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Antibacterial Activity of Certain Members of Chlorophyceae From Warangal District, Telangana State, India

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Abstract

Chloroform and Methanol extracts of three genera of algae (*Oedogonium crispum* (Hassal) Wittrock, *Rhizoclonium hieroglyphicum* (Ag)Kuetz. and *Spirogyra biformis* C-C.Jao.) from fresh water of Warangal District were tested *in vitro* for their antibacterial activities against two strains of gram positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and two strains of gram negative bacteria (*Klebesiella pneumonia* and *Samonella typhi*) by the disc diffusion method. Chloroform was the best solution for extracting the effective antibacterial materials from the algae used in this investigation, with the exception of *O.crispum, for* which methanol was the most effective extraction solution. Chloroform extracts of fresh *S.biformis* and *R.heiroglyphicum* showed effective results against all test organisms. For control sample observations the gentamycin was used. A significant difference between in an antibacterial activity evaluated chloroform and methanol extracts of each alga under investigation was not observed. In addition, as a result of dried and fresh extract antibacterial activity comparison, it was found that all test organisms were more sensitive to fresh extracts of the algae.

Key words: Green algae, antimicrobial activity, methanol, chloroform, zone of inhibition, human pathogens.

Introduction

Cyanobacteria have been identified as the most promising group of the organisms capable of producing bioactive compounds (Fish and Codd, 1994 and Schlegel *et al.,* 1999; Asthana *et al*., 2009). Cyanobacteria are known to produce metabolites with diverse biological activity such as antibacterial, antifungal, antiviral, anticancer, antiplasmodial, algicide, antiplatelet aggregation and immuno-suppressive activities (Borowitzka,1995;Jaki *et al,* 2000; Kajiyama *et al.*,1998; Patterson and Carmeli, 1992; Gerwick *et al.,* 1994; Luesch *et al.,* 2000; Papendorf *et al.,* 1998; Papke *et al.,* 1997, Rho *et al.,* 1996; Koehn *et al.,* 1992 and Ghasemi *et al.,* 2003). The ability to produce bioactive substances may be noticed not only as a defense mechanism but also as a good source of new bioactive compounds from a pharmaceutical point of view (Soltani *et al*., 2005). Recently, Sanaa (2007) has studied bioactive allelochemical compounds from *Oscillatoria* species (Egypitian isolates). Many unique compounds of fresh water origin with various biological activities have been isolated and some of them are under investigation to develop new pharmaceuticals (Lima-Filho *et al.,* 2002; Choudhary *et al*., 2005; Kamble and Chavan, 2010; Abedin and Hala, 2008; Elsie and Dhanarajan, 2010). In recent years, such interest to evaluate plants possessing an antimicrobial activity for various diseases has been growing (Krishnaraju *et al.,* 2005; Raghavendra *et al*., 2006; Selvamaleeswaran *et al*., 2010 and Haripriya *et al*., 2010). Not only plants but some algae have been reported for their antibacterial or antifungal activities against human pathogens. The present study was undertaken to examine antimicrobial effects of methanolic and chloroform extracts of Chlorophyceae species, i.e. *Spirogyra biformis, Oedogonium crispum* and *Rhyzoclonium heiroglyphicum*) against some selected bacterial strains of human pathogens.

Materials and Methods

Three genera of algae viz., *O.crispum*, *R.heiroglyphicum* and *S.biformis* (Figure-1A,B &C) were collected from fresh water bodies of Warangal District and were used for the preparation of different solvent extracts. Algal samples were cleaned and necrotic parts were removed. Then the samples were rinsed with sterile water to remove any associated debris. These cleaned fresh materials were air-dried and then powdered with the help of a blender. The powder (5g) was filled in the thimble and extracted with chloroform and methanol by using a Soxhlet apparatus at the temperature of 60ºC for 8h. From the solvent extracts, the volume of 5 ml. was isolated separately, allowed to dry at a room temperature and weighed to estimate the concentration in 1 ml. Four strains of microorganisms were obtained from Department of Microbiology, Kakatiya University(Telangana State,India),i.e. Gram negative bacteria (*Klebsiella pneumoniae*, *Salmonella typhi*) and the Gram positive ones (*Staphylococcus aureus, Bacillus cereus*).

The antimicrobial activity was evaluated using the agar diffusion technique in Petri dishes. 25 µl of each extract was loaded on sterile filter paper discs with 6 mm in diameter (E-760), and air dried. Indicator microorganisms were spread on Mueller-Hinton Agar plates. After incubation for 24 h at 30ºC, a clear zone around a disc was evidence of an antimicrobial activity. The diameters of the zones of inhibition were measured in millimeters. Each test was prepared in triplicate. The discs loaded with gentamycin served as a standard control.

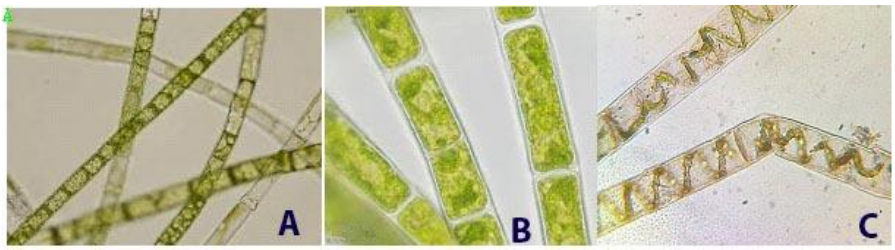

Figure. 1. A, B, C. *Oedogonium crispum* (Hassal)Wittrock (x 225). B. *Rhizoclonium heiroglyphicum* (Ag) Kuetz. (x 600).

C. *Spirogyra biformis* C-C.Jao. (x 400)

Results and Discussion

The chloroform and methanolic extracts were taken for antibacterial activity against four strains of human bacterial pathogens. The degree of activity was varied with reference to the concentration of algal extracts. The chloroform extract of *S.biformis* has shown antibacterial activity against four pathogens viz., *K.pneumoniae*, *S.aureus*, *B.cereus* and *S.typhi* with the inhibition zones of 5, 4, 5 and 4 mm, respectively (Table-1).The methanolic extracts of *S.biformis* exhibited the antibacterial activity against all four strains of bacteria but when compared to chloroform extracts the results were found be less (3, 3, 4 and 3 mm). In the standard control treated samples containing gentamycin, the results were expressed as higher inhibition zones (10, 8, 11 and 7 mm) when compared to the algal crude extracts. The methanolic extracts of *O.crispum* exhibited the antibacterial activity against with the maximum zone of inhibition of 6, 6, 7 and 5 mm. Chloroform extracts of *R. heiroglyphicum* exhibited antibacterial activity with the maximum zone of inhibition of 1, 2, 3 and 4 mm, respectively. Methanolic extracts *R. heiroglyphicum* have shown antibacterial potency against only on three strains of bacteria, i.e. *S.aureus*, *B.cereus* and *S.typhi* with the zone of inhibition of 3, 3 and 2 mm, respectively. The methanolic extracts of *R.heiroglyphicum* failed to show the antibacterial activity against the *K.pneumoniae* .The present results revealed that the chloroform (more effective,Table-1) and methanolic extracts of *S.biformis* have shown the antibacterial activity against *K.pneumoniae.* The same solvent extracts of *O.crispum* also indicated antibacterial efficacy against *K.pneumoniae*, the methanolic extracts have shown larger zone of inhibition than the chloroform ones. On the other hand, the methanolic extracts of *R.heiroglyphicum* have not shown antibacterial efficiency but the chloroform ones expressed lower antibacterial activity against the *K,pneumoniae* pathogen(1 mm).

Table -1. Antibacterial activity of three Green algal extracts against 4 strains of Human pathogens

Algae	Organic solvent	Bacterial strains			
		***Klebsiella pneumoniae* (-ve)**	***Staphylococcus aureus* (+ve)**	***Bacillus cereus* (+ve)**	***Salmonella typhi* (-ve)**
Spirogyra biformis	i). Methanol ii) Chloroform	03 05	03 04	04 05	03 04
Oedogonium crispum	i). Methanol ii) Chloroform	06 05	06 05	07 06	05 04
Rhizoclonium heiroglyphicm	i). Methanol ii) Chloroform	- 01	03 02	03 03	02 04
Gentamycin (10µg)	Control	10	08	11	07

Staphylococcus aureus is a Gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin. The emergence of antibiotic-resistant forms of pathogenic *S. aureus* (e.g. MRSA)

is a worldwide problem in clinical medicine. All chloroform and methanolic extracts of *S.biformis*, *O.crispum* and *R .heiroglyphicum* show the antibacterial activity against *S.aureus* and it suggest that the both the solvent extracts may be used to treat the diseases like skin infections and sinusitis.

Bacillus cereus is an endemic, soil-dwelling, gram-positive, rod-shaped, beta hemolytic bacterium. It is the cause of "Fried Rice Syndrome," as the bacteria is classically contracted from fried rice dishes that have been sitting at room temperature for hours (such as at a buffet). The methanolic and chloroform extracts of *S.biformis*, *O.crispum* and *R.heoroglyphicum* demonstrated the antibacterial activity against the *B.cereus*. It was confirmed that the active bioactive substance presence in three genera of algae can be used as alternative medicine to treat food borne illness in humans.

Salmonella typhi belongs to the same family as *Escherichia*, which includes the species *E.coli*. Salmonellae cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning. Chloroform and methanolic extracts of chosen algal genera have shown the antibacterial activity against *S.typhi* and it could be suggested that the chloroform extracts of *S. biformis* and *R.heiroglyphicum* and methanolic extracts of *O.crispum* may be used to treat the diseases like typhoid fever. Most of the identified components with antibacterial activity extracted from plant groups are aromatic or saturated organic compounds and they are more soluble in both organic media. Similarly, in the present study the chloroform extracts have shown higher activity than the methanolic ones. Current study indicated that the antibacterial property of three genera against the selected strains of human pathogenic bacteria varies depending upon the solvent medium used for the extraction. Such research gave an indication of presence of the promising antibacterial compounds in the selected algae and obtained results indicated that investigated group of selected strains displayed a potential that would warrants further work.

The differences between current results and the conclusions from previous studies might be due to variability in the production of secondary metabolites, occasionally related to seasonal variations (Lima-Filho *et al*., 2002; and Moreau *et al*., 1988). 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 (Gonzalez *et al.*, 2001 and Perez *et al*., 1990).

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