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Evaluation of Antimicrobial Properties of Crude Extracts of *Narcine timlei* (Bloch & Scheider -1801)

V. Ravitchandirane[1]*, A. Chitra[2] and T. Ganesan[3]

[1]Department of Zoology, Centre for Post-Graduate Studies, Puducherry - 605 008, India.
[2]Department of Microbiology, Indira Gandhi College of Arts and Science, Puducherry - 605 006, India.
[3]Department of Plant Science, Tagore Arts College, Puducherry - 605 008, India.

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The crude methanol and petroleum ether fractions of *Narcine timlei* were tested for antimicrobial activity against pathogenic fungi and bacteria by zone of inhibition method. The antimicrobial potential of both the extracts was compared with standard antibiotics. The crude methanol fraction exhibited highest activity indicating the fish as a source of antimicrobial substances.

Key words: Electric ray, Bioactivity, Antibacterial, Antifungal, Crude extract.

Fish constitute almost half the number of vertebrates on earth and are of immense value to human beings in various ways. Fish live in intimate contact with its environment, that is rich in both saprophytic and pathogenic microbes. Development of innate defense mechanism constitutes both physical and chemical barrier to infections and is important for fish good health. The low infection rate of fish is remarkable and has inspired further studies of its defense system (Gudmundur Bergsson *et al.,* 2005).

The integumental secretion of fish, such as the multifunctional skin mucus has been shown to play a significant role in defense against fungi, bacteria and viruses (Chinchar *et al.,* 2004; Ellis, 2001; Ragunath Ravi and Venkatesvaran, 1999; Magarinos *et al.,* 1995). Since fish are unable to synthesize bioactive compounds denovo, they depend on dietary intake. The ingested substances and their metabolites are deposited more efficiently in flesh than in skin (Svjetlana Luterotti, 1999). Even though reports on the antimicrobial properties of skin secretions of fish are ample, the studies pertaining to "entire fish"

* To whom all correspondence should be addressed.
E-mail:vairavit@yahoo.co.in

are scanty. In view of the above facts, the present work has been under taken to study the antimicrobial properties of the organic solvent extracts of a commercially not important spotted electric ray *Narcine timlei.*

MATERIAL AND METHODS

Extraction and Fractionation

Freshly collected adult fish was cut into small pieces and about 500g was homogenized and refluxed twice with hot methanol for 4-6h (Clarke,1969). The combined methanol extract was filtered through muslin cloth and centrifuged at 10,000 rpm for 10 minutes. Resulting supernatant was evaporated under vacuum. The residue obtained was extracted with petroleum ether (60°C-80°C). Both methanol and petroleum ether fractions were used for antimicrobial studies.

Antifungal assay

Three fungi *viz*. *Aspergillus niger* (a common storage fungus), *Trichoderma harzianum* (a bio-fungicide) and *Rhizoctonia solani* (a plant pathogen with broad spectrum host range) were obtained from the Department of Plant Sciences, Centre for PG Studies, Puducherry. The fungal cultures were multiplied and maintained on potato dextrose agar (PDA) slants (Riker and Riker, 1936).

The antifungal activity was determined by poisoned food technique (Grover and Moore, 1962). The crude extracts were incorporated in PDA at 50 and 100 µg/ml concentration. The plates were inoculated in the centre with 6mm dia. mycelial plug punched from 2-5 days old plate cultures. Three replicates were prepared for each concentration and for each fungus. All the inoculated plates, along with suitable control were incubated in dark at 26 ± 2°C for 3-8 days. At the end of the incubation period the difference in colony diameter between control and treated plates was converted to percent inhibition.

$$\text{Percent inhibition} = \frac{\text{Control-Treatment}}{\text{Control}} \times 100$$

Antibacterial assay

Five Gram-negative bacterial strains *viz., Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii, Proteus mirabilis, Pseudomonas aeruginosa* were used to assess the antibacterial activity in the crude extracts. Bacterial species were inoculated at about 10^8cells/ ml in the nutrient broth and incubated at 28 ± 2°C for 24h.

In vitro antibacterial activity was evaluated using the standard Disc-diffusion technique (McCaffrey and Erdean,1985). Crude extracts (50 and 100µg/disc) were impregnated to 6mm dia. Whatman No.1 paper discs. Further, they were aseptically placed on agar plates seeded with the test bacteria. All the plates were incubated at 37 ±1°C for 24h. After 24h incubation the plates were observed for the presence of zone of inhibition around the discs. The inhibition zones around the paper discs were measured, averaged and recorded. The growth inhibition results were compared with standard antibiotic Cefaperazone + sulbactam. All the experiments were carried out in triplicates along with suitable controls.

Table 1. Antibacterial activity of crude petroleum ether and methanol extract of *Narcine timlei*

S. No	Pathogens Tested	Percent Inhibition			
		Petroleum ether extract		Methanol extract	
		50µg/ml	100µg/ml	50µg/ml	100µg/ml
1.	*Aspergillus niger*	-	17	19	41
2.	*Trichoderma harzianum*	-	-	29	71
3.	*Rhizoctonia solani*	-	20	-	32

RESULTS AND DISCUSSION

Antifungal activity

Between the two solvent extracts, methanol extract (Table –1; Plate –1) was more active than petroleum ether extract. Growth of all the three test fungi were suppressed to varying extent at 100 µg/ml, where as only two fungi were inhibited at 50µg/ml. Among the three fungi *Trichoderma harzianum* was more sensitive with 71 % reduction in radial growth at 100µg/ml , followed by *Aspergillus niger* (41%) and (33%). Petroleum ether extract had no effect on any of the test fungi at 50µg/ml. However, strong to moderate growth suppressive effect was noticed against *Aspergillus niger* (71 %) and (20%). *Trichoderma harzianum* was not affected even at 100µg/ml. In addition, both the extracts exhibited growth suppression, sporulation / sclerotia production in treated plates. This observation indicates that the fish extracts interferes with some biochemical reactions connected to asexual reproduction.

Antibacterial activity

A glimpse of the data on antibacterial activity of the two solvent extracts revealed a similar pattern of activity as that of antifungal activity. Methanol extract displayed broad spectrum activity and inhibited growth of all the five bacteria used in this study. The effect was more pronounced at 100µg/disc than at 50µg/disc (Table -2; Plate -2). Yogamoorthi and Srikala (2001) reported antibacterial activity of crude methanol extract of mucus of *Narcine timlei* against *Vibrio cholerae, Salmonella typhi, Shigella flexneri*. The ether extract of *Narcine brunnea* exhibited significant antibacterial activity against *Vibrio cholerae* (Ravitchandirane and Yogamoorthi, 2008). Further, it was also observed that the inhibitory effect of crude methanol extract at 100µg/disc concentration was slightly higher than the inhibitory activity of standard antibiotics *viz.* Cefaperazone + sulbactum used as control in the present study. Cefaperazone is a broad spectrum antibiotic against wide range of aerobic and anaerobic gram positive and gram negative bacteria by disrupting the synthesis of the peptidoglycan of bacterial cell walls (Pegler and Healy, 2007). Out of the two solvents tested methanol was found to be best extractant of the active components and the compounds seem to be polar in nature.

Table 2. Antifungal activity of crude petroleum ether and methanol extract of *Narcine timlei*

S. No	Pathogens Tested	Percent Inhibition			
		Petroleum ether extract		Methanol extract	
		50µg/ml	100µg/ml	50µg/ml	100µg/ml
1.	*Escherichia coli*	-	-	-	9
2.	*Klebsiella pneumoniae*	-	-	-	9
3.	*Citrobacter freundii*	-	16	-	22
4.	*Proteus mirabilis*	-	18	19	25
5.	*Pseudomonas aeruginosa*	-	16	19	25

CONCLUSION

From this study it can be concluded that *Narcine timlei* may be a potential source of antimicrobial substances for the control of pathogenic microbes.

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