

Isolation and Characterization of Biopotential Antimicrobial Compounds from Mangrove Plant Species (*Excoecaria Agallocha*)

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Abstract:- *Excoecaria agallocha* is a milky mangrove widely distributed in India coastal regions. This species has many common names including thillai, milky mangrove, Blind your eye mangrove and river poison tree. It is extremely important medicinal plants for against harmful human pathogenic micro organisms. The crude extractions were prepared by using five different solvents like water, ethanol, methanol, acetone and benzoic acid. The extracted sample was tested against pathogenic bacteria isolates of *E.coli*, *Pseudomonas*, *Bacillus*, *Streptomyces* and *Salmonella* species. The acetone extract was showed against the all bacterial species in minimum zone of inhibition (11-15nm) followed by moderately were ethanol, methanol, benzoic acid and water. The acetone extract of *Excoecaria agallocha* was shown the well activity of all pathogenic bacteria.

Keywords: Mangrove species, Pathogen, Antimicrobial activity, and Zone of inhibition

INTRODUCTION

Mangrove forests are among one of the world's most productive tropical ecosystems and are highly potential because the ecosystem is always under stress which leads to the production of certain compounds for their survival. India harbors some of the best mangrove forests of the world which are located in the alluvial deltas of the major rivers such as the Ganga, Mahanadi, Godavari, Krishna, and Cauvery also on the bay of Andaman and Nicobar Islands. It covers about 6,749 sq km along the 7,516.6 km long coast line, including Island territories (P.Saranraj et al 2015). Mangrove plants require a number of physiological adaptations to overcome the problems of anoxia, high salinity and frequent tidal inundation. These compounds are synthesized by primary or secondary metabolism of living organism.

The genus of *Excoecaria* comprises nearly forty species which are distributed in the mangrove region of Asia, Africa and Northwest Australia. *Excoecaria agallocha* is a milky mangrove widely distributed in India coastal regions. This mangrove species belongs to the genus *Excoecaria* of the family *Euphorbiaceae*. It also known as a back mangrove, is found at higher elevations back away from the ocean where salinity is lower.

This species has many common names including Thillai, milky mangrove, Blind your eye mangrove and river poison tree. Most of the names refer to its poisonous or blinding nature. The milky latex discharges from *E. Agallocha* bark is poisonous and may cause temporary blindness and blistering of skin. The latex is also known for its biocidal effects on marine microbes and phytoplankton. It causes metabolic depression of rice field crab (*Oziotelphusa senex*) and is used as an uterotonic, fish poison, dark poison and contains novel chalcones and piperidine alkaloids.

MATERIALS AND METHODS

The fresh leaves of *E. Agallocha* were collected from coastal region pudhucherry in Tamilnadu. The sample was washed with sterile water for removing dust particles. After shadow drying the leaves were powdered by using mechanical grinder. Then the powder keeps in air tight container.

The powdered sample was extracted by using five different solvent like Water, Acetone, Methanol, Ethanol and Benzoic acid. Twenty five gram of fine powder was added to a soxhlet apparatus along with each solvent one by one for extraction of chemicals. The liquid extracts were evaporated to dryness by vacuum distillation and stored at 4°C for further analysis (Patra et al., 2008).



Figure 1: Extraction of sample

ANTIMICROBIAL ACTIVITY

Here we are using five different microorganisms for anti-microbial activity. They are namely *E.coli*, *Pseudomonas*, *Bacillus*, *Streptomyces* and *Salmonella* species. These species obtained from Department of Marine Science, Bharathidasan University, Tiruchirappalli. These strains are maintained and tested on nutrient agar medium.

In this study we are using Agar well diffusion method for Antimicrobial activity. The agar well diffusion method described by (Smania et al 1995) was adopted for antibacterial assay. 10 ml of nutrient agar medium poured to sterile Petri plates in aseptic condition at room temperature. After solidification each test organisms were streaked on separate agar plates by using inoculation loop. Wells of 6 mm diameter were punched over the agar plates

using sterile gel puncher. 100 µl of five different plant extract (Water, Ethanol, Methanol, Acetone and Benzoic acid) were poured into the wells of separate agar plates.

The plates were incubated at 37°C for 24 hours for bacterial growth. The zones of inhibition were measured with antibiotic zone scale in mm.

RESULTS AND DISCUSSION

Antimicrobial activity:

The antibacterial activity of *Excoecaria agallocha* extracts (Acetone, Benzoic acid, ethanol, water and methanol) against five different microorganisms by agar well diffusion method. The Acetone extract showed considerably more activity than other extracts. Maximum antibacterial activity was shown against *Salmonella* (Table-1).

STRAINS	ACETONE	BENZOIC ACID	ETHANOL	METHANOL	WATER
<i>E. coli</i>	7.5±0.85 mm	ND	6±1.5 mm	4.86±0.76 Mm	6.5±2.76 mm
<i>Pseudomonasputida</i>	5±0.54 mm	5.7±1.43 mm	5.7±1.43 mm	9.3±2.24 Mm	8.65±0.8 mm
<i>Bacillussubtilis</i>	11.5±0.64 mm	6.23±2.4 mm	5.78±0.7 mm	ND	4.78±0.5 mm
<i>Streptomycesgriseus</i>	7.8±1.5 mm	ND	6±1.8 Mm	8.7±0.67 Mm	3.65±0.65 Mm
<i>Salmonellatyphi</i>	13±1.5 mm	9.6±2.1 mm	6.87±0.2 mm	7.98±0.6 mm	9.35±0.76 mm

(mm – zone of inhibition, ND- not detected)

Table 1: Antimicrobial activity of different solvent extract of *Excoecaria agallocha*.



Figure 1: Antimicrobial activity of various extraction of *Excoecaria agallocha*.

In this study Benzoic acid extract given minimum zone of inhibition against *salmonella* ($9.6\pm 2.1\text{mm}$) then *Bacillus* ($6.23\pm 2.4\text{mm}$) species. This extract gives less zone of inhibition ($1>$) against *E.coli* and *Stretomyces* species. Other Ethanol, Methanol and Water extract shows minimum zone of inhibition against test organisms. Only acetone extract gives higher inhibitory on *Salmonella* ($13\pm 1.5\text{mm}$) and *Bacillus* ($11.5\pm 0.64\text{mm}$) strains.

The studied plant was most active against gram-negative bacteria than gram positive bacteria. In case of solution with low activity, a large concentration or volume is needed. In general gram-positive bacteria are considered more sensitive than gram negative bacteria towards different antimicrobial compounds because of the difference in the structure of their cell walls (Scherrer and Gerhardt, 1971) but our result showed that the extracts are effective against both gram-positive and gram-negative bacteria. Earlier report suggests (Agoramoorthy et al., 2007) significant antimicrobial activity of the fatty acid methyl ester extracts of the leaves of *E. agallocha*, which corroborates to our findings. Antimicrobial properties of

substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infections and in food spoilage. The active constituents of plants usually interfere with growth and metabolism of microorganisms in a negative manner (Aboaba et al 2006).

Excoecaria agallocha is a well-known mangrove plant, with reports on its chemical constituents. Investigations on the presence of metabolites from the plant revealed the presence of diterpenoids, triterpenoids, flavonoid, glucoside, polyphenols and phorbol esters. Antibacterial compounds are important as bacteria are responsible for a wide variety of disease conditions including many dental diseases (dental caries, bleeding gum, gingivitis) and prevention of bacteria helps in controlling these diseases.

Present work investigated on antibacterial activity of the extract of *Excoecaria agallocha* various organic solvents were used for the extraction of the bio-active compounds of the plants (Saha et al., 1995). The leaves of *Excoecaria agallocha* have been reported to use for the treatments of eye diseases, tooth hacks, rheumatoid

arthritis, etc., (Das and Das, 1994). In our work the organic solvent of acetone was used to extract the bioactive compounds of leaves of the plant. Mangrove forests have played an important role in the Socio economics life of the people. The mangrove forests have several valuable medicinal plants that are used in medicinal fields (Azariah et. al 1987, Thomas et al 1996, Das and Das 1994). This study has revealed the antibacterial activity of mangrove plants and can be suggested that the bioactive contents of the mangrove plants are promising natural antimicrobial agents that can be harnessed as potential antibacterial toxicants. Further, extensive studies are recommended for these mangrove plants samples to actually identify the bioactive compounds responsible for their antimicrobial activities. From the results it can be concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant microorganisms. *Excoecaria agallocha* showed maximum antibacterial activity and so this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hitherto unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of important lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.

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