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Original Article

Green Purification of Mitragynine from Kratom Leaves (*Mitragyna speciosa*) through Solvent Variation in Acid-Base Extraction

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Abstrak

Kratom (*Mitragyna speciosa*) is a major natural source of mitragynine, an indole alkaloid with potential as an alternative analgesic to opioids. However, conventional chromatographic purification methods are costly and generate large volumes of solvent waste, making them less compatible with green chemistry principles. This study aimed to develop a non-chromatographic purification method using acid–base liquid–liquid extraction (ABLLE) by varying the partition solvent. Kratom leaves were extracted with ethanol and methanol, and the alkaloids were subsequently separated through acidification, basification, and liquid–liquid partitioning using ethyl acetate and dichloromethane. Total alkaloid content (TAC) was determined by UV–Vis spectrophotometry based on the bromocresol green (BCG) reaction, while mitragynine purity was quantified using high-performance liquid chromatography with ultraviolet detection (HPLC-UV). The results showed that the ethanol–dichloromethane system produced the alkaloid fraction with the highest mitragynine purity, reaching 39.09%, whereas the ethanol–ethyl acetate system yielded a purity of 27.47%. Both systems demonstrated a process efficiency of approximately 19%, with lower solvent consumption and reduced process waste compared with column chromatography. These findings indicate that acid–base liquid–liquid extraction has the potential to serve as a simpler, scalable, and more sustainable approach for mitragynine purification, thereby supporting the development of purification processes aligned with green chemistry principles.

Keywords: *Mitragyna speciosa; acid–base liquid–liquid extraction; non-chromatographic purification method; green alkaloid isolation.*

1. Introduction

Kratom (*Mitragyna speciosa* Korth.) is a tropical plant widely cultivated in Southeast Asia, particularly in Indonesia, the world's largest producer of kratom. Kratom leaves contain indole alkaloids, with mitragynine as the major constituent, accounting for approximately 66% of the total alkaloid content [1,2]. Mitragynine exhibits analgesic effects and has potential as a therapeutic agent for opioid withdrawal [1,3,4]. However, Indonesian kratom exports remain largely limited to low-value dried leaf powder with minimal downstream processing [5,6]. Therefore, the development of purification methods capable of enhancing its added value is of considerable importance.

Conventional approaches to mitragynine purification, including column chromatography, preparative high-performance liquid chromatography (preparative HPLC), and fractionation, are resource-intensive, costly, and require specialized expertise [7–9]. Moreover, these techniques generally consume substantial amounts of hazardous solvents and generate significant waste, making them less compatible with green chemistry principles and, in some cases, producing unstable fractions [10]. These limitations highlight the need for alternative purification methods that are simpler, greener, and sustainable for alkaloid isolation.

Acid–base liquid–liquid extraction (ABLLE) is a simple and cost-effective alternative approach that may provide a practical solution to these challenges by exploiting the pH-dependent solubility of alkaloids. Under acidic conditions, alkaloids become protonated and remain soluble in the aqueous phase, whereas under basic conditions, the free-base alkaloids are deprotonated and repartition into the organic phase [11–13]. By avoiding chromatographic techniques that require solid adsorbents and sophisticated instrumentation, ABLLE reduces dependence on costly equipment and offers a more economical process with the potential to generate less waste.

While ABLLE is a well-established method, this study provides a systematic evaluation of how the maceration solvent (methanol vs. ethanol) and partition solvent (dichloromethane vs. ethyl acetate)

affect mitragynine recovery and purity. Moreover, quantitative comparisons between ABLLE and conventional chromatographic methods remain limited, particularly in terms of downstream processing and production sustainability. Therefore, this study aimed to: (1) evaluate the effects of solvent variation on mitragynine recovery and purity, (2) compare the performance of ABLLE with column chromatography, and (3) assess the process in terms of green chemistry principles, particularly solvent consumption and waste reduction potential.

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2. Research Method

This research was conducted in three main stages. The first stage involved optimizing the solvent selection method for maceration. The second stage consisted of the acid–base extraction process along with green metrics analysis. The third stage involved a comparative analysis of the isolation results obtained via the liquid–liquid extraction method and column chromatography.

2.1. Instruments and Materials

Instruments: The equipment used included a set of glassware, an OHAUS analytical balance, a separatory funnel, a CAMAG UV lamp, micropipettes of various volumes, Pasteur pipettes, an IKA RV 10 Basic rotary evaporator, and a UV–Vis spectrophotometer (DLAB SP-UV1100). High-performance liquid chromatography with ultraviolet detection (HPLC-UV) analysis was performed using an Agilent 1200 Series Liquid Chromatography system (Agilent, Mississauga, ON, Canada) equipped with a Kinetex EVO C18 100 Å column (5 µm, Phenomenex, Torrance, CA, USA).

Materials: Kratom leaves (Mitragyna speciosa) were obtained from BRIN PUSPIPTEK Serpong, Indonesia, as the main raw material. Technical-grade ethanol (96%, Multi Kimia Nusantara), technical-grade methanol (96%, Multi Kimia Nusantara), technical-grade dichloromethane (DCM), and technical-grade ethyl acetate were used as solvents. Reagents included Dragendorff reagent (Merck), Mayer reagent (Merck), bromocresol green (Merck), and ammonia. A Certified Reference Material (CRM) of mitragynine was used as the reference standard.

2.2 Method

2.2.1 Selection of Maceration Solvent

Maceration

A total of 100 g of kratom leaf powder was separately macerated with methanol or ethanol at a ratio of 1:5 (w/v) for 72 h at room temperature. The resulting extracts were concentrated using a rotary evaporator.

Determination of Total Alkaloid Content by Bromocresol Green (BCG) Method

Total alkaloid content (TAC) was determined by UV–Vis spectrophotometry using the bromocresol green reagent and calibrated against berberine chloride as the standard [14]. Modifications were made to the berberine chloride volume series (0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mL) and the mass of the tested extract sample (0.02 g). Absorbance was measured at a maximum wavelength of 420 nm in duplicate. Results were expressed as miligrams of berberine chloride equivalent per gram of extract (mg BCE/g extract), calculated according to the following equation:

$$TAC = \frac{C\ x\ Fpx\ V}{m}$$

where C is the concentration, DF is the dilution factor, V is the volume, and m is the initial mass of the extract. Data analysis was performed to determine the most suitable maceration solvent for the subsequent steps based on total alkaloid quantification and toxicological considerations.

2.2.2 Acid–Base Extraction of Alkaloids

Acid–Base Extraction

The acid–base extraction method was adapted from Flores-Bocanegra et al. [7] and Sharma et al. [11]. The crude extract was dissolved in water, acidified with 5% HCl to pH 2–3, and extracted with ethyl acetate (3 × 100 mL) to remove non-alkaloid compounds. The aqueous phase was then basified with NH_4OH to pH 9–10, followed by extraction with dichloromethane (DCM) or ethyl acetate (3 × 100 mL). The resulting alkaloid fraction was dried over Na_2SO_4 and concentrated.

Analytical Method

The presence of alkaloids was confirmed by thin-layer chromatography (TLC) using hexane:ethyl acetate:ammonia (30:15:1, v/v/v) mobile phase. Alkaloids were indicated by the appearance of an orange spot at an R_f value of 0.5 after spraying with Dragendorff reagent [15]. HPLC-UV analysis was performed for mitragynine quantification using gradient elution with acetonitrile and ammonium bicarbonate buffer as the mobile phase, with detection at 226 nm [16,17]. The mitragynine content in solid samples was calculated using the following equations:

$$\text{Purity (\%)} = \frac{\text{Theoritcal concentration}}{\text{Actual concentration}} \text{ x (CRM mitragynine standard content) x } 100$$

$$\text{Efficiency (\%)} = \frac{\text{mass of mitragynine in the fraction}}{\text{initial mass of mitragynine}} \text{ x } 100$$

Analysis and evaluation of the optimal solvent combination were carried out using green metrics through calculation of the E-factor. The E-factor was defined as the ratio of waste mass to product mass [18], according to Sheldon (2017).

$$E - factor = \frac{\text{mass of waste}}{\text{mass of product}}$$

2.2.3 Comparison with Conventional Column Chromatography

Conventional Column Chromatographic Isolation of Mitragynine

Dried *Mitragyna speciosa* leaf powder was extracted with technical-grade methanol at a ratio of 1:3 (w/v) by maceration for 24 h at room temperature (25 °C). The filtrate was collected and

concentrated using a rotary evaporator at 40–45 °C to obtain a dark green viscous extract. A 200 mL portion of the extract was mixed with hot water (1:1, v/v) to remove chlorophyll. The mixture was allowed to stand for 24 h until two layers were formed, consisting of an upper hydroalcoholic layer and a lower chlorophyll-rich layer. The hydroalcoholic layer was further separated and evaporated to obtain a brown aqueous fraction, which was used for further analysis. The aqueous fraction was acidified with CH_3COOH to pH 3–4 to convert the alkaloids into their water-soluble salt forms. Nonpolar compounds were removed by partitioning with *n*-hexane. The solution was then basified with 25% NH_4OH to pH 9–10, allowing the free-base alkaloids to be successively extracted with dichloromethane (DCM) and ethyl acetate (EtOAc).

The DCM fraction was purified by gravity column chromatography using silica gel 60 as the stationary phase. Elution was performed stepwise with 100% DCM, followed by a gradient mixture of DCM: methanol (99:1), yielding fractions A–J. Fraction H, which exhibited the highest mass (200 mg), was selected for further analysis. This fraction was analyzed by TLC under UV light (254 and 366 nm) and sprayed with Dragendorff and Mayer reagents for alkaloid confirmation. Structural characterization was carried out using UV–Vis spectroscopy (λmax ≈ 226 nm) and FTIR to identify the characteristics of its functional groups. HPLC-UV was used for mitragynine quantification using gradient elution with acetonitrile and ammonium bicarbonate buffer as the mobile phase, with detection at 226 nm [16,17].

Comparison of Purification Methods: Conventional Chromatography and ABLLE

The data obtained from purification using conventional chromatography were compared with the results obtained using the ABLLE method. The analysis was conducted based on "green metrics," including the E-factor [17] and solvent consumption.

3. Results and Discussion

3.1 Selection of Solvent for Maceration

Table 1. Effect of maceration solvent on extraction yield and total alkaloid content

Maceration Solvent	Extract Yield (%)	Total Alkaloid Content (mg BCE/g extract)	Environmental Note
Methanol	24.4	9.39 ± 0.447	Toxic, non-food-grade
Ethanol	13.9	16.92 ± 0.363	Safer and more renewable

Based on Table 1, maceration using methanol produced a higher crude extract yield (24.4%) than ethanol (13.9%). However, the quantitative analysis of total alkaloid content (TAC) in each extract using the bromocresol green (BCG)–UV-Vis spectrophotometric method showed the opposite trend. The quantitative analysis of total alkaloids was performed using a BCG calibration curve, which demonstrated a linear relationship (R^2 = 0.9993) with the regression equation $y = 0.0506x - 0.0016$. The complete calibration data are provided in the Supplementary File. The results indicate that the ethanolic extract contained a higher alkaloid content (16.92 ± 0.363 mg BCE/g extract) than the methanolic extract (9.39 ± 0.447 mg BCE/g extract). This pattern suggests that, in the present study, ethanol tended to produce an alkaloid-enriched extract, whereas methanol appeared to extract a broader range of matrix components. These findings highlight that mass extraction efficiency and selectivity toward the target compounds are two parameters that are not necessarily directly proportional.

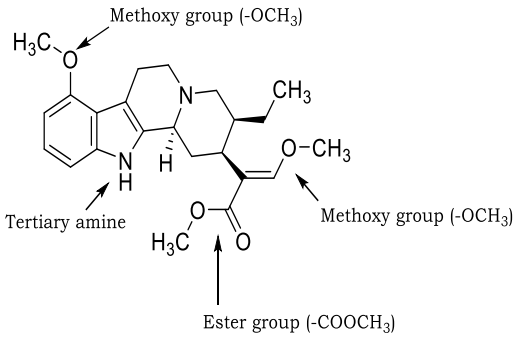

Figure 1. Mitragynine structure

Figure 1 shows the chemical structure of mitragynine, an indole alkaloid containing a tertiary amine as a weakly basic center, along with an aromatic framework and oxygenated substituents (including methoxy and ester groups) which contribute to its semipolar character. During maceration, protic solvents such as methanol and ethanol may interact with mitragynine through hydrogen-bonding interactions with heteroatoms, particularly nitrogen and oxygen atoms, as well as dipole–dipole interactions involving the polar functional groups of the molecule. The differences in extraction performance observed in this study are likely associated with the different polarities of the two solvents. Methanol, which has a higher dielectric constant ($\varepsilon \approx 33$), may extract a broader range of polar and semipolar matrix components, thereby contributing to the higher crude extract yield [21]. In contrast, ethanol, with a more moderate polarity ($\varepsilon \approx 24$), produced a higher total alkaloid content under the present experimental conditions, suggesting its greater potential as an initial extraction solvent for alkaloid enrichment [19].

Table 2. Comparison of extract yield and chemical constituents

Reference	Method	Initial Simplicia (g)	Sample source	Solvent	Extract yield (%)	Total alkaloid content	Mitragynine content
This study	Maceration	100	West Kalimantan, Indonesia	Methanol	24.4	9.39 ± 0.447 mg BCE/g extract	—
				Etanol	13.9	16.92 ± 0.363 mg BCE/g extract	—
[20]	Maceration	600	Nakhon Si Thammarat, Thailand	Methanol	≈ 10.08	52.82 ± 0.85 mg ATR/g extract	35.87 ± 1.01 mg/g extract
				Ethanol	≈ 8.80	88.04 ± 0.15 mg ATR/g extract	58.75 ± 0.21 mg/g extract
				Water	≈ 9.67	5.61 ± 0.13 mg ATR/g extract	3.85 ± 0.17 mg/g extract

Based on Table 2, the findings of the present study are in agreement with those reported by Limcharoen et al. [20], who highlighted that methanol produced a higher crude extract yield than ethanol, with yields of approximately 10.08% and 8.80%, respectively. Conversely, the ethanolic extract exhibited higher total alkaloid and mitragynine contents than the methanolic extract [20]. A similar trend observed in both studies indicates that methanol may extract a broader spectrum of compounds from the kratom leaf matrix, resulting in a higher crude extract yield. Meanwhile, ethanol may provide greater selectivity toward alkaloid-containing constituents, making it a more promising solvent for obtaining alkaloid-enriched extracts.

The findings of Goh et al. [21], using accelerated solvent extraction (ASE), and Karunakaran et al. [22], using ultrasound-assisted extraction (UAE), further support this interpretation. Despite differences in extraction techniques, both studies showed that methanol produced the highest extract yield, while mitragynine content among organic solvents was relatively comparable or not significantly different [21,22]. Conversely, Bayu et al. [23] reported that methanol yielded both a higher extract mass and a higher mitragynine content. This difference suggests that the solvent effect on kratom alkaloid enrichment is context-dependent rather than universal. The observed variation may be associated with biological factors, such as plant variety and age; cultivation conditions, including climate and soil type; and post-harvest handling or storage conditions [24,25]. Thus, the present findings do not negate the results reported by Bayu et al. [23], but rather indicate that solvent-dependent extraction performance is strongly influenced by matrix characteristics and extraction process design.

From a green chemistry perspective, ethanol is considered more favorable than methanol. Ethanol exhibits lower toxicity, is biodegradable, and can be derived from renewable resources [26,27].

Accordingly, the use of ethanol as a maceration solvent may contribute not only to improved alkaloid selectivity but also to better alignment with sustainability principles. The choice of maceration solvent is also critical for downstream processing, as it influences the separation burden during the subsequent acid–base liquid–liquid extraction step.

3.2 Acid–Base Extraction and Alkaloid Fractionation

Table 3. Results of acid–base liquid-liquid extraction

Method	Sample/ Fraction	Basic-stage partition solvent	Mass (g)	Yield (%)	Mitragynine content (%)
I	EtOH-DCM	Dichloromethane	0.486	3.8	39.03
II	EtOH–EtOAc	Ethyl acetate	0.702	5.4	27.47

The alkaloid fractionation results indicate that solvent selection during the basic extraction stage influenced both fraction yield and mitragynine content. Mitragynine was quantified by HPLC-UV using a mitragynine standard calibration curve with excellent linearity (R^2 = 0.9998), based on the regression equation y = 141.76x + 11.319; the complete calibration data are provided in the Supplementary File. As shown in Table 3, the ethyl acetate fraction (EtOH–EtOAc) afforded a higher yield of 0.702 g, corresponding to approximately 5.4% of the initial extract, but contained a lower mitragynine level of 27.47%. Conversely, the dichloromethane fraction (EtOH–DCM) gave a lower yield of 0.486 g, corresponding to approximately 3.8%, while exhibiting a higher mitragynine content of 39.03%. This result suggests that extraction yield and selectivity toward mitragynine do not necessarily increase proportionally.

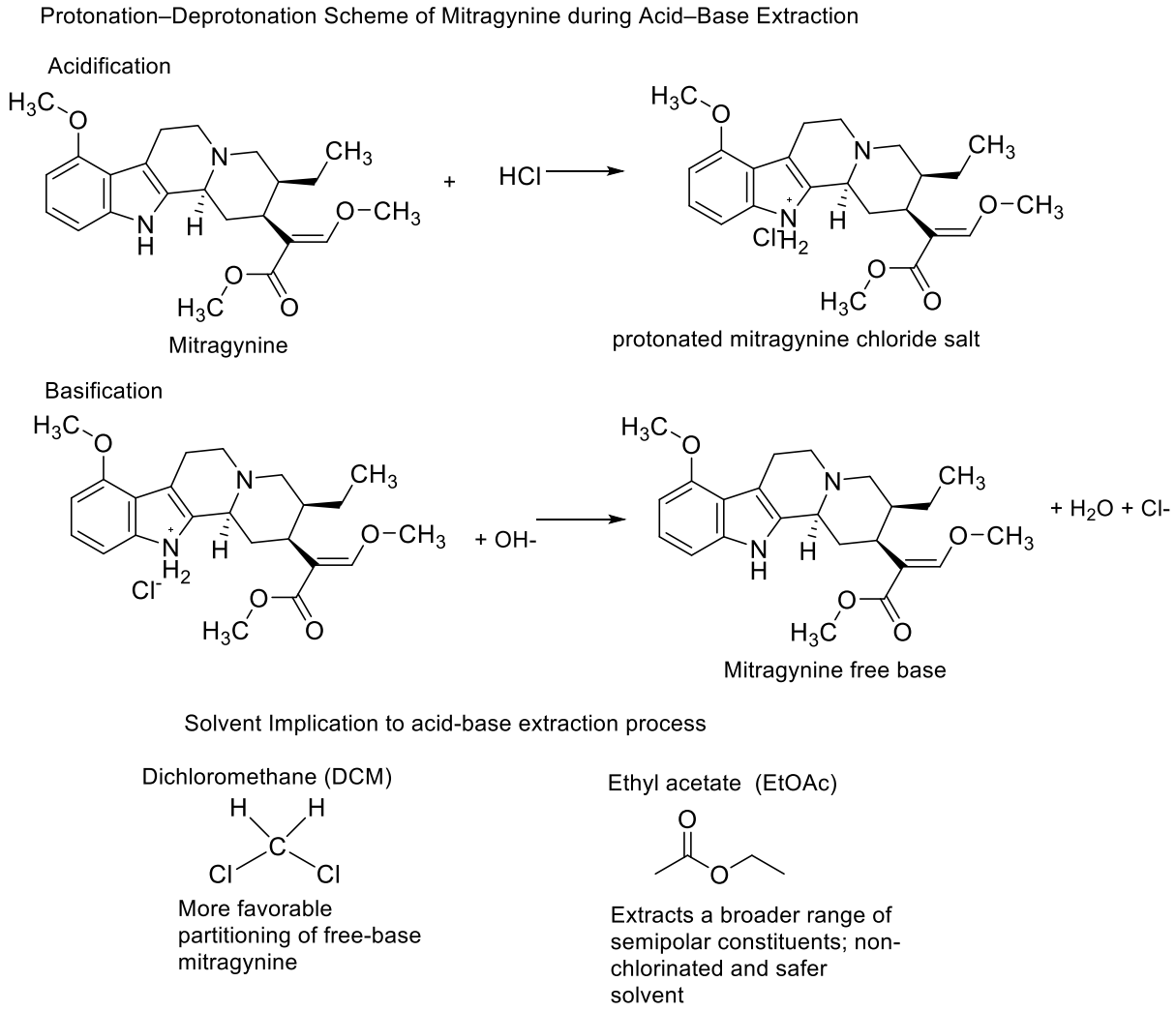

Figure 2. Protonation–deprotonation scheme of mitragynine during acid–base liquid–liquid extraction and the implication of basic-stage solvent selection.

Mitragynine is a weakly basic alkaloid with an approximate pK_a of 8.1 and moderate lipophilicity (log P ~1.7). Under acidic conditions, mitragynine becomes protonated and more water-soluble, whereas under basic conditions it predominantly exists as the neutral free-base form and is more readily partitioned into the organic phase, as illustrated in Figure 2. The difference in mitragynine content between the DCM and ethyl acetate fractions may therefore be related to differences in solvent–solute interactions with free-base mitragynine, including polarity, lipophilicity matching, and weak noncovalent interactions.

Dichloromethane (polarity index ~3.1) [19] may offer a more selective solvation environment for free-base mitragynine through lipophilicity matching, dispersion forces, and weak noncovalent interactions [28,29]. By contrast, ethyl acetate (polarity index ~4.4) [21], which contains a carbonyl oxygen as a hydrogen-bond-accepting site, may dissolve a broader range of neutral semipolar constituents. This may explain why ethyl acetate produced a higher alkaloid fraction yield, whereas DCM resulted in a higher mitragynine content in the present study.

Based on Table 4, the mitragynine content of the dichloromethane fraction in the present study was higher than that reported by Sharma et al. [11]. This difference may be influenced by several factors, including biological characteristics of kratom plants (such as variety and plant age), geographical growing conditions (such as climate and soil type), and the harvesting period [24,25]. Meanwhile, compared with the study conducted by Bayu et al. [23], which used chloroform as the extraction solvent, the alkaloid fraction yield and mitragynine percentage obtained in the present study were lower. This variation indicates that the type of solvent used in the final partition step influences the degree of mitragynine enrichment. In terms of solvent properties, chloroform and dichloromethane are both chlorinated solvents that act as weak hydrogen-bond donors through their C–H protons, with chloroform exhibiting stronger donor ability than dichloromethane [28].

Table 4. Comparison of alkaloid fraction results with previous studies

Reference	Sample origin	Final partition solvent	Acid–base extraction method	Alkaloid fraction yield (% w/w)	Mitragynine content (% w/w)
Method I	BRIN Puspiptek Serpong; material originally from Kalimantan, Indonesia	DCM	The ethanolic extract was acidified with HCl (pH 2–3); the aqueous phase was basified with NH_4OH (pH 9–10), followed by extraction with DCM	3.8	39.03
Method II	BRIN Puspiptek Serpong; material originally from Kalimantan, Indonesia	EtOAc	The ethanolic extract was acidified with HCl (pH 2–3); the aqueous phase was basified with NH_4OH (pH 9–10), followed by extraction with ethyl acetate	5.4	27.47
[11]	Pure Land Ethnobotanicals, Madison, USA	DCM	The ethanolic extract in MeOH was acidified with HCl (pH 2–3); the aqueous phase was basified with NH_4OH (pH 8–9), followed by extraction with DCM	6.15	33.6
[23]	Putussibau, Kalimantan Barat, Indonesia	Chloroform	The methanolic extract in acetic acid was basified with NH_4OH (pH 8–9), followed by partitioning with chloroform	~3.0	~45.0

*Data are presented as the percentage yield of the alkaloid fraction relative to the initial extract.

This property may contribute to the stabilization of free-base mitragynine during partitioning. From a sustainability perspective, the methods reported by Sharma et al. [11] and Bayu et al. [23] rely on chlorinated solvents, namely dichloromethane and chloroform, which are associated with relatively high toxicity risks and unfavorable environmental impacts [30,31]. Therefore, ethyl acetate is a safer and more environmentally benign non-chlorinated solvent alternative. This consideration is consistent with solvent selection guides developed by GlaxoSmithKline (GSK), Sanofi, and Pfizer [32–34]. Although the ethyl acetate fraction showed a lower mitragynine content than the dichloromethane fraction, it provided a relatively comparable overall mitragynine recovery, with a process efficiency of approximately 19%. This finding suggests that solvent selection in extraction should not be evaluated solely on purity or yield but should also consider occupational safety, toxicity, and environmental impact. Accordingly, ethyl acetate may serve as a practical alternative to DCM in the acid–base extraction of mitragynine as it is biodegradable, less toxic, and safer for laboratory handling [35,36].

E-factor Analysis of Methods I and II

The environmental performance of the acid–base liquid–liquid extraction (ABLLE) process for mitragynine isolation from *Mitragyna speciosa* leaves was evaluated based on solvent consumption, product yield, purity, and solvent recovery. The total solvent mass was estimated from the solvent volume and density: ethanol (0.789 g mL^{-1}), ethyl acetate (0.902 g mL^{-1}), dichloromethane/DCM (1.33 g mL^{-1}), and water (1.00 g mL^{-1}). As summarized in Table 5, Method I consumed 3068.33 g of solvent, while Method II consumed 2939.93 g. Method II showed higher solvent recovery because it used only non-chlorinated solvents, specifically ethanol and ethyl acetate. In contrast, DCM recovery in Method I was limited to 80.67%. The semi-purified product masses were 0.4855 g and 0.7022 g for Methods I and II, respectively, corresponding to an estimated mitragynine mass of approximately 0.19 g in both methods.

The E-factor values after solvent recovery were 888.36 for Method I and 556.22 for Method II. This calculation follows Sheldon's definition of the E-factor [18], in which water is excluded to avoid distortion caused by the dominance of water mass. For data transparency, the E-factor values "including water" are also reported; however, these values were not used for comparative evaluation between methods, in accordance with current reporting practices in the pharmaceutical industry [18]. The difference in E-factor values between the

two methods can be attributed to the higher density and toxicity concerns of DCM, as well as its lower recovery rate, which increased the relative waste mass in Method I compared with Method II. These results are consistent with the solvent polarity profile: Method I, which used DCM, produced a higher mitragynine purity but also resulted in a higher E-factor and greater toxicity concern. In contrast, Method II showed a lower E-factor and better solvent recovery, indicating that it is more closely aligned with the principles of green chemistry.

Table 5. Sustainability metrics of DCM- and EtOAc-based ABLLE methods for mitragynine enrichment

Parameter	Method I (EtOH–EtOAc–DCM)	Method II (EtOH–EtOAc)	Notes/Interpretation
Solvent system	500 mL EtOH for maceration; 300 mL EtOAc; 300 mL DCM; 2000 mL aqueous phase	500 mL EtOH for maceration; 600 mL EtOAc; 2000 mL aqueous phase	Acid–base separation using organic and aqueous phases
Total solvent mass (g)	3068.33	2939.93	Calculated from solvent volume × density
Solid residue/waste (g)	69.98	70	Biodegradable plant residue
Product mass (g)	0.4855	0.7022	Semi-purified alkaloid fraction obtained after ABLLE
Mitragynine purity (%)	39.03	27.47	Determined by HPLC-UV
Mitragynine yield (g)	0.18	0.193	Calculated from product mass × mitragynine purity
Solvent recovery (%)	EtOH:90% DCM: 80.67% EtOAc: 90.3%	EtOH : 90% EtOAc: 91.67%	Calculated from the recovered solvent volume
E-factor without solvent recovery, excluding water	Mitragynine basis: 6114.54; alkaloid fraction basis: 2386.50	Mitragynine basis: 5331.63; alkaloid fraction basis: 1464.60	Waste mass/product mass
E-factor without solvent recovery, including water	Mitragynine basis: 16658.30; alkaloid fraction basis: 6501.73	Mitragynine basis: 15989.12; alkaloid fraction basis: 4317.06	Reported for transparency only
E-factor with solvent recovery, excluding water	Mitragynine basis: 888.36; alkaloid fraction basis: 346.73	Mitragynine basis: 556.22; alkaloid fraction basis: 152.79	
E-factor with solvent recovery, including water	Mitragynine basis: 11432.10; alkaloid fraction basis: 4461.95	Mitragynine basis: 11114.80; alkaloid fraction basis: 3000.98	
Relative sustainability profile	Moderate; efficient but uses a chlorinated solvent	Higher; non-chlorinated solvent system with better recovery	Based on toxicity, recyclability, and waste reduction

Note: E-factor was calculated as the ratio of waste mass to product mass. Two calculation bases are presented: mitragynine mass and semi-purified alkaloid fraction mass. Water-inclusive E-factor values are reported for transparency but were not used as the primary basis for method comparison.

Future optimization may focus on replacing DCM with greener solvent alternatives, such as

ethyl lactate or 2-MeTHF (2-methyltetrahydrofuran), to further improve the environmental performance of the ABLLE process [18]. Although Method II resulted in slightly lower mitragynine purity, it showed a more favorable sustainability profile because it eliminated chlorinated solvent use, reduced total solvent consumption, and improved solvent recovery. The reduction in E-factor values, particularly after solvent recovery and water exclusion, further confirms the environmental advantage of the EtOAc-based system.

3.3 Comparison between Conventional Chromatography and the ABLLE Method

Table 6. Comparison between conventional chromatographic purification and the ABLLE method

Parameter	Column Chromatography	ABLLE Method (Present Study)	Green Chemistry Principle
Solvent consumption	~ 3321.6 g of DCM and methanol; often difficult to recover or recycle	~2900 – 3000 g total solvent mass using EtOH–EtOAc or EtOH–EtOAc–DCM systems; 80–92% of the solvent can be recovered	Principle 1: Waste Prevention; Principle 5: Safer Solvents and Auxiliaries
Solid waste	Plant residue, silica gel, and disposable cartridges	Only approximately 70 g of biodegradable plant residue	Principle 1: Waste Prevention
Energy requirement	High; requires pressure-driven systems, stepwise eluent fractionation, and repeated evaporation	Low; maceration at room temperature followed by evaporation	Principle 6: Design for Energy Efficiency
Solvent recycling	Minimal, with low recovery	85–90% for EtOH and EtOAc, and 80–85% for DCM	Principle 1: Waste Prevention
E-factor with solvent recovery	21,585	500 – 888 depending on the method	Principle 1: Waste Prevention
Toxicity/environmental hazard	High, due to the use of DCM, methanol, hexane, and silica waste	Moderate for the EtOH–EtOAc system; higher when DCM is used	Principle 5: Safer Solvents and Auxiliaries
Cost	High, due to the use of chromatography columns, silica gel, large solvent volumes, and longer processing time	Lower, as the process requires only basic laboratory glassware	
Mitragynine purity	47.9% after one to three purification steps; can reach >95% after multiple cycles	27–39% after one cycle	Provides a scalable pre-purification fraction suitable for further refinemen

The acid–base liquid–liquid extraction (ABLLE) method offers a simpler and more environmentally favorable approach compared with chromatography-based purification. Table 6 presents the main comparison between the two methods. Although conventional chromatography can achieve higher mitragynine purity, it requires large solvent volumes, adsorbents such as silica gel, and greater energy input and processing time. In contrast, ABLLE can be performed using basic

laboratory glassware, consumes less solvent, and allows higher solvent recovery, making it more consistent with the principles of green chemistry.
Based on the E-factor values, conventional chromatography showed a substantially higher value of 21,585, far exceeding that of ABLLE (500–888). This value reflects the high mass of waste generated per unit mass of final product, mainly due to the extensive use of solvents and adsorbents. This difference highlights the advantages of ABLLE in terms of solvent efficiency and waste reduction compared with chromatographic purification.

In the laboratory-scale isolation of minor compounds from natural product matrices, E-factor values can be exceptionally high; therefore, the absolute values reported in this study are more appropriately interpreted for relative comparison between methods. This is because process efficiency is strongly influenced by the concentration of the target compound in the starting material [37]. Mitragynine in kratom leaves has been reported to account for only approximately 0.5%–1.5% of the dry weight [4], resulting in a relatively small amount of purified product compared with the mass of solvent used. Sheldon [18] also noted that fine chemical and pharmaceutical processes generally exhibit high E-factor values, typically ranging from 25 to 100 or even higher for small-scale processes, primarily because solvents are the major contributors to waste generation.

The extraction of other natural products with higher active compound contents or simpler sample matrices generally results in lower E-factor values [37]. Therefore, although the absolute E-factor values in this study appear high, they remain reasonable and acceptable, particularly because the analysis focuses on comparison between methods. In this context, ABLLE demonstrated a lower environmental impact than conventional chromatographic purification.

Overall Implications

The development of a non-chromatographic purification route for mitragynine may contribute to more sustainable natural product processing. By prioritizing ethanol- and ethyl acetate-based solvent systems, solvent-related hazards can be reduced while maintaining acceptable extraction efficiency. Furthermore, this approach avoids the use of disposable adsorbents, reduces solvent waste, and lowers energy requirements, suggesting its potential applicability for industrial-scale implementation in resource-limited settings, including Indonesia, a major kratom-producing region. Future research should focus on solvent substitution strategies, optimization of solvent recovery, and life cycle assessment (LCA) to quantitatively evaluate the environmental performance of this approach relative to conventional chromatographic methods.

4. Conclusion

This study shows that acid–base liquid–liquid extraction (ABLLE) can serve as a scalable and sustainable alternative to chromatographic purification for the isolation of mitragynine from Mitragyna speciosa leaves. Ethanol was identified as a more favorable maceration solvent, as it provided higher alkaloid selectivity than methanol. Among the partition solvents, dichloromethane (DCM) afforded the highest mitragynine purity (39.03%), whereas ethyl acetate offered a safer and more environmentally benign alternative, with slightly lower purity (27.47%) but comparable process efficiency (~19%). By reducing reliance on chromatographic purification, this method reduces solvent consumption, avoids the use of disposable adsorbents, and minimizes energy requirements [19].

Accordingly, the proposed approach aligns with several key principles of green chemistry, including waste prevention (Principle 1), the use of safer solvents and auxiliaries (Principle 5), design for energy efficiency (Principle 6), and reduction of derivatives or auxiliary materials (Principle 8). Overall, the ABLLE method provides a cost-effective and environmentally favorable approach for alkaloid enrichment, particularly for application in resource-limited laboratories, such as those in Indonesia. Future studies should focus on replacing hazardous solvents such as dichloromethane with bio-based alternatives, including ethyl lactate or 2-MeTHF, and on conducting life cycle assessment (LCA) to quantitatively evaluate environmental performance.

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