

# Antioxidation, antiglycation, and cytoprotective potential of various kratom (*Mitragyna speciosa*) leaf extracts and its major alkaloid, mitragynine

Rinnapat Boonsut<sup>1</sup>, Juraithip Wungsintaweekul<sup>2</sup>, Charoenwong Premprasert<sup>2</sup>, Narumon Sengnon<sup>2</sup>, Ilham Hama<sup>2</sup>, Supattra Limsuwanchote<sup>1\*</sup> 

<sup>1</sup>Division of Health and Applied Sciences (Pharmacology Program), Faculty of Science, Prince of Songkla University, Hat Yai, Thailand.

<sup>2</sup>Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Thailand.

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## ABSTRACT

Oxidative stress and advanced glycation end-products (AGEs) contribute to cellular damage and the progression of chronic diseases. Kratom (*Mitragyna speciosa* (Korth.) Havil.) has been traditionally used for various therapeutic purposes; however, its antioxidative and cytoprotective properties remain inadequately characterized. This study evaluated the antioxidant, antiglycation, and cytoprotective activities of kratom leaf extracts, including aqueous (KW), methanol (KM), alkaloid (KA), and its major alkaloid, mitragynine (MG), using chemical and cell-based assays. Total tannin, flavonoid, phenolic, and MG contents were quantified. MG exhibited strong radical scavenging activity in 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) ( $IC_{50} = 12.02 \pm 0.43 \mu\text{g/ml}$ ) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) ( $IC_{50} = 677.50 \pm 55.24 \mu\text{g/ml}$ ) assays. In the ferric reducing antioxidant power assay, KW and KM rapidly reduced ferric ions within 5 minutes. KM showed the highest antiglycation activity in the BSA-glucose model ( $IC_{50} = 4.98 \pm 0.76 \mu\text{g/ml}$ ). Notably, KW significantly reduced intracellular reactive oxygen species by 27.72%–98.45% and malondialdehyde levels by 2.9–10.4-fold, protecting HepG2 cells from  $H_2O_2$ -induced cytotoxicity. These findings suggest that KW is a promising natural source for mitigating oxidative damage. Further studies should characterize its bioactive constituents to clarify potential synergistic mechanisms and assess their therapeutic relevance in oxidative stress-related disease models.

## INTRODUCTION

Oxidative stress originates from a disparity between free radicals and antioxidants, leading to cellular damage. Excessive production of free radicals can harm biological molecules such as lipids, proteins, and DNA and disrupt redox signaling. This damage contributes to the development of a wide range of diseases, including metabolic, cardiovascular, and neurodegenerative disorders and cancers [1].

A key consequence of oxidative stress is the formation of advanced glycation end-products (AGEs), which result from

non-enzymatic reactions between amino acids and glucose. AGEs can further amplify reactive oxygen species (ROS) production and impair antioxidant defenses, promoting the onset and progression of various diseases [2]. Compounds that can scavenge free radicals or inhibit glycation reactions are, therefore, considered to have broad therapeutic potential. Natural antioxidants, including flavonoids, phenolics, alkaloids, and terpenes, have demonstrated antiglycation activity, suggesting their potential role in mitigating oxidative stress- and AGEs-related diseases [2,3].

*Mitragyna speciosa* (Korth.) Havil., commonly known as kratom, is classified within the Rubiaceae family. Recently, kratom use has gained considerable attention. The most appealing aspect of kratom is its analgesic properties [4]. In traditional medicine, kratom has also been widely used for managing diabetes [5]. Supporting this ethnomedical use,

\*Corresponding Author

Supattra Limsuwanchote, Division of Health and Applied Sciences (Pharmacology Program), Faculty of Science, Prince of Songkla University, Hat Yai, Thailand. E-mail: [supattra.l@psu.ac.th](mailto:supattra.l@psu.ac.th)

oxidative stress is known to play a significant role in type 2 diabetes mellitus (T2DM), where long-term hyperglycemia enhances free radical production and contributes to the formation of AGEs [6]. Apart from its well-known analgesic effects, kratom also exhibits various pharmacological activities, including antioxidant, antibacterial, anti-inflammatory, and antidepressant effects [7]. The bioactive compounds in kratom, particularly mitragynine (MG) and 7-hydroxymitragynine (7-OH-MG), both indole alkaloids, have been identified as the primary molecules responsible for its analgesic effects [8–10].

Despite reports on the antioxidant and antidiabetic properties of kratom, including its ability to scavenge DPPH and ABTS radicals [11], increase glucose transporter activity in muscle cells [12], inhibit alpha-glucosidase [13], and control blood glucose levels in diabetic rats [14], the specific mechanisms and substances responsible for these effects remain unclear. Moreover, research on kratom's impact on oxidative stress-related conditions is limited, with most studies primarily focusing on methanol extracts and chemical assays. To address these gaps, the present study uniquely evaluates a range of kratom extracts, specifically aqueous (KW), methanol (KM), and alkaloid (KA) extracts separately, along with its major alkaloid, MG. This design allows for a broader investigation into the contributions of both alkaloid and non-alkaloid constituents. The study investigates their antioxidant and antiglycation effects using both chemical assays (DPPH, ABTS, and FRAP assays, BSA-glucose and BSA-methylglyoxal (MGO) models) and the HepG2 liver cell model, which has been relatively underexplored in the existing literature. Furthermore, Pearson correlation analysis between MG content and antioxidant activities was conducted to explore the hypothesis that the bioactivities of kratom may arise not only from MG but also from other bioactive compounds present in kratom. Through this integrated approach, this study aims to provide a clearer understanding of the potential mechanisms or bioactive substances through which kratom may help prevent or mitigate oxidative stress-related complications, thereby contributing novel insights to the field.

## MATERIALS AND METHODS

### Chemicals and reagents

All the chemicals, if not otherwise specified, were obtained from Sigma-Aldrich (St. Louis, USA). Low-glucose Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Thermo Fisher Scientific (Waltham, USA). Gallic acid, 2',7'-dichlorofluorescein diacetate (DCFH-DA), and hydrogen peroxide solution (30%) were obtained from Merck (Darmstadt, Germany). Solvents were of analytical grade. Methanol, ethanol, chloroform, acetonitrile, and glacial acetic acid were purchased from RCI Labscan Limited (Bangkok, Thailand).

### Plant materials

Fully expanded kratom leaves were collected from Surat Thani Province, Thailand. A voucher specimen [Wungsintaweekul, J. N6/006 (PSU)] has been stored at the

Prince of Songkla University Herbarium (PSU Herbarium), situated at the Division of Biological Science, Faculty of Science, Prince of Songkla University, Thailand.

### Preparation of kratom extracts and isolated MG

Kratom leaves were dried at 50°C and then powdered for extract preparation. Three kratom extracts were prepared using different solvents and extraction methods and are hereafter referred to as the aqueous extract (KW), methanol extract (KM), and alkaloid extract (KA). Unless otherwise specified, the term "kratom extracts" refers collectively to these three preparations. KW was prepared by boiling kratom powder (400 g) in water (1 l) for 60 minutes. The filtrates from three separate extractions were combined and lyophilized to yield 68.6 g of KW. KM was obtained by sonicating kratom powder (100 g) in methanol (1 l) for 30 minutes, followed by 16 hours of maceration at room temperature. This process was repeated five times, and the combined extract was concentrated and dried using a rotary evaporator, yielding 14.2 g of KM. KA was prepared using 102 g of kratom powder through an acid–base extraction method, with slight modifications from the procedure outlined by Keawpradub [15]. The resulting yield of KA was 0.18 g. All extracts were stored at 4°C until used.

MG (purity  $\geq 90\%$ ) was isolated using silica gel column chromatography as described by Keawpradub [15].

To prepare the sample stock solution, KW was dissolved in sterile water at 10 mg/ml, whereas KM, KA, and MG were dissolved in DMSO at the same concentration. They were stored at  $-20^{\circ}\text{C}$  until use. In all experiments, the samples were diluted with the appropriate solvents before use.

### HPLC analysis of MG in kratom extracts

The MG content in kratom extracts, including KW, KM, and KA, was analyzed using a previously reported HPLC method [16]. Briefly, the analysis was conducted on a Shimadzu Prominence-*i* LC-2030C 3D Plus system equipped with a VertiSep™ USP C18 HPLC column (4.6  $\times$  250 mm, 5  $\mu\text{m}$ ) (Nonthaburi, Thailand), an autosampler, and a diode array detector. The mobile phase consisted of 20 mM ammonium acetate (pH 6.0) and acetonitrile in a 35:65 ratio, employing isocratic elution at a flow rate of 1 ml/min. Chromatograms were recorded at a wavelength of 225 nm.

### Determination of total phenolic content (TPC)

TPC was determined using the Folin–Ciocalteu method [17]. Each kratom extract (5 mg/ml, 0.5 ml) was mixed with 2.5 ml of 10% (v/v) Folin–Ciocalteu's reagent. After standing at room temperature for 5 minutes, 2.5 ml of 7.5% (w/v)  $\text{NaHCO}_3$  was added to the mixture and incubated for 30 minutes. Methanol was used as a blank. Gallic acid (7.8–125  $\mu\text{g/ml}$ ) was used to prepare the standard curve. The absorbance was then measured at 765 nm. The results were expressed as mg of gallic acid equivalent per gram of kratom extract (mg GAE/g extract). All experiments were performed in triplicate.

### Determination of total tannin content (TTC)

TTC was determined using the gravimetric method [18]. Polyvinylpyrrolidone (PVPP) 100 mg was added

into 1 ml of each kratom extract (5 mg/ml), where the TPC had previously been measured. The mixture was then stirred and allowed to stand at 4°C for 15 minutes. It was subsequently centrifuged at 3000 rpm for 10 minutes. The supernatant was analyzed for non-tannin phenolic content using the Folin–Ciocalteu method. The tannin content was calculated by subtracting the non-tannin phenolic content from the total phenolic content. The results were expressed as mg GAE/g extract. All experiments were performed in triplicate.

#### Determination of total flavonoid content (TFC)

The TFC was evaluated using the aluminum chloride method [19]. Each kratom extract (5 mg/ml) was appropriately diluted, and then 125 µl of each diluted extract was mixed with 75 µl of 5% NaNO<sub>2</sub>. After 6 minutes of incubation, 150 µl of 10% AlCl<sub>3</sub> was added and allowed to stand at room temperature for 5 minutes. Subsequently, 750 µl of 1 M NaOH was mixed in, and distilled water was added to bring the total volume to 2.5 ml. The reaction mixture was incubated for 15 minutes at room temperature, and the absorbance was then measured at 510 nm. The TFC was calculated using a quercetin standard curve ranging from 2.5 to 50 µg/ml and expressed as mg of quercetin equivalents per gram extract (mg QE/g extract). All experiments were performed in triplicate.

#### Antioxidant activity assays

##### DPPH assay

An aliquot of 20 µl of each kratom extract and MG (0.31–5 mg/ml) was combined with 180 µl of 0.15 mM DPPH and allowed to react for 30 minutes at room temperature in the dark. The absorbance was read at 517 nm [20]. Gallic acid, at a concentration ranging from 5 to 50 µg/ml, was used for preparing standard curve. The percentage of inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{[A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank sample}})]}{A_{\text{control}}} \times 100$$

where  $A_{\text{control}}$  refers to the absorbance of the control,  $A_{\text{sample}}$  is the absorbance of the test samples, and  $A_{\text{blank sample}}$  represents the absorbance of the sample without DPPH. The results were reported as IC<sub>50</sub> values, which were derived from plotting the % inhibition against the concentration. All the samples were analyzed in triplicate.

##### ABTS radical scavenging assay

The ABTS solution was prepared by mixing 7 mM ABTS with 7.35 mM K<sub>2</sub>SO<sub>4</sub> at a ratio of 2:1. The solution was then diluted with ethanol to obtain an absorbance of 0.7±0.02 at 734 nm. The 10 µl of each kratom extract and MG (5–100 µg/ml in methanol or water) were gently mixed with 190 µl of the ABTS solution in the 96-well plates. The mixtures were then incubated in the dark at room temperature for 6 minutes [21]. Trolox was used as the positive control. The ABTS scavenging activity was calculated using the following formula:

$$\% \text{ ABTS radical scavenging} = \frac{[A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank sample}})]}{A_{\text{control}}} \times 100$$

where  $A_{\text{control}}$  is the absorbance of the control,  $A_{\text{sample}}$  is the absorbance of the test samples, and  $A_{\text{blank sample}}$  denotes the absorbance of the sample without the ABTS solution. The results were reported as IC<sub>50</sub> values, calculated by plotting the % ABTS radical scavenging against the concentration. All the samples were analyzed in triplicate.

##### FRAP assay

FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM FeCl<sub>3</sub> in a volume ratio of 10:1:1. Subsequently, 10 µl of each kratom extract and MG (10 mg/ml) or gallic acid (15.6–500 µM) were combined with 300 µl of FRAP reagent and thoroughly mixed [22]. The absorbance at 593 nm was then measured at 5, 15, 30, 45, and 60 minutes of incubation. The results were expressed as mM of gallic acid equivalents per gram extract (mM GAE/g extract). All the samples were analyzed in triplicate.

#### Antiglycation assays

##### BSA-glucose model

The antiglycation assay with the BSA-glucose model was conducted as described by Yagi *et al.* [21] with a slight modification. Briefly, each kratom extract, MG (1–100 µg/ml) or aminoguanidine (1–100 µg/ml) as a positive control, was combined with the reaction mixture containing BSA solution (50 mg/ml), 800 mM glucose, and 0.02% (w/v) sodium azide, in a volume of 20 µl each. Following a 14-day incubation at 37°C, the resulting pellets were washed with 5% trichloroacetic acid (TCA) (60 µl) and then dissolved in 10 mM PBS (pH 10, 60 µl). Fluorescence intensity was measured at excitation and emission wavelengths of 370 and 440 nm, respectively, using Varioskan™ LUX multimode microplate reader (Thermo Scientific, Vantaa, Finland). The percentage of AGEs inhibition was calculated as:

$$\% \text{ AGEs inhibition} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

where  $A_{\text{sample}}$  is the fluorescence of the reaction mixture with sample, and  $A_{\text{control}}$  is the fluorescence of the reaction mixture without sample. All the samples were analyzed in triplicate.

##### BSA-methylglyoxal (MGO) model

The BSA-MGO model assay was performed according to the published method [23]. AGEs were analyzed by mixing each sample (1–100 µg/ml) or aminoguanidine (1–100 µg/ml) with BSA solution (20 mg/ml) and 60 mM MGO, with a volume of 50 µl each. The mixture was then incubated at 37°C for 14 days. Sodium azide (0.02% w/v) was used as a preservative. Fluorescence intensity was measured at excitation and emission wavelengths of 340 and 420 nm, respectively. Each sample was analyzed in triplicate. The inhibition of AGEs formation was calculated using the same formula as previously described.



## Cell culture experiment

### Cell culture

HepG2 cells (ATCC® HB-8065™) were grown in low-glucose DMEM supplemented with 10% FBS and maintained at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. The culture medium was changed every 2–3 days. Cells were sub-cultured upon reaching approximately 80%–90% confluence and subsequently used for further studies.

### Cell viability assay

Cell viability assay was assessed using MTT assay [24]. HepG2 cells ( $2 \times 10^4$  cells/well) were treated with various concentrations of kratom extracts and MG (1.56–500 µg/ml) for 24 hours. After washing with 10 mM PBS (pH 7.4), an MTT solution (0.5 mg/ml) was added to the wells. The absorbance of the resulting formazan was measured at a wavelength of 570 nm using SPECTROstar Nano microplate reader (BMG LABTECH, Ortenberg, Germany). All experiments were carried out in triplicate. The concentration of compounds that yielded at least 80% cell viability was considered non-cytotoxic.

### Cytoprotective assay

Cytoprotective effect of kratom extracts and MG were assessed against H<sub>2</sub>O<sub>2</sub>-induced toxicity in HepG2 cells using an MTT assay [25]. Initially, HepG2 cells were incubated for 24 hours in the presence of H<sub>2</sub>O<sub>2</sub> (0.5–5 mM) to determine the appropriate cytotoxic concentration of H<sub>2</sub>O<sub>2</sub>. Once the optimal concentration of H<sub>2</sub>O<sub>2</sub> was obtained, the cells were pre-treated for 24 hours with non-cytotoxic concentrations of kratom extracts and MG and thereafter treated with H<sub>2</sub>O<sub>2</sub> (1.5 mM) for an additional 6 hours. Each experiment was performed in triplicate using epicatechin (100 µM) as a positive control.

### Intracellular ROS formation assay

Intracellular ROS levels were measured using the DCFH-DA assay [25]. HepG2 cells were seeded into 48-well plates at a density of  $2.5 \times 10^4$  cells/well. Following a 24-hour exposure to non-cytotoxic concentrations of kratom extracts and MG, the cells were rinsed with 10 mM PBS (pH 7.4) and then incubated with 10 µM DCFH-DA for 30 minutes at 37°C in the dark. After that, the cells were washed with ice-cold PBS and subsequently treated with 1.5 mM H<sub>2</sub>O<sub>2</sub> in a serum-free medium for 1 hour. The cells were then washed, lysed, and centrifuged at  $13000 \times g$  for 10 minutes at 4°C. Fluorescence intensity in the supernatant was measured at 485 nm for excitation and 530 nm for emission wavelengths. The results were normalized to protein concentration, as estimated by the Bradford assay (Bio-Rad, California, USA), and reported as a percentage of ROS formation relative to untreated cells. Each experiment was conducted in triplicate, with 100 µM epicatechin serving as a positive control.

### Lipid peroxidation assay

MDA levels in HepG2 cells were measured using a thiobarbituric acid-reactive substance (TBARS) assay with modifications to the published method [25]. HepG2 cells were seeded into 6-well plates at a density of  $2 \times 10^5$  cells/well. The

cells were pre-incubated with non-cytotoxic concentrations of kratom extracts and MG for 24 hours, followed by treatment with 1.5 mM H<sub>2</sub>O<sub>2</sub> in a serum-free medium for 6 hours. The cells were then gently scraped and centrifuged at 12000 rpm for 10 minutes. The collected cell pellets were re-suspended in 25 mM Tris-HCl buffer (pH 7.4) and sonicated for 5 minutes. MDA levels were analyzed by combining the supernatant with a TBA solution, followed by heating at 90°C for 20 minutes, cooling on ice for 10 minutes, and then centrifuging at 12000 rpm for 10 minutes. The absorbance was measured at 532 nm. The results were normalized to protein concentration and expressed as nmol MDA equivalents per mg protein.

### Statistical analysis

Data were presented as mean  $\pm$  SEM or mean  $\pm$  SD ( $n = 3$ ), as indicated. Statistical analysis was performed using SPSS (version 25, USA). Differences in the means between two independent groups were examined with the independent samples t-test, while one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test, was used to compare differences among multiple treatment groups. A p-value  $< 0.05$  was considered statistically significant. The correlation between MG content and antioxidant and antiglycation activities was assessed using Pearson's correlation analysis.

## RESULTS AND DISCUSSION

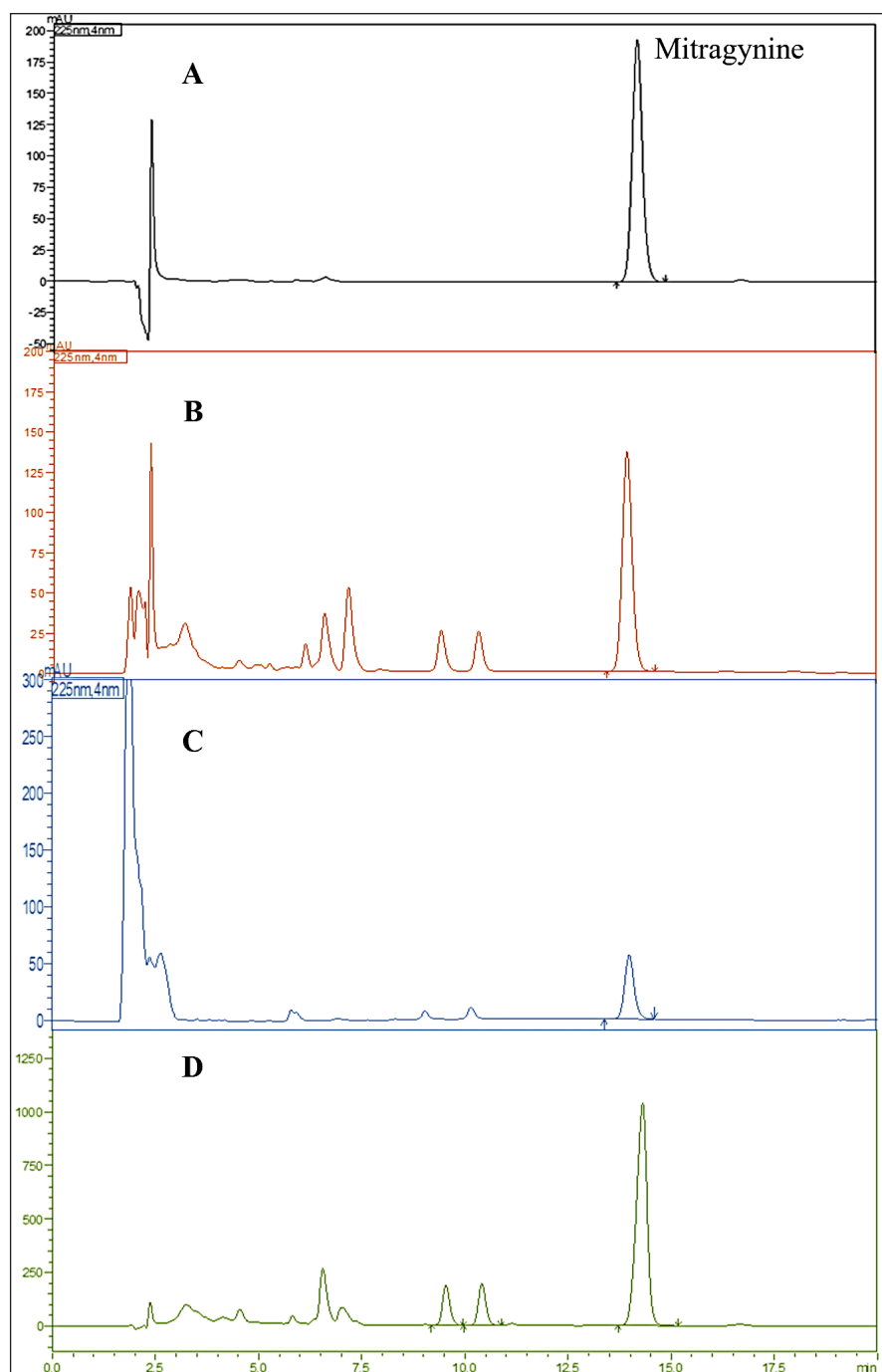
### Phytochemical analysis of kratom extracts

Kratom, a medicinal plant widely utilized across Southeast Asia, has garnered global interest due to its diverse pharmacological activities. Traditionally, it has been used to treat various ailments, particularly pain, diarrhea, and diabetes. The primary active therapeutic substances in kratom are alkaloids, which are predominantly concentrated in its leaves [26,27]. Among these, MG is the most abundant indole alkaloid and has been suggested to be responsible for several pharmacological effects. Additionally, kratom leaves contain flavonoids, tannins, and triterpenoids, which also exhibit important pharmacological activities. However, it has been reported that the phytochemical profile of kratom, particularly its alkaloid content, varies depending on geographical location and climatic conditions, potentially influencing both its therapeutic efficacy and toxicity profile [16,27]. Given these variations, this study extracted kratom leaves using methanol and water and additionally prepared crude alkaloid extracts and purified MG. They were subjected to comparative analyses of their antioxidant, antiglycation, and cytoprotective properties. Additionally, phytochemical screening was performed to identify the chemical constituents present in each extract. The results revealed the presence of phenolics, flavonoids, tannins, and alkaloids in kratom extracts. As shown in Table 1, TPC was observed in higher amounts in KA compared to KM and KW, whereas TTC was more prominent in KM and KW ( $p < 0.05$ ). The TFC was similar among the three extracts. These findings contrast with those reported by Parthasarathy *et al.* [28], which found that the methanolic extract exhibited the highest amounts of TPC and TFC compared to alkaloid and aqueous extracts. Additionally, in this study, the HPLC method was employed to standardize each kratom extract based on

**Table 1.** Phytochemical constituents and MG content in kratom extracts.

Kratom extracts	TPC* (mg GAE/g extract)	TTC* (mg GAE/g extract)	TFC* (mg QE/g extract)	MG* (mg/g extract)
KW	65.69 ± 1.64 <sup>a</sup>	40.08 ± 2.91 <sup>a</sup>	11.01 ± 0.72 <sup>a</sup>	8.28 ± 0.32 <sup>a</sup>
KM	78.86 ± 7.91 <sup>b</sup>	35.18 ± 1.66 <sup>a</sup>	10.72 ± 0.83 <sup>a</sup>	76.46 ± 3.61 <sup>b</sup>
KA	129.40 ± 3.67 <sup>c</sup>	18.04 ± 2.76 <sup>b</sup>	11.25 ± 0.68 <sup>a</sup>	213.83 ± 17.53 <sup>c</sup>

\* Data are presented as mean ± SD ( $n = 3$ ). Different superscript within the column indicates significant difference ( $p < 0.05$ ).



**Figure 1.** HPLC chromatogram: (A) Mitragynine (MG); (B) Methanol extract (KM); (C) Aqueous extract (KW); (D) Alkaloid extract (KA).

its MG content. The HPLC chromatogram displayed a peak corresponding to MG with a retention time of 13.9 minutes, as shown in Figure 1. Among the extracts, the highest amount of MG was found in KA, followed by KM and KW (Table 1). Notably, the acid–base extraction process yielded an MG concentration approximately three times higher than that of KM. The lower MG content in KW is likely due to its lipophilic nature and poor water solubility [29]. These findings suggest that KA is rich in both TPC and MG, while KM and KW contain lower levels of these compounds.

Antioxidant activity of kratom extracts and MG

Preventing oxidative stress-induced cell damage is a promising strategy for managing chronic diseases and their complications. Oxidative stress leads to alterations in biological

Table 2. IC<sub>50</sub> values of kratom extracts and MG in DPPH, ABTS, and antiglycation assays.

Samples	DPPH (µg/ml)	ABTS (µg/ml)	Antiglycation* (µg/ml)
Gallic acid	16.10 ± 0.34 <sup>a</sup>	–	–
Trolox	–	5.43 ± 0.10 <sup>a</sup>	–
KW	1225 ± 50.32 <sup>b</sup>	51.04 ± 2.63 <sup>b</sup>	30.05 ± 0.53 <sup>b</sup>
KM	992.26 ± 26.39 <sup>c</sup>	84.45 ± 4.67 <sup>c</sup>	4.98 ± 0.76 <sup>a</sup>
KA	850.43 ± 74.73 <sup>d</sup>	27.87 ± 3.14 <sup>d</sup>	44.99 ± 11.47 <sup>b</sup>
MG	677.50 ± 55.24 <sup>c</sup>	12.02 ± 0.43 <sup>a</sup>	43.39 ± 14.67 <sup>b</sup>

Gallic acid and trolox were used as positive controls for the DPPH and ABTS assays, respectively. Data are presented as mean ± SD (*n* = 3). Different superscripts within the column indicate significant difference (*p* < 0.05). \* represents antiglycation activity determined by the BSA-glucose assay.

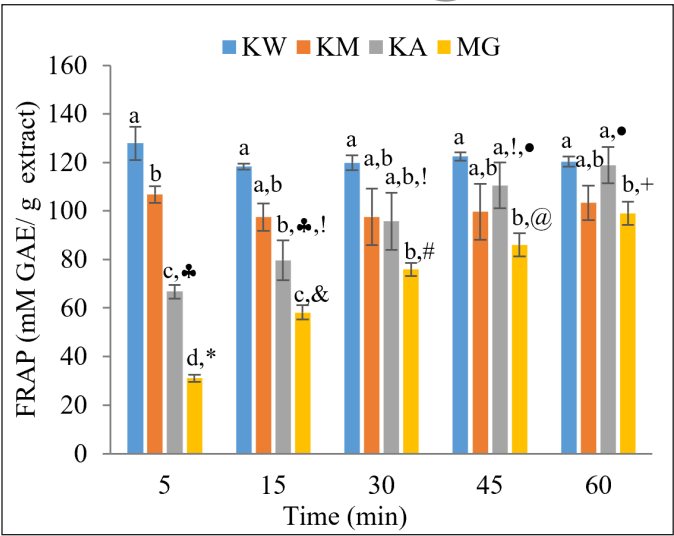
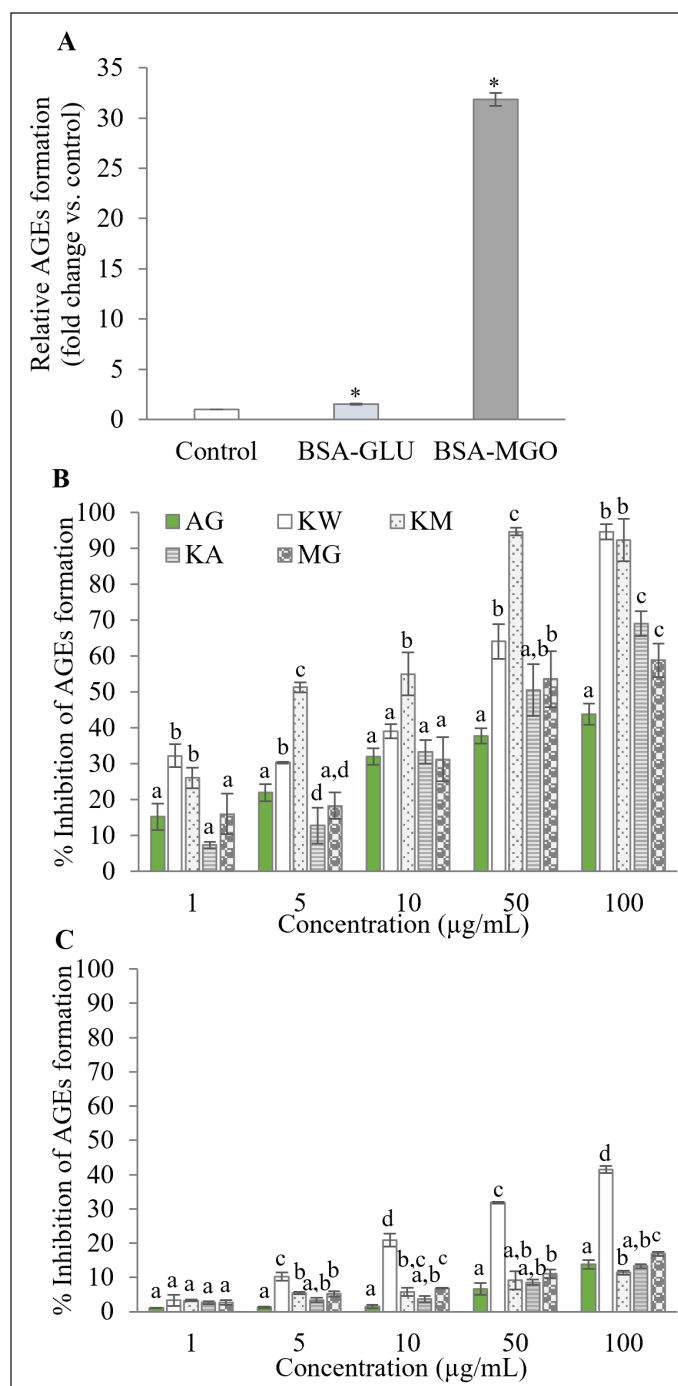


Figure 2. FRAP assay results for KW, KM, KA, and MG at various time points from 5 to 60 minutes of incubation. Data are presented as mean ± SD (*n* = 3). Different symbols represent statistically significant differences between time points within the same sample group, and different alphabetic letters represent statistically significant differences between sample groups at the same time point (*p* < 0.05). KW = Aqueous extract; KM = Methanol extract; KA = Alkaloid extract; MG = Mitragnine.

processes, including impaired cellular function, loss of structural integrity, and DNA damage, which can, in turn, promote further free radical production and perpetuate a cycle of cellular injury [30]. Traditional remedies, such as kratom, have been used in folk medicine for managing chronic diseases, including diabetes, and may offer valuable therapeutic insights [5]. To evaluate the antioxidant capacity of kratom extracts and MG, the DPPH, ABTS, and FRAP assays were used. As summarized in Table 2, it was found that MG exhibited the highest radical scavenging ability, followed by KA, KM, and KW, respectively. However, differences in the IC<sub>50</sub> values between the two assays were observed, suggesting that all tested samples demonstrated greater efficacy in scavenging the ABTS radical compared to the DPPH radical. Compared to these assays, the FRAP assay evaluates antioxidant activity based on the reduction potential of metal ions in various substances. The FRAP reaction may exhibit variability depending on the incubation period [31]; in this study, absorbance was measured over a duration of 5 to 60 minutes. Figure 2 illustrates the antioxidant power of each kratom extract and MG. The results showed that KW and KM rapidly reduced Fe<sup>3+</sup> to Fe<sup>2+</sup> within 5 minutes of the reaction. KW expressed stronger activity than KM, with values of 127.92 ± 6.86 and 106.81 ± 3.44 mM GAE/g extract, respectively. Initially, KA and MG displayed limited antioxidant activity when incubated for 5 minutes; however, with prolonged incubation, their activity increased, nearly matching that of KW and KM after 30 minutes. As reported by Rahmawati [32], the alkaloid extract showed a lower FRAP value compared to crude methanol extract after 10 minutes of reaction. This may be attributed to the weakly basic properties of alkaloids, which may interfere with the redox reaction in the FRAP assay, as it is optimized for an acidic pH. Despite this, the FRAP assay in the present study suggests that kratom extracts and MG exhibited good reducing power. Overall, these antioxidant findings are consistent with previous studies demonstrating the antioxidant activity of kratom extracts and MG [13,28,33]. However, variations in IC<sub>50</sub> values have been observed, which may reflect differences in extraction methods, assay conditions, or phytochemical composition.

Antiglycation activity of kratom extracts and MG

Free radicals play a crucial role in the glycation reaction and subsequent AGEs formation, which can contribute to the onset and progression of various diseases, including diabetes mellitus (DM), Alzheimer’s disease, cardiovascular, liver, and kidney diseases. It has been reported that high levels of AGEs are found in these patients, with further increases observed in those with complications. Therefore, targeting AGEs is a key strategy for the management of a broad range of chronic diseases and their complications [2]. Given that natural compounds possessing antioxidant properties have the potential to inhibit glycation or AGEs formation [34], this study examined the effect of kratom extracts and MG in both BSA-glucose and BSA-MGO models under identical conditions (2-week incubation, same concentration range) to allow direct comparison. In the BSA-glucose model, AGEs formation increases by 1.6-fold compared to the blank control group (Fig. 3A). Kratom extracts and MG exhibited dose-dependent



**Figure 3.** Antiglycation assay: (A) Formation of AGEs after 2 weeks of incubation in the BSA-glucose and BSA-MGO assays. (B-C) Antiglycation activity of AG, KW, KM, KA, and MG at 1, 5, 10, 50, and 100 µg/ml in the BSA-glucose (B) and BSA-MGO (C) assays. Data are expressed as % inhibition relative to control and are presented as mean  $\pm$  SD ( $n = 3$ ). \* indicates significant differences compared to the control group ( $p < 0.05$ ) as determined by the independent samples t-test. Different alphabetic letters represent statistically significant differences between sample groups at the same concentration ( $p < 0.05$ ). AG = Aminoguanidine; KW = Aqueous extract; KM = Methanol extract; KA = Alkaloid extract; MG = Mitragynine.

inhibition of AGEs formation. Comparable to aminoguanidine, a prototype antiglycation drug that inhibits AGEs formation

via scavenging reactive carbonyl groups, kratom extracts and MG showed higher antiglycation activity at the same tested concentration (Fig. 3B). Analysis of  $IC_{50}$  values revealed that KM exhibited the highest antiglycation activity, followed by KW, MG, and KA, respectively (Table 2). In contrast, the BSA-MGO model exhibited substantially higher AGEs formation (31.9-fold compared to the blank control, Fig. 3A) due to the high reactivity of MGO. While kratom extracts and MG demonstrated some inhibitory activity (Fig. 3C), the overall inhibition was much lower, and  $IC_{50}$  values could not be determined within the tested concentration range. This may be attributed to the rapid and highly reactive nature of MGO-mediated glycation, which may limit the effectiveness of test compounds at the concentrations used. These results suggest that kratom extracts and MG may primarily exert their antiglycation effects during the early stages of glycation, as represented by the BSA-glucose model, rather than at intermediate stages modeled by MGO. Although the BSA-MGO model remains useful for evaluating carbonyl trapping capacity, further optimization such as testing higher concentrations may be necessary to comprehensively evaluate the antiglycation potential of kratom in this model.

Currently, it has been reported that several natural products, including polyphenolic, flavonoids, terpenoids, alkaloids, and polysaccharides, exhibit antiglycation activity primarily through mechanisms such as scavenging free radicals and reducing highly reactive glycation agents, such as dicarbonyl compounds [34]. Considering the observed antioxidant activity, kratom extracts and MG may partially inhibit glycation through radical scavenging mechanisms. Therefore, kratom may be considered a promising AGE inhibitor.

#### Correlation between MG content and antioxidant and antiglycation activities

To further explore the contribution of MG content to antioxidant and antiglycation activities, Pearson's correlation analysis was performed (Table 3). MG content showed a strong negative correlation with DPPH radical scavenging activity ( $r = -0.917$ ,  $p = 0.001$ ), suggesting its potential contribution to free radical scavenging. In contrast, correlations with ABTS or FRAP at either 5 minutes or 30 minutes were not statistically significant. A moderate, non-significant positive correlation was found between MG and antiglycation activity. This implies that MG may play a partial role in glycation inhibition. Moreover, ABTS and antiglycation activity showed a strong, significant negative correlation ( $r = -0.949$ ,  $p < 0.001$ ), suggesting that some compounds may possess both activities. Overall, MG appears to be more closely linked to radical scavenging than to ferric reducing or antiglycation capacity. The observed correlations support the notion that antioxidant and antiglycation mechanisms may involve distinct groups of phytochemical constituents.

#### Effect of kratom extracts and MG on viability in HepG2 cells

To assess cytotoxicity, HepG2 cells were treated with kratom extracts and MG for 24 hours, followed by the MTT assay. The results (Fig. 4) demonstrated that KW ranging from 6.25 to 500 µg/ml had no substantial toxic effect on cell viability which ranged from  $94.37\% \pm 3.79\%$  to  $105.07\% \pm$

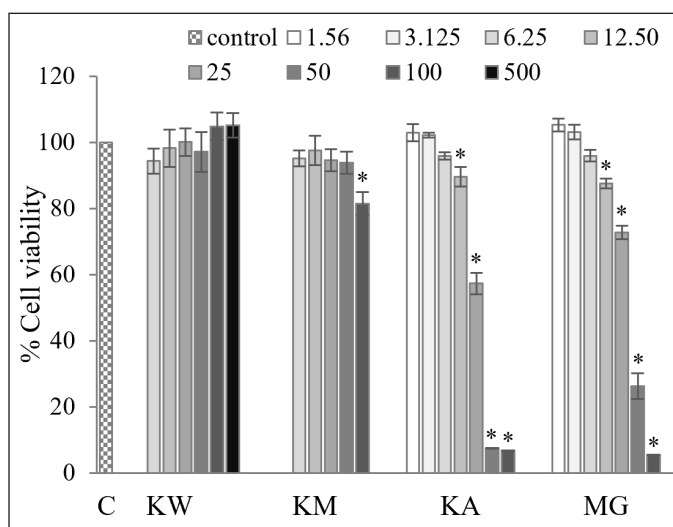


**Table 3.** Pearson correlation coefficients between MG content and antioxidant activities (DPPH, ABTS, and FRAP) and antiglycation activity (BSA-glucose).

	MG <sup>a</sup>	DPPH <sup>b</sup>	ABTS <sup>c</sup>	FRAP <sup>d</sup>	FRAP <sup>e</sup>	Antiglycation <sup>f</sup>
MG	1					
DPPH	−0.917**	1				
ABTS	−0.567	0.230	1			
FRAP5	−0.461	0.721*	−0.448	1		
FRAP30	−0.569	0.633	−0.046	0.698*	1	
Antiglycation	0.503	−0.142	−0.949**	0.457	−0.057	1

<sup>a</sup> MG content (mg/g extract); <sup>b</sup> IC<sub>50</sub> of DPPH; <sup>c</sup> IC<sub>50</sub> of ABTS; <sup>d</sup> FRAP at 5 minutes; <sup>e</sup> FRAP at 30 minutes; <sup>f</sup> IC<sub>50</sub> of antiglycation assay (BSA-glucose model).

\*\* Significant at the 0.01 level (2-tailed); \* Significant at the 0.05 level (2-tailed).



**Figure 4.** HepG2 cell viability after 24-hour exposure to KW (6.25–500 µg/ml), KM (6.25–100 µg/ml), KA (1.56–100 µg/ml) and MG (1.56–100 µg/ml). Data are expressed as % cell viability relative to the untreated control and shown as mean ± SEM (*n* = 3). \* indicates significant differences compared to the control group (*p* < 0.05). C = Control; KW = Aqueous extract; KM = Methanol extract; KA = Alkaloid extract; MG = Mitragynine.

3.68%. In addition, the KM (6.25–100 µg/ml) did not affect cell viability up to 50 µg/ml, with values ranging from 96.76% ± 3.31% to 100.48% ± 4.49%. KA and MG were tested at the same concentration range as KM (6.25–100 µg/ml); however, unlike KM, both KA and MG exhibited significant cytotoxicity at concentrations ≥12.5 µg/ml. KA and MG decreased cell viability with values between 6.77% ± 0.11% to 89.53% ± 3.00% and 5.46% ± 0.12% to 87.56% ± 1.45%, respectively. Therefore, the tested concentration of KA and MG was further reduced to 1.56 µg/ml. At this lower range (1.56–6.25 µg/ml), KA and MG had no significant cytotoxicity effect on cell viability. IC<sub>50</sub> values were calculated to assess the cytotoxic potency. As KW and KM did not reduce cell viability below 50% even at the highest concentration tested, their IC<sub>50</sub> values could not be determined. In contrast, the IC<sub>50</sub> value for KA and MG was calculated as 26.37 ± 0.68 µg/ml and 35.31 ± 2.04 µg/ml,

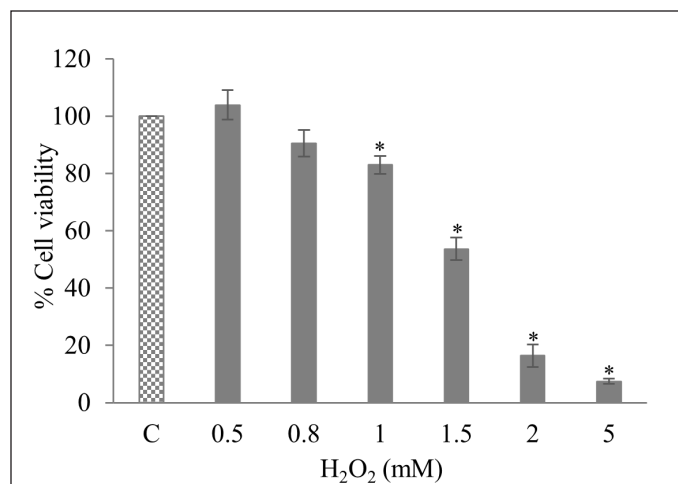
respectively. These findings suggest that KW and KM are likely non-cytotoxic or possess low cytotoxicity toward HepG2 cells, while KA exhibited the highest cytotoxicity among the tested samples. Regarding the obtained results, the working concentration ranges of tested samples for further experiments were as follows: 12.5–500 µg/ml for KW, 12.5–50 µg/ml for KM, and 1.56–6.25 µg/ml for KA and MG. When considered alongside the IC<sub>50</sub> values, these findings indicate that KA and MG possess a narrow therapeutic window, as their cytotoxic concentrations are slightly higher than their effective working ranges.

These results align with previous findings indicating that at high doses of alkaloid extract and MG, over 11.3 µg/ml, they were cytotoxic, as assessed by the trypan blue exclusion method [35]. Several mechanisms underlying MG-induced cytotoxicity have been described. At high concentrations (75 µM), MG has been shown to induce cell cycle arrest at the G1 and S phases, activate caspase-dependent apoptosis, and disrupt membrane integrity in human SH-SY5Y neuronal cells [36]. Additionally, cytotoxicity of MG has been associated with the inhibition of cytochrome P450 (CYP450), particularly CYP2D6, and CYP3A4/5 [37,38]. A more recent study has demonstrated that MG exhibits moderate cytotoxicity toward HepG2 cells, with an IC<sub>50</sub> value of approximately 42 µM while showing non-cytotoxic effects on normal human liver cells (HL-7702) at concentrations below 200 µM. In contrast, paynantheine, the second most abundant kratom alkaloid, exhibited only weak cytotoxicity against both HepG2 and HL-7702 cells [39]. These findings support the view that structural differences among kratom alkaloids contribute to their differential biological activities. Given that the KA contains multiple alkaloids beyond MG, it is possible that synergistic or additive effects among these constituents contribute to the observed cytotoxicity.

#### Effect of kratom extracts and MG on cytoprotective effect in HepG2 cells

The cytoprotective effect of kratom extracts and MG was performed against H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity in HepG2 cells. The concentration of H<sub>2</sub>O<sub>2</sub> which decreased cell viability to about 40%–60% was selected. HepG2 cell viability decreased with an increase in H<sub>2</sub>O<sub>2</sub> concentration. Treatment with 1.5



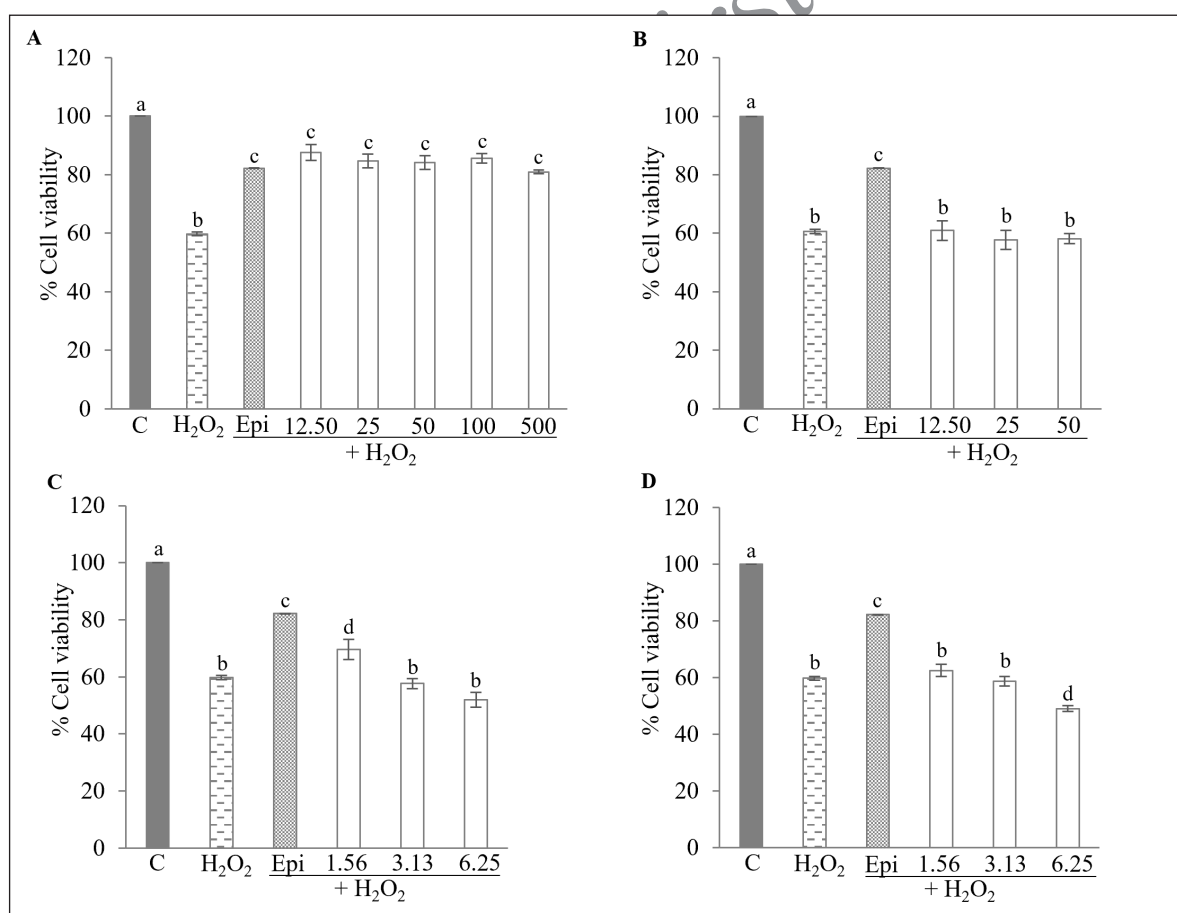


**Figure 5.** HepG2 cell viability after exposure to H<sub>2</sub>O<sub>2</sub> (0.5–5 mM). Data are expressed as % cell viability relative to the untreated control and presented as mean  $\pm$  SEM ( $n = 3$ ). \* indicates significant differences compared to the control group ( $p < 0.05$ ) as determined by the independent samples  $t$ -test. C = Control; KW = Aqueous extract; KM = Methanol extract; KA = Alkaloid extract; MG = Mitragynine.

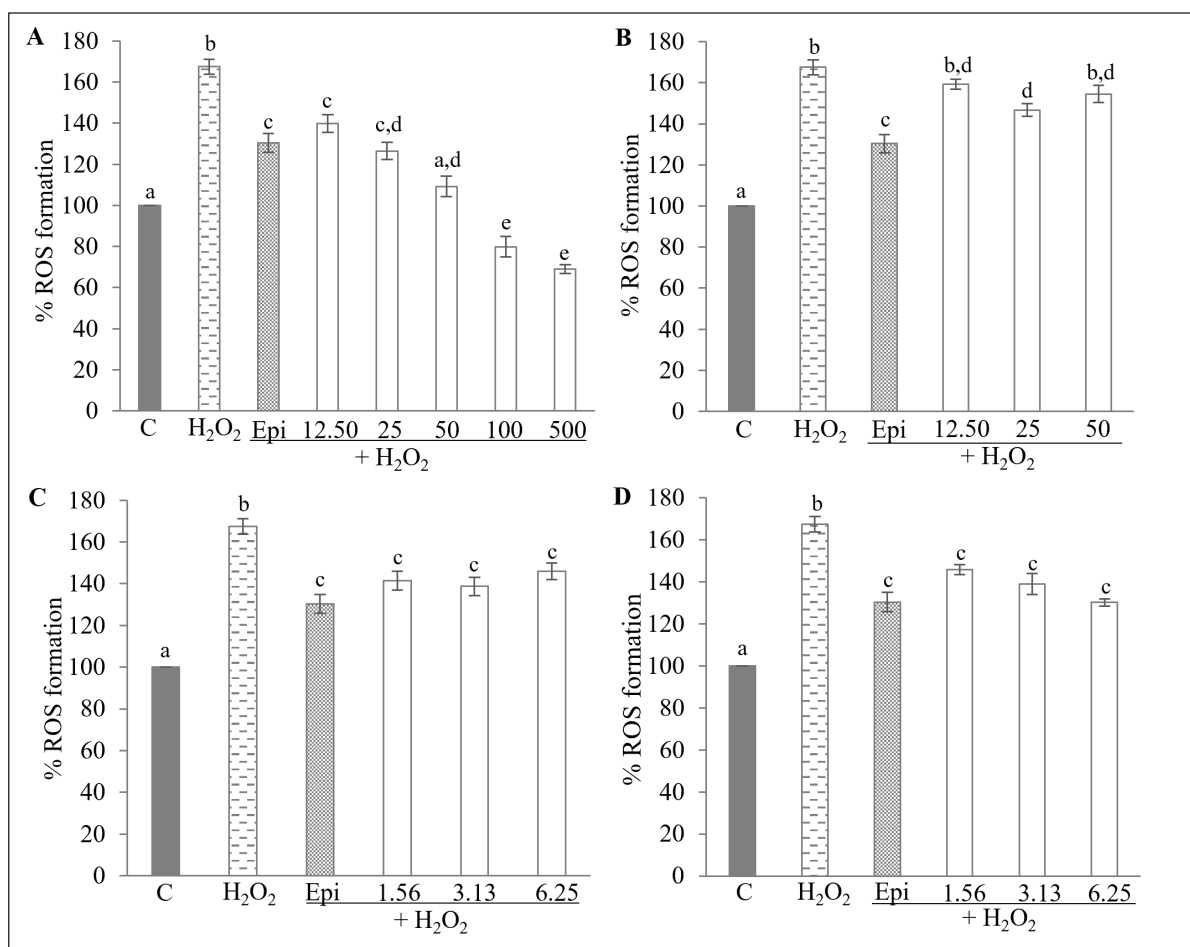
mM H<sub>2</sub>O<sub>2</sub> decreased cell viability to about  $53.64\% \pm 3.90\%$  in comparison to the control group; therefore, this concentration was selected for subsequent cytoprotective experiments (Fig. 5). Figure 6 illustrates the cytoprotective effects of kratom extracts and MG on HepG2 cells exposed to these treatments 24 hours before inducing cytotoxicity with H<sub>2</sub>O<sub>2</sub>. The results show that HepG2 cells treated with KW (12.5–500  $\mu$ g/ml) exhibited a significant ability to prevent H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity by increasing cell viability to about 80.91%–87.55% compared to H<sub>2</sub>O<sub>2</sub> group ( $p < 0.05$ ). This effect was similar to that of epicatechin, a positive standard, which at 100  $\mu$ M increased cell viability to about 82.21% (Fig. 6A). On the other hand, KM (12.5–50  $\mu$ g/ml), KA (1.56–6.25  $\mu$ g/ml), and MG (1.56–6.25  $\mu$ g/ml) did not effective in protecting cells from H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity (Fig. 6B–D). These results suggested that KW exhibits considerable cytoprotective activity in this context.

#### Effect of kratom extracts and MG on ROS formation in HepG2 cells

The effect of kratom extracts and MG on ROS production was evaluated using the fluorescent probe DCFH-DA, which is deacetylated to form DCFH<sub>2</sub> and subsequently



**Figure 6.** Cytoprotective effect of KW, KM, KA, and MG against H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity assay. HepG2 cells were treated with (A) KW (12.5–500  $\mu$ g/ml); (B) KM (12.5–50  $\mu$ g/ml); (C) KA (1.56–6.25  $\mu$ g/ml); and (D) MG (1.56–6.25  $\mu$ g/ml). Data are expressed as % cell viability relative to the untreated control and shown as the mean  $\pm$  SEM ( $n = 3$ ). Different alphabetic letters are significantly different ( $p < 0.05$ ). C = Control; Epi = Epicatechin (100  $\mu$ M); KW = Aqueous extract; KM = Methanol extract; KA = Alkaloid extract; MG = Mitragynine.



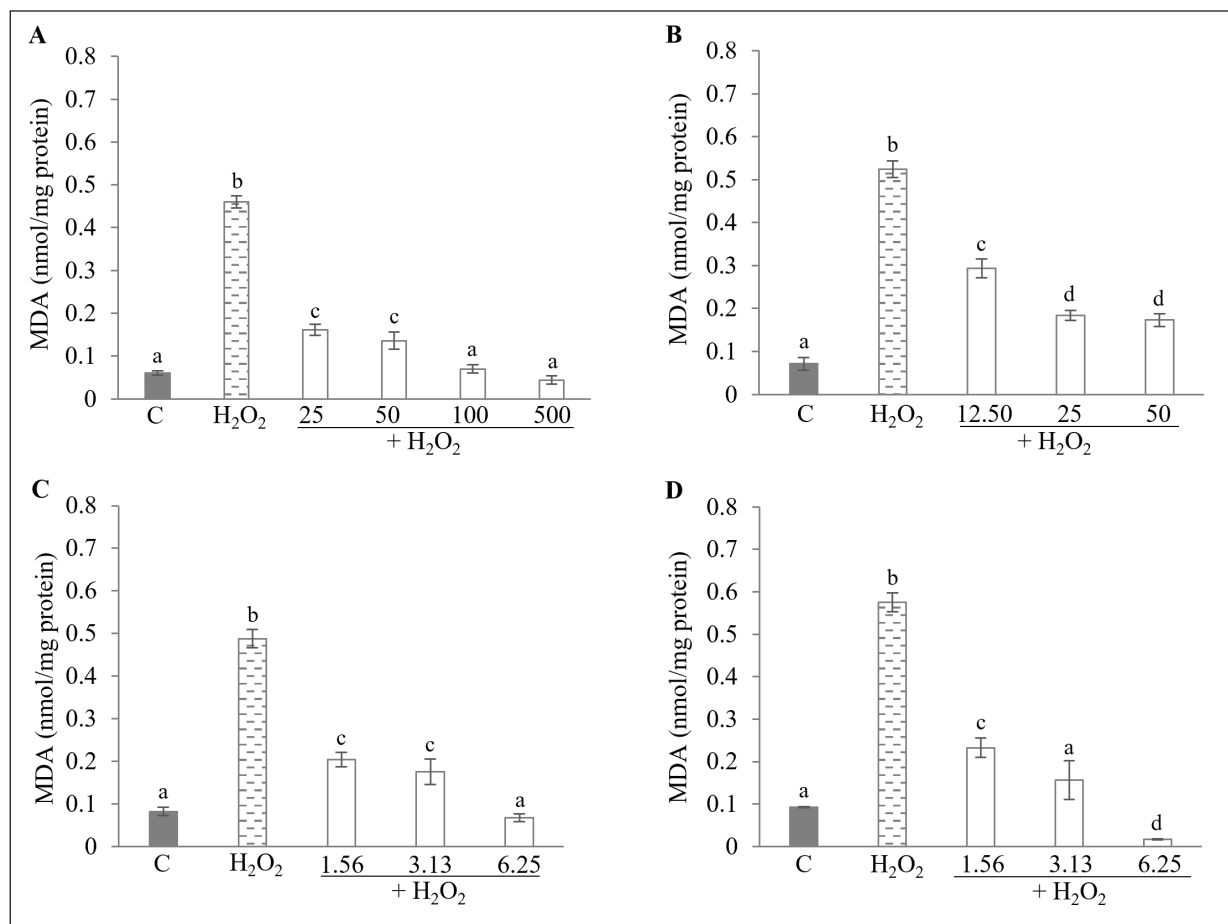
**Figure 7.** Effect of KW, KM, KA, and MG on H<sub>2</sub>O<sub>2</sub>-induced ROS production in HepG2 cells. Cells were treated with (A) KW (12.5–500 µg/ml); (B) KM (12.5–50 µg/ml); (C) KA (1.56–6.25 µg/ml); (D) MG (1.56–6.25 µg/ml). Data are expressed as % ROS formation relative to the untreated control and shown as the mean ± SEM (*n* = 3). Different alphabetic letters are significantly different (*p* < 0.05). C = Control; Epi = Epicatechin (100 µM); KW = Aqueous extract; KM = Methanol extract; KA = Alkaloid extract; MG = Mitragynine.

reacted with intracellular ROS to produce the fluorescent compound dichlorofluorescein (DCF). As shown in Figure 7, treatment with H<sub>2</sub>O<sub>2</sub> led to a 1.7-fold increase in intracellular ROS compared to the control group (*p* < 0.05). However, when HepG2 cells were pretreated with kratom extracts or MG, ROS levels decreased relative to the H<sub>2</sub>O<sub>2</sub>-treated group. In the KW-treated group (Fig. 7A), the level of ROS significantly decreased (*p* < 0.05) in a concentration-dependent manner, with a reduction of 27.72%–98.45% compared to the H<sub>2</sub>O<sub>2</sub> group. The positive control, 100 µM epicatechin, suppressed ROS by 37.16%. KM showed a slight decrease in ROS level at a concentration of 25 µg/ml by 20.78% compared to the H<sub>2</sub>O<sub>2</sub> group (*p* < 0.05) (Fig. 7B). A significant decrease in ROS was observed in HepG2 cells treated with KA and MG by 21.52%–28.78% and 21.77%–37.32%, respectively (Fig. 7C, D). Additionally, it has been reported that both crude methanol and alkaloid extracts can significantly suppress ROS formation in RAW 264.7 macrophages treated with lipopolysaccharides (LPS) and H<sub>2</sub>O<sub>2</sub> at concentrations of 100 and 25 ppm, respectively [32]. These results suggest that kratom extracts and MG effectively mitigate

ROS production to varying degrees, reflecting differences in antioxidant potential among the tested samples.

#### Effect of kratom extracts and MG on lipid peroxidation in HepG2 cells

Lipid peroxidation is a process triggered by oxidative stress, in which polyunsaturated fatty acids (PUFAs) are attacked by ROS. MDA, a primary byproduct of lipid peroxidation, is commonly used as a biomarker to assess oxidative stress. Excessive MDA accumulation can lead to pathological diseases, including diabetes, liver disease, cardiovascular diseases, cancers, and neurodegenerative disorders [40]. Natural compounds that attenuate lipid peroxidation products may serve as promising agents for the prevention and treatment of lipid peroxidation-associated diseases [41]. As shown in Figure 8, HepG2 cells treated with H<sub>2</sub>O<sub>2</sub> alone showed a significant increase in MDA levels, approximately 5.9–7.6 times higher than those in the control group (*p* < 0.05). Interestingly, pretreatment with various kratom extracts and MG substantially reduced MDA production in a dose-dependent manner. In the group treated with KW (25–500 µg/ml), MDA levels decreased



**Figure 8.** Effect of KW, KM, KA, and MG on H<sub>2</sub>O<sub>2</sub>-induced lipid peroxidation in HepG2 cells. Cells were treated with (A) KW (25–500 μg/ml); (B) KM (12.5–50 μg/ml); (C) KA (1.56–6.25 μg/ml); (D) MG (1.56–6.25 μg/ml). Data are expressed as the mean ± SD (*n* = 3). Different alphabetic letters are significantly different (*p* < 0.05). C = Control; KW = Aqueous extract; KM = Methanol extract; KA = Alkaloid extract; MG = Mitragynine.

significantly, with a 2.9–10.4-fold reduction compared to the H<sub>2</sub>O<sub>2</sub>-only group (*p* < 0.05) (Fig. 8A). Similarly, in cells treated with KM (12.5–50 μg/ml), MDA levels were reduced by 1.8–3.0 times relative to the H<sub>2</sub>O<sub>2</sub> group (*p* < 0.05) (Fig. 8B). A comparable protective effect was observed in the KA- and MG-treated groups (1.56–6.25 μg/ml), which showed significant reductions in MDA levels compared to the H<sub>2</sub>O<sub>2</sub> group (*p* < 0.05) (Fig. 8C, D). These findings are consistent with prior research indicating that kratom ethanolic extract can reduce MDA levels and improve oxidative stress biomarkers in diabetic mice [14]. The results from the HepG2 cell model highlight the potential of kratom as a treatment for oxidative stress-related conditions, supporting further investigation, particularly regarding its traditional use for T2DM management.

Based on the experimental results, kratom extracts and MG exhibited antioxidant, antiglycation, and cytoprotective effects against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress in HepG2 cells. Among the tested samples, MG demonstrated the most potent radical scavenging activity, as indicated by the lowest IC<sub>50</sub> in the DPPH and ABTS assays. KW and KM showed rapid metal ion reduction in the FRAP assay and strong antiglycation activity, reflecting their redox-modulating capabilities.

However, cytotoxicity varied among the extracts. KA and MG were more cytotoxic than KW and KM. Notably, although KM, KA, and MG reduced ROS and lipid peroxidation at non-toxic concentrations, only KW could significantly reduce H<sub>2</sub>O<sub>2</sub>-induced cell death, suggesting the presence of additional cytoprotective mechanisms in this extract.

Previous phytochemical analyses have revealed that kratom leaves contain various flavonoids and polyphenols, including rutin, quercetin, epicatechin, procyanidin B2, and chlorogenic acid. These compounds are well-known for their antioxidant and cytoprotective effects [11,13]. The differences in antioxidant activities among the extracts may be attributed to variations in the composition and relative abundance of these compounds. KW stood out for its low toxicity and strong antioxidant activity, despite containing relatively low MG levels. In contrast, the higher cytotoxicity of KM and KA may be linked to increased concentrations of MG or other alkaloids, which can exert pro-oxidant or apoptotic effects depending on the cellular microenvironment [42,43]. This indicates that MG content alone does not predict bioactivity and that interactions among multiple phytochemicals likely contribute to the overall biological effects.

These findings underscore the importance of synergistic or antagonistic interactions between phytoconstituents. The combination of lipophilic and hydrophilic compounds, particularly phenolics, may result in additive, synergistic, or antagonistic antioxidant effects. These interactions depend on factors such as compound ratio, structural compatibility, redox potential, subcellular localization, and bioavailability [42,44].

Based on general structural considerations, the antioxidant potential of phytochemicals such as phenolics and alkaloids is often influenced by the presence and position of functional groups such as hydroxyl and methoxy moieties. These groups are known to facilitate free radical scavenging through mechanisms such as hydrogen atom transfer (HAT), single-electron transfer-proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET) [45]. MG contains methoxy substituents and a polycyclic indole structure which may contribute to moderate radical-stabilizing properties [46]. However, its overall antioxidant capacity, as observed in the current assays, was lower than that of phenolic compounds such as gallic acid and quercetin [33].

Beyond direct antioxidant effects, many phytochemicals activate cellular signaling pathways such as the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) axis, which regulates oxidative stress responses. Nrf2 activation results in its translocation into the nucleus, where it binds to AREs and promotes the expression of antioxidant and phase II detoxifying enzymes, including heme oxygenase-1 (HO-1), catalase, and superoxide dismutase. Polyphenols, such as phenolic acids, flavonoids, terpenes, and tannins, have been reported to modulate this pathway and influence its epigenetic regulation [47]. Some alkaloids have also been shown to inhibit NADPH oxidase, a key enzyme involved in ROS production, activate the Nrf2 pathway, and influence peroxisome proliferator-activated receptors levels [42]. Thus, the observed antioxidant effects may result not only from direct ROS scavenging but also from upstream modulation of oxidative stress responses. However, further validation involving gene expression assays is warranted.

From a clinical perspective, oxidative stress is implicated in numerous chronic diseases including T2DM, cardiovascular diseases, neurodegenerative disorders, and cancer [1]. Preclinical evidence supports the role of kratom and its major alkaloids, particularly MG, in modulating oxidative stress and associated pathways. MG has demonstrated antioxidant, anti-inflammatory, neuroprotective, lipolytic, and enzyme-inhibitory activities relevant to these conditions. For instance, MG inhibits acetylcholinesterase and  $\alpha$ -glucosidase, which may provide therapeutic benefits for Alzheimer's disease and diabetes, respectively. Moreover, clinical studies have suggested its lipid-lowering effects and potential utility in managing metabolic syndrome and cardiovascular diseases [48–50].

The safety profile of kratom extracts and MG have been evaluated in several *in vivo* studies. In diabetic rodent models, ethanolic extracts of kratom leaves administered at 100–400 mg/kg/day improved glucose tolerance and lipid profiles without notable toxicity [14]. Acute toxicity studies using aqueous extracts reported no toxic effects at doses up to 2,000 mg/kg body weight, classifying them as category 5 or

unclassified under the GHS system. Sub-chronic toxicity studies further identified a no observed adverse effect level (NOAEL) of 1,000 mg/kg body weight, indicating a low toxicity profile for the extract [51]. For MG, oral administration at 1 and 10 mg/kg body weight for 28 days did not produce adverse effects in Sprague Dawley rats. However, a high dose of 100 mg/kg body weight induced hepatic stress without lethality [52].

Despite these promising findings, current clinical evidence remains limited, and safety concerns warrant careful consideration. MG has been associated with hepatotoxicity, neurotoxicity, and kratom-related fatalities, particularly at high doses or when used in combination with other substances [53]. Case reports have documented MG-induced cholestatic or mixed-pattern liver injury [54,55]. Forensic investigations have identified MG plasma levels exceeding 1,000 ng/ml in kratom-related deaths [56], while regular users showed a median plasma level of 90.86 ng/ml (range 14.7–380.7 ng/ml) [49]. The  $IC_{50}$  value for MG cytotoxicity observed in this study (35.31  $\mu$ g/ml) is much higher than reported plasma levels, suggesting that the cytotoxic threshold in cell models does not directly reflect fatal exposures. This highlights the need for caution when extrapolating *in vitro* findings to *in vivo* contexts. Systemic toxicity may result from complex pharmacodynamic interactions, metabolite effects, or cumulative exposure. Moreover, due to frequent polydrug use and limited toxicology data, the direct contribution of MG to observed adverse events remains inconclusive [56,57].

Recent reviews have emphasized the complex and evolving safety profile of kratom. While many users report beneficial effects such as pain relief, mood enhancement, and self-management of substance use disorders, some studies indicate risks of dependence, withdrawal symptoms, and adverse events. Most reported side effects are mild and self-limiting, including nausea, dizziness, and constipation. However, severe adverse events are often associated with adulterated products or polydrug use. Notably, kratom appears to pose a lower risk of respiratory depression compared to classical opioids, possibly due to its partial  $\mu$ -opioid receptor agonism and low  $\beta$ -arrestin recruitment. Nonetheless, interindividual variability, lack of standardized formulations and dosage regimens, along with product variability continue to complicate risk–benefit assessments. These findings underscore the need for rigorous quality control, well-defined dosing protocols, and close clinical monitoring to ensure the safe therapeutic use of kratom [56,57].

This study reveals that kratom extracts, particularly KW, exhibit promising antioxidant, antiglycation, and cytoprotective activities, which arise from both individual phytochemicals and their interactions. However, this study has certain limitations. First, the primary bioactive constituents of KW were not specifically identified. Future research should consider LC-MS/MS or GC-MS profiling to comprehensively characterize its chemical composition and identify the compounds responsible for the observed effects. Second, the use of HepG2 cells as the sole *in vitro* model may not fully represent the metabolic characteristics of normal hepatic tissue. HepG2 cells have low expression levels of key CYP450 enzymes, which can limit their response to xenobiotics and oxidative stress [58]. Nonetheless, findings from *in vivo* studies



in diabetic rats have demonstrated consistent metabolic and antioxidant benefits of kratom extract [14], supporting the physiological relevance of the present results and offering preliminary translational insight into its therapeutic potential. Future investigations incorporating non-cancerous hepatic or metabolically relevant cell lines would further strengthen the biological relevance of these findings.

## CONCLUSION

Kratom extracts, including KW, KM, KA, and their major alkaloid MG, exhibited antioxidant and antiglycation activities, as demonstrated by DPPH, ABTS, FRAP, BSA-glucose, and BSA-MGO assays. Pearson correlation analysis indicated that both alkaloid and non-alkaloid constituents contributed to these effects. Among the tested samples, KW demonstrated notable cytoprotective activity in HepG2 cells by reducing ROS and MDA levels while exhibiting low cytotoxicity. Overall, KW emerged as the most promising extract due to its multifaceted antioxidant and cytoprotective properties. Further studies are warranted to identify its active constituents, clarify underlying mechanisms, and evaluate safety and therapeutic potential in oxidative stress-related conditions.

## LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)
AGEs	Advanced glycation end-products
BSA	Bovine serum albumin
DCFH-DA	2',7'-dichlorofluorescein diacetate
DMEM	Dulbecco's Modified Eagle Medium
DPPH	2,2-diphenyl-1-picrylhydrazyl
FBS	Fetal bovine serum
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalents
KW	Kratom aqueous extract
KM	Kratom methanol extract
KA	Kratom alkaloid extract
MDA	Malondialdehyde
MG	Mitragynine
MGO	Methylglyoxal
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide
ROS	Reactive oxygen species

## AUTHOR CONTRIBUTION

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

## CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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## USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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