Comparative Analysis of Mitragynine Content in Kratom Leaves (Mitragyna speciosa Korth) from Kabupaten Kapuas Hulu Using HPLC Method

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Abstract

Opioid drugs are common analgesics for managing chronic pain, but they have significant short- and long-term side effects. Despite this, opioid consumption continues to rise in high-income countries, raising concerns about dependency. Kratom (Mitragyna speciosa Korth), which has opioid-like effects, shows potential as an analgesic with fewer side effects. This study aimed to explore and compare the mitragynine content in Kratom leaves from various regions in Kabupaten Kapuas Hulu, Indonesia, using High-Performance Liquid Chromatography (HPLC). Kratom leaves from 14 regions in Kapuas Hulu were collected, dried, and ground. Mitragynine was extracted using 70% ethanol and analyzed with HPLC. The yield percentage and mitragynine content in both the powdered simplisia and ethanol extract were measured and compared. The study showed that the average ethanol extract yield was 28%, with the highest yield from Elis Menendang (31.20%) and the lowest from Nanga Lauk (23.86%). Mitragynine content was significantly higher in the ethanol extract (3.22%) compared to the powdered simplisia (1.46%). Samples from Lanjak, Jongkong, Bunut, and Putussibau Kota showed mitragynine content exceeding 4% in the ethanol extract. HPLC analysis indicated that ethanol extraction significantly increases the mitragynine content in Kratom leaves compared to powdered simplisia, up to twice as much.

Keywords: chromatography, extraction, kratom, mitragynine, opioid

Introduction

Opioid drugs such as morphine, codeine, hydrocodone, oxycodone, fentanyl, and tramadol are common analgesics used in the management of chronic pain, but they have side effects with both short-term and long-term use (Zhang *et al*., 2020). Despite causing side effects, the consumption of opioid drugs as pain relievers continued to increase in high-income countries from 2009 to 2019 (Jayawardana *et al*., 2021). The increasing consumption of opioids as pain relievers has the potential to cause dependency, necessitating the exploration of other alternatives with minimal side effects. Recent research indicates that Kratom or Mitragyna speciosa Korth has effects similar to opioid drugs, suggesting its potential as an analgesic (Eastlack, Cornett and Kaye, 2020). Another study by Carpenter et al. showed that an extract of 30 mg/kg body weight of mitragynine from Mitragyna speciosa Korth had the same effect as 6 mg/kg body weight of oxycodone when administered either orally or intraperitoneally (Carpenter *et al*., 2016).

Kratom is a plant that grows widely in Southeast Asia, including Thailand, Indonesia, Malaysia, Myanmar, the Philippines, and Papua New Guinea. Kratom is commonly cultivated in Kalimantan, North Sumatra, and Aceh for consumption in the form of tea or ground into powder. Kratom leaves contain high levels of alkaloids, with the main components being mitragynine and 7-hydroxymitragynine (7-HMG), where mitragynine has potential analgesic and antinociceptive effects (Firmansyah, Sundalian and Taufiq, 2021). Research by Saref et al. indicates that kratom can be used to reduce opioid addiction and is believed to be an alternative to heroin and methamphetamine (Saref *et al*., 2019).

Opioid compounds are commonly used in pain management, one of which is through the administration of μ-Opioid

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Receptor (MOR) agonists. Research shows that mitragynine in kratom has activity as an MOR agonist and has the potential to replace morphine as a chronic pain therapy, especially in cancer patients (Singh *et al.*, 2018, Hong *et al.*, 2023). Meanwhile, Foss et al. mention that kratom's mitragynine can reduce neuropathic pain through adrenergic and opioid mechanisms (Foss *et al.*, 2020).

Although various studies show kratom's potential in the health sector, its consumption and cultivation are still limited due to a lack of exploratory research on kratom utilization in Indonesia. One of the largest kratom-producing centers in Indonesia is Regency Kapuas Hulu in Kalimantan Barat (West Kalimantan). In this region, kratom is commonly cultivated and consumed as a beverage, but the investigation of the active mitragynine content of kratom from Kapuas Hulu has never been conducted. Therefore, this study will explore the active mitragynine compound content in kratom leaves from several villages in Kabupaten (Regency) Kapuas Hulu as part of Indonesia's medicinal plant biodiversity using High-Performance Liquid Chromatography (HPLC). HPLC is a method that can separate single compounds from mixtures and is commonly used to detect and isolate mitragynine (Ng and Ha, 2024).

Materials and Methods

Design, Location and Time of Study

This research was an observational analytical laboratory study, with samples consisting of kratom leaves from 14 regions in Kabupaten Kapuas Hulu, namely: Jongkong (JK), Jongkong Kiri (JKK), Bunut (BN), Kampung Baru (KB), Lanjak (LJ), Jajang (JJ), Boyan (BY), Nanga Lauk (NL), Kalis (KL), Mupa (MP), Putusibau Kota (PK), Ulak Paok (UP), Mendalam (MD), and Elis Menendang (EM). The research was conducted from April to October 2023 at the Faculty of Medicine, Tanjungpura University.

Materials and Equipments

The tools and materials used in this research include: High-Performance Liquid Chromatography (HPLC), a rotary evaporator, an oven, a grinding machine, beaker glasses, Erlenmeyer flasks, an analytical balance, a centrifuge, kratom leaves, and 70% technical ethanol.

Sample Preparation

Fresh kratom leaves from various regions in Kapuas Hulu were cleaned and separated from their center veins until finely shredded. Subsequently, they were grounded using a grinding machine with a mesh size of 3500. The ground kratom leaves were then divided into two parts: one part as powdered simplicia and the other part used for extraction materials.

Extraction of Kratom's Leaves

A total of 500 grams of powdered kratom leaf (simplicia) was extracted using 70% ethanol solvent at room temperature for 3 days by maceration method. The solvent (1.400 ml) was replaced every 24 hours, with 1.300 ml used on the second and third days. The macerated extract was then concentrated using a rotary evaporator at 40°C, followed by further heating in an oven at 40-50°C until a thick extract was obtained. On average, approximately 100 grams of thick extract was obtained.

Analysis of Mitragynine Content Using HPLC Method

Approximately 50 mg of powdered simplicia sample was transfered into a volumetric flask. Added exactly 10 mL of sample solution to each volumetric flask containing the sample. Homogenized the sample and standards by shaking at least 400 times. Transfer as much of the sample as possible using a pipette (1.175 mL) into a 2 mL tube for subsequent centrifugation for 1-2 minutes. Mitragynine was analyzed by using a HPLC method developed by Sim et.al. with slightly modification (Sim *et al.*, 2022). Gradient elution was used a Reverse Phase (RP) C_{18} column with a mobile phase comprising acetonitrile (A) and ammonium bicarbonate buffer (B) at a flow rate of 1.5 mL/min. Using a syringe equipped with a filter, slowly inject the sample into an HPLC sample vial for analysis. Perform the same procedure for the ethanol extract.

Yield Percentage (%)

The yield of each sample was quantified using the following formula:

$$\%\,Yield = Area\,Ratio\,x \left[\frac{(standard\ concentration\ (^{mg}/_{mL})\ x\ solvent\ volume\ (mL)}{sample's\ weight\ (mg)} \right] x\ 100\%$$

with:

$$\text{Area ratio} = \frac{\text{area of sample}}{\text{area of standard}}$$

Information of standard concentration, sample's weight and solvent's volume are consecutively as follows : 0.1 mg/mL, 50 mg, and 10 mL

Results

Comparison of Ethanol Extract Yield from Kratom Leaves

Approximately 100 grams of powdered kratom leaf (simplicia) from 14 different regions were extracted until an average final yield weight of 28 grams was obtained. Based on data from Table 1, the average sample experienced a weight loss of 72% from its initial weight. Samples from each region exhibited varying yield percentages, ranging from the lowest in Nanga Lauk (NL) at 23.86% to the highest in Elis Menendang (EM) at 31.20%.

Table 1. Percentage (%) Yield of Ethanol Extract from Kratom Leaves from 14 Regions in Kabupaten Kapuas Hulu

No	Sample	Original Weight (g)	End Weight (g)	Yield (%)
1	NL	100,02	23,86	23,86
2	LJ	100,03	25,15	25,14
3	JK	100,10	26,57	26,54
4	BN	100,00	26,93	26,93
5	KL	100,01	27,20	27,20
6	MP	100,00	27,94	27,94
7	UP	100,04	27,98	27,97
8	JKK	100,02	28,32	28,31
9	JJ	100,00	28,51	28,51
10	BY	100,00	29,60	29,60
11	PK	100,00	30,26	30,26
12	KB	100,31	30,46	30,37
13	MD	100,01	30,63	30,63
14	EM	100,10	31,23	31,20
	Average	**100,04**	**28,00**	**27,98**

Meanwhile, the overall average percentage yield of ethanol extract across all samples was 27.98%. Other samples with yield percentages > 30% came from Mendalam (MD) at 30.63%, Kampung Baru (KB) at 30.37%, and Putussibau Kota (PK) at 30.26%.

Comparison of Mitragynine Content in Kratom Leaf Powder and Ethanol Extract

The next study was conducted to measure the levels of the active compound

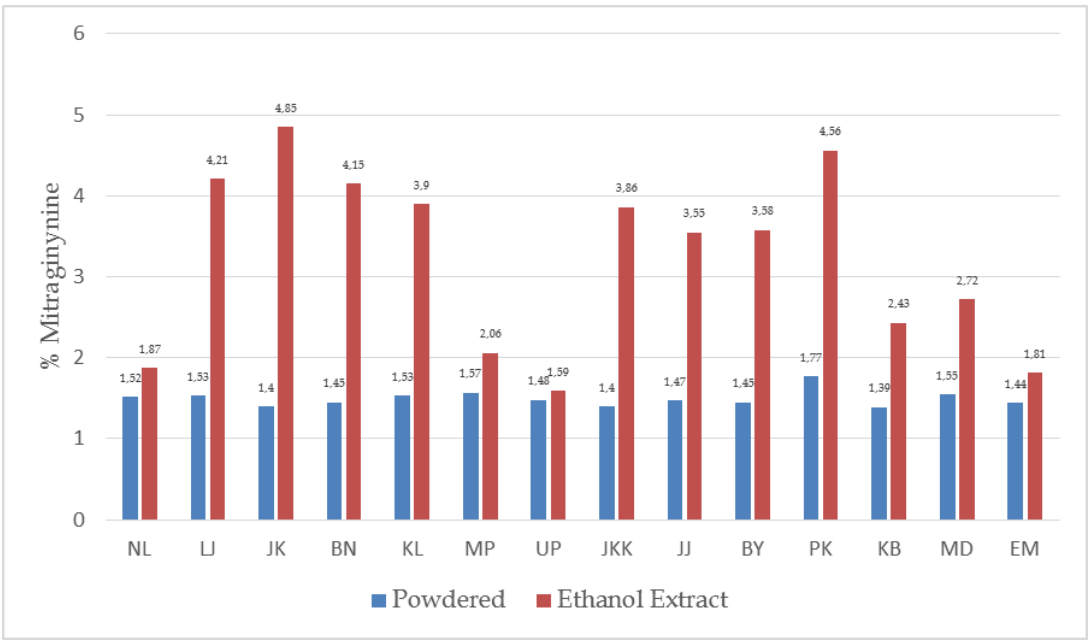

Figure 1. Comparison of Mitragynine Percentage (%) in Kratom Leaf Powder (Simplicia) and Ethanol Extract from 14 Regions in Kabupaten Kapuas Hulu

mitragynine in kratom leaf powder (simplicia) as well as in ethanol extract. The measurement of mitragynine levels was carried out using High Performance Liquid Chromatography (HPLC). The results of the mitragynine content measurements can be seen in Figure 1.

In the analysis of mitragynine content from kratom leaf powder (simplicia), an average percentage of 1.5% mitragynine was obtained for all samples. The powder sample with the highest mitragynine content came from Putussibau Kota (PK) at 1.77%, followed by Mupa (MP) at 1.57%, Mendalam (MD) at 1.55%, Lanjak (LJ) and Kalis (KL) both at 1.53%. On the other hand, for ethanol extract samples, the analysis showed that Jongkong (JK) had the highest percentage of mitragynine at 4.85%, followed by Putussibau Kota (PK) at 4.56%, Lanjak (LJ) at 4.21%, Bunut (BN) at 4.15%, and Kalis (KL) at 3.89%. On average, ethanol extract produced 3.22% mitragynine.

Kratom leaves from Putussibau Kota, Lanjak, and Kalis exhibited high percentages of mitragynine in both powder and ethanol extract samples. However, based on the average values of all 14 samples, it is noted that the percentage of mitragynine in ethanol extract is 2.1 times higher compared to powder extract, specifically 3.22% for ethanol extract versus 1.46% for powder/simplicia extract. The sample from Ulak Paloh was the only sample where the percentage of mitragynine in powder and ethanol extract were relatively close, at 1.48% for simplicia and 1.59% for ethanol extract.

Analysis of Comparison of Mitragynine Levels in Kratom Leaf Powder (Simplicia) and Ethanol Extract

Although the overall mitragynine content in kratom leaf powder (simplicia) was quite high, extraction significantly increases the concentration (yield) of mitragynine. Based on the statistical test results using a paired t-test, it was found that the mitragynine content in ethanol extract significantly higher compared to powder with a p-value $\alpha < 0.001$ (Table 2).

Discussion

Kratom or Ketum (Mitragyna speciosa Korth) is a tropical plant from the Rubiaceae family, native to Southeast Asia and Papua New Guinea. Also known as purik, ithang, kakuan, thom, or biak, this plant was discovered by botanist Pieter Willem Korthals (Raini, 2017). The local population typically uses kratom as traditional medicine to treat various ailments such as fever, malaria, diarrhea, cough, hypertension, diabetes, muscle pain, and worm infections (Ahmad, *et al* 2022; Suhaimi *et al.*, 2019; Mu'amar, 2021; Ningrum *et al.*, 2021). Scientifically, kratom is known to have pharmacological effects such as anti-inflammatory, antinociceptive, anti-obesity, analgesic, antipyretic, sedative, stimulant, depressant, antidopaminergic, effects on memory, antidiarrheal, antioxidant, and antimicrobial properties (Parthasarathy *et al.*, 2009; Ahmad *et al.*, 2022; Janthongkaw *et al.*, 2023; Toklo *et al.*, 2023; Paankhao *et al.*, 2024; Sureram *et al.*, 2024). Various studies have shown that the mitragynine content in kratom has health benefits, one of which is as a pain reliever (Váradi *et al.*, 2016; Chakraborty, Uprety, Daibani, *et al.*, 2021; Chakraborty, Uprety, Slocum, *et al.*, 2021).

Chronic pain affects one-third of the world's population, becoming a health problem because it reduces productivity and causes difficulty in various activities.

Table 2. Paired t-test for Mitragynine Percentage in Kratom Leaf Powder (Simplicia) and Ethanol Extract

Paired Samples Test									
		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	% Tepung - % Ekstrak	-1.72755	1.10337	.29489	-2.36461	-1.09049	-5.858	13	.000

Mitragynine, which is abundantly found in kratom leaves, has activity as a MOR agonist, giving kratom plant products effects similar to other opioid compounds. MOR activation triggers a series of signal transduction processes mainly caused by G-protein dissociation. G-protein is a trimeric protein consisting of three subunits: α, β, and γ. Activated G-protein will dissociate into two, the α subunit and the βγ subunit, which is a dimeric protein (Listos *et al.*, 2019). This G-protein dissociation activates several signaling pathways. The Gα subunit inhibits adenylate cyclase and the synthesis of cAMP and protein kinase A (PKA), which affect ion channels on the cell membrane, such as inhibiting TRPV1 and voltage-gated sodium channels (VGSC). Meanwhile, the Gβγ subunit inhibits calcium channels and opens potassium channels. These mechanisms reduce excitability and neurotransmitter release from presynaptic neurons, causing analgesia induced by MOR agonists (Zhang *et al.*, 2020).

Given the significant potential of kratom leaves as a herbal pain reliever, it is essential to explore this medicinal plant, especially in various kratom-producing regions in Indonesia. This study analyzed the mitragynine content in kratom, comparing simplicia (powder) with ethanol extract. The people of Kapuas Hulu generally consume kratom leaves in powder form, and this study proved that the mitragynine obtained in the powder is 2.1 times lower than when extracted. Ethanol extract can produce an average of 32.22 mg/g of mitragynine, higher than the 14.95 mg/g of mitragynine in simplicia. Extraction is a process carried out to isolate specific components in a plant, such as phenolic compounds (Farahani, 2021). This study proves that almost all samples contain higher mitragynine in ethanol extract than in simplicia.

Kratom leaves contain various types of alkaloids, so the extraction process can extract multiple biomolecules, including various alkaloids. The choice of solvent plays a vital role in determining the biomolecules to be extracted. Mitragynine can generally be extracted using solvents like methanol, hexane, and ethanol, producing different yields (Purwayantie et al., 2022). Often, to achieve optimal results, a combination of several solvents is used, as in the study conducted by Mustafa et al. (2020), which resulted in a mitragynine yield of 75 mg (Mustafa *et al.*, 2020).

The mitragynine content in kratom leaves generally varies based on the location and growing conditions (Mustafa *et al.*, 2020). Research indicates that the mitragynine content in samples from Thailand is influenced by light intensity, humidity, soil water content, soil pH, and calcium (Leksungnoen *et al.*, 2022). However, the samples in this study were from wild kratom trees (not cultivated) from several areas in Kabupaten Kapuas Hulu, so the growing conditions could not be controlled. This study shows that the mitragynine content from ethanol extracts of kratom leaves from 14 regions in Kabupaten Kapuas Hulu ranges from 15.87 mg/g to 48.52 mg/g, higher compared to the mitragynine content in ethanol extracts from Thailand samples, which ranges from 7.5 mg/g to 26.6 mg/g (Leksungnoen *et al.*, 2022). This value is also higher than kratom from two regions in Malaysia, which has 0.094% and 0.105% (Sanagia *et al.*, 2013). The mitragynine content in kratom leaf powder from Kabupaten Kapuas Hulu is also relatively high, with variations in mitragynine levels from 13.94 mg/g to 17.69 mg/g.

Conclusion

The analysis of mitragynine content from 14 regions in Kabupaten Kapuas Hulu using HPLC showed varying results, ranging from 1.40% to 1.77% for leaf powder (simplicia) and 1.58% to 4.85% for ethanol extract. Extraction significantly increased the mitragynine content, with values up to twice as high in ethanol extract compared to simplicia (3.22% versus 1.49%) with a p-value $\alpha < 0.001$. Several regions exhibited mitragynine content exceeding 4% in their ethanol extracts, such as Lanjak, Jongkong, Bunut, and Putussibau Kota kratom leaves.

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