

APST

Asia-Pacific Journal of Science and Technology

<https://www.tci-thaijo.org/index.php/APST/index>Published by Research and Innovation Department,
Khon Kaen University, Thailand**Protective and Therapeutic Effects of Thai Vegetable Extracts Against Oxidative Stress and Apoptosis in RAW 264.7 Macrophage Cells**Adisak Palasap¹, Nisachon Jangpromma^{2,3}, Narintorn Rattanata⁴, Patcharee Boonsiri⁵, Sakda Daduang^{2,6}, Patcharaporn Tippayawat¹, Pornsuda Maraming¹, Jringjai Areemit⁷, Paweena Tunakhun¹, and Jureerut Daduang^{1,*}¹ Centre for Research and Development in Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand² Protein and Proteomics Research Center for Commercial and Industrial Purposes (ProCCI), Department of Biochemistry, Faculty of Sciences, Khon Kaen University, Khon Kaen 40002, Thailand³ Department of Biochemistry, Faculty of Sciences, Khon Kaen University, Khon Kaen 40002, Thailand⁴ Learning Area of Science and Technology, Khonkaen Wittayayon School, Khon Kaen 40000, Thailand⁵ Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand⁶ Division of Pharmacognosy and Toxicology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand⁷ Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand

*Corresponding author: jurpoo@kku.ac.th

Received 22 September 2025

Revised 18 February 2026

Accepted 20 March 2026

Abstract

Consuming vegetables is vital for disease prevention, and Thailand's diverse vegetable resources are of particular interest. This study investigated the antioxidant and anti-apoptotic activities of ethanolic extracts from 48 Thai vegetable species. Antioxidant capacity was evaluated using FRAP and DPPH assays, and total phenolic content was determined by the Folin-Ciocalteu method. *Schinus terebinthifolius* (MT) and *Caesalpinia mimosoides* (KY) exhibited the highest phenolic content and antioxidant activity and were selected for further study. In RAW 264.7 macrophages exposed to H₂O₂ (200 µM), cell viability decreased to approximately 30%, whereas co-treatment with KY extract (12.5-100 µg/mL) restored viability in a dose-dependent manner, reaching 84.65%, while MT extract increased viability to approximately 50%. Flow cytometric analysis revealed that H₂O₂ induced apoptosis in ~90% of cells; MT extract reduced apoptotic cells by up to 20.65%, whereas KY extract produced a modest reduction (≤3.37%). RT-qPCR analysis demonstrated the upregulation of antioxidant-related genes (*CAT*, *SOD1*) and downregulation of pro-apoptotic genes (*p53*, *caspase-9*). Trace-element analysis further suggested that copper, selenium, and zinc may contribute to antioxidant enzyme activity. Collectively, MT and KY extracts exhibit significant antioxidant and anti-apoptotic effects, supporting their potential as non-toxic natural agents for mitigating oxidative stress and apoptosis-related conditions.

Keywords: Antioxidant, Anti-apoptosis, Thai vegetables, *Schinus terebinthifolius* Raddi, *Caesalpinia mimosoides* Lamk.

1. Introduction

Consuming vegetables has a positive impact on health and helps prevent chronic conditions such as cancer [1], cardiovascular diseases [2], diabetes [3], Alzheimer's [4], also age-related decline. Phytochemicals, especially antioxidants, in vegetables are crucial in reducing oxidative stress and preventing these diseases [5]. Oxidative stress, caused by an imbalance in cellular redox, is linked to conditions like inflammation, ischemic injury, atherosclerosis, diabetes, and cancer [6]. ROS, which interfere with cellular operations and trigger apoptotic pathways, are essential in this process. The organism protects itself from ROS with enzymatic antioxidants like

catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), which help neutralize ROS [7]. Phytochemicals, including phenolic compounds, help reduce oxidative stress by neutralizing free radicals, supporting the immune system, and influencing gene expression [8]. In Thailand, various plants used as food and medicine have been researched for their biological effects. For instance, *Schinus terebinthifolius* Raddi (Matoom Saudi; MT) leaf extract shows antioxidant, antimicrobial, antifungal, and anti-mutagenic effects [9]. Various parts of *Caesalpinia mimosoides* Lamk. (Kaya; KY) exhibit antioxidants [10], antimicrobial [11], anti-inflammatory [12], anticancer [13], anti-aging [14], and neuroprotective attributes. While some plants are well-researched, the antioxidant and anti-apoptotic properties of many Thai vegetables remain largely unexplored. This study aims to preserve traditional knowledge and enhance the economic and medicinal value of these resources, specifically by exploring the antioxidant and anti-apoptotic effects of ethanolic extracts from Thai vegetables. These findings could support new dietary or therapeutic approaches for diseases related to oxidative damage and cell demise.

2. Materials and methods

2.1 Chemicals and reagents

Dulbecco's Modified Eagle's Medium-High Glucose (DMEM-HG), fetal bovine serum, penicillin-streptomycin, and trypsin-EDTA were obtained from Gibco BRL (Grand Island, NY). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), crystal violet, ethidium bromide (EtBr), and acridine orange (AO) were purchased from Sigma (SigmaAldrich, USA).

2.2 Sample preparation and extraction

48 different vegetable varieties were purchased from local markets in Khon Kaen, Thailand in May 2020. The edible components were cleaned, combined, and dried at 50°C, homogenized in a Waring blender, and crushed with a mortar and pestle under dimmed light. For 72 h, each 250 g homogenate was extracted using 1000 mL of 70% ethanol at room temperature. Afterward, the mixture was filtered through Whatman filter paper, and the ethanol was removed using a rotary evaporator under reduced pressure. Extracts were stored in desiccators for later experiments.

2.3 Determination of total phenolic content

The total phenolic content of the ethanolic vegetable extracts was determined using the Folin-Ciocalteu spectrophotometric method with minor modifications [15]. Briefly, the dried extracts were first re-dissolved in DMSO. An aliquot of 200 μ L of each extract solution was mixed with 1 mL of 0.2 M Folin-Ciocalteu reagent and diluted with DMSO. The reaction mixture was incubated at room temperature in the dark for 30 min. Subsequently, 800 μ L of 7% (w/v) sodium carbonate solution was added. Gallic acid was used as the calibration standard, and the absorbance of the resulting blue complex was measured at 750 nm using a Shimadzu UV mini-1240 spectrophotometer (Japan). The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract.

2.4 Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay was used to measure total antioxidant capacity. 200 mM iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), 10 mM 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) was prepared in 40 mM HCl, and a 300 mM acetate buffer at pH 3.6 was mixed in a ratio of 10:1:1 (v/v) to produce the FRAP reagent. One milliliter of the vegetable extract was mixed with one milliliter of the FRAP reagent. Following a five-min period, the absorbance of the reaction mixture was assessed at a wavelength of 593 nm using a Shimadzu UV mini-1240. A solution of iron (II) sulfate (FeSO_4) was utilized to generate the standard curve. The findings were reported as $\mu\text{mol Fe (II)}/\text{g}$ of dry weight vegetable extract.

2.5 DPPH Radical Scavenging Assay

To evaluate the activity of scavenging free radicals, DPPH spectrophotometric test was used, with modifications based on the method described by Mensor et al. (2001) [16]. A 0.25 mM DPPH solution was combined with 20 μ L of each dilution of the extracts, using one milliliter of the solution, which had been diluted in 70% ethanol, followed by gentle mixing. After 10 min with dark condition, the absorbance of the combination at 540 nm was determined using a Shimadzu UV mini-1240. The radical scavenging activity was represented by the 50% inhibition concentration (IC_{50}).

2.6 Cell culture

The Protein and Proteomics Research Center for Commercial and Industrial Purposes (ProCCI), Department of Biochemistry, Faculty of Science, Khon Kaen University, supplied adherent RAW 264.7 macrophage and Vero cells. Cells were cultivated in T-flasks with DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin-streptomycin at 37°C in a humidified environment with 5% CO₂.

2.7 Determination of cytotoxicity of vegetable extracts

The MTT assay was utilized to evaluate the cytotoxic impact of vegetable extracts on cells. After being seeded at a density of 1×10^5 cells/mL in 96-well plates, Vero cells were treated with extracts at concentrations between 50 and 200 µg/mL. Cell viability was assessed using the MTT assay after a 24 h incubation at 37°C in a 5% CO₂ environment. 150 µL of MTT solution (0.5 mg/mL) was added to each well, which was then incubated for two hours. After removing the supernatants, formazan crystals were dissolved in DMSO. At 570 nm, absorbance was determined with a Varioskan™ LUX multimode microplate reader. The results were presented as averages $\pm$ standard deviations from three separate studies, and each test was run in triplicate. The negative controls contained 0.5% DMSO.

2.8 Determination of protective effects of vegetable extracts against oxidative damage and cell death

RAW 264.7 cells were seeded at a density of 2×10^5 cells/well in 96-well plates and cultured in high-glucose DMEM supplemented with 10% fetal bovine serum and 1% antibiotics at 37 °C in a humidified atmosphere containing 5% CO₂ for 24 h. Cells were then assigned to three groups: (I) untreated group, cultured in complete medium; (II) control group, treated with 200 µM H₂O₂; and (III) treated group, exposed to 200 µM H₂O₂ in the presence of various concentrations of vegetable extracts. The concentration of 200 µM H₂O₂ was selected based on preliminary optimization, as it reliably induces oxidative stress in RAW 264.7 macrophages without causing excessive cytotoxicity. After 24 h of treatment, cell viability was assessed using the MTT assay. For the anti-apoptosis experiment, RAW 264.7 cells were seeded at a density of 3×10^6 cells/well in 12-well plates and cultured for 24 h prior to treatment. Cells were trypsinized, rinsed with 1×PBS, and centrifuged at 2500 rpm following treatment with H₂O₂ and vegetable extracts. After resuspending the pellets in 200-500 µL of 1× Annexin V binding buffer, 100 µL aliquots were transferred to tubes for flow cytometry. After adding 1 µL of Annexin V-FITC and 2 µL of propidium iodide (PI), the samples were incubated in the dark at room temperature for 15 min. Using BD Accuri C6 software, a flow cytometer (BD Biosciences, San Jose, CA, USA) was employed to analyze apoptosis.

2.9 Quantitative real-time polymerase chain reaction (RT-qPCR)

The expression of apoptotic proteins (*p53*, *caspase-9*) and antioxidant enzymes (*SOD*, *CAT*, *Gpx1*) has been evaluated by RT-qPCR. Following the cultivation of RAW 264.7 cells and their treatment with H₂O₂ and vegetable extracts, TRIzol® reagent was used to extract the RNA. Using the oligo(dT)18 primer and the RevertAid First-Strand cDNA Synthesis Kit, cDNA was generated from 1 µg of RNA. RT-qPCR was conducted in triplicate on a LightCycler® 480 real-time PCR system. Each 20 µL reaction comprised 2 µL of cDNA, 0.2 µM primers, and 10 µL of SYBR® Green PCR master solution. To normalize gene expressions, β -actin was chosen. To determine relative gene expression changes, the $\Delta\Delta$ CT technique (Livak and Schmittgen, 2001) was used [17]. To confirm the PCR specificity, a melting curve study was conducted for 15 seconds at 95°C.

2.10 Determination of some trace elements by Inductively coupled plasma-mass spectrometer (ICP-MS)

An ICP-MS Agilent 7500A (Agilent Technologies, USA) was used to determine zinc, copper, and selenium levels. Briefly, 500 mg of vegetable material was placed in a PTFE digesting vessel and mixed with 7 mL HNO₃ and 3 mL H₂O₂. The vessel was closed and left for 60 min before decomposition in a microwave digestion machine (Milestone, ETHOSTC). To separate the components that had not dissolved, for 10 min, the samples were centrifuged at 4000 rpm. After transferring the extract to a volumetric flask, 25 mL of double-distilled water was added. The same method was used to analyze a certified reference material (GBW07605 tea sample) and blank experiments (n = 3).

2.11 Statistical analysis

ANOVA and t-tests were performed to analyze all experimental results, which are presented as the mean $\pm$ SD. A p-value of less than 0.05 (*p < 0.05) was considered statistically significant.

3. Results

3.1 Total phenolic content and antioxidant activity

We analyzed the total phenolic content and antioxidant activity of fresh vegetable material from 48 species, with the details summarized in the *Supporting Information*. The ethanolic extracts showed total phenolic contents ranging from 19.03 ± 0.00 to 421.94 ± 0.02 mg GAE/g dry weight. The highest phenolic contents were found in MT (421.94 ± 0.02 mg GAE/g), KY (415.97 ± 0.01 mg GAE/g), and E-hin (348.36 ± 0.02 mg GAE/g). The FRAP assay showed values from 13.19 ± 0.01 to 1711.82 ± 0.02 $\mu\text{mol Fe(II)/g dry wt}$, with MT and KY exhibiting the highest reducing power. The DPPH radical-scavenging assay revealed IC₅₀ values ranging from 41.90 ± 0.06 to 11301.30 ± 0.21 $\mu\text{g/mL}$. The strongest radical scavenging capacities were found in MT (41.90 ± 0.06 $\mu\text{g/mL}$), Makok (52.40 ± 0.02 $\mu\text{g/mL}$), and KY (66.50 ± 0.08 $\mu\text{g/mL}$).

3.2 Cytotoxicity of vegetable extracts against Vero cells

The cytotoxicity assay demonstrated the effects of different extracts on Vero cells. MT and KY extracts at all tested concentrations (50, 100, and 200 $\mu\text{g/mL}$) maintained cell viability above 90% relative to the untreated control (Figure 1). No statistically significant differences were observed between treated and control groups ($p > 0.05$), indicating no apparent cytotoxic effects under the experimental conditions.

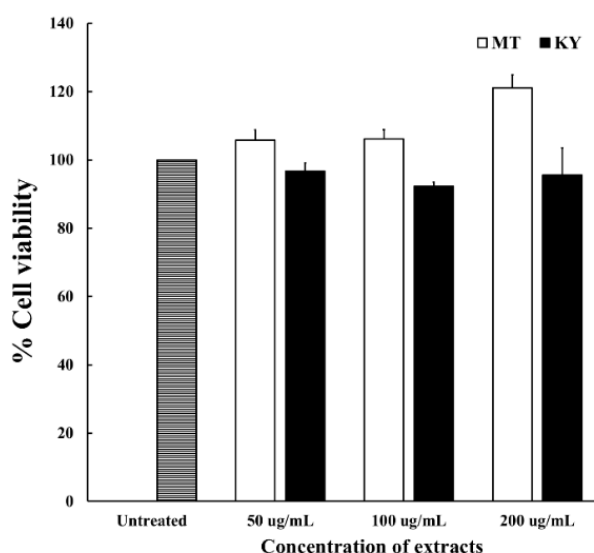


Figure 1 Cytotoxic effects of MT and KY extracts on Vero cells treated at 50, 100, and 200 $\mu\text{g/mL}$. Data is presented as mean $\pm$ SD ($n = 3$). No significant differences were observed compared with the control (one-way ANOVA, $p > 0.05$).

3.3 Protective effects of vegetable extracts against oxidative damage

MT and KY extracts were tested for their ability to shield RAW 264.7 cells from oxidative damage brought on by H_2O_2 . by treating them with 200 μM H_2O_2 and extracts ranging from 12.5 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$ for 24 h. The MTT assay (Figure 2) showed that H_2O_2 exposure reduced cell viability from 100% to 30% ($p < 0.0001$). Co-incubation with KY extract led to a dose-dependent increase in cell viability, reaching 84.65%. In contrast, MT extract resulted in approximately 50% cell viability ($p < 0.05$) compared to KY.

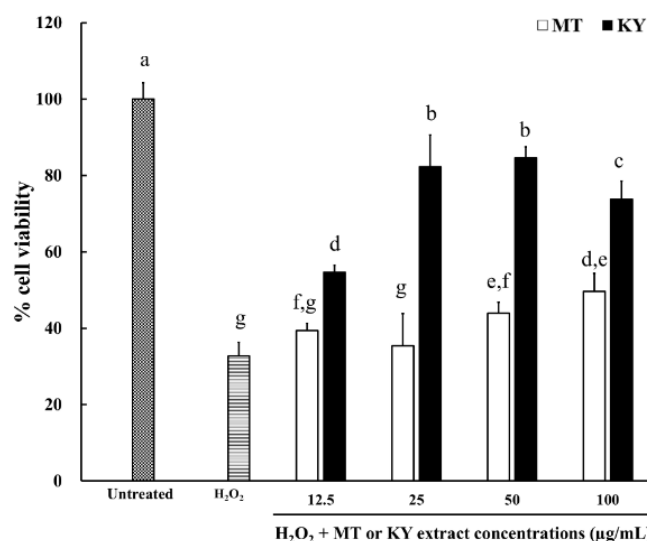


Figure 2 Effects of MT and KY extracts at various concentrations on H₂O₂-induced oxidative stress RAW 264.7 cells, assessed using the MTT assay. Bars sharing the same letter are not significantly different ($p > 0.05$). Data is presented as mean $\pm$ SD ($n = 3$).

3.3 Anti-apoptosis activity of vegetable extracts in RAW 264.7 cells

Applying the Annexin V-FITC/PI double labeling method and flow cytometry, the anti-apoptotic activity of MT and KY extracts on RAW 264.7 cells was evaluated. As shown in Figure 3, exposure to H₂O₂ (200 μ M) increased apoptotic cells to approximately 90%. Co-incubation with MT and KY extracts attenuated apoptosis; MT reduced apoptosis by 11.28% and 20.65% at 25 and 50 μ g/mL, respectively, whereas KY caused only minor reductions (2.57–3.37%). Statistical analysis showed a significant effect for MT at 50 μ g/mL ($p < 0.05$), while MT at 25 μ g/mL and KY at all tested concentrations were not significant ($p > 0.05$).

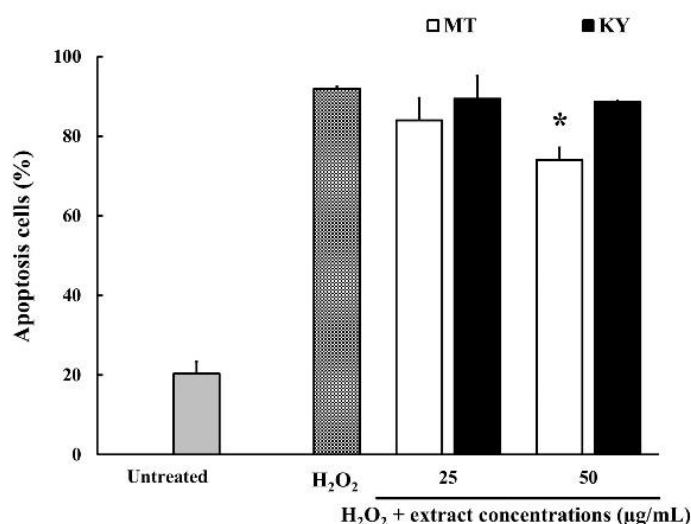


Figure 3 Effects of MT and KY extracts (25 and 50 μ g/mL) on H₂O₂-induced apoptosis in RAW 264.7 cells, as assessed by Annexin V-FITC/PI staining followed by flow cytometry. Data is presented as mean $\pm$ SD from three independent experiments. Statistical analysis was performed using one-way ANOVA followed by a post hoc test; * $p < 0.05$ vs. H₂O₂-treated control.

3.4 Antioxidant gene expression in Raw 264.7 cells quantified by RT-PCR

We investigated how the expression of genes encoding antioxidant enzymes, such as *SOD*, *CAT*, and *GPx1*, was impacted by vegetable extracts, which are essential for cellular defense against oxidative stress. Co-

incubation of RAW 264.7 cells with MT extract led to upregulation of the *SOD1* gene (Figure 4a). In contrast, KY extract resulted in downregulation of *SOD* gene expression (Figure 4d). The *CAT* gene, encoding catalase, was downregulated by KY extracts at concentrations of 25 and 50 μ M (Figure 4e). However, cells exposed to MT extracts showed significantly higher *CAT* gene expression (Figure 4b). Additionally, both MT and KY extract downregulated *GPx1* gene expression, which encodes glutathione peroxidase 1 (Figure 4c and 4f).

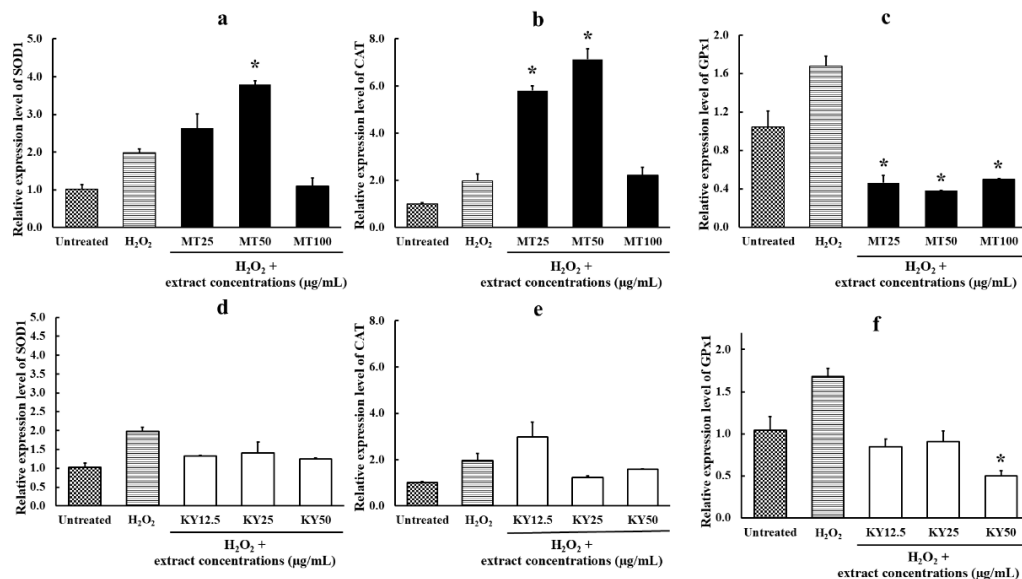


Figure 4 Effect of MT and KY extracts on antioxidant enzyme gene expression in H₂O₂-treated RAW 264.7 Cells. RAW 264.7 cells were co-incubated with H₂O₂ (200 μ M) and varying concentrations of MT (25-100 μ g/mL) or KY (12.5-50 μ g/mL) extracts for 24 h. Gene expression levels of *SOD1*, *CAT*, and *GPx1* were measured by RT-qPCR and normalized to β -actin expression. Panels (a-c) show the modulation of *SOD1*, *CAT*, and *GPx1* expressions in response to MT extract, while panels (d-f) depict the corresponding gene expression changes following KY extract treatment. *p < 0.05 compared to the H₂O₂-treated control group.

3.5 Evaluation of apoptosis gene expression in RAW 264.7 cells by RT-PCR

Treatment with MT and KY extracts at concentrations ranging from 12.5 μ g/mL to 50 μ g/mL led to significant downregulation of the *caspase-9* gene compared to the H₂O₂-treated group. Treatment with the KY extract significantly reduced *p53* expressions at 50 μ g/mL, while the MT extract exhibited a similar significant decrease at 100 μ g/mL.

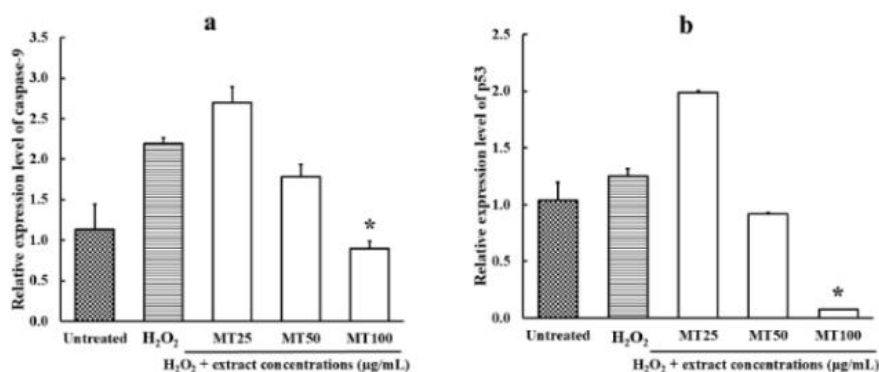


Figure 5 Effect of MT and KY extracts on apoptosis-related gene expression in H₂O₂ treated RAW 264.7 Cells. RAW 264.7 cells were co-incubated with H₂O₂ (200 μ M) and various concentrations of MT (25-100 μ g/mL) or KY (12.5-50 μ g/mL) extracts for 24 h. Gene expression levels of *caspase-9* and *p53* were measured by RT-qPCR and normalized to β -actin expression. Panels (a) and (b) show the modulation of *caspase-9* and *p53* expressions in response to MT extract, while panels (c) and (d) depict the corresponding gene expression changes after KY extract treatment. The mean $\pm$ SD of three separate studies is used to express the results. *p < 0.05 in comparison to the control group that received H₂O₂.

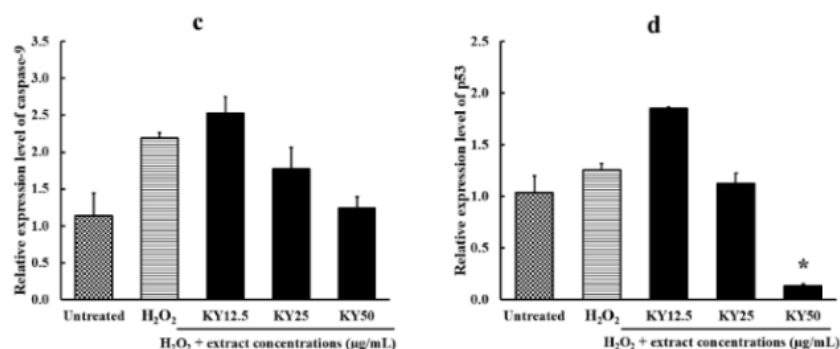


Figure 5 Effect of MT and KY extracts on apoptosis-related gene expression in H₂O₂ treated RAW 264.7 Cells. RAW 264.7 cells were co-incubated with H₂O₂ (200 µM) and various concentrations of MT (25-100 µg/mL) or KY (12.5-50 µg/mL) extracts for 24 h. Gene expression levels of *caspase-9* and *p53* were measured by RT-qPCR and normalized to *β-actin* expression. Panels (a) and (b) show the modulation of *caspase-9* and *p53* expressions in response to MT extract, while panels (c) and (d) depict the corresponding gene expression changes after KY extract treatment. The mean ± SD of three separate studies is used to express the results. *p < 0.05 in comparison to the control group that received H₂O₂. (Cont.)

3.6 Trace elements evaluation by ICP-MS

MT and KY were analyzed for trace elements by ICP-MS, including copper, zinc and selenium, which are the cofactors of antioxidant enzymes. The copper content of MT and KY extracts were 1.054 mg/kg and 0.665 mg/kg, respectively as shown in Table 1. The selenium content was higher in the MT extract (4.606 mg/kg) compared to the KY extract (0.551 mg/kg). In contrast, the zinc content in the MT extract was 0.326 mg/kg, while the KY extract displayed a much higher zinc content of 8.267 mg/kg.

Table 1 Trace element composition of MT and KY extracts

Vegetables	Element (mg/kg)		
	Copper	Selenium	Zinc
MT	1.054	4.606	0.326
KY	0.665	0.551	8.267

4. Discussion

Vegetable extracts have long been used for pharmacological purposes. Phenolic compounds from vegetables exhibit antioxidant activity, reduce lipid peroxidation, and DNA damage [18]. Additionally, they suppress inflammation by controlling pro-inflammatory chemicals such as leukocyte adhesion, cytokine (TNF-α), and nitric oxide (NO) synthesis [19]. Excessive levels of NO and TNF-α can lead to cell death and tissue damage [20]. Numerous biological functions, such as anti-inflammatory, anti-cancer, antibacterial, and antioxidant qualities, are linked to these compounds. For instance, significant antibacterial efficacy against *Escherichia coli* and *Staphylococcus aureus* was demonstrated using MT leaf extract [9]. Meanwhile, the viability of cervical cancer cells was decreased by the methanolic extract of KY in a way that was dependent on both time and dose [13]. Crude methanolic extract of *Limnophila aromatica* exhibited antimicrobial activity [9, 21], and *Polygonum odoratum* leaf extract showed potent anti-inflammatory and antioxidant effects [22]. *Careya arborea* Roxb leaf extract demonstrated anticancer activity against MCF-7 cells [23]. The total phenolic content and antioxidant activity of some local Thai vegetables (Supporting information), with KY and MT extracts having the highest phenolic content. Daduang et al. (2011) also reported high phenolic content, ferric-reducing antioxidant power, and low IC₅₀ for DPPH in MT and KY ethanolic extracts [10]. Cytotoxicity tests on Vero cells showed that MT and KY extracts at concentrations of 50, 100, and 200 µg/mL were non-toxic (Figure 1). Therefore, MT and KY were selected for further investigation. In this study, we investigated the protective effects of MT and KY extracts against H₂O₂-induced apoptosis in RAW 264.7 cells (Figure 2). H₂O₂ at 200 µM significantly increased non-viable cells to 39.4%. This is consistent with Payoungkiattikun et al. (2020) [24], who showed the dose-dependent effects of H₂O₂ on apoptosis in RAW 264.7 cells. Co-incubation with MT extracts (25 and 50 µg/mL) reduced apoptosis by 11.28% and 20.65%, respectively. KY extract (25 and 50 µg/mL) also reduced apoptosis (Figure 3). Previous studies, such as those by Tangsaengvit et al. (2013) [25] and Rocha et al. (2018) [26], reported that antioxidant-rich plant extracts protect against oxidative stress-induced apoptosis by scavenging ROS and enhancing antioxidant activity. Our study supports this, as MT and KY extracts, shown in *Supporting information*,

exhibit high antioxidant activity, which may mitigate H₂O₂-induced ROS damage by directly scavenging ROS, boosting antioxidant defenses, or modulating related signaling pathways. Phytochemicals and antioxidant enzymes like *SODs*, *CAT*, and *GPx* play key roles in detoxifying H₂O₂ and protecting cells from oxidative damage. *SOD1* (Cu/Zn-SOD) is found primarily in the cytoplasm of mammals, *SOD2* (Mn-SOD) is localized in mitochondria, and *SOD3* is predominantly expressed in extracellular spaces and certain tissues such as lung and kidney, reflecting distinct tissue-specific roles of these antioxidant enzymes [27]. These enzymes convert superoxide into oxygen and H₂O₂, defending cells against ROS. Reduced *SOD* levels or mutations impairing their function can cause significant phenotypic effects. *CAT* and *GPx1* neutralize H₂O₂ by converting it into water and oxygen [28].

In our study, KY extract downregulated *SOD* gene expression (Figure 4d), while MT extract upregulated it (Figure 4a), suggesting differing regulatory effects of these extracts on *SOD* gene expression. KY extract, particularly at 25 and 50 µM, downregulated *CAT* gene expression (Figure 4e), suggesting it may reduce catalase production, whereas MT extract significantly increased *CAT* gene expression (Figure 4b), indicating its potential to enhance catalase production. The regulation of *CAT* gene expression by KY and MT extracts could affect cellular antioxidant defense. Both MT (Figure 4c) and KY (Figure 4f) extract downregulated *GPx1* gene expression, indicating a shared impact on this important antioxidant enzyme. The downregulation of *SOD* and *CAT* by KY extract despite improved cell viability is consistent with a “sparing effect,” in which potent antioxidants directly scavenge reactive oxygen species and reduce the need for transcriptional induction of endogenous antioxidant enzymes [29]. In addition, the involvement of glutathione peroxidase-dependent redox regulation, which requires selenium as an essential cofactor, may explain the observed modulation of *GPx1* expression [30]. These findings have implications for dietary or therapeutic strategies targeting oxidative stress-related conditions.

We examined the molecular processes that underlie the actions of extracts of MT and KY. *Caspase-9*, involved in the intrinsic apoptotic pathway, was significantly downregulated after treatment with MT and KY extracts (25–50 µg/mL), suggesting inhibition of the mitochondrial apoptotic pathway. Kim et al. (2018) proposed that plant extracts may reduce apoptosis by modulating key apoptotic mediators [31]. *p53*, a tumor suppressor gene, regulates cell-cycle progression and apoptosis in response to stress. The significant downregulation of *p53* after treatment with 50 µg/mL of KY and 100 µg/mL of MT extracts indicates a reduction in cellular stress responses, suggesting potential protective effects against oxidative damage. Antioxidant enzymes protect cells by neutralizing free radicals and combating oxidative stress, and they require essential cofactors including copper (Cu), zinc (Zn), manganese (Mn), and selenium (Se), which are critical for their catalytic activity during redox reactions [32]. Table 1 shows the mineral content of MT extract: copper (1.054 mg/kg), zinc (0.326 mg/kg), and selenium (4.606 mg/kg), suggesting a role in enhancing antioxidant enzyme activity. KY extract has slightly lower copper content (0.665 mg/kg) but higher zinc content (8.267 mg/kg) and moderate selenium (0.551 mg/kg), indicating its potential to support antioxidant functions and contribute to cellular protection against oxidative stress. Notably, copper and zinc serve as cofactors for superoxide dismutase, while selenium is essential for glutathione peroxidase activity, collectively strengthening enzymatic antioxidant defenses and maintaining redox homeostasis [33].

5. Conclusion

Among 48 species of Thai vegetable extracts, MT and KY extracts showed high total phenolic content, antioxidant activity, and significant anti-apoptotic effects on H₂O₂-induced oxidative stress in RAW 264.7 cells. Both extracts enhanced cell viability and reduced apoptosis. Gene expression analysis suggested modulation of key antioxidant genes, providing insight into their antioxidant mechanisms. Additionally, downregulation of pro-apoptotic genes indicated potential protection against cellular apoptosis. Trace-element analysis revealed variations in copper, selenium, and zinc content, which may support antioxidant enzyme activity.

6. Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the author(s) used ChatGPT (OpenAI) in order to assist with language polishing and improving the clarity of certain sentences. After using this tool, the author(s) thoroughly reviewed and edited the content as needed and take full responsibility for the content of this publication.

7. Acknowledgments

This work was supported by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program and Khon Kaen University (grant no. PHD/0204/2556 to A. Palasap), Basic Research Fund 2565 (project no. 2537102), and NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation (grant no. B38G670003). We thank our colleagues at the Protein and Proteomics

Research Center for Commercial and Industrial Purposes (ProCCI), Department of Biochemistry, Khon Kaen University. Special thanks to Professor David Blair and the KKU publication clinic for manuscript editing and valuable comments.

8. Conflicts of Interest

The authors declare no conflict of interest.

9. Author Contributions

Palasap, A.: Writing – review & editing, Methodology, Writing – original draft; Jangpromma, N.: Conceptualization, Methodology, Supervision; Rattanata, N.: Methodology; Boonsiri, P.: Conceptualization, Writing – review & editing, Supervision; Daduang, S.: Conceptualization; Tippayawat, P.: Methodology; Maraming, P.: Writing – review & editing; Tunakhun, P.: Methodology; Daduang, J.: Conceptualization, Supervision, Project administration.

10. References

- [1] Farooq B, Koul B, Mahant D, Yadav D. Phytochemical analyses, antioxidant and anticancer activities of ethanolic leaf extracts of *Moringa oleifera* Lam. varieties. *Plants*. 2021;10:2348.
- [2] Mellendick K, Shanahan L, Wideman L, et al. Diets rich in fruits and vegetables are associated with lower cardiovascular disease risk in adolescents. *Nutrients*. 2018;10:136.
- [3] Okaiyeto K, Falade AO, Oguntibeju OO. Traditional uses, nutritional and pharmacological potentials of *Clerodendrum volubile*. *Plants*. 2021;10:1893.
- [4] Omar SH, Scott CJ, Hamlin AS, Obied HK. The protective role of plant biophenols in mechanisms of Alzheimer's disease. *J Nutr Biochem*. 2017;47:1-20.
- [5] Williams B, Watanabe CMH, Schultz PG, et al. Age-related effects of *Ginkgo biloba* extract on synaptic plasticity and excitability. *Neurobiol Aging*. 2004;25(7):955-962.
- [6] Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol Rev*. 2014;94:329-354.
- [7] Jena AB, Samal RR, Bhol NK, Duttaroy AK. Cellular red-ox system in health and disease: the latest update. *Biomed Pharmacother*. 2023;162:114542.
- [8] Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J Nutr*. 2004;134:3475S-3489S.
- [9] Uliana MP, Fronza M, da Silva AG, et al. Composition and biological activity of Brazilian rose pepper (*Schinus terebinthifolius* Raddi) leaves. *Ind Crops Prod*. 2016;83:235-240.
- [10] Daduang J, Suk V, Vichitphan A, et al. High phenolics and antioxidants of some tropical vegetables related to antibacterial and anticancer activities. *Afr J Pharm Pharmacol*. 2011;5:608-615.
- [11] Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chem*. 2005;92(3):491-497.
- [12] Yodsauwe O, Karalai C, Ponglimanont C, et al. Potential anti-inflammatory diterpenoids from the roots of *Caesalpinia mimosoides* Lamk. *Phytochemistry*. 2010;71(14-15):1756-1764.
- [13] Palasap A, Limpaboon T, Boonsiri P, et al. Cytotoxic effects of phytophenolics from *Caesalpinia mimosoides* Lamk on cervical carcinoma cell lines through an apoptotic pathway. *Asian Pac J Cancer Prev*. 2014;15:449-454.
- [14] Rangsinth P, Prasansuklab A, Duangjan C, et al. Leaf extract of *Caesalpinia mimosoides* enhances oxidative stress resistance and prolongs lifespan in *Caenorhabditis elegans*. *BMC Complement Altern Med*. 2019;19(1):164.
- [15] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*. 1965;16:144-158.
- [16] Mensor LL, Menezes FS, Leitao GG, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother Res*. 2001;15(2):127-130.
- [17] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods*. 2001;25:402-408.
- [18] Velmurugan BK, Rathinasamy B, Lohanathan BP, Thiyagarajan V, Weng CF. Neuroprotective role of phytochemicals. *Molecules*. 2018;23:2485.
- [19] Gunawardena S, Nadeeshani H, Amarasinghe V, et al. Bioactive properties and therapeutic aspects of fermented vegetables: a review. *Food Prod Process Nutr*. 2024;6:31.
- [20] Chandimali N, Bak SG, Park EH, et al. Free radicals and their impact on health and antioxidant defenses: a review. *Cell Death Discov*. 2025;11:19.

- [21] Nanasombat S, Teckchuen N. Antimicrobial, antioxidant, and anticancer activities of Thai local vegetables. *J Med Plants Res.* 2009;3(5):443-449.
- [22] Chansiw N, Chotinantakul K, Srichairatanakool S. Anti-inflammatory and antioxidant activities of the extracts from leaves and stems of *Polygonum odoratum* Lour. *Anti-Inflamm Anti-Allergy Agents Med Chem.* 2019;18:45-54.
- [23] Buranrat B, Boontha S, Temkitthawon P, Chomchalao P. Anticancer activities of *Careya arborea* Roxb on MCF-7 human breast cancer cells. *Biologia (Bratisl).* 2020;75:2359-2366.
- [24] Payoungkiattikun W, Joompang A, Thongchot S, et al. Evidence of multi-functional peptide activity: potential role of KT2 and RT2 for anti-inflammatory, anti-oxidative stress, and anti-apoptosis properties. *Appl Biol Chem.* 2020;63:5.
- [25] Tangsaengvit N, Kitphati W, Tadtong S, et al. Neurite outgrowth and neuroprotective effects of quercetin from *Caesalpinia mimosoides* Lamk. on cultured P19-derived neurons. *Evid Based Complement Alternat Med.* 2013;2013:838690.
- [26] Rocha PS, Campos JF, Nunes-Souza V, et al. Antioxidant and protective effects of *Schinus terebinthifolius* Raddi against doxorubicin-induced oxidative stress in normal cells. *Pharm Biol.* 2018;56:407-414.
- [27] Kodirov SA. Comparison of Superoxide Dismutase Activity at the Cell, Organ, and Whole-Body Levels. *Cell Biochemistry and Biophysics.* 2025;83(3):2713-2726.
- [28] Azadmanesh J, Borgstahl GE. A review of the catalytic mechanism of human manganese superoxide dismutase. *Antioxidants (Basel).* 2018;7:25.
- [29] Forman HJ, Davies KJA, Ursini F. How do nutritional antioxidants really work: nucleophilic tone and para-hormesis versus free radical scavenging in vivo. *Free Radic Biol Med.* 2014;66:24-35.
- [30] Brigelius-Flohé R, Maiorino M. Glutathione peroxidases. *Biochim Biophys Acta.* 2013;1830(5):3289-3303.
- [31] Kim KB, Lee S, Kang I, Kim JH. *Momordica charantia* ethanol extract attenuates H₂O₂-induced cell death by its antioxidant and anti-apoptotic properties in human neuroblastoma SK-N-MC cells. *Nutrients.* 2018;10:1368.
- [32] Frontiers in Molecular Biosciences. Antioxidant and oxidative enzymes, genetic variants, and cofactors as prognostic biomarkers of COVID-19 severity and mortality. *Front Mol Biosci.* 2025; PMID: 41209585.
- [33] Anam K, Ananyan S, Rishabh M, et al. Zinc, copper, and selenium levels in vitiligo: a systematic review and meta-analysis. *Sci Rep.* 2024;14(1):23700.