The Effectiveness Comparison of Single Bulb Garlic Extract for Antibacterial Agent \textit{P. aeruginosa}

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Abstract. Organosulfur compounds (Allicin, Ajoene) in single bulb garlic are responsible for antibacterial agents. Antibacterial activity by lipopolysaccharide (LPS) inhibiting the formation of the bacterial cell membrane. \textit{P. aeruginosa} is one of the bacteria that has LPS and belongs to the group Gram-negative bacteria. The purpose of this study was to determine the effectiveness ratio of gel preparation and not gel extract of single bulb garlic juice (SBGJ) as an antibacterial agent for \textit{P. aeruginosa}. The method used began by making gel and not gel preparations, stability test of gel preparation, bacterial preparation and culture, antibacterial test, and identification of bacterial morphological damage. The results showed that the gel preparation with SBGJ 100\% has the most appropriate characteristics for a good gel preparation. The bacteria were positively identified \textit{P. aeruginosa} bacteria and characterized by stem-shaped bacteria and red in Gram staining. The gel preparation and not gel have an antibacterial power of \textit{P. aeruginosa} based on the statistical analysis performed. Gel preparations with 100\% concentrations have significantly different antibacterial power levels compared with other preparations, even with control treatment using Ceftazidime. Damage of \textit{P. aeruginosa} bacteria with 100\% gel treatment showed that all bacteria had shrunk so that bacteria as if rounded, known through observation using Scanning Electron Microscope (SEM).

Keywords: Antibacterial agents, organosulfur compound, \textit{P. aeruginosa}, single garlic, SEM

1. Introduction

Burns are injuries obtained by someone unintentionally. Burns can be categorized from mild to severe. Severe categories of burns require a long healing time. During the healing process, burns can experience complications by various microorganisms, one of which is \textit{P. aeruginosa} bacteria. \textit{P. aeruginosa} bacteria is one of the pathogenic microorganisms that can cause serious complications in burns. This group of gram-negative bacteria and have a mechanism of inherent resistance to antibiotics [1, 2] with a range of 12-19\% [3]. The mechanism of resistance is obtained by the presence of LPS, the outermost layer of the negative gram bacterial membrane system [4], which is impermeable [1]. The structure of LPS in the membrane system caused the infection very difficult to treat. Treatment of \textit{P. aeruginosa} infection is still conducted using synthetic antibiotics.

Antibiotics that can be used for the treatment of \textit{P. aeruginosa} infection have a range of resistance between 22.5-50\% [5]. Improper use of antibiotics will cause many side effects, encourage the emergence of resistant bacteria [3], and even death [6]. This phenomenon has attracted researchers to
develop herbal-based antibiotics with minimal side effects. One herb that is widely used is single bulb garlic (*Allium sativum*).

The most important part of garlic is tuber which is widely used in everyday life. Garlic bulbs are divided into two types, tubers consisting of many cloves (Multi-bulb garlic) and one clove (Single bulb garlic). Tubers consisting of one clove are known as single bulb garlic or *bawang lanang* (Javanese). Single bulb garlic formed due to environmental influences that do not support the development of tuber, causing the garlic plant to only develop in one bud, the main shoot, and suppress the growth of other shoots so that a single bulb formed [7].

Garlic (*A. sativum*) has more than 200 chemical compounds such as active compounds (allicin, alliin, and ajoene), enzymes (allinase, peroxidase, and myosinase), carbohydrates (sucrose and glucose), various essential amino acids, and vitamins [8]. Organosulfur compounds are active compounds in garlic which contain the sulfur element. Organosulfur compounds are responsible for the aroma, taste, and pharmacological properties of garlic. Alliin, Allicin, and Ajoene are dominant organosulfur compounds in single garlic [9]. The organosulfur compounds have many pharmacological effects, such as an enhancement of T-regulatory [10] and an antibacterial agent.

Organosulfur compounds in garlic, such as Allicin, and Ajoene are bioactive compounds which are responsible as antibacterial agents [11]. The antimicrobial effects of organosulfur compounds are caused by several inhibitions on various dependent thiol enzymatic systems [12]. Antibacterial activity of organosulfur compounds was also obtained by inhibiting the formation of LPS in the bacterial cell membrane [13]. A study was conducted using ethanol extract of garlic against *P. aeruginosa* with the largest diameter of inhibitory power produced was 10 mm [14]. Accordingly, this study was conducted to determine the antibacterial effect of SBGJ.

2. Methods

2.1. Instruments and Materials

The instruments used in the research was glass beaker, measuring tube, stirring rod, metal spatula, mortar, pistil, pipette, filter cloth, a centrifugation tube, test tube, film bottle, knife, grater, micropipette, autoclave, analytical balance, refrigerator, incubator, and SEM. The materials used were gloves, masks, Carbomer940, TEA, dH2O, ddH2O, glycerol, absolute alcohol, alcohol 70%, MHA medium, *P. aeruginosa* bacteria, distilled water, Ceftazidime injection, garlic, single bulb garlic, tissue, label paper, and heat-resistant plastic.

2.2. Experiment

2.2.1. Preparation of Gel and Non-Gel Single Garlic Extract.

100 grams of SBG were peeled and was washed with distilled water. Single bulb garlic (SBG) cleaned then shredded and filtered using a filter cloth. Juice extracts were made in concentrations of 25%, 50%, 75%, and 100%. Some JESBGs used as the gel. 0.4 grams of carbomer940 were dissolved in 5 mL of ddH2O mixed with extract. Add 0.2 mL Triethanolamine (TEA) into the mixture, let stand for several hours until it expands. The mixture was put into a mortar and then stirred gently until a gel mass was formed. Then, 1.5 mL of glycerol was put into the mixture while stirred slowly. Add residual ddH2O which was mixed with the extract and stirred to form a good gel mass [14].

2.2.2. Stability Assay of Juice Extract of Single Bulb Garlic (JESBG).

The stability assay of gel served to determine the quality of the gel that has been made. The stability assay of gel carried out were (a) Organoleptic test by observing the coloring, smell, and gel form [15], (b) pH test, 2 grams of preparation dissolved in 10 mL of distilled water, ad to 20 mL of distilled water, stirred until evenly distributed and measured with a pH meter, (c) a viscosity test, 20 mL of gel was poured into a tubular container and a spindle 64 was installed, the viscometer was turned on and made sure the rotor was capable of rotating at 60 rpm, observe the pointer from the viscometer that
points to the number on a viscosity scale and multiply by a factor of 100. (d) the scattering power test, 0.5 grams of prepared gel placed on a round glass with a diameter of 15 cm, other glass placed on it and left for 1 minute, measure the diameter of the spreading gel, add 100 grams of additional load and allow to stand for 1 minute, then measure the constant diameter [16].

2.2.3. Preparation of Bacterial Culture.
This preparation was conducted by taking one dose of bacteria from NA. Inoculate on NB medium. Then it was vortexed to be homogeneous, then incubated at 37 °C for 1×24 hours. The test of bacterial suspension on the NB medium that was incubated was measured using a spectrophotometer at a wavelength of 625 nm so that the known Optical Density (OD) was 0.1 equivalent to 108 CFU/mL [17].

2.2.4. Identification of P. aeruginosa.
The identification of P. aeruginosa was done by Gram staining; (1) bacterial colonies were placed on object glass, dripped with sterile distilled water, and then air dried, (2) dried preparations fixed by passing over Bunsen fire, (3) the specimen dripped with crystal violet, allowed to stand for 1 minute, rinsed with running water, (4) the specimen was dripped with Lugol and allowed to stand for 1 minute, rinsed with running water, (5) an absolute alcohol dripped to the specimen and allowed to stand for 5-10 seconds, then rinsed with running water, (6) safranin dripped to the specimen and allowed to stand for half a minute, rinsed with running water, (7) dried with suction paper, poured emersion oil and viewed under a microscope using 100× magnification, and (8) positive results indicated by the presence of red-colored and stem-shaped bacteria.

2.2.5. Antibacterial Assay.
Antibacterial test was carried out using well diffusion method; (1) made wells on Muller Hinton Agar medium which had been inoculated with bacteria, (2) each antibacterial substance included into the well, (3) the specimen incubated at 37 ºC for 1×24 hours, (4) the diameter of the inhibitory zone measured with calipers, and (5) the measurement results were analyzed.

2.2.6. Identification of Morphological Damage of P. aeruginosa.
The preparation of the SEM, (1) pure bacterial suspension centrifuged at 3500 rpm for 15 minutes, (2) the supernatant discarded and the precipitate washed twice with the phosphate buffer and centrifuged again, (3) 2% glutaraldehyde with a pH of 7.3 added on the precipitate and allowed to stand for 1-2 hours, (4) 2% tannin acid added to the sediment, allowed to stand for 1-2 hours, (5) cacodylate buffer added to sediment, allowed to stand for 20 minutes, (6) 1% Osmium Tetroxide added, allow it to stand for 1 hour, (7) 50% alcohol added to the precipitate, let stand for 20 minutes, (8) add 70%, 80% alcohol, respectively 95%, allowed to stand for 10 minutes, added absolute alcohol and allowed to stand for 20 minutes, (9) centrifuge at 3500 rpm for 10 minutes, (10) add t-butanol to the sediment and leave it for 20 minutes, do this step twice , (11) a suspension made in the butanol (12) a thin smear made of suspension on the frozen slip cover, (13) air-dry the slip cover.

2.2.7. Data Analysis.
The data obtained from the research on inhibition zone diameter were first tested for normality and homogeneity test then analyzed with One Way ANOVA at a significance level of 5% to determine whether there was any effect of various concentrations of juice extract of single bulb garlic on P. aeruginosa. Statistical analysis was carried out with the help of statistical software. Damage to bacterial morphology was analyzed by describing the type of damage on the cell membrane of the bacteria using Scanning Electron Microscopes.
3. Results and Discussion

3.1. Stability Assay of JESBG Gel

Table 1 shows in an organoleptic test the basic gel and gel formula has a soft and chewy texture. The basic gel is clear and colorless; the color of gels in various concentrations ranged in yellowish white to slightly bright yellow with a specific odor of SBG. The pH level of the gel (G50%, G75%, and G100%) is in accordance with the criteria for skin pH range by 4.5 to 6.5 [18]. Viscosity test shows that only the basic gel meets the requirements of a good viscosity value of 2000-4000 cps [19]. The viscosity value that does not meet the requirements is due to the practitioner's error who tested the gel 2 weeks after the gel is made. It is known that the higher the viscosity value of the gel, the smaller the dispersion phase separation rate and the more stable the gel is. Viscosity test data is known that the higher the SBG concentration, the higher the viscosity of the gel. The spread of good preparation ranges from 5-7 cm [20]. The spread ability of gel that meets the criteria is the G100% preparations. The greater the spread ability of the gel, the better the distribution of the gel when applied to the skin [20].

Table 1. The result of Stability assay of JESBG Gel.

| No | Specimen code | Texture | Organoleptic | pH | Viscosity (cps) | Dispersibility (cm) |
|----|---------------|---------|--------------|----|----------------|---------------------|
| 1  | BG            | Soft Chewy | Clear, colorless | 7.94 | 3600 | 5.6 |
| 2  | G25%          | Soft Chewy | Yellowish white | 6.65 | 30   | 8.3 |
| 3  | G50%          | Soft Chewy | Yellowish white | 5.75 | 145  | 7.6 |
| 4  | G75%          | Soft Chewy | Pale yellow   | 4.85 | 190  | 7.4 |
| 5  | G100%         | Soft Chewy | Slightly bright yellow | 5.54 | 630  | 6.2 |

BG (basic gel)
G25%-100% (gel used for four different concentration of the treatment)

Figure 1. P. aeruginosa in Gram Staining (100× magnification)
3.2. Identification of *P. aeruginosa*

Figure 1 shows a positive result for *P. aeruginosa* that is characterized by the presence of red stem-shaped bacteria. Gram staining is a differential staining used for characterization and discovery of Gram-positive and negative bacteria [21]. *P. aeruginosa* is a Gram-negative bacterium that absorbs safranin dye, which is observed in bacteria seen as red [22]. The staining results are obtained due to the presence of a different membrane structure in gram-negative bacteria [14].

3.3. Antibacterial effect of Gel and Non-Gel JESBG

**Table 2.** The result of the antibacterial assay

| No | Treatment | Concentration | Inhibitory zone diameter (mm) | Category |
|----|-----------|---------------|-------------------------------|----------|
| 1  | Gel       | 25%           | 14                            | Strong   |
|    |           | 50%           | 18                            | Strong   |
|    |           | 75%           | 19                            | Strong   |
|    |           | 100%          | 21                            | Very Strong |
| 2  | Non-Gel   | 25%           | 0                             | -        |
|    |           | 50%           | 7                             | Weak     |
|    |           | 75%           | 8                             | Weak     |
|    |           | 100%          | 9                             | Weak     |
| 3  | Basic Gel | -             | 0                             | -        |

Table 2 shows that the concentration of 100% JESBG on a gel and the non-gel specimen has the largest diameter of inhibitory 21 and 9 mm. The following is mean and notation of the antibacterial effect of gel non-gel of JESBG from Duncan’s test.

**Table 3.** Means and notations from Duncan’s test

| Treatment* | Mean |
|------------|------|
| NG25       | 3.0000a |
| NG50       | 6.6667b |
| NG75       | 7.6667bc |
| NG100      | 8.6667c |
| G25        | 13.6667d |
| G50        | 17.0000e |
| C+         | 18.0000f |
| G75        | 18.6667c |
| G100       | 21.0000f |

*NG*: Non-Gel  
*G*: Gel  
*C+*: Positive control (*Ceftazidime*)

Statistical analysis shows that all data in the study were normally distributed and homogeneous with p values > 0.05 (Attachment 3). One Way ANOVA test showed that the significance value of antibacterial inhibition was 0.000 (p < 0.05) (Attachment 3) so that it could be concluded that gel and non-gel preparation of JESBG had an antibacterial effect in inhibiting the growth of *P. aeruginosa*. The antibacterial effect of gel with 100% concentration of JESBG had significantly different results compared to other preparations (Table 3).

The diameter of the inhibitory zone is the diameter of the clear zone that formed around the well after treatment with the gel and non-gel of JESBG. The diameter of the inhibitory zone determines the amount of antibacterial effect given from the material being tested. The minimum inhibitory zone for
sensitive and strong categories based on CLSI criteria standards is ≥ 15 mm to ≤ 20 mm [23]. The amount of inhibition obtained in gel specimen with a concentration of 100% JESBG shows that the specimen contains a significant antibacterial effect to inhibit the growth of *P. aeruginosa*.

Allicin is an organosulfur compound as an antibacterial agent by inhibiting RNA synthesis and lipid synthesis [24]. Inhibition of RNA synthesis is done by forming very strong bonds in the Dependent DNA enzyme and RNA bacterial polymerase [25]. Carbomer used as a gel base is a transparent gelling agent and is biodegradable. Bioadhesive is the nature of drug preparations that can be attached to the membrane surface easily. These properties make extracts that have been mixed with a gel base easily attach to the bacterial membrane so that they can inhibit growth and even kill bacteria quickly.

3.4. Morphological Damage of *P. aeruginosa* by SEM

Figure 2 shows that at a concentration of 100% JESBG, *P. aeruginosa* suffered cell wall damage showed by the cell being rounded. These results are inversely proportional to the positive control treatment using Ceftazidime with a concentration of 30 µg/mL and negative control (only distilled water) which showed no changes in the shape of *P. aeruginosa* cells. Changes in the cell shape from the stem-shaped into a round due to a hydrophilic characteristic of the active compounds contained in the extract of a single garlic extract, so that they can pass through the cell membrane system. Changes in cell shape are caused by damage to LPS.

![Figure 2](image)

The increasing permeability of *P. aeruginosa* cell membrane caused shrinkage and phospholipids damage. Antibacterial compounds that attacked phospholipids broke down the phospholipid into several compounds such as glycerol and phosphoric acid. This damage made phospholipids unable to maintain the shape of the cell membrane which resulting leakage in the bacterial cell membrane [26]. The shrinkage of the *P. aeruginosa* cell membrane is causing cell lysis. Cells that experienced morphological lysis appeared partially or completely. Antibacterial compounds could react with the phospholipid component of gram-negative bacteria cells and caused lysis of the cell wall [27].
4. Conclusion
All gels have a soft, chewy form, yellowish white to a slightly bright yellow, and a garlic-specific odor based on high concentration. Gel with a concentration of 100% based on the gel stability test which includes pH, viscosity, and spread ability, have the most suitable characteristics for a good gel. Gram staining shows that bacteria that are positively identified by P. aeruginosa bacteria are characterized by the presence of red rod-shaped bacteria. Statistical analysis showed that all antibacterial inhibitory data were normally distributed and homogeneous, so ANOVA analysis was performed and showed a significance level of p <0.05. This result can be concluded that gel and non-gel JESBG have antibacterial power against P. aeruginosa. Gel with 100% concentration of JESBG has an antibacterial effect which is significantly different from other specimen and even with control treatment using Ceftazidime. Damage to P. aeruginosa caused by 100% gel treatment shows that all bacteria are shrinkage and look like are rounded are known through observation using SEM.

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