Original scientific paper
UDC 582.998.14-16:[547.56:542.613

DETECTION AND EXTRACTION PROCESS OF CHLOROGENIC ACID FROM *TARAXACUM OFFICINALE*

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Abstract

Chlorogenic acid is a phenolic compound with antibacterial, antioxidant and antiviral effects. At present, the research on extraction of chlorogenic acid mainly focuses on natural products such as *Eucommia ulmoides* and honeysuckle. The *Taraxacum officinale* is more widely distributed and produces. However, there were few studies on extraction technology of chlorogenic acid from *Taraxacum officinale*. The aim of this research was to investigate detection and extraction process of chlorogenic acid from *T. officinale*.

Taraxacum officinale was used as the materials, and high performance liquid chromatography (HPLC) was used as a reference method to determine the content of chlorogenic acid in the extract solution. And its extraction process was optimized by orthogonal experimental design method.

The results showed that the HPLC detection method of chlorogenic acid from *Taraxacum officinale* was 5% methanol elution condition 0 ~ 5 min., 5 - 15% methanol elution condition 5 ~ 15min , 15 - 5% methanol elution condition 15 ~ 20min., 5 % methanol elution condition 20 ~ 25 min., and the buffer salt was 1‰ phosphoric acid aqueous solution, and the wavelength was 350 nm. The method was stable and reliable. The extraction technology of chlorogenic acid was researched from multiple factors, the best parameters of the technology were obtained that the ultrasonic temperature is 80 ^{0}C, the solid-liquid ratio is 1 : 30, the solvent concentration is 50%, and the ultrasonic time is 40 min., then the extraction rate can reach 1.921%.

This study obtained the detection method and optimized the extraction process of chlorogenic acid in *Taraxacum officinale*, and it provided a research basis for the development and utilization of *Taraxacum officinale* medicinal value.

***Key words**: Taraxacum Officinale, Chlorogenic Acid, High performance liquid chromatography, Orthogonal, Ultrasonic extraction.*

1. Introduction

Dandelion is a perennial herbaceous plant of the genus *Taraxacum* in *Asteraceae*. *Taraxacum* includes about 2,000 species, mainly produced in the temperate zone of the northern hemisphere to the subtropical region, a few are produced in tropical South America [1]. Dandelion has various chemical components, such as phenolic acids, flavonoids, phytosterols, sesquiterpene lactones, etc. [2 - 4]. Dandelion extract has anti-inflammatory, antibacterial and anti-tumor effects [5, 6]. Studies have shown that dandelion extract has a certain inhibitory effect on the growth of human melanoma cells [7]. 100 μg/mL dandelion extract was found significantly inhibiting IL-1β, IL-6, IL-8, TNF-α and neutrophil chemoattractant protein-2 (GCP) produced by LPS stimulated breast epithelial cells-2) expression. So it had a good anti-inflammatory effect [8]. Chlorogenic acid is one of the important medicinal ingredients of dandelion. At present, the research of chlorogenic acid in dandelion is mostly focused on the research of active function, and there are few research methods using its detection and extraction process [9, 10]. Due to the special biological effect of ultrasound, choosing appropriate ultrasound parameters can form more pores between the cell walls of the plant, thereby enhancing the permeability and selectivity of the cell membrane, and is now widely used to

extract biologically active ingredients in plants [11, 12]. Ultrasonic extraction method has been used in the extraction of active ingredients of *Eucommia ulmoides*, *Lonicera japonica* Thunb., and other plants [13 - 15]. There were few reports on the extraction of active ingredients in dandelion.

Therefore, the aim of this research was to investigate detection and extraction process of chlorogenic acid from T. officinale. This study will provide research basis for the effective extraction and application of chlorogenic acid in dandelion.

2. Materials and Methods

2.1 Reagents and standards

Reference substance of chlorogenic acid (Purity ≥ 98%) was provided by sigma (No 110885-200102). Acetonitrile and methanol (HPLC grade, 99.9%) were purchased from Fisher (USA). HPLC-grade water was made by double-distilling pre-deionized water, and other reagents were analytically pure.

2.2 Raw material

Whole plant of *Taraxacum officinale* were collected in March 2018 in campus of Sumy National Agrarian University. The identities of the tubers were organoleptically confirmed to be *T. officinale* by specialists from the Sumy National Agrarian University. Before extraction, samples were freeze-dried in a freeze-drier (Labconco, USA). Then samples was gridded by a domestic mixed grinder (Yiyong, China), a powder constituted by particles with a size lower than 500 μm was obtained. The freeze-dried powder was stored under vacuum, at room temperature.

2.3 Determination method of chlorogenic acid content in *T. officinale*

2.3.1 Chromatographic conditions and instrumentation

Analysis was performed on a Shimadzu Acquity HPLC system (LC-20A, Shimadzu, Japan). An Inertsil ODS-3 C18 column (5 μm, 250 x 4.6 mm) was applied for all analyses. The mobile phase was composed of A (MeOH) and B (1‰ phosphoric acid solution, adjusted to pH 8 with ammonia-water) with a gradient elution: 0 - 5 min., 5% A; 5 - 15 min., 5 - 15% A; 15 - 20 min., 15% - 5% A; 20 - 5 min. 5% A. The flow rate of the mobile phase was 1 mL/min. And the column temperature was maintained at 30 ^{0}C. Detection wavelength was set at 350 nm. Target peak was identified by comparing their retention time of the respective standard. A standard graph of chlorogenic acid was prepared by plotting concentration versus peak area.

2.3.2 Standard preparation

10 mg chlorogenic acid standard was weighed, and then was dissolved in a 10 mL one-mark volumetric flask with 10% methanol to form the 1 mg/mL stock solution. Draw 5 mL of 1 mg/mL chlorogenic acid solution, add 10% methanol to a constant volume to 10 mL, and then sequentially dilute to obtain 6 concentration levels of the reference mixture 1, 0.5, 0.25, 0.125, 0.0625, 0.0313 mg/mL chlorogenic acid standard solution. The standard solutions were filtered through a 0.45 μm membrane prior to injection. All solutions were stored in a refrigerator at 4 ^{0}C before analysis.

2.3.3 Extraction preparation of sample of *T. officinale*

1 g of sample powder was accurately weighted and 50 mL of extraction solvent 60% ethanol was added. Ultrasonic (power 250 W, frequency 35 KHz) extraction for 40 min. was appled, than suction filtration, the extraction operation was repeated 3 times. The extracts were combined 3 times, concentrated under reduced pressure at 50 ^{0}C by rotary evaporator (N-1300D, EYELA, Japan), made up to 25 mL with 60% ethanol, and stored at 4 ^{0}C in the refrigerator. They were filtered with 0.45 μm filter before injection.

2.3.4 Method validation

Through determining the linearity, LOD, LOQ, precision, repeatability, stability, and accuracy additive recovery rate, the established HPLC method was validated.

2.3.5 Calculation of chlorogenic acid extraction rate

$$\text{Extraction rat (\%)} = \frac{c \times v \times a}{M}$$

Where: "v" is the volume of the extract; "c" is the concentration of chlorogenic acid in the extract; "a" is the dilution factor; "M" is the weight of the sample.

2.4 Single factor experiment for chlorogenic acid content in *T. officinale*

The effect of different temperature (40, 50, 60, 70, and 80 ^{0}C) on extraction rate were tested, while the ethanol volume fraction for extraction was 70%, the material-liquid ratio was 1 : 20 (g/mL), ultrasonic time was 40 min. After confirming that 70 ^{0}C of ultrasonic temperature was better from the above mentioned temperaturse, the effect of the material-liquid ratios (1 : 15, 1 : 20, 1 : 25, 1 : 30, and 1 : 35) on extraction was compared under the following conditions: the ethanol volume fraction

was 70 %, and the ultrasound time was 40 min. After the material-liquid ratio of 1 : 30 was found better, the effect of ethanol volume fractions (20, 40, 60, 80, and 100%) on extraction rate was tested. While ultrasonic time was 40 min., the ultrasonic temperature was 70 ^{0}C and the material-liquid ratio was 1 : 30. When the better volume fraction of ethanol was determined, the effects of extraction of ultrasonic time (10, 20, 30, 40, and 50 minutes) was tested under the conditions as followings: the ultrasonic temperature was 70 ^{0}C and the material-liquid ratio was 1 : 30. Finally the optimal level of each factor were determined. The dandelion sample was 1 g for each test.

2.5 Orthogonal experiment design

According to the single-factor test results, ultrasonic temperature (A), material-liquid ratio (B), ethanol volume fraction (C) and ultrasonic time (D) were selected as the investigation factors with the extraction rate of dandelion chlorogenic acid as the evaluation index. Orthogonal experiment design L9(3^4) was applied for obtaining the best extraction process.

2.6 Statistical Analyses

All statistical analyses, including the design of orthogonal test and independent-sample t test, were carried out with Excel 2010 and PASW (IBM SPSS Statistics) statistical software (version 19.0).

3 Results and Discussion

3.1 High performance liquid chromatography for determination of chlorogenic acid

The mobile phase was composed of A (MeOH) and B (1‰ Phosphoric acid solution, adjusted to pH 8.0 with ammonia-water) with a gradient elution: 0 - 5 min., 5% A; 5 - 15 min., 5 - 15% A; 15 - 20 min., 15% - 5% A; and 20 - 5 min 5% A. The flow rate of the mobile phase was 1 mL/min., and the column temperature was maintained at 30 ^{0}C. Detection wavelength was set at 350 nm. Under this method, the peak shape was good. The detection object and other components were well separated. The high performance liquid chromatogram of chlorogenic acid standard was shown in Figure 1. The standard curve for chlorogenic acid was y = 1.4624 x - 2.5424, and the linear range was 0.00692 ~ 0.44 μg/mL. The linear relationship was good (R^2 = 0.999).

The method was examined from the precision test, stability test, repeatability test, and recovery rate. The reference solution was injected 6 times continuously with a volume of 10 μL. The RSD of the chlorogenic acid peak area was 1.32%, which indicated that the instrument precision was good. The test sample solution from dandelion sample was injected at 0, 2, 4, 8, 12, and 24 h, respectively (Figure 2).

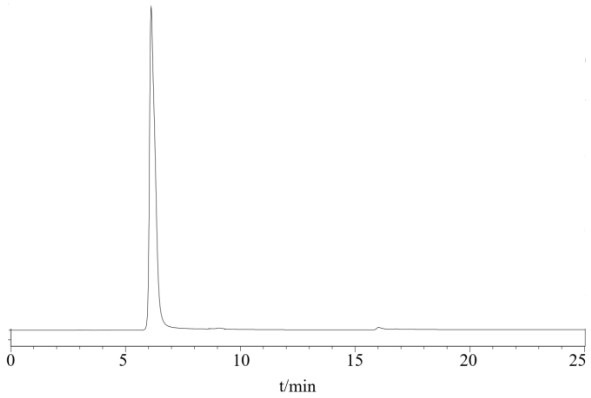

Figure 1 High performance liquid chromatogram of chlorogenic acid standard

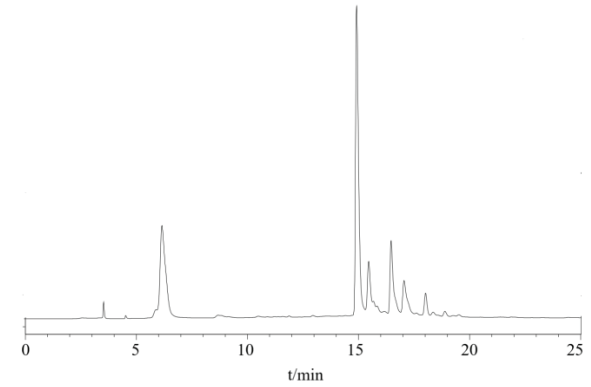

Figure 2 High-performance liquid chromatogram of dandelion sample

The peak area was measured according to the method of "2.3.1". The RSD of the chlorogenic acid peak area was 1.02%, indicating that the test sample solution is stable within 24 h. 6 dandelion sample solutions were used to determine the content of chlorogenic acid in the sample. The RSDs of the measured contents were 1.14%, indicating good repeatability. Through the high-medium-low-concentration chlorogenic acid reference substance addition recovery test, the recovery rate obtained was between 93.8 - 97.6%.

3.2 Single factor analysis

3.2.1 Effect of extraction temperature on extraction rate of chlorogenic acid

As shown in Figure 3, the extraction rate of chlorogenic acid increased first and then decreases slightly with ultrasonic temperature increasing. When the extraction temperature was 70 ^{0}C, the extraction rate of chlorogenic acid was the highest with the rate of 1.41%. When the temperature exceeded 70 ^{0}C, the extraction rate decreased. High temperature is conducive to the dissolution of compound besides the chlorogenic acid, and the temperature is too high, the active ingredients are easily damaged. At the same time solvent can be losed easily [16].

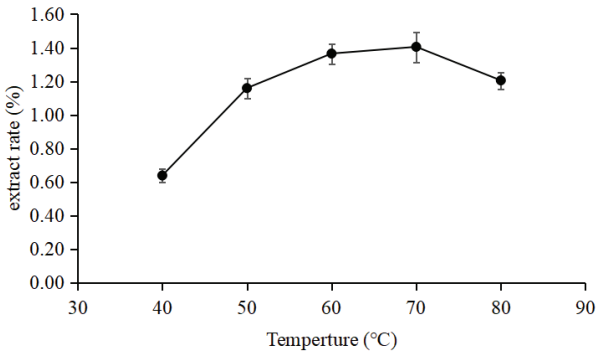

Figure 3. The effect of ultrasonic temperature on the extraction rate

3.2.2 Effect of solid-liquid ratio (right) on the extraction rate

It can be seen from Figure 4 that the chlorogenic acid extraction rate of dandelion rises when the material-liquid ratio was increased at the beginning. The chlorogenic acid of dandelion had basically dissolved out when the solid-liquid ratio reached at 1 : 30 g/mL, and then tends to be stable. For cost considerations, the solid-liquid ratio was 1 : 30 g/mL. When the solid-liquid ratio was 1 : 50 g/mL, the extraction rate decreased slightly. As the amount of solvent increasing, the dissolved amount of alcohol-soluble and fat-soluble components in dandelion increased, which may effected the dissolution of organic acid components. Finally, the extraction rate of chlorogenic acid in dandelion reduced.

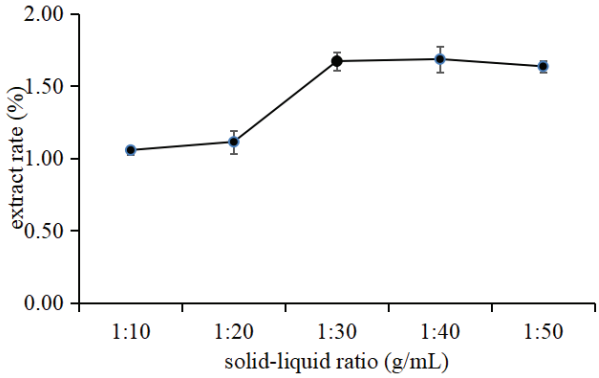

Figure 4. Effect of solid-liquid ratio (right) on the extraction rate

3.2.3 The effect of ethanol content on the extraction rate

As shown in Figure 5, at the beginning, the extraction rate of dandelion chlorogenic acid increased significantly with the increasing of ethanol content in the extraction solvent. When the ethanol content was 60 %(v:v), the extraction rate of dandelion chlorogenic acid reached the highest with the value of 1.51%. However, as the ethanol content continued to increase, the extraction rate of dandelion chlorogenic acid showed a downward trend. When the ethanol content was 80%, the extraction rate decreases slightly. It was speculated that with the ethanol content increasing, the dissolution amount of some alcohol-soluble and fat-soluble in dandelion increased. In addition, the viscosity of the solution increases, the diffusion resistance of chlorogenic acid in the dandelion to the solvent increases, the diffusion slowed down, and the extraction rate decreased [17].

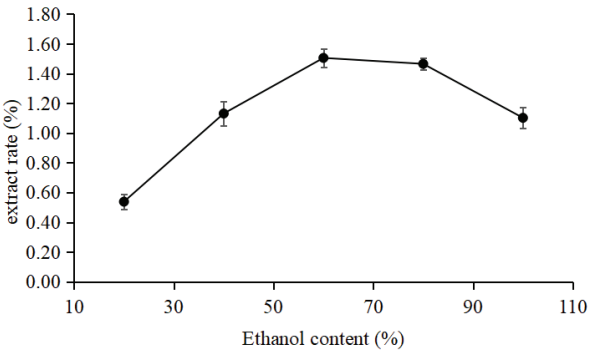

Figure 5. Effect of ethanol content on the extraction rate

3.2.4 The effect of ultrasonic time on the extraction rate

As shown in Figure 6, at the beginning, the extraction rate gradually increased as the extraction time increased. When the extraction time was 45 min., the highest extraction rate was 1.67%. However, with the extraction time continual increasing the extraction rate began to decline. It indicated that when the extraction time is sufficient, chlorogenic acid in dandelion had basically dissolved out. If the ultrasonic time was too long, a large amount of non-organic acids will be dissolved out, resulting in a reduction in the extraction rate of organic acids and increased energy consumption.

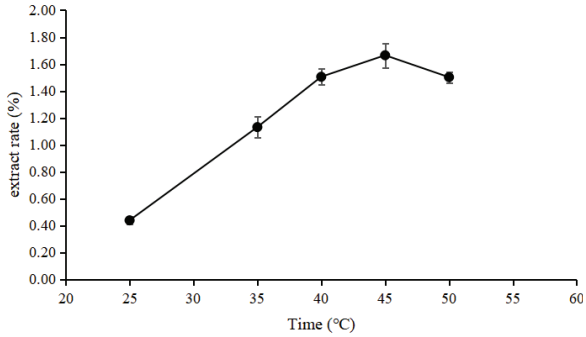

Figure 6. Effect of ultrasonic time on the extraction rate

3.3 Orthogonal experiment result

Based on the single-factor experimental results, L9 (34) orthogonal test was performed on the ultrasonic

time, ultrasonic temperature, material-liquid ratio and ethanol volume fraction. The orthogonal test design was shown in Table 1, and the results were shown in Table 2. The range (R) of the orthogonal test was B > C > A = D, which indicated that the order of the influence of various factors on the extraction rate of dandelion chlorogenic acid was from large to small in order: material-liquid ratio > ethanol content > ultrasonic temperature = ultrasound time. The optimal process conditions for dandelion chlorogenic acid extraction were A2B2C1D2 (solid-liquid ratio was 1 : 30, the ethanol content was 50%, the ultrasonic temperature was 60 ^{0}C, and the ultrasonic time was 40 min). Since this optimal combination did not appear in the orthogonal test, a verification test should be performed on the obtained optimal combination. The dandelion chlorogenic acid extraction rate test was repeated 5 times. The dandelion chlorogenic acid extraction rate was 1.92%, which was higher than the highest chlorogenic acid extraction rate (1.89%) in Table 2. The relative standard deviation was 0.83%, which proved that the process conditions were stable, and suitable for the extraction of dandelion chlorogenic acid.

Table 1. Factors and levels

Level	Factor			
	A Temperture (^{0}C)	B S/L ratio	C Ethanol content (%)	D Time (min.)
1	50	1 : 25	50	35
2	60	1 : 30	60	40
3	70	1 : 35	70	45

Table 2. $L_9(3^4)$ arrangement and results of orthogonal test

NO	A	B	C	D	Extraction rate (%)
1	1	1	1	1	1.41
2	1	2	2	2	1.67
3	1	3	3	3	1.58
4	2	1	2	3	1.39
5	2	2	3	1	1.89
6	2	3	1	2	1.81
7	3	1	3	2	1.53
8	3	2	1	3	1.85
9	3	3	2	1	1.12
$\mathbf{K_1}$	4.66	4.33	5.07	4.42	
$\mathbf{K_2}$	5.09	5.41	4.19	4.81	
$\mathbf{K_3}$	4.50	4.51	5.00	4.82	
$\mathbf{k_1}$	1.55	1.44	1.69	1.47	
$\mathbf{k_2}$	1.70	1.80	1.40	1.67	
$\mathbf{k_3}$	1.50	1.50	1.66	1.61	
R	0.20	0.36	0.29	0.20	

Note: "K" represents the sum of chlorogenic acid extraction rate at the same level of each factor; "k" represents the average value of chlorogenic acid extraction rate at the same level of each factor; "R" determines the magnitude of the influence of factors on the test results.

4. Conclusions

- In the established HPLC method for chlorogenic acid, methanol and 1‰ phosphoric acid were used as the mobile phase for gradient eluted with a 1.0 mL/min flow rate. The detection wavelength was 350 nm. The column temperature was 30 . The injection volume 10 µL. The precision, stability, repeatability, and recovery rate of standard addition indicated that the method was stable and reliable.
- The optimal extraction process was as following: extraction temperature was 60 ^{0}C, material-liquid ratio was 1 : 30, ethanol volume fraction was 50%, and extraction time was 40 min. Under this extraction process, the extraction rate of chlorogenic acid is 1.92%.
- The process conditions are stable and feasible, which is suitable for the extraction of dandelion chlorogenic acid.

Acknowledgement

This research was financially supported by the National Natural Science Foundation of Henan (Grant No.182300410086) and Key Scientific Research Projects of Higher Education in Henan Province (No. 20A180025).

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