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PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.4, No.1, pp 245-249, Jan-Mar 2012

Bioactive potential of *Exoecaria agallocha* collected from Pichavaram Mangrove area, South east coast of India-an *In vitro* study

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Abstract: The present study is on antimicrobial activity of *Exoecaria agallocha* collected from Pichavaram area . Leaves were shade dried and extracted successively with methanol and Chloroform. The antimicrobial activity of the solvent extracts on various microbial human pathogens was tested using agar well diffusion technique. The study proved the medicinal value of *Exoecaria agallocha.*
Key Words: Mangrove plant *Exoecaria*, Methanol, Chloroform, Human pathogens.

INTRODUCTION

Indiscriminate use of antibiotics develop resistance in pathogens. Due to this reason it becomes necessary to discover new antibiotics to control infectious diseases. Higher plants play an important role in providing antibiotic compounds. They are rich in active principles, which are used as therapuetic drugs.[1] India as a mega biodiversity country, many medicinal plants are already in use to control number of diseases. However the biomedical potential of mangrove plant is not well understood. Hence the present study.

MATERIALS AND METHODS

Preparation of extracts

The fresh plant leaves were thoroughly washed and air dried immediately. The 300g of pulverized leaves were soaked with methanol and chloroform at 1:6 ratio and kept for 15-20 days at room temperature for extraction. The above solvent extracts were then evaporated using an evaporator and they redissolved in the same solvents and used as test samples. Solvents were kept as control.

Preparation of inoculum

Microorganisms are grown in nutrient broth at 37°C. The log phase culture was used for the antimicrobial activity.

Composition of Nutrient Broth

Peptone	5.0 g
Beef extract	3.0 g
NaCl	5.0 g
Distilled water	1000 ml
pH	7.1

Composition of Muller Hinton Agar

Beef, infusion	300.00 g
Casein acid hydrolysate	17.50g
Starch	1 .50g
Agar	15.00g
Distilled water	1000 ml
pH	7.3 ± 0.

Antibacterial activity

Nutrient broth was prepared and sterilized. Ten different human pathogens such as *Pseudomonas sp., Bacillus cereus, Aeromonas hydrophila, Shigella flexneri, Vibrio cholerae ElTor, V. cholerae 0139, Salmonella sp., V. cholerae classical, V. cholerae 01790* and *E. coli* (ETEC) were inoculated separately and kept for incubation.

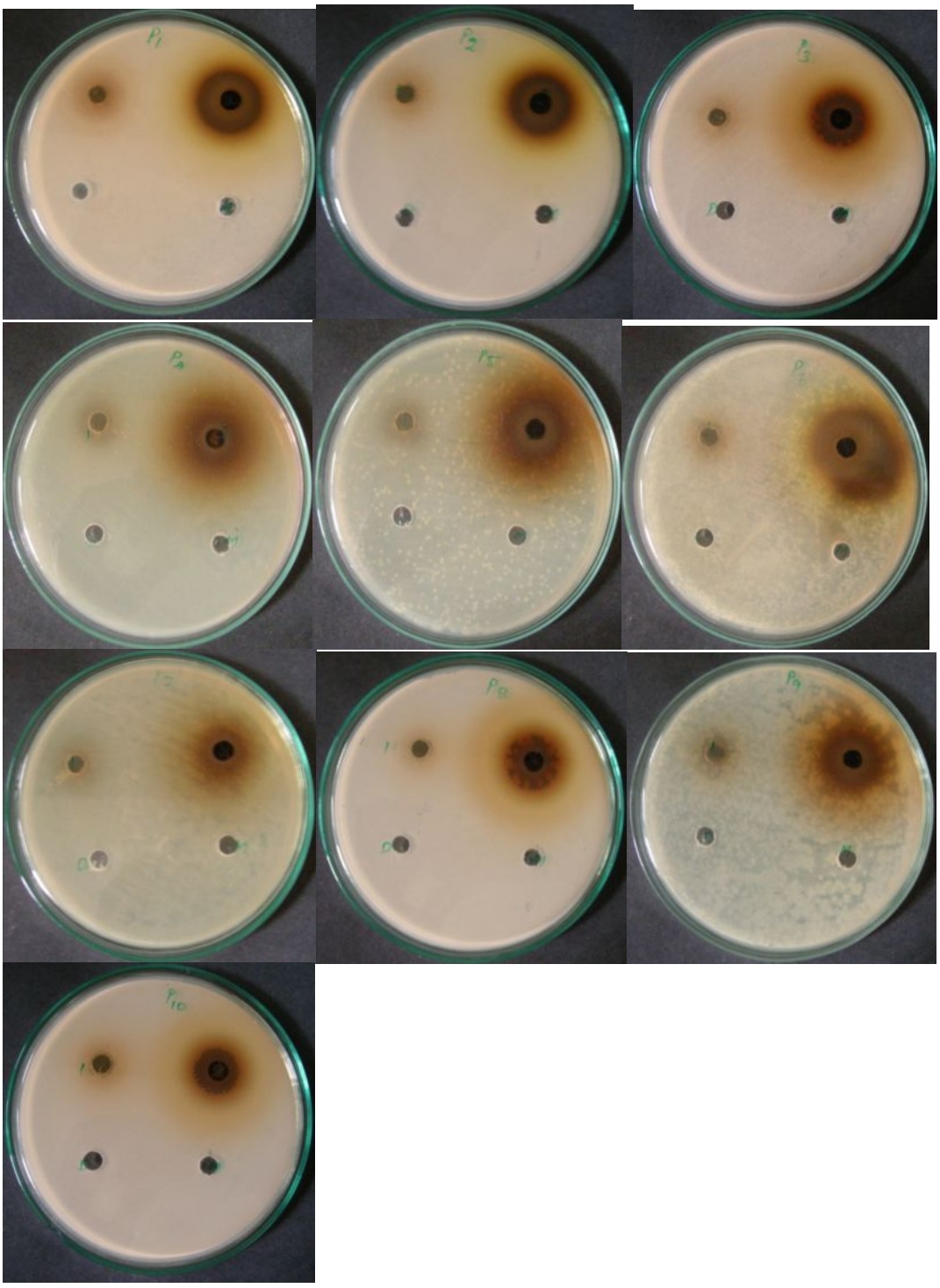
Agar well diffusion method

The agar well diffusion assay as per the procedure of Varahalarao *et al*[2]., was adopted in the present assay. Each bacterial suspension was spreaded over the surface of Muller-Hinton Agar Plates. The plates containing wells of 6mm diameter were filled with 50µl extracts. The plates were then incubated at 37°C for 24 hrs. The results were expressed in terms of the diameter of the inhibition zone.

Table:1 Zone of the inhibition of *Exoecaria agallocha* extract

Name of the organism	**Zone of the clearance (mm)Methanol Chloroform Extract**	
P1-Pseudomonas aeruginosa	15	4
P2-Bacillus cereus	16	5
P3-Aeromonas hydrophila	13	4
P4-Shigella flexneri	7	6
P5-Vibrio cholerae ElTor	13	-
P6-V. cholerae 0139	17	6
P7-Salmonella typhi	8	-
P8-V. cholerae classical	10	4
P9-V. cholerae 01790	12	-
P10-E. coli (ETEC)	16	5

Fig:1 Zone of the inhibition of *Exoecaria agallocha* extract



RESULTS AND DISCUSSION

The study proved that the methanolic extract of the *Exoecaria* leaves was inhibitory to all the ten pathogens tested. Chloroform extract was also inhibited 7 pathogens. However the zone of inhibition was comparatively lesser. The results were significant as *V.cholerae* 0139 strain, the one which caused cholera in India was the most inhibited followed by other enteric pathogens which were already proved as deadly pathogens. Other serious pathogens like ETEC *E.coli* and *Salmonella typhi, Shigella flexneri* were also been inhibited at various levels. The study clearly indicated the plant contained active molecules against gram positive and gram negative pathogens and has the potential to develop new drugs.

The presence of enterobacteria in food and water is a common cause of diarrhea, dysentery and enteric fever especially in developing countries. *V.cholerae* strains like *Vibrio cholerae ElTor, V. cholerae 0139, V. cholerae classical* and *V. cholerae 01790* used in the present study are important as they are the causative agent of cholera epidemics in Asian countries. The methanolic extract seemed to be highly inhibitory to all *V.cholerae* strains tested besides equally killing other enteric pathogens viz. Enterotoxigenic *E.coli* (ETEC) and *Aeromonas hydrophila* eventhough *Pseudomonas aeruginosa* is an opportunistic pathogen, in developing countries especially where HIV and other immune related diseases are prevalent, this pathogen deserves to be controlled.

V.cholerae 0139 strain was most inhibited by methanolic extract *Exoecaria* leaf extract followed by *B.cereus> ETECE.coli> P.aeruginosa >V.cholerae ElTor=A.hydrophila> V.cholerae* 0139>*S.typhi* and *S.flexneri. B.cereus* being a gram positive bacteria and a spore former its inhibition deserves special mentioning.

Pseudomonas aeruginosa was more resistant to most of the antibiotics commonly used in clinical practices[3].It was resistant to many herbal extracts also[4]. However in the present study it was highly susceptible to the methanolic extract of *Exoecaria agallocha.*

The use of plants as a source of medicine in treating disease is an age old practice. Plants produce a variety of bioactive molecules making them rich source of different types of medicines[5]. Compared to conventional pharmacuetics herbal based medicines are believed to have lesser or no side effects and cost effective also. Hence large population of people in developing countries relie on folk medicines for common infections and chronic diseases.There is a growing interest among scientist in identifying the compounds responsible for the cure.

Thus the present study clearly indicated the potential of *Exoecaria agallocha* extract in drug formulation for severe enteric diseases. Further research is going on to identify the active molecules responsible for the therapeutic potential.

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