

Research Article

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Comparative study on the antioxidant activities of ten common flower teas from China

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Abstract: Flower teas are widely consumed across the world because of their beneficial health effects. The antioxidant activities of methanol extracts from ten common flower teas in China were evaluated using four antioxidant assays. The total phenolic (TPCs) and total flavonoid contents (TFCs) were also investigated. Most of these flower teas exhibited potent antioxidant effects, of which *rosae rugosae flos* exerted the strongest antioxidant effects in four assays. *Rosae rugosae flos* also exerted the highest TPC, while *fragrans* showed the highest TFC. Correlation analysis indicated that phenolics play a key role in the antioxidant effect of flower teas, while flavonoids were poorly correlated with antioxidant activity. The results supported the consumption of flower teas as functional foods and their application as sources of natural antioxidants in the food industry.

Keywords: flower teas; *rosae rugosae flos*; antioxidant activity; phenolic; functional food.

1 Introduction

Flower teas, or scented teas, which consist of dried flowers from one or more plants, have been consumed since antiquity worldwide. In China, flower teas with various

kinds of medicinal properties are even more popular than green teas, especially for female consumers [1]. Flower teas are receiving widespread and increasing attention due to their multiple beneficial health effects. The beneficial properties of edible flowers derived products could be attributed to their abundant of phenolic compounds. Phenolics have exhibited multiple physiologic effects on humans, which could reduce the risk of cancers and coronary heart disease, inhibit platelet aggregation, and prevent oxidative damage to low density lipoproteins and lipids [2, 3]. Phenolics possess potent antioxidant effects linked to their capacity to scavenge free radicals, chelate prooxidant metal ions, and break radical chain reactions, which could serve as an important quality index of flower teas [4]. Nevertheless, in contrast to a large number of published studies on the aroma and volatile compounds exist in flower teas, much less attention has been paid to their bioactive phenolics [5, 6].

Therefore, as part of our ongoing research on the active constituents in edible flowers and their derived products [7], the comparative study on the antioxidant effects of ten commonly consumed flower teas in China was carried out, in view of their potential benefits of natural antioxidants for food purposes. This research evaluates the antioxidant effects of ten flower teas extracts by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulphonic acid (ABTS), ferric reducing antioxidant power (FRAP), and cellular antioxidant activity (CAA) assays. Moreover, their total phenolic (TPCs) and flavonoid contents (TFCs) were measured, and the relevance between the phenolic contents and antioxidant effects were surveyed.

2 Materials and Methods

2.1 Reagents and materials

DPPH, ABTS and tripyridyltriazine (TPTZ) were purchased from J&K Scientific Ltd (Beijing, China). Gallic acid, rutin, trolox, and Folin-Ciocalteu reagent were obtained from

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Table 1: Ten common flower teas in China collected from Kunming City.

Flower teas	Common name	Chinese name	Species	Family
	magnoliae flos	Xinyi	<i>Magnolia denudata</i>	Magnoliaceae
	lily	Baihe	<i>Lilium brownie</i> var. <i>viridulum</i>	Liliaceae
	lonicerae flos	Jinyinhua	<i>Lonicera japonica</i>	Caprifoliaceae
	fragrans	Guihua	<i>Osmanthus fragrans</i>	Oleaceae
	carnation	Kangnaixin	<i>Dianthus caryophyllus</i>	Caryophyllaceae
	trollflower	Jinlianhua	<i>Trollius chinensis</i>	Ranunculaceae
	chrysanthemum buds	Taiju	<i>Dendranthema lavandulifolium</i>	Compositae
	rosae rugosae flos	Meigui	<i>Rosa rugosa</i>	Rosaceae
	praecox	Lamei	<i>Chimonanthus praecox</i>	Calycanthaceae
	forget-me-not	Wuwangwo	<i>Myosotis silvatica</i>	Boraginaceae

Aladdin Reagent (Shanghai, China). HepG2 cell, DMEM, antibiotic–antimycotic, DCFH-DA, AAPH were obtained from Keygen Biotech (Nanjing, China). Ultrapure water was acquired using a Youpu water purification system (Chengdu, China). Ten flower teas listed in Table 1 were acquired in Kunming City of China in December 2014, which have been identified by Prof. Le Cai from Yunnan University. The voucher specimens (No. f1401-f1410) were available at Zhuhai Campus of Zunyi Medical University.

2.2 Extraction

The air-dried flower teas were extracted by using a ultrasound-assisted extraction method [8]. Briefly, the mixture of powdered samples (10.0 g) and 80% of aqueous MeOH (100 mL) was sonicated for 20 min under continuous nitrogen gas purging, which was filtered with Whatman #2 filter paper (Kent, England) and subsequently rinsed with MeOH (50 mL). And the residue was extracted under the same conditions. The filtrates were combined and evaporated with a Heidolph rotary evaporator (Schwabach, Germany) at 40°C, then were redissolved in 80% of aqueous MeOH to a concentration of 10 g/L, which were stored at 4°C until the analyses were performed.

2.3 DPPH assay

The DPPH assay was performed by using a formerly described method [9]. The mixture of 0.1 mL DPPH (0.304 mM) and 0.1 mL sample at various concentrations was shaken vigorously and incubated at 25°C in the dark for 30 min. Then the absorbance of the mixture was determined at 515 nm with a Bio-Tek ELx800 microplate reader (Winooski, USA). The DPPH free radical-scavenging capacity was expressed as inhibition (*I*), which could be calculated as follows: $I(\%) = [(A_0 - A_s)/A_0] \times 100\%$, where *A_s* is the absorbance of a sample with DPPH, and *A₀* is the absorbance of DPPH without any sample. The IC₅₀ value (50% absorbance reduction) was acquired by a curve relating the concentration to the absorbance of a sample. Rutin served as a positive control.

2.4 ABTS assay

The ABTS assay was performed by using a formerly described method [10]. The reaction of ABTS (7 mM) and K₂S₂O₈ (2.5 mM) was maintained for 12–16 h at room temperature in the dark to prepare the ABTS⁺ radical

solution, which was diluted with PBS (200 mM, pH = 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. The mixture of 3.9 mL ABTS⁺ and 0.1 mL sample was shaken vigorously and incubated at room temperature in the dark for 6 min. The absorbance of the mixture was determined at 734 nm with a Shimadzu UV–Vis 2550 spectrometer (Kyoto, Japan). The ABTS radical-scavenging capacity was calculated as follows: $I(\%) = [(A_0 - A_s)/A_0] \times 100\%$, where *A_s* is the absorbance of a sample with ABTS, and *A₀* is the absorbance of ABTS without any sample. The calibration curve was prepared from a standard solution of trolox at various concentrations: $A = 0.80911 - 0.02475 \times C_{\text{trolox}}$ ($R^2 = 0.9915$). The results were expressed as trolox equivalent to the antioxidant ability (TEAC, mmol trolox/g Ex). Rutin served as a positive control.

2.5 FRAP assay

The FRAP assay was conducted by using a formerly described method [7]. The mixing of 2.5 mL TPTZ (10 mM in 40 mM HCl), 25 mL sodium acetate buffer (0.3 M, pH 3.6), and 2.5 mL FeCl₃ (20 mM) was accomplished to prepare the FRAP working reagent. A total of 100 μL FRAP reagent were mixed with 25 μL sample, which was incubated at 37°C for 30 min. The absorbance was determined at 595 nm using a Bio-Tek ELx800 microplate reader. A standard solution of Fe²⁺ at various concentrations was utilized to generate the calibration curve: $A = 0.1339 + 0.63873 \times C_{\text{Fe}}$ ($R^2 = 0.9996$). The results were expressed as μM Fe²⁺/g dry extract (Ex). Rutin served as a positive control.

2.6 CAA in HepG2 cells

HepG2 cells were cultured in DMEM which contained a 10% fetal bovine serum and a 1% antibiotic–antimycotic at 37°C and in a 5% CO₂ atmosphere. The CAA assay was performed by using a previously described method [11]. HepG2 cells were seeded in a 96-well microplate with 100 μL of growth medium, which proliferated to 5.5 × 10⁴ cells/well after 24 h. Then, the wells were removed for their growth medium and washed by PBS. Triplicate wells were treated with 100 μL growth medium containing a sample and 30 μM DCFH-DA for 1 h, which were washed with 100 μL PBS and pretreated with samples before AAPH (700 μM in 100 μL DMEM) was added. The 96-well microplate was measured with the emission wavelength at 525 nm after an excitation at 488 nm every 5 min for 1 h using a Bio-Tek ELx800 microplate reader at 37°C. The CAA value was calculated as follows: $CAA = (JCA - JSA)/JCA$, where

f /SA indicates the integrated area under the sample in the fluorescence/time curve, and f /CA indicates the integrated area under the control in the fluorescence/time curve. The EC_{50} value was calculated from the median effect plot of $\log(fa/fu)/\log(\text{dose})$, where fa is the fraction affected by the CAA unit, and fu is the fraction unaffected (1-CAA units) by the treatment.

2.7 TPC

The TPC was tested by using the Folin-Ciocalteu method [12]. The mixture of 2.25 mL prediluted Folin-Ciocalteu and 15.0 μ L sample was shaken and incubated for 5 min. Then, 3.0 mL of Na_2CO_3 (75%, w/v) solution was added. The mixture was incubated at room temperature for 30 min and tested for its absorbance at 765 nm. TPC was expressed as mg gallic acid equivalents (GAE)/g dry extract (Ex), which was calculated by a calibration curve acquired by a standard solution of gallic acid at various concentrations: $A = 0.04165 + 0.1036 \times C_{GA}$ ($R^2 = 0.9992$).

2.8 TFC

The TFC was tested by using a previous described colorimetric method [7]. A total of 1.0 mL prediluted sample was mixed with 0.3 mL of $NaNO_2$ (5%, w/v) and 4.0 mL of deionized water in a 10.0 mL colorimetric tube. And 0.3 mL of $AlCl_3$ (10%, w/v) was added after 5 min. Then, 2.0 mL of NaOH (1.0 M) and 2.4 mL of deionized water was added after 6 min. The mixture was shaken vigorously and determined for its absorbance at 510 nm. A standard solution of rutin at various concentrations was utilized to generate the calibration curve: $A = 0.00956 + 0.00806 \times C_{rutin}$ ($R^2 = 0.9974$), and the results were expressed as mg rutin equivalents (RE)/g dry extract (Ex).

2.9 Statistical analysis

All results are presented as the mean \pm SD for triplicate determinations of each sample. Data were examined with one-way ANOVA test plus a Student–Newman–Keuls test using the SPSS statistical package version 13.0 (Chicago, USA). Statistical significance was defined as $P < 0.05$.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Antioxidant activities

Ten common flower teas that are widely consumed in China (Table 1) were extracted under the same conditions with 80% aqueous MeOH [8], and tested for their antioxidant activities using four experimental models based on different principles.

The DPPH assay is a preferred model to estimate the antioxidant capacity of plant samples due to its high efficiency and sensitivity. The radical-scavenging capacity of the sample is associated with its potential to provide protons [13]. As observed in Table 2, *rosae rugosae flos* exhibited the strongest DPPH radical scavenging capacity (IC_{50} , 22.54 ± 1.25 μ g/mL) among ten selected flower teas, followed by the troll flower (IC_{50} , 41.16 ± 1.24 μ g/mL), *loniceræ flos* (IC_{50} , 57.22 ± 3.12 μ g/mL), *frangrans* (IC_{50} , 66.56 ± 1.35 μ g/mL), and *chrysanthemum buds* (IC_{50} , 69.94 ± 2.36 μ g/mL), respectively. Conversely, *carnation* (IC_{50} , 734.62 ± 15.61 μ g/mL), *lily* (IC_{50} , 531.72 ± 10.13 μ g/mL) and *magnoliae flos* (IC_{50} , 350.80 ± 5.67 μ g/mL) displayed relatively weak DPPH radical-scavenging effects.

The ABTS assay is widely utilized to estimate the antioxidant capacity of plant samples since it can test antioxidant activities of lipophilic and hydrophilic constituents within the same sample. As in the DPPH assay, *rosae rugosae flos* exhibited the strongest ABTS radical scavenging activity (1807.70 ± 193.33 mmol trolox/g Ex), which was more effective than the positive control rutin (920.23 ± 10.12 mmol trolox/g Ex). *Forget-me-not* (873.02 ± 49.96 mmol trolox/g Ex) and the troll flower (776.65 ± 29.71 mmol trolox/g Ex) also exhibited nearly the equal effective activities as rutin, while *magnoliae flos* (270.48 ± 23.11 mmol trolox/g Ex) and the lily (245.23 ± 14.05 mmol trolox/g Ex) showed the lowest ABTS radical scavenging capacities.

The FRAP assay estimates the reducing ability of natural products by reducing Fe^{3+} -TPTZ to Fe^{2+} -TPTZ, which is linked to its potential to break free the radical chain by donating hydrogen atoms [14]. The FRAP values of ten flower teas were in the range of 0.154–2.345 mmol Fe^{2+} /g Ex, with the strongest efficacy rendered by *rosae rugosae flos* (2.345 ± 0.215 mmol Fe^{2+} /g Ex) (Table 2). The troll flower (1.796 ± 0.029 mmol Fe^{2+} /g Ex), *forget-me-not* (1.612 ± 0.030 mmol Fe^{2+} /g Ex) and *loniceræ flos* (1.321 ± 0.052 mmol Fe^{2+} /g Ex) also exhibited relative high FRAP values, while the *carnation* (0.154 ± 0.029 mmol Fe^{2+} /g Ex) and the lily (0.242 ± 0.013 mmol Fe^{2+} /g Ex) showed the lowest FRAP values.

Table 2: The antioxidant activities of ten flower teas.

Flower teas	DPPH (IC ₅₀ , µg/mL)	ABTS (mmol trolox/g Ex)	FRAP (mmol Fe ²⁺ /g Ex)	CAA (EC ₅₀ , µg/mL)
magnoliae flos	350.80 ± 5.67 ^a	270.48 ± 23.11 ^a	0.337 ± 0.019 ^a	218.45 ± 23.56 ^a
lily	531.72 ± 10.13 ^b	245.23 ± 14.05 ^a	0.242 ± 0.013 ^b	92.47 ± 11.01 ^b
lonicerae flos	57.22 ± 3.12 ^c	485.45 ± 31.61 ^b	1.321 ± 0.052 ^c	30.16 ± 4.52 ^c
fragrans	66.56 ± 1.35 ^d	472.88 ± 21.07 ^b	1.115 ± 0.093 ^c	26.78 ± 4.36 ^c
carnation	734.62 ± 15.61 ^e	545.68 ± 14.66 ^c	0.154 ± 0.029 ^d	51.23 ± 9.63 ^d
trollflower	41.16 ± 1.24 ^f	776.65 ± 29.71 ^d	1.796 ± 0.029 ^e	12.69 ± 1.19 ^e
chrysanthemum buds	69.94 ± 2.36 ^g	671.15 ± 46.19 ^e	1.235 ± 0.230 ^f	65.44 ± 2.69 ^f
rosae rugosae flos	22.54 ± 1.25 ^h	1807.70 ± 193.33 ^f	2.345 ± 0.215 ^g	11.38 ± 0.97 ^g
praecox	91.18 ± 3.26 ⁱ	627.16 ± 22.15 ^g	1.022 ± 0.024 ^h	94.34 ± 13.67 ^h
forget-me-not	68.53 ± 1.85 ^g	873.02 ± 49.96 ^h	1.612 ± 0.030 ⁱ	19.69 ± 3.36 ⁱ

Values are expressed as the mean ± SD (n = 3).

Different superscript letters within a column indicated that the values were statistically different from each other at the level $P < 0.05$, and values marked by the same letter were not statistically different.

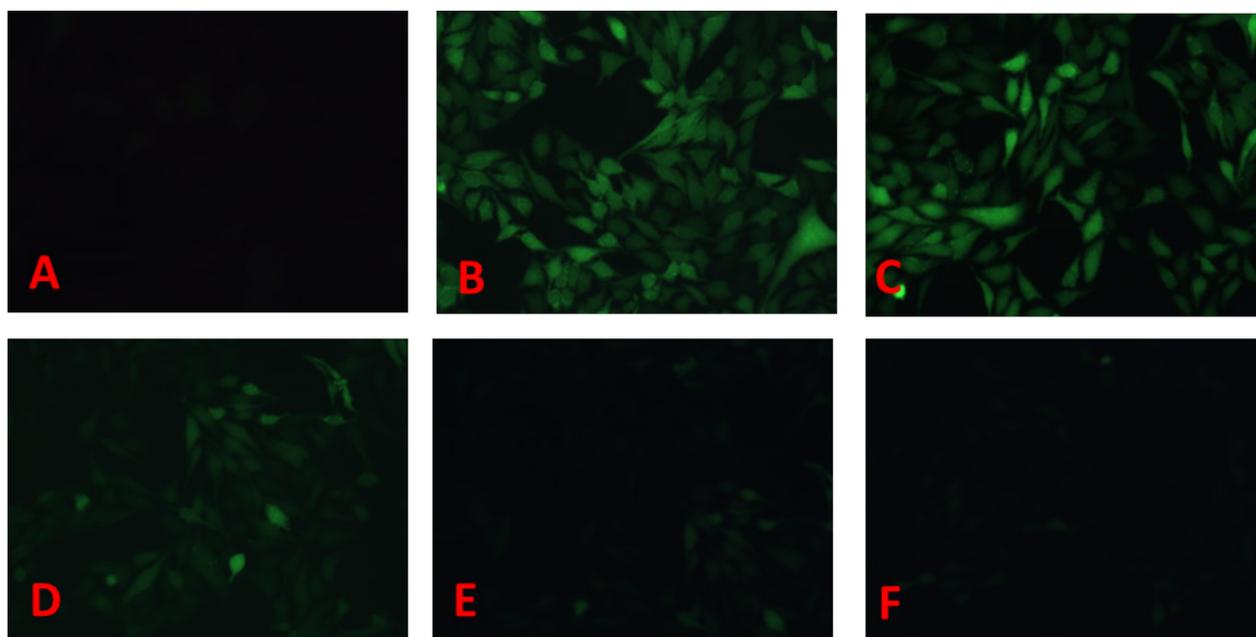


Figure 1: Comparison of ROS in HepG2 cells with DCFH-DA fluorescence staining (A: control group; B: model group; C~F: 5, 10, 25, 50 µg/mL extracts of rosae rugosae flos; × 200).

The CAA assay is a more biologically related model when compared with these chemical assays since it takes into account the biological influence factors such as uptake, metabolism and localization of the antioxidants within cells [11]. The CAA of flower teas were performed using H₂O₂-induced oxidative damage in human liver cancer cells HepG2. Table 2 shows the EC₅₀ values of the

flower teas, which ranged from 11.38 to 218.45 µg/mL. Rosae rugosae flos displayed the highest CAA (IC₅₀, 11.38 ± 0.97 µg/mL), followed by the troll flower (IC₅₀, 12.69 ± 1.19 µg/mL) and the forget-me-not (IC₅₀, 19.69 ± 3.36 µg/mL), which also showed high *in vitro* antioxidant activities. The lily (IC₅₀, 92.47 ± 11.01 µg/mL), praecox (IC₅₀, 94.34 ± 13.67 µg/mL), and magnoliae flos (IC₅₀, 218.45 ± 23.56 µg/

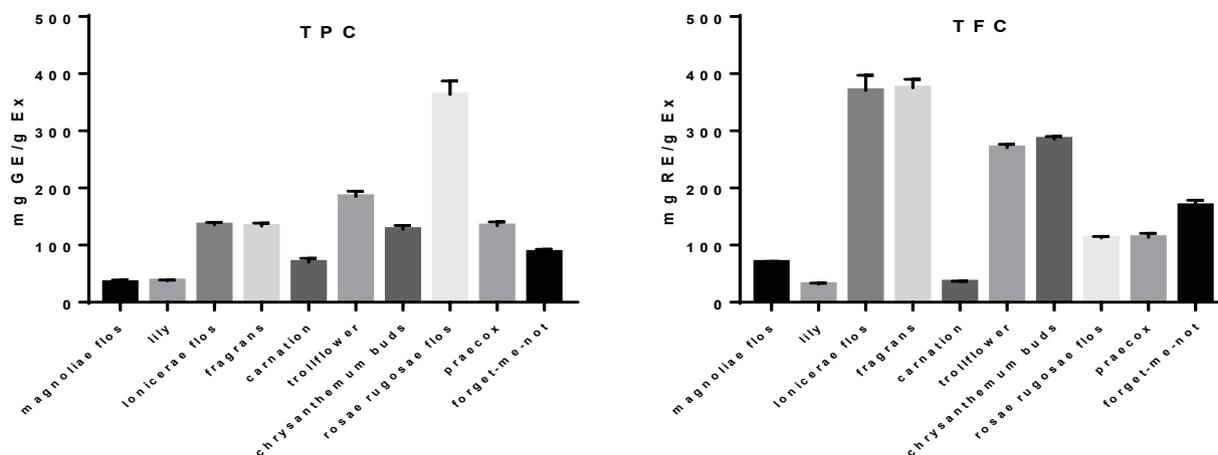


Figure 2: The TPCs and TFCs of ten flower teas.

mL) showed relatively low CAA. In all, the results of the CAA assay were almost identical to those of the chemical antioxidant activity assessment assays. Besides, the ROS in the H_2O_2 -induced oxidative damage in HepG2 cells were observed using a fluorescence microscope (Figure 1). Visually, extracts of *rosae rugosae flos* could reduce the ROS in HepG2 cells caused by H_2O_2 .

The above methods provided similar results, namely *rosae rugosae flos* exerted the highest antioxidant ability in the DPPH, ABTS, FRAP and CAA models, and the troll flower and *lonicerae flos* also exhibited relatively high antioxidant activities. Conversely, the carnation showed the lowest DPPH and FRAP values, while the lily showed the weakest ABTS value, and *magnoliae flos* displayed the lowest CAA value. Chen et al. also determined the antioxidant abilities of thirty fresh flowers and found that *Rosa rugosa* showed the highest DPPH ($612.79 \mu\text{mol trolox/g}$), FRAP ($273.10 \mu\text{mol trolox/g}$) and TEAC ($1013.71 \mu\text{mol trolox/g}$) values [15], the results of which were consistent with the current study.

3.2 TPCs and TFCs

Phenolics are one of the most effective antioxidant constituents and play a vital role in free radical scavenging capacities [16]. Additionally, it was reported that a large number of various kinds of phenolics including phenolic acids, flavonoids, anthocyanins and many other phenolics exist in edible flowers [17, 18], which prompted us to investigate the TPCs and TFCs of ten flower teas. As showed in Figure 2, ten flower teas showed significant differences in TPC, which ranged from 34.82 to $363.40 \text{ mg GE/g Ex}$. *Rosae rugosae flos* had the highest TPC at 363.40

$\pm 19.37 \text{ mg GE/g Ex}$, followed by the troll flower at $185.24 \pm 7.53 \text{ mg GE/g Ex}$, while *magnoliae flos* ($34.82 \pm 3.29 \text{ mg GE/g Ex}$) and the lily ($37.73 \pm 1.14 \text{ mg GE/g Ex}$) showed the lowest TPCs (Figure 2). A previous study also reported that *R. rugosa* showed a relatively high TPC at 57.82 mg GAE/g among twenty-three selected flowers, which was consistent with the current findings [19].

Flavonoids are probably the most important natural phenolics and distribute widely in the plant kingdom [20]. The TFCs of ten flower teas presented huge differences compared with TPCs. *Fragrans* ($375.38 \pm 12.16 \text{ mg RE/g Ex}$) and *lonicerae flos* ($370.41 \pm 21.94 \text{ mg RE/g Ex}$) had the highest TFC value, followed by *chrysanthemum buds* ($285.63 \pm 3.65 \text{ mg RE/g Ex}$) and *trollflower* ($270.54 \pm 4.81 \text{ mg RE/g Ex}$). In addition, lily ($31.36 \pm 1.87 \text{ mg RE/g Ex}$) showed the lowest TFC. The family of phenolics from plants are large and diverse and covers multiple categories of aromatic compounds. The results indicated that most of phenolic compounds in these flower teas might exist in the non-flavonoid form [21].

The above experiments revealed that flower teas with higher contents of phenolics also possess a more potent antioxidant activity, suggesting that phenolics might be responsible for the antioxidant effects of flower teas, which was further demonstrated by the fact that the correlation between TPC and antioxidant activities was found to be remarkable (Table 3). However, there was no significant relevance between the TFC and TPC, antioxidant activity, which implies that the flavonoids were not principal components of phenolics in these flower teas; and that other kinds of phenolics, phenylpropionic acids and tannins, might be responsible for the antioxidant effects of the flower teas. Xiong et al. also reported that phenolics were likely to contribute to the antioxidant activities of

Table 3: Correlations between phenolic contents and antioxidant activities.

Assays	Equations			
	TPC	R^2	TFC	R^2
TFC	$y = 0.2932x + 145$	0.0439	/	/
IC ₅₀ of DPPH	$y = 0.0001313x - 0.00248$	0.9395	$y = 3.04e^{-005}x + 0.009135$	0.0987
ABTS	$y = 4.293x + 115.4$	0.8351	$y = -0.09697x + 695.3$	0.0008
FRAP	$y = 0.006411x + 0.2784$	0.7225	$y = 0.002442x + 0.6699$	0.2054
IC ₅₀ of CAA	$y = 0.0002496x + 0.0219$	0.6509	$y = 5.736e^{-005}x + 0.02435$	0.0674

edible flowers, whereas flavonoids were poorly correlated with antioxidant capacity [21].

4 Conclusions

The antioxidant activities and phenolic contents of ten commonly consumed flower teas in China were investigated in detail. The results showed that these flower teas were rich sources of antioxidant compounds, which could serve as significant dietary sources of natural antioxidants for the prevention of diseases caused by oxidative stress [22]. Especially, the rosae rugosae flos extract possessed the highest TPC and exerted the most powerful antioxidant effects; trollflower also exhibited impressive antioxidant effects and relatively higher TPCs. Positive correlations between the antioxidant capacities and the TPCs indicated that phenolics could be responsible for the antioxidant activities of these flower teas. These findings support the consumption of flower teas in a diet as functional foods and encourage continued component analysis and *in vivo* studies of these flower teas so that more applications can be developed as in dietary supplements, functional ingredients, and additives to prevent food oxidation.

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Conflict of interest: Authors state no conflict of interest.

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