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Research Article

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Antibacterial activity of *Sargassum Ilicifolium* and *Kappaphycus alvarezii*

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ABSTRACT

Microorganisms, of late have developed resistance to existing antibiotics, thereby leading to an increasing demand for new antibiotics. Since seaweeds offer a rich source of bioactive molecules, the present study was carried out to investigate its antibacterial potential. Two species of seaweeds namely, Sargassum ilicifolium and Kappaphycus alvarezii collected from different coastal regions of Rameshwaram (southeastern coast of Tamil Nadu, India) were used in the present study. For microbiological testing of the seaweed extracts, agar well diffusion method was used. The zone of inhibition was measured for all the different crude algal extracts against three strains of microorganisms namely, Escherichia coli, Salmonella sp. and Klebsiella sp. that cause diseases and disorders in human beings, animals and plants. Crude extracts prepared from chloroform, ethanol and methanol revealed a wide range of antibacterial activity against the test pathogens. Maximum inhibition was noticed with ethanol extracts in case of S. ilicifolium but all the three extracts showed varied results in case of K. alvarezii. However, no specific solvent exhibited activity against all the test organisms effectively. The overall antibacterial 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.

Key words: seaweeds, antibacterial activity, ethanol extract, *S. ilicifolium, K. alvarezii.*

INTRODUCTION

Seaweeds provide many vitamins and are rich in iodine, potassium, iron, magnesium and calcium. Many bioactive compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae. Crude extracts of *Gracilaria edulis, Calorpha peltada* and *Hydroclothres sp.* in ethanol were used to screen for their antibacterial activity against six bacterial pathogens namely, *Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus faecalis and Bacillus cereus* [1]. Antimicrobial activities on bacteria and fungi were reported by [2-5].

Presently seaweeds constitute commercially important marine renewable resources which provide valuable idea for the development of new drugs against cancer, microbial infections and inflammations. The algae *S. ilicifolium, Padina tetrastromatica* and the red algae *Gracilaria corticata* collected from different coastal regions were tested for antimicrobial activity against six strains of bacteria and fungi that cause diseases and disorders in man, animals and plants [6].

Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae [7-11]. The bactericidal agents found in algae include aminoacids, terpenoids, phlorotannins, acrylic acid, phenolic compounds, steroids, halogenated ketones and alkanes, cyclic polysulphides and fatty acids [12]. As a

consequence of an increasing demand for biodiversity in the screening programmes seeking therapeutic agents from natural products, there is now a greater interest in marine organisms.

The present study was undertaken to investigate the antibacterial activities of chloroform, ethanol and methanol extracts of two seaweeds *Sargassum ilicifolium* and *Kappaphycus alvarezii* collected from the Rameshwaram coast against three human pathogenic bacteria namely, *Escherichia coli, Salmonella sp.* and *Klebsiella sp.*

EXPERIMENTAL SECTION

Collection of Marine Algae Samples:
For screening of antibacterial activity of marine algae the study area considered was the Rameshwaram coast of Tamil Nadu. Live and healthy marine algae were collected from the coast of Rameshwaram in the month of January. The two collected algae samples were identified by algal experts. The collected algae were rinsed with water to remove epiphytes and necrotic parts. Again samples were thoroughly rinsed with sterile water to remove any associated debris. The algae after rinsing, were dried carefully in shade under room temperature for 10 days and then immediately subjected to extraction.

Preparation of Extracts:
The algae after drying were weighed and then chopped. The chopped samples were finely powdered using mixer grinder. The finely powdered samples were weighed and 5 grams of samples were dissolved in various organic solvents, namely, 80% ethanol, methanol and chloroform. It was kept for 48 hours at room temperature and mixed at regular intervals. After 48 hours the sample dissolved in each solvent was filtered using Whatman filter paper to separate the filtrate for further use in antibacterial testing of algal samples.

Test Microorganisms Used:
Pure cultures of *E. coli, Salmonella sp.* and *Klebsiella sp.* were used as the test microorganism for antibacterial testing.

Plate Assay Method:
Antibacterial activity was assayed using the agar well diffusion test technique [13]. Muller Hinton Agar Medium (MHA) plates were prepared and the pH was maintained at 7.4. A sterile cotton swab was used for spreading the test microorganism from the 24 hours inoculated broth evenly on the MHA plates. Similarly swabbing was done separately for each test microorganism on the MHA plates and left for few minutes to allow complete absorption of the inoculum. In each of these plates three wells of 5mm diameter each were made using an appropriate size sterilized cork borer.

Different concentration of each algal extract was added to the respective wells on the MHA plates. Concentration ranging from 50 µl, 75 µl and 100 µl respectively were placed in the wells and allowed to diffuse at room temperature for 30 minutes. 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 evidence for the presence of antibacterial active compounds in the algal extracts. Diameters of the zone of inhibition were measured in mm.

RESULTS AND DISCUSSION

The main objective of the work was to evaluate and compare the ability of different macro algal species from southwest coast of India to produce bioactive compounds of potential therapeutic interests. The production of antimicrobial activities was considered to be an effective indicator of the capability of the seaweeds to synthesize bioactive secondary metabolites.

Different extracts of *S.ilicifolium* and *K. alvarezi* were tested for their antimicrobial activity against three strains of microorganisms, by agar well diffusion method. The results of antimicrobial activity against tested pathogens were tabulated in Table-1 and Table-2 for the crude extracts of *S.ilicifolium* and *K. alvarezi*, respectively. In case of *E.coli* inhibition, ethanol extracts of both the algal species under study showed better results when compared to methanol and chloroform extracts (Fig-1 and Fig-4). While there was no significant result in the methanol and chloroform extracts of *K.alvarezii*, there was no zone of inhibition only in the chloroform extract of *S.ilicifolium*.

Table- 1: Zone of inhibition of chloroform, ethanol, and methanol extracts for *S.ilicifolium* in MHA plates

Name of the microorganism	Solvents	Zone of inhibition (mm)		
		50 µl	75 µl	100 µl
E.coli	Ethanol	16	18	21
	Methanol	15	17	20
	Chloroform	Nil	Nil	Nil
Salmonella sp.	Ethanol	16	21	32
	Methanol	Nil	10	12
	Chloroform	Nil	Nil	Nil
Klebsiella sp.	Ethanol	15	20	30
	Methanol	15	18	19
	Chloroform	Nil	Nil	Nil

Table-2: Zone of inhibition of chloroform, ethanol, and methanol extracts for *K.alvarezii* in MHA media

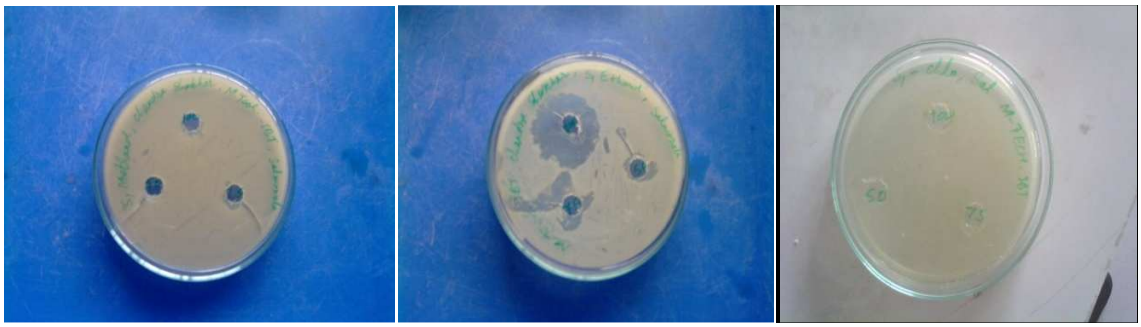
Name of the microorganism	Solvents	Zone of inhibition (mm)		
		50 µl	75 µl	100 µl
E.coli	Ethanol	10	20	25
	Methanol	Nil	Nil	Nil
	Chloroform	Nil	Nil	Nil
Salmonella sp.	Ethanol	10	12	15
	Methanol	12	16	20
	Chloroform	15	19	30
Klebsiella sp.	Ethanol	Nil	Nil	Nil
	Methanol	13	17	18
	Chloroform	Nil	Nil	Nil

methanol **ethanol** **chloroform**

Fig-1: Zone of inhibition of extracts of *S. ilicifolium* against *E.coli*

methanol **ethanol** **chloroform**

Fig- 2: Zone of inhibition of extracts of *S. ilicifolium* against *Klebsiella sp.*



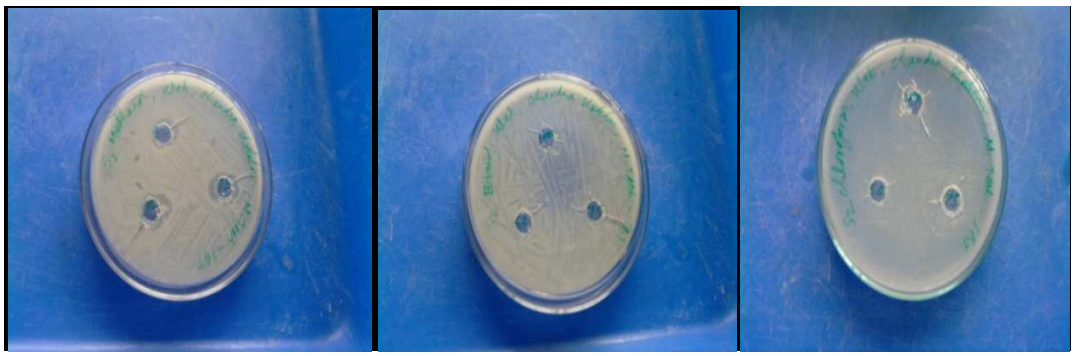
methanol **ethanol** **chloroform**

Fig-3: Zone of inhibition of extracts of *S. ilicifolium* against *Salmonella sp.*

methanol **ethanol** **chloroform**

Fig- 4: Zone of inhibition of extracts of *K. alvarezii* against *E.coli*

There was varied result in both the species concerning inhibition to *Klebsiella sp*. While *S. ilicifolium* showed zone of inhibition in both methanol and ethanol (prominent) extracts, *K. alvarezii* showed result only in the methanol extract (Fig-2 and Fig-5).With respect to *Salmonella sp*. *K. alvarezii* showed zone of inhibition in all the extracts, chloroform being the most prominent. *S. ilicifolium* displayed significant zone of inhibition in the ethanol extract when compared to the methanol extract while there was no result in the chloroform extract (Fig-3 and Fig-6).



methanol **ethanol** **chloroform**

Fig- 5: Zone of inhibition of extracts of *K. alvarezii* against *Klebsiella sp*.

In the present study, it was observed that ethanol was the best organic solvent for extracting the effective antibacterial material from the algae species used in this experiment. The result exhibited by chloroform was less than that exhibited by ethanol and methanol. The best halo-zone produced was in the extract of *S. ilicifolium* in the ethanol extract. Similar results were obtained by different seaweeds collected from the Vedalai coast, Gulf of Mannar, Tamilnadu wherein, some commonly occurring green algae *Codium adherens*, *Ulva reticulata* and *Halimeda tuna* were evaluated for antibacterial activity [14]. However, in another study by [15], methanolic extracts

of seaweed exhibited broad spectrum of antibacterial activity than other extracts. The large diameter of zone inhibition represent the high sensitivity of the microorganisms to the seaweed extracts under study and vice versa. The absence of zone inhibition indicates the resistance to microorganisms to the seaweed extract. Ethanol was found to be the best solvent for *S. ilicifolium* [6]. *Gracillaria edulis* and *Calorpha peltada* inhibited the growth of *E. coli* and *S. aureus*, *S. faecalis* and *P. aeruginosa* [1].

methanol **ethanol** **chloroform**

Fig-6: Zone of inhibition of extracts of *K.alvarezii* against *Salmonella sp.*

The antibacterial and antifungal activities of the extracts of marine algae were found to exhibit seasonal variations [16]. Marine algae are a rich source of fatty acids [17] and antioxidants [18] and 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.

CONCLUSION

In this work, we were able to show that these two marine macro algae of Rameshwaram coast in Tamil Nadu, India can be used for development of anti-pathogenic drugs in the pharmaceutical industries. Our results also revealed that the differential antibacterial activities of these marine macro algae may be attributed to the presence of different antibacterial compounds which are easily extracted with organic solvents. However a detailed study in this field is required for isolation and characterization of the antibacterial compounds from these marine macro algae.

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