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Antibacterial Activity of Ethanol Extract of Red Spinach Leaves (*Amaranthus tricolor* L.) Extracted Using Microwave-Assisted Extraction Against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*

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ABSTRACT

Background: Humans are among the most susceptible living beings to bacterial infections. Infectious wounds on the skin surface are easily colonized by various kinds of bacteria such as *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Red spinach (*Amaranthus tricolor* L.) contains flavonoids with antimicrobial properties.
Objective: The aim of this study was to determine the antibacterial activity of ethanol extract of red spinach leaves (*Amaranthus tricolor* L.) against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*.
Methods: This research includes the preparation, characterization, phytochemical screening of dried powder and extract. Extraction was performed by microwave-assisted extraction (MAE) method using 70% and 96% ethanol variations and then antibacterial activity of ethanol extract of red spinach leaves against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* bacteria was carried out by paper disc diffusion method (Kirby-Bauer test) to obtain the diameter of inhibition.
Results: The 70% ethanol extract of red spinach leaves has the lowest concentrations of 0.78 mg/ml and 25 mg/ml against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* bacteria with diameters of 7.23 mm, and 7.90 mm respectively. While the 96% ethanol extract of red spinach leaves has the lowest concentration of 0.78 mg/ml against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* bacteria with a same diameter of 7.43 mm.
Conclusion: Ethanol extract of red spinach leaves has antibacterial activity against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*.

Keywords: *Antibacterial, Amaranthus tricolor L., disc diffusion, Pseudomonas aeruginosa, Streptococcus pyogenes.*

ABSTRAK

Latar Belakang: Manusia adalah salah satu makhluk hidup yang paling rentan terhadap infeksi bakteri. Luka infeksi pada permukaan kulit mudah terkolonisasi oleh berbagai jenis bakteri seperti *Pseudomonas aeruginosa* dan *Streptococcus pyogenes*. Bayam merah (*Amaranthus tricolor* L.) mengandung flavonoid yang memiliki sifat antimikroba.
Tujuan: Penelitian ini bertujuan untuk menentukan aktivitas antibakteri ekstrak etanol daun bayam merah (*Amaranthus tricolor* L.) terhadap *Pseudomonas aeruginosa* dan *Streptococcus pyogenes*.
Metode: Penelitian ini meliputi persiapan, karakterisasi, dan skrining fitokimia dari serbuk kering dan ekstrak. Ekstraksi dilakukan dengan metode ekstraksi berbantuan gelombang mikro (MAE) menggunakan variasi etanol 70% dan 96%, kemudian aktivitas antibakteri ekstrak etanol daun bayam merah terhadap bakteri *Pseudomonas aeruginosa* dan

Streptococcus pyogenes diuji menggunakan metode difusi cakram kertas (uji Kirby-Bauer) untuk mendapatkan diameter hambatan. Hasil: Ekstrak etanol 70% dari daun bayam merah memiliki konsentrasi terendah 0,78 mg/ml dan 25 mg/ml terhadap bakteri *Pseudomonas aeruginosa* dan *Streptococcus pyogenes* dengan diameter hambatan masing-masing 7,23 mm dan 7,90 mm. Sementara itu, ekstrak etanol 96% dari daun bayam merah memiliki konsentrasi terendah 0,78 mg/ml terhadap bakteri *Pseudomonas aeruginosa* dan *Streptococcus pyogenes* dengan diameter hambatan yang sama yaitu 7,43 mm. Kesimpulan: Ekstrak etanol daun bayam merah memiliki aktivitas antibakteri terhadap *Pseudomonas aeruginosa* dan *Streptococcus pyogenes*.

Kata kunci: Antibakteri, *Amaranthus tricolor* L., difusi cakram, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*.

1. Introduction

The use of plants as a medicine for disease has been done since the time of ancestors in Indonesia; one of the plants often used is red spinach. Spinach plants originated from America and are easy to grow and spread worldwide in tropical and sub-tropical areas [1].

Red spinach (*Amaranthus tricolor* L.) began to be used as phytotherapy. Phytotherapy is an alternative treatment that uses plants as drugs or as a complement to the body's health. Red spinach (*Amaranthus tricolor* L.) is included in the Amaranthaceae family [2].

Spinach plants contain compounds in the leaves, namely vitamins, minerals, chlorophyll, flavonoids, saponins, tannins, alkaloids, and carotenoids, and stems contain polyphenols. Flavonoid compounds are phenol compounds that are efficacious as anti-inflammatory and antimicrobial; tannins are efficacious as astringents, and saponins are efficacious in spurring collagen formation, which plays a role in the wound healing [3].

Infectious diseases are suffered by many people in developing countries, including Indonesia. Humans are among the living things most susceptible to bacterial infections. Various organisms quickly colonize infectious wounds on the skin surface [4]. One of them is the *Pseudomonas aeruginosa* bacteria. These bacteria often cause wound infections on the skin, both incisions and abrasions [5]. *Streptococcus pyogenes* is a Gram-positive bacterium that causes pharyngitis infections, often called laryngitis, and impetigo (skin surface infections) [6].

Based on research by Arif et al., screening results from ethanol extracts of red spinach leaves showed the presence of alkaloid, tannin, flavonoid, and saponin group compounds [7]. Limbong's research (2017) also shows that red spinach leaf extract contains flavonoids, tannins, and steroids/triterpenoids. Secondary metabolite compounds that act as antibacterials are flavonoids and tannins. These compounds are polar and non-polar [8]. Therefore, the appropriate solvent must be used for extraction. Ethanol is a safe and non-toxic solvent. The ethanol solvent was chosen because it is a universal solvent in its use, meaning that the solvent can distill or extract compounds that are either polar or semi-polar, non-toxic, can mix with water, and the heat required for concentration is less [9].

The most common methods used to extract secondary metabolite compounds in spinach leaves are maceration and reflux, commonly referred to as conventional methods. However, these extraction methods require solvents and a lot of time. Microwave-assisted extraction (MAE) is an extraction that utilizes microwaves. Dipole interactions between water molecules and solvents in the microwave cause the temperature and pressure of the solvent to rise, resulting in diffusion from the sample to the solvent with a high extraction rate [10]. Some advantages of extracting with this technique are faster heating rate, low-temperature gradient, smaller equipment size, and high extraction yield [11]. The MAE method has proven more effective because it heats the solvent quickly and efficiently compared to conventional extraction methods. Based on the research of Sulistyono, the minimum inhibitory concentration (MIC) obtained from the MAE method proved to be more effective and produced a more significant inhibition zone than the maceration method [12].

Based on Limbong's research, red spinach leaf extract has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria. Activity and Minimum Inhibitory Concentration (MIC)

of ethanol extract of red spinach leaves against *Staphylococcus aureus* bacteria at a concentration of 6 mg/ml was 6.71 mm and against *Escherichia coli* bacteria at a concentration of 20 mg/ml was 6.56 mm [8]. Based on the description above, researchers are interested in scientifically testing the antibacterial activity of ethanol extracts of red spinach leaves (*Amaranthus tricolor* L.) extracted using the Microwaves-assisted extraction (MAE) method with 70% and 96% ethanol solvents, which are then tested against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* bacteria. This research includes characterization of simplicia, phytochemical screening, making ethanol extract of red spinach leaves, and testing antibacterial activity [13].

2. Method

2.1 Tools

The tools used consisted of autoclave (Dixon), beaker glass (Pyrex), biosafety cabinet (Astec HLF 1200L), hot plate (IKA C-MAG), incubator (Memmert), vernier caliper (RoHS), ose needle, watch glass, microwave (Samsung), microscope (Primo Star), analytical balance (Metler AE 200), oven (Memmert), water bath, rotary evaporator (Heidolph), UV/Vis spectrophotometry (Thermo).

2.2 Materials

The materials used in this study were distilled water, amyl alcohol ($C_5H_{12}OH$), alpha-naphthol ($C_{10}H_8O$), gallic acid ($C7H_6O_5$), glacial acetic acid (CH_3COOH), sulfuric acid (H_2SO_4) 2 N, hydrochloric acid (HCl) 2 N, nitric acid (HNO_3) 0.5 N, test microbes (*Pseudomonas aeruginosa* and *Streptococcus pyogene*s), iron (III) chloride 1% ($FeCl_3$), bismuth (III) nitrate ($Bi(NO_3)3.\ 5H_2O$), ethanol 96% (C_2H_5OH), iodine (I), folin, isopropanol (C_3H_8O), potassium iodide (KI), chloralhydrate (C2H3Cl3O2), chloroform ($CHCl_3$), red spinach leaves (*Amarantus tricolor* L.), Nutrient Agar (NA) media, Nutrient Broth (NB) media, Mueller Hinton Agar (MHA) media, methanol (CH_3OH), sodium hydroxide (NaOH) 2 N, sodium carbonate (Na_2CO_3), sodium chloride (NaCI), anhydrous sodium sulfate (Na_2SO_4), mercury (II) chloride ($HgCl_2$), magnesium powder (Mg), zinc powder (Zn), lead (II) acetate ($Pb(CH_3COO)_2$) 0.4 M.

2.3 Research Procedure

2.3.1 Sampling

The samples used were red spinach leaves (*Amaranthus tricolor* L.), which were fresh and reddish, obtained from Blok Gading Street, Village III Sunggal District, Tanjung Gusta Village, Deli Serdang Regency.

2.3.2 Sample processing into Simplicia

Fresh red spinach leaves were cleaned of dirt with water, drained, and weighed as wet weight. Red spinach leaves were dried in a drying cabinet at a temperature of ± 38 ^oC until dry and brittle, then dry simplicia was sorted again to separate unwanted plant parts, then weighed, and blended until it became powder. The resulting simplicia was sieved using mesh 60 and stored in a tightly closed container at room temperature before extraction [14].

2.3.3 Preparation of ethanol extract of red spinach leaves (Amaranthus tricolor L.)

The extraction process was carried out using the modified MAE method. The extraction used a power of 300 W, and the ratio of the sample to the solvent used was 1:10 (b/v). The red spinach leaf powder was weighed as much as 30 grams and dissolved in 300 mL of 70% and 96% ethanol in a 500 mL flat bottom flask. Then, the solution was extracted using a microwave for 4 minutes. The extraction process was repeated using the same amount of solvent for three repetitions. The extraction results were filtered using filter paper. The filtrate was evaporated using an oven at 45^oC until a thick extract was obtained [15].

2.3.4 Phytochemical screening

Phytochemical screening was performed on simplicia and extracts, including alkaloids, flavonoids, glycosides, tannins, and steroids/triterpenoids.

2.3.5 Characterization of simplicia and extracts

Characterization of simplicia included water content, water and ethanol soluble essence content, total ash content, and acid insoluble ash content. Characterization of extracts included water content, ash content, and total ash content.

2.3.6 Determination of phenol content

Determination of phenol content was performed using the Folin-Ciocalteu method. Determination of phenol content included the preparation of the gallic acid standard master solution, determination of maximum wavelength, plotting the calibration curve, and determination of total phenol content of the extract.

2.3.7 Antibacterial Activity Test of Red Spinach Leaf Ethanol Extract (Amaranthus tricolor L.) against Streptococcus pyogenes

Antibacterial activity testing of ethanol extracts of red spinach leaves with various concentrations was carried out by the diffusion method using paper blocking. A total of 1 ml of test bacterial suspension was poured into a Petri dish, 15 ml of Mueller Hinton agar media was added, and homogenized. After the media had solidified, the agar paper was placed with each concentration of ethanol extract of red spinach leaves, positive control, and negative control. The extract concentrations used were 400; 300; 200; 100; 50; 25; 12.5; 6.25; 3.125; 1.56; and 0.78 mg/mL. The positive and negative controls were 30 μg of chloramphenicol antibiotic disk and DMSO, respectively. They were then incubated at 37 ^{o}C for 24 hours. Furthermore, the diameter of the inhibition zone was measured, characterized by forming a clear zone around the disc. The zone of inhibition was measured using a caliper [14].

2.3.8 Antibacterial Activity Test of Ethanol Extract of Red Spinach Leaf (Amaranthus tricolor L.) against Pseudomonas aeruginosa

Antibacterial activity testing of ethanol extracts of red spinach leaves with various concentrations was carried out by diffusion method using paper blocking. The cotton swab was dipped into the suspension of test bacteria, then pressed to the side of the tube so that the water drained, then scraped over the entire surface of the solidified media. Each concentration of ethanol extract of red spinach leaves, positive control, and negative control was placed on the blotting paper. The extract concentrations used were 400; 300; 200; 100; 50; 25; 12.5; 6.25; 3.125; 1.56; and 0.78 mg/mL. The positive and negative controls were 30 μg of chloramphenicol antibiotic disk and DMSO, respectively. They were then incubated at 37 ^{o}C for 24 hours. Furthermore, the diameter of the inhibition zone was measured, characterized by forming a clear zone around the disc. The zone of inhibition was measured using a caliper [16].

3. Results and Discussion

3.1 Phytochemical screening

The chemical compound group test results showed that the simplicia and red spinach leaf extracts contained chemical compounds of alkaloid, flavonoid, glycoside, saponin, tannin, and steroid/triterpenoid groups.

3.2 Characterization of Simplicia and extracts

The result of determining the water content of red spinach leaf simplicia was 6.6%; it meets the requirements where the requirement is at most 10% [17]. Examination of water content was carried out to provide a minimum limit of water content that can still be tolerated in extracts because high water content will cause bacteria and fungi to grow quickly, and the active ingredients contained therein can be decomposed [18].

Determination of water and ethanol soluble essence content is a quantitative method for the amount of compound content in simplisia that solvents can attract. The results of determining the soluble essence content in water and ethanol are 19.51% and 11.71%. Based on the Indonesian Herbal Pharmacopoeia Edition II, plants that are in the same family as red spinach leaves, namely the spinach duri plant (*Amaranthus spinosus*), state that the requirement for water-soluble essence content is not less than 7.5%. In comparison, the requirement for the value of soluble essence content in ethanol is not less than 7.6% so that the results of water-soluble essence content and ethanol soluble essence content meet the predetermined requirements and leaf Simplicia meets the simplisia quality uniformity test [14]

The total and acid-insoluble ash content in red spinach leaf simplicia was determined to be 5.68% and 0.39%. According to Materia Medika Volume V, plants that have the same family as red spinach leaves, namely spinach thorn plants (*Amaranthus spinosus*), state that the acceptable total ash content value is no more than 10%, so the results obtained in this study, namely 5.68%, have met the requirements. The requirement for acid-insoluble ash content is no more than 1% [14], so the results obtained met the requirements. High total ash content may be partly derived from impurities. If the heavy metal content is too high, it will endanger health, so it is necessary to check the total ash and acid-insoluble ash content to ensure that the extract used does not contain certain heavy metals that exceed the requirements. The determination

of acid-insoluble ash content determines the amount of ash content obtained from external factors derived from impurities derived from sand or soil [19].

The water content of ethanol extract of red spinach leaves in 70% ethanol extract is 9.95%, and in 96% ethanol extract is 8.65%, so the results obtained are categorized as thick extracts. These results indicate that the water content in 70% ethanol extract is higher than in 96% ethanol extract. The increase in temperature during the extraction process causes more water to evaporate in the material, and the remaining water content gets lower. A decrease in the moisture content of the extract after reaching the highest heating time is due to the water content in the material evaporating more and more [20].

The total ash content obtained from ethanol extract of red spinach leaves extracted with 70% ethanol solvent was 5.86%, and 96% ethanol was 6.16%. Based on Herbal Pharmacopoeia edition II, plants that are in the same family as red spinach leaves, namely spinach leaves (*Amaranthus spinosus*), state that the requirements for total ash content in extracts are no more than 9.1% [14], so the results obtained have met the established requirements.

Acid insoluble ash content of ethanol extract of red spinach leaves extracted with 70% ethanol solvent was 0.06%, and 96% ethanol was 0.08%. Based on Herbal Pharmacopoeia edition II, plants that are in the same family as red spinach leaves, namely spinach leaves (*Amaranthus spinosus*), state that the requirements for acid insoluble ash content in extracts are no more than 0.3%, so the results obtained in 76% ethanol have not met the requirements. In comparison, 96% of ethanol has met the requirements. Acid insoluble ash content is determined by determining the amount of ash content obtained from external factors, such as impurities derived from sand or soil [19].

3.3 Determination of Phenol Content

The absorbance value of ethanol extract from red spinach leaves was measured at the 58th minute after adding water, Folin Ciocalteu reagent, and 10% sodium carbonate at a wavelength of 742 nm. Samples were tested and measured as many as three times repetitions to obtain accurate data. The absorbance value obtained was then substituted into the gallic acid regression equation to calculate the total phenol content.

Table 1. Total phenol content

Sample	Average total phenolic content
70% ethanol extract of red spinach leaves	138.72 ± 1.0716
96% ethanol extract of red spinach leaves	259.17 ± 0.9699

The results showed that the higher the ethanol content, the higher the total phenol obtained. In extracting gedi leaves (*Abelmoschus manihot* L.), 96% ethanol is the best solvent for producing total phenolic content and flavonoids [21]. Research conducted by Sari and Triyasmono found that 30%, 50%, 70%, and 96% ethanol yielded 2.60%, 1.88%, 1.88%, and 1.92% extracts, respectively. Total flavonoids in the 30%, 50%, 70%, and 96% ethanol extracts were 12.3, 8.9, 18.0, and 44.7 mg quercetin equivalents/g extract, respectively [22]. These results show that the weight of the yield does not determine the total flavonoid content obtained, where flavonoids are phenol compounds [13].

3.4 Antimicrobial Activity of Ethanol Extract of Red Spinach Leaf

The antimicrobial activity of ethanol extracts of red spinach leaves with various concentrations against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* bacteria was performed using the agar diffusion method using a 6 mm diameter disc paper. The antimicrobial test results are characterized by forming a clear zone around the paper blocking, measured with a caliper.

Table 2. Antibacterial Activity of 70% Ethanol Extract of red spinach leaves against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*

No	**Concentrations (mg/mL)**	**Diameter of Inhibition Area (mm)**	
		Pseudomonas aeruginosa	***Streptococcus pyogenes***
1	400	10.26 ± 0.1527	10.00 ± 0.2000
2	300	10.16 ± 0.0577	8.60 ± 0.1000
3	200	10.13 ± 0.0567	8.50 ± 0.2000
4	100	9.16 ± 0.0577	8.40 ± 0.1732
5	50	9.06 ± 0.0577	8.26 ± 0.0577

6	25	8.60 ± 0.2000	7.90 ± 0.1000
7	12.5	8.26 ± 0.0577	0.00
8	6.25	7.73 ± 0.0577	0.00
9	3.125	7.60 ± 0.1732	0.00
10	1.56	7.40 ± 0.0000	0.00
11	0.78	7.23 ± 0.1527	0.00
12	Negative control	0.00	0.00
13	Positive control	20.53 ± 0.4509	20.33± 0.5773

Note: Negative control using DMSO solvent and Positive control using 30 µg Chloramphenicol

Table 3. Antibacterial Activity of 96% Ethanol Extract of red spinach leaves against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*

No	**Concentrations (mg/mL)**	**Diameter of Inhibition Area (mm)**	
		Pseudomonas aeruginosa	***Streptococcus pyogenes***
1	400	10.86 ± 0.8082	10.53 ± 0.2081
2	300	10.43 ± 0.0577	10.33 ± 0.0577
3	200	10.30 ± 0.1000	9.36 ± 0.1154
4	100	10.30 ± 0.1000	8.90 ± 0.1000
5	50	10.30 ± 0.1000	8.76 ± 0.0577
6	25	10.06 ± 0.2516	8.66 ± 0.0577
7	12.5	9.40 ± 0.3605	8.26 ± 0.2309
8	6.25	8.96 ± 0.4041	8.00± 0.2645
9	3.125	8.66 ± 0.4041	7.90 ± 0.1000
10	1.56	8.33 ± 0.3214	7.70 ± 0.1000
11	0.78	7.43 ± 0.1154	7.43 ± 0.0577
12	Negative control	0.00	0.00
13	Positive control	20.53 ± 0.4509	20.33± 0.5773

Note: Negative control using DMSO solvent
Positive control using 30 µg Chloramphenicol

The results of the antibacterial activity test of ethanol extracts of red spinach leaves extracted using 70% and 96% ethanol solvents inhibited the growth of *Pseudomonas aeruginosa* and *Streptococcus pyogenes* as indicated by the diameter of the inhibition zone formed. The minimum inhibition concentration (MIC) against *Pseudomonas aeruginosa* bacteria in 70% ethanol extract was 0.78 mg/ml with an inhibition diameter of 7.23 mm, and against *Streptococcus pyogenes*, was at the concentration of 25 mg/ml with the inhibition diameter of 8.26 mm while in 96% ethanol extract was at the concentration of 0.78 mg/ml with the inhibition diameter of 7.43 mm and against *Streptococcus pyogenes* was at the concentration of 0.78 mg/ml with the inhibition diameter of 7.50 mm. The increase in the zone of bacterial inhibition is directly proportional to the rise in the concentration of ethanol extract from the red spinach leaves tested.

The 70% ethanol extract of red spinach leaves against *Pseudomonas aeruginosa* with extract concentrations of 200, 300, and 400 mg/ml are categorized as strong. In contrast, extract concentrations of 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 mg/ml are categorized as medium. *Streptococcus pyogenes* bacteria with an extract concentration of 400 mg/ml is categorized as strong. In comparison, extract concentrations of 25, 50, 100, 200, and 300 mg/mL are categorized as moderate, at concentrations of 0.78, 1.56, 3.125, 6.25, and 12.5 mg/mL are categorized as weak. Meanwhile, 96% of the ethanol extract of red spinach leaves against *Pseudomonas aeruginosa* bacteria with extract concentrations of 50, 100, 200, 300, and 400 mg/mL are categorized as strong. In contrast, extract concentrations of 0.78, 1.56, 3.125, 6.25, 12.5, and 25 mg/ml are categorized as moderate. In the inhibition of *Streptococcus pyogenes*, extract concentrations of 300 mg/ml and 400 mg/ml are categorized as strong. In contrast, extract concentrations of 0.78 mg/ml, 1.56 mg/ml, 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml are categorized as moderate. The determination of the antibacterial category is based on Davis and Stout [23], where the diameter of the inhibition zone of 5 mm or less is categorized as weak, the inhibition zone of 5-10 mm is categorized as moderate, the inhibition zone of 10-20 mm is categorized as strong, and the inhibition zone of 20 mm or more is categorized as very strong. The difference in antibacterial activity can be caused by differences in the content of phenol compounds possessed by both types of extracts, where the total phenol content in 96% ethanol extract is higher than in 70% ethanol extract.

The difference in the diameter of the inhibition zone of each concentration is due to the difference in the amount of active substance contained in the concentration; the greater the concentration, the greater the active substance component included in it. So that the greater the inhibition zone is produced [24].

The selection of chloramphenicol as a positive control aims to see the condition of the clear zone that shows the inhibition of antibacterial activity, and negative control using DMSO solvent to know the condition if there is no clear zone that shows no inhibition of antibacterial activity. The selection of chloramphenicol as a positive control is because it is a broad-spectrum antibacterial that can kill Gram-positive and Gram-negative bacteria. Antibacterial tests were carried out against DMSO to show that the solvent used to dilute the extract did not affect the inhibition of the test bacteria; this is because DMSO is an organosulfur compound and is only used to dilute the extract. This liquid is non-toxic, so it does not inhibit the inhibition of bacteria and does not interfere with the observation of antibacterial activity [25].

Antibacterial activity is influenced by several factors, including extract concentration, antibacterial compound content, type of bacteria inhibited, and extract diffusion power. The effect of concentration on the bacterial inhibition zone may be due to the different levels of compound content in the ethanol extract of red spinach leaves (*Amaranthus tricolor* L.), which plays a role in inhibiting the growth of *Pseudomonas aeruginosa* and *Streptococcus pyogenes* bacteria with different mechanisms of action as antibacterial in each compound.

4. Conclusion

Based on the results of the research that has been carried out, it can be concluded that ethanol extract from red spinach leaves has antimicrobial activity against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*.

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6. Conflict of Interest

The authors declare there are no conflicts of interest.

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