Antibacterial Activity of the Extracts of Padina boryana Thivy

A.Sangeetha[1], T. V. Poonguzhali[2]
[1]*Research Scholar, Dept. of Botany, Queen Mary's College, Chennai- 600004*
[2]*Associate Professor, Dept. of Botany, Queen Mary's College, Chennai-600004*

Abstract **- In the marine eco system, seaweeds are directly exposed and are susceptible to ambient micro organisms such as bacteria, fungi and viruses. Seaweed species of padina (brown algae) from the coast of Tamilnadu, India were tested in vitro for their antibacterial activities against different types of bacteria using disc diffusion method. The algal extracts were tested for their antibacterial activity against six strains (Salmonella typhi, Vibrio alginolyticus, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus luteus). Among four solvents tested, methanol exhibited more inhibitory effect followed by ethanol, aqueous and then acetone. Finally, we conclude that marine macro algae from the South coast of Tamilnadu, India are potential sources of bioactive compounds and should be investigated for natural antibiotics. The seaweed extract manifest preferable antibacterial activities, hence in the future, it would be good if it is further taken for treatment of human diseases.**

Index Terms **- Seaweed, bacterial strains, Padina boryana, Antibacterial activity.**

INTRODUCTION

Seaweeds belong to a group of plants known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) depending on their nutrient and chemical composition. Seaweeds constitute a vital part of marine ecosystems. It was estimated that about 90% of the species of marine plant are algae and about 50% of the global photosynthesis is contributed from them (Domettila et al., 2013). Over the past decades, seaweeds have been used by humans as medicine and food and their extracts have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential. The marine environment is a good source of bioactive secondary metaboliets, many of which exhibit structural features not found in terrestrial natural products (Cantillo et al., 2010).

Seaweeds are the reservoirs of carotenoids, pigments, polyphenols, enzymes, diverse functional polysaccharides. Seaweeds are excellent source of vitamin A, B1, B12, C, D and E (Skulberg, 2000). Seaweeds have generated an enormous amount of interest in the pharmaceutical industry as a fresh source of bioactive compounds with immense medicinal potential (Shyamala et al., 2013). Macroalgae produce a wide variety of chemically active metabolites including alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols that have a broad range of biological activities.

Biostimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics. The edible seaweeds contain asignificant amount of the protein, vitamins and minerals essential for the human nutrition (Fayaz et al., 2005). The nutrient composition of seaweed varies and is affected by the species, geographic areas, seasons of the year and temperature of the water. Most of the compounds of marine algae show anti-bacterial activities (Vairappan et al., 2001, Vlachos et al., 1996). Many metabolites isolated from marine algae have been shown to possess bioactive efforts (Oh et al., 2008, Venkateswarlu et al., 2007 and Yang et al., 2006).

Based on the above facts, the present study aims to evaluate the antimicrobial activity of methanol, acetone, and ethanol and aqueous extracts of Padina boryana Thivy.

MATERIALS AND METHODS

The seaweeds were collected from Mandapam coast, Rameshwaram India. Algal sample was handpicked, washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes, extraneous matter and necrotic were removed. Samples were collected in sterilized polyethylene bags, and put in an ice box, then transferred to the laboratory immediately until the experimental work was done. Then seaweeds were blotted on the blotting paper, shade dried at ambient temperature and the samples were grounded into a fine powder using tissue blender. The powdered samples were then stored in the refrigerator for further use. The algae collected was identified as Padina boryana Thivy. and was authenticated by Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The specimen has been submitted to the institute for preservation.

Ten grams of powdered samples were packed in Soxhlet apparatus and extracted with (1:10) solvents like methanol, acetone, and ethanol and aqueous for 8 h, and the filtrate was collected (crude extracts) and stored in the refrigerator until further use.

The algal extracts were tested for their antibacterial activity against six strains (Salmonella typhi, Vibrio alginolyticus, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus luteus). Stock cultures were maintained at 4°C on Nutrient agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated at 24hrs at 37ºC (Elbeshehy et al., 2015).

Antibacterial activity of extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured into the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc were placed in MHA plates and add 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc. The plates were incubated at 37ºC for 24 hrs. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

Seaweeds are broadly screened to isolate drugs or bioactive substances all over the world (Rao, 1991), they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of antimicrobial activities (Cox et al., 2010).

Extracts of brown seaweed were tested against bacteria. The results are presented in Table 1, 2, 3 and 4 and Figs. 1, 2, 3 and 4 respectively. In this study, six test pathogens were considered, namely Salmonella typhi, Vibrio alginolyticus, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus luteus towards study of inhibition of microbial growth. The plates were incubated at 37ºC.

Among four solvents tested, methanol exhibited more inhibitory effect followed by ethanol, aqueous and then acetone. For instance, the methanol extract of Padina boryana Thivy had strong antibacterial inhibition against Salmonella typhi (26 mm, respectively) followed by Escherichia coli (24 mm). The methanol extracts of Padina boryana Thivy showed a broad spectrum of antimicrobial activity.

The solvent system used for the extraction played a major role in displaying the anti-bacterial activity. In the present study, among the different solvent extract, methanol extract of Padina boryana Thivy displayed highest inhibitory activity against the test pathogens.

Ethanol was found to be the best solvent for extracting the active principles in almost all species of seaweeds (Rebecca et al., 2013). Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than n-hexane and ethyl acetate (Tuney et al., 2006). It is clear that using organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activities compared to water-based methods (Lima-Filho et al., 2002). Lim et al., (2011) and Darah et al., (2013) reported that the higher concentration of the extract was needed to kill the microorganism cells than to inhibit the growth of these cells on time-kill profile study.

Table 1. Antibacterial activity of Ethanol extract of Padina boryana Thivy

Organisms	Zone of Inhibition(mm)		
	Sample (1mg/ml)		
	1000 µg	750 µg	500 µg
Pseudomonas aeruginosa	7	-	-
Micrococcus luteus	8	7	7
Salmonella typhi	10	9	8
Vibrio alginolyticus	12	10	8

Escherichia coli	10	8	7
Staphylococcus aureus	10	9	8

Fig 1. Antibacterial activity of Ethanol extract of Padina boryana Thivy

Pseudomonas aeruginosa

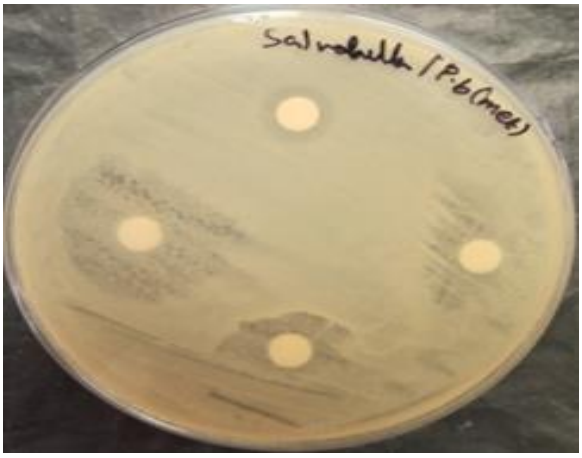
Vibrio alginolyticus

Salmonella typhi

Table 2. Antibacterial activity of Methanol extract of Padina boryana Thivy

Organisms	Zone of Inhibition(mm) Sample (1mg/ml)		
	1000 µg	750 µg	500 µg
Salmonella typhi	26	24	19
Escherichia coli	24	15	8
Pseudomonas aeruginosa	11	9	-
Micrococcus luteus	8	7	-
Vibrio alginolyticus	9	7	7
Staphylococcus aureus	7	7	7

Fig 2. Antibacterial activity of Methanol extract of Padina boryana Thivy



Salmonella typhi

Pseudomonas aeruginosa

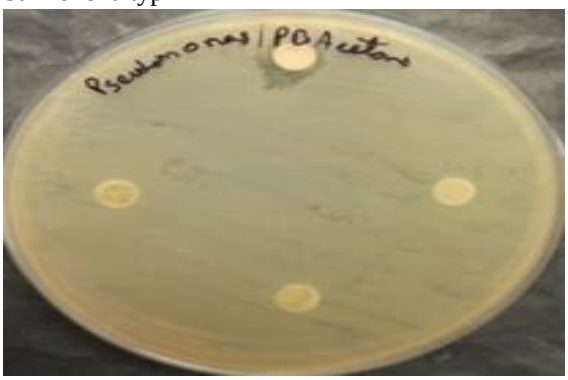
Escherichia coli

Table 3. Antibacterial activity of Acetone extract of Padina boryana Thivy

Organisms	Zone of Inhibition(mm)		
	Sample (1mg/ml)		
	1000 µg	750 µg	500 µg
Salmonella typhi	9	8	-
Vibrio alginolyticus	-	-	-
Escherichia coli	8	8	-
Staphylococcus aureus	7	7	7
Pseudomonas aeruginosa	9	-	-
Micrococcus luteus	7	7	7

Fig 3. Antibacterial activity of Acetone extract of Padina boryana Thivy

Salmonella typhi



Pseudomonas aeruginosa

Escherichia coli

Table 4. Antibacterial activity of Aqueous extract of Padina boryana Thivy

Organisms	Zone of Inhibition(mm)		
	Sample (1mg/ml)		
	1000 µg	750 µg	500 µg
Salmonella typhi	12	9	8
Vibrio alginolyticus	8	9	8
Escherichia coli	7	-	-
Staphylococcus aureus	9	8	9
Pseudomonas aeruginosa	7	7	-
Micrococcus luteus	9	8	-

Fig 4. Antibacterial activity of Aqueous extract of Padina boryana Thivy

Salmonella typhi

Staphylococcus aureus

vibrio alginolyticus

CONCLUSION

The results obtained in the present study clearly demonstrate that the methanol extract of Padina boryana Thivy had strong antibacterial inhibition. The present study concluded that among different solvent extracts, methanol extract of Padina boryana Thivy displayed highest inhibitory activity against the test pathogens. These findings suggested that there may be a potential to utilize such seaweed extracts in food products to act as antimicrobial which would have promising applications in enhancing the food safety. Finally, we conclude that marine macro algae from the South coast of Tamilnadu, India are potential sources of bioactive compounds and should be investigated for natural antibiotics.

REFERENCES

[1] Cantillo-Ciau Z, Moo-Puc R, Quijano L, Freile-Pelegrin Y. The tropical brown alga lobophora variegata: A source of antiprotozoal compounds. Mar. Drugs, 2010; 8: 1292–1304.

[2] Cox S, Abu-Ghannam N, Gupta S. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Int Food Res J, 2010; 17: 205-220.

[3] Darah I., Jain K., Lim S.H., Wendy R. Efficacy of pyroligneous acid from Rhizophora apiculata on pathogenic Candida albicans. J Appl Pharm Sci, 2013; 3: 7–13.

[4] Domettila, J. Joselin and S. Jeeva, 2013. "Phytochemical analysis on some south Indian seaweeds". Journal of Chemical and Pharmaceutical Research, Vol. 5(4).

[5] Elbeshehy EKF, Elazzazy AM, Aggelis G. Silver nanoparticles synthesis mediated by new isolates of Bacillus spp., nanoparticle characterization and their activity against Bean Yellow Mosaic Virus and human pathogens. Frontiers in Microbiology, 2015; 6: 453. doi:10.3389/fmicb.2015.00453.

[6] Ki-Bong Oh, Ji Hye Lee, Soon-Chun Chung, Jongheon Shin, Hee Jae Shin, Hye-Kyeong Kim, Hyi-Seung Lee, 2008. Antimicrobial activities of the bromophenols from the red alga Odonthalia corymbifera and some synthetic derivatives, Bioorganic & Medicinal Chemistry Letters, 18, 104-108.

[7] Lim S.H., Darah I., Jain K., Suraya S. Gallic acid: an anticandidal compound in hydrolysable tannin extracted from the barks of Rhizophora apiculata Blume. J Appl Pharm Sci, 2011; 01 (06): 75-79.

[8] Lima-Filho JV, Carvalho AF, Freitas SM. Antibacterial activity of extracts of six macroalgae from the Northeastern Brazilian Coast. Brazilian J. Microbiol, 2002; 33: 311-313.

[9] Mohamed, Fayaz, K.K. Namitha, K.N. Chidambara Murthy, M. Mahadeva Swamy, R. Sarada, Salma Khanam, P.V. Subbarao and G.A. Ravishankar, 2005. Chemical composition, Iron bioavailability and antioxidant activity of kappsphycus alvarezi (Doty). J. Agric. Food Chemi., 53: 792-797.

[10] Rao P. S. P. Biological investigation of Indian marine algae and screening of some green, red and brown seaweeds for their antimicrobial activity. Seaweed Res Utiln, 1991; 14(1): 37-43.

[11] Rebecca J. L, Dhanalakshmi V, Sharmila S, and Merina P. Das. In vitro antimicrobial activity of Gracilaria SP and Enteromorpha SP. Research Journal of Pharmaceutical, Biological and Chemical Sciences (RJPBCS), 2013 (4) 693-697.

[12] Rui-yun Yang, Chun-yuan Li, Yong-cheng Lin, Guang-tian Peng, Zhi-gang She, Shi-ning Zhou, 2006, Lactones from a brown alga endophytic fungus (No. ZZF36) from the South China Sea and their antimicrobial activities, Bioorganic & Medicinal Chemistry Letters, 16, 16, 4205-4208.

[13] Somepalli Venkateswarlu, Gopala K. Panchagnula, Aditya L. Gottumukkala, Gottumukkala V. Subbaraju, 2007. Synthesis, structural revision, and biological activities of4′-chloroaurone, a metabolite of marine brown alga Spatoglossum variabile, Tetrahedron, 63, 29, 6909-6914.

[14] TuneyI, Çadirci BH, Unal D, Sukatar A. Antimicrobial activities of the extracts of marine algae from the coast of Urla (Azmir, Turkey). Turk J Biol, 2006; 30: 171-5.

[15] Vairappan, C.S., Daitoh, M., Suzuki, M., Abe, T. and Masuda, M, 2001. Antibacterial halogenated metabites from the Malysian laurencia species. Phyto chemistry, 58: 291-297.

[16] Vlachos, V., Critchley, A.T. and Von Holy, A. 1999. Differential anti-bacterial activity of extras from selected southern African macro algal thalli. Bot. Mar., 42: 165-173.