Contents List available at VOLKSON PRESS

New Materials and Intelligent Manufacturing (NMIM)

DOI : http://doi.org/10.26480/icnmim.01.2018.413.416

Journal Homepage: https://topicsonchemeng.org.my/

ISBN: 978-1-948012-12-6

STUDY ON EXTRACTION OF FLAVONOIDS FROM FRUIT OF ROSA DAVURICA PALL BY DIFFERENT METHODS AND ITS ANTIOXIDANT ACTIVITY

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ARTICLE DETAILS

Article History:

Received 26 June 2018
Accepted 2 July 2018
Available online 1 August 2018

ABSTRACT

Taking the extraction yield of total flavonoids from fruit of Rosa davurica Pall. (FRDP) as index, the optimum extraction technology and in vitro antioxidant properties of flavonoid obtained from various modern extraction techniques were explored. The scavenging ability for DPPH· and ABTS+· of FRDP extracts were investigated. The extraction rate of flavonoids using microwave synergistic enzyme method was relatively higher than that of other extraction technique. The scavenging ability for DPPH· and ABTS were employed to evaluate the antioxidant activity of flavonoids, rutin and Vc. Results showed flavonoids had a strong scavenging ability for DPPH· and ABTS+·, and its scavenging activity gradually increased with the increasing mass concentration of flavonoids from FRDP.

KEYWORDS

Fruit of *Rosa davurica Pall.*, flavonoids, extraction technology, antioxidant activity.

1. INTRODUCTION

Based on a study, rose fruit is the dried ripe fruit of *Rosa davurica Pall.*, which has been widely used in beverages, health products and other fields currently [1]. Researchers have shown that the fruit contained plenty of chemical components, such as flavonoids, polysaccharides, saponins, triterpenoic acid, vitamin and so on [2-4]. According to research, pharmacology study indicated that the fruit possessed the functions of promoting digestion, antioxidant, anti-aging, improving the immune regulation and antiradiation [5-7].

Based on a research, compared with traditional extraction methods, microwave extraction possesses the advantages of high extraction efficiency, good reproducibility, time saving, reagent saving, and small pollution [8]. Enzyme-assisted is a new method which can improve the extraction rate. The present study compared the effect of different extraction methods on yield of flavonoids, which provide a basis for subsequent research and development. Moreover, the antioxidant properties of flavonoids were also studied.

2. MATERIALS AND METHODS

2.1 Plant Materials and Chemicals

The plant material was collected from Jilin Province Junlin Chinese Herbal Medicine Co., Ltd. Rutin was obtained from Chengdu Manste Biotechnology Co., Ltd. DPPH was purchased from Shanghai Ruji Biotechnology Development Co., Ltd. Vitamin C was acquired from Tianjin Yongda Chemical Reagent Co., Ltd.

2.2 Calibration curve and determination of total flavonoid content (TFC) of sample solution

As the reference substance, 10.54 mg of rutin was weight and put into a 50 mL volumetric flask, which was made up to the mark by 60% ethanol. Then, the flask was shaken to obtain rutin solution with 0.2108 mg/mL. Sodium nitrite-aluminum nitrate coloration method was applied to the preparation of standard curve. The regression equation was A=9.3004C-0.0074, R^2=0.9992. Results showed the standards showed a good linear relationship in the range of 0.008 to 0.100 mg/mL. 1 mL of the flavonoids extract of FRDP was placed in a 25 mL volumetric flask. The flavonoids extract of FRDP without the chromogenic agent solution was used as a blank. The absorbance was measured at 505 nm according to the above method, and the TFC in the sample was calculated from the standard curve.

2.3 Selection of extraction methods

Using 4 g FRDP powder as raw material, four methods including microwave-assisted extraction, enzymatic extraction, microwave synergistic enzymatic extraction and microwave synergistic surfactant extraction methods were adopted to select the appropriate method.

2.4 Microwave Synergistic Enzymatic Extraction of Single Factor Experimental Design

Taking extraction efficiency of flavonoids as the index, the ratio of material to liquid (1:5, 1:10, 1:20, 1:30, 1:40, 1:50), extraction temperature (30, 40, 50, 60, 70, 80 °C), extraction time (0.5, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0 h), cellulase dosage (0.1, 0.3, 0.5, 0.7, 0.9 %), ethanol concentration (40, 50, 60, 70, 80, 90 %) and pH (2, 3, 4, 5, 6, 7, 8) were selected to investigate the influence of various factors on extraction efficiency of flavonoids.

2.5 Microwave Cooperative Enzymatic Extraction of Orthogonal Experimental Design

Based on the single factor experimental results, four factors including ratio of material to liquid, extraction time, enzyme dosage and ethanol concentration were selected to conduct further study. Orthogonal test of four factors with three levels were designed to determine the optimum extraction conditions of flavonoids.

2.6 In vitro Antioxidant Properties

2.6.1 Removal of DPPH free radicals

The samples of different concentration total flavonoids were prepared first, and the addition of other reagents and coloration operation were done regularly. The absorbance was measured at 517 nm (A sample) using 50 % ethanol as a reference. The sample was replaced with 2.0 mL distilled

water which absorbance was measured at 517 nm (A blank). Based on a study, two different concentrations of the samples (2.0 mL) were taken into 10 mL colorimetric tube, respectively. Then 2.0 mL of anhydrous ethanol was added, and mixed which absorbance were measured also 517 nm absorbance (A control) [9]. The DPPH· clearance rate (η DPPH·) was calculated according to formula (1). The same concentration of Vc and rutin were used as controls.

ηDPPH·=（A blank－A sample＋A control）/A blank × 100 （1）

2.6.2 Removal of ABTS radical cations

The samples which containing different concentration total flavonoids was prepared firstly, and an equal volume of potassium persulfate solution with 2.45 mmol/L and ABTS+·solution with 7 mmol/L were added. The mixture took reaction in dark at 23 ℃ for 12-16 hours. Then, the base solution was diluted (40 to 50 times) until the absorbance of $ABTS^{+}$ working solution was 0.7000 ± 005 (A blank) at 734 nm. 3.9 mL of the working solution were mixed with the sample and protected against light at 23 ℃ for 6 min. according to research, distilled water was used as a blank. The absorbance (A) was measured at 734 nm [10]. The ABTS+· clearance rate (η·ABTS+) was calculated according to eq. (2). The same concentration of Vc and rutin were used as controls.

η ·$ABTS^{+}$=（A blank－A）/A blank × 100 （2）

3. RESULTS AND DISCUSSION

3.1 Selection of Extraction Methods

Four extraction methods were compared to determine the optimal conditions of flavonoids. The absorbance was measured according to the method in section 2.2. The extraction rates of flavonoids were 19.35, 20.46, 23.76 and 21.74 mg/g respectively, which indicated that microwave synergy enzyme extraction was the best among the four methods.

3.2 Single Factor Experimental Results of Microwave-assisted Enzymatic Extraction

3.2.1 Effect of material and liquid ratio

As shown in Figure 1(a), small ratio of material to liquid was not conducive to the dissolution of flavonoids, which might slow the diffusion rate and bring about incomplete extraction. If the ratio of material to liquid increased, the extraction rate of flavonoids gradually increased. However, when the ratio of material to liquid was too high, the extraction efficiency of flavonoid showed decreased trend. Considering a variety factors, the ratio of material to liquid was selected to be 1:40.

3.2.2 Effect of temperature

As shown in Figure 1(b), with the increasing of temperature, the diffusion rate became fast. However, as the temperature increased, the extraction rate increased slowly. When the temperature was higher, it caused high consumption of energy, for which 80 ℃ was selected.

3.2.3 Effect of extraction time

Figure 1(c) showed long extraction time was helpful to the transfer of flavonoids from FRDP to the extract. However, the flavonoids in the solution may degrade at a high temperature. Therefore, the optimum extraction time was chosen to be 3.0 h.

3.2.4 Effect of enzyme dosage

As can be seen from Figure 1(d), with the continuous increase of enzyme dosage, it began to decrease. Hence, the amount of enzyme of 0.5 % was determined to be the best.

3.2.5 Effect of ethanol concentration

From Figure 1(e) we can know: With the continuous increase of ethanol concentration, some flavonoids that were not easily soluble in organic solvents cannot be fully transferred to the extract, resulting in the decrease of extraction rate. So, 70 % ethanol was selected.

3.2.6 Effect of PH

As shown in Figure 1(f), when pH increased, some flavonoids were changed in structure, which resulting in a lower extraction rate.

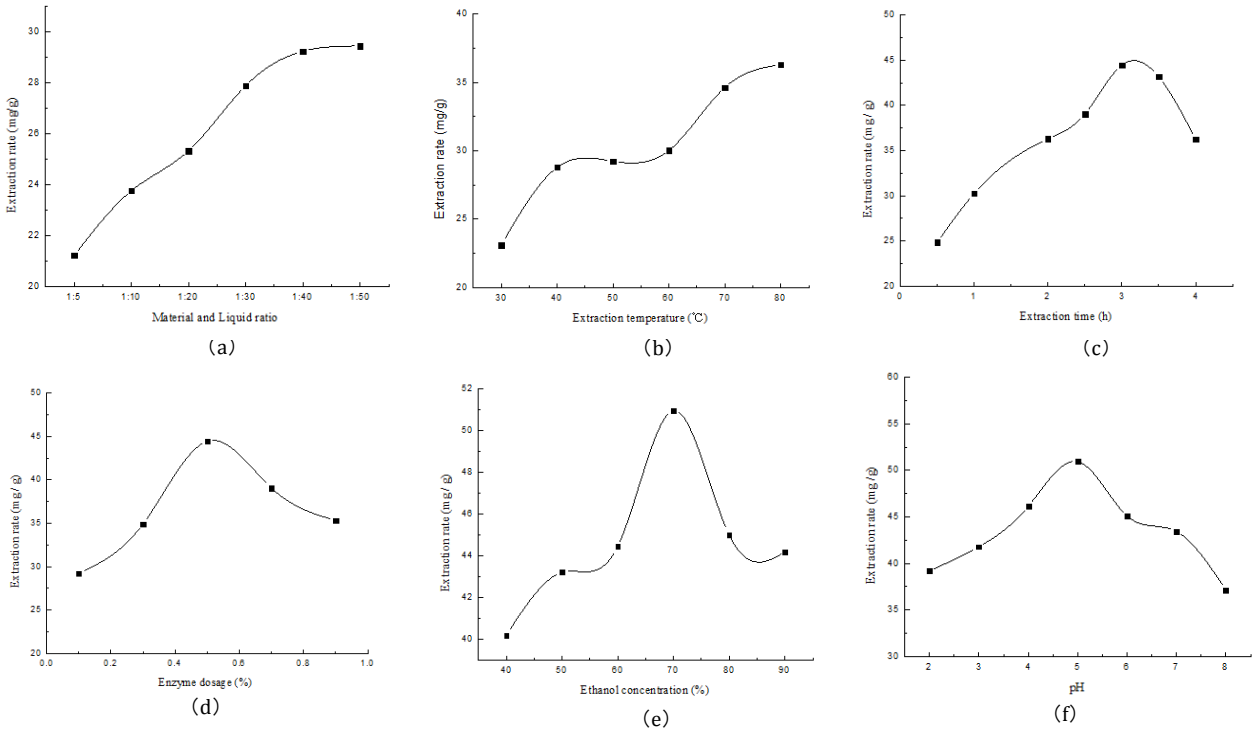


Figure 1: The effect of different conditions on the extraction of flavonoids from RDP

3.3 Microwave-assisted Enzymatic Extraction of Orthogonal Experimental Results

It can be seen from Table 2 the order of influence was A>C>D>Band the best extraction condition was $A_1B_2C_2D_3$. Therefore, the ratio of material to liquid was 1:35. Extraction time was 3.0 h. Amount of cellulase dosage was 0.5 % and ethanol was 75 %.

Table 2: Orthogonal experimental design and results analys

Number	A Ratio of material to liquid (g:mL)	B Time (h)	C Cellulase dosage (%)	D Ethanol concentration (%)	Extraction rate (mg/g)
1	1:35	2.5	0.4	65	49.73
2	1:35	3.0	0.5	70	52.72
3	1:35	3.5	0.6	75	49.44
4	1:40	2.5	0.5	75	51.45
5	1:40	3.0	0.6	60	46.12
6	1:40	3.5	0.4	70	44.63
7	1:45	2.5	0.6	70	44.08
8	1:45	3.0	0.4	75	48.17
9	1:45	3.5	0.5	65	46.73
k_1	50.63	48.42	47.51	47.53	
k_2	47.4	49.00	49.60	47.14	
k_3	46.33	46.93	46.55	49.69	
R	4.30	2.07	3.05	2.55	

In summary, the microwave synergistic enzyme extraction was selected to be the ideal method for extraction of flavonoids from FRDP. The optimum conditions were as follows: microwave power of 500 W for 10 min, material-liquid ratio of 1:35, 75 % ethanol solvent, pH of 5, amount of cellulase of 0.5 % and 80 ℃ water bath refluxed for 3.0 hours.

3.4 Process Stability Verification Test Results

The experiment was repeated for three times according to the above process conditions. The average yields of flavonoids from FRDP was 54.84 mg/g. FRSD was 0.88 %. Average TFC in dry extract was 26.20 %, FRSD was 3.25 %, indicating the optimal microwave synergetic enzyme extraction process was stable and feasible.

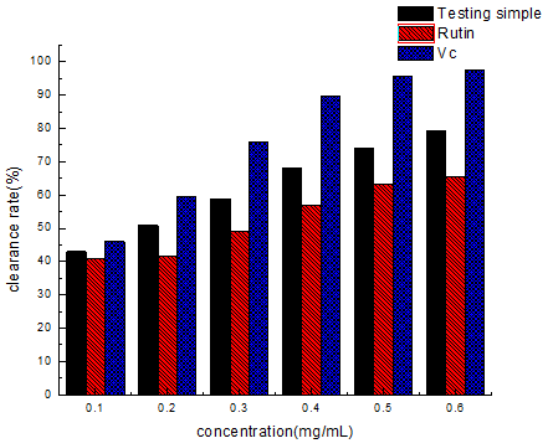
3.5 In vitro Antioxidant Test Results

Removal of DPPH free radical results.

The DPPH· scavenging capacity test results of FRDP extraction, rutin and Vc were shown in Figure 2. As can be seen from Figure 2, scavenging rate of DPPH· gradually increased with the increase of the flavonoid concentration. When the mass concentration was 0.6 mg/mL, the scavenging rate of DPPH· was 79.31 %. The scavenging rate for DPPH· of rutin and VC also increased as the mass concentration increased. The experimental results showed the flavonoid extract possessed higher scavenging capacity for DPPH· than that of rutin.

Removal of ABTS radical cations results

As can be seen from Figure 3, the scavenging rate for ABTS+·of the sample solution, rutin, and Vc gradually increased with the increase of mass concentration. When the TFC in the test solution was 0.6 mg/mL, the scavenging rate for ABTS+·was 88.73 %. Results showed the extract of flavonoids from FRDP possessed a strong scavenging ability against ABTS+·, and its scavenging capacity was significantly higher than of rutin and Vc.



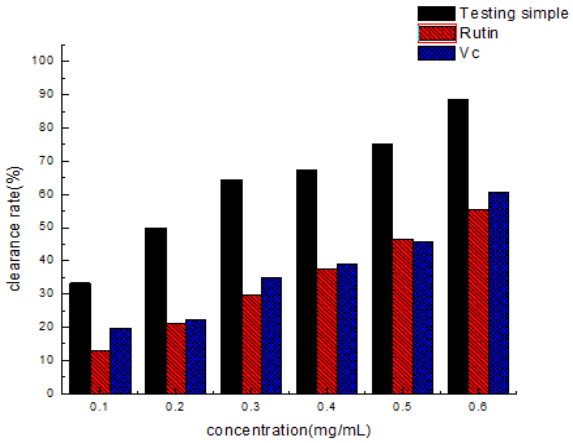


Figure 2: DPPH· scavenging capability **Figure 3:** ABTS+· scavenging capability

4. CONCLUSION

In this study, single factor and orthogonal experiments were used to optimize the extraction technology of flavonoids from FRDP by microwave synergistic enzyme method. Results showed that the optimum conditions were as follows: microwave power of 500 W, extraction time of 10 min, material-liquid ratio of 1:35, ethanol of 75 %, pH of 5, cellulase dosage of 0.5 % and 80 ℃ water bath refluxing 3.0 h. The extraction rate of flavonoids from FRDP was 54.84 mg/g, and the TFC in dried extract was 26.20 %. The scavenging rates for DPPH· and ABTS+·of extracts (0.6 mg/mL) were 79.31 % and 88.73 %, respectively, and increased with the increase of mass concentration of flavonoids. Therefore, flavonoids in FRDP possessed a good in vitro antioxidant capacity, which provided experimental basis for its future use and development.

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