International Journal of Zoology and Applied Biosciences
Volume 1, Issue 6, pp: 267-270, 2016
Research Article

ISSN: 2455-9571
http://www.ijzab.com

SCREENING ON ANTIMICROBIAL ACTIVITY OF *OSCILLATORIA FORMOSA* AND *SPIRULINA SUBSALSA*

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Article History: Received 11th October 2016; Accepted 17th November 2016; Published 2017

ABSTRACT

In this study, totally thirty species of cyanobacteria belonging to twenty-two genera were isolated and identified from Vedaranyam coastal area. Among them cyanobacteria, *Ocillatoria* and *Spirulina* were tested in compliance with the agar well diffusion method for their antibacterial and antifungal agent production on human pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger* and *Candida albicans*). The antimicrobial activity was maintained by using different solvents (ethanol, chloroform, methanol and water). The present study exhibit maximum antimicrobial activity was recorded from methanol extracts of cyanobacterial species. The methanol extract of *Oscillatoria formosa* showed maximum inhibition against all the human pathogens than *Spirulina subsalsa*. Similarly bacterial pathogens were showed maximum inhibition when compared to fungal pathogens

Keywords: Antibacterial activity, Antifungal activity, human pathogens, *Oscillatoria Formosa, Spirulina subsalsa*.

INTRODUCTION

Cyanobacteria are prokaryotic organisms capable of oxygenic photosynthesis (Moore, 1981). They appeared to be a rich source for many useful products and are known to produce a number of bioactive compounds (Carmichael, 2001; Codd, 1997) and also rich source for many useful natural products are used as feed and fertilizer (Bano and Siddiqui, 2004). During the last few decades, cyanobacteria have been described as potentially important source for vitamins, fuels, fine chemicals and many other pharmaceutical products (Chacon-de-Popoici, 2004; De Varies *et al.*, 2004; Miura *et al.*, 1993; Pesando and Bouicha, 1991). To date, more than 10,000 marine derived compounds have been isolated and this is coming from less than 1% of the total marine biodiversity. The range of marine organisms tapped for their natural products production includes sponges, tunicates, bryozoans, nudibranchs, and gorgonians. Of these marine organisms, one particular group that is emerging as a source of important bioactive compounds is the marine blue-green algae or cyanobacteria. Screening of cyanobacteria for antibiotics and other pharmacologically active compounds, has received ever-increasing interest as a potential source for new drugs (Ostensvik *et al.*, 1998, Fish and Codd, 1994; Browitzka, 1995). Cyanobacteria from local habitats seem to be a source of potential new active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms (Mundt and Teuscher 1998).

The use of antimicrobial agents has increased significantly in aquaculture practices. Antibiotics used in both human as well as veterinary medicines have been tried experimentally to treat bacterial infections of animal. Problems including solubility, palatability, toxicity, cost, delivery and governmental restrictions have limited the available antibiotics to a select few, especially in food culture. Decreased efficacy and resistance of pathogens to antibiotics has necessitated development of new alternatives (Ramamurthy and Raveendran, 2009). The aim of the present work was to study the antimicrobial activity of *Oscillotoria formosa* and *Spirulina subsalsa* against bacteria and pathogenic fungi.

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MATERIALS AND METHODS

In order to study the antimicrobial efficiency, cyanobacterial samples were collected from Vedaranyam, Tamil Nadu, India. Cyanobacterial samples were collected from various collection spots in the study site. Standard microbiological methods were followed for isolation and purification of cyanobacterial strains. Algal samples were microscopically examined and plated on solid agar medium. The isolated cyanobacteria were maintained in ASN III medium.

The inoculated plates were incubated in culture room (temperature maintained at 25 ± 2^0C with cool while fluorescent tube emitting 2500 lux for 18 hrs a day) and were regularly examined for the growth of cyanobacteria. Colonies appearing on solid medium were picked up and transferred to liquid medium. By repeated streaking, cultures were made unialgal and maintained in ASN III liquid medium. Identification of algal forms was made with the help of keys given by Cyanophyta manual.

The isolated species were tested for antimicrobial activity with different solvents such as ethanol, chloroform, methanol and water. Among the cyanobacteria tested, *O. formosa* and *S. subsalsa* showed positive results with methanol and hence these two species were selected for further studies.

The test organisms used in the present study were bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa*, and *Bacillus subtilis*) and fungi (*Aspergillus niger and Candida albicans*). The test microbial pathogen cultures were obtained from the stock cultures maintained in nutrient agar medium in bacteria and potato dextrose agar medium in fungi.

Antimicrobial susceptibility assay

Muller Hinton agar plates were inoculated with test organisms by spread plate method. Wells were punched in the agar plate. Microbial assay was carried out by well method in petridishes. Cultures of each microbial strain were swabbed with sterile cotton on the surface of medium. *O. formosa* and *S. subsalsa* extracts were tested with different concentrations (5, 10 and 15µl) for the antimicrobial activity against the pathogenic microbes such as *S. aureus, P. aeruginosa, B. subtilis, A. niger* and *C. albicans*. The plates were incubated for 24 hrs at 37°C; solvent control was performed in each case. After 24 hrs (Bacteria) and 72 hrs (Fungi), areas of inhibited microbial growth were observed as clear zone around the well. Antimicrobial activity was measured as diameter of zone of inhibition, excluding the well diameter.

RESULT AND DISCUSSION

Totally thirty species of cyanobacteria belonging to twenty-two genera were isolated and identified from study site. Among the genera *Ocillatoria, Microcystis* and *Spirulina* with each three species and followed by *Phormidium* and *Lyngbya* with each two species were the dominant genus and other with single one each. Phytoplankton and benthic microalgal communities make important contributions to the functioning of mangrove environments (Table 1). However, their contribution to total estuarine production is relatively small in most regions of Southeast Asia, Australia, Central America and tropical South America. Robertson and Blaber (1992) suggested that the contribution of plankton to total net production in mangrove habitats ranges from 20 to 50%. Careful measurements are verifying that predication for large systems. Phytoplanktons are responsible for 20% of the total production in mangrove estuaries in the Fly River Delta in Papua New Guinea (Robertson *et al.*, 1991) and 20-22% of the total production in the Pichavaram mangroves of south India (Kawabata *et al.*, 1993). In the present studies the 30 species of cyanobacteria were isolated from Vedaranyam coastal area.

Table 1. Cyanobacterial flora.

S. No.	Name of the species
1	*Oscillatoria amoena*
2	*Oscillatoria tenuis*
3	*Oscillatoria formosa*
4	*Anabaena sphaerica*
5	*Nostoc muscorum*
6	*Chamaesiphon sideriphilus*
7	*Xenococcus acervatus*
8	*Phormidium valderianum*
9	*Phormidium fragile*
10	*Trichodesmium erythraeum*
11	*Richelia intracellularis*
12	*Hapalosiphon welwitchii*
13	*Dichothrix bauriana*
14	*Spirulina subsalsa*
15	*Spirulina platensis*
16	*Spirulina subtilissima*
17	*Lyngbya majuscula*
18	*Lyngbya hieronymusii*
19	*Stichosiphon sansibaricus*
20	*Nodularia spumigena*
21	*Microcoleus chthonoplasts*
22	*Myxosarcina concinna*
23	*Merismopedia glauca*
24	*Chroococcus turgidus*
25	*Gomphospaeria aponina*
26	*Microcystis pulverea*
27	*Microcystis lamelliformis*
28	*Microcystis flos-aquae*
29	*Synechocystis pevalekii*
30	*Gloeothece linearis*

The results obtained from the present study concerning the biological activity of the antimicrobial agents produced by some selected cyanobacteria against different species of bacteria and fungi were recorded in Table 2. It is clear from

this table that the diameter of the inhibition zone depends mainly on type of the cyanobacterial species, type the solvent used and the tested bacterial and fungal organisms. Concerning the antibacterial effects, the results cleared that methanol extracts of *O. formosa* gave the highest biological activity against *S. aureus* (21 mm) when compared to methanol extracts of *S. subsalsa* (18 mm). The maximum inhibition zone (21 mm) was observed in the extract (15 µl per well) of *Oscillatoria* against *S. auerus* and the minimum inhibition zone (07 mm) was observed with the extract of (5 µl) *Spirulina* against *C. albicans*. All the extracts showed the inhibitory effect on the test organisms. However, maximum inhibition was noticed in bacterial pathogen when compared to fungal pathogen. Similarly extracts from both *O. formosa* and *S. subsalsa* showed zone of inhibition in all the concentrations tested. In general, the increased concentration of the cyanobacterial extract was more effective against the microbial growth than the minimum concentration.

Table 2. Antimicrobial activity of different concentration of *Oscillatoria formosa* and *Spirulina subsalsa* (zone of inhibition in mm).

S. No.	Name of organism	*Spirulina subsalsa*			*Oscillatoria formosa*		
		5 µl	10 µl	15 µl	5 µl	10 µl	15 µl
1	*Staphylococcus aureus*	11	15	18	11	16	21
2	*Bacillus subtilis*	08	11	15	10	14	17
3	*Pseudomonas aeruginosa*	09	12	15	11	14	18
4	*Aspergillus niger*	08	10	12	10	13	14
5	*Candida albicans*	07	08	10	08	09	11

The ethanol extracts of this cyanobacterial species were tested against different bacterial and fungal pathogens (Ramamurthy and Raveendran, 2009). These results proved also that ethanol extracts of *O. formosa* was the best activity against bacterial and fungal species while ethanol extracts of *S. subsalsa*. In the present pilot screening of *O. formosa* and *S. subsalsa* extracts of two species were found to show species specific activity against the five human pathogens. The details of activity of ethanol extracts of algae along with activity profile with standard commercial antibiotics are tested.

Hornsey and Hide (Hornsey and Hide, 1974) tested 151 species of British marine algae and found that, although antibacterial activity was more evident in some taxonomic groups, it also varied seasonally. They found Gracilaria marked no activity sp., *Enteromorpha* sp. and *Cladophora dalmatica*. But, in our case the alcoholic extract of *O. formosa* and *S. subsalsa* showed good antimicrobial activity. Our results clearly showed that the ethanol solvent system was efficient in extracting the active compounds. The antimicrobial activity found in two extracts showed the success of the non-polar hydrophobic extracts independent of diffusion parameters in the assay method employed.

Padmakumar and Ayyakkannu (Padmakumar and Ayyakkannu, 1986) reported toluene-methanol (1:3) extracts of species belonging to Rhodophyceae exhibited broad-spectrum activity when compared to Chlorophyceae and Phaeophyceae. Vidyavathi and Vidyavathi and Sridha (1991) reported chloroform-methanol extract of fully grown *G. corticata* showed maximum activity against *S. aureus* compared to medium and young stages of growth. The antibacterial activity of *O. formosa* and *Spirulina subsalsa* extracts (methanolic) against *S. aureus*, *P. aeroginosa*, *B. subtilis*, *A. niger* and *C. albicans*

Ramamurthy and Raveendran (2009) reported that the sensitivity of pathogens is more to *Lyngbya majuscula* extracts compared to *S. platensis* extracts with *E. tarda* and *A. hydrophila* showing maximum sensitivity. *P. aeruginosa*, *A. salmonicida V.alginolyticus*, *P. fluorescens*, *A. niger*, *Penicillium sp* and *C. albicans* were moderately sensitive to the algal extracts and *T. viride* were low sensitive to the other organisms. According to Padmakumar and Ayyakkannu (1997), *S. aureus* was the most susceptible bacterial pathogen followed by *Vibrio* sp. whereas *P. aeruginosa* was most resistant.

CONCLUSION

From the results it can be concluded that cyanobacterial extracts have great potential as antimicrobial compounds against bacteria and fungi. Overall, the present study provides a basic idea to show the potential of microalgal extracts for development of anti-pathogenic agents for use in pharmaceutical. The results of this work indicate that this group of organisms displays a potential that warrants further investigations.

ACKNOWLEDGMENT

The authors express sincere thanks to the HOD of Zoology and Principal of Khadir Mohideen College for the facilities provided to carry out this research.

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