

In vitro* bioefficiency of marine mangrove plant activity of *Rhizophora conjugata

***Varahalarao Vadlapudi and K Chandrasekhar Naidu**

Phytochemical lab, Department of Botany, Andhra University, Visakhapatnam-530017, A. P, India.

**Corres. author: vadlapudivr@gmail.com, kcnaidu2002@yahoo.com
Tel: + 91-9985670299**

ABSTRACT: In this present study antimicrobial activity of *Rhizophora conjugata* (Rhizophoraceae), the plant parts of were collected from coringa forest near Kakinada, Godavari-krishna delta area were dried and extracted successively with hexane, chloroform and methanol using the soxhlet extraction apparatus. The antimicrobial activities of the organic solvent extracts on the various test microorganisms, including bacteria and fungi investigated using agar well diffusion technique. Methanol extracts exhibited promising antimicrobial activity than chloroform and hexane extracts. Among all tested microorganisms *L. acidophilus* (22 mm) showing highest susceptibility followed by *S. salivarius* (19 mm) and *A. hydrophila*, *S. mutans* and lowest activity was found with *C. herbarum*, *F.oxysporum*, *S. anginosus* and *S.mitis* with concentration 100 mg/ml. This study, has to some extent, validated the medicinal potential of the mangrove plants.

Key words: *Rhizophora conjugata*, Soxhlet extraction, agar well diffusion technique,

INTRODUCTION

Infectious diseases are the leading cause of death world-wide and at the same time antibiotic resistance has become a global concern (Westh *et al.*, 2004)¹. Therefore there has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. The presence of antibacterial substances in the higher plants is well established (Srinivasan, *et al* 2001)². This has forced scientist to search for new antimicrobial substances from various sources including medicinal plants species which have been serving as the best natural source of drugs and medicines since the beginning of civilization.

Recent research evidenced that Indian mangroves contained antibacterial (Chandrasekaran *et al.*, 2009)³ and antifungal (Bose, S. and A. Bose, 2008)⁴ properties. Until now, more than 200 bioactive metabolites have been isolated from true mangroves of tropical and subtropical populations (Wu *et al.*, 2008)⁵. According to their chemical structure, most of the isolated compounds belong to steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics which having a wide range of therapeutic possibilities (Bandaranayake., 1998)⁶. The aim of the present study was to investigate the antibacterial and antifungal activity of ethanolic extracts of *Rhizophora conjugata*.

MATERIALS AND METHODS

Plant material and extract preparation:

Rhizophora conjugata belongs to Rhizophoraceae was taxonomically identified and the Voucher specimen is stored. The plant parts were collected from coringa forest near Kakinada, Godavari-krishna delta area, Andhra Pradesh, India. The plant material were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container. The powder obtained was subjected to successive soxhlet extraction with methanol.

Microorganisms:

Acremonium strictum (MTCC 2599), *Alternaria alternata* (MTCC) , *Aspergillus flavus* (MTCC 4633), *Aeromonas hydrophila* (MTCC646), *Asperigillus niger* (MTCC 2723), *Bipolaris bicolor* (MTCC2105), *Candida albicans* (MTCC3017), *Cladosporium herbarum* (MTCC2143) , *Fusarium oxysporum* (MTCC1755), *Macrophomina phaseolina* (MTCC2165) , *Pseudomaonas marginales* (MTCC2758), *Rhizoctonia solani* (MTCC 4633), *Streptococcus mutans*(MTCC 890), *Streptococcus salivarius* (MTCC1938), *Streptococcus mitis* (MTCC2696), *Streptococcus anginosus* (MTCC 1929), *Streptococcus mutans* (MTCC 497), *Streptococcus gordonii* (MTCC26950), *Staphylococcus aureus* (MTCC 96), *Lactobacillus acidophilus* (MTCC447), *Ustilago maydis*

(MTCC 1474) . Microorganisms were obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The strains are maintained and tested on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi.

Determination of antibacterial activity: The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion method of (Murray. 1995)⁷ modified by (Olurinola. 1996)⁸.

20 ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated with 0.2 ml 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 and allow diffusing for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The above procedure is allowed for fungal assays but except the media potato dextrose agar instead of nutrient agar and incubates at 25°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 AND DISCUSSION

(Table 1 and Fig 1) *L. acidophilus* (22 mm) showing highest susceptibility followed by *S. salivarius* (19 mm) and *A. hydrophila*, *S. mutans* and lowest activity was found with *C. herbarum*, *F.oxysporum*, *S. anginosus* and *S.mitis*

Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Successive isolation of botanical compounds from plant

material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the plant extracts of methanol provided more consistent antimicrobial activity.

Table 1: Antimicrobial activity of methanol extracts of *Rhizophora conjugate*

Microorganisms	100 mg/ml
<i>Acremonium strictum</i>	7
<i>Aspergillus flavus</i>	8
<i>Aeromonas hydrophila</i>	15
<i>Asperigillus niger</i>	11
<i>Bipolaris bicolor</i>	10
<i>Candida albicans</i>	11
<i>Cladosporium herbarum</i>	7
<i>Fusarium oxysporum</i>	7
<i>Macrophomina phaseolina</i>	9
<i>Pseudomaonas marginales</i>	11
<i>Rhizoctonia solani</i> .	14
<i>Streptococcus mutans</i>	15
<i>Streptococcus salivarius</i>	19
<i>Streptococcus mitis</i>	7
<i>Streptococcus anginosus</i>	7
<i>Streptococcus gordonii</i>	11
<i>Staphylococcus aureus</i>	11
<i>Lactobacillus acidophilus</i>	22
<i>Ustilago maydis</i>	8

Volume per well: 50µl; Borer size used: 6mm;
Extract concentrations in 100, 300 and 500 mg/ml, No zone (-).

(Table 1) summarizes the antimicrobial activities of zone of inhibition of methanol (7 to 22 mm)

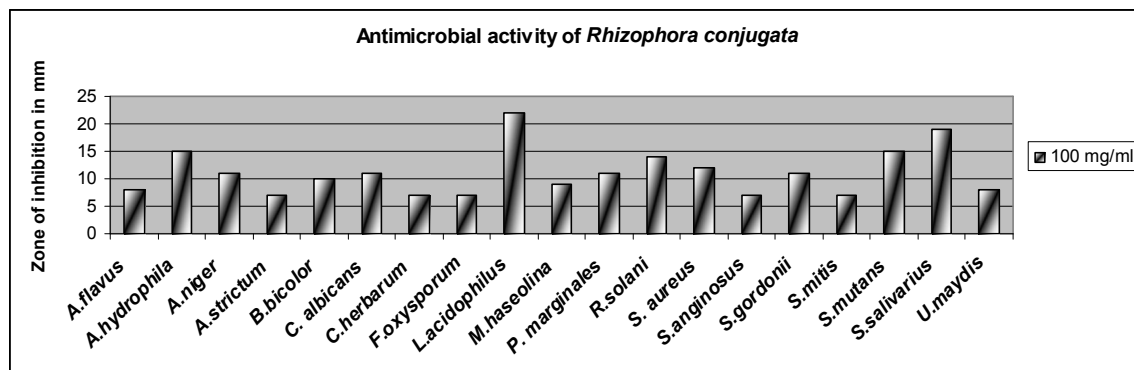


Fig 1

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