



Original article

doi: 10.12980/jclm.4.2016J6-86

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Bioactive potential of some economically important marine gastropods along the Gulf of Mannar region, southeast coast of India

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ARTICLE INFO

Article history:

Received 29 Apr 2016

Received in revised form 17 May, 2nd

revised form 31 May 2016

Accepted 15 Jun 2016

Available online 5 Aug 2016

Keywords:

Marine gastropods

Antimicrobial

Antioxidant

Cytotoxicity

Gulf of Mannar

Southeast coast of India

ABSTRACT

Objective: To analyse the economically important gastropods for prospective antimicrobial, antioxidant and cytotoxic activities from the Gulf of Mannar region, southeast coast of India.

Methods: The bioactive potential of some gastropods *i.e.* *Babylonia spirata* (*B. spirata*), *Phalium glaucum*, *Tonna dolium*, *Hemifusus pugilinus*, *Xancus pyrum*, *Chicoreus ramosus* (*C. ramosus*), *Harpa articularis*, *Ficus ficus* and *Babylonia zeylanica* were analysed. Antimicrobial activity was carried out against 8 human pathogenic bacteria and 3 fungal strains by well diffusion method. Antioxidant and cytotoxic activities were analyzed by standard methods.

Results: In antibacterial and antifungal activities, methanolic extract of *B. spirata* significantly showed the highest inhibition zone against *Aeromonas hydrophila* and *Fusarium* spp. ($P > 0.05$). In the total antioxidant activity, the maximum activity was observed in *B. spirata* (510 µg/mg) and in the 1,1-diphenyl-2-picrylhydrazyl scavenging activity, *B. spirata* showed the highest percentage of inhibition (76.7%). In the case of cytotoxicity *i.e.* brine shrimp lethality tests the methanolic extract of *C. ramosus* showed the lowest percentage of mortality and the LC₅₀ values were found to be 523.9 µg/mL.

Conclusions: The results revealed that all the gastropods in the present study possessed antimicrobial, antioxidant and cytotoxic effects. However, species like *B. spirata* and *C. ramosus* exhibited potent activity and can be used for further clinical studies.

1. Introduction

Marine organisms have important compounds in necessities of pharmaceuticals especially invertebrates which are used in the biomedical area[1,2]. Molluscs are potential organisms for the extract of pharmacologically active drugs and are distributed throughout the world. Molluscs are the largest phylum comprising about 23% of the marine organisms and account for 80% from this gastropod. The Gulf of Mannar is one of the most important beds for gastropods which are associated with seaweed and algae. Numerous bioactive compounds have been studied for their bioactive potential *i.e.* antimicrobial, cytotoxic and anti-tumor[3,4]. In general very less studies have been done on the antimicrobial protein of molluscs while some molluscs have been estimated as whole body homogenate which possesses a range of antimicrobial compounds. The gastropod egg and tissue consists of active secondary metabolites that have more antimicrobial activities because of the protecting embryos inside the capsule[5]. The antioxidant compounds are essential to trap free radicals and

reduce the risk of cancer and heart disorders[6-8]. The large number of works has been done in other organisms, but only a few researches were done on molluscs[9]. The resources of gastropods are not well utilized in the areas around the Gulf of Mannar region. The objective of the present study was to investigate the antimicrobial activity, cytotoxicity and antioxidant potential of the body tissues of some economically important gastropods around the Gulf of Mannar region, southeast coast of India.

2. Materials and methods

2.1. Sample collection and extraction

Live specimens of *Babylonia spirata* (*B. spirata*), *Phalium glaucum* (*P. glaucum*), *Tonna dolium* (*T. dolium*), *Hemifusus pugilinus* (*H. pugilinus*), *Xancus pyrum* (*X. pyrum*), *Chicoreus ramosus* (*C. ramosus*), *Harpa articularis* (*H. articularis*), *Ficus ficus* (*F. ficus*) and *Babylonia zeylanica* (*B. zeylanica*) were collected from the Mandapam coast, southeast coast of India. They were directly brought to the laboratory and identified from marine gastropod hatchery research laboratory, Kamaraj College, Tuticorin, Tamilnadu, India. The 50 g of whole body muscle was taken out from the shell, used for extraction by methanol and stored for 4 days. The crude extracts were filtered through Whatman No.1

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Foundation project: Supported by UGC for BSR fellowship (Grant No. 02.09.2013/4-1/2008 (BSR)/Dt24.11.2009).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

filter paper and the solvents were concentrated by rotary evaporator (VC100A Lark Rotavapor® at 30 °C) with reduced pressure to give aqueous suspension and freeze dried. The final residues were stored at 4 °C for more analysis[10].

2.2. Bacterial strains and fungal strains

The dried methanolic crude extracts were used for antimicrobial assay against human pathogens viz., *Escherichia coli*, *Salmonella typhi* (*S. typhi*), *Salmonella paratyphi A*, *Aeromonas hydrophila* (*A. hydrophila*), *Vibrio cholera*, *Vibrio parahaemolyticus*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Aspergillus niger* (*A. niger*), *Penicillium* spp. and *Fusarium* spp. All the pathogenic microbial strains were obtained from Department of Microbiology, Kamaraj College, Tuticorin, Tamil Nadu, India.

2.3. Antibacterial and antifungal assay

Antibacterial activity was determined by well diffusion method[11]. Methanol was used as negative control and streptomycin was used as positive control. All the plates were incubated at 37 °C for 24 h. Results were calculated by measuring the zone of inhibition in millimetres. Antifungal activity was carried out by using the standard well diffusion method according to National Committee for Clinical Laboratory Standards[12]. The tetracycline discs (30 mg disc) were used as a positive control and methanol were used as negative control. All the extracts were tested in triplicate at attention of accurate results.

2.4. Total antioxidant activity

The total antioxidant activities of methanolic extract acquired from gastropods were determined[13]. The sample volume of 0.3 mL was taken and mixed with the reaction mixture consisting 63.0 mL reagent solution (0.6 mol/L sulfuric acid, 28 mol/L sodium phosphate and 4 mol/L ammonium molybdate). By using the water bath it was incubated at 95 °C for 90 min. At 695 nm the absorbance was measured and total antioxidant activity was expressed as the number of equivalents of ascorbic acid in milligram per gram of extract.

2.5. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging capacity of the methanolic extracts of gastropods was determined[14]. DPPH solution was prepared freshly, added into all test tubes followed by the extract in a serial dilution method (15–250 µg/mL) and made the final volume up to 5 mL. The absorbance was examined at 517 nm in spectrophotometer after 30 min. Gallic acid and methanol were used as the standard and blank

control respectively. This blank was used as control samples.

2.6. Cytotoxicity-brine shrimp lethality assay

Brine shrimp eggs were collected from Kolathur aquaculture industry. The glass chamber was used for hatching shrimp eggs which were filled with filtered seawater. The chamber was partitioned by both dark and light areas. After two days of hatching, 10 brine shrimps were introduced into the tube containing 4 mL of seawater. Test tubes were made in triplicate and 30 brine shrimps were totally used. The volumes of test tubes were made up to 5 mL by adding the methanolic extract of marine gastropods and left uncovered under the lamp. Totally five concentrations were used to assess the LC₅₀ i.e. 0.1, 1.0, 10.0, 100.0 and 1000.0 mg/mL. Every 24 h the number of surviving shrimp were counted and noted. Probit analysis was done to calculate LC₅₀ and 95% confidence intervals. The percentage of mortality was calculated to know the activity of the bioactive compounds presenting in the gastropod extracts.

2.7. Statistical analysis

All experiments were done in triplicate (antimicrobial activity) and One-way ANOVA was calculated using SPSS statistical software. Means of three observations were compared with Duncan's multiple range test at $P > 0.05$ for determining the statistical significance. Probit software version 1.5 (US-EPA) was used to calculate LC₅₀ and 95% confidence limit.

3. Results

3.1. Antimicrobial activity

The crude extracts from the gastropod species against eight human pathogenic bacteria and three fungal strains were tested for the antimicrobial activity. The inhibition zones of methanolic extracts against the treated organisms were shown in Tables 1 and 2. From the antimicrobial result, *B. spirata* significantly showed the highest inhibition zone ($P > 0.05$) [(12.40 ± 0.16) mm] against *A. hydrophila* and *H. articularis* significantly showed the lowest inhibition zone ($P > 0.05$) [(2.20 ± 0.11) mm] against *Vibrio parahaemolyticus*. In fungal assay, *Fusarium* spp. significantly showed high susceptibility ($P > 0.05$) [(18.00 ± 0.13) mm] against methanolic extract of *B. spirata* and *B. zeylanica* significantly showed the lowest inhibition zone ($P > 0.05$) [(1.90 ± 0.04) mm] against *A. niger*.

3.2. Total antioxidant activity

The methanolic extracts of the gastropods were analysed for total antioxidant activity (Figure 1) and the result showed that the

Table 1

Antibacterial activity against extract of different marine gastropods from the Gulf of Mannar region.

Species	Inhibition of zone (mm)										
	<i>P. glaucum</i>	<i>T. dolium</i>	<i>H. pugilinus</i>	<i>B. spirata</i>	<i>X. pyrum</i>	<i>C. ramosus</i>	<i>H. articularis</i>	<i>F. ficus</i>	<i>B. zeylanica</i>	PC	NC
<i>Escherichia coli</i>	2.80 ± 0.20 ^a	3.20 ± 0.14 ^a	2.90 ± 0.11 ^b	3.20 ± 0.87 ^a	-	2.60 ± 0.04 ^a	-	2.80 ± 0.01 ^b	4.90 ± 0.15 ^a	24.00 ± 0.10 ^a	-
<i>S. typhi</i>	2.30 ± 0.14 ^a	4.40 ± 0.08 ^a	-	2.90 ± 0.71 ^b	-	7.20 ± 0.62 ^c	2.60 ± 0.44 ^a	3.40 ± 0.66 ^c	2.70 ± 0.63 ^b	26.00 ± 0.21 ^b	-
<i>Salmonella paratyphi A</i>	2.60 ± 0.03 ^b	6.80 ± 0.04 ^b	-	3.90 ± 0.23 ^a	4.70 ± 0.31 ^c	8.50 ± 0.41 ^a	-	2.90 ± 0.21 ^a	-	28.00 ± 0.61 ^a	-
<i>A. hydrophila</i>	3.10 ± 0.17 ^a	4.80 ± 0.03 ^c	3.30 ± 0.21 ^a	12.40 ± 0.16 ^c	5.40 ± 0.19 ^a	-	2.70 ± 0.12 ^b	-	2.80 ± 0.45 ^a	28.00 ± 0.41 ^c	-
<i>Vibrio cholerae</i>	3.30 ± 0.32 ^a	2.80 ± 0.03 ^b	-	2.90 ± 0.07 ^a	-	-	6.70 ± 0.31 ^a	-	3.50 ± 0.23 ^a	21.00 ± 0.36 ^a	-
<i>Vibrio parahaemolyticus</i>	2.70 ± 0.09 ^b	2.90 ± 0.07 ^c	2.30 ± 0.21 ^a	4.10 ± 0.43 ^b	7.40 ± 0.22 ^a	-	2.20 ± 0.11 ^b	4.80 ± 0.78 ^a	2.30 ± 0.07 ^a	24.00 ± 0.21 ^a	-
<i>Klebsiella pneumonia</i>	4.00 ± 0.05 ^a	2.40 ± 0.03 ^a	-	4.40 ± 0.41 ^b	-	5.40 ± 0.33 ^a	8.90 ± 0.61 ^a	-	7.10 ± 0.41 ^a	20.00 ± 0.12 ^a	-
<i>Staphylococcus aureus</i>	2.70 ± 0.03 ^a	2.40 ± 0.17 ^b	2.90 ± 0.10 ^a	2.60 ± 0.21 ^a	3.40 ± 0.77	2.80 ± 0.12 ^c	-	4.70 ± 0.13 ^a	3.70 ± 0.13 ^b	21.00 ± 0.32 ^a	-

^{a, b, c}: Values (mean ± SD) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$). PC: Positive control (streptomycin 50 µg); NC: Negative control (methanol).

Table 2

Antifungal activities against extract of different marine gastropods form the Gulf of Mannar region.

Species	Inhibition of zone (mm)										
	<i>P. glaucum</i>	<i>T. dolium</i>	<i>H. pugilinus</i>	<i>B. spirata</i>	<i>X. pyrum</i>	<i>C. ramosus</i>	<i>H. articularis</i>	<i>F. ficus</i>	<i>B. zeylanica</i>	PC	NC
<i>A. niger</i>	-	14.00 ± 0.52 ^a	-	8.00 ± 0.51 ^c	2.30 ± 0.03 ^a	2.40 ± 0.23 ^b	-	-	1.90 ± 0.04 ^a	22.00 ± 0.01 ^a	-
<i>Penicillium</i> spp.	-	5.00 ± 0.60 ^a	-	-	6.00 ^a ± 0.51 ^a	11.00 ± 0.47 ^a	-	-	-	21.00 ± 0.01 ^a	-
<i>Fusarium</i> spp.	-	-	2.50 ± 0.02 ^b	18.00 ^a ± 0.13	-	-	2.00 ± 0.89 ^a	7.00 ± 0.21 ^b	16.00 ± 0.47 ^b	22.00 ± 0.03 ^a	-

PC: Positive control (tetracycline 30 µg); NC: Negative control.

maximum antioxidant activity was observed in *B. spirata* (510 µg/mg) and the minimum activity was observed in *T. dolium* (56 µg/mg).

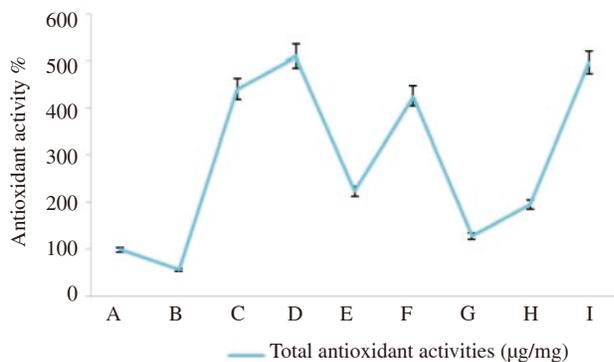


Figure 1. Total antioxidant activities of methanolic extract of gastropods. A: *P. glaucum*; B: *T. dolium*; C: *H. pugilinus*; D: *B. spirata*; E: *X. pyrum*; F: *C. ramosus*; G: *H. articularis*; H: *F. ficus*; I: *B. zeylanica*.

3.3. DPPH scavenging activity

The DPPH radical scavenging activity at different concentration of 20–100 µg were showed in Figure 2. The *B. spirata* extract showed the highest percentage inhibition of 76.7% and the lowest percentage inhibition was observed in *T. dolium* of 13.4%.

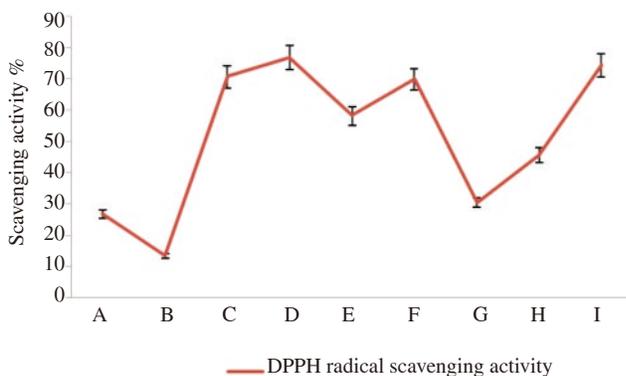


Figure 2. DPPH radical scavenging activity of methanolic extract of gastropods. A: *P. glaucum*; B: *T. dolium*; C: *H. pugilinus*; D: *B. spirata*; E: *X. pyrum*; F: *C. ramosus*; G: *H. articularis*; H: *F. ficus*; I: *B. zeylanica*.

Table 3

Percentage of mortality in brine shrimp lethality test.

Concentration of extracts (µg/mL)	Mortality (%)								
	<i>P. glaucum</i>	<i>T. dolium</i>	<i>H. pugilinus</i>	<i>B. spirata</i>	<i>X. pyrum</i>	<i>C. ramosus</i>	<i>H. articularis</i>	<i>F. ficus</i>	<i>B. zeylanica</i>
0.1	-	-	-	-	-	-	-	-	-
1.0	10	20	-	-	-	10	-	-	0
10.0	20	20	10	10	20	10	20	20	10
100.0	40	30	40	20	40	30	30	20	20
1000.0	80	90	70	70	80	60	80	80	84
Control	-	-	-	-	-	-	-	-	-

3.4. Cytotoxicity-brine shrimp lethality assay

From the result, *C. ramosus* showed the lowest percentage of mortality and the LC₅₀ of 523.9 µg/mL was obtained. And the highest percentage of mortality was observed in *T. dolium* and LC₅₀ of 93.5 µg/mL was obtained. The values were represented in Tables 3 and 4.

Table 4LC₅₀ (µg/mL) and 95% confidence limit of methanolic extract of gastropods.

Samples	LC ₅₀ (µg/mL)	95% Confidence limit
<i>P. glaucum</i>	120.4	30.9–992.8
<i>T. dolium</i>	93.5	22.6–817.2
<i>H. pugilinus</i>	234.3	70.5–1655.0
<i>B. spirata</i>	378.9	114.2–4073.2
<i>X. pyrum</i>	138.5	41.9–724.8
<i>C. ramosus</i>	523.9	95.3–59061.0
<i>H. articularis</i>	176.0	52.8–1063.4
<i>F. ficus</i>	216.8	51.2–3358.9
<i>B. zeylanica</i>	264.0	86.9–1428.2

4. Discussion

Marine natural products gain importance in the pharmaceutical industries. The identification of new compounds is extremely important. From the present study the diverse antimicrobial activities were observed against eight human pathogenic bacteria and three fungal strains. Methanolic extracts of *B. spirata*, *P. glaucum*, *T. dolium*, *H. pugilinus*, *X. pyrum*, *C. ramosus*, *H. articularis*, *F. ficus* and *B. zeylanica* were used. The maximum inhibition zone was found in *B. spirata* and the lowest inhibition zone was found in *H. articularis* and all the nine gastropod showed antimicrobial activities. Previous report revealed that the marine snail *Tibia insulaechorab* possesses potent antimicrobial activity[15]. Mollusc like *Meretrix casta* and *Tridacna maxima* exhibited higher antibacterial activity against twelve human pathogens and they were used in six solvents out of those ethanol and methanol extracts showed higher antimicrobial activity[16]. The antimicrobial activity of green mussel *Perna viridis* (*P. viridis*) and edible oyster *Crassostrea madrasensis* extract of marine bivalves was previously reported[17]. According to Periyasamy et al.[18] the mollusk *P. viridis* showed the highest activity against *Pseudomonas aeruginosa*. The mollusk *B. spirata* showed the maximum activity (12 mm) against *Pseudomonas aeruginosa*[19]. Sugesh and Mayavan[20] reported maximum antibacterial activity

with the solvents like ethanol and methanol extracts of *H. puglinus* against human pathogens. According to Tilaga et al.[21] *P. glaucum* showed wide spectrum activity against *S. typhi* (12 mm). From the reports it was evident that molluscs possess diverse antibacterial activities against eight human pathogenic bacteria and three fungal strains in the present study.

DPPH radical (oil-soluble free radical) scavenging activity assay has been widely used for screening antioxidant activity. Earlier report evidenced that the antioxidant peptide isolated from *Conus betulinus* (body and viscera) shows 20%–25% of scavenging effect[22]. According to methanolic extract from *B. spinosa*, the scavenging activity was found to be 39.43% at 10 mg/mL[23]. In the present study, the scavenging activity was higher in *C. ramosus* and lower in *T. dolium*. Brine shrimp assay was the initial estimation of toxicity test and can be correlated with cytotoxic and anti-tumour properties. The cytotoxic assays revealed the strong cytotoxic nature of ethyl acetate extract of *P. viridis*[24]. According to Subhadrappa et al.[25] cytotoxic activity was observed in three molluscs, namely, *Anadara granosa*, *Placenta placenta* and *Pinctada fucata* and mortality was increased with increasing concentration. It was similar to the present study that cytotoxicity was observed in all the nine molluscs and the mortality was increased with increasing concentration. And the maximum activity was found in *C. ramosus* and the minimum activity found in *T. dolium*.

There are only few studies conducted in the commercial important gastropods. The information on the antimicrobial, antioxidant and cytotoxicity were only limited. Therefore, the present study was focused on it. Brine shrimp lethality test is the initial test to analyse the toxicity profile of the extract against brine shrimp nauplii. However, all the nine gastropods showed antibacterial, antioxidant and cytotoxic activities. By analysing the toxicity they will be further used in cell lines in drug industry.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We express gratitude to UGC for BSR fellowship for financial support (Grant No. 02.09.2013/4-1/2008 (BSR)/Dt24.11.2009).

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