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Synergistic Effect of Antioxidant Phenolic Acids on Human Phenolsulfotransferase Activity

CHI-TAI YEH, PING-HSIAO SHIH, AND GOW-CHIN YEN*

Department of Food Science, National Chung-Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan

Sulfate conjugation by phenolsulfotransferases (PSTs) is an important process in the detoxification of xenobiotics and endogenous compounds. There are two forms of PSTs for the sulfation of small phenols (PST-P) and monoamines (PST-M). Phenolic acids are known to increase the activities of PST-P and PST-M. The purpose of this study is to investigate the synergistic effect of the combinations of phenolic acids on human PSTs activities. The combinations of *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid, and coumaric acid in a random order for their effects on PSTs activities were evaluated at concentrations of 2.5, 5.0, and 7.5 μM. The PST-M activity was significantly increased when gentisic acid was combined with each of the other phenolic acids. When *p*-hydroxybenzoic acid was combined with each of the other phenolic acids, a synergistic effect with respect to the promotion of PST-P activity was obtained. A potential synergistic effect for the PST-P activity was also found in the following combination: *p*-hydroxybenzoic acid + gallic acid + gentisic acid, *p*-hydroxybenzoic acid + gallic acid + *m*-coumaric acid, *p*-hydroxybenzoic acid + *o*-coumaric acid + *p*-coumaric acid, *p*-hydroxybenzoic acid + *o*-coumaric acid + *m*-coumaric acid, gallic acid + gentisic acid + *p*-coumaric acid, and gallic acid + *o*-coumaric acid + *m*-coumaric acid. Therefore, the activities of both forms of PSTs can be promoted by all of these combinations of phenolic acids. These results provide a better understanding regarding the effect of phenolic acids on human PSTs activities, as well as more information on the intake of antioxidant phenolic acids for human health.

KEYWORDS: Phenolsulfotransferases; phenolic acids; synergistic effect; *p*-hydroxybenzoic acid; gallic acid

INTRODUCTION

The phenolsulfotransferases (PSTs) are the main sulfate conjugation enzymes for catecholamines, thyroid hormones, and drugs. At least two major forms of human PST enzymes have been characterized biochemically from liver, blood platelets, and other tissues. The PST-P is relatively specific for the sulfation of small phenols and structurally related neutral compounds, whereas PST-M is primarily responsible for the sulfation of monoamines such as dopamine (*1*). These cytosolic PSTs are particularly active in platelets and are generally present in the intestinal wall, adrenal gland, and in the brain (*2*). However, the PSTs in human platelets, liver, and gut showed great variations in their activities. The levels of PST-P in platelets are particularly interesting because they are highly correlated with the PST-P levels in the human liver, cerebral cortex, and small intestinal mucosa (*3*). Thus, it is feasible to use the PST activity in blood platelets to reflect any drug metabolizing activity in other tissues of interest. Some evidences have shown that harmful substances might accumulate in the body when the PST activity is inhibited (*4*, *5*).

There has been a growing interest in naturally occurring anticarcinogenic substances in plant foods. Plant phenols (e.g., flavonoids and phenolic acids) are currently considered as one of the most promising groups of potential dietary anticarcinogens (*6*). Plant phenolic acids are of current interest due to their important biological and pharmacological properties, especially the antiinflammatory (*7*), oxygen free radical scavenging activity (*8*), and the antimutagenic and anticarcinogenic activities (*9*). The beneficial effects of phenolic acids are found to be attributed to their antioxidant properties (*10*, *11*). Our previous studies revealed that *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid, and coumaric acid all could increase the activities of both PST-P and PST-M. These phenolic acids also possessed antioxidant capacity in the ORAC and TEAC assays (*12*). The overall effects of phenolic acids on the activities of PST-P and PST-M are highly correlated to their ORAC values, suggesting that antioxidant phenolic acids might alter sulfate conjugation. However, there is limited knowledge available about the synergistic action of these antioxidant phenolic acids on the activities of both forms of PSTs.

Therefore, this study used human platelets as a model to explore the systematic combinations of two or three phenolic compounds, by evaluating the interactions of antioxidant

* To whom correspondence should be addressed. Fax: 886-4-2285-4378. E-mail: gcyen@dragon.nchu.edu.tw.

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Table 1. Promotion of P-Form Phenolsulfotransferase Activity by Phenolic Acids[a]

concentration (μM)	promotion activity (%)					
	p-hydroxybenzoic acid	gallic acid	gentisic acid	*o*-coumaric acid	*p*-coumaric acid	*m*-coumaric acid
2.5	1.4 ± 0.1^{g}	12 ± 1^{c}	5 ± 1^{e}	4 ± 1^{f}	3 ± 1^{f}	3 ± 1^{f}
5.0	5 ± 1^{e}	26 ± 0.4^{b}	12 ± 1^{c}	8 ± 0.3^{d}	11 ± 1^{c}	4 ± 1^{f}
7.5	9 ± 2^{d}	28 ± 1^{a}	27 ± 2^{a}	28 ± 1^{a}	27 ± 1^{a}	26 ± 0.4^{b}

[a] Data are given as average percent promotion of triplicate analyses ± SD. PST-P activity of control (without phenolic acids) was 0.40 ± 0.07 pmol/min/mg of protein. Numbers followed by the same letter are not significantly different at $p < 0.05$.

phenolic acids on the activities of PST-P and PST-M. Any possible synergistic effects of these phenolic acids via different combinations will provide a deeper understanding of the effect of antioxidant phenolic acids on human PSTs activities, as well as information on the intake of antioxidant phenolic acids for human health.

MATERIALS AND METHODS

Materials. Gentisic acid, ferulic acid, gallic acid, *o*-coumaric acid, *p*-coumaric acid, *m*-coumaric acid, *p*-nitrophenol, dopamine, sucrose, and Na2EDTA were purchased from Sigma Chem. Co. (St. Louis, MO). [35S]-labeled 3′-phosphoadenosine-5′-phosphosulfate (PAPS35) (1.0−1.5Ci/mmol) was purchased from DuPont New England Nuclear (Boston, MA).

Platelet Preparation. Blood (50 mL) was collected from healthy adult volunteers by venepuncture and added into 1 mL of 5% Na2EDTA with gentle mixing. Samples were kept at room temperature and processed within 2 h. After being centrifuged at 300*g* for 10 min, platelet-rich plasma was carefully aspirated and recentrifuged at 8000*g* for 5 min. The platelet-rich pellet was washed twice with 0.9% sodium chloride, resuspended in 5 mM potassium phosphate buffer (pH 7.0) and then homogenized. The homogenate was stored at −20 °C until analyzed.

Platelet Protein Estimation. Protein content of platelet cytosols was determined by the method of Bradford with bovine serum albumin as a standard (*13*).

Assay of Phenolsulfotransferase Activity. PST activity was measured by the method of Foldes and Meek (*14*) with modifications. Platelet homogenates (20 μL) were incubated with *p*-nitrophenol (3 μM), dopamine (10 μM), and 6.7 μM [35S]-labeled PAPS in a final volume of 180 μL potassium phosphate buffer (pH 7.0) at 37 °C for 20 min. The reaction was terminated by the addition of 0.1 M barium acetate. Protein, unreacted PAPS, and free sulfate were removed by precipitation with the addition of 0.1 M barium hydroxide and 0.1 M zinc sulfate. After centrifugation at 11500*g* for 3 min, 0.5 mL of the supernatant was mixed thoroughly with 4 mL of scintillant, and radioactivity was measured by liquid scintillation spectrometry. Incubation was performed in triplicate with blank (without homogenate addition) being subtracted.

Synergistic Effects of Antioxidant Phenolic Acids on Phenolsulfotransferase Activity. The influence of antioxidant phenolic acids on PST activity was determined according to the method of Foldes and Meek (*14*) and Bamforth et al. (*15*) with a slight modification. The incubation mixture contained 100 μL of 0.1 M potassium phosphate buffer (pH 7.0), 20 μL of the platelet homogenates, 20 μL of the substrate, and 20 μL of phenolic acids (final concentration of 5.0 μM for the individual compound) and 20 μL of [35S]-labeled PAPS (final concentration of 6.7 μM) were subsequently added to tubes at 37 °C in a water bath. After 20 min, the reaction was terminated by the addition of 0.1 M barium acetate (200 μL). Any unreacted PAPS, free sulfate, or protein was precipitated by the addition of 0.1 M barium hydroxide (200 μL), followed by 0.1 M zinc sulfate (200 μL). After centrifugation (11500*g* for 3 min), 500 μL of the supernatant was mixed thoroughly with 4 mL of scintillant, and radioactivity was measured by liquid scintillation spectrometry. All assays were performed in triplicate.

Table 2. Promotion of M-Form Phenolsulfotransferase Activity by Phenolic Acids[a]

concentration (μM)	promotion activity (%)			
	p-hydroxybenzoic acid	gallic acid	ferulic acid	gentisic acid
2.5	4 ± 1^{d}	5 ± 2^{c}	2 ± 1^{d}	6 ± 1^{c}
5.0	7 ± 1^{b}	9 ± 1^{ab}	4 ± 1^{d}	8 ± 1^{b}
7.5	8 ± 1^{b}	10 ± 1^{a}	8 ± 1^{b}	10 ± 1^{a}

[a] Data are given as average percent promotion of triplicate analyses ± SD. PST-M activity of control (without phenolic acids) was 2.71 ± 0.03 pmol/min/mg of protein. Numbers followed by the same letter are not significantly different at $p < 0.05$.

To evaluate the synergistic effects of antioxidant phenolic acids in different combinations, phenolic compounds at concentrations of 2.5, 5.0, and 7.5 μM were mixed in random order in the PST assay. In all of these experiments, the concentration of the phenolic acids were calibrated to add an equal sample size of 20 μL to platelet homogenates in a final volume of 180 μL of potassium phosphate buffer. The results of triplicates were expressed as percent relative promotion (Promotion (%)): Promotion (%) = ((C−S)/C) × 100, where C is the intensity of radioactivity in the control, and S is the intensity of radioactivity in the sample (*12*). The potential synergistic effect was evaluated by comparing the total promotion effect obtained from the individual compound to that obtained from a combination of compounds tested under the same total concentration.

Statistical Analysis. Synergistic interaction between two/or three antioxidant phenolic acids was tested statistically by regular, two-side hypothesis testing at the 5% significance level (*16*). Differences in the synergistic effects of antioxidant phenolic acids were tested by one-way analysis of variance (ANOVA). Significant differences ($p < 0.05$) between means were determined using Duncan's multiple-range test.

RESULTS

Effect of Antioxidant Phenolic Acids on Human Phenolsulfotransferases Activities. In this study, we used human blood platelets as a model to evaluate the effects of antioxidant phenolic acids on the sulfation of *p*-nitrophenol and dopamine in vitro. The effects of the five antioxidant phenolic acids, including *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid, and coumaric acid, were investigated individually at three different concentration levels. The results indicated that the PST-P and PST-M activities of the control (without phenolic acids addition) were 0.40 and 2.71 pmol/min/mg protein, respectively, and that the addition of phenolic acids could influence the activities of both forms of PSTs.

As shown in **Tables 1** and **2**, significant differences in the promotion of the two types of PSTs activities were observed among the five compounds. *p*-Hydroxybenzoic acid, gentisic acid, gallic acid, *o*-coumaric acid, *p*-coumaric acid, and *m*-coumaric acid were found to significantly increase ($p < 0.05$) PST-P activity, while *p*-hydroxybenzoic acid, gallic acid, ferulic acid, and gentisic acid increased the PST-M activity. At 2.5

Table 3. Synergistic Interactions in P-Form Phenolsulfotransferase Activity by Two-Compound Combinations[a]

combination	expected promotion[b] (%)	observed promotion (%)	test statistic t_0	interaction[c] ($p < 0.05$)
p-hydroxybenzoic acid+ gallic acid	13.4 ± 1.1	19.9 ± 1.6	10.4	synergism
p-hydroxybenzoic acid+ gentisic acid	6.4 ± 1.1	14.2 ± 1.2	11.6	synergism
p-hydroxybenzoic acid+ *o*-coumaric acid	5.4 ± 1.1	23.4 ± 1.4	14.5	synergism
p-hydroxybenzoic acid+ *p*-coumaric acid	4.4 ± 1.1	24.3 ± 2.5	16.4	synergism
p-hydroxybenzoic acid+ *m*-coumaric acid	4.4 ± 1.1	23.4 ± 1.0	15.3	synergism
gallic acid + gentisic acid	17 ± 2.0	22.9 ± 2.1	10.3	synergism
gallic acid + *o*-coumaric acid	16 ± 2.0	19.7 ± 0.8	8.6	synergism
gallic acid + *p*-coumaric acid	15 ± 2.0	23.9 ± 1.8	15.4	synergism
gallic acid + *m*-coumaric acid	15 ± 2.0	21.4 ± 2.3	13.6	synergism
gentisic acid + *o*-coumaric acid	9 ± 2.0	16.5 ± 1.2	10.7	synergism
gentisic acid + *p*-coumaric acid	8 ± 2.0	21.7 ± 1.2	17.1	synergism
gentisic acid + *m*-coumaric acid	8 ± 2.0	17.6 ± 2.3	14.4	synergism
o-coumaric acid + *p*-coumaric acid	7 ± 2.0	8.5 ± 1.0	1.6	none
o-coumaric acid + *m*-coumaric acid	7 ± 2.0	7.1 ± 1.8	2.8	none
p-coumaric acid + *m*-coumaric acid	6 ± 1.1	9.1 ± 1.6	3.4	none

[a] Data are given as mean values of triplicate analyses ± SD. Concentration of individual phenol was 2.5 μM, resulting in a total addition level of 5 μM. [b] Calculated by summation of effects due to individual phenolic acid at 2.5 μM addition level (**Table 1**). [c] The obtained test statistic was compared with t_{crit} ($p < 0.05$) = $t_{0.025,4}$ = 2.77.

μM, the increase in the activity of PST-P might vary from 1.4% (with *p*-hydroxybenzoic acid) to 12% (with gallic acid) (**Table 1**). At 7.5 μM, however, no further statistically significant difference in the activity of PST-P was observed among the gallic acid (28%), gentisic acid (27%), *p*-coumaric acid (27%), and *o*-coumaric acid (28%) (**Table 1**). In summary, the activity of PST-P increased by these phenolic acids at 2.5 μM was in the order of gallic acid > gentisic acid > *o*-coumaric acid ≒ *p*-coumaric acid ≒ *m*-coumaric acid ≒ *p*-hydroxybenzoic acid, and at 5.0 and 7.5 μM, the order was gallic acid ≒ *o*-coumaric acid > *p*-coumaric acid ≒ gentisic acid > *m*-coumaric acid > *p*-hydroxybenzoic acid.

At 2.5 μM, gentisic acid and gallic acid exhibited similar promotion effects in PST-M (6 and 5%, respectively), while ferulic acid exhibited only 2% promotion in the PST-M activity (**Table 2**). On the other hand, at 2.5 μM, gentisic acid, and gallic acid were the most active compounds to promote ($p < 0.05$) the activity of PST-M, followed by *p*-hydroxybenzoic acid and ferulic acid. At 5.0 and 7.5 μM, gentisic acid, as well as gallic acid, was also the most active compound to promote the PST-M activity (**Table 2**). In **Tables 1** and **2**, *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid, and coumaric acid exhibited positive, linear dose−response effects in PSTs activities. However, the magnitude of increment in the PSTs activities among the different compounds varied markedly with the increase in concentration. At 5.0 and 7.5 μM, gallic acid showed the highest promotion effect in the activities of both PST-P and PST-M ($p < 0.05$), and *p*-hydroxybenzoic acid showed a moderate promotion effect on the activities of both forms of PSTs.

Synergistic Effects of Antioxidant Phenolic Acids on Human Phenolsulfotransferases Activities. The synergistic effects of different combinations of two or three antioxidant phenolic acids on human PSTs activities are shown in **Tables 3** and **4**. When 2.5 μM *p*-hydroxybenzoic acid was paired with the other compounds to give a total concentration of 5.0 μM, the increased activity of PST-P ranged from 19.9 to 24.3% (**Table 3**). The increased activity of PST-P obtained was generally higher than that of individual compounds (**Table 1**). This significantly higher activity can be defined as a synergistic effect of *p*-hydroxybenzoic acid on the PST-P activity (**Table 3**). In a 1:1 mixture of *p*-hydroxybenzoic acid and gallic acid, the promotion in activity was significantly ($p < 0.05$) higher than the sum of the effects due to the individual compounds. A synergistic effect in the PST-M activities (13−21%) was also observed when 2.5 μM gentisic acid was paired with the other compounds to give a total concentration of 5.0 μM (**Table 4**). Moreover, the increased activity of PST-M obtained from the mixture was generally higher than those of individual compounds (**Table 2**). In a 1:1 mixture of gentisic acid and gallic acid, the promotion in activities was significantly ($p < 0.05$) higher than the sum of the PST-M activity of the individual compounds (**Table 4**).

Table 4. Interactions in Promotion of M-Form Phenolsulfotransferase Activity by Two-Compound Combinations[a]

combination	expected promotion[b] (%)	observed promotion (%)	test statistic t_0	interaction[c] ($p < 0.05$)
gentisic acid + ferulic acid	8 ± 2	13 ± 1	10.8	synergism
gentisic acid + gallic acid	11 ± 3	20 ± 0.3	18.2	synergism
gentisic acid + *p*-hydroxybenzoic acid	10 ± 2	21 ± 0.3	12.9	synergism
ferulic acid + gallic acid	7 ± 3	20 ± 0.3	14.7	synergism
ferulic acid + *p*-hydroxybenzoic acid	6 ± 2	8 ± 0.2	1.2	none
gallic +*p*-hydroxybenzoic acid	9 ± 3	11 ± 1	1.7	none

[a] Data are given as mean values of triplicate analyses ± SD. Concentration of individual phenol was 2.5 μM, resulting in a total addition level of 5 μM. [b] Calculated by summation of effects due to individual phenolic acid at 2.5 μM addition level (**Table 2**). [c] The obtained test statistic was compared with t_{crit} ($p < 0.05$) = $t_{0.025,4}$ = 2.77.

Furthermore, a potential synergistic interaction in PST-P activity was found when a combination of three phenolic acids was used. In that case, *p*-hydroxybenzoic acid and gallic acid may exert a synergistic interaction in all combinations. As shown in **Table 5**, the activity of PST-P was significantly ($p < 0.05$) increased by the following combinations of phenolic compounds: *p*-hydroxybenzoic acid + gallic acid + gentisic acid, *p*-hydroxybenzoic acid + gallic acid + *m*-coumaric acid, *p*-hydroxybenzoic acid + *o*-coumaric acid + *p*-coumaric acid, *p*-hydroxybenzoic acid + *o*-coumaric acid + *m*-coumaric acid, gallic acid + gentisic acid + *p*-coumaric acid, and gallic acid + *o*-coumaric acid + *m*-coumaric acid. This finding is consistent with the synergistic effect of the combination of *p*-hydroxybenzoic acid and gallic acid (**Table 3**). It is speculated that these promotions in PSTs activities are attributed to the synergistic effects due to the *p*-hydroxybenzoic acid and gallic acid.

DISCUSSION

This paper described the synergistic effects of antioxidant phenolic acids on the activities of both forms of PSTs. The results have demonstrated that the activity of PST-P was enhanced ($p < 0.05$) by *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid, and coumaric acid, whereas the activity of PST-M was elevated by gentisic acid, gallic acid, *p*-hydroxybenzoic acid, and ferulic acid (**Tables 1** and **2**). These results are in agreement with our previous findings (*12*), in which gallic acid, gentisic acid, and *p*-hydroxybenzoic acid enhanced the activities of PST-P and PST-M. These phenolic compounds, especially gallic acid, *p*-hydroxybenzoic acid, gentisic acid, and coumaric acid, exhibited strong antioxidant activity in the oxygen radical absorbance capacity (ORAC) assay and trolox equivalent antioxidant capacity (TEAC) assay. Thus, there was a significant correlation between the promotion effect in PST activities and the antioxidant capacity of phenolic compounds.

Table 5. Interactions in Promotion of P-form Phenolsulfotransferase Activity by Three-compound Combinations[a]

combination	expected promotion[b] (%)	observed promotion (%)	test statistic t_0	interaction[c] ($p < 0.05$)
p-hydroxybenzoic acid + gallic acid + gentisic acid	18.4 ± 2.1	25 ± 2	11.4	synergism
p-hydroxybenzoic acid + gallic acid + *o*-coumaric acid	17.4 ± 2.1	18 ± 2	1.7	none
p-hydroxybenzoic acid + gallic acid + *p*-coumaric acid	16.4 ± 2.1	17 ± 2	1.0	none
p-hydroxybenzoic acid + gallic acid + *m*-coumaric acid	16.4 ± 2.1	30 ± 2	23.6	synergism
p-hydroxybenzoic acid + *o*-coumaric acid+ *p*-coumaric acid	8.4 ± 2.2	11 ± 1	4.5	synergism
p-hydroxybenzoic acid + *o*-coumaric acid+ *m*-coumaric acid	8.4 ± 1.1	22 ± 1	25.3	synergism
p-hydroxybenzoic acid + *p*-coumaric acid+ *m*-coumaric acid	6.4 ± 2	7 ± 1	1.7	none
p-hydroxybenzoic acid + gentisic acid + *o*-coumaric acid	10.4 ± 2	12 ± 1	1.9	none
p-hydroxybenzoic acid + gentisic acid + *p*-coumaric acid	9.4 ± 1	10 ± 1	2.2	none
gentisic acid + *o*-coumaric acid + *p*-coumaric acid	12 ± 3	13 ± 1	1.5	none
gallic acid + gentisic acid + *p*-coumaric acid	20 ± 3	24 ± 1	5.6	synergism
gallic acid + *o*-coumaric acid + *p*-coumaric	19 ± 3	20 ± 2	1.6	none
gallic acid + *o*-coumaric acid + *m*-coumaric	19 ± 3	26 ± 1	12.1	synergism
gallic acid + *p*-coumaric acid+ *m*-coumaric	18 ± 4	19 ± 1	1.5	none
o-coumaric acid + *p*-coumaric acid + *m*-coumaric acid	10 ± 3	12 ± 1	1.9	none

[a] Data are given as mean values of triplicate analyses ± SD. Concentration of individual phenol was 2.5 μM, resulting in a total addition level of 7.5 μM. [b] Calculated by summation of effects due to individual phenolic acid at 2.5 μM addition level (**Table 1**). [c] The obtained test statistic was compared with t_{crit} ($p < 0.05$) = $t_{0.025,4} = 2.77$.

Hydroxycinnamic acids and hydroxylbenzoic acids are secondary plant products and are commonly found in plant-derived foodstuffs. Gallic acid, ferulic acid, gentisic acid, and coumaric acid have higher antioxidant activity than trolox ($p < 0.05$) (*12*). Ferulic acid and *p*-coumaric acid are reported to be scavengers of thiol free radicals (*17*). Ferulic acid also possesses potent antioxidant properties in preventing the oxidative DNA damage induced by the Fenton reaction (*18*). Gentisic acid is reported to have an inhibitory action in the myeloperoxidase system and is able to impair the tyrosyl radical catalyzed low-density lipoprotein peroxidation (*19*). In addition, gallic acid has been shown to induce cell death in cancer cells (*20*). In the present study, *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid, and coumaric acid exhibited a positive and linear dose−response by increasing the activities of both forms of PSTs. Gallic acid showed the highest promotion effects on the activities of both the PST-P and PST-M ($p < 0.05$), while *p*-hydroxybenzoic acid showed the lowest effect. Sulfate conjugation by PST is an important pathway for the detoxification of xenobiotics and endogenous compounds. When the PSTs activities are inhibited, harmful substances might accumulate in the body (*4*, *5*). The data from Yeh and Yen (*12*) suggested that antioxidant phenolic acids might alter sulfate conjugation.

The wide mixture of phenolic antioxidants found in wine and plant foods may produce synergistic protection against LDL oxidation (*21*). Stahl et al. (*22*), using a lipid peroxidation assay, have reported that the carotenoid mixtures (lycopene and lutein) have a synergistic protection on multilamellar liposomes against oxidative damage. Genistein, a prominent isoflavone in soy product exhibited a synergistic anticancer effect with vitamin D against human prostatic epithelial cell line, suggesting that dietary intake of genistein in combination with vitamin D may be beneficial for prostate cancer control (*23*). In this present study, the synergistic effects of antioxidant phenolic acids on the increase of human PSTs activities were examined by using different combinations of two or three phenolic compounds. It was found that these antioxidant phenolic acids showed a significant synergistic effect on the increase of human PSTs activities (**Tables 3** and **4**). When gallic acid was combined with other antioxidant phenolic acids, it exerted a synergistic effect on the PSTs activities. A synergistic effect on the PST-M activity was also observed when gentisic acid was paired with other compounds tested in this study. Furthermore, a potential synergistic interaction of PST-P activity was found with a combination of three phenolic acids (**Table 5**). The increased activity of PST-P obtained was generally higher than that of individual compounds (**Table 1**). Meyer et al. (*24*) demonstrated that catechin, cyanidin, caffeic acid, quercetin, and ellagic acid showed a synergistic protection against human LDL oxidation in both two-compound and three-compound combinations, suggesting that high amounts of antioxidant flavonoids and phenolic acids may exert a cardioprotective effect in humans. Information on antioxidant interactions among different phenolic acids could provide more understanding of the effects of phenolic acids on human PST activities as well as information on the intake of antioxidant phenolic acids for human health. Phenolic acids can be derived from two nonphenolic molecules such as benzoic and cinnamic acids. Gallic acid, gentisic acid, and *p*-hydroxybenzoic acids are hydroxy derivatives of benzoic acid, while ferulic and *p*-coumaric acids are hydroxy derivatives of cinnamic acid. In the present study, gallic acid showed the highest promotion effect in the activity of PST-P (26% promotion at 5 μM), than *p*-hydroxybenzoic acid, which had more hydroxyl groups. The differences in their structural features can be applied to explain the observed variations in the increased PST activity among different antioxidant phenolic acids. Therefore, the differences in the synergistic effects among the phenolic acids on the PST-P activity can be attributed to the variations in the hydroxyl groups in the A ring and their antioxidant activities (*12*). The capacity of these phenolic acids to modulate sulfate conjugation may be a factor in the interindividual variation found in xenobiotic metabolism and could make it necessary for volunteers in drug metabolism studies to adhere to a common dietary regime.

In conclusion, our results have demonstrated that *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid, and coumaric acid exhibit a positive and linear dose−response by increasing the activities of both forms of PSTs. Furthermore, in both two-compound and three-compound combinations with each of other phenolic acids, gallic acid, and gentisic acid, they exhibit the potential synergistic effects in the promotion of PSTs activities. The activities of both forms of PSTs can be promoted by those phenolic acids, with two or three being used together. Therefore, as we suggested previously (*12*), the differences in their structural features can be applied to explain the observed variations in the increased PST activity among different antioxidant phenolic acids. Since PST is a key enzyme to catalyze the xenobiotics metabolism, the increased activity of

PST will therefore promote the efficiency of detoxification. The biological implications of these findings could be important, not only for understanding the synergistic effects of antioxidant phenolic acids in human PSTs activities, but also for understanding the possible role of antioxidant phenolic acids as a chemopreventive agent in sulfate conjugation.

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