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Abstract. There is a widespread increase in the number of cases of infectious diseases, including those caused by *Staphylococcus aureus*. *Scylla serrata* haemolymph contains active compounds, including antimicrobial peptide proteins as cofactor molecules in the antibody and immune system which resists infection. This research evaluated the antibacterial activity of *Scylla serrata* haemolymph supernatant and virgin coconut oil (VCO) against the growth of *S. aureus* ATCC 25923 strain. The research used an experimental laboratory design with post test control only and 3 replicates of each treatment. Samples of *Scylla serrata* haemolymph were centrifuged to extract the supernatant, which was divided into 9 treatment groups at different concentrations (100% to 0.39%). There were 2 control groups: positive control (Cefadroxil) and negative control (distilled water). Virgin coconut oil was produced using a traditional method for 9 hours and divided into 5 concentration groups (100% to 6.25%) with a positive control (Erythromycin 0.6%) and negative control (DMSO). The activity test used was inhibition zone diameter on nutrient agar and the minimum inhibition concentration (MIC) of each treatment was determined. The result showed that for *Scylla serrata* haemolymph supernatant the largest inhibition zone diameter was 16.83 mm with strong activity and the smallest was 5.58 mm with medium activity. The MIC of *Scylla serrata* haemolymph supernatant was 12.5%. For 100%, 50%, and 25% VCO the inhibition zone diameters were 2.67 mm, 0.91 mm, and 0.5 mm, respectively, with no inhibition at lower concentrations. We conclude that *Scylla serrata* haemolymph supernatant showed medium to strong inhibitory activity against the growth of *S. aureus*, while virgin coconut oil had weak activity and did not effectively inhibit the growth of *S. aureus* ATCC 25923.

1. Introduction

Infectious diseases result in more than 13 million deaths each year and are a major cause of mortality in developing countries, which are mostly located in tropical and subtropical regions [1]. In recent years, the incidence of infectious diseases has increased, including the incidence of infections caused by *Staphylococcus aureus*. Diseases caused by bacterial infections are a major threat to human health and cause nearly 50,000 deaths around the world every day [2]. *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Vibrio cholerae*, *Salmonella* sp. *Shigella* sp. *Clostridium difficile*, *Campylobacter jejuni*, and *Vibrio haemiliticus* are examples of bacteria that can cause infection in humans [3]. *Staphylococcus aureus* is one of these pathogenic bacteria affecting humans and one of the main causes of infection that causes morbidity and mortality. Invasive *S. aureus* infection is becoming a serious public health problem [4].

Southeast Sulawesi Province in Indonesia is a maritime region with seas that offer potential for the development of fisheries, marine tourism, and industry, including the pharmaceutical industry. Marine biota in Southeast Sulawesi include fish, sea cucumber, shrimp, seaweed, and several types of crab, in particular the mangrove crab *Scylla serrata*[5].

Similarly, Southeast Sulawesi is one of the major coconut production areas in Indonesia, with 55,710 ha of coconut plantations and an annual production of 40,830 tons [6]. This opens up opportunities to develop a variety of useful products from coconuts [7], one of the most important being virgin coconut oil (VCO) [8]. Virgin coconut oil (VCO) is extracted from coconut oil without using heat, thus avoiding deleterious changes in composition and characteristics. VCO is widely recognised as having advantages for health, and has a growing number of uses for medical purposes, in functional nutrition, and in food.

Mangrove crabs (*Scylla* sp.) are an aquatic biological resource with a high economic value and potential for aquaculture [9]. In addition to the use of crab meat and shells, blood or haemolymph from the mangrove crab *Scylla serrata* have been found to contain active compounds called antimicrobial peptide proteins (AMP) which are cofactor molecules in the body's immune system and defence against infection [10]. Research has shown that the haemolymph of *Scylla serrata* can inhibit the growth of *Salmonella typhi* and *Candida albicans* as indicated by the inhibition zone diameter in tests on disc paper [11]. The inhibition zone diameter of 23 mm indicated strong activity against *Salmonella typhi* bacteria, while a diameter of 5.37 mm indicated medium activity against *Candida albicans*. Meanwhile, VCO has several advantages, including a high content of medium-chain fatty acids with low molecular weight. The main medium-chain saturated fatty acid (MCFA) contained in virgin coconut oil (VCO) is lauric acid (C12, 54.61%) [12]. The purpose of this study was to analyse the antibacterial activity of *Scylla serrata* haemolymph supernatant and virgin coconut oil (VCO) on the growth of *Staphylococcus aureus* ATCC 25923.

2. Methods

This research was carried out in the Laboratory of Microbiology, Faculty of Medicine, Halu Oleo University, Southeast Sulawesi. An experimental research method with post test only and three replicates per treatment was used. Mangrove crab (*Scylla serrata*) supernatant was prepared in nine concentrations: 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, 0.39 %, with positive control (Cefadroxil) and negative control (distilled water) groups. Traditionally produced virgin coconut oil (VCO) was prepared in 5 concentrations: 100%, 50%, 25%, 12.5%, and 6.25% with 0.6% Erythromycin as positive control and DMSO as negative control. The strength of inhibitory activity was determined based on the diameter of the inhibition zone against *Staphylococcus aureus* ATCC 25923 on agar nutrient media.

2.1. Sampling and preparation

Mangrove crabs (*Scylla serrata*) were collected and cleaned to remove any dirt. The samples used were adult crabs weighing around 200 grams. Haemolymph (blood) was collected by cutting each leg of the crab using a sterile knife. To avoid clotting, the crab blood was stored in a centrifuge tube to which sodium citrate was added with a pH of 4.6 and a ratio of 2:1. The sample was then dissolved in distilled water and centrifuged at 10,000 rpm for 10 minutes at 4^{o}C. The supernatant was obtained by aspiration using micropipette and stored at 4^{o}C until needed [13].

Coconuts were collected from the coastal area of Bungkutoko Village, Abeli District, Kendari City, Southeast Sulawesi Province, Indonesia. Five fresh, old coconut fruit were collected from 11-12 year old trees [14]. The maturity of the coconut fruit is indicated by brown coir, while size can be chosen randomly [15]. The coconut meat was finely shredded using a grater machine to produce 2.5 kg of grated coconut. The grated coconut was soaked in boiled water (1:2) and pressed/squeezed with clean hands for 30 minutes before being filtered. The coconut milk was collected in a container and allowed to stand for 1 hour until two layers were formed, namely cream (top) and water (below). The cream layer was then separated from the water [14], and allowed to stand for 9 hours at 30^{0}C until three layers were formed, namely oil (VCO), a middle layer referred to as *blondo*, with a watery layer beneath. The VCO layer was then separated and stored [15].

2.2. Antibacterial activity testing

Antibacterial activity was tested using the disc paper diffusion method. The agar diffusion method was carried out by pouring 40µl of *Staphylococcus aureus* suspension onto a compacted nutrient agar (NA) medium in a petri dish, then spreading it evenly. Each treatment sample comprised a 30µl aliquot of *Scylla serrata* haemolymph supernatant dripped onto disc paper with a diameter of 6 mm. Cefadroxil (30µl) as a positive control and distilled water (30µl) were similarly dripped onto discs. The treatment and control disc papers were left to stand for 5 minutes at room temperature to enable absorption by the disc paper. The disc papers were placed on the NA medium surface just above the *Staphylococcus aureus* colony, with 5 treated disc papers evenly spaced in each petri dish.

The antibacterial activity of VCO was tested in a similar manner using the paper disc diffusion method. One millilitre of the prepared *Staphylococcus aureus* suspension was inoculated onto 15 ml NA media which had been allowed to stand until set and level. Virgin coconut oil (VCO) at the appropriate treatment concentrations was dripped onto 6 mm diameter disc papers; Erythromycin 0.6% antibacterial discs (positive control) and DMSO treated discs (negative control) were also prepared. The discs were placed on the surface of the medium just above the Staphylococcus aureus ATCC 25923 colonies as above.

The NA media with the treatment and control discs were incubated at 37ºC in an incubator for 1x24 hours. The presence of an inhibition zone around a disc paper indicates that the growth of *Staphylococcus aureus* has been inhibited by antibacterial compounds in substance added to the disc paper. The diameter of the inhibition zone formed around each disc paper was observed and measured using a ruler. The lowest concentration capable of inhibiting inoculated bacteria as shown by the formation of a clear (inhibition) zone was determined as the Minimum Inhibitory Concentration (MIC) of the substance tested [16].

3. Results

The inhibition zone diameter was largest at the highest concentrations of *Scylla serrata* haemolymph supernatant (Table 1) and VCO (Table 2). In both trials, the positive control had a larger inhibition zone than the treatments, however the *Scylla serrata* haemolymph supernatant showed strong antibacterial activity at concentrations of 25% and above, while VCO activity was weak even at 100%. The test results of the Minimum Inhibitory Level (MIC) of the supernatant Scylla serrata haemolymph were 12.5% with an average inhibition zone diameter of 5.58 mm with a moderate interpretation. The minimum inhibitory concentration (MIC) of *Scylla serrata* haemolymph supernatant was 12,5%, while that of virgin coconut oil (VCO) was 25%.

Table 1. Interpretation of *Scylla serrata* haemolymph supernatant (SSHS) inhibition zone diameters as activity against *Staphylococcus aureus* ATCC 25923 growth

Substance tested	Concentration	Volume (µL)	Inhibition zone (mm)				Interpretation [17]
			I	II	III	Mean	
SSHS	100%	30	16	18	16.5	16.83	*Strong*
SSHS	50%	30	12.75	12.5	13.75	13	*Strong*
SSHS	25%	30	12	9.75	11	10.91	*Strong*
SSHS	12.5%	30	4.25	5.75	6.75	5.58	*Intermediate*
SSHS	6.25%	30	0	0	0	0	-
SSHS	3.125%	30	0	0	0	0	-
SSHS	1.56%	30	0	0	0	0	-
SSHS	0.78%	30	0	0	0	0	-
SSHS	0.39%	30	0	0	0	0	-
Cefadroxil	Control (+)	30	27.58	28	28	27.86	*Sensitive*
Distilled water	Control (-)	30	0	0	0	0	-

Table 2. Interpretation of virgin coconut oil (VCO) inhibition zone diameters as activity against *Staphylococcus aureus* ATCC 25923 growth

Substance tested	Concentration	Volume (µL)	Inhibition zone (mm) I	II	III	Mean	SD	Interpretation [17]
VCO	100%	30	2.75	1.75	3.5	2.67	0.878	*Weak*
VCO	50%	30	-	1.5	1.25	0.91	0.804	*Weak*
VCO	25%	30	-	1.5	-	0.5	0.866	*Weak*
VCO	12.5%	30	-	-	-	-	-	-
VCO	6.25%	30	-	-	-	-	-	-
Erythromycin	0.6%	15	23.0	23.0	23.0	23.0		*Sensitive*
DMSO	Control (+) Control (-)	20	-	-	-		-	-

4. Discussion

The test results of mangrove crab (*Scylla serrata*) haemolymph supernatant activity against the test bacteria showed a positive response with inhibition of the growth of *Staphylococcus aureus* bacteria. This response can be assumed to be due to the presence of active compounds produced by the tested animals, in particular Anionic Antimicrobial Peptide Proteins (AAMP) [18], first reported in 1980. Antimicrobial Peptides (AMP) are known as important components of nonspecific body defences in a wide variety of living organisms including bacteria, fungi, plants, insects, birds, crustaceans, amphibians, and mammals. In crustaceans, the body's defences against microbes, such as adhesion, phagocytosis, nodule formation, and melanisation, depend on cellular activity controlled by the haemolymph [19].

The antibacterial activity of *S, serrata* haemolymph supernatant varied with the concentration used. The lowest concentrations (0.39% to 6.25%) did not show an inhibitory effect against the growth of S*, aureus* bacteria, while moderate activity was detected at a concentration of 12.5% (5.58 mm inhibition zone diameter). with a moderate interpretation. Thus, relatively small amounts of active the substances were not effective. At concentrations of 25%, 50%, 100% the inhibitory effect was strong, positively correlated with concentration (respective mean inhibition zone diameters of 10.91 mm, 13 mm, 16.83 mm), and did not reach a plateau.

One known antibacterial mechanism of action of the active compounds found in mangrove crab haemolymph is through the peptidoglycan contained in AMP which will damage the integrity of the bacterial cell wall, inhibiting the growth of gram-positive bacteria. AMP binds lipids to the bacterial wall, resulting in the formation of pores and causing the release of important bacterial cell components; the lipid bond also disturbs the synthesis of teichoic acid and lipoteichoic acid in the cell wall. If teichoic acid is not formed then the bacteria becomes uncharged because teichoic acid makes bacteria negatively charged [10].

Previous research testing the inhibitory activity of haemolymph extract of Scylla mangrove crabs on the growth of *Candida albicans* and *Salmonella thypi* found inhibition zones of 5.37 mm against *Candida albicans* indicating moderate activity, and 6.23 mm against *Salmonella thypi*, interpreted as strong activity [11]. The results of this study show strong activity against *S. aureus*, and together these results indicate that *S. serrata* haemolymph extract may have potential as a relatively broad spectrum antibacterial agent.

A study on the haemolymph extract of 8 species of crabs (*Charybdis feriata*, *C. lucifera*, *C. amboinensis*, *C. natator*, *Portunus sanguinolentus*, *P. pelagicus*, and *Dromia dehanni*) against 10 bacterial strains (*Staphylococcus aureus, Salmonella typhi, S. paratyphi, Klebsiella oxytoca , Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Lactobacillus vulgaris, Vibrio* sp., *K. pneumonia*) reported a maximum inhibition zone diameter (*P. pelagicus* haemolymph against *S. aureus*) of 15 mm and a minimum inhibition zone diameter (*P. sanguinolentus, C. lucifera*, and *D, dehanni* against *Klebsiella oxytoca*, *Lactobacillus vulgaris, S. aureus*, and *K. pneumonia*) of 4mm [19]. The MIC (12.5% against *S aureus*) and moderate to strong activity of *Scylla serrata* haemolymph supernatant compare favourably with these results from other crab species.

Virgin coconut oil (VCO) was shown to have positive but weak inhibitory activity on *Staphylococcus aureus* ATCC 25923. The diameter of the inhibition zone increased with VCO concentration from 0.5 mm at 25% to 2.67 mm at 100%. The observed antibacterial inhibition is thought to be due to the presence of lauric acid, one of the components of medium chain saturated fatty acids, and indeed the main MCFA, contained in virgin coconut oil (VCO). Lauric acid is a compound with antibacterial properties [12].

One factors influencing the weak antibacterial activity of virgin coconut oil (VCO) is the low concentration of lauric acid, resulting in weak inhibitory activity against bacterial growth. Previous research has found that low concentrations of lauric acid will not be able to inhibit bacterial growth, although they can have an effect through blocking the production of exoezymes, virulence factors and the formation of toxins by bacteria [20]. Lauric acid contains monoglycerides called monolaurins. Monolaurin is a bioactive compound found in lauric acid which is active in the process of inhibiting bacteria. The monolaurin content of lauric acid is about 3%; it can therefore be assumed that, if the composition of lauric acid is very small, the amount of the active (antibacterial) monoglyceride monolaurin compound that is formed will also be very small, so that the inhibitory action of virgin coconut oil will be weak [21].

Another possible reason for the weak bacterial growth inhibition shown by Virgin coconut oil (VCO) in this study is the ability of VCO to diffuse from the disc into the agar medium. The oil structure which is composed of many triglyceride molecules causes very low solubility so that an emulsion tends to form, and thus the compounds may not be dissolved and therefore may not be able to blend. Hydrophilic semipolar DMSO solvents do not mix completely with hydrophobic oil fatty acid components [22]. As expressed by Lahlou, M. (2004) in [20], the complex chemical structure of oils containing triglycerides causes their solubility to decrease so that it can be difficult for these oils to diffuse in the antibacterial test medium. According to [23], VCO is considered less effective in inhibiting Gram positive bacteria such as *S, aureus*. Virgin coconut oil (VCO) is a non-polar molecule that interacts more easily with cell membranes and destroys the lipid layers found in cell membranes which are mostly found in Gram negative bacteria, while *S, aureus* which is a Gram Positive bacterium, has an arrangement of thick peptidoglycan layers making it difficult to initiate lysis [23]. Furthermore, one factor affecting the difference in inhibition zone diameter at concentrations of 100%, 50% and 25%, could be the ability of *Staphylococcus aureus* lipase enzymes to break down triglycerides such as the lauric acid in virgin coconut oil. These factors might be overcome through the application of enzymatic methods.

5. Conclusion

Haemolymph supernatant extracted from the mangrove crab *Scylla serrata* showed medium to strong antibacterial activity against the growth of *Staphylococcus aureus* ATCC 25923. The minimum inhibitory level (MIC) against the growth of *S. aureus* bacteria was 12.5% with an average inhibition zone diameter of 5.58 mm and moderate activity. Traditionally produced Virgin Coconut Oil (VCO) was not effective as an antibacterial agent, with weak inhibitory activity against *Staphylococcus aureus* ATCC 25923 bacteria at 100% (maximum inhibition diameter 2.67 mm) compared with the antibiotic Erythromycin. Further research needs to be done on the isolation of proteins (active compounds) from mangrove crab (*Scylla serrata*) haemolymph and on the antibacterial activity of Virgin Coconut Oil (VCO) using enzymatic methods.

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