) used as control. Results were expressed as mean ± SD and the data were analyzed using one-way analysis of variance (ANOVA) to discover the significant difference at the 5% ($P<0.05$)level.

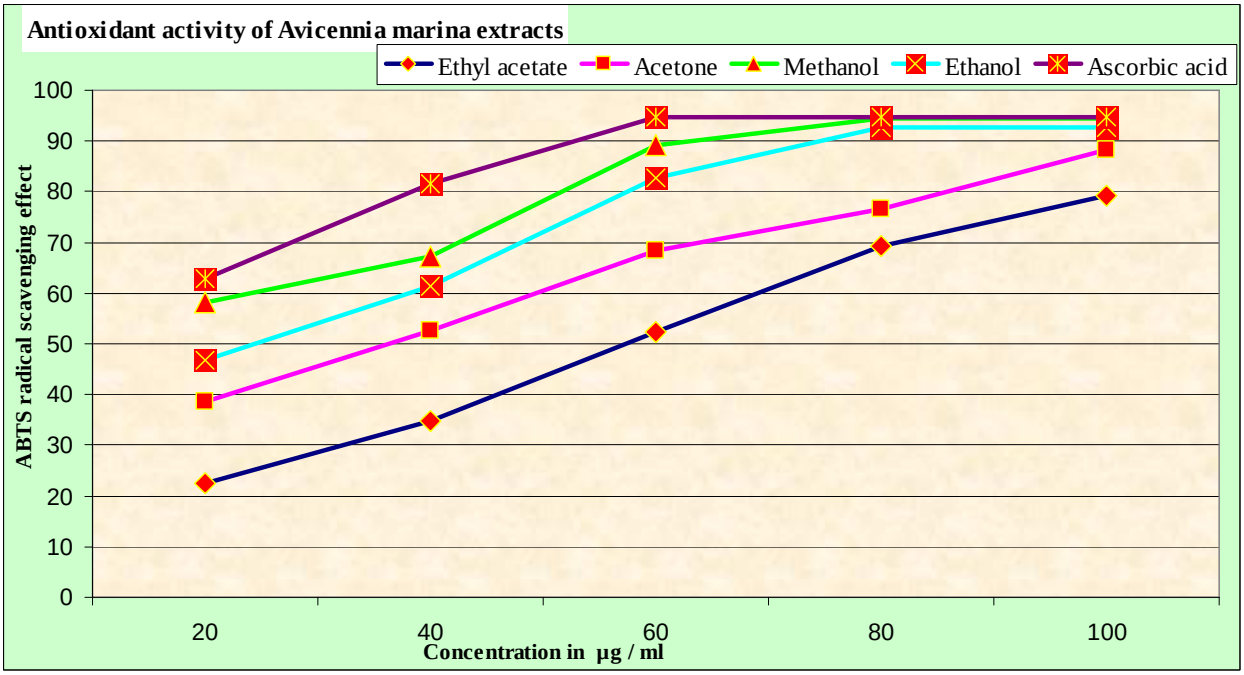
RESULTS

Plants are an important source of potentially useful principles for the development of new bioactive entities. The knowledge of secondary metabolites and their biological activities is desirable, not only for discovery of novel bioactive principles, but also for disclosing new sources of already known biologically active compounds. *Avicennia marina* L is a member of *Avicenniaceae* family and this family includes many plants that are rich source of wide-range of chemical constituents. The bark, leaves, and fruits of *Avicennia marina* are used in folk medicine to treat skin diseases.[11].

ANTIOXIDANT ACTIVITY

Several techniques have been used to determine the antioxidant activity . Free radicals are known to play a definite role in a wide variety of pathological symptoms. Antioxidants fight against free radicals and protect us from various diseases. The non enzymatic antioxidant activity of plants is attributed to the secondary metabolites. The antioxidant activity of the fractions was estimated spectrophotmetrically by ABTS method and the data is given in Fig-1. The outcome of antioxidant activity by ABTS method is high for methanol extract followed by ethanol, acetone and ethyl acetate extracts.

Fig-1 Antioxidant activity by ABTS METHOD



ANTIBACTERIAL ACTIVITY

The efficacy of the leaf extract of *Avicennia marina* L against the test cultures are given in Fig.2. The inhibitory effect of ethyl acetate, acetone, methanol and ethanol extracts are diverse. The methanol extract was active on all the test cultures used, ethanol is active against all the test cultures used *Porteus vulgaris*. Where as the ethyl acetate extracts was active only against *Bacillus cereus* and the acetone extracts are effective only the gram positive test cultures (*Bacillus cereus* and *Enterococcus faecalis*). The zone of inhibiton exerted by these extracts was higher on all the gram positive test cultures used than the gram negative cultures. The efficacy of methanol extracts on *Bacillus subtilis* and *Enterococcus faecalis* (19.33 mm) is more than gentamicin.

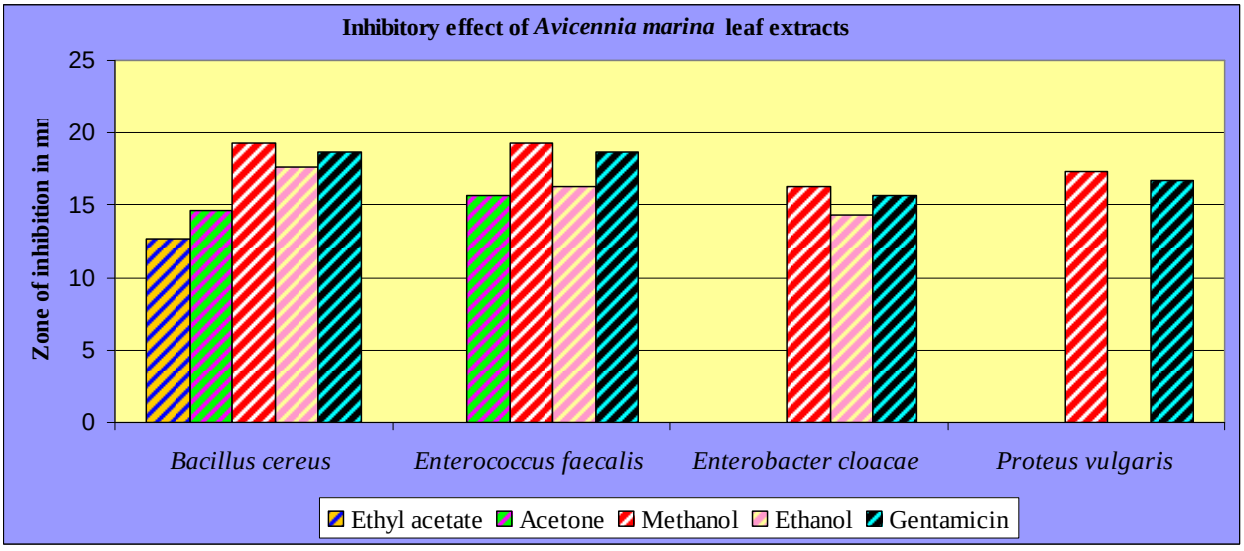


Fig-2 Antibacterial Activity By the *Avicennia marina* Extracts

DISCUSSION

Avicennia marina fruit is used as traditional food. Its flour is used as a base for making cakes [12]. The phytochemical studies of Govindasamy *et.al* [13] revealed that mangroves of India are rich with alkaloids, flavonoids, sterols, tannins and triterpenoid. Shanmugapriya *et.al*[14] reported the methanol extracts of *Avicennia*

marina and *Avicennia officinalis* to be rich in secondary metabolites and possess antioxidant and antibacterial activity. Our results are in concurrence with their study.

Abeysinghe and Wanigatunge[15], reported the antibacterial activity of *Avicennia marina* leaf extracts in petroleum ether, chloroform, ethyl acetate and ethanol against *Escherichia coli, Pseudomonas species, Proteus species, Shigella species and Staphylococcus species*. Satdve *et al*, [16] screened for the antimicrobial activity of *Gymnema sylsestre* leaf extract in ethanol and reported that the leaf extract demonstrated antibacterial activity against *Bacillus pumilis, B.subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results of our present study are in agreement with the above findings. The methanol infusion in our study exhibits highest zone of inhibition on all the test cultures compared to that of other solvent infusions (ethyl acetate, acetone and ethanol). This correlates with the antioxidant activity and this may be due to the presence of bioactive principles present in methanol.

The efficacy of methanol extract is high against the gram positive microorganisms compared to that of the gram negative cultures used. This may be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extract, or these bacterial strains may have some kind of resistance mechanisms, (e.g., enzymatic inactivation, target site modification and decreased intracellular drug accumulation). The variation of antibacterial activity of our extracts might be due to availability of bioactive principle, which assorted from fraction to fraction. The leaf extracts that showed antibacterial activity were compared with broad spectrum antibiotic gentamicin.

From this comparison, it was observed that most of the extracts were less effective than gentamicin, but methanol extracts in the crude form itself is more effective than gentamicin against *Enterococcus faecalis.* In the case of the positive extracts exhibiting equal or less effectiveness in comparison to gentamicin, there is every possibility of having more antibacterial activity than gentamicin when the bioactive compounds of these extracts were purified and tested. So, the leaves of *Avicennia marina* L is strongly recommended for consideration as a valuable source for the study of isolation, identification and characterization of potential bioactive compounds with antibacterial property. Finally, there is a need to explore this area further to understand the potentiality of the mangrove plants towards the development of new era medicines with different solvents against many pathogenic microorganisms, so as to get the lead molecule to combat the diseases caused by the microorganisms.

CONCLUSION

This study has exposed the evidence of antioxidant and antibacterial ability of *Avicennia marina* leaf extracts in specific solvents. Alkaloids, flavonoids, steroids and tannins are known for the antibacterial activity and the phytochemical analysis of different solvent extracts by earlier reports indicates that flavonoids and tannins are common in all the extracts with antibacterial properties. These studies also validate that the *Avicennia marina* leaf extracts are used for the treatment bacterial disorder. The *Avicennia marina* leaf extracts can be used to discover new bioactive natural products and can be used as a potential source that may control microbial disorders. Hence, there is need for further studies on the other parts of the plant in order to isolate, identify, characterize and elucidate the structure of bioactive compounds which possess antioxidant and antimicrobial activity.

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