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BIOAUTOGRAPHY SCREENING OF A MANGROVE *Excoecaria agallocha* L.

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ABSTRACT

The aqueous leaf extracts of *Excoecaria agallocha* were evaluated for antibacterial activity along with their phytochemical screening. The minimum inhibitory concentration (MIC) of gram positive and gram negative organisms were analysed by micro titre plate technique. Preliminary phytochemical screening revealed the presence of tannins, alkaloids, steroids, flavonoids and anthroquinone glycosides which might be the responsible factors for bioactivity against chosen bacterial pathogens. Hence *Excoecaria agallocha* aqueous leaf extracts is more sensitive for gram negative organisms.

Key words: *Excoecaria agallocha*, Antibacterial activity, MIC, Phytochemical screening.

INTRODUCTION

In ancient days, herbal plants were used in the treatment of various diseases (Ravi Kumar, 2009). The plants are still important sources of medicines, especially in developing countries. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs. Herbal medicines are often used to provide first-line and basic health service, both to people living in remote areas where it is the only available health service, and to people living in poor areas where it offers the only affordable remedy.

Herbal plants have high medicinal value as they have the rich source of bioactive compounds and have no side effects. The bioactive compounds from the herbal plants reduce the virulence of the microorganisms, thereby preventing and protecting from the infections.

Mangroves are various types of trees up to medium height and shrubs that grow in saline coastal sediment habitats in the tropics and subtropics – mainly between latitudes 25^{o}N and 25^{o}S. It is an exclusive group of plants with remarkable ecological importance, inhabit mostly the zones washed by the back and forth movement of tides.

Mangrove plants are rich source of bioactive compounds and are utilized in many parts of the world as a renewable resource. Many secondary metabolites like alkaloids, phenolic, steroids, and terpenoids have been characterized from mangroves which have pharmacological importance (Patra and Thatoi, 2011; Choudhury, 2005).

Excoecaria agallocha is a mangrove species belongs to the family Euphorbiaceae. It is commonly known as Thillai and milky mangrove. It is also known as back mangrove, since it is found were salinity is lower. This species is common in mangrove swamps from India in the northwest to Australia in the southwest. This species is a small tree that can grow up to 15m height. Due to its complex chemistry, this plant has many medicinal uses. *Excoecaria agallocha* is rich in diterpenoids, triterpenoids and flavonoids. It is used as folk medicine and has biologically active anti-HIV, anti-cancer, anti-bacterial and anti-viral compounds (Patra, 2012). It is used as purgative and in the treatment of leprosy, rheumatism, epilepsy, flatulence, dermatitis and toothache (Sheikh JH, 2009). Hence this study aimed at the antibacterial activity and phytochemical analysis.

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MATERIALS AND METHODS

Collection of Plant Material

Fresh, young leaves of *Excoecaria agallocha* L. were collected from the estuary of Allapakkam, Cuddalore. The specimen was identified at the centre for advanced studies in marine biology at Annamalai University, Parangipettai, and Tamil Nadu. The leaves were air dried in shade for 15 days and then pulverized to fine powder for further analysis.

ANTIBACTERIAL SCREENING

Microbial Strains Used

Different microbial strains were used to evaluate the antimicrobial effect of which two were gram positive bacterial strains (i.e) *Staphylococcus aureus*, *Bacillus subtilis* and three were gram negative bacterial strains (i.e) *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae.* The strains were obtained from Jamal Mohamed College, Trichy, Tamil Nadu, India and maintained on agar slants.

Disc Diffusion Method

Disc diffusion method was carried out for antibacterial susceptibility testing according to the standard method to assess the presence of antibacterial activities of the plant extract (Ravikumar, 2011; Kim, 1999; Nusrat S, 2008). Muller Hinton agar (MHA) plates were prepared. Overnight nutrient broth culture of test organisms were seeded over the MHA plates using sterile cotton swab so as to make lawn culture. The discs which had been impregnated with aqueous extracts of leaf were placed on the MHA plates with the control disc and subjected to antibacterial screening. The plates were then incubated at 37^{o}c for 18 to 24 hours depending on the species of bacteria used in this test. After the incubation, the plates were examined for inhibition zone.

Chi-Square Test (χ^2)

In this study chi-square test (χ^2) was applied. The purpose of chi – square test (χ^2) was to decide whether the set of observed data (Antibiogram of microorganisms) agrees with the standard antimicrobial disc susceptibility test (NCCLS, 2002).

MIC- Using Micro Titre Plate Technique

Phenol red indicator method

Tryptone soya broth and 0.2% of phenol red solution was prepared. For use 5ml of 0.2% phenol red solution was mixed in 100 ml tryptone soya broth media. Add 50µl of this phenol red mixture media into micro titre wells from 1to9 columns. The plant extract was prepared by 100 grams of *Excoecaria agallocha* leaf powder was dissolved in 50 ml of distilled water and filtered. Add 50µl of plant extract to column 1 and mix well. Now transfer 50µl from column 1 to column 2, likewise follow till column 9 and discard 50µl from column 9. To this add 50µl of bacterial culture and incubate. Mc Farland Nephelometer Standard was prepared to approximate the turbidity of a bacterial suspension.

PHYTOCHEMICAL ANALYSIS

A qualitative phytochemical test to detect the presence of alkaloid, tannin, saponin, flavonoid, glycoside and phenolic compounds were carried out using standard procedures (Brindha *et al.*, 1981).

RESULT

The bacterial infection affects humans throughout their life. The infection depends on the nature of the organisms and interacts with the human body.

In earlier study, Jayanta KP *et al*, (2009) reported that the antimicrobial activity of the aqueous leaf extracts of *Excoecaria agallocha* was more effective against *Streptococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *E. coli* whereas it showed less antimicrobial activity against *Bacillus brevis*, *Shigella flexneri*, *Bacillus licheniformis* and *Staphylococcus aureus*. The methanol extract was more effective against *Staphylococcus aureus* and *E. coli.*

Followed by Sivaperumal *et al*, (2010) obtained that the crude chloroform extracts from *Excoecaria agallocha* was showed maximum activity against *Pseudomonas aeruginosa* (10mm), *Staphylococcus epidermidis* (9mm), and minimum activity (7mm) against *Staphylococcus aureus*, *Streptococcus pneumonia*.

Vundru AK *et al*, (2011) showed that the chloroform and methanolic extracts of Excoecaria *agallocha* showed the antibacterial activity with the zone inhibition range of 14-18mm.

In present study, the antibacterial activity of the aqueous leaf extracts of *Excoecaria agallocha* was assayed in vitro by disc diffusion method against five bacterial strains. Antibacterial activity was done at various concentrations such as 128µg, 64µg, 32µg, 16µg for the test organisms. The gram negative bacterial strains revealed the zone of inhibition that *Escherichia coli(*17mm), *Pseudomonas aeruginosa* (15mm), *Klebsiella pneumonia* (16mm) and gram positive bacterial strains showed *Staphylococcus aureus*(15mm), *Bacillus subtilis*(14mm) at 128µg concentration respectively (Table 1) (Figure 1).

The chi-square values obtained respectively which was less than the calculated table value. X^2 (0.05) = 3.841 at 5% level of significance. Above results lead to the conclusion that the data was consistent with the hypothesis, the diameter of inhibition zone obtained from the observed data showed the similarities with experimental data.

In earlier study, Jayanta KP *et al*, (2009) determined the MIC values for *Streptococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *E. coli* were ranged from 5 to 7 mg/ml.

MIC values for different strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Bacillus subtilis* were obtained using phenol indicator method. Each row represents one organism; a colour change from red (original) to yellow indicates the growth of organism.

The MIC values for 1st row (*Escherichia coli*) was < 2µg, 2nd row (*Bacillus subtilis*) was 128µg, 3rd row (*Staphylococcus aureus*) was 256µg, 4th row (*Pseudomonas aeruginosa*) was128µg and 5th row (*Klebsiella pneumonia*) was 32µg concentrations respectively (Table 2) (Figure 2). Based on these result *Escherichia coli* is more sensitive than other organism to this extract.

In earlier study, (Wickramasinghe M. Bandaranayake, 1994) reported the phytochemical analysis. The aqueous leaf extracts of *Excoecaria agallocha* showed the presence of Alkaloid, Protein and amino acids, Carbohydrates, Cardiac glycosides, Anthroquinone glycosides, Steroids and sterols, Saponins, Flavonoids and the absence of Tanin and Phenolic compound.

In present study, the aqueous leaf extracts of *Excoecaria agallocha* were subjected to suitable chemical tests to confirm the presence of phytochemicals such as Alkaloids, reducing sugar, phytosterol, fixed oil and fats, phenolic compounds, tanins, gums mucilage (Table 3).

Table 1. Zone of inhibition formed by aqueous extract of *Excoecaria agallocha* leaf against bacterial strains

S.NO	Sample	Bacterial strains	µg	Zone of inhibition in Diameter (mm)		$X^2=\sum[(O-E)^2]/E$
				Standard value	Observed value (Aqueous)	Observed crude
1	*Excoecaria agallocha* leaf powder	*E.coli*	128µg	20	17	0.45
2		*P.aeruginosa*	128µg	20	15	1.25
3		*Klebsiella pneumoniae*	128µg	20	16	0.8
4		*S.aureus*	128µg	20	15	1.25
5		*B.subtilis*	128µg	20	14	1.8

Table value $_X^2$ (0.05) = 3.841

Chi-square value significance at 5% level.

Table 2. MIC values of test organisms in aqueous extract of *Excoecaria agallocha*

S.No	Organisms	MIC values
1	*E.coli*	< 2µg
2	*P.aeruginosa*	128 µg
3	*Klebsiella pneumoniae*	32 µg
4	*S.aureus*	256 µg
5	*B.subtilis*	128 µg

Table 3. Phytochemical analysis

S.No	Phytochemical Constituents	Aqueous Extract
1	Alkaloids	Positive
2	Carbohydrates	Positive
3	Glycosides	Positive
4	Phyto sterols	Positive
5	Saponins	Positive
6	Fixed oils and fats	Negative
7	Tannins and phenolic compounds	Negative
8	Protein and amino acids	Positive
9	Gums and mucillage	Positive
10	Flavonoids	Positive
11	Lignin	Positive
12	Volatile oil	Negative

Fig 1. Zone formation formed by aqueous extract of *Excoecaria agallocha* at different concentration

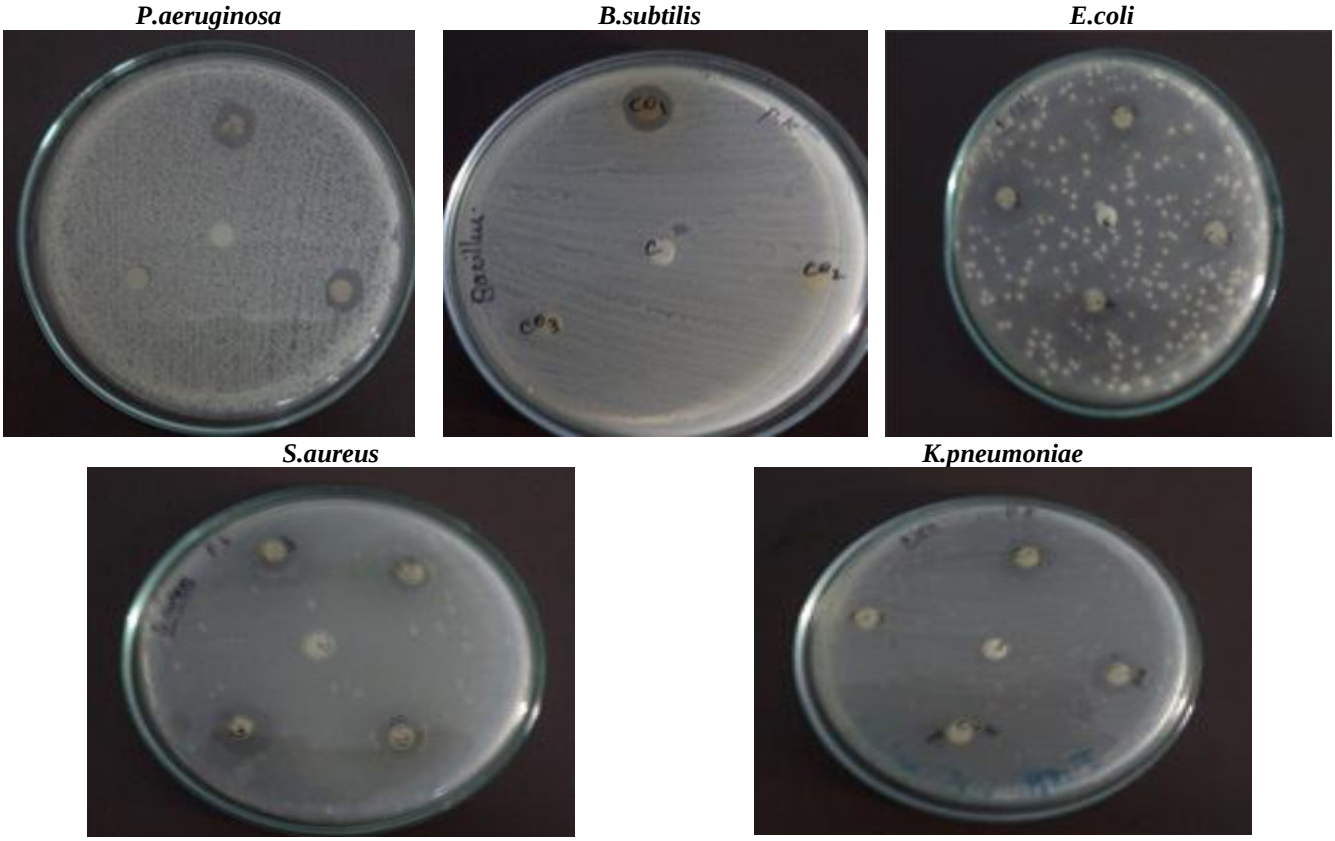
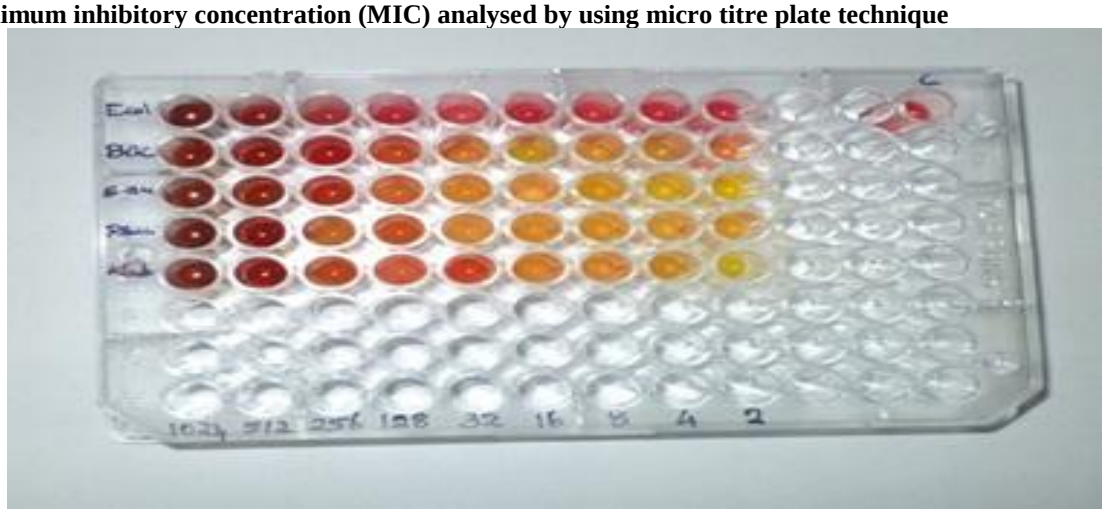


Fig 2. Minimum inhibitory concentration (MIC) analysed by using micro titre plate technique



Yellow colour shows the growth of the organisms.
Red colour shows the growth inhibition.

DISCUSSION

Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infections. 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. The studied plant was most active against gram-negative bacteria than gram-positive bacteria.The presence of such phytochemicals may be correlated with the fact that aqueous extracts showed

maximum activity against the bacterial strains. The active constituents of plants usually interfere with growth and metabolism of microorganisms in a negative manner.

CONCLUSION

The present study showed the antibacterial activity of the leaf extracts of *Excoecaria agallocha* against pathogenic organisms. Hence this plant can be used to cure the infection caused by the tested strains. Further studies are needed to isolate pure compounds from this plant extract and to establish the mode of action of the isolated compounds.

REFERENCES

Brindha P, Sasikala B, Purushothaman K. Phytochemical analysis of *E. alba*. *BMEBR*, 3(1), 1981, 84-96.

Choudhury S, Sree A, Mukherjee SC, Pattnaik P, Bapuji M. *In vitro* activity of extracts of selected marine algae and mangrove against fish pathogens. *Asian Fisheries Science*, 18, 2005, 285-294.

Jayanta KP, Tapan KP, Sakti KR, Nabin KD, Hrudyanath. Phytochemical screening and antimicrobial assessment of leaf extracts of *Excoecaria Agallocha* L.: A mangal species of Bhitarkanika, Orissa, India. *Advances in Natural and Applied Sciences*, 3(2),2009, 241-246.

Kim J, Marshall M.R, Wie C. Antibacterial activity of some essential oil components against five food borne pathogens. *Journal of Agric Food Chem*, 43, 1999, 2839 - 2845.

Nusrat S, Mohammad AA, Firoj A, Israt JS, Lutfun N, Satyajit DS. Bioactivity of *Excoecaria agallocha. Rev. bras. Farmacogn*, 18(4), 2008.

Patra JK, Gouda S, Sahoo SK, Thatoi HN. Chromatography separation, ^{1}H NMR analysis and bioautography screening of methanol extract of *Excoecaria agallocha* L. from Bhitarkanika, Orissa, India. *Asian Pacific Journal of Tropical Biomedicine*, 2012, S50-S56.

Patra JK, Thatoi HN. Metabolic diversity and bioactivity screening of mangrove plants: a review. *Acta Physiol Plant*, 33, 2011, 1051-1061.

Ravi Kumar S, Ramanathan G, Subhakaran M, Jacob Inbaneson S. Antimicrobial compounds from marine halophytes for silkworm disease treatment. *International Journal of Medicine and Medical Sciences*, 1(5), 2009, 184-191.

Ravikumar S, Syed Ali M, Ramu A, Ferosekhan M. Antibacterial Activity of Chosen Mangrove Plants Against Bacterial Specified Pathogens. *World Applied Sciences Journal*, 14 (8), 2011, 1198-1202.

Sheikh JH, Hitoshi A, Magdi El-Sayed, Firoj A. Antioxidative and anti-histamine-release activities of *Excoecaria agallocha* L. *Pharmacologyonline*, 2, 2009, 927-936.

Sivaperumal P, Ramasamy P, Jacob Inbaneson S, Ravikumar S. Screening of antibacterial activity of mangrove leaf bioactive compounds against antibiotic resistant clinical isolates. *World Journal of Fish and Marine Sciences*, 2(5), 2010, 348-353.

Vindru AK, Kandru A, Busi S. *In vitro* antimicrobial activity of leaf extract of certain mangrove plants collected from Godavarin estuarine of Konaseema delta, India. *Int. J. Med. Arom. Plants*, 1(2), 2011, 132-136.

Wickramasinghe M. Bandaranayake. Phytochemical constituents and pigments in mangrove species and mangal associates of Northern Australia. *Australian Institute of Marine Science*, 15, 1994.