Study on the Extraction of Flavonoids from *Hylocereus undatus* (Haw.) Britton & Rose and Their Antioxidant Activity

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Abstract [**Objectives**] This study was conducted to investigate the extraction process, content determination, and antioxidant properties of flavonoids from *Hylocereus undatus* (Haw.) Britton & Rose. [**Methods**] Using *H. undatus* as the raw material, the effects of ethanol concentration, ultrasonic temperature, time, and solid-to-liquid ratio on the total flavonoid yield were investigated through single-factor and orthogonal experiments. [**Results**] All factors had a significant effect on the yield. The optimized conditions were determined as follows: ethanol concentration 75%, ultrasonic temperature 60 ℃, ultrasonic time 30 min, and solid-to-liquid ratio 1∶50 (g/ml). Under these conditions, the total flavonoid yield reached 3.08%. Evaluation of antioxidant activity revealed that the extract exhibited superior scavenging rates against both DPPH and hydroxyl radicals compared with the standard reference compound BHT. [**Conclusions**] This study holds significant importance for elucidating the pharmacological mechanisms of flavonoids in *H. undatus* and for expanding their application in medicine and health products.

Key words *Hylocereus undatus*; Flavonoids; Antioxidant activity

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Hylocereus undatus (Haw.) Britton & Rose is a characteristic medicinal and edible plant from Zhaoqing, Guangdong. It grows on the cliffs of the Seven Star Cave, possesses a sweet taste and cool nature, and enters the lung meridian. It exhibits pharmacological effects such as clearing away heat and toxic materials, and moistening the lungs to arrest cough. Due to its richness in antioxidant components, *H. undatus* has potential application value in delaying skin aging and assisting in the treatment of respiratory diseases[1-4]. Flavonoids, as crucial bioactive substances in plants, feature a basic structure comprising two benzene rings linked by a three-carbon oxygenated bridge. This class encompasses subcategories such as flavonols, isoflavones, and anthocyanins. Relying on functional groups such as phenolic hydroxyls, they exhibit significant bioactivities including antioxidant, anti-inflammatory, and neuroprotective effects[5-9]. Modern research has confirmed the broad prospects of such compounds in areas including cancer prevention. Flavonoids derived from citrus sources, such as hesperidin and naringin, have already been validated by relevant studies[10-14]. Given the characteristics of both, in-depth research on the extraction process and antioxidant properties of flavonoids from *H. undatus* holds significant importance for elucidating its pharmacological mechanisms and expanding its applications in medicine and health products. Currently, research on flavonoids both at home and abroad predominantly focuses on food development and trace element analysis. While their physiological activities have been clarified, systematic studies on the optimization of extraction processes, content determination, and antioxidant activity of these components from specific plants remain relatively scarce. In this study, *H. undatus* was selected as the raw material to investigate the extraction and antioxidant activity of its flavonoids. Single-factor experiments were conducted to investigate the effects of key parameters on extraction efficiency, followed by an orthogonal experiment to optimize the optimal process. Quantitative analysis was achieved by establishing a standard curve using an ultraviolet-visible spectrophotometer. The antioxidant activity was systematically evaluated through DPPH and hydroxyl radical scavenging assays. This study provides technical support for the efficient extraction and industrial application of flavonoids from *H. undatus*, thereby enriching the research framework of flavonoid compounds.

Materials and Methods

Experimental materials

Materials

Table 1 Experimental materials

Name	Specification	Manufacturer
H. undatus	Dried flowers	Commercially available, Zhaoqing City
Ferrous sulfate heptahydrate	Analytically pure	Xilong Scientific Co., Ltd.
Salicylic acid	Analytically pure	Xilong Scientific Co., Ltd.
Butylated hydroxytoluene (BHT)	Analytically pure	Tianjin Huasheng Chemical Reagent Co., Ltd.
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	Analytically pure	Shanghai Macklin Biochemical Technology Co., Ltd.
Sodium nitrite	5%	Shenzhen Bida Environmental Technology Co., Ltd.
Aluminum nitrate	10%	Shenzhen Bida Environmental Technology Co., Ltd.
Rutin	≥98%	Dingzhou Baikesaisi Biotechnology Co., Ltd.

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Zhao LIU (1984 –), male, P. R. China, senior experimentalist, master, devoted to research about food engineering.

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Experimental instruments

Table 2 Experimental instruments

Instrument name	Manufacturer
YP502N Electronic balance	Shanghai Precision Instruments Co., Ltd.
UV-3100 Ultraviolet-visible Spectrophotometer	Hangzhou Junsheng Scientific Equipment Co., Ltd.
Chunlin ultrasonic cleaner	Shenzhen Chunlin Cleaning Equipment Co., Ltd.

Experimental methods

Sample pretreatment A precise 1.0 g sample of air-dried *H. undatus* was weighed and placed into a 100 ml conical flask. A measured volume of ethanol was added to the flask. The ultrasonic bath was filled with water to two-thirds of its capacity and heated to a specified temperature. Subsequently, the flask containing the sample was immersed in the bath and subjected to extraction under defined conditions. The resulting extract was filtered through gauze into a 100 ml volumetric flask and then diluted to constant volume with ethanol for subsequent use.

Construction of standard curve The method of Zhang *et al.* [5] was employed with minor modifications. Briefly, 20.00 mg of rutin standard was weighed into a clean beaker. An appropriate volume of 60% ethanol solution was added and stirred until complete dissolution. The solution was then transferred and diluted to constant volume in a 100 ml volumetric flask. After thorough mixing, a rutin standard solution with a concentration of 0.20 mg/ml was obtained. Six 25 ml volumetric flasks were labeled, and different volumes (0.00, 2.00, 4.00, 6.00, 8.00, and 10.00 ml) of the standard solution were pipetted into the flasks, respectively. Subsequently, 1 ml of a 5% sodium nitrite solution was added to each flask. After shaking, the mixtures were allowed to stand for 5 min. Then, 1 ml of a 10% aluminum nitrate reagent was added, followed by shaking and standing for another 5 min. Finally, 4 ml of a 4% sodium hydroxide solution was added to each flask, followed by dilution to the mark, thorough shaking, and standing for 10 min. Using the first sample as the blank control, the absorbance of each solution was measured at a wavelength of 510 nm. A standard curve was plotted with the concentration of the rutin standard (C) as the x-axis and the corresponding absorbance (A) as the y-axis, from which a linear regression equation was obtained.

Content determination A 2.00 ml aliquot of the sample extract was precisely pipetted and determined for absorbance according to section "Construction of standard curve". The concentration in the sample was calculated by the regression equation. The extraction yield of total flavonoids was then calculated using Equation 1.

$$\text{Extraction yield of total flavonoids (\%)} = kcv/m \quad \text{(Equation 1)}$$

In Equation 1, k is the ratio of the volume of the tested solution to the total volume of the testing solution prepared after sample extraction; c is the concentration of total flavonoids, in mg/ml; v is the volume of the tested solution, in ml; and m is the mass of the weighed sample, in g.

Single-factor experiments

Table 3 Single-factor experimental design

Influencing factor	Fixed conditions	Variable settings	Analysis content
Ethanol concentration	Temperature: 50 ℃, time: 30 min, solid-to-liquid ratio: 1∶40	15%, 30%, 45%, 60%, 75%	Extraction efficiency under various concentrations
Ultrasonic temperature	Ethanol concentration: 60%, time: 30 min, solid-to-liquid ratio: 1∶40	40 ℃, 50 ℃, 60 ℃, 70 ℃, 80 ℃	Extraction efficiency at corresponding temperature points
Ultrasonic time	Ethanol concentration: 60%, temperature: 50 ℃, solid-to-liquid ratio: 1∶40	15 min, 30 min, 45 min, 60 min, 75 min	Extraction efficiency under different time durations
Solid-to-liquid ratio	Ethanol concentration: 60%, temperature: 50 ℃, time: 30 min	1∶20, 1∶30, 1∶40, 1∶50, 1∶60	Extraction efficiency under different solid-to-liquid ratios

Orthogonal optimization experiment Based on the results of single-factor experiments, four key variables, ethanol concentration, ultrasonic temperature, ultrasonic time, and solid-to-liquid ratio, were selected for investigation. Three gradient levels were set for each independent variable. Using the extraction yield of total flavonoids from *H. undatus* as the evaluation index, a four-factor three-level orthogonal experiment $L_9(3^4)$ was designed to systematically optimize the extraction process conditions. The experimental design is presented in Table 4.

Determination of antioxidant activity

Determination of DPPH radical scavenging rate The DPPH radical scavenging rate was determined accroding to the method of Tian *et al.* [15] with minor modifications. The 10 mg/ml sample solution prepared in section "Content determination" was diluted with 60% ethanol solution to obtain a series of concentrations (2.00, 4.00, 6.00, 8.00, 10.00 mg/ml). Exactly, 0.004 g of DPPH powder was weighed and dissolved, and then diluted to constant volume to prepare a 0.004% DPPH stock solution. A 2.0 ml aliquot of each diluted extract solution was mixed with 2.0 ml of the DPPH stock solution. The mixture was shaken thoroughly and incubated in the dark for 30 min. Using distilled water as the blank control and butylated hydroxytoluene (BHT) at corresponding concentrations as the positive control, the absorbance of each mixture was measured at a wavelength of 517 nm. The DPPH radical scavenging rate was calculated using Equation 2.

$$\text{DPPH radical scavenging rate (\%)} = [(A_0 - A_1)/A_0] \times 100\% \quad \text{(Equation 2)}$$

In Equation 2, A_1 is the absorbance of the sample or positive control; and A_0 is the absorbance of the blank control.

Table 4 Factors and levels of the orthogonal experiment

Level	Ethanol concentration A//%	Ultrasonic temperature B//℃	Ultrasonic time C//min	Solid-to-liquid ratio D//g∶ml
1	45	50	30	1∶40
2	60	60	45	1∶50
3	75	70	60	1∶60

Determination of hydroxyl radical scavenging rate Exactly 1.67 g of ferrous sulfate was accurately weighed and added into an appropriate container. Distilled water was added slowly with stirring to prepare a 6 mmol/L $FeSO_4$ solution. Exactly 0.16 g of salicylic acid was weighed into a beaker. An appropriate amount of absolute ethanol was added and stirred until the salicylic acid was completely dissolved. The solution was then transferred and diluted to constant volume in a 200 ml volumetric flask. After thorough mixing, a 6 mmol/L salicylic acid-ethanol solution was obtained. Additionally, a 0.1% hydrogen peroxide (H_2O_2) solution was prepared. Next, 1.0 ml aliquots of the *H. undatus* extract solutions at different concentrations were pipetted into separate clean and dry test tubes. An equal volume (1.0 ml each) of the prepared $FeSO_4$ solution, salicylic acid-ethanol solution, and H_2O_2 solution was then added sequentially to each tube. The mixtures were shaken thoroughly and allowed to stand for 30 min. Finally, the absorbance of each reaction mixture was measured at a wavelength of 510 nm. Using distilled water as the blank control and BHT at corresponding concentrations as the reference control, the hydroxyl radical scavenging rate was calculated according to Equation 2.

Results and Analysis

Construction of standard curve

As shown in Fig. 1, a significant correlation was observed between absorbance at 510 nm and rutin concentration. The correlation coefficient (R^2) was 0.997, and the linear regression equation was $y = 0.054\ 8x + 0.009\ 5$.

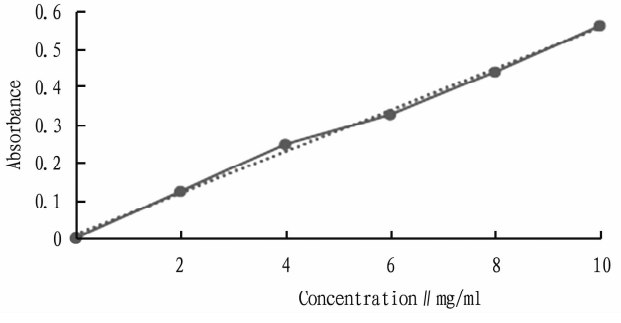

Fig. 1 Rutin standard curve

Analysis of single-factor experiment results

Effect of ethanol concentration on the extraction yield of total flavonoids from *H. undatus* As illustrated in Fig. 2, during the extraction process, the yield of total flavonoids initially increased and then decreased with the gradual rise in ethanol concentration. The yield peaked at 2.21% when the ethanol concentration was 60%. This phenomenon occurred because flavonoids possess both alcohol-soluble and water-soluble properties. The addition of an appropriate concentration of ethanol facilitated the attainment of an optimal balance between these solubilities, which promoted the dissolution of total flavonoids and consequently enhanced the extraction yield. When the polarity of the extraction solvent was significantly altered due to excessively high or low ethanol concentrations, its dissolving power for flavonoids was affected, leading to a reduction in the dissolution amount and a consequent decrease in the final yield of total flavonoids.

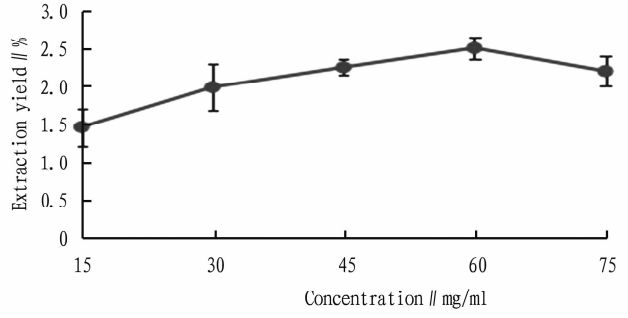

Fig. 2 Effect of ethanol concentration on the extraction yield of total flavonoids from *H. undatus*

Effect of ultrasonic temperature on the extraction yield of total flavonoids from *H. undatus* As shown in Fig. 3, temperature was a key factor influencing the yield during the extraction process. The yield increased as the temperature gradually rose. When the temperature reached 60 ℃, the yield peaked at 2.68%. However, due to the instability of flavonoid chemical structures under high-temperature conditions, further increases in temperature led to alterations in their molecular structure, resulting in a subsequent decline in the total flavonoid yield.

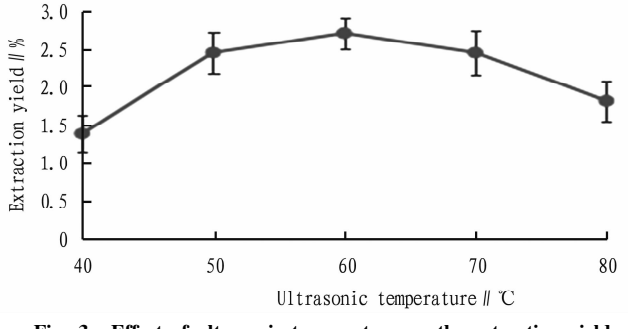

Fig. 3 Effect of ultrasonic temperature on the extraction yield of total flavonoids from *H. undatus*

Effect of ultrasonic time on the extraction yield of total flavonoids from *H. undatus* As depicted in Fig. 4, the extraction yield of total flavonoids exhibited a pattern of initial increase followed by a decrease over the course of the ultrasonic treatment time. The yield reached a peak of 2.76% at the time point of 45 min. However, beyond 45 min, hydrolysis reactions of flavonoids may have occurred, leading to the release of more impurities. These impurities could then be adsorbed by insoluble materials, resulting in

a gradual decline in the total flavonoid yield thereafter.

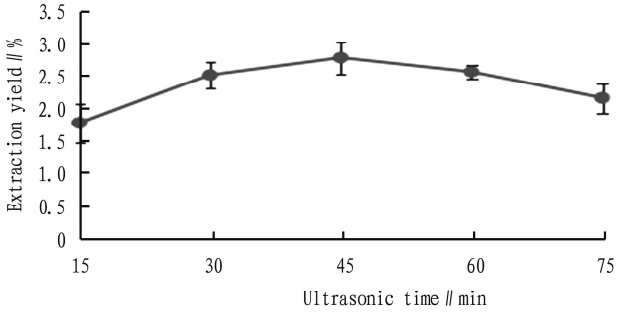

Fig. 4 Effect of ultrasonic time on the extraction yield of total flavonoids from *H. undatus*

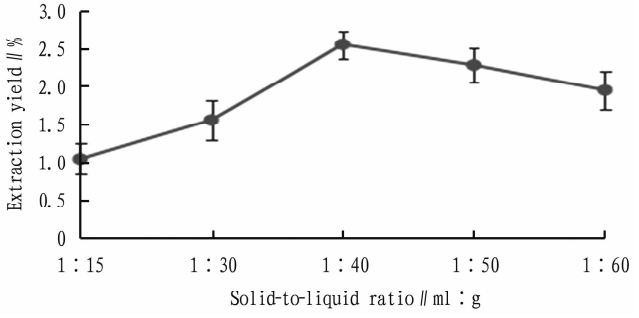

Fig. 5 Effect of solid-to-liquid ratio on the extraction yield of total flavonoids from *H. undatus*

Effect of solid-to-liquid ratio on the extraction yield of total flavonoids from *H. undatus* As shown in Fig. 5, the yield initially increased and then decreased as the solid-to-liquid ratio was progressively increased. The yield reached its maximum of 2.50% at a solid-to-liquid ratio of 1 ∶ 40 (ml/g). When an appropriate amount of solvent was added, it provided more adequate space for the dissolution of total flavonoids, facilitating their release from the raw material and thereby enhancing the extraction yield. However, when an excessive amount of solvent was added, the concentration of the active components in the solution was overly diluted. This excessive dilution could be detrimental as it hindered sufficient contact between the total flavonoids and the solvent and thus their dissolution, ultimately leading to a decrease in the concentration of flavonoids per unit volume and a consequent decline in the extraction yield.

Analysis of orthogonal experiment results

As clearly indicated by the data in Table 5, the effects of ethanol concentration, temperature, time, and solid-to-liquid ratio on the total flavonoid yield varied significantly. The order of their effects, from greatest to least, followed the sequence: ethanol concentration (factor A) > ultrasonic time (factor C) > ultrasonic temperature (factor B) > solid-to-liquid ratio (factor D). Analysis of the data revealed that the optimal parameter combination was $A_3B_2C_1D_2$. It corresponded to an ethanol concentration of 75%, a temperature of 60 ℃, an ultrasonic time of 30 min, and a solid-to-liquid ratio of 1 ∶ 50 (ml/g). Under these optimized conditions, the extraction yield reached 3.08%.

Table 5 Results of orthogonal experiment

No.	Ethanol concentration A // %	Ultrasonic temperature B // ℃	Ultrasonic time C // min	Solid-to-liquid ratio D // g ∶ ml	Extraction yield // %
1	45	50	30	1 ∶ 40	2.17
2	45	60	45	1 ∶ 50	2.28
3	45	70	60	1 ∶ 60	1.92
4	60	50	45	1 ∶ 50	2.43
5	60	60	60	1 ∶ 40	2.68
6	60	70	30	1 ∶ 60	2.90
7	75	50	60	1 ∶ 60	2.68
8	75	60	30	1 ∶ 50	3.08
9	75	70	45	1 ∶ 40	2.54
K_1	2.12	2.43	2.72	2.46	
K_2	2.67	2.68	2.42	2.60	
K_3	2.77	2.45	2.43	2.50	
R	0.65	0.25	0.30	0.14	

Table 6 Results of variance analysis

Factor	Sum of squares (SS)	Degrees of freedom (df)	Mean square (MS)	F-value	F-critical value	Significance
Ethanol concentration	0.722	2	0.361	85.960	6.944	Significant
Ultrasonic temperature	0.116	2	0.581	13.841	6.944	Significant
Ultrasonic time	0.174	2	0.087	20.738	6.944	Significant
Solid-to-liquid ratio	0.028	2	0.014	3.389	6.944	Non-significant
Error	0.017	4	0.004			

Analysis of antioxidant activity results

DPPH radical scavenging rate As illustrated in Fig. 6, both the total flavonoids from *H. undatus* and BHT possessed DPPH radical scavenging capabilities. Within the concentration range of 2 to 8 mg/ml, the DPPH radical scavenging activity of both the *H. undatus* total flavonoids and BHT increased gradually. However, within the range of 8 to 10 mg/ml, the scavenging rates for both substances increased by approximately 20%. Overall, the DPPH radical scavenging capacity of the *H. undatus* total flavonoids was significantly superior to that of BHT.

Hydroxyl radical scavenging rate As shown in Fig. 7, both the total flavonoids from *H. undatus* and BHT exhibited hydroxyl radical scavenging activity. The hydroxyl radical scavenging rate of the *H. undatus* total flavonoids did not vary significantly with increasing concentration. In contrast, the scavenging activity of BHT increased noticeably within the range from 2 to 6 mg/ml, followed by a more gradual increase from 6 to 10 mg/ml. Overall, the hydroxyl radical scavenging capacity of the *H. undatus* total flavonoids remained superior to that of BHT.

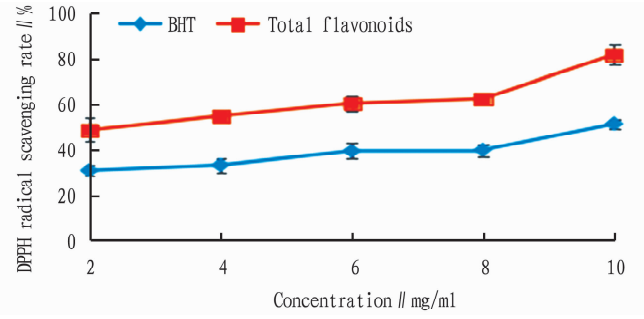

Fig. 6 DPPH radical scavenging activity of total flavonoids from *H. undatus*

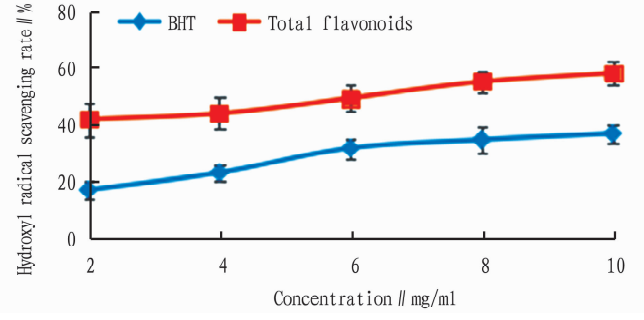

Fig. 7 Hydroxyl radical scavenging activity of total flavonoids from *H. undatus*

Conclusions and Discussion

In this study, *H. undatus* was selected as the raw material to optimize the ultrasonic-assisted extraction process for total flavonoids and investigate their antioxidant activity. The findings provide scientific support for the high-value development and diversified application of flavonoids from this plant. Through single-factor and orthogonal experiments, with the total flavonoid yield as the evaluation criterion, the optimal extraction process was determined as follows: ethanol concentration 75%, extraction temperature 60 ℃, extraction time 30 min, and solid-to-liquid ratio 1∶50 (ml/g). Under these conditions, the total flavonoid yield reached 3.08%. The optimized process significantly reduced the extraction duration while enhancing the flavonoid yield, thereby validating the superiority of ultrasonic-assisted technology in extracting flavonoids from plant materials and providing a feasible reference for potential large-scale production. Antioxidant activity assays revealed that the flavonoid extract obtained under the optimal conditions exhibited superior DPPH and hydroxyl radical scavenging capacities compared with the commonly used antioxidant BHT. This confirms its excellent potential as a natural antioxidant and lays a foundation for subsequent new product development.

References

[1] LIAO WZ, LIU W, ZHOU XQ, *et al.* Study on the antitussive, expectorant, anti-inflammatory, and analgesic effects of the extract of *Hylocereus undatus* flower and its preliminary mechanism[J]. Journal of Li-Shizhen Traditional Chinese Medicine, 2022, 33(4): 821 – 823. (in Chinese).
[2] ZHANG DC. *Hylocereus undatus* (Bawanghua)[J]. China Vegetables, 2017(1): 68. (in Chinese).
[3] DUAN ZF, FU L. The effects on scavenging of free radicals of chemical components in the water extract from *Hylocereus undatus* (Haw.) Britt. et. Rose[J]. Food Science and Technology, 2011, 36(9): 262 – 266. (in Chinese).
[4] YI Y, WU X, WANG Y, *et al.* Studies on the flavonoids from the flowers of *Hylocereus undatus*[J]. Journal of Chinese Medicinal Materials, 2011, 34(5): 712 – 716. (in Chinese).
[5] ZHANG XY. Study on the extraction and biological activity of flavonoids from wild tulips[D]. Yili: Yili Normal University, 2023. (in Chinese).
[6] SHEN N, WANG TF, GAN Q, *et al.* Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity[J]. Food Chemistry, 2022, 383: 132531 – 132531.
[7] HE SY. Extraction, purification of flavonoids from Ampelopsis grossedentata leaves and their application in cosmetics[D]. Changsha: Central South University of Forestry and Technology, 2024. (in Chinese).
[8] DING CL. Study on extraction and purification process of flavonoids from *Tussilago farfara* L.[D]. Zhengzhou: Zhengzhou University, 2009. (in Chinese).
[9] JIANG CY. Study on the extraction and purification process of flavonoids from *Lycopus lucidus* Turcz. roots and their anti-rheumatoid arthritis activity[D]. Wanzhou: Chongqing Three Gorges University, 2024. (in Chinese).
[10] MIN KY, MAHENDRAN S, SENG YW, *et al.* Hesperidin and its aglycone hesperetin in breast cancer therapy: A review of recent developments and future prospects[J]. Saudi Journal of Biological Sciences, 2021, 28(12): 6730 – 6747.
[11] SACHDEVA KA, CHOPRA K. Naringin mitigate okadaic acid-induced cognitive impairment in an experimental paradigm of Alzheimer's disease [J]. Journal of Functional Foods, 2015, 19: 110 – 125.
[12] XU YY, ZHANG YH, QIAN YY, *et al.* Research progress on pharmacological effects of anthocyanins[J]. Drugs & Clinic, 2022, 37(8): 1886 – 1891. (in Chinese).
[13] YANG OY, LI JJ, CHEN XY, *et al.* Chalcone derivatives: Role in anticancer therapy[J]. Biomolecules, 2021, 11(6): 894 – 894.
[14] ZHUANG CL, ZHANG W, SHENG CQ, *et al.* Chalcone: A privileged structure in medicinal chemistry[J]. Chemical reviews, 2017, 117 (12): 7762 – 7810.
[15] TIAN JH, ZHANG CY, WEI L. Study on the extraction technology and antioxidant activity of total flavonoids from the pomace of sea buckthorn [J]. Natural Product Research and Development, 2021, 33(1): 65 – 72. (in Chinese).

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[17] TONG SQ, HUANG J, WANG BL, *et al.* Studies on the chemical constituents of *Sarcandra glabra*[J]. Chin. Tradit. Herb. Drugs, 2010, 41(2): 198 – 201. (in Chinese).
[18] MA WJ, XIAO DJ, DENG SZ. Studies on chemical constituents of the marine sponge *Spheciospongia vagabunda* from the South China Sea (I) [J]. Chin. J. Mar. Drugs, 2004, (2): 15 – 17. (in Chinese).
[19] REN Y, ZHANG DW, DAI SJ. Chemical constituents from *Solanum lyratum*[J]. Chin. J. Nat. Med., 2009, 7(3): 203 – 205.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU