

GC-MS analysis and antibacterial activity of the Sea cucumber (*Muelleria lecanora*) extract

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Abstract

Sea cucumbers are marine invertebrates commonly found in benthic areas and deep seas. On a global scale, they have a high commercial value with an increased level of production and trade. This study aims to analyze the bioactive compound in *Sea cucumber (Muelleria lecanora)* using the Ultrasound-Assisted Extraction Method (UAE) and Gas Chromatography-Mass Spectrometry (GC-MS). Furthermore, it identifies the antibacterial activity in microorganisms *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus*. The bioactive compounds were extracted using methanol, acetone, and n-hexane solvent and were separated by ultrasound-assisted extraction. In the initial stage, phytochemicals were screened using Gas Chromatography-Mass Spectrometry (GC-MS). Disc diffusion method was then used to determine the antibacterial activity against *Salmonella*, *Staphylococcus aureus*, and *Escherichia coli*. The results showed that methanol extract is more suitable

for extracting bioactive compounds of *Muelleria lecanora* than acetone and n-hexane. Meanwhile, acetone solvents are more suitable for the production of flavonoid and steroid compounds than *Muelleria lecanora* samples. Heneicosane compounds that function as a new antiproliferative for inhibition of tumor and cancerous cells are produced from n-hexane. The antibacterial activity of acetone, methanol and n-hexane extract determined by diffusion assay was effective against *Staphylococcus aureus* and *Salmonella* but ineffective against *Escherichia coli*. GC-MS results showed that the major constituents obtained were steroid and flavanoid. From this study, Sea cucumber extract can be considered a healthy nutrient in food and pharmaceutical products.

Introduction

Sea cucumbers are invertebrate found in various marine habitats and are specially cultivated by countries in East Asia such as China and Japan.¹ The majority of countries that consume sea cucumbers are located in Indo-Pacific Asia, including Hongkong, Malaysia, Indonesia, Japan, Singapore, South Korea, Philippines, and China.² Sea cucumbers have complete nutritional content with low-fat, high protein, and rich in essential amino acids, such as lysine, arginine, and tryptophan.³ They have a body wall composed of non-soluble collagen and is utilized as a dietary supplement.³ Furthermore, they reduce arthritis pain because of a rich source of chondroitin sulfate polysaccharides.⁴ Sea cucumbers (*Holothuroidea*) are thorn-skinned marine animals with potential as a source of pharmacology and can be processed as food. Furthermore, they are known as gamat and beche-de-mer, and are used in medical systems of the middle eastern society and Asian people.⁵ They are also recognized as a traditional remedy for treating asthma, rheumatism, hypertension, impotence, constipation, and burns.⁶ Other functions include anti-coagulant, anti-cancer, anti-inflammatory, antithrombotic, antimicrobial, antioxidant, antiangiogenic antihypertensive, anti-tumor, and healing wound. These bioactive compounds of saponin, phenolics lectins, sterols, peptides, glycosaminoglycan, chondroitin sulfate, cerebroside, and sulfate polysaccharides can be used as a potential antibacterial. These substances suppress the growth and development of bacteria in the sea. The need for new antimicrobial materials increases because the growth and development of bacteria are currently resistant to antibiotics in addition to the growing conventional antibiotics.⁷

A study on the sea cucumbers *Holothuria scabra* and *Holothuria leucospilota*, from the northern coast of the Persian Gulf, showed the antibacterial and antifungal effects on *Aspergillus*

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niger, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.^{8,9} In another in vitro study, the antibacterial effects of the sea cucumber *Apostichopus japonicus* against *Micrococcus lysodeikticus*, *Streptococcus dysgalactiae*, *Nocardiosis*, *Pseudoalteromonasnigrifaciens*, and *Shewanella baltica*.¹⁰ Antibacterial activities of extracts from different organs (gonad, body wall, respiratory tree, and digestive tract) and antifouling of the sea cucumber *Holothuria leucospilota* against bacteria *Staphylococcus aureus* was also conducted.¹¹ In the same study, *Stichopus hermanni*, *Thelenota ananas*, *Thelenota anax*, *Holothuria fuccogilva*, and *Actinopyga mauritiana* have potential as antibacterial.¹² These functional materials lead to potential developments in the various food and biomedicine industries. The study presented a general view of the major medicinal and health benefits of functional sea cucumbers from the Asian region.

A few conventional methods (e.g., Maceration, Enzyme-Assisted Extraction (EAE), Ultrasonic Assisted Extraction (UAE), Microwave Assisted Extraction (MAE), Heat Reflux, and Mechanical Rabbling) were utilized for the extraction of target compounds from crude materials.¹³ Ultrasound can hydrate and facilitate swelling of vegetal tissue. It increases mass transfer and allows high diffusion rates across the cell. In contrast, cavitation produced by ultrasonic waves disrupts the cell, then releases contents.¹⁴ Some related studies, such as the use of ultrasound methods, high hydrostatic pressure, and high electric field pulse were widely applied to the rehydration process to improve the mass-liquid displacement. Furthermore, the use of energy produced by high-frequency sound waves above 16 kHz was widely regarded as one of the most effective technologies. A previous study on sea cucumber extracts' antibacterial activity was reported against various pathogenic bacteria such as *Listeria*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella*.¹⁵ A study for antimicrobial activities and antioxidants was significantly conducted, and the potential was examined through ultrasonic-assisted extraction with time variations (30, 60, 80, and 120 min). The efficiency of various solvents (acetone, n-hexane, and methanol) for the phytochemical extraction of *Sea Cucumber Muellaria* and the identification of bioactive compounds were analyzed using GC-MS (Gas Chromatography-Mass Spectrometry). Furthermore, antibacterial efficacy against pathogenic bacteria *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* was conducted using disc diffusion methods.

Materials and Methods

Materials

The sea cucumber phylum *Echinodermata*, family *Holothuriidae* and genus *Muellaria lecanora* (Figure 1) were collected from the coast of Barrang Lompo Island in Makassar, South Sulawesi, Indonesia. During the trip, they were stored in a cooling box that contains an ice pack. The storage, preparation, and analysis of samples were conducted in the Chemical and Instrumental Analysis Laboratory, Chemical Engineering Department, Politeknik Negeri Ujung Pandang, Indonesia.

The solvents used for sample extraction and reagents of analytical grade, aquadestilata, methanol, n-hexane, acetone, McFarland Standard (barium chloride and sulfuric acid), pH paper, antibiotic disc blank (Whatman No.1 and 5), dimethyl sulfoxide, and sodium chloride were supplied by Merck Millipore (Burlington, Massachusetts, United States). Tetracycline hydrochloride was provided by Sigma Aldrich (St. Louis, Missouri, United States).

Bacterial strains *Salmonella* (ATCC 13076), *Escherichia coli* (ATCC 25922), and *Staphylococcus aureus* (ATCC 25923) were obtained from Microbiology Laboratory, Department of Biology, State University of Makassar, South Sulawesi, Indonesia. Furthermore, the isolated bacteria grew at a temperature of 32°C in nutrient broth (DIFCO Laboratories, Detroit, USA) following standard procedures.¹⁶ Media growth nutrient agar and plate count agar were purchased from Oxoid, Basingstoke, United Kingdom.

The tools used include water bath (Memmert WNB 7 Basic control) Hettich Zentrifugen EBA-20, rotary evaporator Buchi, Hitachi centrifuge brands, Ultrasonic Assisted Extraction instrument (Elmasonic P30), and Shimadzu GC-MS 2010 brand Gas Chromatography-Mass Spectrometry plus.

Process for the preparation of extracts

The sea cucumbers were wiped clean and dried in an oven at 70°C, then cropped and minced. Furthermore, 100 g was weighed, homogenized, and extracted using an ultrasonic-assisted extraction method with a ratio volume of 1:2 (V/V) methanol, acetone, or n-hexane for 30, 60, 90, and 120 min. This was conducted in the rotary evaporator at 39°C, followed by a shaker for 24 h at a temperature of 10°C. The supernatant was centrifuged for 10 min, and

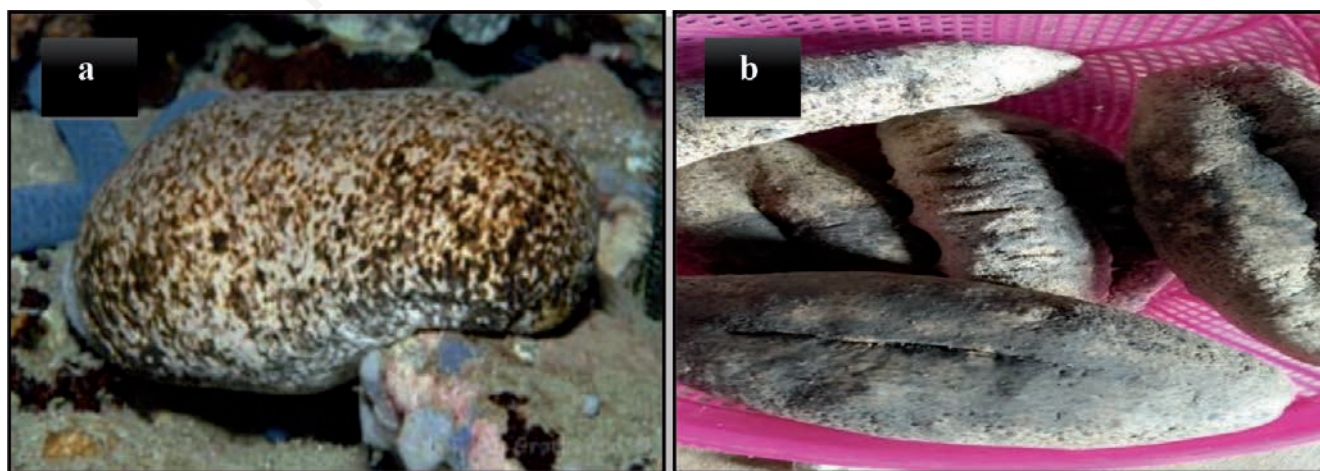


Figure 1. Sea cucumber *Muellaria lecanora*: a) fresh, and b) dried.

its bioactive compounds were analyzed using GC-MS. Furthermore, the antibacterial activity was conducted through the disc diffusion method.

Gas Chromatography-Mass Spectrometry (GC-MS)

Analysis

Flame Ionization Detector (FID) in EI mode at 70 eV and capillary column DB-5 (30 μm , 0.25 mm, 0.25 μm film) were used to test the sea cucumber using GC-MS. 1 mL of the extract was added with 3 mL of methanol 96% in the reaction tube and vortex. The temperature of injectors and the detector was 250°C and 220°C, one sample dissolved with 1 μL methanol was injected and analyzed for 2 min at 60°C and increased to 300°C/min at 3°C with Helium (He) gas carrier at 1 mL/min. This generated two GC data in the form of the chromatogram, which displays the compound's peaks in the methanol, acetone, or n-hexane extract. The current MS (Mass Spectroscopy) data showed the molecular weight at each peak. Any peaks appearing on the GC chromatogram indicated a single molecule and have a fragmentation pattern displayed in the MS spectra. The fragmentation pattern was used to identified organic compounds contained in the sea cucumber sample.

Antimicrobial assay

Disc antibiotic blank (Whatman No. 1) was cut and sterilized with other equipment using autoclaved at 121°C for 15 min. Growth media microorganisms are 5g nutrient agar (NA) with dissolved 250 mL of aquades in the Erlenmeyer 500 mL heated to homogeneous. Furthermore, 0.9 g NaCl was dissolved in a 100 mL volumetric flask, and inserted into the reaction tube of 9 mL. Mc Farland solvent was obtained by mixing a solution of barium chloride (BaCl_2) 1.175% and sulphuric acid (H_2SO_4) 1%. Mc Farland 0.5% was used as standard turbidity (absorbance 600 nm). Media nutrient agar and sodium chloride solvent sterilization was conducted using autoclave at temperature 121°C for 15 min.

Sterile nutrient agar 20 mL was poured in Petri dishes and was inoculated at 37°C evenly with 0.1 mL of a 24 h broth culture of test bacteria.¹⁷ Sea cucumber extract 0.25 g was dissolved

in 1 mL aqueous dimethylsulfoxide (DMSO) with tween 80 (0.5% v/v for easy diffusion) and filtered through a 0.45 μm membrane filter. In addition, each sterile disk (Whatman 6 mm, number 5) was dipped in 20 μL of extracts and carefully put on the agar plate using flame sterilized forceps to ensure the disks were at least 2 cm apart. The plates were inverted after 30 minutes and incubated at 37°C for 48 h. This was followed by measuring the inhibitory zone for each sample and the type of bacteria in mm. The test was performed in duplicate, and the mean area inhibition diameter was reported. Furthermore, negative controls used a 10% DMSO solvent, and one paper disc was given a tetracycline HCl as a positive control. Classification of the antibacterial activity was conducted and 10 mm diameter was considered active, while less was inactive.¹⁸

Results and Discussion

Yield analysis extract of acetone, n-hexane, and methanol

Figure 2 showed that the extraction time affected sea cucumbers production obtained by various solvents (acetone, n-hexane, methanol) and time extraction (30, 60, 90, and 120 min). The study results showed that the highest yield of *Muelleria lecanora* extraction uses solvents (methanol, acetone, and n-hexane). The highest yield on the extraction was with methanol solvent of 11.2% and the lowest was n-hexane of 1.86%. Yield analysis on methanol, acetone, and n-hexane extract obtained a range of 6.04-11.2%, 4.46-6.83%, and 1.1-1.8%. The ultrasonic-assisted method showed a direct proportionality with extraction time and the resulting crude extract. A study by Yusuf *et al.*¹⁹ stated that the length of time for the extraction process is very influential in the resulting extract. Therefore, increasing the time from 30 to 120 min using different solvents significantly increased the yield of sea cucumbers. The yield obtained was directly correlated with an increase in extraction time (Figure 2).

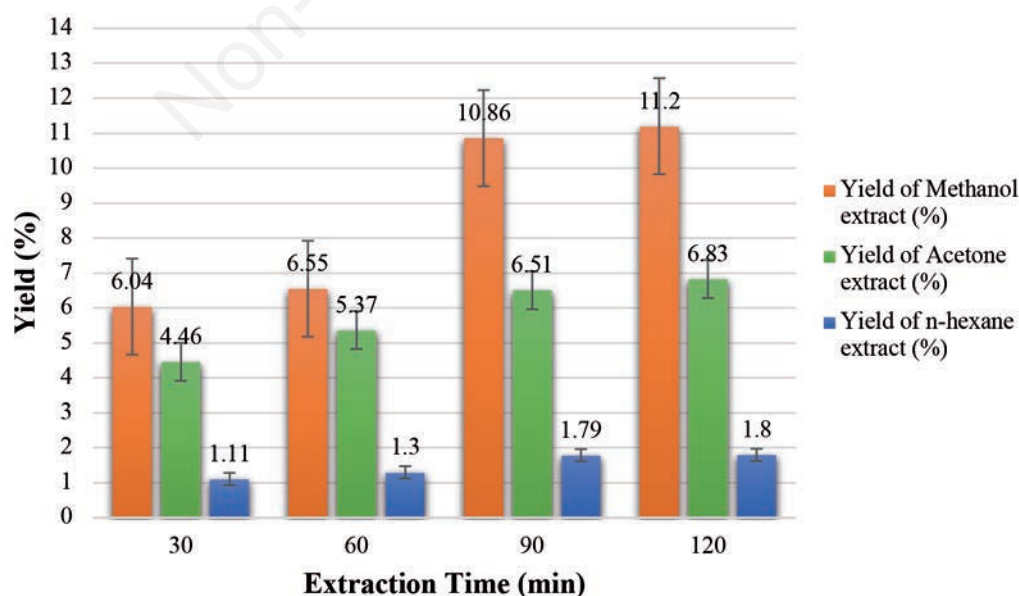


Figure 2. Effect of different solvents and time to extract sea cucumber yield.

GC-MS profile bioactive compound of *Muelleria lecanora*

Table 1 showed the availability of a bioactive compound in the methanol acetone and n-hexane extract of *Muelleria lecanora* characterized by GC-MS. The acetone extract of sea cucumbers *Muelleria lecanora* had a running time of 39 min for GC and MS spectrum (Figure 3). Chromatogram evaluation of the acetone extracts confirmed sixty major peaks and determined the components. Major component of this extract are as follows 9-Octadecenoic acid (Z) -, methyl ester (7.36%), Azulene, 1,2,3,5, 6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.)] (7.53%), Stigmasta-5,22-dien-3-ol, acetate, (3.beta.) (8.86%), Hexadecanoic Acid, Methyl Ester (11.2%), and Cholest-5-EN-3-YL Acetate (13.61%), which is an antioxidant and antibacterial component. Pharmacologically, azulene compounds exhibit antipyretic properties, anti-inflammatory drugs, and cardiac tonic.^{20,21}

Figure 3 showed that the spectrum for GC and MS run time for the *Muelleria lecanora* methanol extract was 39 min. The result analysis includes their component quantity, molecular formula, and composition within the *Muelleria lecanora* methanol extracts. Table 1 showed the percentage composition and list of known compounds. Many of the antioxidants and antimicrobials were present in trace levels, a complex mixture of numerous compounds; 9-Hexadecenoic acid, methyl ester, (Z) (1.54%), Omega 3/5,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (ALL-Z) EPA (2.59%), Ergosta-14,22-Dien-3-OL, Acetate, (3.Beta.,5.Alpha.,22E)- (4.7%), Cholest-5-EN-3-YL Acetate (7.64%), 2-[(Hexadecyloxy)Methyl]Oxirane (9.34%), Octadecanoic acid, methyl ester (13.34%), 9-Octadecenoic acid (Z), methyl ester (stearic acid methyl ester, 14.42%), Hexadecanoic Acid, Methyl Ester (17.36%), performs a crucial role. Furthermore, steroids and flavonoids were the significant components in the sea cucumber, which was rich in glycosides and triterpene with proven antifungal

Table 1. GC-MS report for methanol, acetone, and n-hexane extract *Muelleria lecanora*.

Antibacterial Compound	Molecular formula	Methanol extract (% of Area)	Acetone extract (% of Area)	n-hexane extract (% of Area)	Reported bioactivity
9-Hexadecenoic acid, methyl ester, (Z) (21)	C ₁₇ H ₃₂ O ₂	1.54	1.16	-	Antioxidant, Anti-inflammatory, antimicrobial activity, and antiandrogenic flavor ²²
Hexadecanoic Acid, Methyl Ester	C ₁₇ H ₃₄ O ₂	17.36	11.42	-	Antimicrobial activity ²²
Tetradecanoic Acid, Methyl Ester	C ₁₅ H ₃₀ O ₂	0.44	5.49	-	Larvicidal and repellent activity ²³
Palmitic Acid	C ₁₆ H ₃₂ O ₂	0.87	-	-	Anti-inflammatory, lubricant, antiandrogenic, nematocide, pesticide, flavor, hemolytic 5-alpha reductase inhibitor, antioxidant, and hypocholesterolemic ²⁴
9-Octadecenoic acid (Z) -, methyl ester (Stearic acid methyl ester)	C ₁₉ H ₃₆ O ₂	14.42	7.36	10.44	Antileukotriene, anti-inflammatory, cancer preventive, 5-alpha reductase inhibitor, hypocholesterolemic, insectifuge, anemiagenic, dermatitogenic, irritant, and antiandrogenic ²³
Tetratriacontane	C ₄₄ H ₉₀	4.15	1.39	14.96	Antibacterial and antifungal ²²
Pentacosane	C ₂₅ H ₅₂	0.32	2.04	14.39	Antitumor, antimicrobial activity, antiviral ²³
35,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (ALL-Z) or EPA/Omega	C ₂₁ H ₃₄ O ₂	2.59	0.5	-	Preventing and managing heart disease, reduce triglycerides accumulation and blood pressure, anti-inflammatory complications after surgery, reduce the chance of abnormal heart rhythm, reduce of heart attack and stroke, slow the development of plaque in the arteries ²⁵
214-.Beta.-H-Pregna7890-	C ₂₁ H ₃₆	1.04	-	-	Antibacterial and antifungal effects ²⁶
2-[(Hexadecyloxy)Methyl]Oxirane	C ₁₉ H ₃₈ O ₂	9.33	2.15	-	Antibacterial activity ²⁷
Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)	C ₃₁ H ₅₀ O	5.79	8.86	-	Free radical Scavenging, Anti-diabetic, Anticancer ²³
Cholest-5-EN-3-YL Acetate	C ₂₉ H ₄₈ O ₂	7.64	13.61	-	Antioxidant activity and antimicrobial activity ²⁸
Ergosta-14,22-Dien-3-OL, Acetate, (3.Beta.,5.Alpha.,22E)-	C ₃₀ H ₅₀ O ₂	4.7	5.89	-	Antibacterial activity ²⁹
Stigmast-5-EN-3-OL, (3.Beta.,24S)- / gamma.-Sitosterol	C ₂₉ H ₅₀ O	2.97	5.79	-	Thyroid inhibitory, antiperoxidative, and hypoglycemic effects ²³
Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	13.34	4.37	-	Antimicrobial activity ²³
Caryophyllene	C ₁₅ H ₂₄	-	0.42	-	Anti-inflammatory and Antimicrobial activity ³⁰
Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.)]	C ₁₅ H ₂₄	-	7.53	-	Analgesic, antiasthmatic, anti-inflammatory, and antipyretic properties ²⁰
Heneicosane	C ₂₁ H ₄₄	-	-	32.91	Anticancer ³¹
Docosane	C ₂₂ H ₄₆	-	-	13.44	Anti-inflammatory and anti-atherogenic ³²

and anti-tumor activity.³³ Moreover, they have impressive amounts of lectins, glycosaminoglycans, omega-6, omega-3 fatty acids (EPA and DHA), and sterols.³⁴

GC-MS test showed forty peaks and components in the n-hexane extract of sea cucumber (Figure 3). Only five were important as pharmacological material, namely Heneicosane (32.91%), Tetratriacontane (14.96%), Pentacosane (14.39%), Docosane (13.44%), and 9-Octadecenoic acid (Z)-, methyl ester (10.44%). There are two exciting compounds to be examined and not found by the other two solvents (methanol and acetone), namely Heneicosane. This serves as an anti-cancer and Docosane anti-inflammatory and anti-atherogenic. Isolation of brown algae *Lobophora variegata* from the Brazilian coastal produced polyunsaturated epoxy-heneicosane compounds that serve as antiproliferative, better tumor cell line inhibition compared to fibroblast.³¹ Furthermore, compounds found in brown algae have similarities with those in sea cucumbers *Muellaria lecanora* such as Polyunsaturated Fatty Acids (PUFAs), 5,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (ALL-Z) EPA/Omega 3 (DHA), and Eicosapentaenoic Acid (EPA).^{31,35} Compounds n-3 PUFAs may increase the sensitivity of tumor cells to conventional therapies. These molecules exhibit anti-tumor activity by inducing apoptosis in human cancer cells alone or combined with conventional chemotherapy agents.³⁶⁻³⁸ Microdilution method of Heneicosane compounds as antibacterial activity against two gram-positive bacterial types *Salmonella* (ATCC 29890) and *Staphylococcus aureus* (ATCC 6538P) was also conducted. Bacteria gram-negative strains *Escherichia coli* (ATCC 10536) did not give good results.³¹

Antibacterial activity

Table 2 showed that antibacterial activity in sea cucumber extracts was measured against three bacterial strains (two gram-positive and one gram-negative).

Screening for sensitivity against the three solvents extracts methanol, acetone, and n-hexane extract of sea cucumber leaves was calculated as 20 μ L. Furthermore, *Muellaria lecanora* was screened for antimicrobial activity through the Kirby-Bauer disc diffusion assay on bacterial strains *Escherichia coli* (gram-negative), *Salmonella* (gram-positive), and *Staphylococcus aureus* (gram-positive).³⁹ Antibacterial activity less than 6 mm was classified as inactive, mildly active (6-7 mm), medium active (7-10 mm), and highly active (>10 mm).¹⁸ In addition, the inhibitory zone \geq 6 mm and maximum zone for *Staphylococcus aureus* (10.94 mm) were immune to all strains. Broad-spectrum showed a minimum zone of 6.09 mm for methanol extract at 60 min extraction against bacteria *Escherichia coli* and acetone extract at 30 min extraction against *Staphylococcus aureus* at a maximum zone of 10.94 mm.

Antibacterial activity against *Escherichia coli*

The antibacterial activity in n-hexane solvents with extraction times of 30, 60, 90, and 120 min was not recommended for biological activities, since the resulting diameter spectrum <7.44 mm. All three solvents belonged to the slightly active category with an average inhibitory zone of 6-7 mm (Table 2 and Figure 4).

Antibacterial activity against *Salmonella*

Disc diffusion assay method was used to determine antimicro-

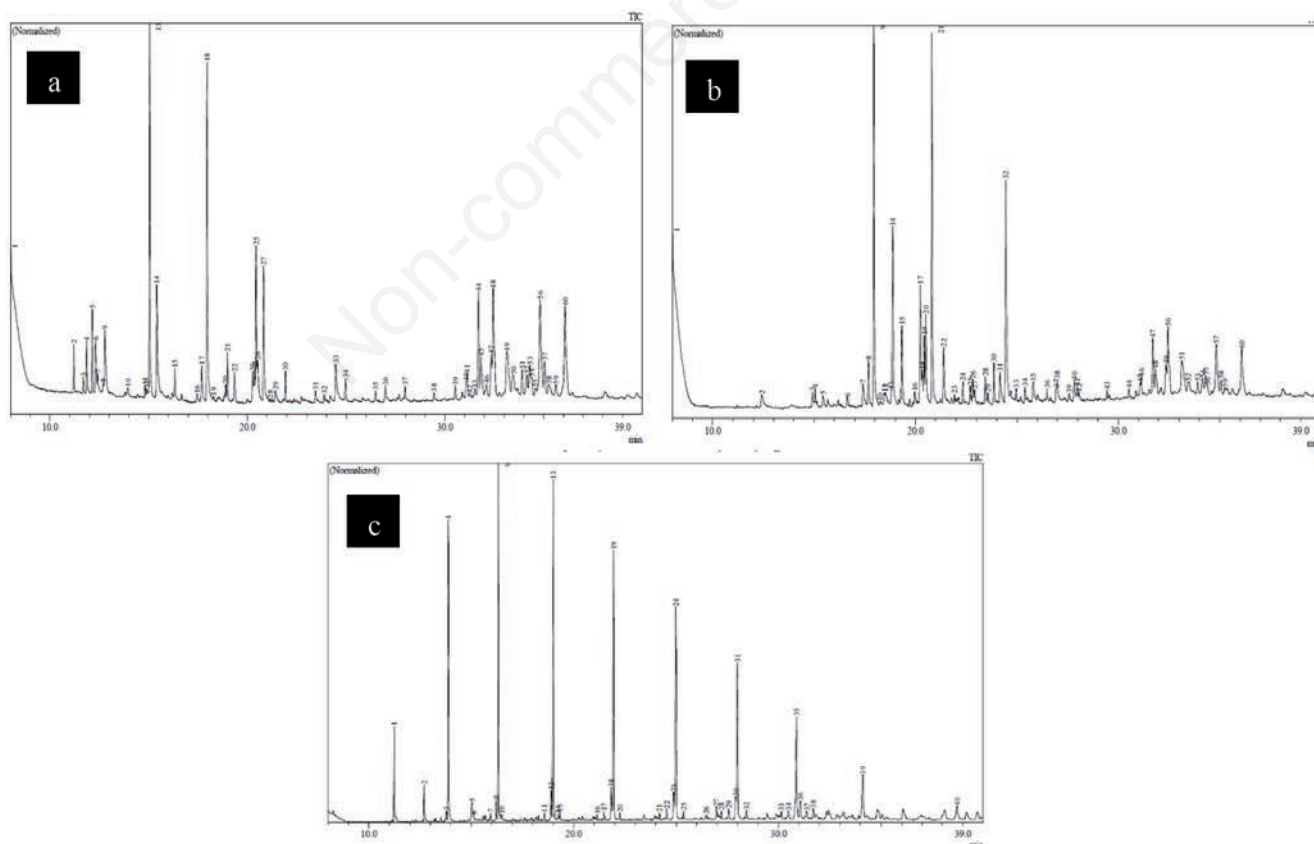


Figure 3. Chromatogram of sea cucumbers *Muellaria lecanora* extract: a. methanol solvent; b. acetone solvent; c. n-hexane solvent.

bial agent activity, through a disc. The antibacterial agent was put on the media to plant microorganisms that will diffuse.¹⁶ Clear areas indicate a growth barrier of microorganisms on the media surface. The advantages of this method are the number of substances used can be arranged. Furthermore, acetone extract with 90 min extraction has a minimum zone diameter of 6.52 mm, and n-hexane with 60 min extraction has a maximum zone diameter of 8.82 mm against *Salmonella*. These results also showed that acetone, methanol, and n-hexane extracts have mild active activity against *Salmonella* with a diameter of inhibitory zone 7 – 10 mm (Table 2 and Figure 5).

Antibacterial activity against *Staphylococcus aureus*

Methanol extract with 120 min extraction has a minimum zone diameter of 6.37 mm. Meanwhile, acetone extract with 30 min extraction has a maximum zone diameter of 10.94 mm against *Staphylococcus aureus*. These results also showed acetone, methanol, and n-hexane extracts have highly active activity against *Salmonella* with a diameter of inhibitory zone >10 mm (Table 2 and Figure 6). Their preliminary antibacterial assay of sea cucumber *Muellaria lecanora* showed different responses to the test strains against bacteria gram-positive (*Salmonella* and *Staphylococcus aureus*). However, they are not recommended for gram-negative bacteria such as *Escherichia coli*.

Conclusions

Ultrasonic-assisted extraction was a practical and valuable method for extracting potential antioxidants from sea cucumber. In summary, acetone, methanol, and n-hexane were excellent solvents for the extraction of bioactive compounds from Sea cucumbers *Muellaria lecanora*. Bioactive compounds extracted using n-hexane solvent served as a new antiproliferative polyun-

saturated epoxy-heneicosane with better inhibition of the tumor cell and anti-cancer. Furthermore, the GC-MS study indicated the existence of a good number of bioactive metabolites, such as flavonoids and steroids. Therefore, sea cucumbers *Muellaria lecanora* showed higher free radical scavenging and may be used in therapeutic applications and food products (functional foods). In addition, it has an effective antibacterial activity on *Salmonella* and *Staphylococcus aureus*, and evaluation showed that the entire extract had antibacterial potential.

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Table 2. Zone of inhibition test extract of sea cucumber *Muellaria lecanora* against different pathogens.

Samples	Extraction time (min.)	Concentration	Inhibition zone (mm)		
			Bacteria <i>Escherichia coli</i>	Bacteria <i>Salmonella</i>	Bacteria <i>Staphylococcus aureus</i>
Methanol extract	30	20 L	6.49	7.19	6.92
Acetone extract			6.33	7.05	10.94
n-hexane extract			6.08	8.03	8.36
Control positive			15.11	23.22	29.40
Control negative			0	0	0
Methanol extract			60	20 L	6.09
Acetone extract	7.12	7.07			8.76
n-hexane extract	6.70	8.82			9.30
Control positive	11.72	24.19			28.10
Control negative	0	0			0
Methanol extract	90	20 L			6.40
Acetone extract			6.87	6.52	7.27
n-hexane extract			6.31	7.22	8.37
Control positive			14.6	24.52	34.91
Control negative			0	0	0
Methanol extract			120	20 L	7.47
Acetone extract	6.27	7.35			6.63
n-hexane extract	7.05	7.34			7.70
Control positive	12.41	26.61			28.31
Control negative	0	0			0

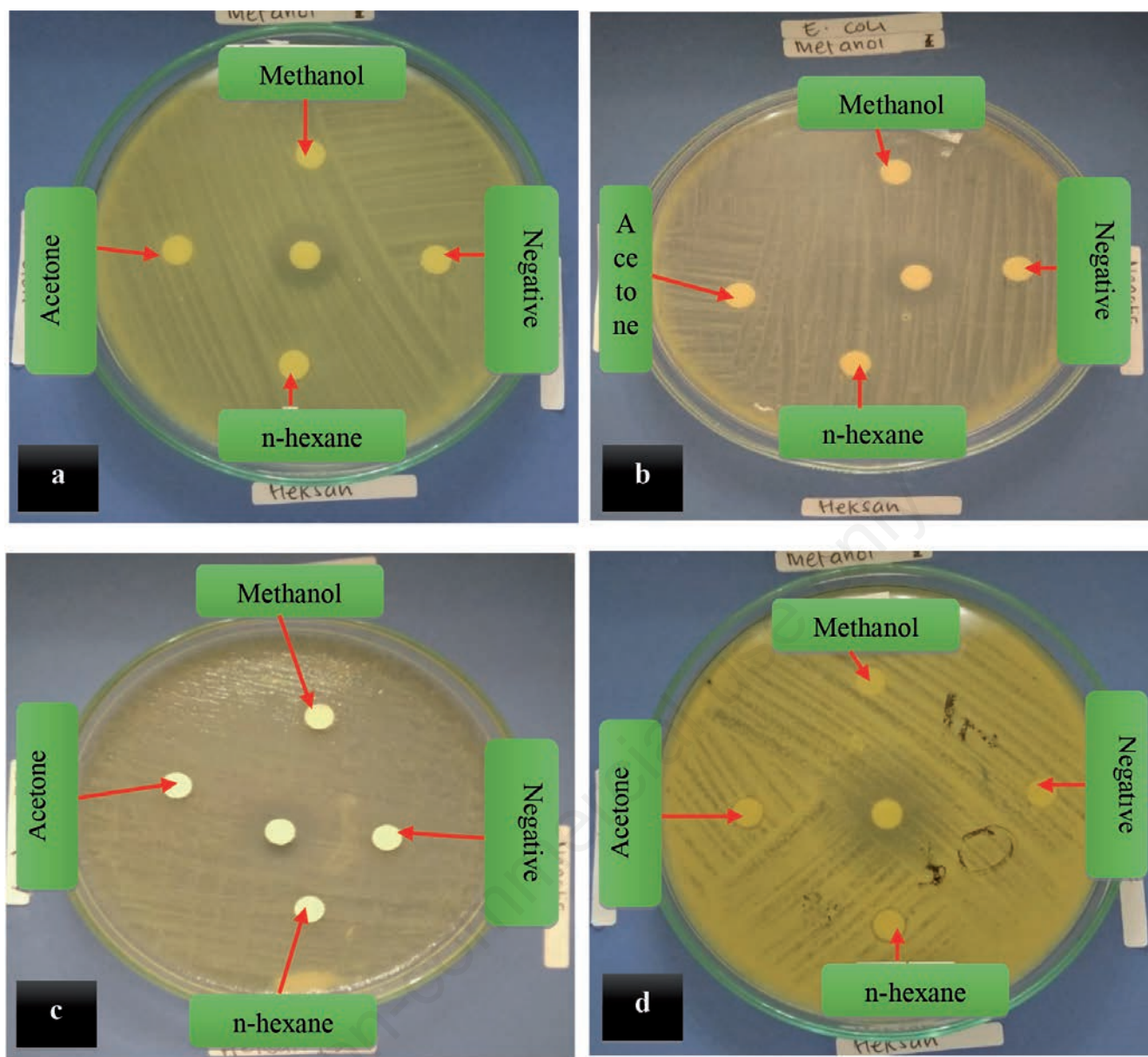


Figure 4. Antibacterial activity of acetone, methanol, and n-hexane extract sea cucumber against *Escherichia coli*: a) 30 min extraction; b) 60 min extraction; c) 90 min extraction, and d) 120 min extraction.

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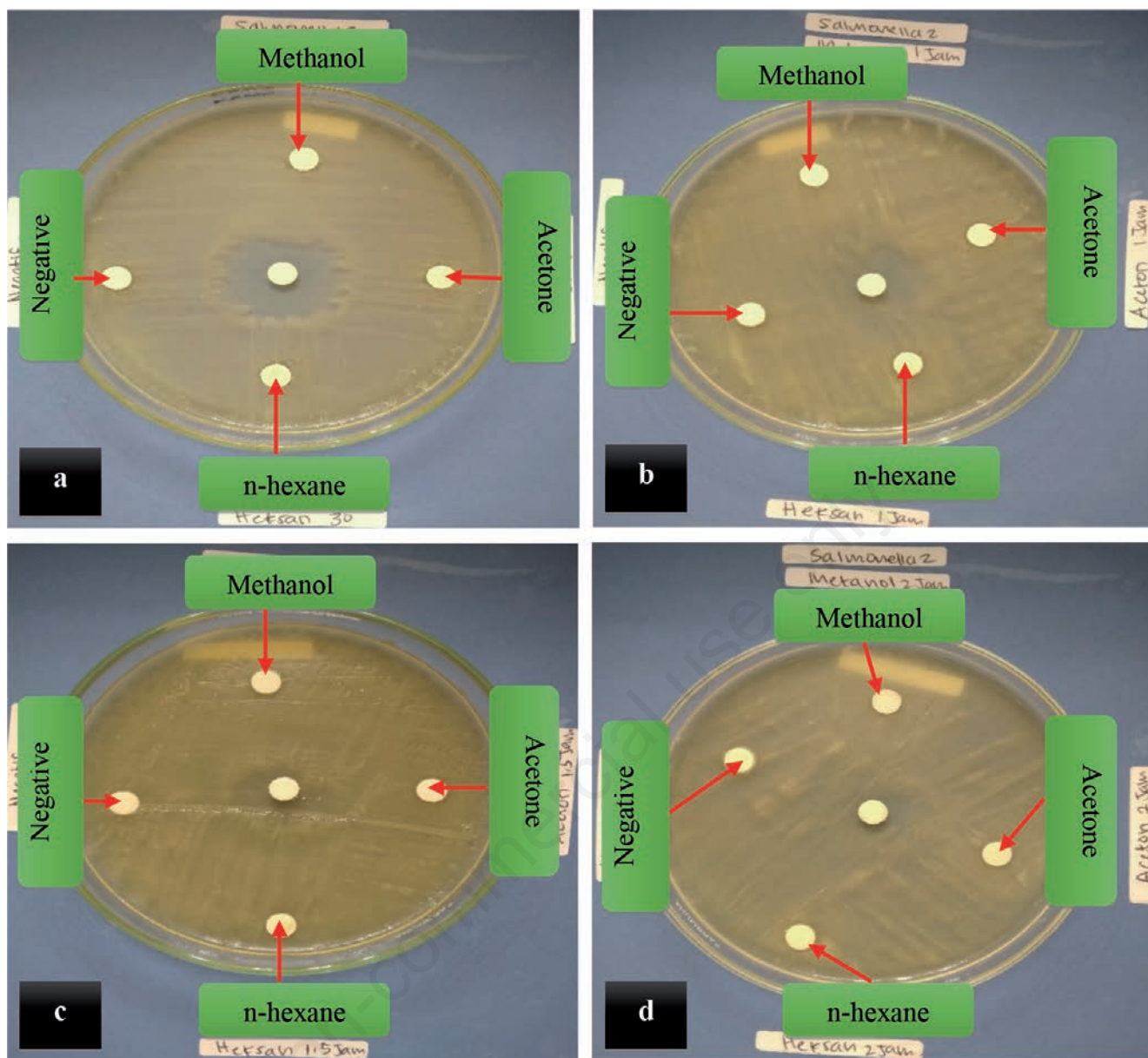


Figure 5. Antibacterial activity of acetone, methanol, and n-hexane extract sea cucumber against *Salmonella*: a) 30 min extraction; b) 60 min extraction; c) 90 min extraction, and d) 120 min extraction.

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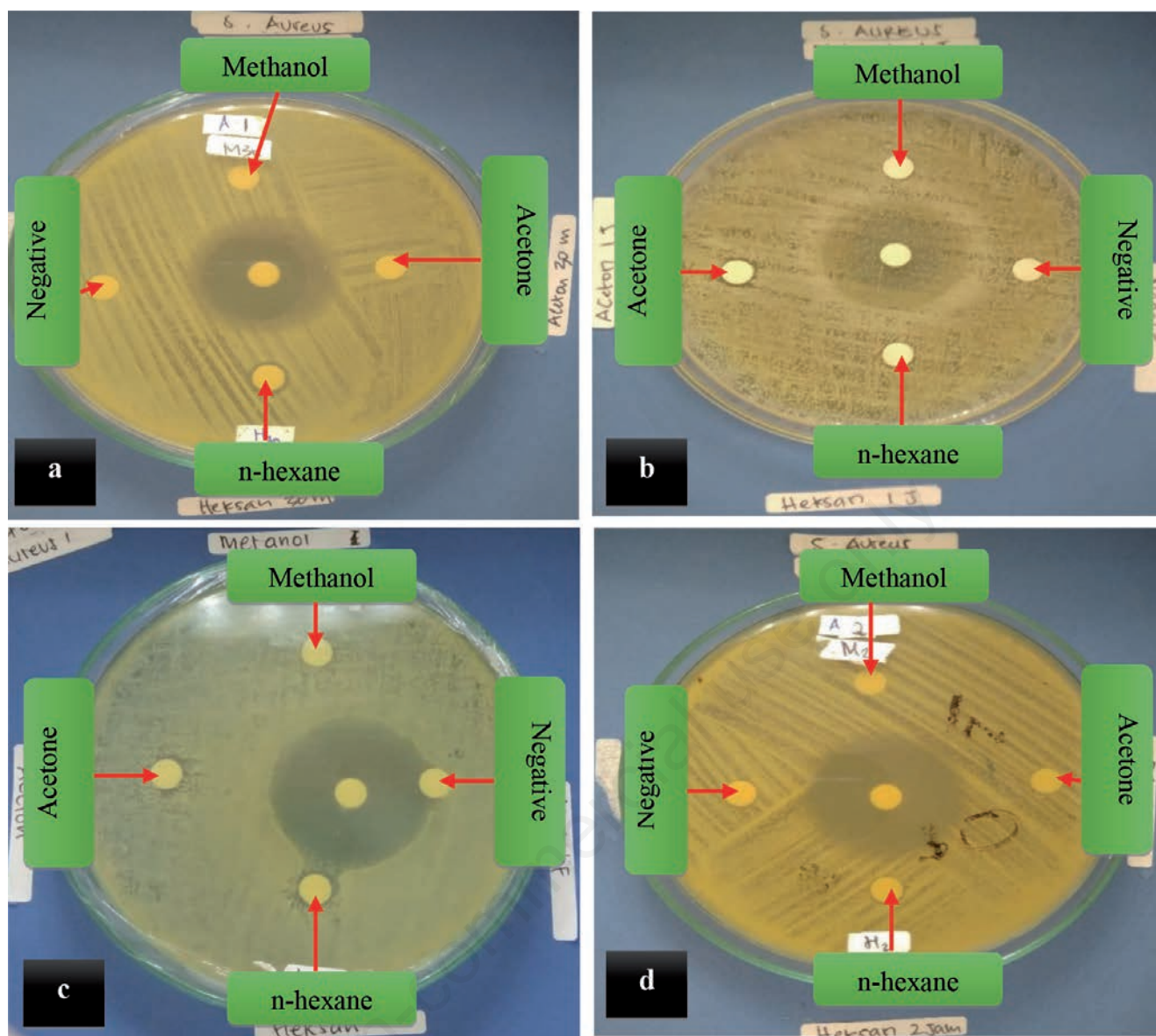


Figure 6. Antibacterial activity of acetone, methanol, and n-hexane extract sea cucumber against *Staphylococcus aureus*: a) 30 min extraction; b) 60 min extraction; c) 90 min extraction, and d) 120 min extraction.

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