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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF *EXCOECARIA AGALLOCHA* L. LEAF IN ACETONE AND PETROLEUM ETHER EXTRACTS

[1]Parasuraman, P. and [2,*] Dr. Manikandan, T.

[1]Govt.Hr.Secondary School, Devikapuram, Tiruvannamalai – 606902, Tamilnadu, India
[2]Department of Botany Arignar Anna Govt. Arts College Villupuram - 605602, Tamilnadu, India

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ABSTRACT

Antibacterial activity of *Excoecaria agallocha*. L. (Euphorbiaceae) was investigated. Leaf extracts of Acetone and Petroleum ether were tested against five bacteria viz. Staphylococcus aureus MTCC3381, Escherichia coli MTCC739, Bacillus cereus MTCC430, Pseudomonas aeruginosa MMTC424, Klebsiella pneumoniae MTCC432. The results of antibacterial activity of acetone extract was highly effective when compared with petroleum ether extract. The gas chromatography and mass spectrometry (GC-MS) analysis was carried out for bioactive components from the acetone and petroleum ether extracts. 24 bioactive compounds are identified. β- Amyrin 8.03 %, Lupeol 7.56%, 4-0 Methylmannose 5.43%, γ-Sito sterol 6.35 %, Tricycloundec 11.39%, 1-1-4a - Trimethyl -5,6-Dimethylene 21.90%, are higher in proportion than other components. Petroleum ether extract showed 20 bioactive compounds,in that α- Amyrin 6.74%,β- Amyrin 6.93%, 1,19-Eicosadine 8.22%, Hentriacontane 8.25%, 2,5,5,8a - Tetramethyl - 6,7-8,8a - Tetra hydro 26.12%, Tricycloundec 19.95% are high in proportion. α - Amyrin and β- Amyrin are good tonic for liver, and is useful for cancer treatment. Lupeol displaying antiprotozoal, antibacterial, antiinflammatory, antitumour and chemopreventive properties.

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INTRODUCTION

Excoecaria agallocha L. is a back mangrove found in lower salinity and back away from the ocean. It belongs to family Euphorbiaceae. This species is naturally growing in abundance in the mangrove belt of pichavaram, on the east coast of Tamil Nadu, India. (11°24'N and 79°44'E) about 13km east of Annamalai University, Chidambaram. This plant is also well distributed in other countries of temperate and tropical Asia. This small tree may grow up to 15m high with acrid milky juice. Flowers are unisexual. The milky latex of *Excoecaria agallocha* L. is very poisonous. It cause blisters and irritation when contact with skin. Many plants are used for treat disease for human beings. Synthetic antibiotics are ineffective against several pathogenic organisms but plant extracts are effective on pathogens. Mangrove plants are useful in traditional medicines (Kokpal *et al*., 1990, Premanathan *et al*., 1996). It cause temporary blindness when contact with eyes. So it is named as "Blind-your-eye mangrove" (Agoramoorthy *et al*., 2007). Thillai, milky mangrove and river poison tree are some of the names of *Excoecaria agallocha* L. The plant parts are used traditionally for treatment of ulcers, leprosy and cancer. The present study was carried out by evaluating the antibacterial activity of two different solvent extracts (Acetone and Petroleum ether) of *Excoecaria agallocha* L. leaf extract.

Corresponding author: Dr. T. Manikandan
PG and Research Department of Botany, Arignar Anna Govt. Arts College, Villupuram - 605602, Tamilnadu, India

MATERIALS AND METHODS

Collection and identification of plant material

Fresh and mature leaves of *Excoecaria agallochaL*. belongs to Euphorbiaceae was collected during monsoon season in the mangrove belt of pitchavaram on the east coast of Tamilnadu (11°24'N and 79°44'E) about 13km east of Annamalai University, Chidambaram. The present study was carried out in the Botanical garden of Arignar Anna Govt. Arts College Villupuram. The collected plant material was carefully examined and identified with the help of "FLORA OF THE PRESIDENCY OF MADRAS" (Gamble, 1954). The leaves were washed under running tap water two to three times to eliminate dust and other foreign particles. Air dried in shadow and them homogenized to fine powder and stored in air tight containers in refrigerator at 4°C for further analysis.

Preparation of plant extracts

For the preparation of leaf extracts soxhlet extractor (Franz von soxhlet in 1879) was used. The powder was successfully extracted using soxhlet apparatus with Acetone and Petroleum ether. This extracts were condensed using rotary vacuum evaporator.

Preparation of inocula

Inoculation was done with the help of MISAT lab Coimbatore. The test organisms were sub cultured by streaking them on nutrient agar followed by incubation for 24h at 37°C. Several colonies of each bacteria species were transferred to sterile nutrient broth. The suspensions were mixed for 15sec and incubate for 24h at 37°C on an orbital incubated shaker. Working concentrations of microbial suspension was prepared in 3ml of sterile saline to turbidity equivalent to 0.5mc land scale (10 adjusting the potical density to 0.1 at 600nm yielding a cell density of 1-2x10^5 CFU/ml.).

Antibacterial assay

Nutrient agar (NA) plates (12cm diameter) were seeded with 8h broth culture of different bacteria. In each of this plates well were (6mm diameter) cut out using sterile cork borer. Using sterilized dropping pipettes different concentrations (500, 1000, 1500 and 2000µg /ml) of plant extract was carefully added in the wells and allowed to diffuse at room temperature for 2h (Well diffusion method Perez *et al*., 1990). The plates were then incubated at 37°C for 18-24h. Chloromphenical (10µg) was used as positive control and DMSO as negative control. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone (Agwa *et al*., 2000)

Table 1. Antibacterial activity of *Excoecaria agallocha* L. leaf in acetone extract

Sample	Conc (µg)	Zone of Inhibition(mm)				
		S.a	E.c	B.c	P.a	K.p
Acetone	500	20.0±0.0	12.0±0.0	13.5±0.7	-	11.5±0.0
	1000	22.5±0.7	15.0±0.0	17.0±0.0	16.0±0.0	14.0±0.0
	1500	25.0±0.0	16.5±0.7	18.5±0.0	18.5±0.7	15.5±0.0
	2000	26.5±0.7	18.0±0.0	20.5±0.7	21.0±0.0	17.0±0.0
Chloromphenicol	10	20.5±0.7	14.0±0.0	20.5±0.7	14.0±0.0	19.5±0.7

Values are means of three independent analysis ± Standard Deviation(n=3)

Table 2. Antibacterial activity of *Excoecaria agallocha* L. leaf in petroleumether extract

Sample	Conc. (µg)	Zone of Inhibition(mm)				
		S.a	E.c	B.c	P.a	K.p
Petrolium ether	500	-	-	-	-	-
	1000	-	-	10.0±0.0	-	-
	1500	11.0± 0.0	-	11.0±0.0	10.5±0.7	12.0±0.0
	2000	12.0±0.0	10.0±0.0	12.5±0.7	12.0±0.0	13.0±0.0
Chloromphenicol	10	20.5±0.7	14.0±0.0	20.5±0.7	14.0±0.0	19.5±0.7

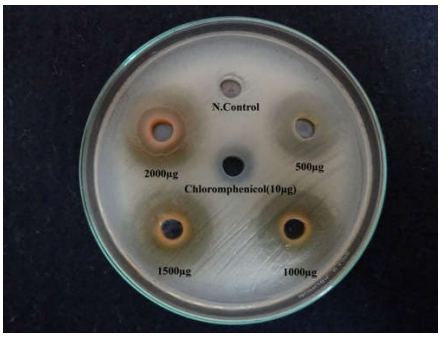
Values are means of three independent analysis ± Standard Deviation (n=3)

RESULTS AND DISCUSSION

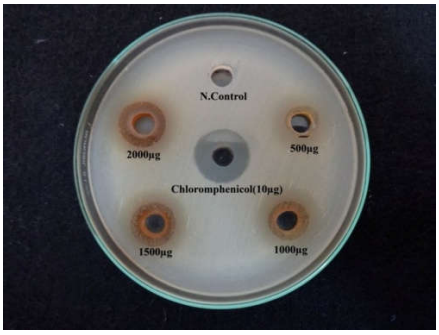
On the basis of gram staining (Christian gram 1884) Staphylococcus aureus and Bacillus cereus are gram positive strains. *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* are gram negative strains. Acetone extract of *Excoecaria agallocha* L. showed high inhibitory effect against *Staphylococcus aureus, Escherichiacoli, Bacillus cereus, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* in 500, 1000,1500,2000 µgs except in 500 µg on *Pseudomonas aeruginosa* Table -1, Plate-1. The observed activities of the two different extracts may be due to varying degrees of active constituents in the solvents. Different solvents have diverse solubility capacity for different phytoconstituents (Marjorie, 1999). From the tested bacteria gram positive bacteria were more susceptible to the extracts than the gram negative bacteria. This finding is similar with the extracts of *Launaea procumbans*, *Vitis vinifera* and *Cyperes rotundus* (Jigna and Sumithra, 2005). *Staphylococcus aureus* was inhibited 14-20mm in 1.8mg (Anas *et al*., 2007).

Antibacterial activity of leaf extract of *Mangifera indica* against *S.aureus* (50mg/ ml) 4mm, 100mg/ml=7mm, 150mg/ml=10mm, 200mg/ml=13mm, 250mg/ml=15mm was recorded by Doughari and Manzara (March, 2008). *S. aureus* (20mm), *B.cereus* (15mm), *P. aeruginosa* (15mm), *E. coli* (10mm), *K. pneumoniae* (10mm) were infected in the Acetone leaf extract of black pepper (Pavithra *et al*., 2009). *Abutilon pannosum* leaf extract of Acetone showed moderate inhibition on *S.aureus* (13.5± 0.4) (Survase *et al*., 2013). Acetone extracts of *Parthenium hysterophorus* showed 75% inhibition on *Bacilus cereus* (Malarkodi and Manoharan, 2013). Petroleum ether extract of *E.agallocha*, L. was inactive against *S.aureus*, *B.cereus*, *E.coli*, *P. aeruginosa*, *K. pneumoniae* at 500mg/ml and 1000mg/ml concentrations (Table 2 and Plate 2). The same result was found by Jayachandran *et al*., 2003 in *Tinospora cardifolia* plant. Antibacterial activity of *Annona squamosa* petroleum ether extract at conc. 1mg/ml against *E.coli, P.aeruginosa* showed mild effect but no inhibition on *S.aureus* (Jayashree D Patel and Vipin kumar July-2008). Tridax erecta showed significant antimicrobial activity against gram positive and gram negative bacteria (Gupta and Vasudeva, 2010). Pet.ether extract of *Aloevera* showed minimum inhibition against *K.pneumoniae* (Thirupathi *et al*., 2010). *Petrolium ether* extract *Clitoria ternatea* showed inhibition on *B.cereus* but ineffect on *K.pneumoniae, S.aureus* (Anand *et al*., 2011). Pet.ether extract of *Tridax procumbans* L, *Phyllanthus niruri* and *Tagetus erecta* L. were not showed antibacterial activity (Dangi *et al*., 2012). Antibacterial activity

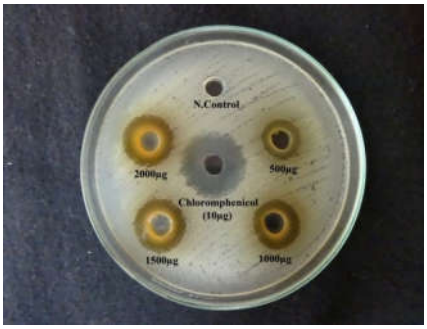
Plate 1. Antibacterial activity of *Excoecaria agallocha* L. in acetone extract



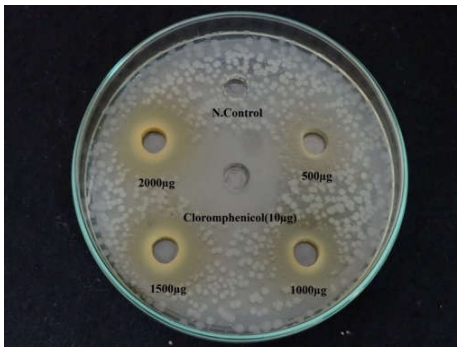
Excoecaria agallocha **Acetone (S.a)**



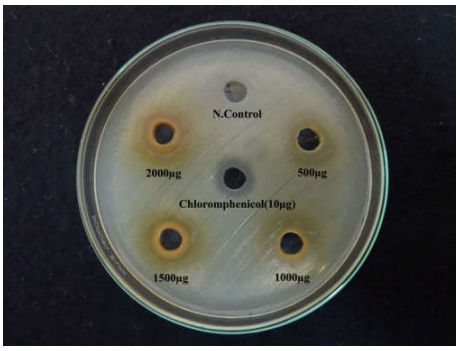
Excoecaria agallocha **Acetone (E.c)**



Excoecaria agallocha **Acetone (B.c)**

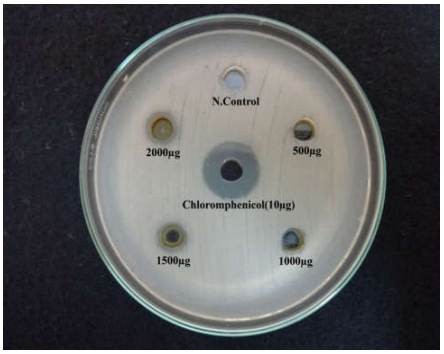


Excoecaria agallocha **Acetone (P.a)**

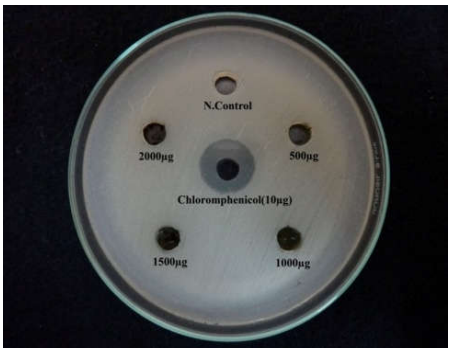


Excoecaria agallocha **Acetone (K.p)**

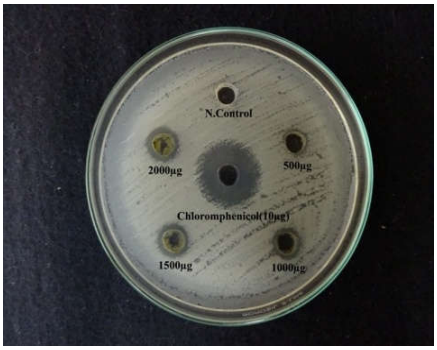
Plate 2. Antibacterial activity of *Excoecaria agallocha* l. in petroleum ether extract



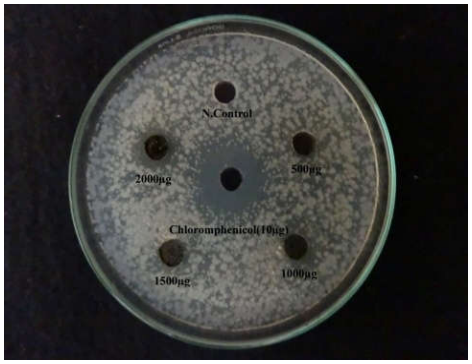
Excoecaria agallocha **Pet.ether (S.a)**



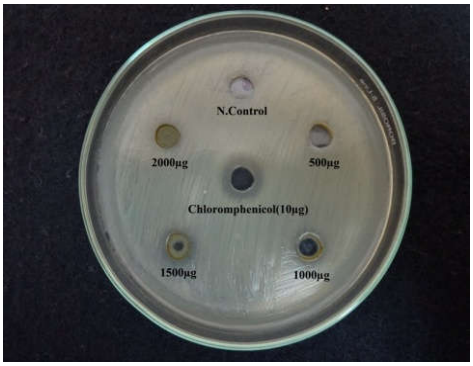
Excoecaria agallocha **Pet.ether (E.c)**



Excoecaria agallocha **Pet.ether (B.c)**



Excoecaria agallocha **Pet.ether (P.a)**



Excoecaria agallocha **Pet.ether (K.p)**

of *Casuarina equisetifolia* extraact of *Petrolium ether* in 100mg/ml concentration was moderately effective On *S.aureus*, *E.coli*, *K.pneumoniae*, *P.aeruginosa* (Nehad M. Gumgumjee *et al*., 2012). Petrolium ether extract of *Mimusops elangi* was not effective at 200mg/ml. but mild effect at 300 mg/ml (Milimita padhi and Sujata mahapatra, 2013).

Conclusion

Acetone extract of *Excoecaria agallocha* L. is exhibited significant inhibition against S.aureus, E.coli, B.cereus, P.aeruginosa K.pneumoniae. The inhibition of pet.ether is 50% against the micro organisms. Further research is needed for *Excoecaria agallocha* L. for a intensive pharmacological uses.

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