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Antibacterial Activities of Green Mussel (*Perna viridis*) and Edible Oyster (*Crassostrea madrasensis*)

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Abstract: In the present study water, ethanol, methanol, acetone, hexane and butanol extracts of two Bivalves, *Perna viridis* and *Crassostrea madrasensis* were screened for antibacterial activity. The extracts were obtained from whole body tissue of the animals and tested against 10 different pathogenic bacteria viz., *Escherichia coli*, *Klebsiella oxytoca*, *K. pneumoniae*, *Lactobacillus vulgaris*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. paratyphi*, *Staphylococcus aureus* and *Vibrio* sp. Ethanol extract of *P. viridis* showed maximum antibacterial activity against *E. coli* and *S. aureus*. Ethanol extract of *C. madrasensis* exhibited highest activity against *S. aureus*. Water extract of *P. viridis* and *C. madrasensis* showed highest activity against *E. coli* and *P. mirabilis*, respectively. The 10:10 (methanol: ethanol) fractionated extracts of *P. viridis* shows highest activity against *P. mirabilis* (8 mm), 14:6, 4:16 and 2:18 fractions showed prominent activity against *P. aeruginosa*, *E.coli* and *K. pneumoniae*. In *C. madrasensis* also 10:10 fraction showed highest activity *E. coli*, *P. aeruginosa* and *S. aureus*. The 18:2, 12:8 and 2:18 fractionated extracts of *C. madrasensis* exhibits effective activity against *S. aureus*, *S. typhi* and *E. coli*. Water, ethanol and methanol extracts showed antibacterial activity against all most all the bacteria tested. Compare to water extracts, ethanol and methanol extracts showed more activity against all pathogens.

Key words: *Perna viridis*, *Crassostrea madrasensis*, antibacterial activity, bioactive compounds, pathogens

INTRODUCTION

Ocean offers a large biodiversity of fauna and flora which is estimated to be over 5,00,000 species more than double of the land species (Kamboj, 1999). There are approximately 5,000 species of Sponges, 11,000 species of Cnidaria, 9,000 species of Annelids, 66,535 species of Molluscs and 6,000 species of Echinoderms were reported (Ruggieri, 1976). Among the mollusks, 50,000 species of Gastropods, 15,000 species of bivalves and 600 species of Cephalopods have been reported to occur (Alfred *et al*., 1998). This rich diversity of marine organisms assumes a great opportunity for the discovery of new bioactive substances. Thus, the marine environment is an exceptional reservoir for bioactive natural products; many of which exhibit structural features that are not found in terrestrial natural products (Joshua, 1999). The marine environment comprises of complex ecosystem with a plethora of organisms and many of these organisms are known to possess bioactive compounds as a common means of defense (Indap and Pathare, 1998). The marine natural products have been investigated predominantly for their antimicrobial, cytotoxic, antitumour and anti-inflammatory properties (Anand and Edward, 2001). The oceans remain as an untapped source for many drugs and contemporary experimental studies which indicate that, pharmacologically active substances could be isolated from marine organisms (Baslow, 1969). In the last decade alone, structures of over 5,000

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marine natural products have been elucidated (Wright, 1998). More than 100 pure compounds of known and new structural types have been isolated and characterized. These compounds belongs to different structural types namely 37% diterpenoids, 18% of steroids/sterol glycosides, 17% sesquiterpenoids and the remaining were alkaloids, amino acids, fatty alcohol esters, glycolipids etc. Of the few bio-evaluated, some showed interesting biological activity (Komboj, 1999).

From 1960's to 1990's, approximately 300 bioactive marine natural products were filed for patent. Approximately 6,500 bioactive compounds were isolated from marine organisms (Komboj, 1999). Among the invertebrates, the Mollusks are highly delicious seafood because of their nutritive value next to fin fishes and crustaceans. They are also very good source for biomedically important products (Shenoy, 1988). Many classes of Molluscs exhibits bioactive compounds like antitumour, antileukemic, antibacterial and antiviral properties have been reported world wide (Kamiya *et al*., 1989; Pettit *et al*., 1987; Anand *et al*., 1997; Rajaganapathy *et al*., 2000). Among the mollusks, some animals exhibited pharmacological activities or other properties which are useful in the biomedical arena. Among the Molluscs, oysters and mussels are very good source for bioactive compounds. Considering the importance of the group and paucity of information in this line present study has been undertaken to ascertain the antibacterial activity of extracts from *Perna viridis* and *Crassostrea madrasensis* against various pathogenic bacteria.

MATERIALS AND METHODS

Extraction of Antibacterial Compounds from Bivalves

P. viridis and *C. madrasensis* were collected from Uppanar estuary (Lat. 11°43'N, long. 79° 49'E), East coast of India. Further analysis was carried out at CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India. Bivalves were brought to the laboratory, the shells were broken and the tissue samples were washed with distilled water. Extraction of bioactive compounds from the tissue samples was done with water, ethanol, methanol, acetone, hexane and butanol. To 5 g of tissue sample, 5 mL of water and solvents were added and ground well with mortar and pestle. Water and solvent extracts were centrifuged at 15000 rpm for 30 min and the supernatants were stored at -20°C until use.

Antibacterial Activity of Bivalve Extracts

Ten species of pathogenic bacteria namely *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Lactobacillus vulgaris*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi*, *Staphylococcus aureus* and *Vibrio* sp. were used to screen the antibacterial activity of the bivalve extracts. Pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37°C for 24 h. Pathogens were swabbed on the surface of the Muller Hinton agar plates and discs (Whatman No. 1 filter paper with 9 mm diameter) impregnated with the 50 μL of bivalve extracts were placed on the surface. Control discs were placed with water and solvents to asses the effect of water and solvents on pathogens. The plates were incubated at 37°C for 24 h and the antibacterial activity was measured accordingly based on the inhibition zone around the disc impregnated with bivalve extract.

Antibacterial Activity of Fractionated Bivalve Extracts

Ethanol and methanol extracts showed potential activity than other extracts and these extracts were fractionated by column chromatography in silica gel (Anand, 2001). Elution were made with, ethanol (E), methanol (M) and ethanol mixed with methanol in various proportions (E:M 18:2, 16:4, 14:2, 12:8, 10:10, 8:12, 8:12, 6:14, 4:16 and 2:18). Eluted fractions were assayed for antibacterial activity as aforementioned disc diffusion method.

RESULTS

Antimicrobial activity of extracts from *Perna viridis* and *Crassostrea madrasensis* are presented in Table 1 and 2. Compare to water extracts, ethanol and methanol extracts showed more activity against all pathogens.

Antibacterial Activity of Extracts from *Perna viridis*

Effect of extracts from *Perna viridis* on pathogenic bacteria revealed that, highest activity was noticed against *E. coli* (4 mm) with water extract. Regarding ethanol extracts maximum activity (8 mm) was found with *E. coli* and *Staphylococcus aureus*. The lowest activity (trace) was found with acetone, hexane and butanol extracts against *K. oxytoca* and *Vibrio* sp. Among the bacteria tested, *Klebsiella pneumoniae* and *Lactobacillus vulgaris* were highly resistant to most of the extracts. Similarly all the extracts of *Perna viridis* showed only trace activity against *Pseudomonas aeruginosa* and *Vibrio* sp. (Table 1).

Antibacterial Activity of Extracts from *Crassostrea madrasensis*

Antibacterial activity of *Crassostrea madrasensis* revealed that, water and methanol extracts showed highest activity against *Proteus mirabilis* (10 mm) and *Staphylococcus aureus* (10 mm), respectively and acetone, hexane, butanol extracts were not effective against these pathogens. Ethanol extract showed highest activity against *Staphylococcus aureus* and *Salmonella typhi*. As noticed in *Perna viridis*, both water and solvent extracts showed no activity against *Klebsiella pneumoniae* and *Lactobacillus vulgaris* except acetone extract (Table 2). Methanol extracts of *Crassostrea madrasensis* exhibited activity against *Pseudomonas aeruginos*a and most of the extracts were not able act against *Vibrio* sp.

Table 1: Antibacterial activity of ethanol and water extracts of *P. viridis*

	Antibacterial activity (mm)					
Name of the bacteria	Water	Ethanol	Methanol	Acetone	Hexane	Butanol
Escherichia coli	4	8	2	1	-	-
Klebsiella oxytoca	T	2	T	-	-	-
K. pneumoniae	-	-	-	T	-	T
Lactobacillus vulgaris	-	-	T	T	T	-
Proteus mirabilis	1.5	4	2	1.5	-	1.5
Pseudomonas aeruginosa	-	T	1	-	-	-
Salmonella typhi	3	7	3	2	1	1
S. paratyphi	1.5	3	1	-	-	-
Staphylococcus aureus	3	8	2	2	1	1
Vibrio sp.	T	T	-	-	-	-

Table 2: Antibacterial activity of ethanol and water extracts of *C. madrasensis*

	Antibacterial activity (mm)					
Name of the bacteria	Water	Ethanol	Methanol	Acetone	Hexane	Butanol
Escherichia coli	3	7	2.5	1	-	-
Klebsiella oxytoca	1.5	4	5	-	T	-
K. pneumoniae	-	-	T	1	-	T
Lactobacillus vulgaris	-	-	-	T	-	-
Proteus mirabilis	10	3	4	1	-	-
Pseudomonas aeruginosa	T	T	1	-	-	-
Salmonella typhi	2	6	3	2	1	T
S. paratyphi	T	3	1	-	-	-
Staphylococcus aureus	4	10	5	1	1	2
Vibrio sp.	T	T	-	-	-	-

Table 3: Antibacterial activity of fractionated extracts of *P.viridis*

Name of the bacteria	E	18:2	16:4	14:6	12:8	10:10	8:12	6:14	4:16	2:18	M
Escherichia coli	2	1	2	2	1	8	2	T	-	9	T
Klebsiella oxytoca	1	-	1	-	T	1	2	-	T	-	1
K. pneumoniae	-	2	1	T	1	2	1	2	1	1	1
Lactobacillus vulgaris	-	T	3	1	-	T	T	-	-	T	2
Proteus mirabilis	2	4	1	2	2	3	T	-	1	-	1
Pseudomonas aeruginosa	T	-	-	1	-	3	1	-	-	5	-
Salmonella typhi	1	-	T	1	7	1	T	-	-	1	-
S. paratyphi	1	-	-	-	T	-	1	-	1	-	T
Staphylococcus aureus	2	1	2	1	-	4	-	1	4	1	1
Vibrio sp.	T	-	-	T	-	-	-	T	-	-	T

Table 4: Antibacterial activity of fractionated extracts of *C. madrasensis*

Name of the bacteria	E	18:2	16:4	14:6	12:8	10:10	8:12	6:14	4:16	2:18	M
Escherichia coli	2	1	2	2	1	8	2	T	-	9	T
Klebsiella oxytoca	1	-	1	-	T	1	2	-	T	-	1
K. pneumoniae	-	2	1	T	1	2	1	2	1	1	1
Lactobacillus vulgaris	-	T	3	1	-	T	T	-	-	T	2
Proteus mirabilis	2	4	1	2	2	3	T	-	1	-	1
Pseudomonas aeruginosa	T	-	-	1	-	3	1	-	-	5	-
Salmonella typhi	1	-	T	1	7	1	T	-	-	1	-
S. paratyphi	1	-	-	-	T	-	1	-	1	-	T
Staphylococcus aureus	T	10	-	T	1	6	T	T	2	1	T
Vibrio sp.	-	T	-	1	-	1	1	-	1	-	T

Control discs (mm): C1-0 (Water), C4-0 (Acetone), C2-T (Ethanol), C5-0 (Hexane), C3-T (Methanol), C6-0 (Butanol) T-trace (<1 mm), -: negative

Antibacterial Activity of Fractionated Extracts

The extracts were fractionated by silica gel column chromatography and highest activities were observed with the extracts of *Perna viridis* against *Proteus mirabilis* (8 mm), *E. coli* (5 mm) and *Staphylococcus aureus* (4 mm) in 10:10 (E:M) fraction. The 14:6, 4:16 and 2:18 fractions showed inhibition against *Salmonella typhi* (3 mm), *Staphylococcus aureus* (4 mm) and *Klebsiella pneumoniae* (6 mm). In *Crassostrea madrasensis*, the fractionation of (E:M) 18:2, 10:10 and 2:18 displayed highest activity against *Staphylococcus aureus* (10 mm), *Proteus mirabilis* (8 mm) and *Escherichia coli* (9 mm). 18:2, 12:8, 2:18 fractions exhibited activity against *Proteus mirabilis* (4 mm), *Salmonella typhi* (7 mm) and *Pseudomonas aeruginosa* (5 mm). Mostly 10:10 fraction showed activity against all pathogenic bacteria. In 6:14 and 14:6 fractions of both animals showed very less activity against all pathogenic bacteria (Table 3 and 4).

DISCUSSION

In the present investigation, distinct antibacterial activity was observed against almost all the pathogenic bacteria. Ethanol extracts of *Perna viridis* showed highest activity against *E. coli*, *S. aureus* and water extracts showed highest activity against *E. coli*, *S. typhi* and *S. aureus*, respectively and other extracts showed lowest activity against *K. pneumoniae* and *L. vulgaris*. Similarly the ethanol extract of *C. madrasensis* exhibited highest activity against *S. aureus* and *E. coli*. Methanol and water extracts displayed highest activity against *K. oxytoca*, *P. mirabilis* and *S. aureus*. *K. pneumoniae* and *L. vulgaris* were highly resistant to all the extracts. Similar study was carried out by Jayaseeli *et al*. (2001), they found antibacterial activity of four bivalves against few pathogens and the extracts showed significant activity against *Bacillus subtilis*. Antibacterial activity of gastropods against *S. typhi* was reported by Rajaganapathi (1996) also supporting present study on antibacterial activity of bivalve extracts. Anand and Edward (2001) studied the antibacterial activities in ethanol extracts of gastropod *Babylonia spirata* and *Turbo brunneus* and observed highest activity against *E. coli*, *K. pneumoniae*, *P. vulgaris* and *S. typhi*. Very similar to the present study, Elizabeth *et al*. (2003) noticed highest antibacterial activity with extracts of *Trochus radiatus* against *S. aureus* and *E. coli*.

Difference in antibacterial activity found with bivalve extracts may depends on extracting capacity of the solvents and the compounds extracted. Most interesting results were found with fractionated extracts of *Perna viridis* against *P. mirabilis*, *E. coli*, *S. aureus* and *P. aeruginosa*. The (M:E) 14:6, 10:10 and 2:18 fractions showed significant activity against *P. aeruginosa*, *E. coli* and *Proteus mirabilis*. Extracts from *Perna viridis* with 10:10 fractions showed significant activity against most of the pathogens tested. Anand and Edward (2001) got similar antibacterial activity with (M:E) 10:10 fraction of *Tibia delicatula* and these results complementing the results of present study. In this study extracts of *C. madrasensis*, with 18:2 and 2:18 fraction showed highest activity against *S. aureus* and *E. coli*. The 10:10 fraction showed prominent activity against *E. coli*, *P. mirabilis*, *P. aeruginosa* and *S. aureus*.

Commercial antibiotics are highly effective to kill the bacterial and fungal pathogens involved in common infection. Water, ethanol and methanol extracts of bivalves used in the present study showed significant antibacterial activity compare with other solvents extraction. It is worthy to note that the product from natural source is good for health and devoid of side effects. However, further investigations involving application of the extracts as drug for human administration need more research. In that angle further research is going with the objectives of extraction of bioactive compounds with different chemical and physical agents and structural elucidation of the purified compound.

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