Biomedical Journal of Indonesia
Journal Homepage: https://bji-fk.ejournal.unsri.ac.id

Total Phenol, Flavonoid Content, and Antioxidant Activity of the Ethanol Fraction of *Arcangelisia Flava* Stem

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ARTICLE INFO

Keywords:
Arcangelisia flava
Phenol content
Flavonoid content
Antioxidant activity

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.32539/BJI.v11i2.225

Received 3 January 2025;
Accepted 4 February 2025

ABSTRACT

Introduction. Oxidative stress caused by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses leads to various diseases. *Arcangelisia flava*, a medicinal plant, contains antioxidant-active phenols and flavonoids. This study evaluates the total phenol and flavonoid content and the antioxidant activity of the ethanol fraction of *A. flava* stem. **Methods.** An experimental study was conducted using 750 grams of *A. flava* stem. The sample underwent fractionation and was analyzed using the Folin-Ciocalteu and $AlCl_3$ methods to determine total phenol and flavonoid content. Antioxidant activity was assessed using the DPPH method. Data were analyzed using regression equations and IC_{50} values. **Results.** The ethanol fraction contained 57.81 ± 3.28 mg GAE/g of total phenol and 114.6 ± 1.02 mg QE/g of total flavonoid. The IC_{50} value for the ethanol fraction was 72.88 ppm, indicating strong antioxidant activity, albeit less potent than ascorbic acid (IC_{50} 7.37 ppm). **Conclusion.** The ethanol fraction of *A. flava* stem is a potential natural antioxidant source.

1. Introduction

Oxidative stress is caused by an imbalance between the excessive synthesis of reactive oxygen species (ROS) and the body's antioxidant defenses, leading to various chronic and inflammatory diseases.[1] Evidence indicates that oxidative stress resulting from increased ROS is closely associated with the onset and progression of numerous diseases, such as cardiovascular system dysfunction caused by hyperlipidemia and coronary heart disease.[2] Furthermore, oxidative stress due to elevated ROS levels can contribute to skin disease development as it plays a critical role in pathological processes. Neurodegenerative diseases, such as Alzheimer's, are also linked to increased ROS, which leads to progressive memory impairment in patients.[3] To counteract the harmful effects of free radicals, the body requires protective mechanisms like antioxidants that can neutralize these radicals.[4]

"Yellow wood" (*Arcangelisia flava*), a medicinal plant widely used in traditional medicine in Indonesia, is known to contain bioactive compounds such as phenols and flavonoids, which exhibit antioxidant activity.[5] Phenols and flavonoids are two groups of compounds proven to have the capacity to neutralize free radicals and protect the body from cell damage caused by oxidative stress.[6] Previous studies have shown that this plant has potential as a natural source of antioxidants that can be utilized in plant-based therapeutics.[7]

However, despite the extensive discussion of the antioxidant potential of *A. flava*, research that examines explicitly the phenol and flavonoid content and the antioxidant activity of the ethanol fraction from *A. flava* stem remains limited. Several previous studies have reported antioxidant activity in *A. flava* extracts, but the results vary depending on the extraction methods and the plant parts used.[8] Therefore, this study aims to comprehensively analyze the total phenol and flavonoid content and the antioxidant activity of the ethanol fraction from *A. flava* stem.

This study's findings are expected to significantly contribute to developing plant-based therapies, particularly those containing natural antioxidant compounds. Furthermore, these findings are anticipated to enrich scientific knowledge regarding the potential of *A. flava* in preventing or mitigating the effects of oxidative stress associated with various degenerative diseases.

2. Methods

This study aims to evaluate the total phenol and flavonoid content and the antioxidant activity of the ethanol fraction of *A. flava* stem. The research design employed is an experimental study conducted at the Basic Chemistry Laboratory of the Faculty of Medicine, Sriwijaya University. The sample used in this study was the stem of *A. flava* obtained from Lubuk Linggau City, South Sumatra. A total of 750 grams of *A. flava* stem with a diameter of 2 cm or more and light green bark and yellow inside the stem. The sample preparation began with cutting the selected *A. flava* stem and cleaning and drying it at room temperature. The dried stem was then ground into simplicia and fractionated through maceration using a solvent of different polarity with three repetitions. The remaining simplicia from the n-hexane extract underwent stepwise fractionation using a semipolar solvent, ethyl acetate, followed by a polar solvent, ethanol. The ethanol fraction filtrate was evaporated in an oven at 40˚C to obtain a concentrated ethanol fraction of *A. flava*. Once the fractionation process was completed, the ethanol fraction was analyzed to determine its total phenol and flavonoid content as well as its antioxidant activity. Total phenol content was measured using the Folin-Ciocalteu method, while total flavonoid content was determined using the $AlCl_3$ method. Both analyses were performed using a UV-Vis spectrophotometer at the appropriate wavelengths.

Antioxidant activity was tested using the DPPH method (2,2-diphenyl-1-picrylhydrazyl), one of the methods to measure the ability of a compound to scavenge free radicals.[9] Antioxidant activity was evaluated based on the decrease in DPPH absorbance caused by its reaction with the ethanol extract sample. Data obtained from the analyses of total phenol, flavonoid content, and antioxidant activity were processed using linear regression equations (x, y) and IC_{50} values to determine the relationships between variables and to estimate dependent variable values, such as total content, in response to changes in the independent variable, precisely the ethanol fraction of *A. flava*.[10]

3. Result

3.1. Ethanol fractionation of *Arcangelisia flava* stem

Seven hundred fifty grams of *A. flava* stem simplicia were fractionated by maceration in stages based on its polarity to produce 27.73 grams of ethanol fraction. The calculated yield, expressed as the percentage of the final ethanolic fraction to the initial simplicia, was 5.03%.

3.2. Phytochemical analysis of ethanol fraction of *Arcangelisia flava* stem

Phytochemical tests were carried out to determine the class of secondary metabolites contained in the ethanol fraction of *A. flava* stem. The results of the phytochemical test can be seen in Table 1.

3.3. Determination of total phenol content in ethanol fraction of *Arcangelisia flava* stem

The total phenol content of the ethanol fraction of *the A. flava* stem was determined using the Folin-Ciocalteu reagent, initiated by measuring the absorbance of the gallic acid standard solution at 760 nm. The absorbance measurements produced a calibration curve with a linear regression equation $y = 0.0128x + 0.0314$ and a coefficient of determination (R^2) of 0.998. The R^2 value, which approaches 1, indicates a linear relationship between concentration and absorbance, where an increase in concentration corresponds to a rise in absorbance, showing a positive correlation between the variables.

Table 1. Phytochemical testing results of the ethanol fraction of A*rcangelisia flava* stem

Type of Test	Observation	Interpretation
Alkaloid		
a. Mayer	Formation of white precipitate	+
b. Dragendroff	Formation of the orange precipitate	+
c. Wagner	Formation of brown precipitate	+
Flavonoid	Formation of yellow layer	+
Saponin	Formation of foam with a height of < 1 cm	-
Tannin	Color change to green-violet	-
Terpenoid	The color changes to green.	-
Steroid	Color change to bluish-green	+

Note: (+) = identified, (-) = not identified

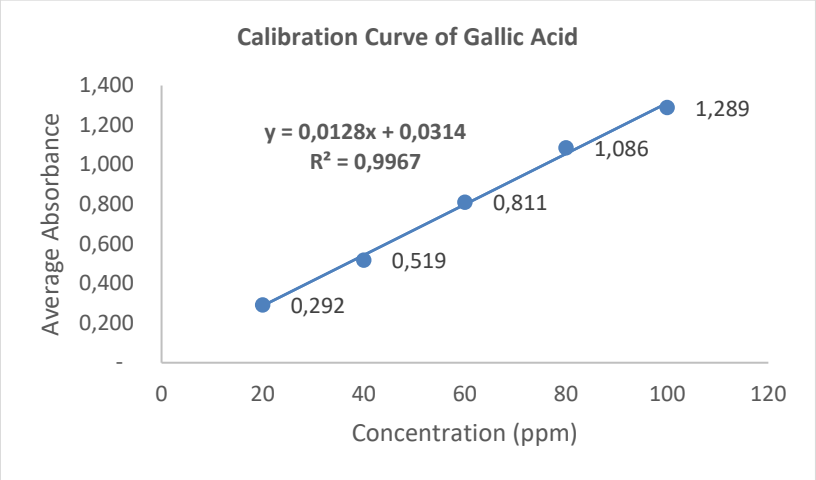

Figure 1. Calibration curve of gallic acid

Enter the absorbance value of this sample into the calibration curve so that the value of c (ppm) is obtained, and to determine the total phenol content, the formula is used: TPC =((c × v) ×DF)/g

Description:

TPC = total phenol content (mg GAE/g)

c = sample concentration (mg/mL)

v = volume of fraction used (mL)

DF = dilution factor

g = sample weight (g)

The weight of the ethanol fraction used was 0.025 g with a dilution factor of 50, and the solution used was 0.5 mL, then recorded at 57.81 ± 3.28 mg GAE/g (Table 2).

3.4. Determination of total flavonoid content in ethanol fraction of *Arcangelisia flava* stem

The total flavonoid content of the ethanol fraction of *A. flava* stem was determined by measuring the absorbance of quercetin standard solution at 510 nm. The absorbance data produced a calibration curve with the linear regression equation y = 0.0041x + 0.1233 and an R^2 value of 0.99.

Table 2. Determination of total phenol content in ethanol fraction of *Arcangelisia flava* stem

Absorbance	Total Phenol Content (µg GAE/g fraction)	Average Total Phenol Content (mg GAE/g fraction)	SD
0.73	54.27		
0.81	60.75	57.81	3.28
0.78	58.41		

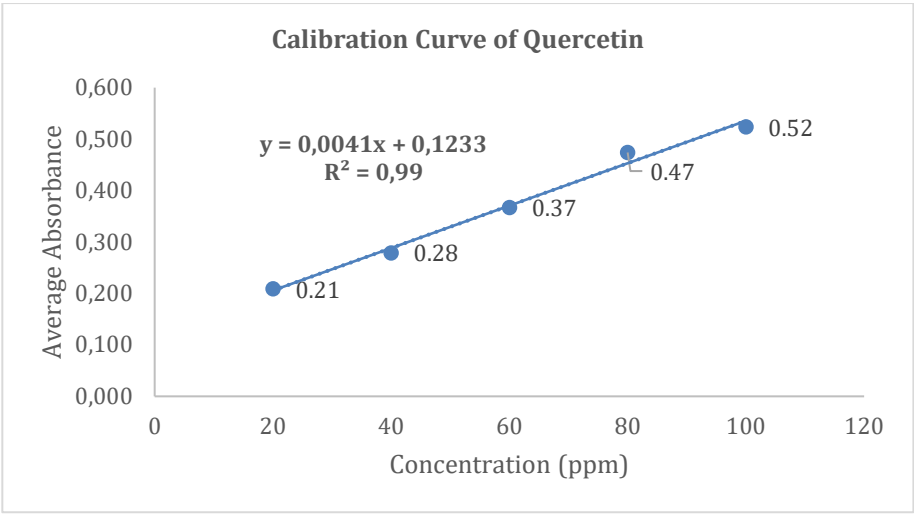

Figure 2. Calibration curve of quercetin

Enter the absorbance value of this sample into the calibration curve so that the value of c (ppm) is obtained, and to determine the total flavonoid content, the formula is used: TFC =((c × v) ×DF)/g

Description:

TFC = total phenol content (mg QE/g)

c = sample concentration (mg/mL)

v = volume of fraction used (mL)

DF = dilution factor

g = sample weight (g)

The weight of the ethanol fraction used was 0.025 g with a dilution factor of 25, and the solution used was 1 mL, then recorded at 114.64 ± 1.02 mg QE/g (Table 3).

3.5. Antioxidant activity in ethanolic fraction of *Arcangelisia flava* stem

The antioxidant activity of the ethanol fraction of *A. flava* stem was evaluated using the DPPH method (1,1-diphenyl-2-picrylhydrazyl) by measuring the absorbance of the ethanol fraction, ascorbic acid, and blank solution with UV-Vis spectrophotometry at 517 nm. A calibration curve was constructed with the linear regression equation, representing sample concentration and y representing inhibition percentage, to calculate the IC_{50} value, indicating the concentration required to inhibit 50% of free radical activity. The regression equations were y = 0.69x – 0.05 (R^2 = 0.98) for the ethanol fraction and y = 1.93x + 35.77 (R^2 = 0.88) for ascorbic acid. IC_{50} values can be seen in the table 4 below.

Table 3. Determination of total flavonoid content in ethanol fraction of *Arcangelisia flava* stem

Absorbance	Total Flavonoid Content (mg QE/g fraction)	Average Total Flavonoid Content (mg QE/g fraction)	SD
0.60	115.78		
0.59	113.83	114.64	1.02
0.59	114.32		

Table 4. Calculation results of inhibition percentage, regression equation, and IC_{50}

Sample	Concentration (ppm)	Inhibition Percentage (%)	Regression Equation	IC_{50} (ppm)
Ascorbic acid	2	27.74	y = 1.93x + 35.77	7.37
	5	46.24		
	10	66.45		
	15	73.55		
	20	73.98		
	25	75.27		
Ethanol fraction of *Arcangelisia flava* stem	2	0.22	y = 0.69x – 0.05	72.88
	5	3.01		
	10	8.17		
	15	11.40		
	20	13.76		
	25	15.91		

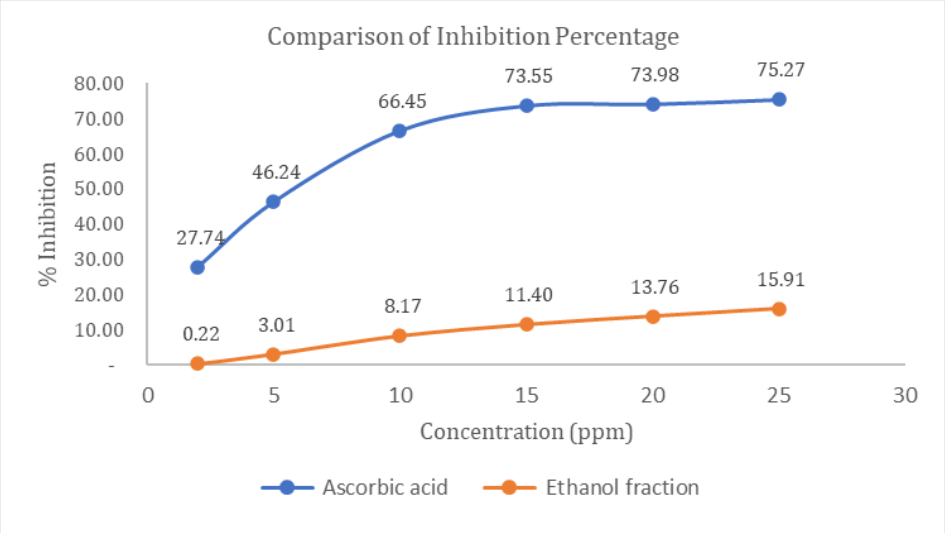

Figure 3. Comparison of inhibition percentage between ethanol fraction of *Arcangelisia flava* stem and ascorbic acid

4. Discussion

A phytochemical analysis of the ethanol fraction of *A. flava* stem was conducted to provide qualitative information on the presence of secondary metabolites that are extractable with a polar solvent, ethanol. The results indicated the presence of alkaloids, flavonoids, and steroids in the ethanol fraction, while saponins, tannins, and terpenoids were not identified. This can be attributed to the separation process based on the principle of "like dissolves like," where compounds dissolve in solvents with similar polarity.[11] Flavonoids, saponins, tannins, and glycosides are polar compounds that dissolve in polar solvents, while terpenoids and alkaloids are nonpolar and dissolve in nonpolar solvents.[5] The absence of saponins and tannins is likely due to repeated separation processes and the low amounts of these compounds in *the A. flava* stem.

This study's identification of secondary metabolites aligns with previous research, which also detected flavonoids, triterpenoids, and alkaloids in the *A. flava* stem. These secondary metabolites exhibit antioxidant activity through various mechanisms, such as flavonoids transferring single electrons (SET) and enhancing antioxidant production, triterpenoids and tannins performing metal chelation, and alkaloids and saponins supporting antioxidant production.[12,13]

This high total phenol content (57.81 ± 3.28 mg GAE/g) indicates the strong antioxidant potential of phenols. Furthermore, the yield of 5.03% demonstrates that even with a low yield, the high total phenol content signifies better potential. In a study by Sari on methanol extract of *A. flava* from Banjar, using the same wavelength, the total phenol content was 6.27 ± 0.06 mg GAE/g.[8] Differences in total phenol content between ethanol and methanol extracts may be attributed to the different polarities of the solvents used during extraction. Ethanol is preferred over methanol due to methanol's higher toxicity despite its better polarity.

External factors such as temperature, soil type, and light intensity during plant growth influence phenol content. Although derived from the same plant species, light intensity significantly affects phenol synthesis, as these compounds rely on light as an energy source during photosynthesis. Indonesia, located near the equator, benefits from relatively stable light intensity throughout the year.[14] However, nutrient-rich soil is also essential in forming secondary metabolites such as phenols.

Flavonoids significantly enhance antioxidant activity; therefore, higher flavonoid content corresponds to greater antioxidant effectiveness. Despite the low yield of 5.03%, the high total flavonoid content suggests better antioxidant potential. A study by Afrizani reported a total flavonoid content of 2.67 ± 11.70 mg QE/g for ethanol extracts of *A. flava* from Aceh under similar wavelength and concentration conditions.[15] Variations in flavonoid content between studies may result from factors such as soil type, temperature, and light intensity. Light dependency is crucial in flavonoid synthesis, as these secondary metabolites require sugars as precursors. Sugars act as sources of phosphoenolpyruvate and erythrose-4-phosphate, which contribute carbon atoms to forming the B-ring in flavonoids and as acetyl units for forming the A-ring. This process occurs in chlorophyll-containing cells via photosynthesis, where light is critical.[14]

The inhibition percentage showed variations across concentrations, with higher concentrations correlating to more remarkable free radical scavenging ability, consistent with prior studies indicating an increase in inhibition percentage with concentration. The IC_{50} value for the ethanol fraction was 72.88 ppm, while ascorbic acid had an IC_{50} value of 7.37 ppm, with lower IC_{50} values indicating more substantial antioxidant capacity. Thus, the ethanol fraction of *A. flava* demonstrates strong antioxidant activity, albeit less potent than ascorbic acid. The lower the IC_{50} value, the stronger the antioxidant capacity. [16]

Panchakul reported a higher IC_{50} value of 43.95 ppm for ethanol extracts of *A. flava* from Nakornpathom, Thailand. This difference occurs because the first is due to differences in the extraction process, and the second is the origin of plants that come from areas with different climates. The higher antioxidant activity of the ethanol extract compared to the ethanol fraction can be attributed to the solubility range of secondary metabolites that can be dissolved in the extraction process, where the ethanol extract can dissolve a broader range of compounds with various polarities. The ethanol fraction only contains polar compounds because nonpolar and semipolar compounds are separated during fractionation.

It is known that climate, exceptionally light intensity, and temperature will affect the photosynthetic process in plants that produce oxygen and primary and secondary metabolites, especially total phenol and flavonoid levels.[17,18] Antioxidant power is influenced by the content of phenols and flavonoids, and we know that flavonoids have radical scavenging activity because they contain phenolic hydroxyl groups. These groups act via the hydrogen atom transfer (HAT) or single electron transfer followed by proton transfer (SETPT) mechanisms. The bond dissociation energy (BDE) of the O−H moiety is a key factor in comparing and explaining the antioxidant properties. The antioxidant activity of 13 natural phenolic compounds from the flavonoid family has been evaluated by using the chemical model (RO)B3LYP/6-311++G(2df,2p)/B3LYP/6-311G(d,p) in the gas phase and two typical solvents: water and ethanol, it was found that the C-H bond (C3-H of flavonoids backbone structure) plays a fundamental role in the antioxidant properties of flavonoids containing 4-carbonyl and/or 3-hydroxyl

groups. These groups release the lone electron on the C3 radical (C3-H bond) into the O-C3-C4-O system and form an intermolecular bond to stabilize the radical, resulting in reduced BDE (C3-H) and enhanced flavonoid antioxidant activity. This suggests these compounds may have antioxidant properties as high as typical phenolic compounds such as quercetin, trans-resveratrol, trolox, and ascorbic acid, especially in isomelacacidin.[19]

5. Conclusion

This study demonstrates that the ethanol fraction of *Arcangelisia flava* stem has the potential to be a strong natural antioxidant source. The total phenol content of 57.81 ± 3.28 mg GAE/g and flavonoid content of 114.64 ± 1.02 mg QE/g significantly contribute to its antioxidant activity, with an IC_{50} value of 72.876 ppm. These findings indicate that the ethanol fraction contains bioactive compounds effective in scavenging free radicals, suggesting its potential to prevent oxidative stress-related diseases. Further studies are needed to explore the antioxidant activity of this fraction in vivo models, identify specific compounds responsible for the activity, and evaluate its therapeutic potential for pharmaceutical applications. With deeper investigation, *Arcangelisia flava* holds promising prospects as a natural active ingredient in health products or preventing degenerative diseases.

6. Author Contribution

F. and H.M. experimented. F. and H.M. wrote the manuscript with support from E.H.O., S.S., and S. helped supervise the project. F. conceived the original idea.

7. Acknowledgments

We would like to express our sincere gratitude to the Faculty of Medicine, Universitas Sriwijaya, for providing grant funding and facilities needed for this research.

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