Optimization Ultrasonic Assisted Extraction (UAE) of Bioactive Compound and Antimicrobial Potential from Sea Urchin (Diadema setosum

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GC-MS investigation of phytochemical composition, in vitro antibacterial potency of *Sea Cucumber Muelleria* Using *Ultrasonic Assisted Extraction (UAE)*

Abstract. Sea cucumber derived pharmacological bioactive compounds have picked up significance in the food and pharmaceutical enterprises. This research aims to identify and study the phytochemicals, antioxidant and antibacterial in the *Sea cucumber Muelleria* using Ultrasound-Assisted Extraction Method (UAE), and also in vitro testing of the microorganisms *Salmonella, Escherichia coli,* and *Staphylococcus aureus*. The *Muelleria* was extracted by methanol, acetone, n-hexane solvent and separated by ultrasound-assisted extraction. Screening of phytochemicals use Gas Chromatography-Mass Spectrometry (GC-MS), and disc diffusion method was followed to determine the antbacterial activity against *Salmonella, Escherichia coli, and Staphylococcus aureus*. The results of this study showed that the acetone extract showed better to produce bioactive compounds of flavanoid and steroid than other chosen solvents. Preliminary screening of phytochemicals was carried out in acetone and methanol extract. Antibacterial activity was determined by disc diffusion assay, acetone, methanol, and n-hexane extract was found effective against *Staphylococcus aureus and Salmonella,* but ineffective on *Escherichia coli*. GC-MS results indicate major constituents were found to be steroid and flavanoid. Results of this study suggested that *Sea cucumber Muelleria* extract could be considered as healthy nutriment in the food and pharmaceutical enterprises.

Keywords: antibacterial, muelleria, phytochemicals, sea cucumber, ultrasound-assisted extraction

1. Introduction

Sea cucumber *Muelleria* is a form invertebrate that can live in a variety of marine habitats and is specially cultivated by countries in East Asia such as China and Japan [1]. The majority of countries whose people consume sea cucumber is located in the Indo-Pacific Asia, including Hongkong, Malaysia, Indonesia, Japan, Singapore, South Korea, Philippines, and China [2]. Sea Cucumber has a complete nutritional content, has a low-fat content, high protein content, and rich in essential amino acids, such as lysine, arginine, and tryptophan[3]. Also, the sea cucumber has a body wall that is composed of non-soluble collagen and has been utilized as a dietary supplement[3]. Sea cucumber can reduce arthritis pain, because rich source of chondroitin sulfate polysaccharides[4]. Sea cucumber *(Holothuroidea)* is a thorn-skinned marine animal that has potential as a source of pharmacology and can be processed as food. Sea cucumber, with other names being gamat, beche-de-mer and sea cucumber, has long been used in medicine systems and food of the middle eastern society and asian people[5,6]. Sea cucumber has been recognized as a traditional remedy for treating asthma, rheumatism, hypertension, impotence, constipation and burns [7]. Other functions of the sea cucumber compounds, among others, anti-coagulant [8,9], anticancer [10], anti-inflammatory [11], antitromotic [12,13], antioxidant [14], antimicrobial [15,16], antihypertensive [17], anti-angiogenic [18,19], antitumor [20,21], and healing Wound [22], due to the role of bioactive compounds display within the sea cucumber mainly the triterpene glycosides (saponin) [23–25], phenolics [26], lectins [27,28], sterols (glycosides and sulfate) [29], peptides [30],

glycosaminoglycan [31,32] , chondroitin sulfate [33], cerberosides [34], and sulfate polysaccharides [35]. This bioactive compound can be used as a potential antibacterial. Antibacterial is a compound that can suppress the growth and development of bacteria, the ability of bioactive compounds in the sea, which makes researchers interested in researching sea cucumber. The need to find new antimicrobial material is increasing, because the growth and development of bacteria are currently able to be resistant to antibiotics, as well as the growing conventional antibiotics [36].

Research [37,38], evaluated antibacterial and antifungal sea cucumber *Holothuria scabra and Holothuria leucospilota*, from the north coast of the Persian Gulf against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger, and Candida albicans*,. Other research [39], in vitro antibacterial analysis of sea cucumber *Apostichopus japonicus against Micrococcus lysodeikticus*, *Streptococcus dysgalactiae*, *Nocardiopsis, Pseudoalteromonas nigrifaciens, and Shewanella baltica*. Recent research on 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* [40].

A few regular extraction methods (e.g., Maceration, Microwave Assisted Extraction (MAE), Ultrasonic Assisted Extraction (UAE), Enzyme-Assisted Extraction (EAE), Heat Reflux, and Mechanical Rabbling) have been utilized for the extraction of target compounds from crude materials[41]. Ultrasound can hydration and facilitate swelling of vegetal tissue. According to research [42], ultrasound can be increase the mass transfer and allowing high diffusion rates across the cell. On the other hand, cavitation produced by ultrasonic waves can also disrupt the cell, then release of contents. Some related studies, such as the use of ultrasound [43] methods, the use of high hydrostatic pressure [44], and utilization of high electric field pulse [45], have been widely applied to the rehydration process to improve the mass-liquid displacement. The use of energy produced by the high-frequency sound waves above 16 kHz [46], is widely regarded as one of the most effective technologies. Previous studies on antibacterial activity of sea cucumber extracts have been reported against several pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Listeria*, and *Salmonella*. Previous literature confirmed that sea cucumber is significantly studied for antioxidant and antimicrobial activities. In this study, Sea Cucumber *Muelleria* is used for investigating antioxidant and antimicrobial potential using ultrasonic-assisted extraction (UAE) with time variations (30, 60, 80, and 120 minutes). The research purpose to analyze the efficiency of various solvents (methanol, acetone and n-hexane) for the phytochemical extraction from *Sea Cucumber Muellaria* and to identification bioactive compound were analyzed by GC-MS *(Gas Chromatography-Mass Spectrometry)* and antibacterial efficacy against pathogenic bacteria *Escherichia coli, Salmonella* and *Staphylococcus aureus* using disc diffusion methods.

2. Materials and Methods

2.1. Materials

Sea cucumber phylum *Echinodermata*, family *Holothuriidae* and genus *Muelleria lecanora* (Fig.1) collected from the coast Barrang Lompo Island in Makassar, South Sulawesi, Indonesia. During the trip, sea cucumber is stored in a cooling box that contains an ice pack and is moved into the freezer after arriving in the laboratory and processed in Food Science and Instrumental Analysis Laboratory, Chemical Engineering Department, Politeknik Negeri Ujung Pandang, Indonesia.

Fig. 1: *Sea cucumber Muellaria lecanora:* a) freshwater and b) dried

All chemicals were of analytical grade, methanol (CAS: 67-56-1), acetone (CAS: 67-64-1), n-hexane (CAS: 110-54-3), aquadestilata (CAS: 7732-18-5), nutrient agar (CAS:105450), plate count agar (CAS: 105463), sodium chloride (CAS: 7647-14-5), McFarland Standard (barium chloride and sulfuric acid), pH paper, disc antibiotic blank (Whatman No.1 and No.5), dimethyl sulfoxide (CAS: 67-68-5) supplied by Merck Millipore (Burlington, Massachusetts, United States), Tetracycline hydrochloride (CAS: 64-75-5) supplied by Sigma Aldrich (St. Louis, Missouri, United States), culture strains *Salmonella, Escherichia coli, and Staphylococcus aureus*. Tools 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.

2.2. Process for the preparation of extracts

Sea cucumbers were wiped clean and dried in an oven at 70°C, then cropped small-minced. Weighed 100 g were homogenized and extracted using ultrasonic-assisted extraction method with a ratio of volume 1:2 (V/V) methanol, acetone or n-hexane for 30, 60, 90 and 120 min, then in the rotary evaporator at 39°C, followed by a shaker at a temperature of 10°C for 24 hours. The Supernatant is produced for each sample, then in centrifugation for 10 min and stored at a temperature of 10°C, for use in analysis bioactive compound in GC-MS and antibacterial disc diffusion method.

2.3. Analysis of Gas Chromatography-Mass Spectrometry (GC-MS)

Sea cucumber was analyzed using GC-MS using Flame Ionization Detector (FID) operated in EI mode at 70 eV and capillary column DB-5 (30 µm, 0.25 mm, 0.25 µm film). 1 mL of the sea cucumber was added with 3 mL of methanol 96% in the reaction tube and vortex. The injectors and detector temperature are set at 250°C and 220°C. One sample was dissolved with 1 µl methanol, then injected and analyzed 2 min at 60°C and increased 3°C to 300°C/min, with carrier gas Helium (He) as 1 mL/min. This analysis will generate two GC data in the form of chromatogram which displays the peaks of the compound contained in the methanol, acetone or n-hexane extract and the current MS (Mass Spectroscopy) data shows the molecular weight at each peak. Any peaks appearing on the GC chromatogram indicate a single molecule and have a fragmentation pattern displayed in the MS spectra. Based on the fragmentation pattern can be identified what compounds are contained in the sea cucumber sample.

2.4. Test microorganisms and culture media

Test microorganisms *Escherichia coli* (gram-negative), *Salmonella* (gram-positive), and *Staphylococcus aureus* (gram-positive), used in these studies were obtained from the Microbiology Laboratory, Department of Biology, State University of Makassar. The isolated bacteria will grow at a temperature of 32°C in nutrient broth (DIFCO Laboratories, Detroit, USA) following standard procedures[47].

2.5. Antimicrobial assay

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

Sterile nutrient agar 20 ml poured in petri dishes, allowed to set at 37°C after inoculate uniformly with 0.1 ml of a 24 hr broth culture of test bacteria [48]. Sea cucumber extract 0,25 g were dissolved in 1 ml aqueous dimethylsulfoxide (DMSO) with tween 80 (0.5% v/v for easy diffusion) and sterilized by filtration through a 0.45 µm membrane filter. Under the aseptic condition, each sterile disc (Whatman 6 mm, number 5) was then dipped in 20 µl of the extracts and carefully placed on the agar plate using flame sterilized forceps, ensuring the discs were at least 2 cm separate from one another. After 30 min, plates were inverted and incubated at 37°C for 48 hr, followed by measuring the inhibitory zone for each sample and the type of bacteria in mm. The experiment test was carried out in duplicate and the averages diameter of area inhibition was recorded. Negative

controls use a 10% DMSO solvent, and one paper disc is given a tetracycline of HCl as a positive control, antibacterial activity was categorised as less than 6 mm was taken as inactive, slightly active (6-7 mm), mild active (7-10 mm), and highly active (>10 mm).[49]

3. Results and Discussion

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

The extraction time has an effect on the yield of sea cucumber obtained using various solvents (methanol, acetone and n-hexane) and time extraction (30, 60, 90 and 120 min.) is shown in Fig.2. Results of the study show that the highest yield of sea cucumber *Muelleria lecanora* extraction uses solvents (methanol, acetone and n-hexane), obtained the highest yield on the extraction using methanol solvent of 11.2% and the lowest of 1.86% for solvent n-hexane. By using ultrasonic-assisted extraction (UAE) method, that the longer the extraction time, then the resulting crude extract is more optimum because the solvent can bind to more extracts. According to Himaya et al., (2010), it confirms that the length of time the extraction process is very influential in the resulting extract. Increasing extraction time from 30 to 120 min using differents solvents significantly increased ($p<0,05$) the yield of sea cucumber. Qin et al., (2018) and D. Chen et al., (2015), reported that the yield of sea cucumber obtained was directly correlated with an increase in extraction time using solvent extraction.

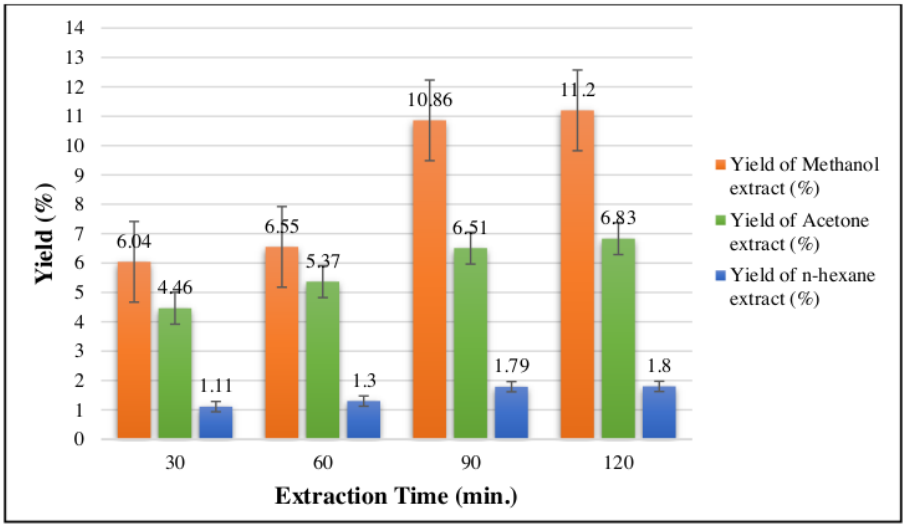

Fig. 2: Effect of different solvents and extraction time to sea cucumber extract yield value

3.2. GC-MS profile bioactive compound of Muelleria lecanora

The availability of a bioactive compound in the methanol acetone and n-hexane extract of *Muelleria lecanora* was characterized by GC-MS analysis (Table 1).

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

Antibacterial Compound	Molecular formula	The methanol extract (% of Area)	Acetone extract (% of Area)	n-hexane extract (% of Area)	Reported bioactivity
9-Hexadecenoic acid, methyl ester, (Z)[53]	$C_{17}H_{32}O_2$	1,54	1,16	-	Antioxidant, Hypocholesterolemic nematicide, Anti-inflammatory, pesticide, anti-androgenic flavour, hemolytic, 5-Alpha reductase inhibitor, potent mosquito larvicide, and antimicrobial activity [54]
Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	17,36	11,42	-	Antimicrobial activty [54]
Tetradecanoic Acid, Methyl Ester	$C_{15}H_{30}O_2$	0,44	5,49	-	Larvicidal and repellent activity [54]
Palmitic Acid	$C_{16}H_{32}O_2$	0,87	-	-	Anti-inflammatory, lubricant, antiandrogenic, nematicide, pesticide, flavour, hemolytic 5-alpha reductase inhibitor, antioxidant[55] and hypocholesterolemic [56]
Stearic acid methyl ester/ 9-Octadecenoic acid (Z) -, methyl ester	$C_{19}H_{36}O_2$	14,42	7,36	10,44	tileukotriene, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge, flavor, anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant[54]
Tetratriacontane	$C_{44}H_{90}$	4,15	1,39	14,96	Antibacterial and antifungal [54]
Pentacosane	$C_{25}H_{52}$	0,32	2,04	14,39	Antitumor, antimicrobial activity, antivirus [54]
EPA/Omega (35,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (ALL-Z))	$C_{21}H_{34}O_2$	2,59	0,5	-	Preventing and mana g heart disease, reduce blood pressure and triglycerides accumulation, slow the development of plaque in the arteries, reduce the chance of abnormal heart rhythm, reduce of heart attack and stroke, antiinflammatory complications after surgery [57]
=-214-.Beta.-H-Pregna7890-	$C_{21}H_{36}$	1,04	-	-	Antibacterial and antifungal effects [58]
2-[(Hexadecyloxy)Methyl]Ox ne	$C_{19}H_{38}O_2$	9,33	2,15	-	Antibacterial activity [59]
Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)	$C_{31}H_{50}O$	5,79	8,86	-	Free radical Scavenging, Anti-diabetic, Anticancer [54]

Cholest-5-EN-3-YL Acetate	$C_{29}H_{48}O_2$	7,64	13,61	-	Antioxidant activity and antimicrobial activity [60]
Ergosta-14,22-Dien-3-OL, Acetate, (3.Beta.,5.Alpha.,22E)-	$C_{30}H_{50}O_2$	4,7	5,89	-	Antibacterial activity [61]
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 [54]
Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	13,34	4,37	-	Antimicrobial activty [54]
Caryophyllene	$C_{15}H_{24}$	-	0,42	-	Anti-inflammatory and Antimicrobial activty [62]
Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.al)	$C_{15}H_{24}$	-	7,53	-	Analgesic, antiasthamatic, anti-inflammatory and antipyretic properties [63]
Heneicosane	$C_{21}H_{44}$	-	-	32,91	Anticancer [64]
Docosane	$C_{22}H_{46}$	-	-	13,44	Anti-inflammatory and anti-therogenic [65]

The GC and MS running time for the methanol extract of *Muelleria lecanora* were 39 min and the spectrum are shown in Fig. 3. GC-MS result analysis includes the active principles with their amount of component, molecular formula and composition inside the methanol extracts of *Muelleria lecanora*. Percentage composition and list of the identified compounds is shown in Table 1. Results showed that many of which were present in trace amounts antioxidants and antimicrobial, a complex mixture of numerous compounds; Hexadecanoic Acid, Methyl Ester (17,36%), 9-Octadecenoic acid (Z) -, methyl ester (stearic acid methyl ester) (14,42%), Octadecanoic acid, methyl ester (13,34%), 2-[(Hexadecyloxy)Methyl]Oxirane (9,34%), and Cholest-5-EN-3-YL Acetate (7,64%), Ergosta-14,22-Dien-3-OL, Acetate, (3.Beta.,5.Alpha.,22E)- (4,7%) , Omega 3/5,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (ALL-Z) EPA (2,59%), 9-Hexadecenoic acid, methyl ester, (Z) (1,54%) performs a crucial role for antioxidant and antibacterial activities. Steroid and flavonoid is the major component in sea cucumber and had good agreement with the results by Silchenko et al., (2008), sea cucumbers are rich in glycosides, particularly triterpene glycosides which are proven to have antifungal and antitumor activities[29]. Moreover, sea cucumbers also have impressive amounts of lectins[67], glycosaminoglycans [68], omega-6 sterols and omega-6 and omega-3 fatty acids (EPA and DHA) and sterols [1,69].

The acetone extract of sea cucumber *Muelleria lecanora* with running time 39 min for GC and MS spectrum is shown in Fig. 4. Chromatogram evaluation of the acetones extract of *Muelleria lecanora* confirmed of sixty major peaks and the components similiar to the peaks were determined[70]. Major component of this extract are as follows Cholest-5-EN-3-YL Acetate (13,61%), Hexadecanoic Acid, Methyl Ester (11,2%), Stigmasta-5,22-dien-3-ol, acetate, (3.beta.) (8,86%)[71], Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.al (7,53%), and 9-

Octadecenoic acid (Z) -, methyl ester (7,36%)[72], which is an antioxidant and antibacterial component. There are Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.al) compounds, which are very good as pharmacological because of use for various herbal formulation exhibiting antipyretic properties, antiasthmatic, anti-inflammatory drugs, and cardiac tonic[63].

GC-MS test has performed showing the presence of forty peaks and components in n-hexane extract of sea cucumber (Fig. 5). Only five main components are important as pharmacological material, namely Heneicosane (32,91%), Tetratriacontane (14,96%), Pentacosane (14,39%), Docosane (13,44%) and stearic acid methyl ester/ 9-Octadecenoic acid (Z) -, methyl ester (10,44%). There are two very interesting compounds to be examined and not found by the other two solvents (methanol and acetone) namely Heneicosane that serves as anticancer and Docosane as anti-inflammatory and anti-atherogenic. Research[64], brown algae *Lobophora variegata* from the Brazilian coastal, results of isolation producing polyunsaturated epoxy-heneicosane compounds that serve as antiproliferative, its better inhibition of the tumour cell lines in comparison to the fibroblast cell line. Similarities with, eicosapentaenoic acid (EPA) or 5,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (ALL-Z) EPA/Omega 3, DHA *and n*-3 polyunsaturated fatty acids (PUFAs) find in sea cucumber *Muellaria lacenora*[73]. Compounds *n*-3 PUFAs may increase tumour cells sensitivity to conventional therapies, these molecules display antitumor activity through induction of apoptosis in human cancer cells alone or combined with conventional chemotherapeutic agents[74–76]. Microdilution method of Heneicosane compounds as antibacterial activity against two types of bacteria *Salmonella* (ATCC 29890, Gram-positive) and *Staphylococcus aureus* (ATCC 6538P, Gram-positive). Bacteria strains *Escherichia coli* (ATCC 10536, Gram-negative), did not give good results [64].

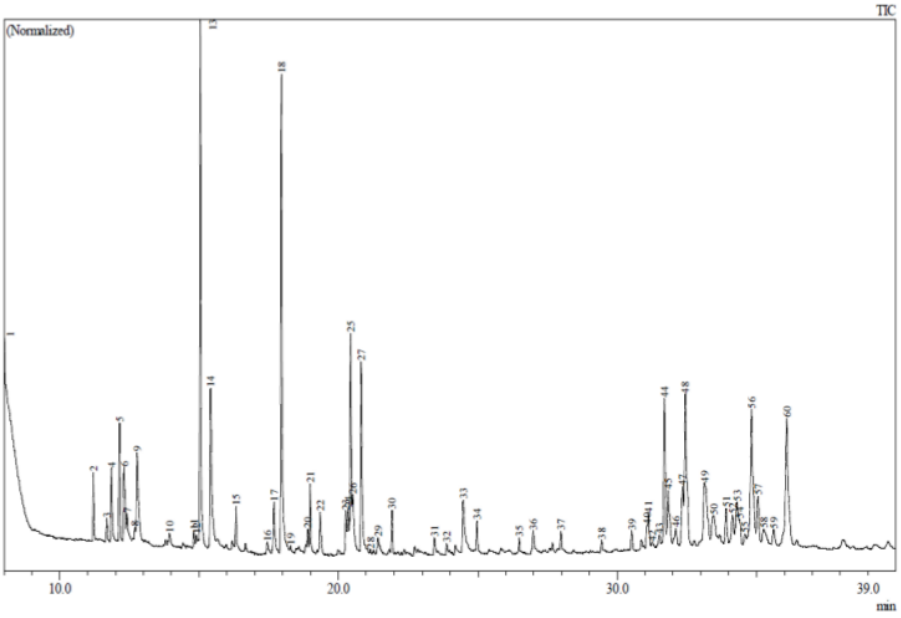

Fig. 3: Profile methanol extract of sea cucumber *Muelleria lecanora* using Gas chromatography-mass spectrometry

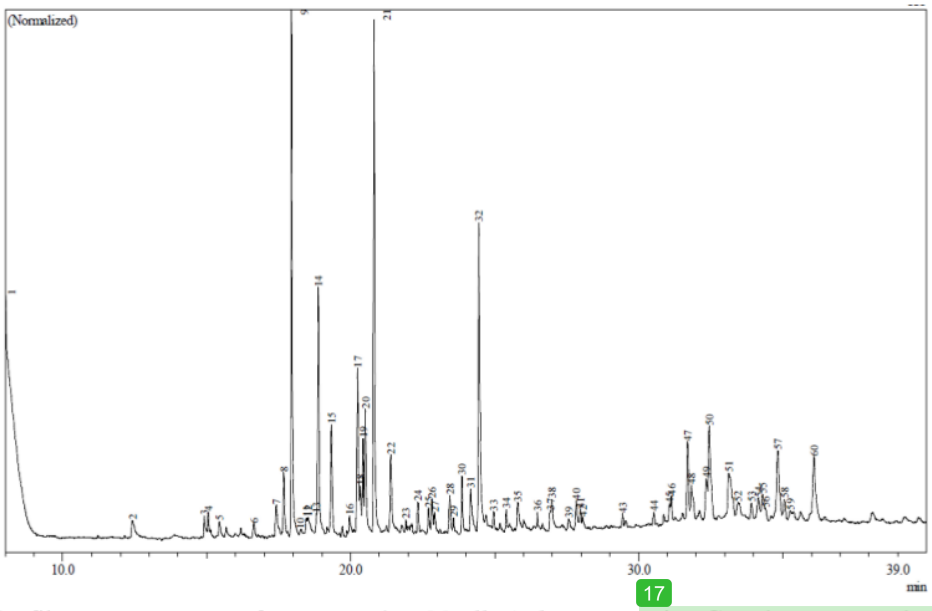

Fig. 4: Profile acetone extract of sea cucumber *Muelleria lecanora* using Gas chromatography-mass spectrometry

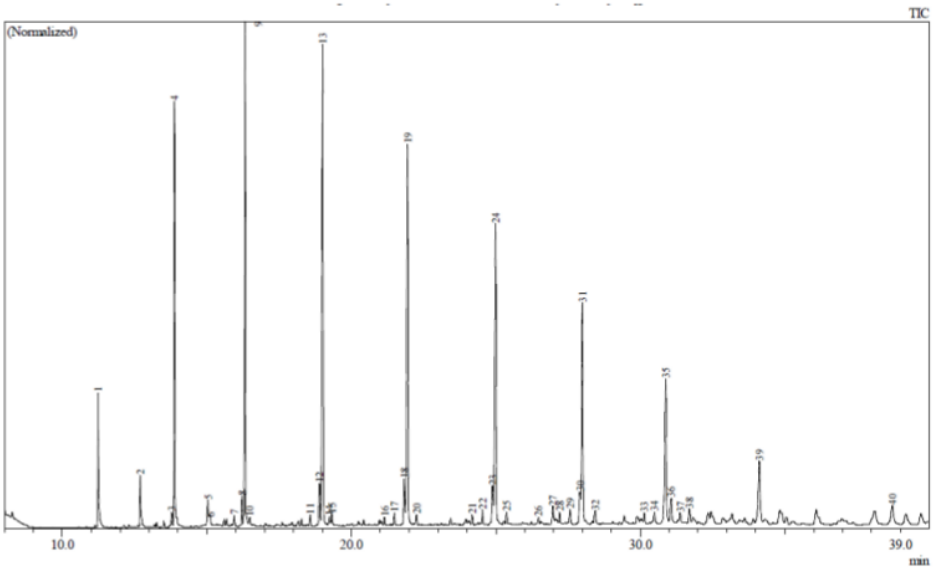

Fig. 5: Profile n-hexane extract of sea cucumber *Muelleria lecanora* using Gas chromatography-mass spectrometry

3.3. Antibacterial activity

Antimicrobial activities of sea cucumber *(Muellaria lecanora)* extracts were evaluated against three bacterial (two gram-positive, one gram-negative) strains as shown in Table 2.

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

Samples	Extraction time (min.)	Concentration	Zone of inhibition (mm)		
			Escherichia coli	***Salmonella***	***Staphylococcus aureus***
Methanol extract	30	20 µL	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 µL	6,09	7,40	7,62
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 µL	6,40	7,01	7,90
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 µL	7,47	6,94	6,37
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

Screening for sensitivity against the three solvents extract methanol, acetone and n-hexane extract of sea cucumber leaves was calculated as 20 µL. Initial screening of *Muelleria lecanora* for antimicrobial activity was carried out via Kirby-Bauer disc diffusion assay on bacterial strains *Escherichia coli* (gram-negative), *Salmonella* (gram-positive), and *Staphylococcus aureus* (gram-positive)[77]. The antibacterial activity was classified as less than 6 mm was taken as inactive, slightly active (6-7 mm), mild active (7-10 mm), and highly active (>10 mm)[49]. All the strains were found to be sensitive with zone of inhibition ≥6 mm and maximum zone obtained for *Staphylococcus aureus* (10,94 mm). Broad-spectrum a minimum zone of 6,09 mm for methanol extract with 60 min extraction against bacteria *Escherichia coli* and acetone extract with 30 min extraction a maximum zone of 10,94 mm against on *Staphylococcus aureus*.

3.3.1. Antibacterial activity against *Escherichia coli*

n-hexane with 30 min extraction, show a broad-spectrum antimicrobial activity with a minimum zone 6,08 mm and maximum zone of 7,40 mm with 120 min methanol extraction against bacteria *Escherichia coli*. All three solvents belong to the antibacterial activity slightly active category with an average inhibitory zone of 6-7 mm (Table 2 and Fig. 6).

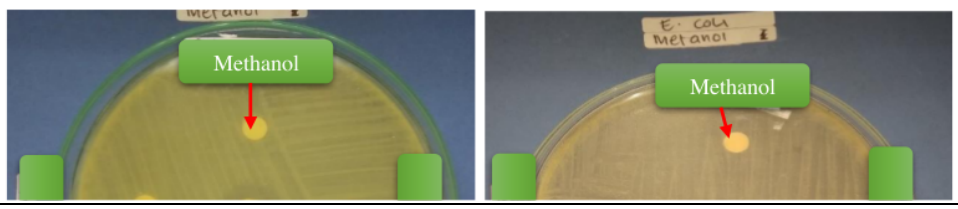

Fig. 6: Antibacterial activity of acetone, methanol and n-hexane extract sea cucumber against *Escherichia coli*: a) 30 min extraction; b) 60 min extraction; c) 90 min extraction, and d) 120 min extraction

3.3.2. Antibacterial activity against *Salmonella*

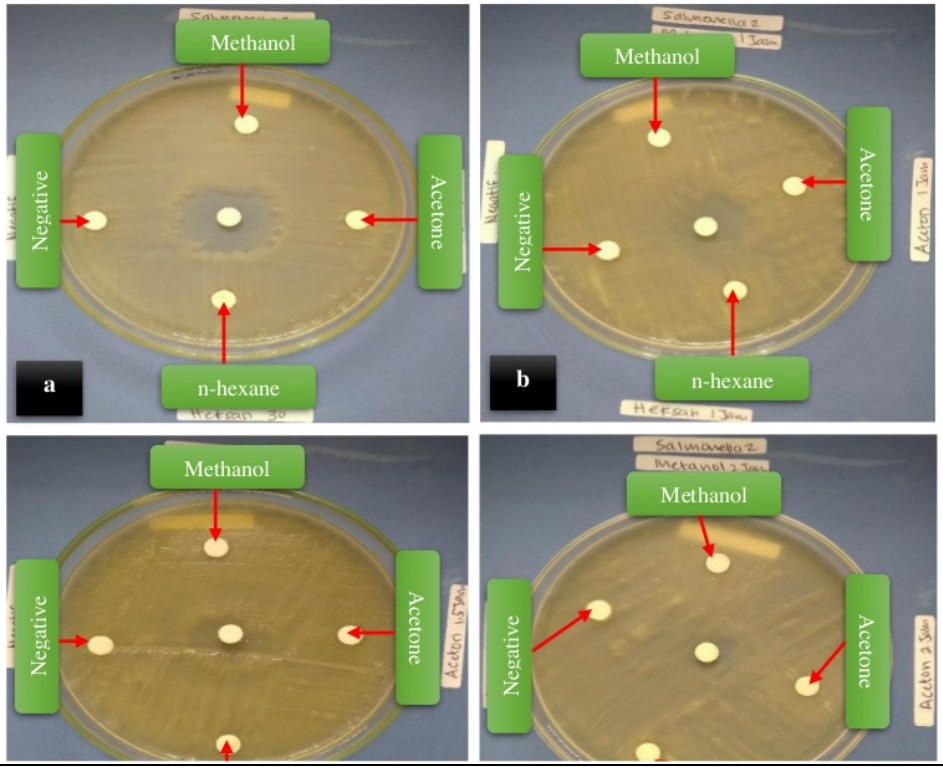

Fig. 7: Antibacterial activity of acetone, methanol and n-hexane extract sea cucumber against *Salmonella*: a) 30 min extraction; b) 60 min extraction; c) 90 min extraction, and d) 120 min extraction

Disc diffusion assay method according to Bauer et al., (1966), determining antimicrobial agent activity, through a disc containing the agent antimicrobial is put on the media so that it has planted microorganisms that will diffuse in the media. Clear areas indicate a growth barrier of microorganisms by antimicrobial agents on the media surface. The advantages of this method are the number of substances used can be arranged. Acetone extract with 90 min extraction has a minimum zone diameter of 6,52 mm and n-hexane with 60 min extraction have a maximum zone diameter of 8,82 mm against on *Salmonella*. These result also showed acetone, methanol and n-hexane extracts as mild active activity against *Salmonella* with a diameter of inhibitory zone 7 – 10 mm (Table 2 and Fig. 7).

3.2.3. Antibacterial activity against *Staphylococcus aureus*

Methanol extract with 120 min extraction has a minimum zone diameter of 6,37 mm and acetone extract with 30 min extraction have a maximum zone diameter of 10,94 mm against on *Staphylococcus aureus*. These result also showed acetone, methanol and n-hexane extracts as highly active activity against *Salmonella* with a diameter of inhibitory zone >10 mm (Table 2 and Fig. 8).

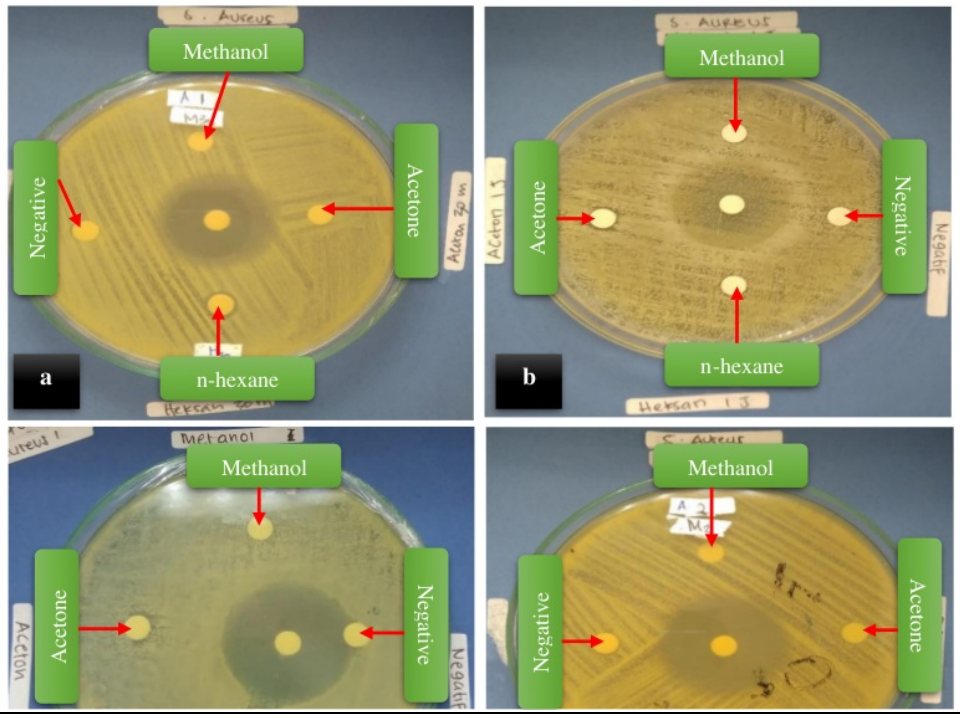

Fig. 8: Antibacterial activity of acetone, methanol and n-hexane extract sea cucumber against *Staphylococcus aureus*: a) 30 min extraction; b) 60 min extraction; c) 90 min extraction, and d) 120 min extraction

The preliminary antibacterial assay of the acetone, methanol and n-hexane extracts of sea cucumber *Muellaria lecanora* showed different responses to the test strains against bacteria gram-positive (*Salmonell*a and *Staphylococcus aureus)*, but not recommended for gram-negative bacteria such as *Escherichia coli*.

4. Conclusion

In summary, acetone, methanol and n-hexane were discovered found to be the excellent solvent for phytochemicals extraction from Sea cucumber *Muelleria lecanora*. The methanol and acetone extract confirmed a most wide variety of bioactive compounds in preliminary phytochemical analysis and a good quantity of antibacterial activity and total phenolics in the antioxidant activity, but n-hexane extract showed a bioactive compound as a new antiproliferative polyunsaturated epoxy-heneicosane its better inhibition of the tumour cell and anticancer. From antibacterial assay results Sea cucumber *Muelleria lecanora* extract was very effective against bacteria gram-positive (*Salmonell*a and *Staphylococcus aureus)*. The bioautography evaluation showed that the whole extract had antibacterial potential and free radical scavenging. GC-MS analysis revealed the presence of the good number of bioactive metabolites such as flavanoid and steroids within the extract. The outcomes of this study implied that Sea cucumber *Muelleria lecanora* have shown higher antibacterial and antioxidant activities which might be used in therapeutic applications and food product (functional food).

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