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ANTIBACTERIAL ACTIVITY OF STEM AND ROOT EXTRACTS OF *Avicennia officinalis* L

N.Sharief Md and UmaMaheswara Rao V *
Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur District,
Andhra Pradesh, India.
* Corresponding author, E-mail: umrvanga@yahoo.co.in

ABSTRACT

Extracts of stem and root of *Avicennia officinalis* L. were prepared in hexane, benzene, ethyl acetate, acetone, methanol and ethanol, and their antibacterial activity was evaluated against *Escherichia coli* MTCC 64, *Enterobacter aerogenes* MTCC 111, *Klebsiella pneumoniae* MTCC 39, *Pseudomonas aeruginosa* MTCC 424, *Bacillus subtilis* MTCC 121, *Lactobacillus delbrueckii* MTCC 911, *Staphylococcus aureus* MTCC 87 and *Streptococcus pyogenes* MTCC 1928 by agar well diffusion method. The stem and root extracts in acetone demonstrated best antibacterial activity. However, extracts prepared in other solvents also showed antibacterial activity against the test organisms. The extracts that possessed antibacterial activity were further used for the determination of the Minimum Inhibitory Concentration (MIC) taking different concentrations viz., 1.25, 2.5 and 5.0mg /100µl . The value of MIC was found to be ranged from 1.25mg/100µl to 5mg/100µl for different test organisms. Further, the extracts that demonstrated the antibacterial activity were checked for their bactericidal and / or bacteriostatic nature. The present study reveals the potential of stem and root extracts of *Avicennia officinalis* L. as antibacterial agent.
Key words: Stem extracts, Root extracts, *Avicennia officinalis* L., Antibacterial activity

[I] INTRODUCTION

Antibiotics since their introduction are one of the most important weapons in fighting against bacterial infections and have largely benefited humans. Many pathogenic organisms are developing plasmid-mediated resistance to the prevailing drugs. Hence, there is a need for novel natural compounds that can be obtained from the plants or microorganisms. Plants, in particular, have been a source of inspiration for novel drug compounds since days immemorable. Plants serve as a reservoir of effective chemotheraputants and provide valuable sources of natural products in the control of several bacterial diseases. Many studies indicate that plants contain bio-active compounds such as peptides, glycosides, alkaloids, saponins, terpenoids, flavonoids etc., with antimicrobial activity against bacterial, fungal and viral infections [1,2,3]. However, the antibacterial activity of mangrove plants has still not been studied as extensively as most other plant species. *Avicennia officinalis* L. plant varies from shrubby stunted individuals to tall trees with broad trunk.

In the present study, stem and root parts of *Avicennia officinalis* L. were collected and extracted in different organic solvents according to their polarity to evaluate antibacterial activity against some MTCC bacterial cultures.

[II] MATERIALS AND METHODS

2.1. Plant material

The stem and root parts of healthy *Avicennia officinalis* L. plants were collected from East Godavari mangroves at Corangi Reserved Forest, (Geographically located between 16^o 39' – 17^o N and 82^o 14'-82^o 23'E) Kakinada, Andhra Pradesh, India. The plant materials were collected in new polythene bags and surface sterilized with 0.01% mercuric chloride solution. The stem and root were chopped separately into small pieces and shade dried at room temperature for seven days.

2.2. Extraction

The extraction of stem and root was carried out by using different solvents in their increasing order of polarity viz., hexane, benzene, ethyl acetate, acetone, and methanol by soaking the plant material in the respective solvents overnight at room temperature one after the other [4]. The contents of each flask were subjected to reflux below the boiling point of the respective solvents viz., Hexane (68^oC), Benzene (80^oC), Ethyl acetate (77^oC), Acetone (55^oC), Ethanol (78^oC) and Methanol (65^oC) for 6-8h in order to extract the active compounds into the solvent. Each extract was vacuum filtered and the filtrates were concentrated by vacuum distillation. The concentrated extracts were incubated at 37^oC for 3-

4 days to facilitate complete evaporation of the volatile solvent leaving behind the dried plant extract. The dried plant extract of 100mg each was dissolved in 1000µl of 1:10 diluted DMSO (in sterile distilled water) to obtain the final concentration of 10mg /100µl [5].

2.3. Determination of antibacterial activity

The bacterial strains viz., *Escherichia coli* MTCC 64, *Enterobacter aerogenes* MTCC 111, *Klebsiella pneumoniae* MTCC 39, *Pseudomonas aeruginosa* MTCC 424, *Bacillus subtilis* MTCC 121, *Lactobacillus delbrueckii* MTCC 911, *Staphylococcus aureus* MTCC 87 and *Streptococcus pyogenes* MTCC 1928 were used in the present study. The antibacterial activity of extracts against the bacteria was tested by agar well diffusion method [6], and zones of inhibition were measured. Each experiment was performed in triplicate and the average value of inhibition and standard deviation were calculated. The zone of inhibition was compared with that of standard Gentaimicin concentration of 1mg/100µl [7].

2.4. Determination of MIC

Minimum Inhibitory Concentration (MIC) as well as bactericidal or bacteriostatic activity were determined by broth dilution assay method. For the determination of MIC, the reconstituted extract in DMSO was serially diluted in Mueller Hinton broth medium to get the concentrations of 1.25, 2.5and 5.0mg/100µl [6].

2.5. Determination of Bactericidal and / or Bacteriostatic activity

For the determination of bactericidal and / or bacteriostatic activity, 0.1ml of culture medium from each broth tube showing no apparent growth was picked upon and sub-cultured on fresh Mueller Hinton agar medium. After incubation at 37^{o} C for 24 hrs, plates showing no visible growth of bacteria were considered for Bactericidal effect and plates with visible growth of bacteria as Bacteriostatic [8].

[III] RESULTS

3.1. Antibacterial sensitivity

The antibacterial activity of stem extracts of *Avicennia officinalis* L. in hexane, benzene, ethyl acetate, acetone, methanol and ethanol along with standard antibiotic, gentamicin against eight test bacteria is given in Figs-1&2. Of the eighth test bacteria, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* were found resistant to hexane extract. All the test bacteria exhibited sensitivity to benzene, ethyl acetate, acetone, methanol and ethanol extracts of stem. *Escherichia coli* and *Pseudomonas aeruginosa* showed greater sensitivity to all solvent extracts of stem, except the hexane, than that of gentamicin. Except *Lactobacillus delbrueckii* and *Staphylococcus aureus*, all the other bacteria were more sensitive to acetone extract than the others. The effectiveness of stem extract in benzene on *Enterobacter aerogenes* and *Klebsiella pneumonia* and methanol extracts on *Enterobacter aerogenes* was found equivalent to gentamicin effect on these two bacteria.

Benzene and methanol extracts showed relatively less effectiveness on *Streptococcus pyogenes* when compared to the effect of gentamicin. The sensitiveness of *Staphylococcus aureus* towards benzene, ethyl acetate and acetone extracts was low when compared to gentamicin. The antibacterial nature of ethyl acetate extract was higher than that of gentamicin against all test bacteria except *Staphylococcus aureus*. The inhibitory action of stem extract in acetone was higher against the four gram negative test bacteria than methanol and ethanol extracts, as well as gentamicin. Except the hexane, all the other solvent extracts of stem showed equal or more effectiveness than gentamicin on all the gram negative bacteria tested. However, with respect to gram positive bacteria tested one or two solvent extracts of stem showed relatively less effectiveness than that of gentamicin.

The root extracts of *Avicennia officialis* L. in hexane, benzene and ethyl acetate were not at all effective on all tested bacteria. The sensitivity of test bacteria towards the acetone, methanol and ethanol extracts of stem along with gentamicin is given in Fig-3. The inhibitory effect of root extracts in acetone, methanol and ethanol was found greater than that of gentamicin, except for acetone extract on *Bacillus subtilis*. Of the gram negative bacteria tested *Escherichia coli* and *Enterobacter aerogenes* were more sensitive to acetone extract of root than methnaol and ethanol extracts, Whereas, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* the other two gram

negative bacteria, were found high sensitive to ethanol extract than the other two extracts. Among the gram positive bacteria tested, the two cocci bacteria viz., *Staphylococcus aureus* and *Streptococcus pyogenes* were comparatively more sensitive to acetone extract, Whereas, *Bacillus subtilis* and *Lactobacillus delbrueckii* were more sensitive to methanol and ethanol extracts respectively.

3.2. Minimum Inhibitory Concentration

The positive extracts for antibacterial activity, were further tested to determine the Minimum Inhibitory Concentration (MIC) at different concentrations viz., 1.25, 2.5 and 5.0 mg /100µl and the data is given in tables 1 and 2. The value of MIC was found to be in the range of 1.25 to 5.0 mg /100µl for stem and root extracts of *Avicennia officinalis* L. against all the bacteria tested. The MIC of stem extracts in benzene, ethyl acetate and methanol was found to be 1.25mg/100µl for all gram negative bacteria tested. The ethanol extract exhibited 5mg/100µl MIC value for all the gram negative bacteria. In the case of gram positive bacteria tested, MIC value of 5mg/100µl for benzene and ethyl acetate extracts and 1.25mg/100µl for methanol and ethanol extracts. However, acetone extract showed varied MIC value with different bacteria. The MIC values of benzene and ethyl acetate extracts were found to be more for gram positive bacteria than gram negative bacteria tested. In contrast the MIC values of methanol and ethanol extracts were relatively higher for gram negative bacteria the gram positive bacteria.

The root extract in acetone showed the same MIC value of 1.25mg/100µl against all the bacteria tested. Similarly, ethanol extract also exhibited same MIC value of 1.25mg/100µl against all bacteria. But methanol extract showed relatively less but same MIC value of 1.25mg/100µl against all gram negative bacteria when compare to that of gram positive bacteria for which the MIC value was 5.0mg/100µl.

3.3. Bactericidal and / or bacteriostatic nature

The bactericidal or bacteriostatic nature of the stem and root extracts that are positive for antibacterial activity are given in tables 3 and 4. The stem extracts of hexane, methanol and ethanol and root extracts in methanol and ethanol were bacteriostatic in nature. Whereas, stem extract in benzene, ethyl acetate and acetone, and root extract in acetone showed bactericidal property.

[IV] DISCUSSION

Stem extract of *Avicennia officinalis* L. in hexane was active against *Klebsiella pneumoniae, Bacillus subtilis, Lactobacillus delbrueckii* and *Staphylococcus aureus*. This finding is in concurrence with the results of Mosquera et al. [9]. They screened the antibacterial activity of Anden Colombian plants by plate diffusion method against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* and reported a mixed result exhibited by the hexane extract. Similarly, the potential antimicrobial activity of the stem bark of *Premna tomentosa* L. in hexane, chloroform, ethylalcohol and water also reported by Anbazhakan and Balu [10].

Stem extract of *Avicennia officinalis* L. in benzene was active against all the test bacteria used with inhibition zones ranging from 11.33mm to 15.33mm. The susceptibility of all the test bacteria to the extracts of *Avicennia officinalis* L. may be a pointer to its potential as a drug that can be used against these microorganisms. Stem extracts in benzene showed relatively lesser zone of inhibition against Gram positive cultures tested. This may possibly be reasoned to the active penetration power of the bioactive principle present in benzene extract through the cell membrane of Gram negative bacteria. The antibacterial activity of benzene extracts of different plants was reported by some earlier workers [11, 12].

Stem extract of *Avicennia officinalis* L. in ethyl acetate was active against all the test bacteria used. This observation draws a good support from the results of Parcha et al. [13]. From their studies, they concluded that the leaf extract of *Alseodaphne keanamia* in ethyl acetate and methanol were active against certain Gram negative and Gram positive bacteria.

Stem and root extracts of *Avicennia officinalis* L. in acetone, methanol and ethanol were effective against Gram negative and Gram positive bacteria tested. Earlier, Puratchikody et al. studied the extracts of *Cyperus rotundus* for antibacterial activity against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus vulgaris* by disc diffusion method and

reported the significant antibacterial activity by the acetone and methanol extracts [14]. With this finding, our result is in great coincidence.

The stem and root extracts that showed antibacterial activity were compared with broad spectrum antibiotic gentamicin at a concentration of 1 mg/100µl. From this comparison, it was observed that majority of the extracts in crude form itself were more effective than gentamicin. With the case of the positive extracts exhibiting equal or less effectiveness in comparison to gentamicin, there is every possibility for having more antibacterial activity than gentamicin when the bioactive compounds of these extracts were purified and tested.

Several reports are documented in literature on determination of MIC values of several plant extracts. Different workers reported different ranges of MIC values with respect to the solvents, plant parts and plants. Nkere and Iroegbu [5] studied the MIC values of the ethanol extracts of root and stem bark of *Picarlima nitida* reported the MIC values ranging from 6.25 to 50mg/ml. Okoli and Iroegbu [6], from their studies reported the MIC value in the range of 3.125 to 12.50 mg/ml for the ethanol root extracts of *Synclisa scabrida*.

In our study, the MIC values for all the positive extracts against the tested bacteria ranged from 1.25mg/100µl to 5mg/100µl. Gram positive test bacteria had lower MIC values than Gram negative test bacteria to the methanol and ethanol extracts of stem. However, benzene and ethyl acetate extracts of stem as well as methanol extracts of root will have lower MIC to gram negative bacteria than gram positive bacteria. These differences may be explained by susceptibility testing condition, physico chemical characters of the bioactive principle present in the extract and even strain to strain difference.

In comparison to some of the earlier reports [15,16] on MIC values of pure compounds, our MIC values may be higher. But this can be substantiated by the argument that this value is for the crude extract. However, the purified form of bioactive compound of the crude extract responsible for antibacterial activity may exhibit the inhibitory effect at a lower concentration.

[V] CONCLUSION

The present study highlights that the *Avicennia officinalis* L can also be strongly recommended for consideration as a valuable source for identification, isolation 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.

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Organism	exane	Benzene	Ethyl acetate	Acetone	Methanol	Ethanol
Escherichia coli MTCC64	-	2.5	2.5	1.25	2.5	5.0
Enterobacter aerogenes MTCC111	-	2.5	2.5	1.25	2.5	5.0
Klebsiella pneumoniae MTCC39	2.5	2.5	2.5	1.25	2.5	5.0
Pseudomonas aeruginosa MTCC424	-	2.5	2.5	2.5	2.5	5.0
Bacillus subtilis MTCC121	1.25	5.0	5.0	1.25	1.25	1.25
Lactobacillus delbrueckii MTCC 911	5.0	5.0	5.0	2.5	1.25	1.25
Staphylococcus aureus MTCC 87	1.25	5.0	5.0	5.0	1.25	1.25
Streptococcus pyogenes MTCC 1928	-	5.0	5.0	2.5	1.25	1.25

Table-1: MIC (mg/100µl) of the Stem extracts of *Avicennia offficinalis* against the test organisms

Organism	Acetone	Methanol	Ethanol
Escherichia coli MTCC64	1.25	1.25	2.5
Enterobacter aerogenes MTCC111	1.25	1.25	2.5
Klebsiella pneumoniae MTCC39	1.25	1.25	2.5
Pseudomonas aeruginosa MTCC424	1.25	1.25	2.5
Bacillus subtilis MTCC121	1.25	5.0	2.5
Lactobacillus delbrueckii MTCC 911	1.25	5.0	2.5
Staphylococcus aureus MTCC 87	1.25	5.0	2.5
Streptococcus pyogenes MTCC 1928	1.25	5.0	2.5

Table-2: MIC (mg/100µl) of the root extracts of *Avicennia offficinalis* against the test organisms

Organism	Solvent					
	Hexane	Benzene	Ethyl acetate	Acetone	Methanol	Ethanol
Escherichia coli MTCC64	-	BC	BC	BC	BS	BS
Enterobacter aerogenes MTCC111	-	BC	BC	BC	BS	BS
Klebsiella pneumoniae MTCC39	BS	BC	BC	BC	BS	BS
Pseudomonas aeruginosa MTCC424	-	BC	BC	BC	BS	BS
Bacillus subtilis MTCC121	BS	BC	BC	BC	BS	BS
Lactobacillus delbrueckii MTCC 911	BS	BC	BC	BC	BS	BS
Staphylococcus aureus MTCC 87	BS	BC	BC	BC	BS	BS
Streptococcus pyogenes MTCC 1928	-	BC	BC	BC	BS	BS

Table-3: Bactericidal /Bacteriostatic activity of Stem extracts of *Avicennia officinalis* against selected bacteria. BS- Bacteriostatic BC- Bactericidal

Graphs

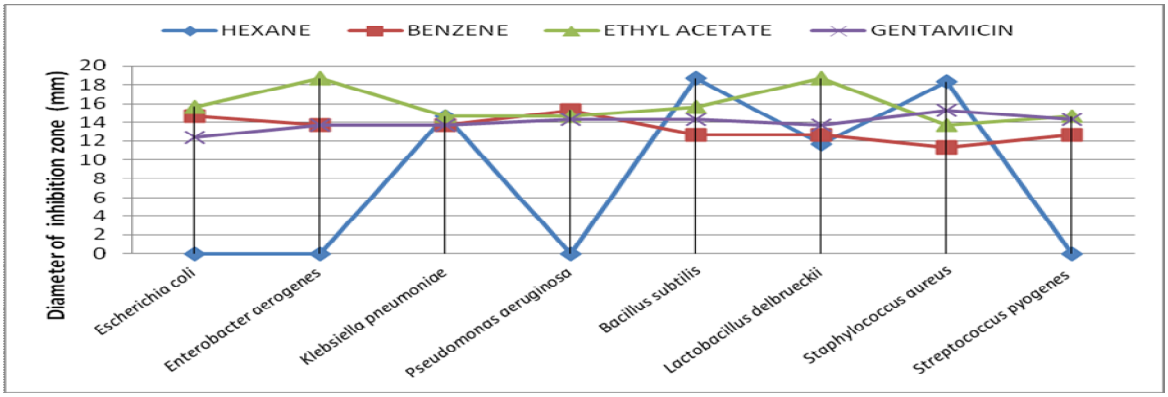

Figure 1: Antibacterial activity of stem extracts in Hexane, Benzene and Ethyl acetate of *Avicennia officinalis* on selected bacteria in comparison with Gentamicin

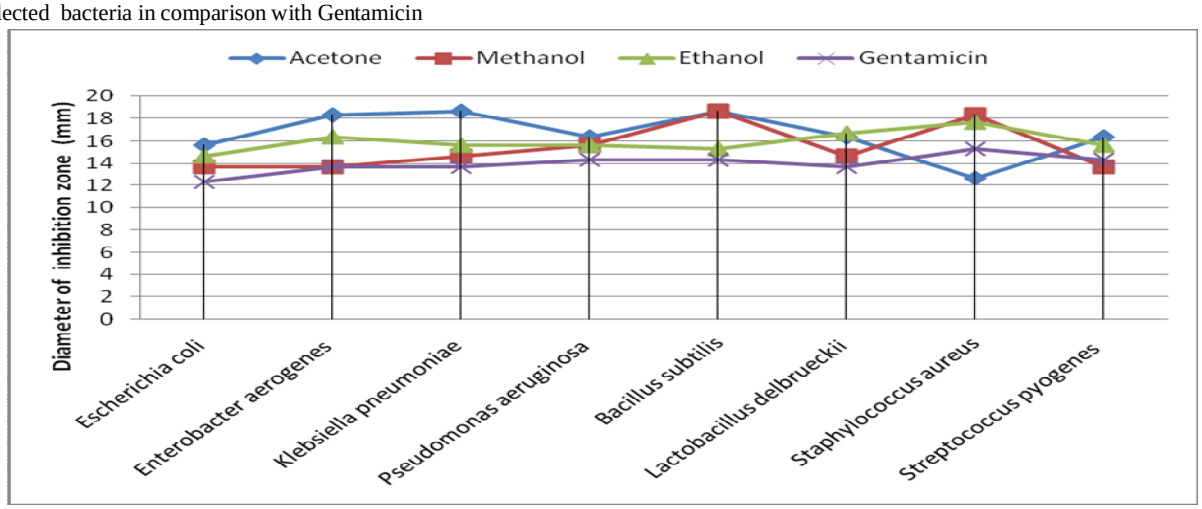

Figure 2: Antibacterial activity of stem extracts in Acetone, Methanol and Ethanol of *Avicennia officinalis* on selected bacteria in comparison with Gentamicin

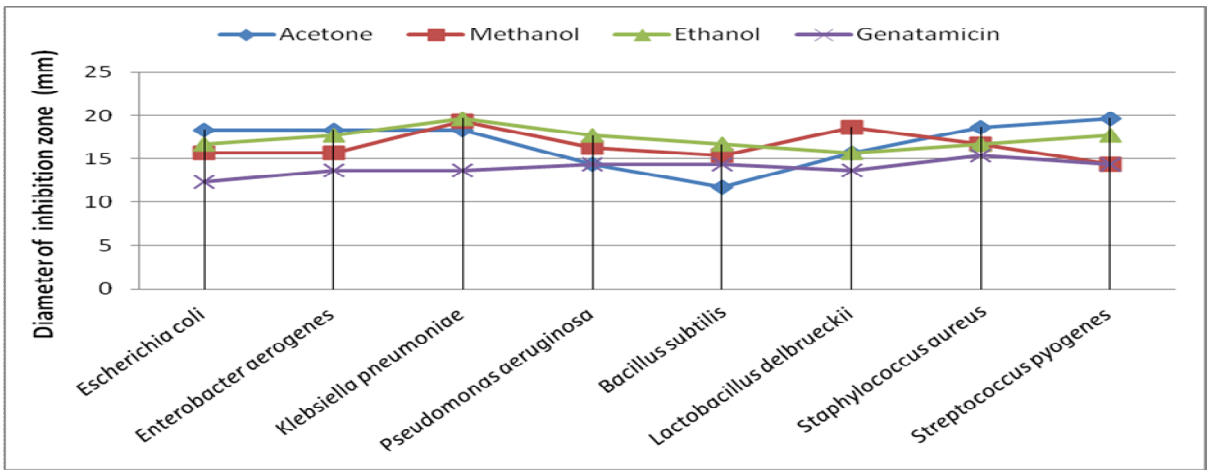

Figure 3: Antibacterial activity of root extracts in Acetone, Methanol and Ethanol of *Avicennia officinalis* on selected bacteria in comparison with Gentamicin