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Bioactive Potential of Coral Associated Gastropod, *Trochus tentorium* of Gulf of Mannar, Southeastern India

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Whole body meats of mollusc, *Trochus tentorium* was extracted with four solvents such as acetone, ethyl acetate, dichloromethane and methanol. Maximum antibacterial inhibition zone was exhibited from acetone crude extract of *T. tentorium* against human pathogen (*Streptococcus pneumoniae* 8 mm). The 100% column purified acetone fraction of *T. tentorium* showed higher activity against human pathogens like *Klebsiella pneumoniae* (7 mm). The minimal inhibitory concentration of the 100% acetone fraction of *T. tentorium* was found to be lower for the pathogens, *E. coli* (0.08 mg), *Streptococcus pnuemoniae* (0.08 mg), *Staphylococcus aureus* (0.09 mg) and *Vibrio cholerae* (0.09 mg), hence 100% acetone fraction of the extract of *T. tentorium* was deemed as potent bioactive compounds against those pathogens.

Key words: Gastropod, antibacterial activity, partial purification, MIC, Tuticorin

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INTRODUCTION

Most of the pathogens are increasingly resistant to the major classes of the routinely used antibiotics. In the late 1960s, the battle against the bacterial infections was considered won in the developed world at least. The reason was that earlier antibiotics could cure lethal infections in a matter of days. Many diseases were initially controlled almost exclusively by the use of antimicrobial drugs. 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 (Riguera, 1997). 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. So far, the majority of the natural products research has been focused on the shell-less snails and works on other classes of gastropoda remain sporadic.

Many studies on bioactive compounds from molluscs exhibiting antitumor, antileukemic and antiviral activities have been reported worldwide (Anand *et al*., 1997; Jayaseeli *et al*., 2001; Kagoo and Ayyakannu, 1992; Rajaganapathi *et al*., 2000). Several studies have reported the bioactivity of the gastropod, like *Drupa margariticola* (Chellaram and Edward, 2009) and reef sponge (Kelman *et al*., 2001) were isolated. The broad and cone shaped shell *Trochus tentorium* is found associated in abundance with live or dead corals along the Tuticorin coast has not been studied for biomedical potential. So, this prosobranchiate mollusc was chosen for the present study with an objective to explore the antibacterial potentiality of its whole body extracts against human pathogens.

MATERIALS AND METHODS

Study area: The Molluskan samples were collected by hand picking using SCUBA diving from the intertidal area at a depth of 5-7 m in Tuticorin coastal waters (Lat 8°45 and Long 78°13'E) of Southeast coast of India. The work was carried out at Suganthi Devadason Marine Research Institute, Tuticorin, India, during June-2007 to April-2008 and one of the author is professionally trained SCUBA diver.

Extraction of molluscs: The Molluscan sample was collected using SCUBA diving from the intertidal area at a depth of 5-10 m. The whole body of the sample (20 g) was cut in to small pieces and air-dried for 24 h at room temperature before extraction with solvents. Then the tissues were rinsed with sterile distilled water and the tissue sample was used for extraction using different solvents such as ethyl acetate, acetone, dichloromethane and methanol. The extracts were cold steeped overnight at -18°C and filtered with Whatman No. 1 filter paper. The filtrate was poured in previously weighed Petri plate and evaporated to dryness in rotary evaporator (Becerro *et al*., 1994; Wright, 1998). The dried crude extracts were used for antibacterial assay against human pathogens.

Antibacterial assay: The antibacterial effect of the extracts was assayed using 15 human pathogens. 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 following the standard Nathan's Agar Well Diffusion (NAWD) technique (Nathan *et al*., 1978). A constant amount of 40 mg of *T. tentorium* was dissolved in 5 mL of Dimethyl Sulfoxide (DMSO) and from this 50 μL DMSO containing 0.4 mg of extracts was loaded onto each well. Wells without the extracts were maintained as control. After 20-24 h of incubation at 37°C, the susceptibility of the test organisms was determined by measuring the diameter of the zone of inhibition around each well to the nearest mm.

Column purification of the active crude extracts: After initial screening, the higher activity was shown by acetone extract and it was fractionated by normal phase silica gel column chromatography by employing a step gradient solvent system from low to high polarity. Sequence of 100% hexane; 20% acetone: 80% hexane, 40% acetone: 60% hexane, 60% acetone: 40% hexane, 80% acetone: 20% hexane, 100% acetone, 80% acetone: 20% methanol, 60% acetone: 40% methanol, 40% acetone: 60% methanol, 20% acetone: 80% methanol and 100% methanol was used for elution. Each fraction thus obtained was once again evaporated, concentrated and assayed for antibacterial activity.

Determination of Minimal Inhibitory Concentration (MIC): Minimal inhibitory concentration was determined by serially diluting the column purified extracts of *T. tentorium* in DMSO so that the concentration of 250, 200, 150, 100 and 50 μg/50 μL extracts were loaded in to each well for the individual pathogenic strains and controls were maintained simultaneously without the extracts used for the experiment.

RESULTS AND DISCUSSION

Antibacterial activity of crude extracts: Four extracts of gastropod was tested against 15 human pathogens. The results of the screening test are summarized in Table 1. In

Table 1: Antibacterial activity of crude extracts of coral reef associated gastropod *Trochus tentorium* against human pathogens

	Radius of the zone of inhibition (mm)			
Pathogens	EA	A	DCM	ME
Escherichia coli	7.0	7.0	7.0	4.0
Shigella dysentriae	6.0	7.0	5.0	3.0
Staphylococcus epidermidis	6.0	7.0	4.0	3.0
S. aureus	5.5	6.5	4.5	3.5
Klebsiella pneumoniae	6.0	7.0	6.0	4.0
Pseudomonas aerogenosa	5.0	6.0	5.0	4.0
Salmonella typhimurium	5.0	5.5	4.5	3.0
S. paratyphi	4.5	5.0	4.0	2.5
Vibrio cholerae	6.0	7.0	6.0	5.0
Strptococcus pneumoniae	7.0	8.0	7.0	4.0
S. faecalis	5.5	6.5	4.5	3.0
Bacillus subtilis	5.0	6.0	4.5	2.5
B. cereus	6.0	7.0	4.0	3.0
Enterobacter aerogenes	5.0	5.5	4.5	3.0
Citrobacter sp.	4.5	5.0	4.0	3.0

A: Acetone; EA: Ethyl acetate; DCM: Dichloromethane, ME: Methanol

Table 2: Antibacterial activity of the column purified fractions of *Trochus tentorium* against human pathogens

	Radius of the zone of inhibition (mm)										
Pathogens	H	80:20	60:40	40:60	20:80	A	80:20	60:40	40:60	20:20	ME
E. coli	-	T	T	2.5	3	6	5	4	3	2	T
S. dysentriae	-	-	T	T	2	5.5	4	2.5	2	T	T
S. epidermidis	-	-	T	2	2.5	6	4.5	3	2.5	2	T
S. aureus	-	-	2	2	3	5	3	2	2	T	T
K. pneumoniae	-	T	-	T	2.5	7	6	5	2	-	2
P. aerogenosa	-	-	2	T	2.5	5	4	3.5	2	2	T
S. typhimurium	-	-	2	2	3	4.5	3.5	2.5	2	-	T
S. paratyphi	-	T	T	T	2	5.5	4	4	3	2	T
V. cholerae	-	T	T	2	2	6	3.5	3	2.5	2	T
S. pneumoniae	-	-	2	T	2.5	5.5	4	2.5	2	2	T
S. faecalis	-	-	2	2	2.5	5	3	3	2	T	2
Bacillus subtilis	-	-	T	2	3	4.5	4	3	2.5	2	T
B. cereus	-	-	2	2.5	3.5	4	3	2	T	-	-
E. aerogenes	-	-	2	2	3	5	3	2.5	T	-	-
Citrobacter sp.	-	-	-	2.5	3	5.5	2.5	2	T	-	T

A: Acetone; H: Hexane; ME: Methanol; T: Trace

the present study, prominent antibacterial activity was conferred by the acetone extract. Of the 4 solvents used, the acetone extracts of *Trochus tentorium* was able to inhibit *Streptococcus pneumoniae* with a maximum zone of 8 mm at concentration of 0.4 mg followed by *E. coli*, *Shigella dysendriae*, *Staphylococus epidermidis*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *B. cereus* with zone of 7 mm each and *Staphylococcus aureus* and *Streptococcus faecalis* with zone of 6.5 mm. On other hand, extracts of ethyl acetate and dichloromethane were able to produce a zone of 7 mm against *E. coli* and zone of 6 mm was produced against *Shigella dysendriae*. *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *B. cereus* by ethyl acetate extracts. However, only slight activity was shown by the crude extracts of methanol.

Antibacterial activity of the column purified extracts: Fractions obtained by column chromatography of the tissue extracts *T. tentorium* exhibited broad spectral activity against human pathogens, when eluted with 100% acetone. Slightly lesser activity was shown by 80% acetone: 20% hexane fractions followed by 60% acetone: 40% hexane fractions. It was noted that the 100% acetone fraction of *T. tentorium* was effective in inhibiting *E. coli* (6 m), *Staphylococcus epidermidis* (6 m), *Klebiella pneumoniae* (7 m) and *V. cholerae* (6 m) at the concentration of 0.5 mg (Table 2).

Minimal Inhibitory Concentration (MIC): Table 3 showed the minimal inhibitory concentration (MIC) of the column fractions of *T. tentorium*. MIC was found to be lower for the human pathogens, *E. coli* (0.082 mg), *Streptococcus pneumoniae* (0.08 mg).

There is growing interest in marine natural products or marine secondary metabolites. This field of research receives the attention of investigators from various fields such as marine biology, marine ecology, biochemistry, chemistry, pharmacology and biotechnology. In the industrialized countries, about 25% of all prescription

Table 3: Minimal Inhibitory Concentration (MIC) of the column purified fractions of *Trochus tentorium* against human pathogens

	Minimal inhibitory concentration (mg)										
Pathogens	H	80:20	60:40	40:60	20:80	A	80:20	60:40	40:60	20:80	ME
Escherichia coli	-	0.45	0.45	0.38	0.35	0.08	0.20	0.25	0.35	0.40	0.45
Shigella dysendriae	-	-	0.45	0.45	0.40	0.12	0.25	0.38	0.40	0.45	0.45
Staphylococcus epidermidis	-	-	0.45	0.40	0.38	0.09	0.25	0.35	0.38	0.40	0.45
S. aureus	-	-	0.40	0.40	0.33	0.13	0.35	0.40	0.40	-	0.45
Klebsiella pneuminiae	-	0.45	-	0.45	0.40	0.12	0.18	0.20	0.38	0.40	0.40
Pseudomonas aerogenosa	-	-	0.40	0.45	0.38	0.13	0.25	0.28	0.40	-	0.45
Salmonella typhimurium	-	-	0.40	0.40	0.33	0.12	0.30	0.38	0.40	0.40	0.45
S. paratyphi	-	0.45	0.45	0.45	0.40	0.12	0.25	0.25	0.38	0.40	0.45
Vibrio cholerae	-	0.45	-	0.40	0.40	0.09	0.30	0.35	0.35	0.40	0.45
Strptococcus pneumoniae	-	-	0.40	0.45	0.38	0.08	0.25	0.38	0.38	0.40	0.45
S. faecalis	-	-	0.40	0.40	0.38	0.10	0.35	0.25	0.40	0.45	0.40
Bacillus subtilis	-	-	0.40	0.40	0.33	0.10	0.25	0.25	0.40	0.40	0.45
B. cereus	-	-	0.40	0.38	0.30	0.12	0.35	0.40	0.45	-	-
Enterobacter aerogenes	-	-	0.40	0.40	0.33	0.10	0.35	0.38	0.45	-	0.45
Citrobacter sp.	-	-	-	0.38	0.33	0.12	0.38	0.40	0.45	-	-

A: Acetate: Hexane; M: Methanol

drugs contain active principles that are still extracted from higher plants. In traditional Indian medicine, especially Sidha medical preparations, the opercula of gastropods are used as an ingredient to combat different diseases. *Trochus tentorium* are benthic organisms and the exact mechanism by which this organism acquires bioactivity substances is not known.

In the present investigation, higher degree of inhibition was confined to acetone phase indicating the substances involved in producing the antibacterial effect could be a medium popular compound. Out of the 6 solvents used in mollusk, *Drupa margariticola*, the extract obtained from acetone exhibited higher antibacterial activity against human pathogens (Chellaram and Edward, 2009), which is in agreement with the present work. Sureshkumar *et al*. (2002) observed that the acetone extracts of different seaweeds showed antibacterial properties against human pathogens. On the contrary, Anand and Edward (2002) noted that the crude methanol extracts of *Cypraea errones* exhibited promising results for antibacterial and antifungal activity. 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 (Anand *et al*., 1997).

The one hundred percent acetone column purified fractions were found to possess highest antibacterial activity. The clear zone of 7 mm was shown by the 100% acetone column purified fractions of *T. tentorium* against *Klebiella pneumoniae* (7 mm) and *Proteus mirabilis* (7 mm) at concentration of 0.225 mg. But in contrast, the crude extract of *Chicoreus virgineus*, after antibacterial assay-guided elution, showed activity only in 100% methanol fraction (Ramasamy and Murugan, 2005). From the comparatively lesser inhibition by the column fractionated extracts to the crude, it could be opined that the active compound may have degraded or modified during the fractionation process. The Minimal Inhibitory Concentration (MIC) was found to be lower for the 100% acetone phases of *T. tentorium* (0.08 mg) for *E. coli*. However, MIC values of 20 μg mL^{-1} were recorded for the metabolites of soft corals, *Caldiella* sp. and *Sinularia* sp. for human pathogenic bacteria. 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 (Chellaram *et al*., 2004). 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 (Gnanambal *et al*., 2005). In conclusion, the present study shows that gastropod, *Trochus tentorium* contain factors with broad spectral antibacterial activity with minimal concentration against human pathogens.

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