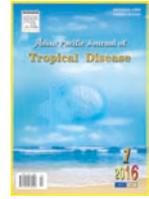




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Antibacterial activity of garlic (*Allium sativum*) againsts Gram-positive bacteria isolated from tiger shrimp (*Penaeus monodon*)

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ABSTRACT

Objective: To report the effectiveness of various concentrations of garlic juice on inhibition zone of Gram-positive bacteria isolated from tiger shrimps.

Methods: Four concentrations of garlic juice used in this research were: 0%, 12.5%, 25% and 50%. Two different bacteria were successfully isolated from tiger shrimps categorized as Gram-positive. The bacterial inhibition zone revealed that garlic juice could inhibit the growth of these bacteria in the form of clear zone on media.

Results: The average diameters of inhibition zone from those two bacteria were significantly increased in garlic juice receiving group compared with the control (untreated) group ($P < 0.05$). For bacteria B, this increase was dose dependent manner.

Conclusions: These findings demonstrated the potential of garlic as a natural alternative to currently used antibacterial agent for Gram-positive infection in the tiger shrimp.

1. Introduction

Tiger Shrimp (*Penaeus monodon*) is one of the fishery and marine products that become the important commodity especially in Southeast Asia, because it has high economic value[1]. This shrimp has several morphological characteristics such as hard skin and large bluish green motif on the body colour. Besides that, tiger shrimp is usually cultivated in a pond with the average length of 20–25 cm and weight of 140 g. The highest nutrient content in a tiger shrimp is protein[2].

The nutrient content of a tiger shrimp could be contaminated by several microorganisms which can endanger human health. Several studies had proved that fungi could cause a disease in a shrimp especially the genus of *Fusarium*[3]. This pathogen may lead to high mortalities by disturbing the osmoregulation of shrimp and this is often found in shrimps cultivated in a pond with poor water quality management[4]. Besides that, other microorganisms that also become the major disease problem in shrimps are bacteria frequently causing a great loss in economics of aquaculture products. In the recent study, several researchers observed the major

decline in commercial production of the tiger shrimp due to the disease outbreaks caused by bacteria[5].

However, this bacteria growth could be inhibited through several ways. Based on its processes, the processes are grouped as physical and chemical processes. Many antibiotics and synthetic chemicals used in shrimp cultivation to prevent the bacterial infection have a residual side effect. The development and discovery of alternative methods to overcome the infection have become the main focus and challenge to keep the sustainability of aquaculture production[3]. Other problem caused by the usage of antibiotics is the growth of antibiotic resistant bacteria[6]. Therefore, the use of natural substances especially from herbs or plants is preferable as an antimicrobial agent to prevent and treat the bacterial infection. This is also recommended by World Health Organization[1].

Garlic is one of the herbs that sparks great interests, since it possesses the antibacterial and antifungal activities. It contains organosulphur compounds (*S*-allylcysteine, *S*-allylcysteinine sulfoxide, *S*-methylcysteine, and *S*-ethylcysteine) and several phenolic compounds[7,8]. The antibacterial compound can inhibit the bacterial growth and is especially used to cure infection. Garlic can be used as antibacterial agent because it contains allicin. Allicin is the main compound that plays an important role in producing a garlic flavour and one of the active compounds which could eliminate germs by impairing the cell walls and inhibiting the protein synthesis[9]. Here, we reported the effect of garlic juice on Gram-positive bacteria isolated from tiger shrimp (*Penaeus monodon*).

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2. Materials and methods

2.1. Sample preparation

Totally 24 tiger shrimps were used in this research. The tiger shrimps were obtained from tiger shrimp cultivation at Dengok Kandangsemangkon Village, Tuban, East Java. The tiger shrimps obtained from cultivation place were weighed about 25 g and then threshed till smooth and put into 225 mL sterile aquades (10^{-1} dilution), continued with serial dilution from 10^{-2} to 10^{-6} . In this research, only dilution 10^{-5} and 10^{-6} were used.

2.2. Garlic juice preparation

Garlic juice was made using simple method. Garlic was shredded and squeezed to obtain the liquid content. Then it was filtered using filter paper to clean the garlic debris from the garlic juice. Several concentrations were made by adding sterile aquades for dilution. Three concentrations of garlic juice used here were 12.5%, 25%, and 50%.

2.3. Bacteria culture

Bacteria were grown using nutrient agar (Sigma) media. About 1 mL sample with dilution 10^{-5} and 10^{-6} were taken and put into Petri dishes, followed by pouring the media into the Petri dishes and shaken for a while to make it homogenous. Bacteria were grown for 24 h. Bacterial colonies observed were purified and continued with Gram staining. Those bacteria were used for bacterial inhibition zone test.

2.4. Bacterial inhibition zone test

One dose of bacteria pure culture was put into reaction tube containing 10 mL nutrient agar liquid. Solution was poured into sterile Petri dish and homogenized. After agar became solid, Petri dish was divided into four areas for four different concentrations (0%, 12.5%, 25% and 50%) of garlic juice treatment. Culture was incubated for 24 h and observed for the appearance of clear zone and the diameter of the clear zone was measured.

2.5. Statistical analysis

Data analysis and processing were done using SPSS for windows. ANOVA test was performed to test the data significance.

3. Results

Two bacteria randomly taken were used in this research. Complete analysed was not conducted to determine the bacteria species. Only morphology analysis and Gram staining were conducted to observe the differences between those two bacteria. First bacteria (bacteria A) had morphological characteristics such as red colour, round shape, flat edge, and convex elevation. Second bacteria (bacteria B) had morphological characteristics that were similar to those of the bacteria A; the only difference was the colour, in which bacteria B had white milk colour. After Gram staining analysis, both bacteria had blue colour that made them classified as Gram-positive bacteria (Figure 1).

Figure 2 summarizes the diameter of clear zone bacteria culture. For bacteria A, the diameter of clear zone was significantly greater in all doses of garlic juice group compared with the untreated control group ($P < 0.05$). There was no significant difference between the effects of these two higher doses. For bacteria B, the diameter of clear zone was significantly greater in all doses of garlic juice group compared with the untreated control group ($P < 0.05$).

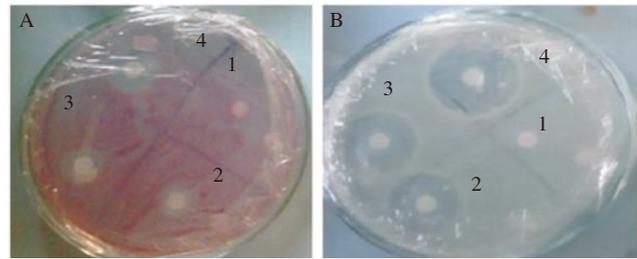


Figure 1. Observation of bacterial growth inhibition analysis.

The appearance of clear zone on culture of bacteria A (A) and bacteria (B). The area seems increasing due to the increased garlic juice concentration. 1: 0% Garlic juice concentration; 2: 12.5% Garlic juice concentration; 3: 25% Garlic juice concentration; 4: 50% Garlic juice concentration.

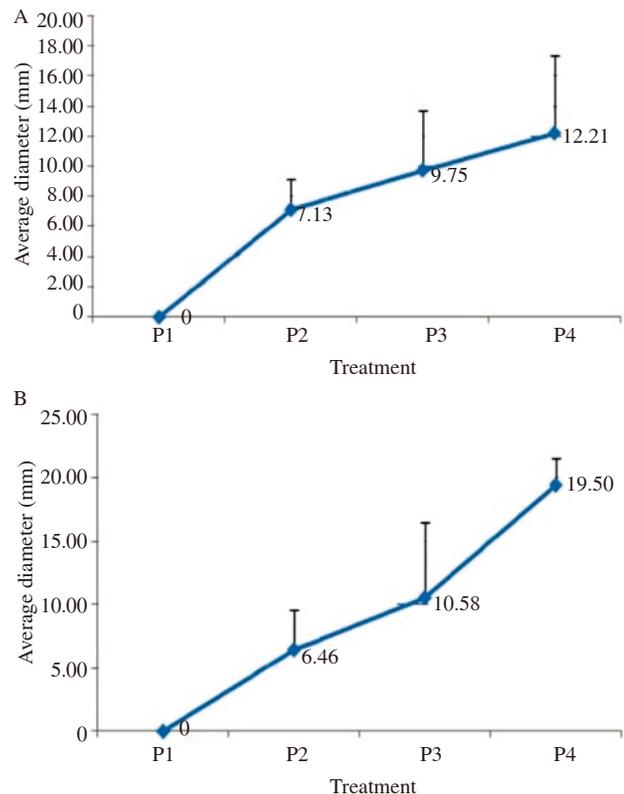


Figure 2. Measurement results of clear zone average diameter.

Clear zones were quantified by measuring the diameter whether in culture of bacteria A (A) or bacteria B (B). The average diameter was also increased in line with the increased concentration. P1: Treatment with 0% garlic juice concentration; P2: Treatment with 12.5% garlic juice concentration; P3: Treatment with 25% garlic juice concentration; P4: Treatment with 50% garlic juice concentration. Significant different ($P < 0.05$).

4. Discussion

In the recent years there have been significant increasing number of researches that focus on the knowledge of the disease infection in the shrimps. However, fishery products are still considered as a commodity with high risk of pathogen content, natural toxins, and many other potential contaminants[10,11]. Although some researches are focusing on the infection caused by Gram-negative bacteria especially from genus *Vibrio*[12], the role of the Gram-positive bacteria on disease infection also could not be ruled out. Generally, the diseases caused by this pathogen are through their invasion and growth within tissues rather than toxin production and dissemination[10,11].

In our study, we have isolated two Gram-positive bacteria from tiger shrimp. Previous studies found that several different bacteria strains are isolated from tiger shrimp muscles such as *Aureobacterium faciens*, *Aeromicrobium erythreum*, *Bacillus subtilis*, *Escherichia*

coli, *Vibrio cholerae*, *Enterobacter aerogenes*, *Micrococcus luteus*, *Pseudomonas putida*, *Pseudomonas aeruginosa* and *Enterococcus pseudoavium*[13]. Although, we did not further investigate the bacteria species, according to their the morphologies, we characterize the two Gram-positive bacteria from tiger shrimps which were closely related to some of the above bacteria.

We used garlic in our study, since its nutritional effect has been extensively investigated as antibacterial agent against a variety of Gram-negative and Gram-positive bacteria. Treatment was conducted using garlic juice or the water extract of juice. Garlic is mainly composed of water. The bacterial growth inhibition using garlic juice generated the positive effect. This result was consistent with several previous studies that showed the inhibitory effect of garlic aqueous extract on numerous bacterial species[14-16].

The inhibition zone analysis was marked by clear zone and increasing average diameter due to the increasing concentrations of garlic juice. We hypothesized that the antibacterial activity of garlic juice on Gram-positive bacteria involves several mechanisms. Anion compounds including nitrates, chlorides, sulfides and organosulphur compounds can be easily resolved in water and are responsible for antibacterial properties[17]. Allicin in garlic is the most important substance that produces antibacterial properties and restricts the speed of RNA synthesis. Lipid is a part of Gram-positive membrane, which helps the easy penetration of allicin into the membrane and consequently influences the RNA[18]. Allicin has been reported to kill pathogens through partial inhibition of DNA and protein synthesis, alteration of the electrochemical ability, induce apoptosis in cells, affect microbial lipid biosynthesis, signal transduction, as well as react with thiol-containing proteins[19-21]. Our finding is consistent with previous studies stating that garlic extract showed a clear zone in the culture of Gram-positive bacteria[22,23].

In conclusion, these findings demonstrate the potential of garlic as a natural alternative to currently used antibacterial agent for Gram-positive infection in the tiger shrimp. Further researches related to the species identification of those two bacteria and increasing number of Gram-positive bacteria need to be carried out.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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