Phytochemical Compound of Garlic (Allium sativum) as an Antibacterial to Staphylococcus aureus Growth

F T Yunus¹, A Suwondo¹ and Martini¹,*
¹Magister Epidemiology, School of Postgraduate Program, Diponegoro University, Semarang, Central Java, 50275, Indonesia

*E-mail: tinihen65@yahoo.co.id

Abstract. Antibiotics use to bacterial infection control causes many side effects from mild to severe. In addition to severe side effects such as shock, antibiotics can cause resistance. Therefore we need an alternative medicine that functions as an antimicrobial derived from plants. Garlic has minimal side effects and can be consumed daily. This study aims to know the phytochemical content and antibacterial effectiveness of Garlic in inhibiting the growth of Staphylococcus aureus. This study used a pure experimental design with randomized posttest. The control group design are giving fresh garlic, garlic extract and garlic powder to bacterial culture media through the dilution method. The phytochemical content in 3 types of preparations are tested using Flavonoid, GCMS, FTIR and HPLC Tests. Data analysis uses Kruskal Wallis test while the Mann Whitney test is used to measure the concentration significance. Out of the 3 types of preparations the one that has more phytochemical compound through the GCMS test is fresh garlic (18 active ingredients) compared to garlic extracts (14 active ingredients) and garlic powders (13 active ingredients). Fresh garlic with a concentration of 50% has more antibacterial activity compared to garlic extracts and garlic powder. When compared with positive control (amoxcilin), fresh garlic has not been able to provide effective results in inhibiting Staphylococcus aureus growth. Phytochemical compound in fresh garlic are more numerous compared to the 2 other preparations and fresh garlic has not been able to provide effective results in inhibiting the Staphylococcus aureus.

Introduction
Infectious disease is one of the biggest health problems not only in Indonesia but also the world. Infectious disease is also one of the leading causes of death in the world. Other than virus, bacteria can also cause infectious disease [1]. Centers for Disease Control and Prevention (CDC), America, estimates that in the United States alone around 23,000 people die each year due to antibiotic-resistant infections. In 2014, World Health Organization (WHO) estimates 20% of people with antibiotic resistance occur in recurrent cases. Antibiotic-resistant bacterial infection with recurrent cases are Staphylococcus aureus (or MRSA, the resistant variant), Pneumonia, Escherichia coli, urinary tract infections, gonorrhea and malaria [2]. With high cases of resistance due to side effects of antibiotics, researchers tried alternative medicines that acted as natural antibiotics from medicinal plants.

Staphylococcus aureus bacterial infectious diseases such as pneumonia, UTI gonorrhea and malaria can be managed with pharmacology, in the form of antibiotics and nonpharmacology. The use of antibiotics has the disadvantage of causing resistance because of incorrect indications and dosage usage [3-6]. On the other hand, the use of non-pharmacology such as
plants or traditional medicine made by natural ingrediants have minimal or no side effects [3-4].

Garlic (*Allium sativum*) is a plant-based food which is consumed everyday and is known to have strong natural antibiotic with a wide spectrum. Phytochemical contents that act as antibacterial include essential oils, allicin, ajoene and flavonoids. Essential oils and allicin inhibits the synthesis of RNA, DNA and bacterial proteins. Ajoene plays a slow role in inhibiting bacteria while flavonoids play a role in denaturing proteins to inhibit bacterial growth. All four of the phytochemical contents above effectively eradicate the bacterial wall [3,7-10]. This study aims to determine the phytochemical content and antibacterial effectiveness of fresh garlic, garlic powder and garlic extract on the growth of *Staphylococcus aureus* bacteria.

**Description of the Model**

This study used a pure experimental design with randomized posttest with control group design that is giving fresh garlic, garlic extract and garlic powder to the bacterial culture media through the dilution method. Data is analyzed using *Kruskal Wallis* test and the concentration significance is determined using *Mann Whitney* test. Both are processed using SPSS.

The independent variable in this study is garlic which is given different treatment and made into 3 forms; fresh garlic, powder form and extract form. The first form is blended fresh garlic. Second, garlic is made into powder using a cabinet dry device at a temperature of 100 F and a pressure of 10 Pa with a particle size of 64 micro. Third, the onion is extracted through a maceration process using a water solvent. All three forms are made into different concentrations; 12.5%, 25% and 50%.

The dependent variable in this study was the inhibitory growth of *Staphylococcus aureus* bacteria which had been isolated on Mannitol Salt Agar (MSA) and incubated at 37 C for 24 hours. Observation and measurement of the inhibition zone diameter is done every 24 hours for 3 days using calipers. The greater the diameter of the clear zone formed, the greater the bacterial inhibition activity. The inhibition of bacteria is classified as strong if the inhibition zone is 10-20 mm. Whereas an inhibition zone of 5-10 mm shows moderate antibacterial activity. Finally, an inhibition zone of less than 5 mm indicates weak antibacterial antactivity.

Phytochemical screening is carried out to determine phytochemical content by flavonoid testing. Flavonoid test is to measure qualitatively the presence or absence of flavonoids in 5 ml of each garlic form using magnesium and hydrochloric acid. The FTIR test measures the similarity of the composition of chemical groups in 10 mg of each garlic form. The GCMS test measures the phytochemical profile contained in 1 µl of each garlic form. The bacterial inhibitory delusion method is carried out to determine the antibacterial activity

**Results and Discussion**

The yield of quarcetin in fresh garlic was 81.181%, lower than that of garlic extract 458.729% and garlic powder 169.409%. Low quarcetin levels indicate a different garlic mass for each treatment. In this study, fresh garlic, garlic extracts and garlic powders shows meaningful results on the first and second days while on the third day only fresh garlic shows meaningful results. If you consider quarcetin as a type of flavonoid that functions as an anti-microbial, the highest should be garlic extract followed by garlic powder. Both of them should still have meaningful results on the third day, but only fresh garlic – which has less Quarcetin compared to garlic extract and powder - still gives a meaningful results on the third day [11].
**Table 1. Flavonoid and Quercetin Test Results**

| No | Forms          | Flavonoid   | Quercetin   |
|----|----------------|-------------|-------------|
| 1  | Fresh Garlic   | Positif (+) | 81,181%     |
| 2  | Garlic Ekstract| Positif (+) | 458,79%     |
| 3  | Garlic Powder  | Positif (+) | 169,49%     |

Based on previous research, quercetin does act as an antibacterial against *Staphylococcus aureus* [12-14]. But studies where the quercetin is high doesn’t show a meaningful result on the third day. This is because garlic among the 4 main types of ingredients (essential oils, allicin, flavonoids and ajoene) the dominant one that plays a role in inhibiting bacterial growth against *Staphylococcus aureus* is allisin. So in other words, even though the increased amount of quercetin in high garlic extract and powder may not necessarily be able to optimally inhibit bacterial growth. This can be seen on the third day, the garlic extracts and powders are static in bacterial inhibition [11, 15-16].

The exact dose of quercetin depends on several factors such as the user's age, health, and several other conditions. At present there is not enough research information to determine the appropriate dosage range for quercetin. Keep in mind that natural products are not always safe and dosage can be important. While from existing research, there are also those who state that the optimal quercetin dose is around 500-1000 mg [11,16].

**Figure 1. FITR Test Result**

Figure 1. shows the purple line (fresh garlic), the green line (garlic powder) and the orange line (garlic extract). The three lines coincide with one another. This shows that the composition of the chemical groups in the 3 treatments of garlic are almost the same or there is no big difference.

FTIR test on fresh garlic, garlic extract and garlic powder showed no difference in
chemical structure even with 3 different treatments. In a previous study, Azeem, 2017, stated that *Allium zativum* (garlic) has phytochemical content, namely glycosods, tannins, alkaloids, saponins and flavonoids which act as an antibacterial agent. The results of this study indicate that the phenol content is higher in fresh garlic compared to garlic powder and extract [17]. Ramesh's research, 2017, shows that there are the same phytochemical content in garlic extracts and powders containing alkaloids, proteins, lipids, oils, flavonoids, phenols, saponins, steroids, tannins, and terpenoids [18]. The results of Lowny's research, 2019, stated that the photochemical content of garlic extract and garlic powder showed a similar chemical structure to this study [19]. In other words, the phytochemical content between fresh garlic, garlic extract and garlic powder shows chemical groups that are almost the same through the FTIR method.

This study uses GC-MS method to show the chemical content of all 3 forms of garlic. The result shows that fresh garlic has the highest chemical content with 17 phytochemical profiles followed by garlic powder (14 phytochemical profiles) and garlic extract (13 phytochemical profiles). A previous research by Manuela Stan, 2017, stated that fresh garlic has better and more the chemical content than garlic powder through the GC-MS method [20].

**Table 2. Recapitulation of Chemical Content Profile of Fresh Garlic, Garlic Extract and Garlic Powder**

| No | Profile of chemical content | Fresh Garlic | Garlic Extract | Garlic Powder |
|----|-----------------------------|--------------|----------------|--------------|
| 1  | O-Butylisourea               | √            | -              | -            |
| 2  | 2-Furancarboxaldehyde (CAS) | √            | -              | -            |
| 3  | 3,3-Dimethoxy-2-Butanone    | √            | -              | -            |
| 4  | 1,2-Cyclopentanediene       | √            | -              | -            |
| 5  | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | √ | - | √ |
| 6  | Formic acid, 2-propenyl ester (CAS) | √ | - | - |
| 7  | Benzeneacetaldehyde (CAS)   | √            | -              | -            |
| 8  | 2-furancarboxaldehyde,5-hydroxymethyl | √ | - | - |
| 9  | Trisulfide,di-2-propenyl    | √            | √              | √            |
| 10 | 1,3-propanediol,2--hydroxymethyl-2-nitro | √ | - | - |
| 11 | Hexadecanoat acid, methyl ester (CAS) | √ | - | - |
| 12 | n-Hexadecanoic acid         | √            | √              | √            |
| 13 | 9,12-octadecadienoic acid (Z,Z) | √ | √ | √ |
| 14 | Octadec-9-enoic acid       | √            | -              | √            |
| 15 | Octadecanoic acid          | √            | √              | √            |
| 16 | 2,6,6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene | √ | - | - |
| 17 | Stimast-5-en-3-ol           | √            | -              | -            |
| 18 | 1,3-Dioxolane-4-methanol,2-ethyl-(CAS) | - | √ | - |
| 19 | Decane                      | -            | √              | -            |
Wanja, 2018, found a different result. He found that there are 10 main phytochemical content in garlic extract by GC-MS method which functions as an antibacterial and antioxidant. The difference in the number of phytochemical content profiles based on the volume of each preparation or type of GC-MS used can affect the number of phytochemical content profiles in the type of garlic preparations [21].

The weakness of this study is that it cannot be ascertained whether the amount of solute taken actually represents the measurement or there might be more of the solvent (water). In addition, ready preparations are not directly measured but take about 1-2 weeks because the equipment used is still in the queue. Although it has been put in the refrigerator but it still affects the phytochemicals in the garlic.

The results shown by 3 treatments on days I, II and III showed significant results at each concentration. There was a difference on day III, especially the treatment using fresh garlic at each concentration which showed a significant difference. The P value was less than / equal to 0.05.

**Table 3. Inhibition for Kruskalis Wallis analysis and Mann Whitney post hoc analysis**

| Treatments                  | Concentration (%) | Average | The Average Inhibition Property Amongst Each Treatment |
|-----------------------------|-------------------|---------|--------------------------------------------------------|
| Negative Control (-)        | 0,23^a            | 2,00^a  |                                                        |
| Fresh Garlic                | 12,5 2,17^e       | 5,67^g  | 10,33^C                                                |
| Garlic Extract              | 12,5 1,63^b       | 6,67^g  |                                                        |
Treatments | Concentration (%) | Average | The Average Inhibition Property Amongst Each Treatment
---|---|---|---
 | 25 | 2.03$^d$ |  
 | 50 | 2.73$^f$ |  
 12.5 | 2.05$^e$ |  
Garlic Powder | 25 | 1.93$^e$ | 7.00$^b$  
 | 50 | 2.43$^e$ |  
Positive Control | 10,60$^h$ | 14.00$^D$ |

Safitri’s research showed different result. The inhibition test of garlic extract on the growth of Staphylococcus Aureus bacteria with water solvent showed significant results [10]. Shorkalso showed significant results on the inhibitory test of garlic bacteria on the growth of Staphylococcus Aureus with concentrations of 4%, 15% and 25%. The difference in this research is that observation and analysis is done every day for 3 days consecutively while the observations and analysis of other studies are accumulative of the specified period [22-24].

The results of this study are almost the same as some previous studies. Lemar’s Research, 2012 states that between fresh garlic with the best garlic powder as an inhibitor of germs in this case is a fungus / anti-candida is fresh garlic. Although this study uses different variables, the results of this study shows that fresh garlic is the best as anticandide / antimicrobial [25]. Uzodike, 2015 and also El mahmood, 2013 found that garlic extract has good inhibitory properties against Staphylococcus aureus which contradicts the result of this study [24, 26]. Meanwhile Ekwenyem found that Garlic extract does not have bacterial inhibition properties against E. Coli and S. Typhi bacteria [23].

Garlic extract and powder on the first and second day of observation can inhibit Staphylococcus aureus but the inhibition zone diameter becomes static or does not change at all on the third day. In contrast, fresh garlic remains subject to change on the first, second and third day. This is what distinguishes from previous studies. From this it can be concluded that the treatment of fresh garlic is quite significant on the growth of Staphylococcus aureus bacteria compared to the treatment of garlic extracts and powders. This result is because the treatment of fresh garlic does not interfere much with its chemical content compared to the treatment of garlic extracts and powders.

Hyunjoo, 2008 conveys that garlic extract with ethanol is more potent as an antibacterial effect than fresh garlic [27]. According to H W Ockerman, 2007 PH content is higher in fresh garlic compared to garlic extract and powder [28]. Likewise, Lemar dkk, 2018 states that fresh garlic has a good antifungal effect [25]. The same was conveyed by Gordon and Lowy, 2010. Fresh garlic has a large inhibition zone diameter for Staphylococcus aureus bacteria compared to garlic extract and garlic powder [29].

In contrast to other studies which say that garlic extract preparations have good antibacterial effects [28], Takashi’s study states that garlic powder has the best antibacterial properties [30].

The antibacterial properties of garlic are high because the acidity level is still high compared to the treatment of extracted and dried onion extracts and powders which affect the chemical composition. Previous studies have found extracts and powders to be quite effective in inhibiting the bacterium Staphylococcus aureus. But in this study it was found that among the preparations of 3 groups under white with 3 treatments; fresh garlic in a blender, garlic
extracted and garlic made in powder; preparations that are effective in inhibiting bacteria that is a group of fresh garlic that is blended with a high concentration of 50%. But in this study it was found that among the 3 groups of garlic preparations with different treatments; fresh garlic in a blender, garlic extracted and garlic made in powder; preparations that are effective in inhibiting bacteria that is a group of fresh garlic that is blended with a high concentration of 50%. When compared with the positive control (amoxicillin) fresh garlic is not effective enough in inhibiting Staphylococcus Aureus bacteria.

Conclusion
The results showed that the garlic preparation which had more phytochemical content through GCMS test was fresh garlic, compared to garlic extract and garlic powder.

The most significant antibacterial property among 3 preparations of garlic is fresh garlic with a concentration of 50%. When compared with the amoxicillin antibiotic as a positive control, fresh garlic with a concentration of 50% has not been able to provide effective / yet significant results as an antibacterial in inhibiting staphylococcus aureus.

References
[1] Alli JA, Boboye BE, Okonko IO, Kolade AF, Nwanze JC 2011 In-vitro Assessment of the effects of garlic (Allium sativum) extract on Pseudomonas aeruginosa and Staphylococcus aureus Pelagia Research Library 2 25–36.
[2] CDC. 2014b. E.coli (Escherichia coli). Access: April 5, 2015. From: http://www.cdc.gov/ecoli/index.html/
[3] Amagase H, Petesch BL, Matsuura H, Kasuga S, Itakura Y 2001 Recent Advances on the Nutritional Effects Associated with the Use of Garlic as a Supplement. JN 131 955S-962S.
[4] Bayan L, Koulivand PH, Gorji A. Garlic: a review of potential therapeutic effects. Avicenna Journal of Phytomedicine, 2014. 4 1–14.
[5] Bennet P, Brown M, Sharma P. Clinical Pharmacology. London: Elsevier. Bentley R, Meganathan R. 1982. Biosynthesis of Vitamin K (menaquinone) in Bacteria. Microbiological Reviews, 2012. 46 241–80.
[6] Borlinghaus J, Albrecht F, Gruhlke MCH, Nwachukwu ID, Slusarenko AJ 2014 Allicin: chemistry and biological properties. Molecules (Basel, Switzerland) 19 12591–12618.
[7] CDC. 2014a. Antimicrobial Resistance. Access: Mart, 6 2015. From: http://www.cdc.gov/drugresistance/
[8] CDC. 2014b. E.coli (Escherichia coli). Access: April, 5 2015. From: http://www.cdc.gov/ecoli/index.html/
[9] CLSI, 2012. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard 11th ed., Pennsylvania: CLSI.
[10] Safitri, Rustama, Kusmor 2014 Efek Ekstrak bawang putih terhadap pertumbuhan bakteri Staphylococcus Aureus, Escherichia Coli, S.agalactia. Med.Journal. 65 72-82
[11] Fujisawa H, Watanabe K, Suma K, Orguchi K, Matsufuji H, Seki T, Ariga T 2009 Antibacterial potential of garlic-derived allicin and its cancellation by sulfhydryl compounds. Bioscience, Biotechnology, and Biochemistry 73 1948–1955.  
[12] Koo Hui Miean 2012 Flavonoid (Myricetin, Quarcetin, Kaempferol, Lutolin and
Apigenin) content of Edible Tropical Plants. Journal of Agricultural and Food Chemistry 49 3106-3112.

[13] Terrace Leighton 2016 Characterization of Active Compounds of Different Garlic (Allium sativum L.) Cultivars. *Journal of Food and Nutrition Sciences*. 68 354-370.

[14] Yoshinura 2018 Bioactive compounds in selected hot spices and medicinal plants. *Journal of agronomy*. 8 32-49.

[15] Fujisawa H, Suma K, Origuchi K, Seki T, Ariga T. Thermostability of allicin determined by chemical and biological assays. *Bioscience, Biotechnology, and Biochemistry*. 72 2877–2883.

[16] Garzoni C, Kelley WL. Staphylococcus aureus: New Evidence for Intracellular Persistence. Trends in Microbiology, 2009. 2 59-65.

[17] Azeem 2017 Biochemical Compounds of Fresh, powder and extract garlic. *Journal of agricultural*. 5 10-25.

[18] Ramesh 2017 Bioactive Compounds and Biological Functions of Garlic (Allium Sativum). *Journal of Microbiology*. 8 1-31.

[19] Lowry T. 2018. Measure biochemical of powder and extract garlic with FTIR. *Indian Journal of Clinical Biochemistry*. 11 37-41.

[20] Manuela, Stan 2017 In vitro Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature. *Electric Journal of Biology*. 5 5–10.

[21] Wanjau 2018 Antimicrobial activity of garlic (Allium sativum L.). *Postepy Fitoterapi*. 11 46-52.

[22] Shokrzadeh 2014 Antibacterial effect of Garlic. *Journal of Microbiology science*. 70 Nr. 1,C93.

[23] Ekwenye 2013 Anti-bacterial effect of garlic (Allium sativum) and ginger (Zingiber officinale) against Staphylococcus aureus, Salmonella thypi, Escherichia coli and Bacillus cereus. *Journal of Microbiology, biotechnology and food sciences*. 2 2481-2491.

[24] Uzodike, Igwe 2012 Efficacy of Garlic (Allium sativum) on Staphylococcus Aureus conjunctivitis. *JNOA*. 12 20-22.

[25] Lemar KM, Turner MP, Lloyd 2018 Garlic (Allium sativum) as an anti-Candida agent: a comparison of the efficacy og fresh garlic and freeze-dried extracts. *Microbiology Journal*. 52 1365-1375.

[26] El-Mahmood Muhammad 2012 Efficacy of Crude extracts of garlic (Allium sativum Linn.) against nosocomial Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae and Pseudomonas aeruginosa. *Journal of Medicinal Plants Research* 3 179-185.

[27] Hyunjoo 2018 Antioxidant and antimicrobial activities of fresh garlic and powder garlic. *Food Sci Biotechnol*. 27 219-225.

[28] Ockerman HW 2016 Antioxidant and antimicrobial effects of garlic. *NCBI*. 37 849-855.

[29] Gordon RJ, Lowy FD 2010 Pathogenesis of methicillin-resistant Staphylococcus aureus infection. *Clinical Infectious Diseases*. 46 350–359.

[30] Takashi 2013 A Comprehensive Survey of Garlic Functionality. *Food SCI Journal*. 21 22-32.