Total Flavonoid Content of Yellow Wood Extract (Arcangelisia Flava (L.) Merr) and Antibacterial Activity Against Staphylococcus aureus

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Abstract
Yellow wood (Arcangelisia flava Merr and (L.)) is a medicinal plant that has received little attention. Yellow wood has antibacterial, antioxidant, anti-hyperlipidemic, and anti-cancer properties. Furthermore, yellow wood is known to contain a range of flavonoid compounds, such as hydroxylarcangelisin, which has broad antibacterial activity. The purpose of this research was to investigate the total flavonoid content of yellow wood extract as well as its antibacterial activity against Staphylococcus aureus bacteria. This step of research included the production of methanol extract, characterization of simplisia, phytochemical screening, and determination of the total flavonoid levels of the yellow wood methanol extract using a visual spectroscopic technique. Yellow wood methanol extract was made using the maceration method using methanol concentrations of 100%, 90%, and 80%, and the extract obtained was concentrated using a rotary evaporator. The total flavonoid content was then determined using the visible spectrophotometric method, and the antibacterial activity test was carried out against Staphylococcus aureus as a positive control. Using chloramphenicol and a negative control using dimethyl sulfoxide (DMSO). The results of this study can be concluded to show that the total flavonoid levels in the methanol extract of yellow wood at a concentration of 80% were 228,314 ± 5,4171 mg QE/g. At a 90% concentration of 245.993 ± 6.7626 mg QE/g and a 100% concentration of 3.0853 mg QE/g. The results of antibacterial activity at the concentration of 100% yellow wood methanol extract against Staphylococcus aureus bacteria have an average blandness of 12.65 mm.

Keywords: Yellow wood; Flavonoids; Spectrofotometri Visible; Staphylococcus aureus;

Introduction
One of the medicinal plants that has not been widely studied is yellow wood (Arcangelisia flava (L.) Merr). Yellow wood has antimicrobial, antioxidant, anti-hyperlipidemic, and anti-cancer properties (Rizki et al., 2022). In addition, yellow wood is also known to contain various flavonoid compounds, such as hydroxylarcangelisin, which is known to have a broad-spectrum antibacterial activity (Mulyani et al., 2020). Yellow wood extract has a broad antibacterial range, including several gram-positive and gram-negative microorganisms (Maryani et al., 2018). One of the secondary metabolites of the yellow wood plant are flavonoids. Flavonoids have a pyran ring that connects a three-carbon chain with one of the benzene rings. Generally, flavonoids are found combined with sugar to form glycosides, which make these compounds more soluble in polar solvents such as methanol (Panca et al., 2022).

The results of the research stated that the yellow wood methanol extract had a total flavonoid content of 1.66% (Rizki et al., 2022). Previous studies have shown that yellow wood extract has the potential to have an antibacterial effect by inhibiting the growth of Staphylococcus aureus bacteria (Dayan et al., 2016). In addition, the yellow root (Arcangelisia flava (L.) Merr) topical gel formulation with Na CMC base had an inhibition of respectively (Mulyani et al., 2020). Several investigations have revealed that the ethanol extract of yellow root stems has outstanding antibacterial and antifungal action (Pratama et al., 2018).

Based on the description above, this prompted researchers to conduct research on the determination of the total flavonoid content of the yellow wood extract at various methanol concentrations and its antibacterial activity against Staphylococcus aureus bacteria.

Methods
Sample
The yellow wood sample used in this study was obtained from

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Preparation of yellow wood extract at various concentrations of methanol

Yellow wood is made into shavings, then dried in the sun for ±3 days. Once dry, grind it in a blender until it becomes powder. Put 10 parts of simplicia in a vessel, pour it with 75 parts of methanol at a concentration of 100, 90, or 80%, cover, and let stand for 5 days, protected from light, while stirring frequently. Squeeze and wash the dregs with each methanol concentration until 100 parts are obtained. Transfer to a closed vessel and store in a cool, dark area for 2 days before filtering (Depkes, 1979). Maserate I and Maserate II are combined, after which they are concentrated by evaporating them in a rotary evaporator at 40°C and Maserate II are combined, after which they stored in a cool, dark area for 2 days before filtering (Depkes, 1979). Maserate I and Maserate II are combined, after which they are concentrated by evaporating them in a rotary evaporator at 40°C to obtain a thick extract (Ningtias and Rani, 2022).

Charactization of Yellow Wood Simplicia

The simplicia characterization includes the determination of water content, water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content (Pulungan et al., 2022).

Phytochemical Screening

Phytochemical screening of yellow wood (Arcangelisia flava (L.) Merr) includes examination of alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, and glycosides. Phytochemical screening was carried out on simplicia and methanol extracts of yellow wood (Arcangelisia flava (L.) Merr) (Nurmazela et al., 2022).

Alkaloid

Powder and methanol extract of yellow wood (Arcangelisia flava (L.) Merr) were each weighed at 0.5 grams, then 1 ml of 2 N hydrochloric acid and 9 ml of distilled water were added, heated over a water bath for 2 minutes, cooled, and then filtered. The filtrate was used for the following experiments: When 3 drops of filtrate were added with 2 drops of Mayer’s reagent, a positive reaction was indicated by the formation of a white or yellow lumpy precipitate. 3 drops of filtrate were added with 2 drops of Bouchardt’s reagent, and a positive reaction was indicated by the formation of a brown to black precipitate. With 3 drops of filtrate added to 2 drops of Dragendorff reagent, a positive reaction is indicated by the formation of a red or orange color. Alkaloids are considered positive if sediment or turbidity occurs in at least 2 reactions from the 3 experiments above (Nurmazela et al., 2022).

Flavonoid

Simplicia powder and methanol extract of yellow wood (Arcangelisia flava (L.) Merr) were each weighed at 10 g, then added to 100 ml of heated water, bubbled for 5 minutes, and separated hot. The filtrate acquired was then taken to 5 ml, then added 0.01 g of Mg powder, 1 ml of concentrated HCl, and 2 ml of amyl liquor, shaken, and permitted to isolate. Flavonoids are positive on the off chance that there is a red, yellow, or orange variety in the amyl liquor layer (Nasution et al., 2022).

Tannin

Simplicia powder and methanol extract of yellow wood (Arcangelisia flava (L.) Merr) were each weighed at 0.5 g of the example was sifted with 10 ml of refined water, and then the filtrate was weakened with refined water until it was dismal. Take 2 ml of the arrangement and add 1 to 2 drops of iron (III) chloride reagent. The appearance of a blue or green-dark tone shows the presence of tannins (Kaban et al., 2022).

Saponin

Simplicia powder and methanol extract of yellow wood (Arcangelisia flava (L.) Merr) each weighed however much 0.5 g of test put in a test tube and added 10 ml of hot refined water, cooled, then shaken vivaciously for 10 seconds. A froth appears, which endures for at least 10 minutes as high as 1–10 cm. If the froth does not disappear after adding 1 drop of 2 N hydrochloric corrosive solution, saponins are present (Rambe et al., 2021).

Examination of steroids / triterpenoids

Simplicia powder methanol extract of yellow wood (Arcangelisia flava (L.) Merr) was each weighed at 1 g, macerated with 20 ml of n-hexane for 2 hours, then, at that point, separated. The filtrate is dissipated in a vanishing cup. To the buildup, 2 drops of anhydrous acidic corrosive and 1 drop of concentrated sulfuric corrosive were added. The appearance of a red-purple tone shows the presence of triterpenoids, while a greenish-blue tone demonstrates the presence of steroids (Rafita et al., 2022).

Glycosides

Examination of glycosides was carried out by the Lieberman reaction. Methanol extract and yellow wood powder (Arcangelisia flava (L.) Merr) were each dissolved in ethanol solvent, evaporated over a water bath then dissolved in 20 ml of acetic anhydride then added with 20 drops of concentrated sulfuric acid. Formation of blue or green color indicates the presence of glycosides (Pulungan et al., 2022).

Preparation of Quercetin Solution

25 mg of quercetin was weighed, then dissolved in a 25 ml volumetric flask, and methanol was added to the limit mark and put into the Standard Stock Solution (C = 1000 µg/mL) LIB I. Then 5 ml of LIB I was pipetted and then put into a 50 ml measuring flask and filled with methanol to the limit mark (C = 100 µg/mL) of LIB II.

Manufacture of Quercetin Maximum Wavelength

0.4 ml of quercetin was pipetted from standard mother liquor II (LIB II), then put into a 10 ml volumetric flask (C = 4 µg/mL), then added 0.1 ml of 10% AlCl3, then added 0.1 ml of sodium acetate 1 M, and 2.8 ml of distilled water, then added methanol to the limit mark. After that it was stirred homogeneously and allowed to stand for 30 minutes. The absorption was then measured at a maximum wavelength of 400–800 nm. Absorbance measurements used Uv-Vis spectrophotometry at a maximum λ of 439.5 nm

Determination of Operating Time and maximum wavelength

Pipette 0.4 ml of quercetin from standard mother liquor II (LIB II), then put it into a 10 ml volumetric flask (C = 4 µg/mL) then added 0.1 ml AlCl3 10%, 0.1 ml sodium acetate 1 M, 2.8 ml distilled water, and methanol to the mark. Then the operating
time of quercetin was measured for 30 minutes at a wavelength of 400–800 nm.

**Quercetin Calibration Curve Measurement**

25 mg of quercetin was weighed, then put into a 25-ml volumetric flask, and methanol was added to the mark (C = 1000 μg/mL (LIB I)). Then pipette 5 ml of quercetin from standard mother liquor I into a 50 ml volumetric flask and add methanol to the mark (C = 100 μg/mL) (LIB II). Then a series of levels were made and then put into a 10 ml measuring flask, then pipetted 0.2, 0.3, 0.4, 0.5, and 0.6 ml from LIB II with concentrations of 2, 3, 4, 5, and 6 μg/mL, then adding ethanol until the limit mark. Pipette 1 ml from each volumetric flask with various concentrations and put it into a 10 ml measuring flask, then add 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M sodium acetate, and 2.8 ml of distilled water. Methanol until the limit mark, then homogenized and left for 6–9 minutes. Then the absorption was measured at a maximum wavelength of 439.5 nm (Wirasti, 2019).

**Determination of Total Flavonoid Content from Methanol Extract of Yellow Wood (Arcangelisia flava (L.) Merr)**

Methanol extract of yellow wood (Arcangelisia flava (L.) Merr) with a concentration of 100%, 90%, and 80% each was weighed as much as 25 mg put into a 25 ml measuring flask with methanol to the limit mark (C = 1000 μg/mL), then pipette 1 ml into a 10 ml measuring flask and then add 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M sodium acetate, and 2.8 ml of distilled water. Methanol until the limit mark, then homogenized and allowed to stand for the operating time. Its absorption was measured at a maximum wavelength of 439.5 nm (Wirasti, 2019).

**Antibacterial Activity Test**

Antibacterial activity was tested using the disc diffusion method by pouring 20 ml of MHA media (Mueller Hinton Agar) into a petri dish, then shaking it and letting it solidify, then Staphylococcus aureus bacterial suspension was swabbed using a sterile swab evenly on the MHA media. Paper discs were soaked with yellow wood methanol extract which had the highest flavonoid content, where the concentrated methanol extract was dissolved in DMSO as much as 10 g in 10 ml, until the extract concentration became 100%, positive control (Chloramphenicol) and negative control (DMSO) were then placed above the media using sterile tweezers, the experiment was carried out 3 times. Then incubated for 18–24 hours at 37°C in an incubator. After which the inhibition zone (clear) was measured.

**Data analysis**

The data to be obtained is the result of measuring the diameter of the inhibition zone of yellow wood methanol extract on the growth of Staphylococcus aureus bacteria using MHA media.

**Results**

**Extraction Results**

The results of macerating with each solvent (80, 90, and 100% methanol) were evaporated using a rotary evaporator, resulting in a viscous extract of 100% methanol with a concentration of 30.3971 g of green-black color (a yield of 15.19%). The viscous extract with a concentration of 90% methanol was yellowish brown in color and weighed as much as 27.8036 g (a yield of 13.90%). The viscous extract with a methanol concentration of 80% was yellowish brown in color and weighed as much as 20.3498 g (a yield of 10.17%).

**Phytochemical Screening**

Test results for extracts and powders of yellow wood simplicia showed positive results for secondary metabolites of alkaloids, flavonoids, saponins, tannins, steroids, and glycosides.

**Examination of Characterization of Yellow Wood Simplicia**

This study shows that the yellow wood simplicia fulfills all the requirements based on the Indonesian Materia Medica (Depkes, 1989).

**Quercetin Maximum Wavelength Determination Results**

The determination of the maximum wavelength has the objective of determining the measurement wavelength where the complex between quercetin and AlCl₃ gives optimum absorbance. The determination of the maximum wavelength is an important factor in chemical analysis using the spectrophotometric method. Measurements at the maximum wavelength will provide the greatest change in absorbance for each content unit, so repeat measurements and replication will minimize measurement errors.

Testing for flavonoids was carried out by measuring the maximum wavelength of the quercetin solution using the visible spectrophotometry method to obtain a wavelength of 439.28 nm with an absorbance of 0.459. The complementary color for testing flavonoids is yellow, which corresponds to the wavelength range of 435–480 nm. The maximum wavelength measurement results are shown in Figure 1. below.

**Quercetin Calibration Curve Measurement Results**

The determination of the standard curve aims to determine the relationship between the concentration of the solution and the absorbance value so that the concentration of the sample can be known. If Lambert-Beer’s law is fulfilled, the standard curve is a straight line.

Measurement of the calibration curve was carried out with different concentrations of solutions, which were pipetted from a 100 μg/mL quercetin solution. Pipette each 0.2, 0.3, 0.4, 0.5, and 0.6 ml to obtain concentrations of 2, 3, 4, 5, and 6 g/mL. Add methanol up to the mark in a 10 ml measuring flask. Pipette 1 ml of each concentration into a 10-mL measuring flask, followed by 0.1 ml of 10% AlCl₃, 0.1 ml of sodium acetate, and 2.8 ml of pure water. Methanol should be added to the mark. After standing for 30 minutes, it was detected at a wavelength of 439.28 nm. From the measurement results, the absorbance of each standard solution is obtained, which is then converted into a linear regression equation.

The regression equation obtained from the standard quercetin solution is y = 0.1169x + 0.0041, with a correlation coefficient of 0.999. The linearity value indicates the correlation between the concentration and the resulting absorbance. Can be seen in figure 2 below:

**Results of Analysis of Total Flavonoid Content of Yellow Wood Extract (Arcangelisia flava (L.) Merr.) at Various Methanol Concentrations**
The determination of total flavonoid levels was calculated using the linear regression line equation $y = ax+b$ obtained from the quercetin calibration curve, so that the concentration $(x)$ was obtained. The value of $x$ is then substituted in the formula for calculating total flavonoid content. The determination of total flavonoid levels was carried out with six repetitions, and the average was taken as shown in the following Table 4.

**Test Results of Antibacterial Activity of Yellow Wood Extract (Arcangelisia flava (L.) Merr) at Methanol Concentration**

In the antibacterial activity test, extracts with a 100% methanol concentration were used, at which concentration the highest levels of flavonoids were obtained, so the antibacterial activity test was continued. The positive control used was chloramphenicol, which aims to determine the presence of inhibition to kill bacteria because it has broad-spectrum antibacterial activity and is bacteriostatic against almost all gram-negative and gram-positive bacteria. The negative control used was DMSO, where it was known that DMSO had no effect on bacteria, then the experiment was repeated 3 times. The results of measuring the diameter of inhibition of a methanol extract of yellow wood (Arcangelisia flava (L.) Merr) at 100% methanol concentration can be seen in Table 5.

**Table 1. Results of Examination of Characterization of Yellow Wood Simplicia Powder**

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Characterization Results (%)</th>
<th>MMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water content</td>
<td>3,33%</td>
<td>≤ 10%</td>
</tr>
<tr>
<td>2</td>
<td>Water soluble essence</td>
<td>3.96%</td>
<td>≥ 2%</td>
</tr>
<tr>
<td>3</td>
<td>Extract content is soluble in ethanol</td>
<td>8.2%</td>
<td>≥ 2%</td>
</tr>
<tr>
<td>4</td>
<td>Total ash content</td>
<td>1,37%</td>
<td>≤ 1,5%</td>
</tr>
<tr>
<td>5</td>
<td>Acid insoluble ash content</td>
<td>0,22%</td>
<td>≤ 0,5%</td>
</tr>
</tbody>
</table>

**Table 2. Results of Phytochemical Screening of Yellow Wood Methanol Extract (Arcangelisia flava (L.) Merr).**

<table>
<thead>
<tr>
<th>No.</th>
<th>Metabolites</th>
<th>Simpilia</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Information:
+: Contains Compound Groups
- : Does not contain Compound Groups

**Table 3. Absorbance Value of Quercetin Standard Solution**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Absorbance</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0,000</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0,259</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0,374</td>
<td>y = 0,1169x + 0,0041</td>
</tr>
<tr>
<td>4</td>
<td>0,488</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0,603</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0,701</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4. Average Value of Total Flavonoids of Yellow Wood Extract (Arcangelisia flava (L.) Merr) at Various Concentrations of Methanol.**

<table>
<thead>
<tr>
<th>Methanol Concentration (%)</th>
<th>Actual Level (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>228.314 ± 5.4171 mg QE/g</td>
</tr>
<tr>
<td>90</td>
<td>245.993 ± 6.7626 mg QE/g</td>
</tr>
<tr>
<td>100</td>
<td>265.953 ± 3.0853 mg QE/g</td>
</tr>
</tbody>
</table>

**Table 5. Results of Measurement of Inhibitory Power of Yellow Wood Extract (Arcangelisia flava (L.) Merr) at Methanol Concentration Against Staphylococcus aureus Bacteria.**

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Average zone of inhibition</th>
<th>category (Dayan et al., 2016)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (+)</td>
<td>21,37 mm</td>
<td>Very strong</td>
</tr>
<tr>
<td>100% methanol extract</td>
<td>12,65 mm</td>
<td>Strong</td>
</tr>
<tr>
<td>control (-)</td>
<td>0</td>
<td>Not inhibit</td>
</tr>
</tbody>
</table>
The results obtained showed that the yellow wood extract at 100% methanol concentration had antibacterial activity. The results of measuring the diameter of the inhibition of Staphylococcus aureus in 3 repetitions were 12.35, 12.8, and 12.8 mm with the strong category of inhibition.

**Discussion**

The results of the yellow wood methanol extract alkaloid test with Bouchardat reagent gave an orange brown precipitate, with Dragendorff reagent it gave an orange precipitate, and with Mayer’s reagent it gave a yellowish white precipitate, whereas in yellow wood simplicia powder with Bouchardat reagent it gave an orange precipitate, with Dragendorff reagent it gave an orange yellow precipitate, and with Mayer’s reagent it gave a white precipitate. Examination of flavonoids after the addition of concentrated hydrochloric acid to magnesium powder and amyl alcohol forms an orange-yellow layer on the amyl alcohol layer (Syahputra et al., 2021).

In the saponin test the extract and yellow wood simplicia powder showed positive results, where saponins have compounds that have hydrophilic and hydrophobic groups. Saponin positive results are indicated by the formation of foam. The appearance of foam indicates the presence of glycosides which have the ability to form foam in water which hydrolyzes into glucose and other compounds (Rani et al., 2022).

Positive results of steroid extracts and yellow wood simplicia powder were indicated by the formation of green color. Testing for steroids or triterpenoids is based on the compound’s ability to form concentrated HSO₄ in acetic anhydride (Robiatun et al., 2022). The results of the yellow wood simplicia extract and powder test showed positive results. The tannin test was carried out by adding 1% FeCl₃. The formation of a black-green color after the addition of 1% FeCl₃ indicates the presence of condensed tannins and forms complex compounds with 1% FeCl₃ (Ridwanto et al., 2023). The color change did not occur with the addition of FeCl₃ because there were no hydroxyl groups present in the tannin compound. The test results for extracts and yellow wood simplicia powder showed positive results with the formation of a green color. The formation of a blue or green color indicates the presence of glycosides (Ratnasari and Handayani, 2018).

Based on the test results, it can be said that the yellowwood extract (*Arcangelisia flava* (L.) Merr) at 100% methanol concentration has the ability to act as an antibacterial against gram-positive *Staphylococcus aureus* bacteria. This could be due to the presence of secondary metabolites in the extract that have antibacterial activity. The ability of the yellow wood extract at 100% methanol concentration to inhibit the activity of *Staphylococcus aureus* bacteria is associated with the bioactive compounds contained therein. In the yellow wood extract at 100% methanol concentration, there are components such as flavonoids, saponins, alkaloids, glycosides, steroids, tannins, and triterpenoids. Flavonoid chemicals act as antibacterials by building complex compounds with extracellular and dissolved proteins, causing damage to the bacterial cell membrane and causing the release of intracellular substances.

**Conclusion**

Total Flavonoid Content of Yellow Wood Extract (*Arcangelisia flava* (L.) Merr) At 100% concentration was 265.953 ± 3.0853 mg QE/g, at 90% methanol concentration was 245.993 ± 6.7626 mg QE/g, and at 80% methanol concentration was 228.314 ± 5.4171 mg QE/g. Yellow wood extract (*Arcangelisia flava* (L.) Merr.) at 100% methanol concentration gave an inhibitory effect on *Staphylococcus aureus* bacteria of 12.65 mm in the strong category.

**Conflict of Interest**

The author declares that there is no conflict of interest in this research.

**Acknowledgement**

The author would like to thank Ridwanto, as the supervisor, who always guided and provided guidance and input so that the author could complete this research.

**Author contribution**

Maryanti Yuza: research conceptualization, data collection, sampling and data compilation study, Ridwanto: research conceptualization and research data compilation, Zulmai Rani: Manuscript Writing and Script Revision.

**References**

Expert review of vaccines 15, 1373–1392.


