Published by SET Publisher

Journal of Pharmacy and Nutrition Sciences

ISSN (online): 1927-5951

Integrated Green Microwave Extraction and Hydrophobic Resin-Based Enrichment of Mitragynine from *Mitragyna speciosa*

Pakorn Suksangsri[1], Weerapat Santinakul[1], Wiwit Suttithumsatid[1,2] and Pharkphoom Panichayupakaranant[1,2,*]

[1]*Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai 90112, Thailand*

[2]*Phytomedicine and Pharmaceutical Biotechnology Excellence Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai 90112, Thailand*

Article Info:

Keywords:
Green extraction,
Hydrophobic resin,
Kratom,
Microwave,
Mitragyna speciosa,
Mitragynine.

Timeline:
Received: December 15, 2025
Accepted: January 03, 2026
Published: March 18, 2026

Citation: Suksangsri P, Santinakul W, Suttithumsatid W, Panichayupakaranant P. Integrated green microwave extraction and hydrophobic resin-based enrichment of mitragynine from *Mitragyna speciosa*. J Pharm Nutr Sci 2026; 16: 1-7.

Abstract:

Kratom (*Mitragyna speciosa* Korth.) has emerged as a promising botanical source of bioactive indole alkaloids, most notably mitragynine, which exerts analgesic activity through partial agonism at μ-opioid receptors. Advancing its pharmacological and clinical potential requires extraction and purification strategies that are efficient, safe, and environmentally responsible, as many conventional methods rely on toxic solvents and produce impure extracts. This study developed an integrated green extraction platform to obtain mitragynine-enriched extracts from kratom leaves with high efficiency and sustainability. Microwave-assisted extraction (MAE), selected for its solvent-saving and energy-efficient performance, produced an ethanolic crude extract with a yield of 28.6% and a mitragynine content of 6.54 ± 0.28% w/w. Initial purification using the low-cost hydrophobic resin SEPLITE® LXA1180 resulted in co-elution of plastid pigments, prompting optimization of both the extraction solvent and elution system. Incorporating 35% glycerin in ethanol effectively suppressed chlorophyll solubilization, while subsequent fractionation using stepwise elution (40%, 50%, and 80% ethanol) markedly reduced polar impurities. The optimized protocol yielded a purified fraction (B2) with a substantially increased mitragynine concentration of 32.41 ± 1.18% w/w. Overall, this integrated MAE–hydrophobic resin approach enhances mitragynine purity while adhering to green-extraction principles through the use of safer solvents and minimized waste. The method provides a practical, scalable, and sustainable framework for preparing standardized mitragynine-rich extracts, supporting future development of kratom-derived analgesic candidates.

DOI: https://doi.org/10.29169/1927-5951.2026.16.01

*Corresponding Author
Tel: +66-74-288980;
E-mail: pharkphoom.p@psu.ac.th

1. INTRODUCTION

Kratom (*M. speciosa*), a tropical evergreen tree of the Rubiaceae family, is native to Southeast Asia, particularly Thailand, Malaysia, and Indonesia [1]. Traditionally, kratom leaves have been used among rural communities, especially in southern Thailand, for enhancing work endurance or as part of local social customs [2-3]. Since its removal from Thailand's Narcotics Schedule V in 2021, kratom has attracted increasing interest not only for traditional and recreational use but also as a promising source of bioactive compounds with potential medicinal and industrial applications.

Mitragynine, the major indole alkaloid in kratom, constitutes up to 66% of the total alkaloid content in the leaves [4-5]. It exhibits moderate lipophilicity (logP = 1.73) and high solubility in ethanol, chloroform, and acetic acid, but poor solubility in water or basic solvents [6-7]. Structurally related diastereomers, speciogynine, speciociliatine, and mitraciliatine, share similar chemical frameworks [4]. Mitragynine displays diverse pharmacological activities, with analgesic effects mediated primarily through partial agonism at μ-opioid receptors [8]. Although less potent than morphine, it presents a more favorable safety profile with markedly reduced respiratory depression [6]. Additional anti-inflammatory and antidepressant effects have also been reported, though largely at the preclinical stage.

Evidence suggests that kratom's analgesic properties may arise from synergistic interactions among multiple alkaloids. Sabetghadam *et al*. (2010) reported that alkaloid-rich extracts produced stronger analgesic responses in animal models than methanolic or aqueous extracts, indicating that minor alkaloids may modulate or enhance mitragynine's activity [9]. Thus, generating mitragynine-enriched extracts while retaining minor alkaloids may support the development of safe, multi-component analgesic formulations.

To enable pharmacological or clinical use, however, extraction and purification methods must be efficient, safe, and environmentally sustainable. Conventional extraction techniques often rely on toxic solvents or yield crude extracts containing chlorophylls, carotenoids, phenolics, and organic acids. Microwave-assisted extraction (MAE) has emerged as a promising green alternative due to its high efficiency, reduced processing time, and compatibility with low-toxicity solvents [10].

Among the expanding range of green solvents, bio-derived and biodegradable alternatives such as ethanol, ethyl lactate, deep eutectic solvents (DESs), glycerin, and supercritical fluids have gained considerable interest for natural product extraction [11]. Each system, however, presents practical limitations, including high viscosity (e.g., glycerin, DESs), narrow polarity range (e.g., supercritical CO_2), elevated cost (e.g., ionic liquids), or requirements for specialized instrumentation. Within this landscape, the glycerin-ethanol binary system has emerged as a particularly promising green solvent. Glycerin, an abundant, low-cost byproduct of biodiesel production, is non-toxic, biodegradable, and recognized as safe for food and pharmaceutical use. Its strong hydrogen-bonding capacity and high polarity facilitate solubilization of various polar phytochemicals, whereas ethanol provides lower viscosity, broader solvation capability, and efficient downstream removal. When combined, glycerin and ethanol form a tunable solvent system with adjustable polarity and improved mass-transfer efficiency as well as suppress pigment extraction.

Despite these advantages, single-step extraction typically produces crude extracts containing undesirable co-extractives, necessitating a subsequent purification step. Hydrophobic macroporous adsorbent resins provide a simple, scalable, and eco-friendly option for enriching mitragynine [12-14]. Febriandi *et al*. (2025) demonstrated that both PAD610 and PAD900 effectively enriched mitragynine content [15]. However, the high cost of these resins remains a major challenge for large-scale or commercial applications. Therefore, employing a low-cost hydrophobic resin, such as SEPLITE® LXA1180, could offer a practical and sustainable alternative.

Integrating MAE with LXA1180-based purification provides a sustainable platform for producing mitragynine-rich extracts with reduced impurities, minimized solvent use, and improved scalability. In this context, the present study employs a glycerin-ethanol solvent system to enhance extraction selectivity while maintaining strong sustainability credentials. Accordingly, the objectives of this work are: (i) to evaluate glycerin-ethanol as a green solvent for MAE of *M. speciosa*; (ii) to optimize LXA1180-based resin purification for mitragynine enrichment; and (iii) to establish an integrated, efficient, and environmentally responsible extraction-purification process. The findings aim to support the development of standardized, high-quality kratom-derived extracts for future pharmacological and industrial applications.

2. MATERIALS AND METHODS

2.1. Plant Material

Dried kratom leaves were purchased from a local market in Songkhla, Thailand. The plant material was taxonomically identified, and a voucher specimen (No. SKP 165-13-19-18) was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University. The dried leaves were coarsely ground, pulverized into fine powder, and sieved through a No. 20 mesh to obtain a uniform particle size.

2.2. Chemicals

Mitragynine standard was obtained from AdooQ Bioscience (Beijing, China). Ethanol (analytical grade), methanol (HPLC grade), ammonium bicarbonate, and other analytical reagents were supplied by RCI Labscan (Bangkok, Thailand). Ultrapure water was prepared using a Milli-Q purification system (Millipore, Bedford, MA, USA). The hydrophobic adsorbent resin SEPLITE® LXA1180 was purchased from Sunresin (Shaanxi, China).

2.3. Microwave-Assisted Extraction

Leaf powders were extracted with either ethanol or 35% v/v glycerin in ethanol using a solid-to-solvent ratio of 1:25 (60 g: 1.5 L). Extractions were performed in a microwave extractor operating at 900 W and 2.45 GHz for 5 min, followed by 1 min of manual stirring. The extraction cycle was repeated once under the same conditions while maintaining the temperature at 70-79 °C.

After extraction, the mixtures were filtered. Ethanol in the crude ethanol extract was removed under reduced pressure using a rotary evaporator, and the residue was dried in a desiccator prior to HPLC quantification of mitragynine.

2.4. Purification of Ethanol Extract

A 200 mL aliquot of crude ethanol extract, prior to evaporation, was diluted with an equal volume of deionized water and loaded onto a chromatographic column (5 × 60 cm) packed with 200 g of LXA1180 resin pre-equilibrated with 50% ethanol. Sequential elution was performed using 50% (1 L), 60% (4 L), 70% (2 L), and 80% (7 L) ethanol. Fractions of 200 mL were collected. Three pooled alkaloid fractions (A1-A3) were identified based on fraction color and the Dragendorff's reagent precipitation test. Each pooled fraction was concentrated by rotary evaporation at 50 °C and dried under reduced pressure.

2.5. Purification of 35% Glycerin Extract

A 200 mL portion of the 35% glycerin extract, prior to evaporation, was mixed with 200 mL deionized water and applied to a column of identical dimensions packed with 200 g LXA1180 equilibrated with 40% ethanol. Elution proceeded with 40% (2 L), 50% (3 L), and 80% (10 L) ethanol. Fractions of 200 mL were collected and combined into two pooled alkaloid fractions (B1 and B2) using the same identification criteria as above. Each pooled fraction was concentrated at 50 °C and dried under reduced pressure.

2.6. Quantitative HPLC Analysis of Mitragynine

Quantification of mitragynine was performed using a previously reported method [16] with minor modifications. Briefly, the analysis was conducted on a Shimadzu LC-20 HPLC system equipped with a photodiode array detector. Separation was achieved on an ACE® 5 C18-PFP column (150 × 4.6 mm). The mobile phase consisted of (A) 5.0 mM ammonium bicarbonate (pH 9.5) and (B) acetonitrile with the following gradient: 0-17 min, 30-70% B; 17-18 min, 70% B; 18-19 min, 70-30% B. The flow rate was 1.5 mL/min, and detection was set at 266 nm.

A standard stock solution (1 mg/10 mL methanol) was serially diluted (6.25-100 µg/mL) and filtered through a 0.45 µm membrane prior to injection. Calibration was performed using peak area versus concentration, yielding the regression equation $Y = 5564.2X - 10{,}775$ ($R^2 = 0.9998$). Sample solutions were prepared by dissolving 1 mg of each fraction in 10 mL methanol and filtering through a 0.45 µm membrane. All measurements were performed in triplicate.

2.7. Statistical Analysis

All experiments were conducted in triplicate. Data are presented as mean ± standard deviation. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$.

3. RESULTS AND DISCUSSION

Mitragynine is relatively stable at temperatures up to 80 °C for short periods, and MAE can be conducted effectively when temperature is carefully controlled to prevent degradation. In this study, MAE was performed

at 900 W for two 5-min cycles while maintaining the temperature between 70-79 °C to ensure efficient extraction while avoiding thermal degradation of mitragynine.

Variation in mitragynine content among kratom leaves cultivated in Thailand (0.75-2.66% w/w) has been previously documented and may influence the quality and pharmacological consistency of kratom-derived products [17]. Therefore, the plant material used in this study was first evaluated for mitragynine content by HPLC. The raw leaf powder contained 1.86 ± 0.48% w/w mitragynine (dry weight), meeting the Thai FDA requirement of not less than 1.0% w/w for use in regulated herbal or food products [18]. These findings confirm that the plant material was of adequate quality for extraction and purification.

MAE using ethanol yielded an extract containing 6.54 ± 0.28% w/w mitragynine and provided a high extraction yield of 28.6%. This represents a substantial enrichment relative to the raw plant material, highlighting the efficiency of MAE in recovering alkaloids. The results align with those reported by Karunakaran *et al*., who found mitragynine levels of 7.2-9.4% w/w in Malaysian kratom extracts prepared using ultrasound-assisted extraction (UAE) [19]. Although UAE also enhances extraction efficiency, it typically requires longer extraction times and larger volumes of solvent compared with MAE.

The superior performance of MAE is attributed to rapid volumetric heating and effective cell disruption, which improve alkaloid solubilization and reduce solvent consumption. These findings are consistent with Subtaeng *et al*. [20], who reported that MAE yields mitragynine-rich extracts more efficiently and preserves compound integrity better than conventional techniques such as reflux extraction.

Following MAE, the crude ethanol extract was subjected to purification using an LXA1180 adsorption column. Three pooled fractions (A1-A3) were obtained based on Dragendorff's reagent testing and fraction color. HPLC analysis (Table **1**) showed that fraction A2 contained the highest mitragynine concentration (16.56% w/w), which was significantly higher than that of fractions A1 and A3. Although column fractionation increased mitragynine content relative to the crude extract, the extent of enrichment remained modest, and A2 and A3 still displayed a brown coloration indicative of co-eluted pigments.

To enhance purification efficiency, the elution strategy was optimized. Given the polarity characteristics of mitragynine and its elution behavior on LXA1180, it was expected to elute predominantly at ethanol concentrations between 60-80% v/v. Therefore, lower ethanol concentrations (< 50%) were used initially to remove polar impurities prior to mitragynine elution. However, the use of 80% ethanol resulted in co-elution of chlorophylls, leading to darker extracts and reduced apparent purity.

To address this issue, 35% glycerin in ethanol was introduced as the extraction solvent. Glycerin effectively suppressed chlorophyll solubilization due to its high polarity and viscosity, producing crude extracts with lighter coloration. The optimized extract was then subjected to LXA1180 column chromatography using the revised 40-50-80% ethanol gradient. Two pooled fractions (B1 and B2) were obtained; B2 appeared as a pale yellow-brownish powder after drying.

HPLC quantification (Figure **1**) revealed that fraction B2 contained up to 32.41 ± 1.18% w/w mitragynine, which was significantly higher than that of fraction B1 (Table **2**), representing a pronounced improvement over both the crude extract and earlier fractions. This enhancement is likely attributed to the more suitable solvent polarity and improved elution efficiency under the optimized conditions. The combined use of a glycerin-ethanol solvent system and stepwise ethanol elution effectively minimized co-extracted pigments and other polar constituents while substantially enriching mitragynine.

Table 1: Extraction Yields and Mitragynine Levels of the Pooled Fractions (A1-A3)

Pooled fractions	Yields of extract (% w/w)*	Mitragynine content (% w/w)
A1 (Fractions 7-15)	0.80	2.97 ± 0.08^{a}
A2 (Fractions 16-51)	0.88	16.56 ± 0.99^{b}
A3 (Fractions 52-97)	1.11	11.41 ± 0.24^{c}

*Values were determined relative to the dry weight of the leaf powder. Values in the same column marked with different letters indicate statistically significant differences ($p < 0.05$).

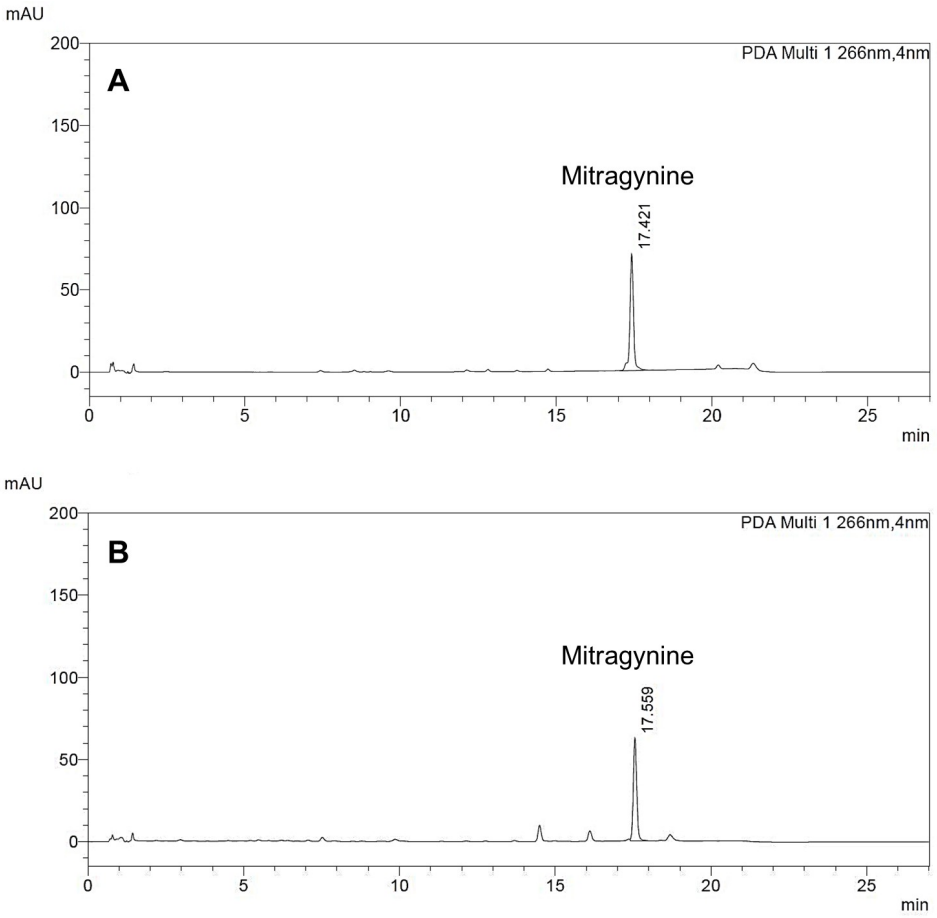

Figure 1: HPLC-chromatograms of standard mitragynine (**A**) and mitragynine-enriched fraction B2 (**B**).

Table 2: Extraction Yields and Mitragynine Levels of Fractions B1 and B2

Pooled fractions	Yields of extract (% w/w)*	Mitragynine content (% w/w)
B1 (Fractions 8-23)	3.61	7.28 ± 0.67^{a}
B2 (Fractions 24-74)	2.94	32.41 ± 1.18^{b}

*Values were determined relative to the dry weight of the leaf powder. Values in the same column marked with different letters indicate statistically significant differences ($p < 0.05$).

In comparison with other green extraction techniques, the integrated MAE-resin approach developed in this study offers several practical advantages. While ultrasound-assisted extraction (UAE) improves mass transfer, it typically requires longer processing times and greater solvent volumes. Supercritical CO_2 extraction provides excellent selectivity for non-polar constituents but depends on high-pressure equipment and co-solvents, increasing cost and operational complexity. Pressurized liquid extraction (PLE) enhances extraction kinetics but may expose thermolabile alkaloids to degradation. In contrast, MAE enables rapid volumetric heating, reduced solvent use, and short extraction times while maintaining temperatures conducive to alkaloid stability [21-23]. When combined with LXA1180 purification and a glycerin-ethanol green solvent system, this workflow delivers higher selectivity, minimal pigment co-extraction, and improved cost-effectiveness, making it a more scalable and industrially viable option.

Overall, the optimized process produced a standardized, mitragynine-rich extract with enhanced purity and reduced pigment contamination. This eco-friendly and scalable methodology supports the development of high-quality kratom extracts for pharmacological research and future commercial applications, with glycerin contributing both improved selectivity and strong alignment with green extraction principles.

Despite the advantages of the optimized extraction, purification workflow, several limitations should be acknowledged. First, natural variability in the alkaloid content of *M. speciosa* leaves, arising from differences in geography, cultivation practices, plant age, and post-harvest handling, may influence the reproducibility of mitragynine yield and purity. Standardization of raw materials or implementation of pre-extraction quality screening will be essential for ensuring consistent product quality in large-scale operations. Second, although the method demonstrates strong potential for scalability, the operational parameters of MAE (such as heat distribution, energy input, and batch size) may require adjustment when transitioning from laboratory units to pilot- or industrial-scale continuous systems. Similarly, resin performance under repeated loading-elution cycles must be evaluated to assess long-term stability, regeneration efficiency, and adsorption capacity. Finally, while LXA1180 is more cost-effective than other hydrophobic resins, the overall cost-benefit ratio will depend on solvent recycling efficiency, resin lifetime, and process throughput. Addressing these considerations in future studies will help strengthen the economic feasibility and industrial applicability of the developed green extraction platform.

4. CONCLUSIONS

This study established a green and efficient protocol for producing mitragynine-enriched extracts from *M. speciosa* by integrating MAE with LXA1180 hydrophobic resin purification. The use of a glycerin-ethanol solvent system in MAE significantly enhanced extraction selectivity by suppressing pigment solubilization while maintaining high extraction efficiency. Subsequent purification using a low-cost LXA1180 resin and a reduced-solvent 40-50-80% ethanol gradient yielded a highly enriched fraction containing 32.41% w/w mitragynine with strong recovery.

Compared with existing MAE-resin approaches, the present workflow introduces three key innovations: (i) a glycerin–ethanol green solvent system that improves selectivity and reduces pigment contamination; (ii) the use of an economical hydrophobic resin suitable for scale-up; and (iii) a streamlined elution strategy that minimizes solvent and chlorophyll co-elution. Together, these advances provide a practical, scalable, and environmentally responsible method for obtaining high-purity mitragynine.

Future work should include bioactivity validation of the enriched fractions, assessment of resin regeneration and reuse, and pilot-scale or continuous-flow extraction trials to evaluate industrial feasibility. These efforts will support the development of standardized kratom-derived ingredients for research, therapeutic development, and potential commercial applications.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This research was financially supported by the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Thailand.

REFERENCES

[1] Zhang M, Lyndon A, Pearson B, McCurdy C, Sharma A, Chen J. Kratom: Botanical insights and cultivation practices for a conspicuous medicinal tree species: ENH1389/EP653, 7/2025. EDIS 2025; 2025(4). https://doi.org/10.32473/edis-ep653-2025

[2] Saingam D, Assanangkornchai S, Geater AF, Balthip Q. Pattern and consequences of krathom (*Mitragyna speciosa* Korth.) use among male villagers in southern Thailand: a qualitative study. Int J Drug Policy 2013; 24(4): 351-358. https://doi.org/10.1016/j.drugpo.2012.09.004

[3] Singh D, Narayanan S, Vicknasingam B, Corazza O, Santacroce R, Roman-Urrestarazu A. Changing trends in the use of kratom (*Mitragyna speciosa*) in Southeast Asia. Hum Psychopharmacol 2017; 32(3). https://doi.org/10.1002/hup.2582

[4] Karunakaran T, Ngew KZ, Zailan AAD, Mian Jong VY, Abu Bakar MH. The chemical and pharmacological properties of mitragynine and its diastereomers: An insight review. Front Pharmacol 2022; 13: 805986. https://doi.org/10.3389/fphar.2022.805986

[5] Takayama H. Chemistry and pharmacology of analgesic indole alkaloids from the rubiaceous plant, *Mitragyna speciosa*. Chem Pharm Bull (Tokyo) 2004; 52(8): 916-928. https://doi.org/10.1248/cpb.52.916

[6] Khamooshi F, Akinnawo AS, Doraji-Bonjar S, Modarresi-Alam A. Mitragynine chemistry: Extraction, synthesis, and clinical effects. Chem Afr 2024; 7: 2907-2920. https://doi.org/10.1007/s42250-024-00921-6

[7] Ramanathan S, Parthasarathy S, Murugaiyah V, Magosso E, Tan SC, Mansor SM. Understanding the physicochemical properties of mitragynine, a principal alkaloid of *Mitragyna speciosa*, for preclinical evaluation. Molecules 2015; 20(3): 4915-27. https://doi.org/10.3390/molecules20034915

[8] Kruegel AC, Gassaway MM, Kapoor A, Váradi A, Majumdar S, Filizola M, Javitch JA, Sames D. Synthetic and receptor signaling explorations of the *Mitragyna* alkaloids: Mitragynine as an atypical molecular framework for opioid receptor modulators. J Am Chem Soc 2016; 138(21): 6754-6764. https://doi.org/10.1021/jacs.6b00360

[9] Sabetghadam A, Ramanathan S, Mansor SM. The evaluation of antinociceptive activity of alkaloid, methanolic, and aqueous extracts of Malaysian *Mitragyna speciosa* Korth leaves in rats. Pharmacognosy Res 2010; 2(3): 181-185. https://doi.org/10.4103/0974-8490.65514

[10] Panichayupakaranant P. Active constituent-rich herbal extracts for development of phytomedicine. Songklanagarind Med J 2017; 35(3): 187-193. https://doi.org/10.31584/smj.2017.35.3.728

[11] Shahbaz M, Riaz M, Momal U, Rasool IFU, Naeem H, Raza N, Moreno A, Khalid W, Esatbeyoglu T. Green solvent extraction and eco-friendly novel techniques of bioactive compounds from plant waste: Applications, future perspective and circular economy. Appl Food Res 2025; 5(2): 101434. https://doi.org/10.1016/j.afres.2025.101434

[12] Saengkhaw K, Arthan N, Panichayupakaranant P. A green method for preparation of cyanidin-3-glucoside from *Carissa carandas* fruits and α-glucosidase inhibitory activity evaluation. J Pharm Nutr Sci 2024; 14: 84-89. https://doi.org/10.29169/1927-5951.2024.14.10

[13] Lateh L, Yuenyongsawad S, Chen H, Panichayupakaranant P. A green method for preparation of curcuminoid‐rich *Curcuma longa* extract and evaluation of its anticancer activity. Pharmacogn Mag 2019; 15: 730-735. https://doi.org/10.4103/pm.pm_162_19

[14] Meah MS, Panichayupakaranant P. α-Mangostin-rich extract: A potential oral antibacterial agent prepared by green extraction. Int J Pharmacogn Chinese Med 2020; 4: 000201. https://doi.org/10.23880/ipcm-16000201

[15] Febriandi R, Rizaldy D, Ishak SSO, Elfahmi. Development of an efficient and eco-friendly method for mitragynine enrichment. Curr Res Biosci Biotechnol 2025; 7(1): 27-34.

[16] Norliana-Izzati MR, Lee CT, Fauziah A, Zunoliza A, Ling SK, Mohd-Hafidz-Hadi A, Ng KKS, Ng CH, Tnah LH, Ng CL, Goh H-H, Lee SL. HPLC-PDA method for the quantification of mitragynine in fresh Kratom (*Mitragyna speciosa*) leaf. J Trop For Sci 2024; 36(4): 434-443. https://doi.org/10.26525/jtfs2024.36.4.434

[17] Leksungnoen N, Andriyas T, Ngernsaengsaruay C, Uthairatsamee S, Racharak P, Sonjaroon W, Kjelgren R, Pearson BJ, McCurdy CR, Sharma A. Variations in mitragynine content in the naturally growing Kratom (*Mitragyna speciosa*) population of Thailand. Front Plant Sci 2022; 13: 1028547. https://doi.org/10.3389/fpls.2022.1028547

[18] AustChamThailand. Thailand's Updated Guidelines on Kratom in Food and Herbal Products. https://www.austchamthailand.com/resources/news/thailands-updated-guidelines-kratom-food-and-herbal-products#:~:text=Raw%20material%20specifications%20and%20standards,necessary%20health%20and%20safety%20standards. 08 Jan 2025. Accessed on November 13, 2025.

[19] Karunakaran T, Goh YS, Santhanam R, Murugaiyah V, Abu Bakar MH, Ramanathan S. RP-HPLC-DAD analysis of mitragynine content in *Mitragyna speciosa* Korth. (Ketum) leaf extracts prepared using ultrasound assisted extraction technique and their cytotoxicity. Separations 2022; 9(11): 345. https://doi.org/10.3390/separations9110345

[20] Subtaeng S, Kruatian T, Jitpreeda P, Wanitsuwan W. Method validation for determination of mitragynine in Kratom leaf extract by high performance liquid chromatography. Bull Appl Sci 2023; 12(2): 10-22. https://doi.org/10.60136/bas.v12.2023.621

[21] Shen L, Pang S, Zhong M, Sun Y, Qayum A, Liu Y, Rashid A, Xu B, Liang Q, Ma H, Ren X. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. Ultrason Sonochem 2023; 101: 106646. https://doi.org/10.1016/j.ultsonch.2023.106646

[22] Kamjam M, Ngamprasertsith S, Sawangkeaw R, Charoenchaitrakool M, Privat R, Jaubert J-N, Molière M. The great versatility of supercritical fluids in industrial processes: A focus on chemical, agri-food and energy applications. Processes 2024; 12(11): 2402. https://doi.org/10.3390/pr12112402

[23] Palos-Hernández A, González-Paramás AM, Santos-Buelga C. Latest advances in green extraction of polyphenols from plants, foods and food by-products. Molecules 2025; 30(1): 55. https://doi.org/10.3390/molecules30010055