Antibacterial Activity of *Xetospongia* sp. and *Spongia* sp. Sponge Extract Against Pathogenic Bacteria from The Waters of Pahawang Island, Lampung

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Abstract: *Sponges are marine benthic invertebrates from the Porifera phylum that have bioactive compounds that can function as antibacterials. The purpose of this study was to compare the extracts of sponges of the Xetospongia sp. and Spongia sp. against pathogenic bacteria. This study was conducted in May-November 2025. Samples of sponges of the Xetospongia sp. and Spongia sp. taken from the waters of Pahawang Island were extracted using methanol, ethyl acetate and hexane. The results showed that the morphological characteristics the Xetospongia sp. had a rough body surface with a rounded shape and reddish color while the Spongia sp. type had a soft body surface with crusts and a dark black color. The yield of sponge extract using ethyl acetate was greater than using methanol and hexane. The antibacterial activity of sponge extracts against pathogenic bacteria of the Xetospongia sp. used hexane while the Spongia sp. used methanol.*

Keywords: Sponge, Yield, Antibacterial Activity

1. INTRODUCTION

Infectious diseases are a significant health problem. They are highly dynamic and can spread directly or indirectly from one person to another. The mortality rate from these diseases remains relatively high (Ristori et. al., 2024). Every year, infectious diseases can cause significant mortality. Treatment for infectious diseases typically involves antibiotics. Inappropriate and uncontrolled antibiotic use accelerates the emergence of antibiotic-resistant bacterial strains, commonly referred to as Multi-Drug Resistance (MDR) (Mancusso et al., 2021). This has become a complex health issue. Concerns about the management of infectious diseases caused by MDR strains have prompted numerous studies exploring new antibiotic compounds. Exploration of bioactive compounds from the seabed has been conducted in various regions and has yielded numerous compounds with potential for use as chemotherapeutic agents (Karthikeyan et. al., 2022). Indonesia's vast waters offer promising marine resources for the exploration of various new chemical compounds

Sponges are marine benthic invertebrates from the phylum Porifera. Their habit of living in extreme conditions has led to their unique defense system, called secondary metabolites (Maslin et. al., 2021). These secondary metabolites are used for self-defense and adaptation mechanisms to extreme environments, competition for space, defense against predation, inhibition of attachment organisms, and defense against other organisms (Lee et. al., 2021). The results of this metabolism are bioactive compounds. These bioactive compounds are compounds from the terpenoid, alkaloid, polypeptide, polyectide, and polyphenol groups (Perdicaris et al., 2013). These bioactive compounds can function as antibacterials, antifungals, antivirals, antitumor, anticancer, antifouling agents, and enzyme inhibitors (Rajendran, 2019). One sponge that possesses bioactive compounds is a sponge from the Demospongiae class, namely the species *Xetospongia* sp. and *Spongia* sp. The aim of this study was to compare the effectiveness of sponge extracts from *Xetospongia* sp. and *Euspongia* sp. against pathogenic bacteria.

2. MATERIALS AND METHODS

Time and Location Research

This research was conducted from May to November 2025 in the waters of Pahawang Island, Pesawaran Regency, Lampung Province (Figure 1). Laboratory analysis was conducted at the Oceanography Laboratory, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Lampung.

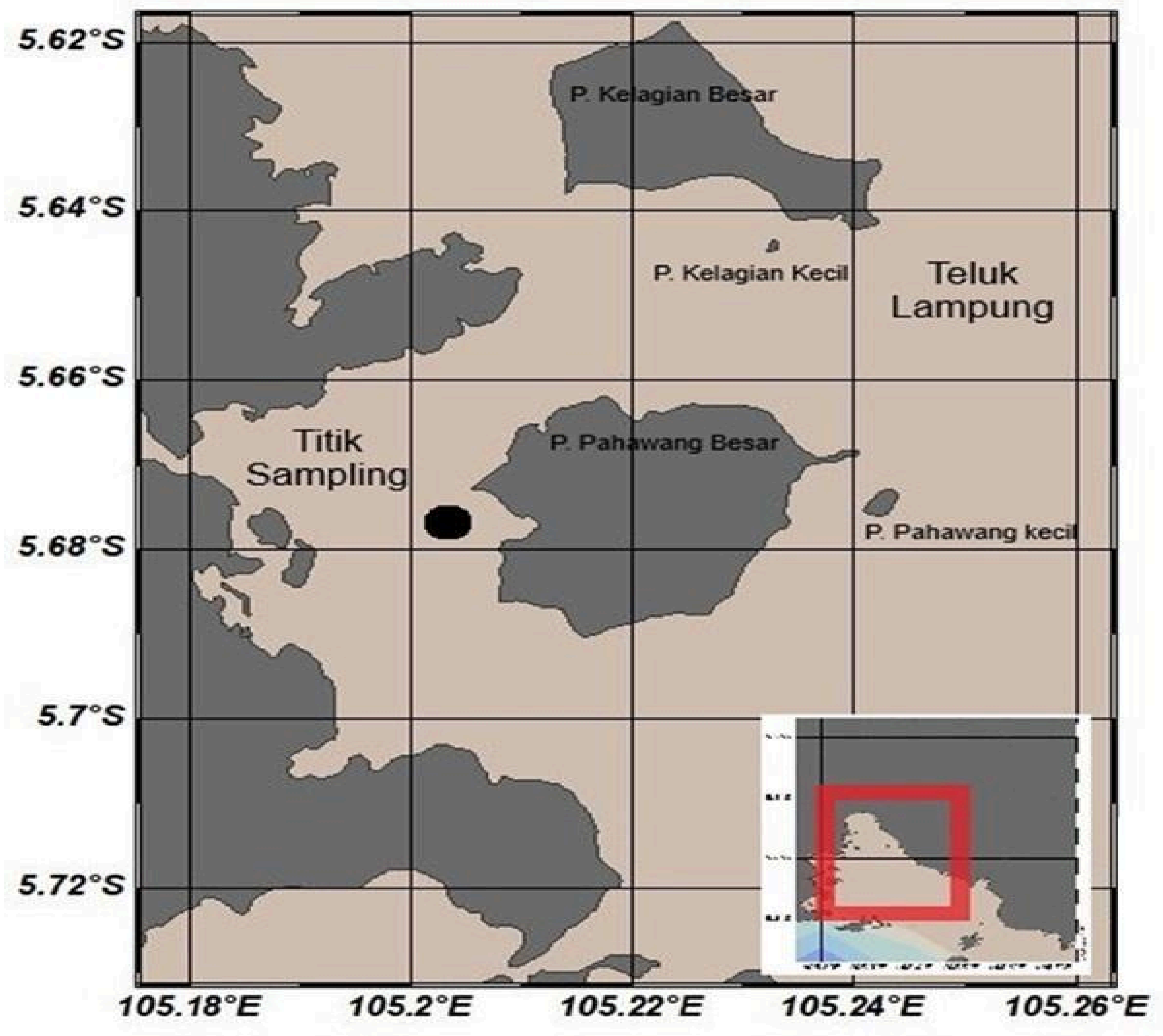

Figure 1. Map of research loaction

Tool and Materials

The tools used in this study include SCUBA (Self-Contained Underwater Breathing Apparatus) diving equipment, masks, gloves, scissors, 600 ml bottles, cool boxes, rulers, knives, Erlenmeyer flasks, rotary evaporators, analytical scales, separating funnels, measuring cups, petri dishes, autoclaves, tweezers, spirit burners, magnetic stirrers, dropper pipettes, micro tubes, hot plates, vortexes, spatulas, laminar air flow, test tubes, underwater camera incubators, and GC-MS while the materials used are sponge identification books, sponge samples, stain pathogenic bacteria *Escherichia coli*, distilled water, methanol, ethyl acetate, hexane, chloramphenicol, disc paper, filter paper and tissue.

Research Procedures

Sponge Sampling

Sampling was conducted using SCUBA diving equipment. Sponge samples were collected and cut using a knife, amounting to 1 kg of each type. They were then wrapped in plastic, placed in a cooler, and transported to the laboratory.

Sponge Ectraction

Extraction was carried out using the principle of maceration in methanol, ethyl acetate, and hexane solvents. 50 g sample was placed in a 100 ml solution of methanol, ethyl acetate, and hexane solvents and left for 48 hours, then filtered using filter paper and a vacuum pump (Kusuma et.al., 2023). The filtrate obtained was then evaporated at 60 °C to obtain a crude extract in the form of a paste. The crude sponge extract was dried at room temperature until dry, weighed, labeled, and stored at 4 °C.

Bacterial Culture

Culture of the pathogenic *E. coli* bacteria was aseptically inoculated into a test tube containing 5 ml of Nutrient Broth (NB) medium. The medium was incubated for 24 hours at 37ºC. Then, the medium was inoculated again from the NB medium into Nutrient Agar

(NA) medium, incubated for another 24 hours, and stored as a stock of the bacteria.

Antibacterial Test

Bacterial effectiveness testing was conducted using the Kirby-Bauer or paper disk method, which involves observing and measuring the inhibition zone of sponge extract. A 1 ml inoculum of pathogenic E. coli bacteria was aseptically taken from NB medium and poured into a Petri dish containing NA medium. Then, a paper disk approximately 0.5 mm in diameter was dipped into sponge extract at specific concentrations with each solvent. The disc was placed using tweezers on top of the NA medium containing pathogenic E. coli bacteria and incubated at 37ºC for 24 hours. All media were then incubated for another 24 hours at 37ºC, and the diameter of the inhibition zone was observed and measured until the third day (Kusuma et al., 2023).

Data Analysis

The yield data was analyzed by comparing the weight of the obtained extract with the weight of the dry raw materials used in the extraction process. Antibacterial activity data was analyzed by calculating the diameter of the inhibition zone, expressed as the clear area around the paper discs for each sponge type and solvent type.

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3. RESULT AND DISCUSION

RESULT

Sponge Identification

Xetospongia sp. has a globular, reddish-colored barrel-shaped body with a rough surface. *Xetospongia* sp. is a type of sponge with a body shape resembling a large vase or barrel, commonly called a barrel sponge. The color of this sponge varies depending on the species and environment, but is typically pink, reddish-brown, purple, or grayish-brown. The outer surface of this sponge is rough or uneven in texture, sometimes with small protrusions. Ostia are found throughout the body surface. This sponge has a large osculum at the top, through which water exits after being filtered. The diameter of the osculum can be quite large and conspicuous. The body skeleton is made of spongin, a protein, and spicules are made of silica. (Hasan et.al., 2022; Khodzori et.al., 2023). *Spongia* sp. has a dark, encrusting body with a smooth surface. *Spongia* sp. is a type of sponge with an irregular shape that can crust, branch, or resemble mounds, so this sponge can be said to lack clear body symmetry. This sponge's color varies, generally light yellow, brown, or yellowish gray depending on the aquatic environment. Ostia of this sponge are scattered throughout the body. The body skeleton is spongin from protein, but the spicules are without silica. (Prastiyanto et.al., 2022).

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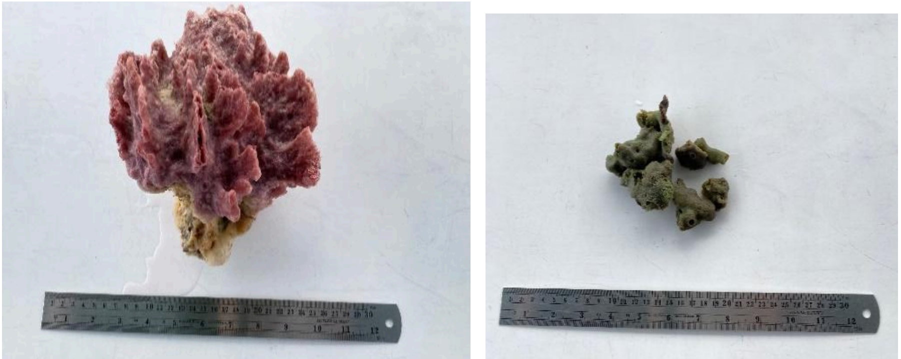

(a) (b)

Figure 2. Marine sponge (a) *Xetospongia* sp. and (b) *Spongia* sp. at research loacation

Sponge Extract Yield

Extraction results showed varying values in methanol, ethyl acetate, and hexane solvents. The yield of *Xetospongia* sp. sponge extract using methanol was 2,24%, ethyl acetate was 8,33%, and hexane was 2,61%, while *Spongia* sp. sponge using methanol was 9,79%, ethyl acetate was 10,08%, and hexane was 1,81%. The yield of *Xetospongia* sp. sponge extract using methanol was 1,01-5,53%, ethyl acetate was 4,43-8,56%, and hexane was 3.34-9.61% (Swantara et. al., 2019; Ibrahim et. al., 2021; Azemi et. al., 2019) while *Spongia* sp. sponge using methanol was 1,01-5,53%, ethyl acetate was 4,43-8,56%, and hexane was 3,34-9,61% (Swantara et. al., 2019; Ibrahim et. al., 2021; Azemi et. al., 2019). using methanol of 2,34-7,78%, ethyl acetate of 5,67-8,89%, hexane of 1,81-9,87%. (Krishnan & Keerthi, 2016; Yang & Liang 2024; Tai et.al., 2022). The yield of sponge extract using ethyl acetate is greater than using methanol and hexane. The yield of sponge extracted using ethyl acetate is higher at 10,08% compared to hexane at 1,81% (Chen et.al., 2022; Majali et.al., 2015). Sponge extract yields at the research site can be seen in Table 1.

Table 1. Sponge extract yield

Marine Sponge	Morphology	Yield (%)		
		Methanol	Ethyl Acetate	Hexane
Xetospongia sp.	*Globular*	2,24	8,33	2,61
Spongia sp.	*Encrusting*	9,79	10,08	1,81

Antibacterial Activity of Sponge Extract Against Pathogenic Bacteria

The antibacterial activity of a bioactive compound is depicted by the clear zone of inhibition formed around the paper disc. The activity of sponge extract against pathogenic bacteria *E. coli* obtained inhibition zone for *Xetospongia* sp. using methanol of 0,6 mm and hexane of 17,3 mm while *Spongia* sp. using methanol of 5,2 mm, ethyl acetate of 4,2 mm and hexane of 3,8 mm. The activity of sponge extract against pathogenic bacteria *E. coli* obtained inhibition zone for *Xetospongia* sp. using methanol of 3 mm and hexane of 25 mm (Edny et.al, 2020) while the antibacterial activity of *Spongia* sp. sponge against pathogenic bacteria *E. coli* with methanol of 8 mm and hexane of 3 mm (Kamaruding et.al, 2020). The antibacterial activity of *Xetospongia* sp. sponge extract is found in hexane solvent while *Spongia* sp. sponge in methanol solvent. This is also the same as what was conveyed in (Hartiadi et. al., 2020; Kalinggi et. al, 2017) where the antibacterial activity of the sponge type *Xetospongia* sp. used hexane while the sponge *Spongia* sp. used methanol (Yang & Liang 2024). The antibacterial activity of sponge extract against pathogenic bacteria at the study site is shown in Table 2.

Table 2. Antibacterial activity of sponge extract against pathogenic *E.coli*

Marine Sponge	Morphology	Inhibition Zone (mm)			
		Methanol	Ethyl Acetate	Hexane	Chloramphenicol (Control)
Xetospongia sp.	*Globular*	0,6	0	17,3	18,4
Spongia sp.	*Encrusting*	5,2	4,2	3,8	20,2

DISCUSSION

The solubility of bioactive substances is highly dependent on the solvent's ability to dissolve a substance. The level of polarity is a very important factor in determining the value of a yield because solvents with different polarities will dissolve different bioactive substances. The yield of sponge extracts from each type of solvent shows varying results. Methanol is an alcohol compound of a carbon group bonded to three hydrogen atoms and one hydroxyl group. Hexane is an alkane compound with a straight chain with six carbon atoms. Ethyl acetate is an ester compound of acetic acid and ethanol. The yield of sponge extracts using ethyl acetate solvent is greater than that of methanol and hexane for each type of sponge as a whole because ethyl acetate has properties between hydrophobic and hydrophilic. Ethyl acetate has a medium polarity that allows for the production of yield extracts. Various forms of bioactive substances in the form of yield extracts in an organism have complex structures that are neither completely polar nor non-polar but are in the middle spectrum so that ethyl acetate solvent is more effective in producing yields compared to methanol and hexane.

The antibacterial activity of sponge extract against the pathogenic bacteria E. coli for the sponge type Xetospongia sp. using hexane solvent was greater than that of methanol and ethyl acetate. Conversely, for the sponge type Spongia sp. antibacterial activity using methanol solvent was greater than that of ethyl acetate and hexane.This proves that the sponge type Xetospongia sp. has bioactive compounds as non-polar antibacterials, while the sponge type Spongia sp. has bioactive compounds as polar antibacterials. The bioactive compounds of the sponge type Xetospongia sp. Non-polar compounds include terpenoid groups such as xestolone, xestosaprol, xestobergsterol, steroid groups such as xetosterol derivatives, 24-methylcholesta-5,22-dien-3β-ol, xestosterol, fatty acid groups such as palmitic acid, stearic acid and oleic acid, and carotenoids (Ke et.al., 2024 & Gamal et.al., 2016). Polar bioactive compounds of Spongia sp. sponges include alkaloid groups such as spongidine a–e, saponin groups such as spongoside, peptide groups such as spongipeptin A–D, amino acid groups such as spongin acid and phenol groups such as spongiaphenol (Maximo et.al., 2016).

CONCLUSION

Sponge found in the waters of Pahawang Island for the type *Xetospongia* sp. have a rounded (globular) reddish body with a rough body surface while the type *Spongia* sp. has a dark encrusting body with a soft body surface. The yield of sponge extract using ethyl acetate is greater than using methanol and hexane. The highest antibacterial activity of *Xetospongia* sp. sponge extract is found in hexane solvent compared to methanol and ethyl acetate while *Spongia* sp. sponge in methanol solvent compared to ethyl acetate and hexane. *Xetospongia* sp. sponge extract with hexane solvent and *Spongia* sp. with methanol solvent can be used as candidates for antibacterial against pathogenic bacteria E. coli**.**

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