Screening Phytochemical Constituency Profiling and Antimicrobial Activity of brown seaweed, *Padina gymnospora* extracts against chosen isolates

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Abstract
Marine based drug discovery had become a fascinating field and nowadays seaweeds play an interesting role. Seaweeds are important source of bioactive molecules with known beneficial effects on human health. In the present study the brown seaweed, *Padina gymnospora* was collected and using Soxhlet apparatus the selected seaweed was extracted with two different solvents, namely chloroform and methanol. Qualitative method of Phytochemical screening was performed and the antibacterial activity was determined by agar well diffusion method. The extracts were administered for the antimicrobial screening against chosen clinical isolates by measuring the zone of inhibition. There was an increase in the diameter of the zone of inhibition when the concentration was increased. These results suggested that the extract of the brown seaweed, *P. gymnospora* have the ability to inhibit the growth of bacteria irrespective of their nature and the results confirm that *P. gymnospora* has the antimicrobial property and on further research it can emerge as a best alternative medicine for various pathogens.

Keywords: *Padina gymnospora,* Seaweed, Antimicrobial activity, Phytocomponents

Introduction

The interest in marine-based drug discovery has been in and out of the limelight in the past few decades (David et al., 2003). The field is being extensively investigated by many research groups and pharmaceutical companies across the globe. Gulf of Mannar Marine Biosphere Reserve (GOMBR) is one of the few of its kind and world renowned for its plenteous and multifarious marine diversity. The Exclusive Economic Zone (EEZ) of Gulf of Mannar covers about 15,000 km² in which, commercial fishing is carried out in about 5,500 km² up to a depth of 50m. Considerable research has been done in the fields of ecosystem preservation, biodiversity, finfish fishery, shellfish fishery, aquaculture, and seaweed industry in Gulf of Mannar (Nammalwar and Joseph, 2002).

Seaweeds or marine algae are primitive non-flowering plants without true root, stem and leaves. Seaweeds have been used as food in Asian countries for centuries as they contain carotenoids, dietary fibres, proteins, vitamins and minerals especially Red and brown algae (Manivannan et al., 2011). Seaweeds are also known to possess valuable medicinal properties such as anticoagulants, antiangiogenic for antiadhesive activities (Cumashi et al., 2007).

Amongst approximately 30,000 species of marine seaweeds, only a small percentage is screened for as potential bioactive compounds (Bhakuni and Rawat, 2005). The chemical forms of these compounds include haloforms, halogenated alkenes, alkenes, alcohols, aldehydes, hydroquinones and ketones that are used in the treatment of many diseases as antibiotics (Lincoln et al., 1991). Recently seaweeds have received significant attention for for their potential as natural antioxidants, antibacterial and cytotoxic properties (Neeta et al., 2012, Kayalvizhi et al., 2012, Mayalen et al., 2007).

In ever-growing antimicrobial resistant world, the prevention and treatment of infectious diseases by marine seaweeds appears to be a possible alternative resource. In the current study the *P. gymnospora* was administered for the evaluation of antimicrobial activity against certain clinical isolates.

Materials and Methods

Selection and Collection of Seaweed

The Seaweed was collected from Mandapam near Rameshwaram, TamilNadu, India. It belongs to the Gulf of Mannar costal line which is generally termed as "Biological paradise". The Brown seaweed, *P, gymnospora* was collected approximately at the Latitude and Longitude locating 9.2838° N, 79.1705° E.

Preparation of Extracts

Seaweeds were washed thoroughly with sterile seawater to remove attached debris and sand particles. The final washing was done using double distilled water and dried under shade. The dried seaweeds were ground to fine powder using the mechanical grinder. About
thirty gram of dried powder of sea weed was reflexed successfully with methanol and chloroform solvents by continuous hot percolation method using soxhlet apparatus. After concentration, methanol and chloroform extracts of *P. gymnospora* residue stored in air tight containers were subjected to further study.

Phytochemical screening

Phytochemical screening of the extract was carried out according to the standard method as described by Trease and Evans (1989).

(i) Test for Saponins

Each of the extract was separately stirred in a test tube, foaming which persisted on warming was taken as an evidence for the presence of saponins.

(ii) Test for Cardiac Glycoside

Small portion of the extract was hydrolyzed with dilute hydrochloric acid for a few hours in a water bath and then the hydrolysate was tested with Legal's reagent, borntrager's reagent and Baljet reagent to detect the presence of various sugars.

(iii) Test for Alkaloids

To a few ml of filtrate, 1 ml of Mayer's reagent (potassium mercuric iodide solution) was added. White or cream-coloured precipitate indicates the presence of alkaloids.

(iv) Test for Flavonoids

The extract was treated with a small piece of magnesium ribbon; this was followed by drop wise addition of concentrated hydrochloric acid. Colours ranging from orange to red indicate flavones, red to crimson indicate flavonols, crimson to magenta indicate flavonones.

(v) Test for Tannins

For tannins identification, one mL of ferric chloride (5% $FeCl_3$) was added to 1 mL of the algal extract. Formation of dark blue or greenish black color indicates the presence of tannins.

(vi) Test for Terpenoids

Two or three granules of tin metal were added in 2 ml thionyl chloride solution. Then the extract was added into test tube and warmed. The formation of pink colored indicates the presence of tri terpenoids.

(vii) Test for steroids

The extract was dissolved in chloroform and equal volume of concentrated sulphuric acid was added. Bluish red to cherry red in the chloroform layer and green fluorescence in the acid layer indicates the steroidal components in the tested extract.

(viii) Test for phenol

The extract was dissolved in 5 ml of distilled water and to this, few drops of neutral 5 % ferric chloride solution were added. A dark green colour indicates the presence of phenolic compounds.

(ix) Test for Coumarin

For coumarins identification, 1 mL of 10 % NaOH was added to 1 mL of algal extract. Formation of yellow colour indicates the presence of coumarins.

Test microorganisms

The chosen Bacterial strains were obtained from Microbial Type Culture Collection centre, IMTECH, Chandigarh which includes Gram positive bacteria such as *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 2057), *Enterococcus faecalis* (MTCC 3159) and *Micrococcus luteus* (MTCC 11948) and Gram Negative Bacteria such as *Yersinia enterocolitica* (MTCC 4865), *Klebsiella pneumoniae* (MTCC 4031), *Escherichia coli* (MTCC 406) and *Vibrio cholerae* (MTCC 3906).

Agar well diffusion assay

The solvents like chloroform and methanol were used to collect the seaweed extract and were tested against the human pathogens at four dose levels (40, 60, 80 and 100). Overnight grown bacterial culture was transferred to sterile Petri plate with Muller Hinton agar medium (Hi Media Laboratories Limited, Mumbai, India) and was spread with sterile spreader to create a lawn. About 5 wells of 6mm diameter were made in each plate with the help of a sterile cork borer. Among the five, four wells placed with different concentrations of the extracts using sterile pipettes and remaining one well was kept as control which had solvent alone. The Petri Dishes were prepared and incubated for 18- 24hrs at 37°C and the zone of inhibition around the well was measured to the nearest millimeter. Each experimental result was determined by the average of triplicate sets.

Results and Discussion

The phytochemicals were screened in *Padina gymnospora* extracts using standard procedures. The methanol and chloroform extracts were assessed for the presence or absence of nine major phytochemicals. It was observed that Phytoconstituents namely Alkaloids, phenols, Steroids, Terpenoids, Cardiac glycosides and amino acids were present in both chloroform and methanolic extracts of *Padina gymnospora*. Whereas other Phytoconstituents namely coumarin was present only in Chloroform extract and Tannins was present only in methanolic extract. Flavonoids and saponins were not found in both the extracts. These phytoconstituents exhibit effective therapeutic potential which enables them to be a good choice for therapeutic intervention.

Seaweeds are nonvascular and photosynthetic plants that inhabit the coastal regions commonly within rocky intertidal or submerged reef-like habitats and have been one of the richest and most promising sources of bioactive primary and secondary metabolites with antimicrobial properties (Faulkner, 2002; Lima-Filho et al., 2002; Fayaz et al., 2005; Tuney et al., 2006; Bansemir et al., 2006; Chew et al., 2008; Cox et al., 2010; Jebasingh et al., 2011). Microorganisms have developed new strategies to evade the action of antibiotics, which results in simultaneous development of resistance to many antibiotic classes making extremely dangerous multidrug resistant (MDR) strains of bacteria such as "superbugs" (Sande-Bruinsma et al., 2008; Thanigaivel et al., 2015).

Synthesis of different metabolites from seaweeds is an indicator of the presence of antimicrobial active compounds (Chiheb et al., 2009). It is also valuable to test these marine antimicrobials for possible synergism with existing drugs (Cheung et al., 2014). Restricting the genetically diverse pathogenic infectious diseases with new novel biologically active sources of potential antimicrobial agents from seaweeds is considered necessary in recent era (Moorthi and Balasubramanian, 2015).

The present study comprises the preparation of solvent extracts and the antimicrobial activity of methanol and chloroform extracts of the brown seaweed was assayed against the chosen isolates. It was observed the increase in Concentration (ul) directly proportional to the inhibition activity against the chosen isolates.The inhibition zone diameter in Fig 1, 2 expresses the state of high inhibition activity of the seaweed extract against the Gram positive strains of Microbes of Clinical Importance.These results suggested that the extract of the brown seaweed, *P. gymnospora*, has the ability to inhibit the growth of bacteria irrespective of their nature. But variations in their activity are accounted for due to the presence of certain phytochemical components based on the concentration that are specific to antimicrobial response.The antimicrobial activity was observed to be significant based on the zone of inhibition influenced by the presence of alkaloids. Thus *P. gymnospora* has better antimicrobial property and further research need to be carried out towards the seaweeds to bring out newer therapeutic agents against several pathogens.

Table Phytochemical screening of methanol and chloroform extracts of ***Padina gymnospora***

Phytochemicals	***Padina gymnospora***	
	Chloroform	Methanol
Alkaloids	+	+
Phenol	+	+
Flavanoids	-	-
Saponins	-	-
Steroids	+	+
Tannins	-	+
Terpenoids	+	+
Coumarin	+	-
Cardiac glycosides	+	+
Amino acid	+	+

Fig 1 Antibacterial activity of chloroform extract of *Padina gymnospora*

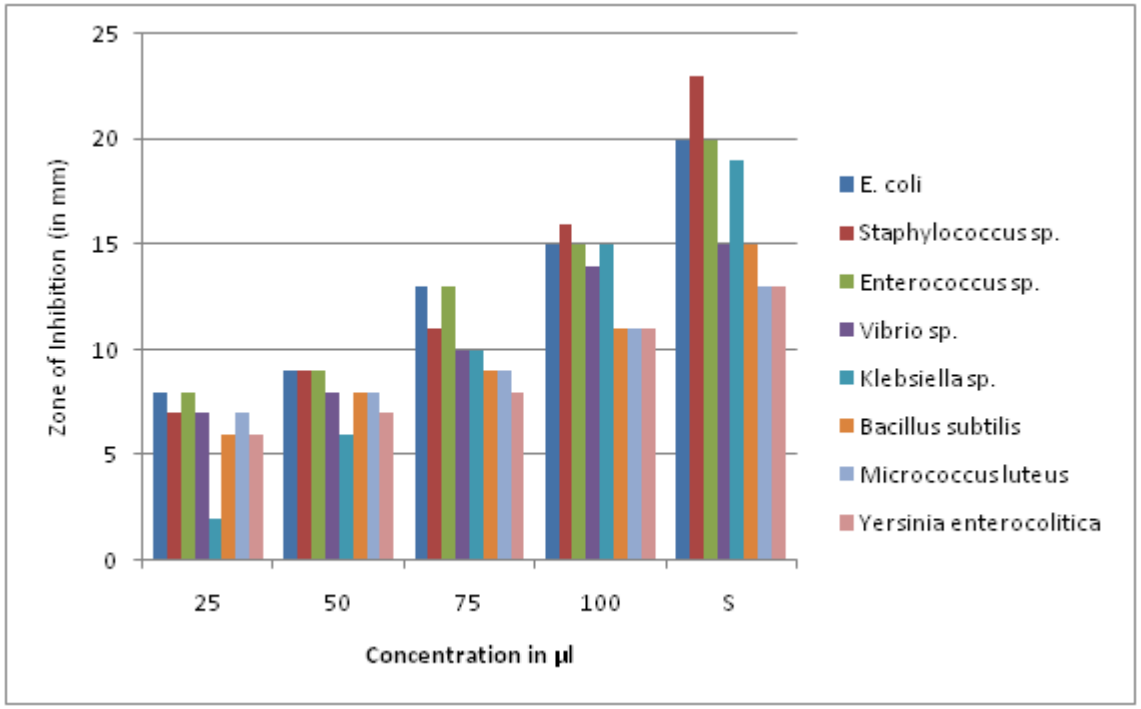
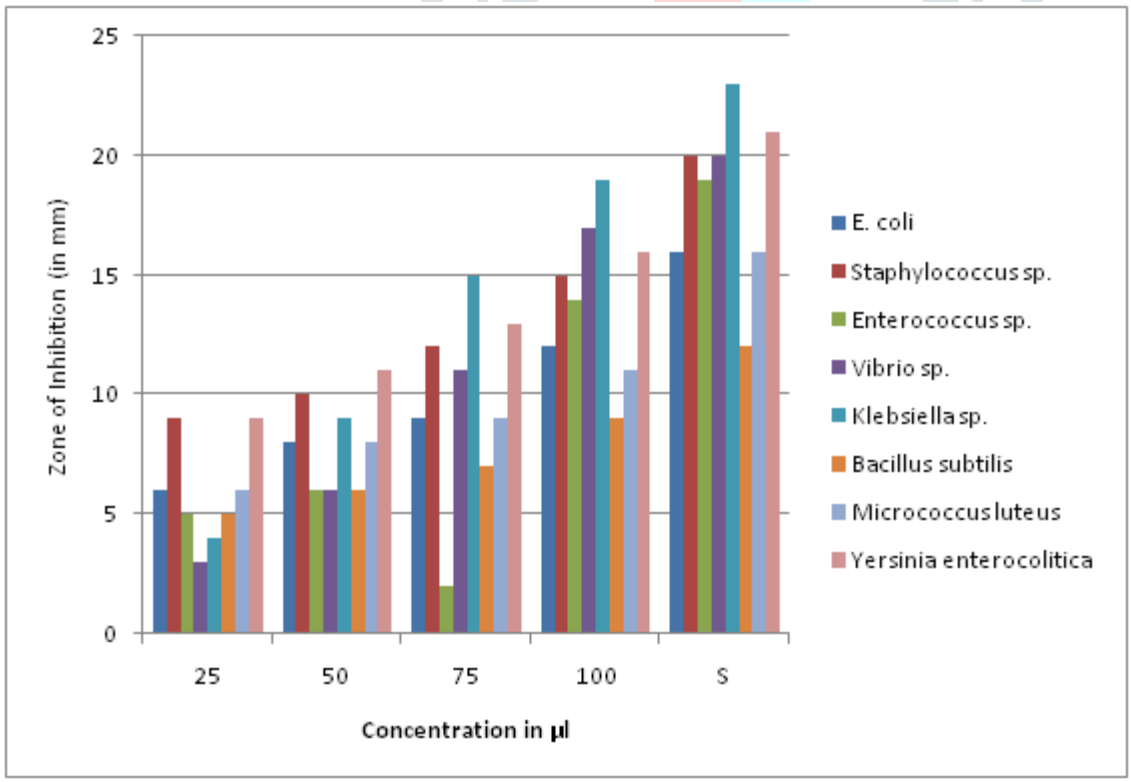


Fig 2 Antibacterial activity of methanol extract of *Padina gymnospora*



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References

1. Bansemir, A., Blume, M., Schröder, S., & Lindequist, U. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture, 252,79-84. (2006).

2. Bhakuni, D., & Rawat, DS. Bioactive marine natural products. New Delhi, India. Anamaya Publishers. (2005).

3. Chander, MP., Veeraragavam, S., & Vijayachari, P. Antimicrobial and hemolytic activityof seaweed Padina gymnospora from South Andaman, Andaman and Nicobar Islandsof India. Int J Curr Microbiol App Sci, 3, 364-369. (2014).

4. Cheung, RCF., Wong, JH., Pan, WL. Chan, YS., Yin, CM., Dan, XL., Wang, H.X., Fang, EF., Lam, SK., Ngai, PH., Xia, LX., Liu, F., Ye, XY., Zhand, GQ., Liu, QH., Sha, O., Lin, P., Ki, C., Bekhit, AA., Bekhit Ael, D., Wan, DC., Ye, XJ., Xia, J., & Nq TB. Antifungal and antiviral products of marine organisms. Applied Microbiology and Biotechnology, 98, 3475- 3494. (2014).

5.Chew, YL., Lim, YY., Omar, M., & Khoo, KS. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Science and Technology, 4, 1067-1072. (2008).

6.Chiheb, I., Hassane, R., Martinez, L., Jose, D., Francisco, G., Antonio, B., Hassan, B., & Mohamed, K. Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. African Journal of Biotechnology, 8, 1258-1262.

7. Cox, S, Ghannam, AN and Gupta, S. (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. International Food Research Journal, 17, 205-220. (2009).

8. Cumashi, A., & Natalia, A. Ushakova, A. comparative study of the anti inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. Glycobiology, 17, 541-552.2007

9. Faulkner, DJ. Marine natural products. Natural Product Reports, 19,1-48, (2002).

10. Fayaz, M., Namitha, KK., Murthy, KNC., Swamy, MM., Sarada, R., Khanam, S., Subbarao PV., & Ravishankar, GA, Chemical composition, iron bioavailability and antioxidant activity of Kappaphycus alvarezzi (Doty), Journal of Agricultural and Food Chemistry, 53, 792-797. (2005).

11. Jebasingh, EJS, Rosenmary, R., Elaiyaraja, S., Sivaraman, K., Lakshmikandan, K., Murugan A., & Raja, P. Potential antibacterial activity of selected green and red seaweeds. Journal of Pharmaceutical and Biomedical Sciences, 5, 1-7. (2011),

12. Kayalvizhi K., Subramanian, V., Anantharaman, P., & Kathiresan, K. Antimicrobial activity of seaweeds from the Gulf of Mannar. Int. J. Pharm. App., 3, 306-314.2012.

13. Lima-Filho, JVM., Carvalho, AFFU., & Freitas, SM. Antibacterial activity of extracts of six macroalgae from the Northeastern Brazillian coast. Brazilian Journal of Microbiology, 33, 311-0313. (2002)

14. Lincoln, RA., Strupinski K., & Walker, JM. Bioactive compounds from algae. Life Chemistry Reports, 8, 97-183.(1991).

15. Manivannan, K., Karthikaidevi, G., Anantharaman, P., & Balasubramanian, T. Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. Asian Pac J Trop Biomed. 1, 114-120.2011.

16. Mayalen, Z., Daniel, R., Yolanda, F. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. J ApplPhycol. 19,449-458.2007.

17. Moorthi, PV., & Balasubramanian, C. Antimicrobial properties of marine seaweed, Sargassum muticum against human pathogens. Journal of Coastal Life Medicine, 3, 122-125. (2015).

18. Nammalwar, P., & Joseph, VE. Bibliography of the Gulf of Mannar. CMFRI Special Publication, 74, 1-204. (2002).

19. Necta, VM., Joshi., & Srisudha, S. Biochemical characterization, haemagglutinating activity and cytotoxic activity of Padina gymnospora(Kutzing) Sonder. Int. J. Bio. Phar. Res., 3,956-961. 2012

20. Newman, DJ., Cragg, GM., & Snader, KM. Natural Products as Sources of New Drugs over the Period 1981-2002. J. Nat. Prod, 66, 1022-1037. 2003.

21. Sande-Bruinsma, N., Grundmann, H., Verloo, D., Tiemersma, E., Monen, J., Goossens, H., & MatusFerech, M. Antimicrobial drug use and resistance in Europe, Emerging Infectious Diseases, 14, 1722-1730. (2008).

22. Thanigaivel, S., Vidhya, SH., Vijayakumar, S., Mukherjee, A., Chandrasekaran, N., & Thomas J. Differential solvent extraction of two seaweeds and their efficacy in controlling Aeromonas salmonicida infection in Oreochromis mossambicus a novel therapeutic approach.Aquaculture 433, 56-64. (2015).

23. Tuney, L., Cadirci, BH., Unal, D., & Sukatar, A. Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). Turkish Journal of Biology, 30, 1-5 (2006),