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FULL PAPER

Phytochemical and antibacterial properties of sea cucumber (*Muelleria lecanora*) from Barrang Lompo Islands, Makassar South Sulawesi

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Abstract

Barrang Lompo Island Waters is home for different species of marine biota of sea cucumber (*Muelleria lecanora*). Many sea cucumber species have been used as health supplements because they contain bioactive compounds that are beneficial to people in Indonesia. Given this, our study was designed to investigate the phytochemical, and antibacterial properties of crude acetone, methanol and hexane extract of sea cucumber using maceration extraction methods. The sea cucumber extract was prepared and the phytochemical profile was studied by analysing Gas Chromatography-Mass Spectrometry (GC-MS). Results showed that the extracts were a complex mixture of numerous compounds; many of which were present in trace amounts antioxidants and antimicrobial; hexadecanoic acid, methyl ester, 9-octadecenoic acid (z) -, methyl ester (stearic acid methyl ester), octadecanoic acid, methyl ester, 2-[(hexadecyloxy)methyl]oxirane, cholest-5-en-3-yl acetate, ergosta-14,22-dien-3-ol, acetate,(3.beta.,5.alpha.,22e), 5,8,11,14-eicosatetraenoic acid, methyl ester, (all-z) epa/omega 3, pentacosane, hexatriacontane, and 9-hexadecenoic acid, methyl ester, (Z). The extract was also evaluated for activity against three pathogenic bacterial strains (*Escherichia coli, Staphylococcus aureus* and *Salmonella*) using the disc diffusion method. The extract exhibited clear zones of inhibition against the tested bacteria. Maximum inhibitory zone concentration values were demonstrated to be: *Escherichia coli = 6.84 mm* , *Staphylococcus aureus* = 7.22 mm, and *Salmonella* = 7.87 mm. These results revealed the significant potential of sea cucumber as a source of antioxidants and antimicrobial agents and also highlight the necessity of further purification and characterisation of solitary bioactive compounds for their prospective applications in pharmaceutical industries, food, and nutraceutical (food functional).

1. Introduction

The increasing number of scientific research and journals published in recent decades has to do with functional materials derived from vegetable and animal natural resources, potentially as food functional, nutraceuticals and health supplement products that support nutritional value needs and improve body health. Among other marine animals, sea cucumber is a source of natural ingredients that contain functional ingredients and bioactive compound that can be used in biomedicine and food processing industries. Sea cucumber is a marine invertebrate of the *Holothuroidea* class, has rough skin on its outer and elongated body and contains a single branched gonad. This marine animal has a wide number of species, ranging from 1716 species, with the largest potential of biological diversity in the Asia-Pacific region. In Indonesia, it is known as "Teripang or Trepang", in France under the name "Beche-De-Mer", which means seafood products, and "Balate" in the islands of Guam or Chomorro. Sea cucumber includes living marine animals with complex environments, making it difficult to live in extreme environments, therefore the sea cucumber can produce biologically active secondary metabolites that can not be found and obtained from other marine animals (Pangestuti and Arifin, 2018).

The sea cucumber has a complete nutritional content, low-fat content, high protein, and rich essential amino

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acids, such as tryptophan, arginine, and lysine, as well as having a body wall consisting of non-soluble collagen which can be used as a nutritional supplement (Chen, 2004). Sea cucumber has been recognized as a traditional remedy for treating asthma, rheumatism, hypertension, impotence, constipation and burns (Wen *et al*., 2010). Other functions, among others, anti-coagulant (Nagase *et al*., 1995; Chen *et al*., 2011), anticancer (Janakiram *et al*., 2015), anti-inflammatory (Olivera-Castillo *et al*., 2018), antithrombotic (Mourão *et al.*, 1998; Pacheco *et al*., 2000), antioxidant (Althunibat *et al*., 2009), antimicrobial (Beauregard *et al*., 2001; Hing *et al*., 2007), antihypertensive (Zhao *et al*., 2007), anti-angiogenic (Roginsky *et al*., 2004; Tian *et al*., 2005), antitumor (Zou *et al*., 2003; Tong *et al*., 2005), and healing wound (San Miguel-Ruiz and García-Arrarás, 2007), due to the role of bioactive compounds present in the sea cucumber mainly the triterpene glycosides (saponin) (Miyamoto *et al*., 1990; Kerr and Chen, 1995; Aminin *et al*., 2010), phenolics (Mamelona *et al*., 2007), lectins (Mojica and Merca, 2004, 2005), sterols (glycosides and sulfate) (Han *et al*., 2009), peptides (Zhou *et al*., 2012), glycosaminoglycan (Kariya *et al*., 1990; Borsig *et al*., 2007), chondroitin sulfate (Ustyuzhanina *et al*., 2016), cerebrosides (Sugawara *et al*., 2006), and sulfate polysaccharides (Luo *et al*., 2013). 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 a 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 (Li *et al*., 2008).

Nguyen *et al*. (2011) reported about potent α-Glucosidase inhibitory activity purified from the body wall of Sea Cucumber (*Stichopus japonicus*). The result shows that sea cucumber fatty acids can potentially be developed as a novel natural nutraceutical for the management of type - 2 diabetes. Other research, indicating that sea cucumber *Apostichopus japonicus, analysis in vitro s*how inhibitory zones on microbes strains *Vibrio splendidus, Vibrio harveyi* and *Staphylococcus aureus* (Jiang *et al.,* 2014). The antibacterial activity of sea cucumber *Actinopyga lecanora* against some common pathogenic Gram-positive bacteria *(Bacillus subtilis and Staphylococcus aureus*) and Gram-negative bacteria *(Escherichia coli, Pseudomonas aeruginosa, and Pseudomonas* spp.), was evaluated. It was indicated that sea cucumber extract was able to inhibit *Staphylococcus aureus* (Ghanbari *et al.,* 2012). Five curvularin macrolides in isolation from sea cucumber *Holothuria moebii,* results in 11-hydroxycurvularins ((11*S*,15*R*)-11-hydroxycurvularin) and ((11*R*,15*R*)-11-hydroxycurvularin) also showed antibacterial activity inhibiting the growth of *Escherichia coli* (Ye *et al*., 2016). Previous studies on antibacterial activity of sea cucumber extracts have been reported against several pathogenic bacteria such as *Escherichia coli, Salmonella, Listeria,* and *Staphylococcus aureus*. Previous literature showed that sea cucumber is extensively studied for antioxidant and antimicrobial activities.

In this study, sea cucumber is used for investigating antioxidant and antimicrobial potential using maceration extraction with three solvents (acetone, hexane and methanol). The present work aimed to investigate the efficiency of different solvents for the phytochemical extraction from sea cucumber and to identify and analyze the bioactive compounds by GC-MS (Gas Chromatography-Mass Spectrometry). The antibacterial efficacy against pathogenic bacteria *Escherichia coli, Salmonella* and *Staphylococcus aureus* using disc diffusion methods was also investigated.

2. Materials and methods

2.1 Materials

Sea cucumber phylum *Echinodermata*, family *Holothuriidae* and genus *Muelleria lecanora* (Figure 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.

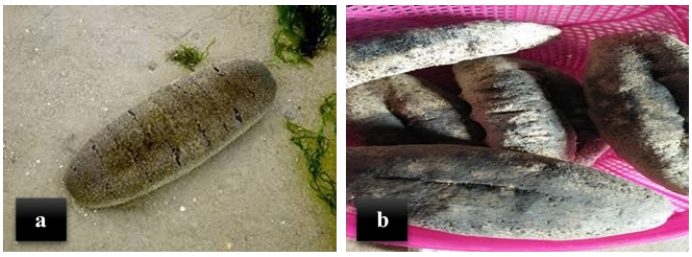

Figure 1. Sea cucumber (*Muellaria lecanora*)*:* a) freshwater and b) dried.

All chemicals were of analytical grade, hexane (CAS: 110-54-3), methanol (CAS: 67-56-1), acetone (CAS: 67-64-1), nutrient agar (CAS:105450), aquadestilata (CAS: 7732-18-5), plate count agar (CAS: 105463), McFarland Standard (barium chloride and sulfuric acid), sodium chloride (CAS: 7647-14-5), 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 *Staphylococcus aureus, Salmonella* and *Escherichia coli.*

2.2 Preparation of extracts

The sea cucumbers were cleaned and dried in an oven at 70°C, then cropped small-minced. About 100 g of the sea cucumbers were homogenized and extracted using the maceration method with a ratio of volume 1:2 (V/V) methanol, acetone or n-hexane. Samples were allowed for 72 hrs with solvent replacement every 24 hrs and were stirring with orbital shakers. The resulting extraction (macerate) is then filtered and concentrated with a rotary evaporator at 40°C until the extract is obtained. The Supernatant is produced for each sample, then in centrifugation for 10 mins and stored at a temperature of 10°C, for use in analysis bioactive compound in GC-MS and antibacterial disc diffusion method.

2.3 Gas Chromatography-Mass Spectrometry(GC-MS)

Sea cucumber was analyzed using GC-MS using capillary column DB-5 (30 µm, 0.25 mm, 0.25 µm film) and Flame Ionization Detector (FID) operated in EI mode at 70 eV. 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 220°C and 250°C. A sample was dissolved with 1 µl methanol, then injected and analyzed at 60°C for 2 minutes and then increased 3°C/min to 300°C, with Helium (He) is used as carrier gas (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 *Salmonella, Staphylococcus aureus* and *Escherichia coli* used in this study were obtained from the Microbiology Laboratory, Department of Biology, State University of Makassar. The bacteria was grown at 32^{o}C in nutrient broth (DIFCO Laboratories, Detroit, USA) following standard procedures (Keagle and Gersen, 2005).

2.5 Antimicrobial assay

Disc antibiotic blank (Whatman No. 1) was cut to size and sterilized with other equipment using autoclaved at 121^{o}C for 15 mins. Growth media microorganisms are 5 g nutrient agar (NA), dissolved 250 mL of aquadest in the Erlenmeyer 500 mL, then heated to homogeneous. The sodium chloride solvent was 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 sodium chloride solvent was sterilized using autoclave at temperature 121^{o}C for 15 mins.

Sterile nutrient agar 20 mL was poured in Petri dishes, allowed to set at 37°C and then inoculate uniformly with 0.1 mL of a 24 hr broth culture of test bacteria (Abubakar *et al.*, 2012). 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 aseptic condition, each sterile disc (Whatman no. 5, 6 mm dia) 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 mins, 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. An experiment was carried out in duplicate and the averages diameter of zone of 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 classified as highly active (>10 mm), mild active (7-10 mm) and slightly active (6-7 mm) and less than 6 mm was taken as inactive (Chandra *et al.,* 2011).

3. Results and discussion

3.1. Yield analysis extract of methanol, acetone and hexane

The effect of extraction time on the yield of sea cucumber obtained using various solvents (methanol, acetone and hexane) is shown in Figure 2. The solvent used is the polar methanol, acetone is semi-polar and the hexane is non-polar. The highest yield of 18.96% by using a methanol solvent, while the lowest yield of 0.14% using hexane solvent was obtained. Acetone solvent obtained a yield of 8.19%. This suggests that the ability of methanol solvent in extracting a compound is very good compared to other solvents.

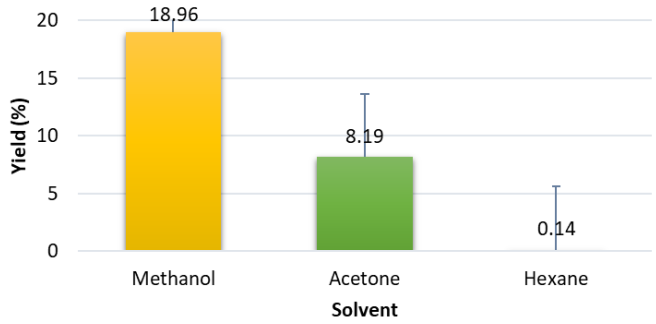

Figure 2. Effect of different solvents to sea cucumber extract yield value.

3.2 GC-MS analysis

The presence of a bioactive compound in the methanol, acetone, and hexane extract of sea cucumber was characterized by GC-MS analysis (Table 1).

The spectrum of GC -MS for acetone, hexane and methanol extract of sea cucumber for 39 mins are shown in Figure 3, 4 and 5. GC-MS result analysis includes the active principles with their, molecular formula, amount of component and composition in the methanol extracts of sea cucumber. List of the identified compounds in the extract and percentage composition is shown in Table 1. Results showed that the extracts were a complex mixture of numerous compounds; many of which were present in trace amounts antioxidants and antimicrobial. Cholest-5-EN-3-YL Acetate (12,6%), Hexadecanoic Acid, Methyl Ester (10.32%), Octadecanoic acid, methyl ester (8,49%), Ergosta-14,22-Dien-3-OL,Acetate, (3.Beta.,5.Alpha.,22E)- (8.18%), Stigmast-5-EN-3-OL, (3.Beta.,24S)-/gamma.-Sitosterol (7.56%), 9-Hexadecenoic acid, methyl ester, (Z) (5.19%), 9-Octadecenoic acid (Z) -, methyl ester (stearic acid methyl ester) (4.11%), 2-[(Hexadecyloxy)Methyl] Oxirane (3.36%%), and,5,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (ALL-Z) EPA/Omega 3 (2.37%), plays a vital role for antibacterial and antioxidant 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 (Han *et al.,* 2009). Moreover, sea cucumbers also have impressive amounts of lectins (Gowda *et al.,* 2008), glycosaminoglycans (Wu *et al*., 2010), omega-6 sterols and omega-6 and omega-3 fatty acids (EPA and DHA) and sterols (Fredalina *et al.*, 1999; Zhong *et al.*, 2007). Two compounds were not obtained from the extraction of acetone solvent and hexane which was Lanost-8-en-3-ol, (3.beta.) and Farnesene epoxide. Lanost-8-en-3-ol, (3.beta.) or Dihydrolanosterol is compounds contained in plant extract of Dangyuja *(Citrus grandis Leaves)*, which serves as an anticancer. Works well in treating human gastric cancer which malignant (cancer) cells form in the lining of the stomach (Moon *et al.*, 2009). Farnesene epoxide is a compound contained in the extracts of plant *Artimisia Herbs-Alba* which is widely acquired in the Mediterranean in North Africa, Western Asia and Southwest Europe. People in the region use this plant as an antiseptic and antispasmodic in herbal medicine. In research (Tilaoui *et al.*, 2011), that the compound content of Farnesene epoxide serves as an antiproliferative activity is the ability of a compound to stop the growth of cells and not allowing the cells to multiply rapidly.

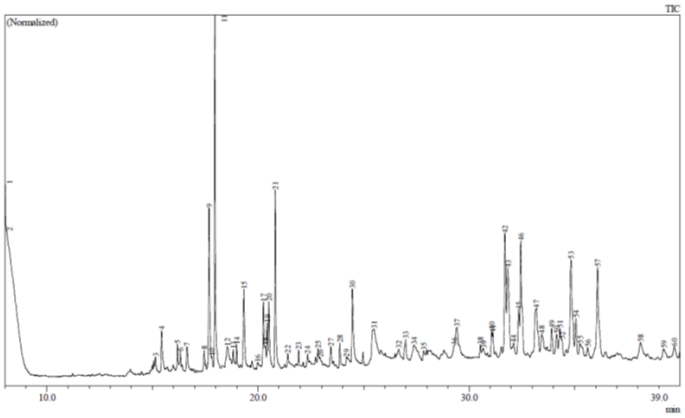

Figure 3. Gas chromatography-mass spectrometry profile for the methanol extract of sea cucumber

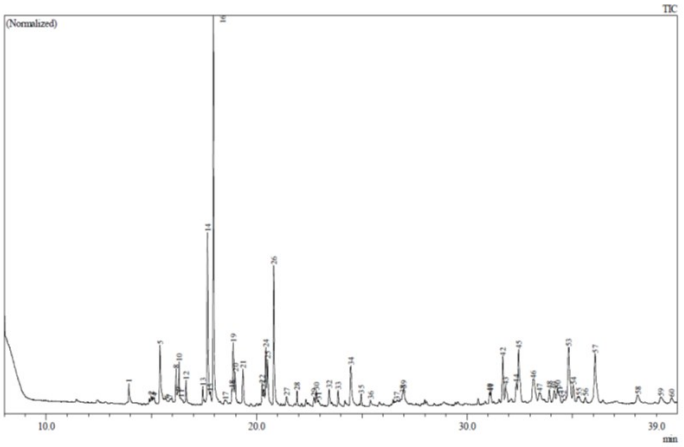

Figure 4. Gas chromatography-mass spectrometry profile for the acetone extract of sea cucumber

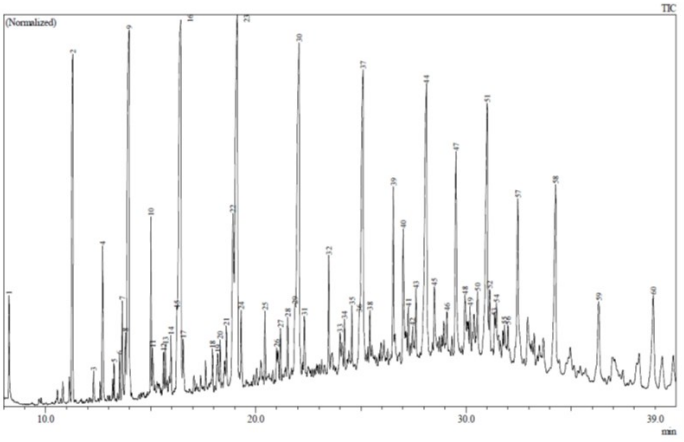

Figure 5. Gas chromatography-mass spectrometry profile for the hexane extract of sea cucumber

Table 1. GC-MS analysis for the methanol, acetone and hexane extract of sea cucumber

Antibacterial Compound	Molecular formula	Methanol extract (% of Area)	Acetone extract (% of Area)	Hexane extract (% of Area)	Reported bioactivity
9-Hexadecenoic acid, methyl ester, (Z)	$C_{17}H_{32}O_2$	5.19	8.3	-	Anti-inflammatory, Antioxidant, Hypocholesterolemic nematicide, pesticide, anti-androgenic flavour, hemolytic, 5-Alpha reductase inhibitor, potent mosquito larvicide, and antimicrobial activity (Abubakar and Majinda, 2016)
Hexadecanoic Acid, Methyl Ester	$C_{17}H_{34}O_2$	10.32	19.29	3.08	Antimicrobial activity (Abubakar and Majinda, 2016)
Tetradecanoic Acid, Methyl Ester	$C_{15}H_{30}O_2$	1.75	4.41	1.45	Larvicidal and repellent activity (Abubakar and Majinda, 2016)
Palmitic Acid	$C_{16}H_{32}O_2$	1.58	-	-	Anti-inflammatory, nematicide, pesticide, lubricant, antiandrogenic, flavour, hemolytic 5-alpha reductase inhibitor, antioxidant, hypocholesterolemic (Anwar *et al.,* 2007)
9-Octadecenoic acid (Z) -, methyl ester (stearic acid methyl ester)	$C_{19}H_{36}O_2$	4.11	7.51	-	Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, antileukotriene—D4, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge, flavor (Abubakar and Majinda, 2016)
Tetratriacontane	$C_{44}H_{90}$	0.95	-	29.95	Antibacterial and antifungal (M. Abubakar and Majinda, 2016)
Pentacosane	$C_{25}H_{52}$	0.32	0.92	3.52	Antitumor, antimicrobial activity, antivirus (Abubakar and Majinda, 2016)
5,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (ALL-Z) EPA/Omega 3	$C_{21}H_{34}O_2$	2.37	3.96	1.62	Preventing and managing heart disease, lower blood pressure, reduce triglycerides accumulation, slow the development of plaque in the arteries, reduce the chance of abnormal heart rhythm, reduce the likelihood of heart attack and stroke, antiinflammatory complications after surgery (Yi *et al.,* 2014)
=-214-.Beta.-H-Pregna7890-	$C_{21}H_{36}$	0.69	1.49	-	Antibacterial and antifungal effects (Dehpour *et al.,* 2012)
2-[(Hexadecyloxy)Methyl] Oxirane	$C_{19}H_{38}O_2$	3.36	0.73	-	Antibacterial activity (Henry Wright *et al.,* 2016)
Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)	$C_{31}H_{50}O$	8.18	7.29	-	Free radical Scavenging, Anti-diabetic, Anticancer (Abubakar and Majinda, 2016)
Cholest-5-EN-3-YL Acetate	$C_{29}H_{48}O_2$	12.06	10.82	-	Antioxidant activity and antimicrobial activity (Singh *et al.,* 2020)
Ergosta-14,22-Dien-3-OL, Acetate, (3.Beta.,5.Alpha.,22E)-	$C_{30}H_{50}O_2$	8.18	5.46	-	Antibacterial activity (Putra and Hadi, 2017)
Stigmast-5-EN-3-OL, (3.Beta.,24S)- / gamma.-Sitosterol	$C_{29}H_{50}O$	7.56	5.5	-	Thyroid inhibitory, antiperoxidative and hypoglycemic effects (Abubakar and Majinda, 2016)

Table 1 (Cont.). GC-MS analysis for the methanol, acetone and hexane extract of sea cucumber

Antibacterial Compound	Molecular formula	Methanol extract (% of Area)	Acetone extract (% of Area)	Hexane extract (% of Area)	Reported bioactivity
Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	8.49	6.64	27.03	Antimicrobial activity (Abubakar and Majinda, 2016)
Caryophyllene	$C_{15}H_{24}$	-	-	-	Anti-inflammatory and Antimicrobial activity (Manjamalai *et al.,* 2012)
Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.al)	$C_{15}H_{24}$	-	-	-	Analgesic, antiasthamatic, anti-inflammatory and antipyretic properties (Hameed *et al.,* 2015)
Heneicosane	$C_{21}H_{44}$	0.48	0.31	3.3	Anticancer (Ávila *et al.,* 2019)
Docosane	$C_{22}H_{46}$	0.34	1.25	0.8	Anti-inflammatory and anti-atherogenic (Uraku, 2016)
Lanost-8-en-3-ol, (3.beta.)	$C_{30}H_{52}O$	1.47	-	-	Anticancer (Moon *et al.,* 2009)
Farnesene epoxide	$C_{15}H_{24}O$	0.35	-	-	Antiproliferative activity (Tilaoui *et al.,* 2011)

Chromatogram GC-MS analysis of the acetones extract of sea cucumber showed the presence of sixty major peaks and the components corresponding to the peaks were determined. Major component of this extract are as follows Hexadecanoic Acid, Methyl Ester (19.29%), Cholest-5-EN-3-YL Acetate (10.82%), 9-Hexadecenoic acid, methyl ester, (Z) (8.3%), 9-Octadecenoic acid (Z) -, methyl ester (stearic acid methyl ester) (7.51%), Stigmasta-5,22-dien-3-ol, acetate, (3.beta.) (7.29%), Octadecanoic acid, methyl ester (6.64%), and Ergosta-14,22-Dien-3-OL, Acetate, (3.Beta.,5.Alpha.,22E)-(5.46%), which is an antioxidant and antibacterial component.

GC-MS test performed showed the presence of sixty peaks and components in the hexane extract of sea cucumber. Only eight main components are important as pharmacological material, namely Tetratriacontane (29.95%), Octadecanoic acid, methyl ester (27.03%), Pentacosane (3.52%), Heneicosane (3.3%), Hexadecanoic Acid, Methyl Ester (3.08%), Tetradecanoic Acid, Methyl Ester (1.45%), 5,8,11,14-Eicosatetraenoic Acid, Methyl Ester, (ALL-Z) EPA/ Omega 3 (1.62%) and Docosane (0.80%). 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 Ávila *et a*l. (2019) find new antiproliferative polyunsaturated epoxy-heneicosane in isolated from the brown alga *Lobophora variegata* from the Brazilian coastal. Heneicosane is an 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 find in sea cucumber *Muellaria lacenora*, DHA *and n*-3 polyunsaturated fatty acids (PUFAs) (Abedi and Sahari, 2014). These molecules display antitumor activity through induction of apoptosis in human cancer cells alone or combined with conventional chemotherapeutic agents, for example, *n*-3 PUFAs may increase tumour cells sensitivity to conventional therapies (Lee *et al.*, 2008; Murray *et al*., 2015; Zhou *et al*., 2017). Microdilution method of Heneicosane compounds as antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* did not give good results (Ávila *et al.*, 2019).

3.3 Antibacterial activity

Antibacterial is a compound that can suppress growth and development of bacterial growth by using bioactive compounds found in sea cucumber, which are generally flavonoids and steroids. Disc diffusion method was followed to determine the antimicrobial 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 and Figure 6.

The concentration of the acetone, hexane and methanol extract of sea cucumber leaves was calculated as 20 µL, were screened for sensitivity against the three solvents extract. Initial screening of sea cucumber for antibacterial activity on bacterial strains *Escherichia coli Salmonella* and *Staphylococcus aureus* was carried out

Table 2. Effect of acetone, hexane and methanol extract of sea cucumber against different bacteria pathogens

Samples	Maceration Extraction time (hrs)	Concentration	Zone of inhibition (mm)		
			Escherichia coli	*Salmonella*	*Staphylococcus aureus*
Methanol extract	72	20 µL	6.2	7.34	7.02
Acetone extract			6.22	7.26	6.88
Hexane extract			6.84	7.87	7.22
Control positive			12.67	25.33	21.87
Control negative			0	0	0

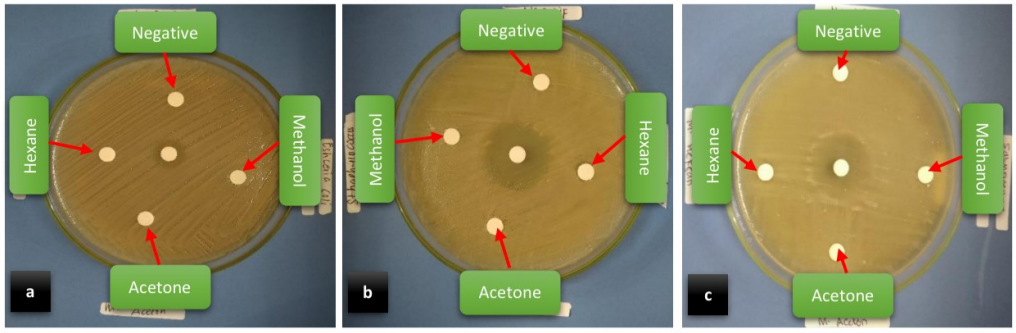

Figure 6. Antibacterial activity against a) *Escherichia coli,* b) *Staphylococcus aureus*, and c) *Salmonella*

via Kirby-Bauer disc diffusion assay. The antibacterial activity was classified as highly active (>10 mm), mild active (7-10 mm) and slightly active (6-7 mm) and less than 6 mm was taken as inactive (Chandra *et al*., 2011).

An antibacterial activity can be known by the formation of a bright zone around the disc paper and the bright zone is the inhibitory zone. Methanol extraction exhibited a broad-spectrum antibacterial activity with a minimum zone diameter of 6.20 mm against bacteria *Escherichia coli* and hexane extraction a maximum zone diameter of 7.87 mm against bacteria *Salmonella*. Methanol extraction belongs to the antibacterial activity slightly active category with an average inhibitory zone of 6-7 mm bacteria *Escherichia coli* and mild active category with an average inhibitory zone of 7-10 mm against bacteria *Salmonella* and *Staphylococcus aureus* (Figure 6). The activity of flavonoids inhibits the growth of bacteria by damaging the cell membrane, thereby inhibiting the synthesis of bacterial cell macromolecules (Dzoyem *et al.,* 2013). The mechanism of the steroid works as an antibacterial by damaging the lipid membrane, so that liposomes leak. Steroids are also known to be interacting with membrane phospholipids, since their permeable nature to lipolytic compounds leads to decreased membrane integrity and morphology of impaired cells membranes, resulting in lysis and fragile cells (Madduluri *et al*., 2013). The preliminary antibacterial assay of the extracts showed different responses to the test strains with the best activity observed for acetone, methanol and n-hexane extracts of sea cucumber 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 and methanol were found to be the best solvent for phytochemicals extraction from Sea cucumber. The methanol and hexane extract showed a maximum number of bioactive compounds in preliminary phytochemical analysis and a good amount of antibacterial activity and total phenolics in the antioxidant activity. Special for methanol extract showed a bioactive compound as an anticancer and antiproliferative activity Lanost-8-en-3-ol, (3.beta.) and Farnesene epoxide. Both of these compounds work well in treating human gastric cancer which malignant cells and ability to stop the growth of cells and not allowing the cells to multiply rapidly. Antibacterial assay results from sea cucumber extract were very effective against bacteria gram-positive (*Salmonell*a and *Staphylococcus aureus*). The bioautography analysis showed that the whole extract had free radical scavenging and antibacterial potential. GC-MS analysis revealed the presence of a good number of bioactive metabolites such as flavanoid and steroids in the extract. The results of this study implied that sea cucumber genus *Muelleria lecanora* have shown better antibacterial and antioxidant activities which could be used in the food and therapeutic applications.

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