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Research Article

Phytochemical Profiling and *In Vitro* Lipoxygenase Inhibitory Activity of Three Kratom Leaf Varieties by GC–MS, HPLC, and LC–HRMS

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Abstract
Kratom (*Mitragyna speciosa* Korth.) is widely used in Southeast Asia, and vein-color phenotypes (red, white, green) are traditionally and commercially associated with different intended uses and perceived effects. Although phytochemical variation among kratom products has been reported, direct comparison of Kalimantan vein-color phenotypes that links standardized alkaloid metrics to a defined anti-inflammatory enzyme target remains limited. Since 15-lipoxygenase (15-LOX) contributes to the formation of pro-inflammatory lipid mediators and is implicated in chronic inflammatory conditions, its inhibition provides a relevant biochemical endpoint to screen anti-inflammatory potential. This study characterized the alkaloid profiles and mitragynine content of red-, white-, and green-vein kratom leaves from Kalimantan and evaluated their *in vitro* 15-LOX inhibitory activity. GC–MS and LC–HRMS indicated broadly similar alkaloid fingerprints across phenotypes, dominated by mitragynine with accompanying constituents, including 7-hydroxymitragynine, paynantheine and the speciogynine/speciociliatine isomeric pair. Mitragynine content in crude methanolic extracts ranged from 22.96 ± 0.24 to 43.87 ± 0.96 mg g^{-1} extract (white > green > red; $p < 0.0001$), whereas alkaloid extracts contained 177.44 ± 0.70 to 258.21 ± 1.00 mg g^{-1} extract (red > green > white; $p < 0.0001$). In the 15-LOX assay, baicalein showed the strongest inhibition (IC_{50} = 9.72 ± 0.07 µg mL^{-1}), followed by mitragynine (28.38 ± 0.39 µg mL^{-1}) and alkaloid extracts (red: 53.01 ± 0.39; green: 56.29 ± 0.79; white: 59.91 ± 0.92 µg mL^{-1}), while crude methanolic extracts exhibited weak activity (IC_{50} > 100 µg mL^{-1}). Overall, Kalimantan vein-color phenotypes share a common monoterpenoid indole alkaloid core profile but differ quantitatively in mitragynine levels and 15-LOX inhibitory potency, supporting further mechanistic and translational investigations.

Keywords: anti-inflammatory; kratom alkaloids; lipoxygenase; *Mitragyna speciosa*; mitragynine

1. INTRODUCTION

Kratom (*Mitragyna speciosa* Korth., Rubiaceae) is a tropical tree native to Southeast Asia and has long been used in traditional medicine in Indonesia, Thailand, Malaysia, the Philippines, Vietnam, and Papua New Guinea [1]. It is known by various vernacular names, including “kratom” in Thailand, “kadamba” in Indonesia, “ketum” and “biak” in Malaysia, “giam” in Vietnam, and “mambog” in the Philippines [2]. The plant typically grows in warm, humid habitats such as riverbanks, freshwater swamp forests, and peat soils [3]. These ecological conditions are characteristic of the Kalimantan region of Indonesia, making it an important area for the natural distribution and cultivation of kratom.

Historically, *Mitragyna speciosa* leaves have been widely used in ethnomedicine across Southeast Asia. In Thailand, fresh leaves are traditionally chewed by laborers to combat fatigue and enhance endurance during manual work, whereas they are commonly brewed as a tea to relieve pain, tiredness, and diarrhea in Malaysia and West Kalimantan. Kratom preparations have also been used in attempts to alleviate symptoms of opioid dependence and as a natural alternative to sedatives. In Thai traditional medicine, kratom has been used for the management of cough, diarrhea, hyperglycemia, and pain [4][5]. These diverse traditional applications suggest the presence of pharmacologically active secondary metabolites in kratom leaves.

Phytochemical studies have shown that *Mitragyna speciosa* contains terpenoids, flavonoids, and a rich array of indole and oxindole alkaloids [6]-[11]. Mitragynine and its oxidized derivative, 7-hydroxymitragynine are recognized as major active constituents, with 7-hydroxymitragynine reported to exhibit markedly

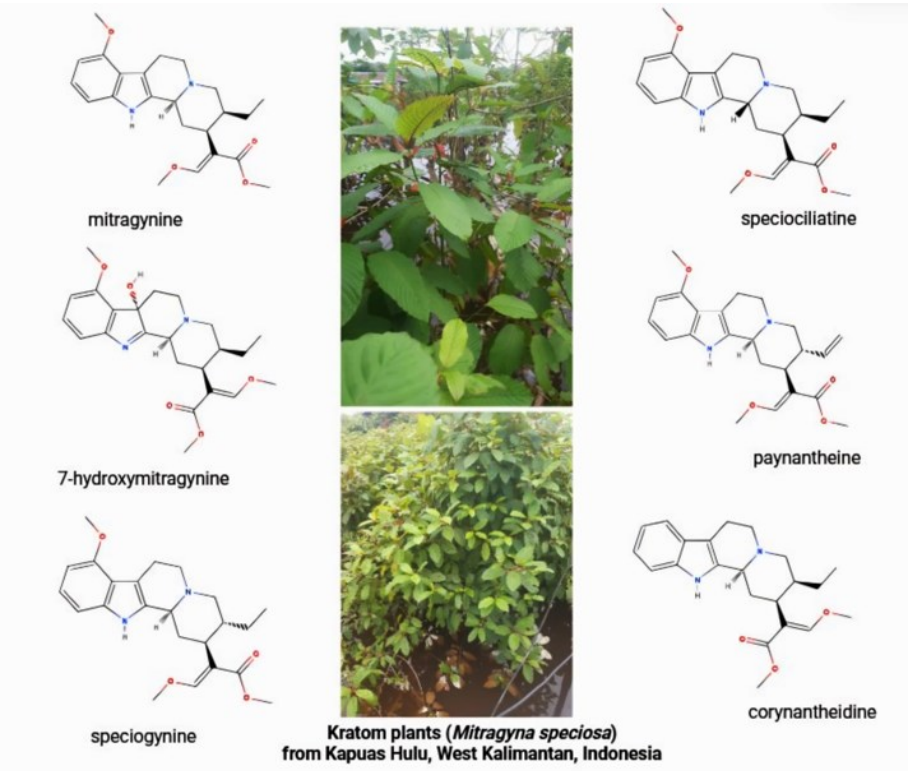


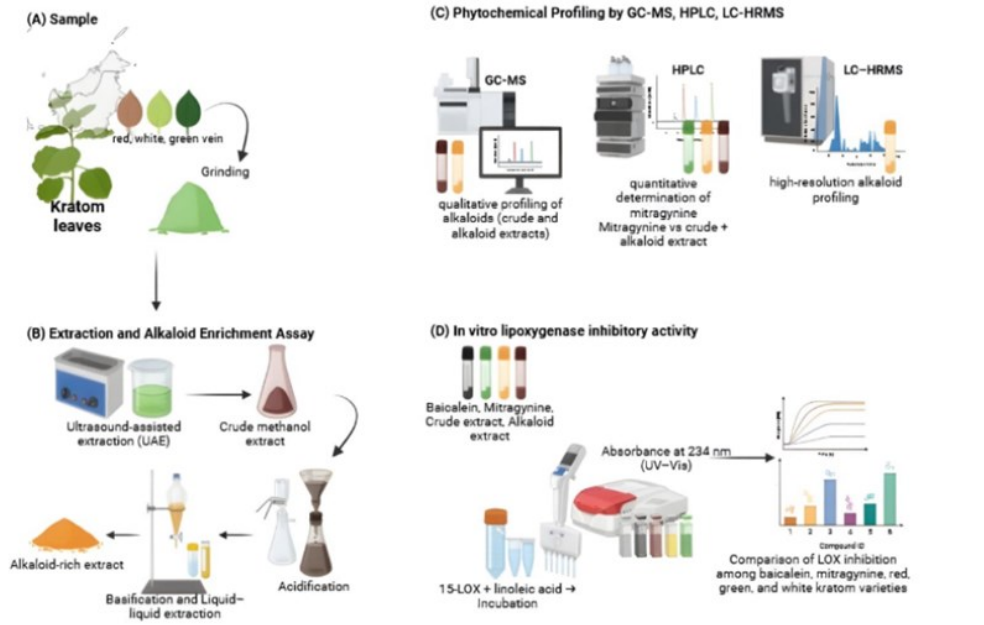
Figure 1. Mitragyna speciosa and major indole alkaloids.

higher analgesic potency than mitragynine and even morphine [12]. Numerous additional alkaloids, such as ajmalicine, paynantheine, speciogynine, isopaynantheine, speciociliatine, mitraciliatine, and several oxindole alkaloids, have also been identified, underscoring the chemical complexity of this species [6][9]. The plant morphology and representative structures of the major indole alkaloids found in *Mitragyna speciosa* are illustrated in Figure 1. The quantitative composition of these alkaloids varies according to genetic background and environmental conditions. For instance, mitragynine levels in Thai specimens are approximately 66% higher than those from Malaysia [13], whereas kratom from Kalimantan, Indonesia, exhibits relatively higher mitragynine concentrations, reaching up to 5.03% in crude extract and 45.86% in alkaloid fractions [14]-[16]. This high alkaloid content highlights the pharmacological potential of Indonesian kratom and justifies further scientific investigation.

Kalimantan kratom also exhibits notable phenotypic variation in leaf vein coloration, which underlies the commonly used red-, green-, and white-vein labels [17]-[19]. Importantly, this vein-color categorization reflects a morphological phenotype rather than a formal taxonomic separation. Therefore, any biochemical differences are plausibly expected to be predominantly quantitative, i.e., shifts in the relative abundance of shared alkaloids and co-metabolites, rather than strictly qualitative presence or absence of the major kratom alkaloids. Users frequently report distinct subjective profiles: red-vein products are often described as more sedative, green-vein as more "balanced," and white-vein as relatively stimulating [20]. In Kalimantan communities, however, red varieties are sometimes used to improve stamina while white varieties are preferred to relieve muscle pain [21]. This apparent mismatch likely reflects context-dependent factors such as dose, preparation practices, and user expectations, underscoring the need for standardized chemical and bioactivity comparisons rather than relying on vein-color narratives alone. However, despite these strong empirical preferences and the widespread use of vein-color terminology, the scientific basis for the perceived differences among kratom varieties remains unclear, and the relationships between varietal identity, alkaloid composition, and biological activity have not been systematically characterized.

Inflammation is a complex, coordinated response of the immune system to infection, injury, or metabolic disturbance [22]. A major component of inflammatory signaling is the arachidonic acid cascade, in which cyclooxygenases (COX) and lipoxygenases (LOX) generate bioactive lipid mediators that regulate inflammatory initiation and resolution [23][24]. LOX comprises several isoenzymes (e.g., 5-LOX, 12-LOX, and 15-LOX) that are classified by their positional oxygenation of polyunsaturated fatty acids. Among these, 15-LOX (human ALOX15) catalyzes lipid hydroperoxide formation that feeds into mediator networks (e.g., 15-HETE and pro-resolving lipid mediators) relevant to inflammatory dynamics. Dysregulation of 15-LOX/ALOX15 has been linked to oxidative lipid signaling and tissue injury pathways and has been implicated in chronic inflammatory and metabolic disorders, including arthritis, asthma/airway inflammation, atherosclerosis, diabetes, and cancer [25]-[28]. In this context, 15-LOX inhibition provides a mechanistically informative screening endpoint for anti-inflammatory potential and is relevant to kratom's traditional use for pain and inflammation-related discomfort, where modulation of lipid-oxidation pathways may contribute to symptom relief. While COX/5-LOX assays are widely used in anti-inflammatory screening, 15-LOX remains comparatively underexplored for kratom, and focusing on this target allows a clearer assessment of whether vein-color-associated chemical variation translates into LOX-pathway modulation.

Several lines of evidence indicate that *Mitragyna speciosa* and its alkaloids possess anti-inflammatory potential. *In vivo* studies have shown that crude and alkaloid extracts reduce edema and modulate inflammatory markers in acute inflammation models [29][30]. *In vitro* and *in silico* studies further suggest that mitragynine and selected kratom alkaloids inhibit COX-2, reduce nitric oxide and proinflammatory cytokines, and modulate NF-κB–related signaling pathways [11][31]-[34]. Notably, purified mitragynine was reported to suppress COX-2 expression (mRNA/protein) and reduce PGE_2 in a dose-responsive manner with minimal impact on COX-1 at lower concentrations [31], and an alkaloid-enriched kratom fraction has been reported to inhibit COX-2/5-LOX more effectively than the corresponding crude extract [11]. These findings support the view that kratom alkaloids may act as multi-target modulators of inflammatory pathways. However, direct experimental evidence for kratom and



(A) Collection of red, green, and white vein Mitragyna speciosa leaves from Kalimantan. (B) Ultrasound-assisted extraction with methanol followed by acid–base liquid–liquid extraction to obtain crude and alkaloid extracts. (C) Phytochemical profiling of extracts using GC–MS, HPLC, and LC–HRMS for qualitative alkaloid screening and quantitative mitragynine determination. (D) In vitro 15-lipoxygenase inhibitory activity of baicalein, mitragynine, and kratom extracts

Figure 2. Schematic workflow of the study.

Table 1. Yields of crude methanolic extract (CME) and alkaloid extract (AE) from red-, white-, and green-vein Mitragyna speciosa leaves obtained by methanol/acid–base extraction.

Sample	Unit	Red	White	Green	P-value (ANOVA)
Crude Methanolic Extract	%	29.41 ± 0.33	28.48 ± 0.17	34.71 ± 0.25	<0.0001
Alkaloid Extract	%	5.43 ± 0.09	7.52 ± 0.13	8.42 ± 0.12	<0.0001

Data are presented as mean ± SD (n = 3), expressed as % (w/w) on a dry leaf basis. For each extract type (CME or AE), yields differed significantly among vein-color varieties (one-way ANOVA, $p < 0.0001$), and Tukey's post hoc test indicated that all pairwise comparisons among varieties were significant ($p < 0.05$).

mitragynine activity on 15-LOX and systematic comparisons across vein-color phenotypes remain limited.

To address this gap, the present study provides a comparative phytochemical and bioactivity evaluation of three kratom vein-color phenotypes (red, green, and white) from Kalimantan. Qualitative alkaloid profiling was conducted using gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–high-resolution mass spectrometry (LC–HRMS), while mitragynine quantification employed validated HPLC–PDA methods. Anti-inflammatory potential was further assessed using an in vitro 15-LOX inhibition assay as a mechanistically relevant screening model. To the best of our knowledge, this is the first study to compare 15-LOX inhibitory activity alongside comprehensive alkaloid profiling and mitragynine quantification across these three vein-color phenotypes from Kalimantan.

2. MATERIALS AND METHODS

2.1. Materials

Leaves of *Mitragyna speciosa* (red, white, and green-vein varieties) were obtained from local kratom farmers in the Jongkong cultivation area, Kapuas Hulu Regency, West Kalimantan, Indonesia, during January–February 2025. The plant material represented harvested leaves collected from cultivated trees within the farming area and was subsequently processed as composite samples for each vein-color variety. The plant material was taxonomically identified as *Mitragyna speciosa* Korth. by the Directorate of Scientific Collection Management, National Research and Innovation Agency (BRIN), Republic of Indonesia (reference no. B-2377/II.6.2/IR.01.02/6/2025). A voucher sample of the dried leaf material was retained and deposited in the Phytochemistry and Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Indonesia (Depok, Indonesia), for future reference. Dried leaf material was powdered and stored in airtight containers at room temperature until extraction.

The chemicals included Methanol (Sigma-Aldrich, USA), chloroform (Smartlab, Indonesia), *n*-hexane (Smartlab, Indonesia), ammonia solution (Smartlab, Indonesia), acetic acid (Sigma-Aldrich, USA), and acetonitrile (HPLC grade, Sigma-Aldrich, USA) were used for extraction and chromatographic analyses. For the lipoxygenase inhibition assay, linoleic acid (Sigma-Aldrich, USA) and lipoxygenase from *Glycine max* (soybean; Sigma-Aldrich, USA) were employed. Mitragynine standard (97.88% purity, HPLC; Markherb, Indonesia) and baicalein standard (96.19% purity, HPLC; Markherb, Indonesia) were used as reference compounds. All reagents were of analytical or HPLC grade and were used without further purification.

Instrumental analyses were carried out using an HPLC system with photodiode array detector (HPLC–PDA; Waters Arc™), a gas chromatography–mass spectrometry system (GC–MS; Agilent Technologies 8890 GC coupled to a 5977C MSD), a liquid chromatography–high-resolution mass spectrometry system (LC–HRMS; Thermo Fisher Scientific Orbitrap Exploris 120), and a UV–visible spectrophotometer (Thermo Fisher Scientific).

2.2. Methods

2.2.1. Preparation of Methanolic and Alkaloid Extract

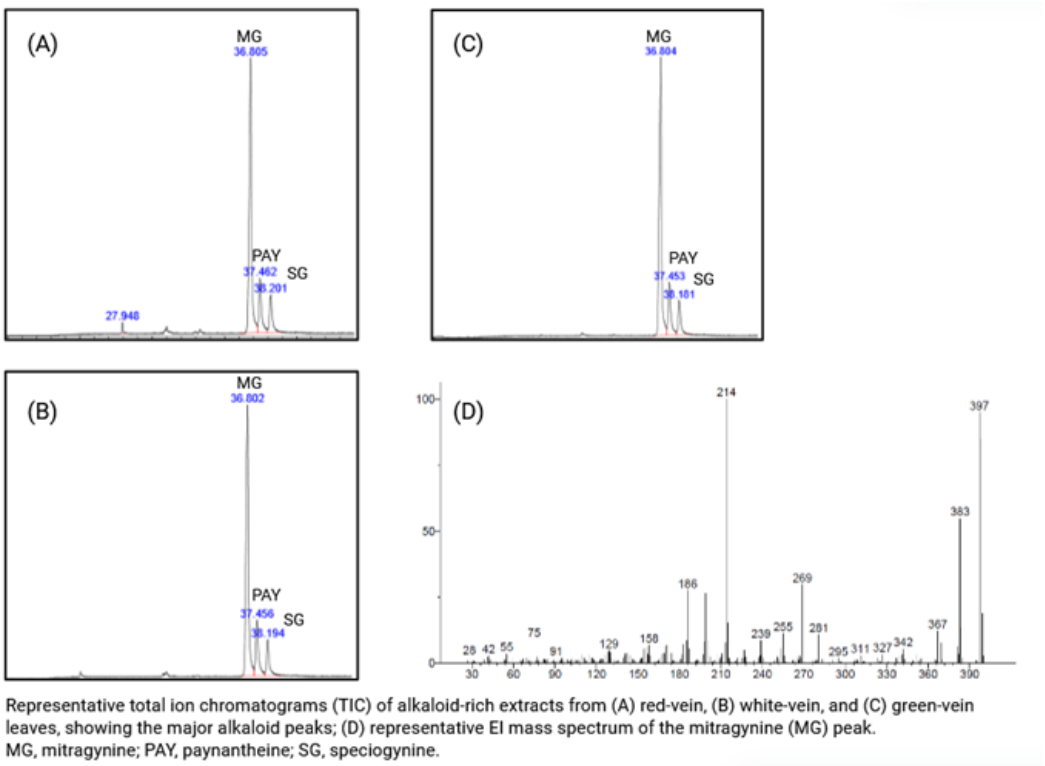
Dried *Mitragyna speciosa* leaf powder from each variety (red, green, and white) was extracted by

ultrasound-assisted extraction using absolute methanol (100%) in an ultrasonic bath (40 kHz; rated ultrasonic power 180 W; heating power 200 W). The extraction was performed at 50 °C for 3 h with a solvent-to-sample ratio of 10:1 (v/w), using the maximum power setting. The resulting extracts were filtered, and the combined filtrates were concentrated under reduced pressure using a rotary evaporator at 45 °C to obtain the crude methanolic extracts (CME). Extraction yield of the methanolic crude extract was calculated on a dry-weight basis relative to the initial mass of dried leaf powder for each vein-color variety and expressed as percentage (w/w). Alkaloid extraction was subsequently carried out from the crude methanolic extracts following the liquid–liquid extraction procedure described by Bayu et al. with slight modifications [15]. Briefly, the methanolic extract was dissolved and acidified with 10% (v/v) acetic acid, then successively extracted with n-hexane to remove non-polar impurities. The aqueous acidic phase was then basified with ammonia solution and extracted with chloroform. The chloroform layer was separated, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to yield the alkaloid extract (AE). Alkaloid extract yield was calculated relative to the mass of methanolic crude extract subjected to the acid–base partitioning (w/w) and expressed as percentage. When required for cross-comparison, the overall alkaloid yield can also be reported on a dry-leaf basis by multiplying the crude-extract yield by the alkaloid-extract yield fraction (Figure 2).

2.2.2. Chromatographic Analysis

2.2.2.1. GC–MS Analysis

GC–MS analysis of crude methanolic and alkaloid extracts was performed using an Agilent 8890 GC system equipped with a 5977C mass selective detector and an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Crude methanolic and alkaloid extracts were dissolved in a methanol:chloroform mixture (4:1, v/v) prior to analysis. Helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. Samples were injected in splitless mode (injection volume: 1 µL). The oven temperature program was set to 50 °C, ramped and held at 280 °C. Mass spectra were recorded in electron ionization (EI) mode, and



Representative total ion chromatograms (TIC) of alkaloid-rich extracts from (A) red-vein, (B) white-vein, and (C) green-vein leaves, showing the major alkaloid peaks; (D) representative EI mass spectrum of the mitragynine (MG) peak.
MG, mitragynine; PAY, paynantheine; SG, speciogynine.

Figure 3. GC–MS chromatographic profiles of alkaloid extracts from three vein-color varieties of Mitragyna speciosa.

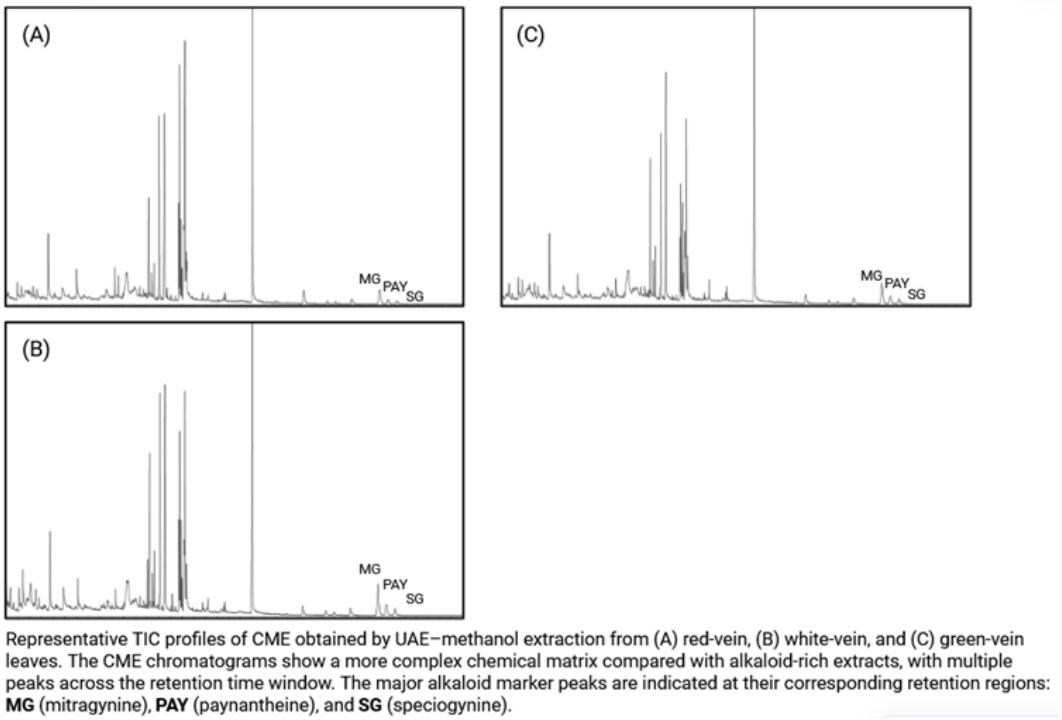


Figure 4. GC–MS total ion chromatograms (TIC) of crude methanolic extracts (CME) from three vein-color varieties of Mitragyna speciosa.

chromatographic peaks were evaluated based on retention times and their mass spectral fragmentation patterns (m/z). Tentative compound identification was carried out by comparing mass spectra with the NIST library, and assignments were accepted when the library match factor was ≥ 99 and consistent with literature data.

2.2.2.2. LC–HRMS Analysis

LC–HRMS analysis of alkaloid extracts was performed using a Thermo Scientific Orbitrap Exploris 120 mass spectrometer coupled to a ZORBAX Eclipse Plus column (2.1 × 100 mm, 1.8 µm). The mobile phases consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile, using a gradient elution program as follows: 0–1 min, 95% A/5% B; 1–16 min, linear gradient to 5% A/95% B; 16–20 min, 5% A/95% B; 20–25 min, linear return to 95% A/5% B; and 25–30 min, 95% A/5% B for re-equilibration. The injection volume was 10 µL, and the total run time was 30 min. Mass spectrometric detection was carried out using electrospray ionization (ESI) in positive mode over an m/z range of 50–750. Putative identification of alkaloids was based on accurate mass measurements, isotopic pattern matching, and comparison against spectral libraries and literature data.

2.2.2.3. HPLC–PDA Quantification of Mitragynine

Quantitative analysis of mitragynine in crude methanolic and alkaloid extracts was performed using an Arc™ HPLC system (Waters, USA) equipped with a photodiode array (PDA) detector. Chromatographic separation was achieved on a Zorbax Eclipse Plus C18 column (250 × 4.6 mm, 5 µm) maintained at 35 °C. The mobile phases consisted of (A) 10 mM ammonium formate in water and (B) acetonitrile, delivered at a flow rate of 1.0 mL min^{-1} under gradient elution. The gradient program was set as follows: 0.01–8.00 min, 55% A/45% B; 8.01–15.00 min, linear gradient to 45% A/55% B; and 15.01–20.00 min, 45% A/55% B. The system was then returned to the initial composition (55% A/45% B) and equilibrated prior to the next injection (re-equilibration). The injection volume was 10 µL, and detection was performed at 260 nm.

2.2.2.4. HPLC–PDA Method Validation

The HPLC–PDA method for mitragynine included assessment of linearity/range, LOD, LOQ, accuracy, and precision. A mitragynine stock solution (1.0 mg mL^{-1}) was prepared by dissolving

2 mg of standard in 2 mL of methanol. Working standard solutions were prepared by serial dilution to obtain six concentration levels in the range of 6.25–200 µg mL^{-1}. Each level was injected in triplicate and linearity was evaluated by linear regression (peak area vs concentration; r^2). LOD and LOQ were estimated using the calibration-curve approach based on the standard deviation of response (σ) and slope (S): LOD = 3.3σ/S and LOQ = 10σ/S. Accuracy was determined by standard-addition recovery at three spiking levels (6.25, 25, and 100 µg mL^{-1}). Precision was evaluated as intra-day repeatability and inter-day intermediate precision at the same levels and expressed as % RSD. Mitragynine content in the samples was calculated from the calibration curve and expressed as mg g^{-1} extract (dry weight basis).

2.2.3. Lipoxygenase Inhibition Assay and IC_{50} Determination

The in vitro lipoxygenase (LOX) inhibition assay was performed according to Listiyani et al. [35] and the EC 1.13.11.12 protocol with minor modifications. Test samples comprised crude methanolic extracts (CME), alkaloid extracts (AE), mitragynine (reference alkaloid), and baicalein (positive control). Stock solutions were prepared in methanol and diluted with borate buffer (0.2 M, pH 9.0) to generate dose–response series, while maintaining the same final methanol concentration across all wells (samples and controls) by adjusting solvent volume. The reaction mixture contained borate buffer, linoleic acid (3 mM) as substrate, and LOX enzyme solution (5000 U mL^{-1}). Samples were pre-incubated with buffer and substrate, the reaction was initiated by enzyme addition, and the mixture was incubated at 25 °C. The reaction was terminated by methanol addition, and the formation of conjugated dienes was monitored at 234 nm using a UV–visible spectrophotometer. Dose–response curves were constructed from five final concentrations for each sample type: 12.5–125 µg mL^{-1} for CME; 12.5–62.5 µg mL^{-1} for AE; 3.125–62.5 µg mL^{-1} for mitragynine; and 2.5–18.75 µg mL^{-1} for baicalein. Percentage inhibition was calculated from absorbance-corrected values relative to the blank, and IC_{50} values were obtained by nonlinear regression using a four-parameter logistic model (variable slope) in GraphPad Prism (version 10).

2.2.4. Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, USA). All experiments were conducted in triplicate, and data are presented as mean ± standard deviation (SD). Data distribution was assessed for normality prior to inferential analysis. Differences among kratom varieties and sample types were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSIONS

3.1. Characteristics and Yields of Kratom Extracts

Ultrasound-assisted extraction (UAE) with methanol generated CME yields that were significantly influenced by vein-color variety, with the green-vein material providing the highest extractable methanol-soluble fraction (Table 1). Relative to prior sonication-based UAE reports, the CME recovery obtained in the present work was higher. For example, a previous UAE–methanol

Table 2. Major alkaloids identified in alkaloid extracts of red, white, and green vein Mitragyna speciosa leaves by LC–HRMS.

Compound	$[M+H]^+$ (m/z)	RT (min)	Detected in
Mitragynine	399.23	8.10–8.12	Red, White, Green
7-Hydroxymitragynine	415.22	7.65–8.37	Red, White, Green
Speciociliatine/Speciogynine*)	399.23	8.48–8.88	Red, White, Green
Paynantheine	397.21	9.01–9.18	Red, White, Green
Rhynchophylline	385.21	7.06–7.20	Red, Green

*) Speciogynine and speciociliatine were not chromatographically resolved under the applied LC conditions and are reported as an isomeric pair.

Table 3. Validation parameters for the HPLC–PDA method used for mitragynine quantification.

Parameter	Unit	Level	Result
Linearity (r^2)	–	–	0.9982
Concentration range	μg mL^{-1}	–	6.25–200
Regression equation	y = ax + b	–	y = 6676.8x + 29351
Limit of detection (LOD)	μg mL^{-1}	–	0.97
Limit of quantification (LOQ)	μg mL^{-1}	–	2.92
Accuracy (recovery, intra-day)*)	%	100	99.99
		25	100.67
		6.25	99.65
Precision (intra-day, %RSD) *)	%	100	1.60
		25	1.47
		6.25	2.57
Inter-day precision (RSD) *)	%	100	0.50
		25	1.07
		6.25	0.76

*) Values are means of replicate measurements at each spiking level.

study reported a dry extract yield of 19.2 ± 2.2% under its operating conditions [36][37], whereas another sonication-based study reported yields of 18.54–22.91% across green, red, and white variants [36][37]. Such cross-study differences are expected because extraction yield is sensitive to operational parameters (e.g., sonication power/intensity, temperature, extraction time, solvent-to-solid ratio, and instrument capacity) as well as biological variability, including geographic origin, harvest time, and post-harvest handling [16][38].

Alkaloid extracts were subsequently prepared from the crude methanolic extracts by acid–base liquid–liquid extraction. In this procedure, the basic alkaloids are first converted into water-soluble salts under acidic conditions, enabling separation from nonpolar constituents, and then recovered in their free-base form by basification and extraction into an organic solvent [39]. Following crude extraction, acid–base liquid–liquid partitioning was applied to concentrate basic alkaloids into the AE. The AE yields also differed significantly among varieties and again showed the highest recovery in the green-vein samples, indicating that the higher CME recovery for green material translated into a greater recoverable alkaloid-enriched fraction. Importantly, the AE yields achieved here were higher than those commonly reported for simpler workflows. For instance, an earlier report described an alkaloid extract yield of 3.27 ± 0.11% [15].

Prior to extraction, the leaf powder of the red vein variety exhibited a slightly reddish-brown color, whereas the green and white vein powders appeared greenish-brown with different color intensities, in agreement with previous reports [18][40]. After UAE with methanol, the crude extracts showed distinct visual differences among varieties. The red vein crude extract formed a reddish-brown powder, while the white and green vein crude extracts appeared brownish-green with varying intensity. In contrast, the alkaloid extracts obtained from all three varieties displayed a more uniform orange-brown color, reflecting the enrichment of alkaloid constituents.

3.2. GC–MS Profiling of Alkaloid Extracts

Targeted GC–MS analysis was carried out on the alkaloid extracts of red, white, and green vein *Mitragyna speciosa* to confirm the presence of the major indole alkaloids commonly reported in kratom. Under the conditions employed, across all three vein-color varieties, the chromatograms exhibited three dominant peaks with closely similar retention times and highly consistent diagnostic ions (Figure 3). These peaks were tentatively identified as mitragynine, paynantheine, and

speciogynine based on NIST library matching and agreement with fragmentation patterns reported in the literature. The spectra of these compounds showed recurring fragment ions in the m/z 397–398 range, together with prominent ions at m/z 383, 269, 214, 200, and 186, which are consistent with reported EI fragmentation of kratom indole alkaloids. The close agreement in retention time and ion patterns between the sample chromatograms and the corresponding NIST library spectra further supported the assignments of these three marker alkaloids.

In all alkaloid extracts, mitragynine appeared as the most intense peak among the three, confirming its role as the dominant alkaloid in *Mitragyna speciosa* leaves. This observation is consistent with multiple previous studies that have identified mitragynine as the primary indole alkaloid and a key contributor to the pharmacological profile of kratom [31][41]. The consistent detection of paynantheine and speciogynine in all three varieties also aligns with earlier GC–MS-based investigations reporting these three compounds as reliable marker peaks in kratom alkaloid fingerprints [8][29][42].

Together, these targeted GC–MS results demonstrate that red, white, and green vein kratom from Kalimantan share a qualitatively similar alkaloid pattern in their alkaloid extract, dominated by mitragynine with paynantheine and speciogynine as co-occurring indole alkaloids. This supports the view that differences among vein-color varieties are more likely to be quantitative (relative abundance of each alkaloid) rather than qualitative (presence or absence of the main kratom alkaloids) and provides a robust chromatographic basis for using these three compounds as chemical markers in future standardization and quality control efforts.

3.3. GC–MS Profiling of Crude Methanolic Extracts

GC–MS analysis of the crude methanolic extracts (CME) from red, white, and green vein *Mitragyna speciosa* revealed a markedly more complex chromatographic pattern than that of the alkaloid extract, reflecting the presence of diverse co-extracted metabolites (Figure 4). Numerous peaks with varying intensities were observed and were tentatively assigned to several major

Table 4. Mitragynine content in crude methanolic and alkaloid extracts of red, white, and green vein Mitragyna speciosa leaves.

Sample	Unit	Variety			P-value (ANOVA)
		Red	White	Green	
Crude methanolic extract (CME)	mg g^{-1} extract	22.96 ± 0.24^{c}	43.87 ± 0.96^{a}	31.08 ± 0.95^{b}	<0.0001
Alkaloid extract (AE)	mg g^{-1} extract	258.21 ± 1.00^{d}	177.44 ± 0.70^{f}	243.13 ± 0.25^{e}	<0.0001
Enrichment factor (AE/CME)		11.25	4.04	7.82	

Values are mean ± SD (n = 3). Within each row, different superscript letters indicate significant differences among varieties (one-way ANOVA followed by Tukey's post hoc test, $p < 0.05$); a–c are used for CME and d–f for AE. Enrichment factor was calculated as the ratio of mean mitragynine contents (AE/CME) for each variety.

compound classes, including indole alkaloids, fatty acids and their esters, terpenoids, sterols, and semi-volatile constituents, based on NIST library matching and fragmentation patterns consistent with literature reports. The major kratom indole alkaloids (mitragynine, paynantheine, and speciogynine) were detected in all three crude extracts, indicating that these marker alkaloids are already present at the crude methanol stage prior to alkaloid enrichment. In addition, several lipid-related components were observed, including fatty acids/esters (e.g., tetradecanoic acid, *n*-hexadecanoic acid, methyl hexadecanoate, methyl linolenate, linolenic acid, and octadecanoic acid) and the diterpene-derived compound neophytadiene, representing typical membrane-associated constituents of leaf matrices. Terpenoid and sterol derivatives (e.g., squalene, tocopherol, and phytosterols such as campesterol, stigmasterol, and β-sitosterol) were also detected. The occurrence of β-sitosterol and stigmasterol is consistent with previous reports on *Mitragyna* species, where these phytosterols frequently occur as major non-alkaloid constituents [43].

Several semi-volatile compounds commonly reported as pigment- and lipid-derived volatiles (e.g., hydroxydihydromaltol, trans-β-ionone, dihydroactinidiolide, and loliolide) were additionally observed in the crude extracts, consistent with the photosynthetic nature of leaf material. Overall, these findings indicate that CME comprises a mixed matrix of alkaloids together with abundant non-alkaloid constituents, in agreement with prior reports describing *Mitragyna speciosa* leaves as containing terpenoids and other non-alkaloid metabolites in addition to indole alkaloids [44]. The chromatographic contrast between CME and AE, diverse multi-class peaks in the former versus alkaloid-dominated profiles in the latter, highlights the effectiveness of acid–base extraction in enriching basic alkaloids while reducing co-extracted non-alkaloid constituents. Moreover, the broadly comparable qualitative CME profiles across the three vein-color varieties suggest that differences among them are more likely driven by relative abundance patterns rather than the exclusive presence or absence of major metabolite classes.

3.4. LC–HRMS Characterization of Alkaloid Extracts

High-resolution LC–MS was employed to more accurately characterize the alkaloid composition of the alkaloid extracts, including constituents that may be difficult to detect by lower-resolution techniques. Separation on a C18 column enabled chromatographic differentiation of several monoterpenoid indole alkaloids based on polarity and interaction with the stationary phase. However, some isomeric or isobaric alkaloids could not be fully resolved under the applied conditions, which is relevant for kratom matrices rich in structurally related MIAs. Subsequent HRMS detection provided accurate $[M+H]^+$ values and diagnostic fragments to support putative structural assignments. Particular attention was given to the detection of 7-hydroxymitragynine, an oxidative metabolite of mitragynine that, although typically present at low levels, is known to exhibit stronger pharmacological potency at opioid receptors [41][45]-[47].

As summarized in Table 2, the LC–HRMS profiles of alkaloid extracts from red, white, and green vein *Mitragyna speciosa* revealed a qualitatively similar set of major alkaloids. Mitragynine was detected in all varieties with a protonated molecular ion at m/z 399.227 and a retention time (RT) of approximately 8.1–8.2 min, and it consistently represented the dominant peak. 7 -Hydroxymitragynine was observed at m/z 415.222 with an earlier RT (7.6–8.4 min), consistent with its higher polarity. Speciogynine and speciociliatine share the same $[M+H]^+$ (m/z 399.227) and, under the current chromatographic conditions, were not baseline-resolved. Therefore, they are reported as an isomeric pair eluting at RT 8.48–8.88 min across all varieties. Paynantheine ($[M+H]^+$ m/z 397.212, RT 9.0–9.2 min) was likewise detected in all varieties. In contrast, rhynchophylline ($[M+H]^+$ m/z 385.212, RT 7.0–7.3 min) was detected in the red and green extracts but was not observed in the white vein sample. Overall, this qualitative profile agrees with prior LC–MS studies reporting mitragynine, 7-hydroxymitragynine, speciogynine/ speciociliatine, paynantheine, and rhynchophylline among characteristic kratom leaf alkaloids [48][49]. The LC–HRMS spectrum of mitragynine showed a dominant $[M+H]^+$ ion at m/z 399.227 and

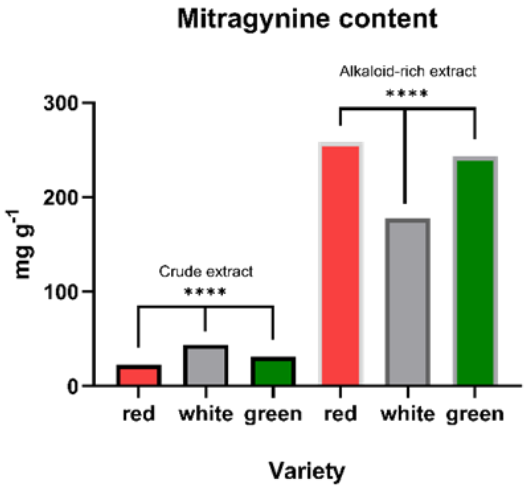


Figure 5. Mitragynine content comparison in crude methanolic and alkaloid extracts of red, white, and green vein Mitragyna speciosa leaves.

characteristic product ions at m/z 174, 226, and 238, consistent with reported fragmentation behavior of monoterpenoid indole alkaloids [6][49]. Collectively, the accurate mass and fragmentation data provided orthogonal support for the putative identification of the major kratom alkaloids in the alkaloid fractions.

From a biosynthetic perspective, the detected compounds fall within the monoterpenoid indole alkaloid (MIA) family derived from tryptamine and secologanin via strictosidine [42][50][51]. Variation in enzyme expression along this pathway, together with environmental influences (e.g., light, temperature, humidity, and soil conditions), may modulate the relative proportions of individual alkaloids among samples [42][49]. The absence of rhynchophylline in the white-vein extract may reflect concentrations below the detection limit under the current conditions, reduced ionization efficiency or matrix-related ion suppression in ESI, co-elution with nearby components, or biological variability (chemotype and/or growing conditions). Accordingly, non-detection in this LC–HRMS screening should not be interpreted as absolute absence and would benefit from targeted confirmation using optimized chromatographic conditions and/or MS/MS-based quantification. Post-harvest handling may further contribute to the observed profiles, particularly for oxygenated metabolites such as 7-hydroxymitragynine, which may originate from a combination of endogenous formation and oxidation during processing and storage [49][52][53]. Thus, the LC–HRMS profiles of the three vein-color varieties reflect integrated genetic, environmental, and post-harvest influences on MIA metabolism and provide a chemical basis for interpreting differences in biological activity observed among the extracts.

3.5. HPLC–PDA Method Validation for Mitragynine Determination

HPLC–PDA is the most widely adopted technique for quantitative determination of mitragynine in *Mitragyna speciosa*, owing to its high separation efficiency, accuracy, and reproducibility, and has become a reference method in phytochemical and quality control studies of kratom extracts [16][36][54]-[56]. In this study, the developed HPLC–PDA method was validated for quantifying mitragynine in both crude methanolic and alkaloid extracts of the three kratom varieties (Table 3).

The calibration curve was linear over 6.25–200 µg mL^{-1} with r^2 = 0.9982 (y = 6676.8x + 29351). This linear relationship indicates that the detector response is directly proportional to mitragynine concentration within the working range, allowing this interval to be defined as the reportable range for quantitative analysis. The method showed good sensitivity with an LOD of 0.9717 µg mL^{-1} and LOQ of 2.9181 µg mL^{-1}. Notably, the LOQ lies well below the lower limit of the calibration range, indicating that the method can provide reliable quantification across the entire working interval and

is sufficiently sensitive for the levels of mitragynine typically encountered in kratom extracts. Accuracy assessed by standard-addition recovery at three levels yielded mean recoveries of 99.65–100.67%. Precision was acceptable, with intra-day and inter-day precision of ≤ 2.57% RSD and ≤ 1.07% RSD, respectively. Overall, these results indicate that the method is fit for purpose for mitragynine determination in the tested kratom extracts.

3.6. Mitragynine Content in Crude and Alkaloid Extracts

The validated HPLC–PDA method was used to quantify mitragynine in CME and AE from red-, white-, and green-vein *Mitragyna speciosa* (Table 4; Figure 5). In CME, mitragynine was highest in the white-vein variety followed by green and red, whereas in AE the ranking shifted to red > green > white. This rank reversal indicates that acid–base extraction does not simply scale the crude composition proportionally, but instead enriches mitragynine in a variety-dependent manner. The enrichment factor analysis (AE/CME) supports this interpretation, showing that mitragynine concentration increased most strongly in the red-vein extract, followed by green and white.

From an extraction-chemistry standpoint, the observed pattern can be rationalized by considering that CME represents a broad pool of methanol-soluble constituents, including not only alkaloids but also abundant non-alkaloid co-extractives (e.g., lipids, sterols, terpenoids, pigments, and other metabolites) that contribute to extract mass and thus influence the apparent mg g^{-1} value. Consequently, a higher mitragynine level in CME may reflect a more favorable mitragynine-to-matrix ratio at the crude stage rather than predicting the efficiency of subsequent alkaloid enrichment. During acidification, basic alkaloids are transferred into the aqueous phase as salts while many neutral components remain separable; defatting further reduces lipophilic co-extractives that dominate the crude matrix. In the basification and back-extraction steps, free-base alkaloids redistribute into the organic phase according to their physicochemical properties, meaning that the final mitragynine enrichment depends on both the starting alkaloid pool and the extent to which non-alkaloid components are removed. Because LC–HRMS profiling indicates that the three varieties share a qualitatively similar set of major kratom alkaloids, the differences observed here are most plausibly driven by differences in relative abundances and matrix composition rather than the presence/absence of major alkaloid markers.

From a mechanistic standpoint, several steps in the acid–base extraction can differentially influence mitragynine recovery among varieties. During acidification, alkaloids are converted into water-soluble salts, while many neutral and non-basic constituents remain separable. Subsequent defatting with n-hexane removes lipids, chlorophyll, and other highly non-polar components observed in the crude GC–MS profiles, which may reduce extract mass more strongly in varieties richer in lipids and pigments. This can indirectly elevate the *relative* mitragynine content in the resulting alkaloid fraction, even when the *absolute* amount transferred is comparable. In the basification and back-extraction steps, free-base alkaloids redistribute between aqueous and organic phases according to their partition coefficients and physicochemical properties. As suggested by LC–HRMS, red, white, and green vein kratom share a common set of major alkaloids (mitragynine, 7-hydroxymitragynine, speciogynine/speciociliatine, paynantheine, and rhynchophylline), but likely differ in their relative proportions. Accordingly, if the red-vein alkaloid pool is more strongly dominated by mitragynine than that of the white-vein variety, the same extraction protocol will yield a higher enrichment factor for mitragynine in the red alkaloid fraction. Taken together, differences in alkaloid composition, removal of non-alkaloid co-extractives, and phase-partition efficiency provide a rational explanation for the non-linear, variety-dependent increase in mitragynine content from crude to alkaloid extracts.

Such behavior is consistent with observations in other alkaloid-producing species, where higher total extract yield does not necessarily translate to higher alkaloid content because less selective protocols can co-extract more non-alkaloid matrix. For example, in *Glaucium corniculatum*, certain extraction conditions produced higher total yield but lower alkaloid-richness compared with more selective procedures [57]. Analogously, the present results support the interpretation that differences in mitragynine levels between crude and alkaloid

fractions reflect the interplay between each variety's intrinsic phytochemical matrix and the selectivity of acid–base extraction toward mitragynine and related alkaloids.

Beyond extraction chemistry, environmental and post-harvest factors are also known to influence mitragynine accumulation [38]. Seasonal variation has been reported, with higher mitragynine levels during the transition from dry to rainy seasons compared with peak rainy season [42]. Sunlight exposure and tropical growing conditions have been associated with increased mitragynine content [58], while soil type (e.g., peat and alluvial soils with higher porosity and organic matter) can influence water and nutrient availability and thereby secondary metabolism [59][60]. Post-harvest factors such as leaf age also play a role, with older leaves containing approximately 1.2-fold higher mitragynine levels than younger leaves [44][61].

Several studies further highlight the wide variability of mitragynine content across kratom varieties, origins, and cultivation conditions. For instance, Janthongkaw et al. [17] reported higher mitragynine in green than red ethanolic extracts [17], whereas Nawaka et al. [19] found comparable levels between red and white Thai kratom [19]. Indonesian samples have been reported to span roughly 26–53 mg g^{-1} in various red and green methanolic extracts [14], and Simamora et al. [62] showed that mitragynine in red and green varieties can fluctuate with distance from river systems, consistent with differences in water availability and soil moisture [62]. Moreover, alkaloid fractionation has been reported to increase mitragynine from low single-digit proportions in crude extracts to several tens of percent in alkaloid extract [15][29][63]. Within this broader context, the variability and enrichment patterns observed here align with literature and underscore that mitragynine content is shaped by a combination of genetic background, environment, post-harvest handling, and methodological factors rather than varietal classification alone.

3.7. In vitro 15-lipoxygenase Inhibitory Activity

15-LOX is a non-heme iron dioxygenase that catalyzes the regioselective oxygenation of polyunsaturated fatty acids (PUFAs) into lipid hydroperoxides that are further converted into inflammatory lipid mediators. Accordingly, 15-LOX inhibition is commonly used as a mechanistic readout in anti-inflammatory screening and is relevant to the broader therapeutic rationale for kratom, which is traditionally used to relieve pain and inflammation-related discomfort [64][65]. Soybean LOX (Glycine max) was selected as a practical screening model because it shares a closely comparable non-heme iron catalytic mechanism and PUFA regioselectivity with human ALOX15 (15-LOX-1), where C-13 oxygenation of linoleic acid (13-HPODE/13-HODE formation) is regiospecifically analogous to C-15 oxygenation of arachidonic acid (15-HPETE/15-HETE) [66][67]. In this assay, enzyme activity was monitored at 234 nm because LOX-catalyzed oxidation of linoleic acid produces conjugated diene hydroperoxides that exhibit a characteristic increase in UV absorbance at this wavelength, allowing direct spectrophotometric tracking of product formation. Thus, decreases in the 234-nm signal in the presence of test samples reflect inhibition of LOX-mediated lipid peroxidation [68].

Table 5. IC_{50} values for in vitro 15-lipoxygenase inhibition by baicalein, mitragynine, and alkaloid extracts of red-, white-, and green-vein *Mitragyna speciosa*.

Sample	Variety	IC_{50} (μg mL^{-1})*
Baicalein (positive control)	–	9.72 ± 0.07
Mitragynine	–	28.38 ± 0.39
Alkaloid extract	Red vein	53.01 ± 0.39
	White vein	59.91 ± 0.92
	Green vein	56.29 ± 0.79

*IC_{50} values are presented as mean ± SD (n = 3), obtained by nonlinear regression of concentration–response curves. One-way ANOVA indicated significant differences among all groups ($p < 0.0001$), and Tukey's multiple comparison test confirmed that the three alkaloid extracts (red, green, white) differ significantly from one another ($p < 0.05$).

3.7.1. Baicalein as a Reference 15-LOX Inhibitor

Baicalein was used as a positive control to verify the performance and responsiveness of the enzyme–substrate system. Under the present conditions, baicalein exhibited potent inhibition with an IC_{50} of 9.72 µg mL^{-1}, which is consistent with reported IC_{50} ranges against soybean LOX systems (typically in the 6–11 µg mL^{-1} range under comparable spectrophotometric designs) [69]-[71]. This agreement supports the validity of the 234-nm conjugated-diene assay and confirms that the enzyme–substrate reaction proceeded appropriately. The high potency of baicalein is also consistent with structure–activity knowledge for classical LOX inhibitors. Polyphenolic flavonoids bearing closely spaced hydroxyl groups can act as strong LOX inhibitors through combinations of iron interaction/redox effects and dense hydrogen-bonding/π-stacking contacts within the active site environment [69][72][73]. These characteristics rationalize why baicalein is routinely used as a benchmark inhibitor and provide a meaningful reference point for comparing the kratom-derived samples.

3.7.2. 15-LOX Inhibitory Activity of Mitragynine

Mitragynine was selected for testing as the principal kratom marker alkaloid because it consistently represents the dominant constituent in alkaloid extract and is widely used as a quality and bioactivity reference for *Mitragyna speciosa* extracts. Its broader anti-inflammatory relevance has been supported by prior evidence showing that purified Malaysian mitragynine suppresses COX-2 expression and reduces PGE_2 production in a dose-dependent manner, with the strongest effect reported at 20 µg mL^{-1} and minimal impact on COX-1 at lower concentrations [31]. However, despite these COX-pathway data, a targeted literature search did not identify prior peer-reviewed studies that directly quantified mitragynine inhibition specifically against 15-LOX, which provides a clear rationale and novelty for evaluating its activity in the present assay [11][74].

Under the applied conditions, mitragynine exhibited a measurable inhibitory effect with an IC_{50} of 28.38 µg mL^{-1}, indicating moderate 15-LOX inhibition in this biochemical system. Its potency was lower than that of baicalein (IC_{50} 9.72 µg mL^{-1}), which is chemically plausible when viewed from a structure–activity perspective. Baicalein is a polyhydroxylated flavonoid with closely spaced phenolic groups that support dense hydrogen-bonding, redox activity, and interaction with the catalytic non-heme iron center, enabling inhibition through disruption of the Fe^{3+}/Fe^{2+} catalytic cycle and/or iron-chelation–assisted stabilization of radical intermediates [73]. In contrast, mitragynine is a monoterpenoid indole alkaloid that lacks a catechol or polyphenolic motif. Therefore, direct redox-type inhibition and strong Fe-centered interactions are expected to be more limited. Its inhibitory profile is more plausibly explained by non-redox mechanisms dominated by shape complementarity and lipophilicity within the LOX fatty-acid binding channel, where the indoloquinolizidine scaffold and indole aromatic system may promote hydrophobic and π–π interactions with residues lining the substrate channel, thereby reducing productive linoleic-acid access and orientation for bis-allylic hydrogen abstraction and subsequent radical propagation. This interpretation is consistent with LOX inhibitor models in which non-phenolic scaffolds inhibit LOX primarily by interfering with the substrate-access pathway rather than by strong iron-chelation/redox mechanisms [23][64][75]. Collectively, the IC_{50} value indicates that mitragynine can directly modulate 15-LOX activity, supporting its role as a functionally relevant contributor to the anti-inflammatory profile of kratom alkaloid fractions, while also explaining its lower potency relative to a polyphenolic benchmark inhibitor.

3.7.3. Comparison of Crude and Alkaloid Extracts

The 15-LOX inhibitory activities of kratom extracts were compared to evaluate the impact of alkaloid enrichment and vein-color variety on potency relative to reference compounds (Table 5; Figure 6). The CME exhibited weak inhibition, with IC_{50} values remaining >100 µg mL^{-1} within the tested range, whereas AE showed consistently stronger inhibition, with IC_{50} values in the mid-50 µg mL^{-1} range. This contrast supports that acid–base extraction increases the relative contribution of basic alkaloids to LOX inhibition while reducing non-alkaloid co-extractives that add mass without comparable inhibitory capacity. This interpretation

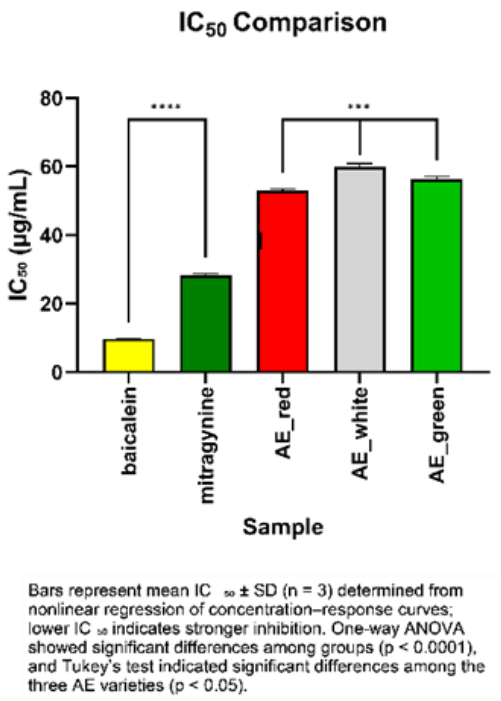


Figure 6. IC_{50} values for in vitro 15-lipoxygenase inhibition by baicalein, mitragynine, and alkaloid extracts (AE) of red-, white-, and green-vein Mitragyna speciosa.

is consistent with the chemical profiles. CME displays a broad non-alkaloid matrix (e.g., lipids/ fatty acids and esters, sterols, terpenoids, pigment-derived semi-volatiles) by GC–MS, while AE is dominated by kratom indole/oxindole alkaloids according to GC–MS/LC–HRMS, providing a compositional basis for the improved AE potency.

This hierarchy indicates that enrichment of indole and oxindole alkaloids markedly enhances 15-LOX inhibitory activity compared with the unfractionated crude extracts. The weak inhibition by crude methanolic extracts likely reflects both their lower alkaloid content and the presence of a wide array of co-extracted metabolites (lipids, sterols, terpenoids, pigments, and other polar constituents) that do not necessarily support, and may even interfere with, alkaloid–enzyme interactions. In contrast, the acid–base extraction protocol concentrates basic alkaloids such as mitragynine, 7-hydroxymitragynine, speciogynine, speciociliatine, paynantheine, and rhynchophylline, while reducing the proportion of non-alkaloid components, as evidenced by the GC–MS and LC–HRMS profiles.

Among AE samples, the IC_{50} values differed significantly (Tukey, $p < 0.05$) and followed the order red < green < white (Table 5). This variety-dependent pattern aligns with the quantitative mitragynine data: HPLC–PDA showed that mitragynine concentration in AE follows red > green > white, and LC–HRMS further indicates that the three varieties share a similar set of major alkaloids but differ in relative composition. Collectively, these findings support that vein-color differences in AE potency are predominantly quantitative, reflecting differences in the relative dominance of mitragynine and co-occurring alkaloids after enrichment, rather than qualitative differences in the presence/absence of marker alkaloids. Nevertheless, vein-color should not be viewed as a single deterministic factor because alkaloid composition and apparent potency can also be shaped by environmental and post-harvest variables and by matrix-dependent extraction behavior, which together can alter the relative proportions of active alkaloids recovered into AE.

A more critical interpretation is warranted regarding biological relevance. IC_{50} values in the 50 –60 µg mL^{-1} range indicate moderate inhibition in a simplified enzyme assay and do not directly translate to in vivo efficacy, where achievable free concentrations are constrained by absorption,

metabolism, protein binding, distribution, and clearance, and where potency may differ across human LOX isoenzymes and cellular contexts. Accordingly, the AE data are best positioned as comparative biochemical screening evidence showing that alkaloid enrichment improves 15-LOX inhibition and that the three varieties differ quantitatively under standardized conditions, while follow-up studies using human ALOX isoforms and inflammation-relevant cellular models are needed to strengthen translational interpretation.

The activity differences not fully explained by mitragynine content are consistent with the multicomponent nature of plant extracts. LOX inhibition by extracts can reflect contributions from co-occurring alkaloids with anti-inflammatory activity and from variety-dependent shifts in alkaloid proportions that may produce additive, synergistic, or antagonistic effects, along with matrix effects that modulate bioactive availability and substrate–enzyme access in the reaction mixture. Supporting evidence indicates that rhynchophylline can suppress inflammatory mediators (e.g., NO/iNOS and pro-inflammatory cytokines) in cellular and in vivo models [76]-[78], while other oxindole/indole alkaloids have also been linked to measurable anti-inflammatory endpoints [79][80]. In addition, in silico studies on LOX isoforms (e.g., 5-LOX) suggest that several kratom alkaloids beyond mitragynine may bind favorably to lipoxygenase active-site regions (e.g., 7-hydroxymitragynine and paynantheine), supporting the plausibility that co-alkaloids contribute to the overall extract response even though such docking results do not constitute direct evidence for 15-LOX inhibition [11].

Although this study did not perform molecular docking or binding-mode analysis specifically against 15-LOX, the integrated evidence from HPLC–PDA quantification and chemical profiling supports a composition–activity relationship at the extract level, suggesting that mitragynine is a major contributor to 15-LOX inhibition under the present assay conditions. Nevertheless, because alkaloid extracts are multicomponent mixtures, the observed activity cannot be attributed to mitragynine alone, and confirmatory mechanistic studies (e.g., docking/MD against 15-LOX and/or testing of additional isolated co-alkaloids) would be required to define specific binding interactions and to resolve potential additive or synergistic contributions.

4. CONCLUSIONS

This study compared red-, white-, and green-vein *Mitragyna speciosa* leaves from Kalimantan in terms of alkaloid profiles, mitragynine levels, and *in vitro* 15-LOX inhibition. Across all vein-color phenotypes, the alkaloid extracts exhibited a broadly similar monoterpenoid indole alkaloid fingerprint dominated by mitragynine, while quantitative differences in mitragynine enrichment and relative alkaloid composition were observed among phenotypes. Alkaloid extraction substantially increased mitragynine content and improved 15-LOX inhibitory potency compared with crude methanolic extracts, and purified mitragynine showed measurable 15-LOX inhibition, although it was less potent than baicalein. Collectively, these results provide a comparative phytochemical and bioactivity dataset for three Kalimantan vein-color phenotypes and support a composition–activity relationship in which mitragynine is a major contributor to 15-LOX inhibition under the present assay conditions, while additional co-occurring alkaloids and matrix effects may modulate extract potency. Future studies should evaluate other kratom alkaloids individually and in defined mixtures to test additivity/synergy, validate activity using human LOX isoenzymes and cell-based inflammatory models, and integrate pharmacokinetic and safety assessments to better define translational relevance.

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Conflicts of Interest

The authors declare no conflict of interest. The funder, Lembaga Pengelola Dana Pendidikan (LPDP), Ministry of Finance of the Republic of Indonesia, had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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DECLARATION OF GENERATIVE AI

Not applicable.

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