Antibacterial activities of Solo garlic

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Abstract. Solo garlic, either in fresh form or its black garlic has a lot of bioactive compounds, such as flavonoids and polyphenols. The aim of this research was to evaluate the antibacterial activity of organic solvents extract’s solo garlic inside the fresh and black garlic form against pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus*, by using agar diffusion and dilution methods. The diffusion test shown that BG4 had the highest inhibitor activity comparing the other treatments. Concentration of BG4 1 mg/ml, either in isopropanol or ethyl acetate was able to inhibit the growth of *E. coli* and *S. aureus*. Based on that results, solo black garlic especially 3 or 4 weeks-fermented solo black garlic could be considered as a therapeutic agent.

1. Introduction

Indonesia is well known around the world for its biodiversity. It is also mention as one of the world’s richest nations in terms of its biodiversity. For centuries, local people have been exploring and utilizing the benefit of this, especially parts of plants as herbal medicine. According to Agarwal and Chauhan [1], herbal medicines are the people's preference because they are cheap and relatively safe. Moreover, WHO reports due to the occurrence of prolonged illness and increased mortality caused by antibacterial resistance affecting people to live with natural products [2]. Secondary metabolites from plants are believed to have antibacterial properties. Some studies mention groups of compounds that are having antibacterial effects, include tannins [3], flavonoids, phenolics [4], alkaloids and saponins [5].

Antibacterial mechanisms are divides into bacteriostatic and bactericidal. Bacteriostatic can inhibit the multiplication of bacterial populations while bactericidal is an antibacterial that can kill bacteria. Bacteriostatics can play a role as bacteriocides in high concentrations [6]. Furthermore, Pelczar and Chan [7] mentioned that the mechanism of antibacterial from compounds in general is done by damaging cell walls, changing membrane permeability, disrupting protein synthesis, and inhibiting the work of enzymes. Antibacterial activity can be tested by two methods, by agar diffusion and dilution. The diffusion method is divided into the disk, wells and trenches. The dilution method is divided into broth dilution and solid dilution. The difference between the methods occurs based on the media used.

In this study, an antibacterial activity test of black garlic was done to *Staphylococcus aureus* and *Escherichia coli*. Solo black garlic is single garlic that is fermented spontaneously at a certain temperature and humidity. Processing with incubation at high temperature and humidity causes garlic to changed its color from white to brown and eventually became black due to the Maillard and Browning reaction process [8,9]. Solo black garlic is known to contain high amounts of flavonoid compounds, antioxidants and polyphenols [10,11].

According to Wang et al. [12], *E. coli* is a pathogenic bacterium in humans which causes digestive disorders and disrupts the working system of the stomach. This bacterium is also a major cause of
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E. coli and S. aureus are the most common pathogenic bacterial which cause of infection in the world, with varying severity of infections, ranging from minor infections of the skin (furunculosis and impetigo), urinary tract infections, respiratory tract infections, respiratory infections and the Central Nervous System (CNS) [13]. The purpose of this study was to investigate antibacterial activity of solo garlic, either in fresh form or black garlic in organic solvent by diffusion and dilution methods.

2. Materials and Methods

2.1. Materials
Garlic samples used in this study were fresh solo garlic (FG), one week fermented solo garlic (BG1), two weeks fermented solo garlic (BG2), three weeks fermented solo garlic (BG3) and four weeks fermented solo garlic (BG4). Solo garlic was fermented spontaneously on an incubator at 72°C and humidity close to 80%. The tested bacteria used were S. aureus ATCC 25923 and E. coli ATCC 25922. Chloramphenicol was used as a positive control. The media used are Nutrient Agar (NA) and Nutrient Broth (NB).

2.2. Extraction of active component of solo garlic
Solo garlic samples both FG and BG were extracted by maceration with isopropanol or ethyl acetate. Forty grams was dissolved in 200 ml of solvent and macerated at room temperature for 24 hours. The maceration solution was evaporated by using vacuum until the volume becomes 40 ml. So, the initial concentration of the extract was 1 g/ml.

2.3. Tested Bacteria Preparation
Tested bacteria were rejuvenated with NB media. One inoculant of tested bacteria was subjected in 5 ml of sterile NB and incubated for 24 hours at 37°C. Rejuvenation is repeated up to 3 times. The fresh culture of the tested bacteria viability was determined by the method of Total Plate Count (TPC).

2.4. Determination of Flavonoid
A mixture of 30 µl ethanol, 50 µl aluminum chloride, 50 µl potassium acetate and 600 ml water were added to a 50 µl of sample. The sample mixture was incubated for 30 minutes. Next, sample absorbance is measured on wavelength of 415 nm. Flavonoids identified as quercetin equivalent (QE) is derived by comparing sample absorbance to dilution absorbance of standard quercetin [14].

2.5. Antibacterial activity of black garlic using diffusion method
One inoculant of tested bacteria was dissolved into 10 ml NB and incubated at 37°C for 24 hours. Bacterial concentration was determined by optical density (OD) and TPC measurements. The concentration of bacteria used was 10⁶ CFU / ml. A hundred µl of bacterial suspension was spread on NA media. Six mm diameter disc paper was prepared and 10 µL fractions with a concentration of 1 mg/ml were dropped. The solvent used to extract solo black garlic was used as negative control, while 1 mg/ml of chloramphenicol (CMP) was served as positive control. Each disc was placed on the surface of the agar medium and incubated at 37°C for 24 hours. Antibacterial activity was determined based on the clear zone produced. Each treatment was carried out three times [15].

2.6. Antibacterial activity of black garlic using dilution method
Solo black garlic samples which showed the highest results in the diffusion method were made in the following dilution series 0.25 mg/ml; 0.5 mg/ml; 0.75 mg/ml and 1 mg/ml, both with isopropanol and ethyl acetate solvents. Antibacterial activity test was done by determining Minimum Inhibitor Concentration (MIC) against S. aureus and E. coli. The results of the antibacterial activity test of each test solution level were compared with controls. Determination of MIC from each series of levels can be seen from the turbidity of the test tube, or by measuring the absorbance at 500 nm absorption [16].
3. Result and Discussion
In this study, a solo garlic, either in the form of fresh garlic or black garlic is extracted using maceration method. The benefit of this method is its feasibility and trait not to utilize heat to perform the process, as a result its natural elements is less likely to deteriorate and break down. Dilution is preferred based on its dilution and polarity level which favorable to separate bio-active compound in the sample. Provided maceration process runs fairly lengthy and static enabling many compounds can be extracted. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity [17]. In this study, an organic solvent i.e. isopropanol and ethyl acetate were chosen considering plant extracts in the organic solvent have been known to have higher bactericidal activities than that of water [18].

Total flavonoids content obtained from different solvents was shown in Table 1. Both fresh garlic and black garlic showed higher number in isopropanol than ethyl acetate solvents. Black garlic fermented in 4 weeks presented 19 times higher than fresh garlic. Heat treatment in black garlic processing has a large influence on flavonoid availability. Ioannou et al. [19] stated that heat treatment possibly increases the total flavonoids contents. This result in accordance with Choi et al. [20] which reported that the total flavonoid content of BG was not only significantly higher than that of fresh garlic, but also increased significantly up to the 21st day of aging, after which the continued to increase slightly for the remainder of the aging period.

Table 1. Flavonoid concentration resulted from solo garlics (fresh and black garlic).

| Sample | The total flavonoid content in solo garlic (mg QE/g) |
|--------|---------------------------------------------------|
| FG     | 20.90 isopropanol 22.84 ethyl acetate            |
| BG1    | 53.56 isopropanol 49.85 ethyl acetate            |
| BG2    | 64.56 isopropanol 62.12 ethyl acetate            |
| BG3    | 375.11 isopropanol 308.49 ethyl acetate          |
| BG4    | 408.62 isopropanol 391.12 ethyl acetate          |

Flavonoids are semi-polar compounds and can be extracted in semi-polar solvents. Therefore, in this study, isopropanol and ethyl acetate were used to compare between polar and non-polar solvents in garlic activities. The highest number of total flavonoids was obtained in BG4 extracted in isopropanol which contain 408.62 mg QE/g. The result was contrary to Bozin et al. [21] and Jang et al. [22], which stated that total flavonoids content in polar solvents were found better than non-polar. According to Bozin et al. [21], total phenol and flavonoids contents of 80% methanol extract from black garlic higher (0.98 mg GAE/g and 6.99 lg QE/g, respectively) than that from fresh garlic. This result was corresponded with Jang et al. [22] with the findings that water extract from black garlic showed the highest total phenols and flavonoid contents. The significant increase in total phenol and flavonoids in black garlic might be due to the conversion of some components in garlic into these highly hydrophilic compounds. Another study revealed that flavonoid compounds in plants extracted in a less amount in another organic solvent such as methanol and petroleum ether [17]. This evidence may be due to another possibility i.e. dielectric constant in ethyl acetate is lower than that in isopropanol solvent [23].

Table 2. demonstrated antibacterial activities of solo garlics in its fresh and black-garlic form. Generally, it can be seen that the sample resistance-zone was relatively bigger in S. aureus than E. coli. The result was possibly due to the structure of cell wall of Gram-positive bacteria which is simpler and enables anti-bacterial compounds to penetrate easily in to the cells and finding a suitable place to operate. On the contrary, the structure of cell wall of Gram-negative bacteria is more complex, comprised of three layers of lipoprotein, peptidoglycan and lipopolysaccharide. Likewise, a similar
A study done by Nguyen et al. [25] mentioned fermented black garlic to have antibacterial activities towards *Listeria monocytogenes* and *Salmonella* sp.

### Table 2. Antibacterial activity of fresh solo garlic and its black garlic by diffusion method

| Bacteria   | Solvent  | Sample (1 mg/ml) | Resistance zone (cm) |
|------------|----------|------------------|----------------------|
| *S. aureus* | Ethyl acetate | FG 1 | 0.50 |
|            |          | BG1  | 0.61 |
|            |          | BG2  | 0.85 |
|            |          | BG3  | 1.79 |
|            |          | BG4  | 2.07 |
|            | Isopropanol | FG1  | 0.58 |
|            |          | BG1  | 0.63 |
|            |          | BG2  | 2.73 |
|            |          | BG3  | 2.71 |
|            |          | BG4  | 2.82 |
| *E. coli*   | Ethyl acetate | FG 1 | 0.74 |
|            |          | BG1  | 0.75 |
|            |          | BG2  | 0.86 |
|            |          | BG3  | 1.52 |
|            | Isopropanol | BG4  | 1.89 |
|            |          | FG1  | 0.70 |
|            |          | BG1  | 1.19 |
|            |          | BG2  | 2.27 |
|            |          | BG3  | 2.20 |
|            |          | BG4  | 2.40 |
| Control    | Positive: CMP (1 mg/ml) | | 1.47 |
|            | Negative 1: Isopropanol | | 0.10 |
|            | Negative 2: Ethyl acetate | | 0.15 |

Among approaches of the sample, BG4 possessed the biggest resistance zone relative to the other samples. This was possibly due to formed flavonoid compounds in BG4 was higher than other samples which was clearly shown in Table 1. A study conducted by Setiyoningrum et al. [10] stated that a longer fermentation period of solo garlic would result in a higher amount of flavonoid compounds. Taken together, antibacterial compounds produced through maceration using isopropanol create relatively wider resistance zone compared to ethyl acetate, both in *S. aureus* and *E. coli*. This may be evident because flavonoid or other anti-bacterial compounds are extracted more in isopropanol than in ethyl acetate. Average resistance zone of BG3 in all solvents was higher than antibiotic chloramphenicol used as positive control. Hence, these finding indicate that fresh or black form of solo garlies could be potentially used as an antibacterial agent.

MIC is defined as the highest dilution or least concentration of extracts that inhibits organism growth. MIC determination is essential in a lab diagnostic since it is pivotal in assisting confirmation process of microorganism-resistance towards anti-microbe genes and monitoring the activities of new anti-microbe agents [26]. The MIC test was performed to BG4 sample which has the largest resistance zone during diffusion method. The MIC BG4 value in isopropanol or ethyl acetate solvent is provided in Table 3. Isopropanol extract of BG4 showed the MIC value of 0.75 mg/ml on *S. aureus* while ethyl acetate extract showed a value of 1 mg/ml. Preethi et al. [27] reported that alcohol extract possesses a trait as a better anti-bacterial solvent than other organic solvents. Isopropanol extract and ethyl acetate extract have the same MIC level with *E. coli* i.e. 1 mg/ml. This was possible because *E. coli* cell wall is more complex.
than *S. aureus* and the antibacterial mechanism of plant extract involves the simultaneous disruption of the cell membrane [28].

| Bacteria   | Solvent       | Concentration of extract (mg/ml) | Turbidity* |
|------------|---------------|---------------------------------|------------|
|            | Ethyl acetate | 1                               | -          |
| *S. aureus*| 0.75          | +                               |
|            | 0.5           | +                               |
|            | 0.25          | +                               |
|            | Isopropanol   | 1                               | -          |
|            | 0.75          | -                               |
|            | 0.5           | +                               |
|            | 0.25          | +                               |
| *E. coli*  | Ethyl acetate | 1                               | -          |
|            | 0.75          | +                               |
|            | 0.5           | +                               |
|            | 0.25          | +                               |
|            | Isopropanol   | 1                               | -          |
|            | 0.75          | -                               |
|            | 0.5           | +                               |
|            | 0.25          | +                               |

4. Conclusion
The BG4 had the biggest resistance zone relative to the other samples, in isopropanol extract or ethyl acetate. Both of extract solvents of BG4 had the MIC level against *E. coli* i.e. 1 g/ml. Otherwise, isopropanol extract of BG4 showed lower MIC against *S. aureus* than ethyl acetate.

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