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Original Research Article

Optimization of Flavonoid, Anthocyanin Content and Antibacterial Activity of *Syzygium myrtifolim* Walp. Leaves with Various Extraction Solvents

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ABSTRACT

Syzygium myrtifolium Walp leaves have antibacterial and natural colourant potential, which are beneficial for health. The purpose of this study was to optimize flavonoid, anthocyanin levels, and antibacterial bioactivity of red shoot leaves with various extraction solvents. Extraction was carried out by maceration using 3 solvents with varying solubility: 70% ethanol, ethyl acetate, and n-hexane. The extract obtained was measured for flavonoid content at 440 nm using UV-Vis spectrophotometry. Anthocyanin content was measured at 510 nm and 700 nm using buffers at pH 1 and 4.5. Antibacterial testing used the paper disk diffusion method, with a negative control using solvent and a positive control using chloramphenicol. The study showed that flavonoid and anthocyanin levels in the ethanol extracts were 24.3 mgEK/g and 1.38 mg/L, respectively. The test results at concentrations of 1, 4, 8, and 16% were best obtained with n-hexane extracts, with inhibition strength values ranging from strong to very strong against *Staphylococcus aureus* (17.25, 20.02, 21.53, and 26.38 mm). For *Escherichia coli*, the inhibitory strength values ranged from strong (10.44, 13.05, 13.43, and 15.27 mm). This study concludes that solvent variation affects the flavonoid and anthocyanin content and the antibacterial bioactivity of *S. myrtifolium* leaf extract.

Keywords: *Syzygium myrtifolium* Walp., Flavonoids. Anthocyanin. Antibacterial

Introduction

Infectious diseases remain a significant health problem in many countries, especially in developing countries. Infectious disease is a condition caused by pathogenic microorganisms with or without clinical symptoms. Some antimicrobial materials used to inhibit disease-causing germs have been developed for a long time at the organism, cellular, and molecular levels. These antimicrobial substances are known as antibiotics.[1]

Antibiotics, drugs used to treat infectious diseases, must be used rationally, appropriately, and safely. Wise use of antibiotics means using appropriate antibiotics for the cause of infection, with an optimal dosage regimen, optimal duration of administration, minimal side effects, and minimal impact on the emergence of resistant microbes.[2] Irrational use of antibiotics will have negative consequences, such as the resistance of microorganisms to some antibiotics and increased side effects of drugs and even death.[3]

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This encourages the search for new and more effective drugs, including those derived from plants that contain active chemicals to inhibit bacterial activity.[4] One of the plants used as an antibacterial is the red shoot plant (*Syzygium myrtifolium* Walp).[5,6]

The use of plants as medicine has been widely studied. Red shoots (*Syzygium myrtifolium* Walp.) are a popular ornamental plant that contains many chemical compounds commonly used by the public. This red shoot plant, especially its leaves, contains flavonoid, phenolic, and terpenoid compounds that have anti-tumour and anti-angiogenic activity.[7,8] The plant is also used as a natural dye with beneficial health effects. In addition to its antioxidant properties, red shoot extract also has antimicrobial potential. Phytochemicals, particularly flavonoids and tannins, are effective in inhibiting the growth of several pathogenic bacterial strains.[9,10,11] This plant is called red shoots because it has young leaves that are red and also has old leaves that are green.[12] The results of a study show that the leaves of the red shoot (*Syzygium myrtifolium* Walp) plant contain several phytochemicals, such as flavonoids and alkaloids.[13] Another study shows that some medicinal plants containing phenolics have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anticancer activities.[14] Phytochemicals, which are thought to be present in the red shoot plant, have the potential to serve as natural antioxidants and sources of natural dyes that are beneficial for health, such as anthocyanins.[15,16] Red shoots are known to contain secondary metabolite compounds and are useful as natural dyes, antioxidants, and for their cytotoxic, antitumor, and antiangiogenetic properties. It is also cytotoxic and has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.[17,18]

Red shoot plants in the total extract inhibited the growth of *S. aureus* and *E. coli* at 0.5% concentration, with inhibitory zone diameters of 7.37 mm and 7.63 mm, respectively. The n-hexane fraction inhibited

the growth of *S. aureus* and *E. coli at* a minimum concentration of 2%, with inhibitory zone diameters of 7.70 mm and 7.87 mm, respectively. The ethyl acetate fraction inhibited the growth of *S. aureus* and *E. coli* at a minimum concentration of 0.5%, with inhibitory zone diameters of 7.63 mm and 7.60 mm, respectively. The ethanol-water fraction inhibited *S. aureus* growth only at 16%, with an inhibition zone diameter of 7.80 mm. In comparison, inhibition of *E. coli* bacteria was observed at a minimum concentration of 1%, with an inhibition zone diameter of 6.97 mm.[18]

The phenolic content contained in a plant is expressed as GAE (gallic acid equivalent), namely, the equivalent number of milligrams of gallic acid (3.4.5-trihydroxybenzoic acid) in one gram. Meanwhile, anthocyanins are found in various plants, both fruits and vegetables, which provide colours ranging from red to purple.[19,20,21] Anthocyanins belong to the group of flavonoid compounds, which are the largest group of natural pigments in plants that are soluble in water and are responsible for providing colour to flowers, fruit, and vegetables[21].

According to research, anthocyanins are best extracted with ethanol, and the best extraction, producing the highest anthocyanin yield, was with a combination of water and ethanol as solvents at room temperature.[16,22] Other research reported an anthocyanin content of 1.31% and antioxidant activity of 23.93%; thus, red cabbage crackers have potential as a source of antioxidants.[23]

The extraction process can use 3 solvents with varying polarities: n-hexane (nonpolar), ethyl acetate (semipolar), and ethanol/methanol (polar). Differences in the solvents used in extraction can affect the total content of bioactive compounds.[24] Research on the anthocyanin, phenolic content, and antibacterial activity of red shoot leaves using a variety of solvents based on polarity is still very limited.[25] Hence, this study aimed to explore the effects of different extraction solvents on the flavonoid and anthocyanin content of *Syzygium myrtifolium*.

Materials and Methods

Plant collection and identification

The samples, red shoot leaves (*Syzygium myrtifolium* Walp.) were collected from the Tamalanrea subdistrict, Makassar city, South Sulawesi Province (5°06'41 "S 119°28'50 "E), on 12 July 2022. *Syzygium myrtifolium* Walp plants were determined at the Biology Laboratory. Faculty of Mathematics and Natural Sciences. Makassar State University (No. Voucher 015/UN36.1.4/LAB.BIO/SKAP/2020). Sampling was done by collecting young red shoot leaves from the shoots, then cleaning them of adhering dirt with running water and air-drying them at room temperature, without exposure to the sun. After drying, the sample was powdered, then sieved until red shoot simplicia (*Syzygium myrtifolium* Walp.) was obtained.[26]

Extraction

The powdered plant material (300 g) was macerated with 3 L of 96% ethanol, and the mixture was stirred and allowed to macerate for 3×24 hours in a tightly closed container protected from sunlight with occasional stirring. The marc was remacerated, and the results of each extraction were dried at room temperature (25 ℃) by aerating until a dry extract was obtained. The procedure was repeated using ethyl acetate and n-hexane solvents using new simplicial.[27]

Qualitative Analysis

Flavonoids

A total of 2 mL of each test solution was transferred into 3 test tubes. Tube 1 serves as a control, while 1 mL of 10% Pb Acetate (lead acetate) solution was added to tube 2 for the flavonoid test. A yellow precipitate indicates the presence of flavonoids. Tube 3 was treated with a few drops of 20% NaOH, which turns yellow in the presence of flavonoids.[26]

Anthocyanin

In this determination, 0.5 g of each extract (ethanol, ethyl acetate, and n-Hexane) from red shoot leaves was mixed with 2 M HCl and heated at 100 ℃ for 5 minutes. A red colour precipitate indicates a positive for anthocyanidin. The above experiment was repeated, with 2 M NaOH added dropwise to the test tubes while observing the colour change. A blue-green colour that fades slowly indicates a positive result.[28]

Quantitative Analysis

Quantitative Analysis of Flavonoid Content

The phenolic content was determined using a previously described colourimetric procedure with some modifications, using quercetin as the standard.[26] In this assay, 10 mg of quercetin is weighed into a 10 mL volumetric flask and dissolved, then ethanol p.a. was added to the upper limit. The solution was prepared at a concentration of 100 ppm by diluting 1 mL, then placing it in a 10 mL measuring flask, and adding a series of solutions at concentrations of 2, 4, 6, 8, and 10 ppm. Each solution was added with 1.5 mL of ethanol p.a and 0.1 mL of 10% aluminium chloride. Subsequently, 0.1 mL of 1 M sodium acetate and distilled water were added to the upper limit. The test solution was shaken and allowed to stand at room temperature for 30 minutes. Absorption was measured at a wavelength of 440 nm using a UV-Vis spectrophotometer.

Each extract (10 mg) was weighed and dissolved in p.a. ethanol to the upper limit in a 10 mL volumetric flask. A total of 0.5 mL of the solution was taken and then added, respectively, to 1.5 mL of ethanol p.a. and 0.1 mL of 10% aluminium chloride. 0.1 mL of 1 M sodium acetate and 2.8 mL of water were added, shaken, and left for 30 minutes at room temperature. The absorption was measured at 440 nm using a UV-Vis spectrophotometer. The levels of each extract were determined in 3 replications.[26]

Quantitative Analysis of Anthocyanin Levels

The ethanol extract of red shoot leaves was weighed at up to 25 mg, then dissolved in 25 mL of pH 1 buffer and left for 15 minutes. Then, 25 mg of the ethanol extract of red shoot leaves was weighed, dissolved in 25 mL of pH 4.5 buffer, and left for 5 minutes. Each was then placed in a cuvette for absorbance measurement in triplicate. Meanwhile. The ethyl acetate and n-hexane extracts were weighed at 25 mg each and dissolved in 1 mL of ethyl acetate and 25 mL of pH 1 buffer, respectively. The mixture was left for 15 minutes. The mixture was then centrifuged (Magal Low Speed Centrifuge M400D) for 15 minutes because ethyl acetate and n-Hexane are insoluble in the buffer solution. Each solution was then placed in a cuvette for absorbance measurement in triplicate. The above procedure was repeated in a pH 4.5 buffer, incubated for 5 minutes, and then centrifuged for 15 minutes. Each solution was then placed in a cuvette to measure its absorbance in triplicate.[28]

In this measurement, blank solutions were used in the form of KCl buffer pH 1 and $CH_3CO_2Na.3H_2O$ buffer pH 4.5. Blank solutions are usually used for calibration purposes as reference solutions in analysis. The anthocyanin content was measured using a UV-Vis spectrophotometer (UV-1900, Shimadzu®) at 510 nm and 700 nm.[28]

Preparation of Buffers

A buffer solution with pH 1.0 was prepared by weighing 1.490 g of KCl, dissolving it in distilled water to make 100 mL, then mixing 76 mL with 1.5 mL of HCl, and measuring the pH to 1. Then, pH 4.5 is achieved by dissolving 4.2028 g of citric acid in distilled water to make 100 mL. Then 5.882 g of Na citrate is weighed and dissolved in distilled water to make a 100 mL solution, 55 mL of citric acid and 45 mL of sodium citrate are mixed, and the pH is measured until pH 4.5.[28]

Antibacterial Activity Testing

Creation of Mueller Hinton Agar (MHA) Media

MHA medium was prepared by adding 38 g of instant MHA powder to 1000 mL of distilled water. Heated until boiling to dissolve the medium. The media was sterilised in an autoclave for 15 minutes at 121℃.[27]

Rejuvenation of Test Bacteria

The test bacteria (*Staphylococcus aureus* and *Escherichia coli*) were taken with a loop needle and inoculated into slanted MHA media in a test tube. Incubate for 1x24 hours at 37ºC.[29] The test bacteria

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(*Staphylococcus aureus* and *Escherichia coli*) were each transferred with a loop needle and suspended in a tube containing 7 mL of 0.9% sterile physiological NaCl solution. The suspension formed was equated to the 5% McFarland standard.[30]

Antibacterial Activity Test Using the Agar Diffusion Method
A total of 0.5 mL of bacterial suspension and 15 mL of MHA were placed in a petri dish, homogenised, and allowed to solidify. A blank paper disk was dropped onto each extract at concentrations of 1%, 4%, 8%, and 16%, with up to 20 µL, and placed on the surface of the agar media. Chloramphenicol was used as the positive control, and the filter was used as the negative control. Petri dishes were incubated upside down at 37 ˚C for 24 hours. The diameter of the clear zone around the paper disk was measured with a calliper and compared with that of the positive control.[29]

Statistical Analysis
All experimental data are presented as the mean ± standard deviation (SD) derived from three replicates. Statistical analysis was carried out using Microsoft Excel 2021. Statistical significance was defined at a threshold of $p < 0.05$.

Result and Discussion

The optimisation of phenolic and anthocyanin contents and their effects on the antibacterial bioactivity of red shoot leaves (*Syzygium myrtifolium* Walp) using various extraction solvents was evaluated in this study. The maceration method was used because of its ability to extract large quantities of samples and the ease of its application.[10] In extraction, the type of solvent used also influences the amount of active compounds in the extract, according to the like dissolves like concept: polar compounds dissolve in polar solvents, and nonpolar compounds dissolve in nonpolar solvents. In this study, 3 solvents with different polarities were used: ethanol (polar), ethyl acetate (semipolar), and n-Hexane (nonpolar). The data in Table 1 show that the ethanol extract has a higher weight and yield than the other extracts, implying that ethanol extracts greater amounts of active compounds.[25] Phytochemical screening helps determine the chemical composition of plant materials. The results are influenced by the choice of solvent and the extraction method used. The content of bioactive compounds in plants varies due to environmental factors, including altitude, soil type, and climate, as well as the formation of secondary metabolites, which is influenced by temperature, pH, water activity, and light intensity. From the phytochemical screening results, many active compounds were found in ethanol extracts, as polar solvents are universal and can solvate all chemical compounds,[31] as shown in Table 2.

Table 1: Percent Yield of Red Shoot Leaf Extract (*Syzygium myrtifolium* Walp) with various extraction solvents.

Extract	Extract Weight (g)	% yield	Extract Colour
Ethanol	300	24.2	Red brown
Ethyl Acetate	300	23.9	Green
n-Hexane	300	18.2	Green

Table 2: Phytochemical Screening Results of Red Shoot Leaf Extract (*Syzygium myrtifolium* Walp) with various extraction solvents.

Compounds	Extracts Ethanol	Ethyl Acetate	n-Hexan
Alkaloids: DragendorffWagner	+	-	-
Mayer	+	-	-
	+	-	-
Flavonoids	+	+	+
Saponin	+	-	-
Phenolic	+	+	+
Tannin	+	+	+
Steroids/Terpenoids	-	-	-

Also, the flavonoid and anthocyanin contents were determined using a UV-Vis spectrophotometer with high sensitivity and accuracy.[14] The results of the spectrophotometer determination of the flavonoid and anthocyanidin content of *Syzygium myrtifolium* are shown in Tables 3 and 5. Flavonoid analysis was carried out using UV-Vis Spectrophotometry because flavonoids contain conjugated aromatic systems, which result in strong absorption bands in the ultraviolet and visible regions. In Table 3, the measurement of the total flavonoid content of the ethanol extract was 24.3 mgEK/g, the ethyl acetate extract was 19.6 mgEK/g, and the n-hexane extract was 19.6 mgEK/g. In this study, to determine the total flavonoid content of the extract, quercetin was used as a standard to generate a calibration curve across several concentration series, yielding the equation, which was used to calculate percent levels. Quercetin is used as a standard because it is a flavonoid in the flavonol group, with a ketone group at C-4 and a hydroxyl group at C-3 or C-5, which is adjacent to the flavone and flavonol groups. Maximum wavelength absorption measurements were carried out over a wavelength range of 400 - 450 nm. The results show that the maximum standard wavelength of quercetin is 440 nm. [32,33]

The anthocyanin compound content of the shoot leaves was greater than that of other parts of the leaves, because this pigment gives the shoot leaves a red color. This color indicates the presence of anthocyanin compounds in that part of the leaf. The activity of forming anthocyanins in plant parts can occur simultaneously with the formation of chlorophyll. In terms of quantity, if a part of a plant is green, it can be seen that that part contains less anthocyanin than chlorophyll or that there is an inhibition of the activity of forming anthocyanin compounds from the formation of chlorophyll. However, the anthocyanin content is not greater than the chlorophyll content in red shoot leaves.[12]
Furthermore, each sample was subjected to a qualitative color test to determine its anthocyanin content. Anthocyanin is a pigment that causes the red-to-blue color in fruits and vegetables. Initial identification of anthocyanins from red shoot leaf extract was carried out using a color test on red shoot leaf extract with the addition of 2 M HCl and 2 M NaOH. The results of the anthocyanin color test for the three samples are shown in Table 4. One factor that influences anthocyanin color is pH. Acidic conditions cause anthocyanins to turn red, while alkaline conditions cause anthocyanins to turn blue. The results of anthocyanin identification from the ethanol and n-Hexane extracts were consistent with the literature.[34,35] When the ethanol, ethyl acetate, and n-Hexane

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extracts were heated with 2 M HCl, they yielded positive red results. Likewise, when 2 M NaOH was added, the results were positive: the color changed to green, and the blue faded slowly.

Table 3: Results of Quantitative Analysis of Phenolic Compounds of Red Shoot Leaf Extract (*Syzygium myrtifolium* Walp) with various extraction solvents.

Extract	AbsorbanceΛ_{440} (nm)	Phenolic content (mgEAG/gram)	Average Level Phenolic (mgEAG/gram)
Ethanol Extract	0.3537	25.5	24.3
	0.3268	23.5	
	0.3302	23.8	
Ethyl Acetate Extract	0.2441	17.2	19.6
	0.2439	17.1	
	0.3438	24.8	
n-hexane Extract	0.2440	17.2	19.6
	0.2439	17.1	
	0.3438	24.8	

Table 4: Results of Qualitative Analysis of Anthocyanin Extract of red shoot leaves (*Syzygium myrtifolium* Walp) with various extraction solvents.

Extract	Test	Discoloration
Ethanol Extract	Heated with 2 M HCl for 5 minutes at 100°C	(+) Red
	Added 2 M NaOH solution	(+) Fading Green
Ethyl Acetate Extract	Heated with 2 M HCl for 5 minutes at 100°C	(+) Red
	Added 2 M NaOH solution	(+) Fading Green
n-Hexane Extract	Heated with 2 M HCl for 5 minutes at 100°C	(+) Red
	Added 2 M NaOH solution	(+)Fading Green

Also, after the color test, the anthocyanin content was measured using a UV-Vis spectrophotometer, providing accurate results. Where the numbers are read, immediately recorded by the detector, and printed as digital numbers or as graphs that have been regressed.[14] The results of the spectrophotometric analysis are shown in Table 5.

Table 5: Results of Quantitative Anthocyanin Analysis of Red Shoot Leaf Extract (*Syzygium myrtifolium* Walp) with various extraction solvents.

Extract	λ(nm)	Absorbance		Absorbance Value	Total Anthocyanins (mg/L)	Average total anthocyanins (mg/L)
		pH 1	pH 4.5			
Ethanol Extract	510	0.223	0.064	0.081	1.35	1.38
	700	0.093	0.015			
	510	0.225	0.065	0.085	1.41	
	700	0.090	0.015			
	510	0.225	0.065	0.084	1.40	
	700	0.091	0.015			
Ethyl Acetate Extract	510	0.047	0.032	0.012	0.20	0.21
	700	0.032	0.029			
	510	0.048	0.031	0.014	0.23	
	700	0.033	0.030			
	510	0.047	0.032	0.013	0.21	
	700	0.032	0.030			
n-Hexane Extract	510	0.043	0.032	0.007	0.11	0.11
	700	0.034	0.030			
	510	0.043	0.032	0.007	0.11	
	700	0.034	0.030			

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510	0.043	0.032	0.007	0.11
700	0.034	0.030		

Factors that influence the stability of anthocyanins include temperature, pH, and storage. pH is one factor that determines the stability of anthocyanins. At a strong acidic pH (below 2), it gives a red color, and at a basic pH (above 4.5), it provides a blue-green color. Apart from pH, another factor that causes anthocyanin degradation is temperature: the higher the temperature and the longer the heating time, the greater the damage to the anthocyanin compound. Apart from pH and temperature factors, the stability of anthocyanins is also influenced by the storage process (light). This is because anthocyanins have a strong tendency to absorb visible light, and the absorbed light energy induces phytochemical effects in the visible spectrum, leading to color changes.[28] In this measurement, a blank buffer solution was used at pH 1 and 4.5. This blank solution aims to determine the absorbance of the solution in the absence of the analyte. Blank solutions are usually used for calibration purposes as reference solutions in analysis. The buffer solution maintains pH stability within the filtration range of anthocyanin compounds. From the results of the anthocyanin content test, the total anthocyanin content of the ethanol solvent was 1.38 mg/L, ethyl acetate (21 mg/L), and n-Hexane was 0.11 mg/L. The results showed that the ethanol extract contains anthocyanidin. From these observational data, it shows that the highest levels of anthocyanin compounds in red shoot leaves tend to be polar, because anthocyanin pigments can dissolve in ethanol, a polar solvent.[36]

Anthocyanin determination was performed using the pH-difference method, namely pH 1 and pH 4.5. At pH 1.0, anthocyanins are in the form of oxonium compounds. Increasingly acidic conditions, especially approaching pH 1, will cause more anthocyanin pigments to exist as colored flavylium or oxonium cations, and absorbance measurements will show greater amounts of anthocyanin. At pH 4.5. i.e., in weak acids, the flavylium cation changes to the more stable colorless hemiketal and chalcone forms.[37] The research results at 510 nm showed a higher absorbance than at 700 nm. The higher the absorbance value of the extract, the more anthocyanin dye (pigment) is extracted.[38,39] The 510 nm wavelength is optimal for cyanidin 3-glucoside, while 700 nm is used to correct the remaining precipitate in the sample. If the sample is completely clear, the absorbance at 700 nm is 0. However, in the results of this study, the absorption value (or absorbance) at 700 nm does not equal 0 because small particles are still present in the sample.[28]

Similarly, antibacterial activity was evaluated using the agar diffusion method. The data obtained is presented in Tables 6, 7, and 8. Ethanol extract inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at 1% concentration, with inhibitory zone diameters of 10.69 mm and 6.80 mm, respectively. Ethyl acetate extract inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at 1% concentration, with inhibitory zone diameters of 6.52 mm and 6.39 mm, respectively. Meanwhile, the n-hexane extract inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at 1% concentration, with inhibitory zone diameters of 17.25 mm and 10.44 mm, respectively. The diameter of the inhibition zone increases with increasing extract concentration. The classification of the inhibitory response to bacterial growth is that an inhibitory zone diameter of more than 20 mm is classified as very strong, 10-20 mm as strong, 5-10 mm as moderate, and less than 5 mm as weak. The effect of the ethanol extract against *Staphylococcus aureus* ranges from strong to very strong, while in *Escherichia coli*, it is classified as moderate. The effect of the ethyl acetate extract on *Staphylococcus aureus* ranges from moderate to strong, while on *Escherichia coli*, it is classified as moderate. The effect of n-hexane extract on *Staphylococcus aureus* ranges from strong to very strong. While on *Escherichia coli*, it is classified as strong.

Table 6: Results of testing the antibacterial activity of ethanol extract of red shoot leaves (*Syzygium myrtifolium* Walp.) against *Staphylococcus aureus* and *Escherichia coli.*

Test Bacteria	Replicates	Concentration (mm) ± SD					
		1%	4%	8%	16%	Control (+)	Control (-)
Staphylococcus aureus	(n=3)	10.69±0.28	13.31±0.13	16.32±2.55	21.26±2.87	44.76±0.61	0
Escherichia coli	(n=3)	6.80±0.48	8.14±1.11	8.90±0.88	9.90±0.78	30.18±2.37	0

Table 7: Results of testing the antibacterial activity of ethyl acetate extract of red shoot leaves (*Syzygium myrtifolium* Walp.) against *Staphylococcus aureus* and *Escherichia coli*

Test Bacteria	Replicates	Concentration (mm)±SD					
		1%	4%	8%	16%	Control (+)	Control (-)
Staphylococcus aureus	n=3	6.52±0.07	9.04±0.93	10.74±0.68	14.07±0.79	41.52±2.13	0
Escherichia coli	n=3	6.39±0.10	6.68±0.32	7.14±0.41	8.42±0.54	33.59±3.21	0

Table 8: Test Results of Antibacterial Activity of n-hexane Extract of Red Pucuk Leaves (*Syzygium myrtifolium* Walp.) Against *Staphylococcus aureus* and *Escherichia coli*.

Test Bacteria	Replicates	Concentration (mm)					
		1%	4%	8%	16%	Control (+)	Control (-)
Staphylococcus aureus	n=3	17.25±2.70	20.02±3.06	21.53±3.57	26.38±1.18	38.44±2.10	0
Escherichia coli	n=3	10.44±0.09	13.05±0.86	13.43±1.18	15.27±0.56	29.69±2.68	0

The best antibacterial activity test results were obtained for n-hexane extract, with inhibitory effect values ranging from strong to very strong for *Staphylococcus aureus* bacteria (17.25 mm, 20.02 mm, 21.53 mm, and 26.38 mm). Meanwhile, for *Escherichia coli*, the inhibitory effect values ranged from strong (10.44 mm, 13.05 mm, 13.43 mm, and 15.27 mm). This is because compounds that have the potential to act as antibacterials are more soluble in nonpolar solvents. The difference in antibacterial activity between ethanol and n-hexane extract of red shoot leaves is that ethanol is a universal solvent that can dissolve most of the compound components contained in the extract. This causes the activity of chemical compounds dissolved in ethanol to work less than optimally.[40,41]

Differences in inhibitory power are caused by differences in organism sensitivity mechanisms, and work in synergy between the active compounds in the extract. Gram-positive bacteria are known to be more sensitive than gram-negative bacteria due to the cell wall structure of

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gram-negative bacteria being more complex compared to that of gram-positive bacteria. The cell wall structure of Gram-negative bacteria consists of three layers, while that of Gram-positive bacteria is a single layer. Apart from that, in gram-positive bacteria, the peptidoglycan is not protected by an outer membrane. The difference in the structure of the membrane layer causes gram-negative bacteria to be less sensitive to antibiotics than gram-positive bacteria.[41] The difference in inhibitory power of various solvents is presented in Figure 1.

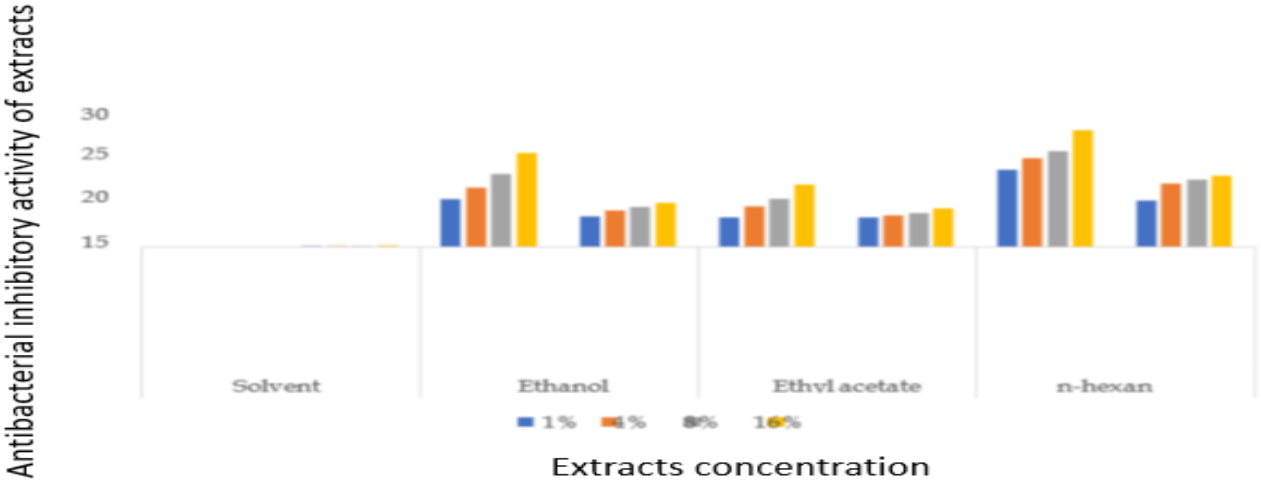

Figure 1: A plot of testing the antibacterial activity of red shoot leaves against concentration

The results of qualitative and quantitative testing of flavonoid and anthocyanin compounds showed that several red shoots leaf extracts, namely ethanol, ethyl acetate, and n-hexane extract, contain flavonoid and anthocyanin compounds. It is suspected that the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* found in red shoot leaf extract (*Syzygium myrtifolium* Walp.) may be due to the presence of alkaloid, flavonoid, anthocyanins, saponin, phenol, and tannin in the plant extract.[27] Alkaloids work as antibacterials by disrupting the peptidoglycan components in bacterial cells so that the cell wall layer does not form completely, causing cell death.[42] The mechanism of action of flavonoids as antimicrobials can be divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism. In inhibiting nucleic acid synthesis, the A and B rings of flavonoid compounds play an essential role in the interchelation or hydrogen bonding process by accumulating nucleic acid bases, thereby inhibiting the formation of DNA and RNA. Meanwhile, the action of flavonoids causes damage to the permeability of bacterial cell wall microsomes and lysosomes, the result of the interaction between flavonoids and bacterial DNA. The mechanism of action of flavonoids is to inhibit cell membrane function by forming complex compounds from extracellular and dissolved proteins, which damage the bacterial cell membrane and are followed by the release of intracellular compounds. In addition, inhibition of bacterial energy metabolism by flavonoids is by inhibiting the bacterial respiration process, so that the inhibited energy will affect the activity of metabolite absorption and biosynthesis of bacterial macromolecules.[43]

Anthocyanins are a group of flavonoid derivatives that have one function as antibacterial. The mechanism of anthocyanins as an antibacterial is the interaction of the cell membrane and intracellular membranes, which disrupts the permeability of the cell membrane, causing anthocyanins to disrupt the function of the cell membrane and cause cell leakage, resulting in cell lysis and eventual cell death.[44]

The mechanism of saponin's action as an antibacterial is by denaturing proteins. Because the surface active substance of saponin is similar to that of a detergent. Saponin can be used as an antibacterial, where the surface tension of bacterial cell walls is reduced, and the permeability of bacterial membranes is damaged. The survival of bacteria will be disrupted due to damage to the cell membrane, and saponin diffuses through the cytoplasmic membrane, disturbing the stability of the membrane, causing the cytoplasmic cell leakage, resulting in cell death.[45]

According to previous research, Syafriana & Wiranti, Red shoot plants are known to contain secondary metabolite compounds such as alkaloids, flavonoids, tannins, triterpenoids, steroids, saponins, and phenolic compounds.[46] These compounds are known to act as natural antimicrobials for plants. Several studies have reported that leaf extracts from this plant have antibacterial activity against several Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*.

Alkaloids work as antibacterials by disrupting the components that make up the peptidoglycan in bacterial cells, inhibiting cell wall formation and causing cell death.[41,47] Tannins have antibacterial activity related to their ability to inactivate bacterial cell adhesins through enzymes and disrupt protein transport in the inner layer of bacterial cells. Tannins also target bacterial cell wall polypeptides, inhibiting the formation of bacterial cell walls.[48,49] The antibacterial mechanism of triterpenoids is to react with porins (trans membrane proteins) on the outer membrane of the bacterial cell wall and form strong polymer bonds, resulting in porin damage. The destruction of porin is the door to the entry and exit of nutrients, so that the inhibitory compound increases the permeability of the bacterial cell wall. The permeability of the bacterial cell wall will interfere with the entry and exit of nutrients and other compounds, so that bacterial growth is inhibited.[50-53] Saponins also have the potential to diffuse through the cytoplasmic membrane, interfering with membrane stability, causing the cytoplasm to leak, resulting in cell death.[54,55]

The mechanism of action of phenol compounds is by increasing the permeability of the cytoplasmic membrane, causing leakage of intracellular components and coagulation of the cytoplasm, resulting in cell lysis. Phenolic compounds have bactericidal activity. Phenolic compounds have broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, so that phenolic compounds can be intensively used as disinfectants.[56,57,58] Phenolic compounds at low concentrations can damage the cytoplasmic membrane and cause leakage of the cell nucleus, whereas at high concentrations, phenolic compounds coagulate with cellular proteins.[59,60] Tannins have antibacterial activity, which is related to their ability to inactivate bacterial cell adhesins through enzymes and disrupt protein transport in the inner layer of bacterial cells. Tannins also target bacterial cell wall polypeptides to stimulate improper bacterial cell wall formation.[61]

Conclusion

Variations in the extraction solvent of red shoot leaves affect the levels of flavonoids, anthocyanins, and their bioactivity as antibacterials.

Flavonoid and anthocyanidin showed varied content in the ethanol extract of red shoot leaves. The study also showed that the antibacterial effects of the extract were obtained using n-hexane as the extracting solvent, which produced the best antibacterial inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*, with values ranging from 17.25-26.38 mm and 10.44 - 15.27 mm, respectively. The study concludes that the solvent extracts of red shoot leaves contain different phytochemicals with potential activities against gram-positive and gram-negative bacterial strains and may have informed its use in ethnomedicine for treating related diseases.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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