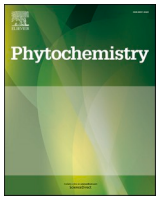
Phytochemistry 247 (2026) 114871

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem



Quantitative analysis of 7-hydroxymitragynine in commercial kratom products and its stability under chemical and physiological conditions

Bharathi Avula [a], Syed Kashif Ali [a], Kiran K. Tatapudi [a], Pankaj Pandey [a], Lochana Cheepilla [b], Islam Husain [a], Shabana I. Khan [a,b], Kumar Katragunta [a], Zulfiqar Ali [a], Ikhlas A. Khan [a,b,*], Amar G. Chittiboyina [a,b,**]

[a] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, Mississippi 38677-1848, United States
[b] Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, Mississippi 38677-1848, United States

ARTICLE INFO

Keywords:
Mitragyna speciosa (Rubiaceae)
Kratom alkaloids
7-Hydroxymitragynine
Mitragynine pseudoindoxyl
Synthetic artifacts
Quantitative analysis
Degradation kinetics

ABSTRACT

7-Hydroxymitragynine (7-OH), a minor alkaloid in kratom (*Mitragyna speciosa* (Korth.) Havil.) (Rubiaceae) and an active metabolite of mitragynine, is a critical transient precursor to mitragynine pseudoindoxyl, which exhibits >2-fold μ-opioid receptor–biased agonism and improved isoform selectivity relative to its precursors. Although present only in trace amounts in kratom leaves, a recent survey identified hundreds of consumer products with semisynthetic 7-OH and mitragynine pseudoindoxyl. Their high potency, ease of access, and risk of addiction have prompted the US FDA to recommend scheduling 7-OH as a Schedule I controlled substance. To address this public health crisis and the associated analytical challenges, a comprehensive analysis of commercially available kratom products was performed. The results revealed substantial inconsistencies between the quantities claimed on the products' labels and the actual 7-OH amount detected in several of these products. Along with 7-OH and mitragynine pseudoindoxyl, several (over)oxidized byproducts were detected, providing crucial insights into their synthetic origins. Analysis of >98% of 7-OH-labeled products indicated a semisynthetic origin, with labeled doses ranging from 0.001 to 33.6 mg per serving. Furthermore, consistent with the prior report, 7-OH degrades into the potentially toxic byproduct 3-dehydromitragynine under simulated gastric conditions. These findings underscore significant safety concerns over oral 7-OH products and necessitate validated analytical monitoring to guide regulatory oversight and consumer protection.

1. Introduction

Mitragynine (**1**) is the principal monoterpene indole alkaloid of *Mitragyna speciosa* (Korth.) Havil. (Rubiaceae), commonly known as kratom. 7-Hydroxymitragynine (7-OH, **2**) is an oxygenated analogue of mitragynine and is often reported as a minor constituent in post-harvest leaf material. Although kratom has been used for centuries, 7-OH was first identified in 1994 as a minor constituent of the plant (Ponglux et al., 1994). Subsequent research has established that 7-OH occurs at trace levels in kratom leaves and is also formed as an active metabolite of **1**, kratom's primary alkaloid. In biological systems, 7-OH (**2**) is produced by hepatic oxidation and exhibits a significantly higher binding affinity for μ-opioid receptors (MOR) than mitragynine itself (Kruegel et al., 2019). The resulting metabolic relationship is particularly relevant in postmortem biological fluids, where the ratio of mitragynine to 7-OH is significantly lower (32.4 to 14.8) (Osawa and Johnson, 2025) compared to ratio reported for original kratom powdered samples (53.84 to 49.32) (Horniakova et al., 2025). While confirming substantial *in vivo* conversion of mitragynine to 7-OH, direct comparison of metabolite ratios across biological and product-based studies should be interpreted cautiously due to differences in sample matrices and analytical context.

Furthermore, 7-OH serves as a key intermediate in the acid-mediated rearrangement of mitragynine, which leads to the formation of mitragynine pseudoindoxyl (**3**) and related spiro compounds. Recent studies

This article is part of a special issue entitled: Prof. David Phillipson published in Phytochemistry.

* Corresponding author: National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, Mississippi 38677-1848, United States.

** Corresponding author: National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, Mississippi 38677-1848, United States.

E-mail addresses: ikhan@olemiss.edu (I.A. Khan), amar@olemiss.edu (A.G. Chittiboyina).

https://doi.org/10.1016/j.phytochem.2026.114871
Received 24 October 2025; Received in revised form 23 January 2026; Accepted 8 March 2026
Available online 11 March 2026

indicate that **3** exhibits higher affinity for MOR than its precursors, 7-OH and mitragynine (Chakraborty et al., 2021; Kamble et al., 2020; Takayama et al., 2002). Hence, biotic oxidative metabolism of **1** into **3** via 7-OH is likely to influence kratom's complex pharmacological effects and potential abuse liabilities (Kamble et al., 2020).

Although fresh kratom leaves contain negligible amounts of 7-OH and **3**, a recent survey identified over 300 consumer products containing these compounds, suggesting that many are likely semisynthetic rather than enriched from minor kratom alkaloids (Hill et al., 2025). Most of these products (82.2%) contained only 7-OH and were sold as chewable or sublingual tablets, shots, and gummies. An additional 14.5% contained both 7-OH and **3**, while only 3.3% contained **3** only. Almost all products containing **3** or a combination of **3** and 7-OH were marketed as "kratom." In contrast, 92% of products containing 7-OH were sold as a standalone ingredient, a significant discrepancy given the low natural abundance of 7-OH in kratom leaf material.

In July 2025, the U.S. Food and Drug Administration formally recommended that 7-OH be classified as a Schedule I controlled substance, a category shared with heroin. In addition to the recommendation, the FDA issued warning letters (U.S. Food and Drug Administration, 2025a, b) to several companies, directing them to stop selling products containing 7-OH. These actions reflect growing public health concerns surrounding the proliferation of highly concentrated, often synthetic, 7-OH products that have received heightened media and regulatory attention. The concentration of 7-OH in "enhanced" products sold online and in convenience stores (Francis, 2024) is significantly higher than levels observed in kratom leaves, and such differences may pose serious inadvertent health risks, including addiction and overdose.

Recent literature has also reported the emergence of a subset of products formulated to contain elevated levels of 7-OH in whole extracts or to enrich specific oxygenated mitragynine-related compounds (Kampmeyer, 2025; Samee et al., 2024). Accurate quantification of 7-OH is therefore essential not only for consumer safety but also crucial for regulators to enforce the law and for scientists to assess the chemical and biological effects of 7-OH. To make the regulatory process more efficient, future efforts should include the quantification of 7-OH congeners, such as **3** and other isobaric compounds (Fig. 1), which are known to interact with opioid receptors with superior potency. Fig. 1

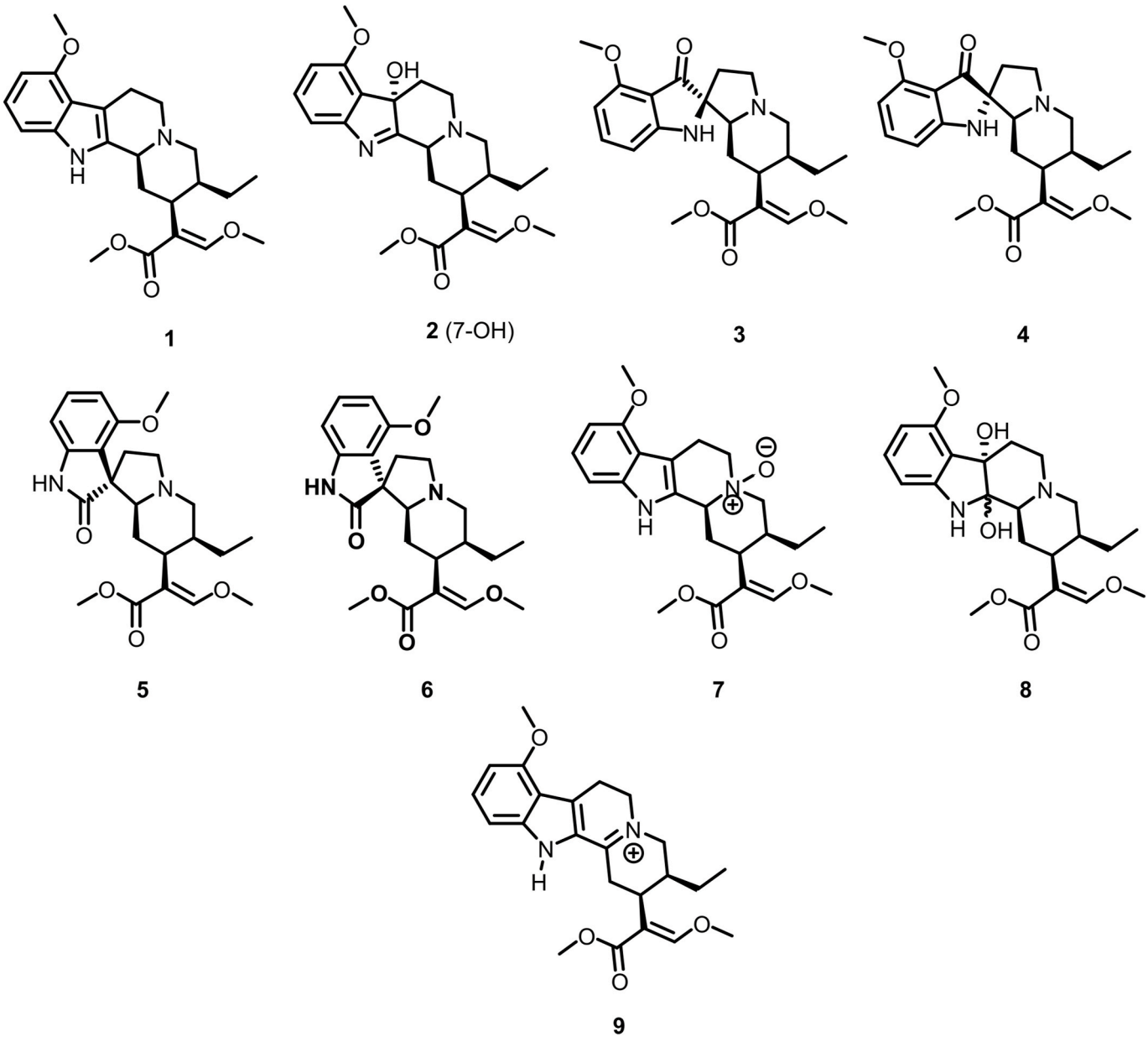


Fig. 1. Structures of mitragynine (**1**), related oxygenated alkaloids (**2**–**8**), and dehydromitragynine (**9**) studied in this work.

shows the structures of mitragynine, related oxygenated mitragynine alkaloids (**2**–**8**), and dehydromitragynine (**9**) investigated in this study. A comprehensive analytical approach would save time and resources by proactively identifying and characterizing semisynthetic substances in various kratom-based consumer products.

In addition to deliberate formulation or enrichment practices, the inherent chemical stability of 7-OH may also influence the composition and chemical profiles of commercial products. Oxidative, acidic, or basic environments can promote degradation or structural rearrangement of mitragynine derivatives, potentially complicating analytical interpretation if not properly considered (Angyal et al., 2023; Chear et al., 2021; Ganasan et al., 2025; Grundmann et al., 2024; Mudge and Brown, 2017; Takayama et al., 2002). Consequently, evaluation of the stability of 7-OH under selected chemical and physiologically relevant conditions is important for distinguishing pre-existing constituents in commercial products from compounds formed during storage, handling, or analysis.

In this study, several commercially available kratom products and specific "7-hydroxy" products, which claim a high percentage of 7-OH , were purchased and analyzed. The results provide evidence of adulteration with structurally related alkaloids as impurities. Furthermore, the stability of 7-OH under chemical and physiological conditions was also investigated to support the interpretation of commercial product profiles.

2. Results

The widespread proliferation of concentrated 7-OH products, such as tablets, gummies, and drink mixes, indicates an elevated concentration of the alkaloid that is not found in naturally occurring kratom. Such an elevated concentration indicates a semisynthetic origin, likely through the oxidative chemical conversion of mitragynine isolates or whole kratom extracts. As only trace amounts of 7-OH are naturally present in kratom leaves, direct extraction from chemically unaltered, full-spectrum extracts would not be economically feasible.

To assess the overall quality of commercial kratom products, especially those marketed with elevated levels of 7-OH , 28 out of 35 products were sourced via online vendors, and seven products (4656-4662) were purchased from a gas station in Oxford, MS, and analyzed using UHPLC-PDA-MS (Table 1). The sample set consisted of seven powders, six capsules, eight liquids, one soft gel, and 13 tablets. To facilitate interpretation of the analytical data, products were grouped by formulation and labeling claims into four functional categories: (i) conventional kratom powders and capsules, (ii) liquid or tincture formulations marketed as kratom extracts, (iii) highly concentrated solid products labeled as "7-OH" or similar terminology, and (iv) other miscellaneous products (Table 2). This classification allowed direct comparison of alkaloid content and artifact profiles across product classes rather than individual products alone. With reference standards and calibration curves, detection limits as low as 50 ppb were achieved using the current analytical methodology, which is expected to enable sensitive and accurate quantification of three specific analytes of interest, mitragynine (**1**), 7-OH (**2**), and mitragynine pseudoindoxyl (**3**). Among the products, 22 claimed the presence of 7-OH, two claimed **3**, and 14 claimed **1**. Additional details pertinent to analytical procedures were outlined under the materials and methods section.

2.1. Quantitative measurement of key kratom alkaloids

2.1.1. Mitragynine (1) content

Mitragynine , the principal alkaloid in kratom, was listed on the labels of 14 out of 35 kratom products analyzed. The content of **1**, as claimed on the manufacturers' label, ranged from ≤7.8 mg per serving in capsule formulations to 40.8 mg per serving in powder-based products. However, analytical testing detected **1** in all 35 products (Table 1). Mitragynine concentrations varied widely by formulation type: solid formulations ranged from 0.02 to 46.3 mg per serving, while liquid formulations ranged from 3.0 to 116 mg per serving.

Discrepancies between labeled claims and analytical concentrations of mitragynine were significant across several evaluated products, with multiple instances exceeding 150% of the stated value. For example, product #4656PR, which specified a range of 20.8–22.5 mg of mitragynine per serving, was determined to contain 40.3 mg upon analysis, which is 186% of the mean labeled claim. Furthermore, dosage forms significantly influenced the concentrations of **1**, with calculated daily intakes ranging from <10 mg to >198 mg based on recommended labeling instructions. Conversely, powder-based kratom products contained quantities closer to their labeled claims (e.g., product #4806 PR claimed 40.8 mg of mitragynine and was determined to contain 40.7 mg). Nevertheless, anomalies in mitragynine's content variability across different formulations highlight the crucial need for rigorous analytical monitoring to ensure label accuracy and safeguard consumer health.

2.1.2. Mitragynine pseudoindoxyl (3) content

The spiro indole alkaloid, **3**, a metabolite with substantially higher MOR agonist activity than either mitragynine or 7-OH, was detected in 33 of the 35 kratom products analyzed. Quantifiable levels were found in most products, with concentrations ranging from 0.01 mg (#4803 PR) to 0.5 mg (#4831PR, 4838 PR) per serving in solid formulations and 0.01 mg (#4659PR, 4660PR) to 8.4 mg (#4814PR) per serving in liquids. Three products (#4656PR, 4661PR, and 4662PR) contained **3** at concentrations below the limit of quantification (LOQ), whereas **3** was not detected in products #4846PR and 4812PR.

Although **3** was frequently detected during analysis, it was specified on the labels of only two products, which claimed 9.0 mg and 10.0 mg per serving, respectively, highlighting significant anomalies in ingredient transparency and quality control within the commercial kratom market.

2.1.3. 7-OH (2) content

7-OH , an oxidative metabolite of mitragynine with superior MOR agonist activity, was declared on the labels of 22 out of 35 evaluated kratom products (most of these products were selected based on 7-OH content). Labeled claims for 7-OH exhibited substantial variance, ranging from ≤0.045% (~0.45 mg/g) in powder formulations to 12.5 mg per serving in tablets. However, analytical quantification detected 7-OH in 34 of 35 products, with measured concentrations ranging from 0.01 to 8.4 mg per serving in solid dosage forms and 0.001 to 0.2 mg/mL in liquid formulations; one product (4842PR) contained no detectable **2**. Calculated maximum daily intakes based on label instructions varied widely, from 0.001 to 33.6 mg. Furthermore, 7-OH concentrations in these formulations significantly exceeded levels typically found in natural dried leaf material (~0.01–0.04% w/w, corresponding to ~0.1–0.4 mg/g) (U.S. Food and Drug Administration, 2025a,b), suggesting that enrichment or targeted alkaloid preparation occurred during manufacturing.

Analysis of 7-OH content revealed significant discrepancies between measured quantities and manufacturer label claims. In products specifying 7-OH content, measured concentrations were consistently lower than advertised, typically ranging from 32 to 81% of the stated amount. Product #4839PR exhibited the lowest levels (32%), while #4832PR showed the highest (81%). Notably, detectable levels of **2** were identified in several products lacking explicit label claims, such as #4814PR (0.1 mg per 10 mL serving).

Alkaloid profiling of 35 commercial kratom products revealed substantial chemical heterogeneity. Of these, 12 products exhibited alkaloid profiles dominated by 7-OH and various structurally related artifacts that are not characteristic of authentic *M. speciosa* leaf extracts. These products consistently displayed chemical signatures indicative of semisynthetic modification rather than simple solvent extraction and enrichment.

The identity of these artifacts was confirmed using reference standards, which identified a consistent suite of oxidation products across

Table 1
Comparison of the label information with the detected quantities of 7-OH (**2**), mitragynine pseudoindoxyl (**3**), and mitragynine (**1**) in commercial kratom samples.

Product code #	Labeled ingredient and amount (mg) per serving size			Label recommended serving size	Label maximum daily servings	Measured ingredients per serving size (mg) (SD[a])			Measured quantity of ingredient in maximum recommended daily servings determined (mg)			Measured *vs* labeled ingredients per serving size (%)		
	7-OH	**3**	Mitragynine			7-OH	**3**	Mitragynine	7-OH	**3**	Mitragynine	7-OH	**3**	Mitragynine
4839PR	10.0	NA[b]	NA	1/2 tablet	1 tablet	3.2 (0.2)	0.3 (0.1)	1.7 (0.04)	6.3	0.5	3.4	31.5	NA	NA
4833PR	7.5	NA	NA	1/2 tablet	1 tablet	4.9 (0.1)	0.2 (0.1)	0.1 (0.001)	9.7	0.3	0.1	64.6	NA	NA
4838PR	7.5	NA	NA	1/2 tablet	1 tablet	5.3 (0.1)	0.5 (0.03)	0.4 (0.03)	10.6	1.0	0.8	70.6	NA	NA
4837PR	12.5	NA	NA	1/4 tablet	1 tablet	8.4 (0.9)	0.2 (0.01)	0.3 (0.02)	33.6	0.7	1.2	67.2	NA	NA
4843PR	7.5	NA	NA	1/2 tablet	NA	6.0 (0.3)	0.3 (0.1)	0.1 (0.001)	NA	NA	NA	79.5	NA	NA
4844PR	10.0	NA	NA	1/2 tablet	NA	6.6 (0.4)	0.1 (0.03)	0.02 (0.001)	NA	NA	NA	66.3	NA	NA
4835PR	10.5	NA	NA	1/2 tablet	2 tablets	7.6 (0.6)	0.2 (0.2)	0.2 (0.1)	30.5	1.0	0.6	72.6	NA	NA
4831PR	7.5	NA	NA	1 tablet	2 tablets	6.0 (0.7)	0.5 (0.3)	1.0 (0.04)	12.1	1.0	2.0	80.1	NA	NA
4834PR	9.0	NA	NA	1/2 tablet	1/2 tablet	5.3 (1.0)	0.1 (0.01)	0.2 (0.03)	5.3	0.1	0.2	59.1	NA	NA
4836PR	9.0	NA	NA	1/2 tablet	1 tablet	5.2 (0.5)	0.2 (0.1)	0.3 (0.1)	10.4	0.3	0.5	57.8	NA	NA
4832PR	7.5	NA	NA	1/2 tablet	1 tablet	6.1 (0.9)	0.2 (0.1)	0.2 (0.003)	12.2	0.3	0.4	81.0	NA	NA
4845PR	7.5	NA	NA	1/4 tablet	NA	5.2 (0.1)	0.1 (0.1)	0.3 (0.4)	NA	NA	NA	69	NA	NA
4842PR	NA	9.0	NA	1/2 tablet	1/2 tablet	ND[c]	3.9 (0.02)	1.1 (0.01)	ND	3.9	1.1	NA	43.2	NA
4814PR	NA	10.0	NA	10 mL	20 mL	0.1 (0.001)	8.4 (0.04)	3.0 (0.1)	0.1	16.8	6.0	NA	84	NA
4846PR	NA	NA	10 mg/mL	NA	NA	0.001[d] mg/mL (0.001)	ND	13.6 [d]mg/mL (0.2)	NA	NA	NA	NA	NA	136
4656PR	<0.04%	NA	20.8-22.5	2.5 mL	2.5 mL	0.001 (0.01)	DUL[e]	40.3 (0.1)	0.001	DUL	40.3	NA	NA	186
4657PR	NA	NA	20.0	28 mL	28 mL	0.03 (0.1)	0.03 (0.002)	30.8 (0.04)	0.03	0.03	30.8	NA	NA	154
4658PR	NA	NA	20.0	14 mL	14 mL	0.2 (0.2)	0.1 (0.001)	45 (0.02)	0.09	0.03	22.4	NA	NA	112
4659PR	NA	NA	NA	1 capsule	2 capsules	0.01 (0.02)	0.01 (0.002)	23.7 (0.2)	0.02	0.01	47.4	NA	NA	NA
4660PR	NA	NA	NA	7.5 mL	7.5 mL	0.07 (0.04)	0.01 (0.01)	116 (0.1)	0.07	0.01	116	NA	NA	NA
4661PR	NA	NA	NA	5 mL	15 mL	0.02 (0.1)	DUL	66 (0.1)	0.1	DUL	198	NA	NA	NA
4662PR	<1.0	NA	<60	5 mL	NA	0.1 (0.01)	DUL	84.4 (0.2)	NA	NA	NA	NA	NA	NA
4808PR	NA	NA	NA	5 capsules	10 capsules	0.08 (0.003)	0.1 (0.002)	31 (0.2)	0.2	0.2	61.9	NA	NA	NA
4810PR	NA	NA	NA	5 capsules	10 capsules	0.1 (0.02)	0.1 (0.002)	33.8 (0.2)	0.2	0.2	67.6	NA	NA	NA
4811PR	NA	NA	NA	5 capsules	10 capsules	0.1 (0.001)	0.1 (0.001)	42.3 (0.2)	0.2	0.2	84.6	NA	NA	NA
4812PR	NA	NA	40.0	1 soft gel	NA	0.1 (0.1)	ND	38.4 (0.4)	NA	NA	NA	NA	NA	96
4809PR	NA	NA	NA	5 capsules	10 capsules	0.1 (0.004)	0.1 (0.002)	46.3 (0.5)	0.2	0.2	92.7	NA	NA	NA
4806PR	0.07	NA	40.8	1 teaspoon = 2.4 g	2 teaspoons	0.1 (0.004)	0.1 (0.001)	40.7 (0.03)	0.2	0.2	81.3	143	NA	99.7
4800PR	<0.045%	NA	1.55%	NA	NA	0.003[d] % (0.003)	0.003[d] % (0.001)	0.9[d] % (0.02)	NA	NA	NA	NA	NA	55.5
4802PR	≤0.35	NA	≤10.5	¼ teaspoon	NA	0.03 (0.01)	0.02 (0.002)	10.3 (0.2)	NA	NA	NA	NA	NA	NA
4805PR	0.17	NA	36.48	1 teaspoon = 2.4 g	2 teaspoons	0.1 (0.01)	0.1 (0.004)	37.3 (0.4)	0.2	0.2	74.6	70.6	NA	102.3
4804PR	0.10	NA	40.56	1 teaspoon = 2.4 g	2 teaspoons	0.1 (0.01)	0.1 (0.001)	39.5 (0.2)	0.2	0.2	79	120	NA	97.3
4807PR	0.36	NA	28.8	1 teaspoon = 2.4 g	2 teaspoons	0.1 (0.001)	0.1 (0.001)	30.7 (0.1)	0.2	0.2	61.5	33.3	NA	106.7
4801PR	≤0.315	NA	≤9.1	¼ teaspoon	NA	0.02 (0.003)	0.02 (0.001)	8.9 (0.1)	NA	NA	NA	NA	NA	NA
4803PR	≤0.27	NA	≤7.8	1 capsule	1 capsule	0.03 (0.01)	0.01 (0.001)	9.4 (0.3)	0.03	0.01	9.4	NA	NA	NA

[a] SD, standard deviation of measurement
[b] NA, not available on product label
[c] ND, not detected
[d] Serving size not specified; data calculated as mg/mL or %
[e] DUL, detected, but under the limit of detection (LOD: 50 ng/mL)

Table 2
Summary of kratom product formulations, labeling claims, and characteristic alkaloid profiles.

Product category	Number of products (n)	Typical label claims	Dominant alkaloid profile	Artifacts detected
Powders	7	Kratom leaf	Dominant: Mitragynine and trace 7-OH	No synthetic artifacts detected
Capsules	6	Kratom leaf	Dominant: Mitragynine and trace 7-OH	No artifacts
Liquids	7	Kratom leaf extract	High mitragynine ± low to moderate 7-OH	No artifacts
	1	Mitragynine pseudoindoxyl shot from *M. speciosa* extract	High mitragynine pseudoindoxyl	No artifacts
Tablets	12	7-OH Mitragynine from *M. speciosa* leaf	High 7-OH ; frequent **3**	Multiple oxidation/ rearranged compounds
	1	Pseudoindoxyl-super alkaloid	High mitragynine pseudoindoxyl	No artifacts
Soft gels	1	Kratom leaf extract	Mitragynine with detectable 7-OH	No synthetic artifacts detected

multiple samples: mitragynine pseudoindoxyl (**3**) and its isomer (**4**), mitragynine oxindoles (**5** and **6**), mitragynine *N* (4) oxide (**7**), 2,7-dihydroxymitragynine (**8**), 2,7-dihydroxypaynantheine, and their corresponding *N*-oxides, and 3-dehydromitragynine (**9**). Compound **9**, a previously reported metabolite of mitragynine (**1**), has been characterized by its distinct pharmacological and toxicological profile (Chakraborty et al., 2021).

Unlike mitragynine or 7-OH , **9** does not display opioid receptor-mediated analgesic activity *in vivo*. However, it demonstrates significant toxicity in C57BL/6J mouse models. Subcutaneous administration of **9** at doses exceeding 50 mg/kg resulted in nearly 100% lethality. Reported LD_{50} values include 48.4 mg/kg (95% CI 71.27–342.3) in 129Sv6 mice and 74 mg/kg (95% CI 48.08–120.5) in CD1 mice (Chakraborty et al., 2021). These data confirm that **9** is toxic across multiple strains through a mechanism independent of opioid receptor activation.

Although specific cytotoxicity assays for **9** in human cell lines are currently limited, broader works on mitragynine metabolites show moderate cytotoxic effects in neuroblastoma (SH-SY5Y), lymphoblastoid (MCL-5), colon adenocarcinoma (Caco-2), and nasopharyngeal carcinoma (NPC/HK-1) lines (Karunakaran et al., 2022; Saidin et al., 2015).

As summarized in Table 1, products in conventional powder and capsule formats exhibited alkaloid profiles dominated by mitragynine, with 7-OH detected only at trace or low levels, consistent with the natural composition of *M. speciosa*. In contrast, products marketed as "7-OH" or "high-potency" formulations displayed markedly elevated concentrations of **2**, frequently accompanied by **3** and additional oxidized or rearranged alkaloid derivatives. These chemical patterns were consistent within their product categories and were notably absent in traditional kratom formulations. A comprehensive summary of formulation types, labeling claims, dominant alkaloids, and artifacts is presented in Table 2, which complements the quantitative findings presented in Table 1.

2.1.4. Chemical profiles across commercial kratom products

Beyond the primary alkaloids quantified in Table 1, several minor alkaloids and oxidative artifacts were identified in a specific subset of products. The distribution of these compounds was formulation dependent. As summarized in Table 3, products marketed as "7-OH" or "high-potency" formulations consistently exhibited a suite of structurally related artifacts. These included mitragynine pseudoindoxyl (**3** and **4**) isomers, oxindoles (**5** and **6**), *N*-oxide (**7**), and advanced oxidation products such as 2,7-dihydroxyanalog (**8**) and 3-dehydromitragynine (**9**). In contrast, these artifacts were notably absent in conventional powders, capsules, and most liquid formulations. The systemic co-occurrence of these artifacts within specific product classes suggests that they arise from shared chemical processing pathways, likely semi-synthetic oxidation rather than natural botanical variability.

2.2. Stability of 7-OH (2)

Given the identification of mitragynine pseudoindoxyl (**3**) and its isomer as artifacts in certain commercial kratom products, we conducted stability studies on 7-OH (**2**). The stability of compound **2** across various conditions was evaluated, including protic and aprotic solvent systems, as well as its pH-dependent stability in simulated gastric fluid and simulated intestinal fluid (Fig. 2). These experiments were designed to model the chemical stability of **2** and the potential formation of additional artifacts following the oral consumption of 7-OH enriched kratom products.

Ultra-high-performance liquid chromatography coupled with photodiode array detection (UHPLC-PDA) was employed to assess the chemical stability of 7-OH (**2**). Dissolution of **2** in methanol resulted in a progressive depletion of the parent compound, concomitant with the emergence of several structurally related alkaloid congeners. These findings indicate nascent structural rearrangements occurring within the protic environment. A comparable degradation pattern was observed in methanol containing 0.1 N ammonia, a solvent system frequently utilized by commercial suppliers of **2** as a reference standard. This observation underscores the inherent instability of **2** in protic environments, which may confound both analytical quantification and interpretation of biological outcomes. Given this instability, we expanded our stability assessment of 7-OH in aqueous buffer systems (pH 4.0, 7.0, and 10.0) and in simulated gastric and intestinal fluids to better mimic oral exposure conditions and compared these results with its stability in an aprotic solvent (acetonitrile). This comprehensive evaluation was designed to elucidate the degradation kinetics and potential structural transformations of 7-OH under physiologically relevant conditions. Samples were collected at 24 h intervals over 8 days. The resulting degradation and formation profiles for each condition are summarized in Fig. 2.

3. Discussion

Expressing 7-OH (**2**) levels as a simple percentage is scientifically debatable, given its status as a minor and highly variable constituent in natural kratom materials (Grundmann et al., 2024; Lydecker et al., 2016; Samee et al., 2024). As an active metabolite of mitragynine (**1**) (Basiliere and Kerrigan, 2020; Kamble et al., 2019; Kruegel et al., 2016), 7-OH is susceptible to abiotic oxidative conversion from its precursor during post-harvesting processes, including drying, storage, and alkaloid enrichment (Karunakaran et al., 2025; Leksungnoen et al., 2022; Sharma et al., 2019). Furthermore, while not yet explicitly reported, specific cytochrome P450 enzymes within the *M. speciosa* could potentially facilitate the biotic oxidation of **1** to produce **2**, mirroring the established human metabolic conversion of mitragynine. However, elevated levels of 7-OH in certain kratom products likely reflect intentional chemical transformation rather than natural occurrence. Although **2** is a transient metabolite of mitragynine, its absolute concentration represents the most direct and toxicologically-relevant metric for assessing consumer exposure and associated safety risks.

Due to the structural relationship between mitragynine and 7-OH ,

Table 3
Artifacts detected across analyzed kratom products claiming to contain 7-OH.

Product code #	3	4	Oxindoles (5)/ (6)	7	Undefined *M*-NOX	Over-oxidation products† (8)	2, 7-Dihydroxypaynantheine	Paynantheine *N*-oxides (tentative)	9
4839PR	✓	✓	✓	✓	✓	✓	✓	✓	✓
4833PR	✓	✓	✓	✓	ND	ND	✓	ND	✓
4838PR	✓	✓	✓	✓	✓	ND	✓	ND	✓
4837PR	✓	✓	✓	✓	ND	ND	✓	ND	✓
4843PR	✓	✓	✓	✓	ND	ND	✓	ND	✓
4844PR	✓	✓	✓	✓	✓	ND	✓	ND	✓
4835PR	✓	✓	✓	✓	✓	✓	✓	ND	✓
4831PR	✓	✓	✓	✓	✓	ND	✓	✓	✓
4834PR	✓	✓	✓	✓	✓	✓	✓	ND	✓
4836PR	✓	✓	✓	✓	ND	✓	✓	✓	✓
4832PR	✓	✓	✓	✓	ND	ND	✓	ND	✓
4845PR	✓	✓	✓	✓	✓	✓	✓	✓	✓

√ = Detected; ND=Not detected.

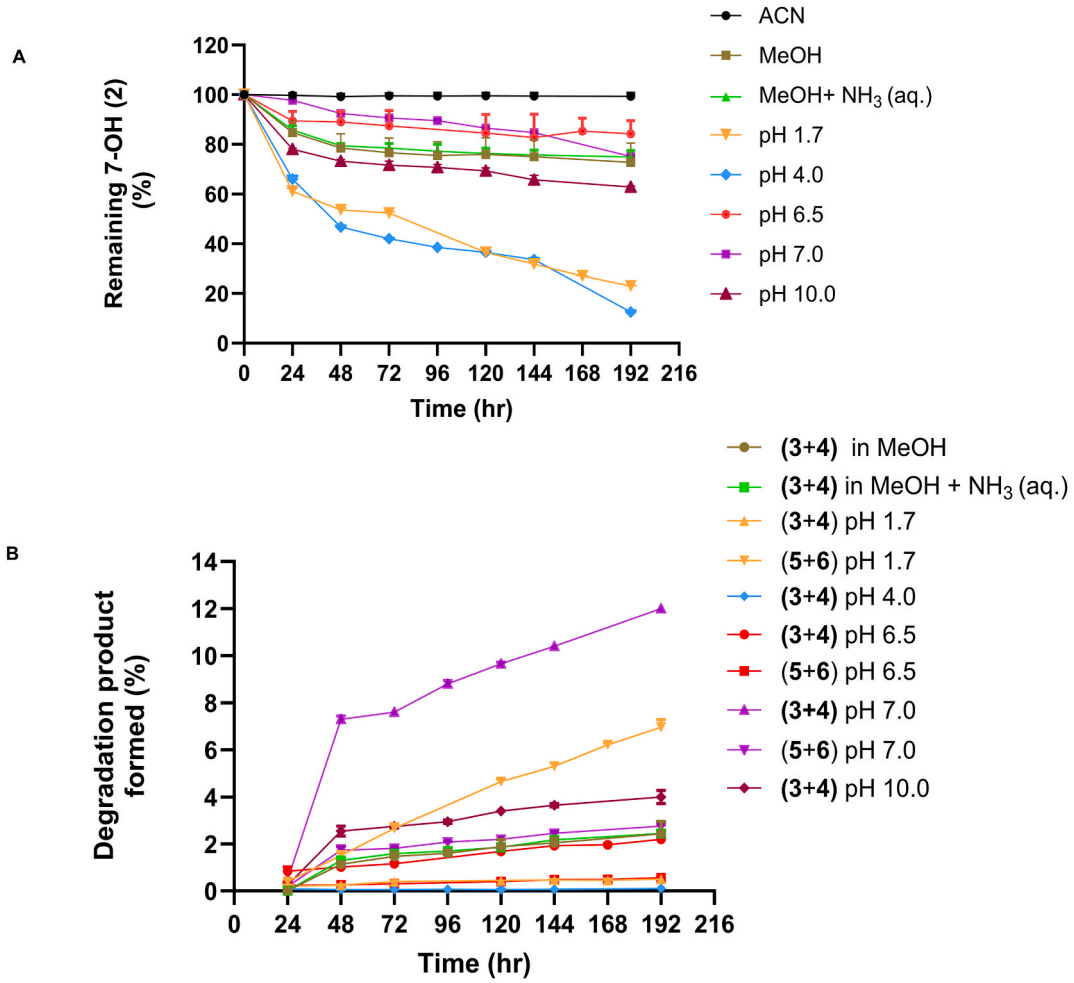


Fig. 2. Time-dependent stability profile of 7-OH under various conditions. (A) Time- and condition-specific degradation of 7-OH under nonprotic and protic solvents along with chemical and physiological conditions; (B) Formation of 7-OH byproducts under different solvents and pH conditions, including physiological conditions.

and oxidative (abiotic or biotic) mechanisms in the conversion of **1** to **2**, the mitragynine :7-OH concentration ratio could serve as a critical diagnostic metric. By normalizing the concentration of minor alkaloid relative to its more abundant precursor, mitragynine, the ratio could provide a standardized framework for distinguishing natural alkaloid profiles from those altered by chemical fortification or semisynthetic modification. Furthermore, the resulting ratio metric enables consistent evaluation of chemical profiles across diverse kratom formulations, regardless of absolute quantities of **1** or **2**. When applied to two previously published data sets on mitragynine and 7-OH (Kikura-Hanajiri et al., 2009; Lydecker et al., 2016), the ratio proves to be a consistent metric across different datasets and timeframes. For instance, a ratio-based analysis of two previous studies (Kikura-Hanajiri et al., 2009; Lydecker et al., 2016) published a few years apart yielded comparable average values of 93 and 85, demonstrating the metric's stability and reliability. In the present study, a notably higher mitragynine :7-OH ratio was observed for the powdered leaf samples and capsules containing powdered leaf. The higher ratio suggests that the

7-OH content in these samples is low, which could be attributed to two main factors: immediate processing and analysis that limited air oxidation of mitragynine, or plant varieties naturally containing a lower 7-OH content (Fig. 3). In contrast, liquid kratom products exhibited a wide range of mitragynine :7-OH ratios, indicating considerable variability and inconsistency in their chemical composition, plausibly due to dissolved oxygen and the presence of antioxidant preservatives.

Tablet products marketed as "7-OH" exhibited a distinctly low mitragynine :7-OH ratio, with values below 0.55. This extremely low ratio indicates a high concentration of 7-OH and a minimal presence of its precursor, mitragynine. Such a composition is not typical of kratom's natural alkaloid profile, indicating that the products were likely produced via economically feasible methods using the more abundant mitragynine as a precursor (Fig. 3).

The chemical profiles observed in these products differ substantially from those expected for traditional kratom leaf material, particularly with respect to elevated levels of oxygenated mitragynine derivatives. Similar findings have been reported in recent studies analyzing the chemical composition of kratom-labeled products, which documented high 7-OH content and additional alkaloids inconsistent with naturally occurring kratom profiles (Alsbrook et al., 2025; Brown et al., 2026; Ganasan et al., 2025; Hill et al., 2025). Analysis of 12 commercial products containing 7-OH revealed consistent artifacts across all samples. The identified artifacts included compounds **3**–**6**, mitragynine *N*(4)-oxide (**7**), **8**, 2,7-dihydroxypaynantheine, their corresponding *N*-oxides, and the potentially toxic (Chakraborty et al., 2021) 3-dehydromitragynine (**9**).

Despite product labeling that claims extraction from *M. speciosa* leaf or aerial parts, the presence of over-oxidation products, such as **8** and its related *N*-oxide, indicates that the oxidation process used to synthesize 7-OH is non-selective. The consistent detection of the potentially toxic **9** is particularly concerning, as it implies that a post-synthesis enrichment step may involve strong acidic conditions.

The pronounced *in vivo* activity of 7-OH observed in these products is particularly concerning in light of recent pharmacokinetic and toxicological studies demonstrating significant biological effects and adverse outcomes associated with 7-OH exposure (Chiang et al., 2025; Pullman et al., 2025; Reif et al., 2025; Zuarth Gonzalez et al., 2025). These reports underscore the importance of accurate quantitative analysis and clear product labeling to support consumer safety and informed regulatory oversight.

The findings from the commercial product analysis prompted a broader investigation into the stability of 7-OH (**2**), which revealed a dependence on both solvent polarity and pH. Compound **2** remained highly stable in acetonitrile, consistent with previous reports (Karunakaran et al., 2025). However, a significant transformation was observed in the methanol or a methanol containing 0.1 N ammonia solvent system, the latter commonly employed by commercial vendors for supplying **2** as an analytical reference standard. These specific conditions were selected to elucidate degradation pathways under laboratory-relevant conditions and are not intended to represent solvents typically used in commercial manufacturing or storage. Under these conditions, a progressive depletion of 7-OH was observed, concomitant with the formation of isobaric artifacts, specifically mitragynine pseudoindoxyls (**3** and **4**). Under alkaline conditions (pH 10.0 buffer), approximately 20% of **2** was depleted within 48 h, with a corresponding formation of pseudoindoxyls (**3** and **4**), approximately 3%; both degradation and isobaric analyte levels remained largely stable thereafter.

Interestingly, even under neutral pH 7.0 buffer conditions, 7-OH exhibited instability and followed first-order degradation kinetics (rate constant (k) = 0.001358 h^{-1}, R^2 = 0.95). Within eight days, >20% of the initial concentration of **2** was consumed, with concurrent formation of ~10% of pseudoindoxyls (**3**/**4**) and ~3% of mitragynine oxindoles (**5**/**6**).

Notably, under acidic conditions, 7-OH was highly unstable. Within eight days, incubation in a pH 4.0 buffer or Fasted-State Simulated Gastric Fluid (FaSSGF, pH 1.7) led to near-complete 7-OH depletion, with **9** identified as the sole byproduct. The rapid loss of stability observed in FaSSGF, mirroring the pH 4.0 results, necessitates further

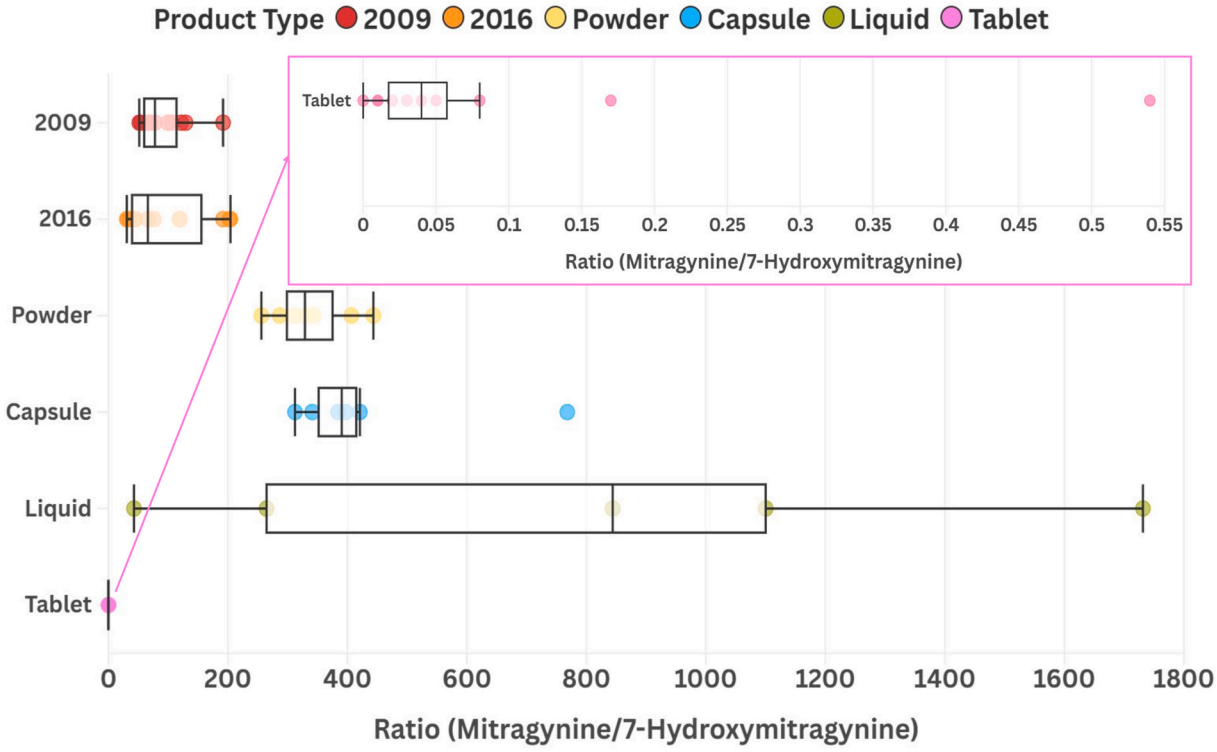


Fig. 3. Comparison of the mitragynine:7-OH ratio in various kratom products with previously reported data (Kikura-Hanajiri et al., 2009; Lydecker et al., 2016). The ratio observed in tablet samples is shown in the inset.

kinetic analysis to characterize the acid-catalyzed degradation mechanism.

The degradation kinetics of 7-OH in FaSSGF (pH 1.7) and at pH 4 were analyzed using zero- and first-order models, with the latter constrained to reflect complete degradation. Although both models yielded comparable R^2 values (~0.978 at pH 1.7 and ~0.899 at pH 4.0), the residual analysis was decisive (Fig. 4). The systematic curvature in the zero-order residuals indicated model misspecification, whereas the first-order residuals were randomly distributed around zero. Therefore, first-order kinetics were selected as the appropriate model, suggesting that the degradation of 7-OH is concentration-dependent under these acidic conditions.

The degradation followed first-order kinetics ($k = 0.005637\ h^{-1}$, $t_{1/2}$ = 123.0 h, 95% CI 111.3 to 136.9 h at FaSSGF (pH 1.7) and $k = 0.006904\ h^{-1}$, $t_{1/2} = 100.4$ h, 95% CI 79.83 to 131.8 h at pH 4.0) and resulted in the proportional formation of **9** (Fig. 5). The apparent rate constant (k) and half-life ($t_{½}$) indicate faster degradation under stronger acidic conditions (pH 1.7) than at pH 4.0, aligning with acid-catalyzed transformation behavior. The transformation of 7-OH to **3** and **4** is particularly noteworthy, as these compounds are reported to be potent MOR agonists with reduced hyperlocomotion compared to **1** or 7-OH (Chakraborty et al., 2021), highlighting the pharmacological significance of the observed conversion. Following acidic degradation of 7-OH, we observed substantial conversion to **9** (Fig. 5). Given the higher toxicity of **9** in animal studies, its cellular impact is likely to be at least as significant as that of mitragynine, although direct comparative data are needed. The potential for conversion of kratom alkaloids like 7-OH into the toxic **9** under acidic or physiological conditions merits attention for further research. Ultimately, the prevalence of 7-OH in kratom products

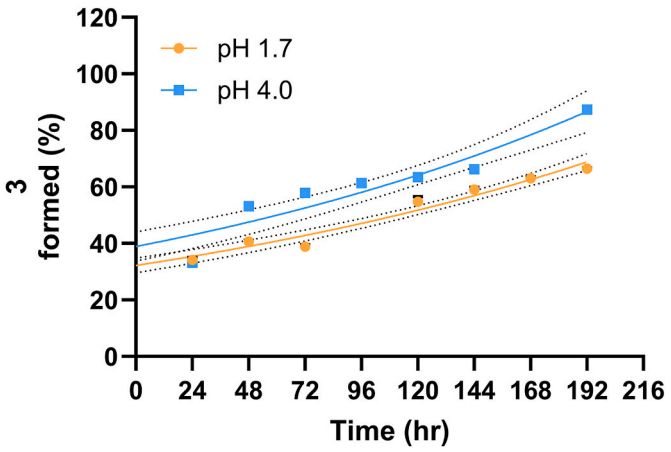


Fig. 5. Formation of 3-dehydromitragynine (**9**) from 7-OH in FaSSGF (pH 1.7) and at pH 4.0 buffer.

will raise substantial health concerns, especially in contexts of high exposure or poor-quality control. Due to its conversion to a toxic byproduct under physiological conditions, regulatory oversight should consider monitoring and limiting 7-OH in commercial kratom preparations.

This study has several limitations. First, the sample size was restricted, with only one product per brand analyzed, and intra-brand batch variability was not assessed. In addition, the analyzed products represent only a subset of the diverse kratom marketplace available via

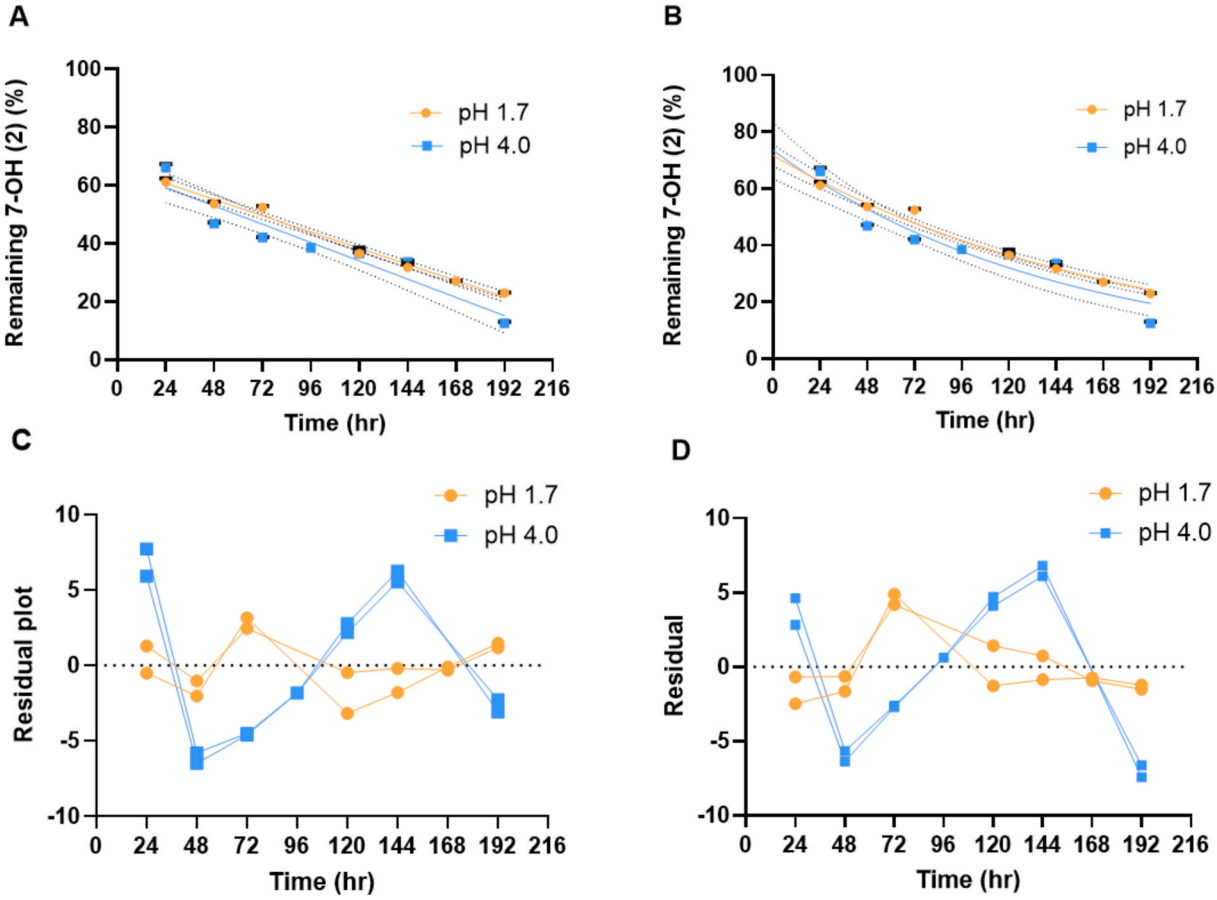


Fig. 4. Degradation kinetics of 7-OH in FaSSGF (pH 1.7) and pH 4.0 buffer with model residuals. (A) Zero-order linear regression of concentration *versus* time; (B) First-order non-linear regression using a one-phase exponential decay model (plateau constrained to zero); (C) Residual plot for the zero-order model (residuals *versus* time); (D) Residual plot for the first-order model (residuals *versus* time).

e-commerce platforms. Importantly, although all products were marketed within the kratom supplement category and frequently labeled as containing *M. speciosa*, our chemical profiling indicates that many products, particularly those labeled as containing 7-OH or **3**, are not representative of chemically unmodified *M. speciosa* leaf material and are best interpreted as semisynthetic formulations derived from mitragynine or enriched extracts. Therefore, these findings primarily apply to commercial products marketed as kratom that may be adulterated or chemically modified, and their broader relevance to other dietary supplement categories remains to be established.

A critical finding of the stability assessment was that 7-OH undergoes approximately 20% degradation within 24 h in methanol, whereas it remains chemically stable in acetonitrile. Although all commercial product analyses in this study were conducted within 12 h of sample preparation, a portion of the minor artifacts detected in methanolic extracts may reflect solvent-induced degradation rather than the original constituent profile of the products. Consequently, acetonitrile is recommended as the extraction and analytical solvent to ensure accurate quantification and minimize artifacts arising from protic degradation.

4. Conclusion

Our comprehensive analysis of commercial Kratom products and subsequent stability studies indicate that formulations marketed as 7-OH (**2**) are unlikely to represent simple botanical extracts. The consistent detection of multiple structurally related compounds, including over-oxidized byproducts and the potentially toxic metabolite **9**, suggests a semisynthetic origin for many of these products. Furthermore, the pronounced instability of 7-OH under selected chemical and physiologically relevant conditions raises significant concerns regarding product shelf life and the potential for consumer exposure to more potent isobaric mitragynine pseudoindoxyls (**3**–**4**) and oxindoles (**5**–**6**). In addition, a substantial conversion of 7-OH to **9** was observed, which raised concerns about the potential for toxic transformation under acidic or physiological conditions. The conversion of 7-OH to the more potent MOR agonist **3**, and the presence of both compounds in commercial kratom products, suggest that their sale in venues like gas stations and e-commerce outlets is inconsistent with the definition of a supplement originating from a botanical source. Collectively, these findings support strengthened product surveillance and validated analytical monitoring of concentrated 7-OH /**3** products and associated byproducts, including **9**, to improve consumer safety and informed regulatory oversight.

5. Experimental section

5.1. Chemicals, alkaloid standards, and supplement products

Mitragynine (**1**) was isolated from botanically verified *M. speciosa* at the National Center for Natural Products Research (NCNPR), University of Mississippi, and used as starting material to produce the following critical chemical standards, 7-OH (**2**), and compounds **6**–**9**. Mitragynine pseudoindoxyl (**3**) (Item No. 20033) was purchased from Cayman Chemical (Ann Arbor, MI, USA). The purity of all reference compounds was greater than 95%. Their identity was confirmed by NMR spectroscopy (Tables S1 and S2), high-resolution mass spectrometry (HRMS), and comparison with prior literature values (Chear et al., 2021; Houghton and Said, 1986; Sharma et al., 2025; Shellard et al., 1978; Smith et al., 2019). HPLC-grade acetonitrile, methanol, and formic acid (Fisher Scientific, Suwanee, GA, USA) were used for extraction and HPLC analysis. Water for the mobile phase was purified using a Milli-Q water purification system (Millipore). Potassium bromide, Oxone®, and *m*-chloroperbenzoic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Reagent-grade anhydrous dichloromethane, chloroform, ethyl acetate, and acetic acid were purchased from Fisher Scientific (Suwanee, GA, USA). Twelve products (#4831PR-4839PR and 4843PR-4845PR) were obtained through an online survey of the '7-hydroxy' kratom market for the current study, conducted between July 2025 and August 2025. Selections were based on product availability and quick access, without a focus on any specific vendor or manufacturer.

5.2. Synthesis of key mitragyna alkaloids

5.2.1. 7-Hydroxymitragynine (7-OH, 2)

To a solution of mitragynine (120.1 mg, 0.301 mmol) in acetone (10 mL) in a 50 mL round-bottom flask, saturated aqueous $NaHCO_3$ (8 mL) was added, and the mixture was cooled to 0 °C. A solution of Oxone® (185 mg, 0.602 mmol) in water (5 mL) was added dropwise over 25 min, and stirring was continued. After 20 min, the reaction was diluted with water (50 mL) and extracted with EtOAc (15 mL × 3) in a separatory funnel. The combined organic layers were collected, washed with water (30 mL), saturated brine solution (15 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum (Kruegel et al., 2019). The resulting residue was subjected to flash chromatography (silica gel) and eluted with hexanes/EtOAc (0 - 70 %), yielding 7-OH product (69.6 mg, 0.168 mmol, 56%) as a pale-yellow solid. $[\alpha]_D^{25} = +57$ (*c* 0.3, $CHCl_3$); ESIMS *m/z* 415.2233 $[M+H]^+$ (calcd 415.2228); ^1H and ^{13}C NMR spectroscopic data: Tables S1 and S2.

5.2.2. 2,7-Dihydroxymitragynine (2,7-DOH (8))

To an ice-cold solution of mitragynine (40 mg, 0.1 mmol) in 5 mL acetonitrile/water (10:1) solvent, 500 µL of 0.01 M KBr (0.6 mg, 0.005 mmol) was added. After 10 min, Oxone® (37 mg, 0.12 mmol) was added in one portion, stirred for 20 min at 0 °C, and gradually warmed to room temperature. After 5 h, the reaction was quenched with saturated $NaHCO_3$ (5 mL), followed by saturated Na_2SO_3 (5 mL). The mixture was transferred to a separatory funnel and extracted with EtOAc (10 mL × 3). The organic layers were combined, washed with saturated brine (5 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced vacuum (Xu et al., 2019). The resulting crude product was subjected to silica gel chromatography with an isocratic mixture of hexanes/acetone/NH_4OH (7:3:0.05) to yield **8** as a viscous liquid (6.9 mg, 0.016 mmol, 16.8 %). $[\alpha]_D^{25} = -76$ (*c* 0.26, $CHCl_3$); ESIMS *m/z* 433.2333 $[M+H]^+$ (calcd 433.2339); ^1H and ^{13}C NMR spectroscopic data: Tables S1 and S2.

5.2.3. 3-Dehydromitragynine (9)

To a 25 mL scintillation vial containing ice-cold 7-OH (25.2 mg, 0.061 mmol) in anhydrous dichloromethane (500 µL) under argon, trifluoroacetic acid (TFA, 25 µL, 0.322 mmol) was added at 0 °C. The reaction mixture was stirred at 0 °C for 2 h, then quenched by further dilution with dichloromethane (5 mL) and concentrated under vacuum (Chakraborty et al., 2021). The resulting residue was redissolved in acetonitrile/water (1:3 v/v, 1 mL) and dried using SpeedVac to yield **9** as a pale-yellow solid (23.5 mg, 0.059 mmol, 97 %). $[\alpha]_D^{25} = +184$ (*c* 0.28, $CHCl_3$); ESIMS *m/z* 397.2123 $[M]^+$ (calcd 397.2122); ^1H and ^{13}C NMR spectroscopic data: Tables S1 and S2.

5.2.4. Mitragynine oxindole B (6)

To a 25-mL round-bottom flask containing an ice-cold, stirred solution of THF/AcOH/H_2O (1:1:1, v/v/v, 3 mL), mitragynine (110 mg, 0.276 mmol) and KBr (3.28 mg, 0.028 mmol) were added. After stirring for 10 min, Oxone® (102 mg, 0.331 mmol) was introduced in three equal portions at 5-min intervals. Following the addition, the ice bath was removed, and the reaction mixture was allowed to warm gradually to room temperature. After 12 h, an additional batch of Oxone® (50 mg, 0.162 mmol) was added, and stirring continued for 1 h. The reaction was quenched by the addition of saturated $NaHCO_3$ (10 mL) and saturated Na_2CO_3 (10 mL) solutions, followed by extraction with EtOAc (20 mL). The organic fraction was separated, and the aqueous phase was further extracted with EtOAc (20 mL × 3). The combined organic fractions were

washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure (Xu et al., 2019). The resulting residue was purified by silica gel-based flash column chromatography with gradient eluents (MeOH/$CHCl_3$, 1:100 to 1:20) to yield **6** as a pale-yellow solid (13.7 mg, 0.033 mmol, 12%). $[\alpha]_D^{25} = -92$ (*c* 0.4, $CHCl_3$); ESIMS *m/z* 415.2229 $[M+H]^+$ (calcd 415.2233); ^1H and ^{13}C NMR spectroscopic data: Tables S1 and S2.

5.2.5. *Mitragynine-N(4)-oxide (7)*

To a stirred solution of mitragynine (51.7 mg, 0.13 mmol) in anhydrous dichloromethane (1.0 mL) under argon atmosphere, *m*-chloroperbenzoic acid (25.0 mg, 0.16 mmol) was added at −50 °C. The reaction mixture was stirred for 30 min, then poured into a chilled 5% $NaHCO_3$ solution, and extracted with $CHCl_3$ (15 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure (Takayama et al., 2002). The residue was purified by silica gel column chromatography (eluent: 5% MeOH in $CHCl_3$), affording **7** as a pale-yellow solid (9.5 mg, 0.023 mmol, 17.7%). $[\alpha]_D^{25} = -37$ (*c* 0.26, $CHCl_3$); ESIMS *m/z* 415.2227 $[M+H]^+$ (calcd 415.2233); ^1H and ^{13}C NMR spectroscopic data: Tables S1 and S2.

5.3. *Preparation of commercial M. speciosa product samples for analysis*

Samples were analyzed across various dosage forms, including tablets, capsules, powders, softgels, and liquids. Solid samples were prepared in their respective form. For tablets, five were weighed, pulverized with a mortar and pestle, and pooled. Capsule samples were prepared by weighing five capsules, opening them, pooling their contents, and triturating the mixture. Soft gel samples were prepared by accurately weighing 5 units, after which the ends of each capsule were manually cut with a knife to release the liquid content. Powder samples (approximately 1.0 g) were homogenized using a mixer mill to obtain a fine powder. All commercial kratom products were finely ground/mixed to ensure homogeneity.

For each solid sample, 100 mg was weighed and suspended in 2 mL of methanol. The methanolic solution was vortexed and sonicated for 30 min at room temperature and then centrifuged for 15 min at 10,000 rpm. The supernatant was transferred to a 10 mL volumetric flask. This extraction procedure was repeated four times, and the combined supernatants were brought to a final volume of 10 mL with methanol.

For liquid samples, a 1.0 mL aliquot of formulation (#4656-4658, 4660-4662, 4814, 4846) was mixed with 1.0 mL methanol, vortexed for 30 s, and sonicated for 30 min. The mixture was vortexed again and then centrifuged for 10 min at 4000 rpm. The clear supernatant was then used for analysis. Prior to LC–MS analysis, a 2.0 mL aliquot was filtered through a 0.45 μm PTFE membrane filter. The first 1.0 mL of filtrate was discarded, and the remaining filtrate was collected in an LC vial. All samples were prepared in duplicate to ensure reproducibility and were further diluted 10-to 50-fold as needed. All samples were freshly prepared and immediately analyzed without storage.

5.4. *Chromatographic conditions*

5.4.1. *Ultra high-performance liquid chromatography-photodiode array Mass spectrometry (UHPLC-PDA-MS) for alkaloids analysis*

Ultra-high-performance liquid chromatography-photodiode array-mass spectrometry (UPLC-PDA-MS) analysis was performed using a Waters Acquity UPLC™ system (Waters Corp., Milford, MA, USA) to separate and identify compounds. The system, which included a quaternary solvent manager, a sample manager, a column compartment, and a PDA detector, was controlled by Waters Empower 3 software. A Waters Acquity UPLC HSS C18 column (150 mm × 2.1 mm I.D., 1.8 μm) with a dedicated guard column (Waters Vanguard LC-18 (2.1 mm × 5 mm)) provided the chromatographic separation at 40 °C, while the samples were kept at 15 °C. The mobile phase, consisting of water (A) and acetonitrile (B) (both with 0.1 % formic acid), was run through a gradient elution at a flow rate of 0.21 mL/min, beginning at 10 % B for 0 min, progressing to 55 % B over 15 min, and subsequently to 100 % B over 17 min. To minimize carryover between runs, a strong and weak needle wash was used, and the column was re-equilibrated following a brief wash step. Finally, a 1 μL sample was injected. Peak identification was confirmed using a combination of mass spectrometry data, retention time matching, and UV-Vis spectral comparison with authentic reference standards.

Detection was performed using a UV detector coupled to a mass spectrometer operating in positive-ion mode. Mass spectrometer conditions were optimized to achieve maximal sensitivity. The probe temperature, capillary voltage, and cone voltage were set at 600 °C, 0.8 kV, and 15 V, respectively. Nitrogen was used as the drying gas. The mass range was set to *m/z* 50–1000.

The method provided excellent baseline resolution for mitragynine , 7-OH , **6**, **9**, and **3**. Peak identification was confirmed by comparing retention times, UV spectra, and mass data with those of reference standards. Specific detection wavelengths were used for quantification: 246 nm for mitragynine , 7-OH , and 233 nm for **3**.

5.4.2. *Method validation*

The UHPLC-PDA method was validated for the quantification of mitragynine , 7-OH , and mitragynine pseudoindoxyl in *Mitragyna* samples. Detection wavelengths were selected based on the UV absorption maxima of each analyte: 246 nm for mitragynine and 7-OH , and 233 nm for **3**, as determined from PDA spectra. Calibration curves were constructed over seven concentration ranges of 0.5–100 μg/mL for mitragynine and 7-OH , and 0.2–100 μg/mL for **3**, with linear regression yielding correlation coefficients (R^2) ≥ 0.99 for all analytes. Limits of detection (LOD) and quantification (LOQ) were determined using signal-to-noise ratios of approximately 3:1 and 10:1, respectively, at the respective wavelengths. The LOD and LOQ were 0.05 μg/mL and 0.2 μg/mL, respectively, providing sufficient sensitivity to reliably detect and quantify these compounds at low concentrations relevant to *Mitragyna* samples. Precision, expressed as intra- and inter-day relative standard deviation (%RSD), ranged from 0.4 to 3% for all analytes. Accuracy, assessed via recovery studies at 5 μg/mL for three commercial products (#4658 PR, 4801 PR, 4839 PR) in duplicate, ranged from 92 to 106%. Specificity was confirmed through analysis of blanks, standards, and spiked samples, verifying both the absence of interfering peaks and the purity of the analyte peaks. Robustness was confirmed as minor variations in column temperature (±2 °C), flow rate (±0.1 mL/min), mobile phase composition, or injection volume did not significantly affect results. Overall, the UHPLC-PDA method is specific, linear, precise, accurate, and robust for the simultaneous quantification of mitragynine, 7-OH, and mitragynine pseudoindoxylin various kratom product matrices.

5.5. *Kinetics of 7-OH (2) degradation*

The degradation kinetics of 7-OH were assessed in fasted-state simulated gastric fluid (FaSSGF) (pH 1.7) and buffer at pH 4.0 by measuring the percentage of the compound remaining at predetermined time intervals (24, 48, 72, 96, 120, 144, 168, and 192 h). Data were analyzed using GraphPad Prism version 10.6.0 (San Diego, CA, USA). Two kinetic models were evaluated: (i) a zero-order model, applied using linear regression of concentration versus time, and (ii) a first-order model, applied using non-linear regression with a one-phase exponential decay equation. For the first-order analysis, the plateau was constrained to zero, reflecting the complete degradation of the compound. Since sample collection began at 24 h, no experimental data were available for t = 0. Therefore, the initial concentration (C_0) was estimated by back-extrapolation of the fitted degradation curve obtained from the subsequent time points.

Credit author statement

Bharathi Avula: Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Syed Kashif Ali:** Synthesis and characterization of mitragyna alkaloids. **Kiran K. Tatapudi:** Method development, validation, analysis, data acquisition, data validation, writing – review & editing. **Pankaj Pandey:** Writing – original draft, Software, Methodology, Formal analysis. **Lochana Cheepilla:** Writing – review & editing, Methodology, Formal analysis. **Islam Husain:** Writing – review & editing, Methodology, Formal analysis. **Shabana I. Khan:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Kumar Katragunta:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Zulfiqar Ali:** Characterization of alkaloids, Writing – review & editing. **Ikhlas A. Khan:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Amar G. Chittiboyina:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Funding

This research is supported by the "Science-Based Authentication of Botanical Ingredients" funded by the Center for Food Safety and Applied Nutrition, US Food and Drug Administration, grant number 5U01FD004246.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix. A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2026.114871.

Data availability

Data will be made available on request.

References

Alsbrook, S., Pro, G., Koturbash, I., 2025. From kratom to 7-hydroxymitragynine: evolution of a natural remedy into a public-health threat. Pharm. Biol. 63, 896–911. https://doi.org/10.1080/13880209.2025.2590311.

Angyal, P., Hegedus, K., Meszaros, B.B., Daru, J., Dudas, A., Galambos, A.R., Essmat, N., Al-Khrasani, M., Varga, S., Soos, T., 2023. Total synthesis and structural plasticity of kratom pseudoindoxyl metabolites. Angew Chem. Int. Ed. Engl. 62, e202303700. https://doi.org/10.1002/anie.202303700.

Basiliere, S., Kerrigan, S., 2020. CYP450-mediated metabolism of mitragynine and investigation of metabolites in human urine. J. Anal. Toxicol. 44, 301–313. https://doi.org/10.1093/jat/bkz108.

Brown, P.N., Chan, M., Zhang, X., Brendler, T., 2026. Elevated 7-hydroxymitragynine levels found in products misbrand as kratom. J. AOAC Int. 109, 124–130. https://doi.org/10.1093/jaoacint/qsaf094.

Chakraborty, S., Uprety, R., Slocum, S.T., Irie, T., Le Rouzic, V., Li, X., Wilson, L.L., Scouller, B., Alder, A.F., Kruegel, A.C., Ansonoff, M., Varadi, A., Eans, S.O., Hunkele, A., Allaoa, A., Kalra, S., Xu, J., Pan, Y.X., Pintar, J., Kivell, B.M., Pasternak, G.W., Cameron, M.D., McLaughlin, J.P., Sames, D., Majumdar, S., 2021. Oxidative metabolism as a modulator of kratom's biological actions. J. Med. Chem. 64, 16553–16572. https://doi.org/10.1021/acs.jmedchem.1c01111.

Chear, N.J.-Y., León, F., Sharma, A., Kanumuri, S.R.R., Zwolinski, G., Abboud, K.A., Singh, D., Restrepo, L.F., Patel, A., Hiranita, T., 2021. Exploring the chemistry of alkaloids from Malaysian *Mitragyna speciosa* (Kratom) and the role of oxindoles on human opioid receptors. J. Nat. Prod. 84, 1034–1043. https://doi.org/10.1021/acs.jnatprod.0c01055.

Chiang, Y.H., Kanumuri, S.R.R., Kuntz, M.A., Senetra, A.S., Berthold, E.C., Kamble, S.H., Mukhopadhyay, S., Hampson, A.J., McCurdy, C.R., Sharma, A., 2025. *In vitro* and *in vivo* pharmacokinetic characterization of 7-hydroxymitragynine, an active metabolite of mitragynine, in Sprague-Dawley rats. Eur. J. Drug Metab. Pharmacokinet. 50, 205–218. https://doi.org/10.1007/s13318-025-00939-2.

Francis, M., 2024. 'Gas-station heroin' targeted in crackdowns by states and cities. https://www.nbcnews.com/health/health-news/kratom-targeted-crackdowns-states-cities-rcna166661.

Ganasan, J., Karunakaran, T., Marimuthu, Y., Rusmadi, N.N., Firouz, N.S., Jenis, J., Kumar, U.S.U., 2025. Chemistry and toxicity of 7-hydroxymitragynine (7-OHMG): an updated review on the oxidized derivative of mitragynine. Phytochem. Rev. 24, 4051–4064. https://doi.org/10.1007/s11101-024-10029-x.

Grundmann, O., Garcia-Romeu, A., McCurdy, C.R., Sharma, A., Smith, K.E., Swogger, M. T., Weiss, S.T., 2024. Not all kratom is equal: the important distinction between native leaf and extract products. Addiction 119, 202–203. https://doi.org/10.1111/add.16366.

Hill, K., Boyer, E.W., Grundmann, O., Smith, K.E., 2025. De facto opioids: characterization of novel 7-hydroxymitragynine and mitragynine pseudoindoxyl product marketing. Drug Alcohol Depend. 272, 112701. https://doi.org/10.1016/j.drugalcdep.2025.112701.

Horniakova, A., Mikus, P., Piestansky, J., 2025. Determination of mitragynine and 7-Hydroxymitragynine in raw kratom plant material by a multisegment injection capillary Zone electrophoresis-tandem mass spectrometry. J. Separ. Sci. 48, e70106. https://doi.org/10.1002/jssc.70106.

Houghton, P.J., Said, I.M., 1986. 3-Dehydromitragynine: an alkaloid from *Mitragyna speciosa*. Phytochemistry 25, 2910–2912. https://doi.org/10.1016/S0031-9422(00)83771-1.

Kamble, S.H., Leon, F., King, T.I., Berthold, E.C., Lopera-Londono, C., Siva Rama Raju, K., Hampson, A.J., Sharma, A., Avery, B.A., McMahon, L.R., McCurdy, C.R., 2020. Metabolism of a kratom alkaloid metabolite in human plasma increases its opioid potency and efficacy. ACS Pharmacol. Transl. Sci. 3, 1063–1068. https://doi.org/10.1021/acsptsci.0c00075.

Kamble, S.H., Sharma, A., King, T.I., León, F., McCurdy, C.R., Avery, B.A., 2019. Metabolite profiling and identification of enzymes responsible for the metabolism of mitragynine, the major alkaloid of *Mitragyna speciosa* (kratom). Xenobiotica 49, 1279–1288. https://doi.org/10.1080/00498254.2018.1552819.

Kampmeyer, P., 2025. Method for Production of spiro-oxindole and Indoxyl Derivatives of Mitragynine. Shaman Pharma, LLC. WO2025123039.

Karunakaran, T., Ngew, K.Z., Zailan, A.A.D., Mian Jong, V.Y., Abu Bakar, M.H., 2022. The chemical and pharmacological properties of mitragynine and its diastereomers: an insight review. Front. Pharmacol. 13, 805986. https://doi.org/10.3389/fphar.2022.805986.

Karunakaran, T., Vicknasingam, B., Chawarski, M.C., 2025. Phytochemical analysis of water and ethanol liquid extracts prepared using freshly harvested leaves of *Mitragyna speciosa* (Korth.). Nat. Prod. Res. 39, 4480–4487. https://doi.org/10.1080/14786419.2024.2362428.

Kikura-Hanajiri, R., Kawamura, M., Maruyama, T., Kitajima, M., Takayama, H., Goda, Y., 2009. Simultaneous analysis of mitragynine, 7-hydroxymitragynine, and other alkaloids in the psychotropic plant "kratom"(*Mitragyna speciosa*) by LC-ESI-MS. Forensic Toxicol. 27, 67–74. https://doi.org/10.1007/s11419-009-0070-5.

Kruegel, A.C., Gassaway, M.M., Kapoor, A., Váradi, A., Majumdar, S., Filizola, M., Javitch, J.A., Sames, D., 2016. Synthetic and receptor signaling explorations of the mitragyna alkaloids: mitragynine as an atypical molecular framework for opioid receptor modulators. J. Am. Chem. Soc. 138, 6754–6764. https://doi.org/10.1021/jacs.6b00360.

Kruegel, A.C., Uprety, R., Grinnell, S.G., Langreck, C., Pekarskaya, E.A., Le Rouzic, V., Ansonoff, M., Gassaway, M.M., Pintar, J.E., Pasternak, G.W., Javitch, J.A., Majumdar, S., Sames, D., 2019. 7-hydroxymitragynine is an active metabolite of mitragynine and a key mediator of its analgesic effects. ACS Cent. Sci. 5, 992–1001. https://doi.org/10.1021/acscentsci.9b00141.

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

Lydecker, A.G., Sharma, A., McCurdy, C.R., Avery, B.A., Babu, K.M., Boyer, E.W., 2016. Suspected adulteration of commercial kratom products with 7-hydroxymitragynine. J. Med. Toxicol. 12, 341–349. https://doi.org/10.1007/s13181-016-0588-y.

Mudge, E.M., Brown, P.N., 2017. Determination of mitragynine in *Mitragyna speciosa* raw materials and finished products by liquid chromatography with uv detection: single-laboratory validation. J. AOAC Int. 100, 18–24. https://doi.org/10.5740/jaoacint.16-0220.

Osawa, K.A., Johnson, R.D., 2025. Postmortem distribution of mitragynine and 7-hydroxymitragynine in 51 cases. J. Anal. Toxicol. 49, 122–128. https://doi.org/10.1093/jat/bkae099.

Ponglux, D., Wongseripipatana, S., Takayama, H., Kikuchi, M., Kurihara, M., Kitajima, M., Aimi, N., Sakai, S., 1994. A New Indole Alkaloid, 7 α-Hydroxy-7H-mitragynine, from *Mitragyna speciosa* in Thailand. Planta Med. 60, 580–581. https://doi.org/10.1055/s-2006-959578.

Pullman, M.K., Kanumuri, S.R.R., Leon, J.F., Cutler, S.J., McCurdy, C.R., Sharma, A., 2025. Cardio-pulmonary arrest in a patient revived with naloxone following reported use of 7-hydroxymitragynine. Clin. Toxicol. 1–2. https://doi.org/10.1080/15563650.2025.2565428.

Reif, B., Adkins, A., Boyer, E.W., Kanumuri, S.R.R., Sharma, A., Smith, K.E., 2025. Substance use disorder following consumption of a novel synthetic 7-hydroxymitragynine product. J. Addiction Med. 10–1097. https://doi.org/10.1097/ADM.0000000000001603.

Saidin, N.A., Holmes, E., Takayama, H., Gooderham, N.J., 2015. The cellular toxicology of mitragynine, the dominant alkaloid of the narcotic-like herb, *Mitragyna speciosa* Korth. Toxicol. Res. 4, 1173–1183. https://doi.org/10.1039/C5TX00113G.

Samee, W., Matra, K., Lakkham, N., Dongkaew, B., Sumkhum, P., Sangwang, W., Nupangtha, W., Promping, J., 2024. Electrical breakdown in liquid-phase processing on an enhancement of 7-hydroxymitragynine conversion from mitragynine in *Mitragyna speciosa* (Kratom). Heliyon 10, e36676. https://doi.org/10.1016/j.heliyon.2024.e36676.

Sharma, A., Kamble, S.H., León, F., Chear, N.J.Y., King, T.I., Berthold, E.C., Ramanathan, S., McCurdy, C.R., Avery, B.A., 2019. Simultaneous quantification of ten key Kratom alkaloids in *Mitragyna speciosa* leaf extracts and commercial products by ultra-performance liquid chromatography− tandem mass spectrometry. Drug Test. Anal. 11, 1162–1171. https://doi.org/10.1002/dta.2604.

Sharma, A., Smith, K.E., Kuntz, M.A., Berthold, E.C., Elashkar, O.I., Guadagnoli, N., Kanumuri, S.R.R., Mukhopadhyay, S., Panlilio, L.V., Epstein, D.H., 2025. Chemical analysis and alkaloid intake for kratom products available in the United States. Drug Test. Anal. 17, 1974–1984. https://doi.org/10.1002/dta.3906.

Shellard, E., Houghton, P., Resha, M., 1978. Mitragyna species of Asia. 30. Oxidation-products of mitragynine and speciating. Planta Med. 33, 223–227. https://doi.org/10.1055/s-0028-1097379.

Smith, L.C., Lin, L., Hwang, C.S., Zhou, B., Kubitz, D.M., Wang, H., Janda, K.D., 2019. Lateral flow assessment and unanticipated toxicity of kratom. Chem. Res. Toxicol. 32, 113–121. https://doi.org/10.1021/acs.chemrestox.8b00218.

Takayama, H., Ishikawa, H., Kurihara, M., Kitajima, M., Aimi, N., Ponglux, D., Koyama, F., Matsumoto, K., Moriyama, T., Yamamoto, L.T., Watanabe, K., Murayama, T., Horie, S., 2002. Studies on the synthesis and opioid agonistic activities of mitragynine-related indole alkaloids: discovery of opioid agonists structurally different from other opioid ligands. J. Med. Chem. 45, 1949–1956. https://doi.org/10.1021/jm010576e.

U.S. Food and Drug Administration, 2025a. FDA Issues Warning Letters to Firms Marketing Products Containing 7-hydroxymitragynine accessed on 2025-10-9. https://www.fda.gov/news-events/press-announcements/fda-issues-warning-letters-firms-marketing-products-containing-7-hydroxymitragynine.

U.S. Food and Drug Administration, 2025b. 7-Hydroxymitragynine (7-OH): an assessment of the scientific data and toxicological concerns around an emerging opioid threat. United States Food and Drug Administration. https://www.fda.gov/media/187899/download?attachment. (Accessed 22 January 2026).

Xu, J., Liang, L., Zheng, H., Chi, Y.R., Tong, R., 2019. Green oxidation of indoles using halide catalysis. Nat. Commun. 10, 4754. https://doi.org/10.1038/s41467-019-12768-4.

Zuarth Gonzalez, J.D., Ragsdale, A.K., Mukhopadhyay, S., McCurdy, C.R., McMahon, L. R., Obeng, S., Wilkerson, J.L., 2025. Mitragynine and 7-hydroxymitragynine: bidirectional effects on breathing in rats. J. Pharmacol. Exp. Therapeut. 392, 103720. https://doi.org/10.1016/j.jpet.2025.103720.