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EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF HALIA MERAH (*Zingiber officinale var. rubrum Theilade*) ETHANOLIC EXTRACT OBTAINED FROM DESA WISATA TUO PARIANGAN, WEST SUMATRA

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Abstract

Halia Merah (*Zingiber officinale var. rubrum Theilade*) is serve as a good source of antioxidants and very useful in medicine field especially in area where it is native such as West Sumatra, Indonesia. This study of antimicrobial and antioxidant activity aims to determine the antimicrobial activity of Halia Merah ethanolic extract using disc diffusion assay and to evaluate the antioxidant activity of Halia Merah in scavenging free radicals. The concentration of Halia Merah ethanolic extract ranging from 50 mg/mL, 100 mg/mL, 200 mg/mL and 400 mg/mL. Based on the result obtained, the highest concentration that is 400 mg/mL showed the largest inhibition zones. The result was 10.00±0.00 mm for *S. aureus*, 28.50±1.06 mm for *B. subtilis,* 9.50±1.50 mm for *P. aeruginosa* and 22.75±1.75 mm for *E. coli*. The antioxidant activity that was measured by using DPPH Radical Scavenging Assay determined that the Halia Merah ethanolic extract scavenging activity increased when the concentration is increased; (80.50% at 50 mg/mL, 88.59% at 100 mg/mL, 91.70% at 200 mg/mL, and 96.27% at 400 mg/mL). By using ANOVA analysis, it indicated that There were significant differences among different concentration used ($p<0.05$). As conclusion, the different concentrations of Halia Merah ethanolic extracts have the ability inhibiting the growth of bacteria from Gram-positive and Gram-negative bacteria. Halia Merah ethanolic extract also showed a positive activity of antioxidant by using DPPH Radical Scavenging Assay. In addition, there were also a high possibility that Halia Merah could be used as an alternative for a prescription and medicine, act as a traditional medicine with a greater cure rate compared to commercial antibiotics and antioxidant.

Keywords: Halia Merah, antimicrobial, antibacterial, antioxidant

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Introduction

Medicinal plants are used to make medicines worldwide, and they serve an essential role in healthcare in several poor nations, including South Africa. Over 80% of people in underdeveloped countries utilize traditional medicines because they are relatively cheap, readily available, and are considered to have minimal bad effects (Kharchoufa et al., 2018). Medicinal plants have various therapeutic qualities depending on the active chemicals they contain. Herbal medicine probably had an important role in the

prevention and treatment of the COVID-19 epidemic, particularly in Asian nations (Liana and Phanumartwiwath, 2022).

The evaluation of the antibacterial activity of Halia Merah extract crude from West Sumatra has great potential and scientific interest in this regard. Halia Merah, often known as red ginger or *Zingiber officinale var. rubrum Theilade*, is an uncommon kind of ginger native to West Sumatra, Indonesia. It has been valued for decades not just for its culinary and therapeutic benefits, but also for Halia Merah's potential as an original source of antibacterial properties. Results revealed that *Zingiber officinale*, *Glycyrrhiza spp*., and *Lamiaceae* family members were frequently used as medicinal sources for COVID-19 therapy (Liana and Phanumartwiwath, 2022).

Additionally, plants like Halia Merah can provide a good supply of antioxidants. Plants produce antioxidants such as carotenoids, flavonoids, cinnamic acid, benzoic acid, folic acid, ascorbic acid, tocopherols, tocotrienols, and many more chemicals (Barkat and Mahmood, 2018). The antioxidants described above also help plants grow and defend themselves. Not only that, but they have beneficial health properties for those who consume them. Although halia species are widely used in traditional medicine and culinary flavoring, little research has been conducted on their antioxidant properties. As a result, their potential as the best antioxidant deserves further investigation.

Zingiberaceae plants have gained a lot of attention since they produce a large number of complex chemicals that can be applied in gastronomy as herbs and spices, flavoring and seasoning, and in cosmetics and medicine as antioxidants and antibacterial agents (Chen et al., 2007). In addition, it is a popular Asian spice. Halia is now generally recognized for its therapeutic advantages and is a crucial component of basic human health care globally.

The current medical business will benefit from research into the antibacterial and antioxidant activities of Halia Merah ethanol extract. Aside from that, the study will be a significant step in ensuring the treatment of non- microbial and microbial-derived foods. The drug suggested to treat the condition is safe and harmless for general usage (Amira, 2023). Although Halia Merah species are widely used in traditional medicine and culinary flavorings, little research has been conducted on their antioxidant properties. The objectives of this study are to determine the antimicrobial activity of Halia Merah (*Zingiber officinale var. rubrum Theilade*) crude extract against pathogenic microorganisms and to evaluate the antioxidant activity of Halia Merah (*Zingiber officinale var. rubrum Theilade*) crude extract by the inhibition of the DPPH radical.

Methods

Materials

Material that have been used in this project were distilled water, Ethanol 95%, α-diphenyl-β-picrylhydrazyl (DPPH) powder, acid, Nutrient agar, Gentamycin and Penicillin. Bacteria involved in the study were two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), while the two Gram-negative bacteria were *Escherichia coli* and *Pseudomonas aeruginosa*. All four bacteria samples were kept in a freezer, sealed in aluminium foil, under controlled laboratory conditions. All of the bacteria were obtained from the Unit Bank Kultur at UiTM Cawangan Negeri Sembilan, Kampus Kuala Pilah.

Apparatus

Apparatus that have been used were disc, cotton swab, hockey stick, filter paper, petri dish, centrifuge tube, micropipette tips, rotary evaporator, incubator, incubator shaker, refrigerator and spectrophotometer.

Preparation of bacteria

Bacteria were standardized to 10^8 cfu/mL. All four bacteria were grown in a falcon tube and incubated overnight at 37°C in an incubator shaker before being spread on Nutrient agar (Akullo et al., 2022).

Antibacterial activity by using disk diffusion assay

The antibacterial properties of the ethanolic crude extract Halia Merah were determined using the disk diffusion technique. In brief, nutritional agar was streaked with a pathogenic bacterial strain under aseptic conditions. Then, four disks of crude extract at various concentrations, one disk of negative control, and one disk of positive control were placed on nutrient agar before being incubated at 37°C for 24 hours (Bubonja-Šonje et al., 2020). Following incubation, the diameter of the growth inhibition zones was assessed. The antibacterial test used ethanolic crude extract Halia Merah at concentrations of 50 mg/mL, 100 mg/mL, 200 mg/mL and 400 mg/mL. The test was run in triplicates. Penicillin and Gentamycin were used as positive control while ethanol was used as negative control. The use of penicillin and gentamycin as positive controls allows for a more precise and bacteria-specific evaluation of Halia Merah extract's antibacterial properties. This method improves the scientific precision of the research and allows for a more detailed evaluation of the extract's potential as a natural antibacterial agent.

Antioxidant radical scavenging assay (DPPH)

The chemical substance used during this activity was α-diphenyl-β-picrylhydrazyl (DPPH). To begin, 2.4 mg of DPPH was dissolved in 100 mL of ethanol to create ethanolic DPPH. Next, 5 µL of each concentration of Halia Merah crude extract were added to 4 mL of ethanolic DPPH solution. The concentrations evaluated in this experiment included 50 mg/mL, 100 mg/mL, 250 mg/mL, and 500 mg/mL. The mixture of ethanolic DPPH and plant extract was rapidly shaken for 30 minutes at room temperature in the dark (Mohd Amin, and Md Yasin, 2023). The maximum wavelength for this assay was measured at 515 nanometres. The positive control was ascorbic acid, whereas the negative control, known as the blank, was ethanol. The test was performed three times with a spectrophotometer.
Equation: % radical scavenging assay = [(A0 – A1)/A0] x 100

Statistical analysis

The inhibition zone for antimicrobial activity assay were conducted in triplicate. The data was shown as mean ± standard error. Data on antioxidant activity assay was reported as mean ± standard error (n=3). The data were examined with one-way ANOVA. The significant different level was chosen at $p < 0.05$. These two statistical analyses were conducted using JMP Pro 17 (Pramiastuti et al., 2020).

Result and Discussion

Antibacterial activity

For the antibacterial activity technique, all four microorganisms were tested using the disk diffusion assay method. Before beginning the antibacterial activity process, a serial dilution of the Halia Merah ethanolic extract at four distinct concentrations was prepared. In this study, ethanolic extracts of Halia Merah at concentrations of 50 mg/mL, 100 mg/mL, 200 mg/mL, and 400 mg/mL were tested against the bacterial.

Antibacterial activity of *Staphylococcus aureus*

According to Table 1, Gentamycin demonstrated an inhibition zone of 17.30 mm, whereas ethanol showed no inhibition zone at all. The largest inhibitory zone by diameter (mm) for *S. aureus* among all concentrations was 15.30 mm, at 400 mg/mL, which is the highest concentration tested. Then followed by 200 mg/mL, 50 mg/mL, and 100 mg/mL. The bigger inhibitory zones at higher concentrations indicate that the extract includes bioactive chemicals, such as flavonoids, capable of affecting bacterial cell wall structure or metabolic activities. *S. aureus* is more vulnerable to plant extracts than Gram-negative bacteria because of its single thick peptidoglycan surface, which allows antimicrobial drugs to penetrate more effectively. The results for all *S. aureus* concentrations are not statistically different. It was obvious that all four concentrations had a lower inhibition zone than the positive control.

Table 1. Inhibition zone (mm) appearing against *S. aureus.*

Ethanolic extract (mg/mL)	Inhibition zone (mm)
400	15.30±0.53
200	8.00±0.50
100	6.67±0.33
50	7.00±0.58
Positive Control (Gentamycin)	17.30±0.17
Negative Control (Ethanol)	-

Data given are the mean and standard error of inhibition (mm) of three readings
Note: (-) = No inhibition

Many medicinal plants have been tested to determine their antimicrobial activity against various pathogenic strains, and many of their bioactive secondary metabolites are active against both Gram-positive and Gram-negative bacteria (Mahlangu, 2019).

Antibacterial activity of *Pseudomonas aeruginosa*

According to Table 2, Gentamycin, a positive control, produced an inhibitory zone of 24.33 mm against *P. aeruginosa*. The largest inhibitory zone for *P. aeruginosa* among all four concentrations was 9.50 mm on average, at 400 mg/mL, which was the highest concentration in this investigation. Next came 50 mg/mL, then 200 mg/mL, and finally 100 mg/mL. Based on the result obtained, the *P. aeruginosa* proved to have a vital position in antibacterial activity and the mechanism of infection. This is because of the ability of *P. aeruginosa* developing efflux activity, formatting biofilm and its structure that are low in membrane permeability (Chakotiya et al., 2017). In addition, *P. aeruginosa* act as microscopic organism that can degrading metals from building of biofilms (Royani et al., 2023). Because of its low membrane permeability and strong resistance mechanisms, *P. aeruginosa* showed only a small inhibition zone even at the highest concentration of Halia Merah extract, confirming its status as a difficult-to-treat pathogen and highlighting the limited efficacy of plant-based antimicrobials against it.

Table 2. Inhibition zone appearing against *P. aeruginosa*

Ethanolic extract (mg/mL)	Inhibition zone (mm)
400	9.50±1.50
200	7.30±0.50
100	6.55±0.55
50	7.75±0.25
Positive Control (Gentamycin)	24.33±1.45
Negative Control (Ethanol)	-

Data given are the mean and standard error of inhibition (mm) of three readings
Note: (-) = No inhibition

Antibacterial activity of *Escherichia coli*

The largest inhibitory zone for *E. coli* was 400 mg/mL, with a mean diameter of 22.75 mm (Table 3). This was also the highest concentration tested. The results for *E. coli* revealed considerable variability in inhibitory zones across concentrations. The results show that the maximum concentration of Halia Merah ethanolic extract (400 mg/mL) has a wider diameter of the inhibitory zone than the positive control. This reveals that Halia Merah extract has a significant concentration-dependent antibacterial effect, exceeding the positive control as stated before. This is most likely due to the increased availability of bioactive compounds at higher concentrations, such as flavonoids, gingerol, and shogaol,

which can break bacterial cell walls and decrease protein production. As the concentration increase, so does the efficacy of the extract's antibacterial properties, until it approaches saturation. The explanation could be a decrease in the solubility of the active ingredients once a chemical can no longer be dissolved, absorbed, or mixed with a solvent (Doye et al., 2023).

Table 3. Inhibition zone appearing against *E. coli.*

Ethanolic extract (mg/mL)	Inhibition zone (mm)
400	22.75±1.75
200	13.67±1.67
100	10.30±0.13
50	7.67±1.20
Positive Control (Gentamycin)	18.17±0.44
Negative Control (Ethanol)	-

Data given are the mean and standard error of inhibition (mm) of three readings
Note: (-) = No inhibition

Antibacterial activity of *Bacillus subtilis*

According to Table 4, the largest inhibition zone seen during a test against *B. subtilis* was 28.50 mm (mean), attained at the highest concentration of 400 mg/mL. The results revealed no significant differences.

Table 4. Inhibition zone appearing against *B. subtilis*

Ethanolic extract (mg/mL)	Inhibition zone (mm)
400	28.50±1.06
200	16.5±2.50
100	7.30±0.13
50	6.50±0.50
Positive Control (Penicillin)	13.00±1.00
Negative Control (Ethanol)	-

Data given are the mean and standard error of inhibition (mm) of three readings
Note: (-) = No inhibition

Penicillin was used, differ from other bacteria that put onto test against Gentamycin. This is because Penicillin is particularly efficient against Gram-positive bacteria, including *Bacillus subtilis*. It prevents bacterial cell wall development by targeting peptidoglycan cross-linking, a key component of Gram-positive bacterial walls. The peptidoglycan layer of Gram-positive bacteria is thick and exposed to the outside world. In contrast, Gram-negative bacteria have a much thinner peptidoglycan layer that is protected by an outer barrier, limiting the penetration of antimicrobial drugs, particularly those with high molecular weight (Zhydzetski et al., 2024). The size of inhibition zone also not influenced by the different concentration range from 400 mg/mL to 50 mg/mL.

Antioxidant activity

The antioxidant activity of ethanolic extracts has been determined using a radical scavenging assay. Similar to antibacterial activity, ethanolic extracts of Halia Merah at concentrations ranging from 400 mg/mL to 50 mg/mL was used. Halia Merah have garnered attention because of its ability in producing compounds that are useful in daily food such as flavouring, spices and also as antioxidant in medicinal sector (Barbosa and Peteros, 2018).

(DPPH) Radical scavenging assay

According to Table 5, 50 mg/mL has the lowest radical scavenging with a percentage of antioxidant of 80.4979, while 400 mg/mL has the highest DPPH radical scavenging with a percentage of antioxidant of 96.2655.

Table 5. DPPH Radical Scavenging Assay of Halia Merah ethanolic extract.

Concentration (mg/mL)	Absorbance at 517 nm	% of antioxidant
400	0.018 ± 0.000333^{c}	96.2655
2001	0.040 ± 0.002730^{b}	91.7012
100	0.055 ± 0.000577^{b}	88.5892
50	0.094 ± 0^{a}	80.4979

Note: Numbers followed by superscript letter (a,b and c) differ to show significant differences.

The 200 mg/mL concentration shows a major rise in its percentage scavenging activity of 0.040, but the 400 mg/mL concentration has the highest percentage of radical scavenging activity of 0.094. The concentration of 100 mg/mL resulted in a significant rise in the percentage of radical scavenging activity, which was more than 200 mg/mL (0.0397), whereas 400 mg/mL resulted in the lowest percentage of radical scavenging activity (0.0183). It is safe to say that the DPPH radical scavenging activity of Halia Merah ethanolic extract increases as the concentration increases. Based on Table 5, it can be seen that Halia Merah has a high percentage of antioxidant activity due to a good flavonoid content and antioxidant activity which could be use in food flavouring and traditional medicine (Ghasemzadeh et al., 2010).

Conclusion

This study concluded that Halia Merah at concentrations ranging from 400 mg/mL to 50 mg/mL have the ability to inhibit the growth of both Gram-positive and Gram-negative bacteria used which were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus subtilis*. *Bacillus subtilis* produced the highest reading of inhibition zone at 400 mg/mL compared to other bacteria because of its role as Gram-positive bacteria that have significantly lower resistance due to their cell composition.

Furthermore, DPPH is considered as first method in evaluating antioxidant activity of an extract (Barbosa and Peteros, 2018). Halia Merah produced a relatively high reading of antioxidant activity percentage because of its potential in scavenging free radicals. Antioxidant have been a popular topic in food and also pharmaceutical company for these past few years, these results give more potential to Halia Merah in being one of natural sources compound that can give good antioxidant effect. Based on the findings, bioactive compounds in Halia Merah ethanolic extracts can be suggested to have potential to be used as an appealing alternative.

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Author Contribution

Muhammad Syazani Shaharin and Aronal Arief Putra – sample collection, data processing and analysis, manuscript writing; Ida Muryany Md Yasin – supervision, manuscript writing; Siti Nur Anisah Aani and Afriani Sandra – editing and review.

Conflict of Interest

Author declares no conflict of interest.

Declaration on the Use of Generative AI

Authors declare Grammarly is generative AI used in the manuscript only for language enhancement.

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