

Identification of new flavonoid compounds in dandelion *Taraxacum mongolicum* Hand.-Mazz. and evaluation of their antioxidant activities

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Abstract

Due to the interest in the potential pharmacological application of dandelion, the chemical constituents and activities of *Taraxacum mongolicum* Hand.-Mazz were studied. Box-Behnken response surface methodology was employed to optimize the protocol for extraction of flavonoids from dandelion. The molecular structures of different flavonoid compounds were acquired and analyzed by liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy. Seven flavonoid compounds were isolated and purified, namely, hesperetin-5'-O- β -rhamnoglucoside (I), quercetin (II), hesperetin-7-glucuronide (III), kaempferol-3-glucoside (IV), baicalein (V), hyperseroside (VI) and rutin (VII), among which the flavonoid compounds II, III, V, VI were extracted for the first time from dandelion, whereas the compound I : hesperetin-5'-O- β -rhamnoglucoside, was identified as a new type of flavonoid that had never reported in the literature. The antioxidant activities of the extracted flavonoids were examined, and the determination of structure-related antioxidant activity could be interpreted based on DFT calculations. As such, we have not only illustrated the rich flavonoid contents in *Taraxacum mongolicum* Hand.-Mazz, but also revealed new types of flavonoids in dandelion in terms of structure and antioxidant properties.

Introduction

Dandelion (*Taraxacum mongolicum* Hand.-Mazz). is a perennial herb which can be used as both medicine and food. It belongs to compositae, alias Huanghuading, or popoding. Nowadays, the value of dandelion has been well-recognized, and widely applied in the food, cosmetics and medicine industries. (González-Castejón et al. 2012). Generally, dandelion contains a variety of biologically active components, including flavonoids, triterpenes, sesquiterpenes, phenolic acids, sterols and coumarins, with high edible and medicinal value.(JinYin et al. 2012). Particularly, dandelion plant extracts and some monomer compounds have antibacterial, anti-inflammatory, antioxidant, hepatoprotective and anti-tumor pharmacological activities.(Astafieva et al. 2015). Flavonoids are one class of the main bio-active components in dandelion, which are closely associated with the pharmacological effects of dandelion. For example, the medicinal effects of dandelion such as anti-cancer, anti-aging, liver-protecting, cholagogic and bacteriostasis properties, are either directly or indirectly related to the activities of flavonoids (Jedrejek et al. 2017).

In general, flavonoids stem from secondary metabolic components in plants, which are very important in phytochemistry.(Y. Zhang et al. 2022; Wen et al. 2017). It has been reported that flavonoids have the pharmacological effects and physiological regulation functions.(Karakaya et al. 2020). Being prominent antioxidants and free radical disruptors, flavonoids can effectively prevent cell damage caused by lipid peroxidation and play a role in preventing and suppressing cancers.(Park et al. 2011). In terms of chemical structure, flavonoids have a series of compounds in which aromatic ring A fuses with pyranone ring C and then connects with another aromatic ring B, and have the basic skeleton characteristics of C6-C3-C6. There are many connecting sites between basic skeleton C ring and B ring, and A ring and B ring often have substituents such as hydroxyl, methoxy, methyl and isopenty, resulting in many different

derivatives and active functions.(Kumar & Pandey et al. 2013). Figure 1 illustrates the flavonoid parent structures, while flavonoids in plants exist mostly in the form of glycosides, that is, hydroxyl groups connected with sugar units and two aromatic rings (A and B). Many studies have proved that the medicinal value of dandelion has a close relationship with the antioxidant activity of dandelion flavonoids.(Park & Song et al. 2013; Lis et al. 2019). However, dandelion flavonoid components are complex, and there is a lack of comprehensive analysis of dandelion flavonoids in terms of structure and antioxidant activity. Therefore, it is of great interest to explore the different forms and antioxidant activities of dandelion flavonoids and analyze the corresponding structure-activity relationship.

In this study, the main flavonoid compounds were extracted from *Taraxacum mongolicum* Hand.-Mazz via optimized response surface methodology, which were purified by preparative high performance liquid chromatography (PHPLC). and analyzed using liquid chromatography-mass spectrometry (LC-MS). and nuclear magnetic resonance (NMR). spectroscopy. Figure 2 shows the schematic plot of our experimental design and procedure. The compound structures were determined based on experimental data and also confirmed by theoretical calculations based on density function theory (DFT). The antioxidant activities of the extracted flavonoids were evaluated based on the assessment of the ability of scavenging DPPH free radicals. The structure-activity relationship of the interested flavonoids was therein explored and discussed.

Materials And Methods

Materials and reagents

Dandelion (*Taraxacum mongolicum* Hand.-Mazz). samples were picked from Wulanchabu City, Inner Mongolia. The species was identified by Yuan Ke Yan (inspector of Hohhot Food and Drug Inspection and Testing Center). Other chemicals were purchased, which include: rutin standard (HPLC purity $\geq$ 98% Shanghai McLean Biochemical Technology Co., Ltd.), absolute ethanol (AR Sinopharm), acetic acid (AR Sinopharm), polyamide resin (14-30 mesh Sinopharm), acetone (AR Xilong), methanol (AR Xilong), DMSO-d₆ (purity > 99.9%, Shanghai Aladdin Biochemical Technology Co., Ltd.).

Extraction of flavonoids from dandelion

At first, single-factor experiments and response surface methodology were applied to optimize the extraction conditions of dandelion. In the single-factor experiments, extraction time (30, 40, 50, 60 and 70 min), extraction temperature (40,50,60 and 80°C), ratio of liquid to material (20, 30, 40, 50 and 60:1 (v/w). mL/g). and ethanol concentration (40%, 50%, 60%, 70%, and 80% (v/w).). were investigated for dandelion flavonoid extract maximization. One factor was changed while the others were held constant in each experiment. Box-Behnken design (BBD). was employed to determine the best combination of different variables to maximize the flavonoid extraction based on the single-factor experimental results. The proper range for the extraction time (A). and the ratio of liquid to material were selected (B), extraction temperature (C). were determined, and then the response surface methodology was utilized to design the experimental conditions. The independent variables and their levels are given in Table 1. On

the basis of the BBD data, a quadratic polynomial model was fitted to correlate the relationship between the independent variables and the response values to predict the optimized conditions .

Table 1 Factors A/B/C adopted in the BBD experiment

level	factor		
	A Extraction time/min	B Liquid-to-material ratio (mL/g).	C Extraction temperature/°C
-1	30	30: 1	50
0	50	50: 1	70
1	70	70: 1	90

With the optimal extraction conditions, the dandelion samples (1 kg). were then extracted 3 times with extraction conditions (70 min, 90 °C, 53:1 mL/g, 75% ethanol). The complex extract sample was concentrated to small volume suspension with water, in turn with n-hexane and ethyl acetate extraction for 5 times. 42.7 g of flavonoid complex extract sample was obtained.

Isolation and purification of dandelion flavonoids

The complex flavonoid extracts were then separated by a polyamide (bottom). + AB-8 (top). macroporous resin column, eluted with 70% methanol, acetone and 60% ethanol (300:1, 0.9 L. 14.2 g)., and concentrated to give 3 groups of eluent components (F1-F3). Preparative high performance liquid chromatography (PHPLC). was carried out on a shimadzu LC-16P instrument with an SPD-20A detector, using a YMC-Pack ODS-A column. The mobile phase was composed of a binary system, in which phase A was water containing 0.1% formic acid and phase B was acetonitrile in water containing 0.1% formic acid.The gradient elution program is as follows: 0–2 min, 20% B; 2–7 min,60% B; 7–10 min, 75% B; 10–14min,35% B; 15–17min, 20% B. The extract sub-components were separated and collected (F1-F3 volume flow rate 4mL/min)., then concentrated under reduced pressure and freeze-dried to obtain components.

Identification of extracted compounds

Different flavonoid compounds were analyzed by AB Triple TOFTM 5600+ LC-MS/MS (SCIEX, ShangHai, China). which was enabled with ultra-fast scanning speed and high mass spectrometry resolution and quantitative sensitivity, ensuring that the system can obtain high accuracy mass spectrometry data and quantitative detection limits. The separation of flavnoid substances in liquid chromatography was performed using C18 (100 mm × 4.6 mm, 5 μm particle size). reverse-phase column with the column temperature of 25 °C. The mobile phase consists of 0.1% formic acid in water (A). and 100% acetonitrile (B). and the flow rate was 0.3 mL/min. The gradient settings for the elution program were as follows: 0–1 min, 95%A; 1–20 min, 95–70% A;20–30 min, 70–10 min A; 30–35 min, 10% A; 35–36 min, 10–95%A; 36–40 min, 95%A. The injection volume was 10 μL. The mass spectrum was obtained over heated electrospray ionization source in negative-ion modes. The key parameters were as follows: the spray

voltage was +3.8 and -2.8 kV and sheath gas flow rate was 35 arbitrary units (arb unit).; auxiliary-gas flow rate was 10 arb unit; capillary temperature was 325 °C with the auxiliary-gas-heater temperature was 350 °C. The scan range was from m/z 10,000 to 100,000. Data acquisition and processing were carried out with Mass Frontier 7.0 and software Xcalibur 4.1 (Waltham, MA, Thermo Scientific)., respectively. For the NMR analysis, the spectra were obtained using Bruker AV-500 spectrometers (500 MHz for ¹H NMR and 125 MHz for ¹³C NMR). (Bruker, Switzerland). In addition, ultraviolet-visible (UV-Vis). spectroscopy was also performed on Shimadzu UV-2550 UV-Vis spectrophotometer (200-800 nm). for the compound analysis.

Assessment of antioxidant capacity

25 mg DPPH was dissolved in 100 mL of absolute ethanol to obtain a stock solution, and a series of extract sample solutions of different concentrations (0, 2, 5, 10, 15, 20, 30, 40, 50 mg/L). were prepared. For the antioxidant activity measurement, the absorbance value at 517 nm was measured after reaction, and all experiment was repeated for three times. The DPPH radical scavenging capacity was calculated as follows: DPPH· scavenging activity (%) = $[1 - (A_s - A_b) / A_d] \times 100\%$. where A_d is the absorbance value of 4 mL 50 mg/L DPPH, A_s is the solution absorbance value of 4 mL 50 mg/L DPPH ethanol solution plus 1 mL sample, A_b is the absorbance value of 4 mL absolute ethanol solution plus 1 mL sample solution. The evaluation and comparison of free radical scavenging capacity of antioxidants is based on IC₅₀ value, which corresponds to the antioxidant solution concentration when DPPH· radical scavenging rate is 50%.

Theoretical calculations

Calculations were performed to assist the understanding and confirmation of the experimental results including structures, antioxidant activities and spectra. All the calculations were carried out using Gaussian 09 package. The calculations in this work were performed by applying the functionals B3LYP with 6-311G++ (d, p). basis set. The calculations gave rise to the energy levels and energy ranges of the highest occupied orbital (HOMO). and the lowest empty orbital (LUMO). of the products with the top three oxidation resistance, as well as the hydrogen extraction enthalpy of B ring substituents. (Bella & Rotondo, 2020). In addition, the NMR ¹H and ¹³C spectra were calculated under the condition of Opt + Freq/GIAO. (Bellocchio et al., 2008).

Results And Discussion

Optimization of extraction condition

All experiments were carried out through Design-Expert design response surface optimization methodology. According to the response surface model, the optimum extraction conditions were as follows: extraction time 70 min, liquid-material ratio 52.56:1 (mL/g)., extraction temperature 90 °C, and the theoretical yield of total flavonoids of dandelion was 13.31%. Considering the simple and practical operation, the optimum extraction process of total flavonoids from dandelion was 70 min, the ratio of

liquid to material was 53:1 (mL/g), and the extraction temperature was 90 °C. The experiment was repeated three times under the same conditions, and the reproducibility of the protocol was confirmed. As a result, the average yield of total flavonoids in dandelion was 14.12%, and the relative error was 5.74%. The prediction ability and feasibility of the model meets the actual expectation (Supporting Information Part 1: Fig. S1, Table S1-S2).

Identification of flavonoid compounds via NMR, LC-MS spectroscopy

Seven compounds were isolated from dandelion extracts, as illustrated in Fig. 3, with one new type of flavonoid that had never been reported in the literature. The identification of the seven compounds are described as follows:

Compound I is a white powdery substance, which can be dissolved in methanol and acetone. Its molecular formula was confirmed as $C_{28}H_{34}O_{15}$ (Fig. 4). by ESI-MS (m/z 609.1, 447.09, 285.04). It was determined that the compound was a flavonoid according to its physicochemical properties and ultraviolet spectrum signal. The 1H NMR (500MHz, DMSO- d_6 , δ ppm). spectrum exhibited signals at 12.03 (s, 1H, -OH phenol), 9.11 (s, 1H, -OH phenol), 6.89 (dd, $J=2.148$, 1H-2'), 6.11 (dd, $J=8.6$, 2.0 Hz, H-6'), 6.94 (m, 2H-3), 5.3 (d, $J=2.0$ Hz, H-8), 3.78 (s, 3H), 5.12-4.03 (m, 13H, -CH ring), 3.65-5.12 (m, 6H, -OH alicyclic), 2.77 (dd, 3H, -CH₂OH). The ^{13}C NMR spectrum showed carbon signals at (HSQC, 125MHz, δ ppm): 145.93, 144.24, 131.81, 127.80, 114.07, 111.90, 103.29, 100.35 and 96.22 ppm (-CH aromatic), 87.32, 82.42, 78.39, 73.00, 71.75, and 70.11 (-CH Alicyclic). It was determined as Hesperetin-5'-O- β -rhamnoglucoside. (2-[3-[3, 4-dihydroxy-6-methyl

-5-[3, 4, 5 - trihydroxy -6-(hydroxymethyl). oxan-2-yl] oxyoxan-2-yl]oxy-4-hydroxy

-5- methylphenyl]-5,7-dihydroxy-2,3-dihydrochromen-4-one). (Fig. 4. Table. 2).

Compound II is a pale yellow needle-like crystalline substance, which can be dissolved in methanol and acetone. Its molecular formula was established as $C_{15}H_{10}O_7$ (Y. G. Zhang et al., 2020). by ESI-MS (m/z 301, m/z 179 and m/z 151). It was determined that the compound was a flavonoid by According to its physicochemical properties and ultraviolet spectrum signal. The compound's 1H NMR (500MHz, DMSO- d_6 , δ ppm). spectrum exhibited signals at δ 12.49 (s, 1H, 5-OH), 9.56 (s, 3-OH), 10.18 (s, H, 7-OH), 7.67 (d, $J = 2.3$ Hz, 1H), 9.33 (s, 4'-OH), 7.54 (dd, $J = 8.5$, 2.2 Hz, 1H, 2'-H), 6.89 (d, $J = 8.5$ Hz, 1H), 6.41 (d, $J = 2.1$ Hz, 1H), 6.19 (d, $J = 2.1$ Hz, 1H), 6'-H, 6.17 (s, H, 6-H). According to the 2D spectrogram of HSQC at C-H intersect, The ^{13}C NMR spectrum showed carbon signals at (HSQC, 125MHz, δ ppm): 176.1 (C-4), 161.8 (C-5), 98.3 (C-6), 163.1 (C-7), 136.5 (C-3), 145.5 (C-4'), 146.5 (C-5'). (Supporting Information Part 2: Fig. S2-S4). so it was determined to be Quercetin (3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one). (Cheng et al. 2020).

Compound III is a pale yellow powder substance, which can be dissolved in methanol and acetone. Its molecular formula was established as $C_{22}H_{22}O_{12}$ 22 by ESI-MS (477.13 [M-H]⁻, m/z 301 and m/z 345.09 MSMS). (Pereira-Caro et al. 2020). It was determined that the compound was a flavonoid according to its

physicochemical properties and ultraviolet spectrum signal. The compound's ^1H NMR (500MHz, DMSO- d_6 , δ ppm). spectrum exhibited signals at δ 12.98 (s, 2H)., which were at 7.27 (1H, d, J = 1.3 Hz)., 6.89 (1H, d, J = 8.7 Hz, 277 H-6'). and 6.81 (1H, d, J = 8.2 Hz, H-5'). 6.43 (1H, s, H-6)., 6.13 (1H, d, J = 1.8 Hz, H-8)., 5.12 (1H, d, J = 7.7 Hz, H-1")., 4.32 (1H, s, H-2)., 4.04 (1H, s, H-3)., 3.62-3.28 (2H, m, H-2", 3"). The ^{13}C NMR spectrum showed carbon signals at (HSQC, 125 MHz, δ ppm): 101.4 (C-10)., 145.3 (C-3', 4')., 132.1 (C-1')., 119.5 (C-6')., 115.5 (C-2')., 115.3 (C-5')., 102.0 (C-1")., 97.4 (C-6)., 96.8 (C-8)., 89.8 (C-2)., 87.4 (C-3")., 86.3 (C-5")., 81.3 (C-2")., 82.7 (C-4")., 76.7 (C-3). so it was determined to be Hesperetin 7- glucuronide (Supporting Information Part 2: Fig. S5-S7). ((2S, 3S, 4S, 5R, 6S).-3, 4, 5-trihydroxy-6-[[5-hydroxy-(3-hydroxy-4-methoxyphenyl).-4-oxo-2,3-dihydrochromen-7-yl]oxy]oxane-2-carboxylic acid).(Yue et al. 2019).

Compound IV is a pale yellow needle-like crystalline substance, which can be dissolved in methanol and acetone. Its molecular formula was established as $\text{C}_{21}\text{H}_{20}\text{O}_{11}$ by ESI-MS (m/z 477.09, m/z 284).(Alvarez-Fernandez, Hornedo-Ortega, Cerezo, Troncoso, & Garcia-Parrilla, 2014). It was determined that the compound was a flavonoid by According to its physicochemical properties and ultraviolet spectrum signal. The compound's ^1H NMR (500MHz, DMSO- d_6 , δ ppm). spectrum exhibited signals at δ 12.43 (1H, s, 5-OH)., 10.73 (1H, s, 7-OH)., 9.66 (1H, s, 3-OH)., 9.37 (1H, s, 4'-OH)., 7.5 (1H, d, J =2.1Hz, H-2')., 7.76 (1H, dd, J =2.1, 8.4Hz, H-6')., 6.94 (1H, d, J =8.4Hz, H-5')., 6.47 (d, J = 2.1 Hz, 1H, H-8)., 6.19 (d, J = 2.1Hz, 1H, H-6)., 5.26 (d, J = 7.5 Hz, 1H, H-1").. 3.41 (m, 1H, H-4").. 3.31(dd, J = 9.7, 5.5, 2.4 Hz, 1H, H-5").. 3.73 (dd, J = 11.9, 5.5 Hz, 1H, H-6a").. 3.89 (dd, J = 11.9, 2.4 Hz, 1H, H-6b"). The ^{13}C NMR spectrum showed carbon signals at (HSQC, 125MHz, δ ppm): 179.1 (C-4)., 165.9 (C-7)., 158.5 (C-9)., 163.2 (C-5)., 150.6 (C-4')., 148.2 (C-3')., 158.8 (C-2)., 134.9 (C-3)., 121.5 (C-6')., 121.7 (C-1')., 105.9 (C-3', C-5')., 111.8 (C-2', C-6')., 103.0 (C-10)., 96.7 (C-6)., 93.5 (C-8)., 104.07 (C-1")., 75.74 (C-2").. 78.05 (C-3")., 71.36 (C-4")., 78.42 (C-5")., 62.63 (C-6"). It was characterized as kaempferol-3-glucoside (Supporting information Part 2: Fig. S8-S10). (5, 7-dihydroxy-2-(4-hydroxyphenyl).- 3-[(2s,3R, 4s, 5s, 6R).-3, 4, 5-trihydroxy-6-(hydroxymethyl).Oxan-2-yl] Oxychromen-4-one).(Sohretoglu et al. 2018).

Compound V is a pale yellow needle-like crystalline substance, which can be dissolved in methanol and acetone. Its molecular formula was established as $\text{C}_{21}\text{H}_{20}\text{O}_{11}$ by ESI-MS (m/z 269.05, 251).(Vedpal, Jayaram, Wadhwani, & Dhanabal, 2020). The compound's ^1H NMR (500MHz, DMSO- d_6 , δ ppm). spectrum exhibited signals at 12.72 (s, 1H, -OH)., 8.90 (s, 1H, OH)., 8.09-7.61 (m, 5H, -CH aromatic)., 6.95 (s, 1H, -CH aromatic)., 6.67 (s, 1H, -CH aromatic). The ^{13}C NMR spectrum showed carbon signals at (HSQC, 125MHz, δ ppm): 182.08 ($>\text{C}=\text{O}$)., 162.83 (C=C)., 153.58, 149.78, 146.90, 131.76, 130.88, 129.28, 129.04, 126.22, 104.40, 104.24, 93.98 (-CH aromatic). It was characterized as baicalein(Supporting Information Part 2: Fig. S11-S13). (5, 6, 7-trihydroxy-2-phenyl-4H-chromen-4-one).(Li Han & Chao et al. 2011).

Compound VI is a Light yellow and light green crystalline powder substance, which can be dissolved in methanol and acetone. Its molecular formula was established as $\text{C}_{21}\text{H}_{20}\text{O}_{12}$ by ESI-MS (m/z 463.1, 301.03, 271.02).(Karakaya et al., 2020).,(Lee et al. 2010). It was determined that the compound was

a flavonoid by According to its physicochemical properties and ultraviolet spectrum signal. The compound's ^1H NMR (500MHz, DMSO- d_6 , δ ppm). spectrum exhibited signals at δ 12.63 (s, 1H, 5-OH)., 10.87 (s, 1H, 7-OH)., 9.72 (s, 1H, 4'-OH)., 9.18 (s, 1H, 3'-OH)., 7.67 (dd, J = 8.5, 2.2 Hz, 1H, 6')., 7.53 (d, J = 2.3 Hz, 1H, 2')., 6.82 (d, J = 8.4 Hz, 1H, 5')., 6.41 (d, J = 2.1 Hz, 1H, 8)., 6.21 (d, J = 2.0 Hz, 1H, 6)., 5.38 (d, J = 7.7 Hz, 1H, 1"). The ^{13}C NMR spectrum showed carbon signals at (HSQC, 125MHz, δ ppm): δ 158.5 (C-2)., 135.6 (C-3)., 179.4 (C-4)., 163.1 (C-5)., 100.1 (C-6)., 166.1 (C-7)., 94.8 (C-8)., 145.8(C-3')., 149.9(C-4')., 71.2, 73.2, 67.9, 75.8, 60.1 (Glu.)., It was characterized as hyperoside (Supporting Information Part 2: Fig. S14-S16). ((3, 4-dihydroxyphenyl).-5, 7-dihydroxy-3-[(2S, 3R, 4S, 5R, 6R).-3, 4, 5-trihydroxy-6-(hydroxymethyl).oxan-2-yl]oxychromen-4-one).(Hicks et al., 2012).

Compound VII is yellow for crystallizationa substance, which can be dissolved in methanol and acetone. Its molecular formula was established as $\text{C}_{27}\text{H}_{30}\text{O}_{16}$ by ESI-MS (m/z 609,285 ,301).(W. Zhang, Xu, Yu, Zhang, & Tang, 2010). The compound's ^1H NMR (500MHz, DMSO- d_6 , δ ppm). spectrum exhibited signals at δ 12.60 (s, 1H, 5-OH)., 10.84 (s, 1H, 7-OH)., 9.66 (s, 1H, 4'-OH)., 9.17 (s, 1H, 3'-OH)., 7.64-7.50 (m, 2H, 2', 6')., 6.85 (d, J = 8.0 Hz, 1H, 5')., 6.39 (s, 1H, 8)., 6.20 (d, J = 2.4 Hz, 1H, 6)., 5.35 (d, J = 6.2 Hz, 1H, 1")., 5.27 (s, 1H)., 5.10 (s, 1H)., 5.06 (d, J = 5.9 Hz, 1H)., 4.52 (d, J = 5.1 Hz, 1H)., 4.41-4.30 (m, 3H)., 3.71 (d, J = 11.0 Hz, 1H)., 3.32 -3.17 (m, 5H)., 3.14-3.03 (m, 2H)., 1.00 (d, J = 6.2 Hz, 3H, 6").The ^{13}C NMR spectrum showed carbon signals at (HSQC, 125MHz, δ ppm): 156.4(C-2)., 133.2(C-3)., 177.2(C-4)., 161.1(C-5)., 98.8(C-6)., 164.7(C-7)., 93.6(C-8)., 156.4(C-9)., 103.6(C-10)., 121(C-1')., 115.2(C-2')., 144.8(C-3')., 148.6(C-4')., 116.1(C-5')., 121.5(C-6')., 101.3-74-76.4-69.9-75.9-66.91(Glc)., 100.7-70.5-70.3-71.8-68.2 (Rha). It was characterized as Rutin (Supporting Information Part 2: Fig. S17-S19). (3, 4-dihydro-xyphenyl).-5, 7-dihydroxy-3-[(2S, 3R, 4S, 5S, 6R).-3, 4, 5-trihydroxy-6-[(2R, 3R, 4R, 5R, 6S).-3, 4, 5-trihydroxy-6-methyloxan-2-yl]oxymet-

hyl]oxan-2-yl]oxychromen-4-one(4).(Yang et al. 2012).

Analysis of the new flavonoid compound I

Fig. 4 shows the total ion chromatogram and MS spectrum of the new compound I. The peak time of compound I is 1.011 min. The liquid chromatography mass spectrum exhibited molecular ion m/z calculated for $\text{C}_{28}\text{H}_{34}\text{O}_{15}$ (610.56 ($[\text{M}-\text{H}]^-$: 609.15)., m/z 447.1, 285.0 (base peak).).

Fig. 5 shows the 1D hydrogen spectrum and the 2D heteronuclear single-quantum correlation (HSQC). spectrum. The singlet peaks at 12.03 ppm and 9.11 ppm stand for two protons from phenolic hydroxyl. The multiplet at 6.94-6.11 ppm for five protons form an aromatic ring and yet another multiplet at 5.52-4.46 ppm accounting for thirteen protons from cyclic - CH (glycone ring). were also observed. The singlet peaks at 3.78-3.64 ppm represent the three protons form -OCH₃ group and a multiplet at 3.66-2.77 for alicyclic hydroxyl group, and a doublet at 2.29 ppm for two protons of -CH₂OH group attached to glycone ring and the singlet representing three protons at 1.15-1.05 ppm for -CH₃ were also found. The ^{13}C NMR spectrum showed carbon signals at (HSQC, 125MHz, δ ppm): 145.93, 144.24, 131.81, 117.80, 114.07,

111.90, 103.29, 100.35 and 96.22 ppm represented aromatic carbon atoms. The peaks at 87.32, 82.42, 78.39, 73.00, 71.75, and 70.11 were assigned for alicyclic carbons. The two carbons from CH₂OH group and a methyl group attached to glycone ring were represented at 28.36 and 18.02 ppm respectively. The oxymethine carbon and aliphatic methylene carbons were represented at 74.11 and 32.51 ppm respectively. The methoxy carbon was represented at 49.33 ppm. It was determined as hesperetin-5'-O- β -rhamnoglucoside.

The structure of the new compound was optimized by DFT with b3lyp/6-311g (d, p). After the optimization of the structure, ¹H and ¹³C data were calculated using the DFT/GIAO method through the Gauss 09 program using b3lyp/6-311+g (2d, p). (Fig. 5. Table 2). The chemical shift values given by DFT/B3LYP are very close to the experimental data, further verifying the determination of the molecular structure of **compound I**.

Table 2 Experimental and calculated ¹H and ¹³C NMR data of new compound.

Position	$\delta H(\text{exp.})$.	$\delta H(\text{cal.})$.	$\delta C(\text{exp.})$.	$\delta C(\text{Cal.})$.
2	4.49	4.39	78.39	78.03
3	6.94	7.28	45.37	43.83
4	—	—		196.44
5	12.03 (s, H, -OH).	12.06	164	166.64
6	6.19 (d, J=1.9HZ, 1H).	6.14	76.49	97.03
7	9.11(s, 2H).	9.23	160.09	166.32
8	5.3 (d, J=2.0HZ, 1H).	5.26	96.20	97.04
9	—	—	161.66	165.44
10	—	—	103.29	103.76
1'	—	—	131.81	131.74
2'	6.89 (d, J=2.148, 1H).	6.75	127.80	124.6
3'	3.85 (s, 3H).	3.73	131.22(-CH ₃).	129.86
4'	9.26 (s, -OH)		145.93(B-Cyclic Phenol).	145.24
5'	—	—	144.24	144.09
6'	6.11 (dd, J=8.6, 2.0Hz).		111.90	—
1"	7.47	7.28	100.35	—
2"	3.31	3.12	71.75	72.90
3"	1.65	1.42	70.11	70.54
4"	—	—	87.32	87.44
5"	1.29 (d, 3H).	1.39	73.00 (-CH ₃).	72.92
1'''	4.46 (dd, 1H).	4.39	114.07 (C=O).	114.63
2'''	3.54 (dd, 1H).	3.56	76.98 (C-OH).	75.03
3'''	4.6 (d, 1H).	4.56	76.20 (C-OH).	76.54
4'''	4.84 (d, 1H).	4.93	69.50 (C-OH).	67.78
5'''	3.85-4.03 (m, 3H).	3.73-4.1	82.42 (-OCH ₃).	82.03

Assesement of antioxidant activity

The antioxidant activities of the flavonoid compounds was assessed through the DPPH radical scavenging assay. DPPH is stable and easy to handle, which is often used to evaluate the activity of free radicals in scavenging antioxidants.(J. Liu et al. 2020). Fig. 6 shows the change of absorption in the reaction process of the new component oxidant reactive with DPPH. The absorbance at 517 nm decreased with the increase of the concentration, showing the scavenging efficiency of the new compound. It was found that there were two main absorption peaks in the 240-400nm region, Band I (at 300-380nm). and Band II (at 240-280nm). (Supporting Information Part 3: Fig. S20). The ultraviolet absorption band I is related to the absorption of the B-ring cinnamoyl system, and the band II is related to the absorption of the A-ring benzoyl system (Supporting Information Fig. S20). The reaction took place rapidly with the increase of compound concentration.

For the comparison of antioxidant capacity, IC_{50} was measured which is the concentration of half inhibition rate, that is, the concentration of scavenger when the free radical scavenging rate is 50%. The smaller the IC_{50} value is, the better is the scavenging effect or antioxidation capacity.(Seyoum Asres & El-Fikyet al. 2006; Nguelefack et al. 2011). Fig.7 shows the results that the IC_{50} of the seven flavonoids in the sequence: quercetin (8.07 mg/L). < hesperetin-5'-O- β -rhamnoglucoside (8.72 mg/L). < kaempferol-3-glucuronide (13.49 mg/L). < baicalein (15.5 mg/L). < hesperetin-7-glucuronide (22.1 mg/L). < hesperidin (31.39 mg/L). < rutin (31.54 mg/L).

Relationship between structure and antioxidant capacity

From the molecular structure analysis, the activity of phenolic hydroxyl group in ring A is the weakest, while that in ring B is the highest. The ortho-disubstituted group in ring B is the necessary antioxidant group of flavonoids, especially when it is substituted by phenolic hydroxyl group (Shen et al. 2022). and the antioxidant activity of 3-OH substitution in C ring is particularly important, among which the glycosylation of 3-OH in C ring is unfavorable, and the stronger the glycosylation degree is, the worse is the antioxidant activity (Wen et al. 2017). Therefore, the antioxidant activity is in the order as follows: quercetin > hesperetin-5'-O- β -rhamnoglucoside > hesperetin-7-glucuronide, as the steric hindrance of large glycoside group plays a major role in shielding or hindering 3, 4-OH of B ring, resulting in its antioxidant activity reduction.(Masuda et al. 2010). Gandow and other research also showed that 3-OH of C ring is very important, which can lead to the decrease of antioxidant activity.(Masuda et al. 2010).

The experimental results showed that C-ring 3-OH glycosides reduced the antioxidant activity of flavonoids in scavenging DPPH free radicals. The more the number of substituted glycosides, the weaker the antioxidant activity, so the antioxidant activity of monoglycosides substituted flavonoids is better than that of diglycosides. As a result, the antioxidant activity is in the order: kaempferol-3-glycoside > hesperidin > rutin. Furthermore, It was found that the position of phenolic hydroxy group had more influence on the difference of antioxidant activity than the number of phenolic hydroxyl group. The existence of B ring phenolic hydroxyl group greatly improved the antioxidant activity, and the addition of B ring glycoside substituent had a positive effect on antioxidant activity (Shen et al. 2022; Tian et al. 2021). so the antioxidant activity of glycoside substitution of C cyclic hydroxyl group would be

unfavorable in the antioxidant capacity. Therefore, the antioxidant activity of baicalein is larger than that of kaempferol-3-glycoside.

Moreover, frontline orbital correlation coefficients were calculated as they can be used to characterize molecular antioxidant activity in quantum chemistry calculations.(Gorgannezhad et al. 2016; Horton et al. 2018). According to molecular orbital theory, frontier orbitals include the highest occupied orbital and the lowest empty orbital, which are closely related to the reactivity of molecules. The highest occupied orbital energy (HOMO). characterizes the electron-giving ability of molecules, that is, the larger the HOMO, the stronger the electron-giving ability of molecules. The lowest empty orbital energy (LOMO). characterizes the ability of molecules to accept electrons, that is, the smaller the LOMO, the stronger the ability of molecules to accept electrons.(Cai Chen et al. 2013; Abuelizz et al. 2021). The frontline orbital energy level difference ΔE ($\Delta E = \text{HOMO}-\text{LOMO}$). characterizes the energy required by molecules from ground state to excited state. The smaller the energy level difference is, the easier is the transition of electrons, so the stronger is the reactivity of molecules.(Borges et al. 2017). The frontier orbital energy levels of three difference molecules with strongest oxidation resistance were calculated. According to the data in Table 3, the HOMO energy level (-5.738656 eV). of hesperetin7-glucuronide is higher than that of the other two compounds, and the electrons in this orbital are the most unstable and easy to lose. From the point of view of $\Delta E = (\text{LUMO}-\text{HOMO})$., the molecular energy range is in the order of ΔE (Hesperetin7-glucuronide). > ΔE (Hesperetin-5'-O- β -rhamnoglucoside). > ΔE (Quercetin). The calculated results are in good agreement with the experimental results (Supporting Information Table S3). The results show that the frontier molecular orbital energy level difference can be used as a reliable theoretical parameter to predict the free radical scavenging activity of flavonoids in the same type of molecules.(W. Zhang et al. 2010).

Table 3 The energy of HOMO, LUMO and $\Delta E = (\text{LUMO}-\text{HOMO})$. of the four flavonoid compounds(eV).

Energy level	Hesperetin-5'-O- β -rhamnoglucoside	Quercetin	Hesperetin 7-glucuronide
E(LUMO). [eV]	-2.139008	-2.133296	-2.188512
E(HOMO). [eV]	-5.803936	-5.738656	-6.084368
$\Delta E = (\text{LUMO}-\text{HOMO})$.	3.664928	3.60536	3.895856

In addition, according to the molecular dynamical theory, the breaking of chemical bond needs to absorb energy for compounds in stable state, so that molecules can move from one potential energy surface to another with higher energy potential energy surface.(Liu Zhang et al. 2019). It can be seen from the molecular orbital (HOMO). diagram in Fig. 8 that there are many electron clouds in B ring, which is the main chemical reaction site.(Abuelizz et al. 2021). The most essential physical and chemical parameter is the difference between the heat of formation of free radicals produced by antioxidants and hydrogen extraction reaction OHF (the dissociation energy of hydrogen extraction reaction)., that is, the antioxidant activity of flavonoids is to generate phenoxy radicals after hydrogen extraction from parent molecules. (Shen et al. 2022). Studies have shown that the B ring of flavonoids is the active site of the reaction, and ortho-substitution can enhance the activity of B ring.(Shen et al. 2022; Abuelizz et al. 2021).

Based on the optimized molecular structures, the comparative analysis of enthalpy of hydrogen extraction reaction was also conducted. , as shown in Fig.9 shows the calculations of the hydrogen extraction enthalpy change of the substituent at the 3' carbon position of the B ring, with the result as follows: quercetin (22.5 Kcal/mol). < hesperetin-5'-O- β -rhamnoglucoside (43.1 Kcal/mol). < hesperetin-7-glucuronide (44.2 Kcal/mol). The results show that the ortho-hydroxyl group of B ring has the strongest activity, mainly because it can carry out the second continuous scavenging reaction of free radicals. (Horton et al. 2018; Vedpal et al. 2020). Therefore, the theoretical prediction results are consistent with the experimental results, showing that DFT method can provide theoretical guidance for the screening of natural flavonoids antioxidants.

Conclusion

seven flavonoids have been extracted and isolated from the whole herb of dandelion *Taraxacum mongolicum* Hand.-Mazz. by optimized extraction protocol. Hesperetin-5'-O- β -rhamnoglucoside is a new type of flavonoid which was for the first time discovered in this work, while quercetin, hesperetin-7-glucuronide, baicalein and hesperidin are the flavonoids that are found and analyzed for the first time in dandelion *Taraxacum mongolicum* Hand.-Mazz. The antioxidant activities against DPPH free radicals have been evaluated and compared. In general, flavonoids have strong activity of scavenging free radicals. The mechanism of scavenging DPPH in vitro is that the substituents on the ring of flavonoids, such as hydroxyl and methoxy, can react with free radicals to generate stable semiquinone free radicals. The molecular orbital energy levels of the flavonoids with stronger antioxidant activity and the hydrogen extraction enthalpy of B ring substituents are reconfirmed by DFT calculation. The theoretical results are consistent with the experimental results, confirming that hesperetin-5'-O- β -rhamnoglucoside has stronger antioxidant activity mainly due to the combined effect of different substituents in B ring and the enhanced hydrogen supply ability. Since these flavonoid compounds are extracted from natural and edible herbal plant, which are safe to use as antioxidants in food and medicine, therefore, this work has not only revealed new flavonoids in dandelion, but also may provide new source of potential pharmacological nutrients.

Declarations

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Data Availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Author Contribution

Rong Wang, conceived and designed the study, acquired data, analyzed and interpreted data, and revised the manuscript; Wei hua Li, helped design the manuscript; Xin xin Zheng and Cao Liu, acquired data and interpreted the results; Qing Huang, drafted the manuscript and interpreted the results; Cao Fang, acquired data and did the statistical analyses. All authors reviewed the manuscript.

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent Participate Informed consent was obtained from all individual participants included in the study.

Conflict of Interest Rong Wang declares that there is no conflict of interest. Wei hua Li declares that there is no conflict of interest. Cao Fang declares that there is no conflict of interest. Xin xin Zheng declares that there is no conflict of interest. Chao Liu declares that there is no conflict of interest.

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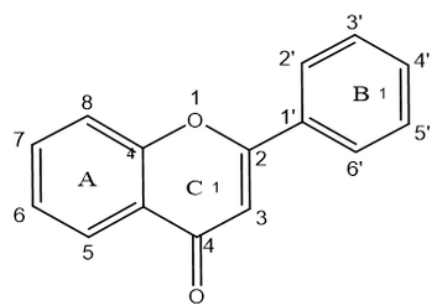
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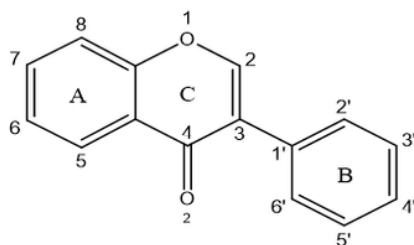
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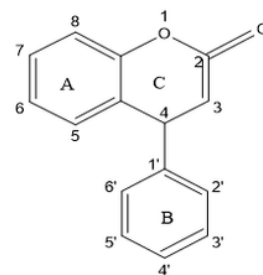
Figures



Flavonoids



Isoflavonoids



Neoflavonoids

Figure 1

Main parent structures of flavonoids

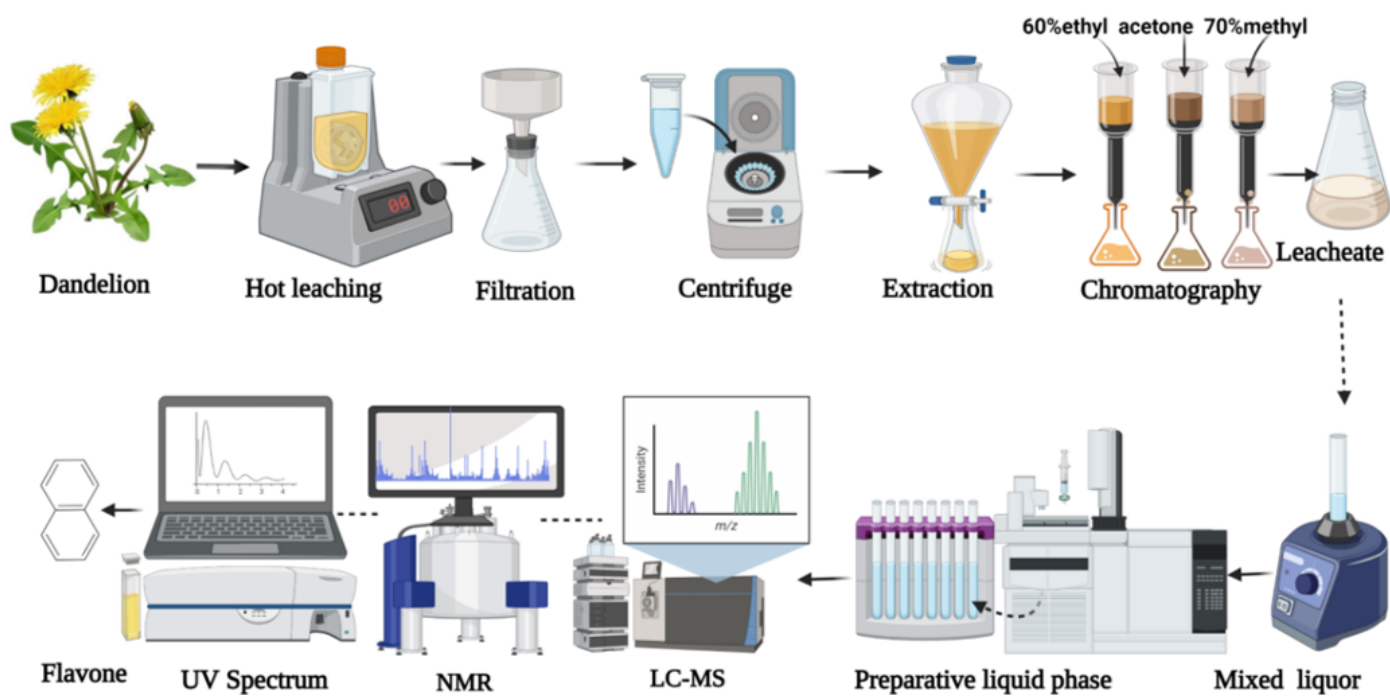
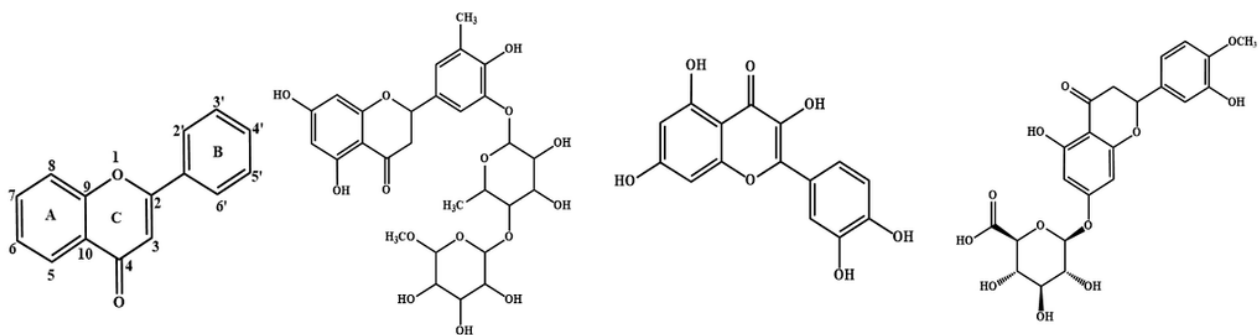


Figure 2

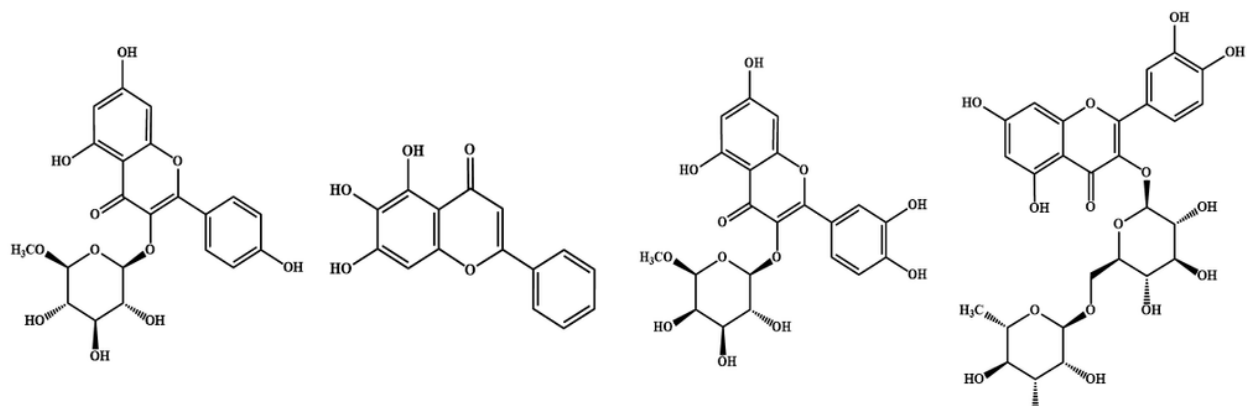
Schematic diagram of extraction, isolation and structural identification of dandelion flavonoids.



Flavonoids maternal Hesneretin-5'-O- β -rhamnoglucoside I

Quercetin II

Hesneretin 7-O-glucuronide III



Kaempferol-3-glucoside IV

Baicalein V

Hyperoside VI

Rutin VII

Figure 3

Structures of the extracted flavonoid compounds

Figure 4

LC-MS Peak Pattern and Corresponding LC-MS² M/Z Pattern.

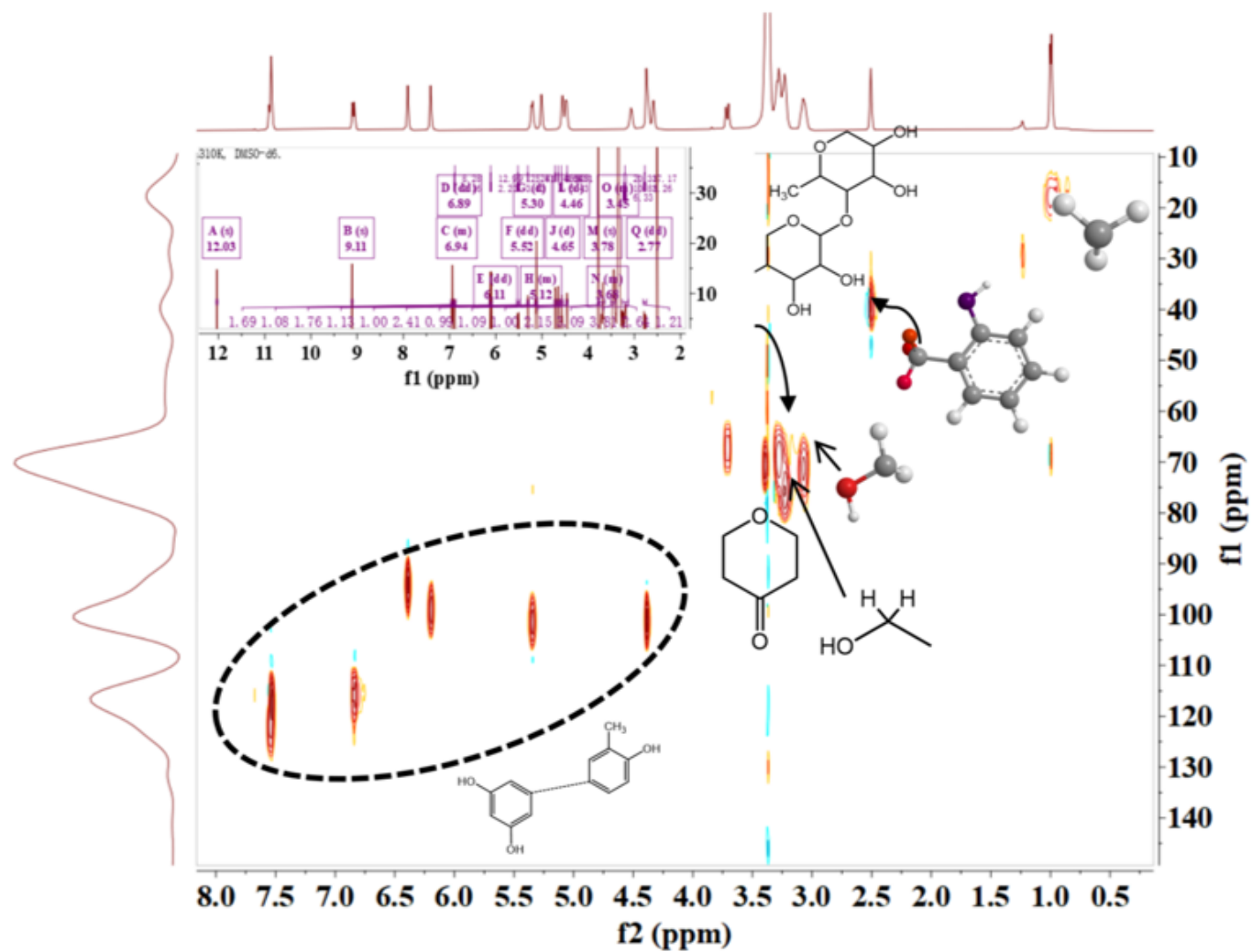


Figure 5

^1H spectra and ^{13}C HSQC NMR spectra of the new compound I.

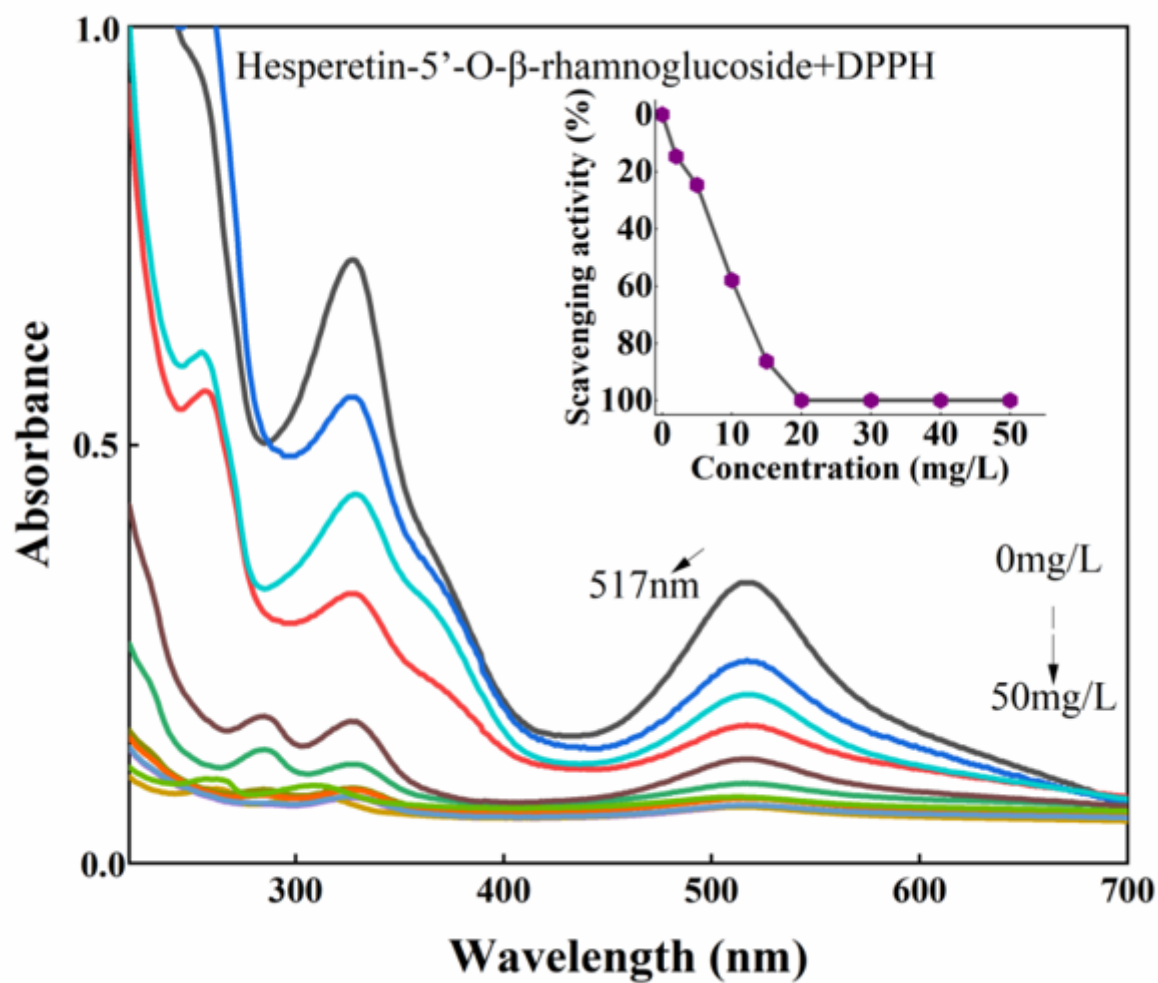


Figure 6

UV-Vis spectra of hesperetin-5'-O-β-rhamnoglucoside-DPPH mixture with change of the antioxidant concentration.

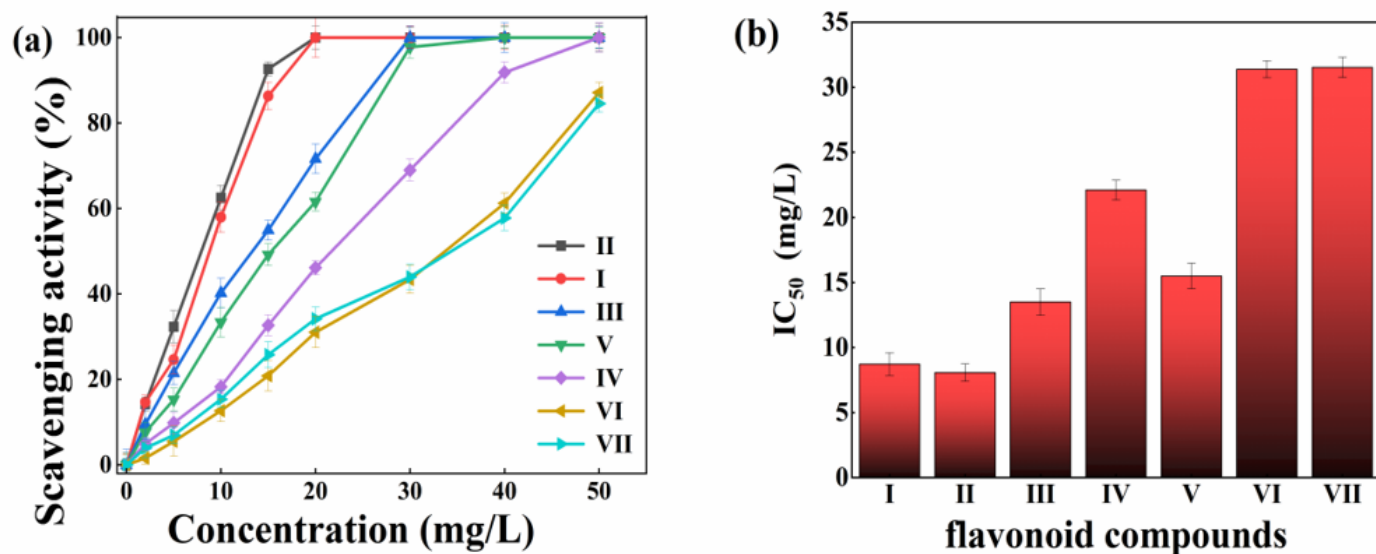


Figure 7

Percentage of antioxidant's radical scavenging rate of seven flavonoid compounds (a). and their corresponding IC₅₀ values (b).

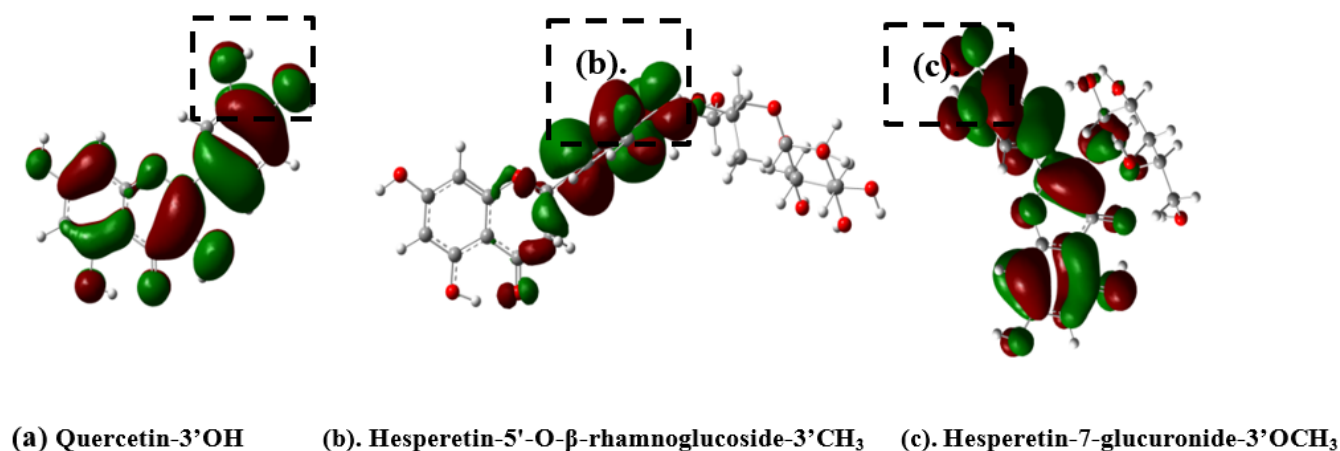


Figure 8

HOMO distribution map of frontier orbital of three flavonoids (red and green colors in the map respectively represent the corresponding phases when two positive and negative charges in molecular orbital are different).

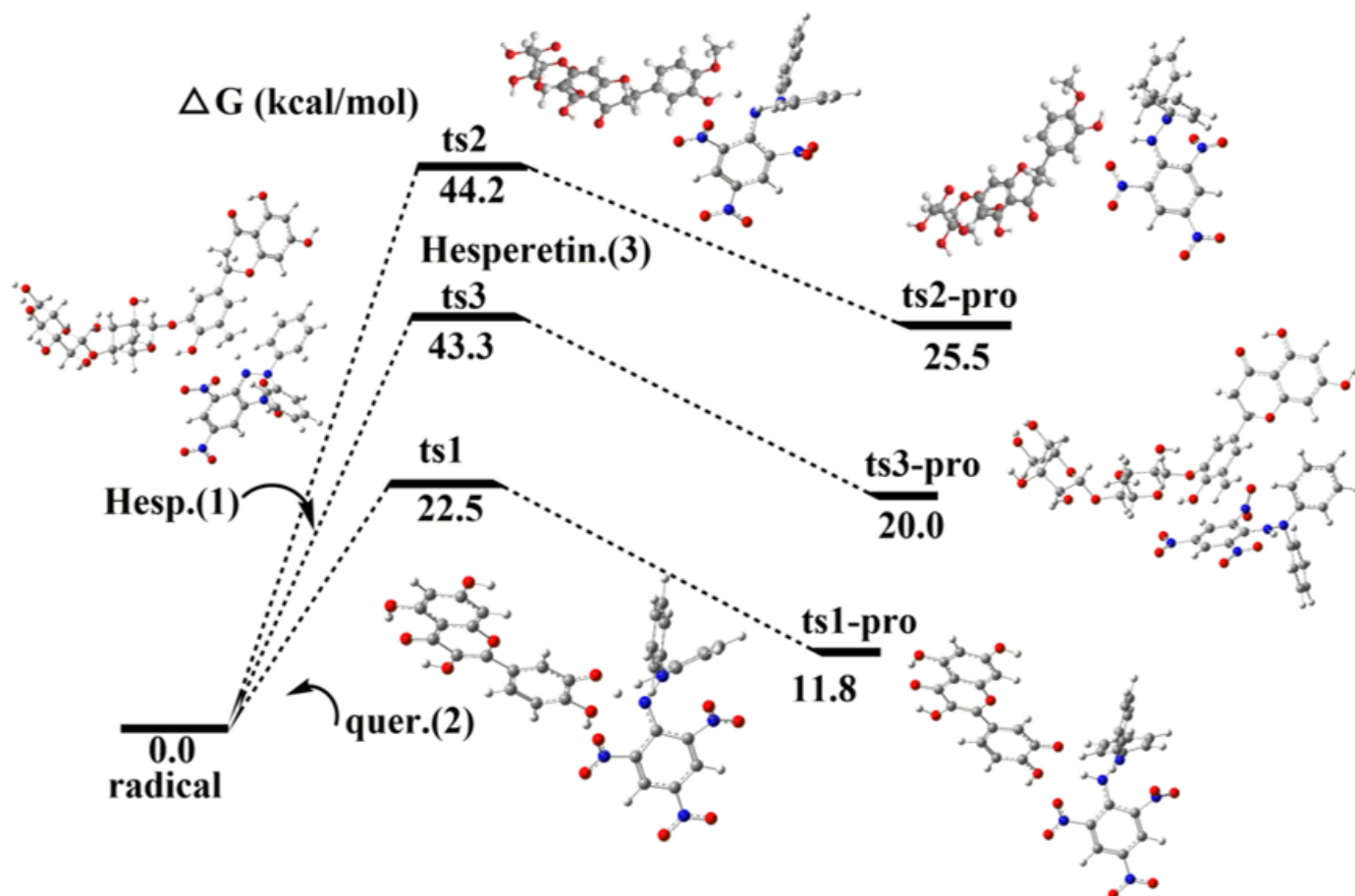


Figure 9

Hydrogen extraction enthalpy of 3'ortho-substituent of B ring

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