

Original Research Article

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Antimicrobial Activity of Microalgae Isolated from Fresh Water Pond, Tamil Nadu, India

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ABSTRACT

Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Hence, a study was attempted in selected algae and cyanobacteria for antimicrobial activity. Among the various algae used, *Spirogyra quinina* had the maximum inhibitory effect followed by *Zygnema stellinum* and *Cyclotella comta*. Among the various extracts, the chloroform extract showed the maximum inhibitory effect followed by diethyl ether, ethanol and methanol for all the selected algae in the present study. Further, each extract had differential effect on various organisms. However, in general, the effects were more with the bacterial pathogens when compared to the fungal pathogens. Among the various extracts of cyanobacteria used, the chloroform extracts showed the maximum inhibitory effect followed by diethyl ether, ethanol and methanol. Further, among the various cyanobacteria, the extracts of *Spirulina major* had the maximum inhibitory effect followed by those of *Oscillatoria limosa* and *Nostoc linckia*. However, all the extracts showed differential inhibitory effect with different pathogens. Nevertheless, in general, all the extracts appeared to be more effective against bacteria than fungi in the present study. In addition, the present study indicates that chloroform can also be used as a solvent for extracting the antibacterial and 204 antifungal agents. Further, among the cyanobacterial and algal extracts, the algal extracts used in the present study showed higher efficacy towards antibacterial activity. The result of the present study clearly indicates the presence of antimicrobial compounds in the selected cyanobacterial and algal species. However, further studies are necessary to elucidate the components responsible for antibacterial and antifungal activities against microorganisms. It can be concluded that some of the extracts obtained using various solvents used in this study had higher antibacterial and antifungal activities and are more effective when compared with some contemporary antibiotics and fungicides.

Keywords

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Introduction

Microalgae constitutes one of the commercially important living and renewable resources. They contain more

than sixty trace elements including minerals, proteins, iodine, bromine and many bioactive substances (Asthana *et al.*, 2009).

To date, many chemically unique compounds of fresh water origin with various biological activities have been isolated (Choudhary *et al.*, 2005; Abedin and Hala, 2008; Desbois *et al.*, 2008; Kamble and Chavan, 2010; Elsie and Dhanarajan, 2010). and some of them are under investigation while some are being used to develop new pharmaceuticals (Limafilho *et al.*, 2002).

Cyanobacteria, the blue green algae are an assemblage of gram negative eubacteria widely distributed throughout the world (Vijayakumar *et al.*, 2011a). Cyanobacteria are emerging as an exciting resource for the discovery of new classes of therapeutics (Pramanik and Mukherjee, 2011). The current increase in scientific and commercial interest in the use of genetic resources is also of significance to international policy makers. Of the gram negative bacterial phyla, proteobacteria and filamentous cyanobacteria are the most prolific producers of potentially novel molecules. If properly developed, cyanobacteria could provide the drugs needed to sustain us for the next 100 years to out battle drug resistant infectious diseases (Pramanik and Mukerjee, 2011). Recent studies have shown that algae are rich sources of structurally novel and biologically active metabolites which are of interest in the pharmaceutical industry. The cell extracts and active constituents have been shown to have antibacterial activity *in vitro* against Gram positive and Gram negative bacteria. In addition, a wide range of results of *in vitro* antibacterial and antifungal activities of extracts of fresh water algae have been reported (Naik Ansari *et al.*, 2012). Thus, plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Hence, the present study was attempted in selected

algae and cyanobacteria for antimicrobial activity.

Materials and Methods

Sample Collection

Water samples containing algae/cyanobacteria were collected from the Naganathar Temple pond, Tiruchirappalli, Tamil Nadu. Samples were isolated and identified by standard microbiological methods (Geilter, 1932; Desikachary, 1959; Rippka, 1988). To study the antimicrobial activity, algal and cyanobacterial species like *Spirogyra quinina*, *Zygnema stellinum*, *Cyclotella comta*, *Oscillatoria limosa*, *Nostoc linckia* and *Spirulina major* were selected.

Microorganisms like *Aeromonas hydrophila*, *Escherichia coli*, *Bacillus subtilis*, *Clostridium perfringens*, *Proteus vulgaris*, *Salmonella typhi*, *Streptococcus faecalis*, *Candida albicans* and *Aspergillus niger* were tested and obtained from Private Hospital, Tiruchirappalli. Bacterial strains were inoculated onto nutrient broth and incubated at 37°C for 24 hours. The fungal strains were also inoculated onto glucose peptone broth and incubated at 30°C for 5 days.

Preparation of the Algal and Cyanobacterial Extracts

Ten day old algal and cyanobacterial cultures were collected, weighed and used for extraction of antimicrobial agents. 0.5 g of each of the five algal / cyanobacterial pellets were extracted in 10 ml of chloroform, diethyl ether, methanol and ethanol respectively. All extracts were preserved at 4°C (Gonzalez *et al.*, 2001) for later use.

Determination of the Inhibition Effect of the Algal and Cyanobacterial Extracts

Antimicrobial and antifungal activities of cyanobacterial extracts were tested by agar well diffusion method. Nutrient agar plates were inoculated with 100 ml of a 24 hour broth culture of the test bacteria or 100 ml of a 5 day glucose peptone broth culture of the test fungi. Four wells (6 mm) were made and filled with 100 ml extract. The plates were incubated for 24 hours at 37°C for bacteria or inoculated for 3 days at 30°C for fungi. The diameter of the inhibition zone was measured with calipers and the result recorded (Attaie *et al.*, 1987). In addition, the antimicrobial activity of cyanobacteria and algae were compared with standard antibiotics (Amoxicillin and Polynoxylin).

Results and Discussion

Results of the antibacterial activities with various cyanobacterial extracts are presented in Table-1. As seen from the table, among the various algal extracts of *Spirogyra quinina*, the chloroform extract showed maximum inhibitory zone followed by those of diethyl ether, ethanol and methanol extracts. While the chloroform extract showed maximum inhibitory zone with *Proteus vulgaris* followed by *Bacillus subtilis*, the least was with *Aspergillus niger* and *Candida albicans*. With regard to diethyl ether extracts, the maximum inhibitory zone was recorded with *Proteus vulgaris* followed by *Bacillus subtilis* while the least was with *Aspergillus niger* and *Candida albicans*. The other two extracts (ethanol and methanol) also showed the same trend.

With regard to the extracts from *Zygnema stellinum*, the chloroform extract showed the maximum inhibitory effect followed by diethyl ether, ethanol and methanol extracts.

While chloroform extracts showed maximum inhibitory effect with *Aeromonas hydrophila* followed by *Proteus vulgaris* the least was with *Cyclotella comta*. The other three extracts (diethyl ether, ethanol and methanol) also showed the same trend. With regard to the extracts of *Cyclotella comta*, the chloroform extract showed maximum inhibitory effect followed by diethyl ether, ethanol and methanol.

While the chloroform extract showed the maximum inhibitory effect with *Enterobacter faecalis* followed by *Bacillus subtilis*, the least was with *Candida albicans*. The other three extracts (diethyl ether, ethanol and methanol) also showed the same trend as that of chloroform extract.

Thus, among the various algae used, *Spirogyra quinina* had the maximum inhibitory effect followed by *Zygnema stellinum* and *Cyclotella comta*. Among the various extracts, the chloroform extract showed the maximum inhibitory effect followed by diethyl ether, ethanol and methanol for all the selected algae in the present study. Further, each extract had differential effect on various organisms. However, in general, the effects were more with the bacterial pathogens when compared to the fungal pathogens.

As seen from the Table-1, among the various cyanobacterial extracts of *Oscillatoria limosa*, the chloroform extract appeared to show the maximum inhibitory effect followed by diethyl ether, ethanol and methanol extracts. While chloroform extract had maximum inhibitory effect on *E. coli* followed by *Aeromonas hydrophila*, the least effect was noticed in the fungi, *Candida albicans*. While diethyl ether extracts of *O. limosa* also showed the maximum inhibitory effect with *E. coli* followed by *Aeromonas hydrophila*, the

least was with *Candida albicans*. The ethanol and methanol extracts also had the maximum inhibitory effect on *E. coli* and *Aeromonas hydrophila* while the least was with *Candida albicans*.

With regard to *Nostoc linckia*, the chloroform extract again showed the maximum inhibitory effect followed by diethyl ether, ethanol and methanol. The chloroform extract showed the maximum inhibitory effect with *Proteus vulgaris* followed by *E. coli*, while the minimum inhibitory effect was with *Candida albicans*.

Among the extracts of *Spirulina major*, the chloroform extract showed the maximum inhibitory followed by diethyl ether, ethanol and methanol. While the chloroform extract showed the maximum inhibitory zone with *A. hydrophila* followed by *B. subtilis* and *E. coli*, the least was with *Candida albicans* and *Aspergillus niger* respectively.

Thus, among the various extracts of cyanobacteria used, the chloroform extracts showed the maximum inhibitory effect followed by diethyl ether, ethanol and methanol. Further, among the various cyanobacteria, the extracts of *Spirulina major* had the maximum inhibitory effect followed by those of *Oscillatoria limosa* and *Nostoc linckia*. However, all the extracts showed differential inhibitory effect with different pathogens. Nevertheless, in general, all the extracts appeared to be more effective against bacteria than fungi in the present study.

It is clear from the above studies that the diameter of the inhibitory zone depends mainly on the type of the algal species, type of the solvent used and the tested bacterial and fungal organisms. In the present study, the highest antimicrobial activity was shown against *Aeromonas hydrophila*, *Bacillus*

subtilis, *Proteus vulgaris* and *E.coli*. These results are in line with the works of Volk and Furkert (2006) and Vijayakumar *et al.* (2011b) who found that cyanobacteria had high biological activities against *A. hydrophila*, *B. subtilis*, *P. vulgaris* and *E. coli*. Among the various extracts, chloroform extract showed the maximum inhibitory zone followed by the extracts of diethyl ether, ethanol and methanol.

Literature reveals that many workers (Noaman *et al.*, 2004; Mayer and Hamann, 2005; Gul and Hamann, 2005; Prashanthkumar *et al.*, 2006; Prasanna *et al.*, 2008; Vijayakumar *et al.*, 2011a, 2011b) showed ethanol and acetone to be a good solvent for extracting antibacterial and antifungal agents from *Oscillatoria latevirens*, *Phormidium corium*, and *Lynbhya martensiana*. However, the present study indicates that chloroform can also be used as a solvent for extracting the antibacterial and antifungal agents. Shimna (2012) while studying antimicrobial activities also suggested chloroform extract to show good results. Thus, the results obtained in the present study are in line with those of Shimna (2012).

The antifungal activities of all the extracts of cyanobacteria tested towards fungi gave positive results but with varying degrees. Results of the experiments done by Prashanthkumar *et al.* (2006), Prasanna *et al.* (2008), Vijayakumar *et al.* (2011a, 2011b) also showed similar results. These observations are in line with those of the present study.

Results using the extracts of algae also showed positive results. Among the various algae used, the extracts of *Spirogyra quinina* showed the maximum efficacy followed by *Zygnema stellinum* and *Cyclotella comta*.

Table.1 Antibacterial and Antifungal Activities of Different Cynobacteria and Algal extracts isolated from Fresh Water (zone of inhibition in cm)

Species	Extracts	<i>Aeromonas hydrophila</i>	<i>E. Coli</i>	<i>Bacillus Subtilis</i>	<i>Clostridium perfringens</i>	<i>Proteus vulgaris</i>	<i>Salmonella typhi</i>	<i>Streptococcus faecalis</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>
<i>Oscillatoria Limosa</i>	Chloroform	3.20	3.80	2.40	2.20	2.60	1.80	1.90	1.90	1.20
	Ethanol	2.80	3.40	2.00	1.80	2.20	1.40	1.50	1.50	0.80
	Diethyl ether	3.00	3.60	2.20	2.00	2.40	1.60	1.70	1.70	1.00
	Methanol	2.60	3.20	1.80	1.60	2.00	1.20	1.30	1.30	0.60
<i>Nostoc linckia</i>	Chloroform	3.00	3.60	2.50	2.20	3.70	1.90	1.80	1.80	1.40
	Ethanol	2.60	3.20	2.10	1.80	3.30	1.50	1.40	1.40	1.00
	Diethyl ether	2.80	3.40	2.30	2.00	3.50	1.70	1.60	1.60	1.20
	Methanol	2.40	3.00	1.90	1.60	3.10	R	1.20	1.20	R
<i>Spirulina Major</i>	Chloroform	4.00	3.80	3.90	3.60	3.60	1.60	2.90	2.00	1.80
	Ethanol	3.60	3.40	3.50	3.20	3.20	1.20	2.50	1.60	1.40
	Diethyl ether	3.80	3.60	3.70	3.40	3.40	1.40	2.70	1.80	1.60
	Methanol	3.40	3.20	3.30	3.20	3.00	1.20	2.30	1.40	1.20
<i>Spirogyra quinina</i>	Chloroform	3.40	3.40	3.80	3.40	4.00	3.60	3.40	2.40	2.40
	Ethanol	3.00	3.00	3.40	3.60	3.60	3.20	3.00	2.00	2.00
	Diethyl ether	3.20	3.20	3.60	3.20	3.80	3.40	3.20	2.20	2.20
	Methanol	2.80	2.80	3.20	3.30	3.40	3.00	2.80	1.80	R
<i>Zygnema stellinum</i>	Chloroform	3.70	3.20	3.20	2.40	3.40	1.80	1.90	2.40	2.20
	Ethanol	3.30	2.80	2.80	2.60	3.00	1.40	1.50	2.00	1.80
	Diethyl ether	3.50	3.00	3.00	2.60	3.20	1.60	1.70	2.20	2.00
	Methanol	3.10	2.60	2.60	R	2.80	1.20	1.30	R	R
<i>Cyclotella Comta</i>	Chloroform	2.00	2.40	2.40	1.80	2.20	1.30	2.60	1.00	0.80
	Ethanol	1.60	2.00	2.00	1.00	1.80	0.90	2.20	0.60	0.40
	Diethyl ether	1.80	2.20	2.20	0.60	2.00	1.10	2.40	0.80	0.60
	Methanol	1.40	1.80	R	R	1.60	R	2.00	0.40	R
Standard antibiotics	Amoxicillin	3.60	1.60	2.80	2.70	2.30	2.40	2.00	1.70	1.70
	Polynoxylin	3.40	3.40	2.80	2.90	2.60	2.60	2.20	1.40	1.50

Among the various extracts, chloroform showed the maximum effect followed by diethyl ether, ethanol and methanol. Further, there was also differential activity towards different organisms with regard to the extracts. Literature reveals that a similar result with different extracts was also noticed by Prakash *et al.* (2011). Among the cyanobacterial and algal extracts, the algal extracts used in the present study showed higher efficacy towards antibacterial activity.

Thus, the result of the present study clearly indicates the presence of antimicrobial compounds in the selected cyanobacterial and algal species. However, further studies are necessary to elucidate the components responsible for antibacterial and antifungal activities against microorganisms. As to the variation in the effectiveness of the extraction methods, it may be attributed to the presence of different antimicrobial substances present in the different organisms as suggested by Lustigman and Brown (1991). The study also indicates that methanol extracts of *Zygnema stellinum*, *Cyclotella comta* and *Nostoc linckia* are also resistant to some of the pathogens (*Candida albicans*, *Aspergillus niger* and *Clostridium perfringens*) used in the present study.

The antimicrobial activities of the test microorganisms against standard antibiotics and fungicides (Amoxicillin and Polynoxylin) are shown in Table-1. It was found that the effect of standard antibiotics was less than that of the selected cyanobacterial and algal extracts on *Proteus vulgaris*, *E. coli*, *Bacillus subtilis*, and *Aspergillus niger*. On the other hand, the effect of the standard antibiotics on *Candida albicans* was variable and was more or less similar to that of the investigated algal extracts. Literature reveals that similar reports were also observed by

Prashanthkumar *et al.* (2006), Prasanna *et al.* (2008), Shimna (2012), Sankarrao (2013) and Mugilan (2014). Thus it can be concluded that some of the extracts obtained using various solvents used in this study had higher antibacterial and antifungal activities and they are more effective when compared with some contemporary antibiotics and fungicides.

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