

In vitro antibacterial activity and potential applications in food of sea urchin (*Diadema setosum*) from Cape of Palette, South Sulawesi

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***In vitro* antibacterial activity and potential applications in food of sea urchin (*Diadema setosum*) from Cape of Palette, South Sulawesi**

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Abstract

Marine invertebrates in support of his life's defence of sea predatory use an innate immune mechanism, namely the cellular component hemocytes by secreting the dissolved antimicrobial and cytotoxic substances. It shows that marine invertebrates are potential sources and promising antimicrobial compounds. This research was aimed at determining the antibacterial activity of sea urchin (*Diadema setosum*) extract against some bacterial isolates and its application to foodstuffs. The gonad and shell of sea urchin extracted by methanol and ethyl acetate and then separated by ultrasound-assisted extraction. Screening of antibacterial compound use gas chromatography-mass spectrometry (GC-MS), and disc diffusion method was followed to determine the antimicrobial activity against *Salmonella*, *Escherichia coli* and *Staphylococcus aureus*. The results of this study showed antibacterial activity against one or more strains. The gonad of sea urchin from methanol extract exhibited significant inhibitory effect and effective against *Salmonella*, *E. coli* and *S. aureus*. Majority of gonad and shell of sea urchin extract showed antibacterial activity against the tested strains. However, gonad of methanol extract was found to be inhibiting microorganism gram-negative (*E. coli*) and gram-positive (*Salmonella* and *S. aureus*). Gonad extract can be a good source of antibacterial agents.

1. Introduction

Sea urchin (Phylum Echinodermata, Class Echinoid) is a commodity traded in many countries, has a hard shell and the inside is five symmetrical. Certain types of Sea urchin have shells coated with black pigments. In addition to having a hard shell, 95% of the body parts of the pig are dominated by very fragile and toxic thorns. These thorns are used to move, protect themselves, and stimulate food, and for certain types contain toxins. The toxins produced by sea urchin can be utilized in the field of treatment that is potentially used as antibiotics new types to be developed in the pharmaceutical field because it contains bioactive compounds (Abubakar *et al.*, 2012). The sea urchin has an innate immune system that is potentially an effective new antimicrobial. 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). The increasing number of studies showing that bacteria can be resistant to conventional antibiotics has encouraged

researchers to find new antimicrobial agents derived from natural substances (vegetable and animal). As invertebrates, sea urchin does not have an adaptive immune system as it belongs to the type of vertebrates (Rast *et al.*, 2006; Smith *et al.*, 2006). The ability to defend itself in the form of resistance to infection attacks from attacking microorganisms, phagocytosis ability (the role of white blood cells in the immune system) and encapsulation (Hildemann and Dix, 1972; Coffaro and Hinegardner, 1977; Ito *et al.*, 1992; Silva, 2000). In recent decades, there has been an increase in marine field research (crustaceans, molluscs and echinoderms) with an object of secondary metabolite content and type of antimicrobial (Haug *et al.*, 2002; Casas *et al.*, 2011).

Research from Rinehart *et al.* (1981), Bryan *et al.* (1997), Haug *et al.* (2002) and Li *et al.* (2008), exposing antimicrobial activity in several species of echinoderms acquired from the Caribbean, Mexico, the Gulf of California, and the Norwegian coast. Studied Prokof'eva *et al.* (2003) obtained antimicrobial compounds such as steroidal glycoside, polyhydroxylated sterols (Zhou *et*

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al., 2014), naphthoquinone pigments (Amarowicz et al., 2012; Vell et al., 2014; Yoon et al., 2019), lysozymes (Cong et al., 2009), and antimicrobial peptides (Li et al., 2010) are in isolation from animal echinoderms. This research suggests that marine animals are potentially developed as potential sources as antibiotics in the pharmaceutical field. Other studies reported by (Haug et al., 2002), showed that antibacterial activity was found in different parts of green sea urchin using test bacteria *Vibrio anguillarum* serotype O2 (FT 1801), *Escherichia coli* (ATCC 15922) and *Staphylococcus aureus* (ATCC 9144) and *Corynebacterium glutamicum* (ATCC 13032). These results indicate that the phylum echinoderms one of them has potential as a natural antimicrobial (Uma and Parvathavarthini, 2010).

Foodborne microbial pathogens contaminating food have been a human concern for a long time. Human attempts to control microbial food contamination have thus also been a long journey; from old-school methods such as heat-processing and cold-processing to chemically induced antimicrobial agents. Consumer awareness, concerns about the potential risk of synthetic food additives to human health, as well as urgent concerns about the freshness of food, have led to renewed interest in exploring more organic and natural methods of food preservation (Si et al., 2006; Rahardiyan, 2019). Research antimicrobial activity on sea urchin has been carried out, from all organs and tissues (gonads and shell). But testing antimicrobial activity by using extracts derived from ultrasonic-assisted extraction (UAE) has not been conducted by other researchers. Potential sea urchin as an antibacterial need to be developed because it can be utilized as a medicinal ingredient in the field of pharmacy. The study is aimed to obtain the swine fleece extract using the ultrasonic-assisted extraction methods, determining antibacterial activity using disc diffusion methods, and bioactive antibacterial components using gas chromatography-mass spectrometry (GC-MS). The research is focused on screening and comparing antimicrobial and hemolytic activity in various organs/tissues of sea urchin *Diadema setosum* (Echinoidea) obtained from Cape of Palette, Bone Regency, South Sulawesi.

2. Materials and methods

2.1 Materials

Sea urchin (*Diadema setosum*) collected from the Cape of Palette, Bone Regency, South Sulawesi, Indonesia. The gonads separated from the sea urchin shell (Figure 1), then washed to remove other components and taken to the laboratory by carrying in the coolbox and stored in the freezer (-10°C) until the

gonads and shell sea urchin processed in Food Science and Instrumental Analysis Laboratory, Chemical Engineering Department, Politeknik Negeri Ujung Pandang, Indonesia.

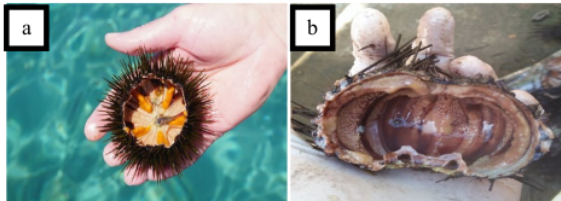


Figure 1. Sea urchin *Diadema setosum*: a) gonad and b) shell

All chemicals were analytical grade, ethyl acetate (CAS: 141-78-6), methanol (CAS: 67-56-1), aquadestilata (CAS: 7732-18-5), nutrient agar (CAS:105450), plate count agar (CAS: 105463), EMB agar (CAS: 101347), 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), beef and fish meat from carrefour, culture strains *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus*. Tools used are 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 Preparation of extracts

Samples (200 g) gonads and shell of sea urchin were homogenized and extracted using Ultrasonic assisted extraction method with a ratio of Volume 1:1.5 (w/v) methanol or ethyl acetate for 30 and 60 mins, then in the rotary evaporator at 39°C temperature, followed by a shaker at a temperature of 10°C for 24 hrs. The Supernatant is produced for each sample, then in centrifugation for 10 mins and stored at a temperature of 10°C, for use in testing the antibacterial disc diffusion method.

2.3 Gas chromatography-mass spectrometry (GC-MS)

Gonads and Shell extract of sea urchin were 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. The injectors and detector temperature are set at 220 and 250°C. About 1 mL extract of sea urchin sample was dissolved in 3 mL methanol 96%, then injected and analyzed at 60°C for 2 mins and then increased 3°C/min to 300°C, with Helium (He) is used as carrier gas (1 mL/

min). The relative number of each component is expressed as a percentage peak area relative to the total peak area. Qualitative identification of different constituents is done by comparison of the relative retention time and the mass spectrum with authentic reference compounds, or by the retention index (RIS) and the mass spectrum.

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) in accordance with standard procedures (Keagle and Gersen, 2005).

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 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 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. Media nutrient agar, chloride sodium solvent and Mc Farland solvent in sterilization using autoclaved at temperature 121°C for 15 min.

Bacteria *Salmonella* sp., *Escherichia coli*, and *Staphylococcus aureus*, as much as 20 mL of sterile nutrient agar was poured in Petri dishes, allowed to set at 37°C and then inoculate uniformly with 0.1 mL of a 24 hrs broth culture of test bacteria (Abubakar *et al.*, 2012; Syahirah and Rabeta, 2019). The gonad and shell extract sea urchin 0.25 g was 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 no. 5, 6 mm dia) was then dipped in 20 µL of the gonad and shell of extracts sea urchin 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 24 hrs, followed by measuring the inhibitory zone for each sample and the type of bacteria in mm. The 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).

2.6 Application in beef and fish meat

Extracts that have the best inhibitory zone against bacteria *E. coli*, *Salmonella sp* and *S. aureus* are applied to beef and fish meat. 0.75 g of samples were dissolved in 3 mL aqueous dimethylsulfoxide (DMSO), beef and fish were cut in rectangular form 4 g, soaked into extract solution, meat was stored on 48 hrs. The sample control is meat without the addition of extracts, as long as storage is observed physical parameters colour, aroma, texture and slime. After 48 hrs of storage, 1 mL each of sterile plate count agar (PCA) and eosin methylene blue agar (EMBA) was poured in Petri dishes, meat dissolved in the NaCl and inoculate uniformly with 0.1 mL (dilution 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4}) at 37°C for 24 hrs of test bacteria.

3. Results and discussion

3.1 GC-MS analysis

The presence of antibacterial components in the methanol and ethyl acetate extract of gonad and shell of sea urchin was characterized by GC-MS analysis (Table 1).

Thirteen constituents were predominantly found in the gonad and shell extract of sea urchin (Table 1). Six of these were from the methanol extract of gonad sea urchin. They include n-hexadecanoic acid (39.66%), Pentadecanoic Acid (1.73%), 9-Octadecenoic acid (Z)-, methyl ester (23.1%), 9-Hexadecenoic acid, methyl ester, (Z)- (3.74%), Tetradecanoic acid (21.01%) oleic acid (0.97%). While the remaining eight—i.e., Cholest-5-EN-3-OL (3-Beta.) (44.19%), n-Hexadecanoic acid (19.44%), Ergosta-5,22-dien-3-ol, (3.beta.,22E) (6.32%), Pentadecanoic acid (1.06%), 1,2-Benzenedicarboxylic Acid (0.10%), 9-Octadecenoic acid (Z)-, methyl ester (4.84%), Arachidonic acid (3.85%) and 9-Hexadecenoic acid, methyl ester, (Z)- (2.9%) were from the methanol extract of shell sea urchin (Table 1). Most of these constituents have been found to show interesting biological activity against certain illnesses and/or pathogens. For instance, the antioxidant, anti-inflammatory, antibacterial, hypocholesterolemic activities reported for n-hexadecanoic acid, 9-Octadecenoic acid (Z)-, methyl ester and Tetradecanoic acid, with the dominant amounts of the three components.

Table 1. Antimicrobial composition of gonad and shell extract of sea urchin

Antibacterial Compound	Molecular formula	Peak area (%)		Peak area (%)	
		Methanol extract of gonad	Methanol extract of shell	Ethyl acetate extract of gonad	Ethyl acetate extract of shell
Cholest-5-EN-3-OL (3. Beta.)	C ₂₇ H ₄₆ O	-	44.2	9.2	51.6
Hexadecanoic acid (Palmitic acid)	C ₁₆ H ₃₂ O ₂	39.7	19.4	8.4	2.7
Ergosta-5,22-dien-3-ol, (3. beta.,22E)	C ₂₈ H ₄₆ O	-	6.3	-	10.7
Tetradecanoic Acid	C ₁₅ H ₃₀ O ₂	1.7	1.1	0.3	-
1,2-Benzenedicarboxylic Acid (Phthalic Acid)	C ₂₄ H ₃₈ O ₄	-	0.1	8.1	0.6
9-Octadecenoic acid (Z) -, methyl	C ₁₉ H ₃₆ O ₂	23.1	4.8	1.2	0.4
Arachidonic acid	C ₂₀ H ₃₂ O ₂	-	3.9	-	-
9-Hexadecenoic acid, methyl ester,	C ₁₇ H ₃₂ O ₂	3.7	2.4	1.1	0.6
Tetradecanoic acid (Myristic acid)	C ₁₄ H ₂₈ O ₂	21.01	-	4.34	1.11
Oleic acid	C ₁₈ H ₃₄ O ₂	0.97	-	-	-
Pentacosane	C ₂₅ H ₅₂	-	-	35.61	15.45
Tetratetracontane	C ₄₄ H ₉₀	-	-	6.88	6.53
Stigmast-5-EN-3-OL, (3. Beta.,24S)-	C ₂₉ H ₅₀ O	-	-	5.9	-

There were ten antimicrobial compounds that were present in considerable amounts (80.93%) found in ethyl acetate extract of gonad sea urchin, and another nine antimicrobial compounds comprised 89.59% (Table 1). A component 9-Hexadecenoic acid, methyl ester, Pentacosane, -Octadecenoic acid (Z) -, methyl ester and Cholest-5-EN-3-OL (3. Beta.) have been reported to have antimicrobial activity. The presence of these compounds makes gonad and shell extract of sea urchin leaves a source of bioactive compound. Other minor compounds like n-Hexadecanoic acid have antibacterial properties, where Tetradecanoic acid, Tetratetracontane and Stigmast-5-EN-3-OL, (3. Beta.,24S) has antimicrobial activity.

3.2 Antibacterial Activity

Disc diffusion method was followed to determine the antimicrobial activity. Antimicrobial activities of gonad and shell of sea urchin (*Diadema setosum*) extracts were evaluated against three bacterial (two gram-positive, one gram-negative) strains with two replicates being tested in the agar plate. The ethyl acetate solutions of the extracts were found to have potent antimicrobial activity against all the gram-positive and gram-negative bacteria tested by disc diffusion assay as shown in Table 2.

3.2.1 Antibacterial activity against *Escherichia coli*

Methanol and ethyl acetate extract of sea urchin for extraction 30 and 60 mins, exhibited a broad-spectrum antimicrobial activity with a minimum zone diameter of 6.5 mm for methanol extract of a shell with 30 mins extraction and ethyl acetate extract of gonad with 30 mins extraction a maximum zone diameter of 7.4 mm

against on *E. coli*. Ethyl acetate and methanol extract showed slightly active activity against *E. coli* with a diameter inhibitory zone 6 – 7 mm (Table 2 and Figure 2). The diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

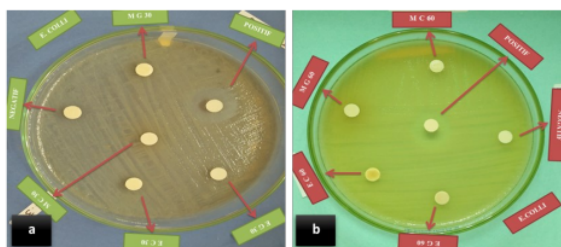


Figure 2. Antibacterial activity of methanol and ethyl acetate extract (gonad and shell) against *Escherichia coli*: a) 30 mins extraction; b) 60 mins extraction. Sample EG30 (ethyl acetate extract of gonad, 30 mins); EC30 (ethyl acetate extract of shell, 30 mins); MG30 (methanol extract of gonad, 30 mins); MC30 (methanol extract of shell, 30 mins); Sample EG60 (ethyl acetate extract of gonad, 60 mins); EC60 (ethyl acetate extract of shell, 60 mins); MG60 (methanol extract of gonad, 60 mins); MC60 (methanol extract of shell, 60 mins).

3.2.2 Antibacterial activity against *Salmonella*

Methanol and ethyl acetate extract of sea urchin for extraction 30 and 60 mins, and two replicates of the samples being tested in the agar plate. Exhibited a broad-spectrum antimicrobial activity with a minimum zone diameter of 8.8 mm for methanol extract of a shell with 30 mins extraction and ethyl acetate extract of gonad with 30 mins extraction a maximum zone diameter of 12.6 mm against on *Salmonella*. These result also showed ethyl acetate and methanol extracts as highly active activity against *E. coli* with a diameter of

Table 2. Effect of gonad and shell extract of sea urchin against different pathogens

Samples	Extraction time (min)	Volume	Zone of inhibition (mm)		
			<i>E. coli</i>	<i>Salmonella</i>	<i>S. aureus</i>
Methanol extract of gonad sea urchin	30	20 µL	6.7	9.6	8.5
	60		6.9	10.5	11.2
Methanol extract of shell sea urchin	30	20 µL	6.5	8.8	7.6
	60		6.8	9.5	11
Ethyl acetate extract of gonad sea urchin	30	20 µL	7.4	12.6	11.8
	60		7.2	12.3	12.1
Ethyl acetate extract of shell sea urchin	30	20 µL	6.8	11.5	10.9
	60		6.9	10.3	10.6
Control positive	30	20 µL	17.3	35.7	32.5
Control negative	30		0	0	0
Control positive	60	20 µL	17.2	33	33
Control negative	60		0	0	0

inhibitory zone greater than 10 mm (Table 2 and Figure 3).

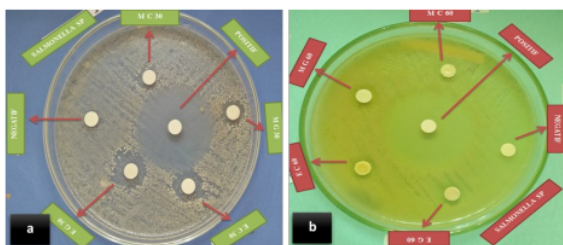


Figure 3. Antibacterial activity of methanol and ethyl acetate extract (gonad and shell) against *Salmonella*: a) 30 mins extraction; and b) 60 mins extraction.

3.2.3 Antibacterial activity against *Staphylococcus aureus*

Methanol and ethyl acetate extract of sea urchin for extraction 30 and 60 mins, exhibited a broad-spectrum antimicrobial activity with a minimum zone diameter of 7,6 mm for methanol extract of a shell with 30 mins extraction and ethyl acetate extract of gonad with 60 mins extraction a maximum zone diameter of 12,1 mm against on *S. aureus*. These result also showed ethyl acetate and methanol extracts as highly active activity against *S. aureus* with a diameter of inhibitory zone greater than 10 mm (Table 2 and Figure 4).

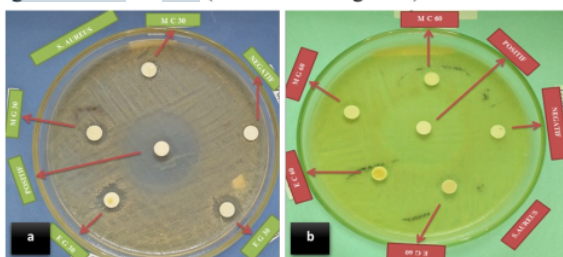


Figure 4. Antibacterial activity of methanol and ethyl acetate extract (gonad and shell) against *Staphylococcus aureus*: a) 30 mins extraction; and b) 60 mins extraction.

The preliminary antimicrobial assay of the extracts showed different responses to the test organisms with the

best activity observed for both methanol and ethyl acetate extracts of gonad and shell sea urchin against bacteria gram-positive (*Salmonella* and *S. aureus*). The gonad and shell sea urchin extracts were observed to show poor activity against the test pathogens bacteria gram-negative (*E. coli*).

3.3 Application in beef and fish meat

The results showed, that ethyl acetate extract of gonads with 30 mins extraction is the best condition to produce antibacterial compounds found in the sea urchin because can highly activity inhibitory zone against bacteria *E. coli*, *Salmonella* and *S. aureus*. The result of this extract will be applied to fresh food i.e. beef and fish meat (Table 3, Figures 5 and 6).

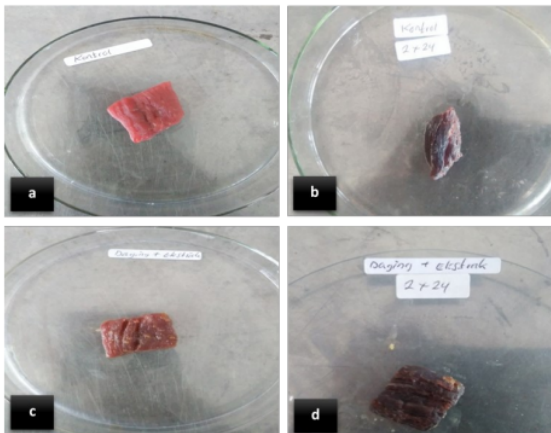


Figure 5. Application ethyl acetate extract of the gonad in beef meat: a) sample of beef control at 0 hrs; b) sample of beef control after 48 hrs; c) sample of beef with ethyl acetate extract of gonad at 0 hrs; and d) sample of beef with ethyl acetate extract of gonad after 48 hrs

The amount of bacteria that grows in beef meats that are not soaked in extracts (sample control) is 2.5×10^5 CFU/g. Beef meat that is not added ethyl acetate extract of gonads sea urchin has suffered damage due to bacterial growth. In samples of beef meat given ethyl

Table 3. Amount of microbial after 48 hrs of storage using Total Plate Count Test (TPC)

Sample	Dilution (CFU/g)				TPC (CFU/g)
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	
Control beef meat	TNTC	TNTC	252	18	2.5 x 10 ⁵
Sample ethyl acetate extract of gonad in beef meat	0	0	0	0	-
Control fish meat	TNTC	TNTC	145	28	1.4 x 10 ⁵
Sample ethyl acetate extract of gonad in beef meat	8	-	-	-	8 x 10 ¹

TNTC = To Numerous To Count

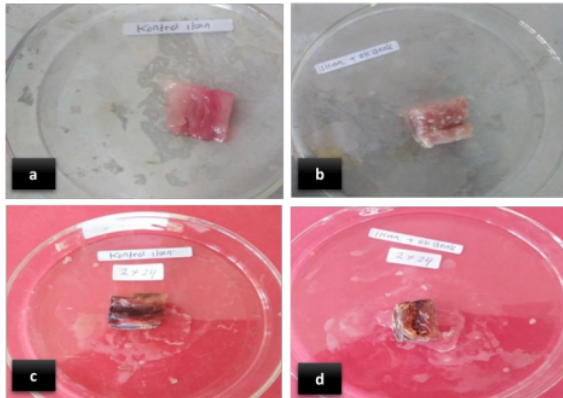


Figure 6. Application ethyl acetate extract of the gonad in fish meat: a) sample of fish control at 0 hrs; b) sample of fish control after 48 hrs; c) sample of fish with ethyl acetate extract of gonad at 0 hrs; and d) sample of fish with ethyl acetate extract of gonad after 48 hrs

acetate extract of gonads, sea urchin is not damaged and overgrown by pathogenic bacteria, due to the presence of antibacterial compounds that inhibit the growth of bacteria during storage of 48 hrs. The same thing on a sample of fish meat (sample control), overgrown by a microbial of 1.4×10^5 CFU/g, while fish meat is given ethyl acetate extract of gonads sea urchin only overgrown microbes amounting to 8×10^1 CFU/g during

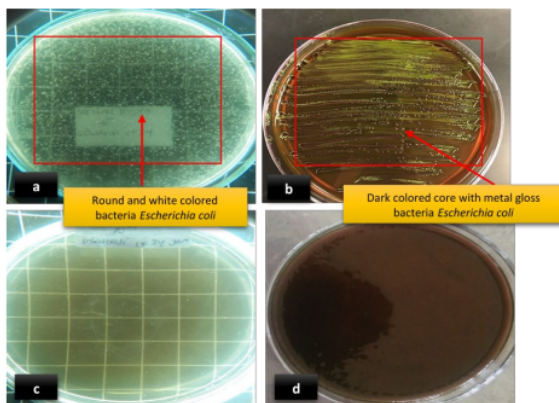


Figure 7. Observation of samples after 48 hrs of storage: a) sample of beef control in the media plate count agar (dilution 10⁻¹); b) sample of beef control in the media eosin methylene blue agar; c) sample of beef with ethyl acetate extract of the gonad in the media plate count agar (dilution 10⁻¹); and d) sample of beef with ethyl acetate extract of the gonad in the media eosin methylene blue agar.

storage 48 hrs. Fish meat is more rapidly damaged than beef because the protein contains very few tendons or binders so that easy to digest the body and easier to decompose (rot). High Protein (about 15 to 20% of other types of meat), as well as meat almost without tendons, makes fish meat a good medium for the growth of the microorganisms (Zhang *et al.*, 2016). Damage by pathogenic microbes can be seen clearly when both samples in isolation on the media plate count agar and eosin methylene blue agar (Figures 7 and 8).

The results showed that Eosin Methylene Blue Agar (EMBA) is a medium used to determine the presence or absence of coliform bacteria in a sample. The medium of EMBA for this has the privilege of containing lactose and serves to distinguish microbes that fermenting lactose such as *S. aureus*, *Pseudomonas aeruginosa*, and *Salmonella*. Microbes that ferment lactose produce colonies with dark nuclei with metal gloss. Other microbes that can grow their colonies are colourless. The functions of eosin and methylene blue help sharpen the colour difference. Nevertheless, if this medium is used in the early stages, other germs can also grow mainly *Pseudomonas aeruginosa*, and *Salmonella* sp., so this

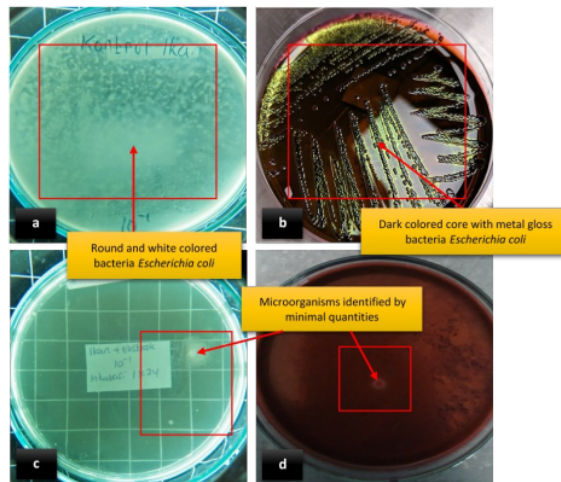


Figure 8. Observation of samples after 48 hrs of storage: a) sample of fish control in the media plate count agar (dilution 10⁻¹); b) sample of fish control in the media eosin methylene blue agar; c) sample of fish with ethyl acetate extract of the gonad in the media plate count agar (dilution 10⁻¹); and d) sample of fish with ethyl acetate extract of the gonad in the media eosin methylene blue agar

can lead to doubts. This media is very good to confirm that the contaminants are *Escherichia coli*. This solid shaped medium is useful for keeping cells from moving places so that it will easily be counted and separated by type when growing into a colony. Solid media also reveals the diffusion of bacterial metabolites to facilitate the testing of a metabolite result.

4. Conclusion

Gonad and shell sea urchin are the sources of secondary metabolites; n-hexadecanoic acid, Pentadecanoic acid, 9-Octadecenoic acid (Z)-, methyl ester, 9-Hexadecenoic acid, methyl ester, (Z)-, Tetradecanoic acid, oleic acid, Cholest-5-EN-3-OL (3. Beta.) (44.19%), Ergosta-5,22-dien-3-ol, (3. beta., 22E), 1,2-Benzenedicarboxylic Acid (0.10%), Pentacosane, Tetratetracontane, and Stigmast-5-EN-3-OL, (3. Beta., 24S)-/ gamma sitosterol, that contributes to the antioxidant and antibacterial properties. Ethyl acetate extracts give the highest number of those antibacterial components for both gonad and shell samples. *S. aureus* and *Salmonella* (gram-positive bacteria) are more sensitive to gonad and shell sea urchin ethyl acetate extract because it has an inhibitory zone diameter greater than 10 mm, and not sensitive to *E. coli* (gram-negative bacteria), with inhibitory zone diameter 6–7 mm. However, further studies on the specific antimicrobial compound activities need to be tested and, possibly, a double or triple combination of the gonad and shell extracts could enhance each of their potencies.

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