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ANTIFUNGAL ACTIVITY OF FRESHWATER GREEN-ALGAE(CHLOROPHYCEAE)

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ABSTRACT

Four green algal members, *Spirogyra biformis, Spirogyra crassa, Rhizoclonium hieroglyphicum* and *Oedogonium crispum* were tested for antifungal activity studies by using pathogenic fungal strains (*Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Trichophyton rubrum*).The present study reveals that the Acetone extract of *S. biformis* expressed with maximum zone of inhibition (12±0.1) against *Trichophyton rubrum* strain with a concentration of 500 mg/ml, the Acetone extract of *S. crassa* showed maximum zone of inhibition (12±0.5) against *Trichophyton rubrum,* with a concentration of 500 mg/ml, The Acetone extract of *R.hieroglyphicum* showed maximum zone of inhibition (9±0.8) against *Aspergillus flavus* with a concentration of 500 mg/ml, The Chloroform extract of *Oedogonium crispum* expressed with maximum zone of inhibition (9±0.6) against *Aspergillus flavus.*Among the four members of chosen green-algae, *S.biformis* has exhibited with maximum zone of inhibition with a solvent of Acetone.The present investigation gives a clear picture that the different solvent extracts of green-algae were having the character of antifungal activity with a concentration of 500 mg/ml.

KEYWORDS: *Spirogyra biformis, Spirogyra crassa, Rhizoclonium hieroglyphicum, Oedogonium crispum,* antifungal activity.

INTRODUCTION

The fresh water green-algae normally locate in various ponds, ditches, lakes, reservoirs and rivers with tangled mass and blanquette of sheets on the water. The microalgae are photosynthetic organisms that play a key role in aquatic ecosystems and they were proved as

potential source for having bioactive compounds to the pharmaceutical industry in drug development. The algal members are rich sources of essential nutrients with a promising source of pharmaceutical values which include alkaloids, phenols, flavonoids, saponins, steroids and the related active metabolites are of great medicinal values which have been extensively used in the drug and pharmaceutical industry. Microalgae are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities(Newman *et al.*,2003).Several studies have been conducted to investigate the products of micro-algal metabolism not only to understand its nature but also to search for substances with possible applications to humans in different fields of interest. Screening of extracts or isolation of metabolites from different micro-algae is a common method for determining the biological activity of these components. Micro-algae have been described as a rich sources of various bioactive compounds of commercial interest (Volk and Furkert, 2006). The bioactive compounds of micro-algal origin can be sourced directly from primary metabolism, such as proteins, fattyacids, vitamins, and pigments, or can be synthesized from secondary metabolism. Such compounds can present antifungal, antiviral, antialgal, anti-enzymatic, or antibiotic actions (Volk,2008).The compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green-algae (Yuan *et. al*., 2005). Although thousands of bioactive compounds have been discovered, the need for novel therapeutic compounds is still urgent in concern of number of new diseases and resistant strains of microorganisms (Chakraborty *et.al.*,2009). These include antimicrobial compounds such as anticancerous/antineoplastic agents, antimitotic agents, muscle-relaxants, immune modulatory agents and enzyme inhibitors (Singh *et.al.*, 2005; Singh *et.al.*, 2011; Prasanna, 2010; Volk and Furkert, 2006; Tan, 2007; Skulberg, 2000; Barios-Llerena, 2007; Patterson *et. al.*,1994; Kamble *et.al.*, 2012; Prashanth Kumar *et.al.*, 2006) .The green-algae of Karimnagar District,Telangana,India have not yet been studied for antifungal activity ,however little work has been done to screen paddy fields of cyanobacteria with regard to their antimicrobial activity.Therefore,the present study was made to find out the antimicrobial activity of selected green-algal extracts with various concentrations(100 mg/ml,300mg/ml and 500 mg/ml) against some pathogenic fungi by using four different solvents.

MATERIAL AND METHODS

In order to find the antifungal efficiency of fresh water green-algae,the samples were collected from different sites of Karimnagar District of Telangana.The collected samples were brought to the laboratory in plastic bags containing water content to prevent evaporation. Samples of micro-algae were isolated by using Chu-10 medium (Chu 1942). The cultures were grown at 24±2°C in a controlled room temperature with white fluorescent lamps at 2-3 k lux and with 16 hours light and 8 hours dark .The algal cultures were harvested after 28 days by centrifugation at 4000 rpm for 10 minutes and washed with distilled water (two times), dried under shade. Dried samples were pulverized by using Mortar and Pistil, the fine grinded powder (400g) extracted with 10 ml of Acetone, Chloroform, Methanol and Water. To get extract compounds with increasing polarity by shaking over night to get complete extraction. The extracts were filtered and the filtrates were evaporated under reduced pressure at room temperature (37^0C), dried crude extracts were weighed and redissolved in 2ml of 0.1% DMSO (Dimethyl Sulphoxide) to get different concentrations (100 mg/ml, 300 mg/ml, 500 mg/ml) and it was stored at 4^0C for further investigation. The pathogens such as, *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Trichophyton rubrum* were obtained from Microbiology Department, Kakatiya university, Warangal, Telangana State, India, and maintained in Algal Biotechnology Laboratory.Department of Botany,Kakatiya University. Four fungal strains, *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Trichophyton rubrum* were tested by using Agar Well Diffusion method.The fungal strains were grown in nutrient broth at 27^0C in Sabouraud's Dextrose Agar (SDA) medium were poured into 15 cm diameter Petri dishes were allowed to cool and solidify and then 100 µl of fungal suspension of the above mentioned test organisms were prepared from 48 hours old cultures and thus transferred into the sterile SDA plates and later made 4 wells in each plate with equal distance with the help of 6 mm borer in the plates. The wells filled with 50 µl of the chosen algal crude extracts with various concentrations(100 mg /ml, 300 mg/ml, 500 mg/ml) were poured with the help of sterile pipette on Sabourad's Dextrose Agar plates. For standard control,Nystatin 50µg/ml was used. Plates were incubated for 48 hours at 27^0C and they were examined by measuring the zones of inhibition and the results were tabulated.

RESULTS AND DISCUSSION

The Acetone extract of *Spirogyra biformis* expressed with maximum zone of inhibition (12±0.1) against the pathogen *Trichophyton rubrum* with the concentration of 500 mg/ml,

followed by, 10±0.4 against *Aspergillus fumigatus*, 10±0.2 against *Aspergillus flavus* and 9±0.2 against *Aspergillus niger*. No zones of inhibition was noticed at 100 mg/ml concentration against *Aspergillus fumigates.*The Chloroform extract of *S. biformis* was expressed with maximum zone of inhibition (10±0.2) against *Aspergillus fumigates* and it was followed by 9±0.2 against *Aspergillus niger*, 8±0.2 against *Aspergillus flavus*, and 8±0.1 against *Trichophyton rubrum* with the concentration of 500 mg/ml. No zone of inhibition were found against the fungal strains of *Aspergillus fumigatus* and *Aspergillus flavus, Aspergillus niger* and *Trichophyton rubrum* with the concentration of 100 mg/ml under study. The Methanol extract of *S.biformis* showed with maximum zone of inhibition (10±0.2) against *Aspergillus niger*, 9±0.2 against *Trichophyton rubrum*, 8±0.0 against *Aspergillus fumigatus* with a concentration of 500mg/ml. No zone of inhibition was noticed against *Aspergillus flavus* in the concentration of 500mg/ml, and the same results were observed in the concentrations of 100mg /ml, 300mg/ml against *Aspergillus fumigatus* and *Aspergillus flavus*(**Table – 1**).In the Water crude extract of *S. biformis*, no zones of inhibition were found against the four fungal strains in the concentrations of 100mg/ml, 300 mg/ml and 500 mg/ml, respectively.

The Acetone extract of *S. crassa* showed maximum zone of inhibition (12±0.5) against *Trichophyton rubrum* with a concentration of 500 mg/ml and followed by 11±0.2 against *Aspergillus fumigatus*, 11±0.1 against *Aspergillusniger* and 9±0.8 against *Aspergillus flavus*. The concentrations such as, 100 mg/ml and 300 mg/ml have shown with less zones of inhibition when compared to the concentration of 500mg/ml under study. The Chloroform crude algal extract was found with maximum zone of inhibition (11±0.4) against *Trichophyton rubrum*, 10±0.2 against *Aspergillus fumigatus*, 8±0.2 against *Aspergillus niger.* No zone of inhibition was observed against *Aspergillus flavus* in the concentration of 100mg/ml, 300mg/ml and 500mg/ml,respectively. In the Methanol extract solvent of *S. crassa* the zone of inhibition was exhibited with 9±0.4 against *Aspergillus flavus*, 8±0.1 against *Aspergillus fumigatus*, 9±0.4 against *Aspergillus niger*, and 7±0.1 against *Trichophyton rubrum* with the concentration of 500mg/ml. There was no zones of inhibition were identified against *Aspergillus fumigatus* at a concentration of 100mg/ml, 300mg/ml and at the same time *Aspergillus flavus*, *Trichophyton rubrum* strains were also not shown antifungal activity at a concentration of 100mg/ml (**Table – 2**). Water solvent extract of *S. crassa* was exhibited the zone of inhibition (7±0.2) against *Aspergillus niger* in concentration

of 500mg/ml and no zones of inhibition in the concentration of 100mg/ml, 300 mg/ml, 500 mg/ml were found in the remaining other three pathogenic fungal strains.

The Acetone extract of *Rhizoclonium hieroglyphicum* showed maximum zone of inhibition (9±0.8) against *Aspergillus flavus* with a concentration of 500 mg/ml, followed by 8±0.6 against *Aspergillus fumigatus,* and 7±0.4 against *Aspergillus niger*. No zone of inhibition found against *Trichophyton rubrum* in the same concentration. The Chloroform algal crude extract was expressed with maximum zone of inhibition (9±0.3) against *Aspergillus flavus* and it was followed by 8±0.4 against *Aspergillus fumigates*, 8±0.4 against *Aspergillus niger* with a concentration of 500 mg/ml. No zones of inhibition were noticed against *Trichophyton rubrum* in the concentrations of 100mg/ml, 300mg/ml and 500mg/ml, respectively. Similarly, no antifungal activity was noticed against *Aspergillus fumigatus* in the concentrations of 100mg/ml and 300mg/ml. The Methanol extract of *R.hieroglyphicum* expressed with only two zones against *Aspergillus niger* and *Trichophyton rubrum* (9±0.2 and 7±0.1) in the 500mg/ml concentration. No zones inhibition were found against *Aspergillus fumigatus, Aspergillus niger* with a concentration of 100 mg/ml, 300 mg/ml, 500 mg/ml and also no zones were found against *Aspergillus niger* and *Trichophyton rubrum* in the concentrations of 100mg/ml, 300mg/ml (**Table – 3**). The Water crude extract of *R. hieroglyphicum* has exhibited with the zone of inhibitions against *Trichophyton rubrum* (7±0.3) and *Aspergillus fumigatus* (7±0.0) in the concentration of 500 mg/ml under study. No zones were observed against the tested pathogenic fungi in the remaining other concentrations. The Chloroform extract of *Oedogonium crispum* expressed with maximum zone of inhibition (9±0.6) against *Aspergillus flavus,* 8±0.5 against *Aspergillus niger* and 8±0.3 against *Aspergillus fumigates* in the 500mg/ml concentration, however, no inhibition zone was found against *Trichophyton rubrum* with the same concentration. No antifungal activity was exhibited against *Trichophyton rubrum* and *Aspergillus niger* at 100 mg/ml, and at the same time *Aspergillus fumigatus* was also failed to show antifungal activity in the concentration of 300 mg/ml. The Methanol extract of alga was exhibited with only two zones of inhibition with a concentration of 500 mg/ml, 9±0.2 against *Aspergillus niger,* 8±0.1 against *Aspergillus flavus,* remaining other concentrations were failed to express the antifungal activity. The Acetone extract of *O. crispum* exhibited with zones of inhibition (8±0.8) against *Aspergillus niger,* 8±0.4 against *Aspergillus fumigatus*, 8±02 against *Trichophyton rubrum,* and 8±0.0 against *Aspergillus flavus* with a concentration of 500mg/ml. The 300mg/ml concentration was also expressed with zones of inhibition almost similar to 500mg/ml concentration but failed to express zone

of inhibition against *Trichophyton rubrum.*The Water crude extract showed only one zone of inhibition (7±0.0) against *Aspergillus flavus* at the concentration 500mg/ml. No zones were observed in the remaining strains of fungi with a concentration of 100mg/ml, 300mg/ml and 500mg/ml, respectively(**Table-4**). The Dimethyl Sulfoxide (DMSO) had no effect on fungal growth. Among all the species studied in the present investigation,it seems *S.crassa* and *S.biformis* were being reported for the first time as more potential for antifungal activity against a pathogen *T.rubrum.*

Table 1: Antifungal activity of *Spirogyra biformis* with different test organisms.

Test Samples	Concentration mg/ml	Diameter of zone of inhibition (mm)			
		An	***Af***	***Afl***	***Tr***
Acetone Extract	100	8±0.1	-	8±00	7±0.1
	300	8±0.1	9±0.2	8±0.2	9±0.1
	500	9±0.2	10±0.4	10±0.2	12±0.1
Chloroform Extract	100	-	-	-	-
	300	8±00	10±0.1	7±0.1	7±0.3
	500	9±0.2	10±0.2	8±0.2	8±0.1
Methanol Extract	100	7±0.1	-	-	8±0.0
	300	8±0.1	-	-	8±0.1
	500	10±0.2	8±00	-	9±0.2
Water Extract	100	-	-	-	-
	300	-	-	-	-
	500	-	-	-	-
DMSO		-	-	-	-

An=Aspergillus niger, Af= Aspergillus fumigatus, Afl= Aspergillus flavus, Tr=Trichophyton rubrum

Table - 2: Antifungal activity of *Spirogyra crassa* with different test organisms

Test Samples	Concentration mg/ml	Diameter of zone of inhibition (mm)			
		An	***Af***	***Afl***	***Tr***
Acetone Extract	100	7±0.2	7±0.4	7±0.2	9±0.1
	300	8±0.3	9±0.1	9±0.5	8±00
	500	11±0.1	11±0.2	9±0.8	12±0.5
Chloroform Extract	100	-	-	-	8±0.1
	300	8±00	8±0.3	-	8±00
	500	10±0.2	10±0.2	-	11±0.4
Methanol Extract	100	7±00	-	-	-
	300	7±0.1	-	8±00	7±00
	500	9±0.4	8±0.1	9±0.4	7±0.1
Water Extract	100	-	-	-	-
	300	-	-	-	-
	500	7±0.2	-	-	-
DMSO		-	-	-	-

An=Aspergillus niger, Af= Aspergillus fumigatus, Afl= Aspergillus flavus, Tr=Trichophyton rubrum.

Table 3: Antifungal activity of *Rhizoclonium hieroglyphicum* with different test organisms.

Test Samples	Concentration mg/ml	Diameter of zone of inhibition (mm)			
		An	*Af*	*Afl*	*Tr*
Acetone Extract	100	7±00	8±0.0	7±0.1	-
	300	7±0.3	7±0.3	8±00	-
	500	7±0.4	8±0.6	9±0.8	-
Chloroform Extract	100	7±0.1	-	8±00	-
	300	7±0.1	-	8±00	-
	500	8±0.4	8±0.4	9±0.3	-
Methanol Extract	100	-	-	-	-
	300	-	-	-	-
	500	9±0.2	-	-	7±0.1
Water Extract	100	-	-	-	-
	300	-	-	-	-
	500	-	7±00	-	7±0.3
DMSO		-	-	-	-

An=Aspergillus niger, Af= Aspergillus fumigatus, Afl= Aspergillus flavus, Tr=Trichophyton rubrum.

Table - 4: Antifungal activity of *Oedogonium crispum* with different test organisms

Test Samples	Concentration mg/ml	Diameter of zone of inhibition (mm)			
		An	*Af*	*Afl*	*Tr*
Acetone Extract	100	7±0.8	7±0.1	7±0.2	-
	300	8±0.4	7±0.1	8±00	-
	500	8±0.8	8±0.4	8±00	8±0.2
Chloroform Extract	100	-	7±0.2	7±00	-
	300	7±00	-	7±0.2	-
	500	8±0.5	8±0.3	9±0.6	-
Methanol Extract	100	-	-	-	-
	300	-	-	-	-
	500	9±0.2	-	8±0.1	-
Water Extract	100	-	-	-	-
	300	-	-	-	-
	500	-	-	7±00	-
DMSO		-	-	-	-

An=Aspergillus niger, Af= Aspergillus fumigatus, Afl= Aspergillus flavus, Tr=Trichophyton rubrum.

Results including diameter of the well(mean ± standard error), (-)= not found

CONCLUSION

This present study proved that micro-algae possess antifungal activity and the results confirms that microalgae *Spirogyra biformis, Spirogyra crassa, Rhizoclonium*

hieroglyphicum and *Oedogonium crispum* were potential source of bioactive compounds against various pathogens and also which can be used as natural non-toxic preservative and may be more acceptable to consumers. Further work is needed to identify the active compounds of these algae.

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