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Screening of antibacterial drugs from marine gastropod *Chicoreus ramosus* (Linnaeus, 1758)

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PEER REVIEW

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Comments

Numerous pathogenic microorganisms have developed their resistance against commonly available antibiotics; hence the need for developing new virulent drugs against these harmful pathogens becomes more important. Chemical drugs may lead to adverse effects and researchers now have focused on pharmacologically active compounds from natural sources. The present study revealed that the acetone and chloroform extract of both the tissues and egg inhibited the growth of the tested pathogenic bacterial strains. The MIC of both extract ranged from 4 to 12 mg/mL. These results suggest that marine gastropods tissue and egg extract contains comparatively good antibacterial activity.

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ABSTRACT

Objective: To screen the antibacterial drugs from different solvent extracts of tissue and egg of marine gastropods *Chicoreus ramosus* against clinically isolated human pathogenic bacteria.

Methods: Different solvent extracts of *Chicoreus ramosus* was screened for their activity against *Vibrio parahaemolyticus* (J13300), *Aeromonas hydrophilla* (IDH1585), *Salmonella typhi* (C6953), *Salmonella paratyphi A* (C6915), *Vibrio cholerae* (IDH5439) and *Escherichia coli* (H10407) using standard well diffusion method and its minimum inhibitory concentration.

Results: The study revealed that the acetone and chloroform extract of both the tissues and egg inhibited the growth of the tested pathogenic bacterial strains. The minimum inhibitory concentration of both the extract ranged from 4 to 12 mg/mL.

Conclusions: These results suggest that marine gastropods tissue and egg extract contains comparatively good antibacterial activity.

KEYWORDS

Antibacterial, Drugs, Marine gastropods, *Chicoreus ramosus*

1. Introduction

Marine organisms comprise approximately a half of the total

biodiversity, thus offering infinite source to discover useful therapeutics. In recent years, a significant number of novel metabolites with potent pharmacological properties have been

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discovered from marine organisms. The phylum Mollusca, which includes soft-bodied invertebrates, the second largest phylum in the animal kingdom makes up a major part of the world's marine invertebrate fauna. Molluscs are the most successful invertebrate group in occupying different habitats. These are the commonest organisms of Indian sea beaches and distributed all over the world in almost all types of habitats. The molluscs have received a considerable amount of research effort, reflecting both their ecological and economic importance, and now gaining importance in deriving drugs^[1].

The first attempt to the screening of antimicrobial activity in marine organisms was initiated around 1950's. Since this time large numbers of marine organisms from a wide range of phyla have been screened for antimicrobial activity^[2]. From 1960's to 1990's approximately 300 bioactive marine natural products were fields of patent. Approximately 6 500 bioactive compounds were isolated from marine organisms. Among the invertebrates, the mollusks are highly delicious seafood because of their nutritive value next to fin fish and crustaceans. They are also a very good source for biometrically important products^[3]. Many classes of molluscs with bioactive compounds exhibiting antitumor, antileukemic, antibacterial and antiviral properties have been reported worldwide^[4]. Many marine molluscs have evolved chemical defense mechanism for their eggs and thus producing secondary metabolites which possess antimicrobial activities^[5].

The marine environment is a huge source for discovering many antibacterial drugs. Apart from the food that is derived from the marine environment, wide varieties of antibacterial drugs are being isolated and characterized with great promise for the treatment of human diseases. Studies on antibacterial screening provide valuable information for new antibiotic discoveries and give new insights into the extract of bioactive compounds from marine molluscs. In the present investigation, an attempt has been made to screen the antibacterial drugs from marine molluscs *Chicoreus ramosus* (*C. ramosus*) against clinically isolated human pathogenic bacteria.

2. Materials and methods

2.1. Sampling and preprocessing

The animals were collected from Tuticorin coast located at southeast coast of Tamil Nadu in the Gulf of Mannar region, which is situated between India and Sri Lanka (latitude 8°48'N and longitude 78°09'E). The tissue and egg samples were washed with tap water until the sand and mud were removed from the shells. After that, the shells were broken using a hammer to remove the soft body tissue. The removed tissues were rinsed with sterile distilled water, cut into small pieces and kept in Petri dishes and dried at a constant temperature of 50 °C for 24 h in a hot air oven. The dried material was powdered thoroughly for solvent extraction.

2.2. Solvent extraction

The powdered tissues and egg mass of *C. ramosus* were extracted with eight different solvents like methanol, ethanol, acetone, acetonitrile, dichloro methane (DCM), chloroform, ethyl acetate and distilled water with the help of soxlet apparatus, and the solvents were concentrated by rotary evaporator (VC100A Lark Rota vapor[®] at 30 °C) with reduced pressure to give a dark brown gummy mass. The resultant residues were stored at 4 °C for further antibacterial screening.

2.3. Bacterial cultures

Six species of bacteria were used for screening the antibacterial activity, including *Vibrio parahaemolyticus* (*V. parahaemolyticus*) (J13300), *Aeromonas hydrophilla* (*A. hydrophilla*) (IDH1585), *Salmonella typhi* (*S. typhi*) (C6953), *Salmonella paratyphi A* (*S. paratyphi A*) (C6915), *Vibrio cholerae* (*V. cholerae*) (IDH5439) and *Escherichia coli* (*E. coli*) (H10407). All the bacterial strains were clinical isolates, obtained from the Microbial Type Culture Collection & the Gene Bank, Institute of microbial technology, Chandigarh, India.

2.4. Inoculums preparation for bacteria

Nutrient broth was prepared and sterilized in an autoclave at 15 pounds pressure for 15 min. All the six bacterial strains were individually inoculated in the sterilized nutrient broth and incubated at 37 °C for 24 h. Mueller Hinton agar (Himedia) was prepared, sterilized in an autoclave at 15 pounds pressure for 15 min and poured into sterile Petri dishes and incubated at 37 °C for 24 h. The 24 hours old bacterial broth cultures were inoculated in the Petri dishes by using a sterile cotton swab.

2.5. Antibacterial screening

The antibacterial screening was investigated against six human pathogenic bacteria by agar well diffusion method followed Ramasamy *et al*^[6]. Twenty four hours old nutrient broth cultures of test bacteria was aseptically swabbed on sterile Mueller Hinton agar plates. Wells of 5 mm in diameter were made aseptically using well cutter, and 50 µL of eight different solvent extracts of tissues and eggs were inoculated. The stock solutions were prepared at a concentration of 20 mg/mL. Positive control well containing 50 µL of tetracycline (1 mg/mL) and negative control containing 50 µL of appropriate solvents were used. The result was calculated by measuring the zone of inhibition in millimeters. For each concentration tested, triplicates were maintained for the confirmation of activity.

2.6. Determination of the minimum inhibitory concentration (MIC)

The solvent extracts of marine gastropods *C. ramosus* which showed significant antibacterial activity were selected for the

determination of MIC followed by the method of Ramasamy *et al*[7]. A stock solution of 20 mg/mL was prepared and serially diluted to obtain various ranges of concentrations between 4 mg/mL and 20 mg/mL. About 0.5 mL of each dilution of different concentrations was transferred into a sterile test tube containing 2 mL of nutrient broth. To the test tubes, 0.5 mL of test organism previously adjusted to a concentration of 10⁵ cells/mL was then introduced. A set of test tubes containing broth alone was used as a control. All the test tubes and control were then incubated at 37 °C for 24 h. After the period of incubation, the tube containing the least concentration of extract showing no visible sign of growth was taken as the minimum inhibitory concentration.

2.7. Statistical analysis

Data on the inhibitory effects of solvent extracts of *C. ramosus* was analyzed by One-way analysis of variance (ANOVA) using SPSS-16 version software followed by Duncun's multiple range test and standard errors±SEM. *P*<0.05 were considered for describing the significant levels.

3. Results

3.1. Antibacterial screening

The inhibition zone in different solvent tissue extracts of *C. ramosus* against clinical isolate human pathogenic bacteria was shown in Table 1. Among the various strains, the maximum zone of inhibition [26.00±1.53 mm] was recorded in acetone extracts against *S. paratyphi* A, followed by (25.00±1.53) mm in *E. coli* strain and the minimum zone of inhibition (7 mm) was noticed

in ethanol, petroleum ether, chloroform and water extracts. In that similar way the inhibition zone in different solvent egg extracts of *C. ramosus* against clinical isolate human pathogenic bacteria was described in Table 1. Among the various strains, the maximum zone of inhibition [26.00±1.53 mm] was recorded in acetone extracts against *V. cholerae*, followed by (25.00±1.53) mm in *E. coli* strain and the minimum zone of inhibition (7mm) was noticed in DCM and acetonitrile. The positive control (tetracycline) was active against all the bacterial strains tested.

3.2. MIC of the active extract against the test organisms

MIC values of acetone tissue and egg extracts against bacterial strains such as *A. hydrophilla*, *S. typhi*, *S. paratyphi* A, *V. cholerae* and *E. coli* were reported as 12, 12, 8, 8, 4 mg/mL and 8, 8, 12, 4, 4 mg/mL respectively. Likewise in chloroform tissue extracts against bacterial strains such as *A. hydrophilla*, *S. typhi*, *S. paratyphi* A and *V. cholerae* was reported as 20, 20, 20 and 20 mg/mL respectively. The chloroform egg extracts against bacterial strains such as *A. hydrophilla*, *S. typhi*, *S. paratyphi* A, *V. cholerae* and *E. coli* were reported as 16, 12, 16, 16 and 20 mg/mL respectively.

4. Discussion

Numerous pathogenic microorganisms have developed their resistance to commonly available antibiotics; hence the need for developing new virulent drugs against these harmful pathogens becomes more important. Chemical drugs may lead to adverse effects and recent researchers have focused on pharmacologically active compounds from natural sources. Marine organisms contain many undiscovered bioactive

Table 1

Antibacterial activity of tissue and egg extracts of *C. ramosus* against clinically isolated human pathogens.

Bacterial strains/solvents	Methanol (mm)		Ethanol (mm)		Acetone (mm)		DCM (mm)		Petroleum ether (mm)		Acetonitrile (mm)		Chloroform (mm)		Water (mm)	
	Tissue	Egg	Tissue	Egg	Tissue	Egg	Tissue	Egg	Tissue	Egg	Tissue	Egg	Tissue	Egg	Tissue	Egg
<i>V. parahaemolyticus</i>	-	-	-	-	-	-	-	7.00±0.58	-	-	-	-	-	-	-	-
<i>A. hydrophilla</i>	-	-	8.00±0.52	-	16.00±0.82	21.00±1.25	-	-	7.00±0.58	-	-	-	8.00±0.58	14.00±0.82	-	-
<i>S. typhi</i>	-	-	-	10.00±0.58	18.00±0.82	20.00±1.25	-	-	7.00±0.52	-	-	7.00±0.58	8.00±0.58	15.00±1.25	-	-
<i>S. paratyphi</i> A	-	-	8.00±0.58	11.00±0.82	26.00±1.53	22.00±1.25	9.00±0.58	7.00±0.58	9.00±0.58	-	-	7.00±0.52	7.00±0.58	14.00±0.82	7.00±0.58	-
<i>V. cholerae</i>	-	-	7.00±0.52	9.00±0.58	20.00±1.25	26.00±1.53	-	-	-	-	-	-	7.00±0.58	11.00±0.82	-	-
<i>E. coli</i>	-	-	9.00±0.58	12.00±0.82	25.00±1.53	25.00±1.53	-	-	11.00±0.58	-	-	7.00±0.58	-	9.00±0.58	-	-

Table 2

MIC of tissue and egg extracts of *C. ramosus* against clinically isolated human pathogens.

Bacterial strains/solvents	Acetone extract										Chloroform extract									
	Tissue (mg/mL)					Egg (mg/mL)					Tissue (mg/mL)					Egg (mg/mL)				
	20	16	12	8	4	20	16	12	8	4	20	16	12	8	4	20	16	12	8	4
<i>V. parahaemolyticus</i>	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>A. hydrophilla</i>	-	-	*	+	++	-	-	-	*	+	*	+	++	+++	+++	-	*	+	++	+++
<i>S. typhi</i>	-	-	*	+	++	-	-	-	*	+	*	+	++	+++	+++	-	-	*	+	++
<i>S. paratyphi</i> A	-	-	-	*	+	-	-	*	+	++	*	+	++	+++	+++	-	*	+	++	+++
<i>V. cholerae</i>	-	-	-	*	+	-	-	-	*	+	*	+	++	+++	+++	-	*	+	++	+++
<i>E. coli</i>	-	-	-	-	*	-	-	-	*	+	+++	+++	+++	+++	+++	*	+	++	+++	+++

* MIC concentration; - No growth; + Cloudy solution (slight growth); ++ Turbid solution (strong growth); +++ Highly turbid solution (dense growth).

compounds; the number of new active compounds isolated from marine organisms are estimated at 10000[8]. Molluscs are considered as one of the important natural sources to derive many bioactive compounds that exhibit antitumor, antimicrobial, anti-inflammatory and antioxidant activities[9]. Molluscs also contain highly rich nutrients, which are beneficial to humans of all ages[10]. Compounds isolated from marine molluscs were also used in the treatment of rheumatoid arthritis and osteoarthritis[11]. Marine mollusc extracts also exhibited antibacterial and antiviral activity against fish pathogenic bacteria and the extract also may be applied in aquaculture[12].

In the present investigation among the various strains tested maximum zone of inhibition [(26.00±1.53) mm] was recorded in acetone extracts against *S. paratyphi* A and minimum zone of inhibition (7 mm) was noticed in ethanol, petroleum ether, chloroform and water extracts. In egg the maximum zone of inhibition [(26±1.53) mm] was recorded in acetone extracts against *V. cholera*, and minimum zone of inhibition (7mm) was noticed in DCM and acetonitrile. Similar observation was made by Suresh *et al*[13]. *Lactobacillus vulagaris*, *Pseudomonas aeruginosa* and *S. typhi* were resistant to a crude methanolic extract of gastropod *Hemifusus pugilinus* with highest activity against *E. coli* (6 mm), followed by *Klebsiella pneumoniae* (4 mm), and the lowest activity against *S. paratyphi* (1 mm), *V. paraheamolyticus* (1 mm).

Suresh *et al.* also reported that the maximum inhibition zone [(10.13±0.13) mm] was observed against *Klebsiella pneumonia* in the ethanol extract of *Babylonia zeylanica* and the minimum inhibition zone (1 mm) was observed against *V. cholerae*[13]. In the case of *Harpa conoidalis*, the maximum inhibition zone [(9.16±0.13) mm] was observed against *S. paratyphi* in ethanol extract and the minimum inhibition zone [(1.03±0.05) mm] was noticed against *Proteus mirabilis*. In the similar way *Babylonia spirata* exhibited the antibacterial activity of ethanol, acetone, methanol, chloroform and water extracts; the maximum inhibition zone (12 mm) was observed against *Pseudomonas aeruginosa* in the crude ethanol extract of *Babylonia spirata* and the minimum inhibition zone (2 mm) was noticed against *Staphylococcus aureus* bacterial strains[14].

The hypobranchial gland extracts of *C. ramosus* was found to inhibit the growth of bacterial strains; among these the broad inhibition zone was formed against *Streptococcus faecalis* and *Staphylococcus aureus*[15]. The ethanol extracts of hypobranchial gland of *Chicoreus virginicus* showed 10 mm of inhibition zone against *S. typhi*, 7 mm against *Shigella flexineri*, 6 mm against *V. cholerae*, 5 mm against *Klebsiella pneumoniae* and 4 mm against *Bacillus subtilis* and *E. coli*, but methanolic extracts exhibited inhibition against *Streptococcus pyogenes*[16]. Although different species and experimental procedure were followed in different studies, they indicated the high degree of antimicrobial activity in marine molluscs. These results encourage the idea that marine molluscs are potent sources for antibacterial drug development.

MIC methods are widely used in the comparative testing of

new drugs. In clinical laboratories they are used to establish the susceptibility of organisms that give equivocal results in disk or well tests, for tests on organisms where disk or well tests may be unreliable and when a more accurate result is required for clinical management. The present study MIC values of acetone tissue and egg extracts against bacterial strains such as *A. hydrophilla*, *S. typhi*, *S. paratyphi* A, *V. cholerae* and *E.coli* were reported as 12, 12, 8, 8, 4 mg/mL and 8, 8, 12, 4, 4 mg/mL respectively. Likewise in chloroform tissue extracts against bacterial strains such as *A. hydrophilla*, *S. typhi*, *S. paratyphi* A and *V. cholerae* was reported as 20, 20, 20 and 20 mg/mL respectively. The chloroform egg extracts against bacterial strains such as *A. hydrophilla*, *S. typhi*, *S. paratyphi* A, *V. cholerae* and *E. coli* were reported as 16, 12, 16, 16 and 20 mg/mL respectively. The study revealed that the acetone and chloroform extract of both the tissues and egg inhibited the growth of the tested pathogenic bacterial strains. Hence the present study indicated that the *C. ramosus* extracts contain compounds with the broad antibacterial activity. However, further investigations involving purification of the active extracts as drugs for humans are needed.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The marine environment is a huge source for discovering many antimicrobial drugs. Apart from the food that is derived from the marine environment, wide varieties of antimicrobial drugs are being isolated and characterized with great promise for the treatment of human diseases. Studies on antimicrobial screening provide valuable information for new antibiotic discoveries and give new insights into the extraction of bioactive compounds from marine molluscs.

Research frontiers

To screen the antibacterial drugs from different solvent extraction of tissue and egg of marine gastropods *C. ramosus*

against clinically isolated human pathogenic bacteria like *V. parahaemolyticus* (J13300), *A. hydrophilla* (IDH1585), *S. typhi* (C6953), *S. paratyphi A* (C6915), *V. cholerae* (IDH5439) and *E. coli* (H10407).

Related reports

Marine molluscs contain many undiscovered bioactive compounds; the number of new active compounds isolated from marine organisms is estimated at 10 000. Molluscs are considered as one of the important natural sources to derive many bioactive compounds that exhibit antitumor, antimicrobial, anti-inflammatory and antioxidant activities. Compounds isolated from marine molluscs were also used in the treatment of rheumatoid arthritis and osteoarthritis. Marine mollusc extracts also exhibited antibacterial and antiviral activity against fish pathogenic bacteria and the extract also may be applied in aquaculture.

Innovations and breakthroughs

The innovative outcome of this research paper is to screen the antibacterial drugs in eight different solvent extractions of tissue and egg of marine molluscs.

Applications

The maximum zone of inhibition [26 ± 1.53 mm] was recorded in acetone extracts against *S. paratyphi A* and the minimum zone of inhibition (7mm) was noticed in ethanol, petroleum ether, chloroform and water extracts. In egg the maximum zone of inhibition [26 ± 1.53 mm] was recorded in acetone extracts against *V. cholera* and minimum zone of inhibition (7 mm) was noticed in DCM and acetonitrile.

Peer review

Numerous pathogenic microorganisms have developed their resistance against commonly available antibiotics; hence the need for developing new virulent drugs against these harmful pathogens becomes more important. Chemical drugs may lead to adverse effects and researchers now have focused on pharmacologically active compounds from natural sources. The present study revealed that the acetone and chloroform extract of both the tissues and egg inhibited the growth of the tested pathogenic bacterial strains. The MIC of both extracts ranged from 4 to 12 mg/mL. These results suggest that marine gastropods tissue and egg extract contains comparatively good antibacterial activity.

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