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Full Length Research Paper

Preparation of productive and highly purified mogrosides from *Siraitia grosvenorii*

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The mogrosides of *Siraitia grosvenorii* are natural sweetener and potential chemopreventive agents. In order to obtain high-yield and good-quality mogrosides, the flash extraction method was employed to extract mogrosides from *S. grosvenorii*. The extraction parameters were optimized by Taguchi's experimental design, and the total yield of mogrosides was 8.6% under the optimum conditions. After purification by the chromatography column, the purity of mogrosides was greater than 92%. The separation technique described here may be applicable to commercial production of high-quality mogrosides.

Key words: Flash extraction, mogrosides, mogroside V, purification, Taguchi's experimental design.

INTRODUCTION

The mogrosides are a class of cucurbitane-type triterpene glycoside in *Siraitia grosvenorii* fruit which has been cultivated in a restricted area of the southern part of China (Kinghorn and Soejarto, 2002). As high-intensity sweetening agent, mogrosides are about hundred times sweeter than sucrose but low-in calorie (Kasai et al., 1989), and have been widely used in sweet beverages. Recently, much attention has been paid on the intriguing pharmacological characteristics of mogrosides, such as anti-cancer and scavenging free radicals (Takasaki et al., 2003; Qi et al., 2008; Pan et al., 2009). Therefore, the mogrosides are potential and valuable source in food additives and medical fields.

Commonly, several methods are available to obtain extracts from vegetal materials, such as Soxhlet extraction, microwave assisted extraction, supercritical fluid extraction, ultrasonically assisted extraction and Flash extraction (Min et al., 2009). As a new technique, the Flash extraction, widely used in herb extraction, could turn the plant tissue into fine particles quickly so that it can improve the efficiency of extraction. However, nowadays, heat reflux extraction with ethanol is, in general practice, applied for the mogrosides large-scale industrial production, despite being time-consuming and labor intensive. To establish efficient and low-cost method for the mogrosides industry, this study aimed to focus on applying Flash extraction techniques to separate mogrosides.

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MATERIALS AND METHODS

Apparatus

Rotary evaporator (RE-52A,Yarong Biochemistry Instrument Co., China); vacuum drying oven (SHZ-3,Shanghai Zhixin Laboratory Instrument Co., China); UV/Vis spectrophotometer (SP-756P, Shanghai Spectrum Instruments Co., China); the Herbal Blitzkrieg Extractor (manufactured by Henan Jinnai Sci-Tech Development Co., China) and Chromatographic system (Waters Co., America) were used for the study.

Reagents and materials

S. grosvenorii was provided by White Pagoda Pharmacy (Beijing, China). Acetic acid and vanillin were purchased from Beijing Chemical Plant (Beijing, China). Acetonitrile, HPLC grade, was from J and K Chemical Co. (Beijing, China). Standard Mogroside V was from Tauto Biotech Co., Shanghai. Chitosan was obtained from Dandong Chemical Factory (Liaoning, China). The Amberlite XAD16 resin was purchased from Rohm and Haas (Rohm and Haas Co., USA), and the D213 resin was obtained from Dandong Chemical Factory (Liaoning, China). All the other reagents were of analytical grade.

Table 1. The factor levels in the experiment design.

Level	Factor A Water/material (v/w)	Factor B Temperature (℃)	FactorC Time (min)
1	10:1	40	4
2	17:1	60	7
3	25:1	80	10

The extraction procedure

S. grosvenorii was washed with water and dried for 12 h at 60℃ in a vacuum oven to 0% moisture; then, a sample of 12.5 g was accurately weighted and extracted with the Herbal Blitzkrieg Extractor. In the extraction procedure, Taguchi experimental design was applied to optimize the extraction conditions for mogrosides. Among various statistical experimental designs, Taguchi experimental design offers distinct advantages by which many factors can be examined simultaneously and much quantitative information can be extracted with a few experimental trials (Stone and Veevers, 1994). The basic principle of this method serves as screening filters which examine the effects of many process variables and identify those factors which have major effects on process, using a few experiments (Dasu et al., 2003). Factors such as temperature, water/material ratio and extraction time were evaluated for their effects on the extraction process (Table 1). After Flash extraction with the Herbal Blitzkrieg Extractor, the crude mogrosides extracts were obtained, and then 5 ml crude extracts were filtered through 0.45 μm membrane for analysis by spectrophotometer.

Purification

The crude extracts was mixed with 37.5 ml chitosan solution and incubated at room temperature for 20 min. Chitosan solution was prepared by dissolving 1 g of chitosan in 100 ml of 1% acetic acid (Teotia et al., 2004). As a cationic polysaccharide, chitosan has the ability to chelate with protein and tannic acid in the mogrosides extract by the active interface made by chitosan's functional groups such as hydroxyl and amine groups.

The slurry was centrifuged (4500 rpm) to remove solids. The obtained supernatant was alkalized (pH 9) with 1.0 mol/L sodium hydroxide; and then the alkalized liquid was applied to affinity chromatography packed with XAD16 resin (3.0 × 40 cm) at a flow rate of 1.5 ml/min, followed by elution with 4 resin bed volumes of 60% aqueous ethanol. The obtained elution was concentrated by vacuum evaporation at 50℃, and then was subjected to ion-exchange chromatography D213 macroporous resin (3.0 × 40 cm) at a flow rate of 1.2 ml/min, so as to decolorize the mogrosides.

Determination of total mogrosides

The total mogrosides were determined by colorimetric method. The standard curve for the yield of mogrosides was obtained as follows: A stock solution consisting of mogrosides (0.34 mg/ml) was prepared. The different volumes of the stock solution with 0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 ml were transferred into a 10-ml test tube. Each tube was combined with 0.5 ml of freshly saturated vanillin-ethanol solution, and the 75% sulfuric acid was added to make a total volume of 6.5 ml. The solution was mixed and incubated at 50℃ in a water-bath for 20 min, then cooled immediately in ice bath. When cooled to room temperature, with a blank solution as reference, the absorbance of solution was scanned by a UV/Vis spectrophotometer in the range of 220 to 700 nm. Scanning results showed that the maximum adsorption was at 587 nm.

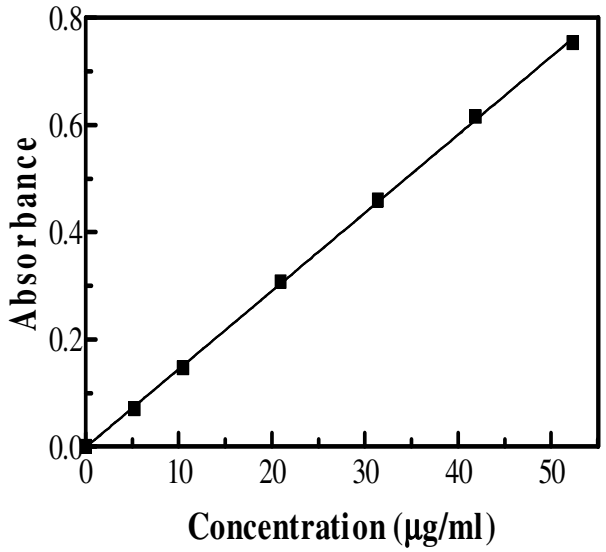

Figure 1. The standard curve of mogrosides.

Analysis of the content of mogroside V

The content of mogroside V as a key indicator of mogrosides was determined by HPLC. HPLC separation was conducted on a Waters Sunfire C18 column (4.6 × 150 mm, 5 μm) at 25℃, and the injection volume was 15 μl. The mobile phase consisted of acetonitrile (A) and water (B) at a flow rate of 1.0 ml/min; a gradient separation was programmed as follows: 0 to 40 min, 5 to 68% (A). The elution was monitored at 203 nm.

Statistical analysis

All tests in this study were carried out in triplicate, and the mean values were presented. Statistics was analyzed using SPSS for Windows, version 11.5 (SPSS Inc, Chicago).

RESULTS AND DISCUSSION

Calibration curves and determination of total mogrosides

According to the linear regressive relationship between the concentration and the absorbance, the calibration curve (Figure 1) for mogrosides could be expressed using the following equation (0 to 52 μg/ml):

$$A = 0.0146\ C\ (R^2 = 0.9996) \quad (1)$$

Where, C (μg/ml) is the concentration of mogrosides solution for colorimetric analysis and A is the absorbance.

The mogrosides yield (%) Y was calculated using the equation:

Table 2. Taguchi's experimental design matrix and corresponding mogrosides yield.

Run	A	B	C	Vacant array	Yield (%)
1	1(10)	1(40)	1(4)	1	6.73
2	1	2(60)	2(7)	2	7.68
3	1	3(80)	3(10)	3	7.64
4	2(17)	1	2	3	7.44
5	2	2	3	1	8.12
6	2	3	1	2	7.93
7	3(25)	1	3	2	7.75
8	3	2	1	3	8.29
9	3	3	2	1	8.12
K 1	7.350	7.307	7.650	7.657	_
K 2	7.830	8.030	7.747	7.787	_
K 3	8.053	7.897	7.837	7.790	_

Table 3. Results of analysis of variance (dependent variable: yield).

Source	Sum of square	df	Mean square	F	Significance
Corrected model	1.719(a)	6	0.287	16.682	0.058
Intercept	539.912	1	539.912	31431.63	
A	0.776	2	0.388	22.579	0.042
B	0.893	2	0.446	25.982	0.037
C	0.051	2	0.026	1.485	0.402
Error	0.034	2	0.017		
Total	541.665	9			
Corrected total	1.754	8			

R squared = 0.980.

$$Y = (A \times DF \times V) / (14600 \times M) \times 100\% \quad (2)$$

Where, V (ml) is the total volume of extraction solvent; DF is the dilution factor; A is the same as in equation (1) and M is the mass of *S. grosvenorii* sample (g).

Optimization of extracting parameters

Orthogonal experiment and variance analysis were used to optimize the extraction parameters. Table 2 presents the experimental design matrix and the yield of mogrosides in each run. The last three rows gave the average yield of each level for the three parameters, where K is the average yield of the parameter at a chosen level. For example, for K1, the value of 7.650 at column "C" is the average of yield at trials 1, 6 and 8, all of which choose level 1 for C; for K2, the value of 7.747 at column "C" is the average of yield at trials 2, 4, and 9, all of which choose level 2 for C. A higher K value indicates a preferred level for the chosen parameter. As the index, the level corresponding to the maximum average yield among the three levels could be chosen for the optimal set of parameters, therefore, it is concluded that the optimal parameters identified were $A_3B_2C_3$.

In order to identify the significant effect of each parameter on the yield of mogrosides, an analysis of variance (ANOVA) was applied to the data. High values for the calculated F mean a greater influence of factor on the experimental results. As shown in Table 3, with respect to the F value, the B (temperature) has the highest F value, meaning that the factor (temperature) is the most important parameter for the yield of mogrosides, and the effects of the three parameters decreases in the following order: B (temperature) > A (water/material ratio) >C (time). The model obtained from ANOVA indicated that the multiple correlation coefficient of R^2 was 0.980, which indicates that the model can explain 98.0% variation in the response, meaning that the applied model is adequate.

To validate the models adequately, extraction was carried out under the optimized conditions as follows: water/material (25:1), time (10 min) and temperature (60℃). Under these conditions, the maximum yield (8.6 %) was obtained, proving the reliability of the statistical analysis.

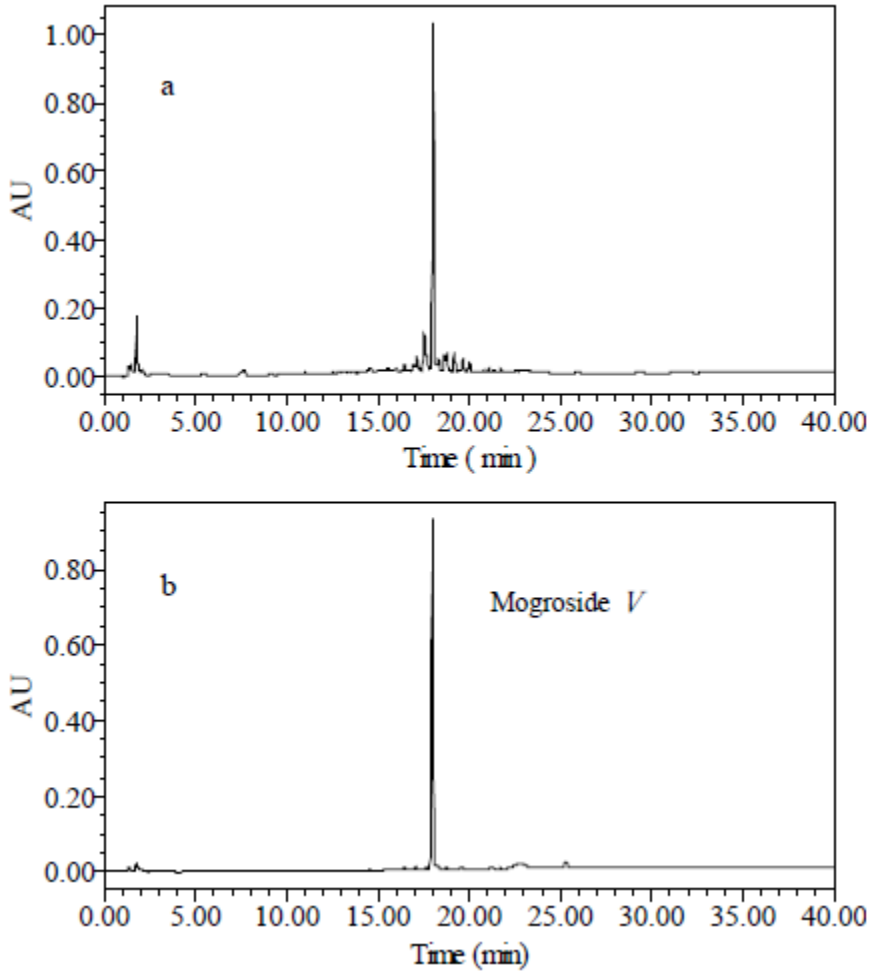

Figure 2. Liquid chromatograms of mogroside V sample (a) and standard mogroside V (b).

The purity of mogrosides and content of mogroside V

Through purification with the chromatography column, the purity of the mogrosides is greater than 92%. Figure 2 shows the chromatograms of mogrosides sample and standard of mogroside V, respectively. A major peak was observed at 18 min in Figure 1(a) which is identical to the retention time of standard (Figure 1b), indicating that the major peak was mogroside V. The amount of the mogroside V in mogrosides, determined by HPLC, was 34.43%, proving the high quality of the mogrosides thus obtained.

Conclusion

In this study, a novel method was established to separate mogrosides from *S. grosvenorii.* The optimum conditions of Flash extraction were obtained as follows: water/biomass ratio 25:1, temperature 60℃, time 10 min, and the high yield (8.6%) of mogrosides was obtained. After two steps of purification including affinity and anion-exchange chromatography, the purity of mogrosides was over 92%. Furthermore, mogrosides was extracted by using water as extraction solvent instead of ethanol. This efficient and lower cost method showed great potential for mogrosides industry.

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