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Study on Antimicrobial Activity of Algal Isolated from a Freshwater Lake

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A B S T R A C T

To study the antimicrobial activity, algal and cyanobacterial species like *Anabaena aequalis*, *Lyngbya aestuarii*, *Oscillatoria angusta*, *Spirulina laxa* and *Synechocystis aquatilis* were selected. Antimicrobial and antifungal activities of algal extracts were tested by agar well diffusion method. In addition, comparing the antimicrobial activity of cyanobacteria and algae with standard antibiotics (Amoxicillin and Polynoxylin) were also done. From the present study, it is clearly evident that all the algal extracts (both methanol and water) had inhibitory effect on the microbes even though the percentage of inhibition varied. Among the methanol extracts, *L. aestuarii* extract showed maximum inhibitory effect against both *E. coli* and *S. faecalis*. However, among the water extracts, even though *L. aestuarii* again recorded maximal inhibitory effect against *E. coli*. Among all the algal species examined, the highest amount of the quercetin was found in *S. aquatilis* (42 mg/l). Estimation of phycocyanin content also suggests that the maximum content was found in *S. aquatilis*. It can be concluded that the antibacterial activity of the algae depends on the content of quercitin and phycocyanin pigments for the alcohol extracts and polysaccharide content for the water extracts.

Introduction

Biologically active compounds present in the plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing (Krishnaraju *et al.*, 2005; Raghavendra *et al.*, 2006; Selvamaleeswaran *et al*., 2010; Haripriya *et al*., 2010).

Aquatic organisms are a rich source of structurally novel and biologically active metabolites (Ely, 2004). Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry.

Algae are a major source of food in the aquatic environment. It is commonly used as a food supplements to the humans and as a feed material to domestic animals. Further, it is also used as a source of biogas production and raw materials for the salad preparation (Sivakumar and Thanigaimalai, 2014).

Fresh water green algae are a group of fast growing autotrophic diversified organisms which transform radiant energy into chemical energy by capturing solar energy which maintain the homoeostasis of ecosystem and biomes. Besides having higher photosynthetic efficiency as compared to terrestrial plants, algae also have high growth rates and biomass production. Tropical conditions such as those in India provide favourable environment for the luxuriant growth of these organisms in nature (Subbaramaiah, 1972; Srivastava and Odhwani, 1992; Thajuddin and Subramanian, 1992; Thajuddin *et al.* 2002; Rajakumar, 2004; Chellappa *et al.* 2004; Goyal S.K, 1962,1964; Bhatnagar and Bhatnagar 2005; Bhatnagar *et al.* 2008; Makandar and Bhatnagar 2010).

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 as well as 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 Mugaiyur lake in Villuppuram District, Tamil Nadu, India. Samples were isolated and identified by standard microbiological methods (Desikachary, 1959; Rippka, 1988). To study the antimicrobial activity, algal and cyanobacterial species like *Anabaena aequalis, Lyngbya aestuarii, Oscillatoria angusta, Spirulina laxa* and *Synechocystis aquatilis* 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 Government 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 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 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, comparing the antimicrobial activity of cyanobacteria and algae with standard antibiotics (Amoxicillin and Polynoxylin*)* were also done.

Results and Discussion

The inhibition percentage of the antibacterial spectrum of methanol algal extracts are presented in Tables 1 and 2. As evident from the table, at a concentration of 0.5 mg/ml, the highest inhibition percentage against *E. coli* was shown by *L. aestuarii* extract (92.6%) followed by *A. aequalis* extract (75%) and the least by *S. trididemi* extract (0.4%). With regard to *S. typhii*, the maximum inhibition was shown by *O. angusta* extract (98.5%) followed by *S. laxa* extract (86.2%) and the least by *L. aestuarii* extract (9.7%). In the case of *S. faecalis*, the maximum inhibition percentage was recorded by the extracts of *L. aestuarii* (94.7%) followed by *O. augusta* (93.3%) and the least by *S. laxa* (10.4%).

The percentage inhibition of water algal extracts are presented in Tables 1 and 2. As seen from the table, against *E. coli*, maximum inhibition was shown by *L. aestuarii* (74.4%) followed by *A. aequalis* (69.8%) and the least by *S. tridedemi* (0.1%), with regard to *S. typhi*, the maximum inhibition was recorded by *S. laxa* extract (94.2%) followed by *L. aestuarii* extract (90.1%) and the least by *O. augusta* extract (59.4%). In the case of *S. faecalis*, the maximum inhibition was recorded by the extract of *A. aequalis* followed by *S. laxa* (79.3%) while the least inhibitory rate was recorded by *L. aestuarii* (60.2%).

Thus, from the present study, it is evident that all the algal extracts (both methanol and water) had inhibitory effect on the microbes even though the percentage of inhibition varied. Among the methanol extracts, *L. aestuarii* extract showed maximum inhibitory effect against both *E. coli* and *S. faecalis* while it was *O. angusta* which recorded maximal inhibitory effect against *S. typhi*. However, among the water extracts, even though *L. aestuarii* again recorded maximal inhibitory effect against *E. coli*, it was *S. laxa* which recorded maximal inhibitory effect against *S. typhi* and *A. aequalis* extract against *S. faecalis*.

The results of the quercetin content is presented in Table-1. From the table it is clear that among all the algal species examined, the highest amount was found in *S. aquatilis* (42 mg/l) followed by *O. angusta* (32 mg/l) and the lowest in *A. aequalis* (4 mg/l).Estimation of phycocyanin content also suggests that the maximum content was found in *S. aquatilis* (4.8 mg/l) and the lowest in *A. aequalis* (0.5 mg/l) (Table-3).

Determination of carotenoid content reveals that the maximum was also noticed in *S. aquatilis* (3.3×10^8 mg/l) while the minimum in *O. angusta* (7×10^7 mg/l) (Table-3). Details of chlorophyll-a content are provided in Table-1.

Table.1 Percentage of inhibition of antibacterial spectrum of different concentrations of algal methanol extracts using Gram-negative and Gram-positive bacteria

Bacteria	Algal isolates	Control	Methanol extract	Inhibition (%)
		Concentrations (0.5 mg ml^{-1})		
Escherichia coli	*Anabaena aequalis*	$1.5\times10^{8} \pm 0.31$	$4.1\times10^{7} \pm 0.46$	75.0
	Lyngbya aestuarii	$2.6\times10^{8} \pm 0.35$	$2.1\times10^{8} \pm 0.12$	92.6
	Oscillatoria angusta	$9.9\times10^{7} \pm 0.72$	$4.4\times10^{7} \pm 0.28$	59.2
	Spirulina laxa	$8.8\times10^{7} \pm 0.14$	$3.1\times10^{7} \pm 0.34$	68.2
	Synechocystis aquatilis	$2.3\times10^{8} \pm 0.02$	$2.4\times10^{8} \pm 0.02$	0.4
Salmonella typhi	*Anabaena aequalis*	$9.6\times10^{6} \pm 0.05$	$8.4\times10^{6} \pm 0.14$	13.9
	Lyngbya aestuarii	$9.6\times10^{7} \pm 0.11$	$9.2\times10^{7} \pm 0.14$	9.7
	Oscillatoria angusta	$4.3\times10^{7} \pm 0.20$	$6.4\times10^{5} \pm 0.13$	98.5
	Spirulina laxa	$6.6\times10^{7} \pm 0.11$	$9.1\times10^{6} \pm 0.33$	86.2
	Synechocystis aquatilis	$4.3\times10^{7} \pm 0.28$	$2.5\times10^{8} \pm 0.14$	27.5
Streptococcus faecalis	*Anabaena aequalis*	$4.1\times10^{7} \pm 0.1$	$3.8\times10^{7} \pm 0.42$	91.0
	Lyngbya aestuarii	$4.1\times10^{7} \pm 0.3$	$2.4\times10^{6} \pm 0.12$	94.7
	Oscillatoria angusta	$3.3\times10^{7} \pm 0.3$	$2.4\times10^{6} \pm 0.04$	93.3
	Spirulina laxa	$3.3\times10^{7} \pm 0.3$	$3.8\times10^{6} \pm 0.06$	10.4
	Synechocystis aquatilis	$7.3\times10^{7} \pm 0.3$	$4.4\times10^{7} \pm 0.44$	42.9

Table.2 Percentage of inhibition of antibacterial spectrum of different concentrations of water algal extracts using Gram-negative and Gram-positive bacteria - Concentrations (0.9 mg ml–1)

Bacteria	Algal isolates	Control	Water extract	Inhibition (%)
Escherichia coli	*Anabaena aequalis*	$4.5\times10^{8}\pm0.11$	$1.7\times10^{8}\pm0.05$	69.8
	Lyngbya aestuarii	$4.5\times10^{8}\pm0.11$	$1.5\times10^{8}\pm0.51$	74.4
	Oscillatoria angusta	$9.7\times10^{7}\pm0.07$	$7.1\times10^{7}\pm0.32$	29.8
	Spirulina laxa	$4.3\times10^{8}\pm0.72$	$2.1\times10^{8}\pm0.52$	58.5
	Synechocystis aquatilis	$4.3\times10^{7}\pm0.43$	$3.4\times10^{8}\pm0.03$	0.1
Salmonella typhi	*Anabaena aequalis*	$4.7\times10^{7}\pm0.06$	$6.5\times10^{6}\pm0.41$	86.4
	Lyngbya aestuarii	$4.7\times10^{7}\pm0.07$	$2.6\times10^{6}\pm0.42$	90.1
	Oscillatoria angusta	$6.6\times10^{8}\pm0.74$	$3.0\times10^{8}\pm0.44$	59.4
	Spirulina laxa	$7.1\times10^{8}\pm0.07$	$4.9\times10^{6}\pm0.32$	94.2
	Synechocystis aquatilis	$6.6\times10^{8}\pm0.72$	$2.7\times10^{9}\pm0.12$	14.7
Streptococcus faecalis	*Anabaena aequalis*	$1.5\times10^{8}\pm0.14$	$1.5\times10^{7}\pm0.07$	91.5
	Lyngbya aestuarii	$1.5\times10^{8}\pm0.13$	$5.6\times10^{7}\pm0.14$	60.2
	Oscillatoria angusta	$1.7\times10^{8}\pm0.07$	$4.1\times10^{7}\pm0.07$	74.0
	Spirulina laxa	$1.7\times10^{8}\pm0.07$	$3.5\times10^{7}\pm0.32$	79.3
	Synechocystis aquatilis	$4.0\times10^{7}\pm0.12$	$1.9\times10^{7}\pm0.14$	60.5

Table.3 Active secondary metabolites contents of all algal species (Concentrations)

Algae	Quercetin (mg l^{-1})	Pigments			Total carbohydrates (mg l^{-1})	Total protein (mg l^{-1})
		Phycocyanin (mg l^{-1})	Carotenoid (mg l^{-1})	Chlorophyll-a (μg l^{-1})		
Anabaena aequalis	4	0.5±0.02	1.6×10^{8} ± 0.1	7728.90 (16^{th} day)	2.12 ± 0.13	0.48 ± 0.49
Lyngbya aestuarii	2.5	0.9±0.04	1.4×10^{8} ± 0.5	3172.30 (6^{th} day)	1.42 ± 0.01	0.66 ± 0.35
Oscillatoria angusta	32	0.7±0.01	7.0×10^{7} ± 0.3	3991.10 (16^{th} day)	1.82 ± 0.14	0.43 ± 0.50
Spirulina laxa	22	0.6±0.05	2.0×10^{8} ± 0.5	411.23 (10^{th} day)	0.85 ± 0.08	0.27 ± 0.04
Synechocystis aquatilis	42	4.8±0.04	3.3×10^{8} ± 0.2	2521.90 (8^{th} day)	3.52 ± 0.22	0.47 ± 0.22

As evident from the Table-1, *A. aequalis* and *O. angusta* continued to grow upto 16 days with maximum chlorophyll-a content reaching 7728.90 and 3991.1 µg/l followed by the stationary phase. With regard to *L. aestuari* chlorophyll-a content at maximum growth phase amounted to 3172.30 (6^{th} day) while the same for *S. taxa* was 2521.9 µg/l (10^{th} day). Thus from the above data, it is clear that it can be concluded that the maximum biomass measured in terms of chlorophyll-a content as well as growth stages differed from each other.

The total carbohydrate and protein content present in each algal species are presented in Table-1. Among the five species, the maximum carbohydrate content was recorded by *S. aquatilis* (3.52 mg/l) followed by *A. aequalis* (2.12 mg/l) and the least by *S. laxa* (0.85 mg/l). On the other hand, the maximum protein content was recorded in *L. aestuarii* (0.66 mg/l) followed by *A. aequalis* (0.48 mg/l) while the lowest was in *S. laxa* (0.27 mg/l).

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; Parekh and Chanda, 2007; Abedin and Hala, 2008; Desbois *et al.,* 2008; Kamble and Chavan, 2010; Elsie and Phanarajan, 2010) and some of them are under investigation while some are being used to develop new pharmaceuticals (Limafilho and Carvalho, 2002). It is also reported that the phenolic content are active as antibacterial against different types of microorganisms like *Salmonella typhi* (Ouattara *et al.,* 2011) and the flavonoids are reported to be they are active against several strains like *Streptococcus* (Shu *et al*., 2011), *E. coli* and *Staphylococcus aureus* (Gao and Zhang, 2010).

Sabarinathan and Ganesan (2008) evaluated the antibacterial effect of Phycocyanin pigment and proved its safety. Results of present study showed that the Phycocyanin content of *Synechocystis aquatilis* was the highest (4.8 mg/ml) in comparison with other algal species.

It was also reported the *E. coli* and *Staphylococcus* are sensitive to polysaccharides (Li-Ya and Chang-Hong, 2010) and *S. aquatilis* showed the highest content of carbohydrates (3.52 mg/l).

The results of the present study showed that methanol extract of the selected algal species had inhibitory activities against gram-positive bacteria. Tuney *et al*. (2006) also showed that the methanol extract of *Gracilaria gracilis* exerted inhibitory effects against gram-positive bacteria *Streptococcus epidermidis* at a concentration of 25 µl. The antimicrobial activity of *Trichodesmium erythraeum*, a genus of filamentous cyanobacteria, showed an inhibitory effect against gram-positive bacteria *Enterococcus faecalis* and *Bacillus subtilis* at a concentration 0.315 µg/ml (Kasinathan *et al*., 2009).

Umamaheshwari *et al.* (2009) found that methanol extract of *Halophila ovalis* exerted antibacterial effects against *Salmonella typhi* and *Salmonella paratyphi-B.* The results of Goud *et al.* (2007) showed that methanol extracts of several species of freshwater algae including *Nostoc* sp., *Lyngbya* sp., *Anabaena* sp. and *Mougeotia* sp. exerted antibacterial activity against Gram-negative bacteria *Salmonella typhimurium*. In

contrast, methanol extract from other freshwater microalgal species such as *Pharmidium* sp., *Cladophora* sp. and *Oscillatoria* sp. showed no inhibitory effects against *Salmonella typhimurium* at concentration 50 mg/ml (Abdo *et al.*, 2012).

The effect of water extracts of the selected algal species showed antibacterial activities against bacterial strains. It is clear that water extracts showed inhibitory effects lower than that of methanol. These results are in harmony with the finding of Goud *et al.* (2007), Sethubathi and Prabu (2010) and Abdo *et al.* (2012). From the present results it could be concluded that the antibacterial activity of the algae depends on the content of quercitin and phycocyanin pigments for the alcohol extracts and polysaccharide content for the water extracts of the species and the type of bacterial strains. The present study, it suggests that *S. aquatilis* can be used for management of gram-positive and gram-negative infections.

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