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Evaluation of Antimicrobial Activity of *Excoecaria Agallocha L*

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ABSTRACT

Excoecaria agallocha (L.) is an important medicinal plant inhabited in mangrove regions. Early researches focused on antimicrobial activity of leaves of concerned plant with various solvents among which ethanol, chloroform and methanol were Used.

KEYWORDS: *Excoecaria agallocha, Medicinal plants, Hexane, Ethyl acetate*

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INTRODUCTION

Mangroves are distinctive group of vascular plants that occur in saline coastal habitats (coringa estuary) and are known to tolerate extreme environmental conditions. Mangrove plants have primary and secondary metabolites such as proteins, carbohydrates, carotenoids, hydrocarbons, aliphatic alcohols, polyunsaturated fatty acids, lipids, pheromones, phorbol esters, phenolics, steroids, terpens, tannins and glycosides etc.[1]. These metabolites were described for bioactive substances as bactericidal, fungicidal, pharmaceutical agents for animal and human beings [2]. Application of chemotherapeutants has created problems via toxicity, resistance, residue leftover and possibly some public health and environmental consequences. Therefore, new drugs have to be found in order to combat such consequences and it is essential to find new compounds that have antimicrobial properties. *Excoecaria agallocha* L. (Euphorbiaceae) is a small mangrove tree found extensively in the tidal forests and swamps of the Krishna-Godavari area. This plant is also well- distributed in a number of other countries of temperate and tropical Asia. The bark oil has been found effective against rheumatism, leprosy and paralysis. This plant also has been traditionally used to treat sores and stings from marine creatures, and ulcers, as a purgative and an emetic. However, the milky sap of this tree can cause temporary blindness if it enters the eyes. The sap can also cause skin blisters and irritation. Clinical trials carried out on this plant have shown its potential anti HIV, anticancer, antibacterial and antiviral properties. The aim of this study was to test this medicinal plant extracts against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria and fungi. Therefore the present study deals with antimicrobial activity of Hexane, Ethyl acetate and methanol extracts of this plant against 4 bacteria and 3 fungal species.

MATERIALS AND METHODS

Plants extraction preparation:

Plant materials of two plant species included in this study were collected from coringa wildlife sanctuary and estuary situated in Andhra Pradesh, India. The collected plants were watery washed, disinfected, rinsed with distilled water and finally dried in shade.

The dried plant material of each plant species was grounded into fine powder to pass 100 mm sieve. 50 g of the fine powder was subjected to soxhlet extraction by using Hexane, Ethyl acetate and Methanol for 48 h resulting extracts in different solvents were evaporated and dried at 40 °C under reduced pressure using rotatory vacuum evaporator. The extract yields were weighted, stored in small bottles in fridge at 5 °*C and* these crude extracts were tested for standard strains of microorganisms.

Antibacterial activity of the plant extracts

Bacterial strains

The antibacterial potency of each plant extract was evaluated using five bacterial strains Two strains of Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and two strains of Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria. Three fungal strains (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*).The bacterial and fungal strains were provided from the microbial type culture (MTCC), Institute of Microbial technology, Chandigarh, India (**Table 1**).

Assay for antimicrobial testing

Isolated test bacteria were grown overnight on nutrient agar plates and fungi were grown on Sabouraud dextrose agar plates. Bacterial inoculums were prepared from overnight grown cultures (24 h) in liquid broth (Hi Media, Mumbai, India), and the turbidity was adjusted equivalent to 0.5 McFarland units (approximately 10^8 CFU/ml for bacteria and fungi inoculums turbidity was equivalent at 10^5 or 10^6 CFU/ml). The microorganisms were inoculated into liquid broth and incubated at 35 ± 2°C for 4 h. The positive control was taken streptomycin (10 µg/ml) for antibacterial activity and Ketocanozole (10 µg/ml) for antifungal activity. The DMSO was taken as negative control to determine possible inhibitory activity of the dilutant of extract. The susceptibilities of the isolated pathogens were determined by the modified Olurinola. 1996 [3] agar well diffusion method with Muller Hinton agar plates. Aliquots of inoculums were spread over the surface of agar plates with a sterile glass spreader. To test the antimicrobial activity all extracts were dissolved in DMSO to make a final concentration of 25 and 50µl. After culture medium poured in the Petri plates remained placed at room temperature for settling and then kept refrigerator for 30 minutes. After this procedure was done, took 3 number cup borer (6 mm) sterilized properly by flaming and then used to make uniform cups/wells in each Petri plate. Then cups/wells filled with different extracts and allow to diffusing the extract into the medium for about 45 minutes. These plates were incubated for a period of 24 h at 37°C in incubator for bacteria and at 30°C for 24-48 h in B.O.D incubator for fungi. Each experiment was done in triplicate and mean values were taken. Antimicrobial activity was measured in the diameter (mm) of the clear inhibitory zone formed around the well.

Results

The antimicrobial activity of E. agallocha extracts are shown in **Table 2** respectively. The results showed that the antimicrobial activities of the crude extracts were increased with increasing concentration. Although the antimicrobial activity of the extracts tested is variable, two gram-positiveve bacteria (*Staphylococcus aureus*, *streptococcus pyogenes*), two gram-*negative*ve bacteria (*E. coli*, *Pseudomonas aeruginosa*) and three strains of fungi (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*) were inhibited by the extracts.

(Table: 2) In E.agallochaall the extracts were found to possess various degree of antimicrobial activity against gram-positive Bacterial, gram-negative Bacterial and fungal strains. Among the tested extracts Hexane exhibited highest antibacterial potential of activity against all the tested bacterial species irrespective of their gram nature. However Ethyl acetate was found to be active after hexane. Methanol exhibited mixed response. Further, the higher zone of inhibition recorded in hexane extract against *Staphylococcus aureus* (2.2 ± 0.12 at 50µl concentration) followed by *streptococcus pyogenes* and E.coli (2.1 ± 0.08 at 50µl concentration). Minimum inhibition was noted in Methanol extract against *Pseudomonas aeruginosa* (0.3 ± 0.08 at 50µl concentration). Antifungal activity of *E.agallocha* the results revealed that Ethyl acetate extract gives somehow better results than Hexane and Methanol. Methanol solvent proved weak solvent than remaining solvents. Highest zone of inhibition noted in Ethyl acetate extracts against *Aspergillus niger* (2.1 ± 0.12 at 50µl concentration) and least also recorded from methanol extract against *Aspergillus niger* (0.8 ± 0.04 at 50µl concentration), *Aspergillus flavus* in methanol. Hexane extracts were resistant and methanol of *Aspergillus fumigatus* also resistant because there were no zones of inhibition observed.

DISCUSSION

Mangroves are inimitable assembly of vascular plants that happen in saline seashore front natural surroundings and are known to endure outrageous ecological conditions. The antimicrobial activity exhibited by the mangrove plant parts could be due to the presence of phytochemicals like alkaloids, tannins, flavonoids and sugars present in the plant extract. Primary and secondary metabolites are very important for the regular mechanism/survival of the species and also it can be used as therapeutic agents. Potential antimicrobial agents from mangrove species were due to the presence of phytoconstituent.

Prakash and Sivakumar 2013[4] reported antimicrobial activity of Excoecaria agallocha. For extraction they used fresh and dried parts of leaves, stem and roots. Ethanol used as solvent and they concluded that dried plant sample of leaf having higher inhibitory activity against pathogenic bacteria compared than the fresh plant extracts. Vadlapudi.et. al., 2009[5] reported antimicrobial activity of E.agallocha, they took leaves as interested parts, and Chloroform and methanol and hexane were used for extraction. They finalize from their findings that chloroform and methanol were found to be effective whereas hexane extracts were inactive. Ravikumar.et.al., 2010[6] reported antibacterial activity of the leaves of E.agallocha against selected fish pathogens. Sahoo et. al., 2012[7] had been reported antibacterial activity of E.agallocha leaf extracts against human pathogens. Among tested solvent ethanol extract showed its activity in all the tested pathogens. Lalith. A. A and Najiah. M 2014 [8] reported antimicrobial activity of *E.agallocha* against selected pathogens. Leaves were obtained via extraction with methanol and they concluded that methanol exhibited strong activity against tested fish pathogens. Parasuraman. P and Rameshwari. R. 2017 [9] reported antibacterial activity o*f E.agallocha* leaf in chloroform and ethanol extracts. Chloroform extract was highly effective on Bacillus cereus strain. So by reviewing all the literature, in this present study whole plant was used, and Ethyl acetate, Methanol and Hexane were utilized for extraction. Among tested solvents Hexane gives better activity against *Staphylococcus aureus* (2.2mm) and for fungi *A.niger (*2.1mm). This is also first record with hexane because no one get better activity with hexane so far. From our finding also concluded that Ethyl acetate was good for the fungal activity of both the plants.

Conclusion

Excoecaria agallocha L. extracts had the ability to inhibit bacterial growth. In the present study whole plant were assessed to test antimicrobial activity against human pathogens. By the results it was concluded that Ethyl acetate for *Excoecaria agallocha* L. Among tested pathogens *P. aeruginosa* and *S. aureus* display highest inhibitory activity for 2 plants respectively. All these extracts were not effective than antibiotics to combat the pathogenic microorganisms studied. This indicated that these plants have potentially antibacterial properties and could be used in the development of novel antibacterial agents.

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Table 1: Details of the bacterial and fungal strains used in bioassay

S. No	Name of the Bacterial/fungal Strains	MMTC Catalogue No.
Gram Positive Bacteria		
1	Staphylococcus aureus	MTCC 3160
2	Streptococcus pyogenes	MTCC 442
Gram Negative Bacteria		
3	Escherichia coli	MTCC 443
4	Pseudomonas aeruginosa	MTCC 424
Fungal pathogens		
5	Aspergillus niger	MTCC 961
6	Aspergillus flavus	MTCC 3396
7	Aspergills fumigatus	MTCC 2584

Table 2: Antimicrobial activity of different solvent extracts of *Excoecaria agallocha L.*

Organism	Solvent	DMSO	25µl	50µl	Control
					Streptomycin (10 µg/ml)
Staphylococcus aureus	EA	1.2±0.08	1.1±0.12	1.6±0.12	ND
	ME	1.7±0.08	1.9±0.12	2.0±0.12	1.0±0.12
	HE	1.6±0.04	1.3±0.08	2.2±0.12	ND
Streptococcus pyogenes	EA	1.2±0.04	0.7±0.08	1.2±0.09	ND
	ME	0.8±0.04	ND	ND	ND
	HE	1.3±0.04	1.6±0.12	2.1±0.08	0.7±0.08
Escherichia coli	EA	1.7±0.04	1.8±0.08	2.1±0.08	0.7±0.04
	ME	1.6±0.08	0.7±0.08	1.0±0.09	ND
	HE	1.4±0.04	1.1±0.12	1.5±0.09	0.4±0.08
Pseudomonas aeruginosa	EA	1.6±2.22	0.83±0.08	1.5±0.12	0.4±0.04
	ME	0.8±0.04	ND	0.3±0.08	ND
	HE	1.8±0.04	0.5±0.04	0.8±0.08	ND
					Ketocanozole (10 µg/ml)
Aspergillus niger	EA	1.4±2.22	0.83±0.12	2.1±0.12	ND
	ME	1.3±0.04	ND	0.8±0.04	ND
	HE	0.8±0.04	0.4±0.08	1.0±0	ND

Note: expand the abbreviations
EA = Ethyl acetate, ME = Methanol, HE = n-hexane, DMSO = Dimethyl Sulfoxide Negative control, ND =, and Control = Positive control

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