

Antibacterial Activity of *Zingiber officinale* Rhizome

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ABSTRACT---Background: The aim of this study was to determine the antibacterial activity and phyto-chemical screening of the *Zingiber officinale* ethanol extract. Fresh rhizomes of *Z. officinale* (Ginger) are collected from Manoko Plantation in Bandung, West Java, Indonesia. **Methods:** Antibacterial activity was determined using the well diffusion method while phytochemical screening was performed to determine the phytochemical composition of the ginger rhizome. **Results:** Results of phytochemical screening revealed phenolic compounds, alkaloids, flavonoids, tannins, and terpenoids. Results for bioassay presented variations in the biological activity of the extract. The extract showed antibacterial activity against all bacteria tested with inhibition diameter zones ranging from 2.67 to 12.47 mm mm. The highest zone was observed in *Staphylococcus aureus* at 400 mg/ml while the smallest inhibition zone was observed in *Pseudomonas aeruginosa* at 100 mg/ml. **Conclusion:** Our research proves that *Z. officinale* rhizome extract has the ability to inhibit bacterial growth. This shows that *Z. officinale* can potentially be used to develop new agents for combat bacteria. However, further studies are needed to explain the mechanism behind these effects.

Keywords: Antibacterial Activity, Manoko Plantation, Bandung, West Java

I. INTRODUCTION

Infectious disease is a cause of death and development in developing countries, including Indonesia. Spread of the source of infection can be known through vectors or known as vectors, namely air, animals, objects and also humans themselves (1). In Indonesia, bacterial infection is still a contagious disease and is also the most common cause of death in children under 5 years of age. Bacterial infections are also a cause of death in children under 1 year by 31%, children aged 1-4 years by 25% (2).

At this time there have been many excessive use of antibiotics and inappropriate usage methods that can cause resistance, so alternative treatments are needed, one of which is ginger. Ginger plant is one example of spice in the form of rhizomes which are often used as traditional medicine. The content of secondary metabolite compounds in ginger plants especially flavonoids, phenols, terpenoids and essential oils can inhibit the growth of pathogens that are detrimental to human life, including bacteria *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Neurospora* sp, *Rhizopus* sp. and *Penicillium* sp (3). From this background, we want to investigate the antibacterial activity of ginger extract *in vitro* in *S. aureus*, *B. subtilis*, and *P. aeruginosa* bacteria.

Plant Determination

Fresh rhizomes of crop determination (7 kg) *Z. officinale* were collected from Manoko Plantation in Bandung, West Java, Indonesia and transported to the Phytochemical and Pharmacological Laboratory, Buana Perjuangan University, for

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cleaning, air drying, grinding, and extraction processes. The plant was later identified as *Z. officinale* by the School of Life Sciences, Bandung Institute of Technology (Code: 6322 / 11.CO2.2 / PL / 2018).

***Z. officinale* rhizome extraction**

Z. officinale (150 g) rhizome powder was macerated thoroughly in 70% ethanol for 72 hours. The liquid extract is obtained and concentrated using a rotary evaporator at 50°C to produce around 15.80% concentrate (the fixed weight of the extract divided by the weight of simplicia multiplied by 100%).

Phytochemical screening

Z. officinale rhizome extract is qualitatively filtered to identify the presence of secondary metabolites including alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, and saponins (4).

Antibacterial Screening

Three different concentration (100, 200, 300 and 400 mg/ml) of ethanolic extracts, were prepared in sterile sample bottles by dissolving 0.1, 0.2, 0.3 and 0.4 g of each extracts in 1 ml of Dimethyl sulfoxide (DMSO).

Antibacterial assay

Antibacterial activity was tested using the agar diffusion method. The media for the prepared Mueller Hinton was poured into a Petri dish with a good label under aseptic conditions and allowed to freeze. Sterile cork borer is used to make four holes in the agar plate in a position quite similar to the other holes in the middle as a control. The plates are inoculated with a standard inoculum containing test bacteria. Each of the three concentrations was ejected in each hole while the control (Erythromycin) was in the center of the plate (5). After 24 hours of the incubation period, the resistance zone is observed with a clear resistance zone, measured and recorded in millimeters using a transparent plastic ruler (6).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of a compound that inhibits the growth of microorganisms. 6.5 g of nutritional stock is prepared by dissolving in 500 ml of distilled water. 5 ml of agar was put into a different test tube and covered with cotton. Agar is autoclaved at 121 ° C for 15 minutes. For each test organism one control isolate and control extract were used at three different concentrations (100, 200, 300 and 400 mg/ml) to make serial dilution by adding 1 ml of extract to each test tube and 0.5 ml of the control isolate diluted. with 3 ml of distilled water and channeled into each test tube and incubated at 37 ° C for 24 hours (7).

Statistical analysis

Data were analyzed using SPSS software (version 22). For statistical analysis, one-way analysis of variance (ANOVA) followed by Tukey was applied. The results are presented as mean ± SD and p-values less than 0.05 are considered significant.

II. RESULT

Phytochemical screening

The phytochemical screening of *Z. officinale* rhizome extract revealed the presence of chemical constituents such as phenolics, alkaloids, flavonoids, tannins, and terpenoids. A summary of the phytochemical screening of extract of *Z. officinale* rhizome is presented in Table 1.

Table 1. Phytochemical screening of extract *Z. officinale* rhizome.

Phytochemical screening	Etanolic extract of <i>Z. officinale</i> rhizome
Phenolics	+

Alkaloids	+
Flavonoids	+
Tannins	+
Terpenoids	+

Antibacterial activity

The results for antibacterial activity are presented in Table 2. Extracts from the ginger rhizome show antibacterial activity against all bacteria tested with inhibition diameter zones ranging from 2.67-12.47 mm. The highest zone was observed in *Staphylococcus aureus* at 400 mg / ml while the smallest inhibition zone was observed in *Pseudomonas aeruginosa* at 100 mg / ml. The general trend observed is an increase in the inhibition zone with an increase in extract concentration. The results of the minimum inhibitory concentration (MIC) showed that ginger ethanol extract showed the most significant effect because it inhibited *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* at 100 mg/ml (Table 3).

Table 2. Results for degree of sensitivity of bacteria against Ginger.

Concentration (mg/ml)	Zones of inhibition in (mm) Organisms		
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>
100	6.67 ± 0.35	5.67 ± 0.76	2.67 ± 2.31
200	8.80 ± 1.49	6.70 ± 0.40	8.33 ± 0.29
300	10,00 ± 0,70	7,47 ± 0,31	10,13 ± 0,40
400	12.47 ± 0.85	8.23 ± 0.29	11.73 ± 0.75
Erythromycin	15.57 ± 2.36	13.93 ± 3.76	21.43 ± 2.96
Negative control	0.00	0.00	0.00

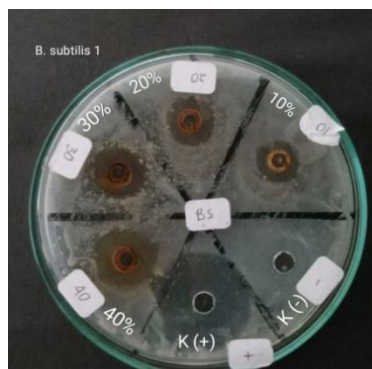
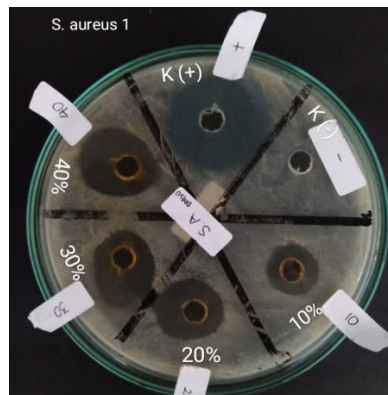


Figure 1. Zones of inhibition of *Z. officinale* rhizome extract against *S. aureus*, *B. subtilis* and *P. aeruginosa*.

Table 3. Results for minimum of inhibition concentration for Ginger.

Concentration (mg/ml)	Zones of inhibition in (mm) Organisms		
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>
100	+	+	+
200	+	+	+
300	+	+	+
400	+	+	+

III. DISCUSSION

The antibacterial activity of *Z. officinale* rhizome extract can be caused by the presence of several secondary metabolite compounds in *Z. officinale* rhizome extract, especially alkaloid, phenolic, flavonoid, tannin, and terpenoid compounds. Some secondary metabolites have been shown to have antibacterial activity. Some flavonoids have been known to inhibit bacterial growth through inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of attachment and biofilm formation, inhibition of porin in cell membranes, changes in membrane permeability, and attenuation of pathogenicity (8). In addition, alkaloids exhibit antibacterial activity and inhibit the transport of ATP-dependent compounds across cell membranes. These alkaloids can function as potential compounds that can act as lead compounds for the development of plant-based antibacterials and/or additional compounds (9).

The antibacterial properties of the phenolic compounds and aromatic alcohols (growth inhibition, lethal effects and cytological damage) were investigated. Furthermore, the antioxidant activity of phenolic compounds and tannins can inhibit bacterial protein synthesis and also neutralize oxidative damage caused by bacteria (10). Our research proves that *Z. officinale* rhizome extract has the ability to inhibit bacterial growth. This shows that *Z. officinale* can potentially be used to develop new agents to fight bacteria. However, further studies are needed to explain the mechanism behind these effects.

IV. ACKNOWLEDGMENT

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V. CONFLICT OF INTEREST

Authors declare no conflict of interest

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