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Potential of *Coelomic Fluid* Extract Sea Cucumber *Acaudina* sp. (Holothuroidea:Caudinidae) as an Antibacterial

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Abstract

Coelomic fluid is the internal medium of sea cucumbers and has multiple functions, including antimicrobial bioactivity, cytotoxicity, reproductive and stress responses, and osmotic volume regulation across sea cucumber species. Coelomic fluid is rich in coelomocytes, proteins, and small molecules that mediate innate immunity. This research aims to investigate the antibacterial properties of the coelomic fluid extract of *Acaudina* sp. This research employed the agar diffusion method as an antibacterial assay, followed by phytochemical screening and GC-MS analysis. The results of this research indicate that the ethanol extract of coelomic fluid exhibits antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The biggest inhibition zone is against *E. coli* at 4.75±0.42 mm. The extract of coelomic fluid was indicated to contain saponin and steroid by phytochemical screening. According to GC-MS analysis, there are 20 bioactive compounds, most of which are groups of fatty acid compounds. The most commonly found bioactive ingredients are n-Hexadecanoid acid, Oleic acid, and Tridecanedial. Based on the results, the coelomic fluid extract of *Acaudina* sp. is not effective enough as an antibacterial agent, but it exhibits a broad-spectrum antibacterial ability.

1. Introduction

The sea cucumber *Acaudina* sp is a marine organism belonging to the phylum Echinodermata and Class Holothuroidea. This sea cucumber is a valuable export commodity with high economic value in both domestic and international markets (Umboh *et al.*, 2018), primarily due to its nutritional content and potential as a source of bioactive compounds. There are 350 species of sea cucumbers in Indonesian waters. Some of which are that most of these sea cucumbers are found in the waters of central and eastern Indonesia (Setyastuti, 2016).

Coelomic fluid is a bodily fluid that occupies the body cavity of some invertebrates, especially sea cucumbers. The coelomic fluid of the sea cucumber Holothuria scabra consists of various cell types, including phagocytic cells, small round cells (SRCs), sterulocytes, fusiform cells, and crystal cells (Wambreuse *et al.*, 2023). Coelomic fluid contains glycoprotein fractions and antigenic components produced by coelomocytes and other tissues, indicating a metabolic relationship between cells, fluids, and gametes in some invertebrates.

In sea cucumber, triterpene glycoside compounds are obtained from coelomic tissue isolation which have a strong membranolytic effect due to the formation of ion channels and pores which are the basic ingredients of hemolytic cytotoxicity, antifungal, and antitumor (Kalinin *et al.*, 2008). In the coelomocyte fluid there are structure-activity relationships such as sulfation patterns and sugar chains that greatly modulate the potential and immunomodulatory properties (Kalinin *et al.*, 2008),

Cell-free coelomic fluid extract (CFE) of *H. tubulosa* induces G2/M cell cycle arrest, oxidative stress, autophagosome depletion, mitochondrial dysfunction, and apoptotic death in HepG2 hepatocellular carcinoma cells (Luparello *et al.*, 2022). Therefore, coelomocyte fluid in *H. tubulosa* has potential as an anticancer agent (Luparello *et al.*, 2022). Furthermore, ethanol and water extracts of *Holothuria tubulosa* (including the coelomic fluid fraction) can inhibit the growth of bacterial and fungal pathogens in vitro,

supporting traditional medicinal uses and motivating extraction efforts (Sellem, *et al.*, 2021).

Furthermore, research on the immune response generated from coelomocyte fluid has been conducted on several types of sea cucumbers, including coelomic fluid in the sea cucumbers *Apostichopus japonicus, H. scabra, H. leucospilota* (Wu *et al.*, 2020; Wang *et al.*, 2022; Wambreuse *et al.*, 2023). Coelomic fluid produced by *H. leucospilota* has a humoral immune response (Wu *et al.*, 2020). Coelomic fluid in *H. scabra* can reveal the presence of a complex immune system at the molecular and cellular levels (Wambreuse *et al.*, 2023).

Rasyid (2012) and Umboh *et al.* (2018) stated that the use and exploration of sea cucumbers, particularly their coelomic fluid, have not been extensively studied compared to other marine products. The various bioactive compounds found in sea cucumbers can be utilized for innovation and improvisation in multiple fields, particularly in the pharmaceutical sector. The antibacterial potential of sea cucumbers can be utilized as an alternative in developing antibiotics to prevent pathogen resistance to antibacterial drugs. Research on the content of amino acids, fatty acids, proteins, fats, carbohydrates, glucosamine content, antibacterials, and antimicrobials has been widely conducted on sea cucumbers, however, research on the importance of coelomic fluid as an antimicrobial, antibacterial, immunity as well as the content of amino acids, fatty acids, and bioactive substances has been conducted on sea cucumbers of the species *H. leucospilota, H. scabra* and *Apostichopus japonicus* has been conducted (Wu *et al.*, 2020; Wang *et al.*, 2022; Wambreuse *et al.*, 2023), but research on coelomic fluid in sea cucumbers Acaudina sp. has not been done much. Therefore, this study aims to investigate and determine the potential antibacterial activity of *Acaudina* sp. against the pathogens *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa,* and *Escherichia coli.*

2. Material and methods

2.1. Sample preparation

Acaudina sp sea cucumber samples were taken from the Delta Wulan Demak waters, Central Java. Ceolomic fluid was collected from 30 *Acaudina* sp. individuals by surgically removing the posterior portion of the body. The ceolomic fluid was to collected with a sterile syringe. The ceolomic fluid was then centrifuged at 4,000 x g at 4ºC for 20 min. (Sellem *et al.*, 2021). The coelomic fluid was then frozen until analysis.

The coelomic fluid sample was macerated with an ethanol solvent, measured to 20 ml, and then macerated with 20 ml of ethanol solvent (v/v, 1:1). The samples were incubated for 24 hours, stirring continuously using a stirrer. The macerate is then filtered using filter paper, and the filtrate is evaporated at a temperature of 35-40ºC using a vacuum rotary evaporator. The extract is then stored in the freezer until the time of testing.

2.2. Antibacterial Test

Two gram-positive bacterial strains (*Bacillus cereus, Staphylococcus aureus*) and two gram-negative bacterial strains (*Escherichia coli, Pseudomonas aeruginosa*) were used as test materials. These four bacteria were chosen because they have a high level of sensitivity to various marine organism extracts (Sellem *et al.*, 2021).

The agar diffusion method (Kirby-Bauer) is an antibacterial test method that uses a paper disc as an extract container. 10 µl extracts from the coelomic fluid were taken with a micropipette and then dropped onto a paper disc. The discs were then placed into Mueller-Hinton agar media containing the test bacteria and incubated at room temperature (32-37°C) for 24 hours (Abraham *et al.*, 2002) After incubation, the diameter of the inhibition zone (from the edge of the disc to the tip of the clear zone) was measured using a caliper. Inhibition zones with a distance of 16 mm or more are considered to have high anti-microbial activity (Sellem *et al.*, 2021). The positive control used in the test is chloramphenicol, a commercial antibiotic with a broad spectrum of activity (effective against both gram-positive and gram-negative bacteria). The purpose of conducting a positive control is to compare and assess the effectiveness of the coelomic fluid extract as an antibacterial agent. The negative control used is ethanol, a solvent from the coelomic fluid samples. The purpose of conducting a negative control test is to determine whether the solvent used to extract the sample affects the antibacterial properties of the extract in the test.

2.3. Phytochemical Screening

Phytochemical screening carried out in this study included alkaloids, flavonoids, saponins, steroids, and tannins. The alkaloid test was performed by adding 1 mL of 2 N HCl solution and 9 mL of distilled water to the extract, then heating for 2 minutes, cooling, and filtering. The filtrate was then collected, and three drops each were added to three test containers. In each subsequent test container, two drops of Mayer, Bouchardat, and Dragendorff reagents were added (one type of reagent each for one test container). Positive results for alkaloid compounds in Mayer's reagent are indicated by the presence of white or yellowish-white precipitates, positive results in Bouchardat's reagent are indicated by brown to black precipitates, and positive results in Dragendorff's reagent are indicated by red or orange precipitates. The flavonoid test was performed by adding 1 mL of 95% ethanol, 0.1 g of magnesium powder, and ten drops of concentrated hydrochloric acid solution to 1 mL of the extract. Positive results for flavonoids in samples are shown in orange, red, and purple, with orange and yellow indicating the presence of flavonoids. The saponin test was performed by adding 10 mL of hot water to 1 mL of extract, then shaking vigorously for 10 seconds and observing the foam that formed. If the foam is stable at a height of 1-10 cm for 10 minutes and does not disappear after adding one drop of 2 N HCl, then the sample is indicated to be positive for containing saponin. The steroid test begins with maceration of the sample in 20 ml of n-hexane for 2 hours, followed by evaporation. The filtrate was then dropped with Liebermann-Burchard reagent, and the color change was observed. The filtrate is positive for steroids if it is greenish-blue or purplish-red. The tannin test was performed by diluting 1 mL of extract with 10 mL of distilled water, then boiling it, allowing it to cool, and filtering. Then, 5 ml of 1% FeCl3 was added to the filtrate, and the color formed was observed. A positive sample contains tannin if a dark blue color forms.

2.4. Compound Identification

The method used to identify compounds contained in coelomic fluid ethanol extract is by chromatography with Gas Chromatography Mass Spectrometry (GS-MS) equipment.

3. Results

3.1 Anti-bacterial Activity

Based on the research results, the inhibition zone of Coelomic fluid extract against E. coli showed a high inhibition zone compared to the other three bacteria (Bacillus cereus, Staphylococcus aureus, and Pseudomonas

aeruginosa). The average inhibition zone formed in *E. coli* was 4.75 ± 0.42 mm, while in *S. aureus* it was 3.78 ± 0.53 mm, in *B. cereus* it was 2.2 ± 0.14 mm, and the lowest occurred in *P. aeruginosa* at 1.7 ± 0.22 mm. The highest antibacterial activity was found against *E.coli*, followed by *S. aureus*, *B. cereus*, and *P. aeruginosa* (Table 1).

Table 1. Zone of inhibition (mm) of the CF ethanol extract against bacterial

Replicate	*E.coli*	*P.aeruginosa*	*B.cereus*	*S.aureus*
1	5.3	2.0	2.0	4,2
2	4.3	1.7	2.2	3.6
3	4.8	1.6	2.3	3.1
4	4.6	1.5	2.3	4.2
Average	4.75 ± 0.42	1.70 ± 0.22	2.20 ± 0.14	3.78 ± 0.53

Average value ± SD (n=4).

The positive control used in this study was chloramphenicol, a broad-spectrum and commonly used antibiotic (Dinos *et al*.., 2016). The positive control served as a comparison for the samples to determine their antibacterial effectiveness. This test showed that chloramphenicol had the highest antibacterial activity against *S. aureus*. The negative controls used in this study were ethyl acetate and ethanol, the solvents used in each test sample. Negative controls were used to determine whether the solvents affected the antibacterial activity of the samples., There is no antibacterial activity was observed in either solvent. (Table 2)

Table 2. Antibacterial activity of chloramphenicol (control +) and ethanol (control +)

Control	*E. coli*	*P. aeruginosa*	*B. cereus*	*S. aureus*
Control +	24,7 ± 0,42	16,6 ± 2,26	26 ± 0,00	37,85 ± 0,21
Control -	0 ± 0,00	0 ± 0,00	0 ± 0,00	0 ± 0,00

Average value ± SD (n=3) Concentration control (+) = 100 µg/ml

Table 2. Phytochemical Screening

Compound	Result
Alkaloid	-
Flavonoid	-
Saponin	+
Steroid	+
Tanin	-

3.2 Phytochemical Screening

Ethanol extract of Coelomic Fluid *Acaudina* sp. is positive for saponins and steroids (Table 2). Furthermore, coelomic fluid extract showed negative results for alkaloids, flavonoids and tannins.

3.3 Compound Bioactive

Based on GC-MS analysis, CF ethanol extract has 20 types of compounds, 3 of which have very wide peak areas, namely Tridecanial, n-hexadecanoic acid, and oleic acid (Table 3). Additionally, Fatty acid compounds are quite dominant in the coelomic fluid *Acaudina* sp extract. Apart from fatty acid compounds, several other compounds have broad-spectrum antibacterial activity.

Table 3. GC-MS Analysis Result

Peak	RT	%Area	Name	MW
1	3.462	0.21	Acetic acid	60
2	7.216	0.30	1,2,3-Propanetriol	92
3	8.136	0.68	2-Bromooctane	192
4	12.925	0.27	Phenol, 2-methoxy-4-(2-propenyl)-	164
5	18.976	0.63	Dodecanoic acid	200
6	23.647	1.12	9-Octadecenoic acid (Z)-	282
7	25.535	1.06	2-Pentadecanone	226
8	26.041	2.28	Hexadecanoic acid, methyl ester	270
9	27.925	30.41	n-Hexadecanoic acid	256
10	29.077	2.76	Oxirane, tetradecyl-	240
11	29.465	5.01	9-Octadecenoic acid, methyl ester	296
12	30.044	3.16	Octadecanoic acid, methyl ester	298
13	31.180	23.49	Oleic Acid	282
14	32.265	1.73	Tricyclo [20.8.0.0 (7,16)] triacontane, 1(22), 7(16)- diepoxy-	444
15	33.018	1.10	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	568
16	38.333	0.45	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	568
17	40.052	1.22	2-Hydroxy-3-(Palmitoyloxy)Propyl(9E)-9-Octadecenoate	594
18	41.716	0.24	Glycerine-1,3-Diolein	620
19	42.590	0.11	Eicosanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester	530
20	45.464	23.77	Tridecanedial	212

4. Discussion

Research by Sellem *et al.* (2021) yielded similar results, where the coelomic fluid ethanol extract from *Holothuria tubulosa* showed that higher activity against *E. coli* than *P. aeruginosa* and *S. aureus*. The results of tests conducted by Adibpour *et al.* (2014) showed that the coelomic fluid ethanol extract from *Holothuria leucospilata* had antibacterial activity against *E. coli, P. aeruginosa*, and *S. Aureus*. Still, they had no inhibitory effect against *B. cereus*. Research conducted by Ibrahim *et al.* (2012) showed that coelomic fluid ethanol extracts from *H.atra, H.scabra*, and *H.leucospilota* had higher activity against *S. aureus* (2.46 ± 1.53 mm) than against *P. aeruginosa* (1.8 ± 0.00 mm), but had no inhibitory effect on *E. coli.*

The effectiveness of coelomic fluid extract against *E. coli* in this study is similar to that of Sellem *et al.* (2021), which is most likely due to the thinner cell wall structure and the absence of endospores. Nimah *et al.* (2012), and Sukmiwati *et al.*. (2018) in their publication stated that the cell wall structure of gram-negative bacteria consists of 10% peptidoglycan, lipopolysaccharide, and lipids (11-22%) so that it is more easily damaged by metabolite compounds which can damage and/or inhibit cell wall synthesis. Additionally, endospores, which gram-negative bacteria do not have, function as a self-protection mechanism against unfavorable environmental conditions. Endospores are retractile and have a thick wall structure in bacterial cells. *B. cereus, one of the bacteria tested in this study, is a bacterium capable of producing endospores and has become a cause of foodborne infections due to* its ability to withstand high temperatures (Basta and Annamaraju, 2021).

Based on the differences in results from several studies above, researchers identified several key factors that influenced the outcomes of antibacterial testing, both in comparison to previous research and within previous studies. These factors include the preparation and extraction process, the method used for anti-bacterial testing, the species used (both test samples and target pathogens), and the content of metabolite compounds in the extract. The main parameters influencing the preparation and extraction process are temperature, incubation duration, and solvent type and concentration (Casagrande *et al.*, 2018). Temperature is known to degrade most of the bioactive compounds contained in the extract (Susanto *et al.*, 2018). Farouk *et al.* (2007) and Abbes *et al.* (2013) said that bioactive compounds tend to be unstable at high temperatures, so the preparation and extraction process is recommended at temperatures <60°C. However, there are exceptions to this rule, particularly for certain metabolite compounds, such as phenolic compounds, which generally act as antioxidants. Research by Soto *et al.* (2019) and Teles *et al.* (2018) indicates that increasing temperature is associated with growing phenolic levels, which in turn enhance the extract's effectiveness as an antioxidant. Likewise, each species of sea cucumber has its unique secondary metabolites, which also depend on environmental conditions. This is because secondary metabolites serve as a mechanism for organisms to respond to their environment, particularly in unfavorable conditions (Mitu *et al.*, 2017). Differences in the types of bioactive compounds and levels/concentrations contained in an extract can influence the test results. Sukmiwati *et al.*. (2020) explained that the number of secondary metabolite compounds can affect the differences in results in each antibacterial test containing the sample.

Phytochemicals are naturally found in plants because they have defense mechanisms against various diseases (Mohan *et al.*, 2021). The phenolic and flavonoid content in an extract exhibits better antioxidant activity (Karimi *et al.*, 2015). Meanwhile, the alkaloid and triterpenoid content is known to have better antibacterial activity (Ibrahim, 2012; Mohan *et al.*, 2021).

Saponins are complex natural compounds composed of steroid or triterpenoid aglycone molecules with one or more sugar/glycoside chains (Narsih *et al.*, 2012; Moses *et al.*, 2014). According to Arabski *et al.* (2012) and Tagousop *et al.* (2018), saponins have cytotoxic, anti-inflammatory, antifungal, antibacterial, and antiviral activities. Saponins work by disrupting the permeability of the outer membrane of bacterial cells, then entering the lipid layer and binding to cholesterol, forming domains that contain cholesterol-saponin complexes, which ultimately lead to the lysis of the bacterial cells. Saponins are known to have higher antibacterial effectiveness against gram-negative bacteria because their lipid content is higher in the cell walls of these bacteria than in those of gram-positive bacteria. This aligns with the results of antibacterial tests, where the coelomic fluid extract has the highest activity against *E. coli*, a Gram-negative bacterium.

Apart from saponins, the coelomic fluid ethanol extract was also positively indicated for containing steroids. Steroids are compounds with 17 carbon atoms arranged in 4 rings linked together (Silva and Salvador, 2020). According to Sari *et al.* (2014) and Fauzia *et al.* (2018), steroids can affect the permeability of bacterial cell membranes. Steroids can interact with cell phospholipid membranes, which are normally impermeable to lipophilic compounds, resulting in membrane instability and potentially altering cell membrane morphology. This causes lysis of the cell membrane and results in the death of the bacterial cell. Research by Fauzia *et a.*. (2018) demonstrated that steroid compounds exhibit better effectiveness against Gram-positive bacteria, with a larger zone of inhibition for *S. aureus* compared to *E. coli*. Based on this, researchers assume that saponins in coelomic fluid extract are more dominant than steroids.

Based on GS-MS analysis, there are 3 types of compounds that are most dominant in coelomic fluid extract, namely n-Hexadecanoic acid with an area percentage of 30.41%, Tridecanedial with an area percentage of 23.77%, and Oleic Acid with an area percentage of 23.49%. The compound n-hexadecanoic acid, commonly known as palmitic acid, is recognized for its broad-spectrum antibacterial activity, as well as antioxidant and anti-inflammatory properties (Willie *et al.*, 2021). A review by Vargas *et al.* (2021) showed that palmitic acid has anti-bacterial activity against *P. aeruginosa* and *S. aureus.*

Likewise, other fatty acid compounds, such as oleic acid, arachidic acid, and stearic acid, are known to have antibacterial activity with higher effectiveness against Gram-positive bacteria (Chandrasekaran *et al.*, 2008; Duraisami and Selvaraju, 2020). Research by Dilika *et al.* (2000) demonstrated that oleic acid exhibited activity against 3 out of 5 gram-positive bacteria, but showed no activity against gram-negative bacteria.

The phenolic compound 2-methoxy-4-(2-propenyl), also known as eugenol, is known to have broad-spectrum antibacterial (Marchese *et al..*, 2017) and antioxidant capabilities (Ashwathanarayana and Naike, 2017). Research by Espineli *et al.*. (2014) demonstrated that eugenol exhibits antibacterial activity against *P. aeruginosa, E. coli*, and *S. aureus*.

Dodecanoic acid, also known as lauric acid, is known to have broad-spectrum anti-bacterial activity

(Matsue *et al.*, 2019). Based on testing by Nitbani *et al.* (2015), lauric acid has anti-bacterial activity against *S. aureus*, *B. cereus*, and *E. coli*. The antibacterial mechanism of action of lauric acid is believed to be mediated through bacterial membranes, where long-chain bases can inhibit cell wall synthesis and interact with bacterial membranes (Fischer, 2020).

However, it is possible that not all constituents were detected by GC-MS, especially in the steroid group. This is based on the working principle of GC-MS, which involves the reading and analysis of volatile constituents (Xu *et al.*, 2017; Matulyte *et al.*, 2019). Meanwhile, the phosphate group in steroid compounds is unstable in thermal analysis (high temperature). Therefore, in several studies, the esterification of the carboxyl group was carried out before proceeding with the GC-MS analysis (Honour, 1997; Hochberg, 1998).

5. Conclusions

Coelomic fluid extract exhibited anti-bacterial activity against all tested bacteria, with the most significant inhibitory power observed against *E. coli* (4.75 ± 0.42 mm). Based on the test bacteria's average value of the inhibition zone, coelomic fluid extract was less effective as an anti-bacterial agent. It can be concluded that the ethanol extract of Coelomic fluid against E. coli, S. aureus, B. cereus, and P. aeruginosa bacteria is classified as weak because the inhibition zone formed is <8 mm. Phytochemical screening revealed that the coelomic fluid extract was positive for saponins and steroids, both of which are known to have antibacterial activity, with varying effectiveness against Gram-positive bacteria. Saponins are effective against gram-negative bacteria, while steroids are effective against gram-positive bacteria. GC-MS analysis revealed that the metabolite compounds present in the coelomic fluid extract exhibited broad-spectrum antibacterial activity, with fatty acid compounds being the dominant compounds found in the extract. There are 3 bioactive compounds that have a large area percentage, namely n-Hexadecanoic acid, Oleic acid, and Tridecanedial

Ethics approval

No permits were required.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contributions

W.W. is responsible for data curation, conceptualization, project administration, funding acquisition, writing – review & editing the original draft. L.M.I.S., R.H and M.T.R are contributed to investigation, resource acquisition, methodology, and formal analysis.

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Declaration of competing Interest

None

References

Abbes, F., S. Besbes, B. Brahim, W. Kchaou, H. Attia, and C. Blecker. 2013. Effect of Concentration Temperature on Some Bioactive Cmpounds and Antioxidant Proprieties of Date Syrup. Food Science Technology, 0(0):1-11.

Abraham T.J., Nagarajan J. & Shanmugam S.A. 2002**.** Antimicrobial substances of potential biomedical importance from holothurians species. *Indian Journal of Marine Sciences*, 31: 161-164

Adibpour, N., F. Nasr, F. Nematpour, A. Shakouri, and A. Ameri. 2014. Anti-bacterial and Antifungal Activity of *Holothuria leucospilota* Isolated from Persian Gulf and Oman Sea. *Jundishapur J Microbiol*., 7(1):1-4.

Akbar, N., N.A. Khan, K. Sagathevan, M. Iqbal, A. Tawab, dan R. Siddiqui. 2019. Gut Bacteria of Cuora amboinensis (turtle) Produce Broad-Spectrum Anti-bacterial Molecules. Nature., 9(17012) Al-mohanna, M.T. 2016. *Morphology and Classification of Bacteria. Module Microbiology*., 1-19.

Arabski, M., A.W. Ciuk, G. Czerwonka, A. Lankoff, & W. Kaca. 2012Effects of Saponins against Clinical *E.coli* Strains and Eukaryotic Cell Line. *Journal of Biomedicine and Biotechnology*., 2012:1-6.

Ashwathanarayana, R. dan R. Naika. 2017. Antioxidant and Cytotoxic Properties of *Pavetta crassicaulis* Bremek Leaf Crude Extract and Its Isolated Pure Compound. *Indian Journal of Natural Products and Resources*., 8(4):335- 350.

Basta M. & P. Annamaraju. 2021. Bacterial Spores. Treasure Island (FL): StatPearls Publishing.

Casagrande, M., J. Zanela, A.W. Junior, C. Busso, J. Wouk, G. Luckervicz, P.F. Montanher, F. Yamashita, & C.R.M. Malfatti. 2018. Influence of Time, Temperature and Solvent on the Extraction of Bioactive Compounds of *Baccharis dracunculifolia*: In Vitro Antioxidant Activity, Antimicrobial Potential, and Phenolic Compound Quantification. *Industrial Crops and Product*., 125:207-219.

Chandrasekaran, M., K. Kannathasan, dan V. Venkatesalu. 2008. Antimicrobial Activity of Fatty Acid Methyl Esters of Some Members of Chenopodiaceae. *Zeitschrift fur Naturforschung*., 63(5): 331-336.

Dilika, F., P.D. Bremner, & J.J.M. Meyer. 2000. Anti-bacterial Activity of Linoleic and Oleic Acids Isolated from *Helichrysum pedunculatum*: A Plant Used during Circumcision Rites. *Fitoterapia*., 71(4): 450-452.

Dinos, G.P., C.M. Athanassopoulus, D.A. Missiri, P.C. Giannopuolo, I.A. Vlachogiannis, G.E. Papadopoulos, D. Papaioannou, D.L. Kalpaxis. 2016. Chloramphenicol Derivatives as Antibacterial and Anticancer Agents: *Historic Problems and Current Solutions. Antibiotics*., 5(20):1-21.

Duraisamy, M. dan R. Selvaraju. 2020. Analysis of Chemical Compounds by Using Gas Chromatography and Mass Spectrum Analysis, In Vitro Antioxidant and Anti-bacterial Activity of Methanolic Extracts of Seaweed *Ulva flexuosa* wulfen (Green Algae). *Aegaeum Journal*. 8(10): 1437- 1457.

Espineli, D.L., E.M.G. Agoo, R.S.D. Fierro, C.C. Shen, C.Y. Ragasa. 2014. Cytotoxic and Antimicrobial

Compounds from *Cinnamomum cebuense* Kosterm (Lauraceae). *Pharmaceutical Chemistry Journal*., 48:598-602.

Farouk, A.E., F.A.H. Ghouse, dan B.H. Ridzwan. 2007. New Bacterial Species Isolated from Malaysian Sea Cucumbers with Optimized Secreted Anti-bacterial. *American Journal of Biochemistry and Biotechnology*., 3(2):60-65.

Fauzia, D.V., D. Kusrini, dan E. Fachriyah. 2018. Isolation and Testing of Bacteria from Steroid Compounds Obtained from Anting-anting Leaf (*Acalypha indica* L.). *Journal of Scientific and Applied Chemistry*., 21(2):64-69.

Fischer, C.L. 2020. Antimicrobial Activity of Host-Derived Lipids. Antibiotics., 9(75):1-17. Harborne, J.B. 1987. Metode Fitokimia. Bandung: Penerbit ITB.

Hochberg, R.B. 1998. Biological Esterification of Steroids. *Endocrine Reviews*., 19(3):331-348.

Honour, J.W. 1997. Steroid Profiling. Ann Clin Biochem., 34:32-44.

Ibrahim, H.A.H. 2012. Anti-bacterial Carotenoids of Three Holothuria Species in Hurghada, Egypt. *Egyptian Journal of Aquatic Research*., 38:185-194.

Kalinin , V.I., D.L. Aminin, S.A.Avilov, A.S. Silchenko, V.A. Stonik. 2008. Triterpene Glycosides from Sea Cucucmbers (Holothurioidea, Echinodermata). Biological Activities and Functions. Studies in natural products chemistry. *Studies in Natural Products Chemestry*. Vol. 35 : 135-196. https://doi.org/10.1016/S1572-5995(08)80006-3

Karimi, E., H.Z.E. Jaafar, A. Ghasemzadeh, M. Ebrahimi. 2015. Fatty Acid Composition, Antioxidant and Anti-bacterial Properties of The Microwave Aqueous Extract of Three Varieties of Labisia pumila Benth. *Biological Research*., 48(9):1-6.

Luparello, C., Branni, R., Abruscato, G., Lazzara, V., Drahos, L., Arizza, V., … Vazzana, M. 2022. Cytotoxic capability and the associated proteomic profile of cell-free coelomic fluid extracts from the edible sea cucumber Holothuria tubulosa on HepG2 liver cancer cells. *EXCLI Journal*, *21*, 722–743. https://doi.org/10.17179/excli2022-4825

Matsue, M., Y. Mori, S. Nagase, Y. Sugiyama, R. Hirano, K. Ogai, K. Ogura, S. Kurihara, dan S. Okamoto. 2019. Measuring the Antimicrobial Activity of Lauric Acid against Various Bacteria in Human Gut Microbiota Using a New Method. *Cell Transplantation*., 28(12):1528-1541.

Matulyte, I., M. Marksa, L. Ivanauskas, Z. Kalveniene, R. Lazauskas, J. Bernatoniene. 2019. GC-MS Analysis of the Composition of the Extracts and Essential Oil from *Myristica fragrans* Seeds Using Magnesium Aluminometasilicate as Excipient. *Molecules*., 24(6):1-12.

Mitu, S.A., U. Bose, S. Suwansa, L.H. Turner, M. Zhao, A. Elizur, S.M. Ogbourne, P.N. Shaw, S.F. Cummins. 2017. Evidence for a Saponin Biosynthesis Pathway in the Body Wall of the Commercially Significant Sea Cucumber *Holothuria scabra*. *Marine Drugs*., 15(349):1-14.

Mohan, E., S. Suriya, S. Shanmugam, K. Rajendran. 2021. Qualitative Phytochemical Screening of Selected Medicinal Plants. *Journal of Drug Delivery and Therapeutics*., 11(2):141-144.

Moses, T., K.K. Papadopoulou, A. Osbourn. 2014. Metabolic and Functional Diversity of Saponins, Biosynthetic Intermediates and Semi-Synthetic Derivatives. *Crit Rev Biochem Mol Biol*., 49(6):439-462.

Narsih, N., S. Kumalaningsih, Wignyanto, S. Wijana. 2012. Identification of Aloin and Saponin and Chemical Composition of Volatile Constituents from *Aloe vera* (L.) *Peel. J Agric Food Tech*., 2(5):79-84.

Nimah, S., W.F. Ma'ruf, dan A. Trianto. 2012. Uji Bioaktivitas Ekstrak Teripang Pasir (*Holothuria scabra*) terhadap Bakteri *Pseudomonas aeruginosa* dan *Bacillus cereus*. *Jurnal Perikanan*., 1(2):1-9.

Nitbani, F.O., Jumina, D. Siswanta, dan E.N. Solikhah. 2015. Isolation and Anti-bacterial Activity Test of Lauric Acid from Crude Coconut Oil (*Cocos nucifera* L.). ELSEVIER., 18:132-140. Ong, J. Y. dan P. S. H. Wong. 2015. Sea cucumbers (Echinodermata: Holothuroidea) from the Johor Straits, Singapore. *Raffles Bulletin of Zoology*., 31:273-291.

Rasyid, A. 2012. Identifikasi Senyawa Metabolit Sekunder Serta Uji Aktivitas Antibakteri dan Antioksidan Ekstrak Metanol Teripang *Stichopus hermanii*. *Jurnal Ilmu dan Teknologi Kelautan Tropis*., 4(2):360-368.

Sellem F., Z. Brahmi, H. Mnasser, S. Rafrafi., dan B.B. Zahar. 2021. Antimicrobial Activities of Coelomic Fluid and Body Wall Extract of the Edible Mediterranean Sea Cucumber *Holothuria tubulosa* Gmelin, 1790. *Cahiers de Biologie Marine*., 58(2):181-188. https://doi.org/10.21411/cbm.a.479839a5

Setyastuti, A. 2016. Timun Laut Teluk Ambon, Maluku. Oseanologi dan Limnologi di Indonesia., 1(3):11-22.

Silva, M. M. C. dan J. A. R. Salvador. 2020. Introductory Chapter: Chemistry and Biological Activity of Steroids-Scope and Overview. IntechOpen.

Soto, E.R., F.J.M. Quijal, A. Cilla, P.E.S. Munekata, J.M. Lorenzo, F. Remize, F.J. Barba. 2019. Influence of Temperature, Solvent and pH on the Selective Extraction of Phenolic Compounds from Tiger Nuts by Products: Triple-TOF-LC-MS-MS Characterization. *Molecules*., 24(797):1-13.

Sukmiwati, M., A. Diharmi, E. Mora, dan E. Susanti. 2018. Aktivitas Antimikroba Teripang Kasur (*Stichopus vastus* Sluiter) dari Perairan Natuna Kepulauan Riau. *Jurnal Pengolahan Hasil Perikanan Indonesia*., 21(2):330-337.

Sukmiwati, M., M. Ilza, A.E. Putri, S.W. Sidauruk. 2020. Antibacterial Activity of Sea Cucumber (*Holothuria atra*) Against *Pseudomonas aeruginosa*. *IOP Conf. Ser: Earth Environ. Sci*., 404:1-6.

Susanto, H., M. Safithri, dan K. Tarman. 2018. Anti-bacterial Activity of *Stichopus hermanii* and *Stichopus variegatus* Methanol Extract. *Current Biochemistry*., 5(2):1-11.

Tagousop, C.N., J.D. Tamokou, I.C. Kengne, D. Ngnokam, dan L.V. Nazabadioko. 2018. Antimicrobial Activities of Saponins from *Melanthera elliptica* and Their Synergistic Effects with Antibiotics Against Pathogenic Phenotypes. *Chemistry Central Journal*., 12(97):1-9.

Teles, A.S.C., D.W.H. Chavez, F.S. Gomes, L.M.C. Cabral, R.V. Tonon. 2018. Effect of Temperature on The Degradation of Bioactive Compounds of Pinot Noir Grape Pomace During Drying. *Brazilian Journal of Food Technology*., 21:1-8.

Umboh, P.M.T., D.S. Wewengkang, dan P.V.Y. Yamlean. 2018. Aktivitas Antibakteri Fraksi Teripang Laut *Holothuria atra* terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus*. *Jurnal Ilmiah Farmasi*., 7(4):88-97.

Vargas, G.C., C.O. Malave, S. Medina, C.M. Guzman, R.G.D. Valle, N.M. Carballeira, dan D.J.S. Rios. 2021. Anti-bacterial fatty acids: An Update of Possible Mechanisms of Action and Implications in the Development of The Next-Generation of Anti-bacterial Agents. *Prog Lipid Res*, 82:1-10.

Wambreuse, N., G. Caulier, I. Eeckhaut, L. Borrello, F. Bureau, L. Fievez, and J. Delroisse. 2023. Morpho-functional Characterisation of Cœlomocytes in the Aquacultivated Sea Cucumber Holothuria Scabra: From Cell Diversity to Transcriptomic Immune Response. *Fish & Shellfish Immunology*. 158. http://dx.doi.org/10.2139/ssrn.4658658

Wang Z, Fan X, Li Z, Guo L, Ren Y, Li Q. 2022. Comparative analysis for immune response of coelomic fluid from coelom and polian vesicle in *Apostichopus japonicus* to *Vibrio splendidus* infection. Fish Shellfish Immunol. 29(4):100074. http://dx.doi.org/10.1016/j.fsirep.2022.100074.

Willie, P., E.A. Uyoh, P.O. Aikpokpodion. 2021. Gas Chromatography-Mass Spectrometry (GC-MS) Assay of Bio-Active Compounds and Phytochemical Analyses in Three Species of Apocynaceae. *Pharmacognosy Journal*., 13(2):383-392.

Wu, X., Chen, T., Huo, D., Yu, Z., Ruan, Y., Cheng, C., Jiang, X., & Ren, C. , 2020. Transcriptomic analysis of sea cucumber (*Holothuria leucospilota*) coelomocytes revealed the echinoderm cytokine response during immune challenge. *BMC Genomics* 21: 306. https://doi.org/10.1186/s12864-020-6698-6

Xu, C., Z. Liang, D. Tang, T. Xiao, M. Tsunoda, Y. Zhang, L. Zhao, S. Deng, Y. Song. 2017. Gas Chromatography - Mass Spectrometry (GC-MS) Analysis of Volatile Components from Guava Leaves. *Journal of Essential Oil Bearing Plants*., 20(6):1536-1546.