



Secretory Structure and Histochemistry of Leaf of Medicinal Plant *Arcangelisia Flava*

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Abstract: This study aimed to identify the tissue-level localization of major secondary metabolites and to describe the secretory structures of *Arcangelisia flava* leaves. Fresh transverse and paradermal hand sections of mature leaves were subjected to histochemical tests for flavonoids, phenolics, alkaloids, terpenoids, and lipophilic compounds. Flavonoid staining showed that flavones were the predominant class, accumulated strongly in the epidermis, while flavanones occurred only in guard cells; flavonols were not detected. Phenolic compounds were abundant in both the epidermis and mesophyll. Alkaloids also localized prominently in these tissues and around vascular bundles. Terpenoid detection using cupric acetate revealed intense deposits in epidermal and mesophyll idioblasts, whereas NADI staining indicated that essential oils were restricted to the epidermis. Lipophilic compounds were similarly concentrated in epidermal idioblasts. A consistent pattern was found in the epidermal cell rows flanking the leaf veins, which simultaneously accumulated flavonoids, phenolics, and terpenoids, suggesting a coordinated defensive zone along vascular pathways. The study also identified glandular hydathode trichomes containing flavones, phenolics, and lipophilic compounds, contrasting with previous descriptions of non-glandular trichomes in this species. This research provides the first detailed histochemical map of *A. flava* leaves and highlights key secretory features relevant to its medicinal value

Keywords: Flavon; Glandular Trichome; Laticifer; Secondary metabolites; Paradermal

Introduction

Arcangelisia flava (L.) Merr. is a medicinal plant belonging to the family Menispermaceae, widely distributed from Hainan (China), Indochina, Thailand, Peninsular Malaysia, various islands of Indonesia, the Philippines, to Papua New Guinea (Mandia et al., 1999). It is a climbing vine with ovate leaves; the young leaves are light green, while the newly emerging shoots are reddish. The inner part of the stem is yellow, and the flowers are arranged in panicles (Forman, 1984). The yellow root of *A. flava* has long been used in traditional medicine to treat liver disease (hepatitis), fever, and diabetes by the Dayak people in West Kalimantan (Tavita et al., 2022). It is also utilized as an antimalarial and anticancer remedy in West Sumatra (Diliarosta et al., 2021). The Enggano Island people in Bengkulu

Province use it to treat eye disorders, enhance stamina, and improve male fertility (Kamilah et al., 2022).

Scientific studies have been conducted to validate the pharmacological potential of *A. flava*. Its antidiabetic activity has been demonstrated by Wahyudi et al. (2016), who reported that the ethyl acetate leaf extract strongly inhibited α -amylase and α -glucosidase activities. The methanolic leaf extract also showed radical scavenging activity against DPPH and hydroxyl radicals. The anti-inflammatory potential of *A. flava* was confirmed by Tavita et al. (2022), who found that ethanolic stem extract stabilized human red blood cells under hypotonic and heat-induced stress conditions. The plant also exhibited antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhii* (Heryani & Nugroho, 2015). The anticancer potential of *A. flava* has been reported by Mutiah et al. (2020), who found that stem extracts from Kalimantan

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exhibited weak to moderate cytotoxicity against WiDr colorectal cancer cells.

These pharmacological properties are closely related to the presence of secondary metabolites within the plant. Phytochemical screening of *A. flava* stem ethanolic extract revealed the presence of flavonoids, polyphenols, anthraquinones, alkaloids, and terpenoids (Rahmah et al. 2024). In addition, berberine, palmatine, and several other important secondary metabolites have been identified from the plant (Pratama et al., 2023).

Despite the extensive pharmacological and phytochemical studies, information regarding the tissue-level localization of secondary metabolites in *A. flava* remains unavailable. Understanding the spatial distribution of these compounds is crucial for elucidating the relationship between secretory structures and their physiological functions, and for supporting further exploration and efficient utilization of its bioactive compounds.

In traditional applications, all vegetative organs—including roots, stems, and leaves—are commonly harvested. However, the continuous use of roots and stems threatens the sustainability of natural populations. Therefore, prioritizing the use of leaves as a source of medicinal compounds is highly desirable.

This study aims to identify the secretory structures and determine the tissue-level localization of secondary metabolites in the leaves of *Arcangelisia flava* through histochemical analysis, providing a scientific basis for utilizing leaves as an alternative source of medicinal compounds.

Method

Plant Material

Two individuals of *Arcangelisia flava* were obtained from the collection garden of the Biopharmaca Research Center, IPB University. Branches and leaves were excised in the field and transported to the laboratory in fresh condition inside water-filled tubes. Mature leaves (approximately the 5th leaf from the apex) were used for analysis.

Histochemical Analysis

Fresh stem and leaf samples were hand-sectioned using a razor blade to obtain transverse and paradermal sections. Histochemical tests included flavonoids, phenolics, alkaloids, terpenoids, and lipophilic compounds.

Flavonoids were detected following Guerin et al. (1971) by treating sections with aqueous AlCl_3 for 10–15 min and observing them under an epifluorescence microscope (Olympus BX51, UV filter). Green fluorescence indicated flavones, yellow indicated flavonols, and blue indicated flavanones.

Phenolics were tested by immersing sections in FeCl_3 solution with a few grains of Na_2CO_3 for 1 h. Brown to greenish-black coloration indicated a positive reaction.

Alkaloids were tested using Wagner and Dragendorff reagents (Demarco, 2017). Leaf sections were immersed for 15–20 min, and stem sections for 1 h. Wagner reagent produced reddish-brown precipitates, whereas Dragendorff produced yellowish-brown coloration. Negative controls were prepared using 5% tartaric acid in 95% ethanol for 48 h before reagent application.

Terpenoids were tested using 5% cupric acetate (2 h) followed by NADI reagent (15 min) (Demarco, 2017). Reddish-brown to brownish-yellow indicated terpenoids, blue indicated essential oils (mono- and sesquiterpenes), and purple indicated resins (di-, tri-, and tetraterpenes). Lipophilic compounds were detected following Boix et al. (2011) with slight modification by staining sections with 0.03% Sudan IV in 70% ethanol at 40°C for 30 min.

All tests were conducted on ~10 samples from two plants. Observations were made using a light microscope (Olympus CX23), and images were taken with a Dino-Lite AM7025X microscope camera.

Observation of Secretory Structures

Paradermal leaf sections were stained with safranin or toluidine blue, or observed directly in water with 30% glycerol to visualize crystals.

Result and Discussion

Flavonoid Accumulation in A. flava Leaves

Histochemical testing with aqueous AlCl_3 revealed flavone accumulation, indicated by greenish fluorescence (Guerin et al., 1971). Flavones accumulated primarily in the leaf epidermis. In the epidermis along the vein, flavones were present on both the cell walls and lumina of epidermal cells and several hypodermal cells (Figure 1B). While in the intercostal regions, accumulation was limited to the epidermal cell walls and the walls of subepidermal sclereids (Figure 1C). In the adaxial epidermis, flavones were restricted to cell walls, whereas in the abaxial epidermis fluorescence extended into the nuclei (Figure 1G–H). Flavone accumulation was also observed in glandular trichomes (Figure 1I). In addition to flavones, flavanones were detected in guard cells through blue fluorescence following aqueous FeCl_3 application (Figure 1H–I).

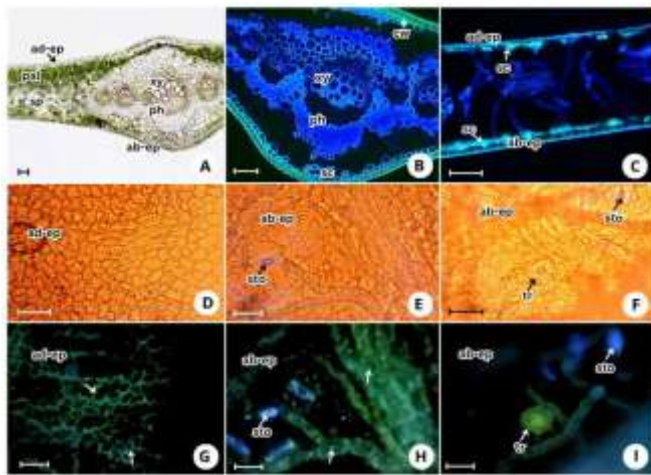


Figure 1. Sites of flavonoid accumulation in *A. flava* leaf: (A, D–F) controls; (B) epidermal cells; (C) sclereids cell walls; (G, H) adaxial and abaxial epidermal cell walls and nuclei; (H, I) stomata guard cells (O) glandular trichome; (A–C) transverse sections; (D–I) paradermal sections. Flavonoid accumulation indicated by arrows. Scale bar: 100 μm . (ad-ep) adaxial epidermis, (pal) palisade parenchyma, (sp) spongy mesophyll, (xy) xylem, (ph) phloem, (ab-ep) abaxial epidermis, (cw) cell walls, (sc) sclereid, (sto) stomata, (tr) trichome

Flavonoids are common plant secondary metabolites that function as antioxidants and protect against UV radiation, as well as bacterial and fungal pathogens (Xie et al., 2015). The presence of flavones in various leaf tissues of *A. flava* is consistent with reports of flavones or flavonols in other Menispermaceae species, such as *Odontocarya vitis* (Ribeiro et al., 2022). Furthermore, LC–MS analysis of *A. flava* stems revealed the presence of 3-hydroxy-3',4',5'-trimethoxyflavone (Pratama et al., 2023), suggesting that the leaves may similarly accumulate this metabolite.

The presence of flavonoids—particularly flavones—in the epidermis likely functions as UV filters and suppressors of reactive oxygen species (ROS). Increases in epidermal flavonoids in response to UV exposure represent a common photoprotective mechanism, including shifts in the quercetin–kaempferol ratio (Falcone Ferreyra et al., 2021). Such dynamic flavonoid accumulation supports the interpretation that epidermal flavones in *A. flava* act as protection against fluctuating UV levels in its habitat. The presence of flavonoids in mesophyll nuclei is also relevant to their regulatory antioxidant roles. Nuclear flavonoids have been reported by Agati et al. (2012), and the flavone tangeretin is known to activate cellular defense pathways that mitigate oxidative stress (Lv et al., 2023). These findings indicate that flavonoids in *A. flava* leaves act not only as direct antioxidants but also as triggers of protective cellular responses.

Flavone accumulation in glandular trichomes and guard cells supports their defensive functions on the leaf surface. Similar patterns occur in *Dionysia tapetodes*, which extrudes flavones as protective layers (Bourdon et al., 2021). The presence of flavonoids in guard cells has also been reported in *Arabidopsis thaliana*, where they function as localized antioxidants that modulate H_2O_2 levels (An et al., 2016). In *A. flava*, the detected flavanones may play comparable roles.

Phenolic Compound Accumulation in *A. flava* Leaves

FeCl_3 staining produced brownish–green to blackish coloration, indicating phenolic presence in *A. flava* leaves. Phenolics were primarily located in idioblasts of the adaxial and abaxial epidermis, with continuous distribution around the vascular bundles (Figure 2B and 2E) and more limited patterns in the intercostal regions (Figure 2C and 2F). Dense deposits occurred in the intercellular spaces of the mesophyll (Figure 2H). Phenolics were also detected in the vein laticifers (Figure 2I), confirming their widespread distribution in leaf tissues.

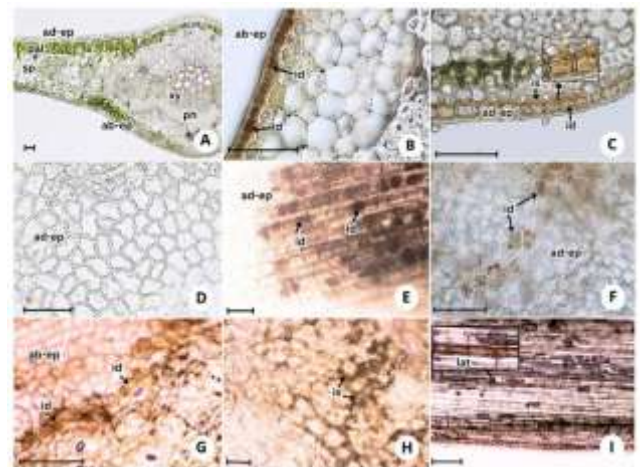


Figure 2. Sites of phenolic compound accumulation in *A. flava* leaf (A,D) control; (B, E) idioblasts in the epidermis around the midrib; (F,G) idioblasts in the adaxial and abaxial epidermis; (H) intercellular spaces in the mesophyll; (I) laticifer in the midrib. (A–C) transverse sections; (D–I) paradermal sections. Phenolic accumulation indicated by arrows. Scale bar: 100 μm . (ad-ep) adaxial epidermis, (pal) palisade parenchyma, (sp) spongy mesophyll, (xy) xylem, (ph) phloem, (ab-ep) abaxial epidermis, (id) idioblast, (is) intercellular spaces, (lat) laticifer.

Histochemical staining revealed strong phenolic accumulation in idioblasts and laticifers of *A. flava* leaves, suggesting a key defensive role against pathogens. This is consistent with the identification of p-hydroxybenzaldehyde and vanillin—both phenolic compounds—in *A. flava* stems (Pratama et al., 2023). p-Hydroxybenzaldehyde inhibits *Fusarium solani* (Xi et al.,

2022), while vanillin suppresses growth of *Botrytis cinerea* and *Alternaria alternata* (Yang et al., 2021). The localization pattern observed here resembles that reported in *Fibraurea tinctoria* (Widuri et al., 2024), suggesting similar defensive strategies within Menispermaceae.

Phenolic accumulation in the vascular tissues indicates a defensive role against pathogen spread. Santoso et al. (2017) reported intensive phenolic accumulation in leaf vascular tissues of cacao infected with *Ceratobasidium theobromae*. Beyond defense, the phenolic content of *A. flava* may contribute to its pharmacological potential. Phenolic compounds, including tannins and phenolic acids, exhibit antiviral activity (influenza, HSV, HPV) and broad-spectrum antibacterial activity (Kaczmarek, 2020), as well as antioxidant properties.

Alkaloid Accumulation with Wagner’s Reagent

Alkaloids were tested using Wagner’s and Dragendorff’s reagents. In leaves, alkaloids accumulated mainly in idioblasts scattered throughout the epidermis and mesophyll. Some epidermal idioblasts stored alkaloids in the cytoplasm, whereas others stored them in vacuoles (Figure 3B and 3C). Alkaloid accumulation was also observed in glandular trichomes on both epidermal surfaces, particularly in the trichome heads (Figure 3B). Idioblasts containing alkaloids also occurred in the palisade (Figure 3E) and spongy mesophyll (Figure 3F). These cells exhibited high concentrations of alkaloids, indicated by the dark brown coloration resulting from Wagner’s reagent.

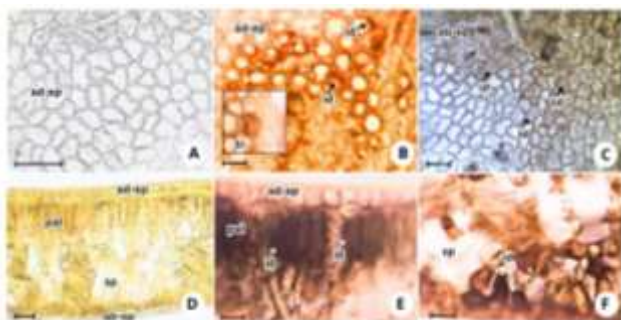


Figure 3. Sites of alkaloid accumulation using Wagner’s reagent in *A. flava* leaf: (A, D) control; (B) glandular trichomes; (B, C) idioblasts in the adaxial and abaxial epidermis; (E) idioblasts in the palisade parenchyma; (F) idioblasts in the spongy mesophyll (A–C, F) paradermal sections, (D–E) transverse sections; Alkaloid accumulation indicated by arrows. Scale bar: 100 µm. (ad-ep) adaxial epidermis, (id) idioblast, (tr) trichome, (ab-ep) abaxial epidermis, (pal) palisade parenchyma, (sp) spongy mesophyll.

Alkaloid Accumulation with Dragendorff’s Reagent

Testing with Dragendorff’s reagent produced a similar pattern, with alkaloids detected in the epidermis

and mesophyll. In the epidermis, alkaloids were stored in idioblasts (Figure 4A and 4B) and glandular trichomes (Figure 4C). In the mesophyll, they were found among palisade cells (Figure 4E) and idioblasts in the spongy tissue (Figure 4F). The two alkaloid storage types—cytoplasmic and vacuolar—observed with Wagner’s reagent were confirmed here as well (Figure 4A and 4B).

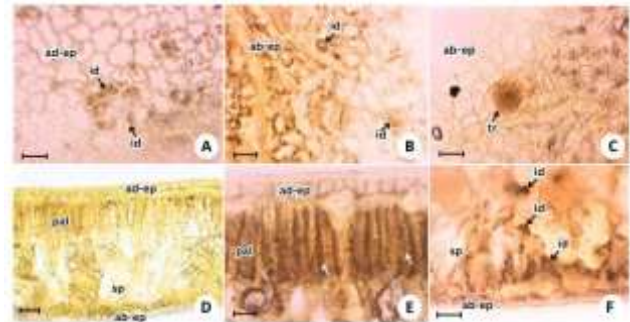


Figure 4. Sites of alkaloid compound accumulation using Dragendorff’s reagent in *A. flava* leaf: (A–B) idioblasts in the adaxial and abaxial epidermis; (C) glandular trichome; (D) control; (E) intercellular spaces of the palisade parenchyma; (O) idioblasts in the spongy mesophyll. (A–C, F) paradermal sections, (D–E) transverse sections; Alkaloid accumulation indicated by arrows. Scale bar: 100 µm. (ad-ep) adaxial epidermis, (id) idioblast, (ab-ep) abaxial epidermis, (tr) trichome, (pal) palisade parenchyma, (sp) spongy mesophyll.

Alkaloid distribution in *A. flava*—detected in idioblasts, laticifers, and glandular trichomes—reflects a characteristic defense-related storage pattern typical of Menispermaceae. Histochemical testing in *Fibraurea tinctoria* showed intense alkaloid distribution in leaves, primarily in the mesophyll and parenchyma of the midrib (Widuri et al., 2024), whereas *A. flava* exhibits a broader range, including the epidermis. The presence of alkaloids in the epidermis and trichomes indicates that these structures function as chemical defense barriers directly exposed to pathogens and herbivores.

Alkaloid accumulation in mesophyll idioblasts also occurs in *Catharanthus roseus*, where idioblasts serve as final sites of vinblastine and vincristine biosynthesis, with precursors synthesized in phloem-adjacent parenchyma and later processed in the epidermis (Guedes et al., 2023). This parallel suggests that idioblasts in *A. flava* act as specialized compartments for storage and possibly final biosynthetic steps of alkaloids. Phytochemical studies show that *A. flava* produces numerous protoberberine alkaloids, including berberine, palmatine, jatrorrhizine, columbamine (Cheng et al., 2021), and dimethylene-berberine (Pratama et al., 2023). These compounds possess antimicrobial, anti-inflammatory, and antiprotozoal activities (Long et al., 2019; Meenu & Radhakrishnan, 2020). Thus, the alkaloid accumulation pattern not only

reflects physiological defense roles but also supports the plant's long-recognized pharmacological use as an anti-infective and anti-inflammatory agent.

Terpenoid Accumulation

Cupric acetate (5%) testing indicated widespread terpenoid distribution in *A. flava* leaves. The epidermal distribution resembled that of flavonoids and phenolics. Near the vascular bundles, terpenoid-accumulating epidermal cells formed continuous layers (Figure 5B), whereas idioblasts appeared sporadically in intercostal areas. Adaxial idioblasts showed dense accumulation throughout the lumen (Figure 5C), while abaxial idioblasts contained terpenoids in the cytoplasm (Figure 5D). Terpenoids also occurred in trichomes (Figure 5D), idioblasts within the palisade (Figure 5E), and vein laticifers (Figure 5F). NADI testing revealed monoterpenes and sesquiterpenes as dark blue coloration. Positive reactions appeared in idioblasts of both epidermal surfaces, while trichomes tested negative (Figure 5G-I). Terpenoid droplets of varying sizes were present in the adaxial and abaxial epidermis (Figure 5G-I).

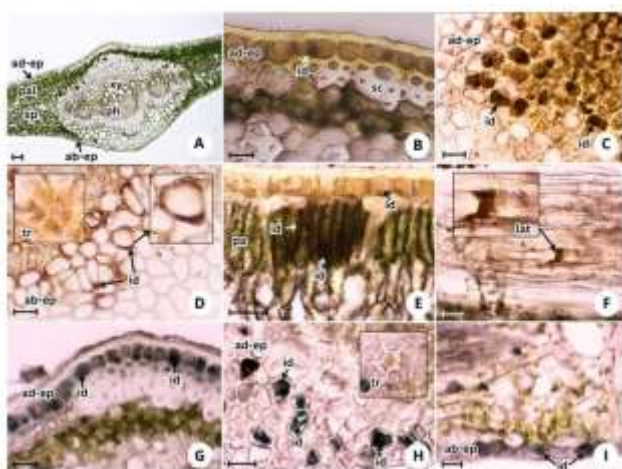


Figure 5. Sites of terpenoid accumulation in *A. flava* leaf: (A) control; (B) epidermal idioblasts; (C, D) idioblasts in the adaxial and abaxial epidermis and trichome; (E) idioblasts among palisade cells; (F) laticifers in the leaf midrib; (B-F) stained with cupric acetate (O-R) idioblasts in the adaxial and abaxial epidermis stained with NADI. (A-B, E,G) transverse sections; (C-D,F,H-I). Terpenoid accumulation indicated by arrows. Scale bar: 100 µm. (ad-ep) adaxial epidermis, (pal) palisade parenchyma, (sp) spongy mesophyll, (xy) xylem, (ph) phloem, (ab-ep) abaxial epidermis, (id) idioblast, (sc) sclereid, (tr) trichome, (pa) palisade parenchyma, (lat) laticifer.

Histochemical testing indicates extensive terpenoid accumulation in idioblasts of the epidermis and mesophyll, as well as in laticifers. NADI testing confirmed monoterpenes and sesquiterpenes in the

epidermis, similar to *Fibraurea tinctoria*, although the latter shows broader distribution (Widuri et al., 2024).

The presence of terpenoids in leaves may parallel metabolites in stems, where LC-MS identified furanoditerpenoids such as fibraurin and arcangelisin (Pratama et al., 2023). Terpenoids function as chemical defenses; essential oil components such as eugenol and terpineol exhibit strong antibacterial activity (Fink, 2022). Terpenoid detection in *A. flava* epidermis via NADI may relate to such defensive roles.

Pharmacologically, fibraurin is antifungal against *Candida* (Hendra et al., 2024), whereas furanoditerpenoids from *Tinospora bakis* exhibit anti-glycation activity (Kabbashi et al., 2024). Mapping terpenoid accumulation in *A. flava* provides a basis for identifying tissues most promising as sources of bioactive metabolites.

Lipophilic Compound Accumulation

Sudan IV staining revealed red-orange accumulation of lipophilic compounds. Lipophilic metabolites were abundant in the leaves. Accumulation was most pronounced in the thick adaxial cuticle and thinner abaxial cuticle (Figure 6B and 6C). In the epidermis, lipophilic compounds accumulated in idioblasts on both surfaces. Droplets were present in abaxial idioblasts (Figure 6C), while adaxial idioblasts showed intense, uniform accumulation throughout the lumen (Figure 6D). Glandular trichomes also accumulated lipophilic compounds, mainly in the head cells near the pore (Figure 6E). In the abaxial epidermis, lipophilic compounds were detected in guard cell walls (Figure 6E). Laticifers in the veins also stored lipophilic metabolites (Figure 6F).

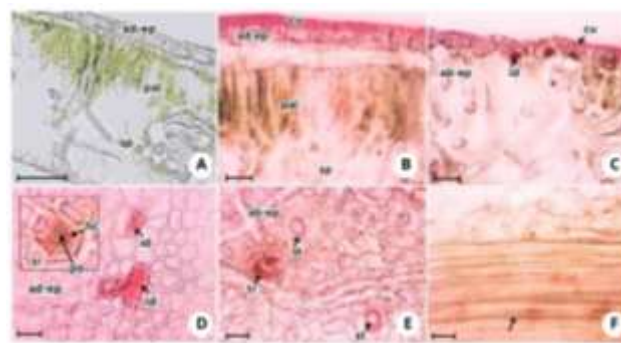


Figure 6. Sites of lipophilic compound accumulation in *A. flava* leaf: (A) control; (B, C) epidermal idioblasts and cuticles of the adaxial and abaxial surfaces; (D) adaxial idioblasts and glandular trichomes; (E) guard cells of stomata; (F) laticifers.

(A-C) transverse sections (D-F) paradermal sections; Lipophilic accumulation indicated by arrows. Scale bar: 100 µm. (ad-ep) adaxial epidermis, (pal) palisade parenchyma, (sp) spongy mesophyll, (cu) cuticle, (ab-ep) abaxial epidermis, (id) idioblast, (tr) trichome, (po) pore, (hc) head cell, (st) stomata.

Various lipophilic compounds occur in plants. Lipophilic substances detected with Sudan IV include fats, waxes, sterols, fat-soluble vitamins, triglycerides, and others (Kromer et al., 2016).

Lipophilic compounds were abundant in the leaves, especially in the adaxial cuticle and epidermal idioblasts. The cuticle plays an important role in preventing non-stomatal water loss. Wax composition can increase in response to drought (Zhang et al., 2023). Compact cutin-wax structures also impede pathogen penetration. Lipophilic compounds in guard cells have also been observed in *Arabidopsis thaliana*, where they may function in energy supply, turgor regulation, and membrane behavior during stomatal movements (McLachlan et al., 2016).

Secretory Structures in *A. flava* Leaves

Plant secondary metabolites are produced and stored within secretory structures. In *A. flava* leaves, these structures include laticifers, idioblasts, and trichomes. Laticifers were found in the vein vascular tissue (Figure 7A) and are of the articulated non-anastomosing type.

Idioblasts were distributed throughout the epidermis and mesophyll. These cells vary in shape sometimes similar to or distinct from neighboring cells and store various metabolites, including flavonoids, phenolics, alkaloids, terpenoids, and lipophilic compounds. Idioblasts containing calcium oxalate crystals (prismatic and raphide forms) were also present. Prismatic crystals were widespread on both leaf surfaces, whereas raphides were fewer (Figure 7B).

Glandular trichomes had apical pores surrounded by a cluster of head-like cells and were present on both adaxial and abaxial surfaces (Figure 7C).



Figure 7. Secretory structures of *A. flava*. (A) Laticifer associated with vascular tissues of the leaf vein; (B) Prismatic and raphide calcium oxalate crystals in epidermal idioblasts; (C) Glandular trichome with apical pore and multicellular head. Scale bar = 100 μ m. (lat) laticifer, (ve) vein, (ad-ep) adaxial epidermis, (pc) prismatic crystal, (rc) raphide crystal, (hc) head cell, (po) pore.

Secretory structures in *A. flava* play crucial roles in the accumulation and distribution of secondary metabolites. Laticifers in the midrib area, of the articulated non-anastomosing type, resemble those reported in *Fibraurea tinctoria* (Widuri et al., 2024). They function as conduits for defensive metabolites. In

addition to idioblasts storing flavonoids, phenolics, alkaloids, and lipophilic compounds, idioblasts in the epidermis also contained prismatic and raphide calcium oxalate crystals. These crystals function in calcium regulation, defense against herbivory, and detoxification of heavy metals (Ajayi et al., 2015; Franceschi & Nakata, 2005). Hilmi et al. (2018) reported that *Reutealis trisperma* grown in 100% gold mine tailings exhibited significantly increased druse crystals in the root cortex, likely as a detoxification mechanism in response to high metal concentrations. Trichomes occurred on both leaf surfaces. Contrary to earlier reports describing non-glandular hydathodal trichomes in *Archangelisia* (Wilkinson, 1978), the trichomes observed here were glandular, with pore-surrounding cells accumulating flavonoids, terpenoids, and lipophilic compounds, but not essential oils or phenolics. This finding suggests that hydathodal trichomes may function similarly to other glandular types such as peltate, capitate, or uniseriate trichomes in *Hyptis capitata* (Rupa et al., 2017), expanding current understanding of secretory structures in Menispermaceae.

Conclusion

Histochemical analyses showed that *Arcangelisia flava* leaves contain diverse secondary metabolites—flavonoids, phenolics, alkaloids, terpenoids, and lipophilic compounds—distributed across the epidermis, mesophyll, idioblasts, trichomes, and laticifers. Flavones dominated the epidermis and trichomes, indicating roles in UV protection and ROS control. Phenolic compounds were concentrated in idioblasts and vascular tissues, supporting their defensive and antimicrobial functions. Alkaloids occurred mainly in idioblasts and trichomes, consistent with the defensive profiles of Menispermaceae. Terpenoids accumulated in idioblasts and laticifers, while abundant lipophilic metabolites were detected in the cuticle and guard cells. These distribution patterns show that *A. flava* leaves possess specialized secretory structures that store key bioactive metabolites, highlighting the leaf's importance in chemical defense and supporting its traditional medicinal use.

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Author Contributions

Conceptualization, YCS; Methodology, YCS; Investigation, YCS and DR; Writing YCS and DR, Writing –Review & Editing, YCS; Supervision, YCS.

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Conflicts of Interest

The authors declare no conflict of interest.

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