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Antibacterial Activity of Ethanol Extract of Red Ginger (*Zingiber officinale* Roscoe) Against *Proteus mirabilis*

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ABSTRACT: Red ginger (*Zingiber officinale* Roscoe) has been extensively utilized in traditional medicine and is reported to possess a wide range of biological activities. *Proteus mirabilis* is a pathogenic bacterium that could cause Urinary Tract Infections (UTIs). Red ginger contains flavonoid compounds, phenolics, essential oils, and tannins that may contribute to its antibacterial properties. This study aimed to evaluate the differences in antibacterial activity of the ethanol extract of red ginger at various concentrations against *Proteus mirabilis* and to identify its phytochemical constituents. Red ginger simplicia was macerated using 96% ethanol and the macerate was evaporated with a rotary vacuum evaporator. Extracts were made in series concentrations of 20%, 40%, 60%, and 80% w/v for the antibacterial activity test. Ampicillin was used as a positive control, while dimethylsulfoxide was used as a negative control. Antibacterial activity was assessed based on the diameter of the inhibition zones, and the data were statistically analyzed using a one-way ANOVA test, followed by a post hoc Tukey test at a 95% confidence level. The results of this study showed that there were differences ($p < 0.05$) in the zone of inhibition values from all series of concentrations of the ethanol extract of red ginger against *Proteus mirabilis*. The extract was found to contain several classes of secondary metabolites, including terpenoids, alkaloids, phenolics, flavonoids, tannins, and saponins.

Keywords: Ethanol extract of red ginger (*Zingiber officinale* Roscoe); antibacterial; *Proteus mirabilis*.

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INTRODUCTION

Ginger is a valuable agricultural commodity. The plant's value is found in the rhizome, which is widely utilized as a component in both fresh and dried herbal preparations, beverages, food flavourings, spices, and medicinal herbs. Ginger rhizomes contain gingerol, oleoresin, and essential oils, which contribute to their extensive use as raw materials in traditional medicinal formulations (Sukmawati et al., 2021). One of the ginger varieties whose potential is widely explored is red ginger (*Zingiber officinale* Roscoe). Research into the antibacterial activity of red ginger needs to be expanded to provide comprehensive information regarding its potential.

According to Setyaningrum & Saparinto (2013), red ginger is characterized by its intensely spicy flavour and intense aroma, which make it a common ingredient in the production of ginger oil and various medicinal preparations. The plant contains essential oils ranging from approximately 2.58% to 3.90% of its dry weight. Furthermore, phytochemical screening of the ethanol extract of red ginger has revealed the presence of several bioactive compounds, including terpenoids, alkaloids, phenolics, flavonoids, tannins, and saponins (Herawati & Saptarini, 2019). The terpenoid, alkaloid, phenolic, flavonoid, tannin, and saponin classes of compounds are recognized for their potential antibacterial properties (Irfan et al., 2014; Othman et al., 2019; Alves et al., 2013; Mailoa et al., 2014; Tagousop et al., 2018).

Shareef et al. (2016) reported that the methanol extract of red ginger has antibacterial activity against *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia*. Research by Martani (2015) reported the Minimum Inhibitory Concentration (MIC) value of ethanol extract of red ginger against *Streptococcus mutans* bacteria at a concentration of 10%. Another study by Widiastuti & Pramestuti (2015) reported that the ethanol extract of red ginger at a concentration of 20% exhibited antibacterial activity against *Staphylococcus aureus*, with a zone of inhibition of 10.17 ± 2.26 mm.

Proteus mirabilis is a Gram-negative, rod-shaped, non-spore forming, and facultatively anaerobic bacterium (Chakkour et al., 2024). Urinary Tract Infections (UTIs) can be caused by bacteria in the urinary tract, including the bladder, prostate, and kidneys (Nuari & Widayati, 2017). The leading cause of UTIs is Gram-negative bacteria that normally inhabit the gastrointestinal tract, including *Escherichia coli* (approximately 80%) and *Klebsiella* spp. (around 5%), while *Enterobacter* and *Proteus* species are detected in about 2% of cases. Untreated urinary tract infections (UTIs) may lead to renal complications, as bacteria can ascend to the kidneys and cause infection, resulting in pyelonephritis, which is characterized by back pain, nausea, fever, and chills. If not treated promptly, kidney infections can lead to permanent kidney damage (Zuliani et al., 2021).

Sari et al. (2017) reported that the ethanol extract of agarwood leaves (*Aquilaria microcarpa* Baill) has antibacterial activity against *Proteus mirabilis*. Another study by Purnami (2012) demonstrated that the ethanol extract of fennel fruit (*Foeniculum vulgare* Mill.) exhibits antibacterial activity against *Proteus mirabilis*. Based on the description above, this study reports the differences in antibacterial activity of ethanol extract of red ginger at several concentration series against *Proteus mirabilis* and also determines the chemical compound groups contained in the extract.

METHODS

Preparation of Red Ginger (*Zingiber officinale* Roscoe) Powder

The red ginger rhizomes used in the study have a slightly firm texture and a reddish-brown color. The red ginger samples were collected from Sendangmulyo Village, Semarang, Central Java Province, Indonesia. Determination was carried out to ensure the authenticity of the plants used in the research. Determination was carried out at the Ecology and Biosystematics Laboratory, Department of Biology, Universitas Diponegoro, Semarang. Red ginger rhizomes are collected and then sorted to remove any dirt or unnecessary parts that are still attached to the ginger. After that, the red ginger is washed clean and chopped. Red ginger (1955 grams) is dried in a drying cabinet at 50°C until it is dry. The dried red ginger rhizomes were pulverised into a fine powder, and then checked for water content, which was found to be less than 10%.

Preparation of Red Ginger Ethanol Extract

Red ginger powder was weighed about 345 grams. The 96% ethanol (2,588 mL) was used to soak the simplicia powder for 3 days, protected from sunlight, at room temperature, with several stirrings. After 3 days, the mixture was filtered to obtain the macerate. The remaceration process is carried out by soaking the dregs in 862 mL of solvent for 2 days, then stirring and filtering again to obtain another macerate. All the macerates were mixed and placed in a closed container in a cool, dark place, protected from light. The filtrate was allowed to stand for 24 hours before being decanted. It was subsequently filtered and concentrated using a rotary vacuum evaporator maintained at 50°C until a viscous extract was obtained.

Preparation of Extract Solution

An 80% w/v stock solution of the red ginger ethanol extract was prepared by accurately weighing 4 g of the extract and dissolving it in 5 mL of dimethyl sulfoxide (DMSO) solvent in a sterile porcelain cup, which was then transferred into a sterile flask. The stock solution was diluted to create four series of concentrations: 20%, 40%, 60%, and 80% w/v.

Antibacterial Activity Test of Red Ginger Ethanol Extract

Clinical isolate of *Proteus mirabilis* obtained from the Integrated Biomedical Laboratory of Sultan Agung University. The culture was re-isolated to obtain pure strain, which was then stored in a refrigerator at 4°C until further use.

The antibacterial activity test was conducted using the pour-plate diffusion method. Approximately 25 mL of sterile Mueller-Hinton Agar (MHA) was mixed with 2.5 mL of a bacterial suspension culture (microorganism population: 1.5 × 10^8 CFU/mL), then homogenized (Banjara et al., 2012). The mixture was poured into a petri dish and then left to solidify. The paper disc was placed on the inoculated solid media. Four paper discs were dripped with 10 µL of extract solutions from all concentration series (20, 40, 60, and 80% w/v), and one paper disc was dripped with 10 µL of DMSO solvent. Five paper discs were placed on the solution until it was saturated (15 minutes). A paper disc containing 10 µg of ampicillin was applied to the same medium. The petri dish was left until the paper disc adhered well to the media. Incubation was carried out in an inverted petri dish at 37°C for 24 hours. Test results were determined by observing the clear zone formed around the paper disc after the incubation period and measuring the zone of inhibition using a vernier caliper. The test was carried out with three replications, and the average of zones of inhibition was calculated.

The zone of inhibition data was subjected to statistical analysis. Normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. Since the data met the assumptions of normality and homogeneity ($p>0.05$),

further analysis was conducted using a one-way ANOVA, followed by Tukey's post hoc test at a 95% confidence level.

Phytochemical Screening of Extract

Terpenoids

Fifty milligrams of extract was ground with ether and then filtered. Then, three drops of Liebermann-Burchard reagent solution were added, and the formation of a blue, green, orange, or red-purple colouration was indicative of the presence of terpenoid constituents (Sarker et al., 2006).

Alkaloids

An accurately weighed 50 mg portion of the extract was dissolved in 10 mL of 2N hydrochloric acid, after which the mixture was filtered. The resulting solution was subsequently distributed into four separate test tubes. Tube 1 served as an untreated control, tube two was treated with Dragendorff's reagent, tube 3 with Mayer's reagent, and tube 4 with Wagner's reagent. A positive result was indicated by the presence of a precipitate in the acidic solution in tubes 2, 3, and 4. A brownish-orange precipitate after the addition of Dragendorf's reagent, a white precipitate after the addition of Mayer's reagent, and a brownish precipitate after the addition of Wagner's reagent indicated a positive result (Farnsworth, 1966).

Phenolics

A quantitative amount of 50 mg of the extract was transferred into a test tube and dissolved in 5 mL of ethanol. The mixture was subsequently filtered, and three drops of ferric chloride ($FeCl_3$) reagent were introduced to the filtrate. The emergence of green, blue-green, red-purple, or blue-black colouration signified the presence of phenolic constituents. In contrast, the formation of a brown precipitate indicated the occurrence of polyphenolic compounds (Farnsworth, 1966).

Flavonoids

About 50 mg portion of the extract was transferred into a test tube and dissolved in 2 mL of 95% ethanol. The resulting solution was treated with a small quantity of magnesium powder, followed by the addition of 2N hydrochloric acid. After filtration, amyl alcohol was introduced to the filtrate. The development of a red, yellow, or orange colouration in the amyl alcohol phase confirmed the presence of flavonoid constituents (Farnsworth, 1966).

Tannins

A measured quantity of 50 mg of the extract was heated with 10 mL of distilled water in a water bath for 30 minutes. The mixture was then filtered, and 1 mL of a 2% sodium chloride solution was subsequently added to the resulting filtrate. If a suspension or precipitate formed, it was filtered through filter paper. To the obtained filtrate, three drops of a 1% gelatin solution were subsequently introduced, and the presence of precipitate indicated a positive result for tannins (Farnsworth, 1966).

Saponins

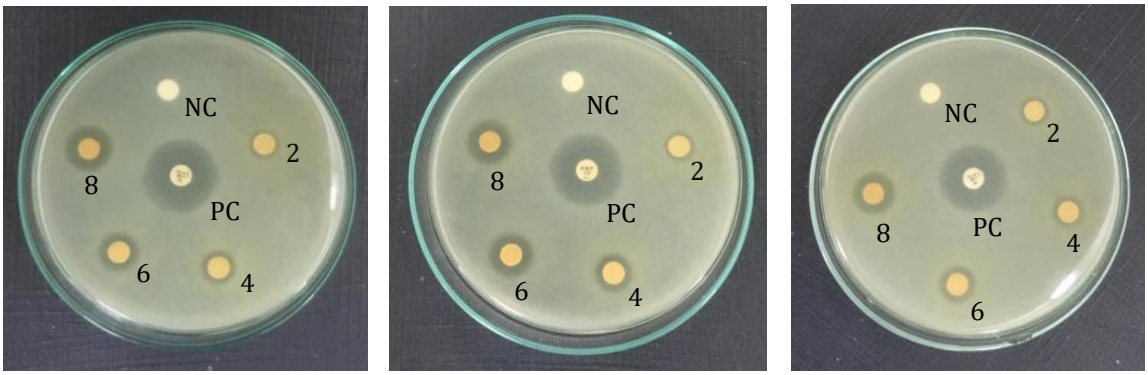
Dissolve 50 mg of the extract in water and shake vigorously for 10 seconds in a vertical direction. If the extract solution produces a foam approximately 1 cm high and persists for 10 minutes, this indicates a positive result for saponins (Farnsworth, 1966).

RESULT AND DISCUSSION

After drying, 370 grams of red ginger powder were obtained, resulting in a yield of 18.92% and the water content of the simplicia was 8%. The ethanol extract obtained from

red ginger was 37.1 grams, corresponding to an extract yield of 10.75%. The red ginger extract was subjected to organoleptic testing to describe its appearance, taste, odour, and colour. The results of the organoleptic evaluation were a thick, extract-like form but still pourable, with a pungent ginger flavour, a characteristic red ginger aroma, and a brownish-yellow colouration.

Ethanol extract of red ginger produced radical inhibition against *Proteus mirabilis*. This was indicated by a clear zone surrounding the disc, indicating no bacterial growth. Similar results were also demonstrated by the Ampicillin disc, used as a control, which exhibited a clear zone. The antibiotic Ampicillin was used because it has a broad spectrum of bactericidal properties, and is also used to treat urinary tract infections. DMSO solvent was used as a negative control to validate the method, demonstrating that the solvent does not exhibit antibacterial activity. This indicates that the antibacterial activity is solely attributed to the red ginger ethanol extract. The results of the antibacterial activity test for several treatments against *Proteus mirabilis* are presented in Figure 1.



(a) (b) (c)

Note: NC=Negative Control (DMSO solvent); PC=Positive Control (Ampicillin 10 µg/disk); 2=Ethanol extract of red ginger 2 mg/disc; 4=Ethanol extract of red ginger 4 mg/disc; 6=Ethanol extract of red ginger 6 mg/disc; 8=Ethanol extract of red ginger 8 mg/disc

Figure 1. Appearance the results of the antibacterial activity test for several treatments on the growth of *Proteus mirabilis*, (a) replication 1; (b) replication 2; and (c) replication 3

Table 1 shows that the zone of inhibition increases as the concentration of the ethanol extract of red ginger increases. Compared to the research of Shareef et al. (2016), the zone of inhibition produced by the ethanol extract was greater than that of the methanol extract against *Proteus mirabilis*. Methanol extract produced a zone of inhibition of 1.99±0.200 mm against *Proteus mirabilis*, while 2 mg/disc of ethanol extract gave a zone of inhibition of 9.14±0.02 mm. Increasing the extract concentration indicates a greater concentration of active compounds in the extract. This could mean that the ability to kill bacteria is also greater. The study is limited to the use of inhibition zone measurements, which provide only semi-quantitative data. To accurately evaluate the antimicrobial potency, it is necessary to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) through the broth microdilution method in accordance with the CLSI protocol. Determination of these values would enable the calculation of appropriate dosages for animal studies, thereby facilitating the evaluation of in vivo antibacterial efficacy.

Table 1. The zone of the inhibition value from several treatments against *Proteus mirabilis*

Treatment	Zone of inhibition (mm)			
	1st attempt	2nd attempt	3rd attempt	Average ±SD
Ethanol extract of red ginger 2 mg/disc	9.12	9.14	9.16	9.14[a] ± 0.02
Ethanol extract of red ginger 4 mg/disc	11.16	11.14	11.18	11.16[a] ± 0.02
Ethanol extract of red ginger 6 mg/disc	12.12	12.16	12.18	12.15[a] ± 0.03
Ethanol extract of red ginger 8 mg/disc	13.14	13.16	13.10	13.13[a] ± 0.03
DMSO solvent	-	-	-	-
Ampicillin 10µg/disk	19.12	19.14	19.16	19.14[a] ± 0.02

SD: Standard Deviation

(-): no inhibition zone was formed

[a]: significantly different ($p<0.05$) to all treatment

The zone of inhibition of red ginger extract was statistically significantly different compared to Ampicillin ($p < 0.05$), indicating a difference in antibacterial activity against *Proteus mirabilis*. The zone of inhibition between the red ginger extract concentration series was also significantly different (2, 4, 6, and 8 mg/disc), indicating differences observed across all concentrations of the extract solution ($p < 0.05$). The difference in concentration resulted in a distinct antibacterial activity profile, despite the type of extract being the same. The antibacterial activity profile of Ampicillin was also different from that of the ethanol extract of red ginger, as indicated by the statistical results ($p < 0.05$).

Phytochemical analysis revealed that the ethanol extract of red ginger comprised several classes of secondary metabolites, including terpenoids, alkaloids, phenolics, flavonoids, tannins, and saponins (Table 2).

Table 2. Phytochemical screening of red ginger ethanol extract

Chemical compounds	Reagents	Observation	Results
Terpenoids	Liebermann Burchard	Red colour	+
Alkaloids	Dragendorf's, Mayer's and Wagner's test	Precipitate formed on all reagents	+
Phenolics	$FeCl_3$	Green colour	+
Flavonoids	Shinoda test	Yellow colour	+
Tannin	1% gelatin	Precipitate was formed	+
Saponin	Froth test	Stable froth	+

These results are similar to those of Herawati and Saptarini (2019), who reported that the ethanol extract of red ginger contains chemical compounds such as terpenoids, alkaloids, phenolics, flavonoids, tannins, and saponins. According to Syafitri et al. (2018), red ginger contains bisabolene, farnesene, sesquiterpene and monoterpene compounds (cineol, citral, and β-sesquiphellandrene). Echeverrigaray et al. (2008) reported that cineol and citral compounds exhibit antibacterial activity against *Proteus mirabilis* with MIC values of 7.5 and 5 µg/mL, respectively. The results of phytochemical screening of the red ginger ethanol extract in this study showed the presence of terpenoid compounds, which most likely act as an antibacterial agent against *Proteus mirabilis*.

CONCLUSION

The ethanol extract of red ginger (*Zingiber officinale* Roscoe) demonstrated inhibition against *Proteus mirabilis* in vitro, with statistically significant differences among the zone of inhibition corresponding to the various extract concentrations. Red ginger ethanol extract contains classes of bioactive compounds comprising terpenoid, alkaloids, phenolics, flavonoids, tannins, and saponins.

AUTHOR CONTRIBUTION

ARJ: Concepts or ideas; design; definition of intellectual content; literature search; experimental studies; data analysis; manuscript preparation.
MCN: Design of experimental; literature search; data analysis; manuscript editing; manuscript review

CONFLICT OF INTEREST

None to declare

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