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Preliminary phycochemical analysis and *in vitro* antibacterial screening of green micro algae, *Desmococcus Olivaceous, Chlorococcum humicola* and *Chlorella vulgaris*

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Abstract

Antibacterial activity of the Acetone, methanolic, ethanolic and DMSO extract of dried green microalgae *Desmococcus olivaceous, chlorococcum humicola and chlorella vulgaris* was assayed against five gram-ve (*Klebsiella pneumoniae*, *Pseudomonas*, *Vibriocholerae*, *Streptococccus pyrogenes* and *Escherichia coli*) and one gram +ve (*Staphylococcus aureus*) bacteria under culture conditions, using the agar disc diffusion technique. Incubation of the Mullar-Hinton agar plates for 24hrs. at 30°C, supplemented with the six test bacteria along with 50ml of acetone, methanolic, ethanolic and DMSO (Dimethyl sulphoxide) extract revealed inhibitory effect. The highest inhibition zone (25 mm& 21 mm) was observed in acetone extract of *Chlorococcum sp* against gram +ve bacteria (*Staphylococcus aureus*) & gram -ve bacteria (*Escherichia coli*). Preliminary phycochemical analysis was also performed on the dried algal sample by employing chemical methods and thin layer chromatography technique to assay the bioactive compounds which revealed the presence of seven principle bioactive compounds *viz.*, phenolic, tannin, flavanoids, saponins, terpenes, carbohydrates & cardiac glycosides.

Keywords: Algal extract, phycochemicals, Antibacterial activity, *Desmococcus olivaceous, Chlorococcum humicola* and *Chlorella vulgaris*

Introduction

Algal organisms are rich sources of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interests in the pharmaceutical industry (Ely *et.al*, 2004; Febles *et.al*, 1995 and Tuney *et.al*., 2006). Algae produce a number of secondary metabolites as a chemical defense against predation, herbivory and competition for space (DeLara-Isassi *et.al.*, 2000 ; De Nys *et. al.*, 1998). In the field of research involving

bioactive substances of plant origin, a greater interest has now arisen in algae. The first investigation on antibiotic activity of algae was carried out by Pratt *et, al.*, (1944). Since algae have been used in traditional medicine for a long time and also some algae have bacteriostatic , bactericidal, antifungal , antiviral and antitumor activity, they have been extensively studied by several researchers (Justo *et, al.*, 2001).

Many investigators have reported antibacterial activities of microalgae as due to fatty acids (Cooper *et. al*., 1983; Findlay and Patil, 1984). Antibacterial activity of volatile extracts of *Spirulina platensis* have been studied by Ozdemir *et.al*., (2004). The present study is aimed at investigations of the phycochemicals and antibacterial properties of the acetone, methanolic, ethanolic and DMSO extracts of fresh water green micro algae, *Desmococcus olivaceous , Chlorococcum humicola* and *Chlorella vulgaris* against six bacterial isolates in order to validate it as an antimicrobial remedy. This study will also hopefully expose new frontiers on the current applications of the algal extract.

Materials and Methods :

Culturing and Growth of Algal organisms

Fresh water green microalgae *Desmococcus olivaceous, Chlorococcum humicola* and *Chlorella vulgaris* were obtained from the Vivekananda Institute of Algal technology (VIAT), Chennai. Algal Biomass was obtained by growing the algal cultures in 20 L of water with NPK fertilizer and a facility to mix the culture with an aeration pump. The algae were grown for 10 days and harvested.

Preparation of Algal extract

Dried algal material (0.5g) was ground in pestle and mortar with acetone, methanol, ethanol and DMSO solvents. The extract was filtered through Whatman no. 1 filter paper to remove all unextractable matter which includes cellular material (Gonzalez del val *et.al*, 2001.) The filtrate was concentrated under reduced pressure by using a rotatory evaporator. The extracts were transferred to a hot air oven, where it was dried at 40°C. Portion of the residue was used for phycochemical analysis while the rest was used for the bacterial susceptibility test.

Phycochemical analysis

Phycochemical analysis of the extract was carried out using chemical methods and confirmation was done by the TLC according to the methodology proposed by Indian Pharmacopeia (1985) and Harborne (1998)

Antimicrobial activity

Acetone, methanol, ethanol and DMSO extracts were tested against a panel of micro organisms including gram-ve *Klebsiella pneumonia*, *Pseudomonas, Vibrio cholerae*, *Streptococcus pyrogenes, Escherichia coli* and gram +ve *Staphylococcus aureus* obtained from Mehta Hospital, Chennai, India. Stock cultures were maintained on nutrient agar medium at 40°C, then sub-cultured in nutrient broth at 37°C prior to each antimicrobial test.

Disc diffusion assay

The sensitivity test of the acetone, methanolic, ethanolic and DMSO extracts were determined using agar disc diffusion method (Bauer *et al.*, 1966). Media were prepared using Muller Hinton Agar poured in Petri dishes and inoculated with test organisms from the broth using cotton swabs. Acetone, methanol, ethanol and DMSO extracts were dissolved in 5ml of DMSO had been impregnated with 50µl of algal extracts and introduced on to the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C. Negative controls were prepared by using DMSO. Penicillin was used as positive reference standard. After incubation, the clear zone around the discs were measured and expressed in mm as a measure of their antibacterial activity.

Results and Discussions

The results of phycochemical screening of acetone, methanolic, ethanolic and DMSO extracts of *Desmococcus olivaceous, Chlorococcum humicola,and Chlorella vulgaris* revealed the presence of flavanoids, saponins, tannins, carbohydrates, phenolics ,terpenes and cardiac glycosides. Steroids and alkaloids were absent in all the extracts. (Table1)

Table 1. Preliminary Phycochemical Screening of *Desmococcus olivaceous, Chlorococcum humicola and Chlorella vulgaris*

Phycochemical compounds	*Desmococcus olivaceous*	*Chlorococcum humicola*	*Chlorella vulgaris*
Alkaloids	-	-	-
Flavonoids	+	+	+
Tannin	+	+	+
Phenolic compounds	++	++	+
Steroids	-	-	-
Terpenoids	++	++	+
Cardiac glycosides	++	++	+
Saponins	+	+	+
Carbohydrates	++	++	+

The results of antimicrobial activities of acetone, ethanolic, methanolic and DMSO extracts of *Desmococcus olivaceous*, *Chlorococcum humicola* and *Chlorella vulgaris* are presented in Table 2 to 4. It was noted from the tables that the diameter of the inhibition zone depends mainly on the type of algal species, type of solvent used and the tested bacterial organism.

Table 2. Antibacterial activity of acetone, ethanolic, methanolic and DMSO extracts of Desmococcus olivaceous on six bacterial strains of varied nature.

Bacterial Strains	**Zone of Inhibition (mm)**			
	Acetone Extract	**Ethanol Extract**	**Methanol Extract**	**DMSO Extract**
Gram-negative *Klebsiella pneumoniae*	10.0 ± 0.5	12.0 ± 0.50	7.5 ± 0.25	8.5 ± 0.25
Pseudomonas	17.2 ± 0.4	12.2 ± 0.4	8.6 ±0.43	8.2 ± 0.6
Vibrio cholerae	8.5±0.5	8.5 ±0.75	8.4 ±0.62	6.8 ±0.4
Streptococcus pyogenes	7.0 ± 0.25	8.0 ±0.5	8.2 ±0.8	7.0 ±0.5

Escherichia coli	7.5 ± 0..5	9.5 ±0.5	8.2 ±0.6	9.6 ±0.4
Gram-positive *Staphylococcus aureus*	16.0 ±0.75	15.2 ±0.6	9.5 ±0.5	9.5 ±0.25

All the values are mean □ standard deviations of three determinations

Table 3 Antibacterial activity of methanolic, ethanolic and acetone extracts of *Chlorococcum* humicola on six bacterial strains of varied nature.

Bacterial Strains	Zone of inhibition (mm)			
	Acetone Extract	**Ethanol Extract**	**Methanol Extract**	**DMSO Extract**
Gram-negative Klebsiella pneumoniae	10.8± 0.6	11±0.25	9±0.6	8±0.25
Pseudomonas	7.4± 0.4	6± 0.4	10±0.25	6±0.8
Vibrio cholerae	8.2±0.6	6+0.42	9±0.25	6.0±0.6
Streptococcus pyogenes	7.0±0.25	6.2±0.4	9±0.5	7±0.6
Escherichia coli	21.4±0.6	11+.0.25	15±0.28	9±0.6
Gram-positive Staphylococcus aureus	25±0.5	14±0.6	9±0.6	6±0.5

All the values are mean □ standard deviations of three determinations.

It was noted that among all the test organisms gram positive bacterial strain *Staphylococcus aureus* and gram –ve *Escherichia coli* registered maximum susceptibility to acetone extract of *Chlorococcum humicola* (Plates 1 & 2) Acetone and ethanolic extract of *D. olivaceous* showed a maximum antibacterial activity against *K.pneumoniae*, *Pseudomonas* and *S. aureus*.(Plates 3 & 4). The results revealed that all the extracts of *Chlorella vulgaris* had moderate activities against the tested microorganisms.

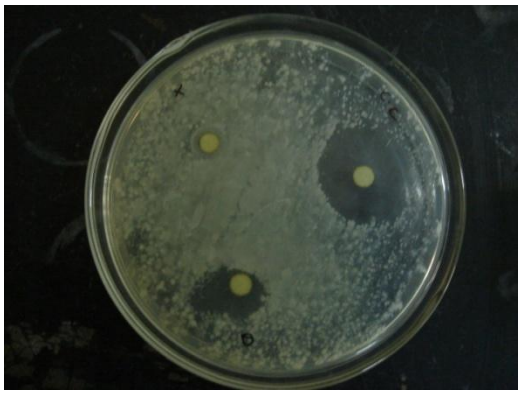

Plate I - Zones of inhibition shown by Acetone extracts of *Desmococcus olivaceous,* and *Chlorococcum humicola* on *S.aureus*

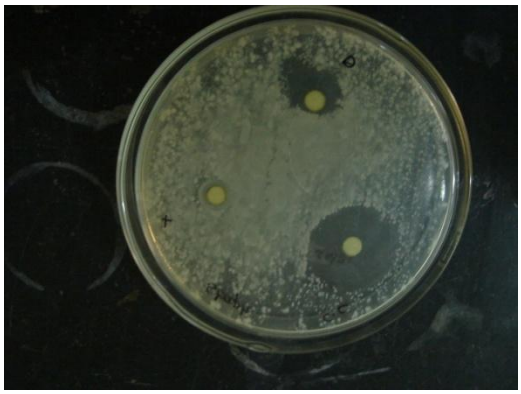

Plate II - Zone of inhibition shown by Acetone extracts of *Desmococcus olivaceous, and Chlorococcum humicola on E-coli*

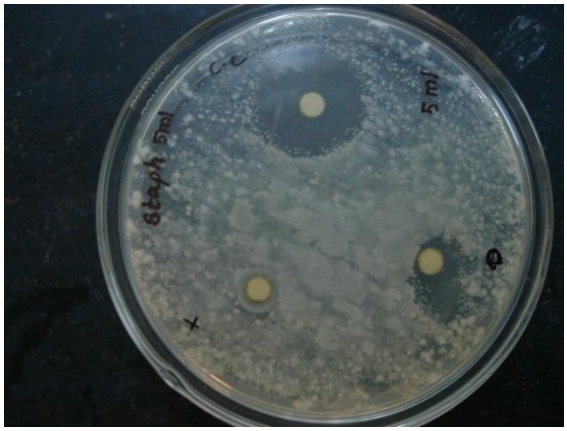

Plate III - Zones of inhibition shown by ethanol extracts of *Desmococcus olivaceous* and *Chlorococcum humicola* on *S. aureus*

Plate IV - Zone of inhibition shown by, ethanol extracts of *Desmococcus olivaceous* and *Chlorococcum humicola on* Klebsiella pneumoniae

Conclusion

In the present study, it was concluded that talgae represents a new source of antimicrobial formulation with stable and biological active compounds. So these bio active compounds will need further studies to identify the chemical structures and to examine their beneficial effects for inhibition of pathogenic bacteria. Antimicrobial metabolites of algae are of

special interest in the development of new harmless environment.

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