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ANTIBACTERIAL ACTIVITY TEST OF 70% ETHANOL EXTRACT GINGER (Zingiber officinale) AGAINST Streptococcus mutans and Escherichia coli BACTERIA

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Abstract
Ginger (Zingiber officinale) is a herbal plant containing bioactive compounds such as flavonoids, alkaloids, saponins, tannins, and terpenoids that have the potential as natural antibacterials. This study aims to determine the antibacterial activity of 70% ethanol extract of ginger against Streptococcus mutans and Escherichia coli bacteria. The study used a laboratory experimental method with a descriptive quantitative approach. The study population was ginger plants that have the potential to be used as natural antibacterials, while the sample was fresh ginger rhizomes obtained by purposive sampling technique. Extraction was carried out using the accelerated maceration method with 70% ethanol solvent. Antibacterial activity testing was carried out using the disc diffusion method at concentrations of 1%, 3%, 5%, and 10%. The research instruments included a rotary evaporator, incubator, autoclave, micropipette, and caliper. Data were analyzed descriptively using Microsoft Excel. The results showed that ginger extract was able to inhibit the growth of Escherichia coli and Streptococcus mutans bacteria with the highest activity at a concentration of 10%. The conclusion of this study is that 70% ethanol extract of ginger has the potential as a natural antibacterial against Gram-positive and Gram-negative bacteria.

Keywords: *Antibacterial Activity, Escherichia Coli, Ginger Extract, Streptococcus Mutans, Zingiber Officinale*

INTRODUCTION

Ginger (Zingiber officinale) is a herbal plant widely used as a cooking spice and traditional medicine because it contains various bioactive compounds such as gingerol, shogaol, flavonoids, phenolics, and essential oils that have potential as antioxidants, anti-inflammatory, and antibacterial. The content of these secondary metabolites gives ginger a broad pharmacological activity and continues to be developed in the fields of health and functional foods. In addition, ginger is known to have a distinctive aroma and spicy taste derived from the active compounds zingerone and gingerol, so it is widely used in modern herbal formulations (Arfan et al., 2024; Ayustaningwarno et al., 2024). Recent research also shows that ethanol extract of ginger can inhibit the growth of various pathogenic microorganisms through the mechanism of cell membrane damage and inhibition of bacterial metabolism, thus potentially used as a source of natural antibacterials (Al-Fatlawi et al., 2024; Ahnafani et al., 2024).

The use of herbal plants as antibacterial alternatives is becoming increasingly important as cases of antibiotic resistance increase due to irrational antibiotic use. Bacterial resistance poses a serious threat to the world of health because it can reduce the effectiveness of infection therapy and increase the risk of disease complications. Inappropriate use of antibiotics can trigger the ability of bacteria to survive despite antimicrobial therapy, so the development of safer and more readily available natural ingredients as antibacterial alternatives is needed (Pratita et al., 2023; Khoirotunnisa et al., 2025). In this context, the use of ginger extract is relevant because its flavonoid and phenolic content is known to have the ability to inhibit the growth of Gram-positive and Gram-negative bacteria, including Streptococcus mutans and

Escherichia coli, which often cause health problems in humans (Ayustaningwarno et al., 2024; Al-Fatlawi et al., 2024).

One method commonly used to obtain active compounds from natural ingredients is maceration because the process is simple and does not damage heat-sensitive compounds. This method works based on the principle of mass transfer, namely the transfer of active compounds from the simple drug to the solvent until equilibrium concentration is achieved. The use of 70% ethanol as a solvent is considered effective in extracting polar and semi-polar compounds such as flavonoids and phenolics that act as antibacterials (C. Lestari, 2022; Aprilia, 2019). Several previous studies have shown that ginger extract has quite strong antibacterial activity. Research by Parumpa et al. (2024) showed that red ginger extract at a concentration of 3% produced an inhibition zone of 11.5 mm against Streptococcus mutans. Meanwhile, research by Azkiyah (2020) reported that 80% ginger ethanol extract was able to inhibit the growth of Staphylococcus aureus by 12.33 mm and Escherichia coli by 14.17 mm. These results prove that ginger has great potential to be developed as a herbal-based antibacterial.

However, research on the antibacterial activity of low-concentration ginger ethanol extract using the accelerated maceration extraction method is still limited, especially against Streptococcus mutans and Escherichia coli bacteria. Therefore, this study was conducted to determine the ability of 70% ginger ethanol extract obtained through the accelerated maceration method to inhibit the growth of these two bacteria and to identify the flavonoid content in the extract. This study is important because it can provide scientific information on the potential of ginger as a natural antibacterial alternative that is more economical and has fewer side effects compared to the use of synthetic antibiotics. The novelty of this study lies in the use of extract concentration variations of 1%, 3%, 5%, and 10% with the disc diffusion method and the application of the accelerated maceration extraction method which has not been widely used in previous studies on Streptococcus mutans and Escherichia coli bacteria.

IMPLEMENTATION METHOD

This research is a laboratory experimental study with a descriptive quantitative approach that aims to determine the antibacterial activity of 70% ethanol extract of ginger (Zingiber officinale) against Streptococcus mutans and Escherichia coli bacteria. The study was conducted through an extraction process using an accelerated maceration method with 70% ethanol solvent, followed by phytochemical screening and testing of antibacterial activity using the disc diffusion method at various concentrations of 1%, 3%, 5%, and 10%. The study was conducted at the Pharmaceutical Preparation Technology, Microbiology, Natural Products, and Pharmacognosy Laboratory of the Tarumanagara Institute, Jakarta from May to July 2025. The experimental approach was chosen because it is able to test cause-and-effect relationships objectively under controlled laboratory conditions (Sugiyono, 2022; Creswell & Creswell, 2023; Che Sulaiman et al., 2020).

The population in this study was all ginger plants (Zingiber officinale) that have the potential to be used as a natural antibacterial agent, while the research sample was fresh ginger rhizomes obtained from Layansari Village, Gandrungmangu District, Cilacap Regency, Central Java using a purposive sampling technique based on certain criteria such as fresh and undamaged conditions. The bacterial samples used were pure cultures of Streptococcus mutans and Escherichia coli because both bacteria are pathogenic bacteria that often cause health problems in humans. Sample selection was carried out to determine the effectiveness of ginger extract against Gram-positive and Gram-negative bacteria (Sugiyono, 2022; Azkiyah, 2020; Parumpa et al., 2024).

The research instruments used included a blender, autoclave, rotary evaporator, incubator, hot plate, magnetic stirrer, micropipette, caliper, petri dish, and other laboratory glassware, while the research materials consisted of ginger simplicia, 70% ethanol, Nutrient Agar media, NaCl, chloramphenicol, and test bacterial cultures. Research data were obtained through observation of phytochemical screening results and measurement of the diameter of the antibacterial inhibition zone using a caliper in millimeters. The data obtained were analyzed using descriptive statistical analysis with the help of Microsoft Excel to

calculate the average value and standard deviation so that the research results could be explained systematically and objectively (Sudaryono, 2022; Creswell & Creswell, 2023; Triyani et al., 2024).

The research procedure began with the determination of ginger plants, processing of the simplicia through washing, drying, and grinding, then continued with the extraction process using the accelerated maceration method using 70% ethanol at a temperature of 40°C. The obtained extract was evaporated using a rotary evaporator until it became a thick extract, then phytochemical screening was carried out to identify the content of flavonoids, alkaloids, tannins, saponins, and terpenoids. Antibacterial activity testing was carried out using the disc diffusion method against Streptococcus mutans and Escherichia coli bacteria with chloramphenicol as a positive control and NaCl as a negative control. After incubation for 24 hours at 37°C, the inhibition zone formed was measured using a caliper to determine the strength of the antibacterial activity of the ginger extract (Ramdhini et al., 2022; Kurama et al., 2020; Harmina, 2021).

RESULTS AND DISCUSSION

Plant Determination

The samples used in this study were ginger collected from local plantations in Layansari Village, Gandrungmangu District, Cilacap Regency, Central Java. The ginger used was fresh and free from pests.

To determine the type of plant used, a plant identification procedure was carried out at the National Research and Innovation Agency in Cibinong, West Java. The results of the plant identification showed that the ginger rhizome belongs to the Zingiberaceae family, with the species Zingiber officinale.

Sample Preparation

Ginger samples were obtained from plantations belonging to residents of Layansari Village, Gandrungmangu District, Cilacap Regency, Central Java. Harvested ginger was cleaned of soil and thoroughly washed. Fresh samples were washed under running water and then thinly sliced. The drying process involved exposing the ginger to direct sunlight, covered with a black cloth, for 3 x 24 hours. Once dry, the ginger was blended and sieved to obtain a fine powder ready for extraction.

Extract Preparation

Extraction was carried out using accelerated maceration method using 70% ethanol. With a ratio of 1:10, namely (300 grams of ginger powder added to 3 liters of 70% ethanol). A total of 100 grams of ginger powder was put into an Erlenmeyer flask, 1 liter of ethanol was added and placed on a hotplate at a temperature of 40º C. Stirred using a magnetic stirrer for 1 hour at a speed of 300 rpm, the extracted filtrate was filtered and stored in a glass container. The second repetition of 100 grams of ginger powder was put into an Erlenmeyer flask, 1 liter of ethanol was added and placed on a hotplate at a temperature of 40º C, stirred continuously using a magnetic stirrer for 1 hour at a speed of 300 rpm, then the extracted filtrate was filtered and combined with the first filtrate. The third repetition of 100 grams of ginger powder was put into an Erlenmeyer flask, 1 liter of ethanol was added, placed on a hotplate at a temperature of 40º C, stirred continuously using a magnetic stirrer for 1 hour at a speed of 300 rpm, then the extracted filtrate was evaporated using a rotary evaporator with a vacuum pressure of 200 bar and an evaporation temperature of 40º C until the desired extract was formed.

A comparison between research conducted by (Azkiyah, 2020) and recent research shows that the accelerated maceration extraction method using 70% ethanol yielded more extract than the conventional extraction method conducted over 5 days using 96% ethanol. This means that this new extraction method is more efficient and yields more extract.

Extract Yield

Yield is the percentage of the extraction yield relative to the weight of the starting material used. Yield values can be influenced by various factors, such as solvent type and concentration, temperature, extraction time, and the method used (Karlina et al., 2021).

Table 1. Results of Material Processing

Fresh Weight	Dry Weight	Powder Weight	Extract Weight	Yield
3.5 kg	1 kg	750 g	21 g	7%

Based on the yield calculation, the ginger extract yield was 7%. This means it meets the yield standard set by the Indonesian Herbal Pharmacopoeia, edition 2, 2017, of at least 5.9%.

*Screening*Phytochemicals

The process of testing the chemical compounds in an extract is called phytochemical screening. The purpose of phytochemical screening is to ensure that the concentrated ginger extract contains alkaloids, saponins, tannins, flavonoids, and terpenoids. Phytochemical screening is conducted independently in the pharmacognosy and chemistry laboratories on the Tarumanagara Institute campus in Jakarta.

Table 2. Phytochemical Screening Results

No.	Phytochemical Compounds	Reagent	Results	Indicator +
1	Alkaloid	*Mayer* *Wagner* *Dragendorff*	White sediment reddish brown Orange	+ + +
2	Saponin	Foam Test (Distilled Water) hot (shaken)	Stable and durable foam more than 30 seconds	+
3	Tannin	$FeCl_3$ (Ferric chloride)	Blackish blue color or dark green	+
4	Flavonoid	Magnesium, concentrated HCl	Red, orange, or bright yellow	+
5	Terpenoid	H2SO4, Acetic acid	Red-brown or purple	+

From the table above, the phytochemical screening results indicate that the ginger extract contains alkaloids, saponins, terpenoids, tannins, and flavonoids.
This is in line with the results of previous research by (Ramdhini et al., 2022), which also conducted phytochemical screening that ginger extract qualitatively contains alkaloid, terpenoid, flavonoid, saponin, and tannin compounds.

Due to the presence of these compounds, ginger extract contains antibacterial compounds, such as flavonoids, which are known to damage cell membranes and inhibit protein formation. Alkaloids interfere with bacterial enzyme function. Saponins are known to disrupt cell function. Meanwhile, terpenoids work by damaging bacterial cell walls and reducing cell activity, ultimately inhibiting bacterial growth and even killing them (Karlina et al., 2021). Furthermore, Table 2 and Appendix 10 also document the results of the phytochemical screening.

Antibacterial Activity Test Results of Ginger Extract (Zingiber officinale)

Antibacterial activity testing was carried out using the disc paper diffusion method after the bacteria were planted on agar media and incubated for 24 hours at 37º C, resulting in the following results.

Table 3. Results of antibacterial activity tests against E. coli bacteria

Concentration	Inhibition Zone Diameter(mm)	Inhibition Zone	Level Antibacterial Activity
10%	20.725 ± 2.35	Formed	Very strong
5%	18,925 ± 1,175	Formed	Strong
3%	17.1 ± 1.7	Formed	Strong
1%	12.9 ± 1.1	Formed	Strong
Control +	38	Formed	Very strong
Control -	0	Not Formed	There isn't any

Table 4. Results of antibacterial activity tests against S. mutans bacteria

Concentration	Inhibition Zone Diameter(mm)	Inhibition Zone	Level Antibacterial Activity
10%	11.975 ± 0.675	Formed	Strong
5%	11.025 ± 0.375	Formed	Strong
3%	9.15 ± 0.35	Formed	Currently
1%	8.525 ± 0.475	Formed	Currently
Control +	36,775	Formed	Very strong
Control -	0	Not Formed	There isn't any

Information :
10% : The concentration of the test solution was 10% ginger extract used was 5% : The concentration of the test solution is 5% ginger extract used3% : The concentration of the test solution is 3% ginger extract used1% : Concentration of test solution 1% ginger extract used
Control + : Chloramphenicol Ointment 20 mg
Control - : NaCl

The test results are shown in Table 3 and Table 4. Based on the above research, 70% ethanol extract of ginger has antibacterial activity, indicated by the presence of a clear zone on the agar medium. The extract concentrations used in this study were 1%, 3%, 5%, and 10%. The positive control used chloramphenicol ointment (chlamycetine brand) with a composition of 20 mg/g chloramphenicol. The negative control NaCl was a comparison that showed no inhibitory effect, so the positive results in other treatments were truly caused by the active compound (Harun et al., 2024).

The data above shows that the greater the concentration of 70% ginger ethanol extract, the larger the resulting clear zone. Comparison between research conducted by (Parumpa et al., 2024) and (Azkiyah, 2020) with the latest research is S. mutans and E. coli bacteria shows that ginger extract has strong antibacterial activity at a concentration of 10%, with an average diameter of the inhibition zone in Escherichia coli of 20.275 ± 2.35 mm in the very strong category and in Streptococcus mutans bacteria with an average diameter of the inhibition zone of 11.975 ± 0.675 mm in the strong category.

CONCLUSION

This study shows that 70% ethanol extract of ginger (Zingiber officinale) obtained through accelerated maceration method has antibacterial activity against Streptococcus mutans and Escherichia coli bacteria. The results of phytochemical screening prove that ginger extract contains alkaloids, flavonoids, saponins, tannins, and terpenoids that play a role in the mechanism of bacterial growth inhibition. The highest antibacterial activity was obtained at a concentration of 10% with an inhibition zone diameter against Escherichia coli of 20.725 ± 2.35 mm in the very strong category and against Streptococcus mutans

of 11.975 ± 0.675 mm in the strong category. These results indicate that increasing the concentration of the extract has an effect on increasing bacterial inhibition so that ginger extract has the potential to be developed as a natural herbal-based antibacterial.

This study still has limitations because the testing was only conducted using the disc diffusion method and did not quantitatively identify the active compound levels or test the toxicity of the extract. Furthermore, the study only used two types of test bacteria, so the scope of antibacterial activity cannot be broadly generalized. Further research is recommended to conduct Minimum Inhibitory Concentration (MIC) tests, identify active compounds using instrumental methods, and develop pharmaceutical formulations based on ginger extract. Practically, the results of this study can serve as a basis for developing natural antibacterial ingredients that are more economical, readily available, and have the potential to reduce the excessive use of synthetic antibiotics.

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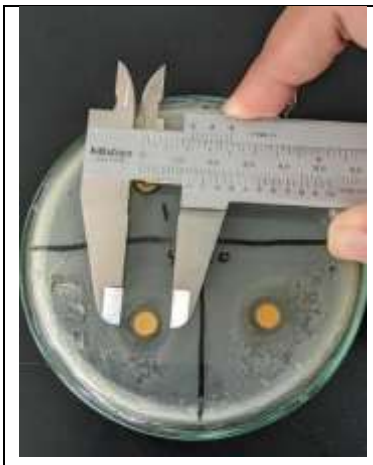
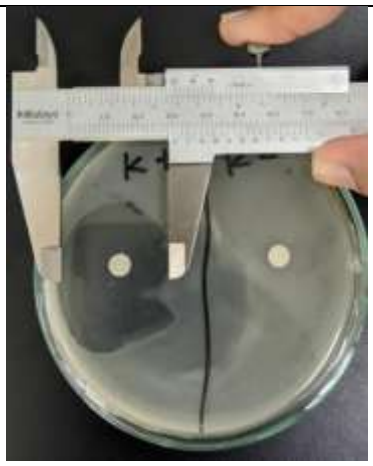
DOCUMENTATION

		
Phytochemical Screening of Alkaloids using Mayer's reagent	Phytochemical Screening of Alkaloids using Wagner's reagent	Phytochemical Screening of Alkaloid Reagents *dragendroff*

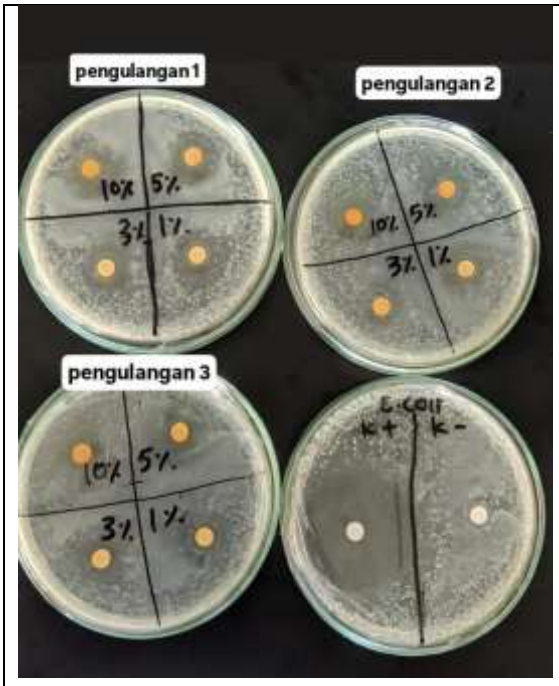
		
Phytochemical Screening of Tannins	Phytochemical Screening flavonoid	Phytochemical Screening of Saponins

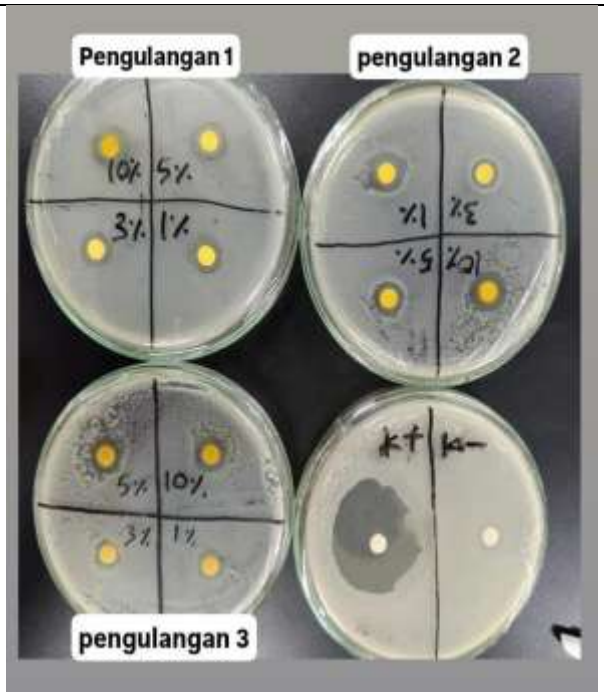

Phytochemical Screening terpenoid





Inhibition Zone Measurement





E. coli Bacteria Inhibition Zone

S. mutans Bacterial Inhibition Zone