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Research Article

Investigating Two Native Algal Species to Determine Antibiotic Susceptibility Against some Pathogens

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Abstract: The aim of present study was to investigate antimicrobial activity of two algal species *Pithophora oedogonium* and *Botrydiopsis arhiza* against four bacterial species: *Salmonella* 1 (egg), Salmonella 4 (meat 2), *Staphylococcus* sp., 4978 and *Staphylococcus* sp., 5034 and four fungal species viz: *Aspergillus niger* 419, *Aspergillus flavus* 1110, *Penicillium viridicatum* 1101 and *Fusarium solini* 1127. The algal cell mass was extracted in 100% ethanol and further concentrations of 2, 4, 6 and 8 mg/mL were made. Antagonistic effects were tested by using disc-diffusion method for bacterial species and agar well technique for fungal species. *B. arhiza* did not exhibit antimicrobial activity against all the test organisms. Whereas the ethanol extract of *P. oedogonium* inhibited the growth of two bacterial and two fungal species viz: *Salmonella 1* (*egg*), *Staphylococcus* sp., 4978, *F. solini* and *P. viridicatum*. The maximum activity was observed against *F. solini* (22.3 mm) at 2 mg/mL conc. applied. It was noted that the growth of *Salmonella 1* (egg) was inhibited maximally at 4 mg/m Lshowing zone of inhibition of 7, 4.67 and 4 mm at 6 and 8 mg/mL conc., respectively. The inhibitory zones of 7 and 3 mm were recorded for *Staphylococcus* sp., 4978 at 4 and 8 mg/mL conc. used. Among fungal species *P. viridicatum* showed maximum Zone of Inhibition (ZOI) of 7.6 mm at 8 mg/mL conc. The ethanolic extract of *P. oedogonium* can be efficiently used in antibiotics production as it proved effective against *Salmonella 1* (*egg*), *Staphylococcus* sp., 4978, *P. viridicatum* and *F. solini*.

Keywords: Anifungal activity, antibacterial activity, *Botrydiopsis arhiza*, microalgae, *Pithophora oedogonium*

INTRODUCTION

The medicinal value of plant and animal extracts can be extensively correlated with the history of mankind. The screening strategies for isolating therapeutic agents from such natural sources have diverted the attention towards algae (Goud *et al*., 2007). The antibiotic activity of algae was initially demonstrated by (Pratt *et al*., 1944).

The huge algal diversity makes these organisms a source of enrichment due to the presence of different compounds growing at various levels of conditions producing bioactives (Plaza *et al*., 2010). Numerous algae and microalgae have proven to be potential sources of such compounds (Mendolia *et al*., 2005; Herrero *et al*., 2006). Many eukaryotic microalgae and cyanobacteria have been reported to produce different biological active compounds that inhibit the growth of different bacteria and fungi in laboratory tests against various human diseases (Kulik, 1995).

Though microalgae have that potential, marine alga had been focused a lot (Jaki *et al*., 2000) as compared to freshwater algae. To extend the knowledge on the freshwater algae for their potential therapeutic interest, the antimicrobial activity of two native algal species viz., *Botrydiopsis arhiza* and *Pithophora oedogonium* against some bacterial and fungal species was determined. *Pithophora oedogonium* belongs to the order Cladophorales, is a "cotton ball" or "horse hair" algae. The freshwater green algae grow as thick mats having filamentous clumps in ponds and shallow lakes. This is also studied as nuisance alga, resistant to many algaecides. The bioactive and nutritive properties of this alga have not been explored (Sukumaran and Thevanathan, 2011). On the other hand *Botrydiopsis arhiza* is a unicellular algae characterized by asexual reproduction through zoosporulation. Sometimes its cells clump together to form masses. Single nucleus and one to two chloroplasts are found in younger cells whereas older cells possess many nuclei and chloroplasts (Guiry *et al*., 2011).

The objectives of the present study were to assess antibacterial and antifungal activity of the cell extracts of *B. arhiza* and *P. oedogonium*.

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MATERIALS AND METHODS

Collection and culturing of algal species: Two algal species were selected as study organisms namely *Pithphora oedogonium* and *Botrydiopsis arhiza. Pithophora oedogonium* was collected from pond maintained in the Botanical garden of Forman Christian College whereas unialgal culture of *Botrydiopsis arhiza* was obtained from the Biological Science Department, F.C.C.U Lahore.

Pithophora oedogonium was washed first with tap and then with sterile distilled water to remove all the debris and waste particles. Afterwards, these were aseptically grown in liquid Bold's Basal Medium (BBM) (Bischoff and Bold, 1963). The culture conditions were maintained at temperature; 25, light intensity; 25 μmole/m^2/sec and pH; 8.0 under (16 h: 8 h) light: dark period (Mubeen *et al*., 2011).

Unialgal culture of *Botrydiopsis arhiza* was maintained on solidified D-11 (Graham *et al*., 1982) media plates. (1.5%) Bacteriological agar No. 1 was added in the medium before autoclaving for the preparation of solid medium. All the culture plates were kept under specific growth conditions which were maintained at temperature; 30°C, light intensity; 200 μmole/m^2/sec, pH; 6.0 (Mubeen *et al*., 2011).

Routine monitoring was done by observing the culture samples under compound microscope. Unialgal colonies were then shifted to sterile liquid D-11 medium to quantify the biomass. These cultures were continuously sub-cultured in liquid D-11 medium after regular intervals of 3 days.

Preparation of algal cell extracts: Aseptically grown filaments of *P. oedogonium* were shade dried at room temperature and kept in oven at 40°C for 12 h to make it further dry. The completely dried algal sample was crushed in mortar and pestle. The powdered algal material was extracted in Soxhlet apparatus using ethanol as organic solvent for 24 h. The resulting extract was evaporated and the final concentrated extract was stored in refrigerator at 4°C.

The unicellular algal culture of *B. arhiza* was centrifuged at 4000 rpm and pellets were weighed. Extraction was carried out by using 10 mL ethanol for 5 g of algal pellet. The residual extract was kept in refrigerator at 4°C (Val *et al*., 2001).

Bacterial and fungal cultures: Four pathogens namely *Salmonella* 4 (Meat 2), *Salmonella* 1 (Egg), *Staphylococcus* 5034, *Staphylococcus* 4978 were collected from Microbiology and Molecular Genetics Department, University of Punjab, Lahore. These cultures were maintained on nutrient agar (Oxoid) and nutrient broth. Four fungal strains *Aspergillus niger* 419, *Aspergillus flavus* 1110, *Penicillium viridicatum* 1101, *Fusarium solini* 1127 were obtained from fungal culture bank of Institute of Agricultural Sciences, University of Punjab, Lahore. These cultures were grown on 2% malt extract and maintained on 2% MEA.

Preparation of bacterial inoculums: The well grown colonies of test bacteria were picked and immersed a loopful of inocula in 15 mL of nutrient broth. It was kept on shaker incubater at 37°C for 24 h. A loopful of the 24 h bacterial broth culture was then diluted in 3 mL of sterile saline (0.9% W/V). The mean bacterial density was counted to be 3.3×10^6 CFU/mL.

Evaluation of antibacterial activity: Disc diffusion method (Bauer *et al*., 1966) was employed for this purpose. Autoclaved discs were loaded with 10 μL of the respective algal extract and air dried for 5 min. The nutrient agar plates were spread with 100 μL of respective culture with the help of glass spreader and the loaded discs were placed onto the surface of agar. The plates were left to dry for 5 min and kept in incubator at 37°C for 24 h. The results were seen as zone of inhibition which was measured in millimeters with the aid of transparent ruler. Streptomycin and kanamycin stocks were also made at 50 mg/mL concentration that served as positive controls. The experiment was done in triplicates.

Evaluation of antifungal assay: Agar-well diffusion method (Attaie *et al*., 1987) was used to test the antifungal activity of both the extracts against test organisms. Sterile malt extract media were inoculated with 100 μL of 5 days old broth fungal cultures separately and 20 mL media was poured in autoclaved petriplates for each of the four fungal species. After solidification, 6 mm wells were created in agar by using sterile borer. One hundred μL of the respective algal extracts were poured into the wells. The fungal culture plates were then incubated at 25°C for 3-5 days. The results were recorded as Zone of Inhibitions (ZOI) measured with the help of transparent ruler in millimeter. Positive control of cyclohexamide-an antifungal agent was also made at 10 mg/mL concentration sidewise. The experiment was carried out in triplicates.

All readings were recorded as mean and the standard errors were also calculated.

RESULTS

The ethanolic extract of *B. arhiza* did not show antimicrobial activity against any of the bacterial and fungal species used in the experiment at all the concentrations of algal extract applied. Whereas, the ethanolic extract of *P. oedogonium* showed considerable antibacterial and antifungal activities for *Salmonella* 1 (egg), *Staphylococcus* sp., 4978, *Fusarium solini* and *Penicillium viridicatum.* On the other hand no antibacterial or antifungal activity was observed for *Salmonella* 4 (meat 2), *Staphylococcus* sp., 5034, *A. niger* and *A. flavus.* In this present study, a zone of 22.3 mm was recorded for *Fusarium solini* at 2 mg/mL conc. used. No activity was observed at any other conc. applied. Maximum activity of 7.6 mm at 8 mg/mL conc. was seen against *Penicillium viridicatum*,

Table 1: Antimicrobial activity of *Pithophora oedogonium*

	Concentrations of extract											
	2 mg/mL			4 mg/mL			6 mg/mL			8 mg/mL		
Bacterial and fungal species	Zone f inhibition (mm)	Avg.	S.E. ±	Zone of inhibition (mm)	Avg.	S.E. ±	Zone of inhibition (mm)	Avg.	S.E. ±	Zone of inhibition (mm)	Avg.	S.E. ±
Salmonella 4 (meat 2)	-	-	-	-	-	-	-	-	-	-	-	-
	-			-			-			-		
	-			-			-			-		
Salmonella 1 (egg)	-	-	-	7	7	0.57	5	4.67	0.33	4	4	0
	-			6			4			4		
	-			8			5			4		
Staphylococcus sp. 4978	-	-	-	7	7	0	-	-	-	2	3	0.57
	-			7			-			4		
	-			7			-			3		
Staphylococcus sp. 5034	-	-	-	-	-	-	-	-	-	-	-	-
	-			-			-			-		
	-			-			-			-		
Fusarium solini	22	22.3	0.33	-	-	-	-	-	-	-	-	-
	22			-			-			-		
	23			-			-			-		
Penicillium viridicatum	-	-	-	2	2.3	0.33	4	4	0	8	7.6	0.33
	-			3			4			8		
	-			2			4			7		
Aspergillus flavus	-	-	-	-	-	-	-	-	-	-	-	-
	-			-			-			-		
	-			-			-			-		
Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	-
	-			-			-			-		
	-			-			-			-		

The data are expressed as mean±S.D.; S.E.: Standard error; Avg.: Average

while at 2 and 4 mg/mL the inhibitory zones were measured as 2.3 and 4 mm, respectively. According to the present investigation a maximum clearing zone was there for *F. soloni* (22.3 mm) among all the test species. The zone of inhibition and values of standard error are mentioned in the Table 1.

DISCUSSION

It had been reported in the present study that among the two freshwater alga one species *P. oedogonium* showed antimicrobial activity while the second *B. arhiza* did not exhibit antimicrobial activity for all the organisms tested. In this attempt to exploit the antimicrobial functions of two native algal species, 100% ethanol was used as the organic solvent for extraction purpose. The earlier reports revealed that using organic solvent systems for the extraction purpose were always better than following aqueous water extraction protocol (Hodgson, 1984).

In the comparison to the present study, many algal species had been reported to contain antibacterial, antifungal and antiviral substances. Different solvent extraction systems were adopted including chloroform, ethanol, methanol, hexane, ethyl acetate, acetone, diethyl ether and toluene for extracting antibacterial compounds from marine alga. The selection of organic solvent for the extraction purpose proved to be restricted for a specific algal species. Such organic solvents had been used individually or in combinations (Allen and Dawson, 1960; Martinez Nadal *et al*., 1966; Bhakuni and Silva, 1974; Debro and Ward, 1979; Rao and Parekh, 1981; Caccamese *et al*., 1980, 1985; Pesando and Caram, 1984; Rao *et al*., 1986; Sastry and Rao, 1994; Rao, 1991, 1995; Centeno and Ballantine, 1999; Dhamotharan, 2002). The choice of solvent for the extraction purpose was a helpful sign in determining antimicrobial activities in freshwater algae. It was reported that the maximum activity was exhibited by methanol followed by ethanol and aqueous water respectively (Goud *et al*., 2007).

The present investigation revealed the first time screening of *B. arhiza* for antimicrobial assessment. The selected bacterial and fungal species were resistant to the algal extract. The possible reasons might include the choice of organic solvent, the extraction method which might not be suited for the efficient extraction of antimicrobial substances. These results can be compared with the previous study presented by Zornitza *et al*. (2000) and Berry *et al*. (2004) which showed antimicrobial activities of some freshwater micro-algal species and at the same time not in other species tested. These differences might include several factors including extraction procedure, seasonal variations which might be posing intra-specific variations in producing biological active antimicrobial compounds.

The present study can also be compared with the study of Abedin and Taha (2008) which reported that some cyanobacterial and green microalgae produced antimicrobial substances against different types of bacteria and fungi. The various zones of inhibition depicted that this variability depended on the particular type of algae, the test organisms and the kind of the organic solvent used in the experiment. They reported that *Spirulina platensis* algal residues mixed in acetone for extraction and the residues of *Anabaena oryzae*

extracted in ethanol gave highest antimicrobial activity against *B. subtilis* and *Pseudomonas aeruginosa.* Their results showed that both extracts were moderately affecting the growth of *E. coli* and *Staphylococcus aureus.*

The use of different solvents and their efficacy can also be seen in a previous study in which *P. oedogonium* methanolic extract showed effective antibacterial activity at 100 µg conc. against *Staphylococcus aureus*, *E. coli*, *Streptococcus faecalis* and *Streptococcus pyogenes*. The same conc. did not exhibit any significant activity for n-hexane extract. A conc. of 250 µg gave inhibitory effect towards *Streptococcus* sp., (Sukumaran and Thevanathan, 2011). An earlier study on two green microalagl species (*Scenedesmus quadricauda* and *Chlorella pyrenoidosa*) and three cyanobacteria (*Tolypothrix ceytonica*, *Anabaena oryzae* and *Spirulina platensis*) were screened for their antifungal activities (Abedin and Taha, 2008).

The ethanolic extract of *P. oedogonium* can be efficiently used in antibiotics production as it proved effective against *Salmonella* 1 (egg), *Staphylococcus* sp., 4978, *P. viridicatum* and *F. solini.* An easy approach to these algae can be used in exploiting its antimicrobial nature commercially. On the other hand unicellular *B. arhiza* can be further exploited for its antimicrobial metabolites by using some other organic solvents against some microbes in future.

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