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Water silk (*Spirogyra bichromatophora*): a Natural Resource for Antimicrobial Phycochemicals

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ABSTRACT

Spirogyra 'water silk' is filamentous green algae grow in slow flowing lotic and shallow lentic water bodies. Intracellular metabolites were evaluated for phycochemicals and antimicrobial activity. Aqueous extract has a significant result against S. aureus followed by acetone and ethanol extract against P. aeruginosa. In the broth dilution method lowest MIC is observed with hexane extract against E. colifollowed by P. aeruginosa and S. aureus. Acetone was found to be bactericidal against all three test pathogens.The presence of carbohydrates, protein, lipids, fat, phytosterols and fixed oil in water silk proves its potency as an antimicrobial drug candidate with broad-spectrum properties.

Keywords: *Phycochemicals, Spirogyra, SBR, Antibacterial activity*

INTRODUCTION

Spirogyra bichromatophora (Randhawa) Transeaupopularly also known as 'water silk' is categorized under the division Chlorophyta, phylum Charophyta, class Conjugatophyceae, order Zygnematales, family Zygnemataceae [1]. It grows in running streams, shallow ponds, ditches, and amongst vegetation at the edges of large lakes and secretes an envelope of mucous that makes it feel slippery, hence also called pond scum because the filaments slip and shine like silk due to the continuous formation of mucilage and due to dissolution of pectin of its cell wall. It is one of the commonest green filamentous algae is named because of the helical or spiral arrangement of the chloroplasts in freshwater lotic and lentic habitats. Abundance is dependent on nutrient levels, particularly phosphorus, in the water. There are more than 400 species of *Spirogyra* reported around the world [2].

Primary or secondary metabolites produced by these organisms may be potential bioactive compounds of interest in feed, bioremediation, biofuel, bioethanol, nanotechnology, and pharmaceutical industries. The genus *Spirogyra* has drawn the attention to researchers due to its allelochemicals inhibiting microalgal growth [3],[4], implying important consequences for the management of aquatic ecosystems. *S. neglecta*is beneficial to the environment as it removes Pb^{2+} from polluted water as well as for its pharmacological importance [5]. Reports are available for its hypolipidemic and hypoglycemic abilities in type 2 diabetic rats induced by streptozotocin and high-fat diet [6]. *Spirogyra neglecta* showed cancer chemo-preventive properties at the early stages of diethylnitrosamine -induced hepato-carcinogenesis in rats [7]. Another species of algae *Spirogyra porticalis* reported for anticancer activity hepG2 and RKO cell lines [8]. *Spirogyra* sp. is consumed as food in northern Thailand. *Spirogyra* contains high amounts of protein, carbohydrate, fat, sulfate and dietary fiber [9], fatty acids, vitamins, and antioxidants [10]. The

cell extracts and active constituents have been shown to have antibacterial activity and antihelmintic properties [11],[12]. Recently, Deethae and co-workers demonstrated *Spirogyra* sp. algal extracts have antiviral properties against herpes simplex virus type 1 and 2 infections [13]. Three decades ago, the identification of pentagalloylglucose as an inhibitor of α-glucosidase, was studied from *Spirogyra varians*, and suggested that polyphenols may be responsible for antimicrobial activity [14]. However, to the best of the knowledge of the authors no reports are available on the antimicrobial properties of *Spirogyra bichromatophora*. So, the authors extracted *Spirogyra bichromatophora* with different solvents and tested it for its antibacterial property against common pathogenic bacteria. Simultaneously, we tested these extracts in a phycochemical screening to achieve information about the nature of the produced compounds.

MATERIAL AND METHODS

Sample collection

Similipal Biosphere Reserve (21° 28" - 22° 08" N; 86° 04"- 86° 37" E) is extended over an area of 5569 km^2 and is located in the central part of the Mayurbhanj district, Odisha. A random sampling procedure based upon the occurrence of *Spirogyra sp.* is adopted to collect the sample from Barehipani waterfall (21.93N 86.38E) flowing over the Meghasani mountain of Similpal Biosphere Reserve (SBR), Odisha, India. Samples were collected using clean sampling bottles, forceps, polythene bags, brushes, petridishes, scalpels etc. Bottles were made airtight after sampling and brought to the laboratory for their preservation. The voucher specimen is deposited at MSCBD University, Odisha, and identified using a monograph [15]. The collected samples were thoroughly washed with tap water, and spread out for shade drying at room temperature. The dried samples were grounded to a fine powder and stored for further experiment.

Solvent extraction

One-gram powdered samples were taken for extraction of bioactive metabolites with organic solvents.e. petroleum ether followed by acetone, hexane, ethanol, methanol, and aqueous with sterile distilled water. After the overnight treatment, the solvents containing bioactive compounds were collected in a pre-weighed tube. The solvents were allowed to air dry. Taking the final weight of those tubes the weight of the extract was calculated. Extracts were then made into a stock concentration dissolving in DMSO (Dimethylsulphoxide).

Screening of antimicrobial activity

Crude solvent extract of *Spirogyra* sp. was screened for antimicrobial activity following agar cup method[16]. Test organisms' include–S1-*Pseudomonas aeruginosa* MTCC 1034, S2 – *Escherichia coli* MTCC 1098, S4 – *Staphylococcus aureus* MTCC 1144.The organisms were incubated overnight in nutrient agar broth to bring them into active condition followed by spreading on the nutrient agar plate. Approximate inoculum size was taken 10^5CFU/ml of active bacterial cells to which crude extract of each solvent was loaded (290 mg/100 µl) per well and standard antibiotic Chloramphenicol and DMSO were considered as control. The plates were incubated at 37°C and the zones of inhibition were measured after 24 hours. More details on antibiogram information and protocols on the maintenance of strain and antibacterial activity can be seen in [16],[17].

Screening for minimum inhibitory concentration (MIC)

A broth microdilution technique was adopted using 96 well micro-titer plates and tetrazolium salt, 2, 3, 5-Triphenyl tetrazolium chloride (TTC) (Eloff, 1998) to determine the MIC with modification [16].

Screening for minimum bactericidal concentration (MBC)

MBC of the effective concentration of the extract was carried out by the colony count method. A sample of 10 µL of the broth from each well of the 96-well microtiter plate exhibiting MIC and from the control wells was taken aseptically mixed with 990µl sterile distilled water and vortexed well. 100 µl of each tube were plated on the nutrient agar plate, incubated at 37°C for 24 hours, and observed for the growth of the bacterial colony. The colonies were counted in each plate to determine the effect of bacteriocidal activity at different concentrations of the [18].

Phycochemical screening and estimation

The presence of phycochemicals such as carbohydrates (Molisch's, Benedict's, and Fehling'sreagent), proteins (Biuret, Xanthoprotein, Ninhydrin, and Millon's reagent), phytosterols (Salkowski and

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LibermannBuchard) was determined using method described by Trease and Evans (1989) [19]. More details can be seen in Panda and Dutta (2011)[20]. Detection of fixed oils and fats was carried out by using a small quantity of the extract pressed between filter papers. Oil stains were obtained which indicated the presence of fixed oils. To detect the presence of fat, a few drops of 0.5 N alcoholic potassium hydroxide were added to the diluted algal extract with a few drops of phenolphthalein. The total carbohydrate content of the sample was estimated by the Anthrone Reagent method using glucose as a standard taken within the range 10-50 µg/ml [21]. The extraction and estimation of the cellular protein of the algae were determined following the Folin-Phenol method [22]. Extraction of lipids is done by gravimetric method [23].

RESULTS & DISCUSSION

In the present study collected water silk was enumerated and identified as *Spirogyra bichromatophora*morphometrically and their bioactive compounds were tested for antimicrobial potential (Plate-1).

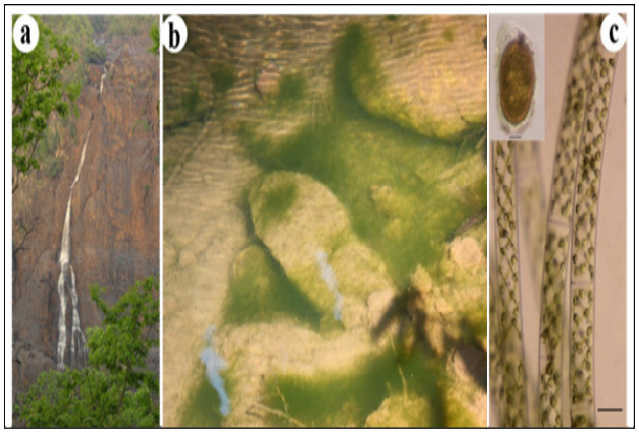

Plate 1(Figsa-c).a.Barehipaniwaterfall, **b.** Occurrence of *Spirogyra* mat, **c.** Microscopic photograph of *Spirogyra bichromatophora*, zygospore (insert); Scale bar - 10µm.

Taxonomic Enumeration

Spirogyra bichromatophora(Randhawa) Transeau, 1944, P. 243

Homotypic synonym: *Spirogyra gallica* var. *bichromatophora* Randhawa 1938, 8:353.
Randhawa (1959), P. 328, Fig. 315

Vegetative cells 60-75×96-160µ, with plane end walls; chloroplasts2, making 4 to 6 turns; conjugation scalariform, large tubes formed by both gametangia; fertile cells cylindric or enlarged; zygospores ellipsoid,54-60×80-90µm; median spore wall smooth, brown.
Habitat- slow running stream, Occurrence – slimy greenish mat in slow running stream, Voucher No. – NOU165.

Antimicrobial activity of crude extracts

Different genera of charophyta were studied for antimicrobial potential where extract of *Spirogyra* sp. showed significant result against pathogenic microbes [24],[25]. Studies on methanolic extracts of *Spirogyra setiformis* and *Navicula* spp. showed potent free radical scavenging and antimicrobial activities where major components were 11,14,17-methyl ester eicosatrienoic and *trans*-geranyl geranol [26]. Algae have been studied extensively for bacteriostatic and bactericidal activity [27],[28]. Bioactive compounds especially fatty acids of diatoms are also studied for antimicrobial potential [29],[30]. It was suggested from earlier studies that the synergistic action of the compound in the crude extract is much more effective than the partially purified compound [31]. The material was extracted with different solvents as well as in aqueous in a decreased gradient of polarity. With agar cup method aqueous extract showed a remarkably higher zone of inhibition (20 mm) against *S. aureus* compared toacetone extract (15 mm) followed by ethanol extract against *P. aeruginosa* (14mm). All other extracts show a lower zone of inhibition ~10 mm (Fig. 1).

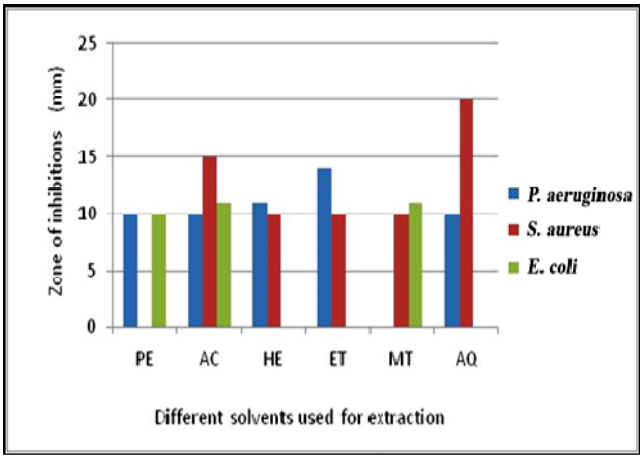

Figure 1 Comparison of antimicrobial activity of different crude extracts against three test pathogens (zone of inhibition in mm including cork borer 6 mm). PE – Petroleum ether, AC – Acetone, HE – Hexane, ET – Ethanol, MT – Methanol, AQ – Aqueous.

Table 1: Determination of MIC (µg/ml) and MBC (mg/ml) of different solvent extracts

Organisms	Acetone	Hexane	Ethanol	Methanol				
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	166	2.65	62.5	2.0	-	-	-	-
P. aeruginosa	166	2.65	125	>2	-	-	-	-
S. aureus	333	2.65	250	>2.0	175	> 2.8	825	>1.65

Table 2: Screening of phycochemicals in *Spirogyra bichromatophora*

Algal taxa	Carbohydrate (mg/g)	Protein (mg/g)	Lipid (mg/g)	Phytosterol	Fixed oil	Fat
S. bichromatophora	+ (2.4)	+ (3.2)	+ (3.3)	+	+	+

(+) presence

The zone of inhibition of extract(s) is well compared with the positive control chloramphenicol. When comparing the results of individual extracts among the bacterial strains acetone extract was active against all three test pathogens (with inhibition zone ranges from 10-15mm), whereas the hexane and ethanol extract showed antibacterial activity against Gram-ve (*E. coil* and *P. aeruginosa* with inhibition zone 10-14mm). Methanol extract and petroleum ether do not show any remarkable zone of inhibition (~10 mm). Aqueous extract showed a maximum zone (20mm) against *S. aureus.* Reports says that methanolic and ethanolic extracts of *Spirogyra* sp. with other freshwater algae through fractional distillation inhibit different bacterial strain, however, more precise study to be standardized as the excessive use of solvent may not be cost-effective [32],[33],[34],[35]. Here the test organism also showed significant antibacterial potential and needed to be followed a supercritical fluid extraction method[36] in interest of mass production.

MIC was further determined by broth dilution method on select extracts (acetone, ethanol, methanol, and hexane) and data presented in Table-1. The MIC of acetone was 166 µg/ml against *E. coli* and *P. aeruginosa* and while 333µg/ml against *S. aureus*. Acetone was found to be bactericidal against all three test pathogens at 2.65 mg/ml. The MIC of hexane extract was observed lowest against *E. coli* (62.5µg/ml) followed by *P. aeruginosa* (125µg/ml) and *S. aureus* (250µg/ml). With a test concentration of 2 mg/ml hexane extract was bactericidal against *E. coli* and *S. aureus* while not able to kill *P. aeruginosa*.Ethanol and methanol extract were also able to inhibit *S. aureus* with MIC 175µg/ml and 825µg/ml respectively (Table-1). Though the reports on anticancer, antiviral, antifungal and antibacterial activity in different algae and cyanobacteria in drugs [37], attentions on commercial production and drug manufacture remained discouraged. The abundant growth of *Spirogyra* in its natural habitat and biosorption capability of heavy metal [38], and pharmaceutical ingredients[39] apparently will create an opportunity for an integrated approach of phycoremediation and extraction of bioactive compounds.

Phycochemical analysis

Total quantity (mg/gm dried biomass) of carbohydrate content was estimated 2.4mg/gm of dried biomass. The protein and lipid content of dried biomass of *Spirogyra* was estimated 3.2mg/gm and 3.3mg/gm respectively. The phycochemical analysis of the algal extracts of *Spirogyra bichromatophora*showed the presence of carbohydrates, proteins, lipids, phytosterols, fats, and oils (Table-2)**.**

Spirogyra and members of filamentous charophyta grow abundantly throughout the world in form of the filamentous mat concerning environmental factors [40],[41], thus easy to harvest. Spirogyra biomass is an efficient energy source for biofuel and lipids, carbohydrates, protein, and inorganic elemental composition were determined in *Spirogyra weberi* [42],[43],[44]. *Spirogyra singularis,* found rich in fat, carbohydrate, and protein contents studied for biochemical and physiological characterization [45]. Recently, isolated mono methyl derivative of lutein from the green algae *Spirogyra rhizopus* was isolated using bioassay-guided purification for antibacterial activity [46]. The presence of volatile compounds, such as long chain hydrocarbons, fatty acids, esters and alcohols in the Methanolic extracts has also been reported from some other Spirogyra spp. [47]. The present hypothesis on phycochemicals in the crude extracts of *Spirogyra* have an antimicrobial

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activity is tested and further investigation is needed on the isolation of bioactive compounds. It could be stated that 'water silk' collected from nature has characteristics of a potential bioresource for antimicrobial phycochemicals.

CONCLUSION

The water silk bloom plenty during summer and winter in most of the water bodies of tropics and subtropics, even though bioprospecting of the biomass has been ever neglected. So, it can be a sustainable, and economical source for drug industries in search of antimicrobial phycochemicals. The study provides some scientific justification that *Spirogyra bichromatophora* could be a potential antimicrobial drug candidate with broad-spectrum properties. However, it is important to point out that the study focused on crude extracts and needs to be further purified through bioassay-guided purification to isolate and identify the compounds responsible for antibacterial activity. In addition, the wide range of distribution of 'water silk' presents an opportunity to obtain valuable compounds.

Consent And Ethical Approval

As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors.

Conflict Of Interest

We wish to declare that there are no conflicts of interest associated with these studies.

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