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Research Article

The Inhibitory Effects of Aqueous Extract from Guava Twigs, *Psidium guajava* L., on Mutation and Oxidative Damage

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This study examines the inhibitory effects of the aqueous extract from guava twigs (GTE), *Psidium guajava* L., on mutation and oxidative damage. The results show that GTE inhibits the mutagenicity of 4-nitroquinoline-N-oxide (4-NQO), a direct mutagen, and 2-aminoanthracene (2-AA), an indirect mutagen, toward *Salmonella typhimurium* TA 98 and TA 100. In addition, GTE shows radical scavenging, reducing activities, tyrosinase inhibition, and liposome protection effects. Meanwhile, GTE in the range of 0.1–0.4 mg/mL protects liver cells from *tert*-butyl-hydroperoxide-(*t*-BHP-) induced cytotoxicity. Furthermore, the cytotoxicity inhibition of GTE in the *t*-BHP-treated cells was demonstrated in a dose-dependent manner. High-performance liquid chromatography analysis suggests that the major phenolic constituents in GTE are gallic acid, ferulic acid, and myricetin. These active phenolic components may contribute to the biological protective effects of GTE in different models. The data suggest that GTE exhibiting biological activities can be applied to antimutation, antityrosinase, and antioxidative damage.

1. Introduction

The production of DNA damage produces a domino effect on mutation and aging diseases. Various mutagens present on food increase oxidative stress and cancer risk through different mechanisms in cells. For example, 4-nitroquinoline-N-oxide (4-NQO), a direct and strong mutagen, is a quinoline derivative carcinogen and can also induce potent intracellular production of oxidative stress [1]. In addition, 2-aminoanthracene (2-AA), a known carcinogenic polycyclic aromatic amine, can induce tumors primarily in the liver [2]. In Ames test, 2-AA requires metabolic activation by the S9 liver preparation. The metabolic products of these mutagens chiefly bind to DNA at guanine residues. The consequence is that whenever these DNA adducts are formed, DNA mutation may increase, eventually increasing the risk of tumor progression.

Other than DNA damage, these mutagens may be metabolized and induce harmful oxidative stress in cells, which also destroys the biological molecules (e.g., lipids) and causes mutations [3]. However, many reports suggest that intracellular oxidative stress derived from reactive nitrogen species (RNS) and reactive oxygen species (ROS) arises during physiological metabolism and after exposure to various chemical encouragements. Oxidative stress can be observed in different pathological states, such as atherosclerosis and cancer. For example, tyrosinase (EC 1.14.18.1) plays an important role in the browning reaction. This enzymatic browning may result in discolouration and reduce the nutritional value of food. In order to prevent the browning, use of food additives including

reducing agent and enzyme inhibitor (such as sulphite) has been recognized [4]. During this complex process, the generation of *o*-quinone will further diminish glutathione and decrease the antioxidant capacity in cells. In the recent approach, the tyrosinase inhibitor from natural sources has become popular not only as a food additive to prevent the browning but also in applied products to decrease oxidative stress.

Guava (*Psidium guajava* L.), a genus of the Myrtaceae family, is a commercially available food crop that is distributed widely in tropical and subtropical regions around the world, such as Taiwan, Vietnam, Thailand, and China. It is a popular fruit with consumers all over the world. It has been used for treating a number of diseases such as inflammation [5], hepatotoxicity [6], and hypertension [7]. Many studies suggested that the fruits and leaves of guava contain bioactive compounds, such as phenolic acids and flavonoids [8]. This is the first study to determine the biological effects of guava twigs, an agricultural waste material, on mutation and tyrosinase activity. The objective of this work is to determine the antimutation and antioxidative damage activity of aqueous extract from guava twigs.

2. Materials and Methods

2.1. Materials. 4-Nitroquinoline-N-oxide (4-NQO), 2-aminoanthracene (2-AA), thiobarbituric acid (TBA), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), *tert*-butylhydroperoxide (*t*-BHP), and mushroom tyrosinase were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-3,4-Dihydroxyphenylalanin (L-DOPA) was obtained from Acros Organic (Geel, Belgium). Culture medium and top agar were prepared as previously described [9]. The twigs of guava were harvested from commercial plantations in Tainan, Taiwan during July 2011.

2.2. Sample Preparation. The guava twigs were grounded after dried in oven at 50°C for 24 h. The powder (100 g) was extracted with water (1000 mL) at 100°C for 30 min and then centrifuged at 10,000 g for 20 min. The extract was filtered and the residue was reextracted under the same conditions. The combined filtrate was then freeze-dried. The yield obtained was 8.1% (w/w). The final sample was named as GTE (the aqueous extract of guava twigs).

2.3. Total Polyphenolics Assay. Total polyphenolics were determined as gallic acid equivalents [10]. The different concentrations of GTE were added to a 10 mL volumetric flask, to which 2 mL sodium carbonate (20% (w/v)) was added. After 5 min, 0.1 mL Folin-Ciocalteu reagent (50% (v/v)) was added and the volume was made up to 10 mL with H_2O. After 1 h incubation at 30°C, the absorbance was measured at 750 nm and compared to a gallic acid calibration curve.

2.4. Total Flavonoid Assay. 1 mL of GTE was incubated with 0.1 mL (2-aminoethyl) diphenyl borate (0.2% in ethanol). After 20 min of incubation, the absorbance was measured at 405 nm. The absorbance of rutin solutions was detected under the same conditions. The amount of flavonoids in GTE (in rutin equivalents) was calculated [11].

2.5. ABTS Radical Cation Inhibition Assay. This assay determined the capacity of GTE to scavenge the ABTS radical cation. The ABTS radical cation scavenging activity was measured as previously described [12]. The ABTS radical cation was generated by reacting 1 mM ABTS with 0.5 mM hydrogen peroxide and 10 units/mL horseradish peroxidase in the dark at 30°C for 2 h. After 1 mL ABTS radical cation was added to samples, the absorbance at 734 nm was recorded after 10 min.

2.6. Reducing Activity Assay. The reducing power of GTE was determined as previously described [13]. Various concentrations of GTE were added to potassium ferricyanide (2.5 mL, 10 mg/mL), and the mixture was incubated at 50°C for 20 min. TCA (2.5 mL, 100 mg/mL) was added to the mixture, which was then centrifuged at 650 g for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 1.0 mg/mL), and then the absorbance was read at 700 nm. The reducing activity was calculated against an ascorbic acid calibration curve.

2.7. Liposome Oxidation Assay. A solution containing the lecithin (580 mg) and phosphate buffer (58 mL, 10 mM, pH 7.4) was sonicated by an ultrasonic cleaner (Branson 8210, Branson Ultrasonic Corporation, Danbury, CT, USA) in an ice-cold water bath for 2 h. The sonicated solution, $FeCl_3$, ascorbic acid, and various concentrations of GTE (0.2 mL) were mixed to produce a final mixture with a concentration of 3.12 μM $FeCl_3$ and 125 μM ascorbic acid and were incubated at 37°C for 1 h. The levels of liposome oxidation were determined as previously described [9].

2.8. Mushroom Tyrosinase Activity Assay. The mushroom tyrosinase was used for the bioassay. The tyrosinase inhibitory activity was determined with the degree of inhibition on tyrosinase-catalyzed oxidation of L-DOPA as previously described [14]. All the experiments were performed in sodium phosphate buffer (pH 6.8) at 25°C. The reaction mixture consisting of 0.1 mL of GTE, 0.1 mL of mushroom tyrosinase (1000 Unit/mL) and L-DOPA (3.8 mM) was determined the absorbance at 475 nm for 5 min. The value in the absence of samples was represented as the control. The inhibition of tyrosinase activity was calculated with the following formula:

$$\text{inhibition}\,(\%) = \left(1 - \left(\frac{\text{OD}_{475}\ \text{in sample}}{\text{OD}_{475}\ \text{in control}}\right)\right) \times 100\%. \quad (1)$$

2.9. HepG2 Cells Viability Assay. HepG2 cells were purchased from Bioresources Collection and Research Center (Shin-Chu, Taiwan) and cultured in minimum essential medium (MEM) containing 10% fetal bovine serum and maintained in humidified 5% CO_2/95% air at 37°C. After cells were

cultured with samples for 2 h, in the presence of 0.2 mM *t*-BHP or not, cell viability was determined by the MTT assay [9].

2.10. Mutagenicity Assay. The mutagenicity of GTE was tested according to the Ames test with a 20 min first incubation at 37°C [15]. The histidine-requiring strains of *Salmonella typhimurium* TA 98 and TA 100 were obtained from Taiwan Agricultural Chemicals and Toxic Substances Research Institute (Taichung, Taiwan). The external metabolic activation system, S9 mix (Molecular Toxicology, Inc., Boone, NC, USA), was prepared from Sprague-Dawley male rats treated with Aroclor 1254. Samples (0.1 mL, 20–100 mg/mL corresponding to 2–10 mg/plate) were added to the overnight cultured *S. typhimurium* TA98 or TA 100 (0.1 mL) and S9 mix (0.5 mL) or 0.1 M phosphate buffer (0.5 mL, pH 7.4) in place of the S9 mix. The entire mixture was incubated at 37°C for 20 min before molten top agar (2.0 mL) was added and then spread out in a Petri dish containing 20 mL of minimum agar. The mixture was counted after incubating at 37°C for 48 h. The toxic effects of GTE on *S. typhimurium* TA 98 and TA 100 was determined as previously described [9].

2.11. Antimutagenic Activity Assay. The antimutagenic activity of GTE was assayed according to the Ames method except for the addition of mutagen before incubation [15]. The concentrations of mutagens were tested as in a previous study [16]. The mutagens used were 4-NQO (0.5 μg/plate), a direct mutagen and 2-AA (2.5 μg/plate), which required S9 mix for metabolic activation. Mutagen (0.1 mL) was added to the mixture of a strain (TA 98 or TA 100), and samples were added with the S9 mix for 2-AA or with phosphate buffer (0.1 M, pH 7.4) for 4-NQO. The mutagenicity of each mutagen in the absence of samples is defined as 100%. The number of spontaneous revertants in the absence of mutagens and samples was used as reference. The inhibition (%) of mutagenicity of the sample was calculated as following:

$$\text{inhibition (\%)} = \left\{1 - \left[\frac{(\text{no. of his}^+ \text{ revertants with mutagen and sample} - \text{no. of spontaneous revertant})}{(\text{no. of his}^+ \text{ revertants with mutagen} - \text{no. of spontaneous revertant})}\right] \times 100\right\}. \quad (2)$$

2.12. High-Performance Liquid Chromatography (HPLC) Assay. HPLC was performed with a Hitachi Liquid Chromatograph (Hitachi Ltd., Tokyo, Japan), consisting of two model L-7100 pumps, and one model L-7455 photodiode array detector. Sample (10 mg/mL) was filtered through a 0.45 μm filter and injected into the HPLC column. The injection volume was 20 μL and the flow rate was 0.8 mL/min. The separation temperature was 25°C. The column was a Mightysil RP-18 GP (5 μm, 250 × 4.6 mm I.D.; Kanto Corporation, Portland, OR, USA). The method involved the use of a binary gradient with mobile phases was previously described [9]. The plot of the peak-area (y) versus concentration (x, μg/mL), the regression equations of the three marker compounds, and their correlation coefficients (r) were as follows: gallic acid, $y = 0.0161x + 0.0102$ ($r^2 = 0.9983$); ferulic acid, $y = 0.0264x + 0.0625$ ($r^2 = 0.9996$); myricetin, $y = 0.0512x + 0.0656$ ($r^2 = 0.9991$).

2.13. Statistical Analysis. All data were presented as means ± standard deviations (SD). Statistical analysis involved use of the Statistical Analysis System software package (SAS Institute Inc.). Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests at a level of $P < 0.05$.

3. Results

The HPLC chromatographic analysis showed that bioactive phenolic components in GTE have been identified as gallic acid, ferulic acid, and myricetin by measuring their retention time and UV absorbance, in relation to standards (Figure 1). Thus, gallic acid, ferulic acid, and myricetin were selected as marker compounds for the HPLC fingerprint chromatograph of GTE.

Table 1 shows the concentrations of the three marker compounds in GTE. The relative amounts of these compounds found in GTE were in the order gallic acid (11.1 mg/g of GTE) > ferulic acid (9.4 mg/g of GTE) > myricetin (3.9 mg/g of GTE). The total levels of polyphenols and flavonoids in GTE, determined as gallic acid and rutin equivalents, were 171.2 ± 8.6 and 27.2 ± 2.1 mg/g of GTE, respectively.

The effects of GTE and the three marker components on radical scavenging and reducing activities are shown in Table 2. The reducing activity of natural products is regarded as their hydrogen donating capacity. The reducing ability of GTE and its three marker components were determined in comparison with ascorbic acid. In the range 0.01–0.04 mg/mL, GTE exhibited a reducing effect that increased, as the extract concentration increased. The reducing capacity of GTE at 0.04 mg/mL was equivalent to 15.9 μg/mL of ascorbic acid. Among the three marker components, gallic acid exhibited a better reducing activity than ferulic acid and myricetin did. The scavenging of ABTS cation radicals is a popular method for determining the antioxidant capacities of natural products. The radical scavenging activity of 0.01–0.04 mg/mL concentrations of GTE was 95.1–99.3%, indicating that GTE was a strong radical terminator. Table 2 also shows the inhibitory effects of gallic acid, ferulic acid and myricetin on radicals being 99.7%, 99.5%, and 99%, respectively. These data indicated that the three marker constituents of GTE might play a part in the antioxidant activity as well as the reducing activity of GTE.

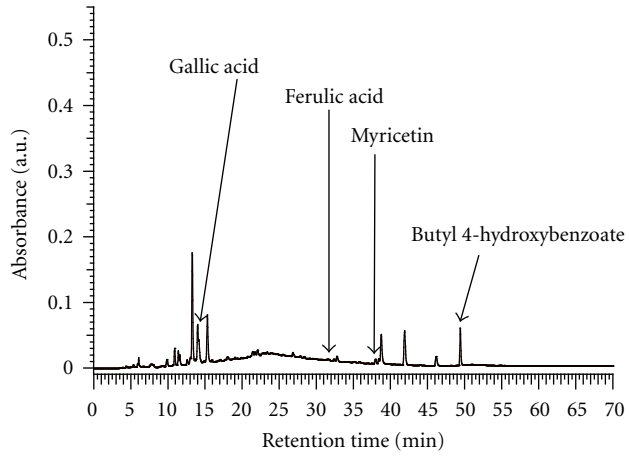

Figure 1: HPLC chromatograms of the aqueous extract of guava twigs (GTE).

Table 1: HPLC analysis of the three marker components in the aqueous extract of guava twigs (GTE).

Components	Retention time (min)	λ_{max} (nm)	Contents (mg/g GTE)
Gallic acid	13.85	218.6	11.1
Ferulic acid	31.95	233.6	9.4
Myricetin	38.06	253.1	3.9

Table 2: Effects of the aqueous extract of guava twigs (GTE) on radical scavenging and reducing activity.

Sample	(mg/mL)	Reducing activity (μg Vit C/mL)	ABTS inhibition (%)
GTE	0.01	4.4 ± 0.3	95.1 ± 0.3
	0.02	8.0 ± 0.6	98.6 ± 0.3
	0.04	15.9 ± 1.9	99.3 ± 0.3
Gallic Acid	0.01	10.8 ± 0.4	99.7 ± 1.0
Ferulic Acid	0.01	3.8 ± 0.1	99.5 ± 0.8
Myricetin	0.01	1.7 ± 0.1	99.0 ± 0.5

Data represent means ± SD for $n = 3$.

Lipid oxidation can increase cellular damage and produce toxic metabolites. In this study, liposome protection was used as a measure of decreasing lipid oxidation provided by GTE. The lower concentrations of GTE (0.01–0.04 mg/mL) did not show obvious effects in the tests of liposome protection, tyrosinase activity, and HepG2 cells viability. Therefore, the higher concentrations of GTE (0.1–0.4 mg/mL) were chosen for further tests. As shown in Table 3, GTE in concentrations of 0.1–0.4 mg/mL exhibited a 92.3–98.4% inhibitory effect on liposome oxidation induced by the Fe^{3+}/H_2O_2 system. Meanwhile, the inhibitory effects of gallic acid, ferulic acid, and myricetin on lipid oxidation were 42.5%, 32.6%, and 42.8%, respectively. These data indicated that GTE and the three marker components provided effective protection from lipid oxidation *in vitro*. Further, as shown in Table 3, GTE also had a potent inhibitory effect on mushroom

Table 3: Effects of the aqueous extract of guava twigs (GTE) on tyrosinase inhibition and liposome protection.

Sample	(mg/mL)	Tyrosinase inhibition (%)	Liposome protection (%)
GTE	0.1	17.7 ± 0.5	92.3 ± 0.9
	0.2	43.8 ± 2.9	96.5 ± 0.9
	0.4	65.6 ± 1.5	98.4 ± 3.4
Gallic acid	0.01	36.9 ± 0.6	42.5 ± 2.4
Ferulic acid	0.01	30.1 ± 2.0	32.6 ± 2.7
Myricetin	0.01	49.4 ± 2.3	42.8 ± 3.7

Data represent means ± SD for $n = 3$.

tyrosinase activity. For concentrations of 0.1–0.4 mg/mL, GTE exhibited 17.7–65.6% inhibition of tyrosinase. In this test, the inhibition of tyrosinase by GTE increased, as the sample concentration increased. Gallic acid, ferulic acid, and myricetin at 0.01 mg/mL, respectively, showed 36.9%, 30.1%, and 49.4% inhibitory effects on tyrosinase activity. Myricetin showed a greater antityrosinase effect than the other two constituents did.

Furthermore, the protective effects of GTE on *t*-BHP-induced oxidative damage in liver HepG2 cells were examined. *t*-BHP is a chemical toxin to produce peroxide intermediates in cells and promotes lipid peroxidation to result in cellular injury. Table 4 shows the protection provided by GTE from cell toxicity in *t*-BHP-induced cytotoxicity. It was found that *t*-BHP at 0.2 mM decreased cell viability down to 25.5% of control group. In the range of 0.1–0.4 mg/mL, GTE protected liver HepG2 cells against oxidative damage in a dose-dependent manner. At 0.4 mg/mL, GTE increased cell viability up to 46.1% of control group in the presence of *t*-BHP. Gallic acid, ferulic acid, and myricetin at 0.01 mg/mL, respectively, increased cell viability up to 51.9%, 58.4%, and 32.1% of control group in the presence of *t*-BHP. These data suggested that the cellular protection of GTE could attribute to its three marker components.

The Ames assay is a common method for determining the mutagenicity of natural products. In the mutation study, if a lethal toxicity occurs in a test treated sample, the results of the mutagenicity could be compromised and the numbers of revertants of TA 98 and TA 100 would be inaccurate. In this study, GTE (2–10 mg/plate) did not show any toxicity against TA 98 or TA 100 (data not shown). The mutagenicity of GTE was determined by comparing the ratio of induced revertants to spontaneous revertants, in the plates. Table 5 shows that GTE (2–10 mg/plate) did not significantly ($P > 0.05$) increase the number of colonies in *S. typhimurium* TA 98 and TA 100, with or without S9 activation.

Furthermore, the antimutagenicity of GTE on 4-NQO and 2-AA induced mutation in *S. typhimurium* TA 98 and TA 100 was examined. As shown in Table 6, GTE displayed dose-dependent protection against 4-NQO induced mutagenicity in *S. typhimurium* TA 98 and TA 100, without S9 activation. GTE at levels of 2–10 mg/plate showed 30–87% inhibition of 4-NQO induced mutagenicity in TA 98 and 31–74% inhibition in TA 100. Table 6 also shows the antimutagenicity of

Table 4: Effects of the aqueous extract of guava twigs (GTE) on *t*-butyl-hydroperoxide-(*t*-BHP-) induced HepG2 cytotoxicity.

Treatment	Sample (mg/mL)	HepG2 cell viability (% of control)
t-BHP	0	25.5 ± 8.6
	0.1	29.4 ± 5.1
t-BHP + GTE	0.2	32.6 ± 2.3
	0.4	46.1 ± 4.3
t-BHP + Gallic acid	0.01	51.9 ± 3.2
t-BHP + Ferulic acid	0.01	58.4 ± 4.2
t-BHP + Myricetin	0.01	32.1 ± 3.8

Data represent means ± SD for $n = 3$.

Table 5: The mutagenicity of the aqueous extract of guava twigs (GTE) toward *S. typhimurium* TA98 and TA100 with and without S9 mix.

Sample (mg/plate)	His^+ revertants/plate (% of spontaneous)	
	TA98	TA100
Spontaneous group	42 ± 2 (100)[a]	194 ± 9 (100)[a]
2	40 ± 5 (95)[a]	186 ± 6 (96)[a]
5	41 ± 3 (98)[a]	177 ± 14 (91)[a]
10	35 ± 3 (83)[a]	175 ± 11 (90)[a]
	TA98 + S9	TA100 + S9
Spontaneous group	27 ± 2 (100)[a]	134 ± 5 (100)[a]
2	25 ± 2 (93)[a]	142 ± 8 (106)[a]
5	27 ± 3 (100)[a]	133 ± 7 (99)[a]
10	24 ± 2 (89)[a]	128 ± 9 (96)[a]

Data represent means ± SD for $n = 3$. Values with different superscripts in a column are significantly different ($P < 0.05$). % of spontaneous = ((no. of his^+ revertants in the presence of sample)/(no. of spontaneous revertants)) × 100. The number of spontaneous revertants was determined without samples and mutagens.

Table 6: The antimutagenicity of the aqueous extract of guava twigs (GTE) toward *S. typhimurium* TA98 and TA100.

Sample (mg/plate)	His^+ revertants/plate (% of inhibition)	
	TA98 + 4-NQO	TA100 + 4-NQO
0	456 ± 32 (0)[d]	1848 ± 55 (0)[d]
2	330 ± 13 (30)[c]	1338 ± 54 (31)[c]
5	144 ± 7 (75)[b]	1049 ± 47 (48)[b]
10	97 ± 6 (87)[a]	632 ± 35 (74)[a]
	His^+ revertants/plate (% of inhibition)	
	TA98 + 2-AA + S9	TA100 + 2-AA + S9
0	534 ± 43 (0)[d]	1780 ± 142 (0)[d]
2	270 ± 19 (54)[c]	1326 ± 86 (28)[c]
5	158 ± 14 (74)[b]	717 ± 32 (65)[b]
10	120 ± 11 (82)[a]	491 ± 27 (78)[a]

Data represent means ± SD for $n = 3$. 4-NQO, 4-nitroquinoline N-oxide. 2-AA, 2-anthramine. Values with different superscripts in a column are significantly different ($P < 0.05$). % of inhibition = (1 − ((no. of revertants with mutagen and sample − no. of spontaneous revertants)/(no. of revertants with mutagen − no. of spontaneous revertants))) × 100. The number of spontaneous revertants was determined without samples and mutagen.

GTE on 2-AA induced mutation in *S. typhimurium* TA 98 and TA 100, with S9 activation. GTE at levels of 2–10 mg/plate showed 54–82% inhibition of 2-AA induced mutagenicity in TA 98 and 28–78% inhibition in TA 100. These observations indicated that GTE could inhibit the mutagenicity of both direct and indirect mutagens *in vitro*.

4. Discussion

In this study, GTE demonstrated multiple biological activities, including antimutation, antioxidation, and antityrosinase. A considerable number of studies suggested that the effects of natural antioxidants, such as polyphenols of plant extract, in the biological systems provided protection because they scavenged radicals, chelated metals, and inhibited the oxidases and then regular cellular redox states [17]. Therefore, phenolic constitutes of GTE such as gallic acid, ferulic acid, and myricetin were examined in this study.

As shown in Table 2, GTE and its three marker components exhibited antioxidant activity by scavenging radicals. It was suggested that any antioxidant capacity was due to the development of a reducing ability, when reacting with free radicals, which terminated the radical chain reaction [18]. Additionally, gallic acid showed greater reducing and radical scavenging effects than the other marker constituents did. The levels of total polyphenols and gallic acid in 0.01 mg/mL of GTE were 0.00171 and 0.00011 mg/mL, respectively. As shown in Table 2, total polyphenols of GTE might play a more important role in radical scavenging effect than the three marker compounds did.

Lipid oxidation occurs in cell membranes; it releases arachidonic acid, which is responsible for long-term oxidative stress in cells. In this study, liposome was prepared from phospholipid and used as a lipid oxidation model to imitate the lipid oxidation of biomolecules. GTE demonstrated a protective effect against the lipid damage caused by the hydroxyl radicals produced from a Fenton-like reaction. In fact, 4-hydroxyl-2-nonenal (HNE), a harmful lipid oxidation product, can bind covalently to cellular DNA, to form the exocyclin etheno-DNA-base adduct [19]. In this study, the GTE provided protection against lipid oxidation, indicating that GTE could protect biolipid molecules from oxidative stress and prevent DNA damage in tissues. The levels of total polyphenols, gallic acid, ferulic acid, and myricetin in 0.1 mg/mL of GTE were 0.01710, 0.00111, 0.00091, and 0.00039 mg/mL, respectively. As shown in Table 3, it was possible that the synergistic effects might exist among total polyphenols and the three marker compounds of GTE in liposome protection.

Polyphenols can form complexes with metal ions and exhibit antityrosinase action. Flavonols possessing a 3-hydroxy-4-keto moiety, such as kaempferol or quercetin, inhibited tyrosinase, because of their ability to chelate the copper in the active site of enzyme [20]. This indicated that GTE decreased tyrosinase activity and prevented the progression of browning in processed food. In addition, tyrosinase not only played a critical role in catalyzing the

melanogenesis, but also promoted the reactive metabolites produced during the process of melanin formation. For example, quinonic compounds generated in the polyphenol oxidation process, which then reacted with the free nucleophilic group of proteins, denatured protein structure, and irreversibly destroyed enzyme function [20]. Meanwhile, the synthesis of melanin caused glutathione depletion and the formation of hydrogen peroxide. This study found that GTE decreased tyrosinase activity implying that GTE could prevent a reduction in glutathione and protect cells against quinone induced damage. The equivalent concentrations of total polyphenols, gallic acid, ferulic acid, and myricetin in 0.4 mg/mL of GTE were 0.06840, 0.00444, 0.00364, and 0.00156 mg/mL, respectively. As shown in Table 3, the three marker constitutes of GTE might contribute more tyrosinase inhibition than total polyphenols did.

t-BHP is a well-known strong oxidant which is often used as a reference compound to induce biological injury during different studies. The toxicity of *t*-BHP is related to its induction of mass peroxide production and reduction of intracellular antioxidant levels. As shown in Table 4, GTE could protect liver cells against *t*-BHP-induced oxidative damage. However, the three marker constitutes of GTE displayed an antioxidative role and reduced *t*-BHP-induced cytotoxicity in liver cells. It was also speculate that the reduction of *t*-BHP cytotoxicity by GTE might be attributable to its three phenolic constituents and other active phytochemicals.

Though a number of studies have suggested that some phytochemicals may exhibit mutagenic and cytotoxic activity [21], GTE shows neither toxicity nor mutagenicity toward *S. typhimurium* TA 98 or TA 100 in the present study. The mechanisms of antimutagenesis have different pathways, including deactivation of mutagens, inhibition of metabolic activation of promutagens and deactivation of activated mutagens. In this study, 4-NQO produced the ultimate mutagenic compound, which bound to DNA and generated stable quinolone-purine mono adducts [1]. On the other hand, 2-AA preferred the biotransformation pathway, where a cytochrome P450-dependent monooxygenase produced a nitrene moiety that bound to DNA [2]. Natural products contain many substances that can likely reduce mutation and cancer. For example, *Origanum vulgare* L ssp. *vulgare* [22] and sesame-seed perisperm [23] also show an antimutagenic effect against different mutagens toward *S. typhimurium* mutation. As shown in Table 6, GTE played an antimutagenic role to suppress the mutagenicity of 4-NQO and 2-AA in the Ames test model. These data implied that the conjugated reaction between GTE and the toxic electrophile was an important detoxification pathway. On the other hand, GTE might play an antimutagenic role by scavenging the active metabolic electrophile of 4-NQO and 2-AA. The antimutagenic effects of the GTE might also be attributable to decrease metabolic activation and the levels of toxic reactive intermediates, which further indirectly reduced cellular oxidative stress and, thereby, prevented mutation.

In summary, as mentioned above, GTE demonstrates antimutation, antioxidation, and antityrosinase effects. These activities may be partially attributable to its polyphenolic constituents. Furthermore, the three marker compounds have similar activities to the extract that contain significantly lower levels of the three marker compounds. This suggests that other polyphenols or unknown active components in GTE could also play critical roles in its protective effects. Although these results demonstrate the protective effects provided by GTE against mutagen-induced mutation and *t*-BHP toxicity, further investigations of the nutritional and physiological effects of GTE are still required.

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