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Research Article

Inhibition of methanol extract snail gonggong *(Strombus urturella)* to pathogenic bacteria *Vibrio alginolyticus*

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ABSTRACT

The purpose of this study was to determine the methanol extract of snail gonggong (Strombus turturella) and the inhibition of methanol extract of gonggong snail meat (Strombus turturella) against the pathogenic bacterium Vibrio alginolyticus. The study used a completely randomized design. The treatment was giving the extract of snail gonggong meat with concentration of 100%, 50%, 25%, positive control using amoxcillin and negative control using distilled water with three replications. The identification of bioactive compounds included alkaloids, saponins, flavonoids, steroids and triterpenoids. The antibacterial activity of the methanol extract of snail gonggong meat (Strombus turturella) was tested by the diffusion method at concentrations of 100%, 50% and 25%. The data collected is then analyzed descriptively qualitatively. The results showed that the antibacterial activity of the 100% concentration of the methanol extract of the gonggong snail meat had moderate inhibition against Vibrio alginolyticus (diameter 7,833 mm). while the concentrations of 50% and 25% did not have an inhibition zone. The identified bioactive compounds included alkaloids, saponins, flavonoids and steroids, while the terpenoid and phenolic compounds were not identified. Thus, the methanol extract of gonggong snail meat has antibacterial potential that can be used to overcome the attack of pathogenic bacteria.

Keywords: antibacterial, bioactive compounds, snail gonggong

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INTRODUCTION

Vibrio is a Gram negative bacteria that has different characteristics from other pathogenic bacteria. Because these bacteria have the ability to survive without oxygen or with oxygen. These bacteria live in watery places such as rivers and bays, therefore these bacteria are very commonly found in seawater (Scoendstadt, 2013). Vibrio alginolyticus is able to act as primary and secondary pathogenic bacteria. Through secondary pathogens, Vibrio alginolyticus attacks organisms previously infected with other diseases, while primary

pathogens Vibrio alginolyticus attack organisms directly which can cause vibriosis (Sukenda et al, 2012). Vibrio alginolyticus infection in humans through foodborne or consumption of seafood products such as raw or undercooked shrimp and fish can result in contamination of Vibrio alginolyticus (Centre for Health Protection, 2010), and damage to blood vessels (Thomson et al, 2004).

The Centers for Disease Control and Prevention in the United States and the states of Alabama, Florida, Louisiana, and Texas report that every year about 30-40 people are infected with Vibrio alginolyticus (Daniels et al, 2000). Arisanti et al (2018), in 2003, 2004 and 2005 found 82 cases of food poisoning in humans through the consumption of seafood products and 60% were caused by contamination of the pathogenic bacterium Vibrio alginolyticus. Data from the Directorate of Food Safety Surveillance and Counseling of the POM Agency of the Republic of Indonesia in 2008, the number of victims of food poisoning through foodborne contamination due to Vibrio alginolyticus in Indonesia reached 25,268 people (Mirawati et al, 2013). Extraordinary events that occurred in Indonesia in 2015 due to contamination of Vibrio alginolyticus in foods such as seafood products consumed resulted in poisoning in humans as many as 1,176 people (Ekawati, 2017). Antibiotics used to inhibit or treat Vibrio alginolyticus infection are chloramphenicol (CP), oxytetracycline (OTC), and furazolidone (FZ). If the use of antibiotics is not effective in inhibiting Vibrio alginolyticus infection, then one alternative used to overcome the problem of resistance due to Vibrio alginolyticus infection is the use of secondary metabolic compounds and bioactive compounds found in plants or animals (Ali, 2006).

Snail 'gonggong' (Strombus turturella) is a marine biota and belongs to the mollusc phylum of the gastropod class which has a very useful function for the aquatic environment in the food chain and acts as an indicator of water quality (Arianti et al, 2013). According to Arularasan et al. (2010) snail meat gonggong is a functional food for Indian society because it has low cholesterol and can treat heart disease. According to (Yoswaty & Zulkifli, 2016) the ethanol extract of the type of snail gonggong (Strombus canarium) contains antibacterial ingredients that can inhibit the growth of pathogenic bacteria A. hydrophila, C. perfringens and Vibrio sp. The groups of bioactive compounds identified were alkaloids and saponins, while the groups of compounds that were not identified were flavonoids, steroids, and triterpenoids. Empirically, the spread of barking snails in Aru Islands Regency is only found in Aru Tengah Timur District, Koijabi Village. The use of snails gonggong by local people as food because in addition to having a very distinctive aroma and texture, it also has the ability to increase endurance and treat diarrhea attacks. This is the main indicator, because so far there has been no exploration of the bioactive content contained in the snail meat gonggong in Koijabi Village and the increase in the use of barking snail meat as a functional food ingredient because it has antioxidant compounds or has antibacterial bioactive content.

The results of this study can be developed for educational development in the form of practical instructions in microbiology courses as a tool to describe the identification of bioactive compounds found in animals and how to see the inhibitory power of these compounds against pathogenic bacteria. The development of this practicum guide aims to direct the microbiology practicum process in the laboratory so that it is more focused on achieving the expected competencies, such as students having to master basic microbiology techniques or procedures in the laboratory.

METHODS

This research is a experimental laboratory, which aims to see the bioactive compounds in the flesh of the snail gonggong *(Strombus turturella)* and to test its antibacterial activity against *Vibrio alginolyticus* bacteria and to measure the diameter of the inhibition zone for each concentration. This research was conducted at the Laboratory of Basic Chemistry, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon and the Laboratory of Fishery Products Technology, Pattimura University, Ambon. The subjects in this study were pure cultures of the pathogenic bacterium *Vibrio alginolyticus* obtained from the Fisheries Product Technology Laboratory, Pattimura University, Ambon. The objects in this study were extracts of snail gonggong 100%, 50%, 25%, bioactive and antibacterial compounds of snails gonggong and the diameter of the inhibition zone.

Tools and materials

The tools used in this research are oven, rotary evaporator, ice box, petri dish, paper disc, test tube, measuring pipette, tweezers, measuring cup, 0.5 mm ose needle, spirit lamp, lighter, scale, marker, laminar flow, cotton, syringe, autoclave, incubator, blender, paper. The materials used in this study were Vibrio alginolyticus bacteria, Methanol, barking snail extract, Amoxilin, NaCl 0.9%, Farland 0.5, Dragendorf reagent, Mayer, Wagner, H2SO4, Amyl alcohol, concentrated HCL, Acetic anhydride, distilled water, Alcohol 70%, DMSO.

Research procedure

1. Sterilization of tools and materials
   Glass utensils were sterilized using an oven at 170^{0}C for 2 hours, media were sterilized using an autoclave at 121^{0}C for 15 minutes. Ose and tweezers are sterilized by bunsen. Luminar air flow cleaned using 70% alcohol, then sterilized by UV lamp for 15 minutes.
2. Extraction
   a. Sampling of snails gonggong from their habitat.
   b. The snail gonggong is separated from its shell using a hammer.
   c. The snail gonggong meat is washed using clean water and then weighed with a base weight of 5 kg and dried under the hot sun until it is completely dry.
   d. The dried snail gonggong meat was then weighed to determine the dry weight. Then sliced or cut and in a blender until it becomes a powder then weighed at 300 grams.
   e. The gonggong snail meat powder weighing 300 grams was soaked using 96% methanol solvent in a 500 ml Elenmeyer container, stirred carefully until evenly distributed and soaked for 48 hours until the solvent above the powder became clear.
   f. The extract is then separated or filtered using a filter cloth between the sample and the solution so that the remaining sample is stored while the 400 ml solution will be evaporated using an evaporator.
   g. Evaporation at a temperature of 50^{0}C and the evaporation time can be adjusted until there is a separation between the methanol solution and the pure compound in the sample.
   h. The resulting percholate was concentrated with rotary evaporate to become a thick extract and put in a mini bottle and then tested for antibacterial activity.
3. Resistance Test
   a. Take the pure isolate that has been prepared using an ose needle, then put the isolate into a test tube containing aquadest.
   b. Homogenize the isolate in a test tube using a vortex.
   c. Prepare a petri dish that already contains TSA medium
   d. Scrape the isolate on the entire surface of the TSA media using a stick that has been given a cotton swab.
   e. Dip the paper disk into each treatment with a concentration of 100%, 50%, 25% then put it into a petri dish.
   f. Mark each treatment with label paper and wrap the edges of the petri dish using plastic warp.
   g. Put the petri dish into the incubator for 24 hours at 37˚C
4. Inhibition zone diameter measurement
   After 24 hours, remove the petri dish from the incubator and measure the clear zone that has formed around the paper disk using a caliper and record the diameter of each treatment into a table.
5. Identification of bioactive compounds snail gonggong
   Bioactive analysis was carried out to determine the active compounds contained in the snail gonggong extract including alkaloid and saponin tests (Yoswaty & Zulkifli, 2016).
   (a) Alkaloids
   The snail gonggong extract was mixed with a few drops of H_2SO_4 and divided into 2 tubes. The first tube was dripped with 2-3 drops of dragendorff's reagent while the second tube was dripped with 2-3 drops of Meyer's reagent. The reaction is positive if an orange precipitate is formed in the first tube and a yellow precipitate is formed in the second tube.
   (b) Saponins (foam test)
   The snail gonggong extract was put into a test tube and added with warmed distilled water, then shaken and added with HCL. The extract contains saponin compounds if a foam is formed which does not disappear for 10 minutes

Data analysis

The data analysis technique used is descriptive qualitative by displaying tables and figures to identify bioactive compounds and test the inhibition of *Vibrio alginolyticus* bacteria.

RESULTS AND DISCUSSION

The bioactive components in the methanol extract of gonggong snail meat were observed by qualitative testing of bioactive compounds. The bioactive tests carried out include; alkaloids, flavonoids, terpenoids,

steroids, phenolics and saponons/tannins. The results of testing the bioactive components can be seen in table 1 below.

Table 1. Bioactive compounds of snail gonggong meat extract

Parameter	Color	Result
Alkaloids	Yellow	+
Flavonoids	Yellow	+
Terpenoids	Red	-
Steroids	Red	+
Phenolic	Yellow	-
Saponins/tanins	Foam	+

Based on the table above, the identification results show that of the six parameters measured, only four groups of bioactive compounds in the methanol extract of snail gonggong meat were identified, namely alkaloids with a yellow color reaction, flavonoids with a yellow color reaction, steroids with brown reactions and saponins or tannins in the reaction that occurs in the form of foam, while terpenoids and phenolics were not identified.

The results of the antibacterial activity test of the snail gonggong meat extract against the growth of Vibrio algonolyticus bacteria can be seen in Table 2 below

Table 2. The results of the power test of snail gonggong meat extract.

Concentration	Replay			mean
	1	2	3	
100%	8,1mm	7,9 mm	7,5 mm	7.833 mm
50%	0 mm	0 mm	0 mm	.000 mm
25%	0 mm	0 mm	0 mm	.000 mm
12,5%	0 mm	0 mm	0 mm	.000 mm
6,25%	0 mm	0 mm	0 mm	.000 mm
3,125%	0 mm	0 mm	0 mm	.000 mm
Amoxilline (+)	32,1mm	29,1 mm	35,1 mm	44.934 mm
Aquades (-)	0 mm	0 mm	0 mm	0 mm

Based on the table above, it shows that each treatment concentration that can inhibit the growth of *Vibrio alginolyticus* bacteria is a concentration of 100% while the concentration of 50%, 25%, cannot have an impact in the form of a clear zone around the petrik dish and can be seen in figure 1 below.

Concentration 100% control (+) and (-)

Figure 1. clear zone growth of *Vibrio alginolyticus*

The picture above is the result of the inhibition test of methanol extract of snail gonggong meat against *Vibrio alginolyticus* bacteria with concentrations of 100%, 50%, and 25%. The 100% concentration treatment with three replications had an impact on the growth of *Vibrio alginolyticus* bacteria with the formation of a clear zone diameter around the petri dish. Each replication gave the diameter of the inhibition zone not too different between them (U1 = 8.1 mm, U2 = 7.9 mm, U3 = 7.5 mm and an average of 7,833 mm including the medium category).

Identification of bioactive compounds is one of the important steps that must be taken to determine whether or not there is a class of bioactive compounds present in a test material (Sa'adah and Nurhasnawati, 2015). This test was carried out qualitatively by dissolving the methanol extract of snail gonggong meat with several reagents in order to obtain the desired bioactive compounds. Snail gonggong meat extract contains bioactive compounds, namely alkaloids, flavonoids, steroids and tannins/saponins. According to Thomas et al. (2014) the diameter of the inhibition zone that can be used as a reference in measuring the effectiveness of a test material in inhibiting bacterial growth is as follows, the inhibition zone 5 mm is categorized as weak, the zone of inhibition is 5-10 mm is categorized as moderate, the inhibition zone is 10-20 mm is categorized as strong and Inhibition zone 20 mm is categorized as very strong. Yoswaty & Zulkifli, 2016, stated that the ethanol extract of the type of snail barking *(Strombus canarium)* contains antibacterial ingredients that can inhibit the growth of pathogenic bacteria A. hydrophila, C. perfringens and Vibrio sp.

The greater the antimicrobial substance in the snail gonggong meat, the greater the opportunity to inhibit the growth of *Vibrio alginolyticus* bacteria by damaging the body's structure and metabolism. In line with the opinion of Rahmawati (2014) that if the concentration of the extract is increased, the diameter of the inhibition zone will also increase because the bioactive components contained in the extract used will also increase. Ajizah (2004) added that the characteristics of the samples used were one of the supporting factors in increasing antibacterial activity. Alkaloids have the ability as an antibacterial by interfering with the peptidoglycan constituent components of bacterial cells, so that the cell wall layer is not fully formed and causes the death of the cell. Polyphenols help fight the formation of free radicals in the body so that it can slow down premature aging. Broadly speaking, polyphenols have antibacterial properties with their mechanism of action by damaging bacterial cell membranes which can induce the formation of complex compound bonds to enzymes or microbial substrates that can increase toxicity (Rachman et al, 2018).

Flavonoids are phenolic compounds that work by denaturing proteins which can cause cell metabolic activity catalyzed by an enzyme which is a protein. Because flavonoids have the ability to form complexes with soluble extracellular proteins and with cell walls, microorganisms cannot attach to and invade cells (Susanti, 2016). Flavonoids are also able to release transduction energy to the bacterial cytoplasmic membrane and inhibit bacterial motility (Manik et al, 2016). In addition, flavonoids can also cause damage to bacterial cell walls through inhibition resulting in the incorporation of glycan chains that are not cross-linked into the peptidoglycan of the cell membrane so that it becomes a weak structure (Sulatstrianah et al, 2014). Nagappan et al (2011) explained that flavonoids will inhibit energy metabolism in bacteria, so that it can inhibit oxygen respiration which then the bacteria will lose the permeability of cell walls, microsomes and lysosomes as an interaction between flavonoids and bacterial DNA. The antibacterial mechanism is to form complexes with cellular and soluble extract proteins and with the microbial wall. Another possibility is that flavonoids play a direct role by interfering with microbial cell function and inhibition of the microbial cell cycle (Ginting et al, 2020). Rimporok et al. 2022, stated that the active compound flavonoid is the largest substance that can act directly as an antioxidant and antibacterial.

Tannins have antibacterial properties. Tannins work as antibacterial by interfering with bacterial surface receptors by binding to the protein adhesin in bacteria which will cause inhibition of protein synthesis for cell wall formation and decrease bacterial adhesion (Mastuti, 2016). Tannins also have chelating properties which are thought to be able to shrink cell walls so that their growth is inhibited and even dead (Sari, 2012). Fitriah et al, 2017, revealed that tannin compounds are known to interfere with peptidoglycan synthesis which causes the formation of bacterial cell walls to be incomplete, resulting in inactivation of bacterial cells in host cells. According to Aisiah (2004), tannins are one type of compound that belongs to the polyphenol group. The mechanism of action of tannins is thought to be able to shrink the cell wall or cell membrane so that it interferes with the permeability of the cell itself. As a result, cells cannot carry out living activities so that their growth is stunted and dies. Steroids act as antibacterial in inhibiting the growth of Porphyromonas gingivalis related to lipid membranes and sensitivity to steroid components that cause leakage in bacterial liposomes (Madduluri et al, 2011). Steroids can interact with cell phospholipid membranes that are permeable to lipophilic compounds, causing decreased membrane integrity and cell membrane morphology to change causing cell brittleness and lysis. The mechanism of action of antibacterial compounds includes inhibiting cell wall synthesis, inhibiting the integrity of microbial cell membranes, inhibiting microbial cell protein synthesis, interfering with microbial cell metabolism and inhibiting nucleic acid and protein synthesis (Rahmadani, 2015).

CONCLUSION

1. The snail gonggong meat extract has bioactive compounds of alkaloids, flavonoids, steroids and saponins/tannins.
2. Concentration of 100% extract snail gonggong meat was able to inhibit the growth of *Vibrio alginolyticus* bacteria with a moderate diameter of inhibition zone.

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