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INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 5, pp.097-101, June, 2010

ISSN: 0975-833X

RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF *Oscillatoria princeps* AND *Lyngbya majuscula* AGAINST PATHOGENIC MICROBES

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ARTICLE INFO

Article History:
Received 11th April, 2010
Received in revised from
17th May, 2010
Accepted 26th May, 2010
Published online 18th, June, 2010

Key words:
Antimicrobial activity,
Oscillatoria princeps,
Lyngbya majuscula.

ABSTRACT

14 species of cyanobacteria belongs to 10 genera were isolated and identified from Athirampattinam coastal area of Tamil Nadu coast. Among this, *Oscillatoria princeps* and *Lyngbya majuscula* were the dominant group selected for the present study. The antimicrobial activity of *Oscillatoria princeps* and *Lyngbya majuscula* was maintained by using different solvents viz. ethanol, acetone, methanol and water. The *Oscillatoria princeps* and *Lyngbya majuscula* were tested against human pathogens like *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Aspergillus niger* and *Candida albicans* to know the antimicrobial activity. The present study shows maximum antimicrobial activity was reported in ethanol extracts of cyanobacterial species. The ethanol extract of *Oscillatoria princeps* showed maximum inhibition against all the human pathogens than *Lyngbya majuscula*. Similarly bacterial pathogens were showed maximum inhibition when compared to fungal pathogens.

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, 1994; De Varies *et al.,*1993; 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 (Gerwick *et al.,* 2001). 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; Borowitzka, 1995). Cyanobacteria from local habitats seem to be a source of potential new bioactive substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms (Mundt *et al.,* 2001).

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Secondary metabolites from cyanobacteria are associated with toxic, hormonal, antineoplastic and antimicrobial effects (Carmichael, 1992; Patterson *et al.,*1994). 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 princeps* and *Lyngbya majuscula* against bacteria and pathogenic fungi.

MATERIALS AND METHODS

The cyanobacteria samples were collected from Adhirampattinam coast of Thanjavur District in Tamilnadu, Southeast coast of India. The epiphytic and extraneous matters present in the samples were removed by washing in marine water followed by distilled water and transported to the laboratory in polyethylene bags at ice temperature. In the present study, 14 species of cyanobacteria were isolated and identified according to Desikachary (1959) and Geitler (1932). Amoung them, *Oscillatoria princeps* and *Lyngbya majuscula* were the dominant group selected to test the antimicrobial activity. The cyanobacterial species such as *O. princeps* and *L. majuscula* were mass cultured using ASN III medium (Waterbury and Stainer, 1981). After 20 days, cultures were harvested by centrifugation then the pellets were washed with distilled water and known volume of pellets

was taken in a pestle and mortar and ground with solvent ethanol. The isolated species were tested for antimicrobial activity with different solvents such as ethanol, acetone, methanol and water. Among the cyanobacteria tested, *Oscillatoria princeps* and *Lyngbya majuscula* showed positive results with ethanol and hence these two species were selected for further studies.

The test organisms used in this work viz., *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Bacillus subtilis*; the fungi like *Aspergillus niger and Candida albicans.* The test microbial pathogen cultures were obtained from pathological laboratory, Sea Horse hospital, Tiruchirappalli and maintained in nutrient agar medium for bacteria and potato dextrose agar medium for fungi. From this culture, sub culture were prepared and used for the present study.

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 (Perez *et al.,*1990). Cultures of each microbial pathogenic strain were swabbed with sterile cotton on the surface of medium. *Oscillatoria princeps* and *Lyngbya majuscula* extracts were tested with different concentrations (10, 20 and 30µl) for the antimicrobial activity against the pathogenic microbes such as *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Aspergillus niger* and *Candida albicans*. The plates were incubated for 24 hrs at 37°C and solvent control was performed in each case. Areas of inhibited microbial growth were observed as clear zone around the well after 24 hour in bacteria and after 48 hours in fungi. Antimicrobial activity was measured as diameter of zone of inhibition, excluding the well diameter.

RESULTS

In the present study, 14 species of cyanobacteria belongs to 10 genera were isolated and identified from Athirampattinam coastal area of Tamil Nadu coast (Table 1). Among the 14 species, Oscillatoria were recorded as the dominant genus with 4 species followed by Lynbya with 2 species. Among this, *Oscillatoria princeps* and *Lyngbya majuscula* were selected based on their dominant occurrence and the effectiveness of extracts for the present study. The results obtained from the present study shows the biological activity of the antimicrobial agents produced by selected cyanobacteria against different species of pathogenic bacteria and fungi were recorded (Table. 2). It is clear that, the diameter of inhibition zone depends mainly on the type of cyanobacterial species, form of solvent used and the tested pathogenic microbes. The antimicrobial activity of *Oscillotoria princeps* and *Lyngbya majuscula* against different pathogens at various concentrations showed the zone of inhibition at diverse level (Fig. 1 and 2). The ethanol extracts of *Oscillotoria princeps* shows the highest antibacterial activity against *Staphylococcus aureus* (21 mm) when compared to ethanol extracts of *Lyngbya majuscula* (18 mm). The maximum inhibition zone (21 mm) was observed in the extract (30 µl per well) of *Oscillatoria* against *S. auerus* and the minimum inhibition zone (07 mm) was observed in the extract of (10 µl) *Lyngbya* against *C. albicans*. All the extracts showed the inhibitory effect on the test organisms.

The comparative antimicrobial activity of *Oscillotoria princeps* and *Lyngbya majuscula* was shown in Fig. 3. However, maximum inhibition was noticed in bacterial pathogen when compared to fungal pathogen. In general the extracts from both *Oscillatoria* and *Lyngbya* showed

Table 1. Shows the list of Cyanobacterial species recorded in Athirampattinam coast

S. No	Cyanobacteria
1	*Chroococcus minor* (kütz.) Nag.
2	*Nostoc punctiforme* (kütz.) Hariot
3	*Gloeocapsa calcarea* Tilden
4	*Microcystis* areuginosa kütz.
5	*Phormidium ambigum* Gomont
6	*Oscillatoria limnetica* Lemm
7	*O.terebriformis* Ag ex Gomont
8	*O. princeps* Vaucher ex Gomont
9	*O. salina* Biswas
10	*Lyngbya majuscula* Harvey ex Gomont
11	L. subconfervoides *Borge*
12	*Synechocytis* sp.
13	*Cyanosarcina sp.*
14	*Cylindrospermum* sp.

Table 2. Antimicrobial activity of *Oscillatoria princeps* and *Lyngbya majuscula* against different pathogenic microbes at various concentrations (Zone of inhibition in mm)

S. No	Name of Organism	*Lyngbya majuscula*			*Oscillatoria princeps*		
		10 µl	20 µl	30 µl	10 µl	20 µl	30 µl
1	*Staphylococcus aureus*	10	13	18	10	15	21
2	*Bacillus subtilis*	08	11	15	09	13	16
3	*Pseudomonas aeruginosa*	09	11	15	11	15	17
4	*Aspergillus niger*	08	10	13	10	12	14
5	*Candida albicans*	07	09	11	08	10	12

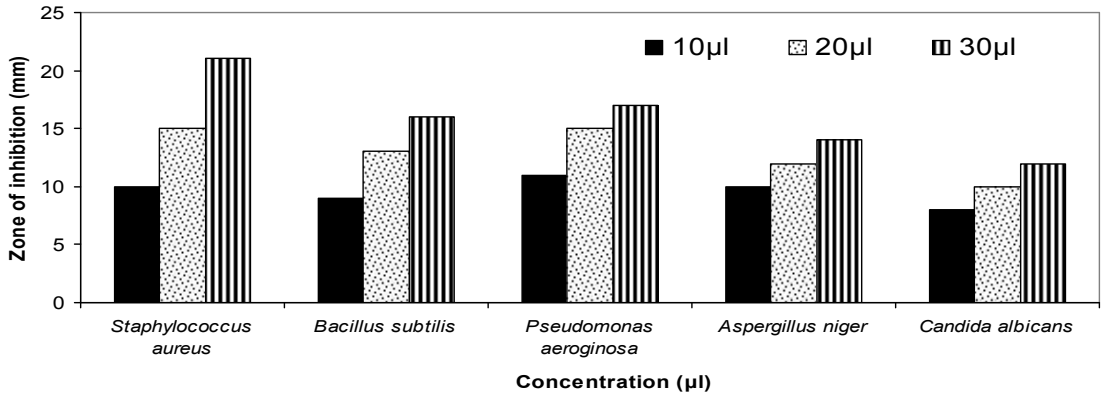

Fig. 1. Antimicrobial activity of *Oscillatoria princeps* against various pathogens

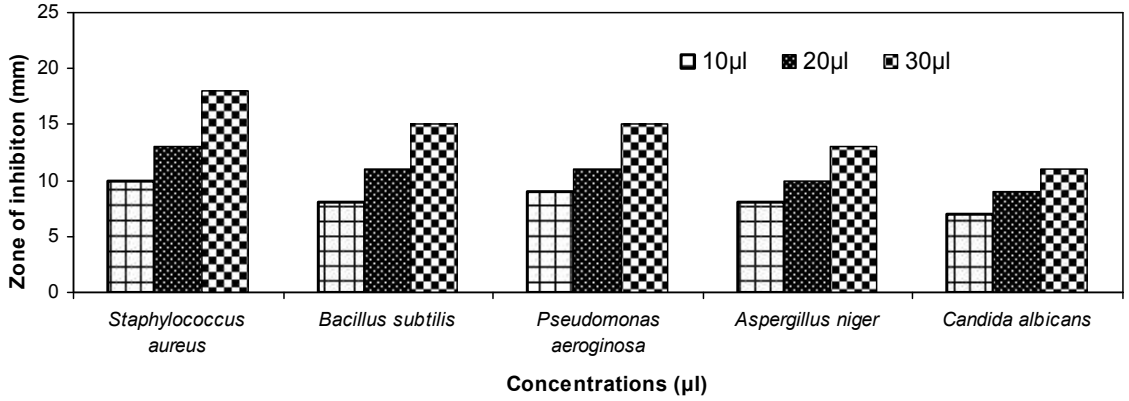

Fig. 2. Antimicrobial activity of *Lynbya majuscula* against various pathogens

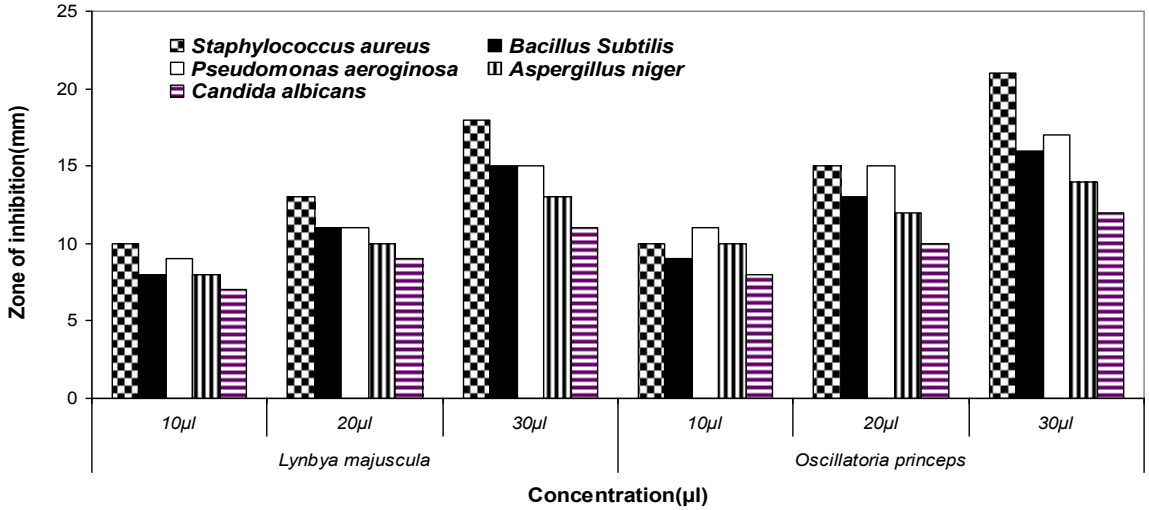

Fig. 3. The comparative antimicrobial activity of *Oscillatoria princeps and Lynbya majuscula* against various pathogens

zone of inhibition in all the concentrations tested. From the present study, the increased concentration of the cyanobacterial extract was more effective against the microbial growth than the minimum concentration.

DISCUSSION

The evaluation of antimicrobial activity of *Oscillotoria princeps* and *Lyngbya majuscule*, particularly ethanol extract of cyanobacterial species showed maximum activity against different bacterial and fungal pathogens (Abedin and Taha, 2008). The ethanol extracts of *Oscillatoria princeps* shows highest inhibition zone to *Staphylococcus aureus* (20 mm) and the results revealed that the antibacterial effects was more in *S. aureus* (20 mm) and *Pseudomona aeroginosa* (17 mm) than *Bacillus subtilis* (16 mm). At the same time, ethanol extracts of *O. princeps* has antifungal effect towards *Aspergillus niger*

(14 mm) and *Candida albicans* (12 mm). In general the increased concentration of the cyanobacterial extracts were more effective against the microbial growth than the minimum concentration. These results also proved that ethanol extracts of *Oscillatoria princeps* were showed best activity against bacterial and fungal species while ethanol extracts of *Lyngbya majuscula*. Where as Ramamurthy and Raveendran (2009) reported that the sensitivity of pathogens is more to *Lyngbya majuscula* extracts compared to *Spirulina platensis* extracts with *E. tarda* and *A. hydrophila* showing maximum sensitivity and *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.

In the present pilot screening of *Oscillotoria princeps* and *Lyngbya majuscula* extracts were found to shows the species specific activity against the five human bacterial and fungal pathogens. 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* sp., *Enteromorpha* sp. and *Cladophora dalmatica* marked no activity. In the present study, the alcoholic extract of *Oscillotoria princeps* and *Lyngbya majuscula* showed good antimicrobial activity and the 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 (1986) reported toluene-methanol (1:3) extracts of species belonging to Rhodophyceae exhibited broad-spectrum activity when compared to Chlorophyceae and Phaeophyceae. Vidyavathi and Sridhar (1991) reported chloroform-methanol extract of fully grown *Gracilaria corticata* showed maximum activity against *S. aureus* compared to medium and young stages of growth. Sreenivasa Rao and Parekh (1981) analyzed *Enteromorpha intestinalis* and *G. corticata* collected from Gujarat coast of India for antibacterial activity and found that the algae were active throughout the year with a peak during the winter season. Padmakumar and Ayyakkannu (1997) found that *S. aureus* was the most susceptible bacterial pathogen followed by *Vibrio* sp. whereas *P. aeruginosa* was most resistant. From the above 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.

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