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Antibacterial Activity and Clinical Potential of Rosella Flowers (*Hibiscus Sabdariffa* L.) Against *Escherichia Coli* ATCC

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ABSTRACT

Secondary metabolites found in rosella flowers (*Hibiscus sabdariffa* L.) include alkaloids, flavonoids, saponins, and tannins, which may have antibacterial and antibiofilm properties. The purpose of this study is to determine whether rosella flower extracts and fractions have antibacterial activity against *Escherichia coli* and to evaluate their potential uses in clinical pharmacy. The maceration method with 96% ethanol was used for extraction, and n-hexane, ethyl acetate, and water were used as solvents for fractionation. To find the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), antibacterial activity testing was carried out utilizing the disk diffusion and dilution procedures. With an inhibitory zone diameter of 16.5 mm and an MBC value of 25 mg/mL against E, the water fraction was found to be the most active. The maceration method with 96% ethanol was used for extraction, and n-hexane, ethyl acetate, and water were used as solvents for fractionation. To find the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), antibacterial activity testing was carried out utilizing the disk diffusion and dilution procedures. With an inhibitory zone diameter of 16.5 mm and an MBC value of 25 mg/mL against E. coli, the water fraction was found to be the most active. It is believed that the antibacterial mechanism of action involves harm to the bacterial cell membrane and cell wall, which increases permeability and causes cell death. Clinically, the water fraction of rosella flowers has the potential to be developed as an adjuvant therapy in the treatment of bacterial infections, particularly those involving biofilm formation such as urinary tract infections. The mechanism of increased bacterial membrane permeability has the potential to enhance the effectiveness of conventional antibiotics. However, the flavonoid content in rosella may also potentially affect drug-metabolizing enzymes and cause drug interactions, particularly with antibiotics and antihypertensive drugs. Therefore, although it shows promising antibacterial activity in vitro, further research is needed to evaluate its pharmacokinetic aspects, safety, drug interactions, and clinical efficacy before it is applied in clinical practice

INTRODUCTION

Because they can spread from person to person, infectious diseases are a health concern that keeps changing throughout time. Numerous microbes, including bacteria, fungus, viruses, and protozoa, can cause infections. *Escherichia coli* is one of the most prevalent bacteria that cause infections (Fiana et al., 2020). The typical gut flora includes the Gram-negative bacterium *E. coli*. Bacteria may develop resistance mechanisms as a result of overuse and improper usage of antibiotics. Human health is at risk due to the increase in antibiotic resistance, which calls for the development of potent substitute antimicrobials. Plants offer an alternative treatment option, such as rosella (*Hibiscus sabdariffa* Linn.), which possesses antimicrobial, antioxidant, anti-inflammatory, antidiabetic, antihypertensive, and antifungal activities (Chofidah et al., 2019). The antimicrobial properties of rosella flowers are attributed to polyphenolic compounds such as flavonoids—specifically anthocyanins and gossypetin—as well as phenolics, tannins, and saponins (Estri & Anggarbeni, 2015).

A research study by Chofidah et al. (2019) demonstrated that tests for antibacterial activity revealed that rosella at concentrations of 20 mg, 30 mg, and 40 mg could effectively inhibit and eliminate bacteria; however, the most effective bacterial inhibition was noted at a concentration of 40 mg of rosella, resulting in an inhibition zone of 6.5 mm against *E. coli*, which is categorized as exhibiting moderate antibacterial activity. Additionally, research by Estri & Anggarbeni (2015) indicated that rosella flowers possess the ability to inhibit the growth of *E. coli* due to their content of phenolic compounds, which include flavonoids, tannins, anthocyanins, and saponins. In clinical pharmacy practice, the use of herbal agents as complementary therapies must consider safety aspects, drug interactions, and patient response variability. Rosella, known for its antihypertensive and diuretic effects, has the potential to interact with medications such as diuretics and anticoagulants, thereby affecting the efficacy and safety of therapy (Nyulas et al., 2024; Dekhoda et al., 2024).

Additionally, interaction studies indicate that *Hibiscus sabdariffa* extract can modify drug pharmacokinetic parameters, such as reducing plasma levels of antihypertensive drugs, thereby potentially diminishing their therapeutic effects (Nurfaradilla et al., 2020). Recent studies also confirm that rosella can affect the bioavailability of certain drugs through pharmacokinetic and pharmacodynamic interaction mechanisms, so its concurrent use with conventional drugs Its concurrent use with conventional medications requires special attention (Heliyon Review, 2026). Therefore, further research is urgently needed before rosella can be widely adopted in clinical practice.

METHODS

Extraction

The ethanol extract of the rosella flower was prepared at a ratio of 1:9: 900 g of rosella flower powder was measured and subsequently immersed in 10 L of 96% ethanol, placed in a sealed container. The soaking process lasted for 3×24 hours with occasional stirring; following this, the extract was gathered, and the filtrate was evaporated using a rotary evaporator at 40°C until a concentrated extract was obtained. To test for antibacterial activity against *E. coli*, the paper disc diffusion method was employed, a sterile Petri dish was prepared, followed by the addition of 15 mL of MHA medium into the dish, which was then evenly spread and allowed to solidify.

Once solidified, a bacterial suspension is streaked onto the surface of the medium. Place 8-mm paper discs (blank discs) with a 60 µL absorption capacity onto the media; Apply a drop of the test solution (extract, n-hexane fraction, ethyl acetate fraction, and water) to each disc, in addition to ciprofloxacin as the positive control and 5% DMSO as the negative control. This procedure should be repeated three times, followed by an incubation period of 24 hours at 37°C.

The antibacterial activity test utilizing the dilution method was performed to ascertain the MIC and MPC of the most effective rosella flower fraction; the medium employed for the test was Brain Heart Infusion (BHI). This approach involves a series of test tubes comprising 10 sterile tubes that contain the active rosella flower fraction at varying

concentrations. The rosella flower ethanol extract was prepared using a 1:9 ratio: 900 grams of rosella flower powder were measured and subsequently immersed in 10 liters of 96% ethanol, placed in a sealed container. The extraction process lasted for 3×24 hours with intermittent stirring; following this, the extract was gathered, and the filtrate was evaporated using a rotary evaporator at 40°C until a concentrated extract was obtained.

To test for antibacterial activity against *E. coli* using the paper disc diffusion method: prepare a sterile Petri dish, pour 15 mL of MHA medium into the dish, distribute it evenly, and allow it to solidify. Once solidified, a bacterial suspension is streaked onto the surface of the medium. Place 8-mm paper discs (blank discs) with a 60 µL absorption capacity onto the media; apply a drop of The test solution, which includes the extract, n-hexane fraction, ethyl acetate fraction, and water, is applied to each disc, accompanied by ciprofloxacin as the positive control and 5% DMSO as the negative control. This procedure is repeated three times, followed by a 24-hour incubation at 37°C.

The antibacterial activity test was performed using the dilution method to ascertain the MIC and MPC of the most effective rosella flower fraction. The medium utilized for this test was Brain Heart

Infusion (BHI). This method involves a series of 10 sterile test tubes, each containing the active rosella flower fraction at different concentrations.

RESULTS AND DISCUSSION

The extraction yield of rosella flower was 24.5%. This yield complies with the standards outlined in the Indonesian Herbal Pharmacopoeia, which stipulate a minimum of 10%. Optimal drying conditions, such as the appropriate temperature and drying time, can increase the yield. When dried at 60°C, the yield is quite high, but the anthocyanin content and antioxidant activity are relatively lower. The appropriate solvent used is also a factor influencing the extraction yield. A study conducted by (Awwalina, 2016) reported a rosella flower extract yield of 36.326% using 70% ethanol, with a sample weight of 300 grams. Research conducted by (Dwiki et al., 2018) showed that 1 gram of rosella flower concentrate macerated with 96% ethanol yielded a yield of 11.07%. Another study conducted by Fita in 2020 on rosella flower concentrate weighing 75.25 g using a 70% solvent yielded a yield of 29.75%.

Table 1. Yield of Thick Extract

No	Powdered Simplex Roselle Flower (g)	Thick Roselle Flower Extract (g)	Yield (%)
1	900 g	221 g	24,5 %

Antibacterial Activity Test of Extract

1. Diffusion Method

Antibacterial activity was assessed through the disk diffusion method on extracts and fractions derived from rosella flowers, as well as ciprofloxacin, targeting *E. coli*. The aim of the diffusion test was to determine the fraction with the highest activity. that inhibits *E. coli* growth and to determine whether a clear zone formed around the disk, indicating the test solution's inhibitory effect on *E. coli* growth on MHA medium.

The antibacterial properties of the rosella flower extract and its fractions were evaluated against *E. coli* at a concentration of 100 mg/ml, using ciprofloxacin as the positive control and 5% DMSO as the negative control. Antibacterial testing of each extract and fraction sample was performed in triplicate utilizing the diffusion technique. The diffusion technique entails measuring or observing the diameter of the clear zone that develops around a disc containing the antibacterial agent.

Table 2. Antibacterial Activity Test of Extract

Sample	Inhibitory Diameter (mm)			Mean ± SD
	Replication			
	1	2	3	
Extract	9	9,6	9,6	9,4±0,28
Positive control	30,3	30	30,6	30,3±0,3
Negative control	-	-	-	-

The results obtained using the diffusion method showed that the extract exhibited inhibitory activity against *E. coli* at a concentration of 100 mg/ml, with an average antibacterial activity of 9.4 mm. The mean diameter of the positive control treated with the antibiotic ciprofloxacin measured 30.3 mm, while the negative control with 5% DMSO did not inhibit *E. coli* because the 5% DMSO solvent lacks the ability to inhibit bacteria.

Observation of the extract's inhibitory activity against the bacteria revealed the influence of the extract concentration administered. The higher the extract concentration administered, the larger the resulting inhibition zone (Maulidie, 2019). Differences in the extract's ability to inhibit the test bacteria are likely due to the presence of secondary metabolites in the extract as well as bacterial resistance properties. Differences in the ability to inhibit bacterial growth are caused by several factors, specifically the antimicrobial properties of the extract, the levels of bacterial compounds, and environmental conditions, including pH and incubation time. The concentration of the fraction can be influenced by various factors, including the diffusion rate of antimicrobial agents, bacterial growth sensitivity, and the reaction between the active ingredient and the medium (Novita, 2016).

After obtaining the data on the inhibition zone diameters of the rosella flower extract, A statistical analysis to assess normality was conducted. The criteria for the normality test stipulate that the data must exhibit homogeneity with a significance level (sig) exceeding $\alpha > 0.05$, thereby allowing for the acceptance of H_0 and classifying the antibacterial data as normally distributed. Subsequently, the

normally distributed data underwent a parametric test, specifically a one-way ANOVA. The significance value derived from the one-way ANOVA results was $0.000 <$, confirming that the data collected were homogeneous. The results from the Tukey test revealed an asterisk (*) in the mean difference column, signifying that the inhibition zone diameters of the ethanol extract, n-hexane fraction, ethyl acetate fraction, and water fraction displayed significant differences. The resulting inhibition zone diameters indicate antibacterial activity that can be clinically categorized as moderate to strong. Based on the classification of inhibition zone diameters, sizes of 10–20 mm generally fall into the strong activity category, while 5–10 mm are categorized as moderate activity (Balouiri et al., 2016; CLSI, 2021). However, when compared to standard antibiotics such as ciprofloxacin, its efficacy remains lower, making it more appropriately positioned as an adjuvant therapy in the treatment of bacterial infections (Venkatesan et al., 2024; Montalvo-González et al., 2022).

2. Dilution Method

Antibacterial activity testing was conducted using the dilution method, also known as the serial dilution test. This method aims to determine the minimum inhibitory concentration (MIC) in the antibacterial activity test by observing the turbidity of a bacterial suspension that has been incubated for 3 days—allowing the bacteria to form a biofilm—and then treated with water fractions. The MIC results were determined by observing the turbidity in the tubes. The turbidity in the tubes was caused by the growth of the test bacteria.

Table 3. Dilution Method

Concentration mg/ml	Replication 1	Replication 2	Replication 3
100	-	-	-
75	-	-	-
50	-	-	-
25	-	-	-
12,5	+	+	+
6,25	+	+	+
3,13	+	+	+
1,56	+	+	+
0,781	+	+	+
0,390	+	+	+
Ciprofloxacin	-	-	-
Fraksi air (-)	-	-	-

The dilution method serves as an effective approach for ascertaining the minimum effective dose of both bacteriostatic and bactericidal agents. The Minimum Inhibitory Concentration (MIC) is identified as the lowest concentration at which no growth of bacterial colonies is detected on the medium. Observational data revealed turbidity at concentrations of 0.390; 0.781; 1.56; 3.13; 6.25; and 12.5 mg/ml; with the MIC of the extract established at 25 mg/ml, signifying that this concentration is the minimum required to eliminate *E. coli* bacteria. The negative control utilized in this investigation was essential for assessing bacterial growth within the medium employed. The positive control for the antibacterial assay was the antibiotic ciprofloxacin. Ciprofloxacin was selected as a benchmark in the evaluation of antibacterial activity due to its classification as a broad-spectrum antibiotic that functions by inhibiting the action of bacterial DNA gyrase. Findings from the research utilizing the dilution method suggest that the extract demonstrates Minimum Inhibitory Concentration (MIC) activity. This efficacy is attributed to the presence of alkaloids, flavonoids, tannins, and saponins within the extract. Saponins exert their effects on bacterial cell walls by lowering surface tension and disrupting membrane permeability. Subsequently, saponins permeate the cytoplasmic membrane, compromising

membrane stability, which results in the leakage of cytoplasmic contents from the cell, potentially leading to cell death (Pratama et al., 2023). A Minimum Inhibitory Concentration (MIC) value of 25 mg/ml indicates that the aqueous fraction derived from rosella flowers possesses bactericidal properties. In a clinical setting, this concentration requires further evaluation regarding the potential for achieving therapeutic levels in the body as well as its pharmacokinetic aspects (Brunton et al., 2018).

Clinical Potential and Safety

Clinically, rosella flowers (*Hibiscus sabdariffa* L.) have potential as a complementary therapeutic agent in the management of bacterial infections, including those caused by *Escherichia coli* (Montalvo-González et al., 2022; Venkatesan et al., 2024). Its antibacterial and anti-biofilm activities suggest potential for use as an adjuvant therapy to enhance the effectiveness of conventional antibiotics, particularly in infections involving biofilm formation (Abass et al., 2022; Suwandi et al., 2025). Biofilms are known to play a crucial role in increasing bacterial resistance to antimicrobial therapy; therefore, agents capable of inhibiting biofilm formation or eradicating biofilms hold significant clinical value (Dwivedi et al., 2020). In addition to antibacterial activity, rosella also possesses various other pharmacological effects

demonstrated in clinical studies, such as antihypertensive, hypolipidemic, anti-inflammatory, and antioxidant effects, as well as protective effects on various organs (Montalvo-González et al., 2022; Zhang et al., 2025). These effects are associated with the content of secondary metabolites such as flavonoids, anthocyanins, and polyphenols, which play a role in the biological activity of rosella (Montalvo-González et al., 2022). From a clinical application perspective, rosella has been developed into various dosage forms, including herbal beverages, capsules, and topical formulations such as mouthwash (Zhang et al., 2025). Recent research indicates that rosella extract exhibits significant antibiofilm activity against oral pathogenic bacteria, making it a potential active ingredient in pharmaceutical formulations such as mouthwash (Suwandi et al., 2025). In terms of safety, the use of rosella in various clinical trials has been reported to be relatively safe and does not cause serious side effects (Montalvo-González et al., 2022).

However, pharmacological effects such as a decrease in blood pressure require attention, particularly in patients undergoing antihypertensive therapy, as they may potentially cause an additive effect in the form of hypotension (Zhang et al., 2025). The secondary metabolites in rosella flowers, such as flavonoids, tannins, and saponins, not only act as antibacterial agents but also possess potential pharmacological effects such as antihypertensive and diuretic properties (Montalvo-González et al., 2022; Zhang et al., 2025). These effects may provide clinical benefits, but they also have the potential to cause side effects such as mild hypotension and gastrointestinal disturbances (Zhang et al., 2025). Additionally, the possibility of drug interactions must be considered, particularly in patients taking antihypertensive medications or antibiotics (Fasinu et al., 2017). Flavonoid compounds are known to influence the activity of cytochrome P450 enzymes, which play a role in the metabolism of various drugs, thereby potentially modifying the pharmacokinetic effects of other medications (Fasinu et al., 2017; Jiang et al., 2020).

The use of herbal agents such as rosella has also been reported to potentially reduce the incidence of antibiotic resistance when used rationally as combination therapy, particularly through mechanisms that increase bacterial membrane permeability and inhibit biofilm formation (Nworu & Akah, 2020). However, in vitro study results demonstrating good antibacterial activity, including Minimum Inhibitory Concentration (MIC) values, still require further investigation to confirm the achievement of therapeutic concentrations in the human body (Venkatesan et al., 2024). Therefore, the development of pharmaceutical formulations and further clinical trials are essential to support the use of rosella as a safe, effective, and scientifically evidence-based phytopharmaceutical agent (Montalvo-González et al., 2022).

CONCLUSION

Rosella flowers (*Hibiscus sabdariffa* L.) demonstrate antibacterial properties against *Escherichia coli*, as evidenced by both diffusion and dilution techniques. The water fraction exhibited the highest activity, with an inhibition zone diameter of 16.5 mm—categorized as moderate to strong activity—and a Minimum Inhibitory Concentration (MIC) of 25 mg/ml, suggesting bactericidal capabilities. The proposed antibacterial mechanism is believed to involve the disruption of the bacterial cell wall and cell membrane, attributed to the presence of secondary metabolites such as flavonoids, tannins, saponins, and alkaloids.

From a clinical perspective, the findings of this research suggest that rosella flowers could be further developed as a complementary or adjuvant treatment option for bacterial infections, especially those caused by *E. coli* and related to biofilm formation. The mechanism of increased bacterial membrane permeability allows rosella to enhance the effectiveness of conventional antibiotics. However, the use of rosella in clinical pharmacy practice must take into account safety considerations and the potential for drug interactions. The flavonoid content has the potential to modulate drug metabolism enzymes, thereby affecting the pharmacokinetics of

other drugs; additionally, pharmacological effects such as antihypertensive properties may produce additive effects when used in combination with certain medications.

Consequently, additional research, especially in vivo studies and clinical trials, is required to confirm its efficacy, safety, and drug interaction profile so that rosella can be developed as an evidence-based phytopharmaceutical that is safe for use in clinical practice.

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