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Research Article

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A comparison between the effects of three algal extracts against pathogenic bacteria

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ABSTRACT

Marine algae of late has been targetted for the analysis of various compounds of pharmaceutical significance. In the present paper we highlight the effects of three species of marine algae collected from the Tamil Nadu coast on the various pathogenic bacteria. All the three species studied, exhibited activity against Pseudomonas fluorescens though neither of them showed activity against Escherichia coli.

Key words: seaweeds, antibacterial activity, methanol extract, plate assay.

INTRODUCTION

Seaweeds are large macro algae that grow in a saltwater or marine environment. They lack true stems, roots and leaves. Most of the bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides [1]. The algae are also used as food, animal fodder, fertilizer, industrial material such as agar and minor medicines. The crude extracts obtained from various seaweeds using different solvent, is subjected to broad based biological screening for antifungal, antiviral, antibacterial, antimalarial, antifilarial, hypoglycaemic and antifertility activity [2-4]. Diethyl ether was the best solvent for extracting the effective antimicrobial components from *Enteromorpha linza*, *Ulva rigida*, and *Gracilaria gracilis* [5]. In another study it was found that the extract of *Ulva pertusa* was more active against *S.aureus* and *B. subtilis*, but less active against *E.coli. Valonia aegrophila* was the most active species against the test organisms whereas the extracts of *Halimeda optunia* and *Halimeda tuna* showed mild activity [6].

In this study the organic solvent extracts of three seaweed species namely, *Centroceiod sp.*, *Chaetomorpha sp.*, and *Enteromorpha sp.*, were tested *invitro* for their antimicrobial activities against seven pathogenic microbes such as *Aeromonas hydrophila*, *Edwardsiella tarda*, *Psuedomonas fluorescens*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* using agar well diffusion method.

EXPERIMENTAL SECTION

Preparation of Extracts:

The powdered algal smples were used for this study. They (5 gm) were mixed with various solvent systems like ethanol, methanol and ethanol: chloroform in the ratio 1:1. After 48 hours the sample was filtered using Whatman No1 filter paper to separate the filtrate for further use in antimicrobial testing of algal samples.

Test Microorganisms:

Antibacterial activity was tested against the pathogenic strains of Aeromonas hydrophila, Edwardsiella tarda, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella typhi, and Staphylococcus aureus. A loopfull of sample from each pure culture was inoculated in sterile nutrient broth and kept overnight at 37°C for growth.

Plate Assay Method:

Antibacterial activity was assayed using the agar well diffusion test technique using Muller Hinton Agar Medium [7]. A sterile cotton swab was used for spreading the test microorganism evenly from the 24 hours incubated broth on the MHA plates. In each of these plates three wells of 5mm diameter each was made using sterilized cork borer and 50 μ l, 75 μ l and 100 μ l of the algal extract was added. The extract loaded plates were kept for incubation at 37°C for 24 hours. After incubation, a clear zone was observed around the well which was the evidence for the presence of antibacterial active compounds in the algal extracts. Diameters of the zone of inhibition were measured in millimetres.

RESULTS AND DISCUSSION

Seaweeds are rich in varied source of bioactive natural products and have been studied as potential biochemical and pharmaceutical agents. The main objective of the work was to evaluate the ability of the three macroalgal species collected from the southwest coast of India to synthesize bioactive compounds of potential therapeutic interests.

Species	Extracts	Zone of inhibition mm						
		AH	ET	EC	PA	PF	ST	SA
Centroceiod sp	Ethanol	-	-	-	-	7	-	3
	Ethanol+Chloroform	-	-	-	-	7	2	-
	Methanol	3	4	-	-	-	5	-
Chaetomorpha sp	Ethanol	-	-	-	-	5	-	-
	Ethanol+Chloroform	-	-	-	-	4	-	-
	Methanol	-	-	-	5	6	8	-
Enteromorpha sp	Ethanol	-	-	-	-	8	-	-
	Ethanol+Chloroform	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	2	2

Table-1 Antibacterial activity of algal extracts

AH- Aeromonas hdrophila, ET- Edwardsiella tarda, EC- Escherichia coli, PA- Pseudomonas aeruginosa, PF- Pseudomonas fluorescens, ST- Salmonella typhi and SA- Staphylococcus aurues.



Fig- 1: Antibacterial activity of the methanol extract of Centroceiod sp against Aeromonas hydrophila

The extracts of Centroceiod sp., Chaetomorpha sp, and Enteromorpha sp., were tested for their antimicrobial activity against seven strains of microorganisms Aeromonas hdrophila, Edwardsiella tarda, Escherichia coli,

Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella typhi and Staphylococcus aurues, by agar well diffusion method. The results of antibacterial activity against the tested pathogens are tabulated in the Table-1.

The methanol extract of Centroceiod sp showed activity against *Aeromonas hydrophila* and *Edwardsiella tarda* (Fig-1 and Fig-2). *Chaetomorpha sp* and *Enteromorpha sp* gave negative results. None of the species showed activity against *Escherichia coli*.



Fig- 2: Antibacterial activity of methanol extract of Centroceiod sp., against Edwardsiella tarda.



Fig- 3: Antibacterial activity of methanol extract of Chaetomorpha sp against Pseudomonas aeruginosa

In case of *Pseudomonas aeruginosa*, the methanol fraction of *Chaetomorpha sp* (5 mm) showed activity. There was no response to other fractions (Fig-3).



Ethanol+chloroform Chaetomorpha sp



Ethanol+chloroform Centroceiod sp



Ethanol Extract
Enteromorpha sp, Centroceiod sp
Chaetomorpha sp



Methanol Extract

Chaetomorpha sp

Fig- 4: Antibacterial activity of Centroceiod sp, Enteromorpha sp, and Chaetomorpha sp against Pseudomonas fluorescens

L. Jevanthi Rebecca et al

In case of *Pseudomonas fluorescens* ethanol was rated the best solvent as all the three species showed activity though there was significant activity in the other two extracts. All the three seaweeds used in this study had shown zone of inhibition for *Pseudomonas fluorescens* irrespective of the solvents used (Fig- 4).



methanol Chaetomorpha sp



methanol Centroceiod sp Enteromorpha sp



ethanol +chloroform Centroceiod sp

Fig- 5: Antibacterial activity of the methanol and ethanol +chloroform extracts of Chaetomorpha sp, Enteromorpha sp and Centroceiod sp., against Salmonella typhi

In case of *Salmonella typhi*, methanol was the best solvent in which *Chaetomorpha* (8 mm) exhibited maximum activity (Fig-5). *Centroceiod sp.*, showed activity in the ethanol+chloroform fraction. All the three algal extracts showed activity against *Salmonella typhi*. In case of *Staphylococcus aureus*, there was very less activity in the ethanol and methanol fractions of *Centroceiod sp.*, and *Enteromorpha sp* respectively.

Seaweed extracts in different solvents exhibited different antimicrobial activities. Various solvents were used for seaweed extractions, maximum inhibition was noticed with ethanol extracts and minimum with chloroform crude extracts. However, no specific solvent exhibited activity against all the test organisms effectively. Results also highlighted that *P.fluorescens* was the most sensitive organism.

The *invitro* screening of organic solvent extracts of three marine algae *viz.*, *Gracilaria cortica*, *Ulva fasciata* and *Enteromorpha compressa* showed species activity in inhibiting the growth of six virulent strains of bacteria pathogenic to fish [8]. Methanol solvent system were efficient in extracting the active compounds. The marine algae *Enteromorpha compressa* is active against *Edwarsiella tarda* whereas the algae *Ulva fasciata* shows positive against *Pseudomonas flurorescens*[8,9]. According to our results, the extract of ethanol + chloroform and methanol of the algae *Enteromorpha* species showed no inhibition against all the test organisms whereas the ethanol extract of the same algae showed inhibition only against *Pseudomonas flurorescens*.

CONCLUSION

Seaweed extracts in different solvents exhibited different antimicrobial activities. The overall antimicrobial activity assessed from the above results indicates the presence of active constituents in the extractions of seaweeds which can be exploited for the production of lead molecules which are of use in pharmaceutical industry.

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L. Jeyanthi Rebecca et al

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