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RESEARCH ARTICLE

Potential Antibacterial Activity of Marine Bivalves *Meretrix casta* and *Tridacna maxima* from South East Coast of India

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ABSTRACT

The whole body extracts of the Meretrix casta and Tridacna maxima with different solvents were assayed for anti bacterial activity using dist diffusion method against 10 human pathogenic bacteria. Among the extracts ethanol and methanol crude extracts exhibited broad antibacterial activity. Highest activity was exhibited against E. coli (8 mm) and Salmonella typhi (8 mm) by the Meretrix casta crude extract of ethanol. Ethanol extract of Tridacna maxima exhibited highest activity against S. aurens (10 mm) and E. coli (7mm) Aqueous extracts of Meretrix casta and Tridacna maxima exhibited highest activity against E. coli and P. mirabilis. The column purified 10:10 (Methanol: Ethanol) extracts of Meretrix casta shows highest activity against P. mirabilis (8 mm), 14:6, 4:16 and 2:18 fractions showed higher activity against P. aeruginosa, E. coli and K. pneumoniae. In Tridacna maxima 10:10 fraction exhibited highest activity against E. coli, P. aeruginosa and S. aureus. The 18:2, 12:8 and 2:18 fractionated extracts of Meretrix casta showed highest activity against S. aureus, S. typhi and E. coli the maximum zone of inhibition value obtained from 10:10 (M:E) fraction of both Meretrix casta and Tridacna maxima.

KEYWORDS*: Meretrix casta, Tridacna maxima, antibacterial activity.*

INTRODUCTION

Ocean offers a large biodiversity of fauna and flora which is estimated to be over 5,00,000 species are more than double of the land species [1]. The marine environment is a huge source for yet to be discovered natural products. Apart from the food that is derived from the marine environment, a wide variety of bioactive substances are being isolated and characterized several with great promise for the treatment of human disease. Molluscs are widely distributed through out the world and have many representatives in the marine and estuarine ecosystem namely slugs, whelks, clams, mussels, oyster, scallops, squids and octopus. This rich diversity to marine organisms assumes a great oppourtunity for the discovery of new bioactive compounds [2]. Many classes of bioactive compounds exhibiting antitumor, antileukemia, antibacterial and antiviral activities have been reported world wide the demand for effective and non toxic antibacterial therapeutics has become even greater with the increased incidence of bacterial infections.

Molluscs in the oceans are a common sight and are virtually untapped resource of novel compounds. Many studies have reported the bioactivity of the Molluscs like *Aphysia* sp. [3], *Phyllialae* sp. [4], bivalves [5], gastropods [6] and their egg masses [7]. Proactive metabolites from molluscs such as sea hare [8], *Chromodoris* sp. [9], Ozhidella [10] were isolated and structurally elucidated. The clams *Meretrix casta* and *Tridacna maxima* usually found attached estuaries in mud soil and they are called as blood clams due to presence of blood. These bivalves were screened for antibacterial activity using whole body tissues extracts obtained from different solvents.

MATERIALS AND METHODS

Live specimens of bivalves (*Meretrix casta* and *Tridacna maxima*) were collected at a depth of 6 m in Muthupet

(10°20' N and 79°32' E) and Tuticorin coastal waters (8°45' N and 78°13 E) South east coast of India. Further analysis was carried out at Biotechnology biology laboratory in Periyar Maniammai University, Vallam, Tamilnadu, India and their soft tissues were removed by breaking the shells. The tissues was cut into small pieces and washed thoroughly with distilled water and air dried. Extraction of bioactive compounds from the tissue samples were done with water, ethanol, methanol,
acetone, hexane and butanol. To 3g of tissue sample with five ml of water and solvents were added and ground well with mortar and pestle seperately. They were centrifuged at 15000 rpm for 30 min and the supernatants were stored at -20°C until use. To test the antibacterial effect of the extracts, against twelve human pathogens (*E. coli, Klebsilla oxytoca, Klebsilla pnenmoniae, Lactobacillus vulgaris, Proteus mirbalis, Psuedomonas aurogenosa, Salmonella typhi, Salmonella paratyphi, Staphylococcus aureus, Vibrio cholera, Bacillus megatorium* and *Proteus vulgaris*).
Bacterial were obtained from Raja Mirasudar Hospital, Thanjavur Tamilnadu and were inoculated in sterile nutrient broth and incubated at 37°C for 24 hrs. Pathogens were swabbed on the surface of Muller Hinton agar plates and discs were Impregnated with extracts were plated on the surface. Control disc were placed with water and solvents to asses the effect on pathogens. The of antibacterial activity was investigated by using the standard techniques [11]. The plates were incubated at 37°C for 24 hours antibacterial activity was expressed interms of diameter of zone of inhibition was measured in mm using vernier caliper scale and recorded (Table 1).

Antibacterial activity of fractioned bivalve extracts

Ethanol and Methanol extracts showed high inhibitory activity than the other extracts and these extracts were fractioned by column chromatography in Silicagel. Elution were made with, ethanol (E) methanol (M) and the ethanol and methanol in different proportion ethanol alone. 18:2, 16:4, 14:6, 12:8, 10:10, 8:12, 6:14, 4:16, 2:18 and methanol alone. Thus 11 fraction were collected separately and tested against twelve bacteria.

RESULTS

From the antibacterial activity six solvents extracts, of *Meretrix casta* and *Tridacna maxima* the ethanol and methanol extracts were able to inhibit all the pathogens exhibiting broad spectral antibiotic activity (Table 1).

Table 1: Antibacterial activity of *Meretrix casta* (Chemintz) against human pathogens

Pathogens	Zone of inhibition					
	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	-	-	-	-
Bacillus megatorium	3	T	2	-	1	1
Proteus vulgaris	-	2	3	2	1	T

- : No activity T : Trace
Ethanolic extracts from *Meretrix casta* on pathogenic bacteria showed, highest activity was against *E. coli* (8 mm). The lowest activity was found with acetone, hexane and butanol extracts against *K. oxytoca* and *Vibrio* sp.
From the antibacterial activity of *Tridacna maxima* water and methanol extracts showed highest activity against *E. coli* (7 mm) and *Klebsiella oxytoca* (10 mm) (Table 2).

Table 2: Antibacterial activity of *Tridacna maxima* against human pathogens

Pathogens	Zone of inhibition					
	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	4	3	4	1	-	-
Pseudomonas aeruginosa	T	T	1	-	-	-
Salmonella typhi	2	6	3	2	1	T
S. paratyphi	T	3	1	-	-	-
Staphylococcus aureus	4	3	1	-	-	-
Vibrio sp.	T	T	T	-	-	-
Bacillus megatorium	3	2	1	T	-	1
Proteus vulgaris	4	2	1	T	-	T

- : No activity; T : Trace

Antibacterial activity of fractioned extracts
The extract were fractionated by silica gel column chromatography and highest activities were observed with the extracts of *Meretrix casta* against *Proteus mirabilis* (8 mm), *E. coli* (5 mm) and *Staphylococcus aureus* (4 mm) and *Klebsiella pneumoniae* (6 mm). In *Tridacna maxima*, the fraction of (E:M) 18:2, 10:10 and 2:18 displayed highest activity against *Staphylococcus aureus* (10 mm), *Proteusmirabilis*(8mm)and *E. coli* (9 mm). 18:2, 12:8, 2:18 fractions exhibited activity against *Proteus mirabilis* (4 mm), *Salmonell 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 twelve pathogenic bacteria. Ethanol extracts of *Meretrix casta* showed highest activity against *E. coli*, *S. aureus* and aqueous 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 *Tridacna maxima* exhibited highest activity against *K. oxytoca*, *P. mirabilis* and *S. aureus*, *K. pneumoniae* and *L. vulgaris* were highly resistant to all the extracts.

Antibacterial activity of four bivalves against few pathogens and the extracts showed significant activity against

Table 3: Antibacterial activity of column purified fractions of *Meretrix casta* (Chemintz) *in* Ethanol and Methanol

Name of the bacteria	Zone of inhibition (mm)										
	(E:M)										
	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
Bacillus megatorium	-	3	1	T	1	2	T	1	2	1	T
Proteus vulgaris											

- : No activity; T : Trace

Table 4: Antibacterial activity of column purified fractions of *Tridacna maxima* in Ethanol and Methanol

Name of the bacteria	Zone of inhibition (mm)										
	(E:M)										
	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	1	-	
Bacillus megatorium	3	1	T	1	2	T	1	2	1	T	
Proteus vulgaris	2	1	T	1	2	T	2	1	1	1	

- : No activity T : Trace

Bacillus subtilis [5] and gastropods against *S. typhi* were reported [12]. Similar antibacterial activities in ethanol extracts of gastropod *Babylonia spirata* and *Turbo brumneus* and observed highest activity against *E. coli, K. pneumoniae, P. vulgaris* and *S. typhi*.

Difference in antibacterial activity found with bivalve extracts may depend extracting capacity of solvents and compound extracted. The most interesting results were found with fractionated extracts of *Meretrix casta* against *P. mirabilis*, *E. coli, S. aureus* and *P. aeruginosa*. the (M:E) 14:6, 10:10 and 2:18 (M:E) fractions showed significant activity against *P. aeruginosa, E. coli* and *Proteus mirabilis*. Extracts from *Meretrix casta* with 10:10 fractions showed significant activity against most of the pathogens tested. Similar antibacterial activity with (M:E) 10:10 fraction of *Tibia delicatula* and these results complementing the results of present study.[13] In this study extracts of *Tridacna maxima* with 18:2 and 2:18 (M:E) 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*. When compared to all solvents used, Ethanol, Methanol and Water elute the antibacterial bioactive compounds from the animals. The hypobranchial glands of *Chicoreus virgineus* and egg capsular of *Rapara rapiformis* extracted with polar solvents like ethanol and methanol also reported to show wide spectract activities. Lesser degree of inhibition by the column fractionated extracts in comparison to the crude could be opined that the active compound may have degraded or modified during the fractionation process.

In conclusion present study indicates the whole body extraction of *Meretrix casta* and *Tridacna maxima* would be a good source of antibacterial agents and would replace the existing inadequate and cost effective antibiotics. Further studies are needed to elucidate structure and mechanism of action of these marine molluscan extracts.

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