SYSTEMATIC REVIEW

Isolation of Phytochemical and Pharmacological Bioactive Compounds From *Mitragyna speciosa* (Korth.): A Scoping Review

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ABSTRACT

Introduction: This scoping review aimed to provide a comprehensive summary and evaluation of solvents and methods for the extraction of bioactive compounds with pharmacological properties from *Mitragyna speciosa* (*M. speciosa*) Korth. **Methods:** The relevant articles were screened on electronic databases such as Scopus, PubMed, and Science Direct and verified their qualities based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guideline. **Results:** We selected 41 articles according to two features; the extraction of bioactive compounds and pharmacological properties of *M. speciosa* extract that involved different solvents and methods. Evidence shows that methanol was the commonly used solvent along with the maceration process in the extraction of *M. speciosa* to obtain valuable bioactive compounds with clinical benefits. Alternatively, Soxhlet provides less exertion to the extraction process with similar value. **Conclusion:** Despite various potential modern techniques and solvents available, the synergy between traditional maceration and Soxhlet and methanol was found to potentially attain pharmacological values and bioactive substances in *M. speciosa*.
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INTRODUCTION

Herbal plants have attracted overwhelming attention among the medicinal research community due to their extensive source of therapeutic phytochemicals (1). *Mitragyna speciosa* (*M. speciosa*) is a herbal plant from the coffee (Rubiaceae) family. It is broadly cultivated across Southeast Asia, particularly in Indonesia, Malaysia, and Thailand, for traditional medicinal and recreational purposes. The plant and its various products and extracts are collectively known by the local inhabitants as biak-biak, ketum, or Maeng Da (2). The popularity of *M. speciosa* as an alternative remedy is no longer confined to Southeast Asian countries, mainly Malaysia, Indonesia, and Thailand, but it has now extended over to European countries and the United States (3,4). Traditionally, the leaves are consumed in the form of juice, tea, capsules, and smoked or chewed to relieve pain, opiate withdrawal, and combat tiredness (5). Putative pharmacological effects attributed to this plant such as antioxidant, antibacterial, anti-diabetic, antiproliferative, anti-inflammatory, and other biological activities (6,7).

The pharmacological properties of *M. speciosa* could be ascribed to the presence of secondary bioactive metabolites, such as flavonoids, alkaloids, saponins, triterpenoids, and glycosides (6,7). Isolation of the secondary metabolites from herbal plants is primarily influenced by the technique and the type of solvent used during the extraction process to retain the quality of the compounds (8,9). Natural phenols or bioactive compounds may have different polarity and characteristic groups which forces the need for a suitable solvent for extraction as different polarity of solvents could affect both biological activity and extraction yield (8,10). Solvents that are primarily used in the extraction process of bioactive compounds as well as their advantages and disadvantages are tabulated in Table I. Generally, non-polar solvents such as hexane and dichloromethane are employed in the extraction of non-polar substances such as fatty acids and steroids. On the other hand, polar solvents including water, methanol, and ethanol are mainly used for polar compound extraction such as phenols, saponins, and glycosides (11-13). On top of

that, other criteria such as low toxicity, boiling point, induction of quick physiologic absorption of the extract, ease of evaporation at low heat, preservation action, and inability to promote extract dissociation or complex should also be considered in determining the most appropriate solvent in plant extractions (14-18).

The conventional approaches include maceration and Soxhlet extraction methods using various solvents, such as water, acetone, methanol, ethanol, or solvent mixtures containing water have been commonly employed to extract bioactive compounds from plants (1,8). The maceration process involves the incubation of coarsely powdered plant material in solvent for at least three days with intermittent stirring to aid in extraction. The extracted material is isolated by separating the liquid from the solid residue by filtration or decantation (11). On the other hand, Soxhlet extraction, which is also known as continuous hot extraction, utilizes a Soxhlet extraction apparatus made of glass, consisting of a round bottom flask, extraction chamber, siphon tube, and condenser. During the extraction process, the finely powdered plant material is placed in a porous bag (thimble). The solvent in the flask and thimble is then placed in the extraction chamber. The heated solvent evaporates, condenses in the condenser, and trickles down to the extraction chamber to extract the material. The process is repeated until the compound is completely extracted and no excess solvent residue in the chamber (11).

Recently, there has been a growing inclination towards advancing and refining contemporary extraction techniques like Ultrasound-assisted Extraction (UAE) that uses high-frequency sound energy (>20 KHz) to disrupt plant cell walls, enhancing the drug's surface area for better solvent penetration and the release of secondary metabolites. In addition, Supercritical carbon dioxide (CO_2) extraction is another modern method utilizing CO_2 in its supercritical state as a solvent to extract compounds from various materials. Under specific pressure and temperature, the CO_2 transforms into a supercritical fluid, offering high solvating power without leaving residues, widely used in extracting oils, flavors, fragrances, and bioactive compounds from natural sources due to its non-toxic, non-flammable, and environmentally friendly nature. The process involves passing CO_2 through the material, dissolving the desired compounds, and then separating them by changing pressure or temperature (18,19). Moreover, Microwave-assisted Extraction (MAE) involves dipole rotation and ionic transfer, displacing charged ions in the solvent and drug material. It's particularly effective for extracting flavonoids (10,11). However, while these modern methods offer certain advantages, they also come with several drawbacks that are listed in Table II. Therefore, applying an appropriate method and solvent is crucial to extract the targeted bioactive compounds accurately (9).

Realizing the impact of solvents and methods in the extraction of plant-derived bioactive compounds, this comprehensive scoping review aimed (i) to provide useful information in selecting the appropriate solvent and method for the extraction of bioactive compounds from *M. speciosa*; and (ii) to analyze the variation and correlation between the type of solvent and extraction method with the pharmacological finding and desirable clinical benefits.

MATERIALS AND METHODS

Screening of literature

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guideline was used to conduct this scoping review (20). A literature search was developed in electronic databases such as Scopus, PubMed, and Science Direct until March 2023. The screening was focused on the studies that are relevant to the solvent and extraction method of bioactive compounds from *M. speciosa* and those with exhibited pharmacological properties. The literature search was limited to peer-reviewed studies published in English and the keywords are restricted to "*Mitragyna speciosa*, *Mitragyna speciosa* AND extraction method, *Mitragyna speciosa* AND solvent, Kratom, *Mitragyna speciosa* AND crude extract".

Criteria for Eligibility and Study Selection

The study criteria include *in vitro* and *in vivo* investigations on the extraction of bioactive compounds and analysis of pharmacological properties of *M. speciosa* utilizing various solvents and methods. Furthermore, other non-relevant investigations including (i) studies that do not involve extraction of *M. speciosa*; (ii) case reports and review articles; and (iii) studies that do not include bioactive compounds and pharmacological findings from *M. speciosa* were excluded from the search.

The screening and selection process of the articles was conducted by three independent reviewers (ASKK, NFZ, and MH). Figure I demonstrates the selection procedure which involved article identification through screening, eligibility, and inclusion criteria. Articles that failed to meet the selection criteria were excluded from the study. The bibliographies of pertinent publications were also scrutinized to find possible papers that were missed during the database search.

RESULTS

Typical Characteristics of the Study

The scoping review consisted of two primary sections. The first section examined the impact of various solvents and extraction techniques on the bioactive compounds in *M. speciosa*. The second section is focused on the effect of different solvents and

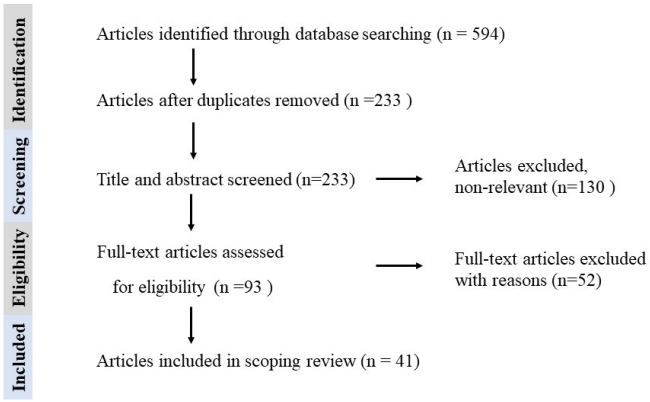

Figure 1 : PRISMA-ScR flow diagram of identification and selection process of inclusion and exclusion criteria of the study.

extraction methods on the pharmacological properties of *M. speciosa*. Based on the electronic databases, the literature search yielded 594 potential articles. As a result of removing the redundant articles, 233 related articles remained. Subsequently, 130 articles were excluded throughout the screening for title and abstract, followed by the exclusion of another 52 articles that did not meet the other criteria of the study. Finally, 41 articles that complied with the requirement of inclusion criteria were retained for this scoping review. Out of the 41 articles screened, 14 articles were focused on the identification of bioactive compounds in the *M. speciosa* extract (6,7,29–32,21–28), while 27 articles were focused on the pharmacological properties (3,6,35–44,7,45,21,23,24,26,28,33,34).

Analysis of Solvent and Methods Used for *M. speciosa*-derived Bioactive Compound Extraction

In the present study, out of 14 research included in this review that reported bioactive compounds found in *M. speciosa*, 11 employed methanol as a solvent in the extraction process while the rest used other solvents including ethanol, water, and mix-solvent. Among 11 methanol users, 7 studies used methanol mainly in the study (7,21,25,26,28,29,32), whereas the other 4 research groups used methanol in comparison with

Table I : Solvents used in plant bioactive compound extraction

Solvent	Chemical formula	Polarity	Boiling point (°C)	Advantages	Disadvantages	Reference
n-Hexane	C_6H_{14}	0.009	68.7	Easy retrieval, nonpolar properties, low latent heat of vaporization (330 kJ/kg), and high selectivity towards solutes.	Air pollution, toxicity, and harmfulness	16,15
Ethyl acetate	$C_4H_8O_2$	0.228	77.1	Low boiling point, low toxicity, relatively polar, highly selective	Inability to dissolve fats, gums, and wax, flammable and volatile.	11
Chloroform	$CHCl_3$	0.259	61.2	Nonpolar, colorless, possesses a pleasant aroma, and exhibits solubility in alcohols. Furthermore, it demonstrates efficient absorption and metabolism within the body.	Sedative effects and the presence of carcinogenic properties	11, 14
Dimethyl sulfoxide (DMSO)	C_2H_6OS	0.444	189	Polar, Less toxicity, easy method compared with acetone for chlorophyll (Chl) extraction	Unstable, high boiling point, the extraction efficiency is affected by temperature and duration of incubation	18
Acetone	C_3H_6O	0.355	58.08	Suitable for extracting antimicrobial compounds, such as saponins and phenols, able to dissolve many hydrophilic compounds, and low in toxicity	Flammable and volatile	14,17
Methanol	CH_3OH	0.762	64.7	High polarity and requires a low temperature to concentrate the extraction	Volatile, toxic at high concentrations, and flammable	10–13
Ethanol	C_2H_5OH	0.654	78.4	Safe for human consumption and polar solvent	Higher boiling point compared to methanol, flammable, and volatile	11,49
Water	H_2O	1.000	100	Highest polarity, ability to dissolve substances, safe, inexpensive, and non-flammable	Cause hydrolysis, easily contaminated by bacterial growth, and requires high temperature to concentrate the extract	11

Table II : Advantages and disadvantages of plant bioactive compound extraction methods

	Method	Advantages	Disadvantages	Reference
Conventional	Maceration	Suitable for heat-sensitive plants	The plant is exposed longer to the solvent and the process uses a large volume of solvent	8,11
	Decoction	Use water as a solvent, which shortens the extraction time	Not suitable for temperature-sensitive and volatile compounds	11,14,47
	Soxhlet	Utilize a smaller quantity of solvent and no filtration is required	Require high-purity solvents with higher cost, non-environmentally friendly, not suitable for temperature-sensitive compounds, and high possibility of thermal decomposition	1,10,47
	Sonication	Enhance cell walls' permeability and induce cavitation to ease dissolving	High cost and ultrasound energy's harmful impact on the vital components of medicinal plants occurs by generating free radicals, leading to undesired alterations in the drug molecules.	14
Modern	MAE	Reduce the extraction time, use less solvent, and achieve high purity of the crude extract	Restricted to phenols with small molecular weight, compounds easily oxidize with additional extraction cycles, and are not suitable for high-temperature sensitive compounds	1,48,50
	UAE	Increase the penetration of the solvent to the surface of the drug, shorter extraction time, less solvent consumption, and high extract yield	Hardly reproducible, high cost, involve a high amount of energy, phytochemical may be degraded by free radicals	1,11,14
	SFE	Use CO_2, which is low-cost, low toxicity, and vaporizes at room temperature, making it suitable for non-polar vehicles	High cost of equipment and polar compounds can only be extracted by polar solvents	1

MAE: Microwave-assisted Extraction, UAE: Ultrasound-assisted Extraction, SFE: Supercritical Fluid Extraction

other solvents such as ethanol and water (6,22,23,30). Furthermore, in the comparison of solvent studies, it was found that methanolic extract produced the highest concentration of mitragynine (15.7%) and 7-Hydroxymitragynine (5.3%), while the methanolic extract using the meseration method had the highest total phenolic content (TPC) 105.58 ± 15.43 mg GAE/g and total flavonoid content (TFC) 91.12 ± 17.27 mg CE/g (7,22,23,30).

The bioactive compounds isolated from *M. speciosa* by different solvents and extraction methods are listed in Table III. Methanol was found to be the most common solvent used in the extraction of secondary metabolites from *M. speciosa*. Out of 11 studies that used methanol, two studies practiced Soxhlet (7,25), three studies with maceration alone (26,28,32), one study with combination of maceration and sonication (21), one study with sonication alone (29), and three studies with modern methods such as UAE, MAE, Supercritical Fluid Extraction (SFE) and/or Accelerated Solvent Extraction (ASE) (6,22,30).

Pharmacological Properties of *M. speciosa* in Different Solvent and Extraction Methods

Based on our pharmacological findings on *M. speciosa* extract, among the 27 studies analyzed, methanol was the most frequently used solvent in 21 studies, followed by ethanol in 4 studies, and water in two studies for the extraction of *M. speciosa*. In terms of extraction methods, traditional maceration was the most commonly employed technique, followed by Soxhlet and other modern methods. Specifically, a combination of maceration with methanol was predominant in 14 studies, compared to ethanol (three studies) in the pharmacological investigation of *M. speciosa*. On the other hand, the Soxhlet method was used along with methanol in three studies. Table IV elaborates on the pharmacological properties of *M. speciosa* extract using different solvents and extraction methods.

DISCUSSION

Due to the variety of bioactive compounds found in *M. speciosa*, the selection of the most appropriate

Table III : Solvent and method in the extraction of bioactive compounds from *M. Speciosa*

Solvent	Extraction Method	Bioactive Compound	Reference
Methanol	Soxhlet	Flavanoid (Rutin, Epicatechin, Quercetin, and Procyanidin B2), Polyphenol (Chlorogenic acid),	7
		Ajmalicine, Corynantheidine, Isomitraphylline, Mitraphylline, Paynantheine, Isocorynantheidine, 7-hydroxymitragynine, Mitragynine, flavonoid (epicatechin), saponin (daucosterol), triterpenoid saponins (quinovic acid 3-O-β-D-quinovopyranoside, quinovic acid 3-O-β-D-glucopyranoside), glycoside derivatives (1-O-feruloyl-β-D-glucopyranoside, benzyl-β-D-glucopyranoside, 3-oxo-α-ionyl-O-β-D-glucopyranoside), roseoside, vogeloside, epivogeloside.	25
	Cold maceration	Alkaloids and flavonoids (high), Saponins (moderate), Tannins and sterols (low)	26
		Caulerpin, Yohimbine, Isospeciofoline, Isorotundifoline, Corynoxine, Corynoxine B, 7-hydroxymitragynine, 7β-hydroxy-7H-mitraciliatine, Paynantheine, 3-Isopaynantheine, Mitragynine, Speciogynine, Speciociliatine, Mitragynaline, Corynantheidaline.	32
	Maceration	Alkaloids, Flavonoids, Saponins, Tannins, Phenol, Steroid/terpenoids.	28
	Maceration and Sonication	Mitragynine	21
	Sonication	7-Hydroxymitragynine, Isospeciofoline, Isospeciofoleine, Isorotundifoline, Corynoxine B, Corynoxine, 7β-Hydroxy-7H-Mitraciliatine, Paynantheine, Mitragynine, Speciogynine, 3-Isopaynantheine, Speciociliatine.	29
Methanol, Ethanol, or Water	UAE, MAE, or SFE-CO_2	Alkaloid (high fraction in MAE), Mitragynine (high yield in UAE)	22
Methanol, or Water	Maceration	High TPC and TFC	23
	Boiling	Low TPC and TFC	
Ethanol	Maceration	Alkaloids, Flavonoids, Triterpenoids / Steroids, Saponin, and Tannin	24
Methanol, Ethanol, or Water	MAE	High Mitragynine (in White Borneo and Bali strain) in methanol,	30
		High 7- hydroxymitragynine (in White Borneo and Red Maeng Da) in methanol	
Methanol, Ethanol, Water, or Ethyl acetate	ASE	Mitragynine (high content in methanol), TPC (high content in ethyl acetate), and TFC (high in ethanol)	6
Mixed solvent ($CHCl_3$–CH_3OH, HCl, and/or aqueous KOH)	Maceration	Mitragynine, Speciociliatine, Speciogynine, Mitraciliatine, Paynantheine, diastereoisomers isopaynantheine, epiallo-isopaynantheine, N(4)-oxides mitragynine-N(4)-oxide, Speciociliatine-N(4)-oxide, Isopaynantheine-N(4)-oxide, Epiallo-isopaynantheine-N(4)-oxide, 9-hydroxylated oxindole, Speciofoline	27
		Isorotundifoleine, Isospeciofoleine, 9-unsubstituted oxindoles corynoxine A, corynoxine B, 3-epirhynchophylline, 3-epicorynoxine B, and Corynoxeine	
Mixed solvent (MeOH, H_2O, and /or HCl)	Maceration with magnetic stirring	Mitragynine, 7- hydroxymitragynine, speciociliatine, Speciogynine, Paynantheine,	27

MAE: Microwave-assisted extraction, ASE: Accelerated Solvent Extraction, UAE: Ultrasound-Assisted Extraction, SFE: Supercritical Fluid Extraction, CO_2: Carbon Dioxide, TPC: Total Phenolic Content, TFC: Total Flavonoid Content

Table IV : Pharmacological findings of M. Speciosa extracted with different solvents and methods

Solvent	Extraction method	Pharmacological findings	Reference
Methanol	Maceration	Antinociceptive effect by extending the nociceptive response latency in a mice hot plate test.	33
		Anti-diarrheal effect investigated in castor oil-induced diarrhea in rats.	34
		Decrease muscle twitch and promote skeletal muscle relaxation.	35
		Alleviate the severity of ethanol withdrawal without causing any side effects related to Rapid Eye Movement (REM) sleep in rat sleep profile.	36
		Gastroprotective effect by reducing the ulcer index in alcohol and acetylsalicylic acid-induced ulcer models, although it showed no protective impact in the reflux esophagitis in mice model.	37
		Anti-depressant effects in the animal behavioral model of depression by reducing the immobility time via the interaction with neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis systems	38
		Antibacterial effect against *Aeromonas hydrophilla, Salmonella typhi*, *Bacillus subtilis, Streptococcus, Pneumoniae* and *Escherichia coli.*	23,28,51
		Reduce ethanol-seeking behavior in mice by reducing dopamine levels in the brain's nucleus accumbens (NAc) region caused by alcohol consumption	3
		Antipsychotic-like effect by inhibiting ethanol-seeking behavior in rats	21
		Strong antioxidant activity compared to aqueous and alkaloid extracts is attributed to their high phenolic and flavonoid contents.	23
		Anti-cancer effect against nasopharyngeal carcinoma.	39
		Alkaloid extracts significantly attenuate morphine withdrawal symptoms	40
	Soxhlet	Antioxidant scavenging activities investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assays and an anti-diabetic effect by inhibiting alpha-amylase activity.	7
		Analgesic activity by targeting opioid receptors at the higher dose.	41
		Improvement in learning but not long-term memory consolidation in a rat model of learning and memory.	42
	Cold maceration	Antipyretic activity in mice pyretic model.	43
		Antinociceptive and anti-inflammatory properties were investigated by inhibiting formalin response and suppressing carrageenan-induced rat paw edema in the mice model.	26
	SFE	Potential anti-inflammatory by reducing excessive Nitric Oxide (NO) production.	44
	ASE	Analgesic and antinociceptive effect in mice model tested by hot plate and tail-flick tests.	6
Water	Boiling	Antioxidant scavenging activities were investigated by DPPH assay, but the activity is weaker compared to methanolic extract.	23
		Enhancing insulin-stimulated glucose transport in muscle cells by increasing activities of key enzymes, and increasing glucose trasporter 1 (GLUT1) content for long-term effects	52
Ethanol	Maceration	Antioxidant scavenging activities were investigated by DPPH assay.	24
		Antidiabetic and antihyperlipidemic agents investigated by strong α-glucosidase inhibitory activity improved various health indicators such as blood glucose level, lipid profile, liver and kidney biomarkers, and oxidative stress indices.	45
		A sedative effect on male mice of BALB/c strain	53
	ASE	Analgesic and antinociceptive effect.	6

SFE: Supercritical Fluid Extraction, ASE: Accelerated Solvent Extraction

solvent and method is important for retaining the efficacy of its pharmacological activity. The effect of solvent polarity on the efficiency of *M. speciosa* extraction was determined using both polar and non-polar solvents, while the varying boiling points of solvents were assessed to understand the effect of temperature on the extraction process. Polar solvents are predominantly utilized in the *M. speciosa* extraction process. Solvents with larger dipole moments exhibit greater polarity. In this case, the polarity of the solvent used for the extract *M. speciosa* decreases from water > methanol > ethanol. Out of 27 studies that involved *M. speciosa* extraction, 21 studies employed methanol, making it the most frequently used solvent, followed by 4 studies on ethanolic extract and two studies on aqueous extract. Moreover, a lower heating process would not only reduce energy consumption but also avoid thermal degradation and damage to the bioactive compounds in *M. speciosa* leaves (46). This has been relevant to methanol as the most chosen solvent for alkaloids, TPC and TFC, and other phytochemical compounds extraction from *M. speciosa* in which it has a comparatively lower boiling point (64.7 °C) than water (100 °C) (11).

Chemical extraction of *M. speciosa* leaves using methanol and ethanol proved that *M. speciosa* contained good proportions of secondary metabolites, especially alkaloids and flavonoids, as well as steroid/terpenoids, phenols, tannins, and saponins (7,24,28). Likewise, high concentrations of alkaloids and flavonoids were detected in the methanolic extraction of *M. speciosa*, while tannins and sterols were identified at low concentrations (26). In addition, other bioactive compounds isolated from *M. speciosa* include flavonoid epicatechin, saponin daucosterol, triterpenoid saponins quinovic acid 3-O-β-D-quinovopyranoside, quinovic acid 3-O-β-D-glucopyranoside, as well as several glycoside derivatives including 1-O-feruloyl-β-D- glucopyranoside, benzyl-β-D-glucopyranoside, 3-oxo-α-ionyl-O-β-D-glucopyranoside, roseoside, vogeloside, and epivogeloside using methanol in Soxhlet method (25). Moreover, scientific evidence reported that methanolic extraction reached the highest level of TPC and TFC (TPC: 105.58 ± 15.43 mg GAE/g and TFC: 91.12 ± 17.27 mg CE/g), compared to mixed solvent extraction (methanol and acetic acid-water) (TPC: 88.37 ± 0.70 mg GAE/g and TFC: 20.03 ± 3.03 mg CE/g), and aqueous extraction (TPC: 66.00 ± 1.23 mg GAE/g and TFC: 28.19 ± 2.28 mg CE/g) (23).

Methanol in both maceration and Soxhlet methods are the predominant conventional practices in many *M. speciosa* extraction studies (22). It has been suggested that utilizing methanol in the Soxhlet conventional method for the *M. speciosa* extraction yielded a high amount of mitragynine in addition to 24 unique peaks, as analyzed using Gas Chromatography-Mass Spectrometer (GC-MS) (42). The disadvantages of both traditional methods are often associated with exposure of the plant to a large amount of solvent over a long period (8,10,11,47). However, Soxhlet differs from the maceration technique in that, it requires a lower amount of solvent and a lesser solvent exposure period in the Soxhlet process.

Meanwhile, several modern extraction methods, including UAE, SFE-CO_2, and MAE (22), have been proposed and compared to assess their direct effect on the alkaloid content of the plant extract yield. In addition, UAE is equipped with an immersion horn in methanol and methanol/water to achieve the best yield of mitragynine and a high quantity of alkaloids, respectively (22). Furthermore, the modern SFE-CO_2 in its supercritical state was used to extract alkaloid compounds from *M. speciosa* with the highest anti-inflammatory (inhibit 60.08% of nitric oxide) and non-toxic activity (retain cell viability at 91.98%) (44). Despite the advantages of modern extraction methods, there are still drawbacks that should be considered, such as the high cost of equipment, its limitation to polar solvents, and the potential for degradation as a result of excessive free radical generation (1,11,14,48).

The use of different solvents and extraction methods in *M. speciosa* bioactive compound extraction revealed various pharmacological findings. Methanol extraction through maceration exhibited antinociceptive, anti-diarrheal, muscle relaxation, gastroprotective, and anti-depressant effects, along with antibacterial properties of *M. speciosa* (3,21,39,40,23,28,33–38). On the other hand, Soxhlet extraction of *M. speciosa* demonstrated antioxidant scavenging activities and anti-diabetic effects (7,26,42). Cold maceration displayed antipyretic activity (43), while sonication showed potential anti-inflammatory effects of *M. speciosa* (44). SFE and ASE methods both demonstrated analgesic and antinociceptive effects, with ASE additionally exhibiting antioxidant scavenging activities of *M. speciosa* (6,24). Ethanol extraction of *M. speciosa* through maceration exhibited antioxidant scavenging activities and antidiabetic and antihyperlipidemic effects (6,24,45). The water only exhibits antioxidant activity of *M. speciosa* but it is weaker compared with methanol (23).

It can be inferred that methanol with maceration and Soxhlet methods have been widely employed in *M. speciosa* extraction, demonstrating their effectiveness in extracting various active compounds and valuable medicinal benefits. Maceration along with methanol on *M. speciosa* extraction exhibits various pharmacological findings including a strong antioxidant (DPPH IC_{50}: 37.08 ± 3.54 µg/mL) and anti-bacterial activities (23). While methanol with the Soxhlet method produced a high amount of TFC (347.72±15.97 mg QE/g) and TPC (167.43±13.50 mg GAE/g) possesses antioxidant (DPPH IC_{50}: 4.34 ± 1.79 µg/mL) and anti-diabetic (α-amylase inhibition IC_{50}: 0.01 ± 7.18 mg/mL) activities (7).

Moreover, Soxhlet offers some beneficial advantages by minimizing the duration of plant exposure to the solvent, which makes it a preferable alternative.

CONCLUSION

The isolation of bioactive compounds from *M. speciosa* has been extensively performed using a myriad of solvents and techniques depending on the pharmacological properties of the plant. The most used traditional method for the extraction of *M. speciosa* was methanol in both maceration and Soxhlet, which can display numerous active compounds in the extract. Although the modern extraction methods are more compelling and produce high efficacy bioactive compounds, they come with high cost compared to traditional methods. At the moment, methanol and Soxhlet were superlative to a certain extent in providing an efficient and uncomplicated procedure. However, the type of compound of interest and its pharmacological properties are taken into account to determine the appropriate extraction solvent and method.

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