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In vitro antimicrobial activity of Gracilaria SP and Enteromorpha SP

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ABSTRACT

Southeast coast of India is a unique marine habitat infested with diverse seaweeds. Some commonly occurring seaweed in southeast coast of India like Gracilaria cortica, Enteromorpha flexuosa and Enteromorpha clathrata were collected from the Covelong Beach and Pulicat Lake region of Tamil Nadu and were evaluated for the antibacterial activity against Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa by well diffusion method. Three different solvents namely methanol ethanol and diethyl ether were used for extraction. The ethanol extract showed better result than other extracts. The maximum antibacterial activity was noted in ethanol extracts of Enteromorpha flexuosa which showed inhibitory activity against Pseudomonas sp. (21 mm) and the minimum was recorded in di-ethyl ether extracts against Pseudomonas sp. (7mm).

Keywords: seaweed, marine algae, antibacterial, Gracilaria, Enteromorpha.

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INTRODUCTION

The seaweed extract were found to be more effective against various human pathogenic microorganisms. Staphylococcus aureus produces various types of diseases like- tonsillities, osteomyelitis, arthritis, pharyngitis etc. [1]. The algal extracts were used as a curative and preventive agent for various diseases such as hypertension, cough, tumour, diarrhea [2, 3]. Seaweed species like Gracilaria, Calorpha and Hydroclothres were screened against six bacterial pathogens namely Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus faecalis and Bacillus cereus [4]. Ethanol extract of Gracilaria edulis inhibited growth of all the test organisms except Bacillus cereus and Enterobacter aerogenes [5].

Selective utilization of marine algae as potential source of pharmaceutical agents has been increasing in recent years. Many of the seaweeds possess bioactive components which inhibit the growth of some of the gram positive and gram negative bacterial pathogens [6]. The macrolgae have a significant attraction as natural source of bioactive molecules with a broad range of biological activities, such as antibiotics, antivirals, antitumorals antioxidant and anti-inflammatories [7]. The elements abundant in seaweeds include: potassium, sodium, calcium, magnesium, zinc, copper, chloride, sulfur, phosphorous, vanadium, cobalt, manganese, selenium, bromine, iodine, arsenic, iron, and fluorine [8].

MATERIALS AND METHODS

Collection and preparation of sample

The seaweed samples were collected from the Pulicat Lake and Covelong beach of Tamil Nadu coast. The collection of seaweeds from the intertidal area was done during the low tide. Samples were collected by random sampling method as per requirement. Collected material were kept in the polyethylene bags and labeled for further preservation and identification at the later stage in the laboratory. These species are identified in the Dr. Krishnamurthy Institute of Algology. The algae after drying were weighed and then chopped. The chopped samples were finely powdered using mixer grinder. The solvent extraction was done using ethanol, methanol and diethyl ether in a Soxhlet apparatus.

Test organisms used

The test organisms used were that of Klebsiella sp, Staphylococcus aureus and Pseudomonas aeruginosa.

Evaluation of antibacterial activity

Antibacterial activity was measured using a well diffusion method [9]. The plates were incubated at 37ºC for 24 hr. The antibacterial activity was assessed by measuring the diameter

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of the area in which bacterial growth was inhibited around the well. The average of three replicates for each extracts and solvent controls were calculated.

RESULTS AND DISCUSSIONS

The antimicrobial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plant, experimental methods, etc., In this study Gracilaria cortica, Enteromorpha flexuosa and Enteromorpha clathrata from Covelong Beach and Pulicat lake,Tamil Nadu were collected, identified and tested against various pathogenic bacteria. It was found that the ethanol extracts of Gracilaria cortica showed maximum activity(18 mm) against Pseudomonas (Table 1) and minimum activity was shown by di-ethyl ether extracts against Staphylococcus aureus (8 mm) as shown in Table 3.The Enteromorpha flexuosa showed maximum activity against Klebsiella (21 mm) when ethanol was used as a solvent (Table 1) and minimum against Staphylococcus aureus in di-ethyl ether extract as shown in Table 3.The Enteromorpha clathrata showed maximum activity against Pseudomonas aeruginosa (21 mm) in methanol extract (Table 2) and minimum activity in di-ethyl ether against Staphylococcus aureus as shown in Table 3.

Table 1: Effect of ethanolic algal extracts on pathogenic bacteria

Species	Test organism	Zone of inhibition (mm)		
		100 µl	150 µl	200 µl
Control	Klebsiella sp	6	8	10
	Pseudomonas aeruginosa	8	10	14
	Staphylococcus aureus	9	13	15
Gracilaria cortica	Klebsiella sp	17	16	15
	Pseudomonas aeruginosa	18	16	15
	Staphylococcus aureus	17	15	14
Enteromorpha flexuosa	Klebsiella sp	21	18	17
	Pseudomonas aeruginosa	20	19	16
	Staphylococcus aureus	16	15	14
Enteromorpha clathrata	Klebsiella sp	11	12	14
	Pseudomonas aeruginosa	10	11	17
	Staphylococcus aureus	13	17	19

Table 2: Effect of methanolic algal extracts on pathogenic bacteria

Species	Test organism	Zone of inhibition (mm)		
		100 µl	150 µl	200 µl
Control	Klebsiella sp	11	12	14
	Pseudomonas aeruginosa	10	11	15
	Staphylococcus aureus	9	11	10
Gracilaria cortica	Klebsiella sp	14	12	11
	Pseudomonas aeruginosa	15	12	10
	Staphylococcus aureus	15	13	Nil
Enteromorpha flexuosa	Klebsiella sp	16	14	13
	Pseudomonas aeruginosa	13	12	Nil
	Staphylococcus aureus	14	13	10

Enteromorpha clathrata	Klebsiella sp	11	15	15
	Pseudomonas aeruginosa	10	21	21
	Staphylococcus aureus	13	16	20

Table 3: Effect of Di-ethyl ether algal extracts on pathogenic bacteria

Species	Test organism	Zone of inhibition (mm)		
		100 µl	150 µl	200 µl
Control	Klebsiella sp	Nil	10	11
	Pseudomonas aeruginosa	Nil	11	14
	Staphylococcus aureus	6	11	14
Gracilaria cortica	Klebsiella sp	11	10	Nil
	Pseudomonas aeruginosa	12	9	8
	Staphylococcus aureus	10	Nil	Nil
Enteromorpha flexuosa	Klebsiella sp	12	10	Nil
	Pseudomonas aeruginosa	11	9	7
	Staphylococcus aureus	Nil	Nil	Nil
Enteromorpha clathrata	Klebsiella sp	8	9	11
	Pseudomonas aeruginosa	Nil	Nil	8
	Staphylococcus aureus	Nil	Nil	Nil

The solvent system used for the extraction played a major role in displaying the anti bacterial activity. Benzene, diethyl ether, acetone, ethyl alcohol and ether were suitable solvents for extracting the antibiotic principle then chloroform [10-12]. However, there are reports that indicate maximum activity in chloroform extracts [13]. Hence the efficiency of chloroform in the extraction of seaweeds remains uncertain. Ethanol was found to be the best solvent for extracting the active principles in almost all species of seaweeds [14].

Antibacterial activities of seaweeds also varied with the species division. The species of Rhodophyta showed the highest activity when compared to Phaeophyta [15, 16]. The reason for this was not explained by these workers but it was suggested that more species have to be screened before coming to definite conclusion. In the present study, the species of Chlorophyta showed the strongest activities against the test bacteria which was in agreement with the findings of Padmakumar and Ayyakannu [16]. It may be probably due to the tested seaweeds vertical distribution. Green algae mostly occur in the intertidal zone lower region, which may be advantage for the protection of the active compounds within the algal plant from degradation.

CONCLUSION

The seaweed extracts possessed significant activity against gram positive and gram negative bacteria when compared with commercial antibiotic ampicillin and gentamycin. It is evident from the present study that the ethanol extracts could be utilized as a good source of antimicrobial agent in pharmaceutical industry.

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