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Optimization of extraction of evodiamine and rutaecarpine from fruit of *Evodia rutaecarpa* using modified supercritical CO_2

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ABSTRACT

Evodiamine and rutaecarpine have been intensively studied due to their pharmacological actions and clinical applications. In this report, supercritical fluid was used to extract evodiamine and rutaecarpine from the unripe fruit of *Evodia rutaecarpa*. Response surface methodology using Box–Behnken experimental design was utilized to optimize parameters for supercritical carbon dioxide extraction with methanol as co-solvent. The effect of various values of dynamic extraction time (30–90 min), temperature (50–70 °C) and pressure (200–400 bar) on extraction yields of the two compounds was evaluated. Determinations of the extracts were performed by high-performance liquid chromatography. The experimental data obtained were fitted to second-order polynomial equations and analyzed by analysis of variance. The highest yields predicted were 1.217 mg/g for evodiamine and 0.969 mg/g for rutaecarpine at the optimal values (time 78 min, temperature 62 °C, pressure 280 bar and co-solvent flow rate 0.4 mL/min), based on the selected range of experimental conditions.

1. Introduction

Evodia rutaecarpa is a medicinal plant and is commonly distributed in East Asia, especially in China. The unripe fruit of *E. rutaecarpa*, called as Wu Zhu Yu in Chinese, has been utilized in traditional Chinese medicine in alleviating pain, stopping vomiting, and stopping diarrhea for a long time [1,2]. The therapeutic effects are considered to be pertinent to the alkaloids occurring in the fruit. The major alkaloids are two indolequinazoline alkaloids evodiamine and rutaecarpine (Fig. 1). In recent studies, it has been reported that evodiamine exerts several pharmacological effects, namely, anti-tumor activities [3,4], antiobesity effects [5], protection against myocardial ischemia-reperfusion injury [6], inhibition of adipogenesis [7], and that rutaecarpine can induce CYP1A1 expression [8]. The ethanol extract has anti-inflammatory action [9]. Both of alkaloids have the effect of suppression of NADPH oxidase activation [10], possess thermoregulation, vascular regulation, anti-allergic, anti-nociceptive and anti-inflammatory activities and inhibit corticosterone production [11].

Supercritical fluid extraction (SFE) with carbon dioxide has gained much attention. It is reported that more than 200 references deal with SFE in the last two years [12]. The interest of SFE is not only as an analytical tool but also for process development, due to its fast and effective extraction of different kinds of compounds in mild conditions and its solvating power being easily manipulated by changes in pressure and temperature. With respect to polar compounds, pure supercritical CO_2 does not have sufficient solvation power, and therefore a polar co-solvent has to be added to CO_2 in order to increase the compounds solubility. Added co-solvents are often methanol, ethanol, 1,2-propanediol, and so on [13–15].

Response surface methodology (RSM) is a statistical method. This method uses quantitative data from an appropriate experimental design to evaluate the response of the statistically designed combinations, to estimate the coefficients by fitting it in a mathematical model that fits best the experimental conditions, to predict the response of the fitted model, to check the adequacy of the mode, and to search optimum condition of factors [16]. As an experimental design, it may minimize assay numbers and time to keep the experimental cost at a minimum level with the possibility of revealing optimum information in studied experimental domain, and it has been applied in various experiments [17–20].

According to Chinese Pharmacopoeia standard, the content of evodiamine and rutaecarpine in the unripe fruit of *E. rutaecarpa* must be higher than 0.15% for medical use [1]. It is necessary to offer a suitable method of quality evaluation for the medicinal plant. A few studies have been reported on extraction and analysis of

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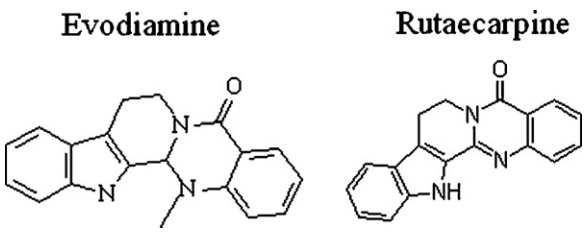

Fig. 1. Structure of evodiamine and rutaecarpine.

the two alkaloids from various materials. For example, evodiamine and rutaecarpine were extracted from plant material and biological sample using organic solvent with subsequent analysis by HPLC [21–23], HPLC–MS [24] as well as counter-current chromatography [25]. As a quick and effective extraction technique, supercritical CO_2 extraction, using ethanol as co-solvent has been reported for extraction of essential oil and alkaloids from *Fructus evodiae* [26,27]. However, the effect of extraction parameters on the yields of the two alkaloids and the optimum extraction conditions has not been subjected to a thorough study with RSM. Furthermore, there has been no report on using methanol as a co-solvent for supercritical CO_2 extraction of the two alkaloids. Therefore, the aim of this study was to develop a SFE process, to optimize the conditions, using RSM, in supercritical CO_2 extraction with methanol as co-solvent, and to evaluate the factors which influence the yields of evodiamine and rutaecarpine for SEF from the fruit of *E. rutaecarpa*.

2. Experimental

2.1. Materials and reagents

The fruit of *E. rutaecarpa* was obtained from Tong-ren-tang Pharmacy (Ningbo, China). The fruit was ground into powder using a herbal pulverizer (FW 100, Tianjin Taisite Instrument Co. Ltd, Tianjia, China) and sieved through a 250 μm filter for extraction later. Evodiamine and rutaecarpine standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). CO_2 (99.5% purity) was from Fangxin Gas Ltd. (Ningbo, China). Acetonitrile of HPLC grade was purchased from Tianjin Shield Company (Tianjin, China). Methanol, tetrahydrofuran and acetic acid were analytical grade and were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Celite (Chemical grade) was from Fengcheng Chemical Ltd. (Shanghai, China).

2.2. Supercritical fluid extraction

A supercritical fluid extractor Spe-ed SFE-2 (Applied Separation, USA) was used, which operates with two pumps, a master pump for delivery of CO_2 and a second pump (Knauer pump, model K-501, Berlin, Germany) for the addition co-solvent. An accurately weighed quantity of grounded sample (about 1 g) was placed in a 10 mL of extraction vessel (60 mm × 15 mm, i.d.) and the void volume was filled with celite. Before the extraction was started, the extraction vessel was preheated in the oven for 10 min. The extraction conditions were as follows: extraction time, static extraction for 5 min and then dynamic extraction up to 90 min; temperature, from 50 to 70 °C; pressure, from 200 to 400 bar, flow-rate of carbon dioxide (gaseous state), 2 L/min; flow-rate of co-solvent methanol, 0.4 mL/min. Collection is at room temperature and atmospheric pressure. The extracts are collected in glass vials (30 mL containing 4 mL of methanol) with a rubber plug at the top. A metal extension to the metering valve is used to pierce the rubber plug and allow collection directly in the collection solvent. A hypodermic needle is pierced through the plug and is connected to a flow meter. The extracts were quantitatively transferred to a 25 mL volumetric flask and made up to the mark with methanol. This solution was analyzed with HPLC. Additionally, the part of solution was dried and weighted for the purity of determination.

2.3. Soxhlet extraction

A known quantity of grounded sample (2.0 g) was accurately weighed into a thimble and was extracted in a 50-mL of extractor with 50 mL of methanol at a syphon rate of 1 cycle/15 min. After 7 h of extraction with the solvent, the extraction solvent was essentially colorless and the extracts were transferred to a 50 mL volumetric flask and made up to the mark with methanol. This solution was analyzed.

All extracts were filtered through a 0.45-μm membrane filter before injecting into the HPLC system.

2.4. HPLC analysis

A high-performance liquid chromatography system (Hitachi, Japan) equipped with a Hitachi pump (model L-2130) and an ultraviolet–visible detector (Hitachi, model L-2400) was used. The column used for separation was a Diamonsil C18 separation column (5 μm, 250 mm × 4.6 mm i.d., Dikma Technology, Beijing, China). The mobile phase was acetonitrile:water:tetrahydrofuran:acetic acid (51:48:1:0.1, v/v/v/v) at a flow-rate of 1 mL/min. Detection was at a wavelength of 254 nm and column temperature was ambient. For all experiments, 20 μL of standards and sample extract were injected.

Evodiamine and rutaecarpine content was determined by referring to the calibration curve established by running evodiamine and rutaecarpine standard at varying concentrations through the HPLC system under the same conditions. The calibration curve was linear from 2.16 to 64.8 μg/mL ($y = -55{,}258 + 37{,}654x$, $r = 0.999$, $n = 5$), and from 2.2 to 66 μg/mL ($y = -64{,}797 + 53{,}080x$, $r = 0.999$, $n = 5$) for evodiamine and rutaecarpine, respectively.

The intra- and interday precision was evaluated by a standard mixture solution of the two alkaloids under the selected chromatography conditions with five replicates in a day for intraday precision and once a day on three consecutive days for interday precision. RSD was taken as a measure of the intra- and interday precisions, which were 2.26 and 3.47% for evodiamine, and 3.17 and 3.35% for rutaecarpine, respectively. Standard addition test was performed to determine recovery in triplicates for each level at two concentrations level. The determined recoveries for evodiamine and rutaecarpine were 98.4 and 103.2% with RSD 3.68 and 4.52%, respectively.

2.5. Experimental design and evaluation

A Box–Behnken experimental design with three variables at three levels was used to determine the response pattern and the interaction effect of the independent variables on the responses. For evaluating the effects of variables at three levels on the extraction efficiency of evodiamine and rutaecarpine with supercritical CO_2 + methanol, the selected three variables and three levels were dynamic extraction time (X_1), extraction temperature (X_2), extraction pressure (X_3). Variables and levels tested are reported in Table 1. The software Design Expert (Stat-Ease Inc., Minneapolis, MN, USA) was employed for experimental design, data analysis, and model building. The experimental design used for the study is shown in Table 2. The experiments were performed at random, while the yields of evodiamine and rutaecarpine obtained were taken as the dependent variables. Five replicates at the center of the design were used to allow for estimation of a pure error sum of squares.

Table 1
Variables and experimental design levels for response surface.

Independent variables	Coded symbols	Levels		
		−1	0	1
Extraction time (min)	X_1	30	60	90
Extraction temperature (°C)	X_2	50	60	70
Extraction pressure (bar)	X_3	200	300	400

For statistical calculations, the relation between the coded values and actual values are described as the following equation:

$$X_i = \frac{Z_i - Z0}{\Delta Z} \qquad i = 1,\ 2,\ 3 \tag{1}$$

where X_i is a coded value of the variable; Z_i is the actual value of variable; Z_0 is the actual value of the Z_i at the center point; and ΔZ is the step change of variable.

The relationship between the response and the independent variables was calculated by the second-order polynomial equation (Eq. (2)). The non-linear computer-generated quadratic model is used for this model:

$$Y = \beta_0 + \sum_{i=0}^{3}\beta_i x_i + \sum_{i=0}^{3}\beta_{ii}X_i^2 + \sum_{i=0}^{3}\sum_{j=1}^{3}\beta_{ij}X_iX_j \tag{2}$$

where Y is the predicted response; X_i and X_j are independent variables which influence the response variable Y; β_0 is the offset term; β_i is the ith linear coefficient; β_{ii} is the ith quadratic coefficient; and β_{ij} is the ijth interaction coefficient.

3. Results and discussion

3.1. HPLC chromatogram of extract

Typical HPLC chromatogram of the sample extracts obtained by supercritical CO_2 + methanol is shown in Fig. 2. Based on the available standard of evodiamine and rutaecarpine, it is possible to identify the both of peaks, which appear at a retention time of approximately 13.4 min and 16.3 min, respectively.

3.2. Selection of co-solvent

Extraction yields of evodiamine and rutaecarpine, using supercritical CO_2 only, are low, due to the two compounds being polar compounds. As a result, polar modifier should be considered. Addition of a small amount of polar co-solvent can enhance significantly the extraction efficiency and, consequently, reduce the extraction time. The method has been used to improve recovery of some polar compounds from natural products, such as alkaloid [28], isoflavone [29], saponin [30].

Since the nature of the co-solvent may influence the extraction yields and the selectivity of the two alkaloids from plant sample, preliminary assays using different co-solvents during supercritical CO_2 extraction were performed at a temperature of 60 °C, pressure of 30 MPa, and extraction time of 60 min except that the extraction with pure supercritical CO_2 was at a temperature of 40 °C, pressure of 40 MPa, and extraction time of 60 min. Fig. 3 shows the extraction yields of evodiamine and rutaecarpine from fruit of *E. rutaecarpa*, and Fig. 4 displays the purity of evodiamine and rutaecarpine in the extracts obtained with various co-solvents (methanol, 50% methanol, 70% methanol, and 95% ethanol). The quality of CO_2 + various co-solvent extracts of *E. rutaecarpa* fruit differed markedly. The solution obtained with CO_2 extraction was clear and colorless. The solution obtained with CO_2 + methanol or

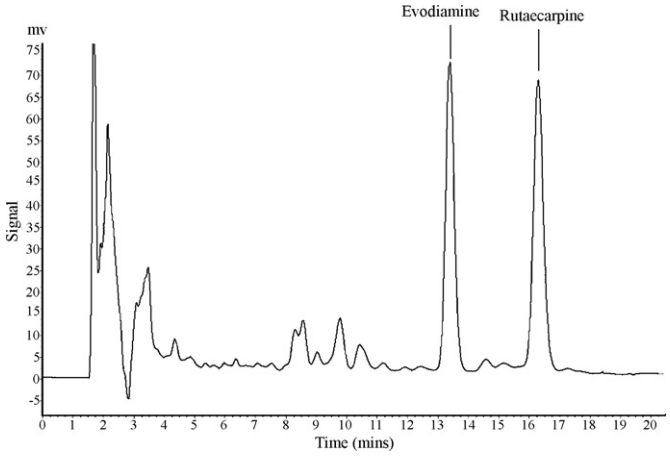

Fig. 2. High performance liquid chromatogram of the extract obtained by supercritical CO_2 + methanol at 280 bar, 62 °C and 78 min.

Table 2
The Box–Behnken experimental design and the responses for the yields of evodiamine and rutaecarpine.

Trail No.	X_1	X_2	X_3	Evodiamine yield (mg/g)	Rutaecarpine yield (mg/g)
1	−1	0	−1	1.015	0.756
2	0	1	1	1.061	0.854
3	0	1	−1	1.105	0.95
4	0	−1	1	0.869	0.674
5	1	1	0	1.147	0.893
6	1	0	−1	1.166	0.925
7	1	0	1	1.183	0.925
8	0	0	0	1.172	0.93
9	0	0	0	1.214	0.939
10	0	0	0	1.086	0.856
11	0	−1	−1	1.112	0.868
12	0	0	0	1.163	0.923
13	0	0	0	1.195	0.938
14	1	−1	0	1.029	0.918
15	−1	−1	0	0.809	0.612
16	−1	0	1	0.835	0.629
17	−1	1	0	1.004	0.799

Experimental values are mean of three determinations.

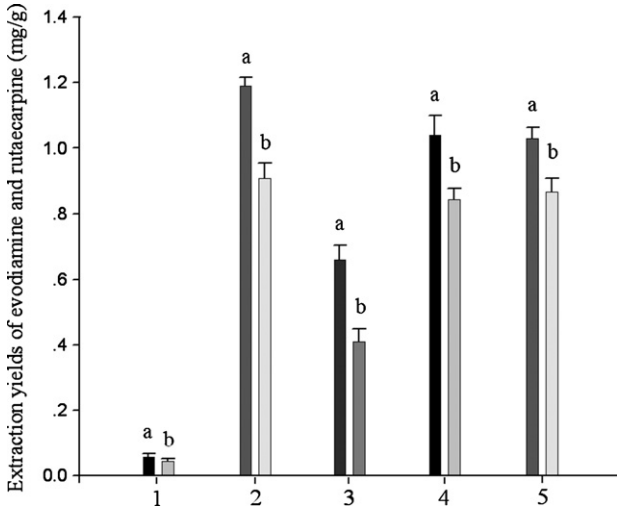

Fig. 3. The effect of different co-solvent on the yields of evodiamine and rutaecarpine. Extraction conditions with CO_2 + co-solvent: pressure of 300 bar, temperature of 60 °C, time of 60 min and co-solvent flow rate of 0.4 mL/min. Extraction conditions with pure CO_2: pressure of 400 bar, temperature of 40 °C and time of 60 min. (n = 3). 1. CO_2; 2. CO_2 + methanol; 3. CO_2 + 50% methanol; 4. CO_2 + 70% methanol; 5. CO_2 + 95% ethanol. (a) Evodiamine and (b) rutaecarpine.

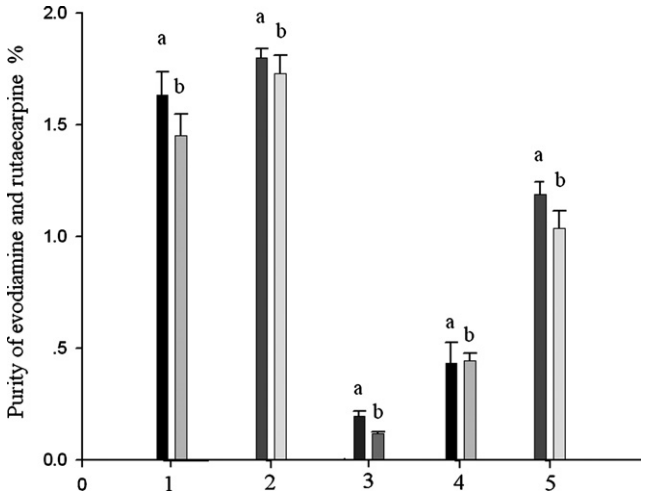

Fig. 4. The purity of evodiamine and rutaecarpine in extracts obtained by supercritical CO_2 with or without co-solvent. Extraction conditions with CO_2 + co-solvent: pressure of 300 bar, temperature of 60 °C, time of 60 min and co-solvent flow rate of 0.4 mL/min. Extraction conditions with pure CO_2: pressure of 400 bar, temperature of 40 °C and time of 60 min. (n = 3). 1. CO_2; 2. CO_2 + methanol; 3. CO_2 + 50% methanol; 4. CO_2 + 70% methanol; 5. CO_2 + 95% ethanol. (a) Evodiamine and (b) rutaecarpine.

CO_2 + 95% ethanol was green with a small amount of precipitation. In contrast, the solution obtained with CO_2 + 50% methanol or CO_2 + 70% methanol was dark brown and more turbid with significant amounts of sediments, indicating co-extraction of more substances. It can be observed that the highest extraction yield (1.19 mg/g and 0.91 mg/g, for evodiamine and rutaecarpine, respectively, in Fig. 3) and the highest purity (1.80% and 1.73%, for evodiamine and rutaecarpine, respectively, in Fig. 4) were obtained using methanol as co-solvent. The extraction yields for the two alkaloids significantly decreased from 1.04 and 0.84 mg/g to 0.66 and 0.41 mg/g for evodiamine and rutaecarpine, respectively, and the purity in the extracts also declined from 0.44% and 0.45% to 0.20% and 0.12% for the two alkaloids, respectively, when water content in methanol increased from 30% to 50%. This behaviour is often observed for the extraction of alkaloids. It might be explained by the solubility of alkaloids in different concentration of methanol. Since the solubility of evodiamine and rutaecarpine in methanol are much higher than in water, the addition of water to methanol will lower the solubility of the two alkaloids and increase the solubility of water soluble impurity, resulting in a decrease in the yield and purity of the two alkaloids.

Flow rate of the co-solvent may influence the recovery of target compounds from plant sample. Assays using different flow rate of methanol as co-solvents during supercritical CO_2 extraction were performed. Fig. 5 shows the effect of flow rate of the co-solvent on extraction yields of the two compounds. It was observed that the flow rate of methanol could influence the amount of evodiamine and rutaecarpine extracted. The yields of the two alkaloids obtained with the co-solvent at flow rate of 0.4 mL/min were 20 times higher than that without the co-solvent. The extraction yield was enhanced with the increase of flow rate up to 0.4 mL/min, and thereafter, the yield was not improved. This could be explained from the balance between co-solvent concentration in CO_2 and solute solubility. Initially, as the flow rate of co-solvent increased, the yield increased, since more alkaloids were solubilized with an increasing co-solvent concentration. However, at certain concentration level, the solubility of the alkaloids in solvent could decrease with a further increase in flow rate, due to a change in fluid phase, and the solvent may leave the system without dissolving all the alkaloids, resulting in decline in the yield. A similar finding about the effect of co-solvent concentration on yield was reported by Sun et al. [31], who found that lutein yield could be increased when canola oil was used as a continuous co-solvent, at low to medium concentrations of canola oil addition in CO_2 (1–3%). However, the yield decreased at higher concentrations of canola oil (4–5%). Therefore, the flow rate of methanol (0.4 mL/min) was selected as the quantity of co-solvent added in supercritical CO_2 extraction.

3.3. Model fitting

The quadratic model from the Box–Behnken design can be used to generate a response surface image for the main interaction among extraction time, temperature and pressure. The mathematical model describing the extraction yield of evodiamine (mg/g) ($Y1$) and rutaecarpine (mg/g) ($Y2$) as functions of the coded independent variables (Table 2) in the selected ranges was given by the following equation, respectively:

$$Y1\ (\mathrm{mg/g}) = 1.17 + 0.11X_1 + 0.062X_2 - 0.056X_3 - 0.078X_1^2 - 0.091X_2^2 - 0.038X_3^2 - 0.019X_1X_2 + 0.049X_1X_3 + 0.05X_2X_3$$

$$Y2\ (\mathrm{mg/g}) = 0.92 + 0.11X_1 + 0.053X_2 - 0.052X_3 - 0.070X_1^2 - 0.042X_2^2 - 0.039X_3^2 - 0.053X_1X_2 + 0.032X_1X_3 + 0.024X_2X_3$$

where X_1, X_2 and X_3 are the coded variables for extraction time, temperature and pressure, respectively.

The results were analyzed using ANOVA (Table 3). The statistical analysis indicated that the proposed model for evodiamine and rutaecarpine extraction was significant with p-value 0.0018 and 0.0008, respectively. The R^2 (coefficient of determination) value of the two models was determined to be 0.9388 and 0.9510, respectively. Meanwhile, the lack of fit was 0.5253 and 0.3648, respectively, which were not significant ($P > 0.05$). These values confirmed that the model was adequate for predicting the yields under any combination of values of the variables inside the experimental domain.

According to ANOVA, extraction time, temperature and pressure were found significant ($P < 0.05$) for evodiamine and rutaecarpine

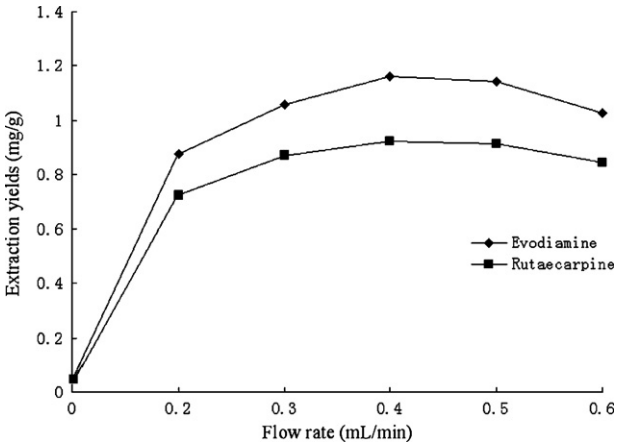

Fig. 5. The effect of flow rate of co-solvent methanol on yields of evodiamine and rutaecarpine at 300 bar, 60 °C and 60 min.

Table 3
Analysis of variance for the response surface quadratic model.

Source	Sum of squares	Degree of freedom	*F*-value	*P*-value	Coefficient of determination (R^2)
For evodiamine					
Model	0.24	9	11.93	0.0018	0.9388
X_1	0.093	1	40.96	0.0004	
X_2	0.031	1	13.67	0.0077	
X_3	0.025	1	11.16	0.0124	
$X_1 X_2$	1.482×10^{-3}	1	0.65	0.4454	
$X_1 X_3$	9.702×10^{-3}	1	4.28	0.0774	
$X_2 X_3$	9.900×10^{-3}	1	4.37	0.075	
X_1^2	0.0256	1	11.26	0.0122	
X_2^2	0.0345	1	15.34	0.0058	
X_3^2	6.201×10^{-3}	1	2.73	0.1422	
Residual	0.016	7			
Lack of fit	6.282×10^{-3}	3	0.87	0.5253	
Pure error	9.59×10^{-3}	4			
Total	0.26	16			
For rutaecarpine					
Model	0.19	9	15.1	0.0008	0.951
X_1	0.093	1	65.8	<0.0001	
X_2	0.022	1	15.8	0.0054	
X_3	0.022	1	15.29	0.0058	
$X_1 X_2$	0.011	1	7.9	0.0261	
$X_1 X_3$	4.032×10^{-3}	1	2.84	0.1360	
$X_2 X_3$	2.401×10^{-3}	1	1.69	0.2349	
X_1^2	0.02	1	14.4	0.0068	
X_2^2	7.418×10^{-3}	1	5.22	0.0563	
X_3^2	6.314×10^{-3}	1	4.44	0.0730	
Residual	9.950×10^{-3}	7			
Lack of fit	5.099×10^{-3}	3	1.4	0.3648	
Pure error	4.850×10^{-3}	4			
Total	0.2	16			

extraction. The interaction between time and temperature was significant for rutaecarpine.

3.4. Effect of the factors

It is usually considered that the yield of target compounds with SFE is influenced by the extraction time, temperature and pressure. Shorter extraction time could cause incomplete extraction and longer extraction time could be time and solvent wasting. Furthermore, time is one of the main factors for exhausted extraction and is an important index for evaluation of extraction efficiency. When considering the effect of temperature on solubility of solid compounds, two different effects can appear by changing temperature. One is the increase of solid volatility with temperature rise, causing an increase of vapor pressure, and another is the decrease of solvent density with temperature rise. The improvement of solubility by temperature is dependent on which effect is more important. If the density effect were predominant, the solubility of compounds in the supercritical phase would have decreased at higher temperatures. In the case that the vapor pressure is overwhelming, the solubility of solid compounds would increase with the increase in the vapor pressure [32]. Moreover, the fluid density can be increased by elevating pressure, but it could cause a descent in the solute vapor pressure. In addition, the solubility of solid compounds in supercritical fluid could be influenced by the repulsive solute–fluid interaction [33]. The solubility of solid compounds generally increases as the pressure began to rise, since the density of the fluid increases with pressure. The main factor affecting the solubility of solid compounds is the density of fluid. As the pressure continues to increase, however, the repulsive solute–fluid interaction becomes more and more. When pressure reaches a certain value for some compounds, the repulsive solute–fluid interaction may become greater than the increase in the solubility obtained from the increased solvent density. In this situation, the solubility

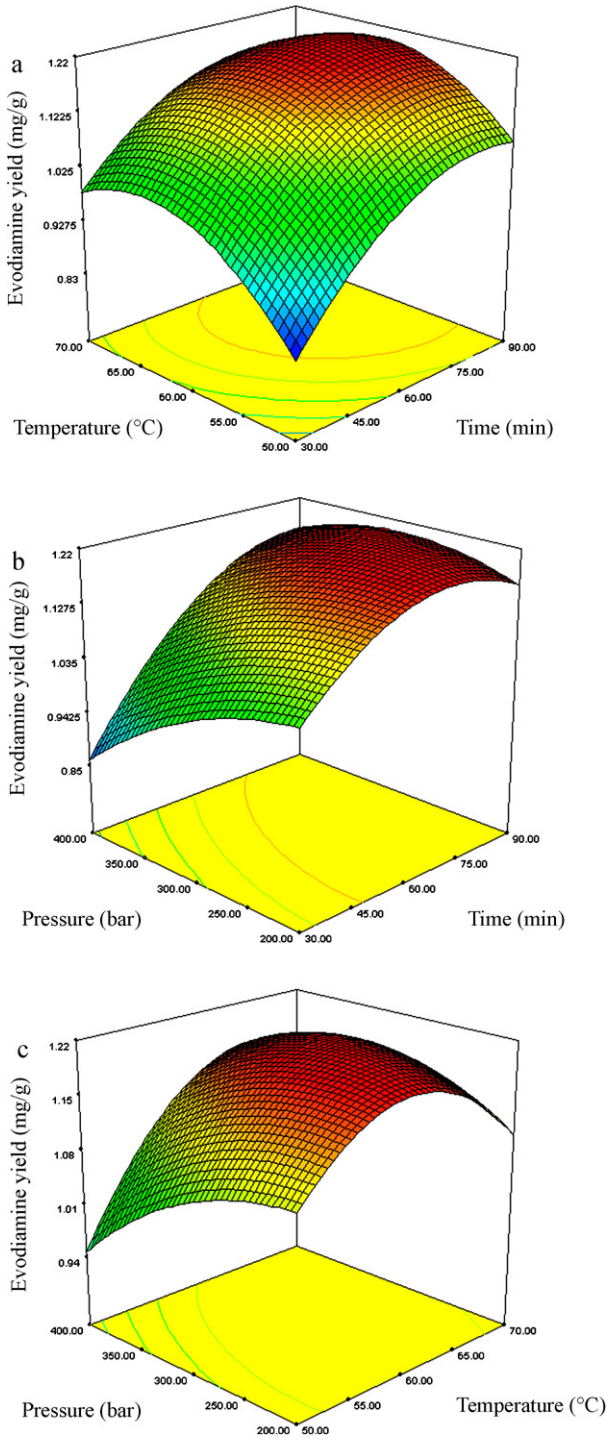

Fig. 6. Response surface plots of evodiamine showing (a) the effect of time and temperature at constant pressure (280 bar), (b) the effect of time and pressure at constant temperature (62 °C), and (c) the effect of temperature and pressure at constant time (78 min).

of the compounds decreases. A lower solubility leads to a decrease in extraction yield. The solubility of solute in supercritical fluid depends on a complex balance among fluid density, solute vapour pressure and the repulsive solute–fluid interaction, which are controlled by temperature and pressure. These three factors were, therefore, chosen and optimized in this study.

Figs. 6 and 7 illustrate the surface plots of the response variables (evodiamine (mg/g) and rutaecarpine (mg/g)), respectively, as a function of extraction time, temperature and pressure, while

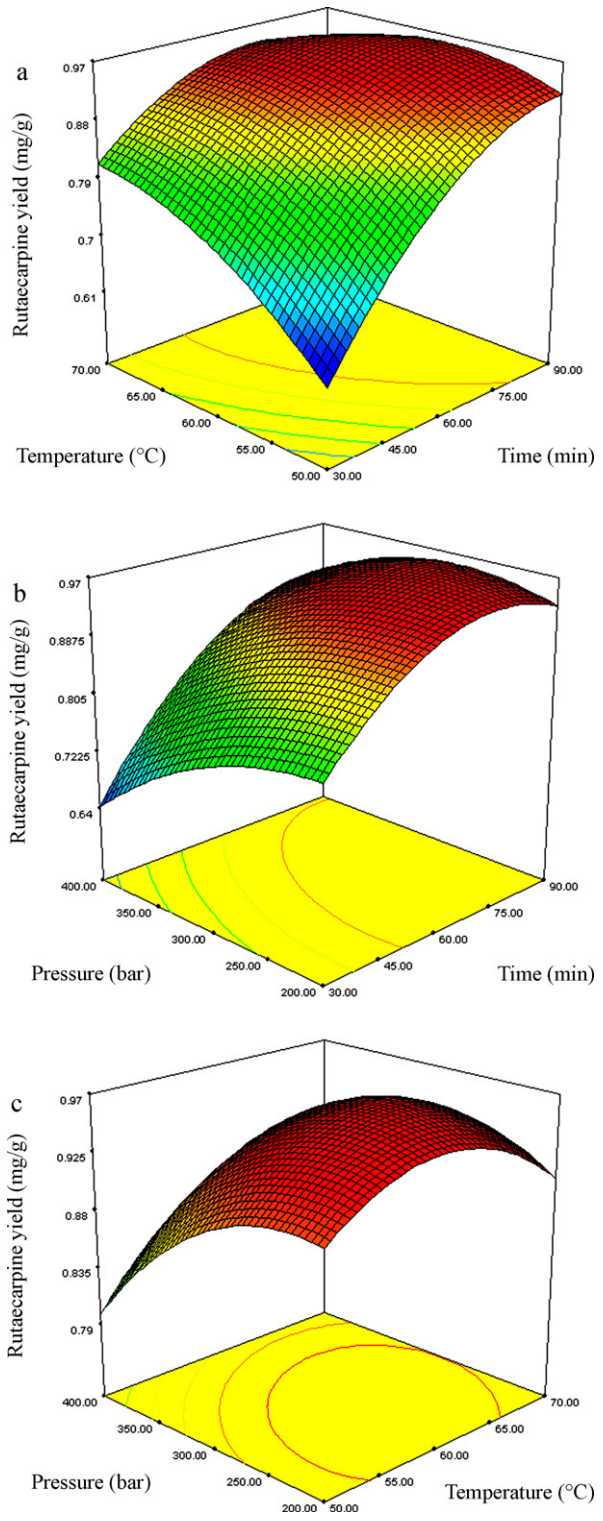

Fig. 7. Response surface plots of rutaecarpine showing (a) the effect of time and temperature at constant pressure (280 bar), (b) the effect of time and pressure at constant temperature (62 °C), and (c) the effect of temperature and pressure at constant time (78 min).

conditions are constant at optimum values (78 min, 62 °C, 280 bar, co-solvent flow rate 0.4 mL/min). These graphs can be used for visually predicting future responses and for determining factor values that optimize the response function.

The effect of different combination of extraction time and temperature on the amount of evodiamine and rutaecarpine yield is shown in Figs. 6a and 7a, respectively. It was observed from Fig. 6a that higher yields (higher than 1.18 mg/g) for evodiamine were attained by setting extraction time longer than 64 min and temperature between 58 °C and 66 °C. When the mathematical model was used to predict the yields that could be obtained using different temperature, the other two variables were kept constant at their optimum values. Fixing time and pressure at optimum values (78 min and 280 bar), the yield of evodiamine increased from 1.01 mg/g at 50 °C to 1.21 mg/g at 65 °C. High temperature conduced to the increase of extraction yield of the compound inside the experimental domain. The effect of increased extraction time on the yield was more noticeable. The extraction yield increased with time. It was noticed that faster extraction was attained within 30 min with the 83% of recovery (1.01 mg/g), based on the yield of 90 min. After 30 min, the yield was slowly increased within the range of 60 min with 98% of recovery (1.18 mg/g), while keeping temperature and pressure constant at optimum condition (62 °C and 280 bar). Similar effect of extraction time and temperature on yields was observed for rutaecarpine (Fig. 7a). The highest yield (0.95 mg/g) was obtained at temperature between 58 and 68 °C, and time longer than 70 min.

Fig. 6b shows the effect of extraction time and pressure on yields of evodiamine. Higher yields were obtained at time above 66 min and pressure between 230 bar to 310 bar. The highest yield of evodiamine (1.214 mg/g) was attained with time 78 min and pressure 280 bar. When the pressure was increased from 200 to 280 bar at optimum values (62 °C and 78 min), the improvement of yields was small from 1.19 to 1.21 mg/g. The increase of pressure from 280 to 400 bar did not enhance the evodiamine yield. In this study, the interaction between time and pressure was not statistically significant ($P > 0.05$). For rutaecarpine extraction, the effect of time and pressure on yields was displayed in Fig. 7b. The highest yields (0.95 mg/g) were attained when time was longer than 67 min and pressure from 200 to 300 bar.

When considering the effects of temperature and pressure on the yields of evodiamine, higher yields were obtained at temperature between 59 and 64 °C, pressure between 224 and 320 bar, as can be seen in Fig. 6c. The yields of evodiamine increased from 1.07 mg/g at 50 °C to 1.21 mg/g at 62 °C, while keeping extraction time and pressure at optimum values (78 min and 280 bar). Also higher yields (about 0.95 mg/g) for rutaecarpine extraction were observed at temperature between 55 °C and 68 °C, pressure between 200 bar and 320 bar in Fig. 7c. The interaction between temperature and pressure was not statistically significant for rutaecarpine and evodiamine extraction ($P > 0.05$). It was also reported that the interaction between pressure and temperature was not statistically significant for stevioside extraction [32] and lutein [33], which means that the synergistic effect between them is weak in the selected experimental range.

Table 4 shows that the suitability between the predictive values, yielded from the estimated models at optimal extraction condition (time 78 min, temperature 62 °C, and pressure 280 bar) and experimental values for evodiamine yields and rutaecarpine yields. Experimental values (1.205 mg/g and 0.949 mg/g for evodiamine and rutaecarpine, respectively) were not significantly different from the predicted values (1.217 mg/g and 0.969 mg/g for evodiamine and rutaecarpine, respectively) within 95% confidence interval. Evodiamine and rutaecarpine purity in the extracts is also compared in Table 4. The purity obtained with methanol modified supercritical CO_2 was 4 times higher than that with Soxhlet extraction. Furthermore, the yield obtained with methanol modified supercritical CO_2 was slightly higher than that with Soxhlet extraction. It reveals that an exhausted extraction was attained with methanol as co-solvent in supercritical CO_2 extraction under selected operation conditions.

Table 4
Extraction yields and purity of evodiamine and rutaecarpine from the powdered fruit of *E. nutaecarpa*.

Extraction condition	Evodiamine yields (mg/g)		Rutaecarpine yields (mg/g)		Evodiamine purity (%)	Rutaecarpine purity (%)
	Experimental	Predicted	Experimental	Predicted	Experimental	
	(mean ± SD, $n = 4$)		(mean ± SD, $n = 4$)		(mean ± SD, $n = 3$)	
Dynamic extraction time, 78 min; temperature, 62 °C; pressure, 280 bar; flow-rate, 2 L/min CO_2 and 0.4 ML/min methanol	1.205 ± 0.059	1.217	0.949 ± 0.029	0.969	1.804 ± 0.042	1.733 ± 0.082
Soxhlet extractor; extraction solvent methanol; extraction time, 7 h	1.192 ± 0.048		0.931 ± 0.016		0.387 ± 0.017	0.373 ± 0.021

4. Conclusions

In this study, the effects of extraction time, temperature and pressure at optimal flow rate of methanol (0.4 mL/min) were evaluated in order to develop an optimized SFE method. The results demonstrated that the change at time, temperature and pressure could affect the yields of the two alkaloids. The estimated models were able to indicate operational conditions, allowing superior extract yield. The highest yields predicted for evodiamine (1.217 mg/g) and rutaecarpine (0.969 mg/g) could be attained at optimal extraction condition. This study shows that the yields and purity of the two alkaloids obtained with methanol as co-solvent were higher than that with containing water of methanol, 95% ethanol or Soxhlet extraction. In the light of these findings, SFE with methanol as co-solvent can be a useful alternative for the extraction of the compounds with high efficiency and reduced extraction time for quantitative recovery. The optimized parameters are helpful for SFE both in analytical and larger scale.

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References

[1] Chinese Pharmacopoeia, Committee, Pharmacopoeia of the People's Republic of China, Chemical Industry Press, Beijing, 2005, pp. 118.
[2] M. Ou, Chinese–English Manual of Common-Used Traditional Medicine, Guangdong Science and Technology Publishing House, Guangzhou, 1992, pp. 255.
[3] T.J. Lee, E.J. Kim, S. Kim, E.M. Jung, J.W. Park, S.H. Jeong, S.E. Park, Y.H. Yoo, T.K. Kwon, Mol. Cancer Ther. 5 (2006) 2398.
[4] S.F. Kan, C.H. Yu, H.F. Pu, J.M. Hsu, M.J. Chen, P.S. Wang, J. Cell. Biochem. 101 (2007) 44.
[5] T. Wang, Y. Wang, Y. Kontani, Y. Kobayashi, Y. Sato, N. Mori, H. Yamashita, Endocrinology 149 (2008) 358.
[6] W.Q. Rang, Y.H. Du, C.P. Hu, F. Ye, K.P. Xu, J. Peng, H.W. Deng, Y.J. Li, Planta Med. 70 (2004) 1140.
[7] T. Wang, Y. Wang, H. Yamashita, FEBS Lett. 583 (2009) 3655.
[8] E.H. Han, H.G. Kim, J.H. Im, T.C. Jeong, H.G. Jeong, Toxicology 266 (2009) 38.
[9] H.C. Ko, Y.H. Wang, K.T. Liou, C.M. Chen, C.H. Chen, W.Y. Wang, S. Chang, Y.C. Hou, K.T. Chen, C.F. Chen, Y.C. Shen, Eur. J. Pharm. 555 (2007) 211.
[10] S.K. Heo, H.J. Yun, H.S. Yi, E.K. Noh, S.D. Park, J. Cell. Biochem. 107 (2009) 123.
[11] P.L. Yu, H.L. Chao, S.W. Wang, P.S. Wang, J. Cell. Biochem. 108 (2009) 469.
[12] M. Herrero, J.A. Mendiolaa, A. Cifuentes, E. Ibanez, J. Chromatogr. A 1217 (2010) 2495.
[13] B. Liu, Y. Chang, H. Jiang, B. Shen, Biomed. Chromatogr. 21 (2007) 79.
[14] F. Montanes, T. Fornari, P.J.M. Alvarez, A. Montilla, N. Corzo, A. Olano, E. Ibanez, J. Supercrit. Fluids 41 (2007) 61.
[15] B. Liu, B. Shen, F. Guo, Y. Chang, Sep. Purif. Technol. 64 (2008) 242.
[16] M.A. Bezerra, R.E. Santelli, E.P. Oliveira, L.S. Villar, L.A. Escaleira, Talanta 76 (2008) 965.
[17] A.L. Ahmad, C.J.C. Derek, M.M.D. Zulkali, Sep. Purif. Technol. 62 (2008) 702.
[18] S.H. Kim, J. Park, B.R. Kim, E.J. Kim, H. Kim, Y. Cho, K.Y. Lee, S.H. Sung, Sep. Sci. Technol. 44 (2009) 1772.
[19] W. Xiao, L. Han, B. Shi, Sep. Sci. Technol. 43 (2008) 671.
[20] C. Turner, L.C. Whitehand, T. Nguyen, T. McKeon, J. Agric. Food Chem. 52 (2004) 26.
[21] F. Pellati, S. Benvenuti, F. Yoshizaki, M. Melegari, J. Sep. Sci. 29 (2006) 641.
[22] M.H. Shyr, L.C. Lin, T.Y. Lin, T.H. Tsai, Anal. Chim. Acta 558 (2006) 16.
[23] M. Zhao, X. Yang, Chromatographia 67 (2008) 543.
[24] Y. Zhou, S.H. Li, R.W. Jiang, M. Cai, X. Liu, L.S. Ding, H.X. Xu, P.P. But, P.C. Shaw, Rapid Commun. Mass Spectrom. 20 (2006) 3111.
[25] R. Liu, X. Chu, A. Sun, L. Kong, J. Chromatogr. A 1074 (2005) 139.
[26] Y. Zhang, Z. Zhang, Food Drug 10 (2008) 27.
[27] W. Liu, J. Li, D. Qiu, Chin. Hosp. Pharm. 23 (2003) 480.
[28] B. Liu, H. Jiang, B. Shen, Y. Chang, J. Chromatogr. A 1075 (2005) 213.
[29] T. Bajer, M. Adam, L. Galla, K. Ventura, J. Sep. Sci. 30 (2007) 122.
[30] J.A. Wood, M.A. Bernards, W. Wan, P.A. Charpentier, J. Supercrit. Fluids 39 (2006) 40.
[31] M. Sun, F. Temelli, J. Supercrit. Fluids 37 (2006) 397.
[32] A. Erkucuk, I.H. Akgun, O. Yesil-Celiktas, J. Supercrit. Fluids 51 (2009) 29.
[33] Q. Ma, X. Xu, Y. Gao, Q. Wang, J. Zhao. Int. J. Food Sci. Technol. 43 (2008) 1763.