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Mitragynine: a review of its extraction, identification, and purification methods

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ABSTRACT

Mitragynine is one of the dominant alkaloids present in *Mitragyna speciosa*. The compound possesses several pharmacological properties such as antinociceptive, anti-inflammatory, and anti-cancer. Studies have reported various methods in extracting mitragynine, both conventional and renewable technology combined with acid-base techniques for the enrichment and purification of mitragynine from *M. speciosa*. Several chromatography and spectroscopy instruments such as HPLC, LC-MS, GC-MS, and NMR have been used to identify mitragynine and its content in both the extract and fraction mixtures. In this review, we aim to provide insight on how the methods of extraction, purification, and identification of mitragynine have been developed over the last few decades. This report shows comparison among the various approaches in extracting mitragynine and points out the facts that different methods gave different yields of the compound.

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1. Introduction

Mitragyna speciosa (Rubiaceae) is an ethnomedicinal herbal plant that is commonly found in Southeast Asia, including Malaysia, Thailand, Myanmar, Papua New Guinea, and Indonesia. In Indonesia, it is known as kratom, while in Thailand it is called kratom, ithang, kakuam, and thom. *M. speciosa* is also known as breed or ketum in Malaysia (Mudge and Brown, 2017; Mustafa et al., 2020; Ramanathan et al., 2015). The plant is consumed daily in the morning by farmers or laborers to improve their performance while working. They drink the decoction of the leaves in divided doses in a day. Additionally, it is also used as a recreational drink in a higher dose for its narcotic effect. These traditional uses then lead to the abuse of kratom in the west, such as United States (Ramanathan and Mccurdy, 2020).

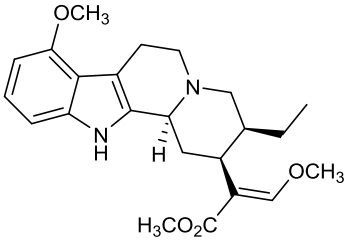
The leaves of *M. speciosa* are known to contain several interesting alkaloids (Shellard, 1989). Mitragynine is the main alkaloid contained in the leaves along with their isomers, including speciogynine, speciociliatine, and mitraciliatine. In the market, mitragynine was reported to be present in the leaves with content ranging from 3.9–62.1 mg/g (Prozialeck et al., 2020). The content of mitragynine in *M. speciosa* leaves varies depending on the geographic location and the harvest seasons (Beng et al., 2011). The mitragynine of *M. speciosa* in Thailand is reported to be 66% of the total alkaloids, whereas in Malaysia, the species contains only 12% mitragynine of the total alkaloids (Takayama, 2004). Table 1 displays the physico-chemical properties of mitragynine.

Mitragynine is the major alkaloid in *M. speciosa* (Suhaimi et al., 2016). It is known to interact with other receptors in the brain to produce stimulant and narcotic effects. A survey on drug use was conducted in Thailand and found that *M. speciosa* was used among 3.76% users aged 12-65 years (Assanangkornchai et al., 2008). *M. speciosa* is one of the plants that are prohibited and is widely abused in Thailand because this plant grows naturally even without human intervention (Singh et al., 2016). Empirically, this plant is used by wider community as a traditional medicine for various illnesses, such as diarrhea, fatigue, muscle aches, and coughs. In addition, it is also used to improve endurance, increase energy, overcome depression and as sexual stimulants. Moreover, the plant is even consumed to overcome withdrawal symptoms of opiate compounds (Eastlack et al., 2020; Raini, 2017; Saingam et al., 2013; Swogger et al., 2015; Vicknasingam et al., 2010).

Over the last few years, numerous pharmacological studies have been carried out to evaluate the biological activities of *M. speciosa* plants, especially the main alkaloid, mitragynine. A number of reports described that mitragynine exhibited analgesic activity at a dose of 30-200 mg/kg. Its antiproliferative effect was reported at concentrations >100 M against the cancer cell line K562 and was more selective against the HCT116 cell line (Goh et al., 2014). Furthermore, the antioxidant activity of mitragynine was also the highest in comparison to the other indole alkaloid compounds contained in *M. speciosa*, such as 7-hydroxymitragynine, paynantheine, and speciociliatine (Elahian et al., 2020). Moreover, a toxicological study of mitragynine

demonstrated that mitragynine was relatively safe at lower subchronic dose but exhibited toxicity at a higher dose (Sabetghadam et al., 2013). In general, the toxic dose that caused death in rats after a single dose of mitragynine was 200 mg/kg (Boto et al., 2008; Suhaimi et al., 2016).

Table 1. Physico-chemical properties of mitragynine

	Mitragynine
IUPAC Name	Methyl 2-(3-ethyl-8-methoxy-1,2,3,4,6,7,12,12*b*-octahydroindolo[2,3-a]quinolizin-2-yl)-3-methoxyprop-2-enoate
Molecular weight	398.5 g/mol
Structure	
Appearance	White amorphous powder
Melting point	104°C
Boiling point	235°C at 5 mmHg
Solubility	Soluble in alcohol, chloroform, acetic acid
Log P	1.73
UV spectra	UV max: 226, 292 nm

Source: National Center for Biotechnology Information (2021); Ramanathan et al. (2015)

2. Extraction methods

The purpose of extraction is to separate soluble metabolites from the insoluble substances using suitable solvents. The selection of the extraction method must meet the required conditions such as selectivity, efficiency, relatively low cost, and safety (Zhang, et al., 2018). During the last few years, intensive research has been carried out focusing on the extraction of compounds using conventional methods and renewable technologies including the use of green solvents (Chua, 2013). Some references on the extraction method for the mitragynine are summarized in Table 2.

Conventional extraction methods use organic solvents, such as methanol (MeOH), ethanol (EtOH), and acetone, or water at atmospheric pressure (Naviglio et al., 2019). Several conventional methods are used to extract compounds from plants, including maceration, percolation, reflux, and Soxhlet extraction. These techniques have both advantages and disadvantages. The advantages of using these methods are the low cost and simple operation. However, these methods require a long time to complete (even up to 7 d) and use a large amount of solvents that is potentially harmful to health and the environment. Moreover, it is rather difficult to completely remove the solvent residues, and the possibility of thermal decomposition (degradation of compounds) due to the high temperature used and the duration of extraction is significant (Easmin et al., 2015).

Due to the limitations of conventional methods in extracting bioactive compounds from plants, more efficient approaches become necessary, one of which the is green extraction method. The method reduces energy consumption, allows the use of a solvent or no solvent, reduces sample degradation, selectivity, and several other advantages (Duistermaat and Kolk 2000). Some examples of green extraction are pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), microwaved-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and accelerated solvent extraction (ASE) (Easmin et al., 2015).

Extraction is an important initial step in the analysis of herbal plants (Fonmboh et al., 2020). For conventional extraction, the use and selection of solvents are crucial. The choice of solvent depends on the type of plant, the part of the plant to be extracted, the nature of the bioactive compounds, and the availability of the solvents. In general, polar solvents such as water, MeOH, and EtOH are used in the extraction of polar compounds, while non-polar solvents such as n-hexane and dichloromethane (DCM) are used for the extraction of non-polar compounds (Abubakar & Haque, 2020). As for the efficiency of the extraction of mitragynine compounds from *M. speciosa*, several types of solvents have been evaluated in different studies.

Mitragynine are the main indole alkaloids that are most commonly found in the leaves of *M. speciosa* (Ya et al., 2019). Parthasarathy et al., 2013) reported that more mitragynine compounds were extracted using MeOH compared to H_2O. Generally, alkaloids are soluble in acidic water, while alkaloid salts are more soluble in aqueous solvents with a neutral pH. Furthermore, alkaloids in the free form will be likely more soluble in polar organic solvents such as chloroform ($CHCl_3$), MeOH, or dimethylsulfoxide (DMSO) (Dewick, 2009). Harizal et al., (2012) carried out the extraction of *M. speciosa* leaves using the Soxhlet method with an organic solvent (MeOH) for 4 h at a temperature of 60°C. The results showed that the MeOH extract of the *M. speciosa* leaves contained 1.6% (w/w) mitragynine. Soxhlet extraction has several advantages, such as the use of a small amount of solvent. Nonetheless, it should be noted that extraction using this technique requires a long duration of extraction (up to 48 h or even 72 h for very strongly retained analytes) and there is a possibility of compound decomposition occurring due to the high temperature used (Luque de Castro and Priego-Capote, 2012).

A good extraction method, especially for mitragynine alkaloid compounds is very important to produce compounds with high purity and yield (Mustafa et al., 2020). A number of studies have tried to obtain greater yields in the extraction of mitragynine. For instance, Mustafa et al., (2020) reported that multilevel extraction using various concentrations of non-polar to polar solvents was able to increase the yield of mitragynine by 75.0 mg/g. In addition, the use of the acid-base method to obtain extracts rich in alkaloids is also often used. Sharma et al., (2019) demonstrated that a combination of maceration method using EtOH and an acid-base process using hydrochloric acid pH 2-3 and 10% ammonium solution, as well as further extraction using dichloromethane, has resulted in about 33.59% (w/w) of mitragynine yield contained in the acid-base extract.

Although organic solvents are widely used for extracting bioactive compounds from plants, their use can cause potential problems for environmental pollution and health risks (Brglez Mojzer et al., 2016) if not handled properly. That said, as mentioned previously, the extraction process development has focused on the use of innovative technologies that allow for optimizing the extraction of bioactive compounds and improving the quality of the extracts (Ameer et al., 2017).

Extraction using innovative technologies such as ultrasound-assisted extraction (UAE) has been carried out by Kikura-Hanajiri et al., 2009) to determine the yield of mitragynine. In said study, they used 13 commercial products with labels made from *M. speciosa* leaves with six in the form of dry leaves, three in powder form, and the other three in the form of resins. They have succeeded in extracting and obtaining extracts with mitragynine content in the 13 commercial products ranging from 0.8 to 62.6 mg/g. The UAE method in solvents facilitate cavitation accelerating the dissolution and diffusion of certain substances as well as heat transfer, which can increase extraction efficiency. Another advantage of UAE is the use of less solvent and a reduction in extraction temperature and duration (Zhang et al., 2018).

Another promising approach is by using the accelerated solvent extraction (ASE) method which requires less solvent than conventional methods. This method is similar to the conventional methods which use several solvents for plant extraction, but ASE can be carried out automatically and consumes less solvent on the device (Giergielewicz-Mozajska et al., 2001). This method was applied in a study conducted by Goh et al., (2021). In their research, stainless steel ASE extraction cells were filled with 10 g of *M. speciosa* leaves. The extraction was carried out at 60°C for 5 min using different solvents, i.e., water, EtOH, ethyl acetate (EtOAc), and MeOH. The results showed that the yield of mitragynine contained in the extract ranged from 1.83 to 7.19% (w/w).

Tabel 2. Various extraction methods of mitragynine

Method of extraction	Sample	Treatment conditions	Yield	Reference
Solvent Extraction	*M. speciosa* leaves	Hot water with a solid-solvent ratio (5:8) for 2 h. Then re-maceration of hot water with the same ratio for 2 h	0.80 mg/g	Parthasarathy et al. (2013)
		MeOH with a solid-solvent ratio (1:5), macerated for 5 d and re-maceration with MeOH solvent for 5 d	4.77 mg/g	Parthasarathy et al. (2013)
		MeOH extract was purified by mixing 90% acetic acid in water (1:35 ratio) and then partitioned with water and petroleum ether. The water layer was separated and moistened with Na_2CO_3 to pH 9 and extracted with $CHCl_3$. The extract was partitioned with water to remove excess alkali and anhydrous Na_2SO_4 was added before evaporation of the solvent.	24.72 mg/g	Parthasarathy et al. (2013)
	Red Malay *M. speciosa*	*M. speciosa* leaves with varietal variants were extracted with a mixture of MeOH/H_2O (1:1 ratio) with the help of a magnetic stirrer at room temperature for 24 h. Purified by several steps using either an alkaline phase or an acid phase ending with precipitation at pH 9 (ammonium hydroxide solution). The residue and alkaline solution were rinsed with CH_2Cl_2.	5.81 mg/g	Boffa et al. (2018)
	Red Bali *M. specioasa*		9.84 mg/g	Boffa et al. (2018)
	Red Thai *M. specioasa*		8.76 mg/g	Boffa et al. (2018)
	White Borneo *M. specioasa*		12.2 mg/g	Boffa et al. (2018)
	Green Malay *M. specioasa*		9.86 mg/g	Boffa et al. (2018)
	M. speciosa leaves	Maceration with cold MeOH for 3 d. The filtrate was added with 10% acetic acid and partitioned with 2 x 50 ml petrol (bp 60-80oC). The water layer was made alkaline by adding sodium carbonate and extracted with 3 x 30 ml chloroform. The chloroform layer was then partitioned with water.	30 mg/g	Houghton et al. (1991)
	M. speciosa leaves	Extraction by Soxhlet method using MeOH solvent for 4 h at 60°C)	16 mg/g	Harizal et al. (2010)
	M. speciosa leaves	Extraction by Soxhlet method using petroleum ether solvent for 8 h at a temperature of 40-60°C	0.88 mg/g	Beng et al. (2011)
	M. speciosa leaves	Maceration using *n*-hexane, chloroform and MeOH as solvent. The residue of each solvent was extracted with a solvent with a higher polarity level.	75 mg/g	Mustafa et al. (2020)
	M. speciosa leaves	Maceration with MeOH for 5 d. MeOH extract was added 10% acetic acid and shaken for 24 h. The acid filtrate was partitioned with petroleum ether then moistened with alkaline pH 9 (25% ammonia solution) and extracted with $CHCl_3$. Then partitioned with water and before drying added anhydrous sodium sulphate.	0.087 (%w/w)	Beng et al. (2011)
	M. speciosa leaves	Maceration with 10 L of $CHCl_3$-MeOH (1:1) and 500 ml of 10% KOH solution in water for 24 h at room temperature. Then the dry extract was added with a solution of 1 M HCl and hexane (1:1). The hexane phase was removed, and the aqueous phase was added with NH_4OH until it reached pH 9. Then extracted with $CHCl_3$. And fractionation using normal-phase flash chromatography	37.5 mg/g	Flores-Bocanegra et al. (2020)
	M. speciosa leaves	Maceration with MeOH for 5 d. Then the MeOH extract was added with 5% sulfuric acid. The acid filtrate was then mixed with sodium carbonate and stirred until it became a grey-green colour with a pH of 11. Then $CHCl_3$ was added to form three layers. The $CHCl_3$ layer is removed from the mixture and anhydrous sodium sulphate is added and then dried	0.087 (%w/w)	Shamima et al. (2012)
	M. speciosa leaves	Maceration with MeOH for 7 d. MeOH extract was dissolved in 10% acetic acid. The acid phase was partitioned with petroleum ether and made alkaline by adding 25% ammonium solution to pH 9 and then extraction with chloroform. The chloroform extract was partitioned with water and before drying added anhydrous sodium sulfate.	15.6 mg/g	Chittrakarn et al. (2010)
	M. speciosa root culture	The dry hairy root cultures were refluxed with 50 ml of MeOH 3 times for 1 h. The extract was then dissolved with	14.25 mg/g	Phongprueksapattana et al. (2008)

		30 ml of 7% acetic acid in water. The acid filtrate was then partitioned with petroleum ether and the solution was added with 25% ammonium sulfate until it reached pH 9. The filtrate was partitioned with 50 ml chloroform three times.		
	M. speciosa leaves	Dried leaves of *M. speciosa* were macerated for 24 h with MeOH (2.5 L) with the help of Soxhlet. The residue is diluted with deionized water in a 1:1 ratio and concentrated	8-10 (%w/w)	Sabetghadam et al. (2010)
	M. speciosa leaves	Maceration with 95% EtOH for more than 3 d and repeated 3 times.	6.24 (%w/w)	Sharma et al. (2019)
	M. speciosa leaves	EtOH extract was dissolved with 20% MeOH and given hydrochloric acid until it reached a pH of 2-3. Partitioned with EtOAc twice. The water layer was then made alkaline with 10% ammonium until it reached a pH of 8-9. Then extracted with dichloromethane three times. Then partitioned with water. The organic layer is dried with sodium sulfate.	33.59 (%w/w)	Sharma et al. (2019)
Ultrasound assisted extraction (UAE)	*M. speciosa* (Big leaves)	Fine powder from each test sample (10-50 mg) was extracted with 10 ml 80% MeOH containing 100 ml of betamethasone valerate solution (0.2 mg/ml) by ultrasonication for 1 h. After that it was stored at room temperature overnight. Each mixture was centrifuged at 3000 rpm for five min and filtered before being injected into the instrument.	23.8 mg/g	Kikura-Hanajiri et al. (2009)
	M. speciosa (Small leaves)		1.6 mg/g	Kikura-Hanajiri et al. (2009)
	M. speciosa (Thirteen kinds of commercial products)		0.8 – 62.6 mg/g	Kikura-Hanajiri et al. (2009)
Accelerated solvent extraction (ASE)	*M. speciosa* Leaves	ASE stainless steel extraction cell was filled with 10 g of milled leaves. Extraction temperature was 60°C for 5 min and was run in two cycles. The solvent used was water, EtOH, EtOAc, and MeOH.	18.3 – 71.9 mg/g	Goh et al. (2021)

There are several factors that affect extraction results other than the use of solvents and extraction methods. These other factors could be the variety of plants used, the region where the plants grow, the temperature and duration of extraction, pH, and several other conditions. To give an example, different vein colors and the origin of *M. speciosa* gave different yields of mitragynine when extracted using the same method. The result showed that the Green Malay (Malaysia) has the highest content of mitragynine 59.7% w/w, followed by the Green Borneo (Borneo), the Red Thai (Thailand), the Red Bali (Bali), and the Red Malay (Malaysia) (Boffa et al., 2018).

Another factor that affects the yield of mitragynine extraction is the temperature. The use of high temperatures also impacts the yield of mitragynine obtained. Extraction using high-temperature methods such as Soxhlet and reflux produce much smaller yields of mitragynine compared to the maceration method (Harizal et al., 2010; Mustafa et al., 2020; Phongprueksapattana et al., 2008). Furthermore, the stability of mitragynine and the other alkaloids is highly dependent on pH and temperature. All of the Mitragyna alkaloids studied were acid labile. Under alkaline conditions, mitragynine undergoes chemical hydrolysis of the methyl ester to produce 16-carboxymitragynine (Basiliere and Kerrigan, 2020), which inarguably decreases the mitragynine yield.

In summary, mitragynine extraction using solvent extraction methods were reported to give the highest yield compared to the other methods. Extraction using UAE method gave mitragynine yield of 0.8-62.6 mg/g, while the ASE method could produce 18.3-71.9 mg/g yield. Ultimately, extraction using the solvent extraction method is an efficient extraction to obtain larger quantities of mitragynine with an easy process.

Up until now, the extraction of mitragynine compounds is still a challenge for future research because the use of simple or conventional extraction methods is still dominant. That being said, developing more effective, high-yielding methods in the extraction of mitragynine by improvising and/or utilising modern approaches may be of significance and can prove to be challenges for future research.

3. Identification and purification methods of mitragynine

Phytochemical characterization of secondary metabolites is the first step in most screening techniques for the identification of bioactive metabolites. This begins with phytochemical screening and is followed by pharmacological testing to determine which components have the desired activity (Cieśla and Moaddel, 2016). The method of identifying a compound should be simple, specific, and sensitive (Prutipanlai et al., 2017). Mass spectrophotometry (MS) and chromatography are techniques that are often used for the identification of a compound (Milman, 2005). In addition, another technique like Nuclear Magnetic Resonance (NMR) spectroscopy is used to identify and characterize the structure of organic compounds (Sharma et al., 2021).

Various methods of mitragynine analysis using liquid chromatography are presented in Table 3. Analysis of mitragynine has been carried out using High-Performance Liquid Chromatography (HPLC) instruments (Fuenffinger et al., 2017; Kikura-Hanajiri et al., 2009; Mudge and Brown 2017), Liquid Chromatography-Mass Spectrometry (LC-MS) (Philipp et al., 2009), Gas Chromatography-Mass Spectrometry (GC-MS) (Oliveira et al., 2016), and NMR (Mustafa et al., 2020). Mudge and Brown (2017) have identified mitragynine compounds using HPLC-Ultraviolet (HPLC-UV). The use of the HPLC method was found to be suitable for the detection of alkaloid compounds such as mitragynine and several other types of alkaloids. HPLC is the most widely used technique for the phytochemical characterization of extracts and is capable of analyzing various metabolites of various plant species (Cieśla and Moaddel, 2016). HPLC adjustments mainly include the use of a C18 reversed-phase column, the use of a binary solvent gradient, and different detection systems such as Diode-Array Detection (DAD) techniques, MS, or NMR (Marston and Hostettmann, 2006).

The GC-MS instrument is widely used in the analysis of volatile compounds (Feng et al., 2020), but research conducted by Oliveira et al. (2016) has succeeded in analyzing the content of the alkaloid mitragynine in 13 commercial products made from *M. speciosa*

leaves. In addition to GC-MS, other spectroscopic instruments, such as UV and infrared (IR) spectroscopy, provide considerable support in the analysis of mitragynine alkaloids. The compound mitragynine has a maximum absorption at 226 and 292 nm (National Center for Biotechnology Information, 2021) which can be identified at that wavelength. Parthasarathy et al. (2013) identified several products containing Kratom *M. speciosa* by comparing the peak of standard mitragynine to the peak of the products containing *M. speciosa* with a test spectrum range of 210-240 nm.

Table 3. Liquid chromatography analysis methods of mitragynine

Sample	Method	Solvent system	Reference
M. speciosa leaves	HPLC – UV	A: 5.0 mM ammonium bicarbonate buffer, pH 9.50; B: Acetonitrile.	Mudge and Brown (2017)
	HPLC-MS/MS	A: 0,1% formic acid in water; B: 0,1% formic acid in Acetonitrile	Fuenffinger et al. (2017)
	UPLC MS/MS	A: aqueous ammonium acetate buffer 10 mM pH 3.5; B: Acetonitrile	Sharma et al. (2019)
	HPLC	A: Water; B: Methanol. with ration (20:80)	Prutipanlai et al. (2017)
	HPLC-DAD	A: Water; B: Methanol. with ration (20:80).	Chittrakarn et al. (2012)
	UPLC	A: 0.1% aqueous formic acid in water; B: Acetonitrile	Casey et al. (2015)
	HPLC-DAD	A: 0.05% trifluoroacetic acid (TFA) in deionized ultrapure water; B: 0.05% TFA in acetonitrile	Ranggasamy et al. (2015)
	HPLC-MS/MS	A: 0,1% formic acid in water; B: Acetonitrile	Fu (2016)
	HPLC-DAD	A: 0,05% formic acid in water pH 5; B: Acetonitrile	Parthasarathy et al. (2013)
	HPLC-DAD	A: 0.1% TFA aqueous solution; 0,1% TFA in Acetonitrile	Boffa et al. (2018)
	HPLC-UV	A: 0,1% Ammonium solution; B: Acetonitrile	Kowalczuk et al. (2013)
	UFLC	A: 0.1 % TFA in MiliQ water; B: Acetonitrile	Kong et al. (2017)
	HPLC-DAD	A: 5 mM phosphate buffer pH 6.0; B: Acetonitrile	Limsuwanchote et al. (2015)

In addition to the methods discussed above, the identification of a compound can also be conducted using NMR instrument. NMR is related to the magnetic properties of certain atomic nuclei (Altemimi et al., 2017). This method is a potential method for identification, especially for organic natural compounds. Until now, this instrument is still the most powerful option to obtain detailed structural information about organic compounds, including mitragynine (Hostettmann et al., 2001; Mustafa et al., 2020).

Table 4. ^{13}C NMR data of mitragynine

Number	^{13}C (ppm)	
	Shamima et al. (2012)	Mustafa et al. (2020)
2	133.6	133.7
3	61.2	61.2
5	53.7	53.7
6	23.8	23.9
7	107.7	107.7
8	117.6	117.5
9	154.4	154.4
10	104.1	104.2
11	121.7	121.5
12	99.6	99.6
13	137.2	137.3
14	29.8	29.8
15	40.6	40.6
16	111.4	111.5
17	160.5	160.4
18	12.8	12.8
19	19.0	19.0
20	39.8	39.9
21	57.7	57.7
22	169.2	169.2
9-OMe	51.3	51.2
17-OMe	55.3	55.3
22-OMe	61.5	61.5

Table 4 and Table 5 show the NMR and MS data of mitragynine, respectively, from several different studies. Based on ^{13}C NMR data, the main characteristic of mitragynine is the presence of eight sp^2 carbon signals (C-2, C-3, C-7, C-10, C-11, C-12, C-13, and C-17) which is characteristic of indole alkaloids. In addition, there are three methoxy groups attached to C-9, C-17, and C-22 (δ_C 51.1, 55.3, and 61.9 ppm) and a methyl group at C-19 (δ_C 19.0) (Flores-Bocanegra et al., 2020).

Furthermore, the purification process of an extract is also crucial, and the level of purity will generally be considered as a factor. Therefore, to obtain bioactive compounds of high purity, additional steps are required (Rostagno and Prado, 2013). Several researchers focus on increasing the purity of the compounds obtained. Sharma et al. (2019) reported that there was an increase in the content of mitragynine alkaloids after carrying out an acid-base process in the previously obtained extract. Acid-base extraction techniques could increase the concentration of mitragynine by converting the compound (as well as other types of alkaloids) into its salt form, which is soluble in water, and then extracting the compound back into the organic layer after neutralization to produce a more concentrated alkaloids extract (Parthasarathy et al., 2013).

To this day, the acid-base technique is the technique of choice for extract enrichment, especially for alkaloid compounds. However, it should be noted that the acid-base method or technique is only one of many purification methods of alkaloids from natural resources. One of the purification techniques that are very easy to do is chromatography. Chromatography is the most widely used separation technique, in which the components in a mixture are separated according to their distribution differences between the mobile and stationary phases (Rostagno and Prado, 2013). such as thin-layer chromatography and column chromatography, which until now still exist and are used for purification of compounds (Altemimi et al., 2017).

Tabel 5. MS parameters of mitragynine

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	References
Mitragynine	399.3	174.1/159.0	Fu (2016)
	399.2	174.1/159.1	Casey et al. (2015)
	399.2	174.1/159.1	Lelono et al. (2021)

In addition to using the acid-base method, the extraction and purification of mitragynine can also be carried out by maceration using the different polarities of the solvent. Solvents that can be used such as n-hexane, chloroform, and MeOH. The residue of each solvent was extracted with a solvent with a higher polarity level (Mustafa et al., 2020). Separation of mitragynine from the crude hexane-chloroform-MeOH extract was carried out using silica gel

column chromatography. The solvent system used in column chromatography is similar to that of TLC, particularly hexane:EtOAc (3:2). Approximately 1.0 g of the crude extract was purified using column chromatography. The obtained fractions were analyzed using TLC, which showed that the 15th to 29th fractions had Rf values and spot characteristics of mitragynine. The amount of mitragynine obtained using this method is about 75 mg (Mustafa et al., 2020).

Mitragynine purification method can also be carried out using flash chromatography using petroleum ether and ethyl acetate as eluent, on a 4 g silica gel column with a flow rate of 18 ml/min and detected at UV 254 nm. The fraction containing the mitragynine was collected and the solvent was removed using rotary evaporator. The purity of the mitragynine obtained was estimated to be 94.17% which was analysed using GC-MS (Orio et al., 2012).

In summary, the best purification technique for obtaining mitragynine compounds is the acid-base method. This technique is the most commonly used in obtaining alkaloid-rich fractions, especially mitragynine. The acid-base extraction technique is quite easy to use and does not involve instruments in the purification process. In addition, the identification of mitragynine compound can be carried out by several identification methods such as HPLC, LC-MS, GC-MS and NMR instruments.

4. Conclusion

Mitragynine is a bioactive compound that can be found in Kratom (*M. speciosa*). Several extraction methods have been reviewed by comparing conventional approaches and methods that have been technologically improvised. Both methods are carried out for the initial extraction of the mitragynine compound which is then purified or purified by acid-base techniques and chromatographic techniques to obtain mitragynine compounds. Identification of mitragynine compounds can be carried out using several instruments such as HPLC, LC-MS, GC-MS or structural identification using NMR spectroscopy. Ultimately, among the approaches known for extracting mitragynine, the solvent extraction methods, particularly the ASE method, has been shown to give the highest yield. That being said, the extraction of mitragynine generally is still carried out using conventional methods. Improvising and/or utilising modern approaches may be of significance in developing more effective, high-yielding methods in the extraction of mitragynine, and can be challenges for future research.

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Conflict of interest

The authors declare there is no conflict of interest in this study.

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