Original Article

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Antioxidant, antiglycation, and anti-inflammatory activities of *Caesalpinia mimosoides*

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SUMMARY Oxidative stress, glycation and inflammation are the main causes of many severe diseases. To date, no single extract has been shown to simultaneously inhibit these three reactions. In this study, the antioxidant, antiglycation and anti-inflammatory activities of ethanol extracts from four edible plants that are commonly used as Thai folk medicine were compared. Among these extracts, *Caesalpinia mimosoides* extract (CME) showed the highest antioxidant potential with Trolox equivalent antioxidant activity (TEAC) of 5.9 ± 0.1 mM/mg followed closely by *Zingiber officinale* extract (ZOE) with a TEAC value of 5.4 ± 0.2 mM/mg. However, CME showed no cytotoxicity, whereas ZOE greater than 60 µg/mL showed cytotoxicity to normal human cells. Antiglycation assay using bovine serum albumin-ribose showed comparable potency between CME and *Spondias dulcis* extract (SDE). However, CME exhibited a high anti-inflammatory activity, significantly higher than SDE and activity depending on the dose. At a concentration of 60 µg/mL, approximately 85% of the interleukin-6 pro-inflammatory cytokine produced from human monocytes, induced by lipopolysaccharides, was completely inhibited by CME whereas SDE showed no inhibition. In summary, CME is the most potential extract with simultaneously activity of these three reactions. CME has the highest total phenolic content expressed as gallic acid equivalent to 301 ± 8 mg/g. Identification using high-performance liquid chromatography revealed the presence of at least four phenolic compounds, gallic acid, syringic acid, *p*-coumaric acid, and ellagic acid are existed in CME. Our finding suggests that CME is a promising natural source for inhibition of oxidative stress, glycation, and inflammation.

Keywords Antioxidant, antiglycation, anti-inflammation, plant extract, chemical composition

1. Introduction

Oxidative stress, glycation, and inflammation are the major causes of several severe chronic non-communicable diseases including diabetes, cardiovascular disorders, and cancers that have become the leading cause of death and morbidity processes (*1–3*). It is known that oxidative stress is a transversal phenomenon in aerobic systems. It occurs when there is an imbalance between the generation of reactive species and inadequate antioxidant defense systems. It has been reported that oxidative stress implicated with the etiology of those severe diseases (*4*). Glycation is a non-enzymatic reaction between the carbonyl group of reducing sugars and the amine group of proteins or nucleic acids to form fructosamine products called Amadori (*5*). The obtained Amadori products continue a series of reactions to form the stable advanced glycation end-products (AGEs) (*6*). These AGEs cause alteration of the structure and function of extracellular matrix proteins (*7*) and generate a severe oxidative stress including reactive oxygen species (ROS) *via* complex biochemical mechanisms (*3*) while the formed ROS in turn can accelerate the rate of AGEs formation (*8,9*). In addition, ROS can stimulate nuclear factor kappa B (NF-κB) to release pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) which play major roles in inflammatory processes (*10*). Phenolic substances from several plants have been reported to inhibit oxidative stress, glycation, or inflammation, however, no compound has all of these pharmacological effects simultaneously (*11–14*). The search for active compounds with multiple targets simultaneously remains challenging for the treatment of these severe chronic diseases. In addition, most reported plants

are inedible and present some toxic for human life (*15,16*). The intake of edible plant extracts should be a much better approach for ones seeking safe and potent bioactive compounds.

In this study, 4 species of edible plants, *Caesalpinia mimosoides*, *Zingiber officinale*, *Spondias dulcis*, and *Dolichandrone serrulata* were compared for inhibitory activities against oxidative stress, glycation, and inflammation. These four plants are commonly used as raw materials in Thai folk medicine. The ethanol extracts from commonly used parts of these plants were investigated. The most active plant extract was selected for constituent analysis using a high-performance liquid chromatography (HPLC).

2. Materials and Methods

2.1. Materials

Bovine serum albumin (BSA), ribose, sodium benzoate, aminoguanidine, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), butylated hydroxytoluene, quercetin, gallic acid, syringic acid, *p*-coumaric acid, ellagic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and lipopolysaccharides (LPS) obtained from *Escherichia coli* (serotype 0111:B4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteu reagent, dimethyl sulfoxide (DMSO), sodium carbonate, ribose, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, and potassium persulfate, were obtained from Merck (Darmstadt, Germany). Ethanol (EtOH), acetic acid, and hydrochloric acid were obtained from RCI Labscan Limited (Bangkok, Thailand). Fetal bovine serum (FBS), complete Roswell Park Memorial Institute (RPMI) 1640 medium, penicillin-streptomycin, and 2-mercaptoethanol were obtained from Gibco BRL Life Technologies (Gaithersburg, MD, USA). AlamarBlue cell viability reagent was obtained from Invitrogen (Merelbeke, Belgium). Human monocytic leukemia (THP-1 cells) were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA).

2.2. Plants and preparation of plant extracts

Fresh plant materials from four plant species (Table 1) were collected or purchased from the northern area of Thailand during July and August 2020. The used parts of each plant were thoroughly washed and cut into small pieces and then dried at 50°C for 24 h. The dried plant materials were pulverized and then macerated with 95% EtOH at room temperature for 48 h. The macerated mixture was subsequently filtered through Whatman No.1 filter paper (GE HealthCare Technologies, Chicago, IL, USA). The residue from filtration was further macerated and filtered in the same manner two more times. The filtrates of each plant material from these three macerations were pooled together and evaporated using a rotary evaporator (Eyela, Tokyo, Japan) until the solvent was completely removed. The obtained crude extracts were transferred into a tight container and stored at 4°C until use.

Table 1. Plant species and their part used

Scientific name	Part used
Caesalpinia mimosoides	Leaf
Zingiber officinale	Rhizome
Spondias dulcis	Stem bark
Dolichandrone serrulata	Leaf

2.3. Determination of total phenolic content

The total phenolic content of the obtained extracts was determined using Folin-Ciocalteu assay described by Sato *et al.* (*17*) with minor modifications. Briefly, the extract was dissolved in EtOH to yield a stock solution of 1 mg/mL. An aliquot of 20 µL this solution was mixed with 45 µL of Folin-Ciocalteu reagent for 2 min, followed by the addition of 135 µL of 20 mg/mL sodium carbonate. Next, the mixture was incubated for approximately 1 h at room temperature. Subsequently the absorbance at 750 nm was measured using microtiter plate reader (BioTek Instruments, Winooski, VT, USA). Gallic acid was used for calibration (10-500 µg/mL in EtOH). The total phenolic content is expressed as gallic acid equivalent (GAE) in mg of gallic acid to 1 g of the extract.

2.4. Determination of antioxidant activity

Free radical scavenging assay was performed according to a method previously described (*18*) with some modifications. Briefly, ABTS and potassium persulfate were dissolved separately in deionized water. The free radical of ABTS was generated by mixing 8 mL of 7 mM ABTS solution with 12 mL of 2.45 mM potassium persulfate solution. The obtained mixture was incubated in the dark at room temperature for 16 h. Then, EtOH was added to the mixture to obtain an absorbance of 0.7 at 750 nm. Stock solutions of the extracts were prepared by dissolving the crude extracts in EtOH at a concentration of 0.5 mg/mL. Then, an aliquot of 20 µL of this ethanol solution was mixed with 180 µL of the ABTS free radical solution. The mixture was incubated in the dark for 5 min at room temperature. Subsequently, the absorbance at 750 nm was measured using a microtiter plate reader (BioTek Instruments). Trolox was used for calibration. Quercetin and butylated hydroxytoluene at 1 mg/mL in EtOH were used as positive controls. Various concentrations (50-500 µM) of Trolox in EtOH were used for calibration. The results are expressed as Trolox equivalent

antioxidant capacity (TEAC) in mM of Trolox which had antioxidant capacity equivalent to 1 mg of the extract. Four standard phenolic compounds (gallic acid, syringic acid, *p*-coumaric acid, and ellagic acid) were investigated for their antioxidant activity using this assay. The IC_{50} value of each standard compound was calculated using GraphPad Prism software version 8.0.1.

2.5. Determination of antiglycation activity

Antiglycation activity of the samples was investigated by determining the inhibition of AGEs formation using BSA-ribose assay as previously described (*19*) with some modifications. Briefly, 10 mg/mL BSA, with or without 0.5 M ribose (and 0.008% sodium benzoate as a preservative), the plant extract in EtOH (0.1 mg/mL) and phosphate buffer (50 mM, pH 7.4) were mixed. The mixtures without extract and with or without ribose were used as negative controls. The reaction mixtures were incubated for 3 days at 45°C. Subsequently, the fluorescence intensity was measured using spectrofluorometer (Molecular Device, San Jose, CA, USA) at excitation and emission wavelengths of 370 and 440 nm, respectively. Aminoguanidine at the same concentration of the extracts (0.1 mg/mL) was used as a positive control. The percentage of AGEs inhibition is calculated using the following equation: AGEs inhibition (%) = $[(F_C - F_{CB}) - (F_S - F_{SB})/(F_C - F_{CB})] \times 100\%$. In which F_C and F_{CB} are the fluorescence intensities of the negative controls with and without ribose, respectively. F_S and F_{SB} are the fluorescence intensities of samples containing the extracts or aminoguanidine with and without ribose, respectively.

2.6. Determination of anti-inflammatory activity

2.6.1. Cell cultures

THP-1 cells were maintained according to ATCC recommendations. The cells were cultured at 37°C under 5% CO_2 in complete RPMI 1640 medium supplemented with 10% FBS, 1% penicillin-streptomycin and 0.05 mM 2-mercaptoethanol for 72 h before the experiments were performed.

2.6.2. Cytotoxicity on THP-1 cells

The possible cytotoxic effect of the obtained plant extracts on THP-1 cells was determined by colorimetric alamarBlue assay according to the manufacturer's protocol (Invitrogen, Merelbeke, Belgium) with slight modifications. Briefly, the concentration of THP-1 cells in the suspension was adjusted to 40×10^4 cells/mL using a hemocytometer for cell counting. Then, 100 µL of this cell suspension was seeded into each well of 96-well plates and incubated at 37°C, 5% CO_2 for 24 h. The extracts were dissolved in DMSO to obtain a concentration of 50 mg/mL, and then diluted with complete RPMI 1640 medium to obtain final concentrations of 0-0.5% v/v. These suspensions were added into the wells containing cells and further incubated in 5% CO_2 at 37°C for 24 h. Cell suspensions without extract or DMSO were used as negative controls. After 24 h, the cells originally in suspension were attached to the surfaces of the wells. An aliquot of 100 µL of culture medium was removed, and 10 µL of 10-fold alamarBlue solution was added to the wells. The cells were further incubated in the dark for 3 h. Subsequently, the plate was read at 562 nm and 600 nm as a reference wavelength using a microtiter plate reader (BioTek Instruments). The percentage of cell viability was calculated using the following equation: Cell viability (%) = $[(OD_{test\ well}) / (OD_{negative\ control\ well})] \times 100\%$. In which OD is the optical density and percentage of cell viability of the negative control is defined as 100%.

2.6.3. Effect of the extracts on inflammatory response

In this experiment, an aliquot of 200 µL of THP-1 cell suspension at a concentration of 40×10^4 cells/mL was seeded into each well of 24-well plates and incubated at 37°C in 5% CO_2 for 1 h. The extracts were dissolved in DMSO to yield a stock solution. This solution was diluted in complete RPMI 1640 medium to obtain a series of nontoxic concentrations to the cells. Then 300 µL of these dilutions or 0.12% DMSO in complete RPMI 1640 medium were added to the cell suspension and further incubated for 4 h at 37°C in 5% CO_2. After that, LPS was added to obtain a final concentration of 1 µg/mL and further incubated at 37°C in 5% CO_2 for 24 h. Next, the media were collected and centrifuged at 6,000 rpm for 3 min to remove non-attached cells. The supernatant was subsequently analyzed for interleukin-6 (IL-6) using an enzyme-linked immunosorbent assay (ELISA). The cells that were not treated with LPS served as a negative control and that were not pretreated with the extracts but incubated with LPS served as a positive control. IL-6 in the cell supernatants (100 µL) was determined by ELISA according to the manufacturer's protocol (BioLegend, San Diego, CA, USA). The optical density of the samples at 450 nm, corrected by the reference wavelength 562 nm, was measured using a microtiter plate reader (BioTek Instruments). Percentage of IL-6 secretion was calculated using the following equation: IL-6 secretion (%) = $[IL\text{-}6_{SAM}/IL\text{-}6_{LPS}] \times 100\%$. In which $IL\text{-}6_{SAM}$ represents the concentrations of the secreted IL-6 from THP-1 cells treated with the extracts or DMSO or untreated with LPS (negative control) and $IL\text{-}6_{LPS}$ represents the concentrations of the secreted IL-6 from the positive control. The percentage of IL-6 secretion of the positive control is defined as 100%.

2.7. HPLC analysis

The selected potential plant extract was analyzed using HPLC Shimadzu L2030 model (Kyoto, Japan) and a reversed phase Eurospher 100, C18 column, 4 mm i.d. × 250 mm, Knauer (Berlin, Germany). The system was conducted with a gradient program as previously described (*20*) with some modifications. Briefly, a gradient eluent composed of 1% acetic acid in water (A) and methanol (B) was used. The gradient program started from 100% of A for 1 min then the ratios of eluent A:B were changed to 70:30 and 40:60 at 10 and 20 min, respectively. After that, the composition of the eluent was put back to 100% of A at 25 min and held on for 10 min. The HPLC condition was operated with an injection volume of 10 µL, a mobile flow rate of 1 mL/min, and running time of 35 min. The eluent was monitored with UV/visible detector at a wavelength of 280 nm. Four standard phenolic compounds, gallic acid, syringic acid, *p*-coumaric acid, and ellagic acid were used as standard solutions for the quantification of phenolic compounds.

2.8. Statistical analysis

All experiments were carried out in triplicate. The results are expressed as mean values ± S.D. To determine statistical different between means ($p < 0.05$), ONE-WAY ANOVA and Tukey's Multiple tests were calculated using SPSS statistical software package v.17.0.

3. Results

3.1. Total phenolic content

The total phenolic content of the ethanol extracts obtained from the four different plants are expressed as GAE value as shown in Figure 1. *C. mimosoides* demonstrated the highest total phenolic content ($p < 0.05$) with a GAE value of 301 ± 8 mg/g, followed by *Z. officinale*, *S. dulcis*, and *D. Serrulata* with the GAE values of 187 ± 4, 159 ± 5, and 43 ± 2 mg/g, respectively.

3.2. Antioxidant activity

The antioxidant activities of the four extracts investigated by ABTS assay were calculated and expressed as TEAC values and shown in Figure 2. It was found that the highest free radical scavenging activity ($p < 0.05$) was obtained from the extract of *C. mimosoides* with a TEAC value of 5.9 ± 0.1 mM/mg, followed closely by that of *Z. officinale* with a TEAC value of 5.4 ± 0.2 mM/mg. The extracts of *S. dulcis* and *D. serrulata* showed low TEAC values of

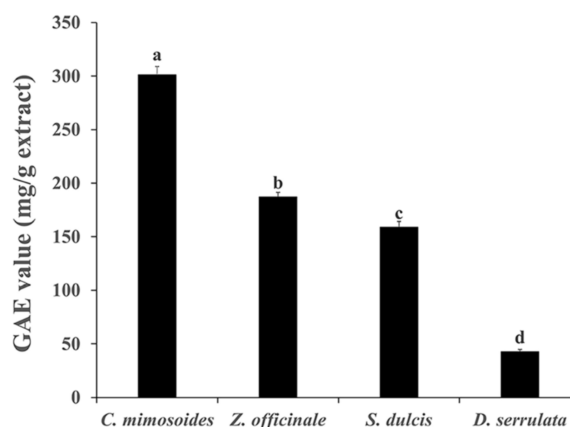

Figure 1. Total phenolic content of four plant extracts. Data represent mean ± SD of three independent experiments. Lowercase letters indicate significant difference between groups ($p < 0.05$).

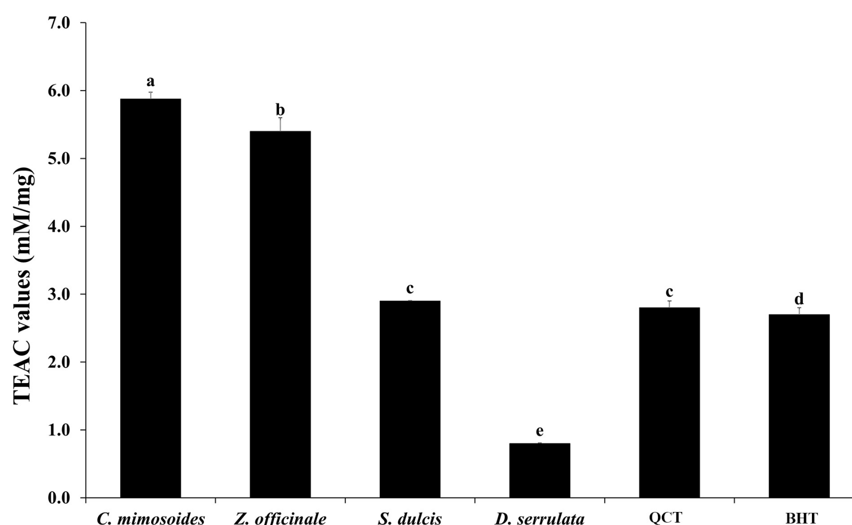

Figure 2. Antioxidant activity of four plant extracts compared to two positive controls, quercetin (QCT) and butylated hydroxytoluene (BHT). Data represent mean ± SD of three independent experiments. Lowercase letters indicate significant difference between groups ($p < 0.05$).

2.9 ± 0.1 and 0.8 ± 0.1 mM/mg, respectively, indicating that the antioxidant activity of these two extracts were significantly less than half of *C. mimosoides*. In addition, the TEAC values of quercetin and butylated hydroxytoluene were 2.8 ± 0.1 and 2.7 ± 0.1 mM/mg, respectively, indicating that the scavenging activity of the two positive controls was significantly lower than *C. mimosoides*.

3.3. Antiglycation activity

In the present study, the antiglycation activity of the extracts was investigated using the BSA-ribose assay. The activity was compared when the final concentration of the extracts and the positive control was the same (0.1 mg/mL). The results are expressed as the percentage of AGEs inhibition as demonstrated in Figure 3. *C. mimosoides* and *S. dulcis* extracts showed the same potential of AGEs inhibition of 11.4 ± 1.1% and 11.5 ± 0.5%, respectively, while that of *Z. officinale* and *D. serrulata* extracts were 5.2 ± 0.4%

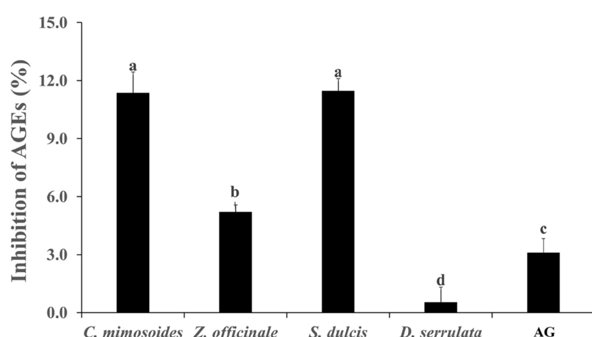

Figure 3. Antiglycation of four plant extracts compared to a positive control, aminoguanidine (AG). Data represent mean ± SD of three independent experiments. Lowercase letters indicate significant difference between groups ($p < 0.05$).

and 0.5 ± 0.8%, respectively. These results indicate that the AGEs inhibition activity of *C. mimosoides* and *S. dulcis* extracts was significantly ($p < 0.05$) higher than *Z. officinale* and *D. serrulata* extracts. In addition, aminoguanidine showed the inhibition power of only 3.1 ± 0.7%. These results indicate that *C. mimosoides* and *S. dulcis* extracts possessed AGEs inhibition activity approximately 3-times higher than the positive control.

3.4. Anti-inflammatory activity

The results from cytotoxicity study indicated that the extracts from different plants possessed different levels of toxicity to THP-1 cells as shown in Figure 4. *C. mimosoides* and *S. dulcis* extracts were found to be nontoxic to THP-1 cells at all concentrations used. The extracts of *Z. officinale* and *D. serrulata* showed some toxicity to THP-1 cells, depending on the concentration used. The higher the concentration, the higher the cytotoxicity. Viable cells after exposure to *Z. officinale* extract at the concentrations of 125 and 250 µg/mL were 66 ± 7.8% and 52 ± 5.7%, respectively, while that after exposure to *D. serrulata* extract at these concentrations were 85 ± 8.1% and 65 ± 9.5%, respectively. The concentrations of all extracts at 60 µg/mL or less were selected for anti-inflammatory activity test. To access the effects of the four plant extracts on THP-1 cells, the cells were stimulated with 1 µg/mL LPS in the absence and presence of the extracts. The results as shown in Figure 5 demonstrate that large amounts of IL-6 (100%) were secreted from the cells upon LPS stimulation, whereas very small amounts of IL-6, near 0%, were observed in the LPS-unstimulated cells. DMSO, used as a solvent for the extracts, showed no effect on IL-6 secretion. The extracts of *C. mimosoides* and *Z. officinale* exhibited extremely potent anti-inflammatory activity in a concentration-dependent

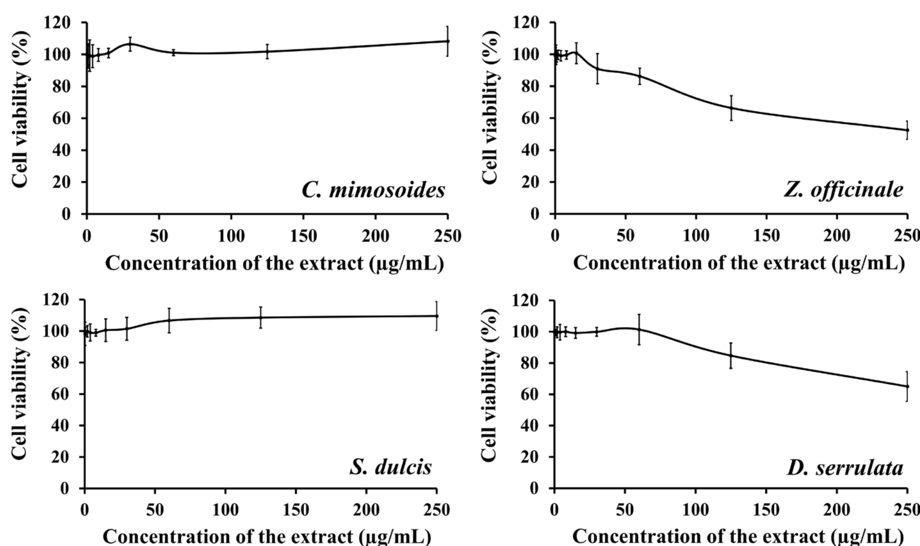

Figure 4. Cytotoxicity of four plant extracts against THP-1 cells. Data represent mean ± SD of three independent experiments.

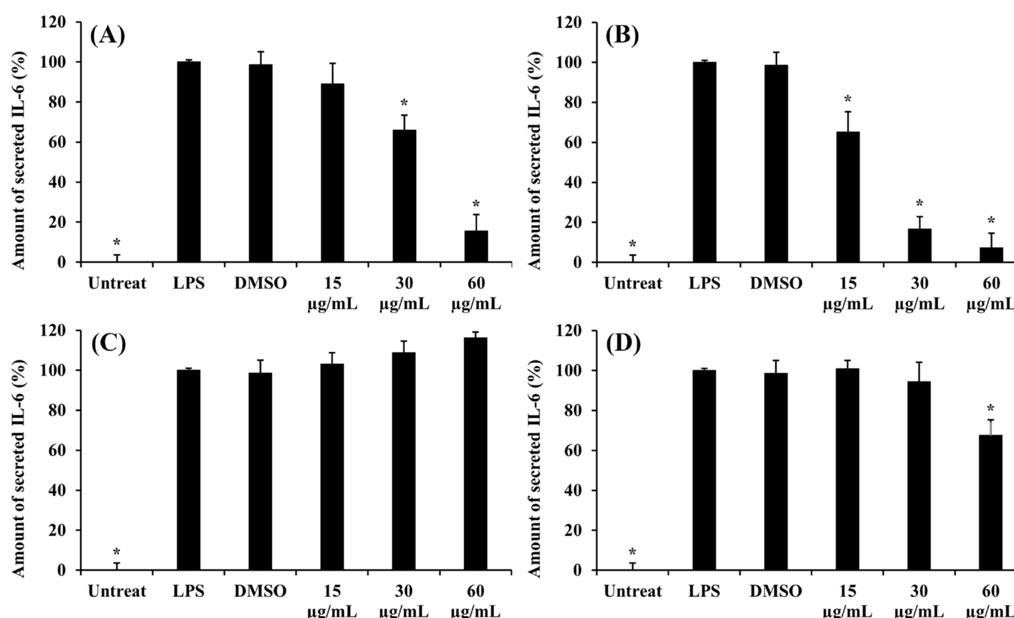

Figure 5. Anti-inflammatory activity of four plant extracts. Data represent mean ± SD of three independent experiments. Significant differences ($p < 0.05$) in comparison with those treated with LPS are indicated by *.

manner. The amount of IL-6 secreted from LPS-treated THP-1 cells was only 15 ± 8.3% and 7 ± 7.2% after the cells were exposed to those two extracts, respectively. This result indicates that both extracts inhibited IL-6 secretion by more than 85%. The inhibitory activity of *D. serrulata* extract also followed a dose dependent manner, but its inhibition power was significantly less than *C. mimosoides* and *Z. officinale* extracts. *S. dulcis* extract at all test concentrations did not inhibit IL-6 secretion. In addition, this extract tended to increase IL-6 secretion.

3.5. HPLC analysis

The above results suggested that *C. mimosoides* extract possessed the highest potential among all tested activities, so this extract was selected for HPLC analysis. Figure 6 shows the HPLC chromatogram of *C. mimosoides* extract in comparison with 4 standard phenolic compounds; gallic acid, syringic acid, *p*-coumaric acid, and ellagic acid. Comparing the retention times of these four standards (Figure 6A) with the extract (Figure 6B), it was observed that the peaks no. 1, 2, 3, and 4 of the extract seemed to be gallic acid, syringic acid, *p*-coumaric acid, and ellagic acid, respectively. To confirm this result, the extract was spiked with standard gallic acid. As expected, the peak height of peak no. 1 was obviously increased as seen in Figure 6C confirming that this peak belongs to gallic acid. Syringic acid, *p*-coumaric acid, and ellagic were added to the extract separately to identify the compounds presented in peaks no. 2, 3, and 4, respectively. The results as shown in Figure 6D, 6E, and 6F, respectively demonstrated that the peak height of peaks no. 2, 3, and 4 were increased accordingly. From this study, our results confirmed that gallic acid, syringic acid, *p*-coumaric acid, and ellagic acid were presented in *C. mimosoides* extract. Quantitative analysis indicated that *C. mimosoides* extract contained approximately 13.48%, 7.86%, 4.16%, and 7.63% of gallic acid, syringic acid, *p*-coumaric acid, and ellagic acid, respectively. From these results, it was considered that high activity of *C. mimosoides* extract might come from these compounds. To confirm this consideration, antioxidant activity of these four compounds was investigated. The results expressed as the percentage of free radical inhibition are demonstrated in Figure 7. From this result, the concentration of each compound that can inhibit 50% of free radicals (IC_{50}) was determined. It was found that among four tested compounds, gallic acid showed the highest antioxidant activity with the lowest IC_{50} value of 1.5 ± 0.1 µg/mL, followed closely by ellagic acid and syringic acid with IC_{50} values of 3.0 ± 0.1 µg/mL and 12.6 ± 0.1 µg/mL, respectively. The lowest antioxidant activity was obtained from *p*-coumaric acid, showing an IC_{50} value of 1.7 mg/mL.

4. Discussion

Oxidative stress, glycation, and inflammation are the major causes of several severe diseases such as cancer, diabetes, and cardiovascular disorders. Many attempts have been made to prevent the processes of these reactions and nowadays, people are interested in prevention using herbal medicines. *C. mimosoides* is a climbing shrub belonging the Fabaceae family and widely grows in various countries such as China,

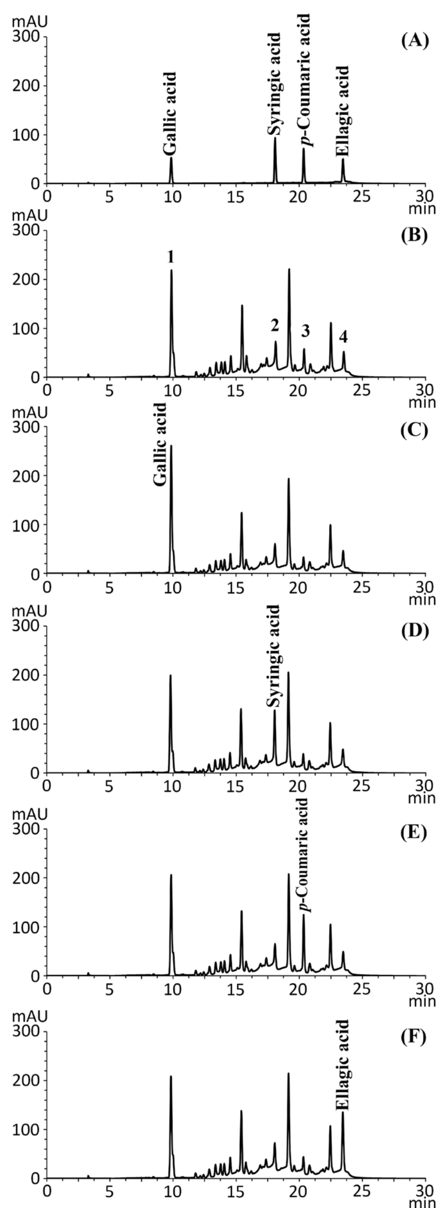

Figure 6. HPLC chromatograms of standard phenolic compounds (A), *C. mimosoides* extract (B), and *C. mimosoides* extract simultaneously spiked with gallic acid (C), syringic acid (D), (*p*-coumaric acid (E), and ellagic acid (F).

Myanmar, India, as well as in northern and north-eastern part of Thailand (*21*). Young shoots and leaves are consumed as vegetable and are traditionally used as a carminative and a remedy against dizziness and skin diseases (*22*). The plant has been reported to provide antioxidant (*23*), antimicrobial (*24*), anti-inflammatory (*25*), anticancer (*26*), and anti-diabetic activities (*27*). In the present study, the antioxidant, antiglycation, and anti-inflammatory activities of *C. mimosoides* were compared with that of *Z. officinale*, *S. dulcis*, and *D. serrulata*. These three plants are also edible and used in traditional medicines for various ailments associated with oxidation, glycation, and inflammation (*28–31*).

Phenolic compounds are good electron donors due to their structures which consist of an aromatic ring with hydroxyl group. Thus, phenolic compounds play pivotal role in antioxidant activity (*32*). Determination of total phenol content in plant extracts is a preliminary step in determining the antioxidant potential. The present study shows that among the four plant ethanol extracts, *C. mimosoides* extract contained the highest concentration of phenolic compounds. This finding is in line with the previous report showing that *C. mimosoides* had the highest phenol content among 33 edible plants (*33*). Furthermore, *C. mimosoides* extract exhibited the highest antioxidant activity *via* free radical scavenging mechanism. While the other three extracts, *Z. officinale*, *S. dulcis* and *D. serrulata* had significantly lower antioxidant activity with lower phenolic contents than *C. mimosoides* extract. These data confirm that phenolic compounds in plant extracts play an important role in antioxidant activity. Our data support the results from various authors. For example, previous report on several phenolic compounds in the leaves and fruits of *S. dulcis* exhibited strong antioxidant activity (*30*) and the previous report on linear relationship between the antioxidant activity and total phenolic content of the citrus fruit extracts (*34*). When *Z. officinale* and *S. dulcis* were compared, the antioxidant activity of *Z. officinale* extract was much higher than that of *S. dulcis*, although the phenolic content of *Z. officinale* extract was only slightly higher than that of *S. dulcis*. This result was considered that the antioxidant activity of plant extracts was not limited to phenolic compounds. The antioxidant activity may also come from the other secondary metabolites contained in plants, such as vitamins, alkaloids, and volatile oils (*35,36*).

AGEs are harmful products that occur in the late stage of glycation. Although several synthetic AGEs inhibitors have achieved promising advances *in vitro* and *in vivo*, but those synthetic compounds also possess dangerous side effects to humans. Therefore, the claims for natural or herbal substances are increasing. In the present study, we compared AGEs inhibition activity of extracts from four Thai medicinal plants using the BSA-ribose assay. As our findings showed that *C. mimosoides* possessed the highest level of antiglycation activity (with the highest phenol content and antioxidant activity), meanwhile, *D. serrulata* leaf extract showed very low antiglycation and antioxidant activities with low phenol content. This can be considered that phenolic compounds of *C. mimosoides* play a role in antiglycation activity due to antioxidation mechanism. Our results were consistent with the results of previous studies which showed a strong relationship between antiglycation and antioxidant properties of plant extracts (*37*). However, *Z. officinale* extract, which had higher phenolic content than *S. dulcis* extract possessed lower antiglycation activity than *S. dulcis* extract. This result suggests that the antiglycation activity of plant extracts may not be ascribed solely by their antioxidant activity or total phenolic content.

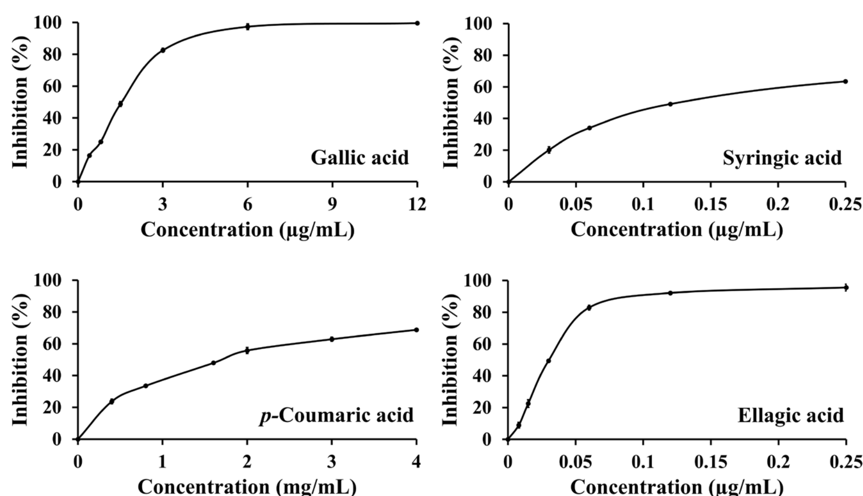

Figure 7. Free radical inhibition of four phenolic compounds presented in *C. mimosoides* extract. Data represent mean ± SD of three independent experiments.

IL-6 is one of pro-inflammatory cytokines that secretes from leukocytes when the cells are stimulated by stimulants such as bacterial LPS. In the present study, we investigated inhibitory effect of the four plant extracts on IL-6 secretion from LPS-stimulated THP-1 cell, the human leukemia monocytic cell line. In this study, the non-toxic concentration (cell viability above 80%) of the extracts was selected based on cell cytotoxicity experiments. It was found that the extracts of *C. mimosoides* and *Z. officinale* showed significant inhibitory effect on IL-6 secretion especially at concentrations of 30 and 60 µg/mL. Our findings supported the data from other reports that phenolic compounds influence anti-inflammatory activities (*38*). The previous studies reported that the anti-inflammatory activity of *Z. officinale* was from several terpenoids including gingerol (*39*). Our results also demonstrate that *D. serrulata* extract tended to inhibit IL-6 secretion at concentrations of 60 µg/mL whereas *S. dulcis* extract had no potential to reduce IL-6 secretion at all tested concentration. The results of this finding show that not only phenol compounds but also other chemical constituents that may be related to the anti-inflammatory process.

Among the four plant ethanol extracts, *C. mimosoides* extract was considered as the most potential extract for inhibition of oxidation, glycation, and inflammation because it has the highest antioxidant and antiglycation activities. This extract also has high anti-inflammatory activity without cytotoxicity. Previous studies have reported that *C. mimosoides* is high in phenolic compounds, vitamins, and carotenoids (*33*). This led us to further analyze the chemistry of this extract. Results performed using HPLC confirmed the presence of four main phenolic compounds: gallic acid, syringic acid, *p*-coumaric acid, and ellagic acid. This result supports previous studies reporting gallic acid and *p*-coumaric acid as the main phenolic compounds found in *C. mimosoides* extract (*21,23,26,27*). Our findings shed new light on syringic acid and ellagic acid in *C. mimosoides* extracts that were not previously reported. In addition, our results show the presence of very low IC_{50} values in free radical scavenging activity of gallic acid, syringic acid, and ellagic acid. This suggests that these phenolic compounds possess very high antioxidant activity and play a pivotal role on the antioxidant activity of *C. mimosoides* extract. As free radicals mainly can enhance glycation and inflammation, it was considered that the high antioxidant activity can lead to the high inhibition of glycation and inflammation. Although further studies of antiglycation and anti-inflammation of these compounds are required.

5. Conclusion

The present study shows a comparative investigation on the antioxidant, antiglycation, and anti-inflammatory activities present in the ethanol extracts of four edible and medicinal plants. All extracts contain phenolic compounds but in different levels. The antioxidant, antiglycation, and anti-inflammatory activities of some plant extracts are not ascribed solely by phenolic compounds, but it is likely affected by other metabolites existed in the extracts. Among these extracts, *C. mimosoides* extract possesses the highest antioxidant and antiglycation activities. It also possesses high anti-inflammatory activity without any cytotoxicity. The main phenolic compounds found in this extract are gallic acid, syringic acid, *p*-coumaric acid, and ellagic acid. We are the first to report that syringic acid and ellagic acid are the phenolic compounds in *C. mimosoides*. It is concluded that *C. mimosoides* extract might be a promising natural source for treatment

of chronic non-communicable diseases caused by oxidative stress, glycation, and inflammation.

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