Antibacterial activity of polyeugenol against *staphylococcus aureus* and *escherichia coli*

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Abstract. Antibacterial activity of polypugenol compounds has been carried out in the form active ingredients and gels against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). Antibacterial activity assay of polyeugenol in active form with varied concentration 0.0025 g/mL, 0.005 g/mL, 0.01 g/mL, 0.02 g/mL, and 0.04 g/mL and gelling form varied concentration 0.01 g/mL, 0.02 g/mL, and 0.04 g/mL. Antibacterial activity assay using agar disk diffusion method by measuring the diameter of inhibition. The result showed that antibacterial activity not presence at *Escherichia coli* while *Staphylococcus aureus* has presence of antibacterial activity at concentration 0.01 g/mL, 0.02 g/mL, dan 0.04 g/mL with inhibition diameter values 15.72; 17.28 and 20.51 mm. Polyeugenol in gelling form were tested against *Staphylococcus aureus* and *Escherichia coli* at concentration 0.01g/mL, 0.02g/mL, dan 0.04g/mL. The presence of antibacterial activity obtained for *Escherichia coli* only at concentration 0.04 g/mL with the value of inhibition diameter is 0.53 mm while concentration 0.01g/mL dan 0.02g/mL not presence antibacterial activity. The presence of antibacterial activity for *Staphylococcus aureus* obtained at concentration 0.01 g/mL, 0.02 g/mL, and 0.04 g/mL with the value of inhibition diameter are 14.46; 15.68 and 18.81 mm that showed relatively strong antibacterial activity. Statistic tested using one way ANOVA in *Staphylococcus aureus* shows significant differences at each concentration from polyeugenol in active form and gelling form with F-count> F-tab.

1. Introduction
Severe bacterial infections are a major cause of morbidity and mortality among newborns and young infants in the developing world [1]. One of the causes of infection due to bacteria is poor hand hygiene. Public awareness of the importance of hand hygiene is still lacking. People are not aware that in their activities, their hands are often contaminated with microbes. One disease that can be caused by not maintaining hand hygiene is diarrhea [2].

Hand hygiene is one of the important aspects to avoid various kinds of diseases caused by bacteria. In general, bacteria such as *Staphylococcus aureus* (S. Aureus) and *Escherichia coli* (E. Coli) are found in parts of human skin, where these bacteria can cause dangerous diseases [3]. Lack of facilities such as limited water availability and soap in public places can be an obstacle to cleaning hands. One alternative solution that can be used is antiseptic gel because its use is easier and more efficient [4].

Antiseptic gel preparations on the market still use alcohol as an antibacterial ingredient, whereas repeated use can cause irritation to the skin [5]. Alcohol can also dissolve layers of fat and sebum on the skin which serves as a protection against microorganism infections [6]. The alternative is to use natural ingredients as antibacterial which can be derived from essential oils from clove leaves (Syzygium
aromaticum). The compound that acts as an antibacterial in essential oils of clove leaves is eugenol. Eugenol has excellent bactericidal activity against various organisms such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa [7] and Listeria monocytogenes [8].

The value of antibacterial activity, eugenol can be increased by various modifications, one of which is to make derivative compounds from eugenol namely 3-(3,4 dimethoxy phenyl)-1-propanol and to test antibacterial activity against gram-negative E. coli bacteria at various concentrations. The results showed that at a concentration of 40% it was more effective at inhibiting the growth of E. coli bacteria with a diameter inhibition zone of 1.9 cm [9]. However, to make derivative compounds from eugenol requires a long processing time of around 70 hours at room temperature [10] and 48 hours at a constant temperature of 45 °C [9]. The length of time needed indicates that the use of eugenol to be antibacterial tends to be ineffective and inefficient. Therefore, another modification of eugenol is needed but it can still increase its antibacterial activity value, one of which is polymerization. This is because polymers can be used as antibacterial compounds, one of which is chitosan which has antibacterial activity against gram-positive and gram-negative bacteria [11].

Making polyethanol itself is easier and more efficient and has been carefully examined. Handayani [12] has synthesized polyeugenol with concentrated H2SO4 catalyst resulting in a yield of 69.31%, insoluble in water but soluble in acetone, chloroform, and benzene. However, the application of polieugenol as an antibacterial compound has never been done. In fact, these compounds have the potential as antibacterial compounds. Therefore, in this research the synthesis of polieugenol was tested for its antibacterial activity against S. aureus and E. coli.

2. Experimental

Antibacterial activity was carried out by testing bacterial growth inhibition against S. aureus and E. coli using agar disk diffusion method (Kirby bauer) [3]. Stages of research include Tool Sterilization, Media Preparation, Bacterial Rejuvenation and Antibacterial Activity Test. The solid media used is 6.8 grams of MHA (Mueller Hinton Agar) which has been autoclaved and heated on a hot plate by stirring using a magnetic stirrer, the process is carried out in the LAF room [13]. Rejuvenation of pure bacterial cultures is carried out on solid media so that it is tilted by scratching an ose needle containing E. coli and S. aureus with a zigzag pattern while being held near to the flame. Each bacterium is made 2 cultures of bacteria. This stage is carried out in a sterile state in the LAF room and incubated at 37 °C for 24 hours [13].

Before testing the bacterial inhibitory activity against polieugenol, the autoclave MHA media was prepared and placed in the Laminar Air Flow (LAF) room. The method used is the agar diffusion method using paper disks (6 mm diameter) [14]. Disc paper was dripped with 20 μL of polieugenol extract in 6 petri dishes with a variation of 0.4 g; 0.2 g; 0.1 g; 0.05 g; 0.025 g and 0.0125 g respectively in 10 mL of methanol solvent [15] and methanol solvents as negative controls [16]. To make a bacterial suspension, one batch of bacteria was dissolved in 4 mL of 9% sterile NaCl with a color ratio according to the standard McFarland 0.5 [17]. After that 100 μL of suspension and 15 mL of sterile bacterial media were vortexed to be homogeneous. Then the solution was put into a sterile petri dish for ± 15 minutes until the solution solidified. Dry discs that have been extracted are placed on the media so that the petri dish is regular. Each type of bacteria is carried out 3 times. Petri dishes are then wrapped in plastic wrap so they remain sterile and incubated for 24 hours at ± 37 °C. The inhibitory power of bacterial growth can be determined by measuring the diameter of the clear zone around the disc paper. The diameter of the inhibitory zone formed was measured using a calipers to determine the effectiveness of antibacterials [18].

After that the gel preparation process was carried out by dissolving the polieugenol compound with several concentrations of concentrations of 0.4 g, 0.2 g and 0.1 g with 5 mL methanol solvent and 5 mL of distilled water for each variation of concentration. After that, CMC 0.5% is slowly added to each variation of its concentration [19] which has been dissolved in distilled water as a gel base in warm conditions, then stirred using mortar [15]. After becoming a gel preparation, anti-bacterial testing was carried out with 3 replications and compared with its antiseptic power with positive control antiseptic power (hand antiseptic gel preparation with ethanol and triclosan active ingredients).
3. Results and Discussion

The antibacterial activity test of polieugenol was carried out in the form of a preparation gel active ingredient. Antibacterial testing was carried out on E. coli bacteria (ATCC 25922) which are gram-negative bacteria [3] and S. aureus (ATCC 25923) which are gram-positive bacteria [20].

The media used in the test is Mueller Hinton Agar (MHA) which is a suitable medium for antimicrobial testing [21]. MHA has been recommended by the FDA and WHO for antimicrobial testing because it contains sulfonamides, trimethoprim and low tetracycline inhibitors so that it can provide good pathogenic growth [22]. Before the testing begins the tools and agar media that will be used must be sterilized first using an autoclave at a temperature of 121°C [23]. Sterilization is carried out so that living microbes and spores can be destroyed so as not to interfere with and contaminate antibacterial testing [24].

The negative control used in the test is methanol, because methanol can dissolve polieugenol and has no antibacterial activity [25]. Natheer [26] said that substances which are used as negative controls are solvents used as compound diluents. The purpose of using negative controls is to compare that the solvent used does not affect the antibacterial test results. The positive control used in the antibacterial activity test is triclosan. The selection of triclosan as a positive control is due to triclosan having a broad spectrum of activity against gram-positive and mostly gram-negative bacteria [27], so it is often used as a mixture that is often found in antiseptic preparations on the market.

Tests are carried out by making several different variations of concentrations, for active ingredients there are 5 variations of concentration namely 0.0025 g/mL, 0.005 g/mL, 0.01 g/mL, 0.02 g/mL and 0.04 g/mL [15], while for sediment gels there are 3 variations of concentration determined from the results of the diameter of the largest inhibition zone in testing active ingredients. Tests took place during the 24-hour incubation period the results of antibacterial activity tests were characterized by the presence of clear areas around the disc that indicated there was no bacterial colony growing in the area, this clear zone called the inhibition area diameter. The results of testing for antibacterial activity and measuring the diameter of inhibitory zones of polieugenol compounds against E. coli and S. aureus can be seen in Table 1.

Table 1. Results of measuring the diameter of the inhibitory zone of the polyeugenol compound against E. coli and S. aureus

| Sample           | Concentration (g/mL) | Average diameter of inhibition zone (mm ± SD) | Escherichia coli ATCC 25922 | Staphylococcus aureus ATCC 25923 |
|------------------|----------------------|-----------------------------------------------|-----------------------------|----------------------------------|
| Active ingredients | 0.0025               | 0 ± 0                                         | 0 ± 0                       | 0 ± 0                            |
|                  | 0.005                | 0 ± 0                                         | 2.12 ± 0.16                 | 0 ± 0                            |
|                  | 0.01                 | 0 ± 0                                         | 15.72 ± 0.22                | 17.18 ± 0.14                    |
|                  | 0.02                 | 0 ± 0                                         | 20.51 ± 0.03                | 23.30 ± 0.32                    |
|                  | 0.04                 | 0 ± 0                                         | 23.58 ± 0.17                | 27.76 ± 0.18                    |
| Trichlosan (positive control) | 0 ± 0             | 0 ± 0                                         | 14.46 ± 0.34                | 18.81 ± 0.34                    |
| Methanol (negative control) | 0 ± 0           | 0 ± 0                                         | 15.68 ± 0.26                | 20.53 ± 0.92                    |
| Preparation gel | 0.01                 | 0 ± 0                                         | 14.46 ± 0.34                | 18.81 ± 0.34                    |
|                  | 0.02                 | 0 ± 0                                         | 15.68 ± 0.26                | 20.53 ± 0.92                    |
|                  | 0.04                 | 0.53 ± 0.92                                  | 18.81 ± 0.34                | 23.58 ± 0.17                    |
| Trichlosan (positive control) | 0 ± 0           | 0 ± 0                                         | 27.76 ± 0.18                | 27.76 ± 0.18                    |
| Methanol (negative control) | 0 ± 0           | 0 ± 0                                         | 27.76 ± 0.18                | 27.76 ± 0.18                    |

Based on Table 1, it can be seen that polieugenol compounds in the form of active ingredients do not have antibacterial activity against E. coli bacteria, while in S. aureus bacteria, polieugenol compounds in the form of active ingredients and preparation gels have antibacterial activity. Polieugenol in the form of active ingredients at a concentration of 0.005 g/mL has an average inhibition diameter of 2.12 mm ± 0.16.
Concentrations of 0.01 g/mL and 0.02 g/mL had a mean inhibition diameter of 15.72 mm ± 0.22 and 17.18 mm ± 0.14, while for a concentration of 0.04 g/mL it had an average average inhibitory diameter of 20.51 mm ± 0.03. Based on Rosyita [28] and Ambarwati [29] the classification of antibacterial activity can be seen from the value of the diameter zone <5 mm including weak, 5-9 mm including the medium category, 10-19 mm including the strong category and >20 mm including the very strong category, so that at a concentration of 0.04 g/mL included in the very strong category, the concentration of 0.01 g/mL and 0.02 g/mL in the strong category and 0.005 g/mL in the very weak category.

The concentration of active ingredients which has the greatest inhibitory power was 0.01 g/mL; 0.02 g/mL and 0.04 g/mL, so this concentration was used in testing the antibacterial activity of polyeugenol compounds in the form of preparatory gels. Preparation of gel preparation from polyeugenol compounds using CMC as a gelling agent, because CMC has no antibacterial activity [30] so it will not affect the testing of antibacterial activity. The measurement results showed that the polyeugenol compounds in the dosage form had almost no antibacterial activity against E. coli bacteria, whereas the polyeugenol compounds in gel dosage forms had antibacterial activity which was classified as strong at a concentration of 0.01 g/mL; 0.02 g/mL and 0.04 g/mL for against S. aureus.

The results of measurements of inhibitory zone diameters generally showed that polyeugenol compounds in the form of active ingredients and dosage gels only had antibacterial activity against S. Aureus (a gram positive) compared to E. coli (a gram negative). This is due to differences in cell wall structure between gram positive and negative bacteria. Gram negative bacteria have two cell membranes, namely the outer membrane and cytoplasmic membrane, whereas gram-positive bacteria only have cytoplasmic membranes [3].

The difference in composition and structure of cell walls in gram negative and gram positive bacteria can affect the antibacterial activity of a chemical compound [31]. The structure of simpler, single layered gram-positive bacterial cell walls contains 90% peptidoglycan layer [32] with low lipid content (1-4%) facilitating bioactive material into cells. Gram negative bacteria itself has more complex cell walls consisting of three layers, namely the outer layer of lipoprotein, the middle layer of lipopolysaccharide which acts as a barrier to the entry of antibacterial bioactive ingredients, and the inner layer of peptidoglycan with lipid content reaching 11-12% [31].

The dominant peptidoglycan content in the cell wall of S. aureus, which includes gram-positive bacteria, is a water-soluble polymer. This characteristic shows that bacterial cell walls are polar. Polyeugenol compounds are also polar so it is easier to penetrate the polar peptidoglycan layer compared to the non-polar lipid layer [28]. E. coli bacteria which are gram negative bacteria contain more lipids, less peptidoglycan and have an outer membrane in the form of a bilayer which serves as a selective defense of compounds that enter or enter cells [33]. This is what causes polyeugenol compounds can not provide antibacterial activity against E. coli bacteria.

The antibacterial activity in polymers is related to the ability to absorb bacterial cell walls. A compound can interact better with gram-positive bacteria compared to gram negative because the negative charge on the cell surface of gram-positive bacteria is more than gram negative. The positive charge of polyeugenol which is distributed to the surface of the cell wall of gram negative bacteria which in turn will inhibit bacterial activity being tested. This also causes greater antibacterial activity of polyeugenol compounds to bacteria than S. aureus with E. coli.

The hydroxyl group (-OH) in this polyeugenol compound has an important role for polyeugenol compounds in inhibiting bacterial growth [35]. The group damages the cell wall so that it can inhibit the metabolism of bacteria. Based on the hypothesis of Pei [36], that the hydroxyl group (-OH) will interact with proteins present in bacteria so that it can prevent the action of enzymes in bacteria. This causes the growth of bacteria to be blocked and bacteria can die.

The testing of antibacterial activity against S. aureus polyeugenol in the form of dosage gel has a smaller inhibition zone value compared to the active ingredient, which indicates that polyeugenol in the form of active ingredients is slightly more effective than polyeugenol compounds in the form of preparatory gel. This is due to the formulation and use of gelling agents that have not been appropriate in preparing gel preparations. According to Selvia [37] the formulation of adding the right concentration and
gelling agent will increase the value of the antibacterial activity of a compound. The selection of gelling agent or gel base used in making gel preparations will affect contact time, speed of release of active substances, and binding capacity to active substances [38]. So that it can affect the spread of power when adding active compounds [39].

Data on the testing of antibacterial activity obtained were then analyzed using statistics. Statistical testing is only done for S. aureus bacteria in the form of active ingredients and preparation gels. The aim is to determine the difference in inhibitory power of each concentration. This statistical test uses the One way Analysis of Variance (ANOVA) test with the results of the results (Table 2) and the following hypothesis.

Ho: There was no significant difference in antibacterial activity between the series of concentrations of polynugenol compounds in the form of active ingredients or in the form of dosage gels against S. aureus.

Ha: There is a significant difference in antibacterial activity between the series of concentrations of polynugenol compounds in the form of active ingredients or in the form of dosage gels against S. aureus.

| Table 2. Oneway ANOVA calculation results of variations in the concentration of polyeugenol to S. aureus. |
|-------------------------------------------------|
| Form of Polyeugenol | F count | F table | Description         |
|---------------------|---------|---------|---------------------|
| Active ingredients  | 778.57  | 5.14    | Significantly different |
| Preparation gel     | 146.15  | 5.14    | Significantly different |

Based on the results of variance analysis with a comparison of tables the value of F-count > F-table is obtained. These results state that Ho is rejected and Ha is accepted, which means that there is a significant difference in antibacterial activity between the series of concentrations of polynugenol compounds in the form of active ingredients or in the form of gel preparations against S. aureus. This indicates that the antibacterial activity of polieugenol in the form of active ingredients and gel preparation against S. aureus in the concentration series was significantly different. Shown by the value of the diameter of the inhibition zone which increases with increasing concentration, according to the research conducted by Baydar [40].

4. Conclusion
Polyeugenol compounds in the form of active ingredients and gel preparations don’t have antibacterial activity against the bacteria Escherichia coli, while Staphylococcus aureus has strong antibacterial activity.

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