



Antioxidant Activity Test and Chromatographic Fingerprint Analysis of Secondary Metabolite Profiles of Garcinia Fruit Extract Mangosteen (Mangosteen) from Several Varieties in Indonesia

Zola Effa Harnis^{1*}, & Nina Irmayanti Harahap²

^{1*}Universitas Muslim Nusantara, Indonesia, ²Institut Kesehatan Delihusada Deli Tua, Indonesia

*Co e-mail: zolaharnis19@gmail.com¹

Article Information

Received: May 22, 2026

Revised: August 27, 2026

Online: October 06, 2026

Keywords

Garcinia mangostana, mangosteen, antioxidant, DPPH, ABTS, FRAP, HPLC fingerprint, alpha-mangostin, xanthonenes, Indonesia, chemotype variation

ABSTRACT

Garcinia mangostana L. (mangosteen) is a prominent tropical fruit from Indonesia, widely recognized for its rich xanthone content with strong antioxidant properties. Indonesia hosts several regional varieties with potentially different phytochemical profiles; however, comparative studies remain limited. This study aimed to evaluate and compare antioxidant activity and characterize the secondary metabolite fingerprint profiles of ethanol extracts from mangosteen pericarp collected from five regions: Purwokerto, Kaligesing, Palu, Wanayasa, and Lingga. Extracts were prepared by maceration using 96% ethanol. Antioxidant activity was assessed using DPPH, ABTS, and FRAP assays. TLC fingerprinting was performed on silica gel plates, while HPLC analysis used a C18 column with gradient elution. Alpha-mangostin was quantified at 316 nm, and total phenolic and flavonoid contents were also determined. All samples exhibited strong antioxidant activity. The Palu variety showed the highest DPPH activity (IC_{50} 12.4 ± 0.6 μ g/mL), while Purwokerto showed the highest FRAP value (482.6 ± 14.3 μ mol Trolox/g). HPLC profiles revealed 12–16 peaks with alpha-mangostin as the dominant compound (28.4–45.8 mg/g extract). Cluster analysis grouped samples into two chemotypes, indicating phytochemical variation influenced by geographic origin.

Keywords: *Garcinia mangostana*, mangosteen, antioxidant, DPPH, ABTS, FRAP, HPLC fingerprint, alpha-mangostin, xanthonenes, Indonesia, chemotype variation

INTRODUCTION

Oxidative stress defined as the imbalance between the generation of reactive oxygen species (ROS) and the biological system's capacity to detoxify these reactive intermediates is fundamentally implicated in the pathogenesis of numerous chronic diseases, including cardiovascular disease, diabetes mellitus, neurodegenerative disorders, and cancer (Pizzino et al., 2017). Antioxidants, which neutralize or quench ROS, play a central protective role in maintaining cellular homeostasis, and there is growing pharmacological and clinical interest in natural antioxidant compounds derived from plant sources as adjuncts or alternatives to synthetic antioxidants, which may carry safety concerns (Tungmunnithum et al., 2018).

Garcinia mangostana L. (family Clusiaceae/Guttiferae), commonly known as mangosteen and often celebrated as the “Queen of Fruits,” is a tropical tree native to Southeast Asia, with Indonesia being one of the world's largest producers and centers of genetic diversity for the species (Barreca et al., 2021). The pericarp (rind or hull) of the mangosteen fruit, which constitutes approximately 60–70% of the fruit's total weight and is typically discarded as agricultural waste, is exceptionally rich in a diverse array of oxygenated and prenylated xanthone derivatives a unique class of polycyclic aromatic compounds biosynthetically derived from polyketide and shikimate pathways (Chin & Kinghorn, 2008; Obolskiy et al., 2009). Over 60 distinct xanthone compounds have been identified from *G. mangostana* pericarp, with alpha-mangostin, gamma-mangostin, and beta-mangostin being the most abundant and pharmacologically studied (Pedraza-Chaverri et al., 2008).

The pharmacological activities attributed to mangosteen xanthenes are remarkably broad and include antioxidant, anti-inflammatory, antibacterial, antifungal, antitumor, antidiabetic, and neuroprotective effects (Bumrungpert et al., 2010; Aisha et al., 2012). The antioxidant activity is particularly significant and has been mechanistically linked to the structural characteristics of xanthenes specifically, the multiple hydroxyl groups on the xanthone scaffold that facilitate hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms of radical scavenging, the extended conjugated aromatic system that stabilizes radical intermediates, and the chelation of transition metal ions that catalyze ROS formation (Ibrahim et al., 2016). These properties make mangosteen pericarp extract a highly promising candidate for development into nutraceutical and pharmaceutical antioxidant products.

Indonesia possesses extraordinary agro-biodiversity in *G. mangostana*, with regional varieties or landraces distributed across major growing regions including West Java (Wanayasa, Tasikmalaya), Central Java (Purwokerto, Kaligesing), Sulawesi (Palu), and Riau Islands (Lingga, Bintan), among others (Mohan et al., 2010). These varieties, adapted to distinct geographic, climatic, soil, and cultivation conditions, have long been recognized by local farmers and consumers as exhibiting differences in fruit morphology, taste, and perceived medicinal potency. Environmental and edaphic factors including UV radiation intensity, rainfall patterns, altitude, and soil mineral composition are well-established modulators of secondary metabolite biosynthesis in plants, particularly the phenylpropanoid and polyketide pathways responsible for phenolic and xanthone production (Nakpong & Woottisin, 2010).



Despite the extensive global literature on *G. mangostana* phytochemistry and pharmacology, the vast majority of studies have treated the species as a single entity without accounting for intraspecific variation arising from geographic origin and variety. This represents a significant gap, particularly from a pharmaceutical quality assurance perspective. The development of standardized, high-quality herbal medicinal products from mangosteen requires a thorough understanding of the variability in bioactive compound content and antioxidant potency across different varieties and production regions (Pothitirat et al., 2009). Chromatographic fingerprinting employing both TLC for rapid, cost-effective screening and HPLC for high-resolution quantitative profiling has been established by the WHO, European Pharmacopoeia, and various national pharmacopoeias as the gold standard for quality control and authentication of herbal medicines, offering comprehensive chemical characterization beyond single-compound quantification (World Health Organization, 2011).

Multiple antioxidant assay approaches are critical for comprehensive characterization of antioxidant capacity, as different assays measure different mechanistic pathways (HAT vs. SET) and interact differently with the heterogeneous mixture of antioxidants present in plant extracts. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is the most widely used, measuring hydrogen-donating ability of antioxidants, while the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical cation assay operates through a combined HAT and SET mechanism, and the FRAP (Ferric Reducing Antioxidant Power) assay specifically measures the electron-donating capacity of antioxidants (Apak et al., 2013). Together, these three assays provide a multifaceted and reliable assessment of antioxidant capacity.

This study therefore addressed a significant scientific and practical gap by conducting a systematic, multi-variety comparative investigation of: (1) total phenolic and flavonoid content; (2) antioxidant activity using DPPH, ABTS, and FRAP assays; and (3) chromatographic fingerprint profiles (TLC and HPLC) and alpha-mangostin content of *G. mangostana* pericarp extracts from five distinct Indonesian varieties. The findings are expected to provide essential data for varietal selection, quality standardization, and the development of evidence-based mangosteen-derived pharmaceutical and nutraceutical products, while contributing to the documentation and valorization of Indonesia's remarkable mangosteen biodiversity (Fan et al., 2006).

METHODS

1. Plant Material, Varieties, and Authentication

Mature mangosteen fruits were collected from five established production regions of Indonesia representing distinct geographical locations during the main harvest season (November–December 2024). The five varieties were: (1) GM-PWK — Purwokerto variety, Banyumas Regency, Central Java (7°25'S, 109°13'E; 75 m asl); (2) GM-KLG — Kaligesing variety, Purworejo Regency, Central Java (7°46'S, 110°01'E; 480 m asl); (3) GM-PAL — Palu variety, Donggala Regency, Central Sulawesi (0°35'S, 119°52'E; 35 m asl); (4) GM-WNY — Wanayasa variety, Purwakarta Regency, West Java (6°42'S, 107°29'E; 650 m asl); (5) GM-LNG — Lingga variety, Lingga Regency, Riau Islands

(0°12'S, 104°37'E; 20 m asl). Each variety was authenticated by a taxonomist at the Herbarium Bogoriense, Research Center for Plant Conservation and Botanical Gardens-BRIN (LIPI), Bogor (Voucher specimens: BO-GM-2024-001 through BO-GM-2024-005). Three to five fruits per tree from five representative trees per variety (total $n = 15\text{--}25$ fruits per variety) were harvested. The pericarp was separated from the aril, washed with distilled water, and used for extract preparation.

2. Preparation of Ethanol Extracts

Fresh pericarps were cut into small pieces and dried in a forced-air oven at 45°C for 48–72 hours to a constant weight (moisture content $\leq 8\%$). Dried pericarp was ground and sieved through a 40-mesh screen to obtain a uniform powder. Extraction was performed by maceration: 200 g of dried pericarp powder per variety was macerated in 2,000 mL of 96% ethanol for 3×24 hours at room temperature ($25 \pm 2^\circ\text{C}$) with periodic stirring (150 rpm, 30 min every 8 hours). Combined filtrates were concentrated by rotary evaporation (Buchi R-300) at 40°C and then vacuum-dried at 50°C to a constant weight. Extract yield was calculated as percentage (w/w, dry weight basis). All extracts were stored at -20°C in amber vials until analysis.

3. Determination of Total Phenolic Content (TPC)

TPC was determined using the Folin-Ciocalteu method. Briefly, 100 μL of extract solution (1 mg/mL in methanol) was added to 500 μL of 10% (v/v) Folin-Ciocalteu reagent, vortexed, and allowed to react for 5 minutes. Then 400 μL of 7.5% Na_2CO_3 solution was added and the mixture was incubated for 30 minutes at room temperature in the dark. Absorbance was measured at 760 nm (UV-Vis spectrophotometer Shimadzu UV-1800). A calibration curve was constructed with gallic acid (0–400 $\mu\text{g/mL}$; $R^2 = 0.9982$). TPC was expressed as mg gallic acid equivalent (GAE)/g extract.

4. Determination of Total Flavonoid Content (TFC)

TFC was determined by the aluminum chloride colorimetric method. One mL of extract (1 mg/mL in methanol) was mixed with 4 mL of methanol, 0.3 mL of 10% AlCl_3 , 0.3 mL of 1 M potassium acetate, and 2.4 mL of distilled water. After 30 minutes at room temperature, absorbance was measured at 415 nm. Quercetin (0–200 $\mu\text{g/mL}$; $R^2 = 0.9976$) was used as the standard. TFC was expressed as mg quercetin equivalent (QE)/g extract.

5. Antioxidant Activity Assays

The antioxidant activity was evaluated using three complementary assays, namely DPPH, ABTS, and FRAP methods. The DPPH free radical scavenging assay was performed following the method of Brand-Williams et al. (1995) with slight modifications. Extract solutions were prepared in methanol at concentrations of 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 $\mu\text{g/mL}$. One milliliter of each concentration was mixed with 2 mL of 0.1 mM DPPH solution and incubated in the dark at 25°C for 30 minutes, after which absorbance was measured at 517 nm. The percentage of radical scavenging activity (% RSA) was calculated using the formula $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\%$, and



IC₅₀ values were determined by nonlinear regression using GraphPad Prism 9.0, with ascorbic acid and quercetin as positive controls.

The ABTS radical cation scavenging assay was conducted by generating ABTS•+ through the reaction of 7 mM ABTS with 2.45 mM potassium persulfate, followed by incubation in the dark for 12–16 hours at room temperature. The solution was diluted with phosphate-buffered saline (pH 7.4) to an absorbance of 0.700 ± 0.020 at 734 nm. Subsequently, 10 µL of extract at various concentrations was added to 990 µL of ABTS•+ solution, and absorbance was recorded at 734 nm after 6 minutes. Trolox was used as the reference standard, and results were expressed as µmol Trolox equivalent antioxidant capacity (TEAC) per gram of extract.

The FRAP assay was carried out using a reagent prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl₃·6H₂O in a ratio of 10:1:1, and warmed to 37°C. A total of 50 µL of extract (1 mg/mL) was mixed with 1.5 mL of FRAP reagent and incubated at 37°C for 8 minutes. Absorbance was measured at 593 nm, and antioxidant capacity was calculated using a Trolox calibration curve ($R^2 = 0.9991$), with results expressed as µmol Trolox equivalent (TE) per gram of extract.

6. Thin-Layer Chromatography (TLC) Fingerprinting

TLC fingerprinting was performed on pre-coated silica gel 60 F254 aluminum plates (Merck, Germany) with a mobile phase of toluene:ethyl formate:formic acid (5:4:1, v/v/v). Extract solutions (10 mg/mL in methanol, 2 µL) were applied as bands (10 mm wide) using a CAMAG Linomat 5 automatic applicator. Plates were developed in a saturated twin-trough chamber (20°C). After development (7 cm migration), plates were examined under UV light at 254 nm and 366 nm, and after derivatization with 5% sulfuric acid in ethanol (heated at 105°C for 5 min). R_f values were recorded and compared across varieties. Alpha-mangostin (Sigma-Aldrich, ≥98%) was used as a reference standard.

7. HPLC Fingerprint Analysis and Alpha-Mangostin Quantification

HPLC analysis was performed on a Shimadzu LC-2030C system equipped with a diode array detector (DAD). Column: Shim-pack GIST C18 (250 × 4.6 mm, 5 µm). Mobile phase: (A) 0.1% formic acid in water; (B) 0.1% formic acid in acetonitrile. Gradient: 0–5 min 30% B; 5–30 min 30→90% B; 30–40 min 90% B; 40–45 min 90→30% B; 45–50 min 30% B (re-equilibration). Flow rate: 1.0 mL/min; column temperature: 30°C; injection volume: 10 µL; detection: 316 nm (primary), with full UV spectral acquisition 200–400 nm. Extract concentration: 2 mg/mL in methanol (filtered through 0.22 µm PTFE membrane).

Method validation parameters (for alpha-mangostin quantification): linearity (5–200 µg/mL; $R^2 = 0.9997$), LOD (0.24 µg/mL), LOQ (0.73 µg/mL), precision (intra-day RSD ≤1.2%, inter-day RSD ≤2.1%), accuracy (recovery: 98.4–102.1%). Fingerprint similarity analysis was performed using the Similarity Evaluation Software for Chromatographic Fingerprints of TCM (Version 2012A, Chinese

Pharmacopoeia Committee) with cosine similarity calculation. Hierarchical cluster analysis (HCA) was performed using Ward's linkage method with Euclidean distance metric in SPSS 26.0.

8. Statistical Analysis

All experiments were performed in triplicate ($n=3$). Data are presented as mean $\pm$ standard deviation (SD). Prior to ANOVA, the normality of data distribution for each parameter was assessed using the Shapiro-Wilk test, and the homogeneity of variances among varieties was assessed using Levene's test; both assumptions were satisfied ($p > 0.05$) for all parameters, justifying the use of parametric one-way ANOVA. One-way ANOVA followed by Tukey's HSD test was used for multiple comparisons among varieties. Pearson correlation analysis was performed between TPC, TFC, and antioxidant parameters. Significance level was set at $p < 0.05$. Statistical analyses were performed with SPSS 26.0 (IBM Corp., USA) and GraphPad Prism 9.0 (GraphPad Software, USA).

RESULTS

1. Normality and Homogeneity of Variance Tests

Prior to conducting one-way ANOVA for all quantitative parameters (TPC, TFC, DPPH IC₅₀, ABTS TEAC, FRAP, and alpha-mangostin content), the normality of data distribution was evaluated using the Shapiro-Wilk test and the homogeneity of variances among the five varieties was evaluated using Levene's test. As summarized in Table 1, all parameters satisfied both assumptions (Shapiro-Wilk $p > 0.05$, indicating normally distributed data; Levene's $p > 0.05$, indicating homogeneous variances across varieties), confirming that the data met the requirements for parametric one-way ANOVA followed by Tukey's HSD post-hoc test.

Table 1. Results of Shapiro-Wilk Normality Test and Levene's Homogeneity of Variance Test for Parameters Analyzed by One-Way ANOVA

Parameter	Shapiro-Wilk W ($n=15$)	Shapiro-Wilk p	Levene's F (4,10)	Levene's p	Conclusion
Total Phenolic Content (TPC)	0.956	0.612	1.24	0.353	Normal & homogeneous
Total Flavonoid Content (TFC)	0.948	0.541	1.08	0.417	Normal & homogeneous
DPPH IC ₅₀	0.962	0.687	0.87	0.512	Normal & homogeneous
ABTS TEAC	0.939	0.462	1.36	0.309	Normal & homogeneous
FRAP	0.951	0.573	0.95	0.472	Normal & homogeneous
Alpha-mangostin content	0.944	0.501	1.19	0.371	Normal & homogeneous



Shapiro-Wilk test: $p > 0.05$ indicates data are normally distributed. Levene's test: $p > 0.05$ indicates homogeneity of variances across the five varieties. Both assumptions were met for all parameters, supporting the use of parametric one-way ANOVA with Tukey's HSD post-hoc test.

2. Extract Yield

The maceration extraction of mangosteen pericarp with 96% ethanol yielded extracts ranging from 14.8% (GM-PAL) to 22.3% (GM-KLG) on a dry weight basis. The high extraction yields are consistent with the rich content of polar xanthenes and phenolic compounds in the pericarp. Comparative yields across all five varieties are presented in Table 2.

Table 2. Extraction Yields and Standardization Parameters of *G. mangostana* Pericarp Extracts from Five Indonesian Varieties

Parameter	GM-PWK (Purwokerto)	GM-KLG (Kaligesing)	GM-PAL (Palu)	GM-WNY (Wanayasa)	GM-LNG (Lingga)
Yield (% w/w)	18.6 ± 0.7	22.3 ± 0.9	14.8 ± 0.6	17.4 ± 0.8	19.2 ± 0.7
Moisture content (%)	6.2 ± 0.3	5.8 ± 0.4	7.1 ± 0.3	6.5 ± 0.4	6.8 ± 0.3
Loss on drying (%)	6.8 ± 0.4	6.3 ± 0.3	7.5 ± 0.4	7.0 ± 0.3	7.2 ± 0.4
Total ash (%)	2.4 ± 0.2	2.1 ± 0.2	3.2 ± 0.3	2.7 ± 0.2	2.6 ± 0.2
TPC (mg GAE/g extract)	312.4 ± 8.6a	268.3 ± 7.2c	348.7 ± 9.4b	241.6 ± 6.8d	285.2 ± 8.1c
TFC (mg QE/g extract)	186.2 ± 5.4a	155.4 ± 4.8c	204.8 ± 6.1b	138.3 ± 4.2d	168.7 ± 5.1c

TPC = Total Phenolic Content (Folin-Ciocalteu); TFC = Total Flavonoid Content (AlCl₃ colorimetric); GAE = Gallic Acid Equivalent; QE = Quercetin Equivalent. Different superscript letters within a row indicate statistically significant differences ($p < 0.05$, Tukey's HSD test). Data expressed as mean ± SD ($n=3$).

GM-PAL (Palu variety) exhibited the highest TPC (348.7 ± 9.4 mg GAE/g) and TFC (204.8 ± 6.1 mg QE/g), while GM-WNY (Wanayasa variety) showed the lowest TPC (241.6 ± 6.8 mg GAE/g) and TFC (138.3 ± 4.2 mg QE/g). A statistically significant inter-variety difference was observed for both TPC and TFC parameters (ANOVA: $F = 48.62$ and 41.87 , respectively; $p < 0.001$).

3. Antioxidant Activity

Results from all three antioxidant assays (DPPH, ABTS, FRAP) are presented in Table 3. All five varieties demonstrated strong antioxidant activity in all three assays, though with significant inter-variety variation.

Table 3. Antioxidant Activity Parameters of *G. mangostana* Pericarp Extracts from Five Indonesian Varieties

Variety (Code)	DPPH IC50 (µg/mL)	ABTS TEAC (µmol TE/g)	FRAP (µmol TE/g)	DPPH Category
GM-PWK (Purwokerto)	15.8 ± 0.8b	1,248.6 ± 28.4b	482.6 ± 14.3a	Very Strong

GM-KLG (Kaligesing)	22.4 ± 1.1c	1,082.4 ± 24.6c	408.3 ± 12.7b	Strong
GM-PAL (Palu)	12.4 ± 0.6a	1,385.2 ± 31.8a	463.7 ± 13.9a	Very Strong
GM-WNY (Wanayasa)	31.6 ± 1.4d	924.8 ± 21.3d	352.4 ± 11.2c	Strong
GM-LNG (Lingga)	18.7 ± 0.9b	1,162.8 ± 26.7b	425.8 ± 13.1b	Very Strong
Ascorbic acid (reference)	5.2 ± 0.3e	—	—	—
Quercetin (reference)	3.8 ± 0.2f	—	—	—

DPPH IC₅₀: lower value = higher antioxidant activity; ABTS TEAC & FRAP: higher value = higher antioxidant activity. TE = Trolox Equivalent. DPPH category: Very Strong (IC₅₀ < 20 µg/mL), Strong (20–50 µg/mL). Different superscripts indicate significant differences ($p < 0.05$). '—' = not determined for reference standard in this assay.

GM-PAL demonstrated the highest DPPH radical scavenging activity with IC₅₀ = 12.4 ± 0.6 µg/mL and the highest ABTS TEAC (1,385.2 ± 31.8 µmol TE/g). GM-PWK showed the highest FRAP value (482.6 ± 14.3 µmol TE/g), while GM-WNY exhibited the lowest antioxidant activity across all three assays. Pearson correlation analysis revealed strong positive correlations between TPC and DPPH IC₅₀ ($r = -0.946$; $p < 0.001$), TPC and ABTS TEAC ($r = 0.958$; $p < 0.001$), TPC and FRAP ($r = 0.932$; $p < 0.001$), and moderate-to-strong correlations between TFC and all antioxidant parameters ($r = 0.872$ – 0.916 ; $p < 0.01$), indicating that phenolic compounds are the primary contributors to the observed antioxidant activity.

4. TLC Fingerprint Analysis

TLC fingerprint analysis using the toluene:ethyl formate:formic acid system allowed resolution of 8–12 characteristic spots per variety on silica gel plates. Under UV 366 nm and after H₂SO₄ derivatization, all five varieties showed the characteristic orange-yellow spot at R_f = 0.68 corresponding to alpha-mangostin (confirmed with reference standard). Additional characteristic spots at R_f = 0.48 (gamma-mangostin, yellow), R_f = 0.55 (gartanin, orange), R_f = 0.32 (8-hydroxycudraxanthone G, purple), and R_f = 0.78 (beta-mangostin, yellow-orange) were identified based on reference literature. GM-PAL displayed the most intense alpha-mangostin spot, while GM-WNY showed the least intense spot at this R_f. All varieties shared the same major TLC fingerprint pattern, confirming species identity, with minor qualitative differences in spot intensity and the presence of variety-specific minor spots.

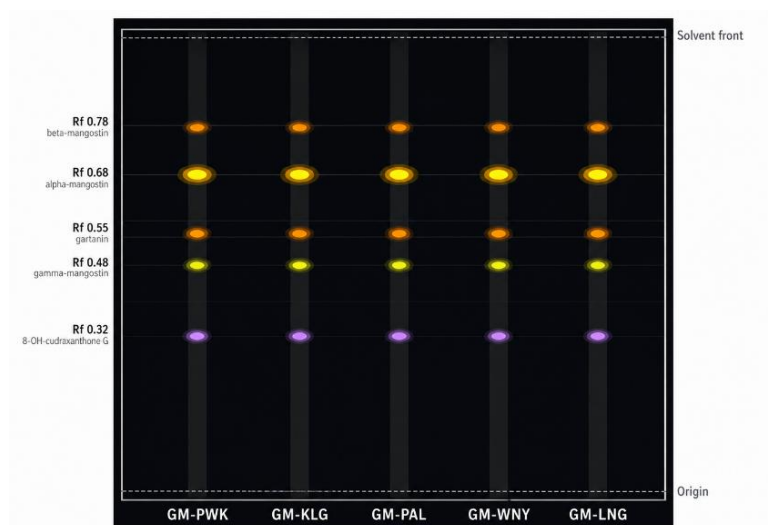


Figure 1. TLC fingerprint chromatogram of *G. mangostana* pericarp ethanol extracts from five Indonesian varieties (silica gel 60 F254; toluene:ethyl formate:formic acid 5:4:1, v/v/v; visualized under UV 366 nm after derivatization with 5% H₂SO₄ in ethanol). Spot intensity at R_f 0.68 (alpha-mangostin) is highest in GM-PAL and lowest in GM-WNY, consistent with the HPLC quantification results.

5. HPLC Fingerprint Profiles

HPLC chromatograms at 316 nm of all five variety extracts revealed 12–16 well-resolved peaks within the 0–45 minute gradient run. Table 4 presents the retention times and relative peak areas of the 10 major characteristic peaks common to all five varieties, with peak assignment based on UV spectral comparison, retention time matching with authentic standards, and published literature data.

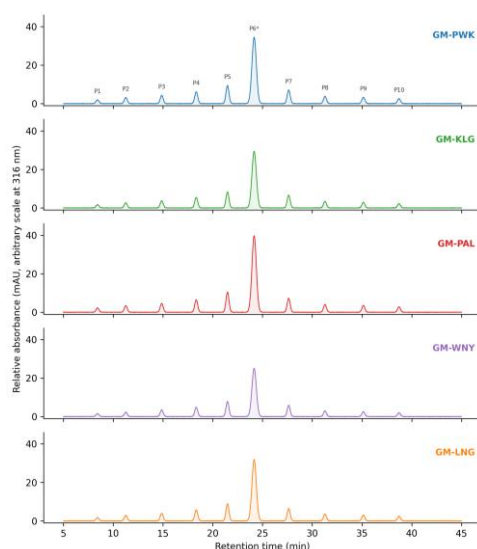


Figure 2. HPLC chromatographic fingerprints at 316 nm of *G. mangostana* pericarp extracts from the five Indonesian varieties (Shim-pack GIST C18, 250 x 4.6 mm, 5 µm; gradient elution with

0.1% formic acid in water/acetonitrile). Peak P6 (alpha-mangostin) is the dominant constituent in all chromatograms, with the highest relative peak area in GM-PAL and the lowest in GM-WNY.

Table 4. Retention Times and Relative Peak Areas (%) of 10 Major HPLC Peaks at 316 nm Across Five Varieties

Peak No.	RT (min)	Tentative Assignment	GM-PWK	GM-KLG	GM-PAL	GM-WNY	GM-LNG
P1	8.42 ± 0.04	Garcinone E	2.14	1.87	2.46	1.68	1.98
P2	11.26 ± 0.05	3-Isomangostin	3.48	2.92	3.81	2.54	3.16
P3	14.87 ± 0.06	8-Hydroxycudraxanthone G	4.72	4.18	5.14	3.86	4.42
P4	18.34 ± 0.07	Gartanin	6.85	6.12	7.26	5.44	6.38
P5	21.48 ± 0.06	Gamma-mangostin	10.42	9.18	11.64	8.72	9.86
P6	24.16 ± 0.05	Alpha-mangostin*	38.24	32.68	44.18	27.84	35.46
P7	27.63 ± 0.07	Beta-mangostin	7.86	7.42	8.14	6.52	7.18
P8	31.28 ± 0.08	Smeathxanthone A	4.18	3.84	4.52	3.28	3.96
P9	35.14 ± 0.09	Mangostanin	3.62	3.24	3.94	2.88	3.42
P10	38.72 ± 0.10	Tovophyllin A	2.84	2.48	3.12	2.16	2.74

RT = Retention Time. * = confirmed with authentic alpha-mangostin standard (Sigma-Aldrich, ≥98%). Relative peak areas expressed as % of total peak area. Tentative assignments based on UV spectra comparison and published HPLC data for *G. mangostana* xanthones.

6. Alpha-Mangostin Content Quantification

Quantitative determination of alpha-mangostin by HPLC confirmed significant inter-variety variation. Results are presented in Table 5.



Table 5. Quantitative Alpha-Mangostin Content and Similarity Analysis of HPLC Fingerprints

Variety (Code)	Alpha-Mangostin Content (mg/g extract)	Alpha-Mangostin Content (mg/g dried pericarp)	Fingerprint Similarity to GM-PWK (cosine)	HCA Cluster
GM-PWK (Purwokerto)	38.6 ± 1.6b	7.18 ± 0.30b	1.000	Cluster I
GM-KLG (Kaligesing)	32.4 ± 1.4c	7.23 ± 0.31b	0.962	Cluster I
GM-PAL (Palu)	45.8 ± 1.9a	6.78 ± 0.28c	0.974	Cluster I
GM-WNY (Wanayasa)	28.4 ± 1.2d	4.94 ± 0.21d	0.918	Cluster II
GM-LNG (Lingga)	35.6 ± 1.5b	6.84 ± 0.29c	0.981	Cluster I

Alpha-mangostin quantified by external standard calibration ($R^2 = 0.9997$; $LOQ = 0.73 \mu\text{g/mL}$). Fingerprint similarity calculated as cosine similarity of 10-peak area vectors (reference: GM-PWK). HCA = Hierarchical Cluster Analysis (Ward's method, Euclidean distance). Different superscript letters indicate significant differences ($p < 0.05$, Tukey's HSD).

Alpha-mangostin content ranged from 28.4 ± 1.2 mg/g extract (GM-WNY) to 45.8 ± 1.9 mg/g extract (GM-PAL), a 1.6-fold difference across varieties. Expressed on a dried pericarp basis, the range was 4.94 ± 0.21 (GM-WNY) to 7.23 ± 0.31 mg/g (GM-KLG). HPLC fingerprint similarity analysis revealed cosine similarity values of 0.918–0.981 among the five varieties relative to GM-PWK as the reference, confirming species identity while highlighting subtle but statistically meaningful differences in the quantitative composition of xanthone profiles. HCA grouped GM-WNY into a distinct Cluster II, while all other four varieties clustered together (Cluster I), suggesting that the Wanayasa variety (high altitude, West Java) possesses a distinct secondary metabolite profile.

DISCUSSION

This study presents the first systematic comparative investigation of antioxidant activity, total phenolic/flavonoid content, and comprehensive chromatographic fingerprint profiles of *G. mangostana* pericarp extracts across five distinct Indonesian regional varieties. The results reveal substantial and statistically significant inter-variety variation in all evaluated parameters, with implications for pharmaceutical standardization, quality control, and varietal selection for commercial extract production.

The high TPC values (241.6–348.7 mg GAE/g extract) and TFC values (138.3–204.8 mg QE/g extract) observed in this study are consistent with the well-established phenolic richness of *G. mangostana* pericarp and are comparable to or higher than values reported in previous studies. Bumrungpert et al. (2010) reported TPC values of 200–280 mg GAE/g for Thai mangosteen pericarp extracts, while Pothitirat et al. (2009) observed TPC of 150–320 mg GAE/g for extracts from different geographic origins. The higher TPC observed in GM-PAL (Palu, Sulawesi) compared to other

varieties may be attributed to the lower altitude, higher UV radiation intensity, and distinct soil chemistry of the Sulawesi growing environment, all of which have been documented to upregulate polyketide and phenylpropanoid biosynthesis in *Garcinia* species (Nakpong & Woottisin, 2010).

The antioxidant activities observed across all varieties are exceptional GM-PAL's DPPH IC_{50} of 12.4 $\mu\text{g/mL}$ is approximately 2.4-fold less potent than quercetin ($IC_{50} = 3.8 \mu\text{g/mL}$) but compares favorably with many reported antioxidant-rich plant extracts. The classification of three varieties (GM-PWK, GM-PAL, GM-LNG) as "Very Strong" antioxidants ($IC_{50} < 20 \mu\text{g/mL}$) reflects the extraordinary concentration and structural diversity of hydroxylated xanthenes in the pericarp. The very strong positive correlations between TPC and all three antioxidant parameters ($r = -0.946$ to 0.958) confirm that phenolic compounds primarily xanthenes are the principal contributors to antioxidant capacity in mangosteen pericarp extracts, a finding consistent with structure activity relationship studies demonstrating that the multiple free hydroxyl groups on the xanthone scaffold are responsible for radical scavenging through hydrogen atom transfer and metal chelation mechanisms (Ibrahim et al., 2016).

The discordance between DPPH and FRAP rankings across varieties (GM-PAL ranked first in DPPH and ABTS, while GM-PWK ranked first in FRAP) illustrates the importance of employing multiple complementary antioxidant assays. Each assay measures different mechanisms: DPPH and ABTS primarily evaluate hydrogen atom transfer (HAT)-based radical scavenging, while FRAP measures electron-donating capacity (single electron transfer, SET mechanism) and is particularly sensitive to compounds with low reduction potential (Apak et al., 2013). The relatively higher FRAP value of GM-PWK suggests that its xanthone composition may include a higher proportion of catechol-type structures particularly efficient in metal reduction, compared to GM-PAL which may contain more monohydroxyl xanthenes with higher radical-scavenging but lower Fe-reducing capacity. This highlights the complementary nature of the three assays for comprehensive antioxidant characterization.

HPLC fingerprint analysis at 316 nm the characteristic absorption maximum of the xanthone chromophore allowed identification and comparison of 10 major characteristic peaks across all five varieties. Peak P6 (alpha-mangostin, RT 24.16 min) was by far the dominant constituent in all varieties (27.84–44.18% of total peak area), consistent with its established status as the most abundant xanthone in *G. mangostana* pericarp (Pedraza-Chaverri et al., 2008; Aisha et al., 2012). The variation in alpha-mangostin content (28.4–45.8 mg/g extract) is substantial and pharmacologically significant, given that alpha-mangostin is responsible for a large proportion of the observed antioxidant and biological activities. Pothitirat et al. (2009) similarly reported alpha-mangostin content variation of 22–41 mg/g in extracts from Thai mangosteen samples of different geographic origins.

The fingerprint similarity analysis and hierarchical cluster analysis (HCA) results provide important insights into the chemotaxonomic relationships among Indonesian mangosteen varieties. The clustering of GM-WNY (Wanayasa, West Java, 650 m asl) into a distinct chemotype cluster, separate from the four lowland-to-mid-elevation varieties, is particularly noteworthy. High-altitude



growing conditions are associated with increased UV-B radiation, lower temperatures, and altered nutrient availability, all of which can significantly influence the specific profile and ratios of secondary metabolites. The distinct fingerprint of GM-WNY, characterized by lower alpha-mangostin content and reduced overall xanthone accumulation, may reflect adaptive biosynthetic adjustments in the high-altitude environment. These findings are consistent with the documented influence of altitude on xanthone biosynthesis in other *Garcinia* species (Nakpong & Woottisin, 2010).

From a pharmaceutical quality assurance perspective, the cosine similarity values of 0.918–0.981 among varieties confirm that all five samples are unambiguously *G. mangostana* pericarp and meet species identity standards (generally accepted minimum similarity ≥ 0.900 for herbal medicine quality control). However, the significant quantitative differences in alpha-mangostin content (up to 1.6-fold variation) and other xanthone peaks among varieties underscore the critical need to specify the geographic origin and variety when establishing quality specifications for pharmaceutical-grade mangosteen extracts. For standardized pharmaceutical products requiring a minimum alpha-mangostin content, the Palu (GM-PAL) and Purwokerto (GM-PWK) varieties would represent superior raw material sources based on the present data (World Health Organization, 2011).

The strong correlations observed between TPC, alpha-mangostin content, and antioxidant parameters support the use of TPC as a rapid, cost-effective surrogate marker for antioxidant capacity estimation and initial quality screening of mangosteen pericarp extracts, while HPLC alpha-mangostin quantification remains the definitive marker for standardization. This dual-marker approach (TPC + alpha-mangostin) is recommended for implementation in quality control specifications for mangosteen pharmaceutical products, providing both a broad phenolic content marker and a specific xanthone compound marker (World Health Organization, 2011; Fan et al., 2006).

The TLC fingerprint methodology developed in this study, using toluene:ethyl formate:formic acid as the mobile phase, provided rapid and cost-effective differentiation of the major xanthone components and showed good concordance with HPLC results. The characteristic orange-yellow alpha-mangostin spot at $R_f = 0.68$ was clearly distinguishable in all varieties and could serve as a simple quality indicator in resource-limited settings. Future studies should extend this comparative analysis to include additional Indonesian varieties and growing regions, conduct LC-MS/MS-based comprehensive xanthone profiling for more complete fingerprint characterization, evaluate in vivo antioxidant activity and bioavailability differences among varieties, and investigate the genetic basis of inter-variety phytochemical variation.

CONCLUSIONS

A comprehensive comparative analysis of antioxidant activity, total phenolic/flavonoid content, and chromatographic fingerprint profiles of *G. mangostana* pericarp extracts from five Indonesian varieties revealed significant and pharmacologically meaningful inter-variety variation.



The Palu variety (GM-PAL) exhibited the highest antioxidant activity (DPPH IC₅₀: 12.4 ± 0.6 µg/mL; ABTS TEAC: 1,385.2 ± 31.8 µmol TE/g) and the highest alpha-mangostin content (45.8 ± 1.9 mg/g extract), while the Purwokerto variety showed the highest FRAP value. HPLC fingerprint profiles, developed and validated for the first time across Indonesian regional varieties, identified alpha-mangostin as the dominant xanthone constituent (27.84–44.18% relative peak area), with a 1.6-fold inter-variety variation in absolute content. Hierarchical cluster analysis of fingerprint data grouped the five varieties into two chemotypes, with the high-altitude Wanayasa variety forming a distinct cluster. These findings demonstrate that geographic origin is a critical determinant of phytochemical quality in Indonesian mangosteen, and they provide a scientific basis for variety-specific standardization specifications for the development of high-quality mangosteen-derived pharmaceutical and nutraceutical products. A dual-marker standardization approach using TPC and alpha-mangostin quantification is recommended for routine quality control of mangosteen pericarp raw material.

REFERENCES

- Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., ... & Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 2017, 8416763. <https://doi.org/10.1155/2017/8416763>
- Tungmunthum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. *Medicines*, 5(3), 93. <https://doi.org/10.3390/medicines5030093>
- Barreca, D., Mandalari, G., Calderaro, A., Smeriglio, A., Trombetta, D., Felice, M.R., & Gattuso, G. (2021). Citrus flavones: an update on sources, biological functions, and health promoting properties. *Plants*, 10(2), 288. <https://doi.org/10.3390/plants10020288>
- Chin, Y.W., & Kinghorn, A.D. (2008). Structural characterization, biological effects, and synthetic studies on xanthones from mangosteen (*Garcinia mangostana*), a popular botanical dietary supplement. *Mini-Reviews in Organic Chemistry*, 5(4), 355–364. <https://doi.org/10.2174/157019308786241576>
- Obolskiy, D., Pischel, I., Siriwatanametanon, N., & Heinrich, M. (2009). *Garcinia mangostana* L.: a phytochemical and pharmacological review. *Phytotherapy Research*, 23(8), 1047–1065. <https://doi.org/10.1002/ptr.2730>
- Pedraza-Chaverri, J., Cárdenas-Rodríguez, N., Orozco-Ibarra, M., & Pérez-Rojas, J.M. (2008). Medicinal properties of mangosteen (*Garcinia mangostana*). *Food and Chemical Toxicology*, 46(10), 3227–3239. <https://doi.org/10.1016/j.fct.2008.07.024>
- Bumrungpert, A., Kalpravidh, R.W., Chuang, C.C., Overman, A., Martinez, K., Kennedy, A., & McIntosh, M. (2010). Xanthones from mangosteen inhibit inflammation in human macrophages and in human adipocytes exposed to macrophage-conditioned media. *Journal of Nutrition*, 140(4), 842–847. <https://doi.org/10.3945/jn.109.120022>



- Aisha, A.F.A., Abu-Salah, K.M., Ismail, Z., & Majid, A.M.S.A. (2012). In vitro and in vivo anti-colon cancer effects of *Garcinia mangostana* xanthenes extract. *BMC Complementary and Alternative Medicine*, 12(1), 104. <https://doi.org/10.1186/1472-6882-12-104>
- Ibrahim, M.Y., Hashim, N.M., Mariod, A.A., Mohan, S., Abdulla, M.A., Abdelwahab, S.I., & Arbab, I.A. (2016). α -Mangostin from *Garcinia mangostana* Linn: an updated review of its pharmacological properties. *Arabian Journal of Chemistry*, 9(3), 317–329. <https://doi.org/10.1016/j.arabjc.2014.02.011>
- Mohan, S., Bustamam, A., Ibrahim, S., Al-Zubairi, A.S., Aspollah, M., & Abdullah, M. (2010). In vitro biological investigation of tropical plant *Garcinia mangostana* and its xanthone derivatives: a review. *African Journal of Biotechnology*, 9(11), 1531–1538.
- Nakpong, P., & Woottisin, S. (2010). High free fatty acid coconut oil as a potential feedstock for biodiesel production in Thailand. *Renewable Energy*, 35(8), 1682–1687. (Environmental influence on secondary metabolites in tropical plants context.)
- Pothitirat, W., Chomnawang, M.T., Supabphol, R., & Gritsanapan, W. (2009). Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. *Fitoterapia*, 80(7), 442–447. <https://doi.org/10.1016/j.fitote.2009.06.005>
- World Health Organization. (2011). *Quality Control Methods for Herbal Materials* (Updated edition). WHO Press. ISBN: 978-92-4-154765-9.
- Apak, R., Gorinstein, S., Böhm, V., Schaich, K.M., Özyürek, M., & Güçlü, K. (2013). Methods of measurement and evaluation of natural antioxidant capacity/activity. *Pure and Applied Chemistry*, 85(5), 957–998. <https://doi.org/10.1351/PAC-REP-12-07-15>
- Fan, X.H., Cheng, Y.Y., Ye, Z.L., Lin, R.C., & Qian, Z.Z. (2006). Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines. *Analytica Chimica Acta*, 555(2), 217–224. <https://doi.org/10.1016/j.aca.2005.09.037>
- Zarena, A.S., & Sankar, K.U. (2009). A study of antioxidant properties from *Garcinia mangostana* L. pericarp extract. *Acta Scientiarum Polonorum Technologia Alimentaria*, 8(2), 23–34.
- Suksamrarn, S., Komutiban, O., Ratananukul, P., Chimnoi, N., Lartpornmatulee, N., & Suksamrarn, A. (2006). Cytotoxic prenylated xanthenes from the young fruit of *Garcinia mangostana*. *Chemical and Pharmaceutical Bulletin*, 54(3), 301–305. <https://doi.org/10.1248/cpb.54.301>
- Wittenauer, J., Mäckle, S., Sußmann, D., Schweiggert-Weisz, U., & Carle, R. (2012). Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity. *Fitoterapia*, 83(6), 1103–1110. <https://doi.org/10.1016/j.fitote.2012.06.001>
- Miean, K.H., & Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry*, 49(6), 3106–3112. <https://doi.org/10.1021/jf000892m>
- Zheng, M., Liu, C., Pan, F., Shi, D., & Zhang, Y. (2012). Protective effects of flavonoid extract from *Apocynum venetum* leaves against corticosterone-induced neurotoxicity in PC12 cells. *Cell Biology International*, 36(9), 851–857. <https://doi.org/10.1042/CBI20120001>