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EURIHALINE MICROALGAE - A NOVEL SUBSTANCE AGAINST POST OPERATIVE PATHOGEN

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ABSTRACT

Objective: To evaluate the antibacterial activity of two saltpan microalgae *Amphora* sp and *Oscillatoria* sp against post operative pathogens

Methods: Samples of microalgae were collected from the puthalam solar saltpan of Kanyakumari district. Two microalgae *Amphora* sp and *Oscillatoria* sp were identified and isolated by serial dilution method. Biomass was produced by culturing the isolated strains in one litre of Walne's medium. The activity was assessed by using ethanol and petroleum ether solvents. The post operative pathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus* were chosen for the present antibacterial susceptibility study. The extracts were tested in compliance with disc diffusion method for their antibacterial activity.

Results: The results showed that the highest zone of inhibition were observed in *Amphora* sp against *E.coli* in ethanol extract and in *Oscillatoria* sp highest zone of inhibition were observed in ethanol extract against *Staphylococcus aureus*. Maximum antibacterial activity were observed in ethanol extracts than petroleum ether extracts.

Conclusion: This result proved that saltpan microalgae possess antibacterial activity therefore, this microalgae is used to treat post operative pathogenic bacteria.

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INTRODUCTION

Post operative pathogen is a serious problem in the field of surgery for a long time. An infected wound complicates the postoperative course and results in prolonged stay in the hospital and delayed recovery (Marjorie Beyers and Susan Dudas, 1977). Today most of the researchers are interested in isolating antibacterial substances from microalgae. Microalgae represent a large and underexplored resource of antimicrobial compounds (Guedes *et al.*, 2011). Microalgae are the biological starting point of aquatic ecosystem. Hypersaline ecosystem possess many unique features different from other aquatic environments. Microalgae act as the sole producer in the saltpan, producing energy by trapping sunlight. Therefore microalgae represent a unique opportunity to discover novel metabolites. Microalgae have meanwhile been found to produce antibiotics. Microalgae have for long been used with

therapeutic purposes; their systematic screening for biologically active principles began in the 1950s. The discovery and development of antibiotics are among the most powerful and successful achievements in modern Science and Technology for the control of infectious diseases (Chanda *et al.*, 2010). Diatoms are the major groups of phytoplanktonic class within algae, in which most organic compounds found in them with antibacterial activity have been pigments, lipids, and carbohydrates (Shimizu, 1993), cyanobacteria (blue-green algae) have unique, biologically active secondary metabolites due to their ecological and morphological diversity (Kreitlow *et al.*, 1999). This study will also hopefully expose new frontiers on the current applications of the algal extract.

MATERIALS AND METHODS

Isolation, Culturing and Growth of Algal organisms

Samples of microalgae were collected from the puthalam solar saltpan of Kanyakumari district. Two microalgae *Amphora* sp and *Oscillatoria* sp were identified and isolated by serial dilution method. Biomass was produced by culturing the isolated strains in one litre of Walne's medium and a facility to

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mix the culture with an aeration pump under laboratory condition. The algae were grown for 1 month and harvested.

Preparation of algal extracts

Freshly dried *Amphora* sp and *Oscillatoria* sp was mixed with solvent : ethanol and petroleum ether (150ml solvent/100g of *Algae*) in soxhlet apparatus and extracted for 1hours. The extracts were filtered and the solvent was removed by air drying. The extracts were stored in an airtight glass bottles in a refrigerator for the analysis of antibacterial activity.

Test pathogens

The post operative pathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and gram positive pathogens namely *Staphylococcus aureus*, collected from Kanniyakumari medical college and hospital (KMCH), Kanniyakumari District, Tamilnadu, India and maintained in our laboratory was chosen for the present antibacterial susceptibility study.

Antibacterial study was carried out by Disc diffusion method using Muller-Hinton Agar (Bauer *et al.*, 1996).

RESULTS AND DISCUSSION

The results of antibacterial activity in two extract of microalgae against post operative pathogens were portrayed in Table 1, Plate 1 and Figure 1.

Table 1. Determination of Antibacterial activity by Disc Diffusion Method against the isolated Microalgae

S.No	Name of the bacteria	Solvent	<i>Oscillatoria</i> sp	<i>Amphora</i> sp
1	<i>P.aeruginosa</i>	Ethanol	10	7
		Petroleum Ether	10	9
2	<i>K.pneumoniae</i>	Ethanol	10	10
		Petroleum Ether	7.5	6.5
3	<i>E.coli</i>	Ethanol	8	13
		Petroleum Ether	7	9
4	<i>P.vulgaris</i>	Ethanol	8.5	9.5
		Petroleum Ether	7	7
5	<i>S.aureus</i>	Ethanol	11.5	10.5

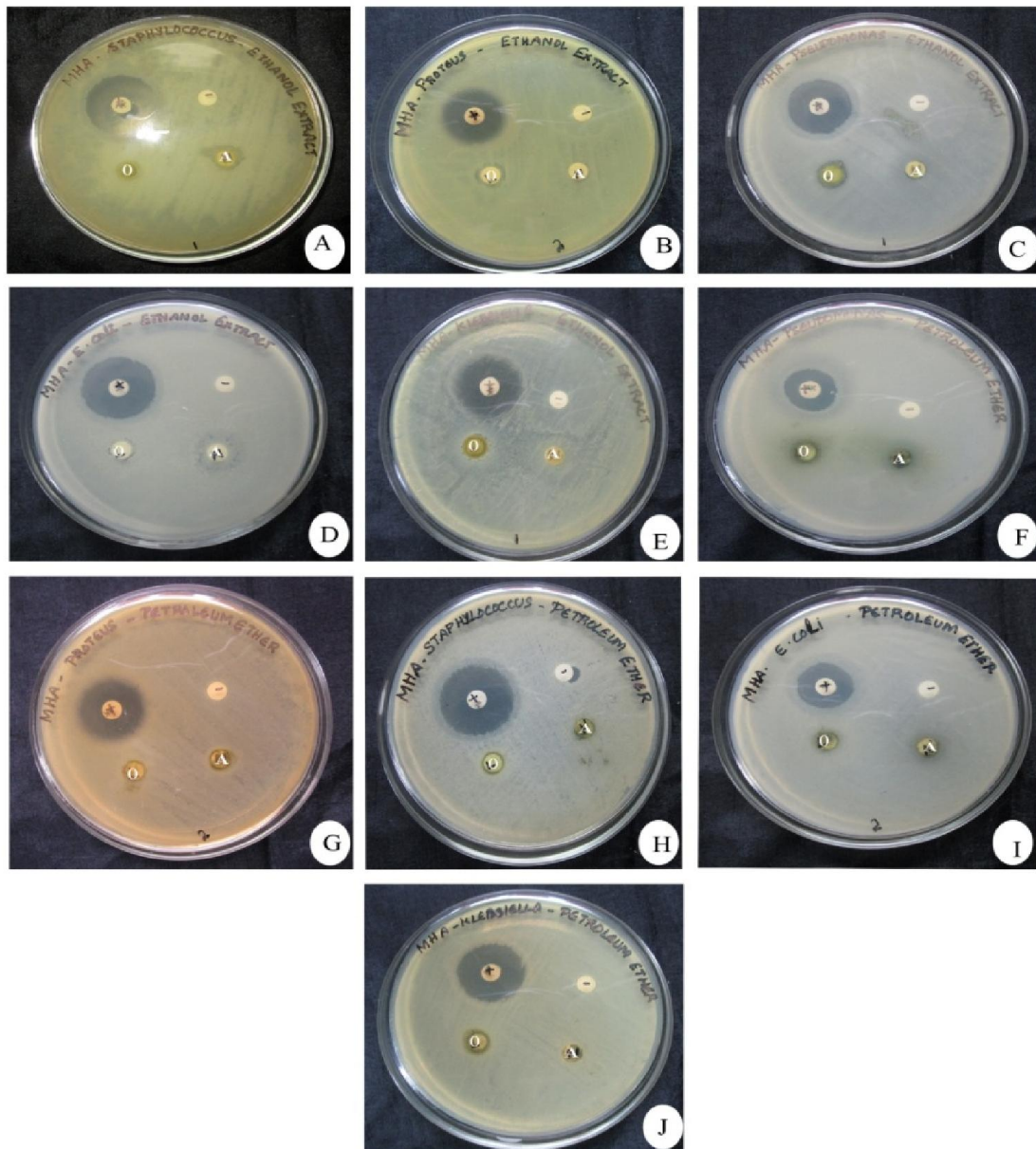


Plate 1. Determination of Antibacterial activity by Disc Diffusion Method against the isolated microalgae from puthalam saltpan

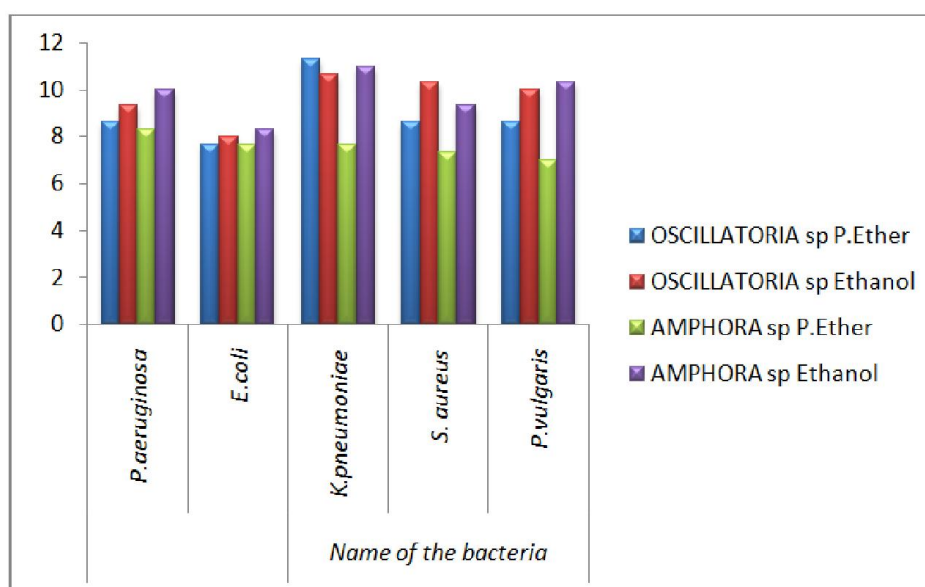


Figure 1. Antibacterial activity of two microalgae in two extracts

The activities of this study showed that the *Oscillatoria* sp. showed highest zone of inhibition (11.5mm) against *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (10 mm), *Proteus vulgaris* (8.5 mm), *E. coli* (8 mm) of which gram positive bacteria (*Staphylococcus aureus*) are more susceptible than gram negative bacteria to ethanol extract. The *Oscillatoria* sp. showed highest zone of inhibition (10mm) against *Pseudomonas aeruginosa* followed by *Klebsiella pneumoniae* (7.5 mm), *Proteus vulgaris*, *Staphylococcus aureus*, *E. coli* (7mm) of which gram negative bacteria (*Pseudomonas aeruginosa*) are more susceptible than gram positive bacteria in petroleum ether extract.

The *Amphora* sp. showed highest zone of inhibition (13mm) against *E. coli* followed by *Staphylococcus aureus* (10.5mm), *Klebsiella pneumoniae* (10mm), *Proteus vulgaris* (9.5mm), *Pseudomonas aeruginosa* (7mm) of which gram negative bacteria (*E. coli*) are more susceptible to ethanol extract. The *Amphora* sp. showed highest zone of inhibition (11mm) against *Staphylococcus aureus* followed by *E. coli*, *Pseudomonas aeruginosa* (9mm), *Proteus vulgaris* (7mm), *Klebsiella pneumoniae* (6.5mm) of which gram positive bacteria (*Staphylococcus aureus*) are more susceptible than gram negative bacteria in petroleum ether extract.

Based on the literature survey concerning the antibacterial activity of microalgae solvents used by earlier researchers to determine antibacterial activity of algal extracts included ethanol (Padmini *et al.*, 1986; Miura *et al.*, 1993; Srinivasakumar and Rajashekar, 2009), petroleum ether (Padmini *et al.*, 1986). (Ganesh Kumar *et al.*, 2011) reported that *Oscillatoria* sp showed maximum inhibition zone. (Lazarus and Valentin Bhimba, 2008) reported that the percentage inhibition of *E. coli* (15.6%) was the highest when compared to other pathogens, the present study also shows that *E. coli* showed the highest zone of inhibition. Experiments of (Walter, and Mahesh 2000) showed that of the eleven marine diatoms screened against bacterial pathogens, 6 showed high antibacterial activity.

(Satsry and Rao, 1994) showed antibacterial activity against gram positive and gram negative pathogenic strains after successive extraction. Likewise, (Marasneh *et al.*, 1995; Prashantkumar *et al.*, 2006) have showed antibacterial activity in organic extracts of six species of marine microalgae against multi antibiotic resistant bacteria. (Vijayakumar Madhumathi *et al.*, 2011) results also proved that ethanol was the best solvent for extracting the antibacterial agents from *Oscillatoria latevirens*. The results showed that the highest zone of inhibition was observed in *Amphora* sp against *E. coli* in ethanol extract and in *Oscillatoria* sp highest zone of inhibition was observed in ethanol extract against *Staphylococcus aureus*.

Conclusion

This study proved that saltpan microalgae possess antibacterial activity. Obviously the present finding opens a new horizons to treat post operative pathogens by using this potent microalgae. Progression of studies in this direction could definitely help to obtain a wide variety of important bioactive natural products from salt tolerant microalgae.

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