Synergistic Effects of *Potentilla fruticosa* L. Leaves Combined with Green Tea Polyphenols in a Variety of Oxidation Systems

Zehua Liu, Ziwen Luo, Caixia Jia, Dongmei Wang, and Dengwu Li

Abstract: *Potentilla fruticosa* L. leaves are widely used as tea in China, with many commercial "Jinlaomei" teas available in markets. It has been confirmed to possess significant antioxidant activity than that of butylated hydroxytoluene. In this study, the synergistic effects of *P. fruticosa* leaves extracts (PFE) combined with green tea polyphenols (GTP) were studied to elucidate their use in combination and find specific combinations with least concentrations that enhance the antioxidant activity. Isobolographic analysis indicated that the combination of PFE and GTP demonstrated extensive synergism (22/28 of the tests showed significant synergy) with 3:1 (PFE:GTP) exhibiting the best synergistic effect. Chemical compositions and content of 7 phenolic compounds in PFE, GTP, and their mixtures were evaluated by reverse-phase-high-performance liquid chromatography. While chemical composition did not seem to change after the combination, as no new peaks appeared in the chromatogram, and no existing peaks disappeared. However, the content of (+)-catechin, (−)-epigallocatechin (EGC), and , (−)-epigallocatechin gallate (EGCG) changed. Besides, antioxidant interactions of extracts and compounds were evaluated, EGC with hyperoside exhibited the greatest synergistic effect and the combination of 3:1 exhibited the strongest synergism (DPPH $\gamma = 0.86$, ABTS $\gamma = 1.12$, FRAP $\gamma = 1.16$). Therefore, interaction of phytochemicals may be one reason for the synergistic effects in PFE + GTP, with EGC + hyperoside likely playing an important role. This report provides a theoretical basis for the concomitant use of *P. fruticosa* blended with GTP, which can be effectively used as a compounded tea, dietary supplements, and substituent of synthetic antioxidant.

Keywords: green tea polyphenols, isobolographic analysis, P. fruticosa L., phenolic compounds, synergistic effects

Practical Application: This result provides a theoretical basis for the concomitant use of *P. fruticosa* leaves blended with green tea polyphenols, which can be effectively used as a compounded tea, dietary supplements, and substituent of synthetic antioxidant.

Introduction

More and more researchers released that it was not adequate to assess the health benefit of food and drug mixtures from their single components because the responsible bioactive compounds seldom work independently (Wang and others 2011). Many plant-based foods are good sources of unique phytochemical antioxidants, which may exert different health-promoting effects (Elham and others 2015). A combination of different plant-based foods may exhibit additive, synergistic, or antagonistic interactions among their different phytochemicals, which may in turn alter their physiological impacts (Wang and others 2011). These effects are not definite properties for plant extracts, but they are related to the way they are explained and their ratios showed that a synergistic effect would enhance the antioxidant activity and thus make it possible to use lower doses of each extracts and prevent the side effects of using large amounts of individual plant extracts (Liao and others 2000; Borgert and others 2005; Jain and others 2011).

Potentilla fruticosa is a species of hardy deciduous flowering shrub in the *Potentilla* genus of the family Rosaceae which has long been applied in traditional Chinese medicine for significantly antioxidant, hypoglycemic, anti-inflammatory, and anti-ulcerogenic potential properties (Miliauskas and others 2004). In Europe and Northern America, it has been used as folk medicinal herbs as a tonic and an antidiarrheal, the leaves were boiled in water and drunk as tea (Syiem and others 2002; Gürbüz and others 2005). Furthermore, *P. fruticosa* has been confirmed to possess relatively high concentrations of phenolic acids and flavonoids with powerful radical scavenging capacity (Zhao and others 2008; Tomczyk and others 2009, 2010). The contents of hyperoside, (+)-catechin, and ellagic acid are extremely high in *P. fruticosa* leaves (Wang and others 2013). The radical scavenging capacities of some extracts were even higher than that of the synthetic antioxidant butylated hydroxytoluene (Miliauskas and others 2004). Moreover, its extracts have been shown to be safe and free of toxic effects in humans and are widely used in applications in the medicine, cosmetic and tea industries (Leporatti and others 2003; Shushunov and others 2009) with many commercial "Jinlaomei" teas available in Chinese markets.

Tea is one of the most popular beverages consumed worldwide, second only to water (Cheng, 2006). Green tea polyphenols (GTP), known as catechins, usually account for more than 30% of the weight of dried tea leaves (Khan and Mukhtar 2007). Plant-based foods are natural and safe when used in small amounts from their natural source, but when used in larger amounts

MS 20152017 Submitted 12/3/2015, Accepted 3/6/2016. *Authors are with College of Forestry, Northwest A&F Univ., Yangling, Shaanxi 712100, China. Direct inquires to authors Wang (E-mail: dmwli@163.com)*

Authors Liu and Luo contributed equally to this work.

doi: 10.1111/1750-3841.13292

Journal of Food Science A Publication of the Institute of Food Technologists

to prevent oxidation, the safety effects are unknown (Elham and others 2015). In our previous study, *P. fruticosa* leaves showed outstanding antioxidant synergy when combined with *Ginkgo biloba* extracts (Wang and others 2015). However, no research has been reported on the antioxidant interactions of *P. fruticosa* with GTP. Therefore, this study aimed at investigating the synergistic effects of *P. fruticosa* extracts (PFE) combined with GTP in a variety of oxidation systems in order to find specific combinations with least concentrations that exhibit synergistic effect. The results provides a theoretical basis for the concomitant use of *P. fruticosa* blended with GTP, which can be effectively used as a compounded tea, a dietary supplements and substituent of synthetic antioxidant.

Materials and Methods

Chemicals and reagents

Sodium borohydride ($NaBH_4$), methanol, hydrochloric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, potassium chloride, potassium dihydrogen phosphate, sodium carbonate, ferric trichloride hexahydrate ($FeCl_3$•$6H_2O$), and potassium persulfate (Tianjin Bodi Chemical Co., Ltd, PR China); 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethyl-benzothiazoline -6- sulphonic acid) diammonium salt (ABTS), 2, 4, 6- Tripyridyl-s-triazine (TPTZ), and 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid (Trolox, Sigma-Aldrich Co., St. Louis, Miss., U.S.A.); gallic acid monohydrate (Kebang Bioscience & Technology Co., Ltd, PR China); ethanol, acetone (Chengdu Kelong Chemical Co., Ltd, PR China); GTP, (–)-epigallocatechin gallate (EGCG), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), (–)-epicatechin (EC), (+)-catechin, hyperoside, and ellagic acid (Shanghai Yuanye Industrial Co. Ltd, PR China). All other reagents and solvents used were of analytical grade. Deionized water (18 MΩ cm) was used to prepare aqueous solutions.

Plant material and preparation of crude extracts

P. fruticosa leaves were collected from Huzhu Northern Mountain, Qinghai during 2014 at an altitude of 2940 m (E 102°21.149′, N 36°55.807′) and were identified by the Herbarium of the Northwest A&F University, Yangling, China. The collected leaves were air-dried under shade at room temperature and stored at −20 °C in the dark for further use. The air-dried leaves (5000 g) were extracted with 80% chilled acetone at room temperature (1 h × 3), and the total filtrate was then concentrated by rotary evaporation under vacuum to obtain the crude extracts (PFE, 1062.59 g; yield, 21.25%).

Determination of total phenolic content

The total phenolic content (TPC) was determined by a modified Folin-Ciocalteau colorimetric method (Burcu and others 2014; Mocan and others 2014). The samples were dissolved in 80% ethanol and then prepared at concentration of 0.2 mg/mL. 100 μL of sample and 400 μL of deionized water were added to a glass culture tube. Meanwhile, 100 μL of Folin-Ciocalteu reagent was added, mixed and let stand for 6 min. Next, 1 mL of 7% sodium carbonate and 0.8 mL of deionized water were added, mixed and let stand for 90 minutes at room temperature. Finally, absorbance was measured at 760 nm with a spectrophotometer (Shimadzu UV-1800). The gallic acid equivalent from the calibration curve of gallic acid standard solutions (10, 20, 40, 60, 80, 100, 200, 300, and 400 μg/mL) was used to calculated the phenolic content of all samples, with all values expressed as millimole gallic acid equivalent per 100 g of dry weight (mmol equiv. GAE/100 g). Data were reported as the mean ± SD for 3 replicates.

Reverse-phase HPLC analysis of phenolic compounds

The HPLC analysis was carried out with an Agilent Series 1260 liquid chromatography equipped with a quaternary gradient pump and a variable-wavelength detector connected to a reverse-phase (RP) SB-C 18 column (5 μm, 4.6 × 250 mm, Agilent, Santa Clara, California, U.S.A.). Data collection was performed using ChemStation software (Agilent).

All samples were filtered through a pinhole and analyzed by RP-HPLC. The 7 phenolic compounds (EGCG, EGC, ECG, EC, (+)-catechin, hyperoside, and ellagic acid) were detected and quantified at ambient temperature. The mobile phases consisted of water with 0.2% trifluoroacetic acid (solvent A) and methanol with 0.2% trifluoroacetic acid (solvent B). The flow rate was kept at 0.8 mL/min and the gradient elution program was set as follows: 5% B (0 min), 20% B (0 to 10 min), 25% B (10 to 15 min), 25% B (15 to 20 min), 30% B (20 to 25 min), 35% B(25 to 30 min), 45% B (30 to 40 min), 70% B (40 to 45 min), 80% B (45 to 55 min), 100% B (55 to 60 min), 100% B (60 to 70 min). The injection volume was 20 μL and the detection wavelength was 254 nm. All the analyses were performed in triplicate.

To ensure the accuracy of the measurements, validation of the method was also performed. The precision of this method was determined by assaying 6 replicates of these compounds. The repeatability of the method was measured by extracting 1 sample 6 times. A recovery experiment was performed by mixing the quantified samples with standard compounds in appropriate amounts. The relative standard deviation (RSD) of the peak area was estimated to be less than 3%, demonstrating the analysis method to be repeatable and accurate.

DPPH free radical-scavenging assay

The DPPH radical scavenging capacity was assayed as described by Yen (Yen and Chen 1995). The samples were diluted with 80% ethanol at the following concentrations: 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.52, and 0.76 μg/mL. Next, amounts of 2.0 mL of test samples and the positive controls were added to 2.0 mL of fresh DPPH methanolic solution (0.1 mM). Finally, the solutions was mixed well and let stand in the dark for 30 min at room temperature, after which their absorbance at 517 nm was measured with a spectrophotometer against a blank. Trolox prepared in the same concentrations was used as the positive control and all measurements were done in triplicate. The DPPH free radical-scavenging activity was calculated by using the following equation:

$$\text{Scavenging Ability} = [1 - (\text{Ai} - \text{Aj})/\text{A}_0)] \times 100\% \quad (1)$$

where A_0 is the absorbance of 80% ethanol (2 mL) and DPPH (2 mL); A_i is the absorbance of tested samples (2 mL sample and 2 mL DPPH radical); A_j is the absorbance of the blank (2 mL sample and 2 mL 80% ethanol). IC_{50} values were the effective concentrations at which DPPH radicals were scavenged by 50% and were obtained from linear regression analysis. A lower IC_{50} value indicates a higher DPPH radical-scavenging activity.

ABTS• + radical cation scavenging assay

The scavenging effects for the ABTS• $^+$ radical cation were monitored according to the method previously described by Mocan with some modifications (Mocan and others 2015). The

ABTS• $^{+}$ solution were stocked by mixing the ABTS solution (7 mM in distilled water) with 2.45 mM potassium persulfate. Before the test, the mixtures were kept in dark for 12 to 16 h at room temperature. For each analysis, the ABTS• $^{+}$ solution was freshly prepared and diluted to an initial absorbance of 0.70 ± 0.02 at 734 nm with pH 7.4 phosphate buffered saline (PBS) solution. Then, 100 μL of sample (0.01 mg/mL, w/v) was added to 3.9 mL of ABTS•$^{+}$ solution and its absorbance was determined at 734 nm. The results were expressed in terms of micromoles trolox equivalent per gram of dry extracts (mmol equiv. Trolox/g). All determinations were carried out in triplicate.

Ferric reducing power (FRAP) assay

The FRAP assay was performed through the method reported by Benzie with some modifications (Benzie and Strain 2008). The FRAP reagent was prepared daily by mixing 300 mM acetate buffer (3.1 g $C_2H_3NaO_2{\cdot}3H_2O$ and 1.6 mL $C_2H_4O_2$), 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3{\cdot}6H_2O$ solution completely at 37 °C. Samples were prepared at a concentration of 0.1 mg/mL. For each analysis, 3 mL of fresh FRAP reagent was added to 400 μL of sample solution. After 30 min of dark incubation at 37 °C in a water bath, the absorbance of the reaction mixture was measured at 593 nm using the spectrophotometer. The trolox was used as the standard solution. The FRAP results were expressed in terms of micromoles trolox equivalent per gram of dry extracts (mmol equiv. Trolox/g). All of the treatment groups were performed in triplicate.

Isobolographic analysis

The isobolographic analysis was used to show the interactions (synergistic, additive or antagonistic) in different combinations (ratios of 7:1, 5:1, 3:1, 1:1, 1:3, 1:5, and 1:7, v/v) of extracts or compounds (all individual extracts or compounds were prepared at 1.0 mg/mL) (Grabovsky and others 2004; Lansky and others 2005; Tallarida 2012). If the observed doses of combination was on the additivity isobole or close to it, this indicates no interaction; measurements below and above the additivity isobole indicates synergism and antagonism, respectively. This interpretation was suitable only for the DPPH assay because a lower IC_{50} value indicates a higher DPPH radical-scavenging activity. For the ABTS and FRAP assays, the interpretation is opposite. In addition, the interaction index, denoted γ, was introduced to further measure the effects of different combinations:

$$(a/A) + (b/B) = \gamma \quad (2)$$

where A and B are the doses of drug A (alone) and B (alone), respectively, that give the specified effect; a and b are the combination doses that produce the effect level. If $\gamma = 1$, the interaction is additive, if $\gamma < 1$, it is synergistic, and if $\gamma > 1$, it is antagonistic. Again, this approach is only suitable for the DPPH assay (smaller γ indicates stronger synergism). For the ABTS and FRAP assays (bigger γ indicates stronger synergism), $\gamma = 1$, $\gamma < 1$ and $\gamma > 1$ correspond to additive, antagonistic and synergistic interactions, respectively.

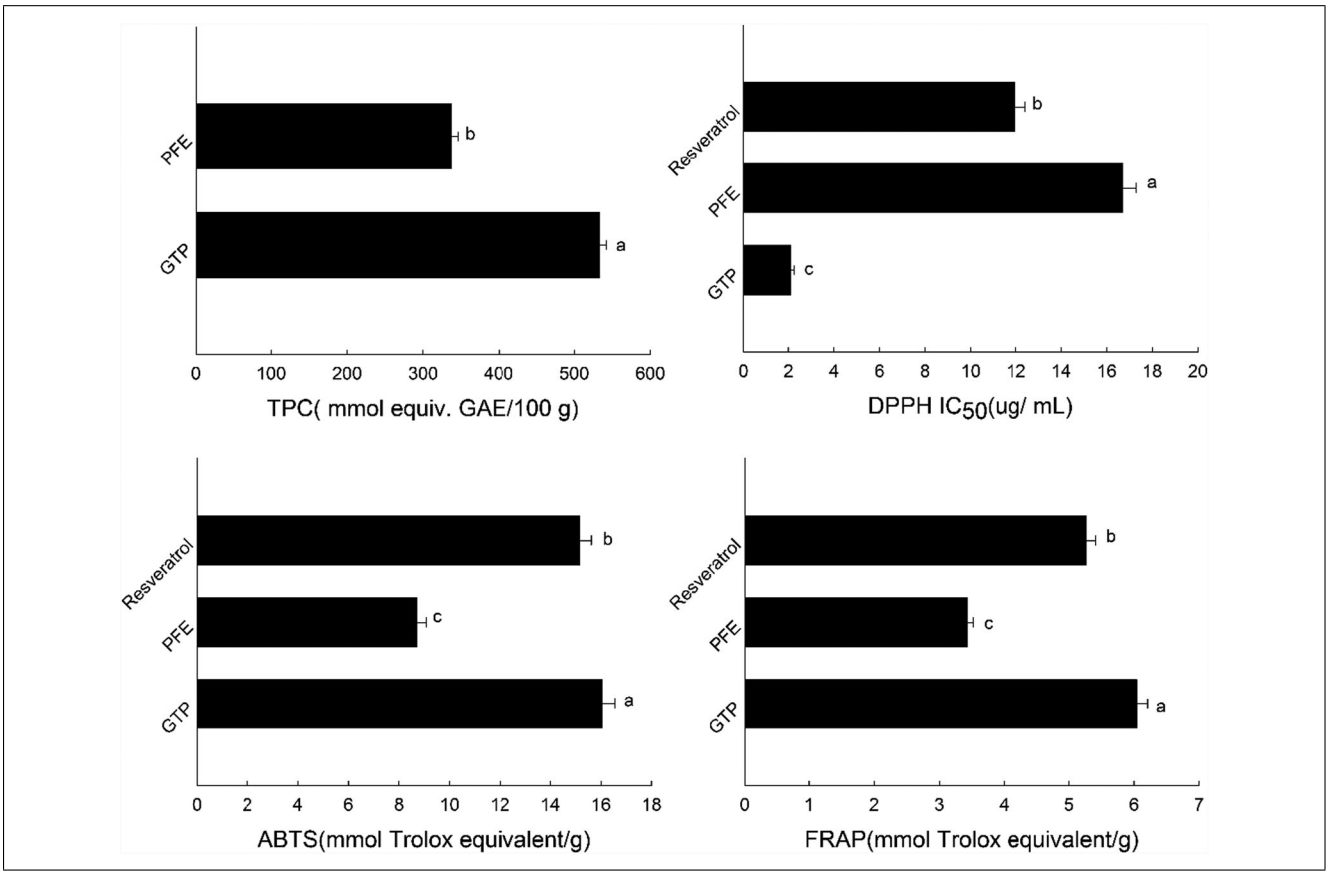

Figure 1–Total phenolic content (TPC) and antioxidant effects (DPPH, ABTS, and FRAP) of PFE and GTP. In each assay, means followed by different letters indicate significant difference by ANOVA ($P < 0.05$). For DPPH, lower IC_{50} values indicate higher DPPH radical scavenging capacity.

Statistical analysis

Expected values (E) were calculated as the average of individual observed amounts for each 1 of 2 combined extracts or compounds, and observed values (O) came through the observed amounts for combined extracts or compounds (Chen and others 2005; Viera and others 2012).

All measurements were carried out in triplicate and every result is given as the mean value of at least 3 independent experiments ± the standard deviation (SD). Statistical analysis were performed using a one-way variance ANOVA followed by Duncan's test; values <0.05 were considered to be significant. Data were analyzed using SPSS 18.0 (SPSS Inc., Chicago, Ill., U.S.A.) for Windows and the figures were made using SigmaPlot 12.0 and Photoshop 7.0 for Windows.

Results and Discussion

Antioxidant activities and TPC of PFE and GTP

Antioxidant activities and TPC of PFE and GTP are presented in Figure 1. Resveratrol was used as the positive control. There significant differences between them ($P < 0.05$). The higher amount of total phenols was given by GTP (533.17 ± 8.91 mmol equiv. GAE/100 g), while the TPC of PFE was 337.21 ± 8.76 mmol equiv. GAE/100 g). For the DPPH free radical-scavenging assay, GTP showed the best activity (IC_{50} = 2.10 ± 0.06 μg/ mL), followed by resveratrol (IC_{50} = 11.96 ± 0.47 μg/ mL) and PFE (IC_{50} = 16.70 ± 0.59 μg/ mL). For the ABTS radical scavenging activity and FRAP activity, GTP also showed the best activity (16.04 ± 0.51 mmol Trolox equivalent/g, 6.04 ± 0.17 mmol Trolox equivalent/g, respectively), followed by resveratrol (15.15 ± 0.47 mmol Trolox equivalent/g, 5.26 ± 0.15 mmol Trolox equivalent/g, respectively) and PFE (8.70 ± 0.38 mmol Trolox equivalent/g, 3.42 ± 0.10 mmol Trolox equivalent/g). In summary, PEF and GTP had rich phenolic compounds and showed very excellent antioxidant capacities.

Interaction effects of PFE combined with GTP on antioxidant capacities

To investigate interactions and identify synergistic combinations, samples were mixed at 7 different ratios. Both the observed and expected values are summarized in Table 1. All types of interactions, including synergistic, additive and antagonistic were observed. In the combination of PFE + GTP, most of the tests showed significant synergy, the last showed additivity and no test showed antagonistic interactions (Table 1 and Figure 2). In other words, most of the tests increased the total antioxidant capacities of the components through synergistic interaction. This indicates an excellent synergistic interaction between PFE and GTP with regard to antioxidant capacities. Within this set, the tests at a ratio of 3:1 (PFE: GTP) exhibited the strongest synergism in ABTS (γ = 1.35) and FRAP (γ = 1.29), indicating that the combination of PFE + GTP at a ratio of 3:1 showed the best antioxidant capacity. The reason behind this requires further research. However, not all of the tests showed synergistic interactions, no matter in TPC, DPPH, ABTS, and FRAP assays. For tests with the same ratio, no obvious corresponding relationship was found among the 4 assays. For instance, tests at a ratio of 5:1 did not show any synergy in the TPC assay, but exhibited outstanding synergistic effect in the DPPH, ABTS, and FRAP assays.

In conclusion, there is an excellent synergistic interaction in PFE + GTP regarding antioxidant activities, for which the combination at a ratio of 3:1 showed the best results. In order to explain this

Table 1–Synergistic, additive and antagonistic antioxidant effects of PFE + GTP.

Combination	Ratio	TPC (mmol equiv. GAE/100 g)			$DPPH_{IC0}$ (μg/ mL)			ABTS (mmol Trolox equivalent/g)			FRAP (mmol Trolox equivalent/g)		
		O	E	γ	O	E	γ	O	E	γ	O	E	γ
PFE + GTP	7:1	369.24 ± 11.37	361.70 ± 9.21	1.02	7.54 ± 0.30*	8.94 ± 0.34	0.84	11.96 ± 0.15*	9.62 ± 0.17	1.24	4.22 ± 0.16*	3.75 ± 0.17	1.13
	5:1	382.07 ± 7.94	369.87 ± 8.90	1.03	5.81 ± 0.21*	7.74 ± 0.29	0.75	12.93 ± 0.29*	9.92 ± 0.23	1.30	4.16 ± 0.10*	3.85 ± 0.09	1.08
	3:1	418.65 ± 15.11*	386.20 ± 9.25	1.08	4.40 ± 0.15*	6.11 ± 0.27	0.72	14.22 ± 0.20*	10.53 ± 0.13	1.35	5.23 ± 0.08*	4.07 ± 0.11	1.29
	1:1	461.13 ± 9.70*	435.19 ± 12.19	1.06	2.66 ± 0.11*	3.74 ± 0.12	0.71	14.76 ± 0.25*	12.37 ± 0.11	1.19	5.20 ± 0.13*	4.73 ± 0.08	1.10
	1:3	506.15 ± 8.95*	484.18 ± 7.74	1.05	2.29 ± 0.09*	2.69 ± 0.09	0.85	16.14 ± 0.14*	14.20 ± 0.21	1.14	6.44 ± 0.21*	5.38 ± 0.09	1.20
	1:5	510.52 ± 8.46	500.51 ± 13.77	1.02	2.12 ± 0.10*	2.46 ± 0.11	0.86	16.27 ± 0.18*	14.82 ± 0.25	1.10	6.00 ± 0.15*	5.60 ± 0.05	1.07
	1:7	534.11 ± 8.24	508.68 ± 12.13	1.04	1.78 ± 0.06*	2.36 ± 0.08	0.75	15.88 ± 0.31	15.12 ± 0.19	1.05	5.94 ± 0.11	5.71 ± 0.12	1.04

Values are the mean of 3 replicates ± SD (n = 3).
The asterisk indicates a significant difference between observed value and expected value ($P < 0.05$). O, observed value; E, expected value; γ, interaction index.

result, the composition change of the phytochemicals before and after the combination and the interactions between individual phenolic chemicals were evaluated.

Phenolic profiles in PFE, GTP, and their combinations analyzed by HPLC

To measure changes in phytochemical composition, the contents of 7 phenolic compounds ((+)-catechin, EGC, ECG, EGCG, EC, hyperoside, and ellagic acid) in PFE and GTP were detected separately and in combination by RP-HPLC. From the chromatogram, any compositional change can be detected. In our preliminary studies, (+)-catechin, caffeic acid, hyperoside, rutin, ellagic acid, and quercetin were detected in PFE, where hyperoside, (+)-catechin, and ellagic acid contents were extremely high, but those of the other 3 compounds were less than 1.0 mg/g. Hyperoside, (+)-catechin, and ellagic acid were therefore considered the main active antioxidant compounds in PFE. In addition, 5 phenolic compounds were detected in GTP and labeled as Peak 1 (EGC), Peak 2 ((+)-catechin), Peak 3 (EGCG), Peak 4 (EC), and Peak 5 (ECG), seen in Figure 3.

From these results, the PFE contained high amounts of hyperoside (64.49 ± 1.11 mg/g), (+)-catechin (25.11±0.93 mg/g), and ellagic acid (5.94±0.09 mg/g), while GTP contained high amounts of EGC (69.98 ± 1.31 mg/g), (+)-catechin (11.03 ± 0.15 mg/g), EGCG (350.60 ± 3.45 mg/g), EC (60.90 ± 0.77 mg/g), and ECG (103.30 ± 2.46 mg/g), as listed in Table 2. After combination, we found that no new peaks appeared in the chromatogram and no existing peaks disappeared, indicating that compositional changes did not occur. However, the content of (+)-catechin, EGC, ECG, and EGCG were changed (Table 3 and Figure 3).

Antioxidant capacities of the 7 phenolic compounds in PFE and GTP

The antioxidant capacities of the 7 phenolic compounds were measured individually (Figure 4). From the results, ECG showed

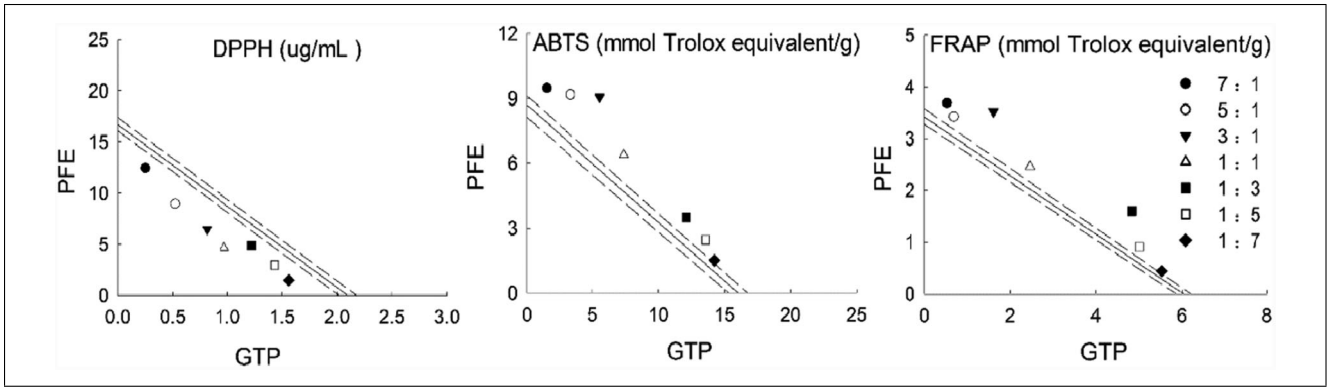

Figure 2–Isobolographic plot of PFE + GTP at 7 different ratios.

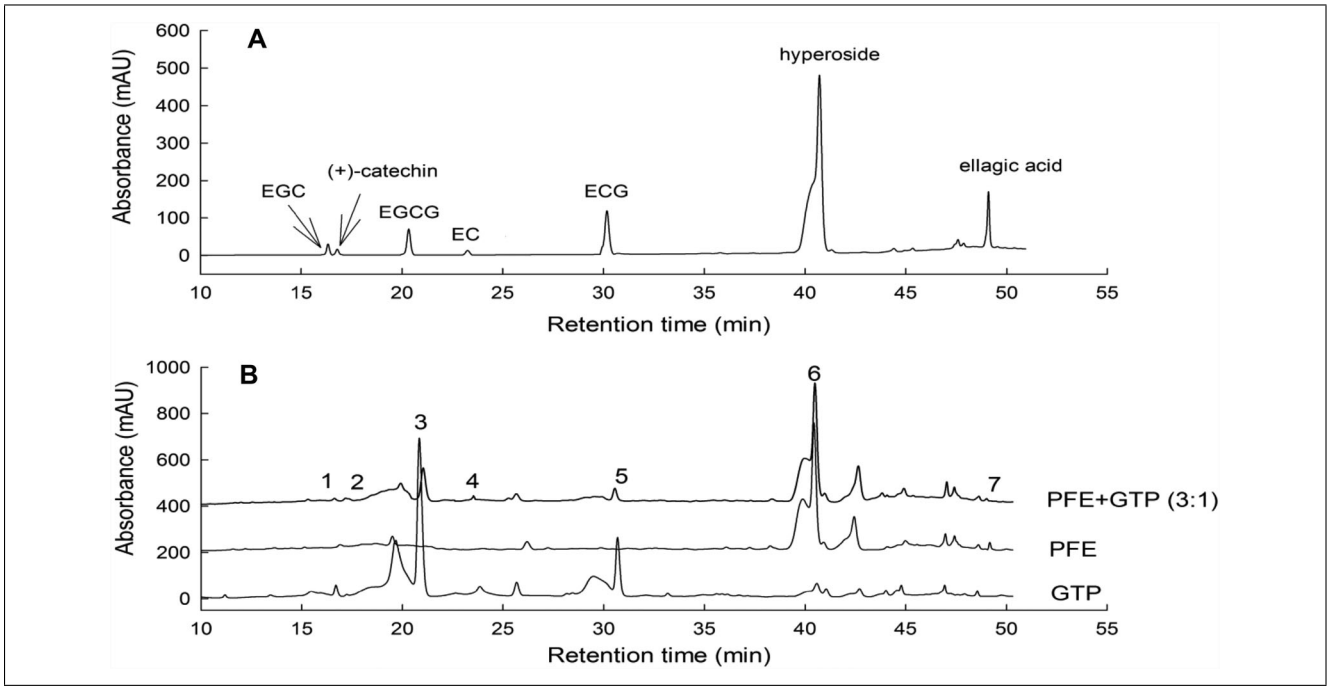

Figure 3–HPLC chromatograms. (A) Chromatography of the 7 standard compounds. (B) Chromatography of PFE, GTP and the combination (3:1) monitored at 254 nm and identified by retention time. Peak 1, EGC; Peak 2, (+)-catechin; Peak 3, EGCG; Peak 4, EC; Peak 5, ECG; Peak 6, hyperoside; Peak 7, ellagic acid.

Table 2–Content of phenolic compounds in PFE and GTP.

Compounds	Retention time (min)	Regression equation	Content (mg/g) PFE	Content (mg/g) GTP
EGC	16.41	$y = 1364.90\chi + 3.294$ ($R^2 = 0.9999$)	ND	69.98 ± 1.31
(+)-Catechin	17.62	$y = 969.44\chi + 2.969$ ($R^2 = 0.9991$)	25.11 ± 0.93	11.03 ± 0.15
EGCG	20.49	$y = 6427.01\chi + 3.277$ ($R^2 = 0.9991$)	ND	350.60 ± 3.45
EC	23.51	$y = 1538.20\chi + 4.494$ ($R^2 = 0.9998$)	ND	60.90 ± 0.77
ECG	30.74	$y = 7334.00\chi + 37.593$ ($R^2 = 0.9999$)	ND	103.30 ± 2.46
Hyperside	39.94	$y = 52742.02\chi + 293.601$ ($R^2 = 0.9993$)	64.49 ± 1.11	ND
Ellagic acid	49.31	$y = 5871.40\chi - 8.614$ ($R^2 = 0.9973$)	5.94 ± 0.09	ND

Values are the mean of 3 replicates ± SD ($n = 3$).
ND, not detectable.

Table 3–Content of phenolic compounds in combinations of PFE + GTP at different ratios.

Compounds		Content (mg/g) 7:1	5:1	3:1	1:1	1:3	1:5	1:7
EGC	O	10.06 ± 0.10*	14.01 ± 0.11*	18.81 ± 0.11*	35.77 ± 0.21	55.11 ± 0.32*	63.73 ± 0.38*	68.58 ± 0.35*
	E	8.75 ± 0.09	11.66 ± 0.19	17.49 ± 0.05	34.99 ± 0.18	52.48 ± 0.31	58.32 ± 0.41	61.23 ± 0.31
(+)-Catechin	O	21.02 ± 0.31*	20.72 ± 0.22*	18.57 ± 0.05*	12.86 ± 0.08*	15.17 ± 0.12	10.92 ± 0.03*	11.13 ± 0.15
	E	23.35 ± 0.23	22.77 ± 0.25	21.59 ± 0.07	18.07 ± 0.15	14.55 ± 0.10	13.37 ± 0.02	12.79 ± 0.14
EGCG	O	46.89 ± 0.24*	68.47 ± 0.22*	99.67 ± 0.34*	189.25 ± 2.11*	265.94 ± 3.37	308.17 ± 5.46*	312.91 ± 6.16
	E	43.83 ± 0.21	58.43 ± 0.30	87.64 ± 0.37	175.30 ± 2.03	262.94 ± 3.25	292.16 ± 6.33	306.78 ± 5.74
EC	O	8.82 ± 0.04*	11.58 ± 0.11	15.81 ± 0.10	32.11 ± 0.25	47.83 ± 0.33	49.88 ± 0.21	54.89 ± 0.33
	E	7.61 ± 0.03	10.15 ± 0.14	15.22 ± 0.11	30.45 ± 0.21	45.67 ± 0.24	50.75 ± 0.29	53.29 ± 0.29
ECG	O	13.69 ± 0.06	19.46 ± 0.17*	25.90 ± 0.17	52.27 ± 0.35	78.42 ± 0.42	88.96 ± 0.35	91.29 ± 0.54
	E	12.91 ± 0.05	17.21 ± 0.20	25.82 ± 0.15	51.65 ± 0.41	77.47 ± 0.50	86.08 ± 0.29	90.39 ± 0.42
Hyperoside	O	56.99 ± 0.34	54.32 ± 0.23	49.27 ± 0.23	33.86 ± 0.21	16.50 ± 0.09	11.06 ± 0.12	8.22 ± 0.08
	E	56.43 ± 0.37	53.73 ± 0.27	48.36 ± 0.31	32.24 ± 0.19	16.12 ± 0.07	10.75 ± 0.07	8.06 ± 0.06
Ellagic acid	O	5.15 ± 0.03	5.05 ± 0.01	4.70 ± 0.02	3.01 ± 0.02	1.45 ± 0.01	0.95 ± 0.01	0.76 ± 0.01
	E	5.20 ± 0.02	4.94 ± 0.01	4.45 ± 0.01	2.97 ± 0.01	1.48 ± 0.01	0.99 ± 0.01	0.74 ± 0.01

Values are the mean of 3 replicates ± SD ($n = 3$).
The asterisk indicates a significant difference between observed value and expected value ($P < 0.05$). O, observed value; E, expected value.

the best DPPH radical scavenging activity (IC_{50} = 1.12 ± 0.03 μg/mL), followed by EGCG (IC_{50} = 1.16 ± 0.02 μg/mL), EGC (IC_{50} = 1.42 ± 0.04 μg/mL), and EC (IC_{50} = 1.63 ± 0.03 μg/mL). (+)-catechin, hyperoside, and ellagic acid also showed excellent DPPH activity (3.98 ± 0.08, 4.59 ± 0.08, and 5.57 ± 0.12 μg/mL). EGCG showed the best ABTS radical scavenging activity (37.53 ± 1.31 mmol Trolox equivalent/g), followed by EC (36.84 ± 0.81 mmol Trolox equivalent/g), ECG (34.77 ± 0.87 mmol Trolox equivalent/g) and hyperoside (32.40 ± 0.79 mmol Trolox equivalent/g). In contrast, the ABTS radical scavenging activities of EGC, (+)-catechin and ellagic acid were more ordinary (25.49 ± 0.53, 23.11 ± 0.48, and 20.10 ± 0.47 mmol Trolox equivalent/g, respectively). ECG showed the best FRAP activity (7.36 ± 0.22 mmol Trolox equivalent/g), followed by EGCG (7.18 ± 0.25 mmol Trolox equivalent/g), EGC (6.88 ± 0.19 mmol Trolox equivalent/g) and EC (6.65 ± 0.21 mmol Trolox equivalent/g), while the FRAP activities of hyperoside, (+)-catechin

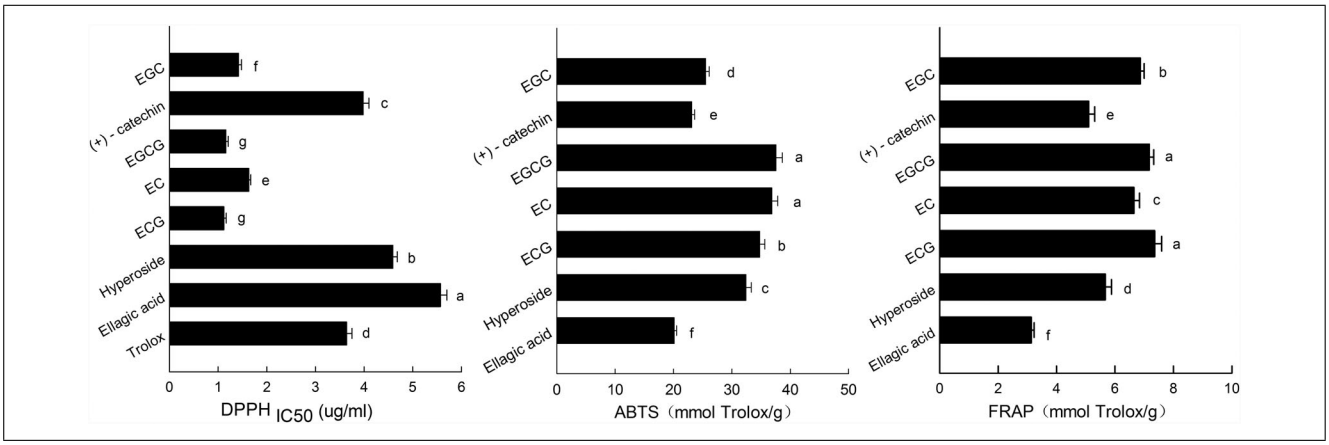

Figure 4–Antioxidant effects of EGC, (+)-catechin, EGCG, EC, ECG, hyperoside, and ellagic acid measured by DPPH, ABTS, and FRAP assays. In each assay, means followed by different letters indicate significant difference by ANOVA ($P < 0.05$). For DPPH, lower IC50 values indicate higher antioxidant capacity.

Table 4–Antioxidant effects of PFE + EGC, PFE + (+)-catechin, PFE + EGCG, PFE + EC and PFE + ECG.

Combination	Ratio	$DPPH_{IC50}$ (μg/ mL)			ABTS (mmol Trolox equivalent/g)			FRAP (mmol Trolox equivalent/g)		
		O	E	γ	O	E	γ	O	E	γ
	7:1	11.98 ± 0.21*	14.79 ± 0.22	0.81	12.74 ± 0.34*	10.80 ± 0.32	1.18	4.43 ± 0.07*	3.85 ± 0.08	1.15
	5:1	12.17 ± 0.24*	14.15 ± 0.26	0.86	13.80 ± 0.31*	11.50 ± 0.29	1.20	4.64 ± 0.08*	4.00 ± 0.07	1.15
	3:1	9.92 ± 0.17*	12.88 ± 0.39	0.77	14.71 ± 0.36*	12.90 ± 0.20	1.14	4.79 ± 0.11*	4.28 ± 0.09	1.12
PFE + EGC	1:1	7.71 ± 0.11*	9.06 ± 0.30	0.85	18.80 ± 0.33*	17.09 ± 0.35	1.10	5.93 ± 0.08*	5.15 ± 0.10	1.15
	1:3	4.62 ± 0.15*	5.24 ± 0.19	0.88	21.03 ± 0.41	21.29 ± 0.31	0.99	6.23 ± 0.12	6.01 ± 0.13	1.04
	1:5	3.35 ± 0.09*	3.97 ± 0.12	0.85	23.06 ± 0.44	22.69 ± 0.49	1.02	6.45 ± 0.15	6.30 ± 0.11	1.02
	1:7	2.99 ± 0.08*	3.33 ± 0.09	0.90	24.33 ± 0.41	23.39 ± 0.40	1.04	6.50 ± 0.14	6.44 ± 0.11	1.01
	7:1	14.20 ± 0.24*	15.11 ± 0.21	0.94	10.71 ± 0.24	10.5 ± 0.20	1.02	3.74 ± 0.10	3.63 ± 0.09	1.03
	5:1	12.73 ± 0.30*	14.58 ± 0.41	0.87	11.43 ± 0.26	11.10 ± 0.31	1.03	3.84 ± 0.09	3.70 ± 0.08	1.04
	3:1	13.63 ± 0.21	13.52 ± 0.38	1.01	11.88 ± 0.28	12.30 ± 0.29	0.97	3.84 ± 0.10	3.84 ± 0.08	1.00
PFE + (+) -catechin	1:1	9.93 ± 0.23	10.34 ± 0.33	0.96	15.06 ± 0.31	15.91 ± 0.33	0.95	4.31 ± 0.06	4.26 ± 0.05	1.01
	1:3	6.88 ± 0.18	7.16 ± 0.25	0.96	18.66 ± 0.40	19.51 ± 0.46	0.96	4.68 ± 0.07	4.68 ± 0.06	1.00
	1:5	6.22 ± 0.20	6.10 ± 0.17	1.02	22.23 ± 0.37*	20.71 ± 0.41	1.07	4.88 ± 0.10	4.82 ± 0.08	1.01
	1:7	5.29 ± 0.21	5.57 ± 0.18	0.95	21.52 ± 0.39	21.31 ± 0.34	1.01	4.79 ± 0.11	4.89 ± 0.07	0.98
	7:1	14.32 ± 0.24	14.76 ± 0.34	0.97	12.56 ± 0.41	12.31 ± 0.35	1.02	3.97 ± 0.10	3.89 ± 0.09	1.02
	5:1	13.98 ± 0.27	14.11 ± 0.41	0.99	14.35 ± 0.39*	13.51 ± 0.40	1.06	4.19 ± 0.11	4.05 ± 0.09	1.04
	3:1	12.18 ± 0.24	12.82 ± 0.34	0.95	17.15 ± 0.35*	15.91 ± 0.40	1.08	4.62 ± 0.12*	4.36 ± 0.11	1.06
PFE + EGCG	1:1	9.32 ± 0.19	8.93 ± 0.20	1.04	24.04 ± 0.44	23.12 ± 0.51	1.04	5.56 ± 0.11*	5.30 ± 0.12	1.05
	1:3	5.05 ± 0.11	5.05 ± 0.18	1.00	29.17 ± 0.45	30.33 ± 0.57	0.96	6.12 ± 0.16	6.24 ± 0.11	0.98
	1:5	3.79 ± 0.06	3.75 ± 0.11	1.01	34.25 ± 0.40	32.73 ± 0.45	1.04	6.67 ± 0.21	6.55 ± 0.17	1.02
	1:7	2.98 ± 0.04	3.10 ± 0.04	0.96	34.27 ± 0.35	33.93 ± 0.40	1.01	6.91 ± 0.18	6.71 ± 0.14	1.03
	7:1	14.38 ± 0.34	14.82 ± 0.31	0.97	12.22 ± 0.24	12.22 ± 0.21	1.00	3.856 ± 0.06	3.82 ± 0.06	1.01
	5:1	13.76 ± 0.32	14.19 ± 0.27	0.97	13.64 ± 0.25	13.39 ± 0.34	1.02	3.97 ± 0.07	3.96 ± 0.09	1.01
	3:1	11.61 ± 0.31*	12.93 ± 0.29	0.90	15.76 ± 0.30	15.74 ± 0.31	1.00	4.22 ± 0.09	4.23 ± 0.11	0.99
PFE + EC	1:1	8.39 ± 0.15*	9.17 ± 0.22	0.92	12.45 ± 0.45	22.77 ± 0.48	1.03	5.06 ± 0.09	5.04 ± 0.08	1.02
	1:3	5.13 ± 0.07	5.40 ± 0.11	0.95	32.37 ± 0.50*	29.81 ± 0.51	1.09	5.77 ± 0.10	5.85 ± 0.08	0.99
	1:5	4.06 ± 0.07	4.14 ± 0.09	0.98	30.48 ± 0.42	32.15 ± 0.50	0.96	5.98 ± 0.13	6.11 ± 0.12	0.98
	1:7	3.50 ± 0.05	3.51 ± 0.05	0.99	31.99 ± 0.41	33.33 ± 0.40	0.96	6.44 ± 0.14	6.25 ± 0.12	1.03
	7:1	14.30 ± 0.30	14.75 ± 0.25	0.97	11.84 ± 0.34	11.96 ± 0.33	0.99	3.99 ± 0.08	3.91 ± 0.05	1.02
	5:1	13.61 ± 0.28	14.10 ± 0.33	0.96	12.80 ± 0.35	13.04 ± 0.29	0.98	4.19 ± 0.09	4.08 ± 0.12	1.03
	3:1	11.65 ± 0.26*	12.80 ± 0.31	0.91	15.37 ± 0.34	15.22 ± 0.30	1.01	4.73 ± 0.11*	4.40 ± 0.09	1.07
PFE + ECG	1:1	8.73 ± 0.13	8.91 ± 0.24	0.98	21.91 ± 0.48	21.73 ± 0.44	1.01	5.67 ± 0.13*	5.39 ± 0.10	1.05
	1:3	5.01 ± 0.11	5.02 ± 0.16	0.99	27.69 ± 0.50	28.25 ± 0.51	0.98	6.65 ± 0.09	6.37 ± 0.14	1.04
	1:5	3.57 ± 0.10	3.72 ± 0.07	0.96	29.62 ± 0.51	30.42 ± 0.49	0.97	6.93 ± 0.12	6.70 ± 0.09	1.03
	1:7	2.95 ± 0.09	3.07 ± 0.08	0.96	32.14 ± 0.49	31.51 ± 0.44	1.02	6.94 ± 0.12	6.87 ± 0.08	1.01

Values are the mean of 3 replicates ± SD ($n = 3$).
The asterisk indicates a significant difference between observed value and expected value ($P < 0.05$). O, observed value; E, expected value; γ, interaction index.

and ellagic acid were also respectable (5.67 ± 0.15, 5.10 ± 0.15, and 3.13 ± 0.09 mmol Trolox equivalent/g, respectively;).

In summary, all 7 phenolic compounds exhibited excellent antioxidant capacities, with the DPPH radical scavenging activity of ECG, EGCG, EGC, and EC, the ABTS radical scavenging activity of EGCG, EC, ECG, and hyperoside, and the FRAP activity of ECG, EGCG, EGC, and EC being of particular note.

Interaction effects between PFE and 5 phenolic compounds in GTP

Studies have shown that synergistic effects may be attributed to particular chemical compounds. In an effort to explain the synergy in the PFE and GTP combination, we measured the interaction effects between the 7 different phenolic compounds, beginning with those between PFE and the 5 chemical compounds in GTP ((+)-catechin, EC, ECG, EGC, and EGCG).

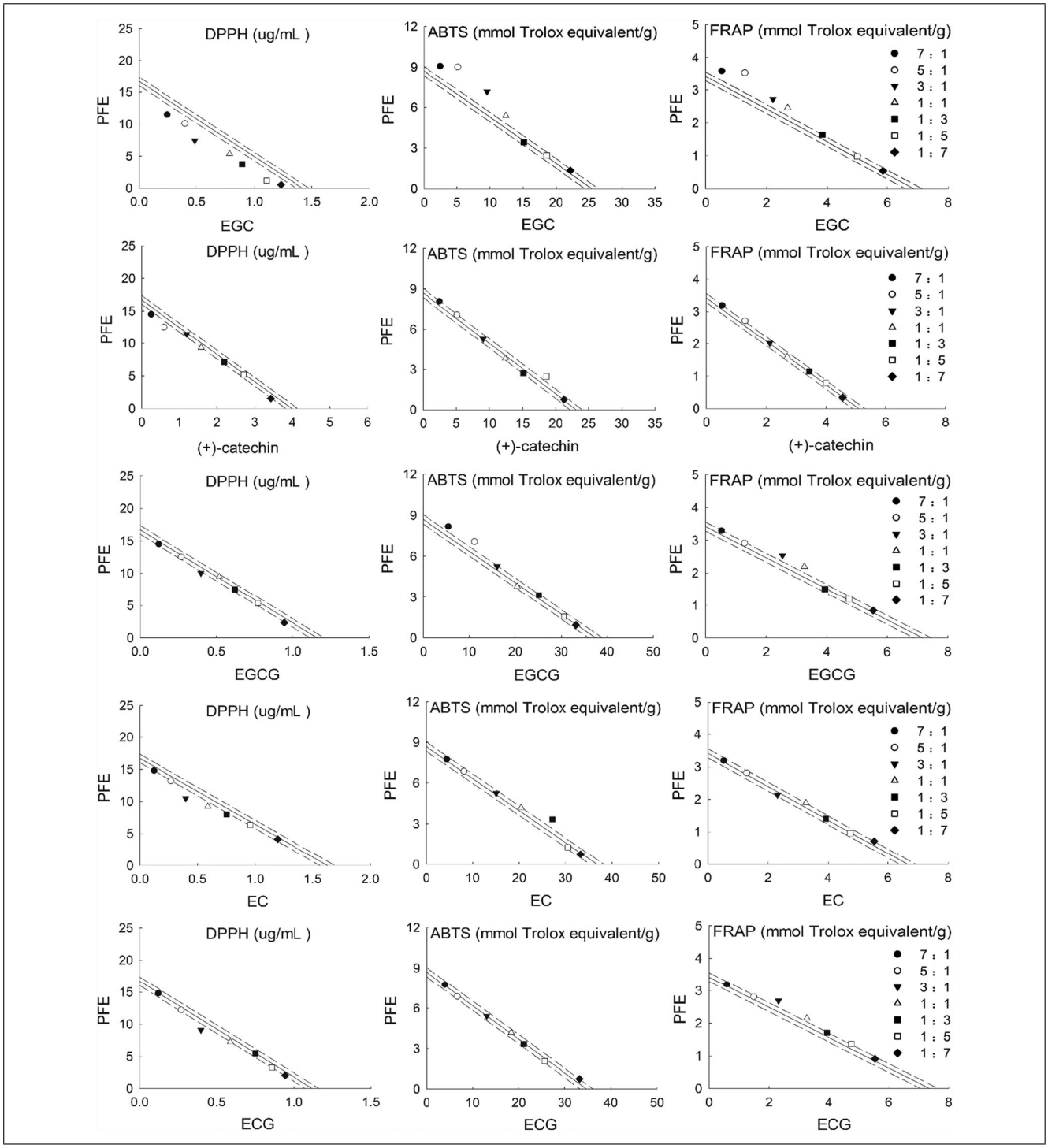

Figure 5–Isobolographic plot of PFE + EGC, PFE + (+)-catechin, PFE + EGCG, PFE + EC, and PFE + ECG at 7 different ratios.

Table 5–Antioxidant effects of EGC + (+)-catechin, EGC + hyperoside, and EGC + ellagic acid.

		$DPPH_{IC50}$ (μg/mL)			ABTS (mmol Trolox equivalent/g)			FRAP (mmol Trolox equivalent/g)		
Combination	**Ratio**	**O**	**E**	**γ**	**O**	**E**	**γ**	**O**	**E**	**γ**
	7:1	1.65 ± 0.03*	1.74 ± 0.02	0.95	25.95 ± 0.33	25.19 ± 0.24	1.03	6.78 ± 0.08	6.65 ± 0.07	1.02
	5:1	1.74 ± 0.03*	1.85 ± 0.02	0.94	25.99 ± 0.31	25.09 ± 0.35	1.04	6.78 ± 0.09	6.58 ± 0.11	1.03
	3:1	1.88 ± 0.02*	2.06 ± 0.04	0.91	25.73 ± 0.43	24.89 ± 0.39	1.03	6.50 ± 0.10	6.43 ± 0.10	1.01
EGC + (+)-catechin	1:1	2.60 ± 0.03	2.70 ± 0.04	0.96	24.79 ± 0.42	24.30 ± 0.31	1.02	5.76 ± 0.10	5.98 ± 0.09	0.96
	1:3	3.23 ± 0.05	3.34 ± 0.03	0.97	23.03 ± 0.42	23.71 ± 0.29	0.97	5.52 ± 0.09	5.54 ± 0.08	1.00
	1:5	3.47 ± 0.06	3.55 ± 0.04	0.98	22.38 ± 0.39*	23.51 ± 0.33	0.95	5.740 ± 0.08*	5.39 ± 0.07	1.06
	1:7	3.62 ± 0.05	3.66 ± 0.04	0.99	22.94 ± 0.40	23.41 ± 0.28	0.98	5.21 ± 0.07	5.32 ± 0.07	0.98
	7:1	1.55 ± 0.02*	1.82 ± 0.01	0.85	29.78 ± 0.45*	26.35 ± 0.24	1.13	7.26 ± 0.08*	6.72 ± 0.05	1.08
	5:1	1.71 ± 0.02*	1.95 ± 0.02	0.88	29.04 ± 0.34*	26.64 ± 0.35	1.09	7.40 ± 0.09*	6.67 ± 0.08	1.11
	3:1	1.89 ± 0.03*	2.21 ± 0.03	0.86	30.52 ± 0.45*	27.21 ± 0.43	1.12	7.66 ± 0.11*	6.57 ± 0.09	1.16
EGC + hyperoside	1:1	2.47 ± 0.04*	3.01 ± 0.03	0.82	31.84 ± 0.47*	28.94 ± 0.41	1.10	7.09 ± 0.10*	6.27 ± 0.08	1.13
	1:3	3.38 ± 0.06*	3.80 ± 0.05	0.89	33.74 ± 0.42*	30.67 ± 0.40	1.10	6.48 ± 0.13*	5.97 ± 0.07	1.09
	1:5	3.86 ± 0.05	4.06 ± 0.05	0.95	32.82 ± 0.45*	31.25 ± 0.39	1.05	6.33 ± 0.10*	5.87 ± 0.05	1.08
	1:7	3.85 ± 0.04*	4.19 ± 0.04	0.92	34.05 ± 0.43*	31.53 ± 0.35	1.08	6.08 ± 0.09	5.82 ± 0.08	1.04
	7:1	1.88 ± 0.04	1.94 ± 0.03	0.97	23.812 ± 0.42	24.81 ± 0.33	0.96	6.40 ± 0.07	6.40 ± 0.05	1.00
	5:1	2.02 ± 0.03	2.11 ± 0.04	0.96	25.08 ± 0.38	24.59 ± 0.37	1.02	6.47 ± 0.14	6.25 ± 0.10	1.03
	3:1	2.31 ± 0.03*	2.46 ± 0.05	0.94	25.17 ± 0.36	24.14 ± 0.35	1.04	6.06 ± 0.13	5.94 ± 0.09	1.02
EGC + ellagic acid	1:1	3.23 ± 0.02*	3.50 ± 0.07	0.93	23.39 ± 0.41	22.79 ± 0.36	1.03	5.41 ± 0.10*	5.00 ± 0.08	1.08
	1:3	4.44 ± 0.05	4.53 ± 0.06	0.98	22.67 ± 0.31*	21.44 ± 0.25	1.06	3.97 ± 0.11	4.06 ± 0.08	0.98
	1:5	4.82 ± 0.07	4.88 ± 0.09	0.99	21.77 ± 0.31	20.99 ± 0.26	1.04	3.90 ± 0.09	3.75 ± 0.07	1.04
	1:7	4.85 ± 0.09	5.05 ± 0.06	0.96	21.19 ± 0.34	20.77 ± 0.22	1.02	3.64 ± 0.10	3.60 ± 0.08	1.01

Values are the mean of 3 replicates ± SD ($n = 3$).
The asterisk indicates a significant difference between observed value and expected value ($P < 0.05$). O, observed value; E, expected value; γ, interaction index.

The expected values, observed values and interaction indexes are shown in Table 4. From these results, all of the tests on the PFE + EGC combinations showed significant synergistic effects. Three combinations (ratios of 5:1, 3:1, and 1:1) showed significant synergistic effects in all 3 antioxidant assays (Figure 5). The combination at a ratio of 5:1 exhibited the strongest synergy in ABTS ($\gamma = 1.20$) and FRAP ($\gamma = 1.15$), as well as very significant synergy in DPPH ($\gamma = 0.86$). As for combinations of PFE with the other phenolic compounds in GTP, the results indicated no obvious synergistic effects, although several tests did show very good antioxidant capacities. This was true of PFE + EGCG, despite it showing good synergy in the ABTS assay. Consequently, EGC was identified as the most efficient compound from GTP acting with PFE.

Previous reports stated that the 3-OH group would significantly increase the radical scavenging power of flavan-3-ols (Amić and others 2003; Seyoum and others 2006). Among all the tested compounds in GTP, only EGC, EC, and (+)-catechin had the 3-OH group while the 3-OH group in EGCG and ECG were all esterified. Furthermore, the chemical structure of EGC consists of 6 phenolic hydroxyl groups, which may determine the highest polarity and strongest polyphenol's antioxidant capacity when compared to the other compounds. Owing to its large amounts of phenolic hydroxyl groups and the activity of phenolic hydroxyl groups, it may be speculated that EGC was most efficient compound in GTP which may stimulated the antioxidant capacity of PFE. However, further study is required to confirm this phenomenon.

Interaction effects between EGC and 3 phenolic compounds in PFE

To assess how the interaction of EGC and PFE contributes to synergistic antioxidant effects, EGC was added to the other 3 phenolic compounds ((+)-catechin, hyperoside, and ellagic acid) in PFE and the antioxidant capacities of the mixtures were analyzed. The expected values, observed values and interaction indexes of the antioxidant capacities are shown in Table 5. From the results, all of the tests on combination of hyperoside + EGC showed significant synergistic effects. The combination at a ratio of 3:1 exhibited the strongest synergism in the ABTS ($\gamma = 1.11$) and FRAP ($\gamma = 1.16$) assays and an excellent DPPH radical scavenging activity ($\gamma = 0.86$). No obvious synergistic effects were found in combinations of EGC with the other 2 phenolic compounds (Figure 6). Hyperoside was therefore identified as the most efficient compound of PFE acting in synergy with EGC.

Similarly, flavonoids are among the most potent natural antioxidants in plants with their chemical structures consist of a backbone with 2 benzene rings linked by a pyran chain (C6–C3–C6) (Burda and others 2001; Cai and others 2006). When compared to (+)-catechin, hyperoside occupied double bond at 2 and 3 position on the C ring which extend the conjugate system between the 2 benzene rings and therefore determined stronger antioxidant activity.

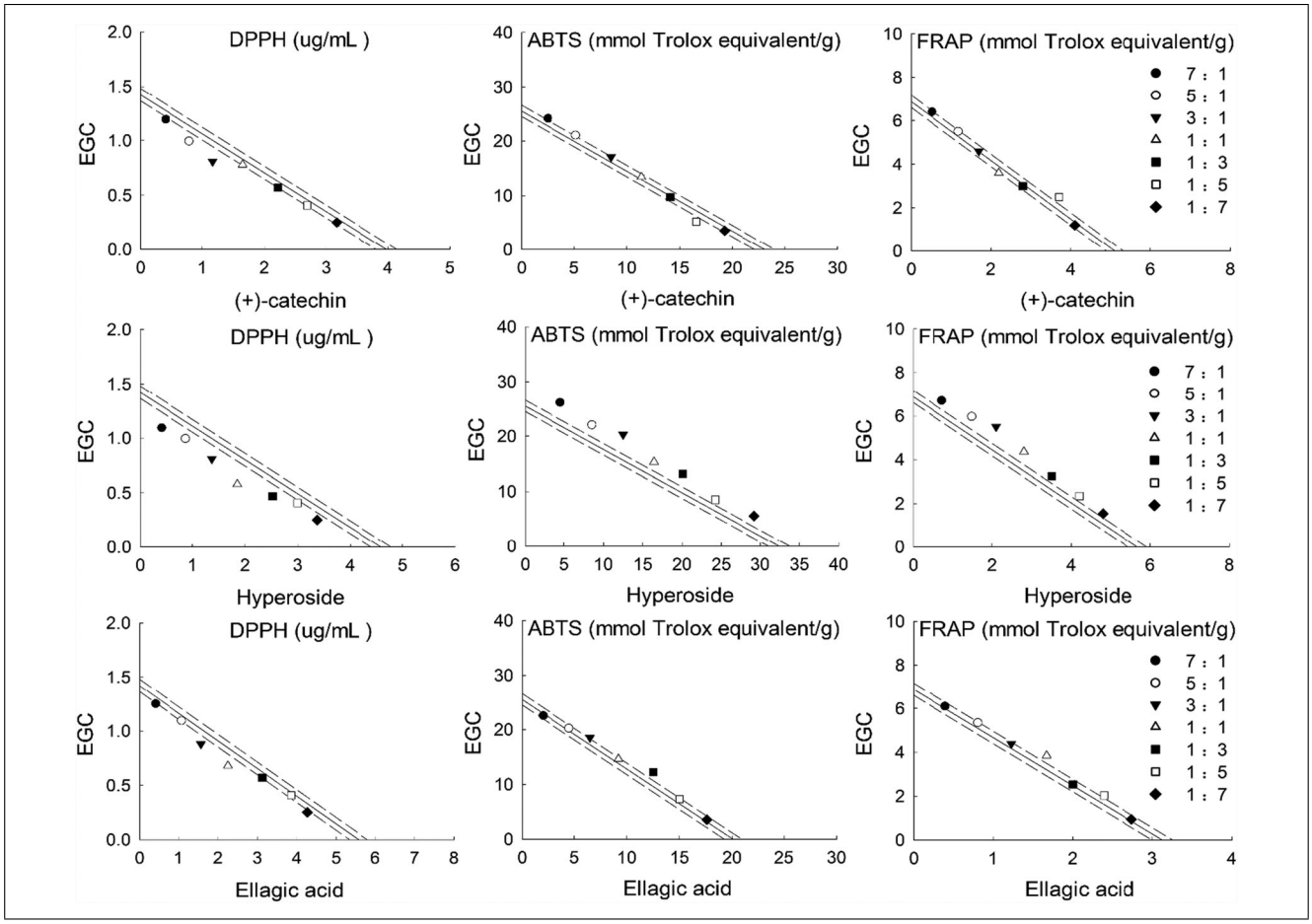

Figure 6–Isobolographic plot of EGC + (+)-catechin, EGC + hyperoside, and EGC + ellagic acid at 7 different ratios.

In addition, hyperoside occupied more overlap area of the conjugate than ellagic acid, which is a possible explanation as to why hyperoside exhibited most antioxidant capacity. In addition, hyperoside not only occupied the highest content in PFE but also have the adjacent –OH groups compared to ellagic acid, which may also explained the fact that hyperoside exhibited most potent antioxidant effects as well as stimulated the antioxidant capacity of EGC. However, this speculation still needed to be proved in future research.

Conclusions

In conclusion, there is an excellent synergistic interaction in PFE + GTP regarding antioxidant activities, especially at a ratio of 3:1. By HPLC analysis, the results indicated that no compositional changes occurred in this mixture. Then the combinational effects of the existing phytochemicals in PFE + GTP were evaluated. From these results, the combination of hyperoside + EGC showed significant synergistic effects, again in the combination ratio of 3:1. Note that the combination of PFE and GTP also showed the best synergistic effect at a ratio of 3:1, where the content of hyperoside was 3 times compare to the content of EGC. The number and position of free phenolic hydroxyl groups on the aromatic rings of EGC and hyperoside may be the main structural features for the effectiveness of free radical scavenging under oxidation and thus may be significantly stimulated their synergy. The synergistic interaction between EGC and hyperoside may therefore be considered the one reason for the antioxidant synergistic effects in combinations of PFE + GTP. In summary, this report provides a theoretical basis for the concomitant use of *P. fruticosa* blended with GTP, which can be effectively used as a compounded tea, a dietary supplements and substituent of synthetic antioxidant.

Acknowledgment

This work was supported by the program from the Fundamental Research Funds for the Central Universities (ZD2013010).

Authors' Contributions

Dongmei Wang and Dengwu Li conceived and designed the experiments. Zehua Liu ,Ziwen Luo and Caixia Jia performed the experiments and analyzed the data. Zehua Liu, Ziwen Luo and Dongmei Wang wrote the paper. Dongmei Wang critically revised the paper.

Competing Interests

The authors declare that they have no competing interests.

References

Amić D, Davidović-Amić D, Bešlo D, Trinajstić N. 2003. Structure-radical scavenging activity relationships of flavonoids. Croat Chem Acta 76:55–61.

Benzie IF, Strain J. 2008. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70–6.

Borgert CJ, Borgert SA, Findlcy KC. 2005. Synergism, antagonism, or additivity of dietary supplements: application of theory to case studies. Thromb Res 117:123–32.

Burcu B, Aysel U, Nurdan S. 2014. Antimicrobial, antioxidant, antimutagenic activities, and phenolic compounds of Iris germanica. Ind Crop Prod 61:520–30.

Burda S, Oleszek W. 2001. Antioxidant and antiradical activities of flavonoids. J Agric Food Chem 49:2774–9.

Cai YZ, Sun M, Xing J, Luo Q, Corke H. 2006. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sci 78:2872–88.

Chen CY, Milbury PE, Lapsley K, Blumberg JB. 2005. Flavonoids from almond skins are bioavailable and act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. J Nutr 135: 1366–73.

Cheng TO. 2006. All teas are not created equal the Chinese green tea and cardiovascular health. Intl J Cardiol 108:301–8.

Elham RN, Alireza SM. 2015. Evaluation of antioxidant interactions in combined extracts of green tea (*Camellia sinensis*), rosemary (*Rosemarinus officinalis*) and oak fruit (*Quercus brantî*). J Food Sci Technol 52(7):4565–71.

Grabovsky Y, Tallarida RJ. 2004. Isobolographic analysis for combination of a full and partial agonist: curved isoboles. J Pharmacol Exp Ther 310:981–6.

Gürbüz I, Özkan AM, Yesilada E, Kutsal O. 2005. Anti-ulcerogenic activity of some plants used in folk medicine of Pinarbasi (Kayseri, Turkey). J Ethnopharmacol 101:313–8.

Jain DP, Pancholi HS, Patel R. 2011. Synergistic antioxidant activity of green tea with some herbs. J Adv Pharm Technol Res 2:177–83.

Khan N, Mukhtar H. 2007. Tea polyphenols for health promotion. Life Sci 81:519–33.

Lansky EP, Jiang W, Mo H, Bravo L, Froom P, Yu W, Harris NM, Neeman I, Campbell MJ. 2005. Possible synergistic prostate cancer suppression by anatomically discrete pomegranate fractions. Invest New Drugs 23:11–20.

Leporatti ML, Ivancheva S. 2003. Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy. J Ethnopharmacol 87:123–42.

Liao K, Yin M. 2000. Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: importance of the partition coefficient. J Agric Food Chem 48:2266–70.

Miliauskas G, Beek TA, Venskutonis PR, Linssen JP, Waard P, Sudhölter EJ. 2004. Antioxidant activity of *Potentilla fruticosa*. J Sci Food Agric 84:1997–2009.

Mocan A, Crisan G, Vlase L, Crisan O, Vodnar DC, Raita O, Gheldiu AM, Toiu A, Oprean R, Tilea I. 2014. Comparative studies on polyphenolic composition, antioxidant and antimicrobial activities of *Schisandra chinensis* leaves and fruits. Molecules 19:15162–79.

Mocan A, Vlase L, Vodnar DC, Gheldiu A, Oprean R, Crisan C. 2015. Antioxidant, antimicrobial effects and phenolic profile of *Lycium barbarum* L. flowers. Molecules. 20:15060–71.

Seyoum A, Asres K, El-Fiky FK. 2006. Structure-radical scavenging activity relationships of flavonoids. Phytochemistry. 67:2058–70.

Shushunov S, Balashov L, Kravtsova A, Krasnogorsky I, Latté KP, Vasiliev A. 2009. Determination of acute toxicity of the aqueous extract of *Potentilla erecta* (Tormentil) rhizomes in rats and mice. J Med Food 12: 1173–6.

Syiem D, Syngai G, Khup P, Khongwir B, Kharbuli B, Kayang H. 2002. Hypoglycemic effects of *Potentilla fulgens* L. in normal and alloxan-induced diabetic mice. J Ethnopharmacol 83:55–61.

Tallarida RJ. 2012. Revisiting the isobole and related quantitative methods for assessing drug synergism. JPET Fast Forward 342:2–8.

Tomczyk M, Latté KP. 2009. *Potentilla*—a review of its phytochemical and pharmacological profile. J Pharmacol 122:184–204.

Tomczyk M, Pleszczyńska M, Wiater A. 2010. Variation in total polyphenolics contents of aerial parts of *Potentilla* species and their anticariogenic activity. Molecules 15:4639–51.

Viera L, Marques A, Barros L, Barriera J, Ferriera I. 2012. Insights in the antioxidant synergistic effects of combined edible mushrooms: phenolis and polysaccharridic extracts of *Boletus edulis* and *Marasmius oreades*. J Food Nutr Res 51(2):109–16.

Wang SN, Meckling KA, Marcone MF, Kakuda Y, Tsao R. 2011. Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. J Agric Food Chem 59:60–968.

Wang SS, Wang DM, Pu WJ, Li DW. 2013. Phytochemical profiles, antioxidant and antimicrobial activities of 3 *Potentilla* species. BMC Complem Altern M 13:321.

Wang SS, Wang DM, Liu ZH. 2015. Synergistic, additive and antagonistic effects of *Potentilla fruticosa* combined with EGb761 on antioxidant capacities and the possible mechanism. Ind Crop Prod 67:227–38.

Yen GC, Chen HY. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 43:27–32.

Zhao YL, Cai GM, Hong X, Shan LM, Xiao XH. 2008. Anti-hepatitis B virus activities of triterpenoid saponin compound from *Potentilla anserine* L. Phytomedicine 15: 253–8.