การศึกษาเปรียบเทียบฤทธิ์ต้านการอักเสบและฤทธิ์ต้านอนุมูลอิสระของสารสกัดจากกระท่อมสายพันธุ์ก้านแดงและก้านขาว

Comparative Anti-Inflammatory and Antioxidant Activities of Extracted from Red- and White-Vein *Mitragyna speciosa* (Korth.) Havil

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บทคัดย่อ

กระท่อม (*Mitragyna speciosa* (Korth.) Havil.) ในประเทศไทยมี 2 สายพันธุ์ ที่มีการใช้บ่อยคือ พันธุ์ก้านแดงและพันธุ์ก้านขาว หมอพื้นบ้านนิยมใช้กระท่อมก้านแดงเป็นยามากกว่าก้านขาว เพื่อให้มีแรงทำงานได้นานขึ้น ทนแดด แก้ท้องร่วง ลงแดง และแก้ปวดเมื่อย ซึ่งมี 3 วิธีหลักในการบริโภคแบบดั้งเดิม ได้แก่ การแช่ในแอลกอฮอล์ การต้มใบ และการเคี้ยวใบสด ดังนั้น งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อเปรียบเทียบฤทธิ์ต้านอนุมูลอิสระชนิด DPPH (DPPH assay) และฤทธิ์ต้านการอักเสบจากการยับยั้งการสร้างไนตริกออกไซด์ ของสารสกัดใบกระท่อมพันธุ์ก้านแดงและก้านขาว ที่ได้จากการแช่หมักในเอทานอล (ร้อยละ 50 และ 95) การต้ม และการคั้นน้ำ ผลการศึกษาพบว่าสารสกัดจากการแช่หมักในเอทานอล ร้อยละ 50 และ 95 ของกระท่อมพันธุ์ก้านแดงและก้านขาวมีค่า EC_{50} อยู่ในช่วงใกล้เคียงกัน (5.24–6.78 µg/mL) และค่า EC_{50} ของกลุ่มดังกล่าวยังต่ำกว่าวิธีการสกัดแบบต้มและการคั้น นอกจากนี้ยังพบว่าสารสกัดจากกระท่อมพันธุ์ก้านแดงที่ได้จากการแช่หมักในเอทานอลร้อยละ 50 แสดงร้อยละการยับยั้งการสร้างไนตริกออกไซด์ได้สูงที่สุด เมื่อเทียบกับวิธีการสกัดอื่น แม้ว่าค่า IC_{50} ของทุกตัวอย่างจะมากกว่า 100 µg/mL โดยสรุปสารสกัดจากกระท่อมพันธุ์ก้านแดงที่ได้จากการแช่หมักในเอทานอลร้อยละ 50 แสดงศักยภาพในการต้านอนุมูลอิสระและต้านการอักเสบสูงที่สุด เมื่อพิจารณาจากการยับยั้งอนุมูลอิสระชนิด DPPH และจากการสร้างไนตริกออกไซด์ ตามลำดับ ผลการศึกษานี้จึงชี้ให้เห็นถึงศักยภาพของวิธีการสกัดและสายพันธุ์กระท่อมที่เหมาะสมสำหรับการพัฒนาเป็นสารออกฤทธิ์ทางชีวภาพ และสนับสนุนการเลือกใช้กระท่อมพันธุ์ก้านแดงในรูปแบบการเตรียมสารสกัดที่สอดคล้องกับการใช้ในภูมิปัญญาพื้นบ้าน

คำสำคัญ: กระท่อม, ฤทธิ์ต้านอนุมูลอิสระ, ฤทธิ์ต้านการอักเสบ, วิธีสกัด

Abstract

Mitragyna speciosa (Korth.) Havil. (kratom) in Thailand is commonly classified into two frequently used varieties: red-veined and white-veined kratom. Traditional healers more commonly use red-veined kratom than white-veined kratom for medicinal purposes, including enhancing work endurance, increasing tolerance to sunlight exposure, relieving diarrhea, alleviating withdrawal symptoms, and reducing muscle pain. Three principal traditional methods of consumption are commonly practiced, namely alcohol maceration, leaf decoction, and chewing fresh leaves. Therefore, this study aimed to compare the antioxidant activity, determined by the DPPH radical scavenging assay, and the anti-inflammatory activity, assessed by inhibition of nitric oxide production, of leaf extracts from red-veined and white-veined kratom prepared by ethanolic maceration (50% and 95%), decoction, and aqueous squeezing. The results showed that the 50% and 95% ethanolic extracts of both red-veined and white-veined kratom exhibited comparable EC_{50} values ranging from 5.24 to 6.78 µg/mL which were lower than those obtained from decoction and aqueous squeezing. In addition, the 50% ethanolic extract of red-veined kratom showed the highest percentage inhibition of nitric oxide production among the different extraction methods, although the IC_{50} values of all samples were greater than 100 µg/mL. In conclusion, the 50% ethanolic extract of red-veined kratom demonstrated the greatest potential for antioxidant and anti-inflammatory activities, as indicated by DPPH radical scavenging activity and inhibition of nitric oxide production, respectively. These findings highlight the importance of appropriate extraction methods and kratom varieties for the development of bioactive agents and support the selection of red-veined kratom in extract preparations consistent with traditional medicinal practices.

Keywords: Kratom, Antioxidant activity, Anti-inflammatory activity, Extraction methods

1. Introduction

Kratom (*Mitragyna speciosa* Korth.) is a tropical plant belonging to the family Rubiaceae and is indigenous to Southeast Asia. In Thailand, kratom was fully removed from the list of Category 5 narcotic substances under the Narcotics Act, and the Kratom Plant Act B.E. 2565 (2022) subsequently legalized its cultivation, possession, and sale. Nevertheless, the manufacture or processing of kratom into foods, herbal preparations, pharmaceuticals, or cosmetic products remains subject to authorization under relevant regulatory frameworks. The import and export of kratom leaves remain under the supervision of the Office of the Narcotics Control Board (ONCB) (Kratom Plant Act, 2022). Following these legal changes, kratom has rapidly gained attention as an emerging economic crop in Thailand.

Previous studies have shown that medicinal plants with strong antioxidant capacity often exhibit anti-inflammatory effects or provide protection against inflammation-related cellular injury, indicating a close link between oxidative stress modulation and inflammatory responses (Arulselvan et al., 2016; Dunkhunthod et al., 2025; Klintong et al., 2024; Sittisart et al., 2020). Nitric oxide (NO) is one of the key mediators linking oxidative stress and inflammation. NO is a reactive free radical molecule with a single unpaired electron and plays a dual role in physiological regulation and pathological inflammation. It contributes to numerous physiological processes, including neurotransmission, smooth muscle relaxation, vasodilation, blood pressure regulation, gene expression, host defense, and immune regulation, and it is also implicated in pathological conditions such as neurodegenerative and cardiovascular diseases (Martemucci et al., 2022).

Traditionally, kratom leaves have been used as a folk medicine and as a stimulant, particularly among manual laborers, to alleviate fatigue, enhance physical endurance, and improve tolerance to heat. The leaves possess a bitter and astringent taste and have been used to treat abdominal pain, diarrhea, and muscle aches (Tangtrongchit, 1978). Various methods of consumption have been reported, including leaf decoction, alcoholic maceration, and chewing fresh leaves. In Thailand, kratom is commonly classified into red-, green-, and white-vein types (Srisakul, 2022). These types are reported to differ in their alkaloid profiles, which may contribute to differences in pharmacological activity (Boffa et al., 2018).

Among the identified alkaloids, mitragynine is the most abundant and is considered a major contributor to the central nervous system (CNS) depressant and analgesic effects of kratom (Kumarnsit et al., 2006, 2007). Mitragynine acts mainly as a partial agonist at μ-opioid receptors, leading to pain relief and mild sedation (Matsumoto et al., 1996). It has also been reported to inhibit the production of prostaglandin E2 (PGE2) through the suppression of cyclooxygenase-2 (COX-2) expression, indicating potential anti-inflammatory properties (Utar et al., 2011). Another major alkaloid in kratom is 7-hydroxymitragynine (7-HMG), which exhibits markedly stronger analgesic potency than mitragynine. Previous pharmacological studies have reported that 7-HMG is approximately 46 times more potent than morphine and about 13 times more potent than mitragynine in terms of analgesic activity (Kruegel & Grundmann, 2018; Matsumoto et al., 2004). This compound acts as a potent agonist at μ-opioid receptors, contributing significantly to the analgesic properties associated with kratom alkaloids (Hemby et al., 2019). Given the high pharmacological potency of 7-HMG, variations in its concentration among kratom types may influence their biological and pharmacological activities. Previous phytochemical investigations have suggested that red-vein kratom may contain relatively higher levels of 7-hydroxymitragynine compared with white-vein kratom, which could partly explain the differences in reported biological effects among kratom types (Dewatisari & Nuryandani, 2025).

Despite increasing interest, comparative studies evaluating antioxidant and anti-inflammatory activities across different kratom vein types, particularly those employing extraction methods that reflect traditional preparation, remain limited. Therefore, the present study aims to compare the anti-inflammatory and antioxidant activities of extracts from red-vein and white-vein kratom leaves, two types commonly used in both traditional and contemporary contexts. Extraction procedures were designed to mimic traditional practices to support evidence-based development of standardized kratom-based herbal products in the future.

2. Materials and methods

2.1 Sample Selection

Two kratom types commonly used by local villagers and traditional healers were selected, namely red-vein kratom collected from Chumphon Province, and white-vein kratom collected from Pattani Province, Thailand.

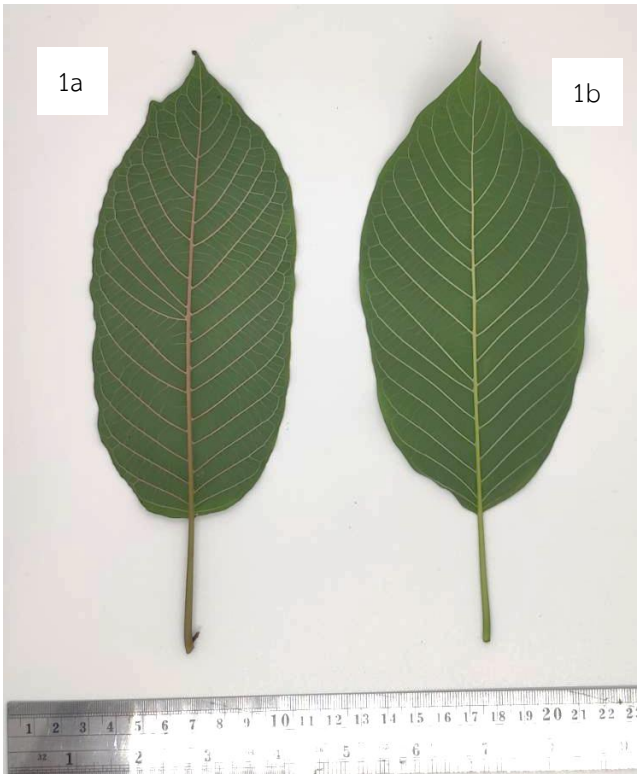

Figure 1. Leaves of red-vein (1a) and white-vein (1b) kratom

2.2 Plant Materials and Preparation

White-vein kratom leaves (1,729 g) were collected from Pattani Province, Thailand, and red-vein kratom leaves (1,765 g) were collected from Chumphon Province, Thailand. The leaves were thoroughly cleaned and subjected to three extraction methods: (1) maceration with ethanol (95% and 50%), (2) decoction, and (3) aqueous extraction by squeezing. The detailed procedures for each method are described below.

2.2.1 Maceration

Fresh white-vein and red-vein kratom leaves were cut into small pieces (approximately 1–1.5 cm) prior to drying. The dried white-vein and red-vein kratom leaves (198.81 g and 137 g, respectively) were macerated with 95% ethanol, while the dried white-vein and red-vein leaves (181.53 g and 142 g, respectively) were macerated with 50% ethanol at a plant material-to-solvent ratio of 1:3 (w/v) at room temperature for 3 days. The extracts were filtered, and the maceration process was repeated three times. The combined filtrates were concentrated under reduced pressure at 45 °C using a rotary evaporator (Büchi, Flawil, Switzerland) to obtain a viscous crude extract, which was further oven-dried to constant weight.

2.2.2 Decoction

Fresh white-vein and red-vein kratom leaves (324 g and 724 g, respectively) were boiled in water at a ratio of 1:3 (w/v) at 100 °C for 15 min. The extract was filtered, and the filtrate was concentrated using a freeze dryer (Lyolab, Barcelona, SPAIN), yielding dry crude powder extract.

2.2.3 Aqueous squeezing

Fresh white-vein and red-vein kratom leaves (80.65 g and 42 g, respectively) were blended with water at a ratio of 1:3 (w/v), filtered, and the filtrates were concentrated using a freeze dryer (Lyolab), yielding dry a crude powder extract. The percentage yield of each extract was calculated.

2.3 DPPH Radical Scavenging Assay

The DPPH assay is widely used for preliminary screening of antioxidant activity because it evaluates the electron- and hydrogen-donating capacity of antioxidant compounds (Brand-Williams et al., 1995). The antioxidant activity of the extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO, USA) radical scavenging assay according to the method described by Yamazaki et al. (1994) with slight modifications. Ethanolic extracts were dissolved in absolute ethanol, whereas aqueous extracts were dissolved in sterile water. Sample solutions were prepared at various concentrations (1, 10, 50, and 100 µg/mL).

A 100 µL aliquot of each sample solution was added to 96-well microplates, followed by 100 µL of DPPH solution. Control solutions consisted of absolute ethanol or distilled water (100 µL), each mixed with 100 µL of DPPH solution. The mixtures were incubated in the dark at room temperature for 30 min.

Absorbance was measured at 520 nm using a microplate reader (The Multiskan GO, Vantaa, FINLAND). Butylated hydroxytoluene (BHT) (Sigma-Aldrich, St. Louis, MO, USA) served as a positive control. The percentage of radical scavenging activity was calculated using the following equation:

$$\text{Inhibition (\%)} = [(OD_{control} - OD_{sample}) / OD_{control}] \times 100 \quad (1)$$

where OD control is the absorbance of the control (without sample), and OD sample is the absorbance in the presence of the test sample. The half-maximal effective concentration (EC_{50}) values were calculated from the dose-response curves using nonlinear regression analysis (four-parameter logistic model) in GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA).

2.4 Cell culture

RAW264.7 macrophage cells (ATCC TIB-71TM, Virginia, USA) were cultured in RPMI-1640 medium (Gibthai, Bangkok, Thailand) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, OK, USA) and 100 U/mL penicillin–streptomycin (Gibco, OK, USA). The cells were maintained at 37 °C in a humidified incubator with 5% CO_2.

2.5 Determination of Nitric Oxide Production in LPS-Stimulated RAW 264.7 Cells

Nitric oxide (NO) production in murine macrophage-like RAW264.7 cells was evaluated following the method of Makchuchit et al., (2017). Briefly, cells were seeded into 96-well plates at a density of 1×10^5 cells/100 µL/well and allowed to adhere for 1 h. The medium was then replaced with fresh RPMI-1640 medium containing 10 ng/mL lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, MO, USA) and the test samples (W-95E, W-50E, W-D, W-Sq, R-95E, R-50E, R-D and R-SQ) at final concentrations of 50 and 100 µg/mL and test prednisolone at a final concentrations of 0.01, 0.1, 1and 10 µg/mL (100 µL/well). Cells were incubated for 24 h, after which NO production was assessed by measuring nitrite accumulation in the culture supernatant using Griess reagent (1% sulfanilamide and 0.1% N-(1- naphthyl) ethylenediamine dihydrochloride in 2.5% phosphoric acid) (Merck, Darmstadt, Germany).

Cytotoxicity was determined in parallel using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, St. Louis, MO, USA) colorimetric assay. After 24 h of incubation with the test samples, 10 µL of MTT solution (5 mg/mL in PBS) was added to each well. Following 2 h incubation, the medium was removed, and 100 µL of isopropanol containing 0.04 M HCl was added to dissolve the formazan crystals formed in the cells. The optical density (OD) was measured at

570 nm using a microplate reader (The Multiskan GO). Test compounds or extracts were considered cytotoxic when cell viability was less than 70% compared with the vehicle-treated control group, according to the ISO 10993-5 guideline. Prednisolone (Sigma-Aldrich, St. Louis, MO, USA) served as a positive control. Stock solutions of 50 mg/mL test samples were prepared in DMSO and diluted with RPMI-1640 medium to ensure a final DMSO concentration of <0.2%. The percentage inhibition of NO production was calculated as equation (2).

$$\text{Inhibition (\%)} = [(OD_{control} - OD_{sample}) / OD_{control}] \times 100 \quad (2)$$

Half-maximal inhibitory concentration (IC_{50}) values were calculated from dose-response curves using nonlinear regression analysis (four-parameter logistic model) in GraphPad Prism software.

2.6 Biosafety

This research project was approved by the Institutional Biosafety Committee (IBC), Thammasat University (Certificate No. 041/2568).

2.7 Statistical analysis

All experiments were conducted in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA). Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD multiple comparison test. A p-value < 0.05 was considered statistically significant.

3. Result

3.1 Extraction yield of kratom leaves obtained by different extraction methods

As shown in Table 1, the extraction yields of kratom leaf extracts ranged from 6.45% to 22.40%, depending on the extraction method and vein type. The highest yield was obtained from 50% ethanol maceration, yielding 22.40% for red-vein kratom (R-50E) and 18.60% for white-vein kratom (W-50E). In contrast, the lowest yields were observed with the decoction method, producing 6.45% for red-vein kratom (R-D) and 8.75% for white-vein kratom (W-D). Overall, ethanol maceration produced higher yields than water-based extractions (decoction and aqueous squeezing) for both vein types.

Table 1. Extraction yield (%) of kratom leaves using different extraction methods

Sample	Vein	Extraction method	Code	%yield
Dried leaves	White	Maceration with 95% ethanol	W-95E	12.50
Dried leaves	White	Maceration with 50% ethanol	W-50E	18.60
Fresh leaves	White	Kratom with Decoction	W-D	8.75
Fresh leaves	White	Kratom with Aqueous Squeezing	W-Sq	15.48
Dried leaves	Red	Maceration with 95% ethanol	R-95E	14.70
Dried leaves	Red	Maceration with 50% ethanol	R-50E	22.40
Fresh leaves	Red	Kratom with Decoction	R-D	6.45
Fresh leaves	Red	Kratom with Aqueous Squeezing	R-Sq	10.68

3.2 Antioxidant activity of kratom extracts determined by the DPPH assay

As shown in Table 2, the results indicated that extracts obtained by maceration with 50% and 95% ethanol, as well as decoction extracts from both red-vein and white-vein kratom exhibited significantly higher antioxidant activity than the standard BHT ($p < 0.05$). Among these, the 50% ethanol extract of red-vein kratom showed the lowest EC_{50} value (5.24±0.30 µg/mL), compared with BHT (EC_{50} = 16.09±2.05 µg/mL). The decoction extracts of white- and red-vein kratom also demonstrated strong antioxidant activity, with EC_{50} value of 6.57±0.12 and 8.01±0.79, respectively. In contrast, the aqueous squeezing extract of white-vein showed no significant difference compared with BHT (EC_{50} = 11.62±3.59 µg/mL). The aqueous squeezing extract of red-vein kratom exhibited substantially lower antioxidant activity than the other extraction methods (EC_{50} = 50.16±2.47 µg/mL).

Table 2. Antioxidant activity of kratom extracts determined by the DPPH assay

Sample	%Inhibition				EC_{50} ±SD
	1 µg/mL	10 µg/mL	50 µg/mL	100 µg/mL	(µg/ml)
W-95E	12.04±6.37	76.04±5.03	92.98±0.88	94.95±0.30	5.86±0.56^{a}
R-95E	9.14±4.09	71.97±9.91	94.48±0.40	94.19±0.24	6.78±0.80^{a}
W-50E	8.85±3.38	86.96±2.64	92.21±0.85	92.83±0.66	5.41±0.29^{a}
R-50E	10.55±6.16	87.96±1.45	93.58±1.07	93.17±3.86	5.24±0.30^{a}
W-D	11.37±5.70	70.22±1.97	81.67±5.13	84.89±5.09	6.57±0.12^{a}
R-D	3.22±3.83	61.48±5.06	78.82±8.19	84.37±5.64	8.01±0.79^{ab}
W-Sq	11.37±3.39	47.70±9.75	81.10±9.95	87.20±4.79	11.62±3.59^{bc}
R-Sq	0.71±2.04	14.48±1.56	49.24±2.39	73.68±2.31	50.16±2.47^{d}
BHT	4.41±2.89	34.71±3.96	77.85±3.04	85.53±0.97	16.09±2.05^{c}

Note: Values are expressed as mean ± SD (n = 3). Different superscript letters (a–d) within the same column indicate statistically significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's HSD multiple comparison test.

3.3 Effects of Kratom Extracts on the Viability of RAW264.7 Cells

As shown in Table 3, the viability of RAW264.7 cells treated with kratom extracts generally remained high at both tested concentrations (50 and 100 µg/mL). Most extracts exhibited cell viability values above 90%, indicating low cytotoxicity toward RAW264.7 cells under the experimental conditions. Among the tested extracts, ethanol maceration extracts (W-95E, W-50E, R-95E, and R-50E) showed the highest cell viability, with several values exceeding 110%, particularly for W-95E and W-50E. In contrast, extracts obtained by decoction and aqueous squeezing tended to show slightly lower cell viability, although the values remained close to or above 90%. When comparing kratom vein types, both white-vein and red-vein extracts showed comparable effects on cell viability at the same concentrations. The decoction extract of red-vein kratom (R-D) showed cell viability values close to 100%, while the aqueous squeezing extract of red-vein kratom (R-Sq) exhibited slightly lower viability compared with other extracts. The reference compound prednisolone showed a concentration-dependent decrease in cell viability, with values decreasing from approximately 101% at 0.01 µg/mL to 75% at 10 µg/mL. Overall, these results suggest that most kratom extracts exhibited minimal cytotoxicity toward RAW264.7 cells at the tested concentrations.

Table 3. Cell viability of RAW264.7 cells treated with different concentrations of kratom extracts

Sample	Concentration (µg/mL)	Cell viability (%)*
W-95E	50	120.31±10.49
	100	125.65±8.48
W-50E	50	109.23±1.72
	100	122.05±6.46
W-D	50	98.72±1.84
	100	100.4±0.05
W-Sq	50	93.72±2.8
	100	95.78±6.20
R-95E	50	111.35±2.54
	100	110.85±4.45
R-50E	50	114.87±7.59
	100	115.40±10.86
R-D	50	94.95±0.34
	100	98.13±2.79
R-SQ	50	89.49±3.30
	100	93.43±1.57
Prednisolone	0.01	101±7.80
	0.1	82.79±4.39
	1	78.00±1.13
	10	75.02±1.19

*Data are presented as mean ± SD (n=3). Cell viability was assessed using the MTT assay.

3.4 Inhibitory effects of kratom extracts on LPS-Induced nitric oxide production in RAW264.7 cells

As shown in Table 4, several kratom extracts demonstrated measurable inhibitory effects on NO production in LPS-stimulated RAW264.7 macrophages. Among the extraction methods, maceration tended to yield extracts with relatively higher NO inhibitory activity. In particular, red-veined kratom extracts obtained by maceration with 50% and 95% ethanol, as well as the white-veined kratom extract obtained by maceration with 50% ethanol, showed the highest levels of NO inhibition compared with other extracts. At a concentration of 100 µg/mL, these extracts exhibited percentage inhibitions of 43.79 ± 10.01%, 43.26 ± 0.69%, and 41.50 ± 10.37%, respectively.

Despite these observations, the overall anti-inflammatory activity of the kratom extracts remained relatively weak, as all tested extracts exhibited IC_{50} values greater than 100 µg/mL. In contrast, the reference compound prednisolone showed strong inhibitory activity, with an IC_{50} value of 0.20 ± 0.01 µg/mL (0.56 ± 0.03 µM). To ensure that the observed inhibition of NO production was not influenced by cytotoxic effects, cell viability was evaluated using the MTT assay (Table 3). The results indicated that all kratom extracts from both white-veined and red-veined varieties were non-cytotoxic to RAW264.7 cells, with cell viability values exceeding 80% at a concentration of 100 µg/mL. These findings suggest that the reduction in NO production observed for the extracts was not attributable to decreased cell viability. In contrast, prednisolone exhibited a strong inhibitory effect on LPS-induced NO production. However, at certain concentrations, approximately 20% reduction in cell viability was observed, which may influence the interpretation of NO inhibition. In such cases, reduced NO levels could result from decreased cell

viability rather than a direct anti-inflammatory effect.

According to ISO 10993-5 (Standard, 2009), a reduction in cell viability greater than 30% is considered cytotoxic. Therefore, to minimize potential confounding effects of cytotoxicity on NO production, only concentrations with cell viability ≥80% (Joo et al., 2014) or ≥90% (Pratama et al., 2024) were considered in the evaluation of NO inhibition. These criteria allowed for a more accurate assessment of the anti-inflammatory activity of kratom extracts, independent of cytotoxic effects.

Table 4. Anti-inflammatory activity of kratom extracts based on inhibition of nitric oxide (NO) production

Sample	Concentration (µg/mL)	% inhibition	IC_{50}(µg/mL)
W-95E	50	11.49±0.88	>100
	100	41.50±10.37	>100
W-50E	50	3.25±9.74	>100
	100	20.99±1.32	>100
W-D	50	6.92±1.91	>100
	100	9.29±3.30	>100
W-Sq	50	6.59±1.59	>100
	100	10.85±2.47	>100
R-95E	50	15.64±7.72	>100
	100	43.26± 0.69	>100
R-50E	50	5.67±10.02	>100
	100	43.79±10.01	>100
R-D	50	0.08±5.68	>100
	100	8.13±1.48	>100
R-SQ	50	4.56±4.04	>100
	100	5.35±4.97	>100
Prednisolone	0.01	12.56±2.70	0.20±0.01
	0.1	50.06±1.58	
	1	68.32±0.52	
	10	74.66±1.20	

*Data are presented as mean ± SD (n=3)

4. Discussion

Kratom (*Mitragyna specosa* (Korth.) Havil) is a medicinal plant traditionally used for relieving pain and muscle soreness, as well as enhancing tolerance to heat. The most commonly used varieties are the white- vein and red -vein types. Kratom has been utilized in various preparation methods, including decoction, ethanolic maceration, and aqueous squeezing extraction. In this study, both kratom varieties were extracted using methods that closely resemble traditional practices. The obtained extracts were then evaluated for their antioxidant activity using DPPH radical scavenging assay and their anti-inflammatory activity through the inhibition of LPS-induced nitric oxide (NO) production in RAW 264.7 cells, both of which are related to inflammatory processes and pain relief. The results revealed that extraction yields were comparable across different methods, with the highest yield obtained from maceration with 50% ethanol.

The antioxidant activity evaluated by the DPPH radical scavenging assay demonstrated that extracts of both kratom types obtained by maceration with 50% ethanol, 95% ethanol, and decoction exhibited significantly stronger antioxidant activity than BHT, which served as the positive control, except for the aqueous squeezing extract. In the present study, the red-veined kratom showed lower antioxidant activity than the white-veined kratom and BHT. A previous study reported that water extraction of kratom at 100 °C for 2 hours, repeated twice, resulted in a relatively high EC_{50} value (Parthasarathy *et al.,* 2009). In contrast, the present study demonstrated lower EC_{50} values and stronger antioxidant activity than BHT, which may be attributed to differences in boiling time. Additionally, variations in the source and harvest season of plant materials may have influenced the concentration of bioactive compounds, leading to discrepancies in the observed results. (Kang *et al.,* 2023)

The extracts from both red-veined and white-veined kratom leaves exhibited comparable antioxidant activities across different extraction methods, including maceration with 95% ethanol, maceration with 50% ethanol, and decoction. However, in the aqueous extraction, the white-veined kratom extract demonstrated significantly stronger antioxidant activity than red-veined extract, with IC_{50} value of 11.62±3.59 µg/mL and 51.49±4.43 µg/mL, respectively.

Regarding anti-inflammatory activity, both extracts showed comparable inhibitory effects at concentrations of 50 and 100 µg/mL across the maceration with 50% ethanol extraction, decoction, and aqueous extraction methods. Notably, in the maceration with 50%, the red-veined kratom extract exhibited greater nitric oxide inhibitory activity at 100 µg/mL than the white-veined extract, with inhibition percentages of 43.79±10.01% and 20.99±1.32%, respectively.

Previous studies have reported that high temperature or heat stress is directly associated with an increase in reactive oxygen species (ROS), leading to oxidative stress in living organisms (Zhang *et al*., 2022). This supports the traditional use of kratom leaves to enhance tolerance to sunlight exposure. Consistently, extracts obtained through decoction, ethanolic maceration, and aqueous squeezing, commonly used in traditional practice, exhibited strong antioxidant activities in this study.

The anti-inflammatory activity of kratom extracts has previously been evaluated by measuring lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 cells. In a previous study, Matrix 5 Step1 (M5S1), a non-alkaloid extract obtained by supercritical carbon dioxide extraction, demonstrated the highest inhibitory effect, with 60.08±10.02% inhibition at a final concentration of 100 µg/mL (Tohar, *et al.,* 2019). This inhibitory activity is comparable to that observed in the present study, in which the 50% ethanolic extract obtained by maceration exhibited anti-inflammatory activity with 43.79 ± 10.01% inhibition at the same concentration (100 µg/mL).

Based on the present findings, ethanolic extracts tended to exhibit stronger antioxidant and anti-inflammatory activity than water-based extracts obtained by aqueous squeezing or decoction. Previous studies have identified mitragynine as a major alkaloid compound with lipophilic properties and poor water solubility. A comparative study reported that the concentration of mitragynine in the water extract was 0.80 mg/g, which was markedly lower than that in the methanolic extract (4.77 mg/g) (Parthasarathy *et al.,* 2013).

However, a previous study reported that mitragynine from kratom leaves exerted anti-inflammatory effects on RAW264.7 macrophage cells by inhibiting prostaglandin E2 (PGE2) production through suppression of cyclooxygenase-2 (COX-2) expression, a key enzyme induced during inflammation, while showing no effect on COX-1 activity. These findings indicate that mitragynine possesses anti-inflammatory potential (Utar *et al.,* 2011). Furthermore, the anti-inflammatory activity of the methanolic kratom extract was demonstrated *in vivo*, where intraperitoneal administration in carrageenan-induced

paw inflammatory mediators via the arachidonic acid pathway, enhanced immune function, and promoted wound healing (Shaik *et al.,* 2009). Therefore, it is suggested that assessing only nitric oxide inhibition may not comprehensively reflect the specific anti-inflammatory mechanisms of kratom extract.

Previous studies have reported that kratom alkaloids exhibit greater biological activity than crude extracts. In silico molecular analyses demonstrated strong binding affinity of kratom alkaloids to the active sites of COX-2 and 5-LOX, supporting their potential as dual COX-2/5-LOX inhibitors. The alkaloid extract suppressed several anti-inflammation parameters, including ROS (64% reduction at 25 ppm), NO (30% reduction at 25 ppm), TNF-α (~ 50% reduction at 25 ppm), and IL-6 production (60% reduction at 6.25 ppm). Cyclooxygenase (COX) and lipoxygenase (LOX) enzymes play crucial roles in the biosynthesis of pro-inflammatory eicosanoids, including prostaglandins and leukotrienes; therefore, simultaneous inhibition of both enzymes may contribute to more effective suppression of inflammatory responses (Rahmawati et al., 2024).

5. Conclusion and suggestions

Both red-veined and white-veined kratom extracts exhibited strong antioxidant activity, particularly when extracted by maceration with 50% and 95% ethanol, as well as by decoction methods, with activities greater than the standard antioxidant BHT under the tested conditions. Among the tested samples, the red-veined kratom extract showed relatively higher anti-inflammatory activity, with 43.79 ± 10.01% inhibition of nitric oxide production at a concentration of 100 µg/mL, although the IC_{50} values remained greater than 100 µg/mL. Overall, the results suggest that ethanolic extraction, particularly with 50% ethanol, may be an effective method for obtaining bioactive compounds from kratom leaves. The findings also indicate that red-veined kratom may have greater potential for applications related to pain relief and inflammatory conditions, which is consistent with its traditional use in folk medicine.

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7. References

Arulselvan, P., Fard, M. T., Tan, W. S., Gothai, S., Fakurazi, S., Norhaizan, M. E., & Kumar, S. S. (2016). Role of antioxidants and natural products in inflammation. Oxidative medicine and cellular longevity, 2016(1), 5276130. https://doi.org/10.1155/2016/5276130

Boffa, L., Ghè, C., Barge, A., Muccioli, G., & Cravotto, G. (2018). Alkaloid profiles and activity in different Mitragyna speciosa strains. Natural Product Communications, 13(9), 1934578X1801300904. https://doi.org/10.1177/1934578X1801300904

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT – Food Science and Technology, 28(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Dewatisari, W. F., & Nuryandani, E. (2025). A review on alkaloid diversity in Mitragyna speciosa (kratom): Influence of strain and geographic origin. Proceeding International Seminar of Science and Technology, 4, 49–62. https://doi.org/10.33830/isst.v4i1.5230

Dunkhunthod, B., Thumanu, K., Teethaisong, Y., Sittisart, P., & Sittisart, P. (2025). Cytoprotective activity of Pogonatherum paniceum (Lam.) Hack. ethanolic extract evaluated by synchrotron radiation-based Fourier transform infrared microspectroscopy. Journal of Integrative Medicine, 23(2), 182–194. https://doi.org/10.1016/j.joim.2025.02.001

Hemby, S. E., McIntosh, S., Leon, F., Cutler, S. J., & McCurdy, C. R. (2019). Abuse liability and therapeutic potential of the Mitragyna speciosa (kratom) alkaloids mitragynine and 7-hydroxymitragynine. Addictive Biology, 24(5), 874–885. https://doi.org/10.1111/adb.12639

Standard, I. (2009). Biological evaluation of medical devices—Part 5: Tests for in vitro cytotoxicity. Geneve, Switzerland: International Organization for Standardization, 10, 9781570203558.

Joo, T., Sowndhararajan, K., Hong, S., Lee, J., Park, S.-Y., Kim, S., & Jhoo, J.-W. (2014). Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells by stem bark of Ulmus pumila L. Saudi Journal of Biological Sciences, 21(5), 427–435. https://doi.org/10.1016/j.sjbs.2014.04.003

Kang, M. H., Han, Y. M., Lee, Y. J., Ji, Y.-J., Seong, H.-A., & Kim, H. D. (2023). Seasonal variations in bioactive compounds and antioxidant activity in the leaves of Sasa quelpaertensis. Horticultural Science and Technology, 41(4), 403–413. https://doi.org/10.7235/HORT.20230037

Klintong, S., Teethaisong, Y., Sittisart, P., Jantarit, N., & Dunkhunthod, B. (2024). Photoprotective effect of Pogonatherum paniceum extract against ultraviolet-B-induced skin aging in HaCaT keratinocytes. Revista Brasileira de Farmacognosia, 34(6), 1363–1376. https://doi.org/10.1007/s43450-024-00586-9

Kratom Plant Act, B.E. 2565 (2022). (2022, August 26). Royal Thai Government Gazette, 139(52G), 1–14.

Kruegel, A. C., & Grundmann, O. (2018). The medicinal chemistry and neuropharmacology of kratom: A preliminary discussion of a promising medicinal plant and analysis of its potential for abuse. Neuropharmacology, 134, 108–120. https://doi.org/10.1016/j.neuropharm.2017.08.026

Kumarnsit, E., Keawpradub, N., & Nuankaew, W. (2006). Acute and long-term effects of alkaloid extract of Mitragyna speciosa on food and water intake and body weight in rats. Fitoterapia, 77(5), 339–345. https://doi.org/10.1016/j.fitote.2006.04.006

Kumarnsit, E., Vongvatcharanon, U., Keawpradub, N., & Intasaro, P. (2007). Fos-like immunoreactivity in rat dorsal raphe nuclei induced by alkaloid extract of Mitragyna speciosa. Neuroscience Letters, 416(2), 128–132. https://doi.org/10.1016/j.neulet.2007.01.061

Makchuchit, S., Rattarom, R., & Itharat, A. (2017). The anti-allergic and anti-inflammatory effects of Benjakul extract (a Thai traditional medicine), its constituent plants and some pure constituents using in vitro experiments. Biomedicine & Pharmacotherapy, 89, 1018–1026. https://doi.org/10.1016/j.biopha.2017.02.066

Martemucci, G., Costagliola, C., Mariano, M., D'Andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free radical properties, source and targets, antioxidant consumption and health. Oxygen, 2(2), 48–78. https://doi.org/10.3390/oxygen2020006

Matsumoto, K., Horie, S., Ishikawa, H., Takayama, H., Aimi, N., Ponglux, D., & Watanabe, K. (2004). Antinociceptive effect of 7-hydroxymitragynine in mice: Discovery of an orally active opioid analgesic from the Thai medicinal herb Mitragyna speciosa. Life Sciences, 74, 2143–2155. https://doi.org/10.1016/j.lfs.2003.09.054

Matsumoto, K., Mizowaki, M., Suchitra, T., Takayama, H., Sakai, S., Aimi, N., & Watanabe, K. (1996). Antinociceptive action of mitragynine in mice: Evidence for the involvement of supraspinal opioid receptors. Life Sciences, 59(14), 1149–1155. https://doi.org/10.1016/0024-3205(96)00411-8

Parthasarathy, S., Azizi, J. B., Ramanathan, S., Ismail, S., Sasidharan, S., Mohd. Said, M. I., & Mansor, S. M. (2009). Evaluation of antioxidant and antibacterial activities of aqueous, methanolic and alkaloid extracts from Mitragyna speciosa (Rubiaceae family) leaves. Molecules, 14, 3964–3974. https://doi.org/10.3390/molecules14103964

Parthasarathy, S., Ramanathan, S., Murugaiyah, V., Hamdan, M. R., Mohd Said, M. I., Lai, C. S., & Mansor, S. M. (2013). A simple HPLC-DAD method for the detection and quantification of psychotropic mitragynine in Mitragyna speciosa (Ketum) and its products for application in forensic investigation. Forensic Science International, 226(1–3), 183–187. https://doi.org/10.1016/j.forsciint.2013.01.014

Pratama, R. R., Sari, R. A., Sholikhah, I., Mansor, H., Chang, H.-I., Sukardiman, & Widyowati, R. (2024). Inhibition of nitric oxide production in RAW 264.7 cells and cytokines IL-1β in osteoarthritis rat models of 70% ethanol extract of Arcangelisia flava (L.) Merr. stems. Heliyon, 10(15), e35730. https://doi.org/10.1016/j.heliyon.2024.e35730

Rahmawati, S. I., Indriani, D. W., Ningsih, F. N., Hardhiyuna, M., Firdayani, F., Ahmadi, P., Rosyidah, A., Septiana, E., Dharmayanti, N. L. P. I., Bayu, A., & Putra, M. Y. (2024). Dual anti-inflammatory activities of COX-2/5-LOX driven by kratom alkaloid extracts in lipopolysaccharide-induced RAW 264.7 cells. Scientific Reports, 14, 28993. https://doi.org/10.1038/s41598-024-79229-x

Shaik Mossadeq, W. M., Sulaiman, M. R., Tengku Mohamad, T. A., Chiong, H. S., Zakaria, Z. A., Jabit, M. L., Baharuldin, M. T. H., & Israf, D. A. (2009). Anti-inflammatory and antinociceptive effects of Mitragyna speciosa Korth methanolic extract. Medical Principles and Practice, 18(5), 378–384. https://doi.org/10.1159/000226292

Sittisart, P., Dunkhunthod, B., & Chuea-nongthon, C. (2020). Antioxidant and anti-inflammatory activities of ethanolic extract from Hoya kerrii Craib. Chiang Mai Journal of Science, 47(5), 912–925.

Srisakul, A. T. (2022). Kratom and the health care among Thais. Journal of Health Research and Innovation, 5(1), 3–6.

Tangtrongchit, K. (1978). Properties of Thai medicine (Part one) regarding various plants, minerals and animals. Bophitphim.

Tohar, N., Shilpi, J. A., Sivasothy, Y., Ahmad, S., & Awang, K. (2019). Chemical constituents and nitric oxide inhibitory activity of supercritical carbon dioxide extracts from Mitragyna speciosa leaves. Arabian Journal of Chemistry, 12(3), 350–359. https://doi.org/10.1016/j.arabjc.2016.09.005

Utar, Z., Majid, M. I. A., Adenan, M. I., Jamil, M. F. A., & Lan, T. M. (2011). Mitragynine inhibits COX-2 mRNA expression and prostaglandin E2 production induced by lipopolysaccharide in RAW264.7 macrophage cells. Journal of Ethnopharmacology, 136, 75–82. https://doi.org/10.1016/j.jep.2011.04.011

Yamazaki, K., Hashimoto, A., Kokusenya, Y., Miyamoto, T., & Sato, T. (1994). Electrochemical method for estimating the antioxidative effect of methanol extracts of crude drugs. Chemical and Pharmaceutical Bulletin, 42, 1663–1665. https://doi.org/10.1248/cpb.42.1663

Zhang, H., Zhang, X., Xu, T., Li, X., Storey, K. B., Chen, Q., & Niu, Y. (2022). Effects of acute heat exposure on oxidative stress and antioxidant defenses in overwintering frogs, Nanorana parkeri. Journal of Thermal Biology, 110, 103355. https://doi.org/10.1016/j.jtherbio.2022.103355