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(RESEARCH ARTICLE)

PHYTOCHEMICAL, ANTIOXIDANT ACTIVITY, AND TOXICITY ASSAY OF STEM BARK FROM *Mitragyna speciosa* Korth

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ABSTRACT

Mitragyna speciosa, a plant belonging to the Rubiaceae family, is commonly found in Southeast Asia and is used as a traditional medicine and as a cosmetic ingredient. This study aimed to determine the phytochemical constituents, antioxidant activity, and toxicity levels of the methanol extract, *n*-hexane fraction, ethyl acetate fraction, and aqueous fraction of *M. speciosa* stem bark. The methods used were maceration extraction, partitioning, phytochemical screening with specific color reagents, determination of total flavonoid content using the $AlCl_3$ colorimetric method, determination of antioxidant activity based on scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, and a toxicity test against *Artemia salina*. The results showed that the methanol extract, *n*-hexane fraction, ethyl acetate fraction, and aqueous fraction of *M. speciosa* stem bark contained phenolics, flavonoids, quinones, alkaloids, triterpenoids, steroids, and saponins, with total flavonoid contents of 83.91, 15.74, 91.38, and 143.71 mg QE/g, respectively. In the antioxidant activity assay, the methanol extract, *n*-hexane fraction, ethyl acetate fraction, and aqueous fraction of *M. speciosa* stem bark had IC_{50} values of 86.97, 311.51, 159.37, and 24.06 µg/mL, respectively, while the toxicity assay showed LC_{50} values of 219.71, 108.09, 272.79, and 231.06 µg/mL, respectively. In conclusion, the methanol extract and fractions of *M. speciosa* stem bark exhibited antioxidant activity and potentially possess cytotoxic activity.

Keywords: *Mitragyna speciosa*, Antioxidant, Phytochemical, Stem Bark, Toxicity.

1 INTRODUCTION

Indonesia has a humid tropical climate with high solar radiation intensity, resulting in exposure to ultraviolet (UV) radiation. Excessive UV exposure can promote the generation of reactive oxygen species (ROS), including free radicals, which may cause oxidative stress. Free radicals can damage cells in the body, contributing to the development of various degenerative diseases, such as cancer. Antioxidant compounds act as free radical scavenging agents that help maintain the balance of chemical reactions within body cells. Accordingly, the utilization of secondary metabolites from plants can be one of the approaches to addressing oxidative stress [1].

Mitragyna speciosa is known as a plant containing 7-hydroxymitragynine and belonging to the Rubiaceae family, which is commonly found in Southeast Asia [2]. In Indonesia, *M. speciosa* can be found on the islands of Sumatra, Papua, and Kalimantan, with local names including kratom, kadamba, ketum, purik, and sapat. The people of East Kalimantan, particularly in Kutai Kartanegara Regency, utilize the stem bark of *M. speciosa* as an ingredient in making face powder. Likewise, the Bentian people in West Kutai Regency use the stem bark of *M. speciosa* as an ingredient for smoothing the skin [3]. A similar practice is also carried out by the people of West Kalimantan, who use the stem bark of *M. speciosa* to smooth facial skin and as an herbal medicine. In addition, its leaves, which are commonly brewed like tea, are empirically used to relieve muscle pain, alleviate stomachache, fever, diarrhea, swelling, and headache [4].

M. speciosa leaves contain alkaloids, steroids, flavonoids, quinones, saponins, and tannins [5]. The leaves exhibit antioxidant activity in the DPPH and ABTS assays, with IC_{50} values of 38.56 µg/mL and 0.29 mg/mL, respectively [6,7]. Furthermore, the leaves also show sunscreen activity, with a sun protection factor (SPF) value of 8.43 [8]. In addition, *M. speciosa* exhibits anti-inflammatory activity based on the carrageenan-induced paw edema method and the xylene-induced ear edema method in mice [9]. The toxicity level of the ethanol extract of *M. speciosa* leaves against *Artemia salina* was determined, yielding an LC_{50} value of 143.50 µg/mL [10]. Methanol extracts of the roots and stems of *M. speciosa* exhibit antibacterial activity against *Propionibacterium acnes* with a moderate inhibitory effect [11,12]. Based on this literature review, the plant has potential for further investigation, particularly as a candidate antioxidant agent.

Although several studies have been conducted on *M. speciosa*, phytochemical and bioactivity studies of the stem bark of *M. speciosa* remain limited. In particular, antioxidant and toxicity assays of the stem bark of *M. speciosa* have not been widely reported. Toxicity screening using the brine shrimp lethality test (BSLT) method can indicate the potential of active compounds in a plant [13]. Based on these considerations, this study was conducted to determine the phytochemical constituents, antioxidant activity, and toxicity levels of the stem bark fractions of *M. speciosa* from East Kalimantan.

2 MATERIAL AND METHODS

2.1 Materials and Equipment

The materials used in this study were kratom stem bark (*Mitragyna speciosa* Korth.) collected from location 0°30'26.0"S 117°00'25.6"E and identified at the Wanariset Samboja Herbarium with specimen number AA 1615, distilled water, methanol, *n*-hexane, ethyl acetate, chloroform-ammonia, H_2SO_4 2 N, Dragendorff's reagent, concentrated H_2SO_4, glacial acetic acid, $FeCl_3$ 1%, concentrated HCl, Mg powder, NaOH 5%, $AlCl_3$ 10%, $NaNO_2$ 5%, *Artemia salina* L. cysts, sea salt, dimethyl sulfoxide (DMSO) 1%, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and quercetin. The equipment used in this study included laboratory glassware, an analytical balance, micropipettes, microplates, a vortex mixer, an incandescent lamp, a retort stand, clamps, cuvettes, a Buchi R-II rotary evaporator, and a Thermo Scientific Orion AquaMate 8100 UV-Vis spectrophotometer.

2.2 Procedure

2.2.1 Secondary Metabolite Extraction

A total of 2.22 kg of *M. speciosa* stem bark was ground and then macerated using methanol as the solvent for 72 hours. The *M. speciosa* stem bark macerate was filtered and concentrated using a rotary evaporator to obtain the methanol extract. Subsequently, the extract was weighed and its yield was calculated. The *M. speciosa* stem bark extract was redissolved in methanol and partitioned with *n*-hexane, followed by ethyl acetate, to obtain the *n*-hexane fraction, ethyl acetate fraction, and aqueous fraction. The resulting fractions were concentrated again using a rotary evaporator, then weighed and their yields were calculated.

2.2.2 Phytochemical Screening

The methanol extract and fractions of *M. speciosa* stem bark were redissolved in methanol. Phytochemical screening was qualitatively performed for phenolics, flavonoids, quinones, alkaloids, triterpenoids, steroids, and saponins using specific reagents based on the modified method of Harborne [14].

2.2.3 Determination of Total Flavonoid Content

The determination of total flavonoid content in the methanol extract and fractions of *M. speciosa* stem bark was carried out based on the modified Chang method [15]. The quercetin standard series was prepared from 5 mg of solid quercetin dissolved in 50 mL of methanol to obtain a 100 µg/mL stock solution. The solution was then diluted to obtain concentrations of 20, 40, 60, and 80 µg/mL. The 100 µg/mL test solutions were prepared by dissolving 1 mg of the methanol extract and fractions of *M. speciosa* stem bark in 10 mL of methanol. The standard and test solutions were each taken at 2 mL and transferred into 10 mL volumetric flasks, followed by the addition of 1 mL of $AlCl_3$ 10% and 0.3

mL of $NaNO_2$ 5%, and then methanol was added to the mark. The solutions were allowed to stand for 1 hour, after which their absorbance values were measured using a UV-Vis spectrophotometer at a wavelength of 432 nm.

2.2.4 In Vitro Antioxidant Activity Assay

The antioxidant activity assay of the methanol extract and fractions of *M. speciosa* stem bark was conducted following the modified Erwin method [13]. A total of 5 mg of solid DPPH was dissolved in 50 mL of methanol to obtain a 100 µg/mL DPPH solution, and this solution was then stored in a dark and closed container. The 500 µg/mL test solutions were prepared by dissolving 25 mg of the extract and fractions of *M. speciosa* stem bark in 50 mL of methanol. The stock solutions were diluted to several concentration variations. The positive control solution was prepared by dissolving 1 mg of solid quercetin as a positive control in 10 mL of methanol to obtain a 100 µg/mL quercetin stock solution. The stock solution was diluted to several concentration variations. Meanwhile, methanol was used as the negative control solution. Each test solution, positive control solution, and negative control solution was taken at 2 mL and then transferred into dark bottles. The solutions were added with 2 mL of DPPH 100 µg/mL solution and incubated for 30 minutes at room temperature. Subsequently, the absorbance of the solutions was measured at a wavelength of 518 nm using a UV-Vis spectrophotometer.

2.2.5 Toxicity Assay against *Artemia salina*

The brine shrimp lethality test (BSLT) of the methanol extract and fractions of *M. speciosa* stem bark was conducted based on the modified Meyer method [16]. The seawater medium was prepared by dissolving 38 g of sea salt in 1000 mL of distilled water. A total of 10 mg of *A. salina* cysts was placed in a compartment containing 500 mL of seawater medium and then left for 48 hours until hatching. The test solutions were prepared by dissolving 1 mg of the extract and fractions of *M. speciosa* stem bark in 100 µL of DMSO 1%, followed by dilution with distilled water to a total volume of 250 µL. A total of 200 µL of the solution was taken and further diluted with distilled water to a total volume of 800 µL to obtain a 1000 µg/mL solution. The stock solution was serially diluted to obtain concentration variations of 500, 250, 125, 62.50, 31.25, 15.63, and 7.81 µg/mL. Subsequently, the same procedure was repeated to prepare the control solution, except that no sample was added. A total of 100 µL of seawater containing 10 *A. salina* larvae was added to a microplate that had been filled with the test solutions. After 24 hours, the number of dead *A. salina* larvae was counted to determine the Lethal Concentration 50% (LC_{50}) value.

2.3 Data Analysis

2.3.1 Data Analysis of Total Flavonoid Content

The analytical technique used to determine the total flavonoid content in the samples was expressed as mg quercetin equivalents per gram of sample weight (mg QE/g). The equation used to determine the total flavonoid content is presented in **Equation 1** as follows.

$$C = \frac{Cq \times V}{m} \times Fp \qquad \textbf{Equation 1}$$

with C = Total flavonoid content (mg QE/g); Cq = Concentration (mg/L); V = Volume (L); m = Sample mass (g); and Fp = Dilution factor.

2.3.2 Data Analysis of the Antioxidant Activity Assay

The analytical technique used to determine the level of antioxidant activity was based on the Inhibition Concentration 50% (IC_{50}), which was determined using regression analysis between concentration and %inhibition using Microsoft Excel. The extent of DPPH radical scavenging was expressed as % inhibition. **Equation 2** for calculating % inhibition is presented as follows.

$$\%\text{Inhibition} = \frac{\text{Abs. Control (DPPH)} - (\text{Abs. Sample} - \text{Abs. Blank})}{\text{Abs. Control (DPPH)}} \times 100\% \qquad \textbf{Equation 2}$$

Furthermore, the determination of the IC_{50} value was performed using **Equation 3** as follows.

$$y = ax + b \qquad \textbf{Equation 3}$$

With y = inhibition (%); x = IC_{50} (µg/mL); a = slope; b = intercept.

2.3.3 Data Analysis of the Toxicity Assay

The analytical technique used to determine the toxicity level against *A. salina* was based on the Lethal Concentration 50% (LC_{50}), which was calculated using Probit Analysis with IBM SPSS. The effectiveness of the samples against *A. salina*

was expressed as LC_{50} (µg/mL) 24 hours after treatment. **Equation 4** for calculating %mortality is presented as follows.

$$\%\text{Mortality} = \frac{\text{Number of dead larvae in the treatment} - \text{Number of dead larvae in the control}}{\text{Total number of larvae}} \times 100\% \quad \textbf{Equation 4}$$

3 RESULTS

3.1 Extraction and Phytochemical Screening

Secondary metabolite extraction from the stem bark of *M. speciosa* was carried out using the maceration method with methanol as the solvent. Methanol was selected as the solvent because it contains hydroxyl and methyl groups that are capable of extracting both polar and non polar compounds. The maceration method was selected because the extraction process is performed without heating, which can minimize damage or changes in the structure of compounds due to the influence of temperature [17]. During the maceration process, the cell walls of the simplicia are disrupted due to the difference in pressure inside and outside the cells, allowing the methanol solvent to extract the secondary metabolites contained within. Stirring was also performed during the maceration process to balance the concentration of the solution so that the extraction results could be maximized. The collected macerate was then concentrated using a rotary evaporator, based on the principle that methanol evaporates at a temperature lower than its boiling point due to reduced pressure [18]. The methanol extract obtained was 146 g, followed by fractionation aimed at obtaining fractions with different levels of polarity. The *n*-hexane fraction obtained was 10 g, the ethyl acetate fraction was 46 g, and the aqueous fraction was 36 g. The yields obtained for the methanol extract, *n*-hexane fraction, ethyl acetate fraction, and aqueous fraction were 6.58, 0.45, 2.07, and 1.62%, respectively. The methanol extract and the three fractions were subjected to phytochemical screening to obtain information on their secondary metabolite constituents, as shown in **Table 1**.

Table 1: Phytochemical Screening Results of *M. speciosa* Stem Bark

Sample Test	Phytochemical Group					
	Phenolics	**Flavonoids**	**Quinones**	**Alkaloids**	**Triterpenoids/ Steroids**	**Saponins**
Methanol extract	+	+	+	+	+	+
n-Hexane fraction	+	-	+	+	+	-
Ethyl acetate fraction	+	+	-	+	+	-
Aqueous fraction	+	+	-	+	+	+

Note: (+) = detected, (-) = not detected.

Based on **Table 1**, it can be seen that phenolics, alkaloids, and triterpenoids were present in the methanol extract and fractions of *M. speciosa* stem bark. These three compounds are commonly found groups of compounds because they are capable of dissolving in polar, semi polar, and non polar solvents. Alkaloids and triterpenoids attracted by polar solvents are presumed to be present in glycoside forms. Quinones were found in the *n*-hexane fraction because these compounds are non polar and can therefore dissolve in *n*-hexane. Flavonoids were found in the ethyl acetate and aqueous fractions. These compounds can be extracted by ethyl acetate and water because flavonoids are semi polar and tend to be polar. Meanwhile, saponins were only present in the aqueous fraction. This is because saponins are polar compounds and therefore dissolve only in polar solvents.

3.2 Total Flavonoid Content

Total flavonoid content represents the total amount of flavonoid compounds present in an extract. This can be determined by reacting the extract to be tested with $AlCl_3$ 10% and $NaNO_2$ 5%, resulting in the formation of a yellow-colored Al^{3+}–flavonoid complex. An increase in the intensity of the yellow color indicates an increase in the concentration of flavonoids in the sample [19]. In general, flavonoid compounds can react with $AlCl_3$ at the C ring, particularly at the hydroxyl and carbonyl groups, forming a complex bond with Al^{3+}. This activates the conjugated system in the flavonoid ring, causing the absorption wavelength to shift to the visible region around 432 nm, which appears as a yellow color. In addition, $NaNO_2$ is added to deprotonate the hydroxyl groups on the B ring, facilitating the formation of a complex bond between Al^{3+} and the flavonoid, thereby enhancing the intensity of the yellow color formed. If $NaNO_2$ is not added, the resulting yellow color may not be sufficiently intense, which can affect the absorbance measurement using a UV-Vis spectrophotometer. In this assay, quercetin was used as the flavonoid standard, prepared at several concentration variations and measured at the maximum wavelength of 432 nm to obtain a standard curve.

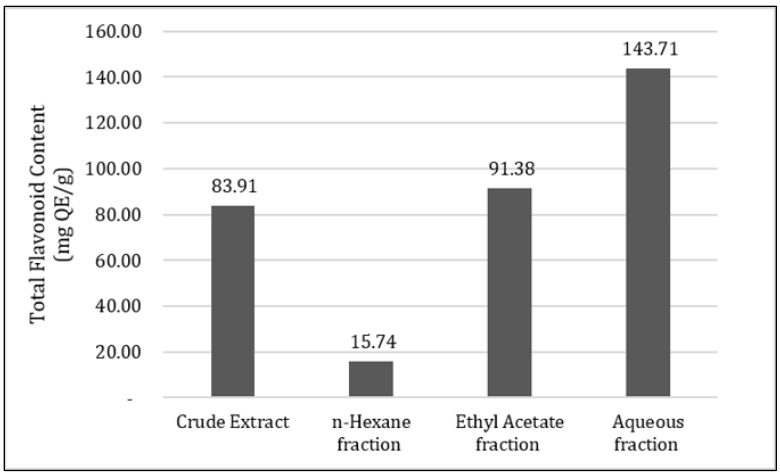


Figure 1: Diagram of Total Flavonoid Content of *M. speciosa* Stem Bark

Based on **Figure 1**, it can be seen that the aqueous fraction had the highest flavonoid content of 143.71 mg QE/g, followed by the ethyl acetate fraction at 91.38 mg QE/g. The distribution of flavonoid content in the fractions of *M. speciosa* stem bark is influenced by the polarity of the solvent. According to Wakeel [20], polar solvents such as methanol are more effective in extracting polar flavonoids such as flavonoid glycosides, while ethyl acetate is semi polar and therefore capable of extracting flavonoids with semi polar properties, such as flavonols or flavanones. *n*-Hexane extracts only a small amount of flavonoids because its non polar nature is less suitable for dissolving flavonoids, and therefore it can only extract flavonoids with polymethoxy or prenylated substituents. Flavonoids are phenolic compounds containing many hydroxyl groups and may also contain glycoside groups, making them highly soluble in solvents that tend to be polar.

3.3 Antioxidant Activity Test

Antioxidants are compounds capable of inhibiting oxidation reactions or free radicals. Antioxidant compounds can donate one electron to free radicals, thereby stabilizing the chain reaction of these radicals. The measurement of antioxidant activity is generally carried out using the DPPH method, in which antioxidants reduce DPPH radicals to stable DPPH–H compounds, as indicated by a color change in the solution from purple to yellow. This color change is measured by determining the absorbance using a UV-Vis spectrophotometer. The IC_{50} value represents the concentration of an antioxidant required to inhibit 50% of the DPPH concentration. The lower the IC_{50} value obtained, the greater the antioxidant activity [13]. The results of the antioxidant activity measurements of the methanol extract and the three fractions of *M. speciosa* stem bark are presented in **Table 2**.

Based on **Table 2**, it is known that the methanol extract of *M. speciosa* stem bark has potential as a source of antioxidants. The total flavonoid content obtained showed a positive correlation with the antioxidant activity of the three fractions. The aqueous fraction had a high total flavonoid content and therefore exhibited very strong antioxidant activity. Meanwhile, the ethyl acetate and *n*-hexane fractions, with medium and low total flavonoid contents, respectively, exhibited lower antioxidant activity than the aqueous fraction. The main pharmacological effect of flavonoids is antioxidant activity, with a mechanism involving electron donation or hydrogen abstraction to •DPPH, thereby stabilizing the radical and rendering it unreactive. In addition, the water fraction contains phenolics, alkaloids, triterpenoids, and saponins, which exhibit antioxidant activity through their respective mechanisms. The methanol extract of *M. speciosa* stem bark also contains similar compounds, but with the additional presence of quinone compounds, which reduces its antioxidant activity. Similarly, quinone compounds are found in the *n*-hexane fraction, reducing its antioxidant activity. Quinone compounds can act as pro-oxidants when present at high doses, generating reactive oxygen species (ROS). Quinones can undergo repeated redox cycling, which increases the number of free radicals in the process [21].

Table 2: Result of Antioxidant Activity of *M. speciosa* Stem Bark

Sample Test	Concentration (µg/mL)	Absorbance			Inhibition (%)			IC_{50} (µg/mL)	Antioxidant category [13]
		I	II	III	I	II	III		
Methanol extract	20	0.589	0.581	0.590	16.76	17.90	16.62	86.97 ±0.67	Strong
	40	0.519	0.520	0.519	26.70	26.56	26.70		
	60	0.443	0.449	0.441	38.35	37.50	38.64		
	80	0.397	0.388	0.387	45.17	46.45	46.59		
	100	0.322	0.323	0.319	56.11	55.97	56.53		
n-Hexane fraction	31.25	0.845	0.849	0.848	13.07	12.65	12.76	311.51 ±1.33	Weak
	62.50	0.791	0.792	0.789	18.72	18.62	18.93		
	125	0.693	0.692	0.691	29.01	29.12	29.22		
	250	0.540	0.537	0.536	44.96	45.27	45.37		
	500	0.294	0.290	0.289	70.58	70.99	71.09		
Ethyl acetate fraction	31.25	0.784	0.787	0.786	20.16	19.86	19.96	159.37 ±0.72	Moderate
	62.50	0.709	0.707	0.707	28.19	28.40	28.40		
	125	0.550	0.545	0.546	44.75	45.27	45.16		
	250	0.323	0.319	0.318	68.72	69.14	69.24		
	500	0.104	0.102	0.101	91.67	91.87	91.98		
Aqueous fraction	20	0.421	0.417	0.421	40.34	40.91	40.34	24.06 ±0.21	Very strong
	40	0.222	0.222	0.218	68.89	68.89	69.46		
	60	0.101	0.099	0.097	86.08	86.36	86.65		
	80	0.076	0.077	0.075	90.06	89.91	90.20		
	100	0.071	0.074	0.071	91.34	90.91	91.34		
Quercetin	2	0.390	0.391	0.390	38.97	38.81	38.97	3.11 ±0.01	Very strong
	4	0.261	0.263	0.265	59.15	58.84	58.53		
	6	0.146	0.146	0.145	77.15	77.15	77.31		
	8	0.066	0.065	0.066	89.83	89.98	89.83		
	10	0.040	0.039	0.039	93.90	94.05	94.05		

3.4 Toxicity Assay

Table 3: Result of Toxicity Assay of *M. speciosa* Stem Bark

Sample Test	LC_{50} (µg/mL)	Toxicity Category [16]
Methanol extract	219.71	Toxic
n-Hexane fraction	108.09	Toxic
Ethyl acetate fraction	272.79	Toxic
Aqueous fraction	231.06	Toxic

The BSLT is a bioactivity screening method conducted to determine the potential of an extract based on the toxicity level of the secondary metabolites contained therein. The BSLT is performed using *Artemia salina* larvae as the test organisms because their cells are still actively developing and have a sensitive response to environmental stress. In the BSLT, the methanol extract and fractions of *M. speciosa* stem bark were dissolved in dimethyl sulfoxide (DMSO) 1% to

facilitate their dissolution in water. This is because DMSO is an aprotic solvent and is non-toxic at low concentrations; therefore, the mortality of *A. salina* is purely caused by the secondary metabolites rather than the solvent used [16]. The test result for *M. speciosa* stem bark are presented in **Table 3**.

The mortality rate of *A. salina* larvae treated with the methanol extract and the three fractions was classified as active, however, it was not directly correlated with the total flavonoid content. The mechanism of mortality in *A. salina* larvae is related to the functions of secondary metabolites present in the methanol extract and the three fractions of *M. speciosa* stem bark, such as alkaloids, flavonoids, and triterpenoids. The mechanism of action of alkaloid compounds is to cause *A. salina* larvae to fail to receive taste stimuli and become unable to recognize their food by inhibiting taste receptors in the larval mouth, resulting in starvation and death of the *A. salina* larvae. Meanwhile, flavonoid compounds act as stomach poisons that can reduce enzyme activity in the digestive system of *A. salina* larvae. In addition, flavonoid compounds inhibit the transport of Na^+ and K^+ ions, causing uncontrolled levels of Na^+ and K^+ ions across the cell membrane, which leads to cell membrane rupture. Furthermore, triterpenoid compounds act by competing with cholesterol, thereby reducing cholesterol absorption in the intestine. The reduction of cholesterol in the body of *A. salina* larvae causes an imbalance in the metabolic system and leads to the death of *A. salina* larvae [22].

4 CONCLUSION

Based on the results of this study, it was found that the methanol extract, *n*-hexane fraction, ethyl acetate fraction, and aqueous fraction of *M. speciosa* stem bark contained phenolics, flavonoids, quinones, alkaloids, triterpenoids, steroids, and saponins, with total flavonoid contents of 83.91, 15.74, 91.38, and 143.71 mg QE/g, respectively. In the antioxidant activity assay, the methanol extract, *n*-hexane fraction, ethyl acetate fraction, and aqueous fraction of *M. speciosa* stem bark had IC_{50} values of 86.97, 311.51, 159.37, and 24.06 µg/mL, respectively, while the toxicity assay showed LC_{50} values of 219.71, 108.09, 272.79, and 231.06 µg/mL, respectively, which were categorized as toxic. In conclusion, the extract and fractions of *M. speciosa* stem bark exhibited antioxidant activity and potentially possessed cytotoxic activity.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

REFERENCES

[1] Pooja G, Shweta S, Patel P. Oxidative Stress and Free Radicals in Disease Pathogenesis: a Review. *Discover Medicine*. 2025;2(1):104.

[2] Sengnon N, Vonghirundecha P, Chaichan W, Juengwatanatrakul T, Onthong J, Kitprasong P, Sriwiriyajan S, Chittrakarn S, Limsuwanchote S, Wungsintaweekul J. Seasonal and Geographic Variation in Alkaloid Content of Kratom (*Mitragyna speciosa* (Korth.) Havil.) from Thailand. *Plants*. 2023;12(4):949.

[3] Mislan, Hendra M, Lariman, Trimurti S, Anwar Y, Rahman N, Iskandar A, Pratama A, Aprianti DA. Laporan Studi Keanekaragaman Hayati Di Danau Kaskade Mahakam Tahun 2022. Dinas Lingkungan Hidup Provinsi Kalimantan Timur; 2022.

[4] Novianry V, Astuti P, Andriani. Comparative Analysis of Mitragynine Content in Kratom Leaves (*Mitragyna speciosa* Korth) from Kabupaten Kapuas Hulu Using HPLC Method. *Sciscitatio*. 2024;5(2):87-93.

[5] Sofia N, Yuniarti, Rosidah. Phytochemical Test on Chratomic Medicine Plants (*Mitragyna speciosa*) at KHDTK ULM. *Jurnal Sylva Scienteae*. 2022;5(2):218–224.

[6] Yuniarti R, Nadia S, Alamanda A, Zubir M, Syahputra RA, Nizam M. Characterization, Phytochemical Screenings and Antioxidant Activity Test of Kratom Leaf Ethanol Extract (*Mitragyna speciosa* Korth) Using DPPH Method. *Journal of Physics: Conference Series*. 2020;1462(1):012026.

[7] Kaewchompoo S, Temkitthawon P, Phumlek K, Waranuch N, Ngamdokmai N, Ingkaninan K. Cosmeceutical Potential of *Mitragyna speciosa* (Kratom): Anti-Adipogenic and Antioxidant Properties of Extracts and Mitragynine. *Molecules*. 2025;30(4256):1-14.

[8] Normaidah, Najahidin M, Rahmah M, Fadlilaturrahmah, Izma H. Uji Sun Protection Factor (SPF) Ekstrak Etanol Daun *Mitragyna speciosa* Korth. *Jurnal Pharmascience*. 2023;10(2):386–393.

[9] Salim HM, Choirotussanijjah, Awwalia ES, Alam IP. Anti-inflammatory Effects and Potential Mechanisms of *Mitragyna speciosa* Methanol Extract on λ-Karagenan-Induced Inflammation Model. *Bali Medical Journal*. 2022;11(3):1172–1175.

[10] Maulia SW, Jubaidah S, Siswanto E. Uji Toksisitas Akut Ekstrak Etanol Daun Kratom (*Mitragyna speciosa* Korth.) dengan Metode Maserasi dan Refluks terhadap Larva *Artemia salina* Leach. *Prosiding Seminar Nasional Kesehatan*. 2021;1:75–85.

[11] Phromchan W, Defri I, Saensano C, Chookaew A, Chlarawipa R, Sriwiriyajan S. Morphological and Physiological Properties of Kratom (*Mitragyna speciosa*) Leaves: Macronutrients, Phytochemicals, Antioxidants, and Mitragynine Content. *Plant Science Today*. 2024;11(2):762-770.

[12] Putri M, Rusmiyanto EPW, Kurniatuhadi R. Potensi Ekstrak Metanol Akar dan Batang Kratom (*Mitragyna speciosa* Korth.) sebagai Antibakteri *Propionibacterium acnes* ATCC 6919 Penyebab Jerawat. *Jurnal Protobiont*. 2023;12(2):43–49.

[13] Erwin E, Pusparohmana WR, Sari IP, Hairani R, Usman U. GC-MS profiling and DPPH radical scavenging activity of the bark of Tampoi (*Baccaurea macrocarpa*). *F1000Research*. 2019;7(1977):1–19.

[14] Harborne JB. *Phytochemical Methods*. 3rd ed. Chapman & Hall; 1998.

[15] Chang CC, Yang MH, Wen HM, Chern JC. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. *Journal of Food and Drug Analysis*. 2002;10(3):178–182.

[16] Erwin, Nuryadi D, Usman. Skrining Fitokimia dan Bioaktivitas Tumbuhan Bakau Api-Api Putih (*Avicennia alba* Blume). *Jurnal Sains dan Kesehatan*. 2020;2(4):311–315.

[17] Rahmawati EM, Marliana E, Ruga R. Phytochemical Analysis and Screening of Antibacterial Activity of White Pacing Flower (*Costus Speciosus* (J. Koening) Sm*.). Proceedings of the International Conference of Tropical Studies and Its Applications*. 2022:266–277. Atlantis Press.

[18] Erwin, Rahmadani IA, Alimuddin, Ridhay A. Penentuan Kadar Flavonoid Total Ekstrak Daun, Kulit Batang, dan Batang Tumbuhan Afrika (*Vernonia amygdalina* Del). *Ulin: Jurnal Hutan Tropis*. 2022;6(2):197–203.

[19] Shraim AM, Ahmed TA, Rahman MM, Hijji YM. Determination of Total Flavonoid Content by Aluminium Chloride Assay: Critical Evaluation. *LWT – Food Science and Technology*. 2021;150:111932.

[20] Wakeel A, Jan SA, Ullah I, Shinwari ZK, Xu M. Solvent Polarity Mediates Phytochemical Yield and Antioxidant Capacity of *Isatis tinctoria*. *PeerJ*. 2019;7:e7857.

[21] Njus D, Asmaro K, Li G, Palomino E. Redox Cycling of Quinones Reduced by Ascorbic Acid. *Chemico-Biological Interactions*. 2023; 373(1):110397.

[22] Karolina A, Pratiwi DR, Erwin. Phytochemical and Toxicity Test of Merung Extracts (*Coptosapelta tomentosa* (Blume). *Jurnal Atomik*. 2018;3(2):79–82.