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Optimization of supercritical carbon dioxide extraction, physicochemical and cytotoxicity properties of *Gynostemma pentaphyllum* seed oil: A potential source of conjugated linolenic acids

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ABSTRACT

In order to further explore the by-products of *Gynostemma pentaphyllum* (Thunb.) Makino, the seed oil was studied for its extraction, physicochemical properties and bioactivity. Supercritical carbon dioxide (SC-CO_2) extraction was the first time to extract *G. pentaphyllum* seed oil (GPSO). The maximum extraction yield of 35.96 ± 0.21% was achieved at the optimal conditions: extraction temperature of 43 °C, extraction pressure of 32 MPa and extraction time of 160 min, which were selected and optimized through response surface methodology. The oil yield and physicochemical properties of GPSO obtained by different extraction methods were analyzed and compared. Fatty acid composition was determined by gas chromatography–mass spectrometry and the structure of seed powder was observed using scanning electron microscope. The results showed that SC-CO_2 extraction was an effective method to improve GPSO quality and tended to extract higher percentage of unsaturated fatty acids (95.69%) especially conjugated linolenic acid (88.17%) in comparison with conventional extraction methods. Furthermore, the cytotoxicity of fatty acids from GPSO (GPFA) was investigated by cell viability assay and cell morphological changes. GPFA significantly inhibited cell viability in a concentration-dependent manner against various cancer cells especially leukemia cells, suggesting its potential benefits as an anticancer agent.

1. Introduction

Gynostemma pentaphyllum (Thunb.) Makino, commonly known in China as Jiao-Gu-Lan, is a perennial liana in the Cucurbitaceae family growing widely throughout Asian countries. It is famous as a traditional medicinal plant with multiple biological functions. The book "Herbs for Famine" published in the Chinese Ming Dynasty (1368–1644 A.D.) described that this herb was used as vegetable, food, and medicinal tea. In traditional medicine, the stem and leaf overground parts of this plant are used as raw material to extract active ingredients for a variety of diseases such as diabetes, hypertension and cancer [1]. Researches have shown that Jiao-Gu-Lan extracts produce various pharmacological effects, including hypolipidemic [2], antidiabetic [3], antioxidant [4] and antitumor [5] effects. Gypenoside tablets are commercially produced from the whole plant and recorded in the Pharmacopoeia Committee of the People's Republic of China in 2010.

There are many active constituents in the leaves of *G. pentaphyllum* have been widely studied, such as saponins [6], flavonoids [7,8], polysaccharides [9,10] and some essential elements. Rhizome transplanting is the main form of *G. pentaphyllum* cultivation, therefore, the seeds become wastes or by-products in the production process and have not yet been fully utilized or commercially explored. Few studies have been reported on the extraction process and characterization of the seed oil from *G. pentaphyllum*. Our previous studies unexpectedly found the seeds contain about 40% vegetable fat and the seed oil was rich in polyunsaturated fatty acids (PUFA) especially some conjugated linolenic acids (CLnA). CLnA are a group of positional and geometric isomers of octadecatrienoic acid (C18:3), which possess double bonds at positions 9, 11, 13 or 8, 10, 12 of their chain. CLnA isomers have been attributed to exhibit several health benefits that are closely associated with the molecular characteristics of conjugated double bonds [11]. The recent research indicate that CLnA could be very effective to prevent and treat many diseases, especially arteriosclerosis [12], diabetes [13] and cancers [14]; and some CLnA have a greater anticancer capacity in humans than their close family member, conjugated linoleic acid (CLA) [15–17]. Moreover, few kinds of

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plant oils have been reported to become the natural sources of CLnA, including *Momordica charantia*, *Punica granatum*, *Calendula officinalis*, *Prunus mahaleb*, *Aleurites fordii*, *Jacaranda mimosifolia*, *Catalpa ovata*, *Trichosanthes kirilowii*, and *Impatiens balsamina* [18], *G. pentaphyllum* can be a potential new source of CLnA.

For high value oils (functional or medicinal oils), the oil extraction process is a key step affecting the oil's yield and bio-functions. Conventionally, we extracted *G. pentaphyllum* seed oil (GPSO) using cold pressing and organic solvent extraction. Mechanical pressing is considered as an advantageous method because the device is simple and easy to operate, and it gives good quality of oil with low yield whereas organic solvent gives higher yield with several disadvantages such as longer extraction period, low capacity and solvent residual in the final product. In recent years, supercritical carbon dioxide (SC-CO_2) extraction method has received considerable attention over the traditional extraction method to be investigated extensively due to its unique properties and numerous advantages. SC-CO_2 is considered as an eco-friendly and green solvent to produce high value oils having pharmaceutical importance [19]. CO_2 is nontoxic, nonflammable, noncorrosive, nonexplosive and bacteriostatic, it is Generally Recognized as Safe (GRAS) to be used in food products [20]. SC-CO_2 has been successfully used to obtain oil from rice bran [21], flaxseed [22], tea seed [23], mango seed [24], pepper [25] and watermelon seed [26].

Response surface methodology (RSM) has been widely acknowledged to optimize the extraction process and determine the effects of process parameters, as well as to investigate their interactions on the relevant variables [27]. It is the most powerful and effective mathematical and statistical techniques compare with other multivariate statistical techniques due to evaluating the effects of multiple parameters, alone or in combination on response variables and reducing the number of experimental trails with high efficiency [28].

The main objective of this study was to optimize the critical SC-CO_2 extraction parameters of GPSO, namely extraction pressure, temperature and time using RSM to achieve the highest yield. In comparison with conventional extraction methods, the physicochemical properties and fatty acid compositions of GPSO obtained from SC-CO_2 extraction and the structure of seed powder were analyzed by GC–MS and scanning electron microscope (SEM) respectively. The cytotoxicity of fatty acids from GPSO against the tested cancer cell lines *in vitro* was evaluated by MTT assay. Our report was probably the first comprehensive description of SC-CO_2 extraction to produce the high-quality and green non-polluting oils from *G. pentaphyllum* seeds.

2. Materials and methods

2.1. Materials

G. pentaphyllum seeds were gathered from their natural habitat in Pingli County (Shaanxi, China) at maturity. They were carefully selected and cleaned to remove impurities and oven-dried at 60 °C for about 5 h, then ground into powder with a blender and the particle size was standardized by passing through a mesh sieves. The powder kept in air tight containers, and stored at 4 °C until used. Carbon dioxide having purity 99.99% was provided by Henglong Gas Corp. (Hefei, China). All chemicals and solvents were of analytical grade.

2.2. Oil extraction

2.2.1. Cold-pressing

G. pentaphyllum seeds were pressed using a spiral oil press at room temperature (25 °C) without any thermal treatment.

2.2.2. Solvent extraction (SE)

5 g of seed powder was refluxed and extracted by Soxhlet extractor with 200 mL of petroleum ether for 6 h. Then the seed oil was collected with a rotary vacuum evaporator at 60 °C. The oil yield percentage was calculated according to the following Eq. (1):

$$\text{Oil yield } (\%) = \frac{\text{Amount of extracts oil (g)} \times 100}{\text{Amount of initial seed powder (g)}} \tag{1}$$

2.2.3. SC-CO_2 extraction

The seed oil extraction process was performed on a supercritical fluid apparatus (HA121-50-01C, Nantong, China) using CO_2 as a solvent. *G. pentaphyllum* seed powder (50 g) was loaded into the extraction vessel with a selected particle size and water content. CO_2 from a cylinder was passed through a chiller kept at 2 °C and pumped into the extractor by a high pressure pump. The pressure and temperature were controlled to an accuracy of ±0.5 MPa and ±0.5 °C, respectively. Each extraction was performed under certain conditions in given extraction pressure, temperature, time and CO_2 flow rate. The extracted oil samples were collected in the separator and measured gravimetrically and the oil yield was expressed as percent weight.

2.3. Experimental design

Response surface methodology (RSM) was adopted to evaluate the effect of independent variables including extraction temperature (°C, X_1), extraction pressure (MPa, X_2) and dynamic extraction time (min, X_3) on the extraction of GPSO yield. The level of each variable was determined based on single-factor experiment. Table 1 shows the minimum, medium and maximum values of independent variable which corresponds to −1, 0 and +1 levels, respectively. The Box–Behnken Design (BBD) of RSM was used to determine the optimal conditions of the extraction process for greatest efficiency. A three-variable, three-level design with a total of 17 experimental runs was performed in triplicate and the average oil yield (%) was taken as response value. The sets of operating parameter as suggested by Design-Expert 8.0.6 Software (DE8) are shown in Table 2. Based on the experimental results presented in Table 2, the predicted value Y, for GPSO yield, was fitted into the equation with a multiple regression procedure and analyzed by DE8 software. The quadratic polynomial regression Eq. (2) was given below:

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

where Y is the predicted response; β_0, β_i, β_{ii}, and β_{ij} are regression coefficients of the intercept, linear, quadratic, and interaction terms, respectively; and X_i and X_j are independent variables. To examine the quality of the fitted model, the coefficient of determination (R^2) and the statistical significance of regression model was performed with F-test to obtain the mathematical relationship between input and output parameters.

Table 1
Independent variables and their levels used in the response surface design.

Independent variables	Levels		
	−1	0	1
Extraction temperature (°C)	25	30	35
Extraction pressure (MPa)	35	40	45
Dynamic extraction time (min)	120	150	180