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Short Communication

Antibacterial activity of the winged oyster *Pteria chinensis* (Pterioida: Pteridae)

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The whole body extracts of the winged oyster, *Pteria chinensis* obtained with different solvents were assayed for antibacterial activity using agar well diffusion technique against human and fish pathogens. The acetone and chloroform crude extracts exhibited broad antibacterial activity. Highest activity was exhibited against *Klebsiella pneumoniae* (5 mm) and *Staphylococcus epidermidis* (5 mm) by the crude extract of acetone and against *Salmonella paratyphi* B (5 mm) by the chloroform extract. Similarly, the crude extract of chloroform was found to inhibit 8 out of 10 fish pathogens tested. The column-purified acetone fractions showed higher activity against *Klebsiella pneumoniae* (5 mm), *Sreptococcus pneumoniae* (4 mm), *Serratia marcescens* (4 mm) and *Proteus mirabilis* (4 mm). The MIC of the 100% acetone fraction was found to be lower for the pathogens, *S. marcescens* (100 µg) and *P. mirabilis* (150 µg) and hence 100% acetonated fraction of the extract of *P. chinensis* can be considered as potent antimicrobial compound against these pathogens.

[**Key words**: Marine natural products, antibacterial activity, *Pteria chinensis*, molluscs]
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The marine environment is a huge source for yet to be discovered bioactive natural products. Apart from the food that is derived from the marine environ, a wide variety of bioactive substances are being isolated and characterized, several with great promise for the treatment of human and fish diseases. For the past two decades, pharmaceutical industry has been relatively successful in overcoming problems due to single resistant determinants; however the advent of multiple resistant mechanism has severely limited the use of many major classes of antimicrobial compounds. The demand for effective and non-toxic antibacterial therapeutics has become even greater with the increased incidence of bacterial infections. Aquaculture has been the world's fast growing food production system for the past decade. On the same line, the impact of diseases in aquaculture is enormous. Therefore, there is a vital interest in discovering new antimicrobial compounds with fewer environmental and toxicological risks to which there is no resistance developed by the pathogens.

Molluscs in the oceans are a common sight and are virtually untapped resource for the discovery of novel compounds. Many studies have reported the bioactivity of the molluscs like *Aplysia* sp.[1], *Phyllidae* sp.[2], bivalves[3], gastropods[4] and their egg masses[5]. Bioactive metabolites from molluscs such as sea hare[6], *Chromodoris* sp.[7], *Onhidella* sp.[8], were isolated and structurally elucidated. The winged oyster, *Pteria chinensis*, is usually found attached to gorgonids and this bivalve was screened for antibacterial activity using the whole body tissue extracts obtained with different solvents.

Live specimens of oyster, *Pteria chinensis* (Pterioida: Pteridae) were collected at a depth of 6 m in Tuticorin coastal waters (lat, 8º45 and long. 78º13′E). They were immediately brought to the laboratory and their soft bodies were removed by breaking the shells. The meat was cut into small pieces, washed thoroughly with distilled water and air-dried. The air-dried meat of approximately 3 g was immersed separately in solvents like acetone, ethyl acetate, methanol, chloroform, butanol and toluene and cold steeped overnight at -18ºC. The extracts from each solvent were filtered separately for three times using Whatman No.1 filter paper. The filtrate was poured in previously weighed petriplates; evaporated to dryness in rotary evaporator and the dried extract was used for all the experiments. To test the antibacterial effect of the extracts, 12 human pathogens [*Salmonella paratyphi* B, *Pseudomonas aeruginosa* (ATCC 29336), *Citrobacter* sp, (ATCC 25405), *Klebsiella pneumoniae* (ATCC 10031), *Staphylococcus epidermidis* (ATCC 12228), *S. aureus* (ATCC 29737), *Shigella dysentriae* (ATCC 13313),

Streptococcus pneumoniae (ATCC 6301), *Vibrio cholerae* (ATCC 15748), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633) and *Enterobacter aerogenes* (ATCC 13048)] and 10 fish pathogens [*Vibrio parahaemolyticus* (ATCC 17802), *V. mimicus* (ATCC 33653), *V. logei* (shrimp isolate), *Serratia marcescens* (MTCC 97), *V. harveyi* (shrimp isolate), *V. vulnificus* (ATCC 27562), *Proteus mirabilis* (MTCC 1429), *V. ordalli* (fish isolate), *Aeromonas hydrophila* (ATCC 7966) and *Micrococcus* sp, (fish isolate)] were used as test strains. The strains were obtained from Christian Medical College (CMC), Vellore. All the test organisms were cultured in Tryptone Soya Broth (TSB) and the 18-24 h old cultures were used for the experiments. The antibacterial activity of the samples was assayed by the standard Nathan's Agar Well Diffusion (NAWD) technique[9] against the test strains on Tryptone Soya Agar (TSA) in petridishes with drilled wells of 6 mm diameter. A constant amount of 0.7 mg of the extract/50 µl (Dimethyl Sulfoxide) DMSO was loaded onto each well. The well at the center served as the control (without the extract). After 22-24 h of incubation at room temperature, the susceptibility of the test organisms was determined by measuring the radius of the zone of inhibition around each well. Partial purification of the extract was carried out following the method outlined by Wright[10]. After initial screening, the extract obtained with acetone was fractionated using normal phase silica gel column chromatography employing a step gradient solvent system from low to high polarity. The step gradient protocol used was: 100% acetone; 80% acetone and 20% heptane; 60% acetone and 40% heptane; 40% acetone and 60% heptane; 20% acetone and 80% heptane; 100% heptane; 80% heptane and 20% methanol; 60% heptane and 40% methanol; 40% heptane and 60% methanol; 20% heptane and 80% methanol and finally 100% methanol. The fractions thus obtained were once again evaporated, concentrated and assayed for antibacterial activity. Minimal inhibitory concentration (MIC) was determined by serially diluting the column purified extracts in DMSO so that concentrations of 100, 125, 150, 175, 200, 225, 250, 275, and 300 µg / 50 µL DMSO were loaded into each well for individual pathogenic strains that were found to be highly susceptible.

Table 1—Antibacterial activity of *Pteria chinensis* against A) human pathogens and B) fish pathogens

Pathogens	Zone of inhibition (mm)					
	A	EA	M	C	B	T
A) Human pathogens						
Salmonella paratyphi B	2	1	1	5	1	-
Pseudomonas aeruginosa	4	-	1	3	-	1
Citrobacter sp.	2	-	1.5	1	-	-
Klebsiella pneumoniae	5	1.5	-	4	-	-
Staplylococcus epidermidis	5	2	-	4	1	-
S.aureus	2	-	-	2	-	-
Shigella dysentriae	2	-	3	4	-	1
Streptococcus pneumoniae	4	2	-	1	-	-
Vibrio cholerae	4.5	-	2	4	1	-
Escherichia coli	2	2	4	4	-	-
Bacillus subtilis	2	-	-	2	-	-
Enterobacter aerogenes	4	-	-	3	1	1
B) Fish pathogens						
Vibrio parahaemolyticus	1.5	1	1	1	-	-
V. mimicus	2	-	-	1.5	-	-
V. logei	2.5	-	-	2.5	-	-
Serratia marcescens	6	1	1	4	-	-
V. harveyi	3	0.5	-	1.5	-	-
V. vulnificus	2.5	-	-	-	-	-
Proteus mirabilis	4	2.5	2	4	1	1
V. ordalli	3	-	-	-	-	-
Aeromonas hydrophila	5	2	3	4	0.5	-
Micrococcus sp.	4	2.5	2	3	-	-

A=Acetone; EA= Ethyl acetate; M= Methanol; C=Chloroform; B=Butanol T=Toluene

Out of the 6 solvents used, the extract obtained from acetone and chloroform exhibited higher activity and that obtained from acetone and chloroform exhibited higher activity and that obtained from toluene and butanol showed mild activities (Table 1 A). Of the 12 human pathogens tested, the acetone and chloroform extracts were able to inhibit all the pathogens exhibiting broad spectral antibacterial activity. Highest activity was exhibited against *Klebsiella pneumoniae* (5 mm) and *Staphylococcus epidermidis* (5 mm) by the extract of acetone and against *Salmonella paratyphi* B (5 mm) by the extract of chloroform. In the case of fish pathogens, acetone extract was found to have a broad spectral activity inhibiting all the test strains used and the extract of chloroform inhibited 8 pathogens (Table 1 B). Extracts obtained from ethyl acetate, methanol, butanol and toluene exhibited mild activities. Fractions obtained by column chromatography of the acetone phase of the tissue extracts exhibited broad spectral activity for both human and fish pathogens when eluted with 100% acetone (Table 2). Slightly lesser activity was shown by 80% acetone and 20% heptane fractions followed by 60% acetone and 40% heptane fractions (Table 2). Higher degree of inhibition was exhibited against *Klebsiella pneumoniae* (5 mm), *Streptococcus pneumoniae* (4 mm) and *Serratia marcescens* (4 mm) and *Proteus mirabilis* (4 mm) by the column fractions of 100% acetonated phase. Fractions in the methanolic phase showed little inhibition. Table 3 shows that the minimal inhibitory concentration (MIC) of the 100% acetone fraction was found to be lower for the pathogens *Serratia marcescens* (100 µg) and *Proteus mirabilis* (150 µg).

Pteria chinensis is sessile organism and the exact mechanism by which this organism acquires bioactive substances is not known. In the present investigation, higher degree of inhibition was confined to acetone phases indicating the substance involved in producing

Table 2—Antibacterial activity column purified fractions of *Pteria chinensis* in acetone, heptane and methanol

Pathogens	Zone of inhibition (mm)										
A) Human pathogens	A	80:20	60:40	40:60	20:80	H	80:20	60:40	40:60	20:80	M
Klebsiella pneumoniae	5	-	-	-	-	-	-	-	0.5	1	0.5
Staphylococcus epidermidis	3	2.5	2	-	-	-	-	-	1	1.5	1.5
Streptococcus pneumoniae	4	3	2	-	-	-	-	-	1	1	1
Vibrio cholerae	3	2	2	-	-	-	-	-	1.5	1.5	1.5
Escherichia coli	3	3	2	2	-	-	-	-	1	1	1
Enterobacter aerogenes	3	2.5	2	-	-	-	-	-	-	-	-
B) Fish pathogens											
Vibrio parahaemolyticus	3	3	2	2	-	-	-	-	-	1	1.5
Serratia marcescens	4	2	2	2	-	-	-	-	-	1	1.5
V. harveyi	3	3	2.5	-	-	-	-	-	1	1.5	1.5
Proteus mirabilis	4	2	3	-	-	-	-	-	-	0.5	-
Aeromonas hydrophila	2	2	2	2	-	-	-	-	-	-	-

A=Acetone, H=Heptane; M=Methanol

Table 3—Minimal inhibitory concentration (MIC) of the acetone fractions of *Pteria chinensis*

Pathogens	Minimal Inhibitory Concentration (MIC) in µg			
	Acetone: heptane			
(A) Human pathogens	100:0	80:20	60:40	40:60
Klebsiella pneumoniae	200	225	250	250
Staphylococcus epidermidis	200	250	275	225
Streptococcus pneumoniae	250	275	300	275
B) Fish pathogens				
Serratia marcescens	100	150	175	200
Proteus mirabilis	150	175	200	200
Aeromonas hydrophila	200	250	275	300

the antibacterial effect could be a medium-polar compound. But, the hypobranchial glands of *Chicoreus virgineus* and egg capsules of *Rapana rapiformis* extracted with polar solvents like ethanol and methanol also have been reported to show wide spectral antibacterial activities[5]. *Cypraea errones* was reported to have antibacterial activity at the non-polar end of the step gradient by the column-purified fractions[11]. 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[12]. In the present experiment, the minimal inhibitory concentration (MIC) of the fraction of 100% acetone was found to be lower for *Serratia marcescens* and *Proteus mirabilis* and so the extract of this particular fraction is now under the process of fractionation and purification, which can be possibly used as antimicrobial compounds against these pathogens.

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