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LIPOPHILIC COMPOSITION OF PLANT *CLIMACOPTERA* SUBCRASSA

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The article provides information about the use of supercritical fluid CO_2 extraction, highlights biologically active complexes from the aerial parts of some plants in the genus *Climacoptera*. By this method of extraction, we can obtain intermediates and products of unique structure which are identified by gas-liquid chromatography with mass spectrometry. By changing the percentage and speed of a co-solvent (15 % ethyl alcohol, the rate of co-solvent 15 g/min), at 400 bar pressure, 40°C temperature, we were able to get extract, which contains polyphenols and saponins in its composition, this extract showed antidiabetic activity.

Keywords: *Climacoptera subcrassa,* supercritical fluid CO_2 extraction, gas-liquid chromatography with mass spectrometry.

Supercritical fluid extraction (SFE) is a relatively new technique in the area of analytical chemistry, having evolved in the last decade as an alternative method of preparing samples prior to analysis. SFE offers many advantages to the analyst that are not inherent in other sample preparation techniques, such as distillation, extraction with liquid solvents, or low resolution liquid chromatography. The most unique property of supercritical fluids for extraction purposes is the ability to adjust their «solubilizing power» primarily via mechanical compression (and additionally, via temperature), thereby providing the possibility of using one supercritical fluid to extract a host of analytes of varying polarity and molecular size. In addition, solute-fluid binary diffusion coefficients are much greater in supercritical fluid media than in liquid-liquid systems, thereby facilitating fast extraction from a variety of sample matrices. The proper choice of supercritical fluid can also provide specific advantages when applied in the sample workup prior to analysis. For example, the low critical temperature of supercritical CO_2, makes it an excellent candidate for extracting thermally labile compounds under conditions slightly above room temperature. In addition, CO_2 provides an extraction environment free from molecular oxygen, thereby limiting potential oxidation of the extracted solutes. Supercritical CO_2, unlike many liquid extraction solvents, is a nontoxic extraction medium; hence, its use in a laboratory environment can eliminate the cost and problems associated with solvent disposal as well as long term exposure of laboratory personnel to potential toxic vapors [1,2].

Nowadays the dominating part of pharmaceutical preparation comes to the Republic of Kazakhstan (RK) from other foreign countries, while an overall objective of the program of pharmaceutical development and drug industry of RK is to systematically decrease the dependence of public health services on the import of medicinal products. The introduction of innovative pharmaceutical practice of chemical engineering processes that encourage a comprehensive and rational use of energy and material resources is an important task. The processing of medicinal plants with liquefied gases and supercritical fluids is among the advanced techniques that can improve production efficiency and quality of herbal medicines. For some types of raw materials containing phenol this technology is considered as an additional or even alternative method for industrial processing of plants pieces [3].

In contrast to traditional methods of extraction, the supercritical fluid extraction using CO_2 provides a virtually complete extraction of biologically active substances from plants. With this method of extraction, it is possible to obtain products and intermediates of unique composition that have no analogues.

Example: The basis of the production of supercritical fluid CO_2 — of the hop extracting Germany, England, USA, Australia, the Netherlands are set supercritical fluid installations as possible to obtain up to 12 types of products from the same plant material. In Asian countries, especially in China supercritical fluid extraction is at the forefront of obtaining sea buckthorn oil by supercritical fluid CO_2 extraction [4, 5].

Excessive pressure used in the supercritical fluid CO_2 extraction eliminates oxidation end products and their destruction due to the fact that the process occurs at 50° C.

Another important factor is the fact that all processes are carried out at moderate temperature regime, up to 89—90° C, which prevents the decay of substances.

These supercritical (or near-critical) parameters dramatically change the selectivity of carbon dioxide as a solvent, which allows small changes in temperature and pressure to regulate the process of supercritical

extraction, providing the most complete extraction of active substances in the extraction of natural raw materials of plant origin [6, 7].

From the literature it is known that the method of subcritical CO_2 can be used for extraction of diglycerides, phospholipids and to tocopherols, with supercritical CO_2 extraction of organic acids, alkaloids, esters, alcohols, aldehydes, ketones, and to highlight the tannins, phenolic compounds and glycosides should be use supercritical CO_2 extraction with solvent.

So far, there is no recent precise data on the possibility of supercritical CO_2-dissolved amino acids (at the pressure of 950—1200 atm). Normal operating parameters of the extraction used today are in the range from 250 to 800 bars (depending on the type of feedstock processed and the requirements to the final extract (or fractions) [8, 9].

The aerial parts of the *Climacoptera subcrassa* collected in the flowering stage in Ile district of Almata region in the Republic of Kazakhstan.

Plants of the genus *Climacoptera* comprise 23 species, 14 species of Kazakhstan. Most of the species have been used for artisanal mining of soda. Kazakh species of the genus *Climacoptera* have not been systematically investigated, therefore it is urgent to study the chemical composition, develop the methods for isolation of biologically active substances and study their biological activity in order to design new drugs and herbal remedies [5, 6]. *C. subccrasa* contains flavonoids and their glycosides, saponins, carbohydrates, tannins, carotene, essential oils, dyes and resins.

To study the chemical constitutes of the plant material we carried out the following activities: processed aerial parts and removed impurities mechanically, dried raw material at room temperature and crushed the stock to 3—5 mm size. Then by supercritical fluid extraction, dry grinded raw material was extracted by varying the processing parameters.

We obtained a dark green extract with brown tint (AK-1) by varying the percentage and speed of the solvent (15 % ethyl alcohol, the rate of the solvent 15 g /min) at the pressure of 400 bars and 40° C.

Dark green shades of extract with ammonia and ferric ammonium alum gave a positive reaction to the polyphenols. The qualitative composition of the extract was studied by using the method of two-dimensional paper chromatography in two solvent systems: butanol-acetic acid-water (40:12,5:29) and 6 % acetic acid (table 1).

Mode, supercritical fluid CO_2 extraction allowed us to extract five monoglycosides flavonoids (substances produced a dirty green color) and four terpenoid compounds (substances manifest a solution of cerium sulphate and detected by a dark purple color). Lipophilic compounds obtained by supercritical fluid CO_2 extraction were analyzed by gas-liquid chromatography with mass spectrometry; the results are shown in table 2.

Table 1

Paper chromatography of the extract

№	R_f, systems		Developers			
	I	II	UV light	ferric ammonium alum	1 % $FeCl_3$	p-nitroaniline diazotized/15 % Na_2CO_3
1	0,57	0,87	yellow	Brown	Dirty-green	violet
2	0,56	0,5	-	-	-	-
3	0,71	0,35	yellow	Brown	Dirty-green	-
4	0,06	0,81	-	-	-	violet
5	0,26	0,58	yellow	Brown	Dirty-green	violet

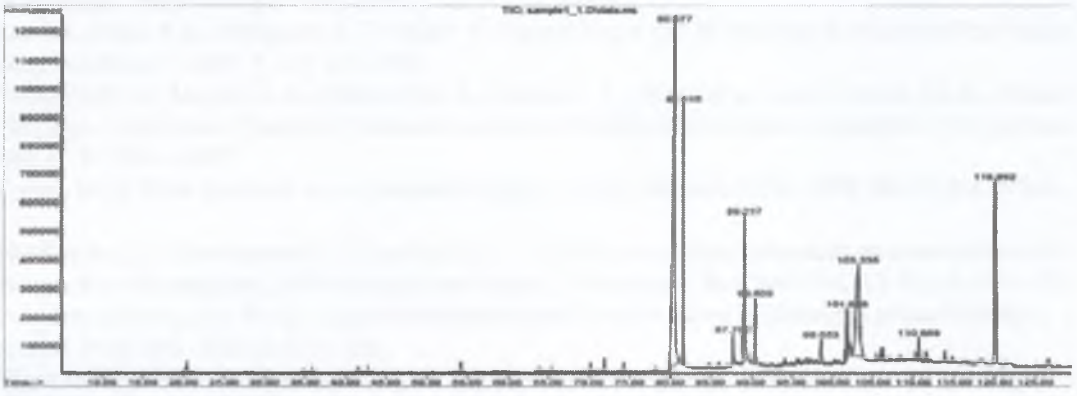

Fig. 1. The gas-liquid chromatography with mass spectroscopy extract of AK-1

Dark green shades of extract were concentrated and purified on Al_2O_3 (powder) and then analyzed by GC/MS–spectroscopy (Center for Physicochemical Methods of Research and Analysis) Almata (Kazakhstan). Results of the analysis indicate that the purified extract substances found 19 of them in sufficient numbers: oleic acid, hexadecanoic acid, ethyl ester, 4-isopropyl-2,2,5,5-tetramethyl-3-imidozolin-1-ol, octadecan.

Table 2

Lipophilic constituents of *Climacoptera subcrassa*

№	Name	Ref	Qual %
1	n-Hexadecanoic acid	102726	99
2	Tridecanoic acid	70150	91
3	Hexadecanoic acid, ethyl ester	124589	98
4	Dodecanoic acid, ethyl ester	81236	68
5	Octadec	122782	94
6	Oleic Acid	122780	93
7	Z-7-Pentadecenol	79825	87
8	Octadecanoic acid	124560	99
9	Octadecanoic acid, ethyl ester	145979	99
10	Tetradecanoic acid, ethyl ester	102760	93
11	4-Isopropyl-2,2,5,5-tetramethyl-3-imidazoline-1-oyle	46743	38
12	2h-Inden-2-one, octohydro-, oxim	26102	30
13	Hentriacontane	204900	83
14	Tetratetracontane	218306	83
15	Dodecane, 2-methyl	47640	90
16	Hentriacontane	204901	90
17	Octadecane	101148	96
18	Heneicosane	133836	96
19	Tetracosane	164290	96

For the first time, using supercritical fluid CO_2 extraction is carried out the work on recruitment conditions for extraction of biologically active substances from *Climacoptera C. subcrassa*.

We were able to obtain the extract by varying the percentage and speed of the solvent (15 % ethyl alcohol, the rate of the solvent 15 g/ min) at 400 bar pressure, temperature 40° C.

The results of the analysis indicate that the purified fragment contain 19 substances of which sufficient oleic acid, hexadecanoic acid ethyl ester, 4-isopropyl-2,2,5,5-tetramethyl-3-imidozolin-1-ol and octadecan.

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