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Antimicrobial activity of Mangrove Plant *Avicennia officinalis* (Lam. Briqvet) on Selected Pathogens

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ABSTRACT

Avicennia officinalis was screened for antimicrobial activity against some clinical and phytopathogens. Plant parts such as leaves and bark of *A. officinalis* were collected from Coringa forest near Kakinada area, dried and extracted successively with hexane, chloroform, methanol and water using the soxhlet extraction apparatus. The antimicrobial activity of the plant extracts on the various test microorganisms, including multiple antibiotic resistant bacteria, was investigated. Antimicrobial activity of the extracts was determined by the Well Diffusion Method. The experimental results concluded the plant extracts of *A .officinalis* have greater potential as antimicrobial compounds against microorganisms and that they can be used in treatment of infectious diseases caused by resistant pathogenic microorganisms.

Keywords: Antimicrobial activity, Antibiotic resistant bacteria, *Avicennia officinalis,* Well Diffusion Method.

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INTRODUCTION

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity in the late 1990s. It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs [1]. In recent years, drug resistance to pathogenic microorganisms has been commonly and widely reported in literature [2]; [3]. Therefore antimicrobials may have significant clinical value in treatment microbial strains [4]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant sciences used in herbal medicines [5]. A large number of plants in different locations around the world have been extracted and semi-purified to investigate individually their antimicrobial activity [6]. [7] Have reported the presence of compounds like tannins, alkaloids, and polyphenols in mangroves which play and important role in the suppression of deleterious microorganisms. [8]; [9]; [10].

Mangrove plant extracts have been used for centuries as popular method for treating several health disorders plant-derived substances have recently become of great interest owing to their versatile application. Numerous studies have been carried out on various natural products screening their antimicrobial activity [11]; [12]; [13]; [14]. The present study was to screen the antimicrobial activity of *A. officinalis* and search for new compounds from this species.

MATERIALS AND METHODS

Plant and extraction:

Mangrove plant *Avicennia officinalis* belongs to family Avicenniaceae, the plant parts were collected from Coringa Mangrove forest, AP. The material was taxonomically identified and the Voucher specimen is stored. The plant materials were dried under shade and then powdered with a mechanical grinder and stored in an airtight container. The powder obtained was subjected to successive soxhlet extraction with the organic solvents with increasing order of polarity.

Test microorganisms:

The microorganisms that were studied including *Bacillus subtilis, Bacillus megaterium*, *Lactobacillus acidophilus* (Gram positive) *Escherichia coli, Enterobacter aerogenes, Enterobacter cloace, Klebsiella pneumonia* (Gram negative) and fungal strains *Candida albicans, Mucar racemosus, Rhizoctonia solani, Rhizopus stolonifer* and *Saccharomyces cerevisiae* through Agar Diffusion Method. The strains are maintained and tested on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi. Active cultures were generated by inoculating a loop full of culture in separate 100 ml nutrient broths and incubating on a shaker at 37^{o} C overnight. The

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cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 minagain and dilute in normal saline to obtain 5 x 10 cfu/Ml.

Determination of antibacterial activity:

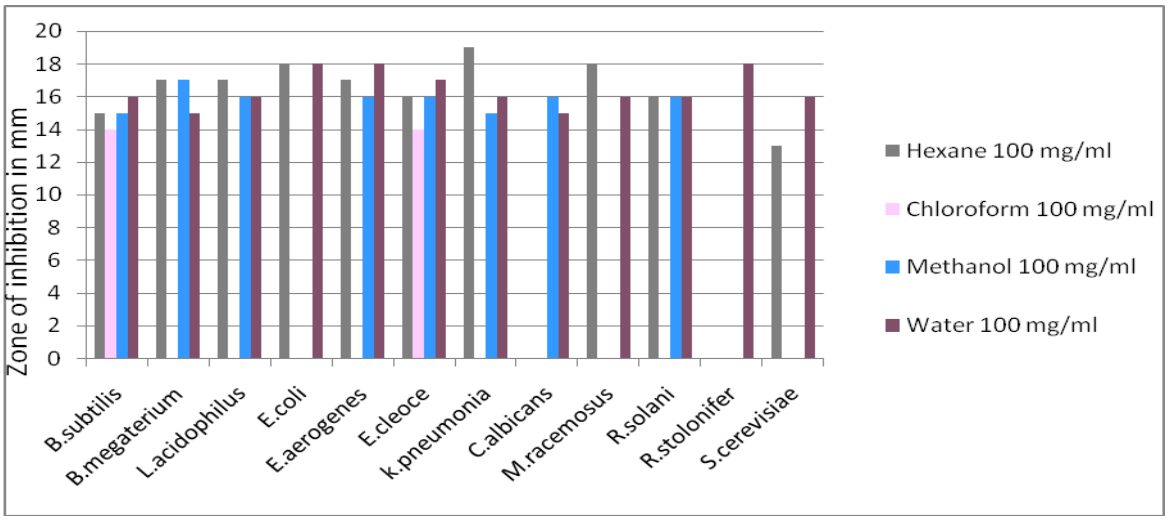
The crude extracts of the different plant parts of this plant were subjected to antimicrobial assay using the Agar Well Diffusion method of [15] modified by [16].

20 ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated with 0.2ml of cultures mixed gently and poured into sterile Petri dishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each Petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50µl of the extract concentration of 100mg/ml 300 mg/ml and 500 mg/ml and allow diffusing for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37^{o}c for 24 hours for bacteria. The above procedure is allowed for fungal assays but expects the media potato dextrose agar instead of nutrient agar and incubates at 25^{o}c for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

RESULTS

Graph 1.1 represents that comparison between four solvents of *A. officinalis* leaves at 100 mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts hexane, methanol and water extracts showed moderate activity whereas chloroform extract showed less activity. Bacterial strains of hexane extract of *K. pneumonia* (19 mm) and fungal strains *M. racemosus* (18 mm) and water extracts of *R. stolonifer* (18 mm) showed moderate activity.

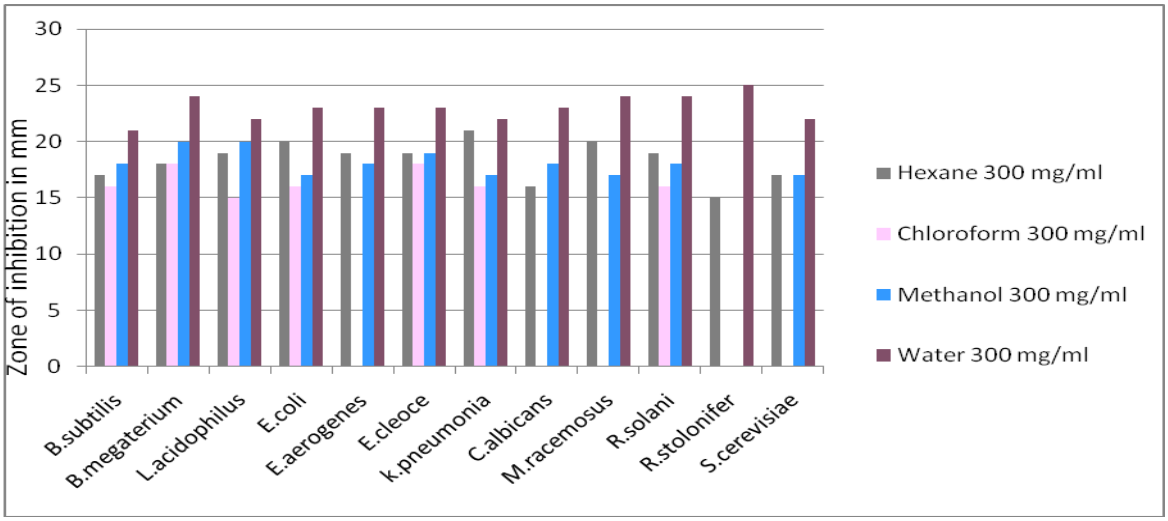
Graph 1.1 Antimicrobial activity of *Avicennia officinalis* leaves 100 mg/ml



ISSN: 0975-8585

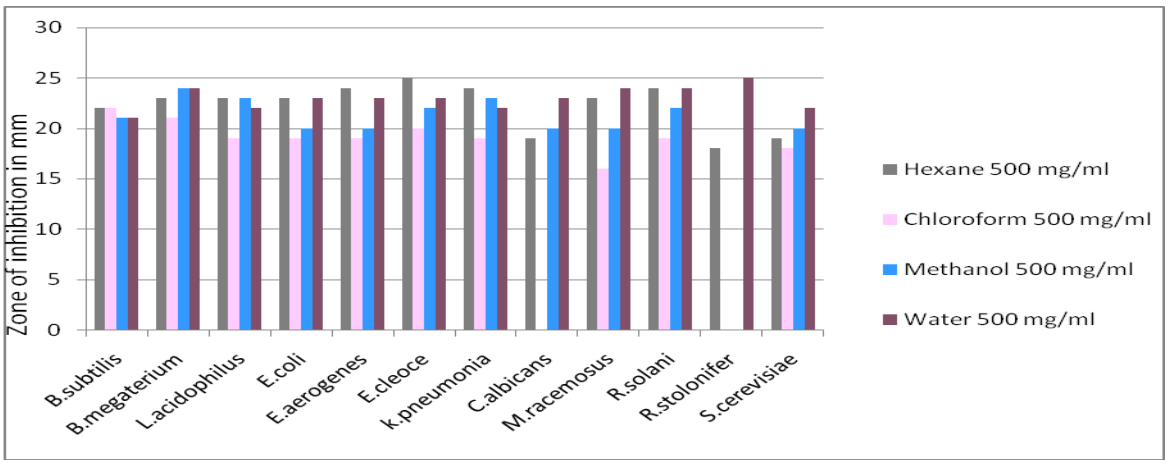
Graph 1.2 represents that comparison between four solvents of *A. officinalis* leaves at 300 mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts water extracts showed high activity. Bacterial strains of water extracts *E. coli* (21 mm), hexane extracts *K. pneumonia* (21 mm) and fungal strains of water extracts *R. solani* (22 mm) and *M. racemosus* (22 mm) showed high activity.

Graph 1.2 Antimicrobial activity of *Avicennia officinalis* leaves 300 mg/ml



Graph 1.3 represents that comparison among four solvents of *A. officinalis* leaves at 500 mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts water extracts showed high activity. Bacterial strains of hexane extract of *E. cloace* (25 mm) and water extracts of *B. megaterium* (25 mm) fungal strains of *R. stolonifer* (25 mm) showed high activity.

Graph 1.3 Antimicrobial activity of *Avicennia officinalis* leaves 500 mg/ml



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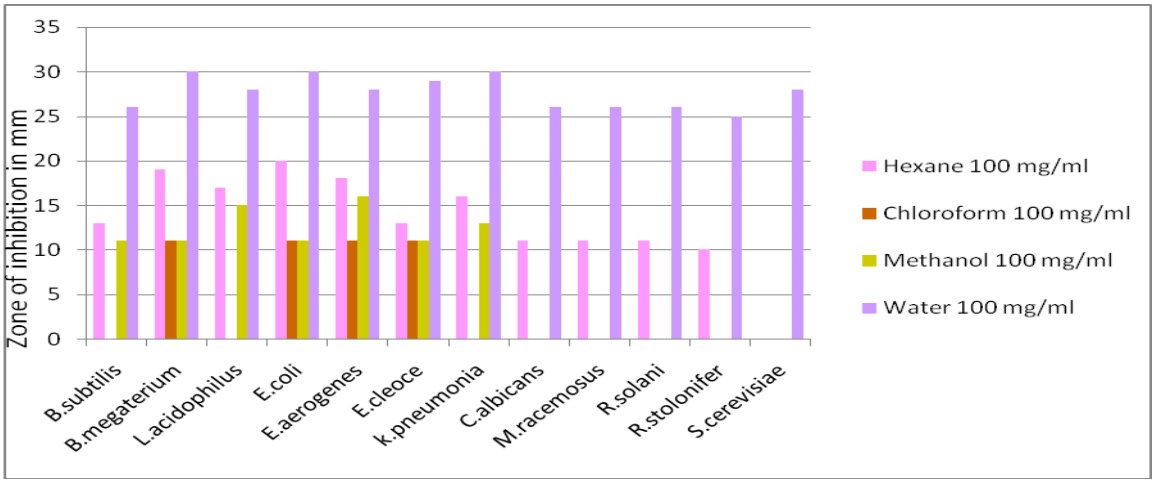
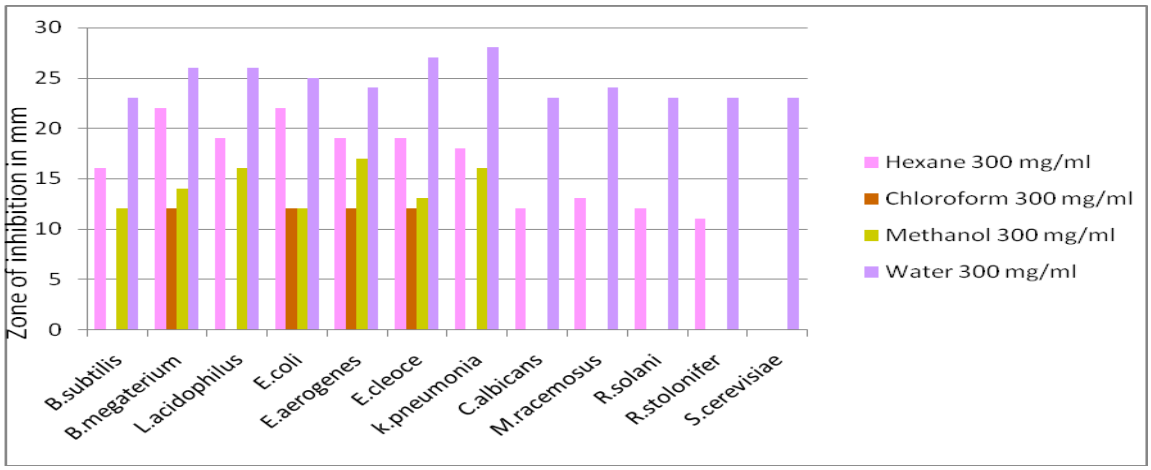
Graph 2.1 represents that comparison among four solvents of *A. officinalis* bark at 100 mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts water extracts showed high activity. Bacterial strains of water extracts of *K. pneumonia* (24 mm) *E. cloace* (24 mm) *L. acidophilus* (24 mm) and fungal strains *M. racemosus* (22 mm) showed high activity.

Graph 2.1 Antimicrobial activity of *Avicennia officinalis* bark 100 mg/ml



Graph 2.2 represents that comparison among four solvents of *A. officinalis* bark at 300 mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts water extracts showed high activity. Bacterial strains of water extracts of *K. pneumonia* (28 mm) and fungal strains *M. racemosus* (24 mm) showed high activity.

Graph 2.2 Antimicrobial activity of *Avicennia officinalis* bark 300 mg/ml



Graph 2.3 represents that comparison among four solvents of *A. officinalis* bark at 500 mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts water extracts showed high activity. Bacterial strains of water extracts of *K. pneumonia* (30 mm), *B.*

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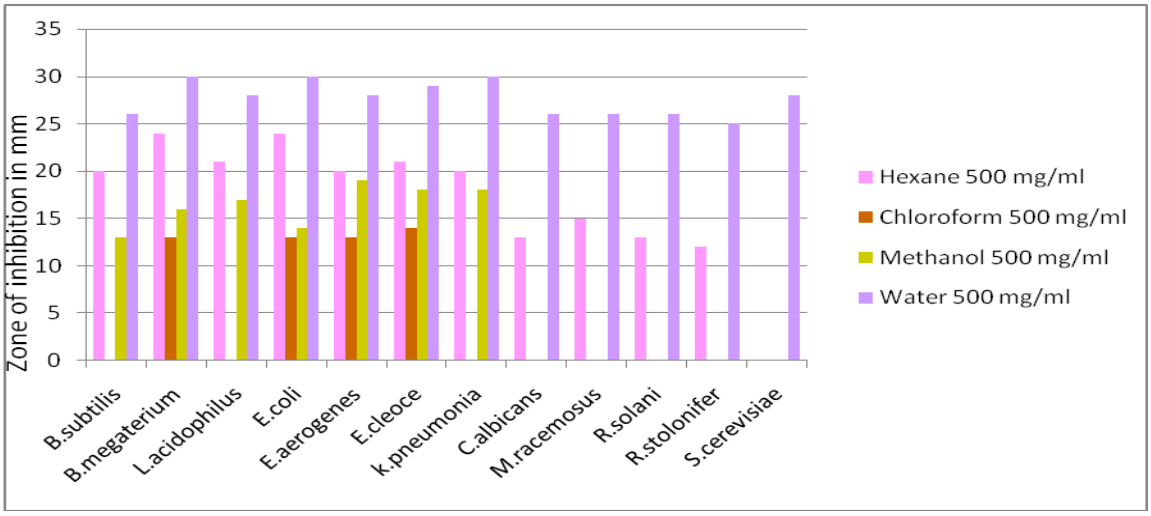
megaterium (30 mm), *E. coli* (30 mm) and fungal strains *S. cerevisiae* (28 mm) showed high activity.

Graph 2.3 Antimicrobial activity of *Avicennia officinalis* bark 500 mg/ml



DISCUSSION

Alkaloids in higher concentration, moderate level of flavonoids and trace amount of terpenoids and cardiac glycosides. Presence of high concentration of alkaloids, tannins and moderate amount of flavonoids and saponins in Rhizophoraceae and Avicenniaceea species has been widely reported by researchers [17].

In vitro antimicrobial screening of mangrove plant *Avicennia officinalis.* Mature leaf extracts of *A. officinalis* in methanol exhibited promising antimicrobial activity [18]. Phytochemical screening revealed that mature leaf of *A. officinalis* contained alkaloids, steroids, triterpenoids and flavonoids. [19] Reported plant extracts of *A. alba* have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms. [20] Reported phytochemical screening, mature leaves of *A. marina* contained alkaloids, strroids and flavonoids.

Water extracts of *A. officinalis* leaves showed high activity against all seven bacterial and five fungal strains. All the four extracts chloroform extract appears to have less antibacterial and antifungal activity than the hexane, methanol and water extracts. Water extracts of *A. officinalis* bark showed high activity against all seven bacterial and five fungal strains. All the four extracts chloroform and methanol extracts did not showed any antifungal activity. When compared to the *A. officinalis* leaves and bark, leaves showed more activity than bark. The above results concluded that plant extracts have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by pathogenic microorganisms.

ISSN: 0975-8585

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