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Full Length Research Paper

Screening for antimicrobial activities of marine molluscs *Thais tissoti* (Petit, 1852) and *Babylonia spirata* (Linnaeus, 1758) against human, fish and biofilm pathogenic microorganisms

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The aim of this study was to screen the presence of antimicrobial activities in the marine mollusc *Thais tissoti* and *Babylonia spirata.* In this study, the human bacterial pathogen *Klebsiella pneumonia* (10.02 ± 0.11 mm) and fungal pathogen *Aspergillus niger* (12.09 ± 0.06 mm) showed most sensitive to ethyl acetate extracts of *T. tissoti.* In *B. spirata* extracts, bacterial pathogen *Proteus mirabilis* (7.02 ± 0.04 mm) and fungal pathogen *Candida albicans* (8.13 ± 0.15* mm) exhibit high zone of inhibition against n-butanol extracts. Among the fish bacterial pathogens assayed, *Aeromonas hydrophila* (9.22 ± 0.03* mm) and fungal pathogen *Ichthyophonus* sp. (5.21 ± 0.11 mm) showed more sensitivity to n- butanol extract of *T. tissoti.* In *B. spirata* extracts, bacterial pathogen *A. hydrophila* (11.2 ± 0.6* mm) and fungal pathogens *A. niger* (15.12 ± 0.23* mm) showed most sensitive to n-butanol extracts. From biofilm bacteria, *Micrococcus luteus* (7.02 ± 0.11* mm), *Micrococcus* sp. (7.25 ± 0.03* mm) showed high sensitivity to n-butanol extracts of molluscs *T. tissoti* and *B. spirata.* These results signify that the marine mollusc extracts express remarkable antimicrobial activity against isolated microbes. The result strongly suggests that the molluscs extracts can be used as antimicrobial agents and further studies for purification and structural elucidation of antimicrobial drugs.

Key words: Antibacterial, antifungal, human pathogen, fish pathogen, biofilm, marine mollusks.

INTRODUCTION

The number of natural products isolated from marine organisms increases rapidly, and now exceeds with hundreds of new compounds being discovered every year (Faulkner, 2002; Proksch and Muller, 2006). Modern technologies have opened vast areas of research for the extraction of biomedical compounds from ocean and seas to treat the deadly diseases. Many classes of natural products exhibiting antitumor, antileukemic, antibacterial and antiviral activities have been reported worldwide. Antimicrobial peptides are important in the first line of the host defense system of many animal species. Marine invertebrates offer a source of potential antimicrobial drugs (Bazes et al., 2009). There is much valuable information for new antibiotic discoveries and gives new insights into bioactive compounds in marine molluscs. Most marine molluscs are sessile soft bodies that inhabit benthic rock environments. A broad range of sessile marine organisms possess antifouling defences, suggesting that biofouling is an important selective pressure in the marine environment. Other selective pressures that could lead to the evolution of bioactive metabolites in the marine environment include predation and surface fouling. They either express constitutively or the expression is induced by exposure to pathogens. Contagious disease is a considerable problem to human health and in the agricultural industry. Disease in

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aquaculture is also predominantly caused by microbially mediated infections and this has lead to the widespread use of antibiotics overseas (Westra, 1998). Of particular concern is the number of human pathogens that have developed multidrug resistance (Stinson, 1996) and some bacterial strains are resistant to all existing antibio-tics (Setti and Micetich, 1998). There is serious concern regarding new and reemerging infectious diseases for which no effective therapies are available (Cragg et al., 1997). Prevention and control of these infectious bacteria will require the development of new antimicrobial agents. Generally fewer extensive, investigations have been made of the antimicrobial proteins of other invertebrate groups and although whole body homogenates of some marine invertebrates have been reported to contain a variety of antimicrobial compounds. Studies of antimicro-bial compounds of marine invertebrates may provide valuable information for new antibiotic discoveries and give new insights into bioactive compounds in marine molluscs. The need for discovery of new and novel antibiotics is imperative because evidence suggests that development and spreading of resistance against any new antimicrobial agents is inevitable. The aim of this work was to investigate the antimicrobial effectors in different extracts of marine molluscs *Thais tissoti* and *Babylonia spirata* against isolated human, fish pathogens and primary biofilm microorganisms.

MATERIALS AND METHODS

Specimen collection and identification

The marine molluscs *T. tissoti* (Petit, 1852) and *B. spirata* (Linnaeus, 1758) were collected from Mudasalodai fishing harbour (Lat 11° 26' N; Long 79°46' E), Tamil Nadu, southeast coast of India. The collected fresh molluscs were preserved with ice and transported to the laboratory and identified by the standard literature of Fernando and Fernando (2002) and Gittenberger and Goud (2003). The animals were carefully removed from the shells.

Extraction

The extraction method was followed by Chellaram et al. (2004). The freshly collected mollusc tissues each 15 g in wet weight were soaked in methanol, acetone, chloroform, n-butanol, ethyl acetate and hexane and maintained for 3 days. The extracts were filtered through Whatman®No.1 filter paper and the solvents were concentrated by rotary evaporator (VC100A Lark Rotavapor® at 30°C) with reduced pressure to give a dark brown gummy mass. The resultant residues were stored at 4°C for further analysis.

Test microorganisms and microbial culture

Human bacterial and fungal pathogens

The reference pathogens used to test antimicrobial assay were, the following gram-positive and gram-negative bacteria such as, *Staphylococcus aureus*, *Streptococcus aureus*, *Escherichia coli*, *Salmonella typhi, Salmonella paratyphi*, *Klebsiella oxytoca, Klebsiella pneumoniae, Vibrio cholera, Vibrio parahaemolyticus* and *Proteus mirabilis,* the fungal pathogens such as *Penicillium* sp., *Candida albicans*, *Candida tropicalis, Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* sp., *Trichophyton rubrum*, *Mucor* sp., *Alternaria alternata* and *Trichophyton mentagarophytes* were used. These microbes were collected from patients of Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai nagar, Chidambaram. Bacterial strains and fungal strains were cultivated maintained on nutrient agar and fungal agar slants at 4°C.

Fish bacterial and fungal pathogens

Following fish pathogens were used for antimicrobial activity, the bacterial pathogens such as *V. cholerae*, *V. parahaemolyticus*, *Vibrio* sp.1, *P. mirabilis, A. hydrophila, Aeromonas* sp., *Streptococcus* sp., *Micrococcus* sp., *Klebsiella* sp. and *Proteus* sp.1 the fungal pathogens such as *Microsporum* sp., *A. fumigatus*, *A. flaves, A. niger, Aspergillus* sp.1, *Aspergillus* sp.2, *Ichthyophonus* sp., *Rhizopus* sp., *Rhizopus* sp.1 and *Fusarium* sp., These fish pathogens were isolated from infected fishes from Cuddalore government fish hatchery during April and May 2010. Isolated pathogens were identified based on the morphological, cultural and biochemical characteristics following Bergey's Manual of Determinative Bacteriology (Holt, 1994) and Manual of Clinical Microbiology (Mahony, 1999). These Fish pathogenic bacterial strains and fungal strains were cultivated at 37°C and maintained on Zobell marine agar and fungal agar slants at 4°C.

Fouling organisms

The reference microbes were used to test antimicrobial assay, *Micrococcus* sp., *S. aureus, Bacillus* sp., *Pseudomonas* sp*., Micrococcus luteus, Micrococcus* sp.1, *Proteus* sp*., Streptococcus* sp.1, *Streptococcus* sp.2 and *Klebsiella* sp. The fouling bacteria used in the antibacterial assay were isolated from the biofilm formed over aluminium, fiber glass and Wood panels by pour plate method of Wahl (1995) and isolated strains were identified based on the morphological, cultural and biochemical characteristics following Bergey's Manual of Determinative Bacteriology (Holt, 1994) and Manual of Clinical Microbiology (Mahony, 1999). These biofilm bacterial strains were cultivated at 37°C and maintained on Zobell marine agar slants at 4°C.

Antibacterial activity

Antibacterial activity was carried out by using standard disc diffusion method (Dulger and Gonuz, 2004; Parekh and Chanda, 2007; Laouer et al., 2009). The test cultures (bacteria 10^8 CFU/ml) were swabbed on top of the solidified media and allowed to dry for 10 min. The human bacteria were maintained on nutrient agar plates, fouling and fish pathogens maintained on Zobell marine agar plates. The different extracts of molluscs were applied on to 6 mm sterile discs in aliquots of 30 µl of solvent, allowed to dry at room temperature, and extract loaded discs were placed on agar plates seeded with isolated microorganisms and incubated at 37°C for 24 h. The susceptibility of the test organisms were determined by radius of the inhibition zone around each disc. All extracts were tested with triplicate at a concentration of 30 mg $disc^{-1}$.

Antifungal activity

Antifungal activity was carried out by using the standard disc diffusion method by (National Committee for Clinical Laboratory Standards, 2002). The extracts applied to 6 mm sterile discs in

Table 1. Antibacterial activity of *T. tissoti* against human bacterial pathogens.

Pathogen	Zone of inhibition (mm) (Mean ± SD)					
	Methanol	E. acetate	Hexane	Acetone	n- Butanol	Chloroform
V. cholera	1.13±0.03*	2.12±0.12	1.02±0.13*	3.02±0.11	7.01±0.02*	1.02±0.05*
S. typhi	2.06±0.15**	1.18±0.06**	1.11±0.04	1.01±0.01*	5.12±0.12**	1.01±0.22
V. parahaemolyticus	1.1±0.18	2.22±0.14*	1.05±0.11**	3.11±0.10	5.02±0.11	1.05±0.15*
Streptococcus aureus	2.1±0.18	1.02±0.12*	1.07±0.18**	4.02±0.15*	7.1±0.12**	4.01±0.11
Staphylococcus aureus	1±0.12***	2.06±0.11	1.03±0.15	3.01±0.25	6.01±0.05	3.01±0.12**
K. oxytoca	1 ± 0.33	3.15±0.05*	1.04±0.33*	4.42±0.12*	5.02±0.11**	1.01±0.22*
P. mirabilis	1.02±0.33*	4.12±0.12	1.06±0.13	3.02±0.16	7.03±0.14	1.01±0.15
S. paratyphi	2±0.11***	1.10±0.33*	1.01±0.13**	2.01±0.18*	6.02±0.11*	3.01±0.12*
K. pneumonia	1.05±0.12*	10.02±0.11	1.02±0.13**	2.05±0.15	5.05±0.17	3.11±0.11
E. coli	1.01±0.02*	1.14±0.02*	1.05±0.02	2.06±0.22*	5.08±0.12*	1.02±0.18*

Values are mean ± SEM; *Values are statistically significant at P< 0.05;**Values are statistically significant at P< 0.01.

Table 2. Antibacterial activity of *B. spirata* against human bacterial pathogens.

Pathogen	Zone of Inhibition (mm) (Mean ± SD)					
	Methanol	E. acetate	Hexane	Acetone	n- Butanol	Chloroform
V. cholera	1.1±0.15*	1.1±0.15*	1.01±0.01*	1.1±0.14*	4.02±0.1**	1.02±0.14**
S. typhi	1.1±0.1***	2±0.04**	1.03±0.03*	1.1±0.11	4±0.05*	2.1±0.13
V. parahaemolyticus	1±0.15*	3.1±0.13*	1.1±0.01	2.3±0.03	4.2±0.04	2.1±0.02*
Streptococcus aureus	1.1±0.16	1.2±0.14	1.2±0.01**	1.06±0.16**	4.2±0.11*	1.01±0.11
Staphylococcus aureus	2±0.15*	2.03±0.01*	1.01±0.01*	1.03±0.04	4.01±0.15	2.01±0.12
K. oxytoca	4.3±0.04	1.2±0.01*	1.03±0.01	1.03±0.05*	4.2±0.01*	1.2±0.31*
P. mirabilis	1.01±0.02*	5.05±0.05**	1.03±0.08	1.2±0.15	7.02±0.04	1.02±0.01
S. paratyphi	1.06±0.04	2.1±0.18*	1±0.11***	1.3±0.11*	5.02 ± 0.12*	2.11±0.12
K. pneumonia	1.1±0.18*	5.1±0.14*	1.02±0.13*	1.03±0.12	4.1 ± 0.12	3.02±0.12
E. coli	2.06±0.15*	3.1±0.06*	1.02±0.01	1.03±0.01*	5.02±0.15*	1.01±0.11*

Values are mean ± SEM; *Values are statistically significant at P< 0.05;**Values are statistically significant at P< 0.01.

aliquots of 30 µl of solvent, was allowed to dry at room temperature and placed on agar plates seeded with microorganisms. The cultures were maintained on fungal agar plates and incubated at 37°C for 24 h. Zones of growth inhibition were measured in millimeters after incubation. All the extracts were tested triplicate at a concentration of 30 mg disc^{-1}.

RESULT AND DISCUSSION

Marine organisms have been found to produce a great diversity of novel bioactive secondary metabolites potential source for new drug discovery. Extensive investigations of molluscs chemically and pharmacologically have been published in sufficient literature. A number of molluscs were ranked very high in the priority list of species exhibiting antimicrobial activity. The benthic invertebrates are potentially vulnerable to microbial infection, which may have led to the evolution of chemical defence mechanisms.

In the present investigation solvent extracts of both *T. tissoti* and *B. spirata* were concentrated under reduced pressure to give a dark brown gummy mass of 2.62 to 1.23 g, respectively. In this study a distinct antimicrobial activity has been observed against isolated bacterial and fungal strains. *T. tissoti* were mostly abundant in rocky shore but *B. spirata* mostly found in sandy shore. Through this study we have compared the antimicrobial activities of different benthic mollusc. In this experiment the solvents methanol, acetone, chloroform, n-butanol, ethyl acetate and hexane were used for extraction and these solvents were selected according to their polarity. The molluscs crude extracts were assayed against isolated human, fish pathogenic and biofilm microbes. From the antimicrobial assay methanol, n-butanol and chloroform extracts showed promising sensitivity against human pathogenic bacteria than fish pathogenic bacteria. The antibacterial activity of *T. tissoti* and *B. spirata* radius of the zone of inhibition around the disc were represented (Tables 1, 2, 3, 4, 5 and 6). From the human pathogenic bacteria tested, *K. pneumonia* showed more sensitivity (10.02 ± 0.11 mm) against ethyl acetate extracts of *T.*

Table 3. Antibacterial activity of *T. tissoti* against Fish pathogens.

Pathogen	Zone of inhibition (mm) (Mean ± SD)					
	Methanol	E. acetate	Hexane	Acetone	n- Butanol	Chloroform
V. cholerae	2.14±0.45**	2.12±0.01*	1.11±0.05*	2.21±0.13**	4.11±0.08*	2.15±0.03*
V. parahaemolyticus	2.21±0.15*	3.16±0.16*	2.15±0.01	3.15±0.12*	5.25±0.16*	3.12±0.11
P. mirabilis	2.21±0.01**	2.37±0.03	1.12±0.02*	4.19±0.05	7.11±0.01	2.22±0.05*
A. hydrophila	3.25±0.12	2.36±0.32**	1.11±0.01	2.34±0.15*	9.22±0.03*	2.15±0.05
Vibrio sp.1	4.26±0.18*	2.12±0.15	1.30±0.11*	2.14±0.11	6.16±0.12**	2.12±0.13*
Proteus sp.1	3.13±0.31**	2.12±0.11*	1.22±0.14**	4.11±0.12*	8.12±0.21**	4.14±0.11
Streptococcus sp.	3.1 ±0.12	2.12±0.18*	1.15±0.13	2.22±0.01	8.16±0.01	2.15±0.1**
Micrococcus sp.	5.11±0.13*	2.11±0.13	3.21±0.11**	3.12±0.12*	8.12±0.25*	4.1±0.1**
Aeromonas sp.1	3.5±0.11**	2.6±0.11**	2.1±0.11	3.2±0.18	6.1±0.18	5.2±0.01**
Aeromonas sp.2	5.2±0.01*	4.4±0.01*	1.2±0.01*	5.14±0.06*	7.3±0.03*	2.2±0.01*

Values are mean ± SEM; *Values are statistically significant at P< 0.05;**Values are statistically significant at P< 0.01.

Table 4. Antibacterial activity of *B. spirata* against Fish pathogens.

Pathogen	Zone of inhibition (mm) (Mean ± SD)					
	Methanol	E. acetate	Hexane	Acetone	n- Butanol	Chloroform
V. cholerae	3.12±0.22*	2.13±0.01**	1.12±0.11*	4.15±0.11*	8.2±0.13*	2.12±0.12*
V. parahaemolyticus	5.22±0.21*	321±0.15	1.03±0.14	3.18±0.22	9.2±0.22*	2.23±0.01
P. mirabilis	4.11±0.15	3.13±0.01*	2.11±0.12*	1.14±0.23**	8.2±0.13*	4.22±0.04*
A. hydrophila	5.02±0.12*	3.16±0.12*	1.05±0.06	2.14±0.12*	11.2±0.6*	5.12±0.05
Vibrio sp.1	5.15± 0.03	5.15±0.01	1.11±0.02*	2.32±0.01	9. 2±0.17**	2.13±0.01*
Proteus sp.1	2.11±0.22*	4.15±0.03**	2.15±0.15**	5.22±0.08*	8.26±0. 1	3.12±0.08
Streptococcus sp.	3.22±0.28	3.02±0.01*	1.12±0.01*	4.21±0.19	11.1±0.01*	4.11±0.01*
Micrococcus sp.	3.22±0.21*	6.1±0.03	2.16±0.10	5.26±0.22*	7.02±0.01	4.13±0.05
Aeromonas sp.1	5.21±0.12	3.2±0.05**	2.17±0.03**	4.11±0.17	8.14±0.12	5.12±0.14*
Aeromonas sp.2	5.01±0.12	4.02±0.02*	1.14±0.01	4.11±0.13**	9.13±0.02**	4.11±0.18*

Values are mean ± SEM; *Values are statistically significant at P< 0.05;**Values are statistically significant at P< 0.01.

Table 5. Antibacterial activity of *T. tissoti* against fouling bacteria.

Pathogen	Zone of inhibition (mm) (Mean ± SD)					
	Methanol	E. acetate	Hexane	Acetone	n- Butanol	Chloroform
Micrococcus sp.	-	-	-	-	5.02±0.02*	-
S. aureus	-	-	-	-	4.2±0.11	1.01±0.12*
Bacillus sp.	-	1.02±0.11*	-	5.02±0.22	5.02±0.15*	2.06±0.11
Pseudomonas sp.	1.15±0.12	2.03±0.04	1.18±0.05*	1.01±0.11**	3 ± 0.12*	1.02±0.14*
Micrococcus luteus	1.16±0.04**	1.02±0.12*	-	1.16±0.16	7.02±0.11*	1.06±0.11
Micrococcus sp.1	-	1.071±0.15	-	1.04±0.18**	4.2±0.22	1.03±0.03*
Proteus sp.	-	2.08±0.21**	2.12±0.08	1.05±0.18*	3.02±0.12**	2.05±0.13
Streptococcus sp.1	-	-	-	3.04±0.11	3.22±0.11	-
Streptococcus sp.2	-	1.05±0.22*	-	1.01±0.22**	3.02±0.03**	1.03±0.02*
Klebsiella sp.	-	2.01±0.22	1.01±0.21	1.06±0.11*	4.02±0.10**	-

Values are mean ± SEM; *Values are statistically significant at P< 0.05;**Values are statistically significant at P< 0.01.

tissoti and *P. mirabilis* showed more sensitivity (7.02 ± 0.04 mm) against n-butanol extract of *B. spirata.* From fish bacterial pathogenic microbes tested, *A. hydrophila* showed most sensitivity (9.22 ± 0.03* mm), (11.2 ± 0.6*

Table 6. Antibacterial activity of *Babylonia spirata* against fouling bacteria.

Pathogen	Zone of inhibition (mm) (Mean ± SD)					
	Methanol	**E. acetate**	**Hexane**	**Acetone**	**n- Butanol**	**Chloroform**
Micrococcus sp.	1.05±0.02*	1.03±0.0.3	1.2±0.15*	1.02±0.12*	7.25±0.03*	1.06±0.04*
S. aureus	3±0.33**	2±0.01***	4.15±0.06	1.01±0.08*	5.02±0.12	4.01±0.07
Bacillus sp.	1.05±0.02	3.06±0.12	1.15±0.03*	2.23±0.13**	4.09±0.03**	2.05±0.03
Pseudomonas sp.	1.18±0.12*	1.04±0.15*	1.22±0.21	1.19±0.01*	4.02±0.08	1.03±0.01**
Micrococcus luteus	-	2.02±0.02	-	-	4.05±0.15*	-
Micrococcus sp.1	1.15±0.06	1.6±0.11**	2.02±0.02*	-	3.05±0.18*	1.04±0.15**
Proteus sp.	2.16±0.08*	2.23±0.13	2.02±0.02	1.01±0.08	4.09±0.03	2.02±0.33*
Streptococcus sp.1	-	1.01±0.05	2.13±0.13*	-	5.03± 0.33*	-
Streptococcus sp.2	2.3±0.01	1.05±0.1	1.23±0.13*	1.15±0.15*	4.01±0.15*	-
Klebsiella sp.	1.12±0.26	2.04±0.03	1.02±0.02	2.02±0.02	4.02±0.01**	1.01±0.3***

Values are mean ± SEM; *Values are statistically significant at P< 0.05;**Values are statistically significant at P< 0.01.

Table 7. Antifungal activity of *Thais tissoti* against human fungal pathogens.

Pathogen	Zone of inhibition (mm) (Mean ± SD)					
	Methanol	**E. acetate**	**Hexane**	**Acetone**	**n- Butanol**	**Chloroform**
C. tropicalis	1.05±0.02*	3.03±0.01*	1.02±0.11	2.2±0.11**	6.03±0.11*	1.02±0.13*
C. albicans	5.05±0.11*	2.01±0.11	1.01±0.03*	5.06±0.01	6.05±0.12*	2.03±0.12*
T. rubrum	1.12±0.26**	5.02±0.01*	4.21±0.01	1.02±0.01	1.04±0.13**	2.21±0.011*
Rhizopus sp.	1.12±0.11**	2.11±0.13*	1.02±0.13*	3.01±0.05*	3.02±0.15*	3.11±0.15**
T. mentagarophytes	1.03±0.18***	3.05±0.05	2.06±0.11*	1.03±0.01**	4.06±0.05	1.21±0.03
A. niger	2.04±0.12*	12.09±0.06	2.04±0.15*	4.02±0.01	6.02±0.5*	2.02±0.05*
Penicillium sp.	1.22±0.11	1.04±0.11*	1.02±0.18	1.04±0.05*	5.01±0.03	2.12±0.01*
A. alternaria	1.03±0.11*	2.02±0.12	1.06±0.09*	1.02±0.01*	5.22±0.04*	1.02±0.08
Mucor sp.	2.13±0.13*	2.01±0.11*	1.33±0.12	2.01±0.02**	5.02±0.03	3.03±0.15*
A. flavus	2.02±0.32*	7.01±0.12**	2.12±0.11*	1.02±0.03	2.03 ± 0.63*	3.04±0.03*

Values are mean ± SEM; *Values are statistically significant at P< 0.05;**Values are statistically significant at P< 0.01.

mm) against both n-Butanol extracts. In the case of biofilm microbes, *M. luteus* (7.02 ± 0.11* mm), *Micrococcus* sp. (7.25 ± 0.03* mm) showed high sensitive to n-butanol extracts of *T. tissoti* and *B. spirata*. Antifungal activities were represented by the zone of inhibition around the disc. In human pathogenic fungal tested (Tables 7, 8, 9 and 10), *A. niger* was the most sensitive (12.09 ± 0.06 mm) against ethyl acetate extracts of *T. tissoti* and *C. albicans* exhibited high zone (8.13 ± 0.15* mm) against n-butanol extracts of *B. spirata.* In fish pathogenic fungal tested, *Ichthyophonus* sp. showed more sensitivity (5.21 ± 0.11 mm) to n-butanol extract of *T. tissoti* and *A. niger* showed high zone (15.12 ± 0.23* mm) formation against n-Butanol extracts of *B. spirata.* These findings are consistent with previous studies on molluscs. Molluscan extracts produced the largest zones of inhibition out of all the marine extracts that were tested against *E. coli* and *P. atrovenetum.* Significantly, different species of mollusc were priority listed for activity in the different assays. Similar result was reported in four bivalves against few pathogens and found that extracts showed significant activity against *Bacillus subtillus* (Jayaseli et al., 2001). Likewise highest activity was observed against *S. typhi* in some gastropods (Prem et al., 1997). The antibacterial activity of ethanol extract of gastropods *Babylonia spirata* and *Turbo brunneus* was observed maximum activity against *E. coli, K. pneumoniae, P. vulgaris* and *S. typhi* (Prem et al., 1997). Very similar to this maximum antibacterial activity against *S. aureus* and *E. coli* on *Trochus radiatus* was reported (Mary et al., 2003a). This study corroborates the results of the present investigation.

Previous studies on the egg masses of marine gastropods, although few in number, have revealed striking contrasts in the biological activity of the species examined. On one hand, the gelatinous egg masses of molluscs in the Aplysiidae, exhibit a broad spectrum of

Table 8. Antifungal activity of *B. spirata* against human fungal pathogens.

Pathogen	Zone of inhibition (mm) (Mean ± SD)					
	Methanol	E. acetate	Hexane	Acetone	n- Butanol	Chloroform
C. tropicalis	3.03±0.02*	4.02±0.02**	2.02±0.15	2.02±0.11*	7.12±0.08*	2.02±0.12*
C. albicans	4.02±0.03	4.01±0.08*	1.03±0.21**	2.01±0.11**	8.13±0.15*	3.05±0.18**
T. rubrum	4.45±0.12	2.03±0.15**	1.15±0.11	1.17±0.13	4.02 ± 0.12	4.15±0.15
Rhizopus sp.	1.12±0.21	2.11±0.05	-	1.14±0.11*	5.15±0.14**	2.01±0.33**
T. mentagarophytes	1.02±0.02*	3.02±0.19*	1.04±0.15**	2.01±0.32*	5.45±0.19*	3.12±0.12
A. niger	2.32±0.01	2.04±0.03*	1.05±0.11	3.06±0.15	6.13±0.2	1.21±0.11**
Penicillium sp.	1.04±0.04*	2.06±0.32	1.07±0.11**	2.01±0.12**	3.42±0.11*	1.11±0.12
A. alternaria	2.05±0.03	3.01±0.12**	1.09±0.21*	2.04±0.22**	6.13±0.18	3.12±0.11**
Mucor sp.	1.02±0.01*	4.02±0.15	2.07±0.15	1.11±0.25	7.15±0.11**	5.02±0.12
A. flavus	2.02±0.12*	2.02±0.17*	1.04±0.11**	2.05±0.12**	6.16±0.12*	7.07±0.13*

Values are mean ± SEM; *Values are statistically significant at P< 0.05;**Values are statistically significant at P< 0.01.

Table 9. Antifungal activity of *T. tissoti* against Fish fungal pathogens.

Pathogen	Zone of inhibition (mm) (Mean ± SD)					
	Methanol	E. acetate	Hexane	Acetone	n- Butanol	Chloroform
Microsporum sp.	1.12±0.16*	1.11±0.12*	1.12±0.12*	1.12±0.13**	3.12±0.11	2.01±0.12*
Aspergillus fumigatus	1.02±0.11	1.01±0.11**	1.13±0.12	1.13±0.11	3.03±0.1	1.12±0.11
Aspergillus flaves	2.33±0.11*	2.06±0.12	1.11±0.12	1.13±0.01	5.11±0.31**	1.04±0.15*
Aspergillus niger	1±0.15*	1.14±0.48	1.02±0.11*	2.15±0.14*	3.12±0.11	5.16±0.11
Ichthyophonus sp.	-	1±0.01**	3.10±0.15	1.12±0.01	5.21±0.11	1.11±0.12*
Rhizopus sp.	1.22±0.13*	1.15±0.02	2.02±0.12	1.16±0.02	4.13±0.12	1.05±0.16*
Rhizopus sp.1	1.12±0.33	1.02±0.22	1.11±0.13*	1.01±0.01	1.02±0.13**	-
Fusarium sp.	3.13±0.02*	1.12±0.11	1.11±0.15	1.06±0.12*	4.14±0.11	1.12±0.15
Aspergillus sp.1	1.12±0.21	11±0.31	1.02±0.03*	1.23±0.08	3.17±0.21	1.16±0.13*
Aspergillus sp.2	2.11±0.11*	1.12±0.12	2.12±0.22	1.12±0.03**	1.14±0.02	-

Values are mean ± SEM; *Values are statistically significant at P< 0.05;**Values are statistically significant at P< 0.01.

Table 10. Antifungal activity of *B. spirata* against Fish fungal pathogens.

Pathogen	Zone of inhibition (mm) (Mean ± SD)					
	Methanol	E. acetate	Hexane	Acetone	n- Butanol	Chloroform
Microsporum sp.	2.01±0.02*	1.1±0.12*	1.03±0.15**	7.02±0.15*	6.12±0.33*	1.12±0.13*
Aspergillus fumigatus	1.12±0.14	1.22±0.21*	-	3.21±0.33	4.15±1.23*	1.14±0.01
Aspergillus flaves	1.11±0.16	2.11±0.15	1.02±0.15	3.12±0.12*	1.02±0.03	1.12±0.01*
Aspergillus niger	1.06±0.01*	1.11±0.08**	1.22±0.12*	1.11±0.14*	15.12±0.23*	1.14±0.08
Ichthyophonus sp.	1.08±0.12	1.01±0.01*	1.32±0.01*	4.04±0.36*	6.11±0.1**	2.14±0.11*
Rhizopus sp.	1.02±0.15*	-	-	1.11±0.15	4.15±0.05*	1.18±0.13
Rhizopus sp.1	1.02±0.25	1.01±0.25*	1.08±0.03	1.13±0.18**	-	1.12±0.15*
Fusarium sp.	1.33±0.15	1.13±0.11	1.09±0.05**	1.25±0.22	1.11±0.15*	2.12±0.12
Aspergillus sp.1	2.12±0.02*	1.14±0.21**	1.01±0.12	2.02±0.15**	8.16±0.21	1.15±0.15*
Aspergillus sp.2	8.02±0.22	14.22±0.13	3.02±0.12*	12.21±0.05*	4.12±1.21**	2.32±0.12*

biological activity (Pennings, 1994). Several glycoproteins from the egg masses of *Aplysia kurodai* and *Aplysia Juliana* were found to have antimicrobial activity (Yamazaki, 1993). Consequently, marine molluscs may

have isolating compounds with specific activity against certain microorganisms or cell types. The benthic marine invertebrates could provide protection against infection, although his assertion does not appear to have ever been tested. However, several compounds with antibacterial activity have been isolated from the egg masses of some marine mollusks (Benkendorff et al., 2000a). These investigations lend support to the present findings of the antimicrobial activity of *T. tissoti* and *B. spirata*.

In the search for novel source of antibiotics it is appropriate to consider the circumstances under which antibiotics might be expected to evolve. Most species of invertebrates suffer disease from a wide range of microbial pathogens and parasites. Certain levels of immunity can be found in all living things. However, invertebrates lack many features of the vertebrate immune system (Beck and Habicht, 1996) and studies on invertebrate immunity have revealed some novel types of defence, such as antibacterial peptides and proteins (Hoffmann and Hetru, 1992). On exposure to foreign material, some molluscs have been shown to exhibit a humoral defence response, including the release of bacteriostatic and bactericidal substances. Some of these molecules could have significant applications in medicine. Molluscs are widely used in world research institution for various studies, but only recently they have been recognized as potential sources of antibacterial and antifungal substances. The potential of marine mollusc as a source of biologically active products is largely unexplored. Hence, a broad based screening of marine molluscs for bioactive compounds is necessary. However, by far the majority of marine organisms are yet to be screened and the potential for discovering a useful antibiotic is sufficient to affirm further research.

Conclusion

From our result showed that the finding of novel bioactive compounds from marine molluscs by screening of different pathogenic microbes. This investigation was followed by the screening tactics based on the ecological knowledge of marine organisms being increasing deployed in the investigation of novel bioactive compounds. However, the result presented here indicated that marine molluscs show antimicrobial properties against human, fish pathogen and biofilm microbes. Our preliminary results reveal that many of these marine organisms produce more or less structurally diverse secondary metabolites which could be of Pharmaceutical interest.

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