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Articles

Influence of supercritical fluid extraction on lignocellulosic plant matrix and extractive substance yield

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Natural substances of plant origin are widely used in pharmacies. However, the quantitative content of biologically active substances in plant cells is insignificant and the currently available extraction methods do not allow for the complete extraction of biologically active substances as a result of the strong lignocellulose matrix of the cell. Therefore, it is necessary to look for new extraction methods that increase the yield of extractive substances from plant raw materials. This paper is devoted to the effect of supercritical fluid extraction on the quantitative content of extractives contained in a plant cell. The study aims to establish the effect of supercritical fluid extraction on the lignocellulose matrix and the yield of extractive substances. The plant biomass of the vegetative part of the narrow-leaved cypress (*Chamaenerion angustifolium* L., Scop., 1771); roots and rhizomes of the stinky bug (*Actaea cimicifuga* Schipcz., J Compton); roots and rhizomes of the golden root (*Rhodiola rosea* L., 1753) were used as research objects. The extractive substances were extracted by sequential liquid extraction, supercritical fluid extraction, and a combination of these methods. To extract the maximum amount of extractives, the following solvents were used: a nonpolar solvent (hexane, 70% ethanol) and a highly polar solvent (water). Extraction of plant materials with supercritical CO_2 was carried out in a SFEU-5/2 laboratory setup with the following parameters: pressure (P), 350 bar; temperature (T), 60 °C; extraction time (t), 180 min. The comparison of the yield of extractive substances obtained by sequential liquid extraction and aqueous alcoholic extraction with the preliminary processing of raw materials with supercritical CO_2 shows that treatment with supercritical carbon dioxide activates the plant matrix; therefore, the content of the extracted components increases.

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Keywords

Vegetable raw materials, extraction, supercritical fluid, biologically active substances

Introduction

Plant cells are a universal source of biologically active substances with a wide spectrum of pharmacological actions. Many clinical studies confirming that biologically active substances (BAS) are synthesized in the cell during life, which can be used to treat various diseases (Newman et al. 2003; Raskin and Ripoll 2004). However, the concentrations of biologically active substances in plant cells are quite low. The main part of the cell is composed of a very strong lignocellulose matrix, which prevents a deeper and more complete extraction of extractives (Hu and Ragauskas 2012).

Therefore, to increase the yield of extractive substances, additional processing is required using chemical, physicochemical, or biological methods. Furthermore, all known methods for extracting extractives, such as extraction with organic solvents or hydrodistillation, do not allow full control of the selectivity of the process (Doronin et al. 2008). Therefore, the development of new alternative methods for the extraction of biologically active substances with high selectivity and efficiency remains an urgent issue.

Supercritical fluid extraction (SCFE) has been widely applied in pharmacy, phytochemistry, and other branches of science as one of the effective methods to meet the requirements of "green chemistry" (Vagi et al. 2005).

The SCFE method is based on the use of a substance in a supercritical fluid state as a solvent. Most often, in industries, including pharmaceuticals, supercritical CO_2 (sc-CO_2) is used as a solvent due to the several advantages of supercritical carbon dioxide over other solvents. First, CO_2 is an inert, nonflammable gas. Second, it is available in a highly pure state. Third, low parameters of the supercritical state of carbon dioxide are easily achievable. The critical boiling point of sc-CO2 was 31 °C and the critical pressure was 73 atm. Such low parameters have a clear advantage when working with thermolabile compounds. Fourth, the properties of supercritical CO_2 can be easily modified by adding organic solvents, such as alcohols and amines (Gumerov and Yarullin 2008).

Supercritical CO_2 is most often used to extract fat-soluble substances from plant cells (Sidelnikov et al. 2003; Vagi et al. 2005).

Carbon dioxide in a supercritical state is a good solvent and, because of its high pressure, can penetrate into a hard-to-reach BAS in the lignocellulose framework of a plant cell. The use of modifiers and cosolvents contributes to the extraction of groups of fat-soluble substances and water-soluble compounds (Huang 2012; Sovová 1994).

The sc-CO_2 extract is much richer in BAS content of BAS than the extract obtained by other methods; also, it does not contain traces of solvent and other impurities. The combination of SCFE and extraction with organic solvents makes it possible to extract BAS and significantly increase the yield of BAS. The aim of this paper is to establish the effect of supercritical fluid extraction on the lignocellulosic plant matrix and the yield of biologically active substances compared to liquid extraction.

Material and methods

The biomass of Golden Root (roots and rhizomes), narrow-leaved fireweed (vegetative part), and black cohosh (roots and rhizomes) were used in the experiment. The research objects were selected on their known therapeutic effects and their widespread use in traditional medicine. The plant material was crushed using a rotary knife mill and sieved on a vibratory drive with sieves. For the analysis, a crushed biomass fraction of up to 1 mm was used.

The particle size of the raw material is of great importance for the duration and yield of SCFE. An experiment was conducted using basil leaves. If the sample particle size was 0.55 mm, 5 h was not sufficient to achieve satisfactory extraction. If the sample was crushed to 0.17 mm particles, 2 h was sufficient to complete the extraction. Grinding the sample into a fine powder can speed up extraction and increase efficiency, but this can also increase the flow rate of supercritical fluid per unit of time.

The extractive substances were extracted by supercritical fluid extraction. The CO_2 extracts were obtained on a SFEU-5/2 unit (Laboratory of Phytopreparation Technology, the Joint Stock Company ‘International Scientific and Production Holding “Phytochemistry”, Karaganda, Republic of Kazakhstan), which is a compact design consisting of two extractors with a capacity of 2.5 liters with a sleeve and 5 liters with a sleeve. The installation diagram is shown in Figure 1. Both extractors are equipped with a casing for the coolant, two high-pressure collectors, and two low-pressure collectors. The high-and low-pressure collectors were equipped with a heat carrier casing. The setting also includes a condenser, storage tank, high-pressure pump on a chassis with a casing, and a heat exchanger for heating and cooling carbon dioxide.

This configuration allows one to work with both subcritical and supercritical CO_2. For the SCF extraction of the studied plant material, the following conditions were used: pressure (P), 350 bar; temperature (T), 60 °C; extraction time (t), 180 min.

The raw material after extraction on a USFE-5/2 unit was subjected to extraction with 70% ethyl alcohol. For this purpose, the meal was placed in a 10 L flask and a water-alcohol mixture was poured into a feed: extractant ratio of 1: 1 and boiled in a water bath for 2 h. Then, the water-alcohol extraction was evaporated on a rotary evaporator until the solvent was removed. The resulting residue was then air-dried.

Sequential extraction was carried out by alternately treating the plant biomass with hexane, 96% ethyl alcohol, and water on a Soxhlet apparatus.

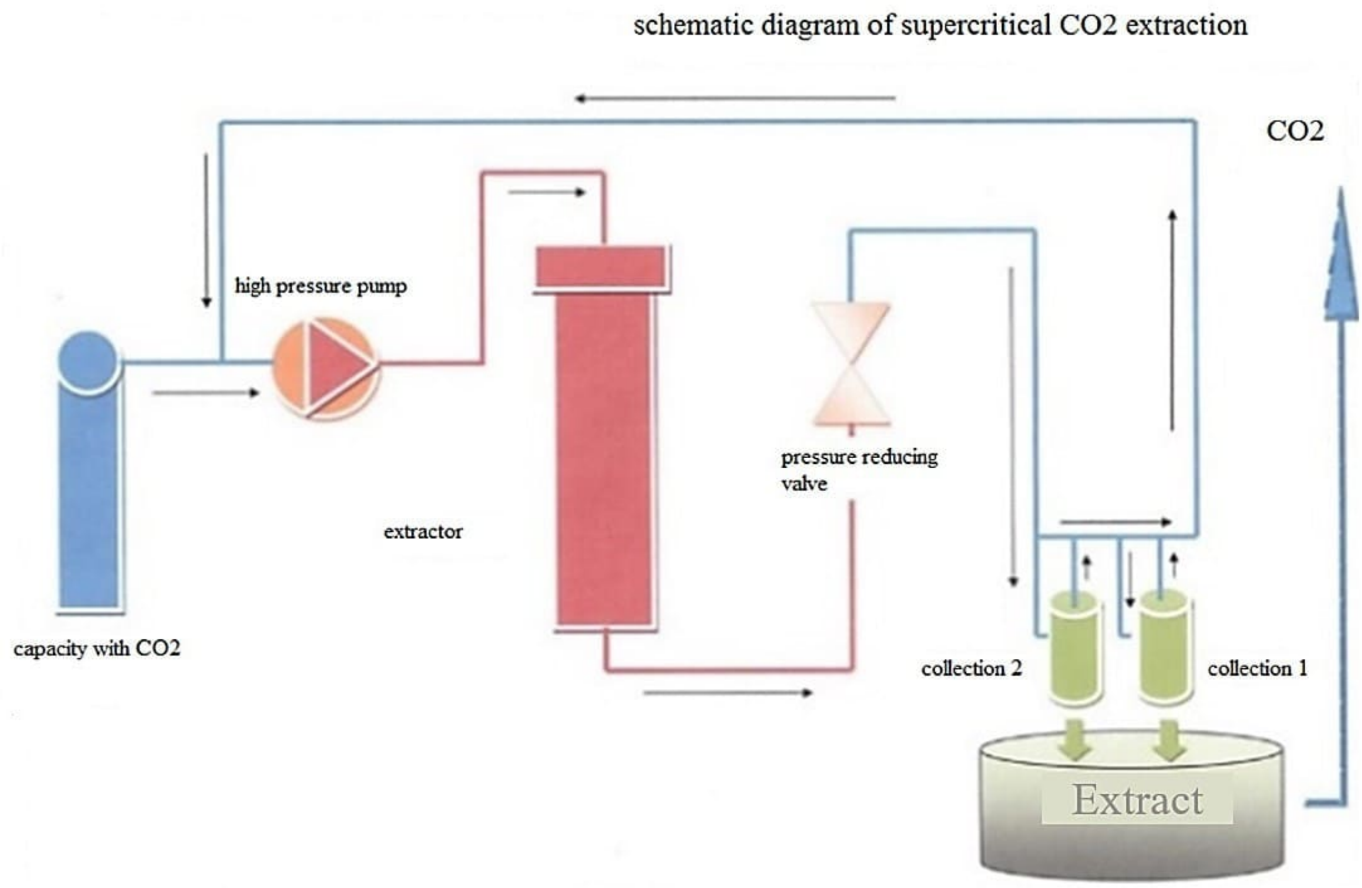

Figure 1. *Localities of Lepidoptera collection in the North Kazakhstan region*

Results

Below we presented the results of sequential supercritical fluid extractions for three plants: narrow-leaved fireweed, black cohosh, and golden root.

The total content of extractives extracted by sequential processing of solvents was significantly lower than the sum of biologically active substances extracted by a combination of supercritical fluid and water-ethanol extractions.

Raw materials	The content of extractives, %						
	Hexane	Ethanol	Water	The total content of extractives, %	Extraction on the unit SFEU-5\2, %	70% water-ethanol extraction, %	The total content of extractives, %
Narrow-leaved fireweed	4.38±0.12	7.64±.023	8.75±0.14	20.77	3.07±0.03	23.65±0.32	26.72
Black cohosh	0.61±0.08	3.97±0.11	4.54±0.17	9.12	0.32±0.06	14.40±0.12	14.72
Golden Root	0.43±0.05	5.03±0.09	6.61±0.14	12.07	0.45±0.04	35.50±0.24	35.95

Table 1. *The yield of extractive substances from raw plant materials extracted by sequential extraction on a Soxhlet apparatus*

We found that the yield of extractives as a result of the traditional treatment with 70% ethanol of the sample, from which 0.32% of black cohosh extractives, 3.07% of Narrow-leaved fireweed, and 0.45% of extractives of Golden Root extractives were previously extracted under the influence of supercritical carbon dioxide was 14.40%, 23.65% and 35.50%, respectively. Compared to sequential extraction, the yield of extractives increased two times for all studied plants. Therefore, supercritical carbon dioxide activates the plant matrix; it can be used as a stage for pre-treatment of plant raw materials to increase the yield of extractives.

Discussion

Biologically active substances of plant origin have become the subject of considerable research and development and are in great demand in the market. When conventional extraction methods (e.g. extraction in a Soxhlet apparatus or extraction with organic solvents) are used to obtain biologically active substances, the disadvantages in the form of a solvent residue, a low extraction rate, or the duration of the extraction process. These problems are solved by studying extraction technology and improving it and the corresponding model. Currently, SCFE technology is used successfully both to extract groups of biologically active substances and to extract individual components, such as caffeine (Baldino et al. 2020). However, the active substances of the plant cell are primarily contained in minor amounts. It is also crucial that the strong lignocellulose wall of the plant cell prevents the extraction of extractive substances.

The use of supercritical fluid extraction to activate the plant matrix and subsequent liquid extraction allows for the extraction of a wide range of BASs.

Supercritical fluid extraction is an alternative to solvent extraction using organic solvents. Supercritical carbon dioxide can have a solvating power similar to that of organic solvents, but with higher diffusion, lower viscosity, and lower surface tension (Dashtianeh et al. 2018). Carbon dioxide is the most commonly used supercritical fluid because it is nontoxic, nonflammable, inexpensive, environmentally friendly and is in mildly critical conditions (T = 31.1 °C and P = 73.8 bar). Due to the low heat of the process and the relatively nonreactive CO_2 used in the extraction, the aromatics produced often closely resemble the original characteristics of the feedstock. Similarly to solvent extraction, sc-CO_2 extracts a large number of nonpolar and polar compounds while maintaining their ‘nativeness’ (Osborne 2014).

An increase in the efficiency of the extraction of biologically active substances in the supercritical mode is characterized by an increase in solubility and better penetration into the lignocellulosic matrix (Zhang et al. 2019). An increase in temperature enhances the interaction of the solvent with the biological matrix, caused by hydrogen bonds, van der Waals forces, active centers in the matrix, and the dipole attraction of solute molecules (Cvjetko Bubalo et al. 2015). The presence of pressure promotes additional extraction of substances from the pores of the matrix (Wijngaard et al. 2012).

Conclusion

The data presented in this document may be of significant interest for the creation of biological products that are promising for use in the food and pharmaceutical industries. Technology has been developed for the extraction of biologically active substances from the raw materials of Narrow-leaved fireweed, black cohosh, and Golden Root under subcritical conditions, followed by treatment with a water-alcohol mixture. We found that the use of SCFE and liquid extraction increases the yield of biologically active substances by 1.3 times; 1.6 times and 3.0 times for narrow-leaved fireweed, black cohosh, and golden root, respectively.

Thus, supercritical fluid extraction is not only an effective method for extracting fat-soluble essential oil compounds but also a promising method for the preprocessing of plant raw materials before extracting polar active substances.

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