

## Screening of Antimicrobial Activity of Sponges Extract from Pasir Putih, East Java (Indonesia)

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Received date: May 26, 2017; Accepted date: September 11, 2017; Published date: September 14, 2017

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### Abstract

The emergence of new infectious diseases, the resurgence of several infections that appeared to have been controlled, and the increase in bacterial resistance have created the necessity for studies directed towards the development of new antimicrobials; considering the failure to acquire new molecules with antimicrobial properties from marine sponges. The objective of this study was to evaluate screening of antimicrobial activity of seven sponges extract from Pasir Putih, East Java against some Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) as well as drug-resistant bacteria (*S. aureus* and *Pseudomonas aeruginosae*) using the agar diffusion method and phytochemical screening of the extract. The findings show that the extract, *Xestospongia testudinaria* has a stronger antibacterial activity against bacterial pathogens *S. aureus*, *E.coli*, *K. pneumoniae*, *Salmonella typhi* and bacteria resistant *P. aeruginosa* MDR and *S. aureus* MRSA compared to other sponge extract. In conclusion, the showed *X. testudinaria* ethanol extract was more active than other sponge extracts.

**Keywords** Antimicrobial activity; Sponges; Pasir putih

### Introduction

Nature has been a very good source of many medical compounds for thousands of years. In the last decades, problem with antibiotic resistant bacteria has emerged. Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents, and nowadays, the need to discover new and more potent of these agents as accessories or alternatives to antibiotic therapy is stronger [1,2].

Marine drugs are compounds obtained from marine plants, animals and microorganisms. About 70% of earth's surface is covered with water and it comprises 5,00,000 live species divided into 30 different phyla. The world ocean has a coastline of about 3,12,000 km and a volume of  $137 \text{ km}^3 \times 106 \text{ km}^3$  making it the largest ecosystem on earth [3]. Indonesia is one of the ten countries with the richest biodiversity, and is often known as a mega-diversity country [4].

Sponges offer a rich source of unique and diverse natural products. Many of these compounds have potent pharmacological activities, including anti-tumor, fungal, viral, and bacterial properties, some of which are currently in preclinical or clinical trials [5]. However, natural products were also found to play important biological and ecological roles for the producing organisms such as defense against predators, competition for space, prevention of fouling, roles in reproduction, and antimicrobial activity. Several antibiotics have been isolated, such as plakortin [6] and manoalide [7] from marine Sponges. Pecaron Bay is located at Situbondo East java. This bay has reef structure which consists of Poriferan and Coelenterata. The study aims at exploring

antimicrobial compounds from sponges taken from Pasir Putih area, Tanjung Pecoran Bay, East Java.

### Materials and Methods

Sponge samples were taken from Pecaron Bay on the last 10-11 March 2016 using scuba diving in 5-20 m depth, 500 m from coast line (Figure 1).

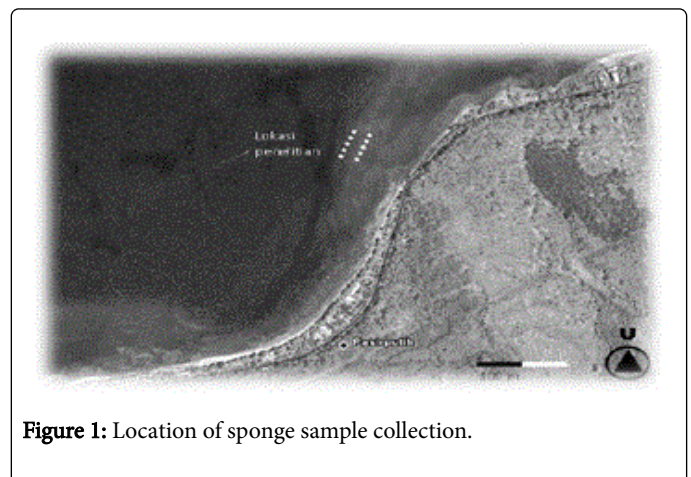


Figure 1: Location of sponge sample collection.

The sponge samples had been photographed underwater to describe the in situ habitat morphology. The samples were preserved by 70% ethanol solution for spicules identification and morphological identification used as the determination key of Van Soest [8] and Hooper and Van Soest [9], whereas for spicules, preservation and

identification were done by bleaching and acid method for rapid sponge survey [10]. The identification of spicule character is used as the key determination of Andri [11,12].

### Extraction

A 1 kg sample was soaked in 100% methanol (MeOH) for a minimum of 24 h to extract the most polar compounds. The MeOH was decanted and reserved and the procedure was repeated two more times.


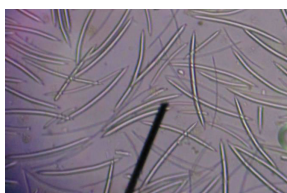

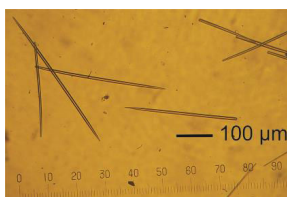

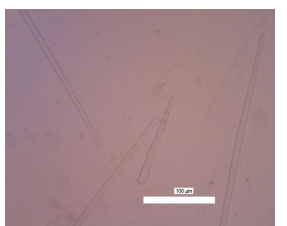
### Antibacterial test


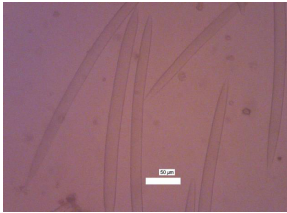




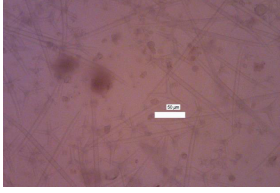

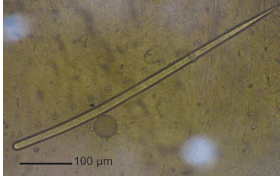
Inhibitory interaction test of extract against pathogenic *Staphylococcus aureus* ATCC, *Escherichia coli* ATCC, *Klebsiella pneumoniae* ATCC, *Salmonella typhi* ATCC, MRSA and MDR was performed by using the agar disk-diffusion method. About 100 µl of the liquid culture of pathogenic bacteria/test bacteria with a density of 109 cell/ml was inoculated into plain agar media Zobell 2216E, and diffused on to the media surface by using a spreader, and left for 15 min. Then, a paper disc was put onto the agar surface that had been

planted with pathogenic bacteria/test bacteria. Then, 30 µl of sponge extract was dropped onto the paper disk, and then incubated under room temperature for 2 × 24 h. Any resulting inhibition zone was closely observed and measured. Antibacterial activity was defined according to Radjasa et al. [13] by the formation of inhibition zones greater than 9 mm around the paper disk.

### Results

In order to explore sponge species diversity, sponge spicules are used as a tool for describing sponges morphology and relationship instead of in situ underwater specimen photograph and microscopic tissue observation. Based on spicules microscopic description, the sponges have been grouped into three classes: Demospongiae (mostly sponges with a skeleton, composed of siliceous spicules), Hexactinellida (always Siliceous skeleton) and Calcarea (always calcareous skeleton) [10]. Almost 90% of sponges species in the world are from Demospongiae Class. In sampling area, 100% of Demospongian classes after morphology identification were based on Van Soest [8] key determination (Table 1).

S. No	Underwater Photograph	Characteristic	Spicula observation	Type spicula	Species (order)
1.		Often giant sized, red-grey, with heavily ridged outer walls, stony hard		Choanosomal unipaucispikular, isotropic skeleton, highly dense network of short longitudinal undivided irregularly parallel tracts	<i>Xestospongia testudinaria</i> (Haplosclereida)
2		Masses of globular osculiferous lobes, orange-brown, rough to the touch but compressible; turns black in alcohol		Megaskleres long oxea	<i>Aaptos aaptos cv Suberitoides</i>
3		GROWTH FORM tubular cylindrical, red color		skeleton multispicular-paucispicular reticulate, spongin fibre abundant	<i>Clathria basilana</i> (Microcionidae)

4.		Massively incrusting with stiff erect branches or laminations, smooth, dark brown, tough/crumblly		Megaskleres single size oxea 250-300x 30-35µm	<i>Neopetrosia exigua</i> (Petrosiidae)
5.		GROWTH FORM yellow inside globular subspherical, large oscules around the body, compressible	-	Skeleton radial, bundles of oxeas issuing from the centre and running spirally to the surface	<i>Cinachyrella australiensis</i> (Tetillidae)
6.		Irregular undulating-conulose surface, often covered by sediment, salmon coloured, compressible		Monactinal Style (monaxon rounded at one end and pointed at the other end with one end blunt and the other pointed)/MS	<i>Clathria reinwardti</i> (Microcionidae)
7.		GROWTH FORM Reddish yellow color, thin lamellate form		Megaskleres long cigarrete oxea 300-350 µmx15x20µm, undifferentiated caltrop	<i>Pachastrella</i> (Pachastrellidae)
8.		GROWTH FORM irregular foliaceous, fleshy		Megaskleres long style 400-500 x25x30µm	<i>Stylissa carteri</i> (Scopalinidae)

**Table 1:** Underwater, photograph, characteristic, type of spicula and species of sponge.

Observations of antimicrobial activity were done by measuring the diameter of inhibition zone formed around the wells using a janka sorong to millimeters (mm). The resulting inhibition zone of each treatment has a different diameter and irregular shapes. Therefore, the

observation was done by measuring the diameter of the horizontal and vertical diameter of inhibition zone formed around the well. The results of measuring the diameter of inhibition zone formed around the well are shown in Table 2.

Extract/drug	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumonia</i>	<i>S. typhi</i>	<i>P. aeruginosa</i> MDR	<i>S. aureus</i> MRSA
Extract <i>Xestospongia testudinaria</i>	20.10 ± 0.26	9.5 ± 0.12	15.25 ± 0.34	15.25 ± 0.26	15.25 ± 0.45	17.50 ± 0.23
Extract <i>Aaptos suberiptoides</i>	6.25 ± 0.15	11.15 ± 0.23	-	-	-	-
Extract <i>Clathria basilana</i>	8.10 ± 0.12	11.0 ± 0.20	12.50 ± 0.26	-	7.0 ± 0.21	15.0 ± 0.28
Extract <i>Neopetrosia exigua</i>	-	10.75 ± 0.68	-	-	8.0 ± 0.32	-
Extract <i>Cinachyrella australiensis</i>	-	10.50 ± 0.58	-	-	13.0 ± 0.65	14.5 ± 0.45
Extract <i>Clathria reinwardti</i>	-	10.75 ± 0.75	-	-	-	-
Extract <i>Pachastrella sp</i>	-	12.65 ± 0.85	-	-	-	10.0 ± 0.46
Extract <i>Stylissa carteri</i>	-	11.70 ± 0.95	-	-	10.0 ± 0.65	8.0 ± 0.50

**Table 2:** Zone inhibition (mm) of extract of sponges.

The results of sponge extracts of antimicrobial activity against pathogenic bacteria (*S. aureus*, *E. coli*, *K. pneumoniae*, *S. typhi*) and 2-resistant bacteria (*P. aeruginosa* MDR and *S. aureus* MRSA) indicate that extracts of the most powerful *Xestospongia testudinaria* antibacterial activity were compared with other extracts and showed broad spectrum antibacterial activity because it showed activity against gram-negative and gram-positive bacteria.

## Discussion

Drug discovery has become a very important field of study within the last few decades. With the increased resistance of the pathogenic microbes that cause human infection, it has become increasingly important to investigate natural products for antimicrobial compounds along with those synthetically derived; along with the increased risk bacterial and fungal infection.

Sponges have few obvious defenses, yet for most species, survival is dependent on their ability to deter predators, inhibit pathogenic microbes, and discourage the formation of a bacterial biofilm and subsequent macrofouling community. Research has indicated that sponge secondary metabolites may play important roles as defenses against some of these biotic challenges [14].

Table 2 shows the result of the in vitro testing of sponge extracts against pathogenic bacteria. This can be observed from the emergence of clear zone around the paper disc. Clear zone around the paper disc indicates the absence of bacteria that can grow after incubation due to the antibacterial compounds in the area. The antibacterial activity of Sponge against gram positive and gram negative bacteria were examined. It was observed that the sponge extract was moderately active against gram positive *S. aureus*, MRSA, and gram negative *K. pneumonia*, *P. aeruginosa* and *P. aeruginosa* MDR bacteria (Table 2). The range of zone of inhibition was 8 to 20 mm for gram positive and 9 to 15 mm for gram negative strains. The maximum activity (20.10 ± 0.26 mm) and minimum activity (6.25 ± 0.15 mm) were recorded against *S. aureus*.

Extract of sponge *Aaptos sberiptoides* have no antibacterial activity against *S. aureus*. Also, extract of *X. testudinaria* shows good activity towards *S. aureus*, but have no antibacterial activity against *E. coli*. This difference in activities is due to diverse chemistry of bioactive compounds in the same sponge. Sponges are primitive marine

invertebrates which contain more natural products than any other marine phylum. Many of their products have strong bioactivities including anticancer, antimicrobial, larvicidal, hemolytic and anti-inflammatory activities and are often applicable for medical use [15].

## Conclusion

Based on this research, it can be concluded that the extract *X. testudinaria* has a stronger antibacterial activity against bacterial pathogens *S. aureus*, *E. coli*, *K. pneumoniae*, *S. typhi* and bacteria resistant *P. aeruginosa* MDR and *S. aureus* MRSA compared with other sponge extract.

## Acknowledgement

This research was supported by grants doctorate in 2016 from the Kemenristek Dikti.

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