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Phycochemical Analysis of Two Members of Order Zygnematales

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Abstract: ***Two members of order Zygnematales, Mougeotia and Spirogyra are vigorously growing in Durg region to an extent that they allow only the compatible microalgae to grow with them. The present study was conducted to investigate the preliminary Phycochemicals present in these algae and antimicrobial tests were conducted to ascertain their compatibility with the other plants in the vicinity and these characteristics make their appearance vital for the water bodies. Presence of carbohydrates, proteins and fats was reported in them along with secondary metabolites as alkaloids and sterols show the active metabolism taking place in them. Antimicrobial study establishes them as potent antibacterial agents as compared to antifungal.***

Keywords: Mougeotia, Spirogyra, phycochemicals, antimicrobial.

1. Introduction

Organisms that are considered as among the most productive organisms due to their high photosynthetic efficiency. They contribute much to the replenishment of oxygen on earth. Their efficiency to fix CO_2 while capturing solar energy is 10 to 50 times greater than that of terrestrial plants [3]. Besides having higher photosynthetic efficiency these algae also have higher growth rates and higher biomass production. Tropical conditions especially found in India are favourable for the luxuriant growth of these organisms in the natural environment [1], [2], [6], [7], [8], [9], [10], [11], [12], [13], [14], [15].

Mougeotia and *Spirogyra*, members of order Zygnemales, are fresh water green filamentous algae, which show high incidence throughout the year in the fresh water bodies of Durg region. The abundance of these members can so high as to obstruct the growth of other major algae in this region. Only those algal species which can co-habitat with them are found growing in the water bodies.

Towards the southwest part of Chhattisgarh plain lies the Durg district, to the west of which lies the river Shivnath. Due to its geographical position it experiences a dry tropical weather which is moderate but a little warmer during summer season. The summer temperatures are quite extreme reaching as much as 48^{o}C, but with equally wet monsoon. The organisms under study were seen to flourish during the extreme summer temperatures and gave shelters to other microalgae as well.

The present study aimed at identifying the characteristics responsible for the vigorous growth and dominance of these members of Zygnematales.

2. Materials and Methods

1. Collection and culture of algae

Mougeotia and Spirogyra were collected from three ponds of Durg region in Chhattisgarh state. The identification of algal members was done by standard books Prescott (1951) [5], Fristch (1937) [4].

Chu 10 medium with pH 7.6 was used for developing monoculture the strains isolated.

2. Preparation of algal extract

The fresh algae obtained by culturing in synthetic media were isolated, washed thoroughly and shade dried at room temperature and then milled into coarse powder in mortar and pestle.

3. Preliminary phytochemical studies

i. Detection of Carbohydrates

Small quantity of aqueous extract was tested qualitatively (Fehling's test) and quantitatively (Anthrone test) for carbohydrate

Fehling's test: A minimum quantity of the extract was treated with 1 ml of Fehling's solution and heated. Formation of a reddish orange precipitate will indicate the presence of carbohydrate.

Anthrone test: quantitative estimation of carbohydrate was done using anthrone reagent.

ii. Detection of proteins

Small quantity of the extract was dissolved in a few ml of water and were subjected to qualitative estimation by Millon's and Biuret test and quantitative estimation by Lowry's test.

a. Millon's test: Millon's reagent was added to the aqueous extract. Appearance of red colour showed the presence of proteins.
b. Biuret test: To the aqueous extract equal volumes of 5 % sodium hydroxide and 1% copper sulphate were added. Violet colour confirmed the presence of proteins.

c. Lowry's test: quantitative test for estimation of protein was done by using folin-cicalteau reagent.

Preparation of extract

The algal powder was dissolved in methanol in an cotton plugged Erlenmeyer flask and kept for 15 days. The flask was periodically subjected to shaking on an electronic shaker. After 15 days the extract was filtered using Whatmann filter paper No. 40.

iii. Detection of alkaloids

A small quantity of the extract was treated with few drops of dilute Hydrochloric acid and filtered. The filtrate was used for the following tests.

a. Hager's reagent: A small quantity of the algal extract was added to saturated solution of picric acid. Yellow precipitate indicated the presence of alkaloids.
b. Mayer's reagent: A small quantity of extract was treated with Mayer's reagent, appearance of cream colour precipitate showed the presence of alkaloids.

iv. Detection of Phytosterols

A small amount of the extract was dissolved in 5 ml. of chloroform separately. Then these solutions were subjected to Salkowski and Libermann Buchard tests for the detection of phytosterols.

a. Salkowski test: To 1 ml of chloroform solution few drops of concentrated sulphuric acid was added. The colour change was observed which showed the presence of phytosterols.
b. Liebermann Burchard's test: The chloroform solution was treated with few drops of concentrated sulphuric acid followed by 1 ml of acetic anhydride solution. Purple colour change showed the presence of phtosterols.

v. Detection of fixed oils and fats

Small quantity of the extract was pressed between filter papers. Oil stains were obtained which indicated the presence of fixed oils.

A few drops of 0.5 N alcoholic potassium hydroxide were added to the algal extract with few drops of phenolphthalein. The mixture was heated in a water bath for 1-2 hours. This resulted in formation of soap.

vi. Detection of phenolic compounds and tannins

To a small quantity of the extract 5% ferric chloride solution was added. To this solution 1% solution of gelatine, containing 10% sodium chloride, 10% lead acetate and aqueous bromine solution, was added. Formation of white precipitate shows the presence of phenolic compounds and tannins.

4. Antimicrobial activity**:** The methanolic extracts of algae were tested for their antibacterial activity by agar well diffusion method. For antibacterial tests the media used was Nutrient agar and for fungal cultures to be tested Potato Dextrose Agar media was used. Amoxicillin was used as standard drug for the antibacterial tests and col-trimazole was used as standard for the antifungal tests.

3. Results

The phycochemical analysis of the algal extracts of *Mougeotia* and *Spirogyra* showed the presence of carbohydtrates, proteins, lipids and fats, alkaloids, sterols and terpenoids, and absence of phenolic compounds in the algae under study.

Table-1 shows the amount of primary metabolites in mg/ml

S. No.	Algal species	Carbohydrates	Proteins
1.	Mougeotia	32	25.4
2.	Spirogyra	20	11.2

Table-2 shows the presence of secondary metabolites in the algae under study

S.No.	Secondary metabolites	*Mougeotia*	*Spirogyra*
1.	Alkaloids	+	+
2.	Sterols	+	+
3.	Fats	+	+
4.	Phenols	-	-
5.	Tannins	-	-

Figure 1-Primary metabolites in *Mougeotia* and *Spirogyra*

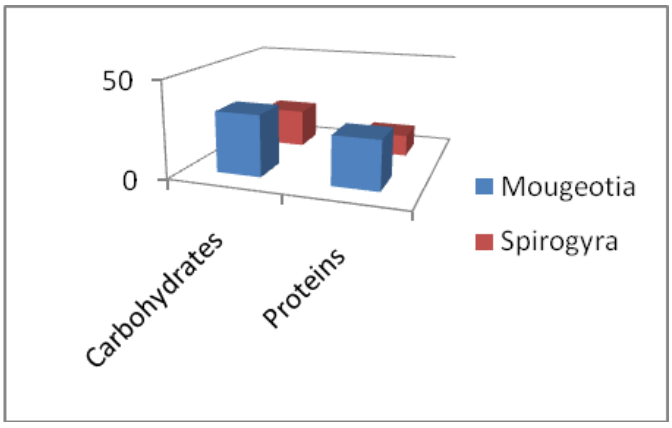
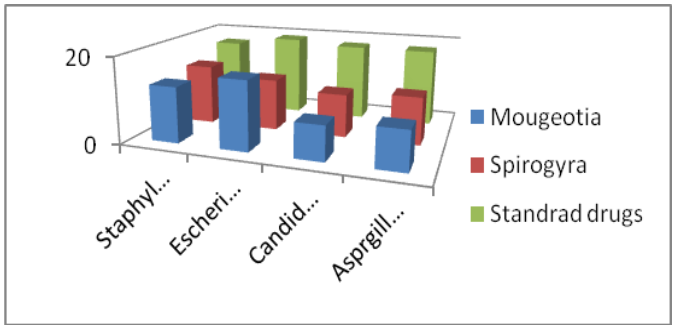


Table-3 Antimicrobial test against bacteria and fungi showed the following results

S. No.	Algal species	Test Microorganisms	MIC (mm)	SD mm
1.	*Mougeotia*	*Staphylococcus aureus*	13	17
		Escherichia coli	16	19
		Candida albicans	8	18
		Asprgillus niger	9	18
2.	*Spirogyra*	*Staphylococcus aureus*	14	17
		Escherichia coli	12	19
		Candida albicans	10	18
		Asprgillus niger	11	18

SD (Standard Drug) : Amoxicillin for Bacteria
: Col-trimazole for Fungi

Figure 2- Comparison of Antimicrobial activity of Mougeotia and Spirogyra to standard drugs.



3. Discussion

Mougeotia and Spirogyra are fresh water green filamentous algae which are actively photosynthesizing organisms, which can be seen as high concentration of carbohydrates and proteins in them. They also show presence of fats. Presence of secondary metabolites as alkaloids and sterols suggest high rate of metabolism in them due to the presence of alternative pathways.

The presence of high concentration of primary metabolites makes them a vital source of food and energy in aquatic ecosystems. Presence of secondary metabolites is probably the reason for their vigorous growth in the water bodies. And that indicates the good health of the water bodies of this region.

Antimicrobial tests conducted using methanolic extracts of *Mougeotia* and *Spirogyra* show them to be equally effective against bacteria and fungi. By comparing the minimum zone of inhibition of the organisms it is revealed that both the organisms show better antibacterial properties than antifungal. Among bacteria *Mougeotia* are more effective on gram (-)ve bacteria than Gram (+) ve whereas *Spirogyra* are more effective on Gram (+) ve bacteria than Gram (-)ve one.

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4 research papers have been published

1)Aeromycofloral diversity of St.Thomas College, Bhilai published in the Proceedings of "Climate change and its effect on Biodiversity".

2)Phycochemical and antimicrobial study of vigorous fresh water alga *Mougeotia* published in International Journal of Current Microbiology and Applied Sciences 3(4): x-xx.

3) Algal biodiversity in fresh water body of Durg. Accepted by Indian Journal of Science,for April 2014 edition .

4)Antimicrobial properties of Spirogyra. Accepted by International Journal of Basic and Applied Science Research (IJBASR) for April 2014 edition

5 Abstracts have been published in various National and International Seminars and Conferences.

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3 International papers

1.*"Physiological behaviour of pesticides in nitrogen fixing Cyanobacterium Nostoc calcicola" by botanical Society of America for Snowbird UTAH, USA from july 25-29'2009 No*

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2. Exogenous carbon supply reverses the inhibitory effects of fungicide bengard on growth and heterocyst forming ability in Cyanobacterium *Nostoc calcicola"* invited to participate in International Congress of Chemistry and Environment ICCE-2009 Conference at Guilin, China from 7th Nov. 2009 to 9th Nov. 2009. Organised by International Congress Of Chemistry And Environment(ICCE - 2009)Research Journal Of Chemistry And Environment, India And Guilin University Of Technology, China
3. Annual Biodiversity and recurrence of Cyanobacterial flora of Bilaspur in Chhattisgarh state of India. Presentation Type: Oral Paper:Papers for sections session: 11 Location: 552A/Convention Center Date: Monday, August 2nd, 2010 Time: 1:30 PM Number: 11001 Abstract ID:*25, Rhode Island USA*

51 Research Papers published so far in different National And International Journals And Proceedings:

127 Abstracts have been published in various national and International seminars and Conferences.

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