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Extraction of *Chrysanthemum morifolium* Extractum by Ethanol Modified Supercritical CO_2

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In this study, ethanol modified supercritical CO_2 extraction of *Chrysanthemum morifolium* extractum was optimized by single factor and orthogonal array design methods with respect to the effects of extracting pressure, extracting temperature, extracting time, flow rate of CO_2, ratio of liquid to solid and ethanol concentration on the yield of *Chrysanthemum morifolium* extractum. The optimum extracting parameters were established with extracting pressure 30 MPa, extracting temperature 45 °C, flow rate of CO_2 10 kg h^{-1}, ratio of liquid to solid 15 mL g^{-1}, ethanol concentration 95 % and extracting time 2 h. Under such conditions, the yield of *Chrysanthemum morifolium* extractum was 29.34 %. The content of flavonoids in *Chrysanthemum morifolium* was 12.46 mg g^{-1}.

Keywords: *Chrysanthemum morifolium*, Supercritical CO_2 extraction, Extractum, Flavonoids.

INTRODUCTION

Chrysanthemum morifolium, which is the dry flower head of *Chrysanthemum morifolium* Ramat., has been widely used in China for over 2000 years. It is one of the important commodities in the medicine market at home and abroad. It is rich in Tongxiang and Haining of Zhejiang Province China, where the planting area of *Chrysanthemum morifolium* is more than 3000 hm^2 and the production of *Chrysanthemum morifolium* is 5×10^6 kg per year. *Chrysanthemum morifolium* has beneficial effects on treating eye diseases, headaches, insomnia and hyperglycemia[1].

Chrysanthemum morifolium extractum is one of the products extracted from *Chrysanthemum morifolium*. The flavonoids are the major component of *Chrysanthemum morifolium* extractum, modern pharmacological studies have shown that flavonoids have significant therapeutic effect on cardiovascular disease[2]. At present, the main methods of extracting flavonoids are ultrasonic-microwave assisted extraction[3], water bath extraction[4], negative-pressure cavitation extraction[5], dynamic high pressure microfluidization-assisted extraction[6], *etc*. Whereas, the study of supercritical carbon dioxide extraction of extractum from *Chrysanthemum morifolium* has not been reported yet. And the major methods of quantitative determining flavonoids are UV spectrophotometry[7] and HPLC[8], *etc*.

In this study, we extracted the extractum from *Chrysanthemum morifolium* by ethanol modified supercritical CO_2 and then determined the content of flavonoids in it by UV spectrophotometry.

EXPERIMENTAL

Supercritical carbon dioxide extraction device (model: HL-0.5/50MPaIIIA; Hangzhou Huali Pump Industry Co., Ltd); Rotary Evaporators (model: RE-52AA; Shanghai Yarong biochemical instrument equipment Co., Ltd); UV spectrophotometer (model: 2450; Shimadzu, Japan); Electronic analytical balance (model: AB204-N; Ohaus, America).

Chrysanthemum morifolium was purchased from Hangzhou Chinese Medicine Factory (Hangzhou, China). Carbon dioxide (99 %) was supplied by Hangzhou Minxing industrial and trading Co., Ltd. (Hangzhou, China). Ethanol (95 %, analytical grade) was supplied by Hangzhou Chemical Reagent Co., Ltd. (Hangzhou, China). Rutin (UV ≥ 98 %) was supplied by Shanghai Jinsui biological technology Co., Ltd (Shanghai, China).

Extraction of *Chrysanthemum morifolium* extractum: 10 g of *Chrysanthemum morifolium* powder was placed in the extraction vessel. After an initial air purge, liquefied carbon dioxide and modifier (ethanol) were pumped into the extraction vessel by a high-pressure pump to a given pressure, the temperature inside the vessel was raised to and maintained at the desired level by a heating jacket encasing the vessel and the CO_2 flow rate was maintained at a given amount. Fractional separation was obtained setting the separator at 5.0 MPa and 40 °C.

After the extraction was completed, the extraction vessel was depressurized and the extract was collected. The extract was transferred to a round bottom flask, spin-dried by a rotary evaporator, then weighed the extractum. The yield is defined in eqn. 1.

$$Y = \frac{\text{g of } \textit{Chrysanthemum morifolium} \text{ extractum}}{\text{g of } \textit{Chrysanthemum morifolium} \text{ powder}} \qquad (1)$$

Preparation of standard stock solution and drawing of calibration curves: The standard stock solution was prepared by dissolving rutin in 30 % (v/v) ethanol to make final concentration of 0.255 mg mL^{-1}. 1 mL of standard stock solutions, added 30 % (v/v) ethanol to 9 mL, 1 mL of 5 % (w/w) $NaNO_2$ were mixed for 6 min and then 1 mL of 10 % $AlCl_3$ (w/w) was added and mixed, after 6 min, 10 mL of 5 % (w/w) NaOH was added, finally, added 30 % ethanol to 25 mL. With 12 min standing, the absorbance of the solution was measured at 510 nm with UV spectrophotometer against the same mixture against 30 % ethanol as blank. Took 0.00-9.00 mL standard stock solution, repeated the operation above. The calibration curve was prepared by plotting absorbance *versus* concentration of rutin. The calibration curve was shown in Fig. 1.

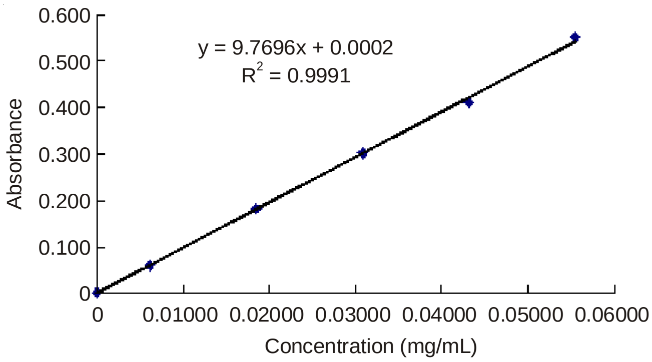

Fig. 1. Standard graph of rutin by UV spectrophotometric method

Determination of flavonides: *Chrysanthemum morifolium* extractum was put into a 100 mL clean dry volumetric flask and dissolved in 30 % ethanol. Took 1 mL of this solution into a 25 mL volumetric flask, then repeated the operation as drawing of calibration curves. The obtained solution was used to determine the concentration of rutin by UV spectrophotometric method. Then the amount of rutin was calculated using the calibration curve. The content of flavonoids was expressed with mg rutin/g *Chrysanthemum morifolium*.

RESULTS AND DISCUSSION

Influence of extracting time: The influence of extracting time on the yield of extractum was given in Fig. 2 under other identical extraction conditions. A gradual rise in yield was seen with increase in duration of extracting time. As seen from Fig. 2, in 1.5 h of extracting time, 16.68 % of yield is obtained and almost no changing thereafter while keeping extracting temperature at 40 °C, extracting pressure at 25 MPa, ratio of liquid to solid at 5 mL g^{-1}, CO_2 flow rate at 8 kg h^{-1} and ethanol concentration at 95 %.

Influence of ethanol concentration: Ethanol was used as an extracting modifier of supercritical CO_2. The influence

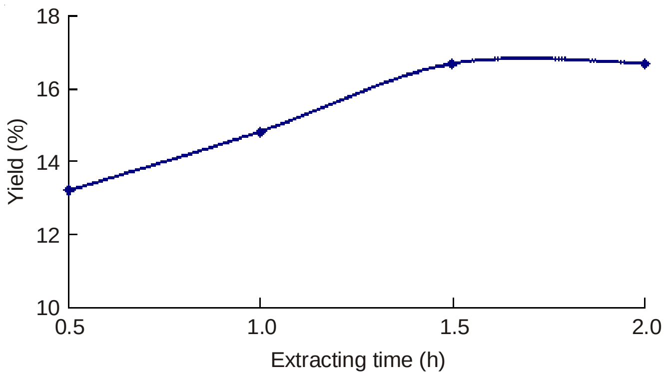

Fig. 2. Effects of extracting time on yield of *Chrysanthemum morifolium* extractum

of ethanol concentration on the yield of *Chrysanthemum morifolium* extractum was shown in Fig. 3 under other identical extraction conditions. The ethanol concentration was varied from 70 to 100 % while keeping extracting temperature at 40 °C, extracting pressure at 25 MPa, ratio of liquid to solid at 5 mL g^{-1}, CO_2 flow rate at 8 kg h^{-1} and extracting time at 1.5 h. The yield of *Chrysanthemum morifolium* extractum increases with ethanol concentration unremittingly, but in consideration of the high price of 100 % ethanol, we selected the 95 % ethanol as extracting modifier.

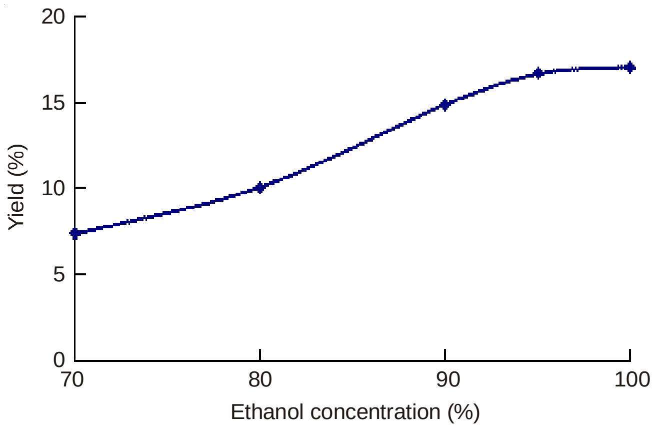

Fig. 3. Effects of ethanol concentration on yield of *Chrysanthemum morifolium* extractum

Influence of extracting pressure: The influence of extracting pressure on the yield of *Chrysanthemum morifolium* extractum was given in Fig. 4 under other identical extraction conditions. The extracting pressure was varied from 15 to 30 MPa while keeping extracting temperature at 40 °C, ratio of liquid to solid at 5 mL g^{-1}, CO_2 flow rate at 8 kg h^{-1}, ethanol concentration at 95 % and extracting time at 1.5 h. With the increase of pressure from 15 to 25 MPa, the yield of *Chrysanthemum morifolium* extractum increases from 10.65 to 16.68 %. With further increase in pressure, little decreased yield was observed.

Influence of extracting temperature: The effect of extracting temperature was shown in Fig. 5. Extracting tempe-rature was varied from 35 to 50 °C keeping extracting pressure at 25 MPa, ratio of liquid to solid at 5 mL g^{-1}, CO_2 flow rate at 8 kg h^{-1}, ethanol concentration at 95 % and extracting time

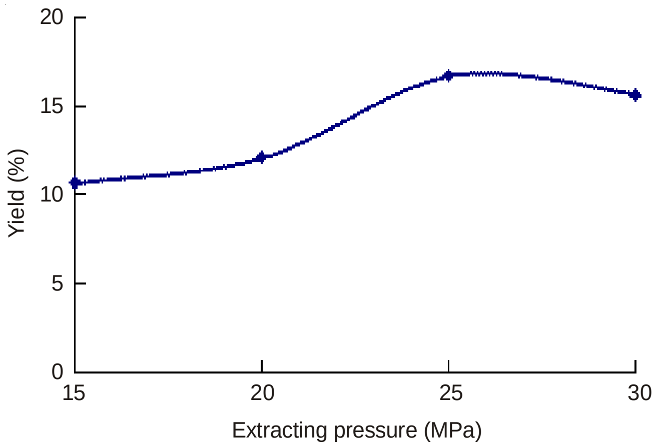

Fig. 4. Effects of extracting pressure on yield of *Chrysanthemum morifolium* extractum

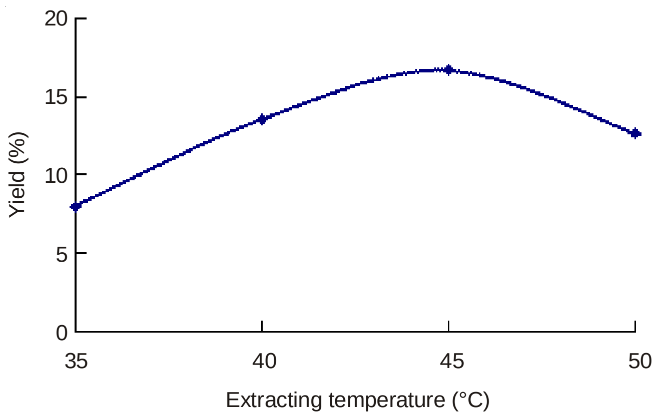

Fig. 5. Effects of extraction temperature on yield of *Chrysanthemum morifolium* extractum

at 1.5 h. With the increase in temperature from 35 to 45 °C, the yield of *Chrysanthemum morifolium* extractum increases from 7.98 to 16.68 %. With further increase in temperature, the yield was decreased.

Influence of CO_2 flow rate: The CO_2 flow rate was varied from 6 to 12 kg h^{-1} keeping extracting temperature at 40 °C, extracting pressure at 25 MPa, ratio of liquid to solid at 5 mL g^{-1}, ethanol concentration at 95 % and extracting time at 1.5 h. The results were shown in Fig. 6. The yield of *Chrysanthemum morifolium* extractum reaches a maximum at 8 kg h^{-1}.

Influence of ratio of liquid to solid: The ratio of ethanol to *Chrysanthemum morifolium* powder (mL g^{-1}) was varied from 5 to 17 keeping extracting temperature at 40 °C, extracting pressure at 25 MPa, CO_2 flow rate at 8 kg h^{-1}, ethanol concentration at 95 % and extracting time at 1.5 h. The results were represented in Fig. 7. The yield of *Chrysanthemum morifolium* extractum reaches a maximum at 13 mL g^{-1} and almost no changing thereafter.

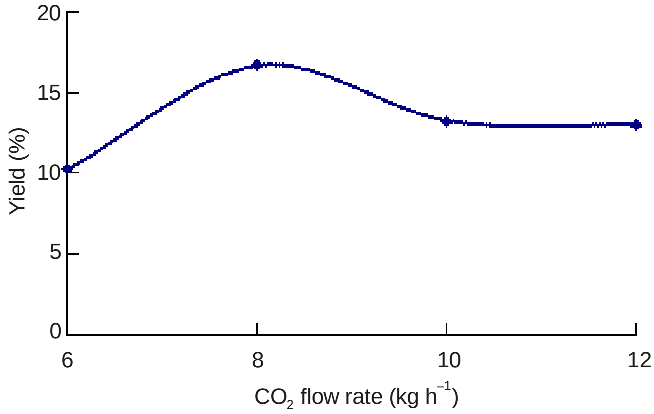

Fig. 6. Effects of carbon dioxide flow rate on yield of *Chrysanthemum morifolium* extractum

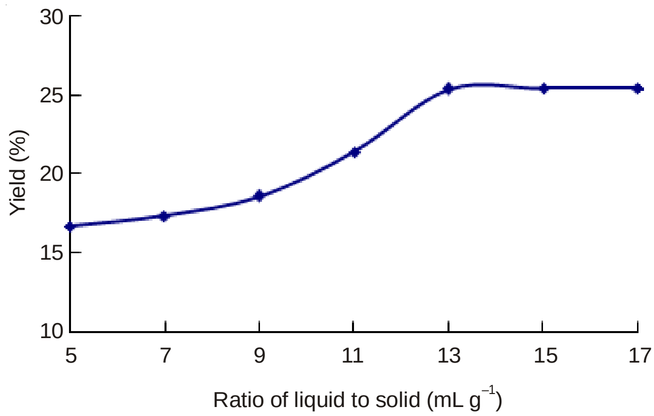

Fig. 7. Effects of liquid to solid ratio on yield of *Chrysanthemum morifolium* extractum

Orthogonal array design (OAD): In order to fully examine the effects of extraction conditions, an orthogonal array design test was employed as a chemometric method for investigating yield of extractum from *Chrysanthemum morifolium* after single-factor experiments. As listed in Table-1, five processing parameters, namely, ratio of liquid to solid (A), extracting time (B), extracting pressure (C), CO_2 flow rate (D) and extracting temperature (E) were studied and optimized.

The experimental results were listed in Table-2. The result was a ranked list of those conditions that make the biggest difference as follow: ratio of liquid to solid > extracting temperature > extracting time > extracting pressure > CO_2 flow rate. The results indicated that the optimal conditions for extraction of extractum from *Chrysanthemum morifolium* was $A_3B_3C_3D_3E_2$, namely 15 mL g^{-1} of liquid to solid ratio, 2 h of extracting time, 30 MPa of extracting pressure, 10 kg h^{-1} of CO_2 flow rate and 45 °C of extracting temperature.

Verification tests: Under the optimal conditions, 10 g sample was extracted by ethanol modified supercritical CO_2

TABLE-1
FACTORS AND LEVELS OF ORTHOGONAL ARRAY DESIGN

Level	A Ratio of liquid to solid (mL g^{-1})	B Extracting time (h)	C Extracting pressure (MPa)	D CO_2 flow rate (kg h^{-1})	E Extracting temperature (°C)
1	11	1	20	6	40
2	13	1.5	25	8	45
3	15	2	30	10	50

TABLE-2
RESULTS OF ORTHOGONAL TEST AND ANALYSIS

No.	A	B	C	D	E	Yield (%)
1	1	1	1	1	1	16.72
2	1	2	2	2	2	18.27
3	1	3	3	3	3	16.41
4	2	1	1	2	2	23.04
5	2	2	2	3	3	25.37
6	2	3	3	1	1	25.54
7	3	1	2	1	3	21.88
8	3	2	3	2	1	28.04
9	3	3	1	3	2	27.44
10	1	1	3	3	2	20.75
11	1	2	1	1	3	13.38
12	1	3	2	2	1	20.65
13	2	1	2	3	1	21.47
14	2	2	3	1	2	26.47
15	2	3	1	2	3	22.69
16	3	1	3	2	3	22.01
17	3	2	1	3	1	26.97
18	3	3	2	1	2	30.93
k_1	17.70	20.98	21.71	22.49	23.23	-
k_2	24.10	23.08	23.09	22.45	24.48	-
k_3	26.21	23.94	23.20	23.07	20.29	-
R	8.51	2.96	1.49	0.62	4.19	-

for three times, the yields of *Chrysanthemum morifolium* extractum were 28.95, 29.02 and 30.05 %, the average yield was 29.34 %. The yield extracted under the optimal conditions was higher than any other set.

Content of flavonoids: Put the products of validation tests into 100 mL clean dry volumetric flasks and dissolved with 30 % ethanol. Took 1 into 25 mL volumetric flask separately, then repeated the operation as drawing of calibration curves. The absorbances of the sample solutions were 0.474, 0.477, 0.510 and then calculated the content of flavonoids in each product were 121.24, 122.01 mg and 130.46 mg according to the calibration curve. The average content of flavonoids in *Chrysanthemum morifolium* was 12.46 mg g^{-1}.

Conclusion

The optimum extracting parameters were established with extracting pressure 30 MPa, extracting temperature 45 °C, CO_2 flow rate10 kg h^{-1}, ratio of liquid to solid 15 mL g^{-1} and ethanol concentration 95 % for an extraction duration of 2 h. The optimum technology for ethanol modified supercritical CO_2 extraction of extractum from *Chrysanthemum morifolium* was high efficient, extraction yield was 29.34 %. The content of flavonoids in *Chrysanthemum morifolium* was 12.46 mg g^{-1}.

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