

## Antibacterial Activity Test of Kersen Fruit Kombucha ( *Muntinga Calabura L.* ) To Bacteria *Staphylococcus Aureus* and *Escherichia Coli* By In Vitro

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### Abstract

Bacterial infections of *Staphylococcus aureus* and *Escherichia coli* cause diarrhea. Kersen fruit can be used as the basis of kombucha, which has antibacterial benefits. This experimental activity aims to determine the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. This study used a laboratory experimental method to test the antibacterial activity of Kersen Fruit Kombucha (KFK) at concentrations of 15%, 25%, and 35%, as well as positive control of cotrimoxazole and normal control of aqua pro injection. Antibacterial activity was measured in vitro using the disc diffusion method to compare affected and unaffected areas. The results of the study on *Escherichia coli* and *Staphylococcus aureus* bacteria showed that the diameter of the inhibition zone in 15% KFK was 7.07-8.63 mm, 25% KFK showed an inhibition zone diameter of 8.11-9.57 mm; and 35% KFK inhibition zone diameter was 12.07-13.07 mm; for the positive control inhibition zone diameter was 13.07-14.72 mm. 35% KFK has a larger inhibition zone diameter than 15% and 25% KFK. Based on the Kruskal-Wallis statistical test, there was a significant difference. This study aims to determine the antibacterial activity of Kersen fruit kombucha against *Staphylococcus aureus* and *Escherichia coli* by measuring the diameter of the inhibition zone.

**Keywords:** antibacterial, kombucha, kersen fruit, inhibitory power

### 1. INTRODUCTION

Every person must have been attacked by an illness, whether acute or chronic. Many factors are causing it, including climate change, lifestyle factors that do not lead to healthy living and unclean environmental factors that often make various body organs susceptible to disease. Diseases often suffered due to these factors are digestive diseases, with symptoms as follows: sick stomach ulcers, diarrhea, and vomiting (Suarnatha IPD & Gunawan IMAO, 2022).

*Staphylococcus aureus* is a gram-positive bacteria consisting of hemolytic, pathogenic, and round. It can

cause mannitol fermentation and is capable of causing coagulase.

*Staphylococcus enterotoxin* is a major cause of foodborne illness. Diarrhea is caused by enterotoxin in the enteric nervous system, especially the nervous system in the digestive tract, which causes damage to the intestines and diarrhea (Zaunit et al., 2019).

*Escherichia coli* bacteria are gram-negative bacteria in the form of short stems. *Escherichia coli* is a type of bacteria that commonly lives around man. *Escherichia coli* is an indicator of food and drink cleanliness. The reason is that *Escherichia coli* in food and drink indicates poor health and is a warning that the air has been

contaminated with human waste (Hutasoit, 2020)

Extract fruit kersen compound phenols, saponins, flavonoids, phenols, and tannins, where phenols own mechanism Work stop with damage protoplasm cells and walls cell as well as precipitate cell proteins microbes. Flavonoids provide effective destructive antibacterial wall cell bacteria, while tannins interfere with the permeability of cells and shrink cell membranes (Subagya, 2019).

For add mark economy tree kersen can be made processed including kombucha tea, viz drink made from fermentation tea and the with the addition of SCOBY (Symbiotic Culture of Bacteria and, Yeasts) specifically *Acetobacter xylinum* and yeast. Fruits are fermented for 10 days to increase antioxidant levels (Nintiasari & Ramadhani, 2022).

## 2. METHOD

### Material Study

Materials used include sugar sand, fruit kersen, Aquadest, Agar media, Acid chloride, Magnesium powder, FeCl 1%, reagents *Wagner* / reagent *Dagendrof*, sour acetate anhydrous, and acid sulfate thick.

The tools used include dropper pipettes, cloth flannel, tube reaction funnel, stove, stem stirrer, glass jar, cloth, spoon, rubber bracelets, shelf storage (Herma), paper strain, and flannel cloth.

Study experimental this is for test activity antibacterial KFK 15%, 25%, and 35%, as well as control positive cotrimoxazole and normal control aqua pro injection. Data was obtained by measuring the largest IZD (Inhibition zone diameter) formed from concentration. The IZD formed with the concentration smallest from capable sample stop growth bacteria is CMB value ( Concentration Minimum Barrier ).

### Formulation

1 kg of kersen fruit taken from the Karanganyar region of Central Java is purplish and washed clean indoors. Making fermented kersen fruit kombucha (*Muntinga Calabura L.*) according to research (Rezaldi et al., Potential of Subang Honey Pineapple (Ananas compass) Fruit as a Gram Positive and Negative Antibacterial Using the Biotechnological Method of Kombucha Fermentation Based on Different Palm Sugar Concentrations, 2022), as following three jar type for sugar for substrate, kombucha as well as kombucha culture (scooby ) as fluid. Add one liter of water and two hundred and fifty grams of fruit kersen, then move 79 grams of sugar to the receptacle up to a volume of 300 ml. The Solution shared becomes three and placed in different glass jars with labels I, II, and III. Next, adjust the percentage with results, namely 15%, 25%, and 35%. After reaching around 25°C, add a kombucha starter of approximately 10% (v/v) each Sunday to every batch. The lid with the top cut off is placed in a covering container so the fermentation process can continue continuously for 14 days at room temperature.

### Activity Test

Testing the antibacterial activity of kersen fruit kombucha ( *Muntinga calabura L.* ) with disc diffusion, which creates NA media of around 15 milliliters in a Petri dish, then clean stick with cotton sterile on the surface place *Staphylococcus aureus* and *Escherichia coli* suspended. Reduce vulnerability bacteria in NA medium. Next, soak test samples for the fruit kombucha fermentation process kersen, with variation of jar I KFK 15%, Jar II KFK 25%, Jar III KFK 35%, cup VI control positive, cotrimoxazole, and control V plate negative, distilled water, done replication three times; After that sample left at 37 °C for 24 hours; Power test resistor For measure the diameter

formed, along with control positive and negative (Fathurrohman *et al.*, 2022).

The highest level of antibacterial activity is triggered by a large diameter zone originating from the transparent zone with the lowest concentration of KFK capable of killing bacteria, called the Minimum Inhibitory Concentration (MIC) of the sample (Fathurrohman *et al.*, 2022).

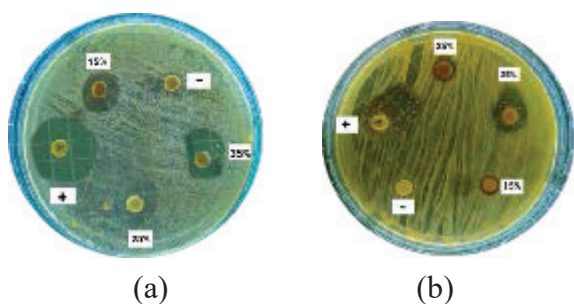
**Data analysis**

The data obtained were processed and statistically analyzed using one-way ANOVA with a significance value of 95%. If data is significantly different, it is passed to the post hoc test.

**3. RESULTS AND DISCUSSION**

Data from the 15%, 25%, and 35% KFK research showed results positively correlated with antibacterial *Staphylococcus aureus* and *Escherichia coli*.

Antibacterial test data for cherry fruit extract (*calabura* L) shows the ability to stop bacterial growth in vitro. The transparent zone around the paper disc indicated inhibitory activity.



**Description:** Antibacterial activity of kersen fruit kombucha ( *Muntinga calabura* L.) against *Staphylococcus aureus* (a) and *Escherichia coli* (b).

**Table 1. IZD Antibacterial Activity of Kersen Fruit Kombucha on *Staphylococcus aureus* and *Escherichia coli* bacteria**

| Bacteria                     | Treatment | Inhibition Zone Diameter |       |       | X̄    |
|------------------------------|-----------|--------------------------|-------|-------|-------|
|                              |           | I                        | II    | III   |       |
| <i>Escherichia coli</i>      | K-        | 0                        | 0     | 0     | 0     |
|                              | K+        | 13.06                    | 13.07 | 13.08 | 13.07 |
|                              | KFK 15%   | 7.07                     | 7.06  | 7.09  | 7.07  |
|                              | KFK 25%   | 8.08                     | 8.10  | 8.15  | 8.11  |
|                              | KFK 35%   | 12.07                    | 12.06 | 12.09 | 12.07 |
| <i>Staphylococcus aureus</i> | K-        | 0                        | 0     | 0     | 0     |
|                              | K+        | 14.50                    | 14.75 | 14.91 | 14.72 |
|                              | KFK 15%   | 8.51                     | 8.73  | 8.4   | 8.63  |
|                              | KFK 25%   | 9.53                     | 9.4   | 9.70  | 9.57  |
|                              | KFK 35%   | 13.02                    | 13.05 | 13.15 | 13.07 |

The transparent zone is formed, different from KFK 15%, 25%, and 35%, which has an IZD value. In the medium zone category, a sugar concentration of 15% produces an average IZD of 8.63mm for *Staphylococcus aureus* and 7.07 mm for *Escherichia coli*. At a sugar concentration of 25%, the average IZD *Staphylococcus aureus* was 9.57 mm, and the average IZD Medium *Escherichia coli* was 8.11 mm. At a sugar concentration of 35%, the average IZD for the growth of *Staphylococcus aureus* was 13.07 mm, including the strong inhibition zone category for *Escherichia coli*, and the average IZD was 12.07 mm. This matter is confirmed by Ma'ruf *et al.*(2022) study that rates different sugars in fermented flower

kombucha, making their own potency bigger. Different pharmacology is used to prevent the development of pathogens of bacteria.

IZD shows the susceptibility of bacteria to a test substance or antibiotic and is expressed as the diameter of the clear zone. IZD at each concentration is different due to the influence of the action of compounds in kersen fruit extract (*Muntinga calabura* L). The greater the inhibitory potential in a bacterial isolate, the greater the transparent zone formed (Molina et al., 2023).

To see the influence of extract fruit kersen (*Muntinga calabura* L.) on the development bacteria *Staphylococcus aureus* and *Escherichia coli*, a data analysis was done with a level significance of 95%. The result analysis shows that no difference means IZD KFK at concentrations of 15%, 25%, and 35% to bacteria *Staphylococcus aureus* and *Escherichia coli*.

Concentration from KFK can stop the development of *Staphylococcus aureus* and *Escherichia coli*. Data shows that a concentration of 35 % KFK forms IZD, the largest in bacteria. IZD value in *Staphylococcus aureus* is 13.07mm, and *Escherichia coli* is 12.07mm in the strong category. Antioxidant activity is high on the extract leaf. Kersen owns a Power resistor with an inhibition diameter of *Escherichia coli* big enough that is 11.73 mm (Mutammimah et al., 2022). The presence of an inhibition zone on the leaves shows that compound chemical nature antibacterial such as flavonoids, triterpenes, steroids, saponins, and tannins (Manarisip et al., 2019).

Difference study This with the previous study previously is kombucha, which can increase antibacterial activity and inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria more optimal, with IZD size almost close to control (+) co-trimoxazole indicating with IZD the strong one (Faizah et al., 2021)

The acetic acid in the KFK that is formed releases a free proton, which causes the pH of the media to decrease (Yanti et al., 2020). Acetic acid is an organic acid formed from kombucha fermentation. The formation of acetic acid in kombucha can inhibit the growth of pathogenic bacteria (Kumar & Joshi, 2016).

Undissociated acetic acid can destroy the lipid bilayer structure of bacteria by injecting protons into the cytoplasm, forming many intracellular protons and leaving the cytoplasm in an acidic state. In addition, it destroys protein and causes energy loss. The greater the level of acetic acid, the greater the ability to stop the development of pathogenic bacteria. The acetic acid in kombucha has the ideal ability to lower the pH of acidic conditions to very acidic, meaning that the low pH of the substrate has an effect on the growth of bacteria and causes serious cell damage because the cytoplasm of pathogenic bacteria becomes unhealthy (Kumar & Joshi, 2016).

The basis of this research is that the sugar content in kombucha fermentation (Yanti et al., 2020) has a significant influence on inhibiting the growth of gram-positive and negative bacterial and fungal microorganisms. Research by Rezaldi et al., (2021) also shows that 40% white sugar content in kombucha made from butterfly pea flowers can stop the development of gram-positive and gram-negative pathogens.

#### 4. CONCLUSION

Kersen fruit kombucha is a pharmaceutical biotechnology product that can stop the development of gram-positive and negative bacteria. 35% KFK in kersen fruit kombucha is the best concentration. It stops the growth of gram-positive and negative bacteria. The IZD obtained from *Staphylococcus aureus* bacteria was 13.07 mm in the strong category. At IZD 12.07



mm, the *Escherichia coli* bacteria were also declared strong.

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