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Research Article

SCREENING OF MARINE GASTROPODS FOR POTENTIAL ANTIBACTERIAL SUBSTANCES AGAINST PATHOGENIC BACTERIA

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ABSTRACT

Marine invertebrate possess many safeguarding secondary metabolites to survive in a microbe-rich benthic ecosystem which are useful in the biomedical area. In the present study, Antibacterial activity was observed in three gastropods - *Mauritia arabica*, *Purpura bufo* and *Pleuroploca trapezium* against five pathogenic bacteria. Antibacterial activity was carried out by disc diffusion method using the tissue and shell extract of benzene and ethanol. The ethanolic tissue extract of *P.bufo* showed a higher inhibitory action (17.67 mm) against gram positive bacteria *Staphylococcus aureus*. Among the two different extract, antibacterial activity was more pronounced in the ethanol extracts than the benzene extracts. The antibacterial potential in the tissue and shell extracts of the molluscs suggest that the different species of gastropods possess distinct antibacterial compounds.

Keywords: Antibacterial activity, *Mauritia arabica*, *Purpura bufo*, *Pleuroploca trapezium*, Molluscan, Gastropods.

INTRODUCTION

Molluscs are the important source of salient natural products. Many of these organisms are known to have safeguarding compounds to protect themselves (Indap & Pathare, 1998). They are widely distributed throughout the world and constitute about 23% of the marine organisms. They are heterogeneous in configuration and diversity, and are represented by amphineura, gastropods, bivalves, cephalopods and scaphopods. Most of these organisms thrives in a harsh and hostile intertidal rocky shores, mudflats and shelf zone where they are exposed to extreme temperatures, pressures and salinity changes along with the inter species and intra-species competitions and microbial attacks. To defend themselves, these organisms rely exclusively on their innate immunity that comprises of humoral and cellular responses, and they lack an adaptive immunity. Humoral immunity in these marine invertebrates is marked by antimicrobial agents in the blood cells and plasma. Cellular immunity in marine invertebrates is mediated by blood cells, motile cells that phagocytize microorganisms and them synthesis bioactive metabolites possessing immunological, antimicrobial and cytotoxic substance into the circulatory fluid. This adaptation helps them to survive in a microbe-rich benthic environment and to overcome different kinds of stress (Gliński & Jarosz, 1997; Loker *et al*., 2004; Mitta *et al*., 2000; Wright & Ermak, 1982). The organisms living in unsanitary and unhygienic conditions evolved a protecting mechanism against the pathogenic microorganisms made researcher to focus on the invertebrates for antimicrobial drug development (Bazes *et al*., 2009; Fischbach & Walsh, 2009; Jayaseeli *et al*., 2001; Mohan *et al*., 2016; Pettit *et al*., 1987; Stix, 2006; Wright & Sutherland, 2007; Yoneyama & Katsumata, 2006).

Antimicrobial compounds research provides a new insights into bioactive compounds in molluscs (Suresh *et al*., 2012). Molluscs are rich source of structurally diverse bioactive compounds with more valuable pharmaceutical and biomedical application (Shanmugam & Mody, 2000). They have more than thousands of secondary metabolite such as peptide, sterols, polypropionate, terpenes, sesquiterpenes, nitrogenous compounds, macrolides, prostaglandins miscellaneous compounds and alkaloids, that exhibits activities like antimicrobial, antitumour, antileukemic, antineoplastic, cytotoxic, anti inflammatory and antiviral (Anand & Edward, 2002; Bazes *et al*., 2009;

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Kamiya *et al*., 1989; König *et al*., 2006; Pettit *et al*., 1987; Ramasamy & Murugan, 2005). In India, 3370 species of marine molluscs have been recorded (Periyasamy *et al*., 2012), still many marine molluscs bioactive compounds are unexplored. Hence a preliminary screening is necessary to explore the bioactive compounds having antimicrobial activity. In the present investigation, three molluscs were screened for antibacterial activity against pathogenic bacteria.

MATERIALS AND METHODS

Specimen collection and extraction

Live specimen of three gastropod was collected from Kadiyapattanam and Muttom, located at south east coast of Kanniyakumari, Tamil Nadu, India. The gastropods are *Mauritia arabica, Purpura bufo* and *Pleuroploca trapezium*. The samples were washed with distilled 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 shell and tissue were dried in a hot air oven at 50ºC for 15 days. The dried sample was ground into powder. 5g of the tissue and shell sample were soaked in 10 ml of benzene and ethanol for 1 week. The extracts were filtered by Whatman No.1 filter paper, concentrated and stored at 4ºC for further analysis.

Antimicrobial assay: Test Microorganisms

The microorganisms used in the antibacterial screening assays were: Two gram-positive bacteria including *Staphylococcus aureus* and *Enterococcus faecalis* and three gram-negative bacteria including *Klebsiella pneumonia, Pesudomonas aeruginosa* and *Enterobacter aerogenes.* These Pathogenic microbial strains were procured from Scudder Lab, Vadasery. Stock cultures of the microorganisms were grown in nutrient agar slants at 37ºC.

Antibacterial activity – Agar Disc Diffusion Assay

Antibacterial activity of each solvent extract was determined using a modified disc diffusion method (Bauer, 1966). Pathogenic bacterial strains were inoculated in sterile nutrient broth and incubated at 37ºC for 24 hours. Sterile Mueller Hinton agar plates were prepared and allowed to solidify. The pathogens to be screened were swabbed on top of the solidified media using sterile swabs. Sterile disc of 6mm, loaded with 50µl of the crude extract. The solvent was allowed to evaporate and the disc was placed on the surface of the plate. The plates were incubated at 37ºC for 24 hours. After incubation, the inhibition zone was measured. Assay was carried out in triplicates and control plates were maintained. The antibiotic disc Kanamycin was used as the positive control and solvent discs were used as the negative control. Zone of inhibition was measured in millimeters and recorded.

RESULTS

The tissue extract of the molluscs showed distinct antibacterial activity on bacterial strains tested. The ethanol solvent tissue extracts showed higher inhibitions when compared to the benzene tissue extracts. The maximum zone of inhibition (17.67±0.47mm) was shown by ethanol tissue extract of *P.bufo* against *S.aureus*. Among the five bacteria tested the benzene extract of the tissue of *P.bufo* failed to inhibit the growth of *E.aerogenes* and *S.aureus* (Table 1).

Table 1. Antibacterial activity of the tissue extracts of the gastropods *M.arabica, P.bufo and P.trapezium* against Gram-positive and Gram-negative bacteria(Zone of inhibition in mm).

Pathogens tested	Bacterial strains	*M.arabica*		*P.bufo*		*P.trapezium*	
		Ethanol	Benzene	Ethanol	Benzene	Ethanol	Benzene
Gram-negative bacteria	*K.pneumoniae*	11.00 ±1.00	6.33±1.52	11.33 ±1.24	4.67 ±0.47	12.67±1.24	12.67±1.24
	P.aeruginosa	14.67±1.16	7.33 ±1.52	12.67±1.24	10.33±0.47	12.00±0.81	8.67±0.47
	E.aerogenes	15.67±1.52	7.67 ±1.52	13.67±1.24	-	13.67±1.24	-
Gram-positive bacteria	*S.aureus*	12.67±1.52	7.00 ±1.00	17.67±0.47	-	14.67±1.24	9.33±1.24
	E.faecalis	11.67 ±1.52	6.33±1.52	10.33±0.94	10.00±1.63	13.33±1.24	12.33±1.69

In shell extracts, ethanol solvent extracts showed significant activity when compared to the benzene extracts. The ethanolic shell extract of *P.bufo* showed maximum antibacterial activity of 14.67±1.24 mm against *P.aeruginosa* and *E.aerogenes.* No zone of inhibition was observed in benzene extract of *P.bufo* against *E.aerogenes* and *S.aureus, and P.trapezium* benzene extract against *S.aureus,* and the ethanol extract of *P.trapezium* showed no antibacterial activity against *E.faecalis* (Table 2).

DISCUSSION

The increasing resistance of numerous bacterial strains against the commercially available antibiotics paved way to develop more potent antibacterial agents from natural source with fewer side effects. Marine invertebrates offers a valuable source of potential antimicrobial drugs (Chakrabort*yet al*., 2016; Jayaraj*et al*., 2008). Molluscs are regarded as one of the most beneficial natural source to derive innumerable active compounds that have a role in

the chemical defense against their predators. Their active compound also exhibits antitumor, antimicrobial, anti-inflammatory and antioxidant activities (Avila, 1995; Benkendorff *et al*., 2011). Many studies worldwide have reported antibacterial and antiviral activities of bioactive compounds from molluscs (Fenical, 1997). In the present study, the ethanol extract of the soft body tissue exhibited a strong antibacterial activity against both Gram positive as well as Gram negative bacteria when compared to the benzene extract. This reveals that polar solvents are capable of extracting compounds that are more effective in controlling bacterial activity than the non-polar solvent extracts. Likewise the ethanol extracts of *Babylonia spirata* and *Turbo brunneus* showed high activity against *E. coli*, *K. pneumoniae*, *P. vulgaris* and *S. typhi* (Anand *et al*., 1997). In a same way the egg white extract of blister beetle, *Mylabris pustulata* (Thunberg) showed antimicrobial activity (Carolyn *et al*., 2019).

Table 2. Antibacterial activity of the shell extracts of the gastropods *M.arabica, P.bufoand P.trapezium*against Gram-positive and Gram-negative bacteria (Zone of inhibition in mm).

Pathogens tested	Bacterial strains	*M.arabica*		*P.bufo*		*P.trapezium*	
		Ethanol	Benzene	Ethanol	Benzene	Ethanol	Benzene
Gram-negative bacteria	*K.pneumoniae*	11.33±1.52	9.33±2.51	12.67±1.24	5.67±0.47	13.33±1.24	9.33±1.24
	P.aeruginosa	13.30±1.52	9.33±1.52	14.67±1.24	11.30±1.24	14.50±0.47	8.33±0.47
	E.aerogenes	14.33±1.52	9.00±1.00	14.67±1.24	-	7.33±0.47	5.33±0.47
Gram-positive bacteria	*S.aureus*	14.00±1.00	11.00±2.00	12.00±0.81	-	14.67±0.94	-
	E.faecalis	13.67±1.52	9.33±1.52	13.67±1.24	7.67±1.24	-	4.33±0.47

Table 3. Effect of Positive and negative control against Gram-positive and Gram-negative bacteria (Zone of inhibition in mm).

Pathogens tested	Bacterial strains	Positive control	Negative control	
		(Kanamycin)	Ethanol	Benzene
Gram-negative bacteria	*K.pneumoniae*	10.00 ± 0.00	5.00 ± 1.00	7.33±1.24
	P.aeruginosa	5.00 ±0.00	10.67±1.52	8.67±0.94
	E.aerogenes	R	14.00±0.81	7.67±0.47
Gram-positive bacteria	*S.aureus*	R	14.00±1.00	7.00 ±0.82
	E.faecalis	R	14.67±0.47	8.00 ±0.82

All values are Mean± Standard deviation, R- Resistant.

In the present finding, the tissue extract exhibited maximum inhibition activity (17.67±0.47mm) against *S.aureus* that agrees well with the result of the whole body ethyl acetate extract of *Villorita cyprinoides* which showed high inhibitory activity against *Streptococcus mutans* and *S.aureus* (Anitha & Rose 2018). Chandramathi and Thilaga (2018) noted that the fractionated whole body tissue extract of marine molluscan gastropod, *Conus achatinus* showed maximum inhibition zone of 20 mm against *S.aureus* at 100mg/ml concentration when tested against ten bacterial pathogens. Similarly Annamalai *et al*., (2007) found that *Perna viridis* showed maximum inhibition against *E. coli* and *S.aureus*. The mucous secretion of *Achatina fulica* inhibited the bacterial growth of both *S. aureus* and *S. epidermidis* (Lorenzi & Martins, 2008; Martins*et al*., 2003). The basis for higher sensitivity of the gram-positive bacteria than gram negative bacteria could be due to their differences in cell membrane constituents. The GCMS analysis of methanolic flesh extract of marine gastropod *T. brunneus* reveals the presence of eight compounds, out of which 7 compounds has antimicrobial properties (Tamil Muthu & Selvaraj, 2015). This shows marine molluscan has the property of inhibiting the bacterial growth. The present investigation shows that the Gram negative bacteria *E.aerogenes* and Gram positive bacteria *S.aureus* and *E.faecalis* which were resistant to the positive control Kanamycin exhibited considerable susceptibility towards ethanolic tissue extract of all the three tested gastropods *M.arabica*, *P.bufo*and *P. trapezium*. The ethanolic tissue extract of three test gastropods showed considerably greater antibacterial activity against Gram negative bacteria *K. pneumonia* and *P.aeruginosa* than the positive control kanamycin so the present study reveals that the antibacterial substance found in tissue sample *M.arabica*, *P.bufo* and *P. trapezium* possess potential antibacterial activity than the commercial antibiotic kanamycin.

CONCLUSION

Many pathogenic microorganisms have developed drug resistance to commercially available antibiotics; hence there is need for developing pharmacologically active compounds from natural source which is devoid of side effects. The present study reveals that marine molluscan

has the antimicrobial property. Each molluscs contains distinct antimicrobial substance that may be used in developing antibacterial compounds since they possess a significant inhibitory activity against pathogenic bacteria. Further investigations have to be carried out in characterizing the active compounds.

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