Antibacterial effect of Padina tetrastromatica against pathogenic Bacteria

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Abstract: ***The antibacterial activities in different solvent extracts of Padina tetrastromatica were screened against human bacterial pathogens Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus epidermis and Streptococcus pyogenes. The maximum zone of inhibition was 14mm recorded from methanol extract of Padina tetrastromatica against Escherichia coli and minimum zone of inhibition was 6mm in acetone extract against Staphylococcus epidermis. The minimum inhibitory concentration (MIC) value of Padina tetrastromatica against bacteria was ranged between 0.2±0.1mm (0.1 mg/ml) to 2.2±0.2mm (6.00 mg/ml)***

IndexTerms ***Algae, Antibacterial, Bacterial pathogen, Methanolic extract, Minimum inhibitory concentration, Padina tetrastromatica , Solvent***.

1. INTRODUCTION

They have been screened extensively to isolate lifesaving drugs or biologically active substances all over the world. In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities and are recognized as a potential source of bioactive natural products. Marine organisms are source material for structurally unique natural products with pharmacological and biological activities (Faulkner, 2001). Antimicrobial compounds that at low concentrations exert an action against microorganisms and exhibit therapeutic toxicity towards them. These can be any substances of natural, synthetic or semi synthetic origin that may be used to kill microorganisms including bacteria, fungi, protozoa and viruses. The antimicrobial activity of algal extracts can be detected by observing the growth of the various micro-organisms that have been placed in contact with the algal extracts. The antimicrobial activity was regarded as an indicator to detect the potent pharmaceutical capacity of macroalgae for its synthesis of bioactive secondary metabolites (Gonzalez *et al.*, 2001). The present study was aimed to screen the pharmacological activity in different solvent extracts of *Padina tetrastromatica* against human pathogenic bacteria.

2. MATERIALS AND METHODS

The collected algae were cleaned with seawater and then freshwater to remove all epiphytes, debris and other foreign materials. The algae were shade dried in room temperature. The shade dried materials were subjected pulverized to get coarse powder. The coarse powdered materials were packed in Soxhlet apparatus separately and successively extracted with methanol, distilled water and acetone. These extracts were collected and concentrated in a rotary evaporator. The concentrated extracts were transferred to air tight containers and stored in a refrigerator for subsequent use.

2.1 Bacterial Pathogens

The antibacterial activity of three different solvent extracts from selected macroalgae were investigated against five bacterial strains *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus epidermis* and *Streptococcus pyogenes*. All the cultures used for the present study were obtained from Microbial type Culture Collection (MTCC) at Chandigarh. The compounds were screened for antibacterial activity using the agar well diffusion method

2.2 Minimum Inhibitory concentration

The compound polycyclic meroditerpenoid was evaluated for *in vitro* growth inhibitory activity against Gram-negative bacteria *Escherichia coli* (ATCC 25922). Antibacterial activities of the compounds were tested by the agar - well diffusion method under standard conditions. The Stock solutions of the compound extracts were prepared in *Dimethyl sulfoxide* (DMSO). Further dilutions were performed with distilled water. The concentration ranges of the tested compounds were 0.1 mg/ml, 0.25 mg/ml, 0.5 mg/ml, 1.5 mg/ml, 3.00 mg/ml, and 6.00 mg/ml.

A set of sterilized petridishes were taken and poured sterilized, molten Muller Hinton agar medium in the petridish, then allowed to cool and solidified. After solidification, wells were plucked, with a sterile cork borer. 50µl of standardized suspension of the test organism was transferred on to each plate and 50 microliters of each concentration of compounds were placed in the well plucked in the pre-sterilized petridish. The control contained only organisms and devoid of tested compounds of *Padina tetrastromatica*. The culture plates were incubated at 27^{o}C for 24 hours. After incubation for 24 hours, the diameters of the inhibition (sterile) zone were measured (mm).

3. RESULT

3.1 Antibacterial activity in different solvent extracts of Padina *tetrastromatica*

The different solvent extracts of *Padina tetrastromatica* was screened against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus epidermis* and *Streptococcus pyogenes.* The zone of inhibition ranged from 6mm to 14mm. The maximum zone of inhibition was 14mm in methanol extract against *Escherichia coli* and minimum zone of inhibition was 6mm against *Staphylococcus epidermis* in acetone extract. **(Fig: 1).**

The aqueous extract showed maximum zone of inhibition 11mm against *Pseudomonas aeruginosa*. The zone of inhibition was 10mm against *Staphylococcus epidermis. Escherichia coli, 8mm* against *Salmonella typhi.* The maximum zone of inhibition was 14mm in methanol extract against *Escherichia coli,* 12mm against *Staphylococcus epidermis,* 10mm against *Salmonella typhi.* The acetone extract of *Padina tetrastromatica* showed maximum zone of inhibition 10mm against *Escherichia coli,* 8mm against *Salmonella typhimurium* and minimum zone of inhibition was 6mm against *Staphylococcus epidermis*. There was no inhibitory effect from the *Streptococcus pyogenes*

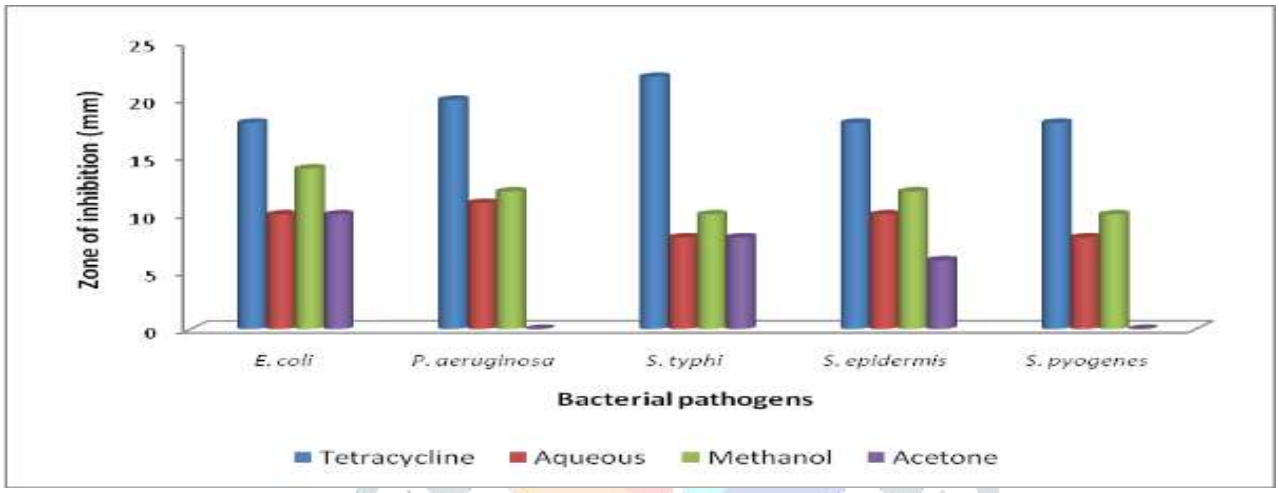

Fig: 1 Antibacterial activity in different solvent extracts of *Padina tetrastromatica*

3.2 Minimum Inhibitory Concentration

The Minimum inhibitory concentration (MIC) of isolated compound (Polycyclic mero diterpenoid) from *Padina tetrastromatica* was done against *Escherichia coli* due to high inhibitory activity. The concentration of compound ranged between 0.1 mg/ml- 6.00 mg/ml. The minimum inhibitory concentration (MIC) of compound extract was 0.1 mg/ml. The zone of inhibition against test organisms were ranged between 0.2 mm to 2.2 mm in different concentration (0.1 mg/ml, 0.25 mg/ml, 0.5 mg/ml, 1.5 mg/ml, 3.00 mg/ml and 6.00 mg/ml). The higher concentration of the tested compounds (6.00 mg/ml) showed maximum growth of tested organism (2.2 ± 0.2mm). The concentration of 3.00 mg/ml showed 1.8 ± 0.0mm. The concentration of 1.5 mg/ml tested compound extract showed 1.4 ± 0.2mm, the concentration of 0.5 mg/ml tested compound extract showed 0.2 ± 0.1mm and the concentration of 0.25 mg/ml showed 0.6 ± 0.2mm. The lowest concentration (0.1 mg/ml) which did not show any growth of tested organism after macroscopic evaluation was determined as Minimum inhibitory concentration (MIC). The tetracycline was used as a control. **(Table: 1).**

Table: 1 Minimum inhibitory concentration of the isolated compound from *Padina tetrastromatica* against *E. coli*

E.coli						
Concentration	**0.1mg/ml**	**0.25mg/ml**	**0.5mg/ml**	**1.5mg/ml**	**3.00mg/ml**	**6.00mg/ml**
Replicate	**___**	**0.6±0.2mm**	**0.2±0.1mm**	**1.4±0.2mm**	**1.8±0.0mm**	**2.2±0.2mm**
Tetracycline	**0.8mm**	**1mm**	**1.2mm**	**1.8mm**	**2.2mm**	**2.3mm**

4. DISCUSSION

Marine algae have been explored as a resource for treating human diseases for centuries, this is because they contain components with therapeutic properties. The antibacterial effect of several macroalgae has been proved (Selvi *et al*. 2001, Tierney *et al*., 2010, Ravi Kumar *et al*. 2002). Rodriguez *et al.*,2010, Bhacuni and Rawat 2005 Priyadharshini *et al* 2011 have reported that seaweeds are an excellent source of components such as polysaccharides, tannins, flavonoids phenolic acids, bromophenols and carotenoids. In the present study three different solvent extracts of *Padina tetrastromatica* inhibited the growth of all the bacterial pathogen tested, but their effect was varied. Depending upon their solubility and polarity, different solvents showed different antimicrobial activity. The inhibitory effect of Escheria coli against methanolic extract was high (14mm) when compared to other extracts. The inhibitory effect of gram negative bacteria E. coli and *Salmonella typhi* were 10mm and 8mm respectively for acetone extracts. Raja *et al.* (2011) indicated that acetone was the best solvent for extracting antimicrobial compounds. But in the present report the effect of acetone was comparatively lower than other extracts. The variation in activity might be due to the loss of compounds during extraction (Kumar et al.,2008). Earlier, studies by Manilal *et al*.,(2016) and Pushpa Raj *et al*.,(2014) also reported the antibacterial activity of *Padina tetrastromatica.* In accordance to the reports given by Christobel *et al* (2011).Aqueous extract of Padina tetrastromatica in the present study also showed considerable antibacterial activity. The results prove that this algal species is an excellent source of bioactive compounds with wide variety of application.

5.References

[1] Bhacuni, D.S, Rawat, D.S. 2005. Bioactive Marine Natural Products. Springer/Anamaya Publishers. 2005. 400 p. ISBN: 978-1402034725

[2] Christabel, J., Lipton, A. P., Aishwarya, M. S., Sarika, A. R., & Udayakumar, A. 2011. Antibacterial activity of aqueous extract from selected macroalgae of southwest coast of India. *Seaweed Research Utilization*, *33*(1 & 2), 67-75

[3] Faulkner. D.J. 2001. Marine natural products. *Natural Product Reports*, 18: 1-49.

[4] Gonzalez del val. A., G. Platas, A. Basilio, A. Cabello, J. Gorrochategui, I. Suay, F. Vicente, E. Portillo, M. Jimenezdelrio, G.G. Reina, and F. Pelaez. 2001. Screening of antimicrobial activities in red, green and brown macro algae from Gran Canaria (Canary Islands, Spain*). Int Microbiol*; 4: 35-40.

[5] Kumar, A., D'Souza, S. S., Gaonkar, S. L., Rai, K. L., & Salimath, B. P. 2008. Growth inhibition and induction of apoptosis in MCF-7 breast cancer cells by a new series of substituted-1, 3, 4-oxadiazole derivatives. *Investigational new drugs*, *26*(5), 425-435.

[6] Manilal, A., Mama, M., Gezmu, T., Merdekios, B., Ameya, G., John, S. E., & Idhayadhulla, A. 2016. An in vitro antibacterial and cytotoxic potentials of bioactive metabolites extracted from *Padina tetrastromatica*. *Biol*, *5*, 65-76.

[7] Priyadharshini,.S, Bragadeeswaran,S, Prabhu, K, Ran, S.S. 2011. Antimicrobial and hemolytic activity of seaweed extracts *Ulva fasciata* (Delile 1813) from Mandapam, Southeast coast of India. In *Asian Pacific Journal of Tropical Biomedicine*, vol. 1, 2011, p. S38–S39.

[8] Pushparaj, A., Raubbin, R. S., & Balasankar, T. 2014. An antibacterial activity of the green seaweed *Caulerpha sertularioides* using five different solvents. *Int J PharmTech Res*, *6*(1), 01-05.

[9] Raja RDA, Jeeva S, Prakash JW, Antonisamy JM, Irudayaraj V. 2011 Antibacterial activity of selected ethnomedicinal plants from South India. *Asian Pac J Trop Med.*;4(11):375–378.

[10] Ravikumar S, L. Anburajan, G. Ramanathan, N. Kaliaperumal. 2002. Screening of Seaweed extracts against antibiotic resistant post-operative infectious pathogens. *Seaweed Res. Utilization*; 24: 95-99.

[11] Rodriguez-Jasso, R. M., Mussatto, S. I., Pastrana, L., Aguilar, C. N., & Teixeira, J. A. 2011 Microwave-assisted extraction of sulfated polysaccharides (fucoidan) from brown seaweed. *Carbohydrate Polymers*, *86*(3), 1137-1144.

[12] Selvi, M., R. Selvaraj and Anandhi Chidambaram. 2001. Screening for antibacterial activity of macroalgae. *Seaweed Res. Utilin.*, 23(1&2): 59-63.

[13] Tierney, M. S., Croft, A. K. & Hayes, M. 2010. A review of antihypertensive and antioxidant activities in macroalgae. *Bot. Mar*. 53:387-408.