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Potential of Wild Ginger (*Zingiber zerumbet*) Rhizome Extract as Antioxidant and Antibacterial

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Abstract. Indonesian people have long used wild ginger (*Zingiber zerumbet*) as an ingredient in traditional medicine and rituals. Its rhizomes have been shown to contain several bioactive compounds with antioxidant and antibiotic activity that are believed to be able to treat typhoid fever. This study aims to evaluate the antioxidant and antibacterial activities of *Z. zerumbet* extract using ethanol and methanol as solvents. *Z. zerumbet* extract was purified, LC-MS tests were conducted to identify active compounds, phenolic and flavonoid content tests were conducted using colorimetry methods, DPPH tests were conducted to measure antioxidant activity, and Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) tests were conducted to measure antibacterial activity. LC-MS analysis proved that *Z. zerumbet* contains active compounds such as flavonoids with their derivatives, such as kaempferol, quercetin, myricetin, etc. *Z. zerumbet* with ethanol solvent had stronger antioxidant activity (IC_{50} = 125.97 μg/mL) than methanol solvent (IC_{50} = 404.91 μg/mL). In contrast, ethanol solvent had lower antibacterial activity than methanol solvent. The findings of this study show that the antioxidant and antibacterial activities of *Z. zerumbet* depend on the solvent used and can be used as an alternative natural remedy for treating typhoid-related infections. This research supports the three goals of the SDGs by strengthening public knowledge of *Z. zerumbet* as a traditional medicinal ingredient, thereby improving disease control and the quality of public health through natural medicinal ingredients.

Keywords: antibacterial activity; antioxidant activity; *Zingiber zerumbet*

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INTRODUCTION

Indonesian people have long used wild ginger (*Zingiber zerumbet*) as an ingredient in traditional medicine and rituals (Jitjum et al., 2025; Pitopang et al., 2019). The use of plants as medicinal ingredients in people's lives is still prevalent today, especially in the Zingiberaceae Family (Abdul Rasyid Fakhrun Gani et al., 2024). Indonesians believe that plants are a natural solution to various health problems and have developed specialized techniques for managing these plants to treat certain diseases (Gani et al., 2022).

Local beliefs about the use of *Z. zerumbet* are supported by phytochemical studies that have proven the plant contains bioactive compounds such as flavonoids and terpenoids (Ghasemzadeh et al., 2016; Ibáñez et al., 2023). Several compounds found in these plants exhibit antibacterial, anti-inflammatory, and immunomodulatory activities, supporting their use as traditional medicinal ingredients (Ahmad et al., 2025; Akhtar et al., 2019). Given its potential, wild ginger is expected to be used as an experimental material in modern pharmacological research to support its use in traditional medicine.

Traditional medicine is one of the fields of ethnomedicine. Traditional medicine provides a foundation for the development of modern pharmacological research (Chung et al., 2021; Ouoba et al., 2022). Pharmacological research provides a scientific basis for traditional medicine (Arman et al., 2022; Hemmami et al., 2024). For example, the *Z. zerumbet* has cultural and health values and is a natural medicinal ingredient (Chan et al., 2023; Sharkawi et al., 2019). The scientific and cultural aspects of the study are also important for identifying other potential uses of *Z. zerumbet* to address health problems.

One of the Indonesian health problems is typhoid fever. Typhoid fever occurs due to contamination of water and food with *Salmonella typhimurium* (Mujiyanto et al., 2025; Nurjayadi et al., 2025). The spread of this disease occurs across several regions of Indonesia. This is very important to overcome so that the Indonesian people can reduce the number of typhoid fever cases. Typhoid fever cases require alternative drugs that are relatively more affordable for the community. In addition, this need is driven by the need to treat patients with antibiotic resistance (Adesegun et al., 2020; Brockett et al., 2020).

Typhoid fever is a challenge for the Indonesian people. This study is designed to address this problem by researching the potential of *Z. zerumbet* as a natural treatment for typhoid fever. The use of plant-based drugs is very beneficial because they can be used sustainably (Nuro et al., 2024; Sánchez et al., 2020). A previous study has demonstrated the potential of *Z. zerumbet* in treating fever due to viral infection (Salleh et al., 2024). Therefore, this study will delve deeper into the potential of *Z. zerumbet* in treating typhoid fever. This study required the identification of the *Z. zerumbet* active compounds, antioxidant activity, and antibacterial activity.

This study aims to evaluate the antioxidant and antibacterial activities of *Z. zerumbet* rhizome extract using ethanol and methanol as solvents. This research is expected to contribute to solving health problems in the community while supporting the SDGs on "good health and well-being". This research can serve as an initial reference for developing Indonesian traditional medicine to address typhoid fever. In addition, this study highlights the wealth of local knowledge of the Indonesian people who use wild ginger as a natural medicine that can be scientifically validated.

METHODS

Study area

The research was conducted in Malang City, East Java, Indonesia. Malang is a highland city well-suited for medicinal plant cultivation. The research sample is wild ginger (*Zingiber zerumbet*), purchased from a spice trader in Malang City.

Procedures

Extraction and Purification

Extraction was performed by repeated maceration with ethanol and methanol as solvents. Maceration was performed three times: the first for 72 hours and the subsequent two for 24 hours each. Extracts were filtered, concentrated under reduced pressure, and further purified by C18 column chromatography. Purified fractions were stored at 40°C until use (Tsvetov et al., 2020).

LC-MS Analysis

The bioactive compounds in wild ginger rhizome extracts (ethanol and methanol) were analysed using a high-performance liquid chromatography system coupled with a quadrupole time-of-flight mass spectrometer. Compound composition was determined from chromatographic peak intensities, with concentrations estimated based on molecular weight and dilution factors. Identification was confirmed by retention time and molecular weight, and further validated through comparison with reference databases (MassBank and NIST) and published literature (Rupprecht et al., 2022).

Total Phenol

Total phenolic content was determined using the modification of the Folin–Ciocalteu colorimetric method with gallic acid as a standard (Khan et al., 2024). 1 mL of wild ginger extract was pipetted and treated as a standard solution to measure its absorbance at 765 nm. The absorbance values were then entered into the gallic acid regression equation to obtain total phenol measurements. Total phenol results are expressed in milligram equivalents per gram of sample (GAE/g).

Total Flavonoid

The measurement of total flavonoids used a colorimetric method with a quercetin standard, with quercetin concentration adjusted. A standard quercetin solution is prepared by dissolving 1mg of raw quercetin in methanol until a concentration of 100 ppm is obtained. Next, a standard solution was prepared at concentrations of 25, 50, 75, and 100 ppm. Each solution is added: 3mL of methanol; 9.2 mL of $AlCl3$ 10%; 0.2 mL of Potassium Acetate; and aquades up to 10mL. The solution is then incubated at room temperature for 30 minutes. The solution is measured by absorbance using UV-Vis at 431nm, so that standard curves and regression equations can be generated. The methanol and ethanol extracts of wild ginger rhizomes are prepared in the same way as standard solutions. The absorbance value results are entered into the standard quercetin regression equation, and the results are expressed in milligrams of quercetin equivalent per gram of sample (QE/g).

Antibacterial Test

Antibacterial test evaluated using the liquid dilution method. This test used seven treatments, namely positive control using chloramphenicol (K1), 400 mg/L wild ginger extract (K2), 200 mg/L wild ginger extract (K3), 100 mg/L wild ginger extract (K4), 50 mg/L wild ginger extract (K5), 25 mg/L wild ginger extract (K6), and negative control using solvent (K7). Each extract with a different solvent is treated the same as 7 treatments. The bacteria were inoculated, and the suspension was standardized using 0.5 McFarland. The wild ginger extract in each treatment is mixed with the same volume of bacterial cultures. Results were observed based on Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Antioxidant Activity Test

The antioxidant activity test of wild ginger extracts (ethanol and methanol) was evaluated using the DPPH method (Oliveira, 2015; Yamauchi et al., 2024). A DPPH stock solution was prepared by dissolving 400 µg DPPH in methanol to a final volume of 10 mL, then mixing until homogeneous. Extracts were dissolved in methanol at concentrations of 25, 50, 75, and 100 ppm. Each 100 µL extract solution was combined with 40 µg/mL DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance was measured using a UV-Vis spectrophotometer at 517 nm, and the percentage of radical inhibition was calculated. The IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals, was determined from the regression curve.

RESULTS AND DISCUSSION

Identification of Active Compounds of Wild Ginger with LC-MS

The dominant bioactive compounds in methanol and ethanol extracts of wild ginger (Figure 1), as determined by LC-MS, are similar and include flavonoid glycosides such as kaempferol, myricetin, and

quercetin, as well as various rhamnoside and acetylated derivatives. This research result indicates that both solvents are capable of dissolving the main flavonoid compounds in ginger. However, there are compositional differences between the two solvents. The dominant compound of wild ginger extract, which uses methanol solvent, is kaempferol 3-(2",4"-diacetylrhamnoside) based on the highest TIC value, followed by other compounds such as kaempferol derivatives, myricetin, and quercetin. Meanwhile, wild ginger extract using ethanol has the same bioactive compound content but with a different distribution, for example, the presence of kaempferol 3-(3",4"-diacetylrhamnoside), which predominantly appears in ethanol solvent.

The solvent differences indicate that solvent polarity influences the successful extraction of various bioactive compounds. Methanol, a more polar solvent than ethanol, can extract a wider variety of flavonoid derivatives with rhamnoside substituents. Meanwhile, the ethanol tends to extract kaempferol derivatives with more complex acetylation groups. These research results show that methanol and solvent can effectively extract flavonoids from wild ginger. However, the solvent affects the type of flavonoid derivative that is dominant, thereby implicating biological activities that can differ, such as antioxidant or antibacterial properties, depending on the specific structure of the compound obtained. Solvents and techniques can influence the active emerging compounds from ginger extract. Table 1 shows the differences in active compounds from ginger based on GC-MS techniques and various compounds from the Zingiberaceae family.

Table 1 shows the comparison of the five dominant wild ginger compounds with the various species of the Zingiberaceae family. The wild ginger bioactive compounds analyzed by LC-MS are similar to those in other Zingiberaceae species. Zingiberaceae generally contains phenolics, flavonoids, and essential oils (Arcusa et al., 2022; Styawan et al., 2022). Turmeric extract contains diarylheptanoids, such as curcumin and cinnamic acid derivatives (Reddy & Lee, 2025). Wild ginger also contains some bioactive compounds such as flavonoids, polyphenols, alkaloids, coumaric acid, organic acids, and other vitamins (Li et al., 2017; Nonglang & Bhan, 2025).

Total Phenol and Flavonoid

The curve of total phenols based on a gallic acid standard (Figure 2) showed a linear relationship between concentration and absorbance at 765 nm. The regression equation is $y = 0.0189x + 0.0550$, with R2 = 0.9991, indicating good linearity over the concentration range of the test results. The slope of the regression line is 0.0189 Abs/ppm; this indicates that a 10-ppm increase in gallic acid concentration can increase absorbance by 0.189. The positive intercept of 0.055 indicates that the absorbance contribution to the measurement system is relatively small, so it does not significantly affect the linearity of the curve.

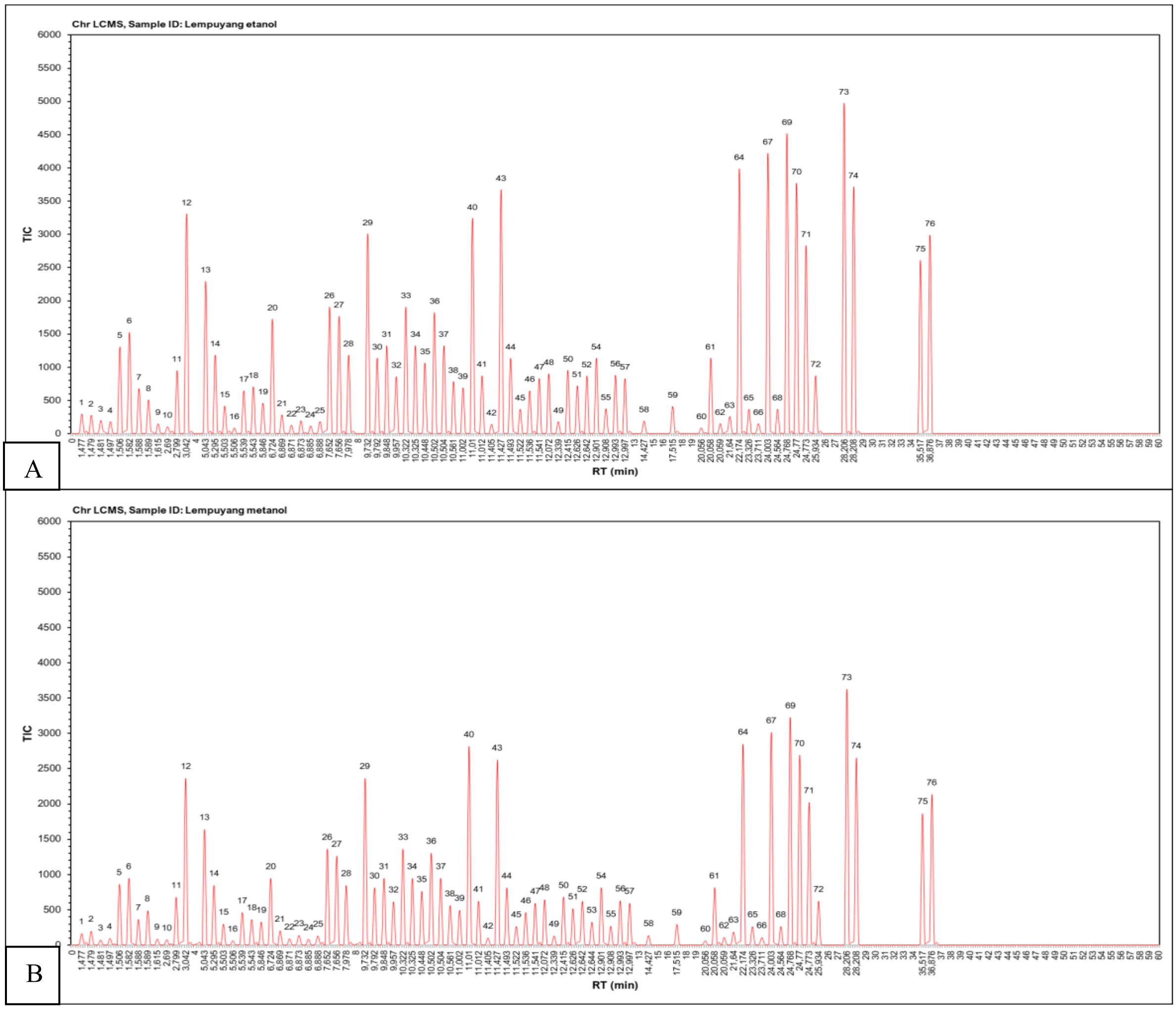


Figure 1. Chromatogram of Bioactive Compounds of Wild Ginger Rhizome with A Different Solvent: (A) Methanol and (B) Ethanol

Table 1. Comparison of 5 dominant compounds in wild ginger with various Zingiberaceae species

Species	Bioactive compound	Analysis techniques	References
Z. zerumbet	Camphor; 1,5,9-Decatiene; 1,6-Heptadine; beta-selinine; and Linalyl acetate	GC-MS	(Helen et al., 2009)
Z. officinale	Zingiberene; alpha-farnesene; b-sesquiphellandrene; alpha-curcumene; and a-farmasene	GC-MS	(Maisaroh et al., 2025)
C. aeruginosa	Ar-turmeron, curzane, furanodiene, curzerenon, and furanodienon	LC-MS	(Septaningsih et al., 2018)
K. galanga	Cyperene, a-gurjunene, heptadecane, 8-heptadecane, and gamma-muurolrnr	GC-MS	(Muzzazinah et al., 2024)
C. zedoaria	1,2,3-Propanetriol; 2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol; 13-Docosenamide; Benzofuran; and 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	GC-MS	(Sasanti et al., 2025)
C. mangga	(E)-Labda-8 (17), 12_dien-15, 16-dial; Neocurdione; Ambrial; and Epicurzerenone	GC-MS	(Sasanti et al., 2025)

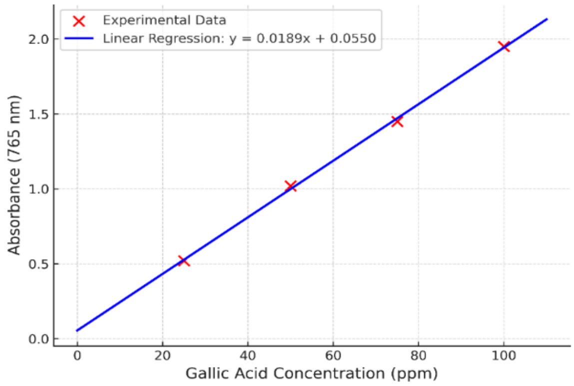


Figure 2. Total Phenol Curve Based on Gallic Acid Standard in Wild Ginger Rhizome Extract

The total phenol curve obtained with the gallic acid standard can be used to determine the total concentration of phenolic compounds using the Folin–Ciocalteu method. Symmetrical and small residual values strengthen the reliability of the resulting linear regression, with a concentration range of 25–100 ppm, considered the optimal working zone. However, the data set is limited, so it is recommended that future research use more data points and conduct repeated tests to increase precision.

The total flavonoid curve based on the quercetin standard (Figure 3) shows a linear relationship between quercetin concentration and absorbance values. The regression equation obtained from this test is $y = 0.0030x - 0.0150$ with $R2 = 0.9441$. This shows that 94% of the data variation can be explained by a linear model, although not as well as the gallic acid standard curve. The slope of the regression line is 0.0030 Abs/ppm, indicating that an increase in quercetin concentration of 10 ppm can increase absorbance by 0.03. The presence of a small negative intercept (-0.015) explains that the absorbance value at concentrations close to zero is almost indistinguishable from the blank.

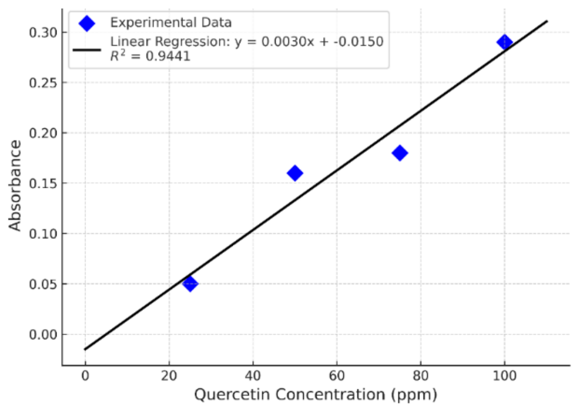


Figure 3. Total Flavonoid Curve Based on Quercetin Standard in Wild Ginger Rhizome Extract

The data points showed good linearity, with the lowest deviations at medium concentrations (50–75 ppm). This decreased the quality of the regression fit. This is due to several factors, including the stability of the standard solution, matrix interference, and pipette variations during testing. Flavonoid compounds in wild ginger rhizome extracts dissolved by methanol and ethanol solvents will produce different flavonoid compound derivatives. Methanol solvent generally dissolves flavonoids better than ethanol, as its polarity is higher (Dong et al., 2017). The herbal extracts with high flavonoid content are directly proportional to their antibacterial activity, particularly in inhibiting the growth of Gram-negative bacteria, such as *S. typhimurium* (Abdullah et al., 2021; Sinaga et al., 2021).

Total phenolics are an important parameter for assessing a plant's antibacterial potential. Phenolic compounds can disrupt bacterial cell membranes, alter bacterial lipid structures, and damage bacterial cell walls (Hossain et al., 2022). Extracts with high phenol content tend to exhibit stronger bacterial growth-inhibitory properties. Differences in solvents also affect the compounds dissolved, so methanol often dissolves more phenolic compounds than ethanol in certain cases (Mitrović et al., 2024). This shows that different solvents can affect the total phenols and flavonoids in wild ginger extracts.

Antioxidant Activity

Figure 4 shows the effect of concentration on the antioxidant activity of wild ginger. The higher the concentration, the higher the attenuation rate. These results explain that wild ginger extract with methanol solvent has a higher inhibition percentage. The linear regression analysis of wild ginger extract dissolved in methanol showed a positive correlation between solution concentration and the percentage of inhibition. An R2 value close to 1 indicates that the variation in inhibition percentage data is influenced by increasing the concentration of the solution extract. In addition, the linear regression results for the ethanol extract indicate a linear relationship between the percentage inhibition and the extract concentration. The high R^2 value indicates that the variation in inhibition data is due to increasing extract concentration. These results demonstrate that the ethanol-dissolved extract is quite stable and can inhibit free radicals, but at a lower rate than the methanol-dissolved extract.

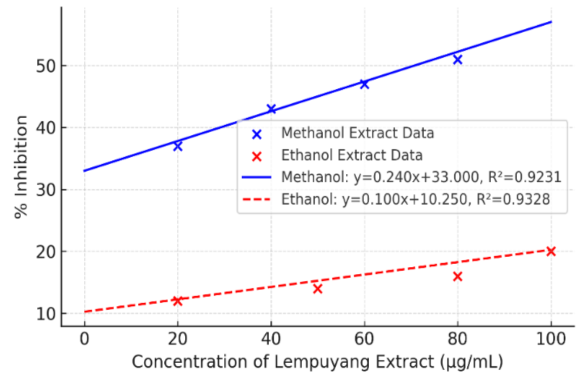


Figure 4. Antioxidant Activity of Wild Ginger Extract

Table 2. IC_{50} Value of Wild Ginger Extract Based on Solvent

Fraction	**IC_{50} Antioxidant Activity (µg/mL)**
Ethanol	125.97
Methanol	404.91

The IC_{50} data (Table 2) explain the difference in antioxidant activity between ethanol and methanol solvents for wild ginger rhizome. The IC_{50} value is the basis for determining the sample concentration to inhibit 50% of free radicals. A lower IC_{50} value indicates stronger antioxidant activity. The wild ginger extract dissolved in ethanol has an IC_{50} of 125.97 µg/mL, which is much lower than that of the methanol extract (404.91 µg/mL). This shows that bioactive compounds dissolved in ethanol are more effective than those dissolved in methanol at inhibiting free radicals.

The difference in IC_{50} results is related to the polarity of the solvent used in extracting wild ginger. Ethanol is more polar than ethanol, allowing it to dissolve phenolic compounds and flavonoids with better antioxidant activity. Therefore, it can be concluded that ethanol is a more optimal solvent for producing wild ginger extract with stronger antioxidant activity than methanol. This may be why ethanol is a more promising candidate as a natural ingredient in healthy foods and traditional medicines to counteract free radicals.

Antibacterial Activity Test

The MIC results (Table 3) for wild ginger extract prepared with ethanol and methanol show that the ethanol extract has higher antibacterial activity against *S. typhimurium*. This can be explained by the clarity of the solution at concentrations K2 to K3: the ethanol solvent yields a clearer extract than the extract with ethanol solvent, which is already cloudy at concentration K3. The clear solution indicates that wild ginger extract inhibits the growth of *S. typhimurium*. The higher the clarity level, the stronger the extract's ability to inhibit bacterial growth. It can be concluded that the extract with ethanol solvent is better at inhibiting the growth of *S. typhimurium* than the extract with methanol solvent.

Table 3. MIC Value of Wild Ginger Rhizome Extract

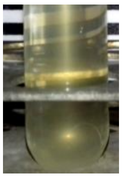
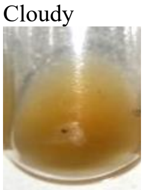
Extract	MIC Value						
	K1	K2	K3	K4	K5	K6	K7
Ethanol	clear 	clear 	Clear 	Fairly Clear 	Cloudy 	Very Cloudy 	Very Cloudy 
Methanol	Clear 	Clear 	Cloudy 	Cloudy 	Cloudy 	Very Cloudy 	Very Cloudy 

Table 4. MBC Value of Wild Ginger Rhizome Extract

Extract	MBC Value						
	K1	K2	K3	K4	K5	K6	K7
Ethanol	0.5	149.5	190	302.5	317	398	613
Methanol	0.5	56.5	82.5	88.5	95.5	138	285

At lower concentrations (K4–K6), the extracts dissolved using ethanol and methanol began to show changes from somewhat clear to very cloudy, indicating that lower concentrations did not completely kill the bacteria. Antibacterial effectiveness decreased as the bioactive compound content decreased. Overall, the extract dissolved in ethanol remained superior to that dissolved in methanol, as it maintained clarity for a longer period. It was concluded that the ethanol extract had greater antibacterial activity against *S. typhimurium*.

The MBC values in Table 4 show that wild ginger extracts dissolved in ethanol and methanol exhibit different levels of inhibition of *S. typhimurium* growth. The extract dissolved in methanol had a relatively lower MBC than the ethanol solvent, for example, at concentrations K2–K5 (56.5–95.5 µg/mL), whereas the ethanol solvent had a higher MBC (149.5–317 µg/mL). The lower MBC value indicates that a lower concentration is required to inhibit S. typhimurium growth. The two extracts showed a clear colour difference at a low concentration (K6). The methanol extract still showed a lower MBC value (138–285 µg/mL) than the ethanol extract (398–613 µg/mL). This data shows that methanol is a solvent that yields a wild ginger extract with more effective antibacterial activity.

The active compounds in *Z. zerumbet* have been shown to inhibit the growth of *S. typhimurium bacteria* (Alwakil et al., 2022; Rahayu et al., 2019). The antibacterial activity of the compounds present in *Z. zerumbet* inhibits bacterial growth. The antibacterial mechanisms include damaging cell walls, inhibiting protein synthesis, and entering bacterial cells and damaging their interiors (Qin et al., 2024; Wong et al., 2021). This shows that methanol and ethanol can be used in the extraction of wild ginger.

The research findings evaluated the comparison of two polar compounds to extract wild ginger rhizomes. The analysis of these two solvents is important for initial identification and for determining a suitable solvent for the extraction of wild ginger rhizomes. This research supports the use of *Z. zerumbet* as a natural medicine through scientific research (Fathi et al., 2022; Noor et al., 2022). The information in this study can be disseminated to the general public. One of them is through ethnobotanical learning at universities by developing interesting learning (Gani et al., 2024). In addition, it is necessary to implement other programs, such as community service, for the use of this plant. The program must be carried out so that this research directly benefits the community and supports health programs aligned with the Sustainable Development Goals (SDGs).

The findings of this study are very important for supporting the SDGs on "good health and well-being". The use of plants as ingredients in traditional medicine has been shown to prevent and treat various diseases (Gitima et al., 2025). Indonesian people also often use other types of plants from the Zingiberaceae Family as medicinal ingredients (Henri et al., 2022; Rahayu et al., 2021). Zingiberaceae plants, especially *Z. zerumbet,* will be readily accepted by the community. Plants with cultural value will support sustainable use and local community conservation (Garcia, 2020). The sustainable acceptance and use of these plants will support the SDGs program by improving public health and preventing the spread of diseases such as typhoid fever. In addition, *Z. zerumbet* is more affordable for the community. These plants are easier to find and more economical. Therefore, the use of plants can support sustainable health programs in the community.

CONCLUSION

Wild ginger extracted with the ethanol solvent exhibited stronger antioxidant activity of wild ginger (IC_{50} = 125.97 µg/mL) than the methanol extract (IC_{50} = 404.91 µg/mL). The methanol extract demonstrated greater antibacterial potency, as evidenced by lower MBC values against *S. typhimurium*. Wild ginger shows significant potential as a phytopharmaceutical candidate, with ethanol more suitable for antioxidant activity, while methanol shows the highest antibacterial effects. Ethanol extract is more suitable for applications requiring antioxidant properties, while methanol extract is better suited for antibacterial purposes. Further studies are recommended to evaluate in vivo efficacy and potential applications in the pharmaceutical and food industries.

AUTHOR CONTRIBUTION STATEMENT

SS conceived and designed the study, conducted the data analysis, and wrote the article. ARFG was responsible for data collection and writing the article. NK was responsible for data collection and proofreading the article. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

Each author has no personal interests or conflicts of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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