ISSN: 0975-766X
Available Online through
www.ijptonline.com
Research Article

ANTIBACTERIAL ACTIVITY OF SEAWEED *ULVA LACTUCA* AGAINST FISH PATHOGENS ISOLATED FROM MARINE FISH *KATSUWONUS PELAMIS*

[1] S. Emmanuel Joshua Jebasingh*, [2] R.P. Rajesh, [1] M. Lakshmikandan
[1]Department of Biotechnology, Sri Paramakalyani Center for Excellence in Environmental Science, Manonmaniam Sundaranar University, Alwarkurichi, Tirunelveli-627412, Tamilnadu, India.
[2] Indian Institute of Science, Molecular Biophysics Unit, Bangaloure, India.
E-mail: singhmicromsu@gmail.com

Received on 05-03-2011 *Accepted on 25-03-2011*

Abstract

The world's oceans, covering more than 70% of the earth surface represent an enormous resource for the discovery of potential chemotherapeutic agents. Marine algae or seaweed the most accessible marine resources of the coastal zone occupy potentially important place as a source of biomedical compounds. To screen antibacterial activity of seaweed *Ulva lactuca* against fish pathogenic bacteria from skip jack tuna, *Katsuwonus pelamis.* The load of bacteria on the outer surface of skip jack tuna is high, when compared with that of gills. Acetone, hexane, methanol and chloroform crude concentration of the seaweed *U. lactuca* extract was tested antibacterial activity against pathogenic bacteria with a range of zone of inhibition is 1 to 2 mm. The partitioned crude methanol extract of the seaweed *U. lactuca* showed highest activity in butanol phase followed by ethyl acetate phase. The zone of inhibition ranged from trace to 4 mm. The chromatographic fraction of the *U. lactuca* also exhibited broad spectral activity against all the pathogenic bacteria. The highest inhibition was found in elution fractions (E.A: M 75:25). The zone of inhibition ranges from 1mm to 6 mm.

Keywords: Marine algae, Fish pathogens, Antibacterial activity, Sea weeds, Natural products.

Introduction

Marine environment is an exceptional reservoir of biologically active natural product, many of which exhibit structural features not been found in terrestrial natural products. Marine algae or Seaweed, the most

accessible marine resources of the coastal zone occupy potentially important place as a source of biomedical compounds[1]. They are the only source for the production of Phytochemical such us agar, carrageenan and algin also contain many trace elements, minerals, protein, iodine, bromine, vitamins and many bioactive substances. A marine

alga was first investigated for the antibiotic activity in 1951 and since then; many works have been carried out on the antibacterial activity of marine plants[2].

Pharmaceutical importance of marine algae is very well known all over the world and extensive efforts were made by research workers from India and abroad to bring out the bioactive substances from seaweeds belonging to Phaeophyceae, Rhodophyceae and Chlorophycea[3]. In India, most of the normal diseases are caused by the foodborn pathogens. One of the main sources of food in the coastal regions of India is fish, which have bacteria in their body, leading to various bacterial diseases. Nowadays, antibiotic treatment of bacterial diseases in fish culture has been widely applied. The prevention and treatment of these infectious diseases by applying products from marine organisms appears as a possible alternative. Hence, the interest in marine organisms as a potential and promising source of pharmaceutical agents has increased during the last year. Seaweeds are considered as such 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[4]. There are numerous reports concerning the inhibiting activities from macro algae against human pathogens, fungi and yeasts, but only few contain data about effects against fish pathogens.

Therefore, the aim of the present study was to investigate the antimicrobial activity of extracts of green seaweed *Ulva lactuca* against fish pathogenic bacteria isolated from the commercially valued skip jack tuna, *Katsuwonus pelamis* that are often the cause of bacterial diseases in aquaculture then to find out novel drug leads.

Materials and Methods

Isolation of pathogenic bacteria

The seaweed *Ulva lactuca* and the fish were collected from Tuticorin coast (Lat. 8°45 N; Long. 78°10 E) of Gulf of Mannar, Southeast Coast of India. The commercially valued skip jack tuna, *Katsuwonus pelamis* was

selected for isolation of pathogenic bacteria. The tuna were purchased from the landing centre in the Therespuram fishing village which is located about 2 km north of Tuticorin Town.

Microbiological analysis:

The bacteria were collected by vigorously swabbing with a sterilized cotton swap on the body surface, gills and tissues of *Katsuwonus pelamis.* Enumeration of the total bacterial load was done using the plate count agar by the conventional pour plate technique.

Extraction of seaweed

The seaweed *Ulva lactuca* was collected fresh, washed thoroughly to remove the adherents and air dried in shade for 24 h at room temperature. The dried seaweed was powdered using a sterile mortar and pestle and extracted (10 ml solvent to 0.1g of powder) with the solvents chloroform, diethyl ether, acetone, and methanol. The extracts were cold steeped overnight at -20°C, filtered with Whatman No. 1 filter paper, evaporated and concentrated [5].

The crude extracts were screened against bacterial pathogens isolated from marine commercially valued fish skip jack tuna, *Katsuwonus pelamis*. Antibacterial assay was carried out by following the standard disc diffusion method[6].

Partitioning of crude extract

The acetone extract of *Ulva lactuca*, was partitioned between ethyl acetate and water, and then this water phase was subsequently partitioned against n-butanol to localize and to assess the polarity of the active substance[7]. The three phases were then collected separately, evaporated, concentrated and screened against marine fish pathogenic bacteria.

Column chromatography

Partial purification of crude extract was carried out following the method outlined[7]. The diethyl ether extract was fractionated using normal phase silica gel (200-400mesh). Column chromatography employing a step gradient solvent system from low to high polarity viz Hexane100%, Hexane75% : Ethylacetate25%, Hexane50% :

Ethylacetate50%, Hexane25% : Ethylacetate75%, Ethylacetate100%, Ethylacetate75% : Methanol25%, Ethylacetate50% : Methanol50%, Ethylacetate25% : Methanol75%, Methanol100%.

Results

In the present investigation, the microbial samples were collected from the body surface and gills of tuna, *Katsuwonus pelamis* by swabbing with a sterile cotton swab and analysed in the laboratory. The estimation of Total Bacterial Count (TBC) and the isolation of some fish pathogenic bacteria. Further, identification of the isolated bacteria from the Medias was done using standard methods. The isolated bacteria's were subjected to antibacterial activity of *Ulva lactuca* seaweed extract.

Microbiological analysis

The microbiological analysis of different parts of the *Katsuwonus pelamis* showed variations in the number of colonies. The changes of total viable bacteria (Total Bacterial Count, TBC) were presented in Table 1. The microbial load was more on the surface of the fish than the gills. As the *K. pelamis* were collected from the Therespuram landing center where the coastal water is polluted by sewage discharge and this may be the main cause for the contaminations. The results indicate good difference in the bacterial load of gills when compared with that of the surface of the fish. The bacterial load decreased from 170 to 20 on the surface of the fish from the initial dilution to the final dilution.

Table 1: Total Bacterial Count (TBC) loads in marine fish *Katsuwonus pelamis.*

S.No	Dilutions	Samples	
		Outer Surface	Gills
		(TBC)	
1	10^{-1}	170	70
2	10^{-2}	120	20
3	10^{-3}	50	5
4	10^{-4}	20	2
5	10^{-5}	-	2

Antibacterial activity of crude extract

The results from the antibacterial assay with the crude concentration of the seaweed *U. latuca* are presented in Table 2. Acetone crude extract showed activity against 7 pathogenic bacteria with the range of inhibition zone of 1 to 3 mm. The Methanol crude extract followed with activity against 6 pathogenic bacteria and with a range of

zone of inhibition of 1 to 2 mm. Chloroform extract also showed activity against 8 pathogenic bacteria and the zone of inhibition varied between trace and 2 mm. and also diethyl ether extract showed activity against 8 pathogenic bacteria zone of inhibition varied between trace and 2 mm.

Table 2: Antibacterial activity of Column purified extract of *U.latuca.*

Fish Pathogens	H	H:EA	H:EA	H:EA	E A	EA:M	EA:M	M
	100	75:25	50:50	25:75	100	75:25	50:50	100
***Pseudomonas* sp.1**	-	-	-	-	-	4	2	1
***Pseudomonas* sp.2**	-	-	-	-	-	6	2	-
***Pseudomonas* sp.3**	-	-	-	-	-	6	2	-
***Salmonella* sp.1**	-	-	-	-	-	6	2	2
***Salmonella* sp. 2**	-	-	-	-	-	5	-	-
***Salmonella* sp. 3**	-	-	-	-	-	1	-	-
***Aeromonas* sp.1**	-	-	-	-	-	6	-	1
***Aeromonas* sp.2**	-	-	-	-	-	6	2	-
E.coli 1	-	-	-	-	-	5	-	-
E.coli 2	-	-	-	-	-	6	2	-

Antibacterial activity of partitioned extract

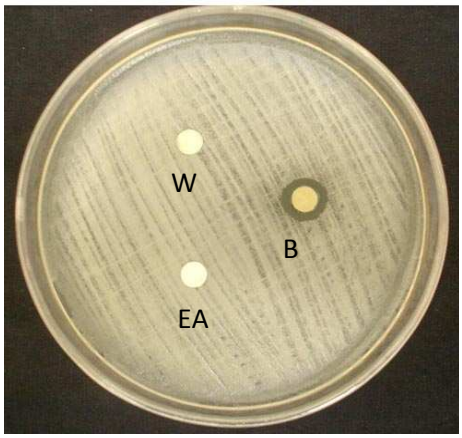
The results from the antibacterial assay with the partitioned crude methanol extract of the seaweed *U. latuca* are presented in Table 3 and Fig 1. The partitioned crude methanol extract of the seaweed *U. latuca* showed highest activity in butanol phase followed by ethyl acetate phase. The zone of inhibition ranged from trace to 4 mm. Butanol phase showed activity against *Pseudomonas* sp.1, *Pseudomonas* sp. 2, *Salmonella* sp.1, *Salmonella* sp.2, *Salmonella* sp.3, *Aeromonas* sp.1, *Aeromonas* sp. 2, *E. coli* sp.1 *and E. coli* sp 2. Highest zone of 4 mm was observed against *Salmonella* sp.2. Ethyl acetate phase showed activity against sp.1, *and Aeromonas* sp.1, Water phase showed trace to 1 mm activity against *Pseudomonas* sp. 2

Table 3: Antibacterial assay of crude extracts of seaweed *Ulva lacuca.*

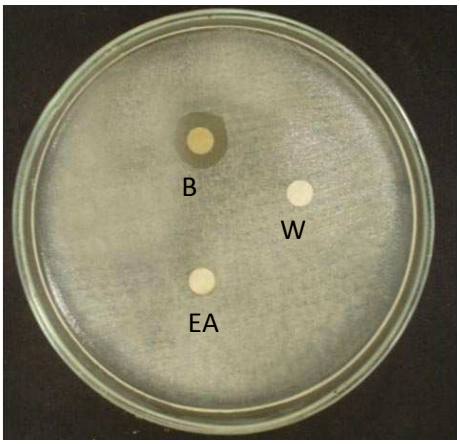
S.No.	Fish pathogens	Different solvents						
		Zone of inhibition in (mm)						
		CH	DE	A	M	EA	B	W
1.	***Pseudomonas* sp.1**	T	1	-	1	-	1	-
2.	***Pseudomonas* sp.2**	1	1	-	-	-	1	-
3.	***Pseudomonas* sp.3**	2	-	2	2	-	2	-
4.	***Salmonella* sp.1**	1	T	3	-	-	1	-
5.	***Salmonella* sp. 2**	-	T	2	2	-	4	-
6.	***Salmonella* sp. 3**	T	1	2	1	-	2	-
7.	***Aeromonas* sp.1**	2	2	2	-	-	2	-
8.	***Aeromonas* sp.2**	-	-	-	-	-	2	-

9.	***E.coli 1***	T	1	2	2	-	3	-
10.	***E.coli 2***	T	T	1	2	-	3	-

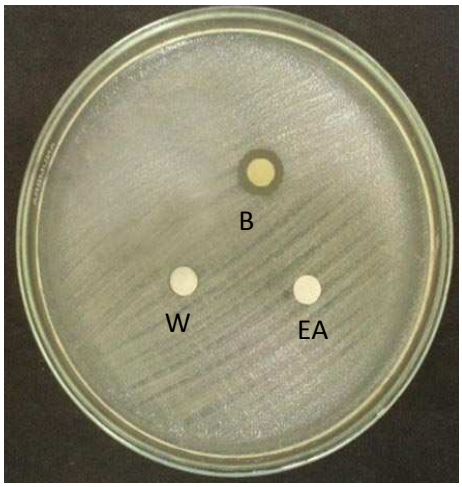
Fig. 1: Antibacterial activities of partitioned crude Acetone extract of *U.lacuca* against fish pathogenic bacteria (EA- Ethyl acetate, B- Butanol, W- Water base)



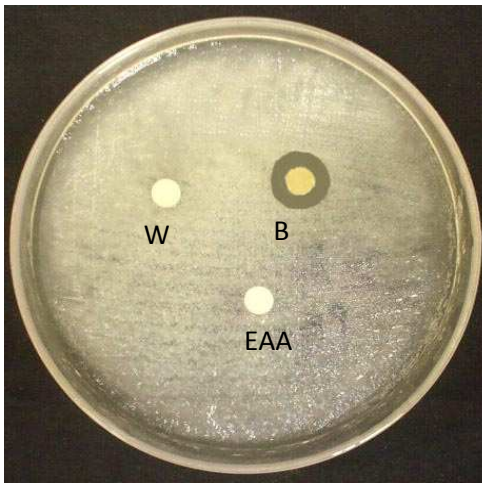
***Aeromonas* sp.1**



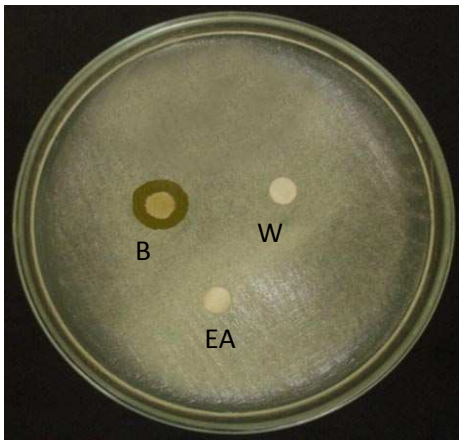
***Aeromonas* sp.2**



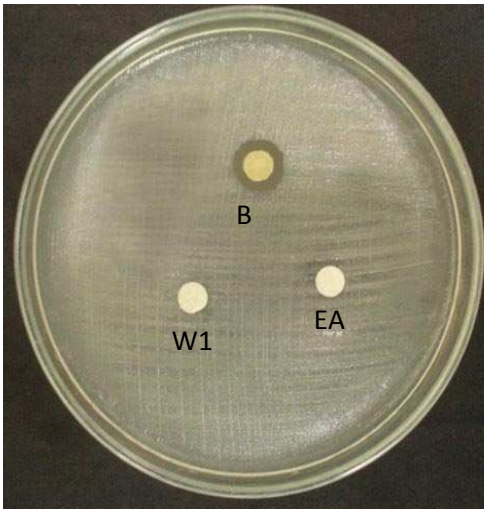
***Salmonella* sp.**



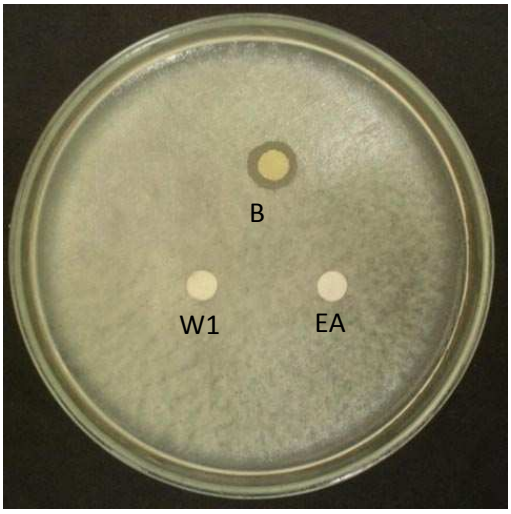
***Salmonella* sp. 2**



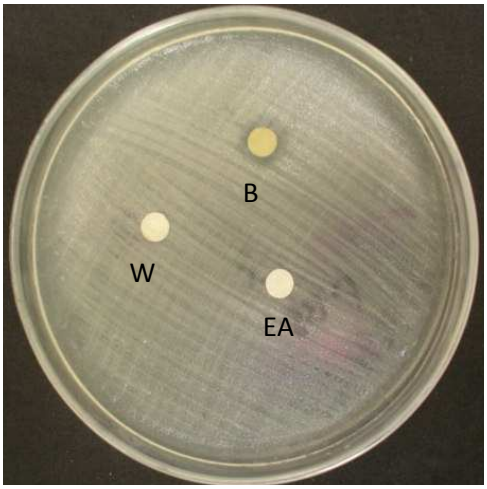
Salmonella



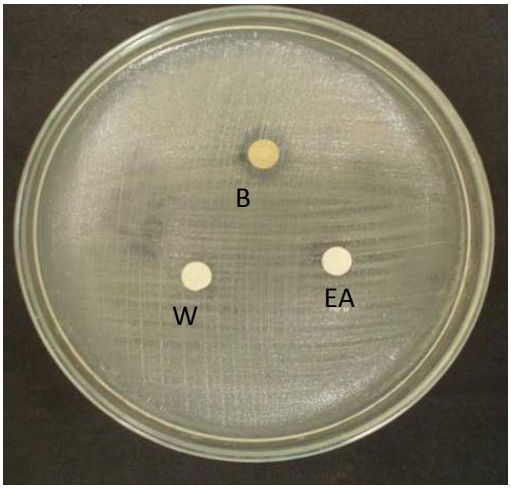
***E. coli* sp 1**



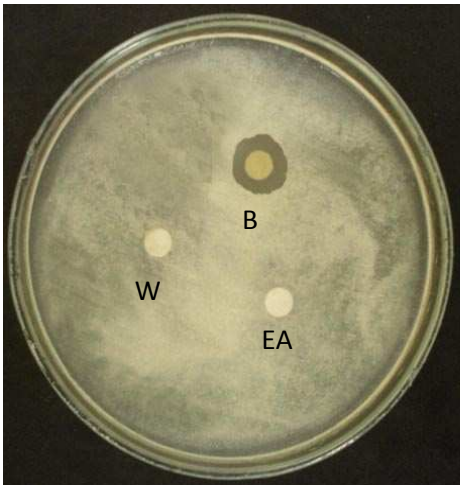
***E. coli* sp2**



***Pseudomonas* sp.1**



***Pseudomonas* sp.2**



***Pseudomonas* sp.3**

The chromatographic fraction of the *U. latuca* also exhibited broad spectral activity against all the 10 pathogenic bacteria. The highest inhibition was found in elution fractions (E.A: M 75:25) the result was presented. The zone of inhibition range from 1mm to 6 mm. In EA: M 50:50 and 100% Methanol fractions have minimum activity

Discussion

In the present investigation the bacterial genera such as, *Pseudomonas* sp, *Salmonella* sp. *Aeromonas* sp. and *E.coli* were isolated from *Katsuwonus pelamis*. The strains were subjected to antibacterial study using *Ulva lactuca* extracts. The seaweeds are known to produce secondary metabolites which help them in defending themselves against bacteria. The study pertaining to antimicrobial activity in marine organisms was initiated around 1950s[8]. In this study acetone extracts of *Ulva lactuca* showed high level activity in the preliminary screening. It showed wide spectrum activity against all the pathogenic bacteria. The seaweed produce a variety of secondary metabolites and many of which exhibits a broad spectrum of bioactivity[9] which coincides with the observation. In the present study, only 50 µg/disc concentrations of the crude and partitioned extracts were used for the assay. The higher activity shown in Butanol phase of *Ulva lactuca* could be attributed to the purity factor. The further purification would reveal more potent activity. This activity may be due to the presence of phenolic compounds. The disc diffusion assay has been followed in the present study to assess the antibacterial activity of extracts.

The partitioning of the crude extract in the present study showed the presence of both non-polar and intermediate polar compounds. Butanol phase showed higher activity against nine bacteria indicating the presence of active compounds in intermediate phase. The present study coincides with the observation of that the activity in the green algae *Udotea flabellum* was localized in ethyl acetate and butanol phases[10].

Conclusions

The methanol Extracts of *Ulva lactuca* showed broad spectrum activity against fish pathogenic bacteria isolated from skip jack tuna, *Katsuwonus pelamis.* Further study needs for isolating the bioactive compounds and identify the exact nature of the compound.

Acknowledgements

The authors acknowledge with thanks the facilities provide by Suganthi Devadason Marine Research Institute, to carry out this work.

References

1. Rao, P. P. S; Rao, P.S; Karmakar, S. M,. Bot. Mar**.** 31, p.295-298, 1988.
2. Selvi, M; Selvaraj, R, Seaweed. Res. Utilin, 22**,** p.161-166. 2000.
3. Padmini Sreenivasa Rao, P; Sreenivasa Rao, P; Karmarkar, S. M, phykos, 25, p.6-11, 1986.
4. Becerro, M. A; Lopez, N. I; turon, X; Uniz, M. J, J. Exp. Mar. Biol. Ecol., 179, p.195-205, 1994.
5. Murugan, A; Santhana Ramasamy, M, Indian. J. Mar. Sci. 32, p.162-164, 2003.
6. Wright, A. E, (1998). Isolation of marine natural products. In: methods of biotechnology. Natural product isolation. Cannell. R.P.J. (ed.). USA. Humana press inc**,** New Jersy. 4, p. 365-408.
7. Burkholder, P. R; Burkholder, L. M, Science**,** 127, p.1174, 1956.
8. Paul, V. J. Seaweed chemical defenses on coral reefs. In; paul, v.j. (ed.). Ecological roles of marine natural products. Comstock publishing associates, ithaca: p.24-50, 1992.
9. Shynisha Begum, M. (2004). Chemical defense in the seaweeds *cheatomorpha linoides* and *dictyota dichotoma* distributed along Tuticorin Coast, Tamilnadu. M. Sc. Dissertation. Cauvery college for women, Trichrapalli.
10. Liju, V. B, antibacterial activity of seaweeds *avrainvillea erecta* (chlorophyceae), *udotea flabellum* (chlorophyceae) and *dictyota* sp. (phaeophyceae) from tuticorin, southeast coast of india. M. Phil. Dissertation, manonmaniam sundaranar university, india, p.59, 2004.

Corresponding Author:

S. Emmanuel Joshua Jebasingh*,

E-mail: singhmicromsu@gmail.com