



ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT FROM SEA CUCUMBER *Holothuria atra*

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ABSTRACT

Holothuria atra is a kind of sea cucumber found in tropical areas such as Indonesia. Limited research has been conducted on the bioactive compound in Indonesian sea cucumbers. This study aimed to determine what compounds and antibacterial properties were present in the methanol extract of *H. atra* obtained from the waters of Lampung, Indonesia. This study utilized maceration with methanol as the extraction solvent. The test for antibacterial activity used the diffusion agar method. The compounds in the methanol extract were identified using Gas Chromatography-Mass Spectrometry (GC-MS). The methanol extract of *H. atra* showed antibacterial activity that was highest against *Escherichia coli* (11.65 mm), followed by *Staphylococcus aureus* (10.56 mm), *Vibrio cholerae* (9.10 mm), and *Bacillus subtilis* (6.38 mm). The major compound identified was 9-octadecenamide (Z)- ($C_{18}H_{35}NO$), followed by 2-methyl-5H-dibenz[b,f]azepine ($C_{15}H_{13}N$), cis-11-eicosenoate ($C_{21}H_{40}O_2$), and tricyclo [6.3.3.0] tetradec-4-ene,10,13-dioxo ($C_{14}H_{18}O_2$). The conclusion is *H. atra* will be an essential source of natural antibacterial agents in the future.

INTRODUCTION

Invertebrates, especially marine invertebrates, are candidates for producing novel bioactive compounds. It is a defense strategy for predator escape and an adaptation for marine invertebrates, particularly soft-bodied species such as sea cucumbers, to survive in the harsh environmental conditions of the ocean ^[1]. In several Asian countries, traditional medicine has also used sea cucumbers. Sea cucumbers are also an essential source of food ^[2].

Holothuria atra is a marine invertebrate belonging to the family Holothuridae, popularly known as the lollyfish or the black sea cucumber. Pearson classified it as a *Halodeima* subgenus in 1914 ^[3]. *Holothuria atra* can be found in the

tropical Indo-Pacific area, between the Red Sea and Australia. Humans commonly harvest and eat *H. atra* by wading or diving at low tide; hence, its commercial worth is modest. According to ^[2], *Holothuria atra* contains bioactive substances such as steroidal sapogenins, lectins, and triterpene glycosides.

Scientists are looking for new sources of bioactive compounds that kill bacteria because those organisms are becoming resistant to antibiotics. Some marine invertebrates can live in places with lots of harmful germs. The condition has allowed them to build up defenses and makes them good choices for getting antibacterial parts ^[4]. Sea cucumbers from Indonesia, especially those Lampung waters, have yet to be studied

much in terms of how they live. Therefore, the purpose of this study was to determine if the methanol extract of *H. atra* could be used in the future as a source of antibacterial agents.

2. Materials and methods

2.1 Sample collection

The sea cucumber (*Holothuria atra*) utilized in this study was collected in Lampung waters, Indonesia. The sample was cleaned of sand and debris that had adhered to the sea cucumber, and the internal organs were removed. The sample was then placed in the labeled plastic bag and packed. They were transported to the laboratory and stored at -20°C until the extraction process was carried out.

2.2. Extraction procedure

The fresh body wall of *Holothuria atra* (500 g) was cut into small pieces and macerated in methanol for three days, with daily stirring. This maceration procedure was done multiple times until the resulting filtrate was colorless. The filtrates were evaporated in a rotary evaporator set to 40°C until a viscous extract was produced. The extracted methanol was then examined for antibacterial activity and chemical content.

2.3 Antibacterial analysis

The agar diffusion method was used to test the antibacterial activity of the methanol extract of *Holothuria atra* against four human pathogenic bacteria. Two gram-positive bacteria strains, *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633), and two gram-negative bacteria strains, *Escherichia coli* (ATCC 25922) and *Vibrio cholerae*, were investigated. All bacterial strains were taken from the Department of Microbiology, Faculty of Medicine, Universitas Indonesia.

Several modifications to the agar diffusion method were utilized to conduct an antibacterial test [5]. The sample was converted into methanol at 1,000 µg/mL. A 20 µL sample was dropped on a 6 mm filter paper disc. The paper disc was then placed in a disposable Petri disc with Mueller Hilton Agar (Himedia) and the microorganism under examination. The microorganism growth inhibition was a clear zone surrounding the paper disc. After 24 hours of incubation at

30°C, the inhibitory zone was determined with a vernier caliper.

2.4 Gas Chromatography-Mass Spectrometry analysis

For the GC-MS analysis, Agilent Technologies 7890 autosamplers, Chemstation data system, and 5975 mass selective detector were utilized for gas chromatography. The column utilized was an HP Ultra 2 capillary with a 0.25 µm film thickness and a column length of 50 mm (30 m x 0.25 mm). The amount of the compounds was calculated using the area under the peak, and the results were expressed as a percentage.

3. Result and Discussion

3.1 Antibacterial activity

Four bacterial strains, namely *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Vibrio cholerae* were used to evaluate the antibacterial activity of the methanol extract of *Holothuria atra* collected from the waters of Lampung, Indonesia. The results indicated that the extract of *H. atra* significantly reduced the growth of *S. aureus* (10.56 mm), *B. subtilis* (6.38 mm), *Vibrio cholerae* (9.10 mm), and *Escherichia coli* (11.65 mm) (Figure 1).

The majority of studies on the antibacterial activities of sea cucumbers have focused on aqueous-methanol, chloroform, and ethanol extracts [6]. According to a prior study, the methanol and ethanol extracts of *H. atra* from the Red Sea Egypt were proven to be effective against *S. aureus* and *Aeromonas hydrophilia*, which are included in [7]. Alcoholic extracts of *H. atra*, *H. scabra*, and *Actinopyga miliaris* from the Tamil Nadu coast effectively against *Pseudomonas aeruginosa* and *V. harveyi*. However, *H. atra* and *A. echiniites* were active against *S. aureus* [8]. Se-Kwon [6] discovered that a methanol extract of *H. atra* from Indian waters inhibited the growth of *S. aureus*, *Klebsiella pneumoniae*, and *Serratia liquefaciens*. Several kinds of sea cucumbers have also been discovered to have antibacterial properties. The antibacterial activity of the aqueous-methanol extract of *H. leucospilota* from the Oman Sea was demonstrated against *E. coli*, *Salmonella typhimurium*, *S. aureus*, and *Pseudomonas*

aeruginosa^[9]. It was stated that *E. coli*, *P. aeruginosa*, and *Enterococcus fecalis* had been tested against the organic extracts (ethanol, methanol, and acetone) of *H. parva* from the Iranian seas. *Staphylococcus aureus* and *S. epidermidis* were also, however, resistant to all tested extracts^[10]. The ethyl

acetate extract of *H. forskali* from the Tunisia coast reduced the growth of *Bacillus subtilis* and *S. aureus*^[11]. N-hexane and chloroform extracts of *H. nobilis*, *B. marmorata*, and *Stichopus chloronotus*, on the other hand, inhibit the growth of *E. coli*^[12].

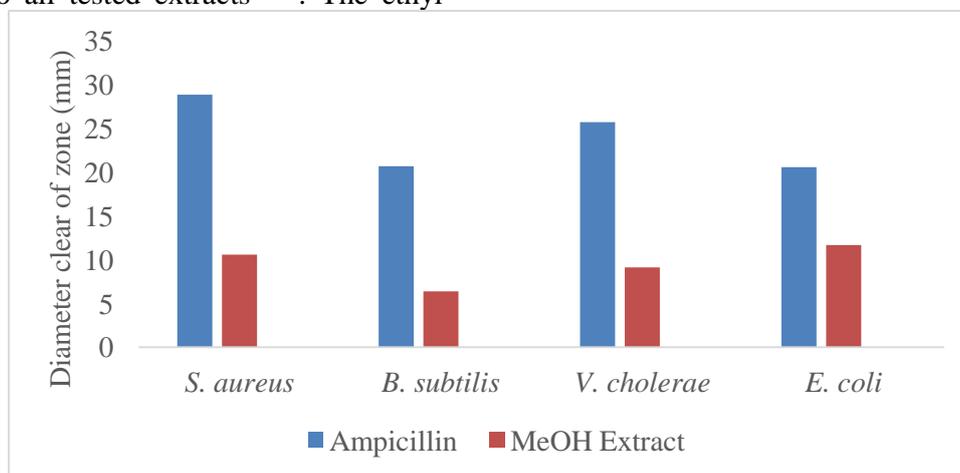


Figure 1. Antibacterial activity of methanol extract of *Holothuria atra*

Several types of sea cucumbers gathered in Indonesian waters were reported to exhibit antibacterial properties. The methanol extracts of *H. atra*, *Actinopyga lecanora*, *S. horrens*, *S. vastus*, and *H. impatiens* exhibited antibacterial activity against *S. epidermidis* and *S. aureus*^[13]. The organic extracts (methanol, n-hexane, and acetone) of *Muelleria lecanora* have antibacterial activity against *S. aureus* and *E. coli*^[14], while *H. hilla*, *A. echinites*, and *H. fuscogilva* were antibacterial against *V. cholerae*, *S. aureus*, *E. coli*, and *B. subtilis*^[15].

3.2 GC - MS analysis

The GC-MS analysis revealed that the methanol extract of *Holothuria atra* contains 10 components (Table 1). The 9-octadecenamide (Z)- ($C_{18}H_{35}NO$) was the primary compound, followed by 2-methyl-

5H-dibenzo[b,f]azepine ($C_{15}H_{13}N$), 11-eicosenoate acid, methyl ester ($C_{21}H_{40}O_2$), and tricyclo[6.3.3.0] tetradec-4-ene, 10,13-dioxo ($C_{14}H_{18}O_2$). Based on Table 1, most of the discovered compounds have antibacterial activity. Previous research has found that the methanol extract of *H. atra* has antibacterial activity, specifically hexadecanoic acid, methyl ester ($C_{17}H_{34}O$), 9-octadecenoic acid (Z)-, methyl ester ($C_{13}H_{36}O$), and octadecanoic acid, methyl ester ($C_{19}H_{36}O_2$)^[16]. Alternatively, the methanol extract of *H. atra* from the Red sea (Egypt) contains compounds with antibacterial activity. The compounds were dodecamethyl-cyclohexasiloxane ($C_{12}H_{36}O_6Si_6$), tetradecamethyl-cycloheptasiloxane ($C_{14}H_{42}O_7Si$), and hexadecamethyl-cyclooctasiloxane ($C_{16}H_{48}O_8Si$)^[7].

Table 1. Compounds identified in the methanol extract of *Holothuria atra*

Peak	RT (min)	Area %	Compounds Name	Reported Activity
1	8.196	2.05	1-nonene	Antibacterial ^[17]
2	15.100	2.64	Hexadecanoic acid, methyl ester	Antibacterial, antifungal, antioxidant, cancer preventives, antidiarrheal, anti-inflammatory, pesticides, and nematocides ^[18]
3	16.887	2.16	Octadecanoic acid, methyl ester	Antioxidants, cancer preventives, antidiarrheal, anti-inflammatory, pesticides, nematocides, and antibacterial ^[18]
4	18.581	8.35	Tricyclo[6.3.3.0]tetradec-4-ene,10,13-dioxo	-
5	18.984	9.24	11-Eicosenoate acid, methyl ester	Antifibrinolytic, antioxidant, lubricant, pesticide, flavor, 5-alpha reductase inhibitor, hemolytic, nematocide, antiallopecic ^[19]
6	20.519	3.63	11-Octadecenoic acid, methyl ester	Absorption and distribution in human plasma and lipoprotein lipids ^[19]
7	24.973	10.59	2-Methyl-5H-dibenzo[b,f]azepine	-
8	31.558	53.54	9-Octadecenamide, (Z)-	Anti-inflammatory and antibacterial ^[20]
9	35.467	6.14	Silicone grease	Antiinflammatory ^[21]
10	38.085	1.66	7-pentadecyne	Antibacterial ^[22]

CONCLUSION

From the above description, it can be concluded that *Holothuria atra* can be utilized as a new antibacterial source. Specifically *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Vibrio cholera* infection. The GC-MS analysis of the compounds identified in the methanol extract of *H. atra* also confirmed this conclusion as well.

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