Antibacterial Activity of Active Compound in Methanol Extract of *Aaptos. sp* Sponge

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***Abstract*—Alkaloids are secondary metabolite compounds found in organisms, both on land and in the sea. These alkaloid compounds play a significant role in human life. The highest concentration of alkaloids is often discovered in marine sponges, which possess abundant alkaloid content. Consequently, research has been conducted to further explore the chemical content of these marine sponges, particularly their activity as antibacterial agents. The objective of this study is to isolate new alkaloid compounds from the *Aaptos sp*. marine sponge, determine their alkaloid structure, and evaluate their in vitro antibacterial activity. The initial phase of this research involves extracting the *Aaptos sp*. marine sponge using a mixture of dichloromethane and methanol in a 1:1 ratio. Subsequently, the methanol extract is partitioned using n-hexane to yield a concentrated extract. The methanol extract is then obtained and tested for antibacterial activity. The anticipated outcome of this study is the discovery of chemical compounds exhibiting antibacterial properties, potentially serving as primary compounds (lead compounds) for developing alternative antibacterial drug candidates. The obtained results exhibit inhibition zones of 9.700 ± 0.374 mm and 7.766 ± 0.694 mm against Gram-positive and Gram-negative bacteria, respectively, indicating the potential of this sponge's methanol extract as an antibiotic.**

***Keywords*—Alkaloid, *Aaptos. sp*, methanol extract, antibacterial**

I. Introduction

Indonesia boasts the world's greatest marine biodiversity due to its distinctive coastal and marine ecosystems. The mapped extent of Indonesia's coral reefs reaches approximately 25,000 square kilometers. Its waters are home to around 8,500 species of fish, 555 species of seaweed, and 950 coral reef biota. Among the notable marine resources are sea sponges, which harbor diverse compounds, both chemically and biologically, including alkaloids [1]. The majority of the biologically active compounds are obtained from sea sponges and microorganisms [2]. Sponges are a diverse group of aquatic animals classified under the phylum Porifera [3]. Sponges are unique as they lack true tissues and organs. Instead, their bodies consist of specialized cells that perform different functions, such as capturing food particles, releasing enzymes, and providing structural support [4].

Sponges are organisms known for their diverse array of secondary metabolite compounds, which are notably the highest among other animals, producing cytotoxic, antibacterial, hemolytic, and various other biological properties [5]. Deep-sea sponges, in particular, have proven to be a prolific source of bioactive metabolites with a wide range of observable structures [6]. Studies focusing on *Aaptos sp*onges have yielded compounds exhibiting highly favorable biological activities. Compounds discovered from sea sponges of the Aaptos genus have been frequently found in rich sources, with alkaloids being a distinctive compound within sponges. Specifically, the Aaptos genus predominantly produces alkaloids, including derivatives such as aaptamine [7].

The majority of active compounds isolated from these sea sponges are alkaloids, particularly basic alkaloid products characterized as cyclic compounds containing nitrogen within their ring systems. While numerous alkaloids are classified based on their molecular frameworks, they are also classified according to the materials used in their synthesis [8]. Alkaloids offer unique compounds that can be considered potential drug candidates. Derivatives of these alkaloids possess inherent properties, such as water solubility, acidity, and lipid solubility, which provide a foundation for their use as medicinal compounds. Alkaloids play a crucial role in both human medicine and the natural defense mechanisms of organisms. Therapeutically, alkaloids are well-known as anesthetic, cardioprotective, and anti-inflammatory agents. Additionally, alkaloids are widely recognized for their clinical applications, such as in antibacterial and anti-malarial treatments [9].

The presence of alkaloid compounds in these sponges enables their antibacterial properties to inhibit the growth of both gram-positive and gram-negative bacteria. In this study, methanol extracts and their fractions were used to determine their antibacterial activity against both types of bacteria. Therefore, the abundant sponges in the Sulawesi ocean can be explored as potential candidates for new antibacterial drugs. This research aims to isolate, characterize, and test the antibacterial activity of secondary metabolites contained in the *Aaptos sp*. sponge extract against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative) bacteria, known as invasive pathogens with high potential to cause various infectious diseases. The study seeks to unveil the secondary metabolite compounds present in *Aaptos sp*. sponge that exhibit antibacterial activity, potentially serving as lead compounds in combating pathogenic microorganisms harmful for humans.

II. Literature Review

Sponges (Phylum Porifera) comprise approximately 8,500 species in the oceans. Marine sponges represent one of the metazoan groups that have persisted for 700–800 million years. Current data on worldwide Porifera records over 8,730 sponge species distributed among 680 different

classes: Calcarea, Hexactinellida, Demospongiae, and the recently recognized Homoscleromorpha. Sponges contribute to coral reef health through several ecological functions, including water filtration, bacterial removal, and organic material recycling, which impact water quality. Additionally, sponges contribute to coral reef health through various ecological functions, such as water filtration and bacterial removal [10].

Sponges, classified as invertebrates within the phylum Porifera, are known to potentially produce bioactive compounds. This is evidenced by the isolation of over 6,000 bioactive substances from marine biota over the past three decades, with 40% of these compounds originating from sponges. Mehbub *et al.* reported on secondary metabolites isolated from the order Dictyoceratida and their biological activities from 2001 to 2012. These compounds exhibited diverse biological activities including cytotoxic, antimicrobial, antiparasitic, antiviral, antioxidant, anti-allergic, anti-inflammatory, atherosclerosis inhibition, and others. Terpenes constitute the major chemical class, comprising 73% of these compounds, including simple terpenes, diterpenes, sesquiterpenes, sesterterpenes, and other terpenes. The second major class consists of nitrogen compounds, constituting 13% of the total, including alkaloids, related alkaloids, and other nitrogen compounds. Research on sponges of the genus Aaptos has yielded compounds with highly promising biological activities. Compounds discovered from marine sponges of the Aaptos genus have been found abundantly in rich sources. In the study by Jang *et al.*, a group of alkaloids known collectively as aaptamines, specifically 1H-benzo[d,e][1,6]-naphthyridine alkaloids, were discovered, exhibiting intriguing biological activities [11].

E. coli is a gram-negative rod-shaped bacterium that does not form spores and belongs to the Enterobacteriaceae family. E. coli bacteria are naturally found in the gastrointestinal tract of humans and animals. E. coli has a diameter of 0.5 μm and a length of 1.0–3.0 μm. Generally, E. coli is motile in fluid and possesses fimbriae [12]. E. coli is one of the most common gram-negative foodborne pathogens, often utilized as an indicator bacterium in testing for fecal contamination in food. This bacterium can lead to diarrhea, gastroenteritis, and a range of complications [13].

S. aureus is a gram-positive bacterium that is anaerobic and pathogenic to humans, causing skin diseases such as boils, impetigo, cellulitis, and burn-related skin disorders [14]. S. aureus grows at temperatures ranging from 6.5–46 °C and at pH levels between 4.2–9.3. This bacterium is commonly found in the air, dust, waste, grows on food, and produces enterotoxins without affecting the outward appearance of the food. S. aureus easily adapts to its environment due to its resistance to antibacterial agents [15]. Refdanita [16] added that the highest consecutive antibiotic resistance of S. aureus is observed for ampicillin, clavulanic acid, amoxicillin, penicillin G, sulbenicillin, chloramphenicol, and ciprofloxacin.

III. Material and Methods

A. Materials

The primary material utilized in this study was the *Aaptos sp*. sponge from South Sulawesi, identified at the Laboratory of Biology, UGM. Chemicals employed for extraction and fractionation included Dichloromethane (DCM), Methanol (MeOH), ethyl acetate, and n-hexane. All organic solvents utilized were of analytical grade. Silica gel with various sizes, Octadecylsilane (ODS) Fuji Sylisia, TLC plates (Merck), and ODS plates were used for purification processes. For visualization, a 10% sulfuric acid in Ethanol (EtOH) staining reagent was employed. The bioactivity testing for antibacterial properties was conducted using Nutrient Agar (NA) medium, Mueller Hinton Agar (MHA) medium, sterile distilled water, E. coli test bacterial cultures, S. aureus test bacterial cultures, physiological saline solution of NaCl, sterile paper discs (6 mm diameter), and chloramphenicol

B. Extraction of Active Compounds from Sponges

300 g sample of *Aaptos sp*. sponge was finely chopped and subjected to maceration using a solvent mixture of DCM:MeOH in a 1:1 (v/v) ratio totaling 400 mL for 2×1 hour at room temperature. The resulting macerate was then filtered using a funnel and cotton, separating it into methanol and dichloromethane extracts. The methanol extract was evaporated using an evaporator below its boiling point, approximately ±35 °C, resulting in a concentrated methanol extract. The concentrated MeOH extract was introduced into a separating funnel, dissolved in n-hexane, and vigorously shaken. After settling, it separated into two layers: the n-hexane layer and the MeOH/water layer. Each layer was then individually evaporated at 77 °C to obtain concentrated n-hexane and MeOH/water extracts. The concentrated MeOH extract was used in this study. This extract underwent separation using column chromatography on silica gel (230–400 mesh), employing a gradient system of n-hexane and ethyl acetate at 10%. Subsequently, fractions were obtained. These fractions were further separated using column chromatography on silica gel (230–400 mesh) with a solvent system of n-hexane: ethyl acetate to yield pure compounds. The extracted fractions were then subjected to antibacterial testing, and their compound content was examined using LC-HRMS.

C. Preparation of Muller Hinton Agar (MHA)

The preparation of Muller Hinton Agar (MHA) begins by weighing 19 grams of MHA and dissolving it in a 500 mL Erlenmeyer flask with distilled water, followed by heating until homogeneous. The media is sterilized using an autoclave at a temperature of 121 °C for 15 minutes. Approximately 25 mL of the media is poured into Petri dishes and allowed to solidify.

D. Preparation of Test Bacterial Suspension

The test colony suspensions of E. coli and S. aureus are prepared by picking a single colony from a solid NA medium and placing it into a reaction tube containing 5 mL of physiological saline (NaCl). The turbidity of the test colony suspension is standardized using a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). These suspensions must be used as inoculum within a 15-minute timeframe.

E. Preparation of Test Bacterial Suspension

The test bacteria suspension is inoculated onto MHA media with 0.1 mL, evenly spread using a hockey stick, and allowed to dry. Discs soaked in the sample for 15 minutes are then aseptically placed onto the media surface. Clear zones around the discs are observed. Subsequently, the inhibition zone data, representing antibacterial activity, is measured thrice using a caliper for analysis [10].

IV. Result and Discussion

A. Aaptos sp. Sponge Extract Yield

The sponge utilized in this study originates from the waters of South Sulawesi. Upon identification, the results revealed:

Kingdom: Animalia
Phylum: Porifera
Class: Demospongiae
Subclass: Heteroscleromorpha
Order: Suberitida
Family: Suberitidae
Genus: Aaptos

The sponge sample underwent extraction via maceration using a solvent mixture of DCM:MeOH in a 1:1 (v/v) ratio, totaling 400 mL for 2 × 1 hour at room temperature. The maceration yielded an extract of approximately 0.4 grams, presenting a dark brownish color. This extract is expected to contain secondary metabolites, which will be subjected to further testing. The extraction outcome denotes the total weight of secondary metabolites successfully obtained from the sample. This information holds paramount importance in the extraction process as it indicates the quantity of extract obtained from the sample during this process. According to Sayuti [17], extraction outcomes are closely related to the active compound content in the sample, implying that higher extraction yields correlate with increased content of active compounds.

Table 1. Phytochemical testing results of methanol extract

Testing	Methanol extract
Alkaloid	+
Flavonoid	−
Terpenoid	+
Steroid	+

Table 2. Inhibition of bacteria against Escherichia coli and Staphylococcus aureus

Bacteria	Sample	Diameter of inhibition (mm) ± SD
Escherichia. coli	Positive control	16.433 ± 0.329
	Negative control	0
	Methanol extract	7.766 ± 0.694
Staphylococcus .aureus	Positive control	18.766 ± 0.169
	Negative control	0
	Methanol extract	9.700 ± 0.374

B. Antibacterial Activity of Methanol Extract from Sponges

Based on the phytochemical test results in Table 1, it's known that the methanol extract contains alkaloids, terpenoids, and steroids. These constituents within the extract are the ones influencing its antibacterial properties. Phenolic compounds and terpenoids primarily target the cytoplasmic membrane due to their hydrophobic nature. Alkaloids possess antibacterial abilities; their mechanism involves interfering with the components forming the bacterial peptidoglycan, disrupting the formation of the cell wall layer and leading to the death of the cell.

The disk diffusion method was used to assess the antibacterial potential of an ethanol extract obtained from Aaptos.sp sponge through the process of maceration. The test outcomes demonstrated the extract's efficacy against both Escherichia coli and Staphylococcus aureus following a 24-hour incubation period using varying concentrations obtained from the extraction process. The evaluation of antibacterial activity was based on the utilization of the methanol extract from Aaptos.sp. as depicted in the Table 2, the methanol extract exhibited the capability to hinder the growth of E. coli and S. aureus.

However, the results also revealed that the methanol extract showed a higher efficiency in impeding the growth of S. aureus, with an average inhibition zone of 9.700 ± 0.374, while the inhibition zone for E. coli averaged at 7.766 ± 0.694. The disparity in response between these two bacterial types is attributed to their distinct sensitivity levels towards the antibacterial compounds contained within the extract. Gram-positive bacteria tend to display greater responsiveness to these antibacterial components due to their simpler cell wall

Structures. Conversely, the more intricate composition of the Gram-negative bacterial cell wall, comprising multiple layers such as lipopolysaccharides, acts as a barrier that restricts antibacterial compounds from penetrating the cell and acting on their intended targets [18].

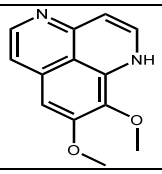
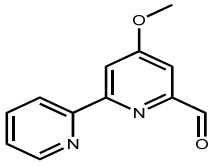
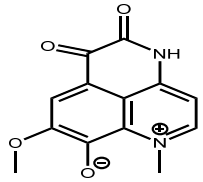
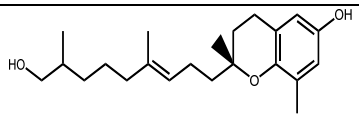
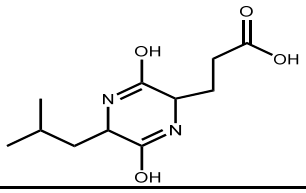
From the antibacterial test results, it can be observed that the methanol extract from Aaptos.sp marine sponges exhibits bacteriostatic properties, meaning it inhibits bacterial growth. According to Goering *et al.* (2013), bacteriostatic refers to an antibiotic property that can inhibit bacterial growth temporary and reversible. The inhibitory concentration is lower than the bactericidal concentration. Bacteriostatic compounds often inhibit protein synthesis or bind to ribosomes. This is demonstrated by the addition of antimicrobial agents to microbial cultures in the logarithmic phase.

C. The Composition of Sponge Extract by LC-HRMS

The bioactive compounds of the sponge extracts were revealed by LC-HRMS, the results were presented in Table 3. Four compounds were identified that may have an impact on antibacterial or antibiotic properties. Among these compounds are aaptamine and its derivatives, such as aaptanon, which are alkaloid derivatives. This finding is consistent with Herlt *et al.* 2004 study, which highlights alkaloids as characteristic compounds in sponges, particularly the genus Aaptos, which predominantly produces alkaloids with aaptamine derivatives [7]. Furthermore, Caerulomycin E, another compound identified, is an antibiotic alkaloid [19]. Additionally, Sargachromanol B, found in these *Aaptos sp*onges, may influence their antibacterial properties. Birringer's research also demonstrates that chromanol derivatives have anti-inflammatory properties [20]. Additionally, one potential new compound was identified, where upon literature review,

no corresponding compound was found at the retention time observed.

Table 3. Compounds from LC-HRMS

Name	Formula	[M+]	RT [min]	Compound
aaptamine	$C_{13}H_{12}N_2O_2$	229.09685	4.664, 4.425 and 3.528	
Caerulomycin E	$C_{12}H_{10}N_2O_2$	215.08038	3.818	
Aaptanone	$C_{13}H_{10}N_2O_4$	259.07114	2.822 and 2.582	
Sargachromanol B	$C_{23}H_{32}O_2$	339.2325	15.868	
NP-016455	$C_{11}H_{18}N_2O_4$	241.11906	4.558	

V. Conclusion

The Aaptos.sp sponge originating from the waters of South Sulawesi contains bioactive components such as alkaloids, terpenoids, and steroids. The methanol extract from the *Aaptos.sp* sponge exhibits robust antibacterial activity, particularly demonstrating a larger inhibition zone against *Staphylococcus aureus* compared to *Escherichia coli.* The *Aaptos.sp* sponge is more sensitive in inhibiting the gram-positive bacterium *Staphylococcus aureus* than the gram-negative bacterium *Escherichia coli*. The extraction method utilized to extract these bioactive compounds involves a combination of two solvents, methanol, and dichloromethane, which effectively separates the polar and nonpolar compounds from this Aaptos.sp sponge.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Wahyuni E. Nanda is responsible for conducting research, formal analysis, investigation, and drafting the initial version. Winarto Haryadi focuses on conceptualization, validation, formal analysis, and reviewing as well as editing the writing. Respati T. Swasono plays a role in validation, formal analysis, and participates in the review and editing of the written material.

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