Biofunctional activity and phytochemical composition by LC-QToF-MS of *Mitragyna speciosa* Korth leaves extracted in Northern Thailand

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ORIGINAL ARTICLE

Abstract

Mitragyna speciosa Korth, a traditional Thai herb, is known for its medicinal properties, including enhancing physical strength and promoting relaxation. However, its misuse can lead to addiction. This study evaluated the antioxidant capacity, anti-inflammatory activity, and chemical composition of *M. speciosa* using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QToF-MS). Leaves were extracted with ethanol, methanol, and water. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay showed that ethanol extraction had the best IC_{50} value (0.23 ± 0.01 mg/mL). Methanol extraction excelled in the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay (2.24 ± 0.33 mg/mL), while water extraction displayed the highest antioxidant capacity in the fluorescence recovery after photobleaching (FRAP) assay (231.87 ± 11.51 mg ascorbic acid equivalent [AAE]/100 g). The water extract exhibited the highest total phenolic content (153.41 ± 0.06 mg gallic acid equivalent [GAE]/g) and total flavonoid content (6.24 ± 0.83 mg QE/g). It also provided the maximum anti-inflammatory protection, with a protection rate of 96.35% and a hemolysis of 3.65%. LC-QToF-MS analysis identified key bioactive compounds, such as stigmatellin Y (99.17%) and mitragynine (98.58%), along with other compounds with antioxidant and anti-inflammatory activities. These findings highlight *M. speciosa*'s potential of being a therapeutic agent. Future research should focus on elucidating the molecular mechanisms of its bioactive compounds, optimizing extraction techniques, and conducting *in vivo* and clinical studies to validate its safety and therapeutic efficacy.

Keywords: Mitragyna speciosa Korth; antioxidant activity; anti-inflammatory; flavonoid; phenolic; LC-QToF-MS

Introduction

The herbal leaves of *Mitragyna speciosa* Korth (MSK) comes from a tree of the Rubiaceae or coffee family. It is a native of Southeast Asia's humid tropical regions, such as Thailand, Malaysia, Indonesia, the Philippines, etc. MSK is a tropical evergreen tree of the genus *Mitragyna* that can grow to a height of 25 m. Its trunk might achieve a

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diameter of 0.9 m. The outer bark is smooth and gray, and the trunk is usually straight. The leaves are glossy and dark green, with an ovate-acuminate form and opposing growth pattern. They can reach a length of over 14–20 cm and a width of 7–12 cm. Their vein count ranges from 12 to 17 (Nakaphan *et al.*, 2016; Veltri and Grundmann, 2019). Additionally, MSK has opioid properties and has some stimulant-like effect to be used as a medicine to treat diseases (Eastlack *et al.*, 2020).

In Thailand, there are three species of kratom: geen, red, and white. Depending on its usage, each species has different flavors and significant compounds. In the industry, the MSK red species are highly sought after because of their potential for industrial processing and application as a medicinal herb. The red variety is known to help manage diabetes and is used to treat dysentery, diarrhea, and stomachache (Goh *et al.*, 2021). Commonly, the leaves are either chewed afresh or grilled and added to food, providing energy to the body and helping individuals endure prolonged exposure to sun without fatigue (Ponglux *et al.*, 1994). The effects usually start within 5–10 min and can last from 2 to 5 h. However, improper use or consumption can lead to addiction and adverse effects, including dry mouth, frequent urination, loss of appetite, constipation, insomnia, dizziness, and vomiting (Azizi *et al.*, 2010).

The legislation of *Mitragyna speciosa* in Thailand marks a significant shift in policy, allowing for its personal use and cultivation. However, safety concerns remain, particularly related to potential misuse, dependency, and the unregulated marketing. The Thai government is moving toward a regulated framework, but public health monitoring and international legal considerations continue to be important with the increase of kratom usage (Khalil *et al.*, 2020).

The leaves of the MSK contain more than 40 structurally related alkaloids along with several flavonoids, terpenoid saponins, polyphenols, and various glycosides. The primary psychoactive compounds in these leaves are mitragynine and 7-hydroxymitragynine, both being effective in pain relief (Racho *et al.*, 2022; Salim *et al.*, 2021). Furthermore, MSK leaf extract has been shown to possess antioxidant and antibacterial properties, effectively targeting Salmonella typhi and Bacillus subtilis. It also effectively inhibits the growth of Escherichia coli and S. pneumoniae. However, research on MSK has been extensively conducted to explore its antioxidant properties, chemical composition, and pharmaceutical potential. This plant is extensively used in various countries, such as Malaysia, Indonesia, Myanmar, and the Philippines (Firmansyah *et al.*, 2021; Sengnon *et al.*, 2023; Zakaria *et al.*, 2023). Therefore, investigating MSK, particularly in Thailand, where it is utilized frequently, is crucial to understand its medicinal benefits.

This study aimed to investigate the phytochemical profile of MSK, a red strain from Chiang Mai Province of Northern Thailand. The research focused on evaluating antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and fluorescence recovery after photobleaching (FRAP) assays as well as quantifying total phenolic content (TPC) and total flavonoid content (TFC). Additionally, the study explored the anti-inflammatory properties of leaves extract. Advanced liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QToF-MS) analysis was applied to identify primary compounds and characterize the chemical composition of Northern Thailand's MSK leaf extracts.

Materials and Method

Plant material

The fresh plant leaves of red species of MSK were collected from Ban Chomphu, subdistrict Chomphu, district Saraphi, Chiang Mai Thailand (latitude: 18.7201203, and longitude: 99.0670545) in June 2021. Authentication of plant leaves of red species was done by the Department of Agriculture, Thailand.

Extraction of plant sample

The method described by Jeeno *et al.* (2022) was used for extraction. Fresh herb leaves were finely chopped, and 1 g of the crushed material was extracted with 30 mL of distilled water, ethanol, and 99% methanol. The prepared combination was kept at room temperature (25°C) for

Figure 1. The fresh plant leaves of red species of *Mitragyna speciosa* Korth.

3 days. After the soaking period, the samples were agitated at 2500 rpm for 5 min and subsequently filtered using Whatman No. 1 filter paper. The filtered samples were concentrated to dryness by using a rotary evaporator. The resulting crude extracts were weighed and reconstituted in dimethyl sulfoxide. Before analysis, the extracted crude material was stored in a refrigerator at 4°C.

DPPH radical scavenging activity

The MSK extract's scavenging activity of free radicals was assessed using the technique outlined by Jeeno *et al.* (2022). With some slight modifications, the DPPH) assay was performed. To make DPPH stock solution, 24 mg of DPPH was dissolved in 100 mL of methanol. By adding 45 mL of methanol to a working DPPH solution that contained 10 mL of DPPH stock solution, the absorbance was changed to 1.1 at 517 nm. On a 96-well plate, 100 µL of DPPH working solution and 100 µL of plant extract solution were combined. A micro-titer-plate reader was used to measure absorbance at 517 nm after 30 min of incubation in the dark. The percentage of DPPH free radical scavenging activity was calculated using the following formula:

$$\text{DPPH} = ([\text{A control} - \text{A sample}]/\text{A control}) \times 100,$$

where A control and A sample are the absorbance reading of control and sample, respectively. The inhibitory concentration at 50% (IC_{50}) value for DPPH free radical scavenging activity corresponds to the sample concentration necessary to inhibit 50% of DPPH free radicals. IC_{50} was determined graphically from the curve plot between the percentages of DPPH scavenging activity and the sample concentration (Kek *et al.*, 2017).

ABTS radical scavenging activity

The ABTS method was used to determine the antioxidant activity potential based on the procedure described by Jeeno *et al.* (2022). The ABTS reagent used trolox as a stable radical in an aqueous solution. The value of correlation between the concentration of the sample and the percentage of ABTS that inhibits it is reported as IC50, or absorbance read at a wavelength of 734 nm. The ABTS radical was created by the reaction of 2.45-mM potassium persulfate with 7-mM ABTS in H_2O, which was then kept at 25°C in the dark for 12–16 h prior to use. After that, an absorbance of 0.7±0.02 at 734 nm was obtained by diluting the ABTS solution with 80% ethanol. The ABTS working solution of 190 µL was added to a 96-well plate containing 10 µL of plant extract. After incubation in the dark for 10 min, the absorbance was measured at 734 nm. Percentage inhibition of absorbance at 734 nm was calculated using the following formula:

$$\text{ABTS radical scavenging effect (\%)} = (\text{Ab} - \text{Aa}/\text{Aa}) \times 100,$$

where Ab is the absorbance of ABTS radical + methanol; Aa is the absorbance of ABTS radical + sample extract/standard. Trolox was used as a standard radical (Arnao *et al.*, 2001).

Ferric ion-reducing antioxidant power

The FRAP assay was performed using the method described by Jeeno *et al.* (2022). Concisely, FRAP reagent was combined with 2.5 mL of 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40-mM HCl, 2.5 mL of ferric chloride, and 25 mL of 300-mM acetate buffer at pH 3.6. The plant extract solution, 1 g, was diluted twice with distilled H_2O, and aliquots of 10 µL of plant extracted solution were combined with 190 µL of FRAP reagent on a 96-well microtiter plate. The sample's absorbance was measured after 30 min in the dark at 593 nm with a microtiter-plate reader. The standard curve was created using ascorbic acid, and FRAP value was determined by comparing it to the standard curve. The result was expressed as "mg ascorbic acid equivalent/100 g" (mg AAE/100 g) of plant extract (Arnao *et al.*, 2001).

Total phenolic content

The TPC was calculated by using Folin–Ciocalteau reagent (FCR) (Dewanto *et al.*, 2002; Jeeno *et al.*, 2022). The method used gallic acid as a standard substance. Different concentrations of gallic acid were prepared in 80% methanol at concentrations of 0.02–0.64 mg/mL, adding 12.5 µL of the sample and 12.5 µL of FCR (diluted with 10 times distilled H_2O) for 6 min; 7% sodium carbonate was added to 100-µL distilled water to prepare 125-µL volume. The solution was allowed to react at room temperature for 9 min. Then the absorbance was measured at a wavelength of 760 nm and the concentration of TPC was estimated using calibration curves. Results were expressed as "mg of gallic acid equivalent/gram" (mg GAE/g) of of dry sample extract.

Total flavonoids content

The TFC was measured by the color test method as described by Jeeno *et al.* (2022). With modification, 25-µL sample solution was put into a 96-well plate, and 7.5-µL sodium nitrite solution (7%) and 12.5-µL distilled H_2O were added and mixed thoroughly. The solution was allowed to stand at 25°C for 5 min. Then, 15 µL of 10% aluminum chloride solution was added, and the mixture was mixed thoroughly and kept at 25°C for 5 min. Finally, 50 µL of 1-M sodium hydroxide solution and 27.5-µL

distilled water were added, mixed thoroughly and kept at 25°C for 5 min. The absorbance was measured immediately at 510 nm using a spectrophotometer measured against H_2O blank, and the concentration of flavonoids was estimated using calibration curves. Results were expressed as "mg of Quercetin equivalent/gram" (mg QE/g) of sample.

Anti-inflammatory properties

The human red blood cell (HRBC) membrane stabilization method was adapted from Chippada *et al.* (2011). Plant solution was prepared at 100-µg/mL concentration in buffer solution (pH 7.4). Alsever solution was added to the blood samples collected from volunteers. The mixture was gently shaken and centrifuged at 3000 rpm for 20 min at 4°C. RBCs were collected, and sodium chloride (NaCl) solution was added to the pellet. After gently shaking to mix, the sample was centrifuged again at 3000 rpm for 20 min at 4°C. Then the washed RBCs were collected and stored for analysis. To prepare 10% v/v HRBC solution, 9 mL of NaCl solution was added to 1 mL of washed RBCs, and the mixture was slowly shaken to achieve uniformity. For sample analysis in a test tube, the following were added: 1 mL of buffer solution (pH 7.4), 2 mL of NaCl solution, 0.5 mL of 10% v/v HRBC solution, and 0.5 mL of plant sample solution. The contents were slowly mixed and incubated at 37°C for 30 min in a water bath. After incubation, the sample was centrifuged at 3000 rpm for 10 min. The absorbance of supernatant was measured at 560 nm. Control samples consisted of 10% v/v HRBC solution and distilled water. The percentages of protection and hemolysis were calculated using Equation (1) and Equation (2), respectively.

Calculation method:

$$\text{Protection (\%)} = 100 - ([A1/A0] \times 100), \quad (1)$$

$$\text{Hemolysis (\%)} = ([A1/A0] \times 100), \quad (2)$$

where A0 = absorbance of the control, and A1 = absorbance of the sample.

LC-QToF-MS analysis of chemical composition

The qualitative dataset of the extracted sample was characterized by using LC-QToF-MS analysis positive mode where molecules are ionized by gaining a positive charge according to the method used by Jeeno *et al.* (2022). In brief, the extracted samples were dissolved in 1-mL methanol (LC-MS grade) and cleaned by using a Dispersive Kit, (Fruits and Vegetables with Pigments and Fats, AOAC method, 2 mL, 100/pk. Contains: 50 mg PSA, 50 mg GCB, 50 mg C18EC, 150 mg MgSO4, Agilent Technology, Santa Clara, CA, USA). The sample solution was filtered through a 0.22-µm filter. The qualitative dataset was performed by using an Agilent 6500 series Q-TOF instrument (Agilent Technology) consisting of a degasser, binary pump, column oven, and thermostat autosampler. The instrument settings were optimized as follows: LC conditions, 0.2 mL/min flow rate; injection volume of 10 µL; and a gradient mobile system starting with 5% ACN and 95% water (1% formic acid) for 30 min. The chromatographic separation was accomplished using a ZORBAX Eclipse Plus C18 (2.1×150 mm, 1.8 µm). The MS conditions involved an electrospray ionization (ESI) probe in positive mode. The nebulizer was operated at 20 psi with 7 L/min nitrogen flow. The capillary temperature was kept at 300°C, and the flow rate was set at 8 µL/min. The range of mass divided by charged number of ions (m/z) was 50–1000, the capillary voltage was 4500 V, and the dry heater temperature was 280°C. The data were used to score the match of ions with the MassHunter METLIN Metabolomics Database. MassHunter Qualitative Analysis software (version 10.0, Agilent Technologies, Santa Clara, CA, USA) was used to perform a find-by-formula search within the databas. The chemical parameters of compounds were determined using the Medlin Library.

Results

Antioxidant activity

The antioxidant activity of MSK was evaluated using DPPH, ABTS, and FRAP assays. DPPH results showed that ethanolic extract had the strongest free radical scavenging ability (IC50 = 0.23 mg/mL), followed by methanolic extract (0.29 mg/mL), while the water extract was less effective (1.81 mg/mL). ABTS assay results indicated methanolic extract had the highest radical scavenging activity (IC50 = 2.24 mg/mL), compared to ethanolic and water extracts. In FRAP assay, water extract showed the greatest reducing power (231.87 mg AAE/100 g), surpassing both ethanolic and methanolic extracts (Table 1).

Total phenolic content and total flavonoid content

The TPC and TFC of MSK leaf extracts are presented in Table 2. The water extract exhibited the highest TPC value at 153.41 ± 0.06 mg GAE/g, followed by the methanol extract at 135.17 ± 0.10 mg GAE/g and the ethanol extract at 131.66 ± 0.04 mg GAE/g. Regarding TFC, the methanol extract showed the highest value at 2.20 ± 0.58 mg QE/g, followed by the ethanol extract at 4.97 ± 0.01 mg QE/g and the water extract at 6.24 ± 0.83 mg QE/g.

Table 1. DPPH scavenging activity, ABTS radical scavenging activity, and FRAP extract of MSK.

Mitragyna speciosa Korth	DPPH (mg/mL) IC_{50}	ABTS (mg/mL) IC_{50}	FRAP (mg AAE/100 g)
Ethanol extracted	0.23 ± 0.01[a]	3.14 ± 0.70[a,b]	113.48 ± 3.79[a]
Methanol extracted	0.29 ± 0.01[a]	2.24 ± 0.33[a]	53.05 ± 6.77[a]
Water extracted	1.81 ± 0.40[b]	3.97 ± 0.62[b]	231.87 ± 11.51[a]

DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2-casino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP: ferric ion reducing antioxidant power.

The results are presented as mean values ± standard deviation.

Different superscripted letters within the same column indicate a significant difference in antioxidant activity between extraction method using the one-way ANOVA statistical test.

Table 2. Total phenolic content and total flavonoid content of MSK extracts.

Sample	*Mitragyna speciosa* Korth		
	Ethanol extracted	Methanol extracted	Water extracted
Total phenolic compound (mg GAE/g)	131.66 ± 0.04[a]	135.17 ± 0.10[a]	153.41 ± 0.06[a]
Total flavonoid content (mg QE/g)	2.20 ± 0.58[a,b]	4.97 ± 0.01[a]	6.24 ± 0.83[b]

The results are presented as mean values ± standard deviation.

Different superscripted letters within the same column indicate a significant difference in TPC and TFC between extraction method using the one-way ANOVA statistical test.

Anti-inflammatory properties

The results of the anti-inflammatory efficacy study, measured by the suppression of hypotonicity-induced hemolysis in HRBC membranes, indicate that the water extract of MSK leaves exhibited the highest protective effect at all tested concentrations (Table 3). At 100 µg/mL, the water extract achieved the maximum protection of 96.35% with only 3.65% hemolysis. Comparatively, the ethanol extract showed 94.73% protection with 5.27% hemolysis at 100 µg/mL, and the methanol extract displayed 92.85% protection with 7.15% hemolysis at the same concentration. These findings suggested that the water extract of MSK leaves was effective in stabilizing HRBC membranes and preventing hemolysis, thereby indicating its superior anti-inflammatory potential, compared to ethanol and methanol extracts.

Table 3. Anti-inflammatory potential of MSK leaf extracts.

Mitragyna speciosa Korth	Concentration (µg/mL)	Protection (%)	Hemolysis (%)
Ethanol extracted	100	94.73 ± 2.29[a]	5.27 ± 2.29
Methanol extracted	100	92.85 ± 2.46[a]	7.15 ± 2.46
Water extracted	100	96.35 ± 0.15[a]	3.65 ± 0.15

The results are presented as mean values ± standard deviation.

Different superscripted letters within the same column indicate a significant difference in anti-inflammatory efficacy between extraction methods using the one-way ANOVA statistical test.

Chemical composition by LC-QToF-MS

The LC-QToF-MS analysis of active MSK compounds extracted with methanol showed the precise mass of molecular formula and the anticipated isotope pattern for that formula. The MassHunter qualitative analysis software find-by-formula search of the Agilent MassHunter METLIN Metabolomics Database used this data to score the match of ions with positive mode database. Phytochemical analysis of MSK leaf extracts was conducted using LC-QToF-MS, and the results are summarized in Table 4, and the LC-QToF-MS chromatograms are shown in Figure 2. The top nine compounds with a higher matching score (93.02–99.17%) were cinnamtannin A1, procyanidin B1, 6-hydroxykaempferol 7-rutinoside, biorobin, rhodomycin D, kaempferol 3-O-β-D-glucosyl-(1->2)-β-D-glucoside, alpinine, mitragynine, and stigmatellin Y. MSK leaf extracts showed a potential of metabolite, anti-inflammatory agent, antioxidant, and lipoxygenase inhibitor.

Discussion

Evaluation of antioxidant properties

The antioxidant properties of MSK extracts showed significant variation depending on the solvent used for extraction. The ethanol extract exhibited the highest antioxidant activity in the DPPH assay (IC50 = 0.23 ± 0.01 mg/mL), probably because of its efficient extraction of phenolic compounds. In contrast, the methanol extract demonstrated superior ABTS radical scavenging activity (IC50 = 2.24 ± 0.33 mg/mL), suggesting it may extract a different set of phenolic compounds with greater ABTS activity. Although the water extract exhibited lower activity in the DPPH and ABTS assays, it demonstrated the highest reduction power in FRAP assay (231.87 ± 11.51 mg AAE/100 g), indicating a strong electron-donating capacity. Additionally, it yielded the

Table 4. Compounds identified from the extract of MSK methanolic leaves according to LC-QToF-MS.

No.	Compound	Formula	RT (min)	Matching score (%)	m/z	Mass
1.	Cinnamtannin A1	$C_{45}H_{38}O_{18}$	0.684	96.44	867.21287	866.20554
2.	Procyanidin B1	$C_{30}H_{26}O_{12}$	0.693	96.03	579.14948	578.14227
3.	6-Hydroxykaempferol 7-rutinoside	$C_{27}H_{30}O_{16}$	0.694	97.49	611.16068	610.15351
4.	Biorobin	$C_{27}H_{30}O_{15}$	0.697	94.95	595.16581	594.15879
5.	Rhodomycin D	$C_{28}H_{31}NO_{11}$	0.702	93.02	575.22429	557.19032
6.	Kaempferol 3-O-β-D-glucosyl-(1->2)-β-D-glucoside	$C_{27}H_{30}O_{16}$	0.718	95.7	633.14237	610.15322
7.	Alpinine	$C_{23}H_{29}NO_6$	0.736	98.55	433.23365	415.19979
8.	Mitragynine	$C_{23}H_{30}N_2O_4$	0.818	98.58	399.2284	398.22114
9.	Stigmatellin Y	$C_{29}H_{40}O_6$	2.742	99.17	579.14993	484.28274

highest values in the analysis of TPC (153.41 ± 0.06 mg GAE/g) and TFC (6.24 ± 0.83 mg QE/g). These results were consistent with the results of the previous studies on plant leaf extracts, where solvent polarity significantly influenced extraction efficiency and antioxidant activities (Benjakul *et al.*, 2014; Tagrida and Benjakul, 2021). The strong antioxidant activity of MSK extracts, particularly in the FRAP assay, proposed their potential for health benefits related to oxidative stress mitigation. These findings underscored the importance of solvent selection in optimizing the extraction of bioactive compounds from plant materials.

Analysis of TPC and TFC

The comparison of TPC and TFC in MSK leaf extracts from different regions illustrated the significant role of geographical origin, extraction methods, and environmental conditions in shaping phytochemical profiles. In this study, MSK leaf extracts from Northern Thailand exhibited a higher TPC (135.17 ± 0.10 mg GAE/g) and lower TFC (4.97 ± 0.01 mg QE/g), compared to the prior findings of Parthasarathy *et al.* (2009), who reported a TPC of 105.6 mg GAE/g and a TFC of 91.1 mg CAE/g in samples collected from Perak, Malaysia. This variation could be attributed to Northern Thailand's unique climatic and soil conditions that could promote higher phenolic content, enhancing the antioxidant potential of MSK leaf extracts. Furthermore, the aqueous extract from Northern Thailand demonstrated a superior TPC (153.41 ± 0.06 mg GAE/g), highlighting water's efficacy as an extraction solvent, as it yielded higher phenolic content than previously reported in studies, such as Kang *et al.* (2010). These findings emphasized the need to optimize extraction techniques and consider regional differences to fully harness the therapeutic potential of MSK, making it a promising source of natural antioxidants with potential applications in health and medicine.

Assessment of anti-inflammatory potential

The anti-inflammatory potential of MSK leaf extracts, especially the aqueous extract, was demonstrated by their ability to stabilize RBC membranes under osmotic stress. This extracellular antioxidant effect, which was discovered by Shaik Mossadeq *et al.* (2009), showed that the methanolic extract of MSK exhibited anti-inflammatory and analgesic effects via inhibition of cyclooxygenase and lipoxygenase. Our study highlighted the role of phenolic compounds in aqueous extract, which was consistent with the anti-inflammatory activity of flavonoids and saponins reported in previous studies. It is well recognized that these biocomponents suppress pro-inflammatory mediators, which aid in the stabilization of cell membranes. Previous studies linking phenolic content to improved anti-inflammatory efficacy (Hassan *et al.*, 2013) further corroborated this finding, emphasizing the MSK aqueous extract's ability to stabilize outer membranes. These findings highlighted the potential for versatile approaches to treat inflammation by MSK, making it a strong candidate for more comprehensive therapeutic applications. Our findings elaborated the therapeutic potential of MSK and offered potential therapeutic advantages for diseases associated with impaired cell membrane integrity.

Phytochemical profiling by LC-QToF-MS

The main substances and chemical components of MSK were investigated using the LC-QToF-MS technique and it was found that stigmatellin Y had a matching rate of 99.17%. Stigmatellin Y is a powerful inhibitor that targets the quinol oxidation (QO) site in the cytochrome bc1 complex within mitochondria and the cytochrome b6f complex in thylakoid membranes.

Mitragynine had a matching rate of 98.58%. Mitragynine, an indole-based alkaloid, is a primary active compound

found in the Southeast Asian plant *Mitragyna speciosa*, commonly known as kratom. The dried leaves of kratom contain a total alkaloid concentration ranging from 0.5% to 1.5%.

In Thai varieties, mitragynine is the predominant component, making up as much as 66% of the total alkaloid content (Handa *et al.*, 2008). Mitragynine has garnered interest for its potential medicinal properties. It is an

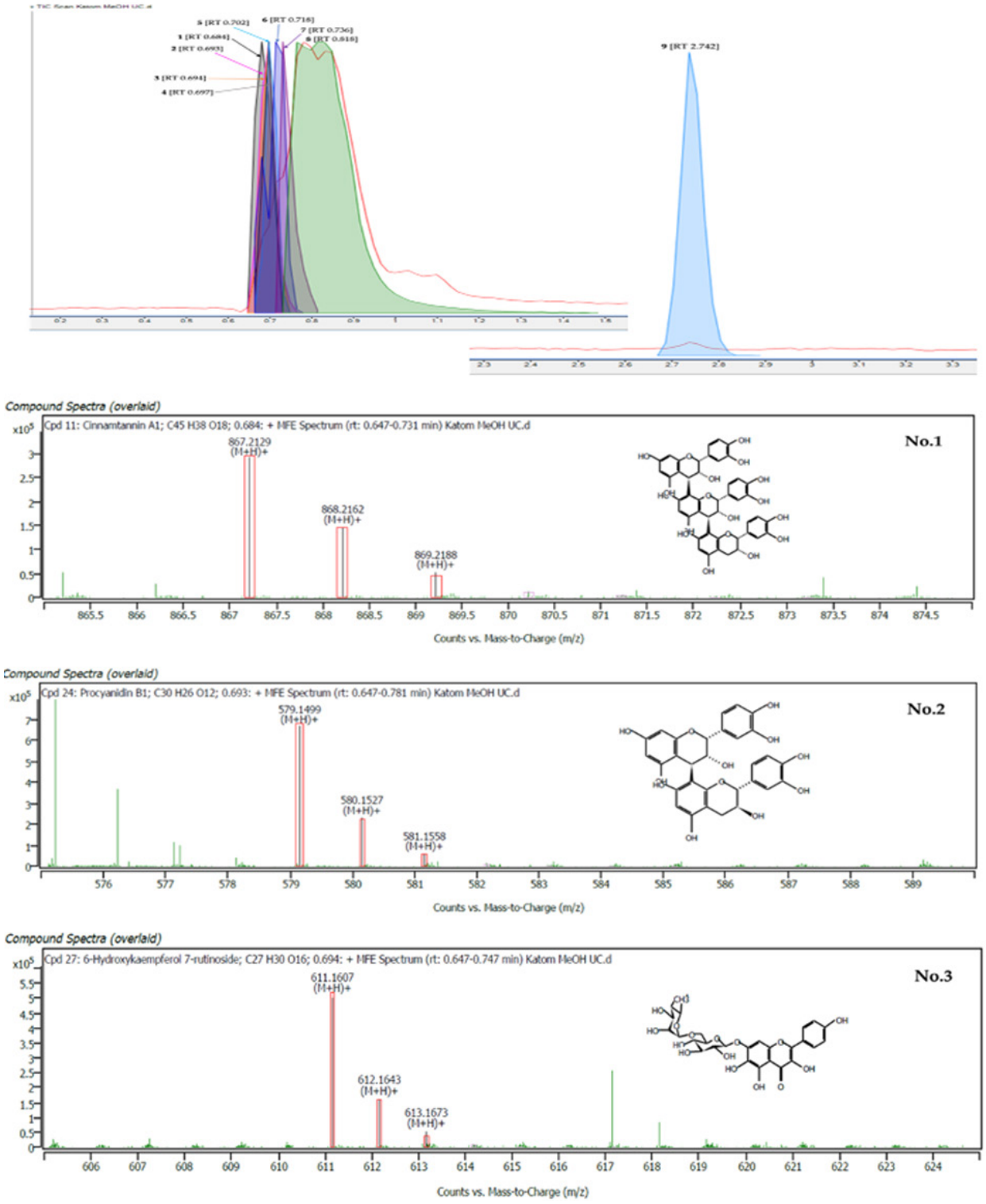

Figure 2. LC-QToF-MS analysis of the top nine compounds in terms of matching scores of MSK methanolic extract. (A) LC-QToF-MS chromatogram; No. 1. mass spectrum ofcinnamtannin A1; No. 2. procyanidin B1; No. 3. 6-hydroxykaempferol 7-rutinoside.

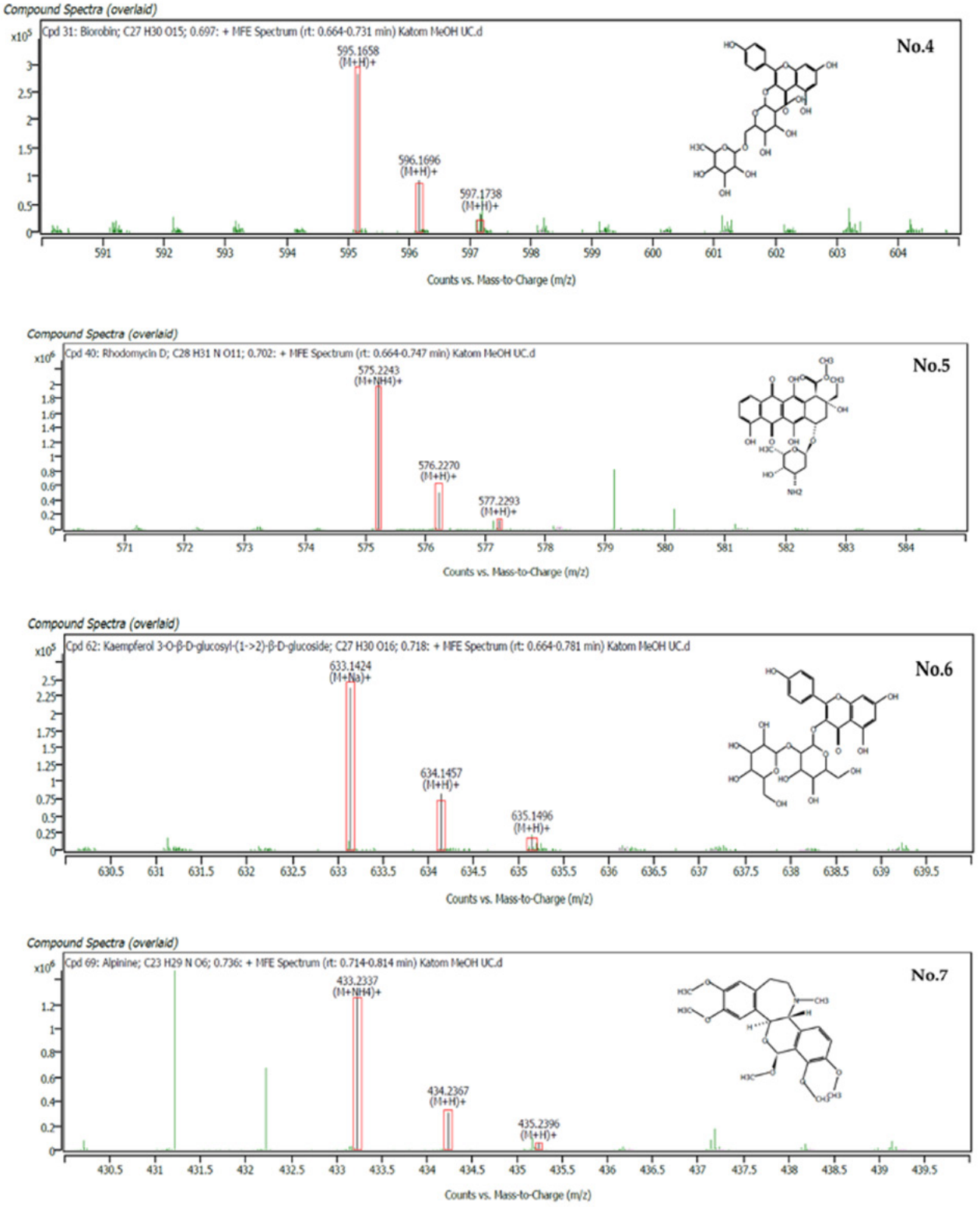

Figure 2. (Continued) LC-QToF-MS analysis of the top nine compounds in terms of matching scores of MSK methanolic extract. (A) LC-QToF-MS chromatogram; No. 4. biorobin; No. 5. rhodomycin D; No. 6. kaempferol 3-O-β-D-glucosyl-(1->2)-β-D-glucoside; No. 7. Alpinine.

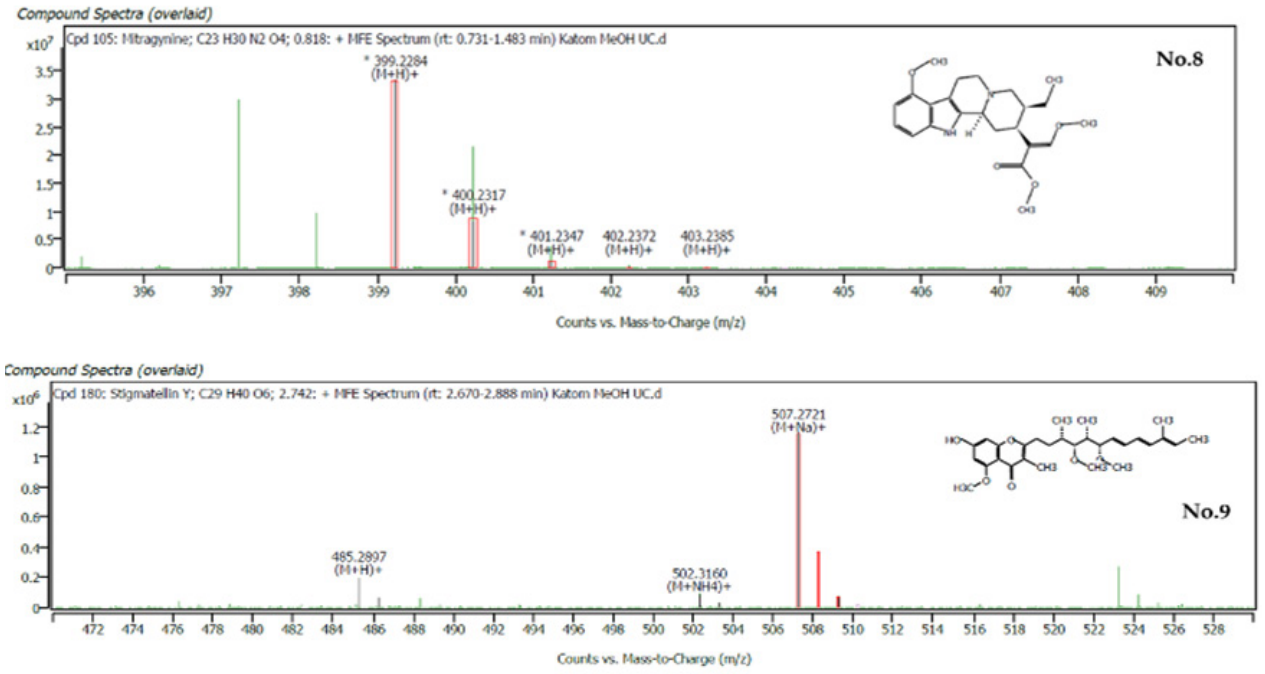

Figure 2. (Continued) LC-QToF-MS analysis of the top nine compounds in terms of matching scores of MSK methanolic extract. (A) LC-QToF-MS chromatogram; No. 8. mitragynine; and No. 9. stigmatellin Y.

in-dole-based compound that offers various therapeutic properties, such as pain relief, mood enhancement, and opioid withdrawal symptom alleviation (Rech et al., 2015). However, despite its potential benefits, the use of mitragynine in medicine is still under study, and it is important to consider its legal status and possible adverse effects. In a previous study characterizing mitragynine using LC-QToF-MS, it was found that it has analgesic properties. Its mechanism of action involves interaction with opioid receptors, leading to opioid-like effects, such as sedation, euphoria, and pain relief. Consequently, for testing in the opioid group, the *M. speciosa* was utilized instead of specific substances or medications targeting the nervous system. (Chear *et al.*, 2021; Matsumoto *et al.*, 2006).

Additionally, MSK has been shown to have anti-inflammatory and antioxidant properties. The bioactive compounds, such as Cinnamtannin A1, Procyanidin B1, and 6-hydroxykaempferol 7-rutinoside, were identified in MSK, all of which possess antioxidant properties and offer health benefits (Colucci *et al.*, 2019). Procyanidin B1 can be transformed into procyanidin A1 through radical oxidation by DPPH radicals under neutral conditions. Alpinine has also been studied for its diverse pharmacological activities, such as potential antioxidant, anti-inflammatory, and antimicrobial effects. However, variations in chemotypes, climate, environmental stressors, and soil types can significantly alter the concentration of mitragynine, contributing to differences in the primary ingredient content (Handa *et al.*, 2008).

Conclusion

This study highlighted the antioxidant and anti-inflammatory properties of MSK. Water extraction showed the highest antioxidant capacity and anti-inflammatory protection, while ethanol and methanol extracts excelled in specific antioxidant assays. The key bioactive compounds, including stigmatellin Y and mitragynine, were identified through LC-QToF-MS, underscoring the herb's medicinal potential. The findings suggested that *Mitragyna speciosa* could be a valuable source of natural antioxidants and anti-inflammatory agents. Future research should focus on understanding its bioactive compounds' molecular mechanisms, optimizing extraction methods, and conducting *in vivo* and clinical studies to validate its therapeutic potential and safety.

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Author Contributions

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Conflict of Interest

The authors declared no conflict of interest.

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