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SHORT COMMUNICATION

Antimicrobial activity of organic solvent extracts of three marine macroalgae from Chilika Lake, Orissa, India

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ABSTRACT

In vitro study of antibacterial activity of organic solvent extracts of three marine macroalgae viz., *Chaetomorpha linum* (Mell) Kuetzing, *Enteromorpha compressa* (L) Greville and *Polysiphonia subtilissima* Mont. showed specific activity in inhibiting the growth of three Gram-negative bacteria (*Shigella flexneri, Vibrio cholerae* and *Escherichia coli)* and two Gram positive bacteria (*Bacillus subtilis* and *Bacillus brevis)*. The results revealed that the chloroform and ethyl acetate extracts were active against most of the pathogens whereas methanol and ethanol extracts were active only against *S. flexneri*.

Keywords: macroalgae, antibacterial activity, Chilika Lake

INTRODUCTION

There is an increasing demand of biodiversity from natural resources for therapeutic drugs. The potential contribution of marine organisms to the discovery of new bioactive molecules is increasingly challenging (Sponga *et al*., 1999; Skulberg, 2000). The macroalgae have a significant attraction as natural source of bioactive molecules with a broad range of biological activities, such as antibiotics, antivirals, antitumorals, antioxidant and anti-inflammatories (Gil-Rodriguez and Alfonoso Carrillo, 1981; Scheuer, 1990; Sreenivasa Rao, 1995; Fleurence, 1999; Dera *et al.,* 2003; Vineela and Elizabeth, 2005; Tuney *et al.,* 2006; Patra *et al.,* 2008). Evidence of phycochemical and pharmacological studies on algae is available in the literature with special reference to terpenoids and steroids (Parameswaran, 1944 and Patterson, 1968). Algae are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes and cyclic polysulphides (Mtolera and Semesi, 1996 and Taskin *et al.,* 2007).

Chilika lagoon (Figure 1) in the Orissa coast is rich in economically and commercially important marine macroalgae such as *Ulva lactuca, Enteromorpha intestinalis, Enteromorpha compressa, Chaetomorpha linum* and *Polysiphonia subtilissima* (Sahu and Adhikary, 1999). The *Enteromorpha* sp. have been used as a source of bioactive compounds similar to those which cause an inhibitory effect against the bacterium *Xanthomonas oryzae*, which causes leaf blight disease in paddy crops (Manimala and Rengasamy, 1993). Besides this, it is also used as a source of methane generation through anaerobic digestion and bioactive compounds (Harron *et al.,* 2000). The *Chaetomorpha* sp. and *Polysiphonia* sp. are also rich in many bioactive compounds such as terpenoids, steroids and aminoacids (Sambamurty, 2005). So far no work has been done on antimicrobial activity of marine macroalgae from Chilika Lake.

In the present work an attempt has been made to evaluate the antimicrobial characteristics of organic solvent extracts (chloroform, ethyl acetate, methanol and ethanol) of three dominant marine macroalgae collected from the brackish water Chilika Lake located at the east coast of Orissa, India.

MATERIALS AND METHODS

Collection of algae samples

Three dominant marine macroalgae such as *E. compressa, C. linum* and *P. subtilissima* were collected at a depth of 50-100 cm from the Chilika Lake (situated between Lat 19° 28'- 19° 54' and Long 85° 05' - 85° 38' E) (Figure 1) during Pre-monsoon season (February- April 2008); out of which two are green algae (*Chlorophyceae*) and one is red algae (*Rhodophyceae*). Collected samples were washed thoroughly with tap water to remove epiphytes, debris and other marine organism, then dried with tissue paper and kept at –20 °C till further analysis. The algal samples were identified by following standard

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procedures (Dhargalkar and Kavlekar, 2004; Sambamurty, 2005).

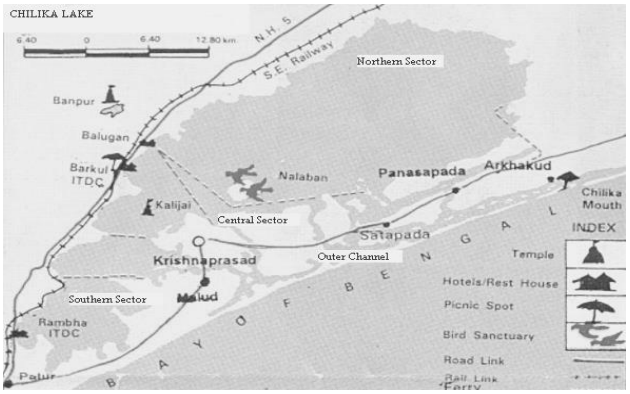

Figure 1: Map of Chilika Lake

Preparation of organic algae extracts

The algae samples were shade dried for 15 days and then pulverized into fine powder using pestle and mortar. The extraction was done by Soxhlet extraction techniques. Different solvents were used successively with gradient polarity (chloroform, ethyl acetate, methanol and ethanol). The extracts were evaporated to complete dryness by vacuum distillation and stored in refrigerator for further use (Akinyemi *et al.,* 2000; Mohanta *et al.,* 2007; Patra *et al.,* 2008).

***In vitro* screening for antibacterial activity of organic extracts**

For screening for antibacterial properties of organic (chloroform, ethyl acetate, methanol and ethanol) algae extracts, five pathogenic bacterial cultures were used. Three Gram-negative namely *Shigella flexneri* (Lab isolate), *Vibrio cholerae* (Lab isolate) and *Escherichia coli* (MTCC-1089) and two Gram-positive bacteria namely *Bacillus subtilis* (MTCC-7164) and *Bacillus brevis* (MTCC-7404). The pure strains were obtained from IMTECH, Chandigarh and some are the laboratory isolates. The organisms were maintained on agar slopes at 4 ºC and sub cultured for 24 h before use. Agar cup plate method (ACPM) of Khalid *et al.* (1999) was followed to ensure the antibacterial activity of the extracts against the test pathogen. Overnight nutrient broth culture of the test organisms were firmly seeded over the nutrient agar plates using sterile cotton swabs. A 100 µL (50 mg/mL) of extract was poured into a 0.6 cm diameter well and left for 2 h for complete diffusion. The plates were incubated at 37 ºC for 18-24 h. Clear zones of inhibition were measured in millimeters and the diameter of clear zones were used as an indication of antibacterial activity.

Each test was made in triplicate, dimethylsulfoxide (DMSO) was tested as negative control and Gentamycin (10 µg/disc) was taken as positive control (standard). The following formula was used for comparison of the antimicrobial activity of the sample with that of the standard (antimicrobial index).

$$\text{Antimicrobial index} = \frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of the standard}} \times 100$$

RESULTS AND DISCUSSION

The antibacterial activities of organic extracts of three dominant marine macroalgae species from the brackish water lake of Chilika were evaluated by agar well diffusion method and the results are summarized in Table 1. Chloroform extract of *C. linum* was effective against most of the pathogens whereas the *P. subtilissima* extract was effective against only *S. flexneri* and *B. subtilis* (Table 1). The chloroform extract of *E. compressa* was effective against *E. coli* and *B. subtilis* only. The ethanol and methanol extract did not show any remarkable activity against the pathogens tested except against *S. flexneri*.

Antibacterial activity depends on both, algal species and the efficiency of the extraction method. For instance, the chloroform extract of *C. linum* was effective against three test organisms; however, the chloroform extract of *P. subtilissima* and *E. compressa* showed different response (Table 1). The chloroform and ethyl acetate extracts were more effective than the methanol and ethanol extracts. This result reflects the presence of bioactive metabolites of the algae which are soluble in these solvents. Del Val *et al.* (2001) found that *E. compressa* do not have antimicrobial activities against *B. subtilis* but in our result we found that the chloroform extract has antibacterial

Table 1: Antibacterial activity of macroalgae extracts against pathogenic bacteria

Bacterial strains	Organic solvent extracts (50 mg/mL)												Gentamycin (10 µg/disc)
	Chloroform extract			Ethyl acetate extract			Methanol extract			Ethanol extract			
	A	B	C	A	B	C	A	B	C	A	B	C	
S. flexneri	18*	12	-	-	-	18	19	-	-		17	-	23
B. brevis	-	-	-	-	-	15	-	-	-	-	-	-	22
V. cholerae	-	-	-	-	-	-	-	-	-	-	-	-	26
B. subtilis	13	11	14	-	-	-	-	-	-	-	-	-	19
E. coli	10	-	12	13	13	12	-	-	-	-	-	-	18

***** = Inhibition zone in mm; - = no inhibition zone; **A** = *C. linum*; **B** = *P. subtilissima*; **C** = *E. compressa*

Table 2: Antibacterial index of macroalgae extracts against standard antibiotics (Gentamycin)

Bacterial strains	Organic solvent extracts (50 mg/mL)											
	Chloroform extract			Ethyl acetate extract			Methanol extract			Ethanol extract		
	A	B	C	A	B	C	A	B	C	A	B	C
S. flexneri	78.3*	52.1	-	-	-	78.3	82.6	-	-	-	73.9	-
B. brevis	-	-	-	-	-		-	-	-	-	-	-
V. cholerae	-	-	-	-	-	-	-	-	-	-	-	-
B. subtilis	68.4	57.9	73.9	-	-	-	-	-	-	-	-	-
E. coli	55.5	-	66.6	72.2	72.2	66.6	-	-	-	-	-	-

***** = antimicrobial index in percentage; - = no activity; **A** = *C. linum*; **B** = *P. subtilissima*; **C** = *E. compressa*

activity against *B. subtilis* with 14 mm inhibition zone (Table 1). Del Val *et al.* (2001) also demonstrated the antibacterial activities of the methanol extract of *Enteromorpha* sp. In contrast in the present study, chloroform and ethyl acetate extracts of *E. compressa* showed a broad spectrum of inhibition as compared to methanol and ethanol extracts. There have been reports on significant antimicrobial activities of marine macroalgae by several workers (Del Val *et al.,* 2001; Choudhury *et al.,* 2003; Tuney *et al.,* 2006; Goud *et al.,* 2007; Patra *et al.,* 2008; Martin *et al.,* 2008) which confirms our findings.

In this study some of the bacterial strains such as *V. cholerae* and *B. brevis* did not respond to all the organic extracts whereas other strains showed some activity. Such unusual response could be attributed to masking of antibacterial activity by the presence of some inhibitory compounds in the extract as observed by Sastry and Rao (1994). Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields higher antimicrobial activity than n-hexane and ethyl acetate (Rosell and Srivastava, 1987; Moreau *et al.,* 1988; Sastry and Rao, 1994), whereas others reported that chloroform is better than methanol and benzene (Febles *et al.,* 1995). It is clear that extraction by organic solvents always provide a higher efficiency for antimicrobial activities as compared to water extracts (Masuda *et al.,* 1997; Lima-Filho *et al.,* 2002).

The experimental study revealed that chloroform and ethyl acetate extracts caused bigger clear zones than methanol and ethanol extracts. The inhibition zones obtained by the organic solvent extracts were compared with standard antibiotic, Gentamycin and the antimicrobial index was calculated from it (Table 2). The variation of antibacterial activity of the organic solvent extracts might be due to the presence of different antibacterial substances among these species as suggested by Lustigman and Brown (1991).

CONCLUSIONS

In this work, we were able to show that these three marine macroalgae of Chilika Lake can be used for development of anti-pathogenic agents in pharmacology and medicine industries. Our results also revealed that the differential antibacterial activities of these marine macroalgae may be attributed to the presence of different antibacterial compounds which are easily extracted with organic solvents. However a detail study in this field is required for production of antibacterial compounds from these marine macroalgae.

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