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In vitro bioactivity against important phytopathogens of *Rhizophora mucronata* (Lam.) and *Acanthus ilicifolius* Linn

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Abstract

Mangroves are the only trees that are capable of thriving in salt water. Mangroves are biochemically unique, producing a wide array of novel natural products. In this present study antimicrobial activity of Rhizophora mucronata (Rhizophoraceae) and Acanthus ilicifolius (Acanthaceae) the plant parts of were collected from Kakinada, Godavari-krishna delta and other areas of Andhra Pradesh 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. This study, has to some extent, validated the potential of the mangrove plants against phytopathogens.

Keywords: Rhizophora mucronata, soxhlet extraction, agar well diffusion technique, mangrove plants.

INTRODUCTION

Plant diseases caused by pathogenic organisms (infectious diseases) including fungi, oomycetes, bacteria, viruses, viroids, virus-like organisms, phytoplasmas, protozoa, nematodes and parasitic plants. Since the beginning of agriculture, generations of farmers have been evolving practices for combating the various plagues suffered by our crops. Following our discovery of the causes of plant diseases in the early nineteenth century, our growing understanding of the interactions of pathogen and host has enabled us to develop a wide array of measures for the control of specific plant diseases. [1] reported that the total number of plant chemicals may exceed 400,000 and out of it more than 10,000 are secondary metabolites whose major role in plant is defensive in nature. Thus, plant based secondary metabolites, which have defensive role may be exploited for the management of diseases. Disease management through plant extracts has been reported by different workers in different crops. But very little is known about the antimicrobial affects of

plant extracts against these diseases. An antimicrobial agent from marine halophytes and medicinal plants is immediate need of ethno pharmacological science in developing novel marine pharmaceuticals. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections in the present work an attempt has been made to carry out screening for the preliminary antimicrobial activity of the mangrove plants used in Indian folk medicine for centuries which may be useful in developing lead compound to combat important plant diseases. The goal of this study was to increase the knowledge of the bioactivity of aerial parts of *Rhizophora mucronata* (Rhizophoraceae) and *Acanthus ilicifolius* (Acanthaceae) mangrove plants 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 applications [2]. it is relatively lesser-known, yet important medicinal plant of Herbal Materia Medica. Numerous studies have been carried out on various natural products screening their antimicrobial activity [3-6]

MATERIALS AND METHODS

Plant material and extract preparation

The *R. mucronata(*Rhizophoraceae) is very rare plant vernacular name is Uppu Ponna and Grows 15-25 m tall Leaves of are stipule yellowish, Tiny black spots on the underside, Bark and leaves are uses for Astringent, Angina, Hemorrhage, Diabetes, Diarrhea, Dysentery and Hematuria. **.**Aerial parts were collected from collected from Kakinada, Godavari-krishna delta and other areas of Andhra Pradesh.

A. ilicifolius (Acanthaceae) common names are Holly-leaved Acanthus, Holly Mangrove, sea holly and it is relatively lesser-known, yet important medicinal plant of Herbal Materia Medica. The plant is used in traditional systems of medicine, including Traditional Indian Medicine (TIM) or Ayurveda and Traditional Chinese Medicine (TCM). It occurs in tropical Asia and Africa, through Malaya to Polynesia. It is a viny shrub or tall herb, upto 1.5 m high, scarcely woody, bushy, with very densegrowth. Shallow tap roots, but occasionally stilt roots are conspicuous. Leaf simple, opposite, decussate, cauline, exstipulate, petiole short, flattened, glabrous, pulvinous to sheathing base.Flower bisexual, typically zygomorphic, complete, erect, sessile, hypogynous. Fruit 1 cm green and 2.5 - 2.0 cm long, kidney shaped 4 seed drupe, Seed 0.5 - 1.0 cm long. Traditional Medicinal Uses.In Ayurveda, the plant is known as Sahachara. According to Nadkarni the drug is astringent and makes a good nervine tonic, expectorant, and stimulant. He says that the root is expectorant, and is used in coughs and asthma. The root, boiled in milk, is largely used in leucorrhoea and general debility. Loureiro says that the Siamese and Indo-Chinese consider the roots to be cordial and attenuant, and useful in paralysis and asthma. The tender shoots and leaves are used in India for bite. In Goa, the leaves, which abound in mucilage, are used as an emollient fomentation in rheumatism and neuralgia.The material was taxonomically identified and the Voucher specimen is stored. 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 the organic solvents with increasing order of polarity i.e. Hexane (60-80°), Chloroform (59.5-61.5°), Methanol (64.5-65.5°) respectively.

Test microorganisms

The phytopathogens *Alternaria alternate, Asperigellus flavus, Asperigellus niger, Macrophomina phaseolina, Pseudomonas syringae, Rhizoctonia solani Xanthomonas campestris* were procured from Microbial Type Culture Collection (MTCC), Chandigarh were used as test organisms. 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 loopful of culture in separate 100mL nutrient broths and incubating on a shaker at 37^{O}C overnight. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted in normal saline to obtain 5 x 10^5 cfu/mL.

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].

Minimum inhibitory concentration (MIC) assays:

Based on the preliminary screening chloroform andmethanolic extracts were found to have potent antimicrobial activity and Minimum Inhibitory Concentrations (MIC) of the extracts wasd etermined according to Elizabeth, 2001 [9].

RESULTS AND DISCUSSION

Table 1: Antimicrobial activity of methanol extracts of *R. mucronata* and *A. licifolius*

Phytopathgens	*R.mucronata*				*A. ilicifolius*			
	100	300	500	MIC	100	300	500	MIC
A. alternata	7	10	13	100	11	15	20	75
A.flavus	-	-	-	-	-	-	-	-
A .niger	12	15	17	90	16	18	23	45
M. phaseolina	-	-	-	-	13	15	16	65
P. syringae	-	-	-	-	10	12	14	85
R. solani	-	-	-	-	8	10	12	100
X. campestris	-	-	-	-	7	9	12	125

(0-19) zone of inhibition in mm, Volume per well: 50µl, Borer size used: 6mm used
(-) indicates no activity, Extract concentration (100, 300, and 500) / ml DMSO

The antimicrobial activities of methanolic extracts were represented in **table 1** according to the results *R. mucronata* and *A.licifolius* shown highest antimicrobial activity against *A .niger* with all concentrations .*M. phaseolina, P. syringae, R. solani* and *X. campestris* are resistant with *R.mucronata* extracts. Both plants *R. mucronata* and *A.licifolius* have no activity with *A.flavus. A.licifolius* showing lowest activity against *X. campestris.*

MIC values are 90 and 100 mg/ml for *R. mucronata* where as 45-125 mg/ml for *A.licifolius.* Earlier antimicrobial activities of *A.licifolius* were reported by [10] and [11]. The differences in antifungal activity is due to the potential difference in the susceptibility of conidia, germinated

conidia and hyphae to antifungal compound and the time duration for the expouser of the compound. Although, the tested plant extracts may contain anti-microbial constituents, further phytochemical and pharmacological studies will be necessary to isolate the active constituents and evaluate the anti-microbial activity against a wide range of microbial population. Negative results do not indicate the absence of bioactive constituents, nor is that the plant inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed. Lack of activity can thus only be proven by using large doses.

CONCLUSION

From the results, it could be concluded that the *R. mucronata* and *A.licifolius* methanolic extracts may be useful as a broad-spectrum antimicrobial agents against phytopathogens. Although, the tested plant extracts may contain anti-microbial constituents, further phytochemical and pharmacological studies will be necessary to isolate the active constituents and evaluate the anti-microbial activity against a wide range of microbial population.

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