Industrial Crops & Products 111 (2018) 430–445

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Research Paper

Antioxidant capacities and total phenolic contents of 30 flowers

Guan-Lin Chen[a], Song-Gen Chen[a], Yi Xiao[b], Nan-Lin Fu[b,*]

[a] Foshan Institute of Occupational Disease Prevention and Control, Foshan 528000, China
[b] The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou 510080, China

ARTICLE INFO

Keywords:
Antioxidant capacity
Alkaline hydrolysis
Phenolic profile
Flavonoids
Flower

ABSTRACT

In order to find new sources of natural antioxidants, total phenolic content (TPC) and total flavonoid content (TFC) of 30 flowers in their free, esterified and insoluble-bound forms were determined. *Rosa rugosa Thunb* (pink) showed the highest TPC, and *Osmanthus fragrans* had the highest TFC. The antioxidant activities of 30 flowers were determined by 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), and trolox equivalent antioxidant capacity (TEAC) assays. *Rosa rugosa Thunb* (purple) and *Rosa rugosa Thunb* (pink) had the highest DPPH (612.79 and 544.75 μmol Trolox/g DW), FRAP (273.10 and 301.14 μmol Trolox/g DW) and TEAC (1013.71 and 937.19 μmol Trolox/g DW) value. Furthermore, several phenolic compounds were detected using ultra performance liquid chromatography (UPLC) technique, and (+)-catechin, ferulic acid, isoquercitrin, and quercitrin were widely found in these flowers. The results implied that the flowers were important natural sources of bioactive components with higher antioxidant capacities for use in the food and pharmaceutical industries.

1. Introduction

Phenolic compounds are a large and diverse group of phytochemicals, which include many different families of aromatic secondary metabolites in plants (Harborne and Williams, 2000), and these compounds have the ability to scavenge numerous radicals (Albishi et al., 2013a; Benavente-García et al., 2000; Chen et al., 2017, 2016, 2015, 2014; Shahidi and Ambigaipalan, 2015; Wang et al., 2016). Phenolics exist in three forms, namely, free, esterified and insoluble bound forms (Jung et al., 2002; Krygier et al., 1982; Alshikh et al., 2015). Fruits and vegetables are natural sources of phenolic compounds, high intake of fruits and vegetables significantly reduce the risk of numerous chronic diseases, such as cardiovascular disease (Hu, 2003; Ikram et al., 2009) and cancer (Riboli and Norat, 2003; Ikram et al., 2009). Flower is an important part of plant, which contains a great variety of natural antioxidants, such as flavonoids, phenolic acids, anthocyanin and many other phenolic compounds (Youwei et al., 2008; Kaur et al., 2006). Flowers have been used in the culinary arts in China for centuries, and some flowers have been eaten since ancient times. In addition, some flowers, such as *Chrysanthemum morifolium Ramat.*, *Bombaxceiba* and *Lonicera Japonica Thunb.* have been used in traditional Chinese medicine. Xiong et al. (2014) analyzed 10 common edible flowers from China, and found that flowers are good sources of phenolics and antioxidants. Li et al. (2014) reported that total phenolic content and antioxidant capacities from 51 edible flowers in water-soluble fraction and fat-soluble fraction were higher than those of insoluble-bound fraction. However, Kaisoon et al. (2011) reported that bound phenolics from *A.leptopus*, *B. hybrid*, *C. sulphureus*, *M arboreus*, *I. chinensis*, *N. nucifera*, *P. obtuse* and *T. minor* were higher than those of soluble phenolics. Although numerous publications have reported the chemical components and bioactivities contained in flowers (Kaisoon et al., 2011; Hung et al., 2012; Lee et al., 2010; Li et al., 2014; Xiong et al., 2014), there is still limited literature on the contents of free, esterified and insoluble-bound phenolics and antioxidant capacity of flowers. The objective of this study was to determine the TPC existing in free, esterified and insoluble-bound forms, to determine the antioxidant capacity of free, esterified and insoluble-bound phenolics isolated from 30 flowers. In addition, polyphenols in free, esterified and insoluble-bound forms of flowers were also identified and quantified by UPLC.

2. Materials and methods

2.1. Materials

Formic acid was of HPLC grade and purchased from CNW Technologies GmbH (Shanghai, China). Acetonitrile was also of HPLC

Abbreviations: FRAP, ferric reducing antioxidant power; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; ABTS, 2, 2-azinobs-(3-ethylbenzothiazoline-6-sulphonic acid); UPLC, ultra performance liquid chromatography

* Corresponding author.
E-mail address: nanlinf@163.com (N.-L. Fu).

http://dx.doi.org/10.1016/j.indcrop.2017.10.051
Received 2 June 2017; Received in revised form 3 September 2017; Accepted 29 October 2017

grade and purchased from Merck (Darmstadt, Germany). Gallic acid, protocatechuic acid, (+)-catechin, caffeic acid, epicatechin, ferulic acid, vanillic acid, quercetin, *p*-coumaric acid, isoquercitrin, quercitrin, rutin, syringic acid, 2,4,6-Tri(2-pyridyl)-*S*-triazine (TPTZ), 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH·), and 2, 2-azinobs-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were purchased from Aladdin Industrial Inc (Shanghai, China). The 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), and the Folin–Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used in the experiments were of analytical grade, and deionized water was used.

2.2. Sample preparation

Thirty flowers were studied, namely, *Amygdalus persica L*, *Bombaxceiba*, *Camellia japonica L, Chaenomeles sinensis*, *Chimonanthus praecox (L.) Link*, *Chrysanthemum morifolium Ramat.*, *Dendranthema indicum* (*Linn.*) *Des Moul.*, *Dendranthema morifolium (Ramat.) Tzvel* (*Gong ju*), *Dianthus caryophyllus*, *Eriobotrya japonica*, *Globe amaranth*, *Gomphrena globsa Linn*, *Hibiscus sabdariffa L*, *Jasminum sambac (L.) Ait*, *Lagerstroemia speciosa*, *Lavandula angustifolia Mill*, *Lilium brownie*, *Limonium sinuatum*, *Lonicera Japonica Thunb.*, *Magnolia soulangeana*, *Osmanthus fragrans*, *Perennial chamomile*, *Plumeria rubra L. cv. Acutifolia*, *Pueraria lobata (Willd.)Ohwi*, *Radix Gentianae*, *Rosa rugosa Thunb* (pink), *Rosa rugosa Thunb* (purple), *Rosmarinus officinalis L*, *Siraitia grosvenorii* and *Sophora japonica Linn*. The flowers were collected from the local markets and supermarkets in Fo-shan, China. The dry flowers were ground to a fine powder with a special grinder for medicinal herbs and passed through a 20-mesh sieve. The particle size was 0.8 mm. The materials were stored at room temperature in a desiccator for use within a month.

2.3. Extraction of phenolics

The free, esterified and insoluble-bound phenolic acids from flowers were prepared using the procedure described in the literature (Ambigaipalan et al., 2016; Krygier et al., 1982) with a slight modification. Twenty millilitres of methanol-acetone-water (7:7:6,v/v/v) were added into 1.0 g of the powder, and then samples were ultrasound for 10 min to extract phenolics at room temperature (23–25 °C). The mixture was centrifuged at 1540*g* for 15 min, and the supernatant was collected. The residue was extracted with the same solvent twice, and the supernatants were combined. The combined supernatants were evaporated at 50 °C using a rotary evaporator until all of the organic solvent was removed from the supernatant. After evaporation, the water phase was acidified to pH 2 with 6 M HCl. Free phenolics were extracted 3 times with ethyl acetate at a solvent to water phase ratio of 1:1 (v/v). The combined extracts were evaporated to dryness in vacuo at 50 °C, and subsequently dissolved in methanol to obtain the fraction containing free phenolics. The supernatant containing esterified phenolics (water phase) was subsequently hydrolyzed with 30 mL of 4 M NaOH under N_2 for 4 h at room temperature. The resultant hydrolysate was acidified to pH 2 using 6 M HCl followed by extracted with ethyl acetate 3 times. The ethyl acetate extracts were combined and evaporated to dryness in vacuo at 50 °C and subsequently dissolved in methanol to obtain phenolics released from their esterified form. The solid residues were treated with 20 mL of 4 M NaOH and hydrolyzed for 4 h at room temperature under a stream of N_2. The resulting slurry was acidified to pH 2 with 6 M HCl and centrifuged at 1540*g* for 15 min. The supernatant was extracted with ethyl acetate 3 times. The combined extracts were evaporated to dryness at 50 °C in vacuo and subsequently dissolved in methanol to obtain phenolics released from their insoluble-bound form.

2.4. Determination of total phenolic content

Total phenolic content (TPC) were determined by using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, 1 mL of the diluted sample was added to 2.5 mL 1:10 diluted Folin-Ciocalteu reagent, and then 2 mL of saturated sodium carbonate solution (75 g/L) was added after 4 min. After incubation at room temperature (23–25 °C) for 120 min, the absorbance of the mixture was measured at 760 nm using a Rui Li U9600 UV–vis spectrophotometer (Beijing, China). Gallic acid was used as the standard for a calibration curve, and the results were expressed as mg of gallic acid equivalents dry weight of flowers (mg GAE/g DW).

2.5. Determination of total flavonoid content

Total flavonoid content (TFC) was determined by using colorimetric method described previously (Xiong et al., 2014) with slight modifications. Briefly, 0.5 mL of the extract (diluted with distilled water to a suitable concentration) was mixed with 0.30 mL of 5% $NaNO_2$ solution. After 6 min, 0.30 mL of 10% Al $(NO_3)_3$ solutions was added and allowed to stand for another 5 min before 2.0 mL of 1 M NaOH was added. The mixture was vortexed well for 10 s and was then recovered with water to a final volume of 10 mL. The absorbance was measured immediately at 510 nm using a spectrophotometer. The results are expressed as mg of rutin equivalents dry weight of flowers (mg RE/g DW).

2.6. Ferric-reducing antioxidant power assay

Ferric-reducing antioxidant power (FRAP) assay was carried out according to the procedure described in the literature (Benzie and Strain, 1996). Briefly, the FRAP reagent was prepared from 20 mM iron (III) chloride solution, 10 mM TPTZ solution in 40 mM HCl and 300 mM sodium acetate buffer (pH 3.6) in a volume ratio of 1:1:10, respectively. FRAP reagent should be prepared fresh daily and warmed in a water bath at 37 °C before use. Then 0.2 mL of the diluted sample was added to 3 mL of the FRAP solution and the absorbance was determined at 593 nm after 2 h (Ozgen et al., 2006). Trolox was used as a reference standard, and the results were expressed as μmol Trolox/g dry weight of flowers (μmol Trolox/g DW).

2.7. DPPH radical scavenging activity

Antioxidant activities of the samples were analyzed by investigating their abilities to scavenging the DPPH· (Cai et al., 2003). The 3.9 mL of 60 μM DPPH· solution in 80% ethanol were added to 0.2 mL of the diluted sample, then the tube was vortexed for 15 s and reacted for 2 h, and the decrease in absorbance of DPPH· was measured at 515 nm using a Rui Li U9600 UV–vis spectrophotometer. Ethanol (80%) was used as a blank solution, and DPPH· solution without test samples (3.9 mL of DPPH· + 0.1 mL of 80% ethanol) served as the control, inhibition (%) of DPPH· absorbance = $(A_{control} - A_{test}) \times 100/A_{control}$. Trolox was used as a reference standard, and the results were expressed as μmol Trolox/g dry weight of flowers (μmol Trolox/g DW).

2.8. Total antioxidant capacity by equivalent antioxidant capacity (TEAC) assay

Antioxidant activities of the samples were analyzed by investigating their ability to scavenge the $ABTS^{\cdot+}$ using the method as described previously in the literature (Ozgen et al., 2006). The $ABTS^{\cdot+}$ (7 mM in 20 mM/L sodium acetate buffer, pH4.5) was mixed with 2.45 mM potassium persulfate, and this mixture was allowed to stand for 12–16 h at room temperature in the dark to create a stable, dark blue–green radical solution. The solution was then diluted with 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.001 at 734 nm to form the test reagent. One hundred microlitres of the test sample were