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A Review on Isolation, Characterization, Biosynthesis, Synthesis, Modification, Pharmacological Activities and Toxicology of Mitragynine

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Highlights

- This paper focuses on the methods that used for isolation and characterization of mitragynine.
- This paper also focuses on the biosynthesis and synthesis process of mitragynine.
- This paper showed the pharmacological activities and toxicology of mitragynine.

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Abstract

Mitragynine is a natural compound found in the leaves of the *Mitragyna speciosa* tree, commonly known as kratom, which is primarily sourced from Southeast Asia. This review article highlights the methodologies of extraction techniques for isolating mitragynine, purification, characterization, and biosynthesis, including the complete synthesis of mitragynine and its derivatives, and briefly summarizes their biological activities and toxicology of mitragynine. The study was conducted by searching several scientific databases. There were extraction methods for mitragynine, including organic solvent extraction (hexane, chloroform, and methanol), green solvent extraction (distilled water), ultrasound-assisted extraction, and accelerated solvent extraction. The purification process of mitragynine using column chromatography with various eluen, including n-hexane, ethyl acetate, and petroleum ether. The natural mitragynine is mainly generated from the shikimate pathway and monoterpenoid secoiridoid pathway. Furthermore, there were several methods for the complete synthesis of mitragynine and the alteration of its structure. Mitragynine and its derivatives possess various pharmacological properties, including anticancer, Analgesic effects, gastrointestinal effects, antidepressant effects, Impact on cognitive function, antioxidant, and antidiabetic. The higher doses of mitragynine (100 mg/kg) in rats led to changes in hematology and the histopathological examination of the liver and brain indicates signs of toxicity.

1. INTRODUCTION

Mitragynine is the primary alkaloid discovered from *Mitragyna speciosa* (kratom, ketum). Studies related to mitragynine have concluded that mitragynine compounds and their derivatives have efficacy in managing pain. The compound mentioned is known to act as a partial opioid agonist, leading to the production of certain effects similar to morphine but with less risk of respiratory depression and addiction, and its use and distribution are severely restricted in many countries. It also acts as a stimulant, producing increased alertness and energy. In recent years, there has been a surge of interest in exploring the potential applications of mitragynine as a natural alternative to opioid painkillers. It is necessary to conduct additional research to gain a comprehensive understanding of its impacts and possible hazards; however, mitragynine holds promise as a novel therapeutic agent for a variety of medical conditions [1]. Mitragynine is an alkaloid compound with the IUPAC name Methyl 2-(3-ethyl-8-methoxy1,2,3,4,6,7,12,12b-octahydroindolo[2,3-a]quinolizin-2-yl)-3-methoxyprop-2-enoate. The compound possesses a molecular formula of $C_{23}H_{30}N_2O_4$ with a molecular weight of 398.5 g/mol. Its melting point is recorded at 104 °C, and the boiling point is 235 °C at 5 mmHg. Mitragynine possesses a logP value of 1.73 and demonstrates solubility in methanol, ethanol, chloroform, and acetic acid [2]. The compound Mitragynine (Figure 1) demonstrates limited solubility in aqueous and basic media, while being susceptible to acidic conditions. The solubility of mitragynine increased from 18.7 ± 0.4 µg/mL to 64.6 ± 1.2 µg/mL in pH 9 buffer and to 88.9 ± 1.6 µg/mL

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in pH 7 buffer. The highest concentration is observed in the pH 4 buffer, with a concentration of 3.5 ± 0.01 mg/mL [3]. *M. Speciosa* generally functions as an analgesic, antidiarrheal, antipyretic, adrenergic, euphoric, antimalarial, and antitussive compound [4, 5].

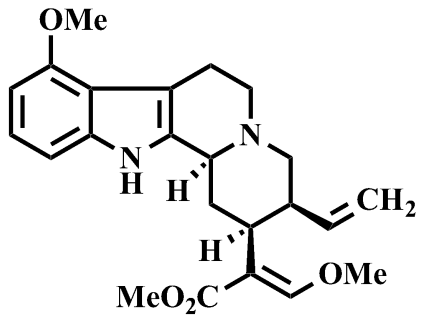

Figure 1. *Chemical structure of mitragynine*

2. ISOLATION OF MITRAGYNINE

2.1. Conventional Extraction Method of Mitragynine

Mitragynine and its derivatives have been frequently extracted from *M. speciosa*, a medicinal plant belonging to the Rubiaceae family and commonly found in Southeast Asian countries including Indonesia, Thailand, and Malaysia, where it is referred to as Kratom [6-8]. *M. speciosa* leaves contain the largest level of mitragynine, with concentrations ranging from 3.9 to 62.1 mg/g [9]. Factors that determine its mitragynine content include the plant's growing habitat, harvest season, age, and cultivation methods [10, 11]. The total amount of mitragynine-associated alkaloid fractions in *M. speciosa* from Indonesia, Malaysia, and Thailand revealed that kratom leaves coming from Indonesia had the highest levels of mitragynine, which were 53% of total alkaloids when compared to kratom leaves originating from Malaysia (33% of total alkaloids) and Thailand (31.25% of total alkaloids) [12].

The initial isolation of mitragynine in 1921 was carried out by Field [13]. In recent times, extensive research has been dedicated to extracting mitragynine through both traditional techniques and sustainable approaches that employ green chemistry [14]. Traditional extraction methods of mitragynine involve the use of organic solvents like acetone, hexane, methanol, ethanol, chloroform, and petroleum ether, employing processes such as maceration, percolation, reflux, and soxhlet extraction [2]. The conventional extraction method of mitragynine that provided high yields (30 mg/g) was using the maceration method with cold methanol for three days [15]. Furthermore, methanol was also used for the extraction of mitragynine by purification using 90% acetic acid in water to produce mitragynine of 24.72 mg/g [16]. In 2020, Mustafa et al. used a maceration method with various solvents, including n-hexane, chloroform, and methanol. The best solvent combination for mitragynine extract was n-hexane-chloroform-methanol which produced 75 mg/g of mitragynine [17]. Flores-Bocanegra et al. also employed a maceration procedure, in which a mixture of 10 L of chloroform-methanol (1:1) and 10% potassium hydroxide (KOH) solution in 500 ml of water was utilized for 24 hours at room temperature. The final mitragynine yield was 37.5 mg/g. [18]. Maceration, which involves combining organic solvents and an acid-base process, has also been utilized to extract mitragynine. Sharma et al. demonstrated in 2019 that the maceration method using ethanol and an acid-base process with hydrochloric acid (HCl) at pH 2-3 yielded a mitragynine content of 33.59% (%w/w) after fractionated with ethyl acetate. Subsequently, the pH of the water layer was modified to a range of 8-9 by adding 10% ammonium [19].

Conventional extraction methods offer several benefits, including cost-effectiveness and ease of operation. Nevertheless, these methods have drawbacks, including prolonged extraction durations and the utilization of significant quantities of solvents that may pose potential risks to both the environment and human health. Therefore, the development of green extraction methods for mitragynine becomes necessary. In 2021, Khunnawutmanotham et al. conducted isolation of mitragynine using water as a green solvent. The leaves

of *M. speciosa* were cut into small fragments and subjected to boiling in 200 mL and 400 mL of distilled water for 30 minutes each. As a result, the extract obtained contained a mitragynine content of 64.63% (w/w) [20]. Additionally, alternative environmentally friendly methods of extraction encompass ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE), and microwave-assisted extraction (MAE).

2.2. Modern Extraction Method of Mitragynine

In 2009, Kikura-Hanajiri et al. carried out mitragynine extraction using the UAE method. Mitragynine concentrations in 13 different commercial products ranged from 0.8 to 62.6 mg/g [21]. In 2013, Haris demonstrated mitragynine extraction using UAE at various temperatures and pH levels. The solvent combination of chloroform-methanol 1:4 (v/v) at pH 9.5 and 60 °C was the best condition for this method [22]. In 2012, Orio and colleagues carried out research to evaluate the efficiency of different extraction methods including UAE, MAE, and supercritical carbon dioxide, using a variety of solvents such as water, methanol, ethanol, and combinations of these, to determine the alkaloid and mitragynine content in *M. speciosa* leaves. According to LC/ESI-MS analysis, the MAE method, which used a 1:1 methanol-water mixture, produced the maximum amount of alkaloid fraction, whereas UAE provided the highest extraction of mitragynine [10]. In 2022, Karunakaran et al. used the UAE technique for the isolation of mitragynine at various times (20, 40, and 60 minutes) and solvents (water, methanol, ethanol, and ethyl acetate). The best conditions were 20 minutes in an ethanol solvent to yield 9% mitragynine [23]. In 2022, Isnaine et al. also reported that the best extraction conditions were 2 hours with methanol as the solvent and ultrasonication time for 10 minutes. This method gave a 16% higher yield of mitragynine compared to extraction without UAE [24].

The ASE method is another technique of green extraction. In 2021, Goh, et al. succeeded in extracting mitragynine using ASE. The leaves of *M. speciosa* were placed in ASE extraction cells. The extraction procedure was completed at a temperature of 60 °C for 5 minutes, utilizing a range of solvents including water, methanol, ethanol, and ethyl acetate. This approach yielded a mitragynin content ranging from 18.3 to 71.9 mg/g [25]. The methods for extracting mitragynine are outlined in Table 1.

Table 1. *The methods for extracting mitragynine from M. speciosa*

Method	Condition	Mitragynine Yield	References
Organic extracsolvention	Cold methanol was used to extract the dry leaves of *M. speciosa* for three days. Afterward, the extract was treated with 10% acetic acid and then washed twice with 50 mL of petroleum ether. Subsequently, the acid layer was subjected to treatment with sodium carbonate, followed by extraction using 30 mL of chloroform, repeated three times.	30 mg/g	[15]
	Methanol was used to extract the dry leaves of *M. speciosa* for three days at room temperature. The extract underwent treatment with a solution of 90% acetic acid in a ratio of 1:35 (w/v), followed by partitioning using petroleum ether and water. The aqueous layer was adjusted to a pH of 9 using sodium carbonate and then subjected to extraction with 3 sets of 30 mL chloroform.	24.72 mg/g	[16]
	Sequential extraction of *M. speciosa* leaves powder using hexane, chloroform, and methanol as solvent.	75 mg/g	[17]
	The process involved macerating the kratom plant at room temperature for a duration of 24 hours using a	37.5 mg/g	[18]

	mixture of 10 L of chloroform-methanol (1:1) and 500 mL of 10% aqueous KOH.		
Combining organic solvents and acid-base extraction	Maceration using ethanol 95% for 3 days. The extract was treated with 20% methanol and added HCl until pH 2 to 3. The solution was then partitioned using ethyl acetate. Subsequently, the aqueous layer was treated with 10% ammonium hydroxide until reaching a pH of 8-9.	33.59%	[19]
Green solvent extraction	*M. speciosa* leaves were chopped into small pieces and boiled for 30 minutes in 200 and 400 mL of distilled water, respectively.	64.63%	[20]
UAE	In order to obtain fine powder from 13 commercially available products, we employed ultrasonication with a solution consisting of 10 milliliters of 80% methanol and 100 milliliters of betamethasone valerate solution (0.2 mg/ml).	0.8 to 62.6 mg/g	[21]
	The kratom leaf powder was immersed in 300 mL of ethanol and subjected to ultrasonication for 20 minutes. A Branson 5510R-MT Ultrasonic extraction chamber with a 40 kHz output and 135 W was employed in the process.	9% (w/w)	[23]
	M. speciosa dry leaves were extracted with methanol at room temperature for 2 hours. The methanol extract was subsequently subjected to ultrasonication for 10 minutes.	16% (w/w)	[24]
ASE	The leaves of *M. speciosa* were placed into extraction cells specifically designed for the ASE method. The sample underwent extraction for 5 minutes at 60 °C using different solvents such as water, ethyl acetate, methanol, and ethanol.	18.3 to 71.9 mg/g	[25]

2.3. Purification of Mitragynine

Mitragynine extract is necessary for the purifying process to obtain high-purity mitragynine. Chromatography is a frequently utilized purifying process. In 2012, Orio et al. conducted a purification process where they utilized flash chromatography to purify an alkaloid extract. Purification was carried out using petroleum ether and ethyl acetate solvents on a column made from silica gel with a capacity of 4 g and a flow rate of 18 mL/minute. UV light with a wavelength of 254 nm was used for detection [12]. Another chromatography method is column chromatography. The purification of mitragynine from the raw extract involved using silica gel column chromatography using a solvent mixture consisting of ethyl acetate and n-hexane in a 2:3 ratio. Column chromatography yielded 34 mitragynine residue fractions. The Rf values and mitragynine spot characteristics in the 15th to 29th fractions closely matched the results of the prior thin-layer chromatography. Subsequently, the fractions were incorporated and evaporated using a rotary evaporator. The mitragynine obtained had a purity of 98% [17]. In 2021, Khunnawutmanotham et al. also purified the mitragynine extract using column chromatography with a silica gel extract ratio of 1:200 and an isocratic solvent of n-hexane/ethyl acetate (7:3) [20].

Purification of mitragynine by crystallization is another method for separating mitragynine from the crude extract. The crude mitragynine was dissolved in methanol, followed by the addition of 5 mL of picric acid solution that had been saturated with methanol. The mixture was subsequently cooled in a refrigerator for

20 minutes to yield crystals of mitragynine picrate with an orange hue. The crystals underwent filtration and were subsequently cleansed with methanol followed by acetone. The crystals of mitragynine picrate were dissolved in a heated, saturated acetone solution, followed by the addition of an excess of dilute ammonia. Diethyl ether was used to extract the solution at the end [6].

3. CHARACTERISTIC OF CHEMICAL IDENTIFICATION OF MITRAGYNINE

The structural characterization of mitragynine was carried out using Fourier Transform Infrared Spectroscopy (FTIR), ultraviolet (UV), mass spectrometry (MS), proton, and carbon nuclear magnetic resonance (NMR) spectrum analysis (Table 2). The application of spectroscopic techniques is essential for accurately determining the molecular structure of mitragynine. The FTIR spectrum is used to identify some important peaks of the mitragynine functional groups, including secondary amine (N-H), was found at 3359–3363 cm^{-1}, carbonyl functional group (C=O) were observed at 1662–1698 cm^{-1}, while C=C alkene and aromatic moieties appeared at 1400–1600 cm^{-1}. On the other hand, ether (C-O-C) and ester (O-CH3) groups presented at 1000–1200 cm^{-1} (Table 2) [26].

Table 2. *A summary of mitragynine elucidation structure utilizing spectroscopy methods*

Mitragynine Structure	IRFT (cm^{-1})	UV (nm)	^{1}H-NMR ppm (*J* in Hz)	^{13}C-NMR (ppm)
N-H amine	3359–3363		7.74, s	-
C-H sp^3	2900–2950		C3-H: 3.20, d (8.4) C18-H: 0.87, t (7.3)	C3: 61.3 C5: 53.7 C6 : 23.8 C14: 29.8 C15: 40.6 C18: 13.0 C19: 19.0 C20: 39.8 C21: 57.7
C=O	1662–1698		-	C22: 169.45
C=C alkene	1620–1650		C17-H: 7.43, s	C16: 11.4 C17: 160.7
C=C aromatic	1400–1600	224–292	ArH10: 6.45, d (7.7) ArH11: 7.00, t (7.9) ArH12: 6.90, d (8,1)	C2: 133.6 C7: 107.7 C8: 117.6 C9: 154.4 C10: 99.9 C11: 122.0 C12: 104.3 C13: 137.2
C-O-C ether	1100–1200		C9-OCH_3: 3.87, s C17-OCH_3: 3.73, s	C9-OCH_3: 51.3 C17-OCH_3: 55.3
O-CH_3 ester	1000–1100		C22-OCH_3: 3.71, s	C22-OCH_3: 61.5

The ultraviolet spectrum of mitragynine demonstrates the absorption characteristics of the mitragynine chromophore within the ultraviolet region, specifically between 224 and 292 nm [27]. In addition, the MS spectrum is employed to ascertain the molecular weight of mitragynine. The molecular ion fragments ([M]+) of mitragynine were identified at 399.2 [28, 29]. The NMR spectrum is a valuable tool for ensuring the precise structure of mitragynine. Mitragynine's aromatic protons typically exhibit chemical shifts in the range of 6.45 to 7 ppm, while the protons of amine and alkene are observed at 7.74 and 7.43 ppm, respectively (refer to Table 2) [18]. The ^{13}C-NMR spectrum of mitragynine can be utilized to determine the total number of carbon atoms, as evidenced by the presence of 23 carbon signals in Table 2. Aromatic carbon (C2, C7-C13) and carbonyl carbon (C=O) are the main characteristics of mitragynine structure [30].

Utilizing X-ray crystallography is a further method that is very helpful in determining the 3D structure of mitragynine [31].

4. BIOSYNTHESIS PATHWAY OF MITRAGYNINE

The natural mitragynine is mainly generated from the shikimate pathway and monoterpenoid secoiridoid pathway (Figure 2). In 2013, Charoonratana et al. studied the mitragynine biosynthesis pathway from *M. speciosa* using ^{1}H-NMR-based and HPLC-based analyses. The formation of 3-deoxy-D-arabinoheptulosonate-7-phosphate from D-erythrose 4-phosphate and phosphoenolpyruvate occurs as an intermediate step in the shikimate pathway, ultimately leading to the production of chorismate. Then, the Chorismate intermediate with the enzyme anthranilate synthase forms an anthranilate intermediate. The anthranilate intermediate was further transformed to produce tryptophan as a tryptamine intermediate [32].

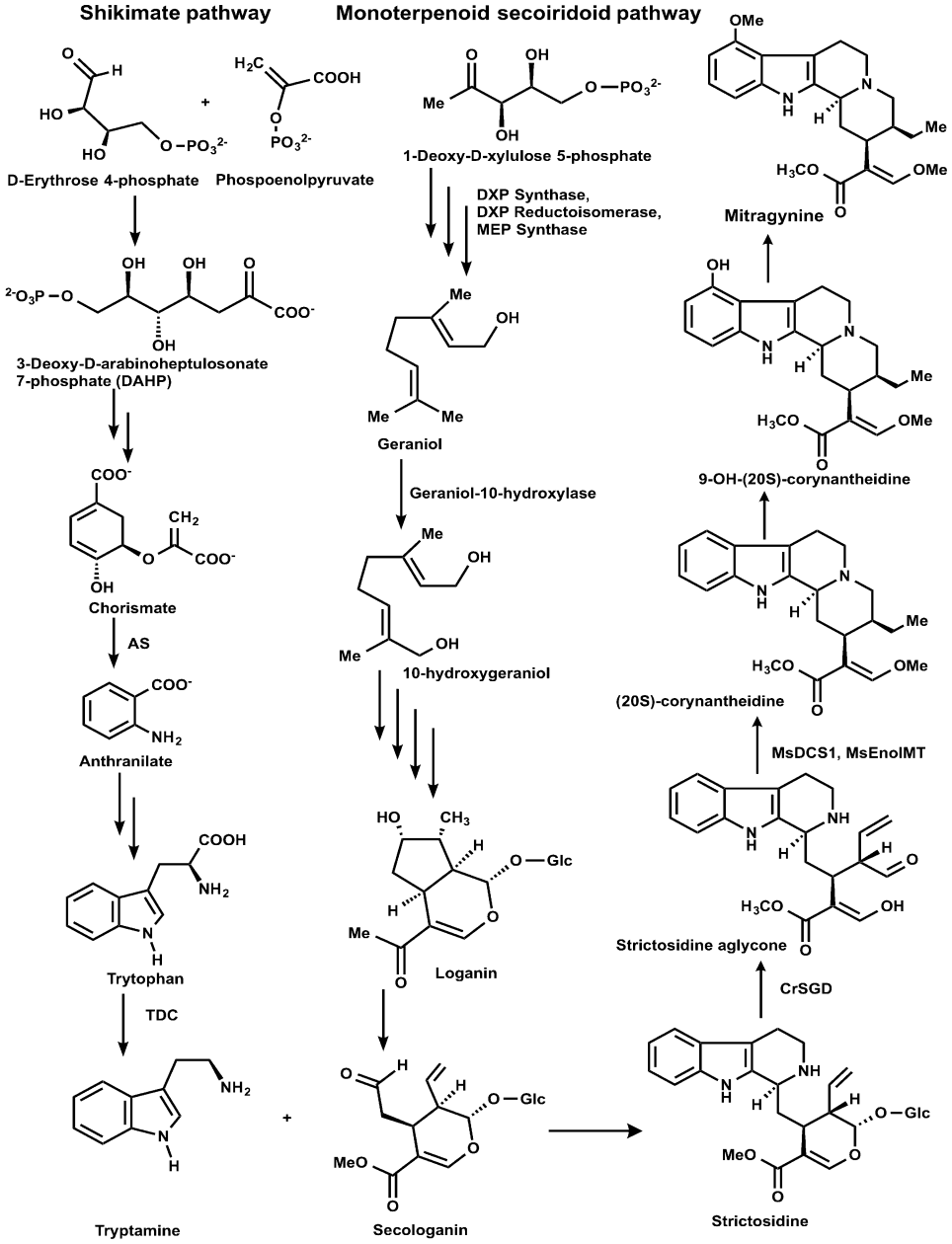

Figure 2. *A brief biosynthesis pathway of mitragynine*

Meanwhile from monoterpenoid secoiridoid pathway produced secologinin as the building blocks of mitragynine (Figure 2). It was reported that secologanin was primarily produced from geraniol. The geraniol with enzyme geraniol-10-hydroxylase form 10-hydroxygeraniol. Then, the 10-hydroxygeraniol

underwent the went ring-closing reaction with oxidation, glycosylation, esterification, hydroxylation to form the loganin as the intermediate of secologanin [33]. The enzymatic process of combining tryptamine and secologanin, known as condensation, is regulated by strictosidine synthase enzyme, resulting in the formation of a strictosidine intermediate [34]. Then strictosidine intermediate with strictosidine glucosidase form strictosidine aglycone. Strictosidine aglycone is central intermediate that can be reductively trapped into numerous isomers [35]. Scooter et al. (2022) studied the key enzyme that regulated the formation of mitragynine or speciogynine. The result showed that the enzyme *M. speciosa* dihydrocorynantheine synthase together with catharanthus roseus strictosidine synthase, catharanthus roseus strictosidine glucosidase, and MsEnolMT were key enzyme to produce mitragynine through (20S)-corynanteidine and 9-OH-(20S)-corynanteidine as intermediates [36].

5. SYNTHESIS OF MITRAGYNINE

The isolation of mitragynine is a lengthy process and results in a low yield, which is why extensive research has been conducted on synthesizing mitragynine. Takayama was the first to report the complete synthesis pathway of mitragynine in 1995 [37]. This synthesis route involved combining racemic 6-chloronicotinic acid with the enzyme lipase SAM II to obtain pure (R)-(+)-3 alcohol. Then, the mitragynine was obtained after eight additional reaction steps. Later in 2007, Cook and coworkers demonstrated another synthesis route of mitragynine via two key steps: The Pictet-Spengler reaction, executed with asymmetric methodology, and the $Ni(COD)_2$-mediated Heck-type cyclization were utilized as illustrated in Figure 3B. Synthesis was conducted using a combination of 2-iodo-3-methoxyaniline (**2**) and TMS-propargyl-substituted Schöllkopf chiral auxiliary (**3**) in the presence of a catalyst ($Pd(OAc)_2$), After undergoing a hydrolysis reaction, an intermediate is formed, which is the 4-methoxy-D-tryptophan benzyl ester (**5**). Afterward via the Pictet–Spengler reaction, the primary amine underwent a reaction with allyl bromide, followed by a reaction with an aldehyde, resulting in the formation of tetrahydro-β-carboline (**7**). The cyclization synthesis proceeded through multiple stages, with a crucial step involving the utilization of Et_3N in CH_3CN and $Ni(COD)_2$. The Corynanthe skeleton (**8**) is created by adding Et_3SiH. Furthermore, the mitragynine (**1**) was obtained after four additional reaction steps (Figure 3B) [38].

Another synthesis route of mitragynine was reported by Sun and Ma in 2011 (Figure 3A). The synthetic approach demonstrated that by mixing alkylidene malonate (**9**), n-butanal, and an O-TMS-protected diphenylprolinol catalyst, an optically active aldehyde (**10**) could be obtained through a Michael addition reaction. Reductive amination of aldehyde (**10**) with 4-methoxytryptamine (**5**) in CH_2Cl_2 gave the desired product enamine (**11**) as a single diastereomer. The enamine (**11**) was subsequently hydrogenated using PtO_2, followed by alkaline hydrolysis and decarboxylation to yield lactam **12**. The intermediate (**13**) was acquired via a sequence of four successive reaction steps, which included debenzylation, primary alcohol oxidation, Pinnick oxidation, and methyl esterification. The Bischler–Napieralski reaction occurred by heating of ester (**13**) with $POCl_3$ and followed by reduction with $NaBH(OAc)_3$ in methanol to give ester (**8**) intermediate. The ester intermediate product (**8**) of this step is the same as the intermediate products from the synthesis route described above. Furthermore, following the reaction steps that were conducted by Ma et al. in 2007, the desired product mitragynine (**1**) was formed [38, 39]. A Brief total synthesis of mitragynine is shown in Figure 3.

In 2012, the Kerschgens group proposed an alternative method for the synthesis of mitragynine. This involved the application of the enantioselective thiourea-catalyzed Pictet-Spengler reaction alongside the Pd-catalyzed Tsuji-Trost allylic alkylation. As shown in Figure 3C, the synthesis pathway commenced with the combination of 4-methoxytryptamine (**5**), nosyl chloride, and triethylamine, followed by the reaction with allyl bromide (**14**) in the presence of dimethyl sulfoxide (DMSO) and potassium carbonate to form the secondary amine (**15**). Afterward, the Pictet–Spengler reaction was conducted by mixing secondary amine (**15**) and dithioacetal in the presence of quinine-derived thiourea as a catalyst, followed by shielding atom nitrogen using Boc_2O, 4-dimethylaminopyridine (DMAP), and toluene to provide tetrahydro-β-carboline (**16**). To create α-ketoester, compound 16's dithioacetal group was hydrolyzed with AgOTf and DMSO before to the cyclization procedure. The Tsuji-Trost cyclization was accomplished by introducing a catalyst of $[Pd(allyl)Cl]_2$ in the presence of bis-1,2-diphenylphosphinoethane, diethylisopropylamine, and cesium carbonate in tetrahydrofuran (THF), resulting in the formation of the tetracyclic compound (**17**).

Furthermore, the mitragynine (**1**) was obtained after three additional reaction steps, including the Wittig reaction using methoxymethyl triphenylphosphonium chloride, deprotection of the Boc-group reaction using trifluoroacetic acid (TFA), and trifluoroacetic anhydride (TFAA) in CH_2Cl and hydrogenation with H_2, Pd/C in ethyl acetate (Figure 3C) [40].

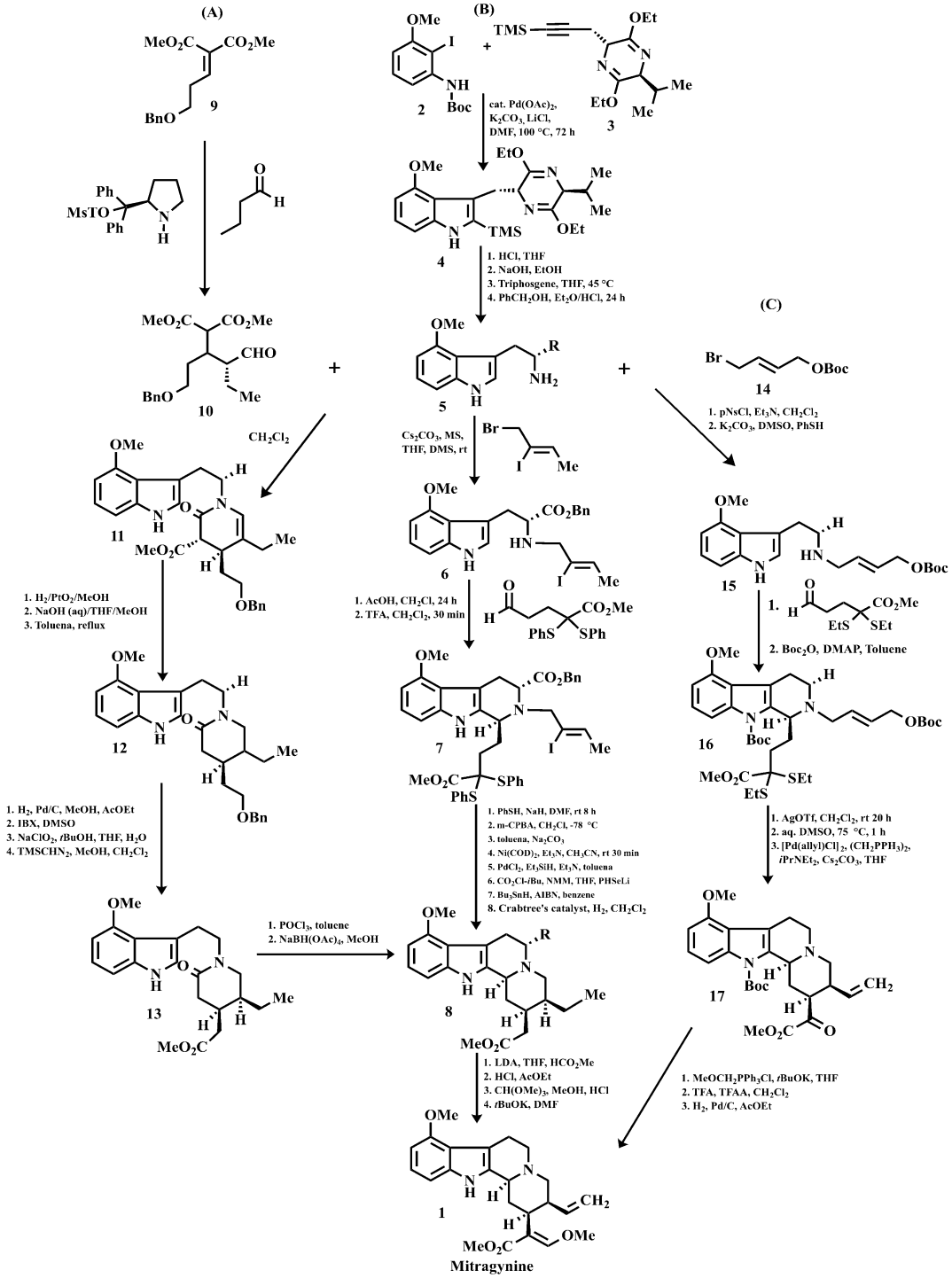

Figure 3. *Brief total synthesis of mitragynine*

6. MITRAGYNINEN DERIVATIVES WITH SUBSTITUENT MODIFICATION

Isolated or synthetic mitragynine can be utilized to create mitragynine derivatives by interconverting functional groups in the mitragynine skeleton as shown in Figure 4. The conversion functional group of

mitragynine at position 9 was reported by Takayama et al. in 2002 using EtSH, $AlCl_3$, and CH_2Cl_2. The methoxy group (-OCH_3) is converted into a hydroxy group (-OH), then the hydroxy group (-OH) is reacted with several alkyl halides (EtI, i-PrI, CH_3OCH_2Cl) and acetic anhydride to become -OEt, -OiPr, -OCH_2OCH_3 and -OAc groups [41]. Another method demonstrated that the hydroxy group was converted to a triflate group (-OTf) by using $PhNTf_2$, Et_3N, and dichloromethane. Then, the triflate intermediate was reacted with phenylboronic acid, 3-furanylboronic acid, and DABAL-Me_3 in the presence of palladium-catalyzed coupling reactions to obtain mitragynine derivatives with various functional groups at C9 position (-Ph, -CH_3, and -Furan-3-yl) [42].

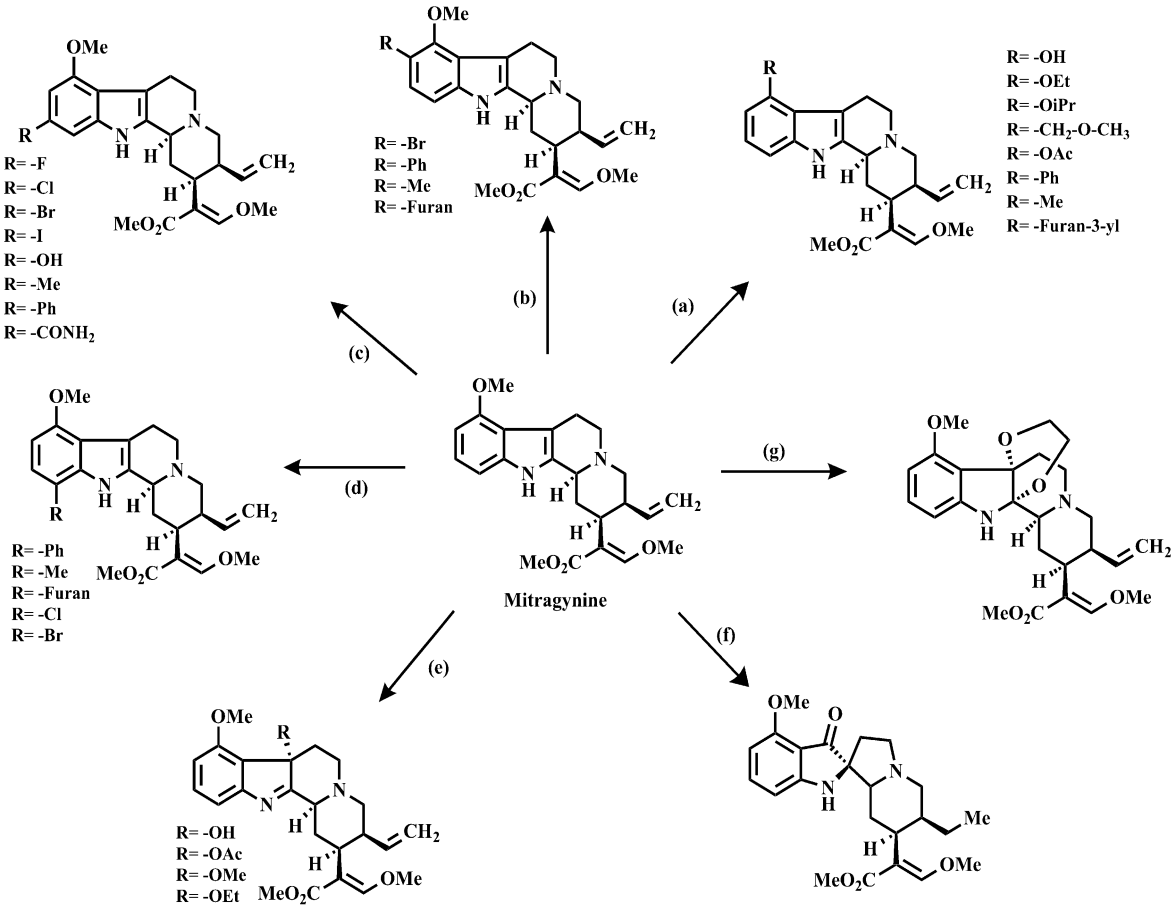

Figure 4. *The interconversion of mitragynine functional group*

In 2021, Chakraborty et al. also demonstrated the nucleophilic substitution reaction at the C10 position. (Figure 4b). Mitragynine was first converted to 10-bromomitragynine in a three-step reaction sequence. Then, 10-bromomitragynine intermediate was reacted with phenylboronic acid, 3-furanylboronic acid, and DABAL-Me_3 in the presence of palladium-catalyzed coupling reactions to form C10 mitragynine analogs [42]. In 2021, Bhowmik et al. demonstrated the successful functionalization of the mitragynine scaffold's C11 and C12 positions by conducting a borylation reaction utilizing bis(pinacolato)diboron, 4,4'-di-tert-butyl-2,2'-dipyridyl, and catalytic $[Ir(COD)OMe]_2$ as shown in Figure 4c, 4d. Before borylation at the C11 position, the mitragynine was first converted into mitragynine-ethylene glycol. In contrast, the borylation reaction at the C12 position can be directly performed. Subsequently, the borylated product underwent nucleophilic substitution reactions, resulting in the synthesis of mitragynine analogs with modifications at both the C11 and C12 positions [43].

Furthermore, the functionalization of the C7 position on the mitragynine structure was accomplished through various reactions, as depicted in Figure 4e. The mitragynine derivatives, such as 7-methoxy and 7-ethoxy were obtained by reacting mitragynine with methanol or ethanol in the presence of iodobenzene diacetate. Then, 7-acetoxymitragynine was obtained by mixing mitragynine with tetraacetate $Pb(OAc)_4$, and 7-hydroxymitragynine was formed via alkaline hydrolysis of mitragynine [44]. Another synthesis

method of 7-hydroxymitragynine was carried out by mixing mitragynine with [bis(trifluoroacetoxy)iodo] benzene (PIFA). Furthermore, the conversion of mitragynine to 7-hydroxymitragynine was successfully achieved using three methods, including with PIFA, second with potassium peroxymonosulfate, and singlet oxygen (O_2) as effective oxidizing agents [1]. The mixture of 7-hydroxymitrgynine and $Zn(OTf)_2$ in toluene, giving mitragynine pseudoindoxyl (Figure 4f). Meanwhile, the mixture of mitragynine with ethylene glycol in the presence of PIFA, and CH_3CN, giving mitragynine-ethylene glycol (Figure 4g**)** [43].

7. PHARMACOLOGICAL ACTIVITY OF MITRAGYNINE

7.1. Anticancer

Mitragynine has been intensively investigated for its anticancer activity and very promising scientific evidence has been brought up. In 2014, Goh and colleagues conducted a study on the potential anticancer effects of mitragynine against colon cancer (HCT116) and leukemia cancer (K562) cell lines. They investigated the mechanism by which mitragynine inhibits cell proliferation. Mitragynine inhibited K562 cells similarly to the positive control, Betulinic acid (IC_{50}= 24.4 μM), with an IC_{50} value of 25.2 μM. However, when compared to 5-fluorouracil, another positive control, mitragynine demonstrated less activity against HCT116 cancer cells, displaying an IC_{50} value of 42.2 μM. Nevertheless, mitragynine exhibited a notable preference for HCT116 cells, as indicated by its selectivity index value of 4.47 when compared to 5-fluorouracil [45]. In 2021, Domnic et al. reported that the anticancer effects of combining mitragynine with cisplatin were investigated in Nasopharyngeal carcinoma (NPC) cell lines (NPC/HK1 and C666-1). The findings revealed that the combination of mitragynine and cisplatin successfully inhibited the migration of both NPC cell lines. Specifically, the combination exhibited a much stronger inhibitory activity (IC_{50} = 2.3 μM) towards the NPC/HK1 cancer cell compared to cisplatin alone (IC_{50} = 9.7 μM). The mixture of mitragynine and cisplatin (IC_{50} = 6.6 μM) demonstrated improved potency against C666-1 cells compared with cisplatin alone (IC_{50} = 14.8 μM) [46].

In 2022, Karunakaran et al. examined the anticancer activity of mitragynine toward HEK-293 kidney and HeLa cervical cancer cell lines. The result showed that mitragynine was inactive (IC_{50}>100 μM) against HEK-293 and HeLa cancer cell lines [23]. Priatna et al. conducted a molecular docking study of mitragynine against breast cancer that expresses estrogen receptor alpha (ERα) and MDM2 proteins in 2022. It was found that mitragynine gave weaker binding energies (-7.90 kcal/mol) than native ligand 4-hydroxytamoxifen (-12.36 kcal/mol) against ERα protein. Similarly, the mitragynine also gave weaker binding energies (-6.57 kcal/mol) than the native ligand against MDM2 protein (-10.54 kcal/mol). The results showed that mitragynine had lower inhibitory activity than the native ligands of both proteins [47].

7.2. Analgesic Effects

Currently, there is ongoing research on the development of mitragynine and its derivatives as potential analgesics. Recent studies suggest that certain derivatives of mitragynine, such as 7-hydroxymitragynine and 7-acetoxymitragynine, exhibit potent analgesic effects with a lower risk of side effects compared to traditional opioids. Matsumoto et al. reported in 2004 that 7-hydroxymitragynine has opioid effects and induces an antinociceptive effect. The alkaloid has a strong analgesic activity effect compared to the compound mitragynine [5]. Takayama evaluated the compound mitragynine and its derivatives for pharmacological activity at the opioid receptor and found that the mitragynine compound had a lower analgesic effect than morphine. However, within *M. speciosa*, a plant known as kratom, there exists a lesser-known compound called 7-hydroxy mitragynine. This compound exhibits analgesic properties that are thirteen times more effective than morphine and forty-six times greater than mitragynine [41].

In 2005, Matsumoto et al. discovered that mitragynine reduces the constriction of the vas deferens caused by nerve stimulation, possibly by blocking neuronal Ca^{2+} channels [48]. Furthermore, Kruegel et al. demonstrated in 2019 that mitragynine is converted to 7-hydroxymitragynine, a more powerful mu-opioid receptor agonist, in rodents. and human liver preparations *in vitro*. This process is mediated by the cytochrome P450 3A isoform [1]. Obeng's research in 2021 found that the efficacy of 7-hydroxymitragynine was much stronger than mitragynine for *in vitro* efficacy for m-opioid receptors. In

the study conducted by Obeng, it was discovered that mitragynine exhibits low affinity for the human μ-opioid receptor (MOR) when tested *in vitro*. In fact, it acts as an antagonist. On the other hand, 7-hydroxymitragynine demonstrated a 9-fold higher affinity compared to mitragynine and functioned as a partial agonist of the MOR [49].

7.3. Gastrointestinal Effects

Tsuchiya et al. investigated the effects of mitragynine in 2002, which resulted in anorexia and weight loss. These effects may be related to the direct inhibition of neurons in the lateral hypothalamus [50]. Subsequently, the impact of subcutaneous administration of 7-hydroxymitragynine was examined, revealing its ability to inhibit gastrointestinal transit in rats [51].

7.4. Antidepressant Effects

In 2011, Idayu et al. reported the antidepressant properties of mitragynine, which were observed at the behavioral level. This effect may be attributed to the recovery of monoamine neurotransmitter levels, particularly noradrenaline, dopamine, and serotonin, as well as interactions with the hypothalamic-pituitary-adrenal axis, which is involved in the regulation of neuroendocrine functions. Additionally, it was demonstrated that mitragynine can reduce corticosterone levels in rats subjected to the forced tail suspension test and swim test. Elevated corticosterone levels are indicative of and a response to natural stressors, thus a decrease in corticosterone concentrations can be inferred as an antidepressant effect [52].

7.5. Impact on Cognitive Function

The study in 2010 conducted by Apryani et al. revealed divergent effects on cognitive function in rats following chronic and acute treatment of mitragynine. In the case of chronic administration (5-15 mg/kg; i.p.) over 28 days, mitragynine notably decreased locomotor activity in open field tests and impaired object recognition and working memory in rats [53]. In contrast, Hazim's findings contradicted those of Apryani. The acute oral exposure to mitragynine did not produce a significant impact on short-term memory or motor coordination in rats, as assessed through the Y-maze and rota-rod tests, respectively. However, it did result in an increase in exploratory activity within the Y-maze [54].

7.6. Antioxidant

Antioxidants stabilize free radicals and inhibit the formation of free radicals. Mitragynine from Kratom (*M. speciosa*) could be promising as an antioxidant [45]. Mitragynine demonstrates middle antioxidant value with ABTS and DPPH assays. Trolox equivalent antioxidant capacity was 1.96 ± 0.04 mmol Trolox/mmol and the IC_{50} was 2.28 ± 0.02 mg/mL. This antioxidant activity was relatively lower than Quercetin (4.86 ± 0.02 mmol Trolox/mmol) and BHT (3.64 ± 0.03 mmol Trolox/mmol). Likewise, the radical scavenging activity of mitragynine is lower than that of quercetin and BHT. The radical scavenging value of quercetin is 0.02 ± 0.00 mg/mL and BHT is 0.28 ± 0.00 mg/mL. The antioxidant capacity of DPPH from the methanol extract of the leaves was higher than the methanol extract of *M. speciosa* stems [55]. This is consistent with the total phenol content, where the leaves have a higher total phenol level than the stems. Ethanolic extract of kratom leaves can reduce DPPH free radicals with an IC_{50} value of 91.86 ppm, lower than vitamin C with an IC_{50} value of 17.45 ppm. The evaluation of antioxidant properties revealed that the extract of ethanol derived from Kratom exhibited a notable IC_{50} of 38.56 μg/ml. These findings clearly indicate that the extract of ethanol obtained from Kratom leaves possesses remarkably strong antioxidant properties [56].

7.7. Antidiabetic

Mitragynine was compound from Kratom has a lower IC_{50} (81.86 ± 1.70 μg/mL.) than acarbose as a positive control. The quantitative analysis of mitragynine in Kratom leaf extracts showed mitragynine contents were in line with the activity of α-glucosidase inhibition. Mytraginine appeared to be the major compound that a found in three of the solvent extracts. The ethanol extract has the highest amount of Mytraginin (58.75 ± 0.21 mg/g extract), followed by methanol extract (35.87 ± 1.01 mg/g extract) and aqueous extract (3.85 ±

0.17 mg/g extract). In addition, the levels of quercetin and rutin in the extract also affect the activity of α-glucosidase inhibition [57].

The research was employed by Niyomdecha et al. in 2022 regarding the potential antidiabetic activity of extracts with various solvents, including hexane, methyl acetate, ethyl acetate, dichloromethane, methanol, and ethanol. The fraction containing mitragynine at a dosage of 5 mg/mL could inhibit 1.54 ± 4.50%, while the fraction that did not contain mitragynine did not show inhibitory activity. This investigation revealed that the ethyl acetate extract had the highest activity, comparable to acarbose [58]. Besides that, *M. speciosa* crude extract is harmful to the kidneys and liver, so treating diabetes with *M. speciosa* requires further research.

7.8. Toxicological Profile of Mitragynine

Mitragynine was typically administered in concentrations ranging from 1 mg/kg to 2000 mg/kg to induce stimulant reactions and counteract restlessness. In the past few years, numerous studies have investigated the harmful effects of Mitragynine [59-61]. Mitragynine's toxicity in animal models was reported to be relatively low. In 1972, Macko et al. reported no toxicity, including tremors and convulsions, at doses up to 920 mg/kg in dogs [62]. In 2007, Janchawee et al. investigated toxic doses of mitragynine in Wistar rat males weighing 220 to 290 g. A single oral dose of 200 mg mitragynine per kg body weight resulted in death in rats. In the meantime, at a concentration of 40 mg/kg, mitragynine exhibited no adverse effects throughout oral administration [63]. Subsequently, the plant extract and mitragynine exhibited cytotoxicity towards human nerve cells, but no genotoxicity in mouse lymphoma gene mutation assay. Furthermore, no mutagenic effects were seen in testing using the Ames test [64].

In 2013, Sabetghadam et al. conducted subchronic exposure experiments of mitragynine at concentrations of 1, 10, and 100 mg/kg on female and male Sprague-Dawley rats 5 weeks old for 28 days. Mitragynine given at level of 100 mg/kg resulted in brain abnormalities including necrosis, local vacuoles, and neuronal degeneration. Mitragynine treatment at level of 10 and 100 mg/kg resulted in hypertrophy of liver cells, sinusoidal dilation, and hepatocyte hemorrhage. Additionally, giving rats 100 mg/kg of mitragynine for 28 days caused hepatotoxicity, as evidenced by an increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In addition, white and red levels of blood revealed a significant decrease. This corresponds to mitragynine's toxicity in toxicological and hematological systems. Female rats exhibited glomerular capsule swelling and an increase of red blood cells in the lumen, indicating early stage renal toxicity. In male rats' kidneys, there was no indication of toxic effects, including foreign body deposition, inflammatory cell infiltration, and necrosis [65].

Another study carried out by Sabetghadem et al. with male Swiss albino rats weighing 25-30 g that had free access to water, and food, and had been acclimatized for 1 week in a holding room before conducting the experiment, showed that alkaloid extract (20–22% of mitragynine content) at dose of 1300 and 2000 mg/kg mitragynine caused death in treated rats in less than 1 hour. Mortality was preceded by perioral tremor, restlessness, and generalized convulsions. Meanwhile, a concentration of mitragynine and alkaloid extract of 175 mg/kg caused the survival of animals with no toxic effects (short-term observations). Further, there is no delayed behavioral toxicity (long-term effect), such as reduced feeding habits or weight loss. [66]. In mice, the LD_{50} of mitragynine is 477 and 591 mg/kg for the kratom alkaloid fractions [67].

The effects of mitragynine on a hERG (human Ether-a-go-go-Related Gene) current channel protein and mRNA expression in hERG-transfected Xenopus oocytes and HEK293 were utilized to investigate the potential risk of cardiotoxicity. The hERG potassium pathways regulate the rapid component of delayed rectifier potassium current (IKr), which is required for the action potential repolarization. Medicines that block hERG may increase the cardiac action potential and QT interval, causing ventricular arrhythmias [68]. Mitragynine inhibited hERG-transfected HEK293 cells' current with an IC_{50} value of 1.62 μM. Mitragynine inhibited IKr expression in oocytes at an IC_{50} of 1.03 μM, demonstrating hERG channel blockage to some extent. Furthermore, mitragynine was also discovered to had an IC_{50} concentration of 3.32 μM for inhibiting muscarinic-gated atrial potassium currents. The findings show that the G protein

inward rectifier channel (GIRK) is significantly inhibited by mitragynine. Blocking both hERG and GIRK channels may increase the risk of cardiotoxicity [69].

Toxicologists have struggled to understand the role of mitragynine in a medicolegal assessment due to the absence of clinical investigations with associated data monitoring. However, there were 1001 mitragynine blood incidents with quantitative results from forty-six different states of the United States and three Canadian provinces. Blood mitragynine concentrations at 800 and 980 ng/mL are considered high and within the limits of fatalities. Furthermore, concentrations of mitragynine greater than 1,000 ng/mL tend to be associated with deaths and may be more causative [70].

8. CONCLUSION

Mitragynine, with its alkaloid structure features isolated from *M. speciose* (kratom). The extraction methods of mitragynine from the dried leaves of *M. speciosa* that produced high yield were organic solvent extraction (hexane, chloroform, and methanol) and green solvent extraction using 200 and 400 mL of distilled water. The highest purity of mitragynine was obtained with silica gel column chromatography using a solvent mixture consisting of ethyl acetate and n-hexane in a 2:3 ratio. The natural mitragynine is mainly generated from the shikimate pathway and monoterpenoid secoiridoid pathway. Furthermore, several methods have been employed to completely synthesize mitragynine and modify its structure. Mitragynine and its derivatives possess various pharmacological properties, including anticancer against HCT116, K562, and NPC (combined with cisplatin) cancer cells. Then, analgesic effects, gastrointestinal effects, antidepressant effects, Impact on cognitive function (5-15 mg/kg), antioxidant, and antidiabetic. However, the higher doses of mitragynine (100 mg/kg) in rats led to changes in hematology and histopathology of the liver and brain, indicating toxicity. Further, based on 1001 mitragynine blood incidents with quantitative results from forty-six different states of the United States and three Canadian provinces. Blood mitragynine concentrations at 800 and 980 ng/mL are considered high and within the limits of fatalities. Furthermore, concentrations of mitragynine greater than 1,000 ng/mL tend to be associated with deaths.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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