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# Optimization of extraction technology for determination of caffeic and chlorogenic acid in dandelion

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***Abstract.*** In order to obtain the optimum extraction technology for rapid and accurate determination of phenolic acids from dandelion in laboratory, methanol was used as extractant, and response surface methodology (RSM) was used to optimize the extraction process combined with the quality evaluation by the fingerprint analysis. Results showed that the input of cellulase was beneficial to the stability of the extraction. The liquid–material ratio promoted the extraction rate, and the methanol with the extraction time or the liquid–material ratio showed synergistic effect on the increase of extraction rate. Chlorogenic acid and caffeic acid were the main compounds and their match degree with the control fingerprint map were all 100 % according to the HPLC fingerprint analysis. Thus, the two compounds were used as main evaluation indexes to obtain the optimal extraction conditions, namely that liquid–material ratio of 300, methanol concentration of 40% and ultrasonic extraction time of 120 min. The obtained extraction process reduced the operation cost and maintained the stability of detection results. It could be directly used for the determination of phenolic acids from dandelion in laboratory.
***Key words:*** caffeic acid, chlorogenic acid, dandelion, fingerprint, optimization.

## Introduction

Dandelion is a common Chinese herbal medicine, rich in polysaccharides, chlorogenic acid, caffeic acid, flavonoids and other active ingredients [WANG, 2016; WANG *et al.*, 2017].

In recent years, with the improvement of people's health awareness, dandelion has gradually become a kind of medicinal and edible plant [MARTINEZ et al., 2015].

Modern pharmacological studies have shown that dandelion has broad–spectrum bacteriostasis, antioxidant, anti–tumor and immune promotion effects, and these effects have a certain relationship with phenolic acids [IDDRISU *et al.*, 2016; WANG *et al.*, 2017].

At present, dandelion not only attracts attention in the field of medicine and health, but also becomes a hotspot in the research and development of functional food [MARTINEZ *et al.*, 2015; TAN *et al.*, 2017]. And so, the extraction of phenolic acids has become an important basis for the application and development of dandelion industries.

Caffeic acid and chlorogenic acid, generally, are the main compounds in dandelion [WANG *et al.*, 2017].

While the China Pharmacopoeia (2015 Edition) stipulates that the content of caffeic acid of dandelion should not be less than 0.02 %.

In addition to species and cultivation conditions [ARIAS *et al.*, 2016], the contents of dandelion compounds are also related to extraction technology.

The extraction methods could be divided into water extraction and organic solvent extraction according to the extractants, and the extraction rate can be improved by dissolving raw materials in extractants and breaking cell walls with physical or enzymatic methods [EGGERT *et al.*, 2018; MARTINEZ *et al.*, 2015; WANG, 2016].

The extraction process usually includes hot water extraction, ultrasonic assisted extraction, reflux extraction and so on [DUAN and LIANG, 2017; TAN *et al.*, 2017].

The factors affecting the extraction rate include liquid–solid ratio ratio, extraction time, extraction temperature, solvent concentration, etc.

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Different extraction conditions may lead to changes in the compounds, and then affect the efficacy of dandelion extracts [JING–HUA *et al.*, 2014].

Therefore, how to balance the maximum extraction rate and extraction quality was the focus of this study.

## Material and methods

### Materials and reagents.

Dry dandelion (*Taraxacum antungense*) leaf powder was provided from the Institute of Coastal Agriculture, Hebei Academy of Agriculture and Forestry Sciences.

Samples were dried at 60 °C and sieved by 60 mesh (diameter=0.42 mm).

Caffeic acid, chlorogenic acid, ferulic acid and luteolin were purchased from National Institutes for Food and Drug Control (China); anhydrous methanol was analytical purity; cellulase activity was 10000 U/g, purified water was prepared by Institute of Coastal Agriculture.

### Extraction and optimization.

A certain of dry dandelion samples were put into the 50 mL plugged centrifugal tube mixed with 5 mL cellulase solution (0.1 %, pH =4) and hatched in hot water at 60 °C for 30 min [WANG, 2016; ZHANG *et al.*, 2017].

After that 20 mL methanol solution was added into the tube and placed into the ultrasonic cleaner (power was 120 W, water temperature was 60 °C) for a certain time.

The extract was centrifuged at 4000 rpm for 5 min and the supernatant was for HPLC check.

The details of dry sample weight, methanol concentration and extraction time were referred to the Box–Behnken design form (Table 1).

**Table 1.**

Box–Behnken design form for the optimization of phenolic acid extraction from dandelion

| No. | Methanol | Liquid–material rate | Time | Caffeic acid | Chlorogenic acid | Total yield* |
|---|---|---|---|---|---|---|
| | % | mL:g | min | mg/g | mg/g | mg/g |
| 1 | 55 | 100 | 30 | 4.66 | 2.59 | 6.07 |
| 2 | 55 | 200 | 75 | 5.69 | 3.11 | 7.38 |
| 3 | 70 | 100 | 75 | 6.07 | 2.87 | 7.63 |
| 4 | 40 | 300 | 75 | 3.31 | 2.64 | 4.75 |
| 5 | 40 | 200 | 120 | 7.70 | 3.40 | 9.55 |
| 6 | 55 | 300 | 120 | 5.77 | 3.11 | 7.46 |
| 7 | 70 | 200 | 30 | 4.93 | 2.72 | 6.41 |
| 8 | 40 | 100 | 75 | 3.31 | 2.48 | 4.66 |
| 9 | 55 | 200 | 75 | 5.21 | 2.98 | 6.83 |
| 10 | 40 | 200 | 30 | 4.79 | 2.78 | 6.31 |
| 11 | 55 | 200 | 75 | 5.92 | 3.07 | 7.59 |
| 12 | 70 | 300 | 75 | 6.39 | 3.24 | 8.15 |
| 13 | 55 | 300 | 30 | 6.06 | 3.09 | 7.74 |
| 14 | 70 | 200 | 120 | 5.91 | 3.04 | 7.56 |
| 15 | 55 | 200 | 75 | 6.41 | 3.14 | 8.12 |
| 16 | 55 | 100 | 120 | 4.18 | 2.59 | 5.59 |
| 17 | 55 | 200 | 75 | 5.32 | 3.00 | 6.95 |

*Total yield = Chlorogenic acid × 0.544+ Caffeic acid

### Measurement methods

The extract was filtered by 0.45 μm membrane then checked by Agilent 1200 High Performance Liquid Chromatography (HPLC) which was equipped with Diamonsil $C_{18}$ column (4.6 mm × 250 mm, 5 μm).

The samples were analyzed under given chromatographic conditions that mobile phase of A (0.02 % phosphate buffer): B (methanol)=50:50, examine wave length of 350 nm, column temperature of 35 °C, flow rate of 1 mL·min$^{-1}$ and sampling rate of 10 μL.

The contents of chlorogenic acid and caffeic acid were calculated from the standard curves which were obtained according to the simulation from peak area and concentrations of standard samples.

The standard curves were:

$$y_1=16.835x_1 - 16.971,\ R^2=0.99$$

and

$$y_2 =22.173x_2 - 13.042\ R^2=0.99,$$

respectively, where $y_1$ and $y_2$ were the peak area of chlorogenic acid and caffeic acid, and $x_1$ and $x_2$ were the contents of chlorogenic acid and caffeic acid, their validate measure scope were 0–0.1 mg/mL.

**Data analysis**. The contents of chlorogenic acid and caffeic acid from each treatment were weighted as the total caffeic acid yield to evaluate the fitted phenolic acid extraction technology through the software of Design Expert 8.0.6.

The weighted method was as follows: with the arithmetic average ratio of chlorogenic acid to caffeic acid as coefficient ($k$) which was 0.544 through the calculation (Table 1).

The total caffeic acid yield was calculated as follows:

*The total yield=Chlorogenic acid content× 0.544+ Caffeic acid content,*

the unit was mg/g.

In order to comprehensively determine the optimal extraction technology, all the HPLC data were carried out the fingerprint analysis by the software of The Similarity Evaluation System for Chromatographic Fingerprint of TCM 2012.130723.

Each treatment was set four repetitions and the software of SPSS 16.0 was used to perform the Duncan (D) significant difference analysis and cluster analysis. The data were shown as mean ± standard err, and the images were created with Origin 8.0 software.

## Results and discussion

**Optimization analysis for caffeic acid extraction.**

Based on the model fitting, the Cubic model fitting result showed the significance (F=6.85, $p$=0.039), however, part of cross–terms exceeded the credibility range.

And so the modified Cubic model was:

$$y=5.71–0.41x_1+0.74x_2–0.19x_3+0.078x_1x_2–0.48x_1x_3+0.048x_2x_3–0.14x_1^2–0.8x_2^2+0.26x_3^2–0.66x_1^2x_2+1.17x_1^2x_3+1.88x_1x_2^2,$$

$R^2$=0.95, where $y$ was caffeic acid content, and $x_1$, $x_2$ and $x_3$ was methanol concentration, liquid–material ratio and extraction time, respectively.

According to the ANOVA analysis (Table 2), the liquid–material ratio significantly promoted the extraction rate (F = 9.42, $P$ = 0.04), the other two factors had no significant impact on the extraction rate, however, the synergistic effects of methanol concentration and extraction time ($x_1^2x_3$, F = 11.54, $P$ = 0.03), methanol concentration and liquid–material ratio ($x_1x_2^2$, F = 29.85, $P$ = 0.01) were shown and significantly promoted the extraction rate.

The influence order on the extraction rate was liquid–material ratio > methanol concentration > extraction time.

According to the actual values, the fitting function was:

$$y=0.88428x_1+0.22173x_2+0.3618x_3–0.00171x_1x_2–0.01338x_1x_3+0.00001x_2x_3–0.00335x_1^2–0.00077x_2^20.00013x_3^2–0.00003x_1^2x_2+0.00012x_1^2x_3+0.00001x_1x_2^2–36.27606$$

Thus, 25 solution schemes with the highest extraction rate were obtained (Appended Table 1).

Among them, the optimized methanol concentration was mainly around 40 %, 60 % and 70 %; the

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extraction time was around 30 minutes and 120 minutes; the liquid–material ratio was around 200 and 300.

**Table 2.**

ANOVA analysis for the Cubic model fitting

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Model | 19.386 | 12 | 1.616 | 6.855 | 0.039 |
| A | 0.687 | 1 | 0.687 | 2.917 | 0.163 |
| B | 2.219 | 1 | 2.219 | 9.416 | 0.037 |
| C | 0.150 | 1 | 0.150 | 0.638 | 0.469 |
| AB | 0.024 | 1 | 0.024 | 0.104 | 0.764 |
| AC | 0.924 | 1 | 0.924 | 3.919 | 0.119 |
| BC | 0.009 | 1 | 0.009 | 0.039 | 0.854 |
| $A^2$ | 0.081 | 1 | 0.081 | 0.342 | 0.590 |
| $B^2$ | 2.708 | 1 | 2.708 | 11.491 | 0.028 |
| $C^2$ | 0.285 | 1 | 0.285 | 1.209 | 0.333 |
| $A^2B$ | 0.881 | 1 | 0.881 | 3.737 | 0.125 |
| $A^2C$ | 2.719 | 1 | 2.719 | 11.536 | 0.027 |
| $AB^2$ | 7.035 | 1 | 7.035 | 29.851 | 0.006 |
| Pure Error | 0.943 | 4 | 0.236 | | |
| Cor Total | 20.329 | 16 | | | |

A represented the methanol concentration; B represented the liquid–material ratio; C represented the extraction time

The solution scheme corresponding to the highest extraction rate was as follows: 40 % of methanol, 200.7 of liquid–material ratio, 120 min of the extraction time and the expected caffeic acid was 7.7 mg/g.

**Optimization analysis for chlorogenic acid extraction.**

With the same analysis method, chlorogenic acid extraction technology was optimized and the fitted model was:

$$y=3.059–0.107x_1+0.255x_2+0.005x_3+0.055x_1x_2–0.075x_1x_3+0.004x_2x_3–0.055x_1^2–0.196x_2^2–0.019x_3^2–0.122x_1^2x_2+0.228x_1^2x_3+0.356x_1x_2^2,$$

$R^2=0.98$, where $y$ was chlorogenic acid content, and $x_1$, $x_2$ and $X_3$ was methanol concentration, liquid– material ratio and extraction time, respectively.

According to the ANOVA analysis (Appended Table 2), the methanol concentration (F=9.66, $p$=0.036) and the liquid–material ratio (F=54.96, $p$=0.002) both significantly promoted the extraction rate, and the synergistic effects of methanol concentration and extraction time ($x_1^2x_3$, F = 22.01, $p$=0.009), methanol concentration and liquid–material ratio ($x_1x_2^2$, F=53.55, $p$=0.002) were shown and significantly promoted the extraction rate. According to the optimization model, 22 solution schemes with the highest extraction rate were obtained (Appended Table 3). Among them, the optimized methanol concentration was mainly around 40 % and 70 %; the extraction time was around 120 minutes; the liquid–material ratio was around 200 and 300.

The solution scheme corresponding to the highest extraction rate was as follows: 40 % of methanol, 203.7 of liquid–material ratio, 120 min of the extraction time and the expected chlorogenic acid was 3.4 mg/g.

**Optimization analysis for the phenolic acid extraction.** Similarly, the fitted model for the weighted contents of caffeic acid and chlorogenic acid was obtained, as follows:

$$y=7.374–0.4725x_1+0.885x_2–0.19x_3+0.1075x_1x_2–0.5225x_1x_3+0.05x_2x_3–0.167x_1^2–0.9095x_2^2+0.2505x_3^2–0.7325x_1^2x_2+1.2875x_1^2x_3+2.065x_1x_2^2,$$

*R2=0.96*, where *y* was total caffeic acid yield, and $x_1$, $x_2$ and $X_3$ was methanol concentration, liquid – material ratio and extraction time, respectively.

According to the ANOVA analysis (Appended Table 4), the liquid–material ratio (F=11.61, *p*=0.03) significantly promoted the extraction rate, and the synergistic effects of methanol concentration and extraction time ($x_1^2x_3$, F =12.29, *p*=0.025), methanol concentration and liquid–material ratio ($x_1x_2^2$, F=31.62, *p*=0.005) were shown and significantly promoted the extraction rate. According to the optimization model, 27 solution schemes with the highest extraction rate were obtained (Appended Table 5).

Among them, the optimized methanol concentration was mainly around 40 %, 60 % and 70 %; the extraction time was around 30 min and 120 minutes; the liquid–material ratio was around 200 and 300.

The solution scheme corresponding to the highest extraction rate was as follows: 40 % of methanol, 202.7 of liquid–material ratio, 120 min of the extraction time and the expected total caffeic acid yield was 9.55 mg/g.

**Fingerprint analysis and cluster analysis.**

The HPLC data of all treatments were imported to the similarity evaluation system of TCM chromatographic fingerprints.

After data matching, the control fingerprints were established by median method (Figure 1).

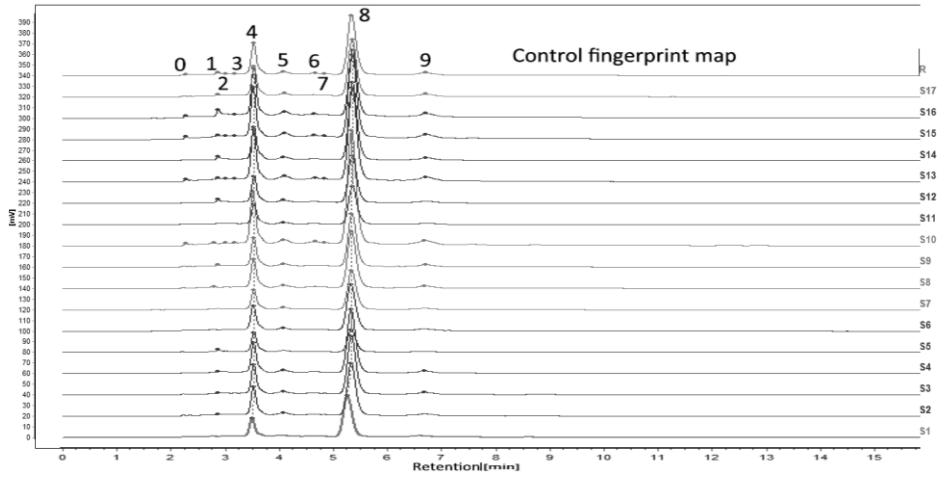


***Figure 1.*** Control fingerprints of dandelion based on the 17 extraction treatments

The RSD of retention time from all peaks was less than 1 %, which indicated that all extraction processes had good consistency. A total of 10 compounds were detected from dandelion samples (Table 3).

Chlorogenic acid (No. 4) and caffeic acid (No. 8) were found from all treatments, and their matching degree were both 100 %.

The other components, like No. 1, No. 5 and No. 9 also showed high matching degree.

The three chemicals would be esculetin, luteolin–7–O–β–D glucoside, and ferulic acid (tested from the standard samples).

By fingerprint similarity analysis (Appended Table 6), the similarity of all treatments were above 0.98, which indicated that all extraction processes have high similarity in quality.

In order to further screen the extraction process, inter–group Association and Euclidean clustering method was carried out by the software of SPSS 16.0 based on the peak area of caffeic acid and chlorogenic acid (Figure 2).

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**Table 3.**

Compounds from the dandelion samples based on the fingerprint analysis

| No. | Retention time | Control fingerprint | Retention time RSD (%) | Matching number | Tentative identification |
|---|---|---|---|---|---|
| 0 | 2.267 | 2.653 | 0 | 4 | |
| 1 | 2.857 | 29.23 | 0.99 | 13 | Esculetin* |
| 2 | 3.009 | 4.65 | 0 | 3 | |
| 3 | 3.173 | 5.875 | 0.08 | 4 | |
| 4 | 3.523 | 261.989 | 0.23 | 17 | chlorogenic acid |
| 5 | 4.084 | 57.745 | 0.23 | 15 | luteolin–7–O–β–D glucoside* |
| 6 | 4.657 | 14.995 | 0.06 | 4 | |
| 7 | 4.826 | 5.405 | 0.06 | 3 | |
| 8 | 5.333 | 657.199 | 0.38 | 17 | caffeic acid |
| 9 | 6.698 | 31.783 | 0.22 | 10 | ferulic acid |

* the compound was speculated from the references (Wei et al, 2007), others compounds were identified by the standard samples

When the threshold T = 10, all extraction processes were classified into two categories, of which treatment 10 was classified as a single category. As the threshold decreases, when T = 5, all extraction processes were divided into five categories, of which treatment 5 was a separate one. According to the above response surface optimization model, the extraction rate of treatment 5 was the highest, followed by treatment 2, 3, 4, 6, 8, 9, 12 and 17.

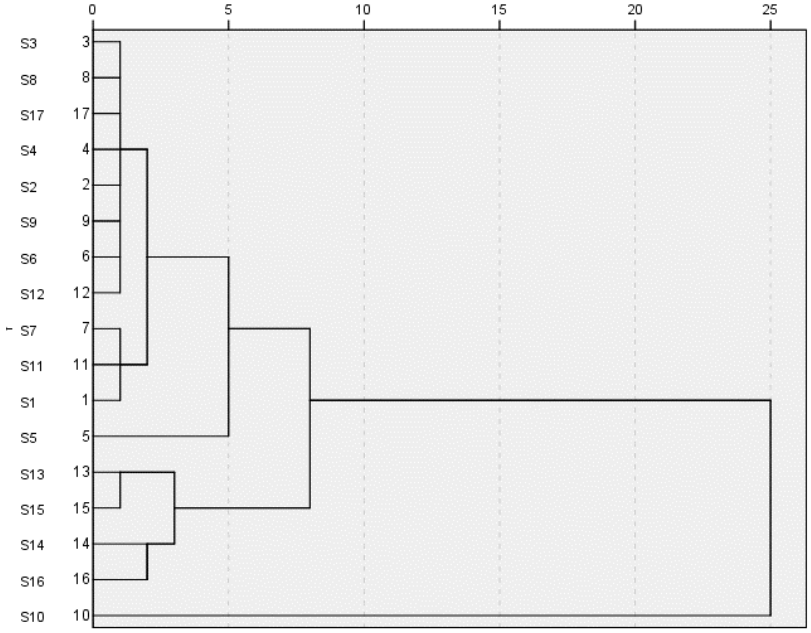


***Figure 2.*** Cluster analysis based on peak area of caffeic acid and chlorogenic acid

During the process of extraction method screening, fingerprint analysis and multi–index were used to evaluate the quality of dandelion under different extraction methods, which had certain scientific rationality for the evaluation of traditional Chinese medicines with complex compounds [JING–HUA *et al.*, 2014].

This study provided an idea for comprehensive evaluation of the quality of traditional Chinese medicine, namely through the different weight coefficients to evaluate their quality according to the role of each compound in the pharmacodynamics, and avoid the conflict between multiple indices when evaluating individually [ZHENG *et al.*, 2017].

At the same time, the extraction optimization method also provided a reference for the optimization for other plant components extraction.

**Screening and validation of extraction process.**

Based on the results from above response surface optimization model, three solution scheme with the highest extraction rate were obtained, however, their conditions were very closed to each other, therefore, the extraction process (I) was confirmed, namely 40 % of methanol, 200 of liquid–material ratio and 120 min of the extraction time.

Meanwhile, combined with the cluster analysis results, the relatively medium level extraction process (II) and low level (III) were selected as the comparison.

Conditions were 70 % of methanol, 300 of liquid–material ratio and 120 min of the extraction time for the extraction process (II) and 60 % of methanol, 300 of liquid–material ratio and 30 min of the extraction time for the extraction process (III), respectively.

Their actual checking values were shown in Figure 3. The actual value was closed to the expected value, indicating that the optimization model could predict the optimal extraction process.

Besides, LSD analysis showed that the value in the process (I) was significantly higher ($p$<0.001) than that in process (III), but there was no significant difference ($p$=0.785) between process (I) and process (II), which meant that process (I) and process (II) could be both the optimal extraction technology.

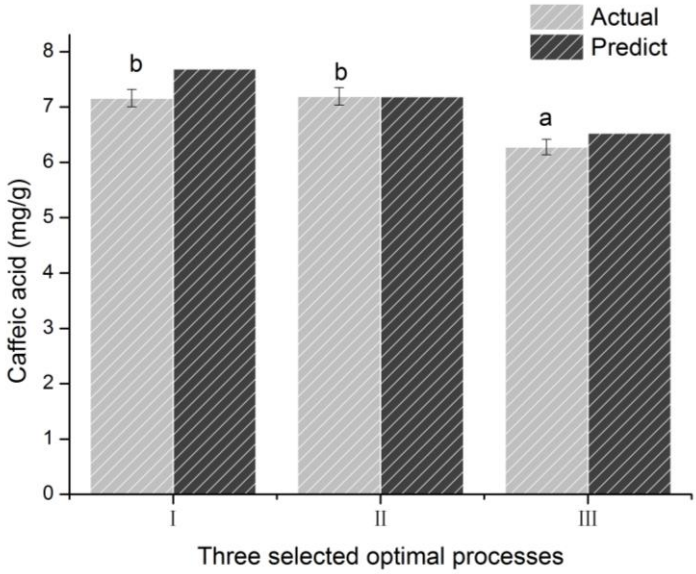


***Figure 3.*** Caffeic acid content in three selected optimal extraction process

The extraction of dandelion components for commercial production mostly adopted water extraction method because of the low cost of extractant and the ease of extraction equipment [WANG, 2016]. While the small–scale extraction mostly used ethanol solvent extraction [WANG *et al.*, 2017] due to the phenolic acids had big dissolution rate in organic solvents.

In this study, methanol was used as the solvent because the main Chinese national standard, like GB/T 22250–2008 (Determination of Chlorogenic Acid in Health food) and Pharmacopoeia 2015 and so on, all recommended the methanol as the mobile phase when checking by HPLC. Therefore, the methanol was directly to extract the dandelion compounds in this research, omitting the steps of rotating evaporation of ethanol extract and secondary dissolution of methanol [XIAO–LING *et al.*, 2017; YAN *et al.*, 2017], which not only decreased the pretreatment time before HPLC injection, but also reduced the experimental operation error.

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At present, the dry dandelion samples in most researches were often at around 1 g and liquid–material ratio was 10–30 [WANG, 2016].

However, the actual conditions in this study showed that the low liquid–material ratio may liable to cause insufficient dissolution.

Besides, the dry sample weight used was around 0.1 g, and methanol solution was 25 mL, which decreased the material cost. In addition, the main compounds from dandelion could be determined within 15 min through the fingerprint analysis, which could decrease the HPLC checking time.

The extraction in this study was based on the enzymatic hydrolysis and ultrasound–assisted extraction [WANG *et al.*, 2017; ZHANG *et al.*, 2017].

According to the optimization results, in view of cost–saving, the process (I) would be the optimum extraction technology, i.e. methanol 40%, liquid– material ratio 200, and extraction time 120 minutes; if considering the actual extraction yield and comprehensive quality, the process (II) would be the optimal extraction technology, i.e. methanol 70 %, liquid–material ratio 300 and extraction time 120 minutes.

**Effect of cellulase and centrifugal operation on the extraction results.**

Considering the incomplete enzymatic hydrolysis or water loss during the process of water bath may lead to the inaccurate of the extraction results, the caffeic acid contents the recommended standard in the China Pharmacopoeia (2015 Edition), were compared under different operations (Table 4).

**Table 4.**

Caffeic acid content under different extraction operations

| | Twice fixed volume after centrifugation | Non– volume fixed |
|---|---|---|
| Adding cellulase | (0.48±0.017) % | (0.59±0.018) % |
| Non–cellulase | (0.35±0.05) % | (0.39±0.064) % |

Results showed that the deviation in the treatments of the adding of cellulase were the lowest regardless of whether twice fixed water volume after centrifuge. It indicated that the cellulase enhanced the stability of the extraction process.

The content of caffeic acid was decreased when fixing the water volume after centrifuge, which meant that the operation of twice fixed water volume after centrifuge increased the absolute error. Therefore, the operation of twice fixed water volume after centrifuge was unnecessary, but it should decrease the loss of evaporation as much as possible in the process of water bath.

## Conclusions

This study optimized the phenolic acid extraction method for providing a high efficiency extraction method for the determination of phenolic acids from dandelion. The key parameters for the recommended extraction conditions were that: the dry dandelion leaf powder of 0.083 g, liquid–material ratio of 300, methanol concentration of 40 % and ultrasonic extraction time of 120 min at 60 °C and the input of 5 mL cellulase solution (0.1 %, pH =4).

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**Appendices**

Appended Table 1: 25 solution schemes with the highest extraction rate for caffeic acid

Appended Table 2: ANOVA analysis for the Cubic model fitting for chlorogenic acid

Appended Table 3: 22 solution schemes with the highest extraction rate for chlorogenic acid

Appended Table 4: ANOVA analysis for the Cubic model fitting for total caffeic acid

Appended Table 5: 27 solution schemes with the highest extraction rate for total caffeic acid

Appended Table 6: Fingerprint similarity analysis for the 17 extraction treatments

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**Appended** Table 1.

25 solution schemes with the highest extraction rate for caffeic acid

| Number | Methanol | Liquid–material | Time | Caffeic acid | Desirability |
|---|---|---|---|---|---|
| 1 | 40 | 200.7 | 120 | 7.7 | 1 |
| 2 | 40 | 202.7 | 120 | 7.7 | 1 |
| 3 | 40 | 206.5 | 120 | 7.69 | 0.998 |
| 4 | 40 | 194 | 120 | 7.69 | 0.997 |
| 5 | 40.2 | 201 | 120 | 7.67 | 0.993 |
| 6 | 41.2 | 218.3 | 120 | 7.41 | 0.934 |
| 7 | 70 | 300 | 120 | 7.19 | 0.884 |
| 8 | 70 | 297.8 | 120 | 7.14 | 0.872 |
| 9 | 69 | 300 | 120 | 7.09 | 0.858 |
| 10 | 69.4 | 297.8 | 120 | 7.06 | 0.856 |
| 11 | 70 | 300 | 109.7 | 6.96 | 0.832 |
| 12 | 70 | 100 | 119.9 | 6.77 | 0.789 |
| 13 | 70 | 277.7 | 120 | 6.72 | 0.777 |
| 14 | 69.5 | 100 | 120 | 6.64 | 0.759 |
| 15 | 62.7 | 300 | 30 | 6.58 | 0.745 |
| 16 | 62.8 | 300 | 30 | 6.58 | 0.745 |
| 17 | 63.1 | 300 | 30 | 6.58 | 0.744 |
| 18 | 61.8 | 299.5 | 30 | 6.57 | 0.742 |
| 19 | 63.2 | 300 | 31.2 | 6.56 | 0.741 |
| 20 | 61.1 | 300 | 30 | 6.56 | 0.74 |
| 21 | 62.5 | 297.1 | 30 | 6.55 | 0.739 |
| 22 | 64.6 | 300 | 30 | 6.557 | 0.738 |
| 23 | 63.7 | 300 | 32.2 | 6.54 | 0.737 |
| 24 | 62.9 | 300 | 33.4 | 6.53 | 0.735 |
| 25 | 60.3 | 300 | 30 | 6.53 | 0.733 |

**Appended Table 2.**

ANOVA analysis for the Cubic model fitting for chlorogenic acid

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Model | 1.06 | 12 | 0.088 | 18.69 | 0.0061 |
| A | 0.046 | 1 | 0.046 | 9.66 | 0.036 |
| B | 0.26 | 1 | 0.26 | 54.96 | 0.002 |
| C | 8.62E–05 | 1 | 8.62E–05 | 0.018 | 0.899 |
| AB | 0.012 | 1 | 0.012 | 2.54 | 0.186 |
| AC | 0.022 | 1 | 0.022 | 4.75 | 0.0947 |
| BC | 5.20E–05 | 1 | 5.20E–05 | 0.011 | 0.9215 |
| A^2 | 0.013 | 1 | 0.013 | 2.73 | 0.1738 |
| B^2 | 0.16 | 1 | 0.16 | 34.33 | 0.0042 |
| C^2 | 1.47E–03 | 1 | 1.47E–03 | 0.31 | 0.6062 |
| A^2B | 0.03 | 1 | 0.03 | 6.27 | 0.0664 |
| A^2C | 0.1 | 1 | 0.1 | 22.01 | 0.009 |
| AB^2 | 0.25 | 1 | 0.25 | 53.55 | 0.002 |
| Pure Error | 0.019 | 4 | 4.72E–03 | | |
| Cor Total | 1.08 | 16 | | | |

Note: A represented the methanol concentration; B represented the liquid–material ratio; C represented the extraction time.

**Appended Table 3.**

22 solution schemes with the highest extraction rate for chlorogenic acid

| Number | Methanol | Liquid–material | Time | chlorogenic acid | Desirability |
|---|---|---|---|---|---|
| 1 | 40 | 203.7 | 120 | 3.4 | 1 |
| 2 | 40 | 209.2 | 119.8 | 3.4 | 1 |
| 3 | 40 | 200 | 120 | 3.4 | 1 |
| 4 | 40 | 210.9 | 120 | 3.4 | 1 |
| 5 | 40 | 207.7 | 119.7 | 3.4 | 1 |
| 6 | 40 | 211.9 | 120 | 3.4 | 1 |
| 7 | 40 | 205.9 | 119.7 | 3.4 | 1 |
| 8 | 40 | 204.7 | 120 | 3.4 | 1 |
| 9 | 70 | 300 | 120 | 3.39 | 0.986 |
| 10 | 70 | 300 | 119.5 | 3.39 | 0.985 |
| 11 | 40 | 225.7 | 120 | 3.38 | 0.983 |
| 12 | 40.6 | 212.4 | 120 | 3.38 | 0.982 |
| 13 | 70 | 300 | 118.7 | 3.38 | 0.982 |
| 14 | 70 | 298.7 | 120 | 3.38 | 0.979 |
| 15 | 69.4 | 300 | 120 | 3.37 | 0.972 |
| 16 | 69.1 | 300 | 120 | 3.37 | 0.965 |
| 17 | 70 | 292.1 | 120 | 3.35 | 0.943 |
| 18 | 70 | 280.7 | 120 | 3.29 | 0.886 |
| 19 | 65.89 | 300 | 96.7 | 3.27 | 0.864 |
| 20 | 66.7 | 300 | 90.5 | 3.27 | 0.864 |
| 21 | 70 | 300 | 70.1 | 3.23 | 0.812 |
| 22 | 63.5 | 300 | 53.6 | 3.21 | 0.794 |

**Appended Table 4.**

ANOVA analysis for the Cubic model fitting for total caffeic acid

| Source | SS | df | MS | F | p |
|---|---|---|---|---|---|
| Model | 24.21 | 12 | 2.02 | 7.48 | 0.0331 |
| A | 0.89 | 1 | 0.89 | 3.31 | 0.1429 |
| B | 3.13 | 1 | 3.13 | 11.61 | 0.0271 |
| C | 0.14 | 1 | 0.14 | 0.54 | 0.5049 |
| AB | 0.046 | 1 | 0.046 | 0.17 | 0.7001 |
| AC | 1.09 | 1 | 1.09 | 4.05 | 0.1145 |
| BC | 0.01 | 1 | 0.01 | 0.037 | 0.8567 |
| A^2 | 0.12 | 1 | 0.12 | 0.44 | 0.5454 |
| B^2 | 3.48 | 1 | 3.48 | 12.91 | 0.0229 |
| C^2 | 0.26 | 1 | 0.26 | 0.98 | 0.3783 |
| A^2B | 1.07 | 1 | 1.07 | 3.98 | 0.1168 |
| A^2C | 3.32 | 1 | 3.32 | 12.29 | 0.025 |
| AB^2 | 8.53 | 1 | 8.53 | 31.62 | 0.005 |
| Pure Error | 1.08 | 4 | 0.27 | | |
| Cor Total | 25.28 | 16 | | | |

Note: A represented the methanol concentration; B represented the liquid–material ratio; C represented the extraction time.

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**Appended Table 5.**

27 solution schemes with the highest extraction rate for total caffeic acid

| Number | Methanol | Liquid–material | Time | Total yield | Desirability |
|---|---|---|---|---|---|
| 1 | 40 | 202.7 | 120 | 9.55 | 1 |
| 2 | 40 | 200 | 120 | 9.55 | 1 |
| 3 | 40 | 200.9 | 120 | 9.55 | 1 |
| 4 | 40 | 203.6 | 120 | 9.55 | 1 |
| 5 | 40 | 194.3 | 120 | 9.53 | 0.997 |
| 6 | 40 | 204.2 | 118.3 | 9.47 | 0.984 |
| 7 | 70 | 300 | 119.9 | 9.03 | 0.894 |
| 8 | 69.9 | 300 | 120 | 9.02 | 0.891 |
| 9 | 70 | 298 | 120 | 8.98 | 0.884 |
| 10 | 69.1 | 300 | 120 | 8.91 | 0.87 |
| 11 | 69.2 | 298.9 | 120 | 8.9 | 0.866 |
| 12 | 70 | 300 | 106.8 | 8.72 | 0.831 |
| 13 | 70 | 100 | 120 | 8.4 | 0.765 |
| 14 | 70 | 100 | 118.9 | 8.38 | 0.762 |
| 15 | 62.6 | 300 | 30 | 8.31 | 0.746 |
| 16 | 62.9 | 300 | 30.1 | 8.3 | 0.746 |
| 17 | 62.4 | 300 | 30 | 8.3 | 0.746 |
| 18 | 63 | 300 | 30.1 | 8.3 | 0.746 |
| 19 | 62.7 | 300 | 31.3 | 8.29 | 0.743 |
| 20 | 62.4 | 300 | 32.8 | 8.27 | 0.739 |
| 21 | 62.6 | 300 | 33.4 | 8.27 | 0.738 |
| 22 | 60.3 | 300 | 30 | 8.25 | 0.735 |
| 23 | 61.5 | 300 | 33.8 | 8.24 | 0.733 |
| 24 | 62.8 | 300 | 35.8 | 8.24 | 0.732 |
| 25 | 65.8 | 300 | 30 | 8.21 | 0.727 |
| 26 | 60.8 | 285.3 | 30 | 8.19 | 0.723 |
| 27 | 65.8 | 300 | 61.4 | 8.09 | 0.701 |

**Appended Table 6.**

Fingerprint similarity analysis for the 17 extraction treatments

| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 | **CM** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S1 | 1 | | | | | | | | | | | | | | | | | |
| S2 | 0.995 | 1 | | | | | | | | | | | | | | | | |
| S3 | 0.994 | 0.998 | 1 | | | | | | | | | | | | | | | |
| S4 | 0.995 | 0.997 | 0.999 | 1 | | | | | | | | | | | | | | |
| S5 | 0.985 | 0.989 | 0.983 | 0.98 | 1 | | | | | | | | | | | | | |
| S6 | 0.996 | 1 | 0.997 | 0.998 | 0.986 | 1 | | | | | | | | | | | | |
| S7 | 0.997 | 0.998 | 0.997 | 0.998 | 0.982 | 0.999 | 1 | | | | | | | | | | | |
| S8 | 0.995 | 0.998 | 1 | 1 | 0.983 | 0.997 | 0.998 | 1 | | | | | | | | | | |
| S9 | 0.993 | 0.998 | 0.999 | 0.999 | 0.987 | 0.998 | 0.997 | 0.999 | 1 | | | | | | | | | |
| S10 | 0.99 | 0.992 | 0.997 | 0.997 | 0.975 | 0.992 | 0.993 | 0.997 | 0.996 | 1 | | | | | | | | |
| S11 | 0.997 | 0.999 | 0.997 | 0.998 | 0.985 | 1 | 1 | 0.998 | 0.998 | 0.993 | 1 | | | | | | | |
| S12 | 0.992 | 0.999 | 0.998 | 0.996 | 0.989 | 0.998 | 0.996 | 0.997 | 0.998 | 0.992 | 0.997 | 1 | | | | | | |
| S13 | 0.99 | 0.996 | 0.998 | 0.996 | 0.985 | 0.995 | 0.994 | 0.997 | 0.998 | 0.998 | 0.994 | 0.996 | 1 | | | | | |
| S14 | 0.995 | 0.995 | 0.999 | 0.999 | 0.976 | 0.995 | 0.997 | 0.999 | 0.997 | 0.997 | 0.996 | 0.994 | 0.995 | 1 | | | | |
| S15 | 0.985 | 0.994 | 0.995 | 0.993 | 0.987 | 0.992 | 0.99 | 0.995 | 0.996 | 0.995 | 0.991 | 0.995 | 0.999 | 0.99 | 1 | | | |
| S16 | 0.967 | 0.983 | 0.982 | 0.977 | 0.983 | 0.979 | 0.974 | 0.98 | 0.984 | 0.981 | 0.976 | 0.985 | 0.99 | 0.972 | 0.994 | 1 | | |
| S17 | 0.993 | 0.998 | 1 | 0.999 | 0.985 | 0.997 | 0.996 | 1 | 1 | 0.997 | 0.997 | 0.998 | 0.998 | 0.997 | 0.996 | 0.984 | 1 | |
| CM | 0.994 | 0.998 | 0.999 | 0.999 | 0.986 | 0.998 | 0.997 | 0.999 | 0.999 | 0.997 | 0.998 | 0.998 | 0.999 | 0.997 | 0.997 | 0.986 | 1 | 1 |

Note: CM meant Control map, generated according to the comparison of each extraction treatment