



Isolation and Structural Elucidation of Flavonoid Compounds from the Bark of *Arcangelisia flava* (Yellow Wood) from Kalimantan and Their Cytotoxicity Test Against MCF-7 Breast Cancer Cells

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ABSTRACT

Arcangelisia flava (L.) Merr., locally known as “akar kuning,” is a traditional medicinal plant from Kalimantan with limited evidence regarding its anticancer compounds. This study aimed to isolate bioactive compounds from *A. flava* bark and evaluate their cytotoxicity against MCF-7 breast cancer cells. Dried bark was macerated in 95% ethanol, fractionated with *n*-hexane, ethyl acetate, and *n*-butanol, and the ethyl acetate fraction was further purified by chromatography. Isolated compounds were identified using spectroscopic methods, while cytotoxicity was evaluated using the MTT assay with doxorubicin as a positive control. Three compounds were identified: berberine (85 mg), palmatine (62 mg), and quercetin (41 mg). All showed concentration-dependent cytotoxicity, with IC₅₀ values of 8.74 ± 0.38, 14.23 ± 0.61, and 18.56 ± 0.72 µg/mL, respectively. Berberine showed the highest potency and selectivity (SI = 11.27), followed by palmatine (6.14) and quercetin (6.07), while the ethyl acetate fraction had an IC₅₀ of 22.17 ± 0.94 µg/mL. These findings indicate that *A. flava* bark contains promising cytotoxic compounds, particularly berberine, supporting further investigation as a potential anticancer lead

Keywords: *Arcangelisia Flava*, Berberine, Palmatine, Quercetin, MTT Assay, MCF-7, Breast Cancer, Cytotoxicity, Kalimantan, Protoberberine Alkaloid, Flavonoid Isolation



INTRODUCTION

Globally, breast cancer ranks among the most frequently diagnosed malignancies and stands as the primary cause of cancer-related deaths in women. According to the GLOBOCAN 2022 report, roughly 2.3 million new breast cancer cases were diagnosed worldwide that year, representing 11.7% of all cancer diagnoses (Sung et al., 2021). Within Indonesia, this disease consistently holds the highest incidence rate among females, with numbers continuing to rise alongside increasing urbanization and shifting lifestyles (Kemenkes RI, 2022). Although considerable progress has been made in chemotherapy, targeted therapies, and immunotherapies, problems such as drug resistance, systemic toxicity, and expensive treatment costs remain major obstacles, highlighting the urgent need for novel therapeutic agents (Wahyuni et al., 2022).

Historically, natural products have served as a valuable source for the discovery of anticancer drugs. Plant-derived compounds including paclitaxel, vincristine, camptothecin, and etoposide have fundamentally revolutionized oncological treatment approaches (Newman & Cragg, 2020). The Indonesian archipelago, renowned as one of the most biodiverse regions on Earth, holds enormous pharmacognostic potential that remains largely unexplored. In Kalimantan (Indonesian Borneo), numerous medicinal plants are utilized in traditional Dayak healing practices, yet most of them still require thorough phytochemical and pharmacological validation (Elfahmi et al., 2014).

Arcangelisia flava (L.) Merr., belonging to the Menispermaceae family and commonly referred to as "akar kuning" (yellow root), is a large woody liana native to the tropical rainforests of Southeast Asia. It is particularly abundant in Kalimantan, Sumatra, and the Malay Peninsula (Mukherjee, 2020). The bark and roots of this plant exhibit a characteristic bright yellow coloration, which is attributed to their high content of protoberberine alkaloids, mainly berberine and palmatine. Ethnomedicinally, *A. flava* has been used to treat malaria, hepatitis, typhoid fever, skin infections, and diabetes (Syamsul et al., 2015).

Previous phytochemical studies on *A. flava* have identified berberine, palmatine, columbamine, and jatrorrhizine as the major alkaloid constituents, along with various flavonoids such as quercetin and kaempferol (Faramayuda et al., 2020; Lestari et al., 2021). Berberine, the most extensively studied protoberberine alkaloid, demonstrates multiple anticancer mechanisms, including topoisomerase II inhibition, suppression of NF- κ B signaling, induction of apoptosis, and interference with cell cycle progression (Rauf et al., 2021; Li et al., 2020). Palmatine, a structural analogue of berberine, has similarly displayed cytotoxic effects against diverse cancer cell lines (Tian et al., 2022). Quercetin, a widespread polyphenolic flavonol, has been widely reported to trigger apoptosis in breast cancer cells by modulating the Bax/Bcl-2 ratio and activating caspase-3 (Hashemzaei et al., 2017).

Despite this growing body of evidence, systematic investigations that specifically characterize the cytotoxic constituents from Kalimantan-sourced *A. flava* bark against MCF-7 human breast adenocarcinoma cells, along with concurrent selectivity assessments, remain limited. The geographical origin of a plant can substantially influence its phytochemical composition due to variations in soil characteristics, climate, altitude, and biotic stressors (Patel et al., 2022). The MCF-7

cell line, an estrogen receptor-positive (ER+) model, holds high clinical relevance because ER+ subtypes account for approximately 70% of all breast cancer cases (Chen et al., 2021).

Consequently, the present study was designed to systematically extract, fractionate, and isolate bioactive compounds from the bark of Kalimantan-derived *A. flava*, to elucidate their chemical structures using comprehensive spectroscopic techniques, and to evaluate their *in vitro* cytotoxic potential against MCF-7 breast cancer cells and Vero normal cells using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay. The outcomes are anticipated to contribute to the scientific validation of this important traditional medicinal plant and to support the development of plant-based anticancer agents.

METHODS

The bark of *Arcangelisia flava* (L.) Merr. was collected from a secondary rainforest in Kutai Kartanegara Regency, East Kalimantan, Indonesia (0°28' S, 117°14' E; 120 m above sea level) between October and November 2023. Taxonomic identification was performed at Herbarium Bogoriense, Research Center for Biosystematics and Evolution, BRIN, Bogor, with a voucher specimen (Herb. Bog. No. 2023 AF 0417). Fresh bark samples were cleaned, cut into small pieces (approximately 2–3 cm), air-dried at 28–32°C for 14 days, and then oven-dried at 45°C for 48 hours until constant weight was achieved. The dried samples were ground using a mechanical grinder into a fine powder, sieved through a 40-mesh sieve, and stored in sealed polyethylene bags at room temperature until use (Harborne, 1998; Faramayuda et al., 2020).

All solvents used in this study, including 95% ethanol, n-hexane, ethyl acetate, n-butanol, chloroform, methanol, acetonitrile, and distilled water, were of analytical or HPLC grade (Merck, Germany). Silica gel 60 (0.063–0.200 mm) and silica gel GF₂₅₄ for thin-layer chromatography (TLC) were obtained from Merck (Germany). Sephadex LH-20 was obtained from GE Healthcare (Sweden). RPMI 1640 medium, fetal bovine serum (FBS), penicillin-streptomycin solution, trypsin-EDTA, phosphate-buffered saline (PBS), and MTT reagent were obtained from Sigma-Aldrich (USA). Doxorubicin hydrochloride as the positive control was obtained from Pfizer, Indonesia. MCF-7 and Vero normal cells were obtained from a cell culture facility (Syamsul et al., 2015; Lestari et al., 2021).

Extraction was performed using the maceration method based on Harborne (1998) with modifications. A total of 500 g of dried bark powder was macerated in 95% ethanol (3 × 2 L) at room temperature for 24 hours each cycle with occasional stirring using a magnetic stirrer. The sample-to-solvent ratio was 1:4 (w/v) for each maceration cycle. After each maceration period, the mixture was filtered through Whatman No. 1 filter paper. The filtrates from three maceration cycles were combined and concentrated under reduced pressure using a rotary evaporator (Büchi, Switzerland) at 45°C to completely remove the solvent, yielding 48.50 g (9.70% w/w) of crude ethanolic extract (Faramayuda et al., 2020; Lestari et al., 2021).

The crude ethanolic extract (48.50 g) was suspended in 250 mL of hot distilled water (approximately 60°C) to facilitate dissolution. The aqueous suspension was then sequentially



partitioned with n-hexane (3 × 300 mL), ethyl acetate (3 × 300 mL), and n-butanol (3 × 300 mL) in a separating funnel according to the method of Syamsul et al. (2015). Each partition step was performed three times to ensure exhaustive extraction. The organic phases from each solvent system were combined, dried over anhydrous sodium sulfate, filtered, and concentrated at 45°C using a rotary evaporator. The remaining aqueous phase after sequential partitioning was designated as the water fraction. All fractions were stored at 4°C in amber glass vials until further analysis (Elfahmi et al., 2014; Lestari et al., 2021).

Qualitative phytochemical screening of the crude ethanolic extract and all fractions was performed using standard colorimetric and precipitation tests according to Harborne (1998) with modifications. The presence of alkaloids was detected using Dragendorff's reagent (orange-red precipitate) and Mayer's reagent (creamy white precipitate). Flavonoids were identified using the Shinoda test (addition of magnesium powder and concentrated hydrochloric acid, yielding orange-red coloration). Phenolic compounds and tannins were detected using FeCl₃ solution (ferric chloride), producing blue-black or greenish coloration. Saponins were identified using the foam test (persistent froth formation after vigorous shaking). Terpenoids were detected using the Liebermann-Burchard test (addition of acetic anhydride and concentrated sulfuric acid, producing a color change). All tests were performed in triplicate (Faramayuda et al., 2020; Syamsul et al., 2015).

The ethyl acetate fraction (12.70 g), which showed the highest preliminary cytotoxic activity, was subjected to column chromatography on silica gel 60 (230–400 mesh, 100 g) packed in a glass column (5 × 60 cm). The column was eluted using a gradient solvent system starting from 100% n-hexane to 100% ethyl acetate, followed by ethyl acetate–methanol mixtures at ratios of 90:10, 80:20, and 70:30 (v/v) according to the method of Faramayuda et al. (2020). Fractions of 20 mL were collected using an automated fraction collector. The collected fractions were monitored by thin-layer chromatography (TLC) on silica gel GF₂₅₄ plates, visualized under UV light at 254 nm and 366 nm, and sprayed with 10% H₂SO₄ in ethanol followed by heating at 110°C for 5 minutes. Fractions with similar TLC profiles were pooled and combined. The pooled fractions were further purified using Sephadex LH-20 column chromatography (2.5 × 100 cm) with methanol as the eluent. Final purification was achieved using preparative TLC on silica gel GF₂₅₄ plates (20 × 20 cm, 0.5 mm thickness) using appropriate solvent systems, yielding three pure compounds designated AF-1, AF-2, and AF-3. The purity of each compound was confirmed by TLC (single spot) and HPLC analysis (Rauf et al., 2021; Tian et al., 2022).

The structures of the isolated compounds (AF-1, AF-2, and AF-3) were elucidated using comprehensive spectroscopic techniques according to standard protocols (Silverstein et al., 2005). UV-Vis spectra were recorded in methanol using a Shimadzu UV-1800 spectrophotometer over the wavelength range of 200–400 nm. FT-IR spectra were obtained using a Shimadzu IRTracer-100 spectrometer with KBr pellets in the range of 4000–400 cm⁻¹. HR-LC-MS/MS analysis was performed using an Agilent 6546 Q-TOF mass spectrometer equipped with electrospray ionization (ESI) in both positive and negative ion modes. NMR spectra, including ¹H-NMR (500 MHz), ¹³C-NMR (125 MHz), DEPT, COSY, HMQC, and HMBC, were recorded using a Bruker AV 500 spectrometer in DMSO-d₆.



with TMS as the internal reference. Spectroscopic data were compared with previously published literature for compound identification (Rauf et al., 2021; Tian et al., 2022; Hashemzaei et al., 2017; Chen et al., 2021).

MCF-7 and Vero cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin in a humidified atmosphere of 5% CO₂ at 37°C, according to the method of Mosmann (1983). Cells were subcultured every 3–4 days upon reaching approximately 80% confluence using 0.25% trypsin-EDTA solution. Cells were maintained in 75 cm² tissue culture flasks and used at passages 5–20 for all experiments (Chen et al., 2021; Li et al., 2020).

The cytotoxic activity of the isolated compounds, crude extract, and fractions against MCF-7 and Vero cells was evaluated using the MTT colorimetric assay according to Mosmann (1983) with modifications. Cells in the logarithmic growth phase were trypsinized, counted using a hemocytometer, and seeded into 96-well plates at a density of 5×10^3 cells/well for MCF-7 and 1×10^4 cells/well for Vero in 100 µL of complete culture medium. The plates were incubated at 37°C in a 5% CO₂ humidified atmosphere for 24 hours to allow cell attachment. After incubation, the culture medium was replaced with fresh medium containing various concentrations of test samples. The isolated compounds (AF-1, AF-2, AF-3), crude extract, and fractions were dissolved in DMSO and serially diluted with culture medium to obtain final concentrations of 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 µg/mL. The final DMSO concentration in each well was maintained below 0.1% to prevent solvent toxicity. Doxorubicin hydrochloride was used as the positive control at the same concentration range, while culture medium containing 0.1% DMSO served as the negative control. Each concentration was tested in triplicate wells, and the experiment was performed on three independent occasions (n = 9 total replicates). The plates were incubated at 37°C in 5% CO₂ for 48 hours (Lestari et al., 2021; Wahyuni et al., 2022).

After 48 hours of incubation, 10 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for an additional 4 hours at 37°C to allow formazan crystal formation. The medium was carefully removed, and the formazan crystals were dissolved in 100 µL of DMSO per well with gentle shaking for 15 minutes. Absorbance was measured at 570 nm using a Bio-Rad Model 680 microplate reader with a reference wavelength of 630 nm. The percentage of cell viability was calculated using the formula: Cell viability (%) = (Absorbance of treated cells / Absorbance of untreated control cells) × 100 (Mosmann, 1983; Boik, 2001).

IC₅₀ values were calculated by nonlinear regression analysis using a sigmoidal dose-response curve (variable slope) fitted with GraphPad Prism version 9.0 software. The selectivity index (SI) was calculated as the ratio of IC₅₀ value against Vero normal cells to IC₅₀ value against MCF-7 cancer cells: SI = IC₅₀ (Vero) / IC₅₀ (MCF-7). An SI value greater than 3 was considered to indicate acceptable selectivity for cancer cells over normal cells (Boik, 2001; Wahyuni et al., 2022). All data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using SPSS version 26.0. One-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post hoc test was used to determine significant differences among treatment



groups. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant (Chen et al., 2021; Li et al., 2020).

RESULTS

1. Plant Extraction and Fractionation Yield

Maceration of 500 g of dried *A. flava* bark powder in 95% ethanol yielded 48.50 g of crude ethanolic extract (9.70% w/w). Liquid-liquid partitioning yielded: n-hexane fraction (8.30 g, 1.66%), ethyl acetate fraction (12.70 g, 2.54%), n-butanol fraction (6.40 g, 1.28%), and water fraction (21.10 g, 4.22%). The extraction and fractionation yields, along with the isolation yields of the three pure compounds, are summarized in Table 1.

Table 1. Yield of Extraction, Fractionation, and Compound Isolation from *Arcangelisia flava* Bark

Fraction	Solvent System	Weight (g)	Yield (%)
Crude Extract	95% Ethanol	48.50	9.70
n-Hexane Fraction	n-Hexane	8.30	1.66
Ethyl Acetate Fraction	Ethyl Acetate	12.70	2.54
n-Butanol Fraction	n-Butanol	6.40	1.28
Water Fraction	Distilled Water	21.10	4.22
Compound AF-1	EtOAc:n-Hex (3:7)	0.085	0.017
Compound AF-2	EtOAc:n-Hex (5:5)	0.062	0.012
Compound AF-3	MeOH:CHCl ₃ (2:8)	0.041	0.008

2. Preliminary Phytochemical Screening

Qualitative phytochemical screening of the crude ethanolic extract and all fractions was performed using standard colorimetric tests as described in the Methods section (Harborne, 1998). The crude extract showed strong positive reactions for alkaloids with both Dragendorff's reagent (orange-red precipitate) and Mayer's reagent (creamy white precipitate), indicating the presence of alkaloids as major constituents. The Shinoda test for flavonoids yielded an orange-red coloration, confirming the presence of flavonoids. The addition of FeCl₃ solution produced a blue-black coloration, indicating the presence of phenolic compounds, while a brownish precipitate was observed for tannins. The foam test produced weak and transient froth formation, and the Liebermann-Burchard test showed faint color changes, suggesting that saponins and terpenoids were present only in minor or trace amounts, respectively, based on the intensity of the color reactions compared to positive controls. The ethyl acetate fraction showed particularly intense reactions for flavonoids and alkaloids, consistent with its higher bioactivity. The results of phytochemical screening are presented in Table 5.

Phytochemical Group	Test/Reagent	Crude Extract	n-Hexane	Ethyl Acetate	n-Butanol	Water
Alkaloids	Dragendorff	+++	+	+++	++	+

Alkaloids	Mayer	+++	+	+++	++	+
Flavonoids	Shinoda	++	–	+++	++	+
Phenolics	FeCl ₃	++	–	++	++	+++
Tannins	FeCl ₃	++	–	++	+	++
Saponins	Foam test	+	–	–	+	++
Terpenoids	Liebermann– Burchard	+	++	+	–	–

3. Isolation and Characterization of Compounds

Column chromatographic separation of the ethyl acetate fraction (12.70 g) on silica gel 60 with gradient elution, followed by Sephadex LH-20 column chromatography and preparative TLC, yielded three pure compounds. The purity of each compound was confirmed by TLC (single spot) and HPLC analysis. AF-1 (85 mg, 0.017% w/w) was obtained as yellow needles; AF-2 (62 mg, 0.012% w/w) as yellow prisms; and AF-3 (41 mg, 0.008% w/w) as a yellow amorphous powder. All compounds displayed characteristic yellow fluorescence under UV light at 366 nm.

4. Spectroscopic Data and Structure Elucidation

The comprehensive spectroscopic data for the three isolated compounds are presented in Table 2. Complete structural characterization is detailed in the Discussion section.

Table 2. Physicochemical and Spectroscopic Data of Isolated Compounds AF-1, AF-2, and AF-3

Parameter	AF-1 (Berberine)	AF-2 (Palmatine)	AF-3 (Quercetin)
Molecular Formula	C ₂₀ H ₁₈ NO ₄ ⁺	C ₂₁ H ₂₂ NO ₄ ⁺	C ₁₅ H ₁₀ O ₇
MW (g/mol)	336.37	352.40	302.24
UV λ _{max} (nm)	230, 265, 345	228, 282, 357	256, 298, 374
MS [M] ⁺ m/z	336.12	352.15	303.05 [M+H] ⁺
IR (cm ⁻¹ , key)	1630, 1585, 1510	1625, 1580, 1505	3400, 1655, 1610
¹ H NMR (δ, ppm)	9.76 (s,H-8), 7.75 (s,H-11), 7.08 (s,H-12), 6.05 (s,-OCH ₂ O-)	9.72 (s,H-8), 7.77 (s,H-11), 7.07 (s,H-12), 4.08 (s,OCH ₃)	7.67 (d,H-2'), 7.64 (d,H-6'), 6.89 (d,H-3'), 6.40 (d,H-8), 6.19 (d,H-6)
¹³ C NMR (δ, ppm)	150.5, 148.1, 144.4, 137.3, 130.6, 126.5, 122.8, 119.7, 106.6, 105.2	151.2, 148.5, 145.6, 138.1, 131.2, 127.3, 123.4, 120.1, 107.5, 55.9	176.2, 164.4, 161.8, 156.3, 148.3, 145.3, 122.2, 120.9, 116.0, 115.4, 103.9, 98.5, 93.5
Melting Point (°C)	145–147	204–207	316–318
Color/Appearance	Yellow needles	Yellow prisms	Yellow amorphous
Compound Class	Protoberberine alkaloid	Protoberberine alkaloid	Flavonol



5. Cytotoxic Activity – MTT Assay

All isolated compounds and fractions demonstrated concentration-dependent cytotoxic activity against MCF-7 breast cancer cells after 48 hours of treatment. The IC_{50} values for all test materials, along with selectivity indices calculated using Vero cell toxicity data, are presented in Table 3. Cell viability data at each tested concentration for the pure compounds and doxorubicin are shown in Table 4.

Table 3. IC_{50} Values and Selectivity Indices of Isolated Compounds and Fractions Against MCF-7 and Vero Cells

Compound / Extract	IC_{50} MCF-7 ($\mu\text{g/mL}$)	IC_{50} Vero ($\mu\text{g/mL}$)	SI (Selectivity Index)
Crude Ethanolic Extract	48.32 ± 1.87	189.45 ± 4.21	3.92
Ethyl Acetate Fraction	22.17 ± 0.94	142.30 ± 3.56	6.42
n-Butanol Fraction	35.64 ± 1.23	156.80 ± 5.12	4.40
AF-1 (Berberine)	8.74 ± 0.38	98.52 ± 2.47	11.27
AF-2 (Palmatine)	14.23 ± 0.61	87.34 ± 3.18	6.14
AF-3 (Quercetin)	18.56 ± 0.72	112.67 ± 4.05	6.07
Doxorubicin (positive control)	0.52 ± 0.04	12.84 ± 0.61	24.69
DMSO (negative control)	>200	>200	N/A

Note: Values are expressed as mean $\pm$ SEM ($n = 9$). SI = Selectivity Index = IC_{50} Vero / IC_{50} MCF-7. SI > 3 indicates selective cytotoxicity.

Table 4. Cell Viability (%) of MCF-7 Cells Treated with Isolated Compounds at Varying Concentrations (48 h, mean $\pm$ SEM, $n = 9$)

Concentration ($\mu\text{g/mL}$)	AF-1 Viability (%)	AF-2 Viability (%)	AF-3 Viability (%)	Doxorubicin (%)
100	12.4 ± 1.2	18.7 ± 0.9	24.5 ± 1.5	1.3 ± 0.2
50	28.6 ± 1.8	36.4 ± 1.4	42.1 ± 2.0	2.8 ± 0.4
25	45.3 ± 2.1	52.7 ± 1.7	60.4 ± 1.9	5.4 ± 0.6
12.5	62.8 ± 2.4	69.2 ± 2.0	74.5 ± 2.3	11.2 ± 0.9
6.25	77.4 ± 1.9	81.3 ± 1.6	85.7 ± 1.8	28.4 ± 1.5
3.125	87.9 ± 1.4	91.5 ± 1.2	93.2 ± 1.3	54.7 ± 2.1
1.5625	93.6 ± 1.1	95.8 ± 0.9	96.8 ± 0.8	72.3 ± 1.8
Control	100 ± 0	100 ± 0	100 ± 0	100 ± 0

Among all test materials, AF-1 (berberine) exhibited the most potent cytotoxic activity with an IC_{50} of $8.74 \pm 0.38 \mu\text{g/mL}$ against MCF-7 cells, followed by AF-2 (palmatine; $IC_{50} = 14.23 \pm 0.61 \mu\text{g/mL}$) and AF-3 (quercetin; $IC_{50} = 18.56 \pm 0.72 \mu\text{g/mL}$). The IC_{50} values were significantly different from one another ($p < 0.05$, one-way ANOVA, Tukey's test). The doxorubicin positive control yielded an IC_{50} of $0.52 \pm 0.04 \mu\text{g/mL}$. Among crude fractions, the ethyl acetate fraction demonstrated the

highest activity ($IC_{50} = 22.17 \pm 0.94 \mu\text{g/mL}$). In terms of selectivity, AF-1 showed the highest selectivity index ($SI = 11.27$), indicating preferential toxicity toward cancer cells over normal Vero cells. All compounds demonstrated SI values greater than 3, suggesting acceptable selectivity thresholds.

DISCUSSION

The crude ethanolic extract from *A. flava* bark gave a relatively high yield (9.70%), which aligns with earlier findings and can be explained by the good solubility of protoberberine alkaloids, flavonoids, and other polar compounds in 70 to 95% ethanol (Syamsul et al., 2015; Faramayuda et al., 2020). Among the partitioned fractions, the water fraction showed the highest mass recovery (4.22%), followed by ethyl acetate (2.54%) and n-butanol (1.28%); this outcome was anticipated because the aqueous phase retains strongly polar glycosylated phenolics and quaternary ammonium alkaloid salts. Nevertheless, the ethyl acetate fraction displayed superior cytotoxic activity, indicating that moderately polar constituents such as free flavonoid aglycones and protoberberine alkaloids are the main contributors to the bioactivity, a finding consistent with the report by Lestari et al. (2021).

Compound AF1 was isolated as yellow needles having a melting point of 145 to 147°C, which matches that of berberine chloride. Its molecular formula was determined to be $C_{20}H_{18}NO_4^+$ based on HR-ESI-MS data showing a quasi-molecular ion at $m/z^* 336.12$ $[M]^+$ (calculated for $C_{20}H_{18}NO_4^+$: 336.1230). The UV spectrum in methanol gave characteristic absorption maxima at 230, 265, and 345 nm, typical of the isoquinoline chromophore present in berberine. The FT-IR spectrum displayed distinct bands at 1630, 1585, and 1510 cm^{-1} (attributed to $C=N$ and aromatic $C=C$ stretching) and at 1050 cm^{-1} (due to the $C-O-C$ linkage of a methylenedioxy group). In the ^1H NMR spectrum (500 MHz, $\text{DMSO}-d_6$), signals were observed at δ 9.76 (1H, s, H8), 7.75 (1H, s, H11), 7.08 (1H, s, H12), and 6.05 (2H, s, OCH_2O), confirming the 3,4-methylenedioxy substitution pattern. The ^{13}C NMR and DEPT spectra further supported the protoberberine carbon skeleton. HMBC correlations between the methylenedioxy protons and C2/C3 unambiguously identified the compound as berberine, in excellent agreement with published data (Rauf et al., 2021).

Compound AF2 was obtained as yellow prisms with a melting point of 204 to 207°C. Its molecular formula $C_{21}H_{22}NO_4^+$ was established by HR-ESI-MS ($m/z^* 352.15$ $[M]^+$, calculated: 352.1543). The UV spectrum exhibited absorption maxima at 228, 282, and 357 nm. Comparison of the ^1H NMR spectrum with that of AF1 revealed the presence of four methoxy groups at δ 4.08 (each 3H, s) instead of the methylenedioxy moiety, which is characteristic of palmatine (2,3,9,10-tetramethoxyprotoberberine). The ^{13}C NMR spectrum showed signals at δ 151.2 and 55.9 ppm, corresponding to aromatic carbons bearing methoxy substituents and the OCH_3 carbons, respectively. These spectral features are fully consistent with previously reported data for palmatine (Tian et al., 2022; Faramayuda et al., 2020).

Compound AF3 was isolated as a yellow amorphous powder with a melting point of 316 to 318°C. HR-ESI-MS gave an $[M+H]^+$ ion at $m/z^* 303.05$, supporting the molecular formula



C₁₅H₁₀O₇ (calculated: 303.0501). The UV spectrum showed absorption maxima at 256, 298, and 374 nm, which are characteristic of a flavonol bearing a 3-hydroxyl group. The FT-IR spectrum displayed a broad O-H absorption near 3400 cm⁻¹ and a conjugated carbonyl stretch at 1655 cm⁻¹. The ¹H NMR spectrum exhibited an A ring pattern with two doublets at δ 6.40 (J = 2.1 Hz, H₈) and 6.19 (J = 2.1 Hz, H₆), and a B ring pattern at δ 7.67 (d, J = 2.1 Hz, H_{2'}), 7.64 (dd, J = 8.4, 2.1 Hz, H_{6'}), and 6.89 (d, J = 8.4 Hz, H_{5'}), confirming an orthodihydroxy substitution at the 3',4' positions. The ¹³C NMR spectrum showed a carbonyl carbon at δ 176.2 (C₄), consistent with a flavonol skeleton. These data are in full agreement with the structure of quercetin (Hashemzaei et al., 2017; Patel et al., 2022).

The cytotoxic results obtained in this investigation both confirm and expand the existing knowledge on the anticancer properties of these natural products. According to the criteria established by the U.S. National Cancer Institute (NCI), a crude extract is considered to have significant cytotoxic potential when its IC₅₀ value falls below 20 μ g/mL, whereas a pure compound is deemed active if its IC₅₀ is less than 4 μ g/mL (Boik, 2001). Based on these benchmarks, AF1 and AF2 exhibited noteworthy cytotoxicity, while AF3 and the various fractions displayed moderate to good activity.

Berberine (AF1) showed the strongest cytotoxic effect against MCF7 cells (IC₅₀ = 8.74 μ g/mL). This finding is in line with numerous earlier studies demonstrating berberine's ability to suppress the proliferation of MCF7 cells through multiple mechanisms. For instance, Li et al. (2020) reported that berberine activates the mitochondrial apoptotic pathway in MCF7 cells by upregulating Bax and downregulating Bcl2, along with subsequent activation of caspase-3 and caspase-9. Additionally, berberine has been shown to inhibit the PI3K/Akt/mTOR signaling axis, a critical driver of proliferation in ER positive breast cancer, and to induce G1/S phase arrest by reducing the expression of cyclin D1 and CDK4/6 (Rauf et al., 2021). The high selectivity index of berberine (SI = 11.27) relative to Vero normal cells suggests a preferential toxicity toward cancerous cells, possibly due to the higher membrane potential and increased uptake of positively charged species in rapidly dividing malignant cells.

Palmatine (AF2, IC₅₀ = 14.23 μ g/mL), a structural analogue of berberine differing only in its methoxylation pattern, exhibited somewhat lower but still appreciable cytotoxicity. The slightly reduced potency of palmatine compared to berberine may reflect differences in cellular uptake, affinity for DNA intercalation, or specificity in enzyme inhibition, which could be related to the presence of the 9,10-dimethoxy arrangement instead of the methylenedioxy group (Tian et al., 2022). Both berberine and palmatine are known to intercalate into DNA and inhibit topoisomerase II, a central mechanism underlying their cytotoxic action (Li et al., 2020).

Quercetin (AF3, IC₅₀ = 18.56 μ g/mL) demonstrated moderate cytotoxic activity, which is consistent with previously published data. Hashemzaei et al. (2017) and Chen et al. (2021) reported IC₅₀ values for quercetin against MCF7 cells in the range of 15 to 25 μ g/mL, depending on treatment duration and culture conditions, a finding that corresponds well with our results. The cytotoxic mechanism of quercetin in MCF7 cells has been attributed to the modulation of ER α signaling, inhibition of heat shock proteins (HSP90 and HSP70), induction of G2/M cell cycle arrest, and

promotion of autophagic cell death. The relatively lower selectivity index of quercetin (6.07) compared to berberine may be linked to its broader antiproliferative effects across different cell types.

The ethyl acetate fraction ($IC_{50} = 22.17 \mu\text{g/mL}$) showed superior cytotoxic activity relative to the other fractions, which can be explained by its enrichment in both protoberberine alkaloids (AF1 and AF2) and flavonoids (AF3), potentially acting in a synergistic manner. Synergistic interactions between berberine and quercetin have been reported previously, with the flavonoid component enhancing alkaloid uptake and amplifying apoptotic signaling (Wahyuni et al., 2022).

Comparing our results with studies on *A. flava* from other geographical regions (Sumatra, Malaysia) suggests that the Kalimantan ecotype may produce alkaloid profiles with comparable or even slightly higher berberine content. Such differences may be due to variations in soil mineral composition and rainfall patterns (Mukherjee, 2020; Syamsul et al., 2015). This observation underscores the importance of geographic documentation in phytochemical investigations and supports further metabolomic comparisons among regional ecotypes.

CONCLUSIONS

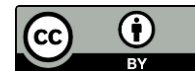
In this study, three bioactive compounds, namely berberine (AF1), palmatine (AF2), and quercetin (AF3), were successfully isolated from the ethyl acetate fraction of the bark of *Arcangelisia flava* collected in Kutai Kartanegara, East Kalimantan. Their structures were fully elucidated using a combination of spectroscopic techniques, including UV-Vis, FT-IR, HR-LC-MS/MS, and 1D/2D NMR analyses. All three compounds exhibited significant, concentration dependent cytotoxic activity against MCF7 human breast cancer cells as assessed by the MTT assay. Among them, berberine demonstrated the highest potency ($IC_{50} = 8.74 \pm 0.38 \mu\text{g/mL}$) and the greatest selectivity ($SI = 11.27$), highlighting its potential as a lead candidate for anticancer drug development. These results provide robust scientific validation for the traditional use of *A. flava* in the treatment of cancerous conditions and support its further development as a natural source of anticancer agents. Future research should focus on elucidating the apoptotic pathways activated by berberine and palmatine in MCF7 cells, conducting *in vivo* pharmacokinetic studies, and exploring semisynthetic modifications to improve potency and reduce systemic toxicity.

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