Radhika D. *et al.* / *International Journal of Phytopharmacology*, 5(1), 2014, 27-30.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

A COMPARATIVE STUDY OF BIOPOTENTIAL OF CRUDE AND FRACTIONATED EXTRACTS OF SOME SEA WEEDS FROM TUTICORIN COAST

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ABSTRACT

Marine algae are known as a potential source of bioactive substances. In the present work, we used four seaweeds *Ulva lactuca, Padina tetrastromatica, Caulerpa scalpelliformis, Stoechospermum marginatum* which were collected from Hare Island in the Gulf of Mannar of Tuticorin coast. Ethanol was taken as the solvent for extraction. The crude extract was purified using column chromatography. Antibacterial activity of crude and column purified fractions were tested against *Klebsiella, Aeromonas, Staphylococcus, Escherichia* and *Pseudomonas* using well diffusion method. Maximum zone of inhibition (8mm) was exhibited by *Padina tetrastomatica* against *Klebsiella*. Minimum zone of inhibition (1mm) was exhibited by *Ulva fasciata*. Highest antibacterial activity was obtained in brown seaweed, whereas, green seaweed showed less antibacterial activity. From this study, we can conclude that column purified fraction showed higher antibacterial activity than crude extracts. The extracts were also analyzed for the secondary metabolites. When the zone of inhibitions was statistically tested (ANOVA) there was a significant difference among the different pathogens only for the fraction of *Padina tetrastomatica.*

Key words: Marine algae, Seaweeds, Antibacterial activity, Secondary metabolites.

INTRODUCTION

Marine environment is an exceptional reservoir of biologically active natural products, many of which exhibits structural features not been found in terrestrial natural products. Marine algae or seaweed, the most accessible marine resource of the coastal zone occupy potentially important place as a source of biomedical components Rao *et al*., 1988.

They are the only source for the production of photochemical such as agar, carrageenan and algin also contain many trace elements, minerals, protein, iodine, bromine, vitamins and many bioactive substances. A marine algae was first investigated for the antibiotic activity in 1950 and since then; many works have been carried out on the antibacterial activity of marine plants Selvi *et al*., 2001 since algae have been used in traditional medicine for a long time Fitton *et al*., 2006 and also some algal substances have bacteriostatic and bactericidal activity they have been extensively studied by several researchers Ghosh *et al*., 2004.

Biostimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics. Seaweeds have some valuable medicinal components such as antibiotics, laxatives, anti-coagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contain a significant amount of the proteins, vitamins and minerals, which are essential nutrition for human Fayaz *et al*., 2005. Most of the compounds of marine algae show antibacterial activities Thirumaran *et al*., 2006.

Antimicrobial activities against bacteria and fungi were reported by Hellio *et al*., 2000. Marine algae

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show pharmacological activities and bioactive compounds primarily for treating deadly disease like Cancer, Acquired Immuno deficiency syndrome (AIDS) Arthritis etc., Several works have been carried out on the extracts from marine algae. Extracts of marine algae were reported to exhibit antimicrobial activity Siddhananta *et al*., 1997.

Taskin *et al*., 2001 and Tuney *et al*., 2006 reported that Gram positive bacteria were more effectively controlled by extracts of algae than Gram negative bacteria. The greater antibiotic activity of the brown algae against pathogen was supported by the recent findings of Veeragurunathan and Geetha, 2009. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities P. Rajasulochana *et al*., 2009. Considering the scenario of the availability on very few records on the antibacterial activity of the macro algae, the present study was made to examine the efficacy of extracts of selected marine macro algal species collected from Tuticorin coast.

MATERIALS AND METHOD

Collection of Seaweed

Live samples of the seaweeds *Ulva lactuca, Padina tetrastromatica, Caulerpa scalpelliformis, Stoechospermum marginatum* were collected by handpicking during low tide from Hare Island in the Gulf of Mannar of Tuticorin coast (08º46' 2.15"N lat; 78º 11' 16.05" E long). Shade dried seaweed were ground to fine powder using electric mixture. The powdered samples were then stored in refrigerator for further use.

Preparation of extracts by Soxhlet extraction method

The Secondary metabolites from the powdered samples were extracted by using soxhlet apparatus. Ethanol was taken as the solvent for extraction.

Phytochemical screening

The different extracts were tested for alkaloids, anthroquinone, catachins, sugar, terpenoid, flavenoid, phenol, quinone and Saponin. Phytochemical screening of the extracts was carried out according to the standard method described by Harborne, 1998.

Well diffusion method

The antibacterial activity of extracts of *Ulva lactuca, Padina tetrastromatica, Caulerpa scalpelliformis* and *Stoechospermum marginatum* against five species of bacteria namely *Klebsiella pneumonia, Staphylococcus aureus, Aeromonas hydrophilla , Escherichia coli* and *Pseudomonas aerugenosa* were determined by well diffusion method.

Statistical analysis

Results are expressed as mean ± SD. The statistical analysis was performed by using one way ANOVA for comparing the zone of inhibition against different pathogens.

RESULTS

The seaweed extracts were analysed for their secondary metabolites. *Ulva fasciata* contained sugar and quinone, *Caulerpa scalpelliformis* had only quinone, *Padina tetrastromatica's* the secondary metabolites were alkaloids, quinone and saponin. Alkaloids, phenol and quinone were present in *Stoechospermum marginatum* (Table 1).

When the fractions of *Ulva fasciata* was tested against different pathogens like *Klebsiella pneumonia, Aeromonas hydrophilla, Staphylococcus aureus, Escherichia coli* and *Pseudomonas* sp. highest zone of inhibition was found in *Aeromonas hydrophilla* in F2 fraction. *Caulerpa* inhibited *Staphylococcus* and *E.coli*. In this sample also the F2 fractions were effective. The Phaeophyceae member *Padina* showed a high antibacterial activity against *Klebsiella pneumonia* and *E.coli*. This was proved by using one way ANOVA. Similarly the other member *Stoechospermum marginatum* also inhibited *Klebsiella* and *Staphylococcus*. When the crude extracts of the four seaweeds were tested against the bacteria *E.coli* was inhibited the most by all the four seaweeds. Least activity was found against *Aeromonas*.

The present study clearly proves that when the antibacterial activity of crude and column purified fractions were tested, the purified fractions showed higher activity than the crude extracts (Table 2-5).

Table 1. Showing the secondary metabolites present in seaweeds

Secondary Metabolites	*Ulva fasciata*	*Caulerpa scalpelliformis*	*Padina tetrastromatica*	*Stoechospermum marginatum*
Alkaloids	-	-	+	+
Anthroquinone	-	-	-	-
Catachins	-	-	-	-
Sugar	+	-	-	-
Terpenoid	-	-	-	-
Flavenoid	-	-	-	-
Phenol	-	-	-	+
Quinone	+	+	+	+
Saponin	-	-	+	-

Table 2. Showing the Zone of inhibition (mm) of different fraction of *Ulva fasciata* against different bacterial pathogens

Fractions	*Klebsiella pneumonia*	*Aeromonas hydrophilla*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas hydrophilla*
F1	1 ± 0	2 ± 0.81	1 ± 0	1 ± 0	2 ± 0.81
F2	4 ± 2.44	6 ± 4.08	1 ± 0	1 ± 0	2 ± 0.81
F3	2 ± 0.81	2 ± 0.81	2 ± 0.81	3 ± 1.63	3 ± 1.63

Table 3. Showing the Zone of inhibition (mm) of different fraction of *Caulerpa scalpelliformis* against different bacterial pathogens

Fractions	*Klebsiella pneumonia*	*Aeromonas hydrophilla*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas hydrophilla*
F1	1 ± 0	1 ± 0	2 ± 0.81	1 ± 0	3 ± 1.63
F2	2 ± 0.81	3 ± 1.63	4 ± 2.44	4 ± 2.44	1 ± 0
F3	2 ± 0.81	1 ± 0	4 ± 2.44	2 ± 0.81	1 ± 0

Table 4. Showing the Zone of inhibition (mm) of different fraction of *Padina tetrastomatica* against different bacterial pathogens

Fractions	*Klebsiella pneumonia*	*Aeromonas hydrophilla*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas hydrophilla*
F1	4 ± 2.44	2 ± 0.81	3 ± 1.63	3 ± 1.63	1 ± 0
F2	6 ± 4.08	4 ± 2.44	1 ± 0	4 ± 2.44	3 ±1.63
F3	8 ± 5.71	5 ± 3.26	1 ± 0	6 ± 4.08	1 ± 0

Table 5. Showing the Zone of inhibition (mm) of different fraction of *Stoechospermum marginatum* against different bacterial pathogens

Fractions	*Klebsiella pneumonia*	*Aeromonas hydrophilla*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas hydrophilla*
F1	3 ± 1.63	3 ± 1.63	1 ± 0	2 ± 0.81	3 ± 1.63
F2	6 ± 4.08	4 ± 2.44	6 ± 4.08	5 ± 3.26	2 ±0.81
F3	7 ± 4.81	4 ± 2.44	2 ± 0.81	3 ± 1.63	5 ± 3.26

Table 6. Showing the Zone of inhibition (mm) of Crude extracts of *Ulva fasciata, Caulerpa scalpelliformis, Padina tetrastomatica, Stoechospermum marginatum* against different bacterial pathogens

Seaweeds	*Klebsiella pneumonia*	*Aeromonas hydrophila*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas hydrophila*
Ulva fasciata	4 ± 2.44	3 ± 1.63	6 ± 4.08	7 ± 4.89	4 ± 2.44
Caulerpa scalpelliformis	2 ± 0.81	3 ± 1.63	2 ± 0.81	4 ± 2.44	2 ±0.81
Padina tetrastomatica	2 ± 0.81	2 ± 0.81	2 ± 0.81	3 ± 1.63	3 ± 1.63
Stoechospermum marginatum	3 ± 1.63	4 ± 2.44	1 ± 0	2 ± 0.81	3 ± 1.63

DISCUSSION

In our present study we fractioned the seaweed extracts and the column purified fractions were used for antibacterial studies. Ethanol was used as the solvent. The brown algae (Phaeophytes) were successful in restricting the bacterial pathogens and the most susceptible species was *Klebsiella pneumonia.*

In the present investigation brown algae (*Padina*) inhibited most of the pathogen when compared to green algae. This was in accordance to that of Padmini, 1991 who reported a higher antibacterial activity in brown algae than green algae. Lavania and Veerappan, 2011 also reported red and brown algal extracts inhibited the growth of pathogen higher than the green algae from Gulf of Mannar of Southeast Coast of India. Antibacterial activity of nine species of seaweed belonging to brown, red and green algae collected from Okha West coast of India revealed that red and brown seaweeds had higher antibacterial activity than green algae Rao, 1995.

Johnsi Christobel *et al*., 2011 reported that extracts of *Padina tetrastromatica* inhibited the growth of *E.coli* and *Pseudomonas aeroginosa* responsible for causing the nosocomial infection, effectively. When one way ANOVA was performed between the various seaweeds and bacterial pathogens, the brown algae significantly inhibited the growth of pathogens. In the present study third fraction of *Padina* inhibited *Klebsiella pneumonia* effectively and showed mild activity against *Staphylococcus aureus and Peudomonos aerogenosa.*

Vijayabaskar and Shiyamala, 2011 reported strong inhibition in the growth of *Aeromonas hydrophila* by *Sargassum wightii* from Gulf of Mannar. In the present study *Aeromonas hydrophila* was highly inhibited by second fraction of *Ulva lactuca* and third fraction of *Padina tetrastromatica.* Apart from these, the result of the present study has brought to light that Gram negative organisms (*Klebsiella, Aeromonas, Escherichia, Pseudomonas*) were more suspectible to the extract of brown seaweeds.

In conclusion, differences between the results of the present investigation and the results of other studies may be due to the production of bioactive compounds related to organic solvents used for the extraction, habitat and the season of algae collection, different growth stages of plants etc. The seaweed collected varied in the bioactivity which may be influenced by available nutrient, temperature, effect of pollution etc. In our present study, the antibacterial activity of the crude and fractions were compared. The fractionated seaweeds showed higher activity compared to crude. So in future, these purified fractions can be used for GCMS and NMR studies. The compounds responsible for the activity can be obtained from GCMS. Their structure can be found out using NMR and they can be further synthesized chemically to form antibacterial drugs.

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