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Antibacterial Potential of Methanol Extract of Red Guava Leaves (*Psidium guajava* L.) Against *Salmonella typhi*

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ABSTRACT

Background: Typhoid fever is a fever caused by an infection of the gastrointestinal tract, where the infection is caused by the bacterium *Salmonella typhi*. This infection is generally found in environmental conditions with dense populations and poor hygiene levels. Various plants have been used from generation to generation to treat typhoid fever, one of which is red guava leaves. This study aims to further explore the potential of methanol extract of red guava leaves (*Psidium guajava* L.) on the activity of *Salmonella typhi* in vitro. **Methods:** This study is an in vitro experimental study using *Salmonella typhi* culture. The phytochemical screening test was carried out on the methanol extract of red guava leaves. Furthermore, an assessment of the diameter of the inhibition zone between groups was carried out. Univariate and bivariate analyzes were performed to compare between groups. **Results:** There was an increase in the diameter of the inhibition zone along with an increase in the dose of methanol extract from red guava leaves. A dose of 100 mg/mL of red guava leaf extract showed a maximum inhibition diameter of 11.85 cm. **Conclusion:** Methanol extract of red guava leaves (*Psidium guajava* L.) has the antibacterial activity of *Salmonella typhi* along with increasing concentrations.

1. Introduction

Typhoid fever is a fever caused by an infection of the gastrointestinal tract, where the infection is caused by the bacterium *Salmonella typhi*. This infection is generally found in environmental conditions with dense populations and poor hygiene levels. This causes this infection to become one of the main infection problems that are often found in big cities. Big cities are synonymous with very massive development on the one hand but leave the other side with slum conditions, dense population, and lack of cleanliness. This is what makes this infection often found in marginal communities in big cities. The prevalence of typhoid fever in Indonesia is 350-810 per

100,000 population or around 1.6%, and is ranked 5th for infectious diseases for all ages in Indonesia.¹⁻⁴

Various efforts, both preventive and curative measures, have been taken to suppress this infection, but optimal results have not been found to minimize cases of this infection. The use of antibiotics such as chloramphenicol is one of the curative measures that has become the current medical standard. The use of this antibiotic itself is not without risk, and chloramphenicol causes various side effects that are not comfortable for users. Side effects in the form of resistance, gastrointestinal disturbances, and disturbances in the growth of bones and teeth in

children make the use of these antibiotics less comfortable and safe for patients in the long term. The uncomfortable effect of using antibiotics causes user non-compliance to consume antibiotics according to the rules and triggers the effects of antibiotic resistance. Where is antibiotic resistance, causing the ineffectiveness of antibiotics to suppress the infection and leading to less optimal eradication of typhoid fever.⁵⁻¹⁰

Indonesia is a country with the second-largest biological wealth in the world. This makes Indonesia has a large enough biological potential to be explored as a new therapeutic modality. Various plants have been used from generation to generation to treat typhoid fever, one of which is red guava leaves. Several previous studies evaluated the antibacterial effect of ethanol extract from guava leaves on *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella typhi*, *Shigella sonnei*, *Bacillus subtilis*, and *Bacillus cereus* bacteria. Not only as an antibacterial, but the ethanol extract from guava leaves also has an antifungal effect against the fungi *Saccharomyces cerevisiae* and *Aspergillus niger*.¹¹⁻¹⁴ This study aims to further explore the potential of methanol extract of red guava leaves (*Psidium guajava* L.) on the activity of *Salmonella typhi* in vitro.

2. Methods

This study is an in vitro experimental study. Samples of guava leaves obtained from one of the guava plantations in Berastagi Regency, North Sumatra, were identified at the Medanense Herbarium at FMIPA, Universitas Sumatera Utara. The guava leaves that have been obtained are then cleaned with running water, then cut into small pieces, then dried. When the simplicia is dry, then it is crushed and sieved, and it can be put in a clean and dry plastic bag. The extraction process was carried out by maceration with 98% methanol solvent. By taking 20 grams of simplicia powder from guava leaves, then put it in a glass container, put 100 ml of 98% methanol, cover it, and leave it for 3 days protected from light. After 3 days, the bath was filtered and re-macerated with the

same amount of solvent. Meanwhile, the filtrate from the first maceration was collected in a vessel and stored. The remaceration process was carried out 2 times. The filtrate from the results of maceration and remaceration is then concentrated using a rotary evaporator at a temperature of 40-50°C until the majority of the solvent has evaporated and followed by the evaporation process over a water bath to form a thick extract.

A phytochemical examination was carried out to explore secondary metabolites. Examination of flavonoids; 10 grams of simplicia powder added to 100 ml of hot water, then boiled for 5 minutes and filtered hot. The filtrate obtained is then taken 5 ml and added with 0.1 gram of Mg powder and 1 ml of concentrated HCl, and 2 ml of alcohol, shaken, and let separately. Flavonoids are positive when a yellowish-red or orange color is formed on the amyl alcohol layer. Alkaloid examination; 0.5-gram simplicia powder was then added with 1 ml of 2 N hydrochloric acid and 9 ml of distilled water and heated over a water bath for 2 minutes, cooled, and filtered. The filtrate obtained is used for the alkaloid test: a. 3 drops of filtrate and added with 2 drops of Mayer's reagent solution, it will form a precipitate that is white or yellow in color, b. 3 drops of filtrate and added with 2 drops of Bouchardat reagent solution will form a thick precipitate. The color is brown to black, c. 3 drops of filtrate and added with 2 drops of Dragendorff reagent solution to form a red/orange color. Alkaloids are positive if they form a precipitate/turbidity in at least 2 of 3 reactions. Saponin Examination; 0.5 gram of simplicia powder and put it in a test tube, then add 10 ml of hot water, cool it, and shake it vigorously for 10 seconds. If it forms foam with a height of 1-10 cm, which is stable for not under 10 minutes and does not disappear when 1 drop of 2 N hydrochloric acids is added, it is concluded that saponins are present. Tannin inspection; 0.5-gram simplicia powder is extracted with 10 ml of distilled water, then filtered, dilute the filtrate obtained until it is colorless. Then take the solution up to 2 ml and add 1-2 drops of 1% iron (III) chloride reagent. If a blue/green-black color is formed,

it indicates the presence of tannins. Examination of glycosides; 3 grams of simplicia powder and 30 ml of 95% ethanol mixed with distilled water (7:3) and 10 ml of 2 N sulfuric acids, refluxed for 1 hour, cooled and filtered. Then take 20 ml of the filtrate plus 25 ml of distilled water and 25 ml of 0.4 M lead (II) acetate, shake and let stand for 5 minutes, and filter. The filtrate was extracted with 20 ml of a mixture of isopropanol and chloroform (2:3), repeating this step up to 3 times. Collect water extract and evaporate at a temperature not exceeding 50°C. Dissolve the remainder in 2 ml of methanol. Put the remaining solution into the test tube and vaporize it in the water bath. In the remainder, add 2 ml of water and 5 drops of Molisch reagent. Then 2 ml of concentrated sulfuric acid is added through the wall of the tube. If a purple ring forms at the boundary of the two liquids, it indicates a glycoside. Steroid/triterpenoid examination; 1 gram of simplicia powder is macerated with 20 ml of ether in 2 hours and filtered. The phytate obtained is then evaporated in an evaporating cup. In the residue, add 2 drops of acetic anhydride and 1 drop of concentrated sulfuric acid (Lieberman-Bourchard reagent), and drop them while reacting to the test sample. If it forms blue or blue-green, then it indicates the presence of steroids, while red, pink, or purple indicates the presence of triterpenoids.

Making variations in the concentration of guava leaf extract were carried out by making a mother liquor of 1,000 mg/ml. Then, this solution was diluted by graduated dilution to 100 mg/ml, 80 mg/ml, 60 mg/ml, 40 mg/ml, and 20 mg/ml. Preparation of mother liquor and various variations of extract concentrations was carried out using DMSO (Dimethyl sulfoxide) as a solvent. Preparation of NA (Nutrient Agar) and NB (Nutrient Broth) media was made in accordance with the work instructions of each brand

media. The ratio in NA media is 38 grams in 1 liter of distilled water, while the ratio in NB media is 13 grams of media in 1 liter of distilled water. Each media is heated and dissolved with a hot stirrer. Then the media must be sterilized by autoclaving at 121°C for 15 minutes. Colonies *Salmonella typhi* from pure cultures was cultured in NB medium according to the McFarland standard turbidity 0.5. By taking 0.1 inoculums of each bacteria from NB according to the standard 0.5 McFarland turbidity, put it into a petri dish and mix it with 20 ml of sterile NA media that has been diluted. Then the petri dish was homogenized and, on its surface, added disc paper which had been soaked in various variations of red guava leaf methanol extract, standard, and control. Then do the same work on the petri dish with disc paper that has been soaked in methanol only. Each concentration, standard, control, and methanol were repeated 3 times. Then the media was incubated at 35-37°C for 18-24 hours. The amount of antimicrobial activity was measured by measuring the diameter of the inhibition zone formed using a vernier caliper.

Data analysis was performed with the help of SPSS software version 25. Univariate analysis was performed to present the distribution of research variable data in the form of mean and standard deviation. Bivariate analysis was performed to compare the mean between the test groups and determine the difference in mean between the treatment groups, with a p-value <0.05.

3. Results

Table 1 presents the results of the phytochemical screening of the methanol extract of red guava leaves. The test results showed that the methanol extract of guava leaves contained tannins, flavonoids, alkaloids, saponins, glycosides, and triterpenes.

Table 1. Results of phytochemical screening of methanol extract of red guava leaves (*Psidium guajava L.*).

Group of compounds	Color formed	Result
Tannins	Blackish green	+
Flavonoid		
Shinoda	Brick red	+
Pb (CH ₃ COO) ₂	yellow	+
Alkaline reagent test	Colorless	+
Alkaloids		
Mayer	No Precipitate	-
Dragendroff	No Precipitate	-
Wagner	No Precipitate	-
Saponins	Formed thick foam	+
Glucaloid	Purple ring	+
Triterpenes/steroids	Reddish ring	+

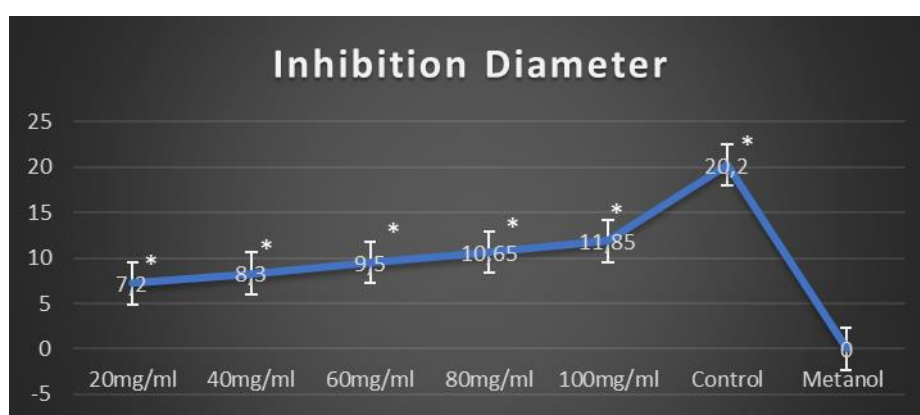


Figure 1. Comparison of inhibition diameter between groups. * Bonferroni post hoc, VS Methanol, $p < 0.05$.

Figure 1 shows the comparison of minimum inhibition diameters between groups. Figure 1 shows that there was an increase in the diameter of the inhibition zone with an increase in the dose of methanol extract from red guava leaves. A dose of 100 mg/mL of red guava leaf extract showed a maximum inhibition diameter of 11.85 cm. The diameter of the inhibition zone for each concentration showed a significant difference compared to the group that only received methanol. However, the diameter of the inhibition zone of the methanol extract of guava leaves was still less than optimal compared to the control group which received chloramphenicol.

4. Discussion

Red guava leaves (*Psidium guajava L.*) have been widely used as a medicine for dysentery. These leaves are also known to have antioxidant effects and can inhibit the formation of dental caries. The results of the phytochemical screening of red guava leaves were positive for flavonoids, saponins, steroids/triterpenoids, tannins, and glycosides. Tannin compounds have antibacterial properties. Its nature as an antibacterial builds stable bonds with proteins so that coagulation of bacterial protoplasm occurs. Saponins can also act as antibacterials, and they cause disruption of the surface tension of the cell

walls. When this is the case, the antibacterial substances will enter easily into the cells and can cause disruption of metabolism and lead to the destruction of bacteria. Flavonoid compounds are polyphenolic compounds with 15 carbon atoms formed in the C6-C3-C6 configuration, which act as antioxidants that can inhibit auto-oxidation by capturing free radicals.¹⁵⁻¹⁶

Based on the research results, it is known that there are differences in the diameter of the inhibition zone of *Salmonella typhi*, which are formed due to the difference in the concentration of guava leaf extract. The higher the concentration of guava leaf extract, the larger the diameter of the inhibition zone *Salmonella typhi* and the greater its inhibitory ability. This agrees with studies showing that methanol extract from red guava leaves has an antibacterial effect against *Salmonella typhi* at a small concentration of 50 mg/ml using the good diffusion method. However, the bacterial inhibition zone at a concentration of 100 mg/ml was greater than that at a concentration of 50 mg/ml. Methanol is said to be more effective as a solvent for making extracts than ethanol. Flavonoids belong to the largest group of natural phenols, and as organic compounds and are polar in nature, so the extraction of these compounds must also use organic solvents and polar solvents as well. Methanol is an organic solvent capable of activating groups of organic compounds with a polarity level similar to that of methanol. By using the principle like dissolves like, namely, a compound that will dissolve totally in a solvent that has the same properties. Methanol can activate the compounds contained in red guava leaves so that the methanol extract of red guava leaves can work more effectively as an antibacterial.¹⁷⁻²¹

5. Conclusion

Methanol extract of red guava leaves (*Psidium guajava* L.) has the antibacterial activity of *Salmonella typhi* along with increasing concentration, where the concentration of 100 mg/mL is the concentration with the maximum inhibition zone diameter.

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