ANTIBACTERIAL ACTIVITY OF MARINE SPONGE COLLECTED FROM SUNHARI BEACH

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Abstract

The antibacterial activity against eleven gram positive and seventeen gram negative bacteria were explored in this study. All the bacterial isolates were checked and identified on the basis of conventional methods for purity and maintained for further work. Antibacterial activity of crude extract of sponge against the test organisms were determined by using agar-well method. Autoclaved Muller Hinton broth was used to keep the bacterial culture in log phase for 2 hrs with constant agitation and subsequently wells were dug onto Muller Hinton Agar. All plates were incubated and after incubation diameter of zone of inhibition was measured.

Introduction

Since marine organisms live in a significantly different environment from those of the terrestrial organisms, it is reasonable to expect that their secondary metabolite will differ considerably. Sponges (Porifera) have been recognized as a rich source of compounds that are of potential interest to mankind. They were determined as potential source of novel antimicrobial agents (Krishnan *et al*., 2014). Some sponges seem to produce potentially useful antifouling agents (Imhoff and Stohr, 2003). Although many bioactive compounds have been discovered in sponges Pedpradab *et al*. (2010), only a few of these compounds have been commercialized (Belarbi *et al*., 2003).

Many sponge or sponge symbiont–derived metabolites are potent antibacterial, antifungal, antifeeding and antifouling compounds (Becerro *et al*., 1997), a number of bacteria associated with sponges were found to be the sources of antibiotics and other bioactive compounds in the marine environment (Bewley *et al*., 1996). In the present study, we report the antibacterial potential of marine sponges collected from Sunahri beach (25°54' N 61°44' E).

Materials and Methods

Sample Collection and extract preparation: *Sponge* was collected from Sunhari beach during low tide in February, 2012. The collected samples were kept into plastic bags containing water to avoid discoloration and brought in the Marine Reference Collection and Resource Centre, University of Karachi for further studies. The sponge surface was washed with sterilized distilled water to remove surface contaminants such as small marine organisms, sand and other debris, then the samples were dried at 55°C for 48 hrs and ground into a fine powder. The powdered samples were later stored in the refrigerator at 4 C until used. Extracts were prepared, with some modifications, following Senevirathne *et al*. (2006). In brief, sponge was extracted with distilled water in the concentration of 5% in a shaking incubator at 25°C for 3 days. The extracts were filtered with Whatman's No. 1 filter paper and re-extracted three times. The extract was concentrated, evaporated and lyophilized to acquire a dry extract. The dry extract was kept in desiccators and then was stored in airtight glass containers sealed further with parafilm protected from sunlight till further studies.

Screening of antibacterial activity: The test organisms for this study were isolated, identified, maintained and stored in the Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi-Pakistan. The antibacterial activity of *Sponge* against eleven gram positive and seventeen gram negative bacteria were examined. All the bacterial isolates were checked for purity and maintained on nutrient agar at 4^0c in the refrigerator until required for use. Antibacterial activity of crude extract against pathogenic bacteria was determined by using agar-well method. Autoclaved Muller Hinton broth was used to freshen the bacterial culture, later wells were punched into Muller Hinton Agar and 10 microliters of culture were poured into the wells (Perez *et al.,* 1990). All plates were incubated at 28$\pm$ 2°C for 24 -48 hours and after incubation diameter of zone of inhibition was measured.

Determination of Minimum inhibitory concentration (MIC): MIC of crude extract was determined by Micro broth dilution method using 96-well microtitre plate (Samie *et al*., 2005)**.** Stock solution of 100 mg/ml of crude extract was prepared in distilled water. Two fold serial dilutions of extracts was made in 100 µl broth and subsequently 10 µl of two hours old fresh culture matched with 0.5 Mac Farland index was added to each well. One well served as antibiotic control while other served as culture control. Microtitre plate was incubated for 24 hours at 37 ºC.

Bio-chemical analysis of the extract: The qualitative bio-ochemical analyses were also carried out for the determination of alkaloids, flavanoids, terpenoids, phenols, saponins, tannins and others (Brindha *et al*, 1977, Harborne, 1973 and Doughari *et al*, 2007)

Results and Discussion

Screening of antibacterial activity: The antibacterial activity of *Sponge* against eleven gram positive and seventeen gram negative bacteria were examined. It was observed that the sponge extract was moderately active against four gram positive *Corynebacterium diptheriae, Corynebacterium hofmanii, Corynebacterium xerosis* and *M. smegmatis* and four gram negative *Klebsiella pneumonia, Serratia marcesens, Acinetobacter baumanii* and *Vibrio cholerae* bacteria (Table 1). The range of zone of inhibition was 15mm to 33mm for gram positive and 15mm to 20mm for gram negative strains. The maximum activity (33±2mm) was recorded against *M. smegmatis* and minimum (15mm) against *orynebacterium xerosis*. The results indicated that this species may have selective response mechanism as suggested by Pedpradab *et al*., 2010. Many researches (Proksch, 1994; Belarbi *et al*. 2003; Mayer and Gustafson 2003, Newman and Cragg, 2004) also indicated that the secondary metabolites of sponges play an important role in defense against infectious microorganisms.

Table 1. Antibacterial potential of *sponge* was determined by Agar well diffusion method in terms of zone of inhibition (mm).

Gram positive bacteria	Zone of inhibition in mm (mean ± S.D)	Gram negative bacteria	Zone of inhibition in mm (mean ± S.D)
Bacillus cereus	-	*Enterobacter aerogenes*	-
Bacillus subtilis	-	*Escherichia coli ATCC 8739*	-
Bacillus thruingiensis	-	*Escherichia coli*	-
Corynebacterium diptheriae	20+0	*E. coli multi drug resistance*	-
Corynebacterium hofmanii	18+1	*Klebsiella pneumonia*	20+2
Corynebacterium xerosis	15+3	*Salmonella typhi*	-
Staphylococcus epidermidis	-	*Salmonella paratyphi A*	-
Streptococcus saprophyticus	-	*Salmonella paratyphi B*	-
M. smegmatis	33+2	*Shigella dysenteriae*	-
Streptococcus fecalis	-	*Serratia marcesens*	19+1
Streptococcus pyogenes	-	*Acinetobacter baumanii*	15+2
		Campylobacter jejuni	-
		Campylobacter coli	-
		Helicobacter pylori	-
		Hemophilus influenza	-
		Vibrio cholera	20+1
		Aeromonas hydrophila	-

Determination of Minimum inhibitory concentration (MIC): Minimum Inhibitory Concentration were also determined by Microdilution method to search the therapeutic concentration which was found in the range for gram positive (MIC 20-70 microgram/ml) for gram negative strains (MIC 34-98) (Table 2). All the other bacterial strains were resistant to these extracts. Though, s*ponge extract* was effective against all tested bacterial strains both gram positive and gram negative but the results depicted that the extract showed better antibacterial property 98 mg/ml for *Serratia marcesens* (gram negative) in vitro gram positive strains. No activity was observed against some of the bacterial pathogens tested probably due to the reason of using crude extract because it might have some inhibitory substances that interfere the antibacterial activity range (Sastry and Rao, 1994)**.** There is also a need to employ all possible techniques to search the biocactive compound that exhibits the antibacterial action.

Table 2. Minimum Inhibititory Concentration (MIC) *of sponge* in mg/ ml was determined by Microdilution method.

Bacteria \ Extract	MIC (mg/ mL)
Gram positive bacteria	
Corynebacterium diptheriae	70
Corynebacterium hofmanii	40
Corynebacterium xerosis	54
M. smegmatis	20
Gram negative bacteria	
Klebsiella pneumonia	34
Acinetobacter baumanii	74
Serratia marcesens	98
Vibrio cholera	72

Bio-chemical analysis: As far as qualitative photochemical analysis is concerned, the extract of *Sponge* possessed alkaloids, flavonoids, phenols and saponins (Table 3). *Soulange et al., (2014)* also reported alkaloids and tannins in marine sponge.

Table 3. Qualitative bio-chemical analysis of *sponge extract.*

Compounds	Presence/absence
Alkaloids	+
Flavonoids	-
Total phenol	+
Saponins	+

Note: + ve indicates presence, - ve indicates absence

The results showing the activity of sponge against some potential human pathogens. Further and comprehensive analysis is needed to introduce it in the world of therapeutics and to combat the load of infectious diseases.

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