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Antibacterial Effectiveness Test of Kencur Rhizome Ethanol Extract (*Kaempferia galanga* L.) against *Escherichia coli* and *Staphylococcus aureus*

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A B S T R A C T

Indonesia is a country with abundant biodiversity. Increased bacterial resistance *Escherichia coli* and *Staphylococcus aureus* antibiotics require alternative treatment, one of which is medicinal plants. Kencur (*Kaempferia galanga* L.) is a medicinal plant that contains saponins, flavonoids, phenols, and tannins, which can inhibit bacterial growth. This study aimed to determine the antibacterial effect of kencur rhizome ethanol extract (*Kaempferia galanga* L.) against *Escherichia coli* and *Staphylococcus aureus*. This research is an experimental laboratory design post-test only control group design. The rhizomes of kencur were extracted using 70% ethanol and tested for antibacterial activity using the well method. There were 6 groups in the study, namely the positive control group using ciprofloxacin, the negative control using distilled water, kencur rhizome ethanol extract concentrations of 12.5%, 25%, 50%, and 100%. The largest diameter of the inhibition zone was found at a concentration of 100%, with an average of 10.18 mm in the bacteria *Escherichia coli* and 22.47 mm in the bacteria *Staphylococcus aureus*. In conclusion, the ethanol extract of kencur rhizome (*Kaempferia galanga* L.) can inhibit the growth of bacteria *Escherichia coli* and *Staphylococcus aureus*.

1. Introduction

The development of science and technology in the health sector has experienced major leaps, bringing rapid progress in efforts to prevent and treat disease. In the midst of increasingly complex challenges of antibiotic resistance, research on the potential of natural compounds as antimicrobial agents has received increasing attention. In this context, pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* stand out as major causes of various infections in humans, from gastrointestinal infections to skin infections. *Escherichia coli*, which is commonly found in the human digestive tract, can be a major cause of disease through its pathogenic variations. On the other hand, *Staphylococcus aureus*, a gram-positive bacterium, can cause infections ranging from boils to serious infections like pneumonia and sepsis. The challenge in dealing with this bacterial infection is increasing due to the development of resistance to conventional antibiotics.[1-5]

Kencur rhizome (*Kaempferia galanga* L.) is a plant that has played an important role in various traditional medicinal systems in various cultures for many years. This plant is known for being rich in natural compounds that have broad health potential. One of the main ingredients that make it attractive in this context is the various phytochemical compounds it has. Flavonoids, alkaloids, and other compounds contained in kencur have attracted the attention of

researchers in the health sector. Flavonoids are compounds known for their antioxidant and anti-inflammatory potential. Alkaloids, on the other hand, have a variety of pharmacological effects and are frequently found in various plants. Both have the potential to interact with microorganisms, including pathogenic bacteria. In the context of antimicrobial potential, these compounds play an important role. The ability of flavonoids to inhibit bacterial enzyme activity and disrupt cell membranes has been studied extensively. Alkaloids, on the other hand, have properties that can influence the growth and development of microorganisms.[6-10] Therefore, the presence of these compounds in kencur has the potential to inhibit the growth and development of pathogenic bacteria, including *Escherichia coli* and *Staphylococcus aureus*. This study aimed to determine the antibacterial potential of kencur rhizome ethanol extract (*Kaempferia galanga L.*) against *Escherichia coli* and *Staphylococcus aureus*.

2. Methods

This study is an in vitro experimental study. This study uses bacterial cultures *Escherichia coli* and *Staphylococcus aureus* on petri dishes obtained from the microbiology laboratory of the Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia. kencur rhizome (*Kaempferia galanga L.*) as the test material, the extraction process was carried out using 96% ethanol solvent by maceration for 1x24 hours. The macerate resulting from maceration is thickened into an extract using a rotary evaporator. This study was approved by the medical and health research ethics committee at the Faculty of Medicine, Universitas Muhammadiyah Surakarta, Surakarta, Indonesia.

Bacterial culture *Escherichia coli* and *Staphylococcus aureus* standardization of bacterial concentrations was carried out using McFarland 0.5 solution. The similarity of turbidity levels showed the same concentration of bacteria between test groups. A total of 24 petri dishes that had been added 1-2 ose of bacterial culture *Escherichia coli* and *Staphylococcus aureus,* and MHA (Mueller Hinton Agar) were used in this study. There are 6 test groups, namely ciprofloxacin as a positive control (K1), negative control, aquadest (K2), a treatment group of kencur rhizome extract concentrations of 12.5%, 25%, 50%, and 100%, respectively as K3-K6. A total of 4 test petri dishes were used in each group. Furthermore, the inhibition of bacteria was measured by measuring the diameter of the inhibition zone of each treatment group. Data analysis was carried out using SPSS software version 25. Univariate analysis was performed to present the data distribution for each test variable. Bivariate analysis was carried out to see statistical differences in each test variable, where $p < 0.05$.

3. Results and Discussion

Table 1 presents the average diameter of the bacterial inhibition zone of *Escherichia coli* in each group. The positive control given ciprofloxacin was the group with the highest inhibition compared to the treatment group with kencur rhizome extract. The group given 100% kencur rhizome extract had the highest inhibition among the other treatment groups.

Table 1. The average diameter of the bacterial inhibition zone *Escherichia coli* in each group.

Treatment	Mean±SD
12,5%	0±0
25%	0±0
50%	6,49±0,52
100%	10,18±0,40
K+ Ciprofloxacin	29,43±0,12
K- Aquadest	0±0

Table 2 presents the average diameter of the bacterial inhibition zone of *Staphylococcus aureus* in each group. The positive control given ciprofloxacin was the group with the highest inhibition compared to the treatment group with kencur rhizome extract. The group given 100% kencur rhizome extract had the highest inhibition among the other treatment groups.

Table 2. The average diameter of the bacterial inhibition zone *Staphylococcus aureus* in each group.

Treatment	Mean±SD
12,5%	16,64±0,45
25%	17,46±0,34
50%	20,82±0,09
100%	22,47±0,11
K+ Ciprofloxacin	33,73±0,10
K- Aquadest	0±0

The antibacterial effect of the ethanol extract of kencur rhizome is related to the content of saponins, flavonoids, phenols, and tannins, which are factors inhibiting bacterial growth. The mechanism of action of saponins as an antibacterial is that they can cause leakage of proteins and enzymes from inside the bacterial cell. Flavonoids are known to have antibacterial properties where the mechanism of action is to make complex compounds with dissolved extracellular proteins so that they can damage the bacterial cell membrane and be followed by the release of intracellular compounds. Phenol increases the permeability of the cytoplasmic membrane, causing the release of intracellular components and coagulation of the cytoplasm resulting in cell lysis. Tannins interfere with peptidoglycan synthesis so that the formation of the cell wall becomes less. The results of this study are in accordance with other studies which state that the ethanol extract of kencur rhizomes can inhibit bacterial growth of *Escherichia coli* and other studies which state that the ethanol extract of kencur rhizome can inhibit bacterial growth *of Staphylococcus aureus.*[11-15]

The ethanol extract of kencur rhizome has more effect on *Staphylococcus aureus* from *Escherichia coli.* Gram-positive bacteria generally have a higher sensitivity to antimicrobial compounds than gram-negative bacteria. Differences in the sensitivity of gram-positive and gram-negative bacteria can be influenced by differences in the structure of the cell wall in the two groups of bacteria. Gram-negative bacteria have a cell wall that contains more lipopolysaccharide, so the lipid content in these bacteria is high, whereas gram-positive bacteria contain little lipopolysaccharide and lots of peptidoglycans so that the extract can more easily penetrate the cell wall of gram-positive bacteria.[16-20]

4. Conclusion

Kencur rhizome ethanol extract (*Kaempferia galanga* L.) has an effect in inhibiting the growth of bacteria *Escherichia coli* and *Staphylococcus aureus*. The highest diameter of the inhibition zone in the kencur rhizome ethanol extract was at a concentration of 100% with a value of 10.18 mm at *Escherichia coli* and 22,47 mm at *Staphylococcus aureus.* The antibacterial effect of kencur rhizome ethanol extract at concentrations of 12.5%, 25%, 50%, and 100% was not able to match the antibacterial effect of ciprofloxacin.

5. References

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