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Antibacterial Potential of *Kaempferia parviflora* Rhizome Extract against *Staphylococcus aureus* ATCC 25923

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Abstract: Bacteria are one of the organisms that cause infectious diseases. One of the bacteria that can cause infection is *Staphylococcus aureus*. Treatment of bacterial infections is by using antibiotics, but using the antibiotics for a long term can cause resistance. It is necessary to look for alternative compounds that can inhibit the growth of *Staphylococcus aureus*. In the bioprospecting process of medicinal plants, it is not only necessary to rely on empirical information but also to have scientific evidence proven through scientific testing. One of the plants with potential as a medicine is black ginger (*Kaempferia parviflora*). The aim of this study is to determine the effectiveness of *Kaempferia parviflora* rhizome in inhibiting the growth of *Staphylococcus aureus* bacteria. The concentrations of the *Kaempferia parviflora* extract tested were 25%, 50%, 75%, and 100%. Based on the results, it can be concluded that the ethanol extract of *Kaempferia parviflora* rhizome has antibacterial activity against the growth of *Staphylococcus aureus* ATCC 25923 at concentrations of 25%, 50%, 75%, and 100%, with strong antibacterial inhibition. This study is expected to contribute to the field of health in general and pharmacy in particular by developing the potential of Indonesian medicinal plants. The findings of this study will be directed toward the development of formulations with antibacterial activity.

Keywords: Bioprospecting, Antibacterial, Black Ginger, *Kaempferia parviflora*, *Staphylococcus aureus*

1. INTRODUCTION

Bacteria are one of the organisms that cause infectious diseases. One of the bacteria that can cause infection is *Staphylococcus aureus*. Treatment of bacterial infections is by using antibiotics, but using the antibiotics for a long term can cause resistance. It is necessary to look for alternative compounds that can inhibit the growth of *Staphylococcus aureus*. In the bioprospecting process of medicinal plants, it is not only necessary to rely on empirical information but also to have scientific evidence proven through scientific testing.

It is necessary to optimize the medicinal properties of these plants. Scientific evidence regarding the therapeutic properties of medicinal plants is essential as raw material for the development of innovative products, both as modern medicine and cosmetics. Given the importance of scientific information on the active compounds contained in medicinal plants, bioprospecting research is needed to facilitate the selection of plants as raw materials for pharmaceutical products. This bioprospecting activity focuses on exploring the medicinal properties of biological resources as raw

materials for pharmaceutical products, ultimately leading to the commercialization of health innovation products [11].

The initial step in the bioprospecting activities of this research involves testing the efficacy or medicinal potential of plant parts that have the potential to act as antibacterial agents. One such plant part is the rhizome of *Kaempferia parviflora*. The selection of *Kaempferia parviflora* rhizome for testing its antibacterial potential is based on literature studies showing that ginger plants contain flavonoids, terpenoids, essential oils, and phenols, which have the potential to inhibit the growth of several pathogenic bacteria that can cause diseases in humans. This ability has been demonstrated in studies showing that *Kaempferia parviflora* extract has antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Cutibacterium acnes* [1]. Other studies have also shown antibacterial activity of *Kaempferia parviflora* extract against *Cronobacter* spp. and *Enterohemorrhagic Escherichia coli* (EHEC) [2]. In this study, the *Kaempferia parviflora* rhizome is tested on *Staphylococcus aureus*. *Staphylococcus aureus* is a bacterium that can cause infections, particularly of the skin. Additionally, the 2022 Global Burden of Disease publication reports that *Staphylococcus aureus* is a leading cause of death in 135 countries worldwide, especially the Methicillin-resistant *Staphylococcus aureus* (MRSA) strain, which is difficult to treat with antibiotics [3].

Based on the above discussion, the author will conduct research on the bioprospecting of ethanol extract from *Kaempferia parviflora* rhizomes as an antibacterial agent against *Staphylococcus aureus*. The aim of this study is to investigate the bioprospecting of secondary metabolite compounds and the antibacterial effects of *Kaempferia parviflora* rhizome extract on the growth of *Staphylococcus aureus*. This research is expected to provide information on the antibacterial effectiveness of ethanol extract from *Kaempferia parviflora* rhizomes against *Staphylococcus aureus*. The solvent used for extraction is 70% ethanol, because it's characteristic, it dissolve polar compounds such as flavonoids that contain in *Kaempferia parviflora* rhizomes that has antibacterial activity.

2. MATERIALS AND METHODS

2.1. Place and time of research

This research was conducted at the Biology Pharmacy Laboratory of Universitas Jenderal Ahmad Yani Yogyakarta.

2.2. Tools and materials

The materials used include the *Kaempferia parviflora* Rhizome, ethanol 70%, DMSO, *Staphylococcus aureus*, Nutrient Agar (NA) media, Mueller Hinton Agar (MHA), paper disc blank, chloramphenicol paper disc. The tools used are Class II BSC, autoclaves, petri dishes, micropipette (Eppendorf), analytical balance (Ohaus), and other glasses.

2.3. Sampling and Plant Determination

Kaempferia parviflora Rhizome were obtained from Sleman, Yogyakarta. The determination of samples was carried out at the Biology Learning Laboratory, Ahmad Dahlan University.

2.4. Preparation and sample extraction of Kaempferia parviflora Rhizome

Kaempferia parviflora Rhizome were washed, then chopped into small pieces. After that, its dried in an oven at 40^{o}C and reduced in particle size using a grinder and 35-mesh sieves. The extraction method used in the research is the maceration method. The maceration method in this study used 70% ethanol solvent ratio 1:10 for 48 hours [4].

2.5. Sterilization

The tools were sterilized in an oven at a temperature of 171 °C for 1 hour. The materials used were sterilized by autoclave at a temperature of 121 °C for 15 minutes [5]. Nutrient agar (NA) media was dissolved in an aqueous solution and homogenized with a stirrer at a temperature of 100 °C until boiling. The NA was sterilized by an autoclave at 121 °C for 15 minutes. Continuously, the NA was cultured with *Staphylococcus aureus.*

2.6. Inoculation of Staphylococcus aureus

In the Bio Safety Cabinet (BSC), *Staphylococcus aureus* were inoculated by the spread plate method on NA media and then incubated for 24 hours at 37 °C. *Staphylococcus aureus* were suspended in a tube containing 10 mL of a 0.9% NaCl solution. Then McFarland standard 0.5 measured turbidity with a turbidimeter.

2.7. Antibacterial Activity Test by Disc Diffusion Method

The serial concentrations of *Kaempferia parviflora* Rhizome extract were dissolved in DMSO 10%. The blank paper disc was soaked in samples for 10 minutes. 100 µL of bacterial suspension was inserted into a petri dish containing *Muller Hinton Agar* media. The petri dish media was divided into two parts, such as control and samples. As a positive control, paper discs containing chloramphenicol 1%. Blank paper discs contained DMSO 10% as negative controls. After that, the petri dish was incubated at 37 °C for 24 hours, and the clear zone formed was measured by their zone inhibition diameter. This test was repeated three times modified by [6].

3. RESULTS AND DISCUSSION

3.1. Extraction of Kaempferia parviflora Rhizome

Plant determination carried out at the Biology Learning Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University, Yogyakarta, showed that the plant used was *Kaempferia parviflora* Wall. ex Baker. The extraction process uses a maceration technique with 70% ethanol solvent with a maceration time of 48 hours with a weight ratio of simplicia: solvent of 1:1. The maceration method chosen was because this method is good for active compounds that are not resistant to heating and simple in the processing process. The extraction results can be seen in Table 1.

Table 1. Results of Black Ginger Rhizome Extraction (*Kaempferia parviflora*)

Replication	Simplicia (g)	Extract (g)	Yield (%)
1	200	69.68	34.84
2	200	59.88	29.94
3	200	64.50	32.25

Maceration is the extraction method used in this study, aimed at extracting flavonoids from *Kaempferia parviflora* rhizome. The principle of extraction using the maceration method involves soaking powdered plant material (simplicia) in an appropriate solvent. The solvent used for maceration is 70% ethanol. Ethanol 70% is polar, allowing it to dissolve polar compounds such as flavonoids. In addition, the 70% ethanol solvent contains 30% water, which is expected to wet the plant material, allowing the extracting agent to penetrate the cell walls of the plant material [7]. The yield data from the extraction process shows that the yield from three repetitions of the extraction was greater than 25%. This result meets the standards set by the Indonesian Herbal Pharmacopoeia,

which requires that the yield percentage should not be less than 10%. The yield percentage indicates the amount of compounds contained in the *Kaempferia parviflora* rhizome simplicia that can be extracted using the 70% ethanol solvent.

3.2. Qualitative analysis

3.2.1. Phytochemical screening

Phytochemical screening is carried out using test tubes by adding various reagents. The results of phytochemical screening showed that *Kaempferia parviflora* Rhizome contains Flavoind, Phenolic, Tannin, Alkaloids, and Steroids.

Table 2. Phytochemical Screening Results

No.	Active Compounds	Screening Result
1.	Flavonoid	+
2.	Phenolic	+
3.	Saponin	-
4.	Tannin	+
5.	Alkaloids	+
6.	Steroids	+

Notes: (+) Positive; (-) Negative

3.3. Antibacterial activity using disk diffusion method

Antibacterial activity was evaluated the inhibition zone in various concentration of *Kaempferia parviflora* Rhizome extract. The reference antibiotic in this test is 1% chloramphenicol. Chloramphenicol is a broad-spectrum antimicrobial agent with a bacteriostatic effect against both gram-positive and gram-negative bacteria. In this study, 10% DMSO is used as the solvent for the extract to be tested. The 10% DMSO solution was chosen because it does not have antibacterial activity that could affect the test results, ensuring that the inhibition zones observed are solely due to the ethanol extract of *Kaempferia parviflora* rhizome and not influenced by the solvent. The test results show that the ethanol extract of *Kaempferia parviflora* rhizome can inhibit the growth of *Staphylococcus aureus* at all concentrations tested. The diameter of the inhibition zones of the ethanol extract of *Kaempferia parviflora* rhizome can be seen in Table 3.

Table 3. Inhibition Zone Diameter (mm) Evaluated Antibacterial Activity

Treatments	Concentration (%)	Replication (mm)			Mean ±SD	Interpretation
		I	II	III		
Kaempferia parviflora Rhizome extract	100	13.6	13.9	12.6	13.37 ±0.68	Strong
	75	12.0	15.6	8.8	12.13±3.40	Strong
	50	12.9	13.0	10.3	12.07 ±1.53	Strong
	25	10.2	9.0	11.8	10.33±1.40	Strong
Chloramphenicol	1	34.2	36.3	34.6	35.03±1.12	Powerful
DMSO	10	0	0	0	0.00 ±0.00	None

Based on **Table 3**, the results of the inhibition zones show that the concentrations of 25%, 50%, 75%, and 100% exhibit a strong inhibitory effect. The results indicate that the higher the concentration

used, the better the ability to inhibit the growth of *Staphylococcus aureus*. This can be seen from the increasing size of the inhibition zones. These findings are also supported by a study by Husnia [8], which states that the higher the concentration of the extract tested, the greater the active compounds present, resulting in larger inhibition zones.

The ability of the ethanol extract of *Kaempferia parviflora* rhizome pounds that act as antibacterial agents contained in the rhizome. Based on phytochemical testing, the ethanol extract of *Kaempferia parviflora* rhizome contains flavonoids, phenolics, alkaloids, tannins, and steroids. Flavonoid compounds act as antibacterial agents by forming complex compounds with extracellular proteins, leading to damage to the bacterial cell membrane, which results in cell lysis [9]. Another compound with antibacterial activity is tannin. Tannins work by inactivating bacterial enzymes and disrupting the protein pathways within the cells, leading to cell death[10]The antibacterial mechanisms of each secondary metabolite compound in the ethanol extract of *Kaempferia parviflora* rhizome can inhibit the growth of *Staphylococcus aureus*.

3.6. Research limitations

The limitation of this study was that the raw material of *Kaempferia parviflora* Rhizome extract might not be easily available in several areas.

4. CONCLUSION

Based on the research results, it can be concluded that the ethanol extract of *Kaempferia parviflora* rhizome has antibacterial activity against the growth of *Staphylococcus aureus* ATCC 25923 with strong antibacterial inhibition category with sequentially the best inhibition zone from 100%, 75%, 50% and 25%.

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Conflicts of interest: The authors declare no conflict of interest.

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