Studies on the Antioxidative Activities of Hsian-tsao (*Mesona procumbens* Hemsl) Leaf Gum

Lih-Shiuh Lai, Su-Tze Chou,* and Wen-Wan Chao

Department of Food and Nutrition, Providence University, 200 Chungchi Road, Shalu, Taichung 433, Taiwan

This study aimed at evaluating the antioxidative activity of crude hsian-tsao leaf gum extracted by sodium bicarbonate solutions and precipitated by 70% ethanol. The antioxidative activities, including the radical-scavenging effects, Fe^{2+}-chelating ability, and reducing power as well as the inhibition of $FeSO_4-H_2O_2$-induced malondialdehyde formation in rat tissue homogenate were studied in vitro. It was found that the antioxidative effect provided by hsian-tsao leaf gum was strongly concentration dependent. In general, the antioxidative activity increased with increasing gum concentration, to a certain extent, and then leveled off with further increase in gum concentration. A concentrtaion-dependent kinetics for the rate of change in antioxidative activity was proposed. The antioxidative activity constant (k) and the half-inhibition concentration (IC_{50}) for each antioxidative reaction studied were calculated. From a comparison of the IC_{50} values for different antioxidative reactions, it seemed that hsian-tsao leaf gum was more effective in scavenging superoxide radicals than chelating Fe^{2+} or scavenging α,α-diphenyl-β-picrylhydrazyl (DPPH) radicals. As compared to the commercial antioxidants, hsian-tsao leaf gum showed less scavenging effect on the DPPH radical and reducing power but better superoxide radical-scavenging effect and Fe^{2+}-chelating ability than α-tocopherol and BHT.

Keywords: *Hsian-tsao; antioxidative activity; reducing power; free radical scavenger*

INTRODUCTION

The importance of active oxygen and free radicals as an exacerbating factor in cellular injury and the aging process has attracted increasing attention over the past 20 years (*1*). In addition, these molecules are considered to induce lipid peroxidation causing the deterioration of foods (*2*). Active oxygen species in the forms of superoxide anion ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) are generated by normal metabolic processes or from exogenous factors and agents. In the past few years, there has been increasing interest in finding natural antioxidants because they can protect the human body from free radicals and retard the progress of many chronic diseases (*3*, *4*) as well as retard lipid oxidative rancidity in foods. The most effective ones seem to be rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*), two plants belonging to the Labiatae family (*5*). Some other plants of the same family have also been tested in lard stored at 75 °C and showed protective action. Oregano extract was found to be the most effective in stabilizing lard, followed by thyme, dittany, marjoram, and lavender extracts, in decreasing order (*6*).

Hsian-tsao (*Mesona procumbens* Hemsl) is also a plant belonging to the Labiatae family. The polysaccharide gum in hsian-tsao can be extracted by using sodium bicarbonate or sodium carbonate and is reported to interact with starch synergistically, which results in a marked increase in viscosity and the formation of a thermoreversible gel (*7*, *8*). The unique aroma and several health benefits of starch/hsian-tsao gum mixed gel, such as lowering blood pressure and diuretic effect, made it a quite popular dessert in Taiwan and southern China (*9*). Although hsian-tsao is a member of the Labiatae family, no literature on the antioxidant activity of hsian-tsao leaf gum is presently available. To characterize a substance as an antioxidant, its interaction against a wide range of species more directly responsible for oxidative damage should be assessed. In particular, a complete screen of antioxidative ability should include assessments on the capacity of a putative antioxidant to scavenge superoxide, hydroxyl radical, and ferryl species. Thus, the objectives of this work were to investigate the antioxidative activity including the radical-scavenging effect, reducing power, and Fe^{2+}-chelating ability of hsian-tsao leaf gum. In addition, results would be compared with those of commercial antioxidants commonly used in the food industry [butylated hydroxytoluene (BHT) and α-tocopherol (α-T*oc*)]. The ability of hsian-tsao leaf gum to prevent the formation of $^{\bullet}OH$-induced malondialdehyde (MDA) in rat tissue homogenate was also investigated.

MATERIALS AND METHODS

Chemicals. BHT, nitroblue tetrazolium (NBT), α-T*oc*, α,α-diphenyl-β-picrylhydrazyl (DPPH), phenazine methosulfate (PMS), dihydronicotinamide adenine dinucleotide (NADH), $FeCl_2$, $FeCl_3$, potassium ferricyanide, [4,4′-[3-(2-pyridinyl-1,2,4-triazine-5,6-diyl] bisbenzenesulfonic acid] (ferrozine), and trichloroacetic acid were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade or purer.

Preparation of Crude Hsian-tsao Leaf Gum. The dried hsian-tsao leaves were purchased from a specific contracted farmer. The moisture content of hsian-tsao leaves was deter-

* Author to whom correspondence should be addressed (telephone 886-4-6328001, ext. 5327; fax 886-4-6318407; e-mail stchou@pu.edu.tw).

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mined prior to polysaccharide gum extraction using AOAC Method 934.01 (*10*). Extraction of polysaccharide gum from hsian-tsao leaf was performed using the method of Lai and Chao (*7*, *8*). Hsian-tsao leaves (4% dry matter) in a sodium bicarbonate solution (0.14 M) were refluxed at 95 °C for 4 h. The polysaccharide gum in the extract was then precipitated with 70% ethanol and vacuum-dried.

Compositions of Hsian-tsao Leaf Gum. The proximate compositions of hsian-tsao leaf gum were analyzed using the methods of the AOAC (Method 934.01 for water, Method 954.02 for crude fat, Method 984.13 for crude protein, Method 962.09 for crude fiber, and Method 900.02 for ash content determination) (*10*). The total phenol content (gallic acid equivalents) was analyzed using the Folin−Ciocalteu reagent method (*11*).

Measurement of Radical-Scavenging Activity. The effect of hsian-tsao leaf gum on the DPPH radical was estimated according to the method of Yamaguchi et al. (*12*). An aliquot of hsiao-tsao leaf gum (200 μL, 0.31−2.5 mg/mL), α-Toc (0.04−1.25 mg/mL), or BHT (0.04−1.25 mg/mL) was mixed with the 100 mM Tris-HCl buffer (800 μL, pH 7.4) and then added to 1 mL of 500 μm DPPH in ethanol (final concentration of 250 μm). The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophometrically at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{scavenging effect (\%)} = \left[1 - \frac{\text{absorbance of sample at 517 nm}}{\text{absorbance of control at 517 nm}}\right] \times 100 \quad (1)$$

Measurement of Superoxide Radical-Scavenging Activity. Effects of hsian-tsao leaf gum, BHT, and α-Toc on superoxide radical were determined by the PMS−NADH superoxide generating system (*13*). The hsian-tsao leaf gum, BHT, or α-Toc (0.25∼2 mg/mL) was added to a solution mixture that contained 200 μM NBT, 624 μM NADH, and 80 μM PMS in 0.1 M phosphate buffer, pH 7.4. After 2 min of incubation at room temperature, the absorbance was measured at 560 nm. The capability to scavenge the superoxide radical was calculated using the following equation:

$$\text{scavenging effect (\%)} = \left[1 - \frac{\text{absorbance of sample at 560 nm}}{\text{absorbance of control at 560 nm}}\right] \times 100 \quad (2)$$

Measurement of Reducing Power. The reducing powers of the hsian-tsao leaf gum, BHT, and α-Toc were determined according to the method of Yen and Chen (*14*). The leaf gum (0.062−6.0 mg/mL), BHT, or α-Toc (0.02−0.625 mg/mL) was mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6, and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then an equal volume of 1% trichloroacetic acid was added to the mixture, which was then centrifuged at 6000 rpm for 10 min. The upper layer of solution was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 1:1:2, and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Measurement of Fe^{2+}-Chelating Ability. The Fe^{2+}-chelating ability was determined according to the method of Decker and Welch (*15*). The Fe^{2+} was monitored by measuring the formation of ferrous iron−ferrozine complex at 562 nm. The hsian-tsao leaf gum (0.031−1.5 mg/mL) was mixed with 2 mM $FeCl_2$ and 5 mM ferrozine at a ratio of 10:1:2. The mixture was shaken and left to stand for 10 min at room temperature. The absorbance of the resulting solution was measured at 562 nm. The lower absorbance of the reaction mixture indicated, the higher the Fe^{2+}-chelating ability. The capability to chelate the ferrous iron was calculated using the following equation:

$$\text{chelating effect (\%)} = \left[1 - \frac{\text{absorbance of sample at 562 nm}}{\text{absorbance of control at 562 nm}}\right] \times 100 \quad (3)$$

$FeSO_4$−H_2O_2-Stimulated Lipid Peroxidation in Rat Tissue Homogenate. The effect of hsian-tsao leaf gum on rat tissue (brain, heart, and liver) homogenate induced with $FeSO_4$−H_2O_2 and lipid peroxidation was determined by MDA−TBA adduct according to the method described by Yang et al. (*16*). Sprague−Dawley (SD) rats (300−350 g) were obtained from the National Laboratory Animal Breeding and Research Center of the National Science Council, Taiwan, and used for $FeSO_4$−H_2O_2-induced lipid peroxidation. Tissues (brain, liver, or heart; 0.3 g) from SD rats and 20 mL of 50 mM sodium phosphate buffer with a pH value of 7.4 were homogenized using a Heidolph homogenizer. A mixture containing 0.5 mL of tissue homogenate, 0.05 mL of potassium phosphate buffer, pH 7.4, 0.025 mL of 5 mM $FeSO_4$, 0.025 mL of 0.3% H_2O_2, and 0.05 mL of various concentrations (5−40 mg/mL) of hsian-tsao leaf gum was incubated for 10 min at 37 °C. After incubation, the incubation solutions were mixed with 2′-thiobarituric acid (0.4% in 0.2 M HCl) and BHT (0.2% in 95% ethanol) at a ratio of 1:2:0.3. The mixture was heated at 90 °C for 45 min. Once cooled, 5 mL of *n*-butanol was added, and the mixture was again shaken vigorously. The *n*-butanol layer was separated by centrifugation at 1000*g* for 10 min, and MDA production was measured at 532 nm. Tetramethoxypropane was used as an external standard. The capability to inhibit MDA formation by adding hsian-tsao leaf gum was calculated using the following equation:

$$\text{inhibition effect (\%)} = \left[1 - \frac{\text{MDA in tissue homogenate with hsian-tsao gum}}{\text{MDA in tissue homogenate without hsian-tsao gum}}\right] \times 100 \quad (4)$$

Statistical Analysis. All data were expressed as mean ± standard deviation. Analysis of variance was performed by ANOVA procedures. Duncan's new multiple-range test was used to determine the difference of means, and $p < 0.05$ was considered to be statistically significant.

RESULTS

Hsian-tsao leaf gum used in this study contained crude fat, 0.52 ± 0.01; crude protein, 10.04 ± 0.21; crude fiber, 1.47 ± 0.11; and ash, 26.20 ± 0.16 (%, dry basis). The total phenol content of hsian-tsao leaf gum was found to be 43.47 ± 1.87 mg/g, as gallic acid.

Figure 1 shows the dose−response curve for the radical-scavenging activity of hsian-tsao leaf gum, α-Toc, and BHT by the DPPH coloring method. It was found that the scavenging effects of various antioxidants on the DPPH radical were strongly concentration dependent. In general, the scavenging effects on the DPPH radical increased with increasing antioxidant concentration to a certain extent and then leveled off with further increase in antioxidant concentration. The scavenging percentage on the DPPH radical was found to be 68.6% for crude hsian-tsao leaf gum at a dose level of 1.25 mg/mL and ∼71.7% for α-Toc and BHT at a dose level of 0.31 mg/mL. In other words, to reach a similar extent of DPPH scavenging effect, the concentration required for hsian-tsao leaf gum was ∼4-fold that required for BHT or α-Toc.

Figure 2 shows the dose−response curve for the superoxide-scavenging activity of hsian-tsao leaf gum by the PMS−NADH superoxide generating system. Apparently, the scavenging effects on the superoxide radical also increased with increasing hsian-tsao leaf

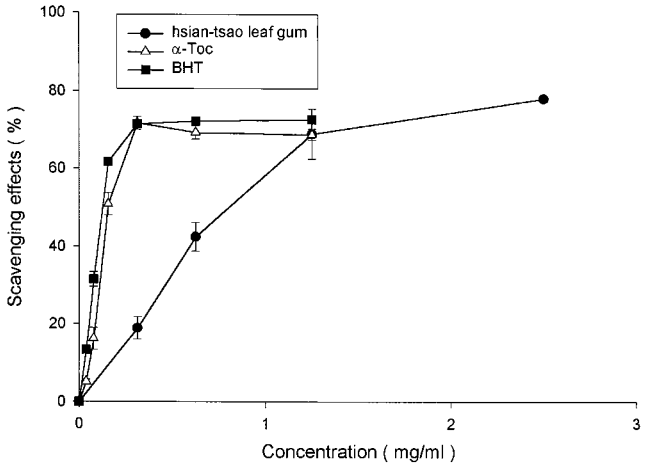

Figure 1. Scavenging effects of crude hsian-tsao leaf gum, α-Toc, and BHT on DPPH radical. Each value represents mean ± standard deviation ($n = 6$).

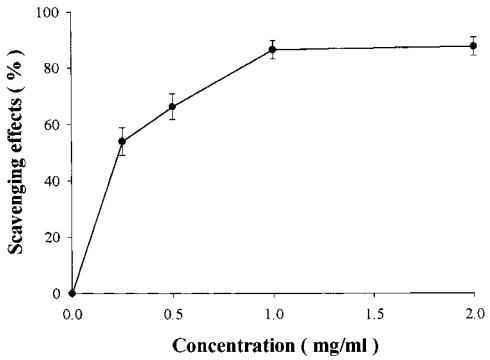

Figure 2. Scavenging effect of crude hsian-tsao leaf gum on superoxide radical. Each value represents mean ± standard deviation ($n = 6$).

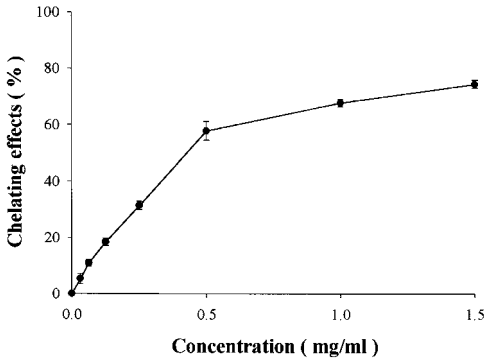

Figure 3. Chelating effect of crude hsian-tsao leaf gum on Fe^{2+} ion. Each value represents mean ± standard deviation ($n = 6$).

gum concentration to a certain extent and then leveled off with further increase in hsian-tsao leaf gum concentration. The scavenging effect on the superoxide radical was found to be 86.5% for hsian-taso leaf gum at a dose level of 1.0 mg/mL. α-Toc and BHT showed no detectable superoxide radical-scavenging effect.

Figure 3 shows the chelating effect of hsian-tsao leaf gum on ferrous ions. Similarly, the ability of chelating ferrous ions also increased with increasing hsian-tsao leaf gum concentration to a certain extent and then leveled off with further increase in hsian-tsao leaf gum concentration. The equilibrium chelating effect was found to be 74.4% for hsian-tsao leaf gum at a dose level

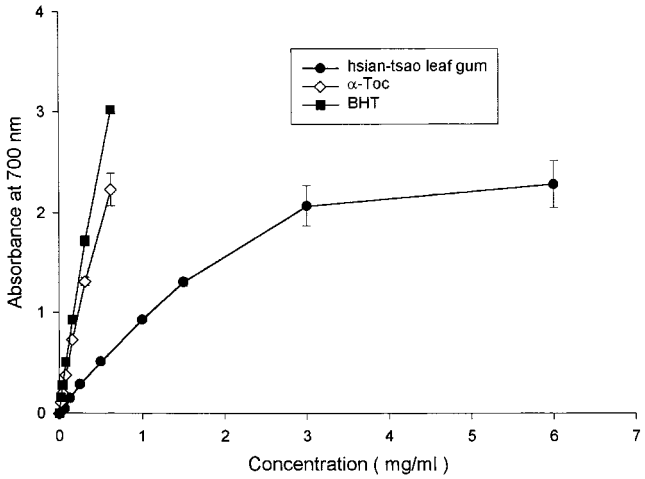

Figure 4. Reducing power of crude hsian-tsao leaf gum, α-Toc, and BHT. Each value represents mean ± standard deviation ($n = 6$).

Table 1. Inhibition Effect of Hsian-tsao Leaf Gum on $FeSO_4-H_2O_2$-Induced Lipid Peroxidation (MDA Production) in Rat Tissue Homogenate in Vitro

	inhibition effect[a] (%)		
concn (mg/mL)	brain	liver	heart
5	15.86 ± 3.50[a]	29.93 ± 4.77[a]	24.69 ± 3.22[a]
10	36.63 ± 4.38[b]	57.08 ± 3.57[b]	53.14 ± 2.26[b]
20	48.00 ± 5.09[c]	79.30 ± 2.36[c]	68.83 ± 1.93[c]
40	75.65 ± 1.71[d]	83.68 ± 2.69[c]	85.21 ± 2.53[d]

[a] Each value represents mean ± SEM ($n = 6$). Letters (a–d) denote significant difference within columns.

of 1.5 mg/mL. α-Toc and BHT showed no detectable ferrous ions chelating effect.

Figure 4 shows the reducing powers of hsian-tsao leaf gum, α-Toc, and BHT. The reducing power (as indicated by the absorbance at 700 nm) also increased with increasing hsian-tsao leaf gum concentration to a certain extent and then leveled off with further increase in hsian-tsao leaf gum concentration. However, the reducing powers of BHT and α-Toc were found to be significantly more pronounced than that of hsian-tsao leaf gum. For example, the absorbances at 700 nm were found to be 2.07 for hsian-tsao leaf gum at a dose level of 3.0 mg/mL, 2.2 for α-Toc at a dose level of 0.625 mg/mL, and 1.72 for BHT at a dose level of 0.312 mg/mL. In other words, to reach a similar extent of reducing power, the concentration required for hsian-tsao leaf gum was ~5–10-fold that required for BHT or α-Toc.

The effect of hsian-tsao leaf gum addition on the $FeSO_4-H_2O_2$-induced MDA formation in rat tissue homogenate was also investigated. The MDA levels in the normal control without $FeSO_4-H_2O_2$ were 0.16 ± 0.01, 0.06 ± 0.01, and 0.06 ± 0.01 μmol/g of wet weight in brain, liver, and heart, respectively. After induction by $FeSO_4-H_2O_2$, the MDA levels in brain, liver, and heart significantly increased to 0.40 ± 0.02, 0.22 ± 0.01, and 0.19 ± 0.01 μmol/g of wet tissue, respectively. However, as shown in Table 1, adding 5–40 mg/mL hsian-tsao leaf gum to rat tissue homogenate significantly reduced MDA formation in tissue homogenate, indicating significant anti-lipid peroxidation activities of hsian-tsao leaf gum. The inhibition percentages were in the range of 15.86–83.68%. It was interesting to note that the inhibition effect provided by hsian-tsao leaf gum was more pronounced for liver and heart tissue homogenate than brain tissue homogenate.

DISCUSSION

The present results provide evidence for potent antioxidative effect of hsian-tsao leaf gum under in vitro conditions.

Scavenging of DPPH Radical by Hsian-tsao Leaf Gum. The proton radical-scavenging action is known to be one of the various mechanisms for antioxidation. DPPH is one of the compounds that possess a proton free radical and shows a characteristic absorption at 517 nm (purple). When DPPH encounters proton radical scavengers, its purple color would fade rapidly (*12*). As shown in Figure 1, at a dosage of 0.31−2.5 mg/mL, hsian-tsao leaf gum showed 18.9−78.0% scavenging effect on DPPH radical. These results imply the antioxidative activity of hsian-tsao leaf gum may be attributed to its proton-donating ability.

Scavenging of Superoxide Radical by Hsian-tsao Leaf Gum. Superoxide ($\cdot O_2^-$), the one-electron reduced form of molecular oxygen, is a precursor to active free radicals that have the potential of reacting with biological macromolecules and thereby inducing tissue damage (*17*). According to the data of Figure 2, the marked inhibitory effect of hsian-tsao leaf gum on superoxide radicals was in a dose-dependent manner. These results reveal that hsian-tsao leaf gum is a potent scavenger of superoxide radicals and has SOD-like ability.

Fe^{2+}-Chelating Ability of Hsian-tsao Leaf Gum. A transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions and may be implicated in cardiovascular disease (*18*). Because Fe^{2+} also has been shown to cause production of oxyradicals and lipid peroxidation, minimization of Fe^{2+} concentrations in the Fenton reaction affords protection against oxidative damage. As shown in Figure 3, hsian-tsao leaf gum had the Fe^{2+}-chelating effect. In other words, hsian-tsao leaf gum may be able to afford protection against oxidative damage.

Reducing Power of Hsian-tsao Leaf Gum. In the reducing power assay, the presence of reductants (antioxidants) in the sample would result in the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. The Fe^{2+} can therefore be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increased absorbance at 700 nm indicates an increase in reducing power. As shown in Figure 4, although the greatest reducing power was observed in α-Toc or BHT relative to hsian-tsao leaf gum, the reducing power of hsian-tsao leaf gum was evident. These results reveal that hsian-tsao leaf gum is an electron donor and could react with free radicals, convert them to more stable products, and terminate radical chain reaction (*14*).

Inhibition of MDA Formation in Rat Tissue Homogenate by Hsian-tsao Leaf Gum. Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acid in the cell membranes that generates a number of degradation products. MDA, one of the major products of lipid peroxidation, has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress (*19*). As shown in Table 1, our results also demonstrated that treatment with hsian-tsao leaf gum successfully inhibited the tissues' MDA formation in the $Fe^{2+}-H_2O_2$ system. In the presence of Fe^{2+} and H_2O_2, the Fenton reaction could proceed and generate hydroxyl radical ($\cdot OH$). The $\cdot OH$ is the most toxic oxyradical that has the potential to proceed the lipid peroxidation. Moreover, lipid peroxidation is believed to be an important cause of cell membrane destruction and cell damage (*20*). Addition of hsian-tsao leaf gum to the $Fe^{2+}-H_2O_2$ system resulted in a concentration-dependent decrease in the formation of tissue MDA levels, suggesting that hsian-tsao leaf gum was a scavenger of $\cdot OH$. Therefore, the $\cdot OH$-scavenging activity observed in this study extends the antioxidative properties of hsian-tsao leaf gum and indicates a possible application for the hsian-tsao leaf gum in the treatment of diseases involving free radicals and oxidative damage, such as lipid peroxidation. Among the three tissue homogenates studied, the brain tissue contained the highest MDA concentration (0.16 μmol/g of wet weight as compared to 0.06 and 0.06 μmol/g of wet weight for liver and heart tissues, respectively). The brain is an aerobic organ that has one of the highest oxygen consumption rates on the basis of its weight and will probably possess high production rates of free radicals. Thus, the brain may be relatively more susceptible to oxidative damage (*21*). Differences in the protection of hsian-tsao leaf gum on lipid peroxidation in the different tissue homogenates are significant. However, the reasons for such a phenomenon are currently unclear and require more studies.

Concentration-Dependent Kinetics for Antioxidative Activity. As shown in Figures 1−4, the antioxidative effect provided by various antioxidants was strongly concentration dependent. In general, the antioxidative activity increased with increasing antioxidant concentration to a certain extent and then leveled off with further increase in antioxidant concentration. Therefore, particularly in this study, we assumed that the concentration-dependent rate of change in the antioxidative activity was proportional to the difference between equilibrium antioxidative activity and antioxidative activity at a given antioxidant concentration as follows

$$dA/dC = -k(A^* - A) \tag{5}$$

where A is the antioxidative activity at any given antioxidant concentration, A^* is the equilibrium antioxidative activity measured as the antioxidative activity that remained constant over a large antioxidant concentration, C is the antioxidant concentration, and k is proportional constant. Then by defining the fractional antioxidative activity (X_A) as

$$X_A = A/A^* \tag{6}$$

and combining eqs 5 and 6, it can be shown that

$$dX_A/dC = -k(1 - X_A) \tag{7}$$

Separating the variables in eq 7 and integrating eq 7, we obtained

$$\int_0^{X_A} \frac{dX_A}{1 - X_A} = -k\int_0^C dC \tag{8}$$

or

$$\ln(1 - X_A) = -kC$$

or

$$1 - X_A = e^{-kC}$$

or

$$1 - (A/A^*) = e^{-kC} \quad (9)$$

Equation 9 was therefore used to simulate the concentration-dependent antioxidative activity of hsian-tsao leaf gum, α-Toc, or BHT evaluated by various reactions. The proportional constant (k) can be obtained from the slope of a $\ln[1 - (A/A^*)]$ against antioxidant concentration (C) plot. The proprortional constant (k) has therefore the unit of reciprocal concentration and can be viewed as an indication for the antioxidative activity. A higher k value represents a higher antioxidative activity for a specific reaction. In addition, for a specific antioxidative reaction, the half-inhibition concentration (IC_{50}) can be calculated as the antioxidant concentration required for providing 50% of the antioxidative activity.

As shown in Tables 2 and 3, it is evident that the coefficients of determination (r^2) are high in all cases (0.90−0.99), indicating the proposed concentration-dependent kinetics (eq 5) for the rate of change in antioxidative activity was appropriate. On the basis of the antioxidative activity constant (k) and half-inhibition concentration (IC_{50}) results, BHT was found to show the most effective DPPH radical-scavenging ability, followed by α-Toc and hsian-tsao leaf gum in decreasing order. The difference was statistically significant ($p < 0.05$). The k values for BHT or α-Toc were ∼4−6 times that for hsian-tsao leaf gum, and the IC_{50} values for BHT or α-Toc were about one-fourth to one-sixth that for hsian-tsao leaf gum. In other words, to reach a similar extent of DPPH-scavenging effect, the concentration required for hsian-tsao leaf gum was significantly higher than that required for BHT or α-Toc. Similarly, for the reducing power assay, the k values for BHT or α-Toc were ∼4 times that for hsian-tsao leaf gum, and the IC_{50} values for BHT or α-Toc were about one-fourth that for hsian-tsao leaf gum. Therefore, to reach a similar extent of reducing effect, the concentration required for hsian-tsao leaf gum was significantly higher than that required for BHT or α-Toc. However, BHT or α-Toc did not exhibit detectable superoxide-scavenging and Fe^{2+}-chelating capabilities. As compared with the IC_{50} values for different antioxidative reactions, it seemed that hsian-tsao leaf gum was more effective in scavenging superoxide radicals than chelating Fe^{2+} or scavenging DPPH radicals.

As shown in Table 3, in the assay of the antioxidative effect on $FeSO_4-H_2O_2$-induced lipid peroxidation in rat tissue homogenate, the k value for liver homogenate was ∼3 times that for brain homogenate and was ∼2 times that for heart homogenate, respectively. On the other hand, the IC_{50} value for liver homogenate was about one-third that for brain homogenate and was about half that for heart homogenate. Therefore, the protection effect provided by hsian-tsao leaf gum was more pronounced for liver, followed by heart and brain tissue homogenate. Reasons for such a phenomenon are currently unclear and require more studies.

Conclusion. This study demonstrates that hsian-tsao leaf gum is a potent polar antioxidant that interacts with a wide range of species directly responsible for oxidative damage. The antioxidative activity of hsian-tsao leaf gum may be attributed to its proton-donating ability and SOD-like activity as evidenced through DPPH and superoxide radical-scavenging results. In addition, hsian-tsao leaf gum can also be viewed as an electron donor that could react with free radicals, convert them to more stable products, and terminate radical chain reactions. In light of these antioxidative effects, although hsian-tsao leaf gum is not currently used as a food additive, it possesses broad prospects for potential application and exploitation for the food industry. However, the components responsible for the antioxidative activity of hsian-tsao leaf gum are currently unclear. Therefore, it is suggested that further work could be performed on the isolation and identification of the antioxidative components in hsian-tsao leaf gum.

Table 2. Antioxidative Activities of Hsian-tsao Leaf Gum, α-Toc, and BHT As Expressed by Antioxidative Activity Constants (k) and Half-Inhibition Concentrations (IC_{50})

antioxidative reaction	antioxidant	k^a (mL/mg)	$IC_{50}{}^a$ (mg/mL)	$r^{2\,b}$
DPPH radical	leaf gum	2.19 ± 0.71^a	0.51 ± 0.19^a	0.99
	α-Toc	8.69 ± 0.86^b	0.15 ± 0.02^b	0.90
	BHT	12.74 ± 0.46^c	0.09 ± 0.00^b	0.95
superoxide radical	leaf gum	3.44 ± 1.11	0.27 ± 0.12	0.97
	α-Toc	NA[c]	NA	NA
	BHT	NA	NA	NA
Fe^{2+}-chelating ability	leaf gum	2.42 ± 0.10	0.46 ± 0.02	1.00
	α-Toc	NA	NA	NA
	BHT	NA	NA	NA
reducing power	leaf gum	0.79 ± 0.03^a	NA	0.98
	α-Toc	2.85 ± 0.30^b	NA	0.99
	BHT	2.65 ± 0.14^b	NA	0.99

[a] k and IC_{50} values are given as mean ± standard deviation ($n = 6$). Means with different letters in the same antioxidative reaction differ significantly ($p < 0.05$). [b] r^2 is the correlation of determination. [c] NA, not applicable.

Table 3. Antioxidative Effect of Hsian-tsao Leaf Gum on $FeSO_4-H_2O_2$-Induced Lipid Peroxidation (MDA Production) in Rat Tissue Homogenate in Vitro As Expressed by Antioxidative Activity Constants (k) and Half-Inhibition Concentrations (IC_{50})

tissue homogenate	k^a (mL/mg)	$IC_{50}{}^a$ (mg/mL)	$r^{2\,b}$
brain	0.05	21.00	0.97
liver	0.15	6.04	0.98
heart	0.08	10.62	0.98

[a] k and IC_{50} values are calculated by using the mean values of inhibition effect data of six replications. [b] r^2 is the correlation of determination.

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