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Antimicrobial activity of certain fresh water microalgae from Thamirabarani River, Tamil Nadu, South India

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ABSTRACT

Objective: To evaluate the antimicrobial potential of fresh water microalgae *viz.*, *Oscillatoria sancta* (*O. sancta*) (Kuetz) Gomont., *Lyngbya birgei* (*L. birgei*) Smith G.M., *Oedogonium echinospermum* (*O. echinospermum*), *Spirogyra decimina* (*S. decimina*) (Muller) Kuetz., *Spirogyra grantiana* (*S. grantiana*) Transeau., *Spirogyra crassa* (*S. crassa*), *Spirogyra biformis* (*S. biformis*) Jao. and *Spirogyra condensata* (Vaucher) Kuetz against human bacterial pathogens. **Methods:** Antimicrobial study was carried out by disc diffusion method against the pathogens *viz.*, *Escherichia coli* (*E. coli*) (ATCC 35218), *Staphylococcus aureus* (*S. aureus*) (ATCC 6538), *Salmonella typhi* (*S. typhi*) (MTCC 733), *Proteus vulgaris* (*P. vulgaris*), *Proteus mirabilis* (*P. mirabilis*) and *Streptococcus pyogenes*. **Results:** The methanolic extract of *O. sancta* showed the antibacterial activity against three pathogens *viz.*, *P. mirabilis*, *P. vulgaris* and *S. aureus* with the inhibition zones of 10, 8 and 8 mm, respectively. The methanolic and ethanolic extracts of *L. birgei* exhibited the antibacterial activity against two pathogens *i.e. P. mirabilis* and *P. vulgaris* with the maximum zone of inhibition of 8 and 8 mm, respectively. The ethanolic extracts of *O. echinospermum* displayed the antibacterial activity against *S. typhi* and *P. mirabilis* with the maximum of zone of inhibition (7 mm). The methanolic extracts of *S. decimina* exhibited the antibacterial activity against *S. aureus* and *P. mirabilis* with the maximum zone of inhibition of 12 and 9 mm, respectively. The ethanolic extracts of *S. grantiana* showed the anti-bacterial activity against three organisms *i.e. E. coli*, *P. vulgaris* and *P. mirabilis* with the zone of inhibition of 9, 10 and 9 mm, respectively. The methanolic extracts of *S. crassa* exhibited the antibacterial activity against *P. mirabilis* with the maximum zone of inhibition (9 mm). The methanolic extracts of *S. biformis* exhibited the antibacterial activity against *P. vulgaris* with the maximum zone of inhibition (8 mm). **Conclusions:** These results give an indication of the presence of promising antibacterial compounds in the plants under studied. Further phytochemical studies are needed to elucidate the components responsible for antibacterial activity of these extracts against bacteria.

1. Introduction

Aquatic organisms are a rich source of structurally novel and biologically active metabolites[1]. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. 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[2-5]. The antimicrobial substances include unsaturated lactones, cyanogenic glycosides, sulphur containing compounds, phenols and phenolic glycosides, saponins and phytoalexins[6]. The use of algae for therapeutic purposes has a long history and the systematic examination of algae for biologically active substances especially antibiotics began in the year 1950. The aqueous and solvent extracts from algae were tested against gram positive and gram negative bacteria[7-10]. The important compounds identified as antimicrobial are fatty acids, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing hetero cyclic compounds, carbohydrates and phenols[11]. A wide range of results of

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in vitro anti-fungal activities of extracts of green algae, diatoms, and dinoflagellates have also been reported[12,13]. Microalgae constitutes one of the commercially important living and renewable resources. They contain more than sixty trace elements incuding minerals, proteins, iodine, bromine and many bioactive substances[14]. To date, many chemically unique compounds of fresh water origin with various biological activities have been isolated, and some of them are under investigation and some are being used to develop new pharmaceuticals[15]. Many bioactive and pharmacologically active substances have been isolated from algae[16-20]. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials[21]. With this knowledge the present study was aimed to screen the antimicrobial potential of *Oscillatoria sancta* (*O. sancta*) (Kuetz) Gomont., *Lyngbya birgei* (*L. birgei*) Smith G.M., *Oedogonium echinospermum* (*O. echinospermum*), *Spirogyra decimina* (*S. decimina*) (Muller) Kuetz., *Spirogyra grantiana* (*S. grantiana*) Transeau., *Spirogyra crassa* (*S. crassa*), *Spirogyra biformis* (*S. biformis*) Jao. and *Spirogyra condensata* (*S. condensata*) (Vaucher) Kuetz against human bacterial pathogens.

2. Materials and methods

Eight species of microalgae *viz.*, *L. birgei* Smith G.M., *O. echinospermum*, *O. sancta* (Kuetz) Gomont., *S. decimina* (Muller) Kuetz., *S. grantiana* Transeau., *S. crassa*, *S. biformis* Jao. and *S. condensata* (Vaucher) Kuetz. were collected from Thamirabarani River and used for the preparation of different solvent extracts. Algae samples were cleaned and necrotic parts were removed. Then the samples were rinsed with sterile water to remove any associated debris. These cleaned fresh materials were air-dried and then powdered with the help of a blender. The powder (5 g) was filled in the thimble and extracted with methanol, ethanol and hexane by using a Soxhlet apparatus at the temperature of 60 ℃ for 8 h. From the solvent extracts 5 mL was isolated separately, allowed to dry at room temperature and weighed to estimate the concentration in 1 mL. The dry extracts were completely dissolved in 5 mL of 0.5% Tween 80 and preserved at 5 ℃ in airtight bottles until further use[22] and the extracts were used for antimicrobial studies. Antimicrobial study was carried out by disc diffusion method[23] against the pathogens *viz.*, *Escherichia coli* (*E. coli*) (ATCC 35218), *Staphylococcus aureus* (*S. aureus*) (ATCC 6538), *Salmonella typhi* (*S. typhi*) (MTCC 733), *Proteus vulgaris* (*P. vulgaris*), *Proteus mirabilis* (*P. mirabilis*) and *Streptococcus pyogenes* (*S. pyogenes*).

3. Results

The methanolic, ethanolic and hexane extracts were tested for antibacterial activity against six human bacterial

Table 1
Antibacterial activity of the various extracts of microalgae from the Thamirabarani River.

Algal species	Extracts	Concentration (mg/mL)	Inhibition zone diameters (mm)					
			E. coli	*S. aureus*	*S. typhi*	*P. vulgaris*	*P. mirabilis*	*S. pyogenes*
O. sancta	Methanol	0.30	–	8	–	8	10	–
	Ethanol	0.35	–	7	–	–	–	–
	Hexane	0.23	–	9	–	–	–	–
L. birgei	Methanol	0.21	–	–	–	–	8	–
	Ethanol	0.16	–	–	–	8	–	–
	Hexane	0.30	–	–	–	–	–	–
O. echinospermum	Methanol	0.24	–	–	7	–	–	–
	Ethanol	0.40	–	–	7	–	7	–
	Hexane	0.18	–	–	7	–	–	–
S. decimina	Methanol	0.20	–	12	–	–	9	–
	Ethanol	0.42	–	–	–	–	–	–
	Hexane	0.37	–	9	–	–	–	–
S. grantiana	Methanol	0.43	–	–	7	–	–	–
	Ethanol	0.36	9	–	–	10	9	–
	Hexane	0.40	–	–	–	–	–	–
S. crassa	Methanol	0.50	–	–	–	–	9	–
	Ethanol	0.34	–	8	–	–	–	–
	Hexane	0.66	–	–	–	–	–	–
S. biformis	Methanol	0.19	–	–	–	8	–	–
	Ethanol	0.21	–	–	–	–	–	–
	Hexane	0.23	–	8	–	–	–	–
S. condensata	Methanol	0.53	–	–	–	–	8	–
	Ethanol	0.39	–	–	–	–	–	–
	Hexane	0.55	–	–	–	–	–	–
Control	Tween 80	0.50%	–	–	–	–	–	–

pathogens. The degree of activity was varied with reference to concentration of algal extracts. The methanolic extract of *O. sancta* showed the antibacterial activity against three pathogens *viz.*, *P. mirabilis*, *P. vulgaris* and *S. aureus* with the inhibition zones of 10, 8 and 8 mm, respectively (Table 1). The ethanolic and hexane extracts of *O. sancta* exhibited the antibacterial activity against only one bacterium *S. aureus* with the inhibition zones of 7 and 9 mm, respectively. *E. coli*, *S. typhi*, *P. mirabilis*, *P. vulgaris* and *S. pyrogenes* were resistant to hexane and ethanolic extracts of *O. sancta* (Table 1). The methanolic and ethanolic extracts of *L. birgei* exhibited the antibacterial activity against two pathogens each *i.e. P. mirabilis* and *P. vulgaris* with the maximum zone of inhibition of 8 and 8 mm, respectively (Table 1). The hexane extract failed to show inhibition zone against any other pathogen used for this antibacterial assay.

The methanolic extracts of *O. echinospermum* exhibited the antibacterial activity against only one bacterium *i.e. S. typhi* with the maximum zone of inhibition (7 mm) (Table 1). *E. coli*, *S. pyrogenes*, *S. aureus*, *P. vulgaris* and *P. mirabilis* were resistant to the methanolic extracts of *O. echinospermum* (Table 1). The ethanolic extracts of *O. echinospermum* displayed the antibacterial activity against *S. typhi* and *P. mirabilis* with the maximum of zone of inhibition (7 mm). The hexane extracts of *O. echinospermum* displayed the antibacterial activity against *S. typhi* with the maximum of zone of inhibition (7 mm).

The methanolic extracts of *S. decimina* exhibited the antibacterial activity against *S. aureus* and *P. mirabilis* with the maximum zone of inhibition of 12 and 9 mm, respectively. The hexane extracts of *S. decimina* exhibited the antibacterial activity against only *S. aureus* with maximum of zone of inhibition (9 mm). The ethanolic extracts of *S. decimina* failed to show the bio-efficacy against the selected bacteria. The methanolic extracts of *S. grantiana* exhibited the anti-bacterial activity against *S. typhi* with the maximum zone of inhibition (7 mm). The ethanolic extracts of *S. grantiana* showed the anti-bacterial activity against three organisms *i.e. E. coli*, *P. vulgaris* and *P. mirabilis* with the zone of inhibition of 9, 10 and 9 mm, respectively. The hexane extracts of *S. grantiana* failed to demonstrate the anti-bacterial activity against the selected pathogens. The methanolic extracts of *S. crassa* exhibited the antibacterial activity against *P. mirabilis* with the maximum zone of inhibition (9 mm). The hexane extract of *S. crassa* failed to show the bio-activity against the selected bacteria. The methanolic extracts of *S. biformis* exhibited the antibacterial activity against *P. vulgaris* with the maximum zone of inhibition (8 mm). The hexane extracts of *S. biformis* exhibited the antibacterial activity against only *S. aureus* with maximum of zone of inhibition (8 mm). The ethanolic extract of *S. biformis* failed to show the bio-activity against the selected bacteria. The methanolic extract of *S. condensata* showed the antibacterial activity against *P. mirabilis* with the maximum zone inhibition of 8 mm. The ethanolic and hexane extracts of *S. condensata* failed to show the antibacterial activity against the selected pathogens.

4. Discussion

Many fresh water microalgae have been recognized as potential source of antibacterial substances. *E. coli* is a gram negative, straight, rod shaped bacterium arranged singly or in pairs. They cause mainly four types of clinical syndromes, urinary tract infections, diarrhoea or gastroenteritis, pyogenic infections and septicemia. The present study results revealed that the ethanolic extracts of *S. grantiana* showed the antibacterial activity against *E. coli*. The results of the present study suggest that the ethanolic extracts of *S. grantiana* may be used to treat urinary tract infections, diarrhea, pyogenic infections and septicemia. *S. aureus* is a gram positive bacterium causing diseases like sepsis in wounds and burns, septicemia, pharyngitis, sinusitis, and tonsillitis. The hexane extracts of *S. biformis* and *S. decimina*, ethanolic extracts of *O. sancta* and methanolic extracts of *S. decimina* showed the anti-bacterial activity against *S. aureus* and suggest that the hexane extracts of *S. biformis* and *S. decimina*, ethanolic extracts of *O. sancta* and methanolic extracts of *S. decimina* may be used to treat the diseases like sepsis in wounds and burns, septicemia, pharyngitis, sinusitis, and tonsillitis. *S. typhi* is a gram negative rod shaped bacterium. *S. typhi* can cause the enteric fever, septicemia with or without local suppurative lesions, and gastroenteritis in human beings. The hexane, methanolic and ethanolic extracts of *O. echinospermum* and methanolic extracts of *S. grantiana* showed the antibacterial activity against the pathogen *S. typhi*, it confirms the active principles against the *S. typhi* in hexane, methanolic and ethanolic extracts of *O. echinospermum* and methanolic extracts of *S. grantiana*. It suggests that in addition to the available drugs, alternatively we can use the extracts of *O. echinospermum* and *S. grantiana* against the enteric fever, septicemia with or without local suppurative lesions, and gastroenteritis. *P. vulgaris* is a rod-shaped gram negative bacterium. It causes urinary tract infections and wound infections in human beings. The methanolic extracts of *L. birgei*, *S. biformis* and ethanolic extracts of *S. grantiana* demonstrated the antibacterial activity against the pathogen *P. vulgaris*. It confirms the active principle presence in *L. birgei*, *S. biformis* and *S. grantiana*. *P. mirabilis* is a gram-negative bacterium. *P. mirabilis* causes urinary tract infections and wound infections. *P. mirabilis* is responsible for about 90% of all *Proteus* infections. The methanolic extracts of *L. birgei*, *S. crassa* and ethanolic extracts of *S. grantiana* and *O. echinospermum* demonstrated the anti-

bacterial activity against the *P. mirabilis* and suggested as alternative medicine to treat urinary and wound infections. Most of the identified components with antimicrobial activity extracted from plant groups are aromatic or saturated organic compounds and they are more soluble in methanol[24]. Similarly, in this study the methanol extracts exhibited higher activity followed by ethanol and the hexane extracts.

The present study indicates that the antibacterial property of the eight algal species against the selected strains of human pathogenic bacteria varies depending upon the solvent medium used for extraction. The most sensitive bacteria were *S. aureus* and *P. mirabilis*, which were inhibited by methanol, ethanol and hexane extracts. *S. pyrogenes* is the most resistant bacteria used for this study. These results give an indication of the presence of promising antibacterial compounds in the plants under studied. Further phytochemical studies are needed to elucidate the components responsible for antibacterial activity of these extracts against bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Ely R, Supriya T, Naik CG. Antimicrobial activity of marine organisms collected off the coast of South East India. *J Exp Mar Biol Ecol* 2004; **309**: 121-127.

[2] Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *Int J Appl Sci Eng* 2005; **2**: 125-134.

[3] Raghavendra MP, Satish S, Raveesha KA. Phytochemical analysis and antibacterial activity of *Oxalis corniculata*, a known medicinal plant. *My Sci* 2006; **1**(1): 72-78.

[4] Selvamaleeswaran P, Wesely EG, Johnson M, Velusamy S, Jeyakumar N. The effect of leaves extracts of *Clitoria ternatea* Linn against the fish pathogens. *Asian Pac J Trop Med* 2010; **3**(9): 723-726.

[5] Haripriya D, Selvan N, Jeyakumar N, Periasamy RS, Johnson M, Irudayaraj V. The effect of extracts of *Selaginella involvens* and *Selaginella inaequalifolia* leaves on poultry pathogens. *Asian Pac J Trop Med* 2010; **3**(9): 678-681.

[6] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol* 2005; **4**: 685-688.

[7] Prashanthkumar P, Angadi SB, Vidyasagar GM. Antimicrobial activity of blue green algae and green algae. *Indian J Pharm Sci* 2006; **88**(6): 647-648.

[8] Singh AP, Chaudhary BK. Preliminary phytochemical analysis and *in vitro* anti-bacterial activity screening of *Pithosphora oedogonia* (Mont.) Wittrock-a fresh water green alga forming mats in the water bodies. *J Algal Biomass Util* 2010; **1**(2): 33-41.

[9] Goud MJP, Seshikala D, Charya MAS. Antibacterial activity and biomolecular composition of certain fresh water microalgae from River Godavari (India). *Sci World J* 2007; **2**(3): 19-23.

[10] Marhivanan K, Ramamurthy V, Rajaram R. Antimicrobial activity of *Oscillatoria princes* and *Lyngbya majuscule* against pathogenic microbes. *Int J Curr Res* 2010; **5**: 97-101.

[11] Kannan RRR, Arumugam R, Anantharaman P. *In vitro* antioxidant activities of ethanol extract from *Enhalus acoroides* (L.F.) Royle. *Asian Pac J Trop Med* 2010; **3**(11): 898-901.

[12] Moreau JD, Pasando P, Bernard P, Caram, B, Pionnat JC. Seasonal variation in the production of antifungal substrates by dictyotales (brown algae) from the French Mediterranean coast. *Hydobiologia* 1988; **2**: 1097-1132.

[13] Renu A. Antibacterial activities of freshwater algae *Chlorella ellipsoidea*. *J Basic Appl Biol* 2010; **4**(1&2): 22-26.

[14] Asthana RK, Tripathi MK, Srivastava A, Singh AP, Singh SP, Nath G, et al. Isolation and identification of a new antibacterial entity from the Antarctic cyanobacterium *Nostoc* CCC 537. *J Appl Phycol* 2009; **21**(1): 81-88.

[15] Limafilho JVM, Carvalho AFFU, Freitas SM. Antibacterial activity of extracts of six macro algae from the Northeastern Brazilian Coast. *Braz J Microbiol* 2002; **33**: 311-313.

[16] Choudhary S, Sree A, Mukherjee SC, Patnaik P, Bapuji M. *In vitro* antibacterial activity of extracts of selected marine algae and mangroves against fish pathogens. *Asian Fish Sci* 2005; **18**: 285-294.

[17] Kamble SM, Chavan AM. Antibacterial activity of some fresh water algae. *J Exp Sci* 2010; **1**(2): 5-6.

[18] Abedin RMA, Hala MT. Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by Plackett-Burman design for antimicrobial activity of *Spirulina platensis*. *Glob J Biotechnol Biochem* 2008; **3**(1): 22-31.

[19] Elsie HB, DhanaRajan MS. Evaluation of antimicrobial activity and phytochemical screening of *Gelidium acerosa*. *J Pharm Sci Res* 2010; **2**(11): 704-707.

[20] Desbois AP, Lebl T, Yan L, Smith VJ. Isolation and structural characterisation of two antibacterial free fatty acids from the marine diatom, *Phaeodactylum tricornutum*. *Appl Microbiol Biotechnol* 2008; **81**(4): 755-764.

[21] Parekh J, Chanda VS. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk J Biol* 2007; **31**: 53-58.

[22] Lin J, Opoku AR, Keller MG, Hutchings AD, Terblanche SE, Jager AK, et al. Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. *J Ethnopharmacol* 1998; **68**: 267-274.

[23] NCCLS. *Performance standards antimicrobial disc susceptibility tests*. 5th ed. PA: Villanova; 1993, p. M2-A5.

[24] Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999; **12**: 564-582.