

Original Article

Antioxidation and active constituents analysis of flower residue of *Rosa damascena*Wen-ya Liu^{a,1}, Lin-yu Chen^{a,1}, Ying-ying Huang^a, Ling Fu^{a,b}, Le-yuan Song^a, Yun-yu Wang^a, Zhe Bai^a, Fang-feng Meng^a, Yue-feng Bi^{a,b,*}^aSchool of Pharmaceutical Sciences, Zhengzhou University, 100 Kexue Avenue, Zhengzhou 450001, China^bCollaborative Innovation Center of New Drug Research and Safety Evaluation, Zhengzhou 450001, China

ARTICLE INFO

Article history:

Received 18 March 2020

Revised 20 May 2020

Accepted 28 May 2020

Available online 11 July 2020

Keywords:

antioxidant activity

Damask rose flower residue

HPLC

phenolic acids

phenolics

Rosa damascena Mill

ABSTRACT

Objective: To make full usage of resource and turn waste into treasure, the chemical constituents and bioactivity were firstly investigated on Damask rose (*Rosa damascena*) flower residue (DRFR).**Methods:** DPPH and ABTS experiments were applied to assess the antioxidant activity of DRFR. Then, column chromatography was used to purify compounds from an antioxidation extract (DRFR-A), and the chemical structure was identified using NMR. The total phenolic acid content was measured by Folin-Ciocalteu colorimetric method, and the content of gallic acid of the indicator ingredient was detected by HPLC.**Results:** DRFR-A was found to show a high activity both on DPPH (IC₅₀: 2.760 μg/mL) and ABTS (IC₅₀: 2.258 μg/mL) compared to positive control V_C. Ten compounds were isolated and identified as quercetin (1), kaempferol (2), gallic acid (3), protocatechuic acid (4), pyrogallol (5), 2-phenylethyl 3,4,5-trihydroxybenzoate (6), methyl gallate (7), *p*-hydroxybenzoic acid (8), *p*-hydroxyphenethyl alcohol (9) and astragalin (10) from DRFR-A. Among them, pyrogallol, 2-phenylethyl-3, 4, 5-trihydroxybenzoate, *p*-hydroxybenzoic acid and *p*-hydroxyphenethyl alcohol are obtained from the plant for the first time. The content of total phenolic acids and gallic acid, main ingredient in DRFR-A was determined as 63.73% and 24.67%, respectively.**Conclusion:** This study provides a reliable data and lays the foundation for the development and utilization of rose residue, and hence for the full utilization of rose resources.© 2020 Tianjin Press of Chinese Herbal Medicines. Published by ELSEVIER B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Damask rose (*Rosa damascena* Mill.), belongs to Rosaceae family, has been widely distributed in Bulgaria, Turkey, Spain, France, India, Syria, Morocco, Tunisia, Saudi Arabia and China (Chevallier, 1996). Roses are usually used to make tea (Wang, Li, & Cao, 2008), wine (Ren, 2007), flowers jazz (Sun, Xiao, & Tao, 2014) and candies (Ye, Hu, Chen, & Gao, 2015). In addition, rose flowers have been used as traditional Chinese medicines to treat various diseases for over a thousand years due to its effects of anti-inflammation, easing depression, improving blood circulation and regulating endocrine system (Nonato et al., 2012). In recent years, modern studies have shown that roses are rich in polyphenols and flavonoids, which possess significant antioxidant level

(Achuthan, Babu, & Padikkala, 2003; Ulusoy, Boşgelmez, & Seçilmişcanbay, 2009). However, up to now, the main researches on *R. damascena* have been focused on the extraction and the utilization of essential oils, which only accounts for 3%–5% of ten thousand in rose (Commission, 2015). And in the process of rose essential oil, a large amount of residue is produced, which is usually discarded as industrial waste without effective utilization. This not only results in environmental pollution, but also leads to a large amount of waste of resources. And any systematical investigation on residue has not been carried out, which seriously affects the development and utilization of rose residue.

Antioxidant refers to antioxidant free radicals. Scientific research has shown that excessive free radical production is closely related to aging and many diseases including cancer, multiple sclerosis, Parkinson disease, senile dementia, autoimmune diseases and asbestosis (Guo, Guo, Li, Fu, & Liu, 2017; Morry, Ngamcherdtrakul, & Yantasee, 2017). Antioxidants can scavenge free radicals. The more intensive antioxidants are common pheno-

* Corresponding author.

E-mail address: zzubyf@126.com (Y.-f. Bi).¹ These authors contributed equally to this work.

lic or polyphenolic compounds, flavonoids, phytic acid substances, and nitrogenous compounds (Wei et al., 2015). At present, antioxidants are mainly used in the prevention and treatment of many diseases (Serafini, 2006), nutrition and health care, and food preservation, etc. In a word, antioxidants, especially from edible plant, have broad development prospects.

The previous experiment of our study hints that the water extract of Damask rose flower residue (DRFR) contains abundant phenolic acids and reflects a certain anti-oxidation effect. And the antioxidant active site from the water extract of DRFR, DRFR-A, was obtained with solvent gradient extraction. In the present study, it is the first time to assess and analyze the antioxidant activities of the bioactive constituents and quality of DRFR-A systematically. The results suggested that DRFR-A had good anti-oxidation, and the most active constituents in DRFR-A were phenolic acids, including quercetin (**1**), kaempferol (**2**), gallic acid (**3**), protocatechuic acid (**4**), pyrogalllic acid (**5**), 2-phenylethyl 3,4,5-trihydroxybenzoate (**6**), methyl gallate (**7**), *p*-hydroxybenzoic acid (**8**), *p*-hydroxyphenethyl alcohol (**9**) and astragalol (**10**). Among them, compounds **5**, **6**, **8** and **9** are obtained from damask rose for the first time. Furthermore, the total phenolic acid content was measured as 63.73% and the content of gallic acid, the indicator ingredient, was detected as 24.67%. This study provides reliable science data and lays the foundation for the development and utilization of rose residue, hence for the full utilization of rose resources.

2. Materials and methods

2.1. Plant materials

Damask rose used in the experiment was collected in Nanyang city, Henan Province, and it was authenticated by the vice Prof. Chengxue Pan, Department of Pharmacognosy, School of Pharmaceutical Sciences, Zhengzhou University. The voucher specimen (No. Damask rose 20170527) has been deposited in our laboratory.

2.2. Apparatus and reagents

ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)], DPPH (2,2-diphenyl-1-picrylhydrazyl) and kojic acid were purchased from Sigma-Aldrich (U.S.A.). Vitamin C (purity 99%), K₂S₂O₈, DMSO, methanol and phosphate buffer were of analytical grade (Tianjin Chemical Plant, China). Microplate reader (BioTek, Instruments, USA) was used to determine absorbance. The NMR spectra was recorded on the Bruker DPX-400 (TMS as internal standard), and the melting points were determined using an X-4 micro-melting point apparatus (Beijing Taike Co., Ltd., China), uncorrected. In order to complete the process of smashing tissue extraction, the Flash Extraction Machine JHBE-100A (Henan Jinnai Science and Technology Co., Ltd., China) was used. HPLC protocol of DRFR-A was detected by a Waters e2695 HPLC Series System that had an automatic injector, a Waters 2489 UV/Vis detector and with Empower ChemStation software. (Waters, USA). All solvents used were of analytical grade (Tianjin Chemical Plant, China). Silica gel GF254 plates (Qingdao Marine Chemical Co., Ltd., China). Silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Company, Ltd., China) were used.

2.3. Sample preparation

The DRFR material (2.5 kg), was pulverized and extracted three times with water (10 min each time) by using smashing tissue extraction at room temperature. The extract was concentrated under reduced pressure to produce DRFR (426 g). Then, the extract

was suspended in water and extracted using ethyl acetate-ethanol gradient. The ethyl acetate extraction site was named DRFR-A (67.4 g).

2.4. Antioxidant capacity

2.4.1. DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging capacity

The method described by (Lu, Yuan, Zeng, & Chen, 2011) was used to assess DPPH radical scavenging activity of samples with modification. Crude extract of each sample was dissolved in ethanol and the results were given as IC₅₀, which is the amount of extract required to scavenge the initial DPPH radical by 50%. Vitamin C (V_C) was used as a positive control. The percent inhibition of DPPH radical was calculated according to the equation as shown below:

$$\text{DPPH radical scavenging capacity}(\%) = (A_{\text{DPPH}} - A_s) / A_{\text{DPPH}} \times 100 \quad (1)$$

where A_{DPPH} is the absorbance of the control solution (containing only DPPH), and A_s is the absorbance of the test solution (including V_C).

2.4.2. ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] radical scavenging capacity

The method described by Kim, Lee, Lee, & Lee (2002) was used to assess the ABTS radical scavenging activity of samples with modification. Crude extract of each sample was dissolved in ethanol and the results were given as IC₅₀, which is the amount of extract required to scavenge the initial ABTS radical by 50%. V_C was used as a positive control. The percent inhibition of ABTS radical was calculated according to the equation as shown below:

$$\text{ABTS radical scavenging capacity}(\%) = [(A_{\text{ABTS}} - A_s) / A_{\text{ABTS}}] \times 100 \quad (2)$$

where A_{ABTS} is the absorbance of the control solution (containing only ABTS), and A_s is the absorbance of the test solution (including V_C).

2.5. Isolation and identification of active constituents

DRFR-A (35 g) was subjected to ODS column chromatography and eluted with H₂O, 10%, 20%, 30%, 40%, 50% MeOH successively to afford six fractions (fractions 1 to 6). Column chromatography was carried out on silica gel (CH₂Cl₂/MeOH 30:1–1:1), and then purified by recrystallization. At last, compounds **3** (11 mg), **4** (3.2 mg) and **5** (11 mg) were obtained from fraction 1; compounds **6** (2.5 mg), **7** (6 mg), **8** (3.6 mg) and **9** (4.1 mg) were isolated successfully from fraction 2; Compound **1** (31 mg), **2** (6.8 mg) and **10** (3.9 mg) were isolated from fraction 5. The chemical structures were identified using NMR with the physical and chemical properties.

2.6. Total phenolic and gallic acid content determination

2.6.1. Total phenolic content

The total phenolic content was measured according to the method of Yuan, Lu, Eskridge, Isom, and Hanna (2018) and Muddathir, Yamauchi, Batubara, Mohieldin, and Mitsunaga (2017) with modification. The extract was dissolved in methanol to 0.20 mg/mL and 100 μL was transferred into test tubes, followed by 200 μL 1 mol/L Folin-Ciocalteu reagent, then 1.0 mL of sodium carbonate solution (75 g/L) was added to the content. After 1 h of incubation at 25 °C water bath in the dark, 200 μL of the mixture was transferred into the designated well of a 96-well microplate.

The absorbance was read at 725 nm, using a microplate reader (BioTek Instruments, U.S.A.). Gallic acid standard solutions (0.2 mg/mL) were used for calibration. The results of total phenolic content were expressed as mg gallic acid equivalents (GAE)/g of DRFR extractions.

2.6.2. Gallic acid content

The content of main ingredients gallic acid was determined by HPLC according to Huang et al. (2018). Briefly, chromatographic separation was performed on a Waters 2695 HPLC (Waters, USA) equipped with auto-sampler and a waters 2489 UV/Vis detector. Chromatographic analysis consisted of an Innovation Explorer C₁₈ column (4.6 mm × 250 mm, 5 μm) and a mobile phase of Acetonitrile-0.5% H₃PO₄ (Gradient elution) was adopted and elution was at a flow rate of 0.8 mL/min at 30 °C. The UV (λ) of 250 nm was used for gallic acid detection. The injection volume was 10 μL.

2.7. Statistical analysis

The IC₅₀ values of antioxidant activities were expressed as the mean (mean ± standard deviation). The significant differences between extracts and positive controls were assessed by SPSS 18.0 software. The antioxidant Curve equation was made by Microsoft Excel. Values were determined to be significant when *P* was less than 0.05 (*P* < 0.05).

3. Results and discussion

3.1. Total phenolic and gallic acid content determination

3.1.1. Total phenolic content

The total phenolic content of DRFR and DRFR-A were shown in Table 1. The amount of total phenolic content in the DRFR was about one-third [Total phenolic content was (386.4 ± 6.5) mg GAE/g, it means (38.64 ± 6.5)% phenolic of DRFR], and the total phenolic content of DRFR-A was 63.73%, indicating that ethyl acetate extraction can be effectively rich in polyphenols in DRFR. This experiment provides a theoretical basis and potential sites for fur-

Table 1

Total phenolic and gallic acid content in DRFR and DRFR-A (mean ± SD, *n* = 3).

Extracts	Yield/%	Total phenolic content/ (mg GAE/g*)	Total phenolic content/%
DRFR	17.04	386.4 ± 6.5	5.03 ± 0.14
DRFR-A	2.697	637.3 ± 14.6**	24.67 ± 0.24**

Note: ***P* < 0.01 vs DRFR group.

ther exploration of its pharmacological activities (antioxidant, anti-aging, whitening, anti-tumor).

3.1.2. Gallic acid content

The previous work (Demir, Yildiz, Alpaslan, & Hayaloglu, 2014) revealed that DRFR was rich in phenolic compounds, and gallic acid was the main ingredient. In this study, the HPLC-UV was applied by Huang et al. (2018) to determine the content of gallic acid in DRFR and DRFR-A. Consequently, as shown in Table 1 and Fig. 1, the content of gallic acid as the main indicator ingredient in DRFR and DRFR-A were (5.03 ± 0.14)% and (24.67 ± 0.24)%, respectively. Investigations have shown that gallic acid has many biological activities, such as anti-oxidation, anti-bacterial, anti-inflammatory and anti-viral, etc. (Zheng, Yang, & Yang, 2017), and are widely used in biology, medicine, chemical industry and other fields. Obviously, DRFR can be used as a raw material for extracting gallic acid.

3.2. Identification of chemical constituents

Compound **1** was obtained as yellow powder from methanol, m.p. 315–318 °C, positive to FeCl₃ testing (shows deep blue) and HCl-Mg reaction, indicating to be a flavones. ¹H NMR (400 MHz, CD₃OD) δ: 7.73 (1H, d, *J* = 2.0 Hz, H-2'), 7.63 (1H, dd, *J* = 2.0, 8.4 Hz, H-6'), 6.88 (1H, d, *J* = 8.8 Hz, H-5'), 6.38 (1H, d, *J* = 1.6 Hz, H-8), 6.18 (1H, d, *J* = 2.0 Hz, H-6); ¹³C NMR (400 MHz, CD₃OD) δ: 148.1 (C-2), 137.3 (C-3), 177.3 (C-4), 158.2 (C-5), 99.1 (C-6), 165.6 (C-7), 94.6 (C-8), 162.7 (C-9), 104.6 (C-10), 124.2 (C-1'), 116.0 (C-2'), 146.3 (C-3'), 148.9 (C-4'), 116.3 (C-5'), 121.8 (C-6'). Compound **1** was identified as quercetin by comparison of the physical and spectral data with those reported in literature (Hung, Song, Liu, Zhao, & Jia, 2013).

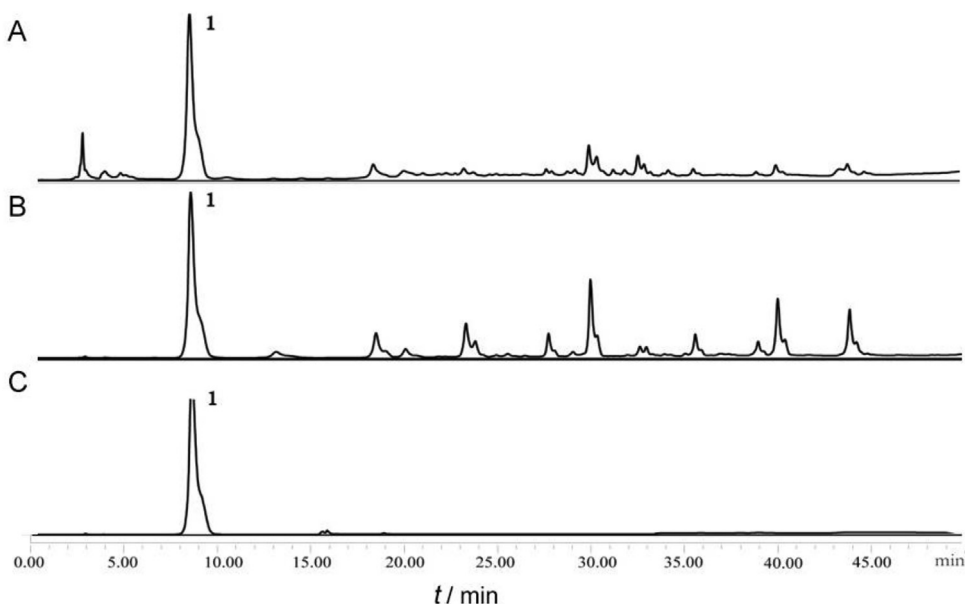


Fig. 1. HPLC chromatograms of fractions of DRFR (A), DRFR-A (B) and reference solution (C); **1** gallic acid.

Compound **2** was obtained as yellow powder from methyl alcohol, m.p. 280–282 °C, positive to FeCl₃ testing (shows deep blue) and HCl-Mg reaction, indicating to be a flavone. ¹H NMR (400 MHz, CD₃COCD₃) δ: 8.16 (2H, d, *J* = 8.8 Hz, H-2', 6'), 7.02 (2H, d, *J* = 9.2 Hz, H-3', 5'), 6.54 (1H, d, *J* = 1.6 Hz H-8), 6.27 (1H, d, *J* = 1.6 Hz, H-6). Compound **2** was identified as kaempferol by comparison of the physical and spectral data with those reported in literature (Hu, Zhu, Liu, & Tu, 2003).

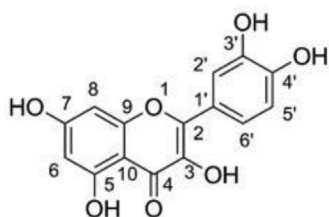
Compound **3** was obtained as white needles from ether-ethyl acetate, m.p. 238–240 °C, positive to FeCl₃ testing (shows deep blue) and Bromocresol green (shows yellow), indicating to be a phenolic acid. ¹H NMR (400 MHz, CD₃COCD₃) δ: 7.15 (2H, s, H-2, 6). Compound **3** was identified as gallic acid by comparison of the physical and spectral data with those reported in literature (Yuan et al., 2001).

Compound **4** was obtained as colorless needles from methyl alcohol, m.p. 198–200 °C, positive to FeCl₃ testing (shows deep blue) and Bromocresol green (shows yellow), indicating to be a phenolic acids. ¹H NMR (400 MHz, CD₃COCD₃) δ: 7.53 (1H, d, *J* = 2.0 Hz, H-2), 7.47 (1H, dd, *J* = 8.4 Hz, 2.0 Hz, H-6), 6.89 (1H, d,

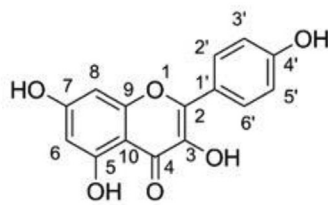
J = 8.4 Hz, H-5). ¹³C NMR (400 MHz, CD₃COCD₃) δ: 167.6 (C-7), 150.7 (C-4), 145.5 (C-3), 123.6 (C-6), 123.0 (C-1), 117.4 (C-2), 115.7 (C-5). Compound **4** was identified as protocatechuic acid by comparison of the physical and spectral data with those reported in literature (Yue, Chen, Yuan, Cui, & Kang, 2011).

Compound **5** was obtained as white needles from methyl alcohol, m.p. 133–134 °C, positive to FeCl₃ testing (shows deep blue), indicating to be a phenol compound. ¹H NMR (400 MHz, CD₃COCD₃) δ: 6.52 (1H, t, *J* = 8.0 Hz, H-5), 6.37 (2H, d, *J* = 8.0 Hz, H-4, 6). Compound **5** was identified as pyrogallol by comparison of the physical and spectral data with those reported in literature (Zhang, Liao, Moore, Wu, & Wang, 2009).

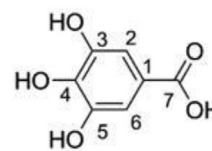
Compound **6** was obtained as white powder from methyl alcohol, m.p. 289–292 °C, positive to FeCl₃ testing (shows deep blue), indicating to be a phenol compound. ¹H NMR (400 MHz, CD₃COCD₃) δ: 7.31 (4H, m, H-2', 3', 5', 6'), 7.22 (1H, H-4'), 7.11 (2H, s, H-2, 6), 4.42 (2H, t, *J* = 6.8 Hz, H-8), 3.05 (2H, t, *J* = 6.8 Hz, H-9). ¹³C NMR (400 MHz, CD₃COCD₃) δ: 166.7 (C-9), 146.1 (C-3, 5), 139.4(C-4), 138.8(C-1'), 129.9 (C-2'), 129.3(C-6'), 127.3(C-3', 5'), 122.0 (C-4'), 109.9 (C-2, 6), 65.8 (C-7), 35.9 (C-8). Compound **6**



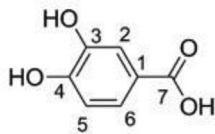
1 Quercetin



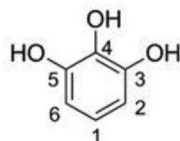
2 Kaempferol



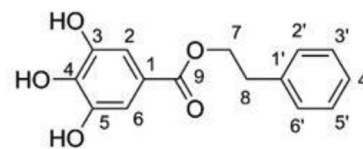
3 Gallic acid



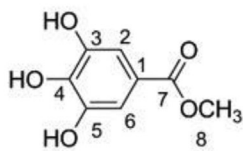
4 Protocatechuic acid



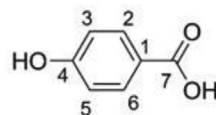
5 Pyrogallol



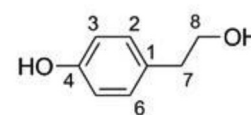
6 2-Phenylethyl 3,4,5-trihydroxybenzoate



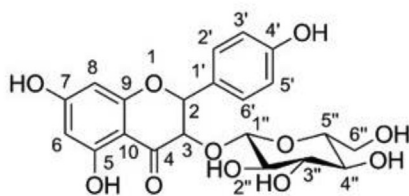
7 Methyl gallate



8 p-Hydroxybenzoic acid



9 p-Hydroxyphenethyl alcohol



10 Astragalin

Fig. 2. Chemical structures of compounds isolated from DRFR.

was identified as 2-Phenylethyl 3, 4, 5-trihydroxybenzoate by comparison of the physical and spectral data with those reported in literature (Tillekeratne et al., 2002).

Compound **7** was obtained as white powder from methyl alcohol, m.p. 202–204 °C, positive to FeCl₃ testing (shows deep blue), indicating to be a phenol compound. ¹H NMR (400 MHz, CD₃COCD₃) δ: 7.12 (2H, s, H-2, 6), 3.79 (3H, s, -OCH₃). ¹³C NMR (400 MHz, CD₃COCD₃) δ: 167.2 (C-7), 146.1 (C-3, 5), 138.7 (C-4), 121.8 (C-1), 109.8 (C-2, 6), 51.9 (C-8). Compound **7** was identified as methyl gallate by comparison of the physical and spectral data with those reported in literature (Tan et al., 2010).

Compound **8** was obtained as white crystal from methyl alcohol, m.p. 214–217 °C, positive to FeCl₃ testing (shows deep blue), indicating to be a phenol compound. ¹H NMR (400 MHz, CD₃COCD₃) δ: 7.93 (2H, d, J = 8.8 Hz, H-3, 5), 6.93 (2H, d, J = 8.8 Hz, H-2, 6). ¹³C NMR (400 MHz, CD₃COCD₃) δ: 167.5 (C-7), 162.6 (C-4), 132.7 (C-2, 6), 122.7 (C-1), 116.0 (C-3, 5). Compound **8** was identified as *p*-hydroxybenzoic acid by comparison of the physical and spectral data with those reported in literature (Feng et al., 2011).

Compound **9** was obtained as white crystal from methyl alcohol, m.p. 89–93 °C, positive to FeCl₃ testing (shows deep blue), indicating to be a phenol compound. ¹H NMR (400 MHz, CD₃COCD₃) δ: 7.05 (2H, d, J = 8.4 Hz, H-2, 6), 6.74 (2H, d, J = 8.4 Hz, H-3, 5), 3.69 (2H, t, J = 7.2 Hz, H-8), 2.71 (2H, t, J = 7.2 Hz, H-7). ¹³C NMR (400 MHz, CD₃COCD₃) δ: 131.0 (C-3), 130.7 (C-5, 7), 115.9 (C-4, 8), 64.2 (C-1), 39.4 (C-2). Compound **9** was identified as *p*-hydroxyphenethyl alcohol by comparison of the physical and spectral data with those reported in literature (Zheng et al., 2012).

Compound **10** was obtained as light yellow crystal from methyl alcohol, m.p. 224–229 °C, positive to HCl-Mg reaction and α-naphthol testing, indicating to be a flavone glycoside. ¹H NMR (400 MHz, DMSO *d*₆) δ: 12.62 (1H, s, 5-OH), 10.87 (1H, brs, 7-OH), 10.19 (1H, brs, 4'-OH), 8.04 (2H, d, J = 7.2 Hz, H-2', 6'), 6.88 (2H, d, J = 6.8 Hz, H-3', 5'), 6.44 (1H, d, J = 2.0 Hz, H-8), 6.21 (1H, d, J = 2.0 Hz, H-6), 5.46 (1H, d, J = 7.6 Hz, H-1''), 5.36 (1H, d, J = 4.8 Hz), 5.07 (1H, d, J = 4.4 Hz), 4.96 (1H, d, J = 5.2 Hz), 4.28 (1H, t, J = 5.6 Hz). ¹³C NMR (400 MHz, DMSO *d*₆) δ: 177.5 (C-4), 164.1 (C-7), 161.2 (C-5), 160.0 (C-4'), 156.4 (C-9), 156.2 (C-2), 133.2 (C-3), 130.9 (C-2', 6'), 120.9 (C-1'), 115.1 (C-3', 5'), 104.0 (C-10), 100.8 (C-1''), 98.7 (C-6), 93.7 (C-8), 77.5 (C-5''), 76.4 (C-3''), 74.2 (C-2''), 69.9 (C-4''), 60.8 (C-6''). Compound **10** was identified as astragaloside by comparison of the physical and spectral

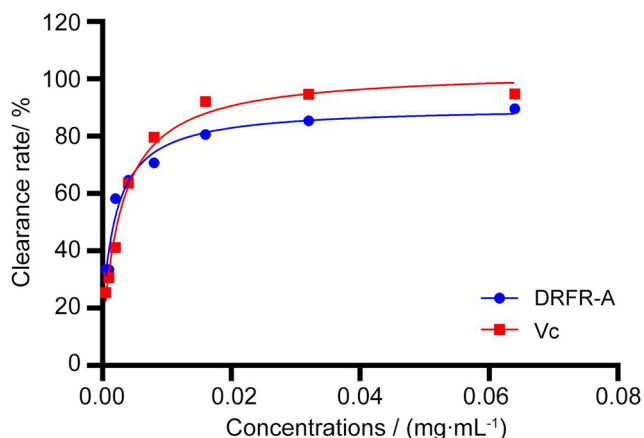


Fig. 3. DPPH scavenging capacity of DRFR-A and positive control (V_c).

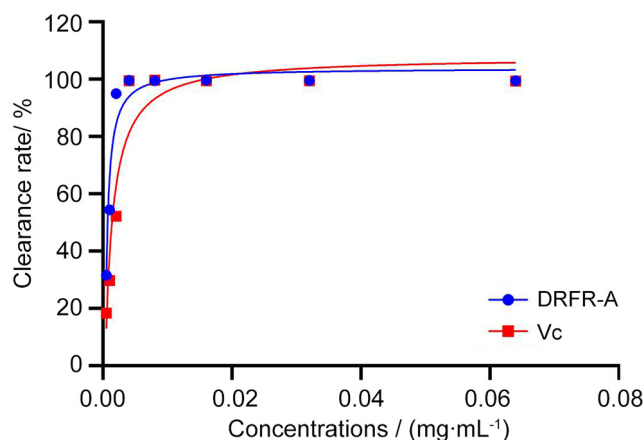


Fig. 4. ABTS scavenging capacity of DRFR-A and positive control (V_c).

data with those reported in literature (Shu, Li, Hu, & Zhang, 2010). All the compound structures were shown in Fig. 2.

3.3. Antioxidant capacity

As showed in Figs. 3 and 4, the same concentration of DRFR-A and V_c on DPPH scavenging capacity and ABTS clearance were almost similar, and in the experimental concentration range, with the increasing concentration of each component, the DPPH free radical scavenging effect and ABTS clearance showed an increasing trend, suggesting that DRFR-A had obvious antioxidant capacity. It implies that DRFR-A is a potential resource of natural antioxidants and has high value for development and has high value for development and application.

4. Conclusion

Above all, this study reveals that rose residue is rich in phenolic acids and has demonstrable antioxidative effect, which indicates the prosperous application prospect of the by-product in the production of rose essential oil, in food, medicine and cosmetics, etc., lays the foundation for turning waste into treasure and also suggests the other flower dregs, the by-product in the process of essential oil, should be carried out intensive investigation and thereby could be make full of use.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors wish to thank associate professor Cheng-xue Pan from Zheng-zhou University for his assistance in the plants identification and authentications. This project was supported financially by the National Natural Science Foundation of China (Grant No. 331/313400531021).

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