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Phenolic contents and antioxidant activities of solvent extracts from four edible flowers

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Abstract

In order to identify new sources of natural antioxidants, the antioxidant activities of various solvent extracts from four edible flower samples [*Wisteria sinensis* (Sims) DC., *Benincasa hispida* (Thunb.) Cogn, *Luffa cylindrica* (L.) Roem, and *Cucurbita pepo* L.) were systemically investigated. The total phenolic content (TPC) and total flavonoid content (TFC), and individual phenolic profile of each extract were investigated, and antioxidant activities were measured by the DPPH radical scavenging activity, superoxide radical scavenging activity, total reduction capability, and ferrous ions chelating activity. Results revealed that all flower extracts exhibited antioxidant activities, and contained certain amounts of phenolic compounds. Specifically, different solvents exhibited different efficiencies in the extraction of phenolics, flavonoids, and compounds with antioxidant activities. The 70% ethanolic extract from *B. hispida* yielded the highest TPC (49.92 mg GAE/g DW), superoxide radical scavenging activity (IC_{50}, 0.073 mg/mL), and FRAP value (18.05 mg of GAE/g DW). The highest TFC was obtained with the ethanolic extract of *W. sinensis* (30.39 mg QE/g DW), and the contents of apigenin, luteolin, and myricetin in the ethanolic extract of *W. sinensis* were significantly higher than those in the other extracts. The 40% ethanolic extract of *L. cylindrica* yielded the highest DPPH scavenging capacity (IC_{50}, 0.340 mg/mL), and water extract of *B. hispida* yielded the highest chelating activity (0.027 mg/mL). Correlation analysis indicated that total phenolics and flavonoids in the extracts were the major contributors to the DPPH scavenging activities and FRAP activities. Overall, results demonstrated that these edible flowers could serve as useful source of natural antioxidants, and be used as functional food ingredients.

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Introduction

Edible flowers have been consumed globally for centuries due to their pleasing colours, delicate flavours, and attractive visual appearance in foods (Trinh *et al.*, 2018; Zheng *et al.*, 2018). Besides their attractive sensorial properties, edible flowers have attracted attention as abundant sources of nutraceutical and bioactive compounds that are beneficial for human health (Fernandes *et al.*, 2017; Janarny *et al.*, 2021). These compounds have various functions such as protecting cells against oxidative damage, preventing chronic degenerative diseases, and reducing the risk of many types of cancer. Numerous studies have revealed that edible flowers are rich in vitamins, carotenoids, flavonoids, anthocyanins, and many other phenolic compounds (Fernandes *et al.*, 2017; Pires *et al.*, 2018; Chensom *et al.*, 2019). Moreover, the content of total phenolics in some edible flowers is higher than that in common fruits and vegetables (de Morais *et al.*, 2020). Many of these compounds have been confirmed to exhibit high antioxidant activities (Petrova *et al.*, 2016; Chen *et al.*, 2018; Zheng *et al.*, 2018), and these natural antioxidants may reduce the risk of oxidative damage and prevent chronic conditions such as cardiovascular disease, cancers, diabetes mellitus, and Alzheimer's disease (Chew *et al.*, 2019; Yamagata, 2019; de Morais *et al.*, 2020). Many recent studies have focused on characterising the phytochemicals and

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antioxidant activities of edible flowers, and found that edible flowers could be a good potential source of natural antioxidants (Janarny *et al.*, 2021; Rivas-García *et al.*, 2021).

The flowers of *Wisteria sinensis* (Sims) DC., *Benincasa hispida* (Thunb.) Cogn, *Luffa cylindrica* (L.) Roem, and *Cucurbita pepo* L. have been consumed in China for decades, and are commonly considered to be vegetables. Previous studies have mainly focused on the phytochemical composition of *W. sinensis* leaves (Rokosz *et al.*, 2018), *B. hispida* fruits (Han *et al.*, 2013; Islam *et al.*, 2021), *L. cylindrica* seeds (Yoshikawa *et al.*, 1991; Al-Snafi, 2019), and *C. pepo* fruits (Kulczyński and Gramza-Michałowska, 2019). Phytochemical analysis has revealed that these plants are rich in flavonoids, phenolic acids, tannins, α-tocopherol, and other antioxidants. Huang *et al.* (2021) found that a flavonoid-rich extract from the edible flowers of *W. sinensis* possessed anti-diabetic effects via the activation of the IRS-1/PI3K/Akt/GLUT4 pathway. Several other studies have shown that the extracts from *C. pepo* fruits (Kopczyńska *et al.*, 2020), and *L. cylindrica* (L.) fruits (Du *et al.*, 2006) and leaves (Al-Snafi, 2019) exhibited antioxidant activities. However, there are few studies on the antioxidant activities and constituents of the four plant flowers (*W. sinensis*, *B. hispida*, *L. cylindrica*, and *C. pepo*). In addition, the antioxidant compounds in plants have different polarities and solubilities, and different extraction solvents may have a significant impact on the antioxidant activities of extracts (Lim *et al.*, 2019; Vural *et al.*, 2020).

Therefore, the aim of the present work was to evaluate the utilisation of several edible flowers as sources of natural antioxidants. Several solvent systems were used to extract antioxidants from these four edible flowers. The total phenolic contents, total flavonoid contents, individual phenolic profiles, and antioxidant capacities of the four edible flower extracts were determined. In addition, the composition of phenolic components was also identified and quantified by HPLC.

Materials and methods

Materials

Gallic acid, 3,4-dihydroxybenzoic acid, *p*-hydroxybenzoic acid, (±)-catechin hydrate, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, epicatechin, *p*-coumaric acid, ferulic acid, sinapic acid, rutin, myricetin, quercetin, luteolin, kaempferol, apigenin, 2,4,6-tripyridyl-s-triazine (TPTZ), nitro blue tetrazolium (NBT), and formic acid of HPLC-grade were purchased from Aladdin Industrial Inc. (Shanghai, China). Folin-Ciocalteu phenol reagent, acetonitrile of HPLC-grade, and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The other chemicals were of analytical grade, and purchased from Sinopharm Chemical Reagent Co., Ltd.

Sample preparation

Four edible flowers were studied, namely *W. sinensis*, *B. hispida*, *L. cylindrica*, and *C. pepo*. The flowers were collected from the local parks and farms in Huai'an, China. The air-dried flowers were ground to 20-mesh size, and later extracted by absolute ethanol, 70% ethanol, 40% ethanol, and water. The flower powders (50 g) were separately mixed with 400 mL of these solvents, and then the suspended solutions were sonicated (250 W, 40 kHz) for 30 min at 50°C. The mixtures were passed through filter papers, and the residues were extracted with the same solvent twice. The pooled filtrates were evaporated at 50°C using a rotary evaporator, and the remaining water was removed by lyophilisation. The dry extracts were kept at -20°C for subsequent analyses. The samples were re-dissolved in DMSO for the assessment of antioxidant capacities.

Determination of total phenolic content

Total phenolic content (TPC) was determined in 96-well plates using the Folin-Ciocalteu method (Singleton *et al.*, 1999) with slight modifications. Briefly, 10 μL of the diluted sample and 60 μL of H_2O were mixed well with 50 μL of 1:5 diluted Folin-Ciocalteu reagent. Five minutes later, 60 μL of 20% aqueous sodium carbonate was added, the solution was mixed well and incubated at room temperature for 2 h. Absorbance at 765 nm was measured using a Multimode Plate Reader (TECAN Infinite M200 PRO, Switzerland). Results were expressed as mg gallic acid equivalents per gram dry weight of extract (mg GAE/g DW) using the standard curve constructed earlier. All assays were performed in triplicate.

Determination of total flavonoid content

Total flavonoid contents (TFC) was determined using the method described previously

(Chen *et al.*, 2018) with slight modifications. Briefly, 20 µL of the diluted sample was mixed with 12 µL of 5% $NaNO_2$ solution in a 96-well plate. After 5 min, 12 µL of 10% $AlCl_3$ solution was added, and the solution was mixed well. After 6 min incubation, 80 µL of 1 M NaOH was added, followed by the addition of 76 µL of water. The mixture was vortexed for 30 s, and absorbance at 510 nm was measured using a Multimode Plate Reader. Results were expressed as mg quercetin equivalents per g dry extract (mg QE/g DW) using the standard curve constructed earlier. All assays were performed in triplicate.

HPLC analysis of phenolic compounds

HPLC analysis was performed using an Agilent HPLC system (1260 series, Agilent Co., USA). HPLC separation was performed on a Megres C_{18} (4.6 × 250 mm, 5 µm) analytical column (Jiangsu Hanbon Sci. & Tech. Co. Ltd., China) at 35°C. The mobile phase consisted of 0.1% formic acid in water (v/v) (solvent A) and methanol (solvent B). The injection volume was 5 µL, and the flow rate was kept at 0.8 mL/min for a total run time of 110 min. Absorbance at 270 nm was detected. The gradient solvent system was as follows: 0 - 5 min isocratic 97% A, 5 - 50 min from 97% A to 75% A, 50 - 95 min from 75% A to 30% A, 95 - 100 min remaining at 30% A, 100 - 110 min from 30% A to 97% A. The phenolic compounds of the different extracts from the four edible flowers were identified based on their retention times relative to the retention time of standards, and quantified based on their peak areas. The concentrations were expressed as milligram per g dry extract (mg/g DW).

DPPH radical scavenging activity

DPPH radical scavenging activity was determined following a previously reported method (Cheng *et al.*, 2006; Cheung *et al.*, 2018) with slight modifications. First, 10 µL of the extracts in DMSO in different concentrations and 190 µL of DPPH solution (0.2 mM in methanol solution) were mixed well in a 96-well plate, and left at ambient temperature. The absorbance was measured at 517 nm after 30 min using a Multimode Plate Reader. The inhibition ratio was calculated using Eq. 1:

$$\text{Inhibition (\%)} = (A_C - A_S) / A_C \times 100 \quad \text{(Eq. 1)}$$

where, A_C = absorbance of the control (absorbance of the solution with no sample), and A_S = absorbance of the extract. Quercetin served as positive control. All assays were performed in triplicate. The half inhibitory concentration (IC_{50}) of DPPH radicals was calculated as the concentration of each sample that scavenged 50% of the DPPH radicals.

Superoxide radical scavenging activity

The superoxide radical scavenging activities of the different extracts were determined following a modified method described by Li *et al.* (2005) and Siddhuraju (2007). Briefly, 180 µL of solution containing methionine (10 mM), riboflavin (3 µM), and NBT (0.1 mM) was prepared with 50 mM of phosphate buffer (pH 7.8), and 20 µL of various concentrations of the extracts were added in a 96-well plate. The reaction solution was mixed well and illuminated (1000 lux) using two 20 W fluorescent lamps for 25 min. Absorbance at 560 nm was measured before and after irradiation using a Multimode Plate Reader. The percentage inhibition was determined as the mean of triplicate analyses using Eq. 2:

$$\text{Inhibition (\%)} = (A_C - A_S) / A_C \times 100 \quad \text{(Eq. 2)}$$

where, A_C = absorbance of the control (absorbance of solution with no samples), and A_S = absorbance of the extract. Quercetin served as positive control. Half inhibitory concentration (IC_{50}) of superoxide radical was the concentration of each sample that inhibits 50% of NBT reduction in the assay system.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed following a previously described method with slight modifications (Zhang *et al.*, 2015). Briefly, 10 µL of the diluted sample was mixed with 190 µL of fresh FRAP solution, which was prepared by mixing 0.3 M of acetate buffer (pH 3.6), 10 mM of TPTZ in 40 mM HCl, and 20 mM of $FeCl_3$ solution at a ratio of 10:1:1 (v/v/v), and the mixture was allowed to react in darkness for 30 min. Absorbance at 593 nm was measured using a Multimode Plate Reader. The antioxidant activity was expressed as mg gallic acid equivalents per gram of extracts (mg GAE/g DW). All assays were performed in triplicate.

Ferrous ion chelating activity

The ferrous ion chelating activity of each sample was estimated following the method reported by Gallegos-Tintoré *et al.* (2011) and Yan *et al.*

(2011) with slight modification. Briefly 10 μL of sample solution was mixed well with 150 μL of methanol and 10 μL of $FeCl_2$ (2 mM). Following 5 min incubation, the reaction was initiated by the addition of 20 μL of ferrozine (5 mM). The mixture was shaken well, and incubated at ambient temperature for 10 min. The absorbance at 562 nm was measured using a Multimode Plate Reader. The ferrous ion chelating activity was calculated using Eq. 3:

$$\text{Metal chelating effect (\%)} = (1 - A_S / A_C) \times 100 \quad \text{(Eq. 3)}$$

where, A_C = absorbance of the control (absorbance of the solution with no sample), and A_S = absorbance of the extract. EDTA-Na_2 was used as the positive control. All assays were performed in triplicate. The IC_{50} value was the concentration at which the chelating activity was 50%.

Statistical analysis

The final data were presented as the mean ± SD (n = 3). Statistical significance was tested by one-way ANOVA and Duncan's multiple-range tests. Differences were considered to be statistically significant when the p-value was lower than 0.05 ($p < 0.05$).

Results and discussion

Total phenolics and flavonoid contents

Natural phenolic compounds, the main source of natural antioxidants, are widely distributed in plants. The TPC and TFC of the extracts from four edible flowers are shown in Table 1. Results showed that TPC varied significantly among the different extracts, ranging from 7.59 to 49.92 mg GAE/g DW. The TPC values of the extracts from the four edible flowers were much higher than those of most edible and wild flowers reported by Li *et al.* (2014). The various solvents exhibited significant effects on total phenolics extraction. The highest TPC was observed in 70% ethanolic extract of *B. hispida*, while the water extract of *B. hispida* had the lowest TPC. It seemed that 70% ethanol was the most effective solvent to extract TPC from these four edible flowers. The effect of extraction solvent was similar for TFC; the extracts with higher TPC also had higher TFC, which is consistent with a previous study (Chen *et al.*, 2018). The TFC in the various extracts ranged from 1.97 to 30.39 mg QE/g DW, accounting for 22.0 - 80.0% of TPC. The highest content was obtained in the ethanolic extract of *W. sinensis*, while the water extract of *C. pepo* had the lowest TFC. Results indicated that ethanol could be more suitable for the extraction of flavonoids from these four edible flowers.

Table 1. Total phenolic and flavonoid contents in extracts of four edible flowers.

Name	Extraction solvent	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg QE/g DW)
Wisteria sinensis	EtOH extract	31.54 ± 0.14^{g}	30.39 ± 0.28^{a}
	70% EtOH extract	37.83 ± 0.62^{f}	16.55 ± 0.05^{h}
	40% EtOH extract	32.32 ± 0.91^{g}	17.42 ± 0.27^{g}
	Water extract	19.77 ± 0.44^{j}	4.35 ± 0.23^{l}
Benincasa hispida	EtOH extract	46.05 ± 0.27^{b}	28.96 ± 0.31^{b}
	70% EtOH extract	49.92 ± 0.87^{a}	27.64 ± 0.10^{c}
	40% EtOH extract	18.08 ± 0.89^{k}	8.43 ± 0.43^{k}
	Water extract	7.59 ± 0.34^{l}	1.97 ± 0.19^{m}
Luffa cylindrica	EtOH extract	26.05 ± 0.93^{i}	20.85 ± 0.33^{f}
	70% EtOH extract	43.01 ± 0.35^{d}	26.24 ± 0.73^{d}
	40% EtOH extract	44.52 ± 0.32^{c}	25.33 ± 0.08^{e}
	Water extract	28.87 ± 0.53^{h}	14.27 ± 0.17^{i}
Cucurbita pepo	EtOH extract	18.80 ± 0.44^{jk}	13.98 ± 0.18^{i}
	70% EtOH extract	40.86 ± 0.18^{e}	14.30 ± 0.57^{i}
	40% EtOH extract	38.48 ± 1.21^{f}	14.29 ± 0.62^{i}
	Water extract	26.96 ± 0.65^{i}	12.28 ± 0.16^{j}

Values (mean ± standard error, n = 3) in the same column followed by different lowercase superscripts are significantly different ($p < 0.05$).

Profiles of individual phenolic compounds

The individual phenolic compounds of the different extracts and their contents were identified and quantified as presented in Figure 1 and Table 2. Fifteen phenolic compounds were measured in these flowers, syringic acid, (±)-catechin hydrate, and epicatechin were not found in any of the four edible flowers. Among the targeted compounds, only *p*-hydroxybenzoic acid was detected in all the tested flowers. Four compounds (vanillic acid, *p*-coumaric acid, rutin, and myricetin) appeared in three edible flowers. Four compounds (3,4-dihydroxybenzoic acid, ferulic acid, luteolin, and apigenin) were shared between two edible flowers. However, gallic acid, and caffeic acid were detected only in the flower of *L. cylindrica*, chlorogenic acid and sinapic acid were detected only in the flower of *C. pepo*, and quercetin was found only in the flower of *B. hispida.* Among the identified compounds, the contents of apigenin, luteolin, and myricetin in the ethanolic extract of *W. sinensis* were significantly higher than those in the other extracts. Large quantities of these three flavonoids have already been reported in many flowers and *Stachys cretica* subsp. Vacillans (Kirkan, 2019; Zheng *et al.*, 2019). These flavonoids possess many beneficial properties including antioxidant, anti-tumour, and anti-inflammatory effects (Rice-Evans *et al.*, 1996; Nabavi *et al.*, 2015). Rutin, a well-known antioxidant, was found in all extracts from *W. sinensis*, *B. hispida*. and *C. pepo*; but, the rutin contents in the water extracts were significantly lower than those in 70% ethanolic extract and 40% ethanolic extract. Although *p*-hydroxybenzoic acid was the most widely occurring phenolic acid, the contents of *p*-hydroxybenzoic acid in the extracts of *C. pepo* were much higher than those of the other flowers extracts.

In the four extracts of *W. sinensis*, the total individual phenolic compound content (TIPCC) ranged from 22.921 to 1.355 mg/g DW; the ethanolic extract had the highest TIPCC, followed by the 70% ethanolic extract and 40% ethanolic extract, while the water extract had the lowest TIPCC. For *B. hispida* and *L. cylindrica*, the TIPCC in the different extracts

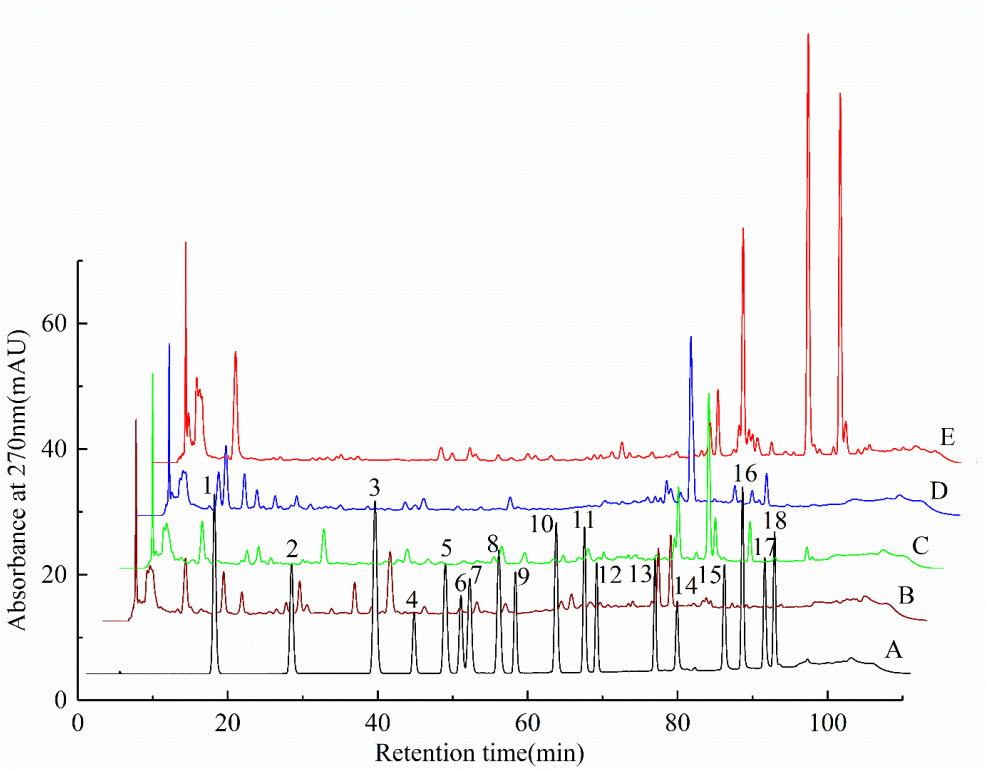

Figure 1. HPLC profile of extracts from four edible flowers. A = standards; B = 70% ethanol extract of *C. pepo*; C = 40% ethanol extract of *L. cylindrica*; D = 70% ethanol extract of *B. hispida*, and E = ethanol extract of *W. sinensis*. Identification peaks: 1 = gallic acid; 2 = 3,4-dihydroxybenzoic acid; 3 = *p*-hydroxybenzoic acid; 4 = (±)-catechin hydrate; 5 = chlorogenic acid; 6 = vanillic acid; 7 = caffeic acid; 8 = syringic acid; 9 = epicatechin; 10 = *p*-coumaric acid; 11 = ferulic acid; 12 = sinapic acid; 13 = rutin; 14 = myricetin; 15 = quercetin; 16 = luteolin; 17 = kaempferol; and 18 = apigenin

Table 2. Phenolic composition in extracts of four edible flowers.

Name	Extraction solvent	Gallic acid	3,4-Dihydroxybenzoic acid	*p*-Hydroxybenzoic acid	(±)-catechin hydrate	Chlorogenic acid	Vanillic acid	Caffeic acid	Syringic acid	Epicatechin	*p*-coumaric acid
		mg/g DW									
Wisteria sinensis	Ethanol	N.D.	N.D.	0.299 ± 0.006^{fg}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.376 ± 0.003^{b}
	70% ethanol	N.D.	N.D.	0.323 ± 0.004^{f}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.049 ± 0.004^{h}
	40% ethanol	N.D.	N.D.	0.198 ± 0.003^{i}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.227 ± 0.002^{f}
	Water	N.D.	N.D.	0.039 ± 0.002^{k}	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.037 ± 0.004^{h}
Benincasa hispida	Ethanol	N.D.	N.D.	0.246 ± 0.002^{h}	N.D.	N.D.	0.632 ± 0.016^{b}	N.D.	N.D.	N.D.	N.D.
	70% ethanol	N.D.	N.D.	0.272 ± 0.007^{gh}	N.D.	N.D.	0.517 ± 0.020^{c}	N.D.	N.D.	N.D.	N.D.
	40% ethanol	N.D.	N.D.	0.084 ± 0.004^{j}	N.D.	N.D.	0.146 ± 0.008^{h}	N.D.	N.D.	N.D.	N.D.
	Water	N.D.	N.D.	0.048 ± 0.004^{jk}	N.D.	N.D.	0.071 ± 0.012^{i}	N.D.	N.D.	N.D.	N.D.
Luffa cylindrica	Ethanol	0.121 ± 0.004^{c}	1.176 ± 0.011^{a}	0.475 ± 0.010^{e}	N.D.	N.D.	0.440 ± 0.014^{e}	0.741 ± 0.024^{a}	N.D.	N.D.	0.401 ± 0.003^{b}
	70% ethanol	0.179 ± 0.002^{b}	0.902 ± 0.022^{c}	0.259 ± 0.013^{gh}	N.D.	N.D.	0.214 ± 0.013^{g}	0.626 ± 0.010^{b}	N.D.	N.D.	0.292 ± 0.002^{e}
	40% ethanol	0.213 ± 0.003^{a}	1.006 ± 0.016^{b}	0.299 ± 0.012^{fg}	N.D.	N.D.	0.264 ± 0.013^{f}	0.773 ± 0.013^{a}	N.D.	N.D.	0.293 ± 0.022^{e}
	Water	0.087 ± 0.001^{d}	0.598 ± 0.020^{e}	0.205 ± 0.005^{i}	N.D.	N.D.	0.183 ± 0.013^{gh}	0.374 ± 0.008^{c}	N.D.	N.D.	0.169 ± 0.004^{g}
Cucurbita pepo	Ethanol	N.D.	0.380 ± 0.019^{f}	4.203 ± 0.052^{a}	N.D.	0.701 ± 0.020^{a}	2.218 ± 0.063^{a}	N.D.	N.D.	N.D.	1.072 ± 0.025^{a}
	70% ethanol	N.D.	0.732 ± 0.010^{d}	1.459 ± 0.031^{b}	N.D.	0.394 ± 0.031^{b}	0.476 ± 0.023^{de}	N.D.	N.D.	N.D.	0.365 ± 0.007^{bc}
	40% ethanol	N.D.	0.767 ± 0.024^{d}	1.336 ± 0.022^{c}	N.D.	0.215 ± 0.018^{d}	0.494 ± 0.014^{c}	N.D.	N.D.	N.D.	0.333 ± 0.002^{cd}
	Water	N.D.	0.568 ± 0.009^{e}	1.099 ± 0.041^{d}	N.D.	0.286 ± 0.011^{c}	0.469 ± 0.019^{de}	N.D.	N.D.	N.D.	0.304 ± 0.049^{de}

Table 2. Continued.

Name	Extraction solvent	Ferulic acid	Sinapic acid	Rutin	Myricetin	Quercetin	Luteolin	Kaempferol	Apigenin	Total individual phenolic compound contents
		mg/g DW								
Wisteria sinensis	Ethanol	0.225 ± 0.007^{b}	N.D.	2.365 ± 0.010^{ef}	6.923 ± 0.096^{a}	N.D.	7.628 ± 0.118^{a}	0.099 ± 0.011^{b}	5.007 ± 0.106 ^{a}	22.921 ± 0.112^{a}
	70% ethanol	0.182 ± 0.010^{cd}	N.D.	2.270 ± 0.030^{fg}	4.401 ± 0.046^{d}	N.D.	2.878 ± 0.047^{b}	0.270 ± 0.024^{a}	1.551 ± 0.035^{b}	11.924 ± 0.091^{c}
	40% ethanol	0.199 ± 0.001^{c}	N.D.	2.159 ± 0.023^{h}	4.192 ± 0.055^{e}	N.D.	2.770 ± 0.053^{c}	0.296 ± 0.012^{a}	1.389 ± 0.038^{c}	11.429 ± 0.072^{d}
	Water	0.071 ± 0.010^{f}	N.D.	0.341 ± 0.012^{k}	0.650 ± 0.046^{h}	N.D.	0.121 ± 0.010^{d}	0.065 ± 0.011^{b}	0.031 ± 0.004^{f}	1.355 ± 0.033^{o}
Benincasa hispida	Ethanol	N.D.	N.D.	3.418 ± 0.053^{b}	N.D.	0.380 ± 0.024^{b}	N.D.	N.D.	N.D.	4.676 ± 0.058^{k}
	70% ethanol	N.D.	N.D.	8.103 ± 0.070^{a}	N.D.	0.757 ± 0.019^{a}	N.D.	N.D.	N.D.	9.649 ± 0.094^{e}
	40% ethanol	N.D.	N.D.	2.267 ± 0.065^{g}	N.D.	0.241 ± 0.014^{c}	N.D.	N.D.	N.D.	2.739 ± 0.081^{n}
	Water	N.D.	N.D.	0.494 ± 0.005^{j}	N.D.	0.045 ± 0.010^{d}	N.D.	N.D.	N.D.	0.658 ± 0.017^{p}
Luffa cylindrica	Ethanol	N.D.	N.D.	N.D.	1.253 ± 0.049^{g}	N.D.	0.121 ± 0.010^{d}	N.D.	0.287 ± 0.004^{d}	5.013 ± 0.074^{j}
	70% ethanol	N.D.	N.D.	N.D.	4.772 ± 0.069^{c}	N.D.	0.079 ± 0.012^{d}	N.D.	0.162 ± 0.012^{e}	7.487 ± 0.068^{g}
	40% ethanol	N.D.	N.D.	N.D.	5.428 ± 0.036^{b}	N.D.	0.074 ± 0.002^{d}	N.D.	0.179 ± 0.008^{e}	8.529 ± 0.056^{f}
	Water	N.D.	N.D.	N.D.	1.658 ± 0.082^{f}	N.D.	0.071 ± 0.008^{d}	N.D.	0.063 ± 0.003^{f}	3.407 ± 0.041^{m}
Cucurbita pepo	Ethanol	0.514 ± 0.005^{a}	0.326 ± 0.005^{a}	3.223 ± 0.092^{c}	0.270 ± 0.032^{i}	N.D.	N.D.	N.D.	N.D.	12.906 ± 0.062^{b}
	70% ethanol	0.231 ± 0.010^{b}	0.352 ± 0.009^{a}	2.726 ± 0.033^{d}	0.139 ± 0.007^{j}	N.D.	N.D.	N.D.	N.D.	6.872 ± 0.088^{h}
	40% ethanol	0.152 ± 0.008^{e}	0.272 ± 0.022^{b}	2.466 ± 0.083^{e}	0.125 ± 0.011^{j}	N.D.	N.D.	N.D.	N.D.	6.161 ± 0.106^{i}
	Water	0.164 ± 0.016^{de}	0.194 ± 0.019^{c}	1.076 ± 0.023^{i}	0.106 ± 0.004^{j}	N.D.	N.D.	N.D.	N.D.	4.266 ± 0.090^{l}

Values (mean ± standard error, n = 3) in the same column followed by different lowercase superscripts are significantly different ($p < 0.05$). N.D. = not detected.

ranged from 9.649 to 0.658 and 8.529 to 3.407 mg/g DW, respectively. However, *B. hispida* and *L. cylindrica* exhibited different trends; the highest TIPCCs were detected in the 70% ethanolic extract and 40% ethanolic extract, respectively. TIPCCs in the extracts of *C. pepo* were significantly lower than those in the extracts of *W. sinensis*, while showing a similar tendency. The water extracts exhibited the lowest TIPCCs, which indicated that water could not be suitable for the extraction of phenolic compounds from these four edible flowers.

DPPH radical scavenging activity

The DPPH radical scavenging activities of the extracts from the four different flowers are presented in Table 3. Significant differences were observed in the DPPH radical scavenging activities of the different solvent extracts with the IC_{50} values varied from 1.822 to 0.340 mg/mL. The 40% ethanolic extract of *L. cylindrica* (IC_{50}, 0.340 mg/mL) showed the highest scavenging capacity, followed by the 70% ethanolic extracts of *B. hispida* (IC_{50}, 0.350 mg/mL) and *L. cylindrica* (IC_{50}, 0.358 mg/mL). The water extract of *W. sinensis* (IC_{50}, 1.822 mg/mL) had the weakest DPPH radical scavenging activity. The DPPH radical scavenging capacities of the 40% ethanolic extracts and 70% ethanolic extracts from three flowers (*W. sinensis*, *L. cylindrica*, and *C. pepo*) were higher than those extracted with ethanol and water. It appeared that polar solvents (40% ethanol and 70% ethanol) could be more suitable for the extraction of compounds with radical scavenging properties from these edible flowers. While for *B. hispida*, the DPPH radical scavenging activities of the ethanolic extract and 70% ethanolic extract were significantly higher than those of the 40% ethanolic extract and water extract. Moreover, the extracts with high TPC and TFC showed high DPPH radical scavenging activities, thus suggesting that the phenolics and flavonoids in these extracts might be the critical compounds for the DPPH radical scavenging activities.

Table 3. Antioxidant capacities of four edible flowers extracts.

Name	Extraction solvent	IC_{50} - DPPH radical scavenging activity (mg/mL)	IC_{50} - superoxide radical scavenging activity (mg/mL)	FRAP (mg GAE/g)	IC_{50} - ferrous chelating activity (mg/mL)
Wisteria sinensis	Ethanol	0.726 ± 0.004^{e}	0.969 ± 0.006^{b}	7.46 ± 0.13^{c}	0.2617 ± 0.005^{g}
	70% ethanol	0.513 ± 0.005^{h}	0.429 ± 0.004^{d}	6.41 ± 0.08^{e}	0.1259 ± 0.002^{Ij}
	40% ethanol	0.530 ± 0.003^{h}	0.234 ± 0.003^{ef}	6.71 ± 0.08^{de}	0.1251 ± 0.001^{Ij}
	Water	1.822 ± 0.011^{a}	0.831 ± 0.046^{c}	1.75 ± 0.22^{j}	0.1162 ± 0.003^{Ij}
Benincasa hispida	Ethanol	0.391 ± 0.006^{i}	1.015 ± 0.053^{b}	17.82 ± 0.12^{a}	1.6645 ± 0.009^{a}
	70% ethanol	0.350 ± 0.002^{j}	0.073 ± 0.004^{h}	18.05 ± 0.16^{a}	0.3804 ± 0.022^{e}
	40% ethanol	1.070 ± 0.020^{c}	0.245 ± 0.019^{e}	6.68 ± 0.06^{de}	0.1871 ± 0.018^{h}
	Water	1.597 ± 0.015^{b}	0.144 ± 0.006^{g}	2.60 ± 0.26^{i}	0.0274 ± 0.001^{k}
Luffa cylindrica	Ethanol	0.742 ± 0.004^{e}	1.825 ± 0.089^{a}	7.32 ± 0.32^{c}	1.5260 ± 0.030^{b}
	70% ethanol	0.358 ± 0.002^{j}	1.022 ± 0.017^{b}	11.95 ± 0.34^{b}	0.5137 ± 0.007^{c}
	40% ethanol	0.340 ± 0.004^{j}	0.177 ± 0.003^{fg}	12.12 ± 0.11^{b}	0.3012 ± 0.003^{f}
	Water	0.526 ± 0.008^{h}	0.400 ± 0.004^{d}	5.32 ± 0.22^{f}	0.1281 ± 0.001^{i}
Cucurbita pepo	Ethanol	0.864 ± 0.011^{d}	N.A.	3.27 ± 0.08^{h}	0.4240 ± 0.003^{d}
	70% ethanol	0.692 ± 0.010^{f}	N.A.	7.27 ± 0.11^{c}	0.2527 ± 0.003^{g}
	40% ethanol	0.636 ± 0.007^{g}	0.459 ± 0.011^{d}	6.92 ± 0.10^{d}	0.1173 ± 0.001^{Ij}
	Water	0.863 ± 0.005^{d}	0.286 ± 0.011^{e}	4.47 ± 0.11^{g}	0.1028 ± 0.001^{j}
Quercetin*		0.011 ± 0.000	0.021 ± 0.000	/	/
EDTA-Na_2*		/	/	/	$0. 015 \pm 0.000$

Values (mean ± standard error, $n = 3$) in the same column followed by different lowercase superscripts are significantly different ($p < 0.05$). N.A. = not available; * positive control.

Superoxide radical scavenging activity

Results of the superoxide radical scavenging activities of the different extracts from the four edible flowers are given in Table 3. The IC_{50} values of the ethanolic extract and 70% ethanolic extract of *C. pepo* could not be calculated. The IC_{50} values of the other extracts ranged from 0.073 to 1.825 mg/mL, with the highest scavenging activity being observed in the 70% ethanolic extract of *B. hispida*, and the lowest in the ethanolic extract of *L. cylindrica*. The solvents had significant effects on the superoxide radical scavenging activities of the extracts. The highest superoxide radical scavenging activities were observed in the 40% ethanolic extract, 70% ethanolic extract, 40% ethanolic extract, and water extract of *W. sinensis*, *B. hispida*, *L. cylindrica*, and *C. pepo*, respectively. The ethanolic extracts exhibited the lowest scavenging capacities, thus suggesting that ethanol could be unsuitable for the recovery of compounds with superoxide radical scavenging activities from these edible flowers.

Ferric reducing antioxidant power (FRAP) assay

As shown in Table 3, significant differences were observed among the extracts with the FRAP activities ranging from 1.75 to 18.05 mg of GAE/g DW. The ethanolic extract (17.82 mg of GAE/g DW) and 40% ethanolic extract (18.05 mg of GAE/g DW) of *B. hispida* yielded significantly higher FRAP values than the other extracts. The 70% ethanolic extract (11.95 mg of GAE/g DW) and 40% ethanolic extract (12.12 mg of GAE/g DW) of *L. cylindrica* also yielded high reducing power activities. In contrast, the water extract (1.75 mg of GAE/g DW) of *W. sinensis* exhibited very poor reducing power activity. Similarly, 40% ethanolic extracts and 70% ethanolic extracts from these flowers exhibited a relatively higher reducing power activities than those extracted with ethanol and water. Therefore, 40% ethanol and 70% ethanol could be better solvents for the extraction of compounds with high FRAP activities from these four edible flowers. All water extracts exhibited the lowest scavenging capacities among the four extracts of each flower. This might indicate that water could not be an effective solvent for the extraction of compounds with reducing power activity from these edible flowers.

Ferrous chelating activity

The ferrous chelating activities of the extracts are shown in Table 3. The IC_{50} values of the different extracts ranged from 0.027 to 1.665 mg/mL. Water extract of *B. hispida* yielded the highest chelating activity, while the lowest activity was observed in the ethanolic extract of *B. hispida*. It was clear that for all flowers, the chelating activities of the water extracts were significantly higher than those of the ethanolic extracts; the observed chelating activities were in the following order: water extract, 40% ethanolic extract, 70% ethanolic extract, and ethanol extract. This indicated that higher chelating activity of extract could be obtained by increasing polarity of the solvent used. Yan *et al.* (2011) also reported that water was appropriate solvents for the extraction of chelating activity substances from *Eupatorium lindleyanum* DC.

Correlation analysis

To better understand the relationships between the antioxidant activities of the extracts and their TPC, TFC, TIPCC, and individual phenolic compound contents, Pearson correlation coefficients were calculated (Figure 2). The TPC and TFC of the extracts showed good linear relationships with the DPPH radical scavenging activities (r = -0.830 and -0.804, respectively). Similar results were also reported by He *et al.* (2015) for the edible flowers of *Pyrus pashia*. Many studies (Tai *et al.*, 2011; Fernandes *et al.*, 2017) have reported a positive correlation between DPPH radical scavenging activities and antioxidant capacity. These results agree with the assertion that the higher DPPH radical scavenging activities may be due to the rich TPC or TFC in the extracts. However, a lower correlation between TIPCC and DPPH radical scavenging activities was observed (r = -0.422), thus suggesting that those individual phenolic compounds might not be the principal antioxidants in the extracts of the four flowers. Correlations between superoxide radical scavenging activity and TPC, TFC, or TIPCC were weak, as shown in Figure 2. This indicated that the TPC, TFC, and TIPCC in these extracts had less contribution towards superoxide radical scavenging activity. These results were consistent with the results of the superoxide radical scavenging activity analysis, where the ethanolic extracts exhibited low scavenging capacities. Ethanolic extracts of flowers mainly contain lipophilic compounds, and most do

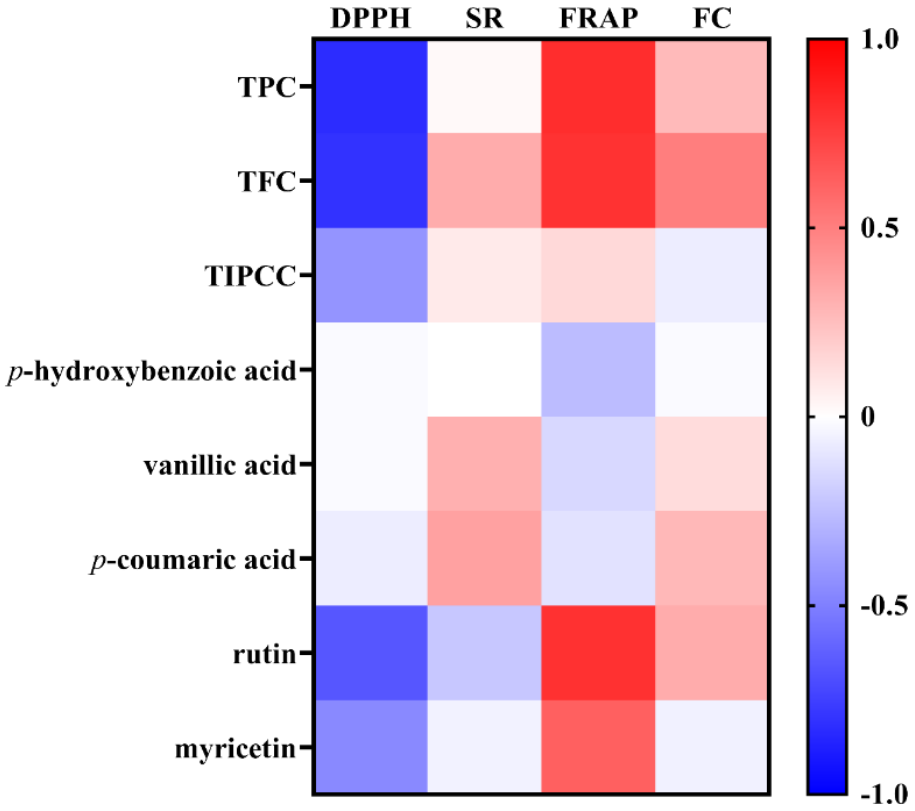

Figure 2. Pearson' correlation (*r*) analysis between the contents of phytochemicals and the antioxidant capacities. DPPH = DPPH radical scavenging activity, SR = superoxide radical scavenging activity, FRAP = ferric reducing antioxidant power, and FC = ferrous chelating activity.

not have antioxidant activities. In the present work, the ethanolic extracts showed low superoxide radical scavenging activities when TPC and TFC showed low antioxidant potential. The correlation analysis indicated that the FRAP values of the different extracts showed a good correlation with TPC or TFC (r = 0.822 and 0.803, respectively). A weak correlation was found between the TIPCC and FRAP activities. This suggested that phenolics and flavonoids were the major contributors to the FRAP activities of the extracts. Similar results have been reported previously (Lahouar *et al.*, 2014; Chen *et al.*, 2015). However, the correlation coefficients were lower than those reported by Patial *et al.* (2019), in which the FRAP values were strongly correlated with the TPC and TFC with r of 0.99. As shown in Figure 2, the correlations between the chelating activities of the different extracts and the TPC, TFC, or TIPCC were poor. This result is consistent with previous studies that the chelating activity and TPC or TFC were not tightly related (Damiani *et al.*, 2014; Islam *et al.*, 2016). For the individual phenolic compounds, the number of samples affects the value of the Pearson correlation coefficient; more samples could provide a more representative correlation. Therefore, Pearson correlation coefficients for the individual phenolic compounds only found in one or two flowers were not calculated. As shown in Figure 2, there was a positive correlation between the FRAP value and the content of rutin (r = 0.804). In addition, moderate correlations were observed between DPPH scavenging activity and the content of rutin (r = -0.666), and furthermore between the FRAP and the content of myricetin (r = 0.620). It has been reported that rutin and myricetin possess significant antioxidant activity (Arora *et al.*, 1998; Pekkarinen *et al.*, 1999), and these results suggested that rutin and myricetin were the important compounds related to the DPPH scavenging activities and FRAP activities of these extracts. The other correlation coefficients for the individual phenolic compounds were much lower, thus suggesting that there were other compounds with antioxidant activities in the flower extracts.

Conclusion

In the present work, water and ethanol at various concentrations were used to prepare antioxidant extracts from four flowers, and the TPC, TFC, and antioxidant capacity of each extract were studied. Results showed that these edible flowers had high TPC, TFC, and antioxidant capacities. The extracts from *B. hispida* exhibited relatively stronger antioxidant capacities. However, the highest level of TPC, TFC, TIPCC, and antioxidant capacities was found in different solvent extracts. 70% ethanol was the most efficient in the extraction of total phenolics, while the extraction of total flavonoids was best with ethanol. 40% ethanol and 70% ethanol were better solvents for the extraction of compounds with high DPPH radical scavenging activities and FRAP activities from these four edible flowers, and compounds with high chelating activities could be best extracted using water as the extraction solvent. Eighteen phenolic components were identified and quantified by HPLC. *p*-hydroxybenzoic acid was widely found in all of the extracts form these four edible flowers, and the contents of individual phenolic compounds varied considerably. Correlation analysis indicated that the antioxidant capacities as measured by DPPH and FRAP methods showed good correlations with TPC, TFC, and the content of rutin and myricetin. The present work indicated that these edible flowers were rich in phenolics and have good antioxidant activities, thus suggesting that they may serve as potential functional foods against oxidative damage.

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