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ANTIMICROBIAL ACTIVITY OF *PTERIA PENGUIN* AGAINST HUMAN PATHOGENS FROM THE SOUTHEAST COAST OF INDIA

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ABSTRACT

The penguin wing oyster *Pteria penguin* whole body extract was obtained with different solvents. The whole body extracts were assayed for antibacterial activity using agar well diffusion technique against human pathogens. The extracts derived from acetone and chloroform exhibited broad antibacterial activity. Highest activity was exhibited against *Klebsiella pneumoniae* (6 mm) and *Staphylococcus epidermidis* (5.5 mm) by the crude extract of acetone and against *Salmonella paratyphi* B (5.5 mm) by the chloroform extract. The column-purified acetone fractions showed higher activity against *Klebsiella pneumoniae* (6 mm), *Streptococcus pneumoniae* (4.5 mm). The MIC of the 100% acetone fraction was found to be equally lower for the pathogens *Klebsiella pneumoniae* (250µg) and *Staphylococcus epidermidis* (250µg). Therefore 100% acetonated fraction of the extract of *P. penguin* can be concluded as potent antimicrobial compound against these human pathogens.

KEY WORDS

Marine bioactive products, Antibacterial activity, *Pteria penguin,* Human pathogens

INTRODUCTION

Marine organisms represent a valuable source of new compounds. The biodiversity of the marine environment and the associated chemical diversity constitute a practically unlimited resource of new active substances in the field of the development of bioactive products. Marine environment provides different biosynthetic conditions to organisms that live in it. With marine species comprising approximately a half of the total global biodiversity, the sea offers an enormous resource for novel compounds, and it has been classified as the largest remaining reservoir of natural molecules to be evaluated for drug activity. The biological activity of an extract of marine organisms or isolated compounds could be assessed in several ways. Several marine bioactive compounds were isolated and characterized with better effect on treating diseasing agents. Biosynthesis of bioactive marine natural products provides many challenging problems[1]. Very different kinds of substances have been obtained from marine organisms among other reasons because they are living in a very exigent, competitive, and aggressive surrounding very different in many aspects from the terrestrial environment[2]. The massive use of antimicrobials for disease control and growth promotion in animals increases the selective pressure exerted on the natural emergence of bacterial resistance[3]. Many classes of bioactive compounds exhibiting antitumour, antileukemia, antibacterial and antiviral activities have been reported worldwide. The demand for effective and non toxic antibacterial therapeutics has become even greater with the increased incidence of bacterial infections[4]. So there is an urgent need for the discovery of new and novel antimicrobial drugs to effectively combat not only the drug resistance but also the new disease producers. Hence, the search for active drugs from alternative sources including marine environment, obviously becomes imperative.

Bivalvia (class) includes clams, oysters, mussels, scallops, and many other families of molluscs that have two hinged shells. The class was known for some time as Pelecypoda, which is a reference to the soft parts of the animal, whereas the name Bivalve simply describes the shell, which has two valves. *P.penguin* is byssally attached to the rocks, corals, gorgonians and other hard objects. It lives from low tide levels to a depth of 35 m[5]. This species are collected for food and pearl trade in Thailand and in the central Philippines.

MATERIALS AND METHODS

Pteria penguin (Pterioida: Pteridae) were collected at a depth of 10 - 12 m from Andaman waters. The valves of the collected samples were opened carefully and the whole body meat was removed. The removed meat was cut into small pieces, washed thoroughly with distilled water and air-dried. The air-dried meat of approximately 2-3 g was plunged separately in solvents like acetone, ethyl acetate, methanol, chloroform, butanol and toluene and cold stored at -18°C for 12 hrs. The drawn out of each solvent were filtered separately for three times using Whatman No.1 filter paper. The filtered extract was poured in weighed petriplates and evaporated to dryness in rotary evaporator[6]. The dried extract was used for all the experiments. To test the antibacterial effect of the extracts, 11 human pathogens *Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella paratyphi* B, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Shigella dysentriae*, *Streptococcus pneumoniae* and *Vibrio cholerae* were used as test strains. 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 against the test strains on Tryptone Soya Agar (TSA) in petridishes with drilled wells of 6 mm diameter[7]. A constant amount of 0.7 mg of the extract / 50 (Dimethyl Sulfoxide) DMSQ 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[8]. After initial screening, the extract obtained with acetone was fractionated using normal phase silica gel column chromatography employing a step gradient solvent system. 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% methanol; 20% heptane and 80% 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 g / 50 l DMSO were loaded into each well for individual pathogenic strains that were found to be highly susceptible.

RESULTS AND DISCUSSION

The extract obtained from acetone and chloroform shows greater activity and the extracts from toluene and butanol showed lesser activities. In the above experiment, the extracts from acetone and chloroform were able to inhibit all the human pathogens exhibiting broad spectral antibacterial activity. Highest activity was exhibited against *Klebsiella pneumoniae* (6 mm), *Staphylococcus epidermidis* (5.5 mm) and *Vibrio cholerae* (5.5 mm) by the extract of acetone and against *Salmonella paratyphi* B (5.5 mm) by the extract of chloroform. Fractions obtained by column chromatography of the acetone phase of the tissue extracts exhibited broad spectral activity for human pathogens when eluted with 100% acetone. Slightly lesser activity was shown by 80% acetone and 20% heptanes fractions followed by 60% acetone and 40% heptanes fractions (Table 2). Higher degree of inhibition was exhibited against *Klebsiella pneumoniae* (6 mm), *Streptococcus pneumoniae* (4.5 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 equally lower for the pathogens *Klebsiella pneumoniae* (250µg) and *Staphylococcus epidermidis* (250µg).

In the present study, higher degree of inhibition was confined to acetone phases indicating that the substance involved in producing 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[9]. But in contrast, the crude extract of *Chicoreus virgineus*, after antibacterial assay-guided elution, showed activity only in 100% methanol fraction[10]. The anti bacterial activity of a marine mollusc *Babylonia spirata* was screened against bacterial pathogens. Earlier studies indicated that the acetone extract of the winged oyster, *Pteria chinensis* was found to have a broad spectral activity inhibiting all the fish pathogenic strains tested and the extract of chloroform inhibited 8 pathogens[6]. The inhibitory action of the methanol fraction of *Perna* viridis was reported against bacterial and fungal strains. Four species of bivalves exhibit significant activity against *Bacillus subtilis* through methanol extract. The difference in antibacterial activity found in the molluscan extracts may depend on the solvents used for extraction and the compound extracted. 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[11]. The ethyl acetate extracts of *Trochus radiatus* was found to possess MIC values of 0.07 and 0.15 mg for *Proteus mirabilis* and *Serratia marcescens*, respectively[12]. The bioactive compounds of *Aplysia sp, Chromodoris* sp and *Onhidella sp* were isolated[13, 14].

Table 1
Antibacterial activity of Pteria penguin against human pathogens

Pathogens	Zone of inhibition (mm)					
	A	EA	M	C	B	T
Human pathogens						
Bacillus subtilis	2.5	-	1	2.5	1	-
Escherichia coli	3	2.5	5.5	5	-	-
Enterobacter aerogenes	5	1.5	-	3.5	2	1.5
Klebsiella pneumoniae	6	2	-	4.5	-	-
Pseudomonas aeruginosa	4.5	-	2	4	-	2
***Salmonella paratyphi* B**	3	1.5	2	5.5	1.5	-
Staphylococcus epidermidis	5.5	2.5	-	4.5	2	-
Staphylococcus aureus	2.5	-	1	3.5	-	-
Shigella dysentriae	2	-	3.5	5	-	2
Streptococcus pneumoniae	4.5	3	-	1.5	-	-
Vibrio cholera	5.5	1	2.5	4.5	1.5	-

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

Table 2
Antibacterial activity column purified fractions of Pteria penguin in acetone, heptanes and methanol

Pathogens	Zone of inhibition (mm)										
Human pathogens	**A**	**80:20**	**60:40**	**40:60**	**20:80**	**H**	**80:20**	**60:40**	**40:60**	**20:80**	**M**
Escherichia coli	3.5	3.5	3	2.5	-	-	-	-	1.5	1.5	2
Enterobacter aerogenes	4	3	2.5	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	6	-	-	-	-	-	-	-	1	1.5	1
Staplylococcus epidermidis	3.5	3	3	-	-	-	-	-	2	2	2
Streptococcus pneumoniae	4.5	3.5	2.5	-	-	-	-	-	1.5	1.5	2
Vibrio cholera	4	3	2.5	-	-	-	-	-	2	2	2

A=Acetone, H=Heptane; M=Methanol

Table 3
Minimal inhibitory concentration (MIC) of the acetone fractions of Pteria penguin

Pathogens	Minimal Inhibitory Concentration of *Pteria penguin* Acetone: Heptane			
(A) Human pathogens	100:0	80:20	60:40	40:60
Klebsiella pneumoniae	250	250	300	300
Staphylococcus epidermidis	250	275	325	275
Streptococcus pneumoniae	275	300	350	325

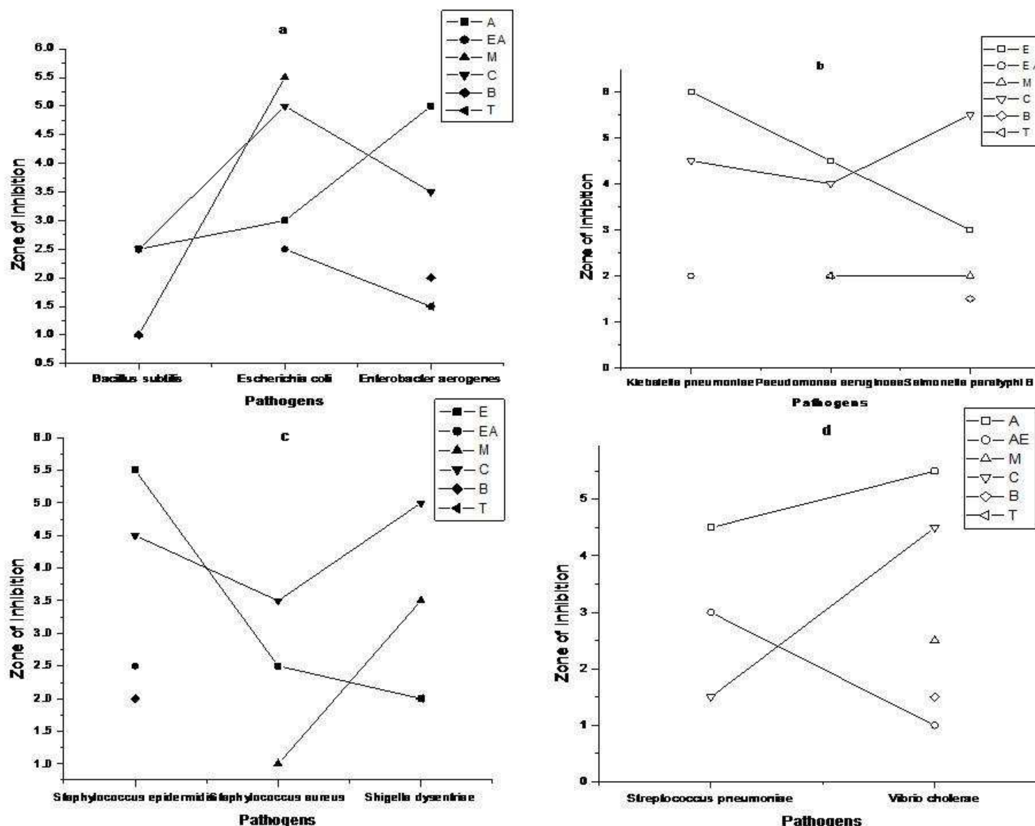

Figure . 1
Antibacterial activity of Pteria penguin against human pathogens. A Acetone; EA Ethyl acetate; M Methanol; C Chloroform; B Butanol T Toluene.

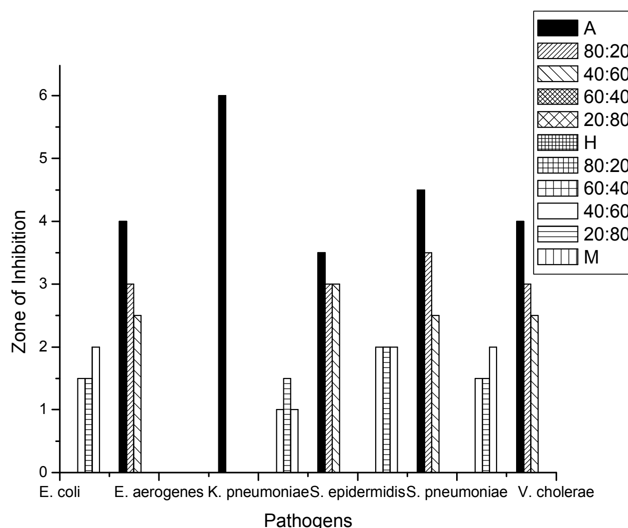

Figure. 2
Antibacterial activity column purified fractions of Pteria penguin in acetone, heptanes and methanol. A Acetone, H Heptane and M Methanol.

CONCLUSION

The active components were polar and 100% acetone washed most of it off the column. This fraction contains highest activity. With the increase of heptanes, which is pretty unpolar, active components are harder to be eluted out. So the activity is decreasing. When methanol was used as eluent, active components were washed out again although it is lower than the acetone fraction. In the present experiment, the minimal inhibitory concentration (MIC) of the fraction of 100% acetone was found to be lower for *Klebsiella pneumoniae*, *Staphylococcus epidermidis* (Table. 3) 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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