PRELIMINARY PHYTOCHEMICAL INVESTIGATION AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF *SONNERATIA APETALA* PLANT

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ABSTRACT

The aim of present study is to investigate the phytochemical constituents and antimicrobial activity of Ethanolic extract of *Sonneratia apetala*. Preliminary Phytochemical tests revealed the presence of alkaloids, flavonoids, tannins, saponins, phytosterols and carbohydrates. Antimicrobial activity was evaluated by Agar well Diffusion method. *In vitro* screening of *Sonneratia apetala* mangrove Ethanolic plant extract showed species specific activity in inhibiting growth of bacteria and fungi. The Ethanolic plant extract showed good activity against selected gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus wernerii*), gram-negative bacteria (*Pseudomonas putida*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia*) and fungal strain (*Candida albicans*). The observations revealed significant zone of inhibition and supports to antimicrobial activity. The bioactive compounds responsible for these antimicrobial activities could be isolated and identified to develop a new drug of pharmaceutical interest.

Keywords: Mangrove plant, *Sonneratia apetala*, antimicrobial activity, Agar well diffusion method.

INTRODUCTION

Medicinal plants, used in the traditional treatments of various diseases on an empirical basis¹⁻⁵. Herbal medicines have been widely practiced throughout the world from ancient time. These medicines are safe and environment friendly. Nearly 80% of the world population depends upon traditional systems of health care⁶. In India, 9500 herbal medicinal plants and also 8000 higher plants have been used in the Indian medicinal industries. The potential for developing antimicrobials from higher plants appears rewarding as it might lead to the development of phytomedicine, which would play a prominent role against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve purpose with lesser side effects that are often associated with synthetic antimicrobials⁷. Several plants and herb species used traditionally have potential antimicrobial and antiviral properties^{8,9} and this has raised the optimism of scientists about the future phyto-antimicrobial agents¹⁰. In recent years secondary plant metabolites and phytochemicals, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents¹¹. Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of many bacterial infections¹². Several phytochemical surveys have been published, including the random sampling approach which involved some plant accessions collected from all parts of the world. The major chemical substances of interest in these surveys were the alkaloids, steroids and saponins, however other diverse are naturally occurring phytocomponents such as flavonoids, tannins, unsaturated steroids, triterpenoids and essential oils¹³. Mangroves are a collection of plants growing in saline coastal habitats and are rich sources of secondary metabolites which possess interesting bioactive properties especially antimicrobial activity. *Sonneratia apetala* Buch. Ham, also known as mangrove apple plant belongs to Sonneratiaceae¹⁴ family. It is grown as a tree or shrub along seaward fringes and intertidal areas like Coringa, Kakinada (Andhra Pradesh, India). This plant has been traditionally used to treat

hepatitis¹⁵. The literature also reports that the leaf part of the plant is widely used for dysentery, sprain and bruises, in treatment of eye troubles (such as cataract) and open sores in children ears and also in heart troubles¹⁶. However, very little is known about the specific chemical constituents present in this plant. So far biological activities have not carried out on the plant and the present study aims to determine Phytochemical constituents and antimicrobial activity on selected gram positive, gram negative bacterial and fungal strain.

MATERIALS AND METHODS

Plant material

Plant parts (leaves, bark, pneumatophores) of *Sonneratia apetala* were collected randomly from mangrove forest, Coringa near to Kakinada East Godavari district, Andhra Pradesh, India. The freshly collected roots of plant were cleaned from dirt, dried under shade and then coarsely powdered manually.

Extraction of plant material

Known amount of coarsely powdered plant material was macerated in ethanol for a period of 7 days and then subjected to hot percolation for 8h. The solution was then filtered concentrated and dried in desiccators. The yield of Ethanolic extract was found to be 8.3%w/w.

Bacterial and fungal strains

The antimicrobial activity of *Sonneratia apetala* was carried on *Staphylococcus aureus* (ATCC BAA1026), *Bacillus subtilis* (ATCC 11774), *Staphylococcus wernerii* (ATCC 27836), *Escherichia coli* (ATCC 10536), *Klebsiella pneumonia* (ATCC 33495), *Pseudomonas putida*, *Pseudomonas aeruginosa* (ATCC 10662), *Proteus mirabilis* (ATCC 14153) and *Candida albicans*.

Phytochemical investigation

Phytochemical¹⁷ tests were carried out on all extracts for qualitative determination of phytochemical constituents¹⁸.

Table1: Phytochemical screening of Ethanolic extract of *Sonneratia apetala*

Chemical constituent	Chemical test	Ethanolic extract
Alkaloids	Dragendroff's test	+
	Mayers test	+
	Hagers test	+
Carbohydrates	Molisch test	+
	Benedict test	+
	Fehling's test	+
Flavonoids	Lead acetate test	+
	Zn-HCl reduction test	+
	NaOH test	+
Glycosides	General test	-
	Mod.Borntragers test	-
Proteins	Xanthoprotein test	-
	Millions test	-
Saponins	Foam test	+
	Froth test	+
Tannins	Lead acetate test	+
	Salkowskii test	+

+ (present),- (absent)

Table 2: Zone of inhibition of Ethanolic extract of *Sonneratia apetala*

Microorganism	Zone of inhibition(mm)			
	Ethanolic Extract			Standard
	25mg/ml	50mg/ml	100mg/ml	25µg/ml
Gram-positive bacteria				
<i>Bacillus subtilis</i>	15±0.23	16±0.11	19±0.17	18±0.15
<i>Staphylococcus aureus</i>	15±0.12	16±0.15	19±0.17	18±0.26
<i>Staphylococcus wernerii</i>	15±0.32	17±0.16	20±0.31	17±0.14
Gram-negative bacteria				
<i>Pseudomonas putida</i>	14±0.15	16±0.13	17±0.21	19±0.21
<i>Escherichia coli</i>	14±0.12	16±0.16	18±0.15	18±0.15
<i>Pseudomonas aeruginosa</i>	13±0.31	14±0.21	16±0.24	19±0.32
<i>Proteus mirabilis</i>	14±0.41	15±0.17	17±0.13	19±0.18
<i>Klebsiella pneumonia</i>	12±0.14	14±0.23	17±0.41	19±0.17
Fungal strain				
<i>Candida albicans</i>	9±0.15	11±0.15	15±0.12	15±0.21

All the values are expressed as Mean ± SEM

Figure 1: Zone of inhibition of Ethanolic extract of *Sonneratia apetala* **Zone of inhibition antimicrobial activity**

Figure 2:(a) shows Antibacterial effect of std Gentamicin (25µg/ml), test sample of concentrations (25,50,100mg/ml) on *staphylococcus warneri* (b) shows anti fungal effect of fluconazole (25µg/ml), test sample (25,50,100mg/ml) on *Candida albicans*

Antibacterial assay

Antibacterial activity¹⁹⁻²¹ of crude extract was tested through agar well diffusion method. For this, agar plates were prepared separately and overnight culture of test bacterial strains were seeded individually over the the surface of agar plates. Approximately 6mm diameter wells were punctured over the agar plates using sterile cork borer. The extracts were then prepared at a concentraions of 25,50,100mg/ml by dissolving in DMSO. The wells were then filled with 50µL of extract. Gentamicin (25µg/ml) was used as positive control. The plates were then incubated at 37°C for 24h. The assay was carried out in triplicate. The antibacterial activity of the extract was determined by measuring the the diameter of inhibition zone around the well filled with extract.

Antifungal assay

Antifungal activity of crude extract was tested through agar well diffusion method. For this, Sabouraud dextrose agar plates (SDA) were prepared. Then with a sterile cotton swab, 72h culture of fungal strain were seeded over the the surface of SDA plates. Approximately 6mm diameter wells were punctured over the agar plates using sterile cork borer. The extracts were then prepared at a concentraions of 25,50,100mg/ml by dissolving in DMSO. The wells were then filled with 50µL of extract. Fluconazole (25µg/ml) was used as positive control. The plates were then incubated at 37°C for 48h. The assay was carried out in triplicate. The antibacterial activity of the extract was determined by measuring the the diameter of inhibition zone around the well filled with extract.²⁰

RESULTS

Preliminary Phytochemical tests revealed the presence of alkaloids, flavonoids, tannins, saponins, phytosterols and carbohydrates Table 1. Antimicrobial activity was evaluated by Agar well Diffusion method. *In vitro* screening of *Sonneratia apetala* mangrove Ethanolic plant extract showed species specific activity in inhibiting growth of bacteria and fungi in a concentration dependent manner and was comparable with standard drug Table 2 and Figure 1, 2. The gram-positive bacteria were more susceptible than gram-negative bacteria. This may be due to the presence of an outer membrane in gram-negative bacteria which acts as a barrier to many environmental substances including antibiotics. Results from present study indicate potential use of *Sonneratia apetala* in treatment of various pathogenic diseases as described in Ayurvedic literature.

DISCUSSION

In present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization²². Although, a significant number of studies have been carried out to programmes have been initiated on crude plant materials. It also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants²³. In this investigation, the active phytocomponents of *Sonneratia apetala* activity of the plant extracts was assayed *in vitro* by agar well diffusion method against 8 bacterial and 1 fungal species. Table 2, summarizes the microbial growth inhibition of ethanol extracts of the plant. The traditional plants may represent new sources of anti-microbial with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine²⁴. Preliminary Phytochemical tests revealed the presence of alkaloids,

flavonoids, tannins, saponins, phytosterols and carbohydrates Table 1.

CONCLUSION

Preliminary Phytochemical tests showed the presence of several Phytochemical constituents in *Sonneratia apetala*. The results obtained from the antibacterial study concludes that the Ethanolic extract of *Sonneratia apetala* possesses good antibacterial indicating its effectiveness against both gram positive and gram negative microbes. The antifungal activity was evaluated by using Sabouraud dextrose agar well diffusion method and the results shows test sample has significant antifungal activity.

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