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# Evaluation of antimicrobial property of Spirogyra species

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**Abstract**
*Spirogyra species* is an important genus of filamentous green algae (Chlorophyta) in showing its antimicrobial activity against number of bacteria and fungi. In present study the effect of Petroleum ether, methanol and chloroform extract of *Spirogyra species* was screened against three bacteria and three plant pathogenic fungi for their level of antimicrobial potential. Thin layer chromatography was used to assay for the compounds and pure fractions obtained were tested for their antimicrobial property and were found to be effective on the entire test organism except for *Clavibacter sp.* and *Curvularia sp*.

**Keywords:** *Spirogyra species,* algae, antimicrobial property

## INTRODUCTION

Man relied on natural products in general and plants in particular to promote and maintain good health and fight sickness, pain and disease since times immemorial.India is an important country in the world where ancient systems of medicine such as Ayurveda, Siddha and Unani have been in practice for many years. The use of extracts from plants and animals for medicinal purposes is a practice as old as the history of mankind. Freshwater algae are a rich source of structurally novel and biologically active metabolites. Primary or secondary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. To date, many chemically unique compounds with various biological activities have been isolated and some of them are under investigation and are being used to develop new pharmaceuticals. The cell extracts and active constituents of various algae have been shown to have antibacterial activity in vitro against Gram-positive and Gram-negative bacteria. A wide range of results of in vitro antibacterial and antifungal activities of extracts of freshwater as well as marine algae have been reported.

*Spirogyra* is a genus of filamentous green algae of the order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus.It is commonly found in freshwater areas and there are more than 400 species of *Spirogyra* in the world. *Spirogyra* is very common in relatively clean eutropic water, developing slimy filamentous green masses.

As an efficient strategy of investigation, organic solvents have been used to extract the possible active principles from freshwater green algae *Spirogyra sp*. Since the western ghats area of Karnataka has an extensive coast where algae from virtually all groups are present, the goal of the present work was to test the extracts of the algae for antimicrobial activity.

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In the present study we describe the antimicrobial characteristics of Methanol, Petroleum ether and chloroform extracts of *Spirogyra sp*., a green algae obtained from the Bhadra reservoir, Lakkavalli, Shimoga district, Karnataka.

## MATERIALS AND METHOD

### Collection of sample

Samples were collected in the floating benthic conditions from the surface at a depth of 5-6 cm from the slow running freshwater at the banks of Bhadra River and adjacent lakes and were taxonomically identified. The collected algal thalli were cleaned of epiphytes and dried parts were removed. Then the samples were rinsed with sterile water to remove any associated debris and were air-dried. Some of it was preserved in 4% formalin solution for taxonomic studies.

### Preparation of algal extracts

The sample was powdered and weighed.100g of fresh and air-dried algal sample were used for cold extraction and hot extraction.

### Cold extraction method

Approximately 50g of the powdered sample was soaked in methanol taken in a conical flask and placed on a shaker for about 5 hours and then allowed to stand overnight at room temperature. The extract of the material thus obtained was filtered to remove undissolved materials and evaporated in rotary flash evaporator under reduced pressure. A thick, dark coloured paste was eluted which was transferred into a clean petridish and stored for further analysis to determine their antimicrobial property.

### Hot extraction method

In this method the extraction was carried out using three different solvents viz., Petroleum ether, Chloroform and Methanol. 100g of powdered algal material were extracted in Soxhlet extractor at $60^0$C containing 250ml of solvent separately using all the three solvents. The material was refluxed for about 36 to 48 hours until

saturation and the resulting extracts were evaporated in a rotary flash evaporator. The obtained extracts were collected in a clean petridish and weighed.

**Thin layer chromatography**

Each of the algal extracts obtained using different solvents were subjected to thin layer chromatography to analyse the crude residue for different compounds. TLC was carried out for all the four extracts obtained by cold and hot extraction method. Different solvents were used as a mobile phase starting from petroeum ether, Carbom tetrachloride, Ethyl acetate, Chloroform and Methanol in various ratios to obtain a good separation in order to elute a compound in its pure form by subjecting the crude residue to column chromatography.

**Column chromatography**

The residue of crude extracts was weighed and accordingly a suitable sized column silica gel of grade (60-120 mesh) was chosen as the stationary phase. The column was filled with activated silica gel. The crude residue was loaded on to the column and eluted using different solvents. The obtained fractions were analysed after being checked on TLC cards for purity.

**Antibacterial activity**

Antibacterial activity of the crude extract and the isolated fractions was evaluated using agar well diffusion technique in petridishes.

The antibacterial activity was screened by the agar well diffusion method against three bacterial strains *Pseudomonas solanacearum, Escherichia coli* and *Clavibacter michiganense*. Indicator organisms, *Pseudomonas solanacearum, Escherichia coli* and *Clavibacter michiganense* were evenly spread on the respective agar plates with sterile spreader and the well were made using sterile cork borer and filled with extract and incubated for 24 h at $37^0$C. Diameters of the zones of inhibition were meared in millimeters.

**Antifungal activity**

Antifungal activity was evaluated using agar well diffusion method against *Fusarium oxysporum*, *Curvularia species* and *Aspergillus niger* that were taken as test organisms.

**Chemical analysis of the compounds**

After acertaing the purity of the compound by TLC technique, the obtained pure fractions were analysed further physiochemically, both quantitatively and qualitatively. Their structures were chemically elucidated through LCMS and $^1$H NMR, Mass spectroscopy and by determining their melting points.

**RESULT**

**Column chromatography**

Two isolates were recovered using Hot methanol extract. Three isolates were recovered using Hot chloroform extract. One fraction was recovered using Hot Petroleum ether extract.

**Antibacterial activity**

The antibacterial activity was screened by the agar well diffusion method against three bacterial strains *Pseudomonas solanacearum, Escherichia coli* and *Clavibacter michiganense.* Chloroform crude extract showed maximum inhibition zone against *Pseudomonas solanacearum* and *Escherichia coli* when compared to standard. There was no effect on *Clavibacter michigegensis* (table 1).

Table 1. Growth inhibition zone (mm) of extracts against different bacteria in agar diffusion method

| Extracts | Growth inhibition zone (in mm) | | |
|---|---|---|---|
| | ***Clavibacter michiganense*** | ***Pseudomonas solanacearum*** | ***Escherichia coli*** |
| Methanol extract I | 0 | 35 | 20 |
| Methanol extract II | 0 | 20 | 24 |
| Chloroform extract I | 0 | 33 | 25 |
| Chloroform extract II | 0 | 29 | 0 |
| Chloroform extract III | 0 | 30 | 45 |
| Petroleum ether extract | 0 | 14 | 12 |
| Streptomycin (standard) | 5 | 35 | 40 |
| Methanol extract (Crude) | 20 | 14 | 12 |
| Chloroform extract (Crude) | 0 | 36 | 46 |

**Antifungal activity**

Antifungal activity was evaluated using agar well diffusion method against *Fusarium oxysporum, Curvularia species and Aspergillus niger.* Methanol and Chloroform crude extract showed maximum inhibition zone against *Aspergillus niger* and *Fusarium oxysporum*.There was no effect on *Curvularia species* (Table 2)

Table 2. Growth inhibition zone (mm) of extracts against different fungi in agar diffusion method

| Extracts | Growth inhibition zone (in mm) | | |
|---|---|---|---|
| | *Aspergillus niger* | *Curvularia species* | *Fusarium oxysprorum* |
| Methanol extract I | 26 | 0 | 0 |
| Methanol extract II | 19 | 0 | 39 |
| Chloroform extract I | 26 | 0 | 16 |
| Chloroform extract II | 0 | 0 | 07 |
| Chloroform extract III | 18 | 0 | 28 |
| Petroleum ether extract | 18 | 0 | 37 |
| Bavistin (standard) | 12 | 0 | 09 |
| Methanol extract (Crude) | 36 | 0 | 43 |
| Chloroform extract (Crude) | 37 | 0 | 32 |

**DISCUSSION**

The overall data presented gives us the indication that the antimicrobial activity is present in the isolated constituents of *Spirogyra spp.* All the compounds have shown high activity against *Pseudomonas solanacearum*. Moderate activity was seen against *E.coli* and no effect on *C.michiganense*. Among fungi *Fusarium oxysporum* and *Aspergillus niger* were more susceptive to the isolated compounds while *Curvularia species* was resistant. Recent investigations on the antimicrobial efficiency was conducted by Goud *et al*. (2007) and reported that maximum antibacterial activity was observed in methanol extracts. Prashant *et al*. (2006) investigated the methanolic extract of a blue green alga and two green algae which have shown good antimicrobial activity. Similar activities were carried out by Tuney *et al*.(2006), Yasmeen *et al.* (2003), Vitor *et al.* (2002) and Helio *et al*.(2002). Marian Stangenberg (1968) has shown that in some stages of algal development *Spirogyra sp.* in their cells contain bacteriostatic substances.

## CONCLUSION

Antibiotic activity may be associated with the presence of unsaturated fatty acids, organic acids and Phenolic compounds present in the extracts of Spirogyra sp. It also depends on both algal species and efficiency of extracting their active principles. Environmental factors also play a key role which can be associated with intra specific variability in the production of secondary metabolites and occasionally related to seasonal variations. Secondly, there may also be differences in the capability of the extraction protocols to recover the active metabolites and differences in the assay methods that would result in different susceptibilities of the target strains.

The chemical nature of active principles in lipid-soluble extracts of algae is not so far totally explored. Our preliminary results suggest that antimicrobial activity observed against Gram-positive as well as Gram-negative bacteria and some pathogenic forms of fungi could be due to more than one active principle. This hypothesis is to be further investigated and aimed for isolation and purification of phyco-constituents responsible for antimicrobial activity.

Finally to conclude, one can justify that the *Spirogyra sp.*,of Bhadra reservoir, Western Ghats, Karnataka are potential sources of bioactive compounds and should be further investigated for natural antibiotics.

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