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PHYTOCHEMICAL PROFILING AND ANTIOXIDANT EVALUATION OF *ARCANGELISIA FLAVA* STEM EXTRACT

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ABSTRACT

Arcangelisia flava (locally known in Ifugao as "hopal") is a medicinal vine belonging to the Menispermaceae family and is traditionally used in the Philippines and other parts of Southeast Asia for the management of liver disorders and inflammatory conditions. This study aimed to conduct phytochemical profiling and evaluate the antioxidant activity of *A. flava* stem extract using the DPPH radical scavenging assay. Phytochemical profiling was carried out primarily using Thin Layer Chromatography (TLC), supported by qualitative test tube reactions. The analysis revealed the presence of alkaloids, flavonoids, phenols, tannins, coumarins, anthraquinones, anthrones, fatty acids, triterpenes, sterols, steroids, essential oils, and carbohydrates (excluding trioses and tetroses), while amino acids were not detected. Antioxidant activity was assessed by measuring the reduction in absorbance at 517 nm following reaction with DPPH, and the stem extract demonstrated strong radical scavenging activity with 81.00% inhibition. These findings contribute additional phytochemical and antioxidant data on *A. flava* stem and support its potential as a source of bioactive compounds. However, the study is limited to qualitative chromatographic profiling and a single antioxidant assay, without quantitative analysis, compound isolation, or further biological validation. Future research employing advanced analytical techniques and comparative evaluation of other plant parts is recommended.

Keywords: *Arcangelisia flava, phytochemical profiling, thin layer chromatography, DPPH assay, antioxidant activity*

INTRODUCTION

Arcangelisia flava is a perennial liana belonging to the Menispermaceae family and is widely recognized for its ethnomedicinal importance across Southeast Asia (Diliarosta et al., 2021). It is a large, woody, glabrous, and dioecious vine that can reach lengths of up to 20 meters, with stems measuring up to 5 cm in diameter and characterized by distinctive yellow wood and sap (Diliarosta et al., 2021; Ramadani, 2017). The species is indigenous to regions including Kalimantan, Sumatra, Sulawesi, Java, and Papua, where it climbs tall trees in primary and secondary forests (Pratama et al., 2018; Ramadani et al., 2018). It has also been reported to occur in forest ecosystems up to approximately 1500 meters above sea level, demonstrating broad ecological adaptability (Hapid et al., 2021). In the Philippines, particularly in Ifugao Province, *A. flava* is locally known as "hopal," and its stem is traditionally boiled to prepare a medicinal decoction used for preventive and therapeutic purposes.

The longstanding ethnomedicinal use of *A. flava* has prompted extensive phytochemical investigations. Studies have revealed a chemically diverse profile dominated by alkaloids, flavonoids, tannins, polyphenols, and terpenoid-related compounds (Diliarosta et al., 2021). Additional reports highlight the contribution of flavonoids and terpenes to the plant's biological activity (Afrizani et al., 2023). Sequential extraction analyses further confirmed the presence of alkaloids and saponins across extracts, while ethyl acetate fractions contained terpenoids, flavonoids, and cardiac glycosides (Delica et al., 2024), underscoring the influence of solvent polarity on phytochemical distribution.

The antioxidant potential of *A. flava* has also been documented. Phytochemical screening has identified flavonoids, polyphenols, anthraquinones, terpenoids, and saponins in stem extracts, with Thin Layer Chromatography (TLC)-bioautography indicating that flavonoids, polyphenols, and anthraquinones contribute significantly to antioxidant activity (Rahmah et al., 2024). Quantitative evaluation revealed IC_{50} values of 78.8 ± 1.2 µg/mL for aqueous extracts and 142.8 ± 2.9 µg/mL for 70% ethanol extracts, alongside substantial total phenolic content (Rahmah et al., 2024). Similarly, ethanol fractions demonstrated strong antioxidant activity with an IC_{50} value of 72.88 ppm and high phenolic and flavonoid concentrations (Fatmawati et al., 2025). Sequential extract analysis also reported antioxidant capacity of 0.714 ± 0.006 TEAC/g (Delica et al., 2024). These findings collectively suggest that the antioxidant properties of *A. flava* are largely attributable to its phenolic and flavonoid constituents.

Given its chemical diversity and documented bioactivity, further localized phytochemical profiling remains valuable, particularly in regions where traditional use is well established. Thin Layer Chromatography provides a practical and cost-effective approach for preliminary identification of major secondary metabolite classes, while the DPPH radical scavenging assay offers a rapid and reliable method for evaluating antioxidant activity. Accordingly, this study aimed to conduct phytochemical profiling of *Arcangelisia flava* stem extract using Thin Layer Chromatography and confirmatory qualitative reactions, and to evaluate its antioxidant activity using the DPPH assay. By generating phytochemical and antioxidant data from samples collected in Ifugao, this research

contributes to the regional scientific documentation of *A. flava* and supports its continued evaluation as a potential source of bioactive compounds.

Research Questions

This study aimed to conduct phytochemical profiling and evaluate the antioxidant activity of *Arcangelisia flava* stem extract. Specifically, it sought to answer the following questions:

1. What phytochemical constituents are present in the stem extract of *Arcangelisia flava*?
   1.1 What secondary metabolite classes are detected through Thin Layer Chromatography (TLC)?
   1.2 Do qualitative test tube reactions confirm the presence of selected phytochemical constituents identified through TLC profiling?
2. Does the stem extract of *Arcangelisia flava* exhibit antioxidant activity as determined by the DPPH radical scavenging assay?
3. What is the percentage radical scavenging activity (%RSA) of the stem extract, and how does it compare with that of the positive control (ascorbic acid) under the same tested condition?

METHODOLOGY

A. Plant Collection and Sample Preparation

Fresh stems of *Arcangelisia flava* were collected from Holowon, Lamut, Ifugao, Philippines. The plant material was authenticated by Agustin B. Lunag, Associate Professor V, Department of Plant Science – Crop Science, College of Agriculture, Nueva Vizcaya State University, on 30 July 2025. Identification was based on diagnostic morphological characteristics, including the distinct bright yellow stems. Harvesting was conducted carefully to minimize environmental disturbance, and the collected samples were immediately transported to the laboratory for processing. A map of the collection site is presented in Figure 1, while Figure 2 illustrates the morphological features of the plant used in this study.

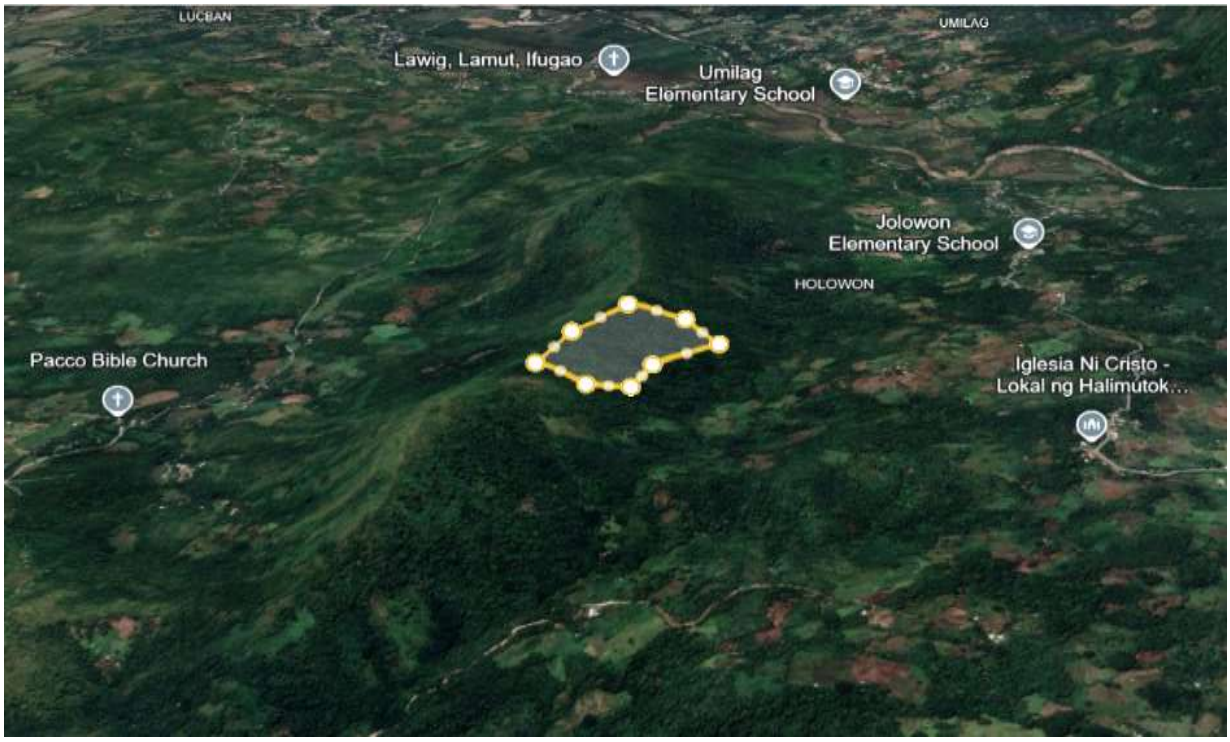

Figure 1. *Geographical location of Arcangelisia flava specimen*

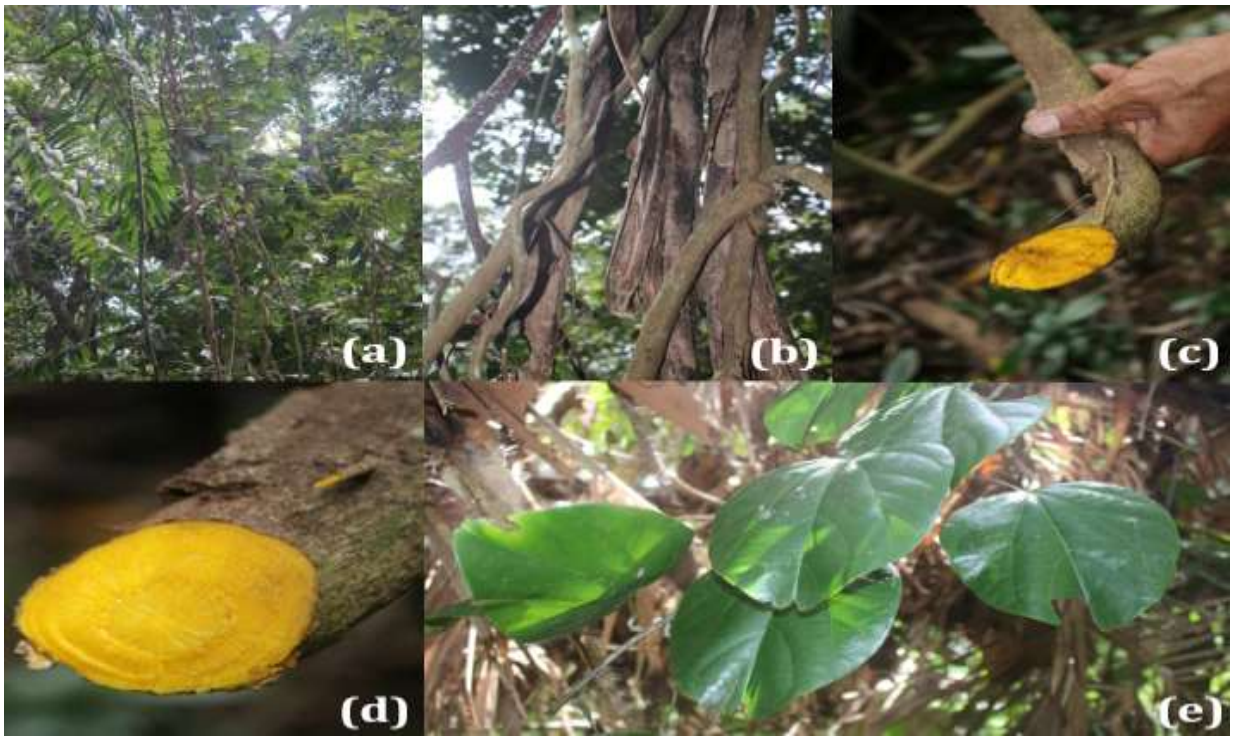

Figure 2. *(a) Full view of the vine intertwined among the vegetation. (b) Close-up of its woody stem structure. (c) Freshly cut stem of Arcangelisia flava showing its distinct bright yellow interior. (d) Close-up of the cross-section. (e) Close-up of A. flava leaves, showcasing their broad, heart-shaped structure.*

Upon arrival at the laboratory, the stems were thoroughly washed with running water to remove adhering soil and contaminants. The samples were air-dried at room temperature under shaded conditions to prevent degradation of thermolabile compounds. Once completely dried, the plant material was pulverized into a fine powder using a mechanical grinder and stored in airtight containers until further extraction and analysis.

B. Extraction

The air-dried and pulverized stem material weighing 100 g was subjected to maceration in 1 L of ethanol following a 1:10 weight-to-volume ratio for 48 hours at room temperature ranging from 25 to 28 °C. The mixture was placed in a tightly sealed glass container and intermittently agitated to facilitate efficient extraction of bioactive constituents. After maceration, the mixture was filtered using Whatman No. 1 filter paper to remove residual plant material. The filtrate was concentrated using a water bath maintained at 40 to 45 °C to allow controlled evaporation of ethanol and prevent degradation of thermolabile compounds until a semi-solid crude extract was obtained. The concentrated extract was transferred into an amber glass container and stored at 4 °C until further phytochemical profiling and antioxidant analysis.

C. Thin-Layer Chromatography Analysis

Thin-Layer Chromatography (TLC) was used as the primary method to identify the presence of bioactive compounds in *Arcangelisia flava* stem extract. The concentrated extract was applied to silica gel plates serving as the stationary phase, while ethanol solvent facilitated the movement of compounds through capillary action. The TLC plates were then placed in a developing chamber to allow the solvent to move through the stationary phase and separate the compounds based on their affinity for the mobile and stationary phases. Various chemical reagents were used in the TLC process to identify specific bioactive compounds including H_2SO_4, vanillin sulfuric acid, @napthol-sulfuric acid, methanolic potassium hydroxide, potassium ferricyanide + ferric chloride, Dragendorff's reagent, antimony (III) chloride, magnesium acetate, and ninhydrin. After applying the reagents, the plates were observed under UV light and visible light to detect color changes and fluorescence.

D. Test Tube Analysis

The Test Tube Phytochemical Analysis of *Arcangelisia flava* stem extract was performed as a confirmatory method to identify the presence of alkaloids and phenols/tannins through simple chemical reactions. A 1 mL aliquot of the concentrated plant extract was added to separate test tubes. For alkaloid detection, 2-3 drops of Dragendorff's reagent were introduced to the extract. The formation of a red-orange precipitate confirmed the presence of alkaloids. To test for phenols and tannins, 2-3 drops of ferric chloride solution were added to another test tube containing 1 mL of the extract. A dark blue or green color indicated the presence of phenols/tannins. These reactions were observed visually, and any color changes or precipitate formations were noted as qualitative indicators of the specific phytochemicals. The results from the test tube reactions served as the primary

evidence for the presence of alkaloids and phenols/tannins, providing further validation of the compounds identified through TLC analysis.

E. DPPH Assay for Antioxidant Activity

The antioxidant activity of *Arcangelisia flava* stem extract was evaluated using the DPPH radical scavenging assay. A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and filtered to obtain a clear working solution. The control absorbance of the DPPH solution at 517 nm was recorded as 0.754 prior to the addition of samples.

For the assay, 3 mL of the DPPH working solution was mixed with 100 µL of the stem extract in a test tube. The control consisted of 3 mL DPPH solution mixed with 100 µL of methanol, while the positive control contained 3 mL DPPH solution combined with 100 µL of ascorbic acid solution. The mixtures were vortexed and incubated in complete darkness at room temperature for 30 minutes. After incubation, absorbance was measured at 517 nm using a UV–Visible spectrophotometer. The stem extract was analyzed in five independent replicates under identical assay conditions, while the control and positive control (ascorbic acid) were measured within the same analytical run.

The percentage radical scavenging activity (%RSA) was calculated using the equation:

$$\%RSA = \frac{A_c - A_s}{A_c} \times 100$$

where A_c represents the absorbance of the control reaction and A_s represents the absorbance of the test sample or standard. The procedure was adapted from Baliyan et al. (2022).

RESULTS AND DISCUSSION

Phytochemical Profiling of Arcangelisia flava Stem Extract

Table 1 Thin Layer Chromatography (TLC) Profiling

Reagent Used	*Compound Tested*	*Observed Result*	*Presence/ Detection*
Vanillin Sulfuric Acid + Heat	Triterpenes and Sterols	Blue spot when exposed to UV light	+
	Essential Oils	Wide range of colors under UV light	++
	Phenols	Brown spot under visible light	+
	Fatty Acids	Yellow spots under visible light	+
@Napthol-sulfuric acid + Heat	All carbohydrates (except tetroses and trioses)	Violet spot under visible light	+

Reagent Used	*Compound Tested*	*Observed Result*	*Presence/ Detection*
Methanolic potassium hydroxide	Anthraquinones	Orange colored spots under visible light	+
	Coumarins	Blue colored spots under UV light	++
	Anthrones	Yellow spots under UV light	+
Potassium ferricyanide + ferric chloride	Tannins	Dark blue color under visible light	++
	Flavonoids	Dark blue spots under visible light	++
	Phenols	Blue spots under visible light	++
Dragendorff's reagent	Alkaloids	Orange spots under visible light	+
Antimony (III) chloride	Flavonoids	Yellow spots under visible light	+
	Steroids	Fluorescent spots under UV light	++
Magnesium Acetate + Heat	Anthraquinones	Orange to violet spots under visible light	+
Ninhydrin	Amino Acids	Not detected	–
H_2SO_4 + Heat	Essential Oils	Violet spots slowly appear under visible light	+

Legend: (+) detected; (++) strongly detected; (−) not detected.

The phytochemical profile obtained in this study revealed the presence of multiple secondary metabolite classes in *Arcangelisia flava* stem, including alkaloids, flavonoids, anthraquinones, anthrones, coumarins, tannins, phenolic compounds, fatty acids, terpenoid derivatives such as triterpenes and steroids, sterols, volatile terpenoid-related constituents, and carbohydrates detected under the applied screening conditions. This compositional diversity is consistent with previous reports identifying *A. flava* as a medicinal plant rich in bioactive compounds such as flavonoids, alkaloids, and terpenoids (Fatmawati et al., 2025; Delica et al., 2024). Prior phytochemical analyses have similarly confirmed the presence of alkaloids, flavonoids, anthraquinones, and related biologically active constituents in stem extracts (Rahmah et al., 2024; Pratama et al., 2023). In addition, reports of antioxidant activity in *A. flava* stem extracts, attributed primarily to flavonoids, polyphenols, and anthraquinones (Rahmah et al., 2024), provide a plausible biochemical basis for the radical scavenging activity observed in the present study.

Table 2 Confirmatory Qualitative Test Tube Reactions

Compound Tested	*Test Used*	*Reagent Applied*	*Observed Result*
Alkaloids	Dragendorff's Test	Dragendorff's Reagent	Reddish-orange precipitate formed.
Phenols/Tannins	Ferric Chloride Test	Ferric Chloride Solution	Dark blue/green color

The qualitative test tube analysis confirmed the presence of alkaloids and phenolic constituents, including tannins, in the stem extract of *Arcangelisia flava*. The formation of a reddish-orange precipitate indicated a positive reaction for alkaloids, while the

development of a dark blue–green coloration confirmed the presence of phenols and tannins. These results are consistent with the Thin Layer Chromatography findings, which likewise indicated the presence of corresponding phytochemical classes. Previous studies have reported the presence of alkaloids, flavonoids, saponins, and terpenoid compounds in *A. flava* stems (Nursyam et al., 2013; Rahmah et al., 2024). Notably, Nursyam et al. (2013) did not detect tannins in their phytochemical screening, in contrast to the present findings. This discrepancy may be attributed to differences in solvent systems, extraction procedures, plant origin, or analytical conditions, which are known to influence the detectability of specific secondary metabolites. The concurrence of TLC profiling and confirmatory qualitative reactions in the present study strengthens the reliability of the observed phytochemical profile and provides a preliminary chemical basis for the antioxidant activity demonstrated.

Antioxidant Activity of Arcangelisia flava Stem Extract

Table 3 Raw Absorbance Values in the DPPH Assay (n = 5)

Replicate	*Absorbance (As)*
1	0.148
2	0.143
3	0.141
4	0.142
5	0.143

Control Absorbance (Ac): 0.754

The absorbance values obtained from five replicates demonstrate minimal variation, indicating good repeatability of the DPPH assay under the tested conditions. The consistently lower absorbance of the extract compared with the control indicates effective reduction of DPPH radicals.

Table 4 Percentage Radical Scavenging Activity of *Arcangelisia flava* Stem Extract and Ascorbic Acid

Sample	*Mean Absorbance ± SD*	*% Radical Scavenging Activity*
A. flava Stem Extract	0.1434 ± 0.0027	81.00%
Ascorbic Acid	0.077	89.79%

The stem extract exhibited 81.00% radical scavenging activity, with a mean absorbance of 0.1434 ± 0.0027, indicating substantial radical scavenging capacity at the tested concentration. Although slightly lower than the positive control, ascorbic acid at 89.79%, the extract demonstrated considerable activity under the same assay conditions. These findings are consistent with studies reporting antioxidant activity in *A. flava* stem extracts using the DPPH assay (Rahmah et al., 2024; Suratno et al., 2019). Variations in reported antioxidant capacities may be attributed to differences in extraction solvents, plant material, and assay conditions. For example, Delica et al. (2024) reported lower antioxidant activity in hexane extracts, underscoring the influence of solvent polarity on the extraction of phenolic and flavonoid constituents. The present findings suggest that ethanol effectively extracts polar antioxidant-active compounds from *A. flava* stem.

Conclusions

The findings of this study demonstrate that the stem extract of *Arcangelisia flava* contains multiple phytochemical constituents, including alkaloids; phenolic compounds such as flavonoids, tannins, and coumarins; anthraquinone derivatives (anthraquinones and anthrones); terpenoid-related compounds including triterpenes, sterols, steroids, and essential oils; as well as carbohydrates (except tetroses and trioses). Amino acids were not detected. Confirmatory qualitative test tube reactions verified the presence of alkaloids and phenols or tannins, supporting the chromatographic results. The ethanolic stem extract exhibited substantial antioxidant activity as determined by the DPPH radical scavenging assay, achieving 81.00% inhibition at the tested concentration. Although slightly lower than the positive control, ascorbic acid at 89.79%, the extract demonstrated considerable radical scavenging capacity under the same assay conditions. Overall, the results confirm the phytochemical richness and antioxidant potential of *A. flava* stem extract, providing preliminary scientific support for its reported medicinal relevance and establishing a basis for further quantitative and compound-specific investigations.

Recommendations

Based on the findings of this study, further research is recommended to expand the understanding of the phytochemical composition and antioxidant activity of *Arcangelisia flava* stem. Future studies may conduct comparative investigations using different extraction conditions and solvents may also be undertaken to assess their influence on antioxidant yield and activity. In addition, examining other plant parts such as leaves and roots could provide insight into possible variations in phytochemical profile and bioactivity. Studies involving larger sample sizes and samples collected from different geographical locations are likewise recommended to evaluate environmental influences on secondary metabolite expression. Finally, further biological evaluation may be conducted to better understand the functional relevance of the observed antioxidant activity.

Compliance with Ethical Standards

This study was conducted in full compliance with established ethical research standards to ensure scientific integrity and responsible laboratory practice. The research involved in phytochemical profiling and antioxidant evaluation of *Arcangelisia flava* stem extract and did not involve human participants or animal subjects; therefore, informed consent and related ethical clearances were not required. All experimental procedures were carried out in accordance with standard laboratory safety and biosafety protocols to ensure proper handling of chemicals and prevent contamination. No conflicts of interest were present in the conduct of this study. Academic integrity was strictly observed through proper citation of all referenced works, and plagiarism was avoided at all stages of manuscript preparation. The interpretation of results was performed objectively and without bias, and all findings were reported accurately and transparently. The data generated were used solely for academic and research purposes to contribute to the scientific understanding of the phytochemical composition and antioxidant potential of *A.*

flava. Artificial intelligence tools were utilized only for language refinement and structural guidance, and full responsibility for the scientific content remains with the researcher.

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