INTERNASIONAL CONFERENCE ON
MULTIDISCIPLINARY APPROACHES IN HEALTH SCIENCE
VOLUME 3, No 1. Tahun 2025 , ISSN 3032-4408 (Online)
https://ejournal.poltekkes-denpasar.ac.id/index.php/icmahs

Antimicrobial Resistance Test of Elephant Ginger (*Zingiber officinale var. Officinarum*) Ethanol Extract Against *Streptococcus pyogenes* Bacteria Using the Disc Diffusion Method

Anak Agung Mas Adinda Iswari[1],I Nyoman Purna[1], Nyoman Mastra[1], Ida Bagus Oka Suyasa[1*],Fransiska Dwi Apriliani[1]

[1] Jurusan Teknologi Laboratorium Medis Politeknik Kesehatan Kemenkes Denpasar

Article history
Posted : 2025-09-20
Reviewed : 2025-12-05
Received : 2025-12-13

ABSCTRACT

Abstract: Background of this study is acute respiratory tract infection (ARI) caused by the bacterium *Streptococcus pyogenes*. The use of antibiotics is not always effective due to the emergence of resistance. Therefore, natural treatment alternatives such as elephant ginger, which contains active compounds like tannins, saponins, phenols, alkaloids, and flavonoids, are needed. Purpose of this study to measure the diameter of the inhibition zone, analyze, and categorize the effect of ethanol extract of elephant ginger on the growth of *Streptococcus pyogenes*. Research method is a true experimental design with Posttest Only Control Design, and data analysis with One Way ANOVA and Least Significant Deference (LSD). The ethanol extract of elephant ginger was prepared in concentrations of 15%, 17.5%, 20%, and 22.5%, and tested against *Streptococcus pyogenes* bacteria with three repetitions. Results showed that the inhibition zone increased with the volume of extract used. The 15% concentration produced 8.83 mm (moderate), 17.5% resulted in 9.08 mm (moderate), 20% in 10.52 mm (strong), and 22.5% in 11.06 mm (strong). The conclusion of this study is that there is a significant difference in the inhibition zone diameters of *Streptococcus pyogenes* growth at each concentration of 15%, 17.5%, 20%, and 22.5%.

Keywords: *Streptococcus pyogenes*, elephant ginger, antimicrobial

Corresponding author: okasuyasa@poltekkes-denpasar.ac.id

INTERNASIONAL CONFERENCE ON
MULTIDISCIPLINARY APPROACHES IN HEALTH SCIENCE
VOLUME 3, No 1. Tahun 2025 , ISSN 3032-4408 (Online)
https://ejournal.poltekkes-denpasar.ac.id/index.php/icmahs

INTRODUCTION

Acute Respiratory Tract Infection (ARTI) remains one of the leading public health issues in Indonesia, with a national prevalence of 9.3% and a slightly higher prevalence of 9.7% reported in Bali Province (Badan Penelitian dan Pengembangan Kesehatan, 2018). This disease continues to be a significant cause of morbidity and mortality, particularly in developing countries where access to adequate healthcare may be limited. One of the primary bacterial pathogens responsible for ARTI is Streptococcus pyogenes, a gram-positive, β-hemolytic bacterium classified under Group A (Group A Streptococcus). This microorganism is well known as the leading cause of acute pharyngitis, tonsillitis, and superficial skin infections such as impetigo and cellulitis. In more severe cases, S. pyogenes can also lead to life-threatening conditions including necrotizing fasciitis, pneumonia, meningitis, and endocarditis (Sari & Nasuha, 2021).

Management of ARTI caused by bacterial infections such as S. pyogenes typically involves the use of antibiotics, with amoxicillin being one of the most commonly prescribed options. However, recent studies have highlighted the irrational use of antibiotics in Indonesia. Dosage errors have reached approximately 14%, with inappropriate treatment duration accounting for 2.67% and under-dosing for 11.33% of cases (Tobat et al., 2015). Such mismanagement contributes significantly to the growing threat of antibiotic resistance, wherein previously effective treatments become ineffective due to bacterial adaptation. Resistance genes can be transferred horizontally among bacteria through mobile genetic elements such as plasmids and transposons, further complicating treatment options and potentially leading to prolonged illness and higher healthcare burdens (Rostinawati et al., 2022).

The global escalation of antibiotic resistance poses serious challenges by increasing patient morbidity, mortality, and healthcare costs. As a result, there is an urgent need to develop alternative therapeutic agents that are both effective and safe, without promoting resistance. One promising avenue involves the use of natural products rich in secondary metabolites, which have

Corresponding author: okasuyasa@poltekkes-denpasar.ac.id

demonstrated antimicrobial properties. These natural compounds are generally regarded as safer due to their minimal side effects and longstanding use in traditional medicine practices (Newman & Cragg, 2020).

Ginger (Zingiber officinale Rosc.) is a well-known medicinal herb commonly used both as a culinary spice and in traditional remedies. Its rhizome contains a variety of bioactive constituents, including essential oils, flavonoids, terpenoids, and phenolic compounds, all of which exhibit antimicrobial potential. Active components such as zingiberene, zingerone, sesquiphellandrene, and oleoresin have been shown to disrupt bacterial cell membranes and interfere with microbial metabolic processes (Sari & Nasuha, 2021). Several previous studies have reported that ginger extract is capable of inhibiting the growth of pathogenic bacteria such as Streptococcus mutans, a species closely related to S. pyogenes (Dianawati & Manisha 2023; Fibryanto et al., 2022)

One particular variety of ginger with high development potential is elephant ginger (Zingiber officinale var. officinarum), which contains a higher concentration of bioactive compounds compared to common ginger. This variety is easy to cultivate and widely available throughout Indonesia. Given these promising characteristics, scientific investigation is necessary to evaluate the antimicrobial activity of elephant ginger extract specifically against Streptococcus pyogenes.

Based on the above rationale, this study aims to assess the inhibitory effect of ethanol extract derived from elephant ginger rhizome on the growth of Streptococcus pyogenes using the disk diffusion method. The findings are expected to serve as a foundation for the development of plant-based antimicrobial agents as potential alternatives to conventional antibiotics, contributing to the prevention of antimicrobial resistance.

Corresponding author: okasuyasa@poltekkes-denpasar.ac.id

VOLUME 3, No 1. Tahun 2025 , ISSN 3032-4408 (Online)
https://ejournal.poltekkes-denpasar.ac.id/index.php/icmahs

METHOD

This research was conducted from October 2024 to April 2025 at several locations: at Pusat Pengolahan Pasca Panen Tanaman Obat (P4TO, Post-Harvest Processing Center for Medicinal Plants) in Tabanan for elephant ginger powder preparation, P4TO Karangasem for extract evaporation, and the Bacteriology Laboratory, Department of Medical Laboratory Technology, Poltekkes Kemenkes Denpasar for antimicrobial testing. A pure isolate of *Streptococcus pyogenes* was obtained from the Microbiology Laboratory of Udayana University.

The study employed a true experimental design using a post-test only control group method, with randomly assigned groups. The experimental group consisted of ethanol extracts of elephant ginger (*Zingiber officinale* var. *officinarum*) at concentrations of 15%, 17.5%, 20%, and 22.5%, while the control group used 96% ethanol as a negative control and 30 µg Vancomycin antibiotic discs as a positive control.

The extract was prepared using the maceration method. 50 grams of dried ginger powder was soaked in 500 mL of 96% ethanol for 72 hours, filtered, and remacerated with fresh solvent before evaporation. Different extract concentrations were prepared by diluting the concentrated extract in 96% ethanol to a total volume of 1 mL using volumetric calculations. Sterile paper discs (6 mm) were saturated with 20 µL of each extract concentration.

Mueller-Hinton Agar (MHA) media enriched with 5% blood was prepared and sterilized according to standard protocol. *S. pyogenes* was rejuvenated on blood agar and incubated anaerobically at 37°C for 24 hours. A bacterial suspension equivalent to 0.5 McFarland (1.5×10^8 CFU/mL) was prepared in sterile 0.9% NaCl solution, and inoculated onto the MHA plate using a sterile cotton swab.

After bacterial inoculation, the extract-impregnated discs, along with the positive and negative controls, were placed on the agar surface with 15 mm spacing between discs, then incubated anaerobically at 37°C for 24 hours. The inhibition zones were observed and measured using a digital caliper. Data collection involved measuring the clear zone diameters around the discs, expressed in millimeters. The instruments used included a digital caliper, sterile discs, McFarland densitometer, analytical balance, micropipettes, and standard laboratory equipment.

Corresponding author: okasuyasa@poltekkes-denpasar.ac.id

VOLUME 3, No 1. Tahun 2025 , ISSN 3032-4408 (Online)
https://ejournal.poltekkes-denpasar.ac.id/index.php/icmahs

The collected data were analyzed using the Shapiro-Wilk test for normality and Levene's test for homogeneity. If assumptions were met, a One-Way ANOVA was applied to assess significant differences between groups, followed by a Least Significant Difference (LSD) post hoc test to identify pairwise group differences. This analysis was conducted to determine the antimicrobial efficacy of elephant ginger ethanol extract and its potential as an alternative to conventional antibiotics.

RESULTS

This study shows that elephant ginger ethanol extract has antimicrobial potential against *Streptococcus pyogenes*, as indicated by the formation of an inhibition zone around the disc diffusion test. The test was conducted at concentrations of 15%, 17.5%, 20%, and 22.5%, and compared to Vancomycin 30 µg as a positive control and 96% ethanol as a negative control.

Table 1. Results of the inhibition zone diameter of elephant ginger ethanol extract and control

Concentrations	Bacterial Growth Inhibition Zone (mm)			Mean ± SD
	1	2	3	
Control (+)	22,8	22,1	22,78	22,56 ± 1,0
Control (-)	0	0	0	0,00 ± 0,00
15%	8,1	9,8	8,6	8,83 ± 0,8
17,5%	9,7	8,44	9,1	9,08 ± 0,6
20%	10,82	10,16	10,6	10,52 ± 0,4
22,5%	11	10,5	11,7	11,06 ± 0,3

Statistical analysis of the inhibition zone diameter using One-Way ANOVA showed a significance value of $p = 0.000$ ($p < 0.05$), indicating a statistically significant difference between concentration groups. Prior normality testing (Shapiro-Wilk) and homogeneity testing (Levene) confirmed that the data met the assumptions for ANOVA. Further LSD post hoc analysis revealed that the 22.5% concentration had a significantly greater inhibitory effect than the lower concentrations.

Corresponding author: okasuyasa@poltekkes-denpasar.ac.id

VOLUME 3, No 1. Tahun 2025 , ISSN 3032-4408 (Online)
https://ejournal.poltekkes-denpasar.ac.id/index.php/icmahs

DISCUSSION

The formation of inhibition zones in this study demonstrates that elephant ginger ethanol extract has significant antimicrobial activity against *Streptococcus pyogenes*. This effectiveness is likely due to the presence of secondary metabolites such as flavonoids, phenols, terpenoids, saponins, alkaloids, and tannins in the extract.

The measurement of the inhibition zone using a caliper showed that increasing the concentration of the extract was correlated with an increase in the diameter of the inhibition zone, reflecting enhanced antimicrobial effectiveness. Vancomycin used as a positive control, produced an average inhibition zone of 22.56 mm, indicating bacterial susceptibility. In contrast, 96% ethanol used as the negative control, did not produce any inhibition zone (0 mm), confirming that the antimicrobial activity originated from the extract and not from the solvent.

To eliminate the potential antimicrobial effects of ethanol, discs treated with 96% ethanol were left for one hour at room temperature to allow complete evaporation. Afterward, no inhibition zone was observed, strengthening the validity of the results.

The choice of 96% ethanol as the extraction solvent was strategic due to its ability to effectively extract these bioactive compounds. It has better volatility and absorption compared to 70% ethanol, allowing for more efficient extraction and concentration of active compounds (Dianawati & Manisha 2023). Additionally, its use as a negative control further validated the role of the extract itself in antimicrobial activity.

The addition of 5% blood in Mueller Hinton Agar and incubation using the candle jar method followed CLSI standards to optimize the growth of *S. pyogenes*, which requires increased CO_2 conditions (Dwijastuti & Dewi, 2023). The presence of clear inhibition zones supports the idea that the active compounds diffused outward from the disc into the agar, with the highest concentration of activity near the disc.

The antimicrobial activity of elephant ginger ethanol extract is attributed to the presence of various active compounds, each of which works through different mechanisms. Flavonoids play a role by disrupting bacterial cell membranes and inhibiting essential enzymes, thereby interfering with the microorganism's metabolic processes. Tannins act by altering the permeability of bacterial cell membranes, making them more

Corresponding author: okasuyasa@poltekkes-denpasar.ac.id

susceptible to lysis (Alouw et al., 2022). Phenols contribute to antimicrobial effects by denaturing bacterial proteins, leading to the loss of their biological function (Utami et al., 2018). Saponins compromise bacterial cell wall integrity, ultimately causing cell rupture. Meanwhile, alkaloids inhibit the synthesis of peptidoglycan, an essential component of the bacterial cell wall, thereby hindering bacterial growth and replication (Magvirah et al., 2020). These synergistic mechanisms highlight the potential of elephant ginger as a natural antimicrobial agent.

Although the inhibition zones were smaller than those of Vancomycin (a potent standard antibiotic), the results suggest that elephant ginger could serve as a natural alternative antimicrobial agent. Discrepancies with previous studies (Sitepu, 2023) may be due to differences in extraction methods, ethanol concentration, or biological variation in plant materials. Experimental variables like pH, temperature, and microbial strain sensitivity were well-controlled in this study (Wilapangga & Syaputra 2018), ensuring the reliability of findings.

CONCLUSIONS

The average diameter of the inhibition zone of elephant ginger ethanol extract (*Zingiber officinale* var. *officinarum*) against *Streptococcus pyogenes* at concentrations of 15%, 17.5%, 20%, and 22.5% were 8.83 mm, 9.08 mm, 10.52 mm, and 11.06 mm, respectively. Statistical analysis showed a significant difference in the inhibition zone diameters between all tested concentrations ($p < 0.05$), indicating a concentration-dependent antibacterial effect. Further research is needed to create a product that can be added with elephant ginger ethanol extract so that it is more acceptable to the public.

CONFLICT OF INTEREST

There is no conflict of interest regarding the publication of this paper.

ACKNOWLEDGMENT

We acknowledge to lecturer of Politecnic Health Denpasar Indonesian Republic of Healthy Ministry Department for supporting this research.

Corresponding author: okasuyasa@poltekkes-denpasar.ac.id

REFERENCES

Badan Penelitian dan Pengembangan Kesehatan. (2018). *Laporan nasional Riskesdas 2018*. Kementerian Kesehatan Republik Indonesia.

Sari, D., & Nasuha, A. (2021). Kandungan zat gizi, fitokimia, dan aktivitas farmakologis pada jahe (*Zingiber officinale* Rosc.): Review. *Tropical Bioscience: Journal of Biological Science, 1*(2), 11– 18.

Tobat, S. R., Mukhtar, M. H., & Pakpahan, I. H. D. (2015). Rasionalitas penggunaan antibiotika pada penyakit ISPA di Puskesmas Kuamang Kuning I Kabupaten Bungo. *Scientia: Jurnal Farmasi dan Kesehatan, 5*(2), 79.

Rostinawati, T. R., Rikadianan, R., & Wicaksono, I. A. (2022). Sensitivity assay of resistant *Escherichia coli* to fosfomycin, kanamycin, rifampicin, ceftriaxone, imipenem, and vancomycin. *Indonesian Journal of Pharmaceutical Science and Technology, 1*(1), 1.

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products, 83*(3), 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285

Dianawati, N., & Manisha, R. (2023). Perbedaan antara ekstrak etanol jahe emprit (*Zingiber officinale* var. *amarum*), jahe gajah (*Zingiber officinale* var. *officinarum*), dan jahe merah (*Zingiber officinale* var. *rubrum*) dalam menghambat bakteri *Streptococcus mutans*. *Bhakta Dental Journal, 1*(1), 13– 18.

Fibryanto, E., Stefani, R., & Winaldy, B. (2022). Pengaruh ekstrak jahe gajah (*Zingiber officinale* var. *officinarum*) terhadap jumlah koloni *Streptococcus mutans* (in vitro). *Jurnal Kedokteran Gigi Universitas Padjadjaran, 34*(2), 136–142.

Rostinawati, T. R., Rikadianan, R., & Wicaksono, I. A. (2022). Sensitivity assay of resistant *Escherichia coli* to fosfomycin, kanamycin, rifampicin, ceftriaxone, imipenem, and vancomycin. *Indonesian Journal of Pharmaceutical Science and Technology, 1*(1), 1.

Dwijastuti, N. M. S., & Dewi, G. A. A. S. (2023). Perbedaan hasil kultur anaerob dengan candle jar dan gas generating sachet untuk bakteri asam laktat *Lactobacillus plantarum* E12.1. *Jurnal Biologi Makassar, 8*(1), 92–101.

Sumilat, D. A. (2019). Antibacterial screening activity of several sponges against *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus saprophyticus*, dan *Pseudomonas aeruginosa*. *Jurnal Ilmiah Platax, 7*(2), 455.

Herawati, I. E., & Saptarini, N. M. (2020). Studi fitokimia pada jahe merah (*Zingiber officinale* Roscoe var. *sunti* Val). *Majalah Farmasetika, 4*(Suppl. 1), 22–27.

Alouw, G., Fatimawali, F., & Lebang, J. S. (2022). Uji aktivitas antibakteri ekstrak etanol daun kersen (*Muntingia calabura* L.) terhadap bakteri *Staphylococcus aureus* dan *Pseudomonas aeruginosa* dengan metode difusi sumuran. *Jurnal Farmasi Medica/Pharmacy Medical Journal (PMJ), 5*(1), 36.

Corresponding author: okasuyasa@poltekkes-denpasar.ac.id

Utami, D. E. R., Krismayanti, L., & Yahdi, Y. (2018). Pengaruh jenis sirih dan variasi konsentrasi ekstrak terhadap pertumbuhan jamur *Candida albicans*. *Biota, 8*(2), 142–156.

Magvirah, T., Marwati, M., & Ardhani, F. (2020). Uji daya hambat bakteri *Staphylococcus aureus* menggunakan ekstrak daun tahongai (*Kleinhovia hospita* L.). *Jurnal Peternakan Lingkungan Tropis, 2*(2), 41.

Sitepu, J. N. (2023). Uji aktivitas antibakteri ekstrak jahe merah (*Zingiber officinale* var. *rubrum*) dan ekstrak bawang putih (*Allium sativum*) terhadap *Streptococcus pyogenes* dengan metode difusi cakram. *Nommensen Journal of Medicine, 8*(2), 77–81.

Wilapangga, A., & Syaputra, S. (2018). Analisis antibakteri metode agar cakram dan uji toksisitas menggunakan BSLT (brine shrimp lethality test) dari ekstrak metanol daun salam (*Eugenia polyantha*). *Indonesian Journal of Biotechnology and Biodiversity, 2*(2), 50–56.

Corresponding author: okasuyasa@poltekkes-denpasar.ac.id