FULL LENGTH RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY AND BIOMOLECULAR COMPOSITION OF CERTAIN FRESH WATER MICRO-ALGAE FROM RIVER GODAVARI (INDIA)

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ABSTRACT
Twenty four fresh water algal species were screened for their antibacterial activity and biomolecules. Bactericidal activity was tested against two gram +ve and four gram -ve bacteria. Maximum antibacterial activity was observed in methanol extracts and least in aqueous extracts. Maximum activity (14mm) was observed in the extracts of *Nostoc, Lyngbya, Mougeotia* and *Pithophora* sp. Gram +ve bacteria were more susceptible than gram -ve bacteria. Thirteen algal species are associated with tannins and phenols, 11 species with steroids, 5 species with flavonoids and 8 species with saponins. Maximum chlorophyll-a was recorded with *Ulothrix* (5.6 mg/g) and least in *Tolypothrix* (0.5 mg/g). Chlorophyll-b was recorded maximum in *Vaucheria* (4.2 mg/g) and least in *Cylindrospermum* (0.6 mg/g). Maximum carotenoid content was recorded in *Ulothrix* (4.5 mg/g) and least in *Tolypothrix* and *Oscillatoria* (0.6 mg/g). Range of protein content was 4-20% with maximum yield in *Cylindrospermum* sp. (20%) and least in *Hydrodictyon* (4%). Carbohydrate content ranged from 14-35% with maximum yield in *Mougeotia* (35%) and least in *Tolypothrix* (14%). Their pharmacological activities and bioactive molecules can be highly exploited.

***Key words*:** Fresh water Algae, antibacterial activity, phycochemicals, phycopigments.

INTRODUCTION
The use of extracts from plants and animals for medicinal purposes is a practice as old as the history of mankind. Following the increase in demand for biodiversity in the screening programs seeking therapeutic drugs from natural products, there is now a greater interest in algae. Algae have been harvested by man for centuries, particularly in Japan and China, where they form a part of the staple diet (Stella Roslin 2003a). The first investigation on the antibiotic activity of algae was carried out by Pratt *et al.* (1944). Apart from that a number of workers both from India and abroad have done investigations on the various aspects of antibiotic activities of marine algae (Bukholder 1960). The exploration of marine algae for nutritional purpose is primarily based on the biochemical constituents.

Evidence of phycochemical and pharmacological studies on algae is available in the literature with special references to terpenoids and steroids (Parameswaran 1944; Patterson 1968). The microalgae have a significant attraction as natural source of bioactive molecules, because they have the potential to produce bioactive compounds in culture, which are difficult to produce by chemical synthesis (Borowitza & Borowitza 1989). Despite this potential, attention has been centered on marine algae (Jaki 2000), with very little on fresh water algae. The present study compares the phycochemical and pharmacological activities of twenty four fresh water algae aimed at exploring their antimicrobial activity and biomolecules of potential therapeutic interest.

MATERIALS AND METHODS
Glassware: All the Glassware used is of borosil type. They are thoroughly cleaned with 5% extran MA 02 neutral (Merck) and rinsed with distilled water before use. Methanol is procured from universal laboratories (Mumbai), Chemicals are purchased from Merk (Mumbai) and all the media ingredients are from Himedia (Mumbai).

Plant Materials: Twenty four algal species were collected from river Godavari. The algae was brought to the laboratory and repeatedly cleaned with fresh water to remove all extraneous matter. Their botanical identities were determined and authenticated. The algal species were oven dried at a temperature of 60^0C for 1 week, grounded into fine powder and stored in airtight containers.

Preparation of algal extracts: The extraction method used in this study was a modification of Akinyemi *et al.* (2000), using 3 solvents (methanol, ethanol and water). 10 g of each of the powdered plant materials were extracted in a soxhlet extractor containing 40 ml of the solvent and the resulting extracts evaporated in a rotary evaporator. The dried extracts were weighted to obtain the required concentration of the crude antibiotic for the study. ater before use. Methanol is procured from universal laboratories (Mumbai), Chemicals are purchased from Merk (Mumbai) and all the media ingredients are from Himedia (Mumbai).

Bacterial cultures: Six bacterial cultures were used for the bioassay, two gram-positive and four gram-negative namely, *Bacillus subtilis* Gislene, 2000, *B. cereus* Geopfert, *et al*. 1972, *Enterobacter aerogens* Gislene, 2000, *Salmonella typhimurium* Cowan, 1991, *Escherichia coli* Migula, 1895 and *Pseudomonas aeruginosa* Schroeter, 1872. The pure strains were obtained from the microbial type culture collection and gene bank of (MTCC), Institute of microbial Technology, Chandigarh, India. The organisms were maintained on agar slopes at 4^0C and sub cultured for 24hr before use.

Bacterial susceptibility testing: The Agar plate well-diffusion method was used as described by Desta (2005). A standardized inoculum 1-2 x 10^7 cfu/ml 0.5 MC Farland standards was introduced onto the surface of sterile agar plate, and evenly distributed the inoculum by using a sterile glass spreader. Simultaneously 8 mm wells were cut from the plate using a sterile cork borer. 70µl of extract at a concentration of 50 mcg/ml were introduced into each well. The agar plates were incubated aerobically at 37^0C and the inhibition zones measured with a ruler and compared with the control well (well containing only the respective solvent) after 24 hr.

Phycochemical tests*:* Phycochemical test was conducted as described by Gibb (1974) for tannins, phenols, steroids, flavonoids and saponins using the methanol extract of the selected algae. Chlorophyll-a, Chlorophyll-b and carotenoid estimation was made by Arnons formula (Arnon 1949). The protein estimation was made by Lowry's method (Lowry 1951) and the carbohydrate estimation was by Anthrone method (Jermyn 1975).

Statistical analysis: All the values or readings are the result of mean of three replicates.

RESULTS

The main objective of this work is to evaluate microbial activity and biomolecular composition of fresh water microalgal species from river Godavari, therefore, the production of antimicrobial activity was considered an indicator of the capability of these algae to synthesize bioactive secondary metabolites.

Result of the antibacterial activity is giving in Table 1. Of the 24 algal species used, *Melosira*, *Microspora*, *Cylindrospermum*, *Oscillatoria* and *Tolypothrix* did not show any antibacterial activity while maximum activity of 14 mm was observed in *Nostoc, Pithophora* , *Mougeotia* and *Lyngbya* sp. *Nostoc* showed maximum activity towards Gram-positive *Bacillus subtilis* in addition to *Escherichia coli* that also showed sensitivity for *Nostoc* extract.

Activity against gram-negative bacteria was less common than against gram-positive. However, among the gram positive bacteria, not all the target strains tested were equally susceptible to the antimicrobial metabolites produced. The two most susceptible organisms were *B. subtilis* and *B. cereus*, which were inhibited by extracts of 18 and 17 species respectively.

Maximum chlorophyll-a was recorded in the species of *Ulothrix* (5.6 mg/g) followed by *Scopulonema, Pithophora* and *Oedogonium* (3.9mg/g), and least recorded in *Tolypothrix* (0.5mg/g).

Maximum Chlorophyll-b was recorded in *Vaucheria* (4.2 mg/g) followed by *Desmidium* (3.2 mg/g) and *Pithophora* (2.9 mg/g). Carotenoid content was maximum in *Ulothrix* (4.5 mg/g) followed by *Melosira* (3.5 mg/g) and *Mougeotia* (3.1 mg/g) and low in *Tolypothrix* and *Oscillatoria* (0.6 mg/g) (Table 1). A variety of fine chemicals such as pigments, vitamins and enzymes with varied applications can be obtained on a commercially viable scale from cyanobacteria. Lowest carbohydrate content was recorded in *Tolypothrix* (14%) (Table 1).

DISCUSSION

Results from the present study shows that about 50% micro algae screened showed some evidence of microbial activity. Cannel *et al.* (1988), screened more than 100 cynobacterial cultures and obtained positive results in less than 10%. When compared with similar studies, it should be noted that the number of species tested in this work is fewer.

Zornitza *et al*. (2000) had shown that a broad spectrum antimicrobial antibiotic is produced by *Nostoc* sp which inhibits the growth of bacteria, notably multiresistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The same authors tested the methanol extract of *Spirogyra* against wide range of bacteria and *Spirogyra varians* showed inhibitory activity against gram positive and gram negative bacteria equally comparable to our study where methanol extracts of *Spirogyra*, inhibited both Gram-positive and Gram-negative bacteria. Berry *et al.* (2004) observed that several species of fresh water green algae belonging to the order Zygnematales (*spirogyra* sp.) had a specific composition of the volatile fraction with antibacterial properties. *Nostoc, Mougeotia* and *Lyngbya* inhibited all the bacterial species in the present study suggesting them as producers of broad spectrum antibiotics. *Lyngbya* has been found to be a rich source of bioactive metabolites, with Pahayokolide A, a bioactive compound inhibiting growth of several representatives of gram positive and gram negative bacteria (Vepritskii 1991).

Previous studies (Zornitza *et al*. 2000; Berry *et al*. 2004) on microalgae have detected similar antimicrobial activities in some of the species tested in this work as well as other activities not detected in our screening .These differences between our results and those from other studies may be due to several factors, that may include intraspecific variability in the production of secondary metabolites occasionally related to seasonal variations, differences in the extraction protocols used to recover the active metabolites as well as differences in the assay methods.

Among the three extracts used in the present study, the activity was in the order methanol >ethanol >aqueous extract (Table 1) agreeing with observations of Vijaya Parthasarathy *et al*. (2004) that methanol is a better solvent for algal extraction and separation of variety of phycochemicals that produce maximum inhibitory effect on both gram positive and gram negative bacteria. The present study revealed the poor activity of water extract in all the algal species, agreeing with earlier reports (Hodgson 1944) that the use of organic solvents is always better for extraction compared with water extraction.

The results of the phycochemical study revealed that tannins and phenols are associated with 13 algal species. The results also show that 11 algal species were positive for steroids, 5 species with flavonoids and 8 species with saponins. Out of the 5 chemical tests, *Pithophora* and *Desmidium* were positive towards 4. *Microspora*, *Enteromorpha*, *Oedogonium*, *Chaetophora* and *Cladophora* and *Anabaena* were positive only towards one chemical tested. Apart from the *Microchaete*, *Nitella* and *Sphaeroplea,* phenols have also been detected in various other algal species, raising further interest in their pharmacological properties.

TABLE 1 : ANTIMICROBIAL ACTIVITY AND BIOMOLECULE COMPOSITION OF FRESH WATER ALGAE.

Algal species	Antibacterial activity (one of inhibition mm)																		Phycochemicals					Phycopigments (mg/g)			Protein content (%)	Carbohydrate content (%)
	B.s.			B.c			E.a			S.t			E.c			P.a			T_t	P_t	St_t	F_t	Sa_t	Chl-a	Chl-b	Car		
	M	E	A	M	E	A	M	E	A	M	E	A	M	E	A	M	E	A										
Oedogonium	5	4	3	5	5	4	5	--	3	4	4	3	--	-	4	7	-	3	--	--	--	--	+	3.9	2.1	2.1	13	30
Nostoc	14	8	5	12	9	5	9	6	4	7	4	5	13	8	6	10	8	4	--	--	--	+	+	2.2	1.3	2.2	5	30
Enteromorpha	5	4	--	5	-	--	5	--	--	3	5	--	--	-	--	5	-	--	+	--	--	--	--	2.5	2.0	2.5	14	15
Hydrodictyon	7	4	--	8	5	--	--	--	--	--	5	--	--	-	--	--	-	--	+	+	--	--	--	1.9	1.3	1.7	4	19
Ulothrix	9	5	--	7	3	--	--	--	--	--	6	--	--	3	--	--	-	--	+	+	--	--	--	5.6	3.1	4.5	10	19
Vaucheria	10	4	--	6	3	--	10	--	--	5	4	--	7	4	--	6	-	--	+	--	+	--	+	3.7	4.2	2.8	6	15
Spirogura	4	--	--	7	--	--	7	--	--	5	--	--	6	--	--	7	-	--	+	+	--	--	+	2.1	1.4	1.9	11	20
Scopulonema	7	4	4	7	5	6	7	4	4	10	4	6	-	-	-	7	4	4	+	--	+	--	--	3.9	2.8	2.9	11	30
Pithophora	14	5	--	5	5	--	10	--	--	6	5	--	12	4	--	11	3	3	+	+	+	--	+	3.9	2.9	2.8	9	24
Lyngbya	14	8	3	12	7	4	6	4	3	7	5	3	10	6	3	9	6	3	--	--	+	+	+	1.0	1.3	1.2	8	31
Anabaena	6	--	3	6	4	3	4	4	--	7	3	3	10	--	--	7	--	--	--	+	--	--	--	3.2	2.5	2.8	11	34
Microcoleus	7	3	--	8	5	--	--	4	--	7	5	--	5	--	--	4	6	--	+	--	+	--	--	2.8	2.5	2.3	13	31
Mougeotia	14	12	4	14	10	3	9	5	3	9	5	3	10	7	3	8	6	3	--	--	+	--	+	3.5	2.9	3.1	9	35
Desmidium	7	3	4	6	4	3	4	7	3	10	7	3	7	5	3	5	3	--	+	+	+	+	--	3.8	3.2	2.7	10	30
Phormidium	5	5	--	5	--	--	--	--	--	--	--	--	4	3	--	--	--	--	--	--	+	+	--	1.8	1.4	1.5	19	19
Tribonema	5	--	--	--	--	--	--	--	--	--	--	--	3	--	--	--	--	--	--	+	--	+	+	2.2	1.3	1.5	15	20
Chaetophora	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	--	--	--	--	1.9	1.4	1.8	15	22
Cladophora	5	--	--	3	--	--	4	--	--	--	--	--	--	--	--	--	--	--	+	--	--	--	--	1.8	1.2	1.6	17	22
Microcoleus	4	--	--	3	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	+	--	--	--	1.8	1.6	1.4	15	14
Tolypothrix	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	+	--	--	0.5	2.1	0.6	15	14
Oscillatoria	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	+	--	--	0.6	0.8	0.6	15	25
Cylindrospermum	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	+	--	--	--	0.7	0.6	1.2	20	31
Microspora	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	--	--	--	2.1	1.3	2.5	15	25
Melosira	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+	+	--	--	1.8	2.1	3.5	17	19

B.s. - *Bacillus subtilis,* B.c -*Bacillus cerius;* E.a. - *Enterobacteria aerogens,* S.t.-*Salmonella typhimurium; E.c - Escherichia coli; Pa - Pseudomonas aeruginosa;* M - Methanol; E - Ethanol; A - Aqueous; T_t - Tanin test; P_t – Phenol test; St_t - Steroid test; F_t - Flavonoids; Sa_t - Saponin test; Chl-a-Chlorophyll-a; Chl-b - Chlorophyll-b; Car - Carotenoid. + : Present; -- : Absent

For example, Glombitza *et al.* (1977) isolated phenols from red algae *Polysiphonia lanosa* where they show antibacterial activity. Similarly, steroids have been identified in various algae (Patterson 1968) including the red (Gibbons 1967) and blue green algae (Reitz 1968). The algal species in the present study were individually positive to one or more phycochemicals, which might have contributed to their antibacterial efficacy.

The carotenoids and phycobiliproteins, characteristic of cyanobacteria have high commercial value and used as natural food colourants and additives as well as in livestock production to improve the health and fertility of cattle (Emodi 1978). The carbohydrate content observed in the present study in marine algae (*Mougeotia* 35%, *Anabaena* 34%, *Lyngbya* and *Microcoleus* 31%) falls within the range of 14%-59% reported by Stella Roslin (2003b). Furthermore, Stella Roslin (2003a) observed that protein content in marine algae varied between 1.5-24.8%, agreeing with the present results where protein content varied between 4-20% with maximum yield recorded in *Cylindrospermum* (20%) and lowest in *Hydrodictyon* (4%). Algal protein either as a supplement or as an alternative source has received worldwide attention. Some strains of *Anabaena* and *Nostoc* are consumed as human food in Chile, Mexico, Peru and Philippines. An alga with high amount of fibre and moderate protein has potential use as a new dietary fibre source and can play an important physiological and nutritional role in human diet (Jeraci 1986). The protein rich algal species could provide supplementary diet and so should be made palatable and popularised.

Results from the present work indicate that the species of fresh water micro algae from River Godavari examined showed a variety of antimicrobial activities and presence of bioactive molecules. Further isolation and identification of the active ingredients need to be done in order to understand their bioprospects.

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