Effectiveness of Eugenol as An Antibacterial Toward 
*Staphylococcus epidermidis*

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**Abstract.** Eugenol is generally used as raw materials of perfumes, but now eugenol was developed as an antioxidant, antifungal, antibacterial and antiseptic. This study aims to determine the effect of eugenol concentration and pH and the effectiveness of eugenol as an antibacterial against *Staphylococcus epidermidis*. The experiments were carried out at concentrations of 1%, 2%, 4%, 6% and 8% with pH 6, 7 and 8. Data were collected and analyzed by two-way ANOVA method with interaction level of 5% (α = 0.05). The results showed that eugenol has an antibacterial effect in inhibiting the growth of bacteria *Staphylococcus Epidermidis* and included in the category of strong to very strong antibacterial. The optimum yield diameter of the inhibitory zone is 22.27 mm, obtained at pH 8 and the concentration of eugenol is 8%

**1. Introduction**  
Indonesia is the world's largest producer of cloves, which are used for the majority to the cigarette industry. Cloves (*Syzygium aromaticum L*) is a plant essential oil of cloves can be used optimally in terms of agribusiness and for education. The main content of clove oil is 78-98% eugenol (*Eugenia aromaticum*) and usually used as raw materials of perfumes.

The research was done by Juvensius, Paulina and Aurelia indicate that extracts of clove (eugenol) have antibacterial activity against *Streptococcus* mutans. Utilization of eugenol as antibacterial continue to be developed, the experiment does the increased variety of bacterial species that can be suppressed growth.

The purpose of this study to determine the effectiveness of eugenol as an antibacterial against *Staphylococcus epidermidis*. The results of this study are expected to add to the economic value of eugenol which is usually used as raw material for the manufacture of perfumes, can now be used as an antibacterial, especially *Staphylococcus epidermidis*.

**2. Methodology**  
The research method used in this study is the 2-way classification method with interaction (two way ANOVA with interaction) with a mean level of (α) 5%. The experiment will test the hypothesis about the effect of 2 independent variables and each of them has a character of adding each other by repeating the test. The material used was eugenol 99.55%, gram-positive bacterium *Staphylococcus epidermidis*, Antibiotik medium No.1 (A1), Tryptone Soya Agar (TSA), Pepton Water, Mannitol Salt Agar (MSA), ethanol 96%, dyes for identification of Gram and aquadest.

How it works: Preparation according to the instructions, measuring media (A1) at pH 6, 7 and 8. All equipment and media to be used must be under sterile conditions.
Making the stock of bacteria: *Staphylococcus epidermidis* upgrades performed by inoculating the agar medium slant and incubation at a temperature of 35°C for 24 hours.

The suspension of bacteria is done by suspending 1 tube of *Staphylococcus epidermidis* by inserting 5 ml of peptone water that has been sterilized. Enter the glass ball 3 pieces into the tube which has been given a solution of peptone water and shake until the perfect suspended bacteria into a solution of peptone water. Take the bacterial suspension and remove the empty tube identification of bacteria carried in the media Mannitol Salt Agar (MSA), by scraping *Staphylococcus epidermidis* bacteria from agar slant (TSA), which has been inoculated for 24 hours in an incubator at a temperature of 35°C.

Identification of the second by way of a gram. The bacterial suspension was taken a bit by using oese, scratched in glass object, wait for it to dry. Apply a drop of *gentian violet* dye to scratches closed and wait for 1 minute. Rinse with distilled water, drops with Lugol let stand 2 minutes, then rinse with distilled water, rinse with alcohol 96% until the paint runs out leached. Drops with a solution *fuchin* wait 30 seconds and rinse with distilled water. After drying cover with a cover glass is then observed using a microscope.

Pouring media in a petri dish with the way the media A1 incorporated into a sterile petri dish of 20 ml as the base layer. Wait until solidified and then coated with 10 ml of media A1 which has been given a stock solution of *Staphylococcus epidermidis* bacteria (ratio of 100: 1) and then flattened. After condensing for 6 wells each petri dish by using the pit. Antibacterial activity test: pitting on the contents of the media in the sample and 70% alcohol as a blank 0.1 ml interchangeably. Incubation at 35°C for 24 hours, and measuring the diameter of the clear zone is formed.

### 3. Results and discussion

Test identification of *Staphylococcus epidermidis* bacteria on media Mannitol Salt Agar (MSA) is shown in Figure 1, resulting in the growth of colonies yellowish white indicating the fermentation of mannitol that is the acid produced, causing changes in phenol red in order that changes from red to colorless yellow [1].

Gram identification tests in Figure 2 shows *Staphylococcus epidermidis* purple, do not move, round, live in pairs and in groups. Petunia identifies *Staphylococcus epidermidis* belongs to the gram-positive bacteria for bacterial cell walls that are thick and resistant to alcohol is able to maintain the first dye (*gentian violet*) so that the addition of another color is not able to penetrate the cell wall.

![Figure 1. Identification of *Staphylococcus epidermidis* with MSA.](image1)

![Figure 2. The results of the identification of *Staphylococcus epidermidis* with painting Gram.](image2)

The negative control test was conducted to determine differences in media treated with a solution of eugenol with the media without being given a solution of eugenol. Results in the can that there is no clear zone is formed, in which there is a white layer evenly on media surface as shown in Figure 3.
Figure 3. The test results negative control

Test form 70% alcohol as solvent eugenol is performed to determine whether alcohol 70% can inhibit the growth of *Staphylococcus epidermidis*. The result was no clear zone formed at all concentrations of eugenol, both in media of pH 6, pH 7 and pH 8. This shows that 70% alcohol cannot inhibit the growth of *Staphylococcus epidermidis* bacteria so that it does not affect to recitation the eugenol sample as shown in Figure 4.

Figure 4. The test results of Blanko and Samples eugenol

Difference pH on A1 media produces different textures on the media. Media with a pH of 6, the texture is mushy, being right media with a pH of 8, the texture is denser. The more alkaline the texture A1 become denser. The texture is denser due to the lower water content. This is because the nature of the base (alkali) more easily form lumps and NaOH has water absorbing properties so that the texture is denser.

Figure 5. Effect of pH media to the diameter of clear zone
Differences enough media pH affects diameter clear zone is formed. This is shown in Figure 5, the highest clear zone diameter at pH 8 with a concentration of 8% amounting to 222.7 mm. According to Baehaki 2005, Staphylococcus epidermidis have an optimum pH 8 where pH effect on rough protease activity of pathogenic bacteria [2]. Referring to Figure 6, the higher the concentration of eugenol seen an increase in growth inhibition of antibacterial against Staphylococcus epidermidis.

![Figure 6. Effect of Concentration of eugenol against the clear zone diameter](image)

This is in accordance with the opinion of Pelezar and Chan (1988) which states that the higher the concentration of a substance antibacterial the faster the microorganisms have been killed and stunted [7], but according to Ganiswara (1995) an increase in the concentration of a substance will be followed by an increase in the inhibition of the growth of bacteria, but at maximum concentration would decline in the inhibition of bacterial growth [4]. According to Nurainy (2008), high concentrations will produce a viscous solution [6]. A solution that is too thick will be difficult to diffusion compared with a more dilute solution. This led to a concentration of 4% to 8% increase in the diameter of the inhibition zone is small compared to the concentration of 1% to 4% with an average increase of 18.2mm. The results of two-way ANOVA with a level of meaning (α) of 5% FA> Fα, FB> Fβ. It shows the difference in media pH and the concentration of eugenol affect diameter clear zone is formed.

According to Davis and Stout (1971), the strength of the inhibition of bacteria are categorized into four, namely: Very strong (> 20mm), strong (10-20mm), medium (5-10mm) and weak (<5mm) [3]. The classification of the strength of bacterial inhibition is shown in table 1.

| No | Concentration | pH  | Diameter clear zone (mm) | Category  |
|----|---------------|-----|---------------------------|-----------|
| 1  | 1%            | 6   | 13.7                      | Strong    |
| 2  | 1%            | 7   | 12.53                     | Very Strong |
| 3  | 1%            | 8   | 12.9                      | Strong    |
| 4  | 2%            | 6   | 20.47                     | Very Strong |
| 5  | 2%            | 7   | 16.1                      | Strong    |
| 6  | 2%            | 8   | 16.73                     | Strong    |
| 7  | 4%            | 6   | 21.23                     | Very Strong |
| 8  | 4%            | 7   | 20                        | Strong    |
| 9  | 4%            | 8   | 20.53                     | Very Strong |
| 10 | 6%            | 6   | 21.73                     | Very Strong |
| 11 | 6%            | 7   | 20.5                      | Very Strong |
|    |     |     |     |     |
|----|-----|-----|-----|-----|
| 12 | 6%  | 8   | 22  | Verry Strong |
| 13 | 8%  | 6   | 22.1| Verry Strong |
| 14 | 8%  | 7   | 21.03| Verry Strong |
| 15 | 8%  | 8   | 22.27| Verry Strong |

From Table 1 at a concentration of 1% of media pH 6, 7 and 8 diameter clear zone resulting in the category of antibacterial strong with the average diameter of 13.04 mm.

Very strong antibacterial category to pH 6 begins at a concentration of 2% by 20.47mm, while extremely powerful antibacterial category at pH 8 start at a concentration of 4% of 20.53 mm and a pH of 7 starts at a concentration of 6% 21.23 mm.

4. Conclusion
From the results of the study, it was concluded that the eugenol concentration and the pH of the media affected the diameter of the inhibition zone produced. The optimum results were obtained at media pH 8, with a eugenol concentration of 8%. Eugenol is effectively used as an antibacterial for Staphylococcus Epidermidis and included a very strong antibacterial category.

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