Standardization of Calamus CO_2-extract and study of individual fractions of herbal preparations

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Abstract: This paper is devoted to the study of dense extract of calamus rhizomes (Acorus calamus L.), obtained in low-temperature subcritical extraction regimen by liquefied carbon dioxide from air - dry raw material at a pressure of 70 atm and a temperature range up to 30.5°C. There was determined chemical composition of the CO_2-Calamus extract, obtained in subcritical conditions. As part of the extract by gas chromatography - mass spectrometry there were revealed 40 individual substances, including various structures terpenes, ethers, aliphatic aldehydes, carboxylic acids and sterols. The most characteristic groups are camphor, linalool, otsimen and pinene. Therapeutic anti-inflammatory, analgesic and antiseptic effect of CO_2-extract of Calamus was experimentally confirmed, that due to the large content of essential oils that are represented as the main active components of terpenes.
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1. Introduction

In the structure of the modern pharmaceutical market steadily increasing share of drugs based on medicinal plants. A wide range of action of these drugs is explained by multicomponent composition containing different classes of chemical compounds, a unique combination of compounds of different nature and mild specific effect due to natural power contained ingredients [1, 2, 3].

Recognition of these drugs in modern medical practice and the wider application of herbal drugs in medical practice - this is not a complete list of the main advantages over synthetic drugs of similar action [4, 5].

Of special interest are herbal preparations of Calamus rhizome obtained in non-standard conditions in the low-temperature subcritical extraction of liquefied carbon dioxide mode. Method gets wide dissemination through the most appropriate and most versatile ability to extract the sume of substances and the transfer of the fractions into extracts and further defining of complex of terpene hydrocarbons, carotenoids, tocopherols, sterols, fatty acids and other biologically active substances [6, 7, 8].

Standardization of obtained herbal medicines and studying of their chemical composition were carried out for several fractions of CO_2 -extracts [9].

2. Material and Methods

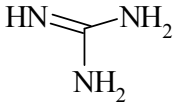
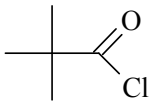
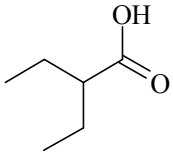
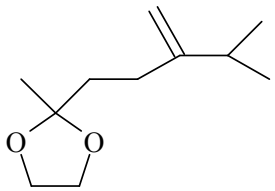
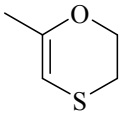
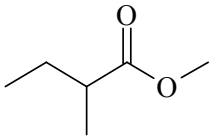
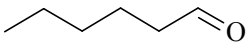
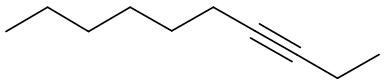
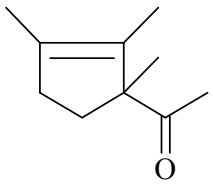
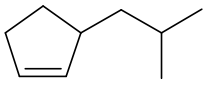
The chemical composition of the CO_2 - extracts was determined by gas chromatography-mass spectrometry method using gas chromatography-mass spectrometer Agilent 7890 GC with mass spectrometric detector 597 Agilent GC/MSD. For qualitative analysis of the test substances used method of absolute calibration. Identified structure was found according to the standard base library of the mass spectra NIST (database 150000 mass spectrum) and by retention indices of the components. Component composition was determined for 40 different substances. Among the large group of compounds were found terpenes of different structure, esters, aliphatic aldehydes, carboxylic acids, and sterols. Percentage composition identified for each CO_2 - extract Calamus component, a component of the resulting. Sequence of components release displayed based on retention time (Tab. 1).

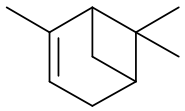
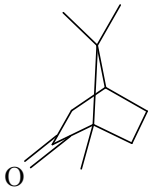
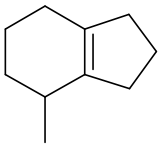
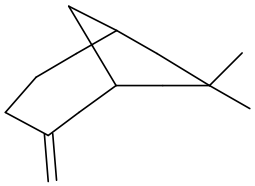
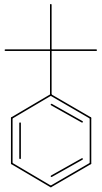
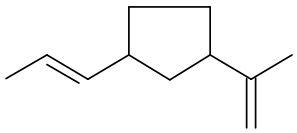
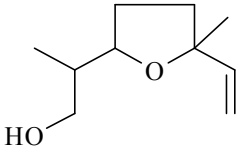
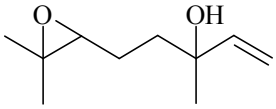
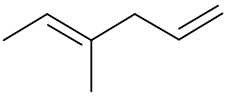
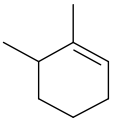
3. Results

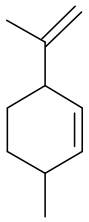
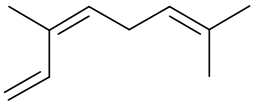
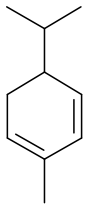
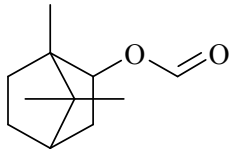
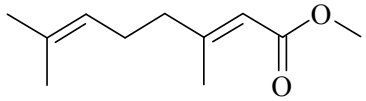
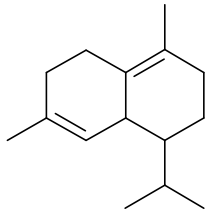
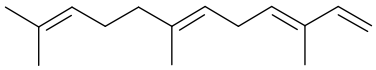
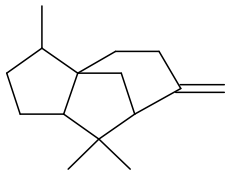
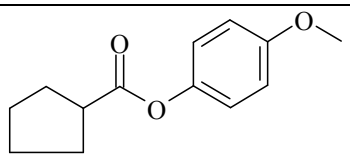
These data of chemical composition are confirmed by the physico-chemical measurement. Was done preliminary separation of the extract on a lipophilic and hydrophilic fraction. Extractives were in the range of 28 to 31 % and takes 1/3 of the herbal preparation bulk. Lipophilic extract fraction consisting of the hexane extraction was tested spectrophotometricaly in UV spectrum. Absorption region corresponding to a wavelength 229 nm shows a slight absorption corresponding to the absorption of π-electrons of unsaturated bonds. A number of bends on the absorption curve corresponding to the absorption region at wavelengths 232 and 242 nm, indicates the presence of triterpene compounds and steroid nature. Area from 267 nm to 292 nm corresponds to the absorbance maximum of aromatic compounds. In the area from 310 nm to 340 nm observed absorbance of oxygenated heterocyclic compounds. At fractionation by hexane and ethyl alcohol appear expressed band

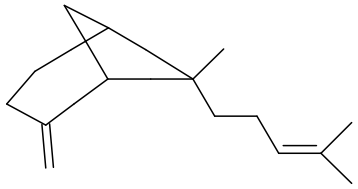
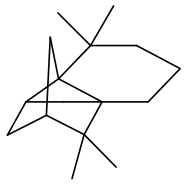
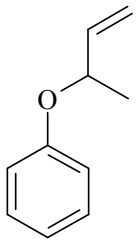
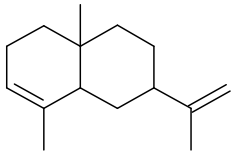
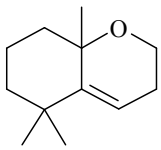
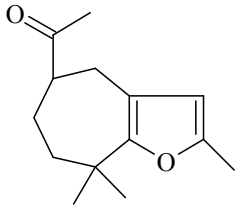
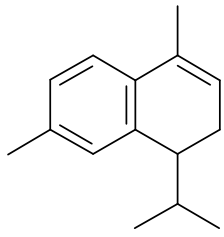
with maximum absorption at 309 nm and inflection at 338 nm, which corresponds to the sum of triterpenoids and phytosterols. In the visible absorption area observed an absorption maximum at 417 nm, indicating the presence of oxonium salts, ethers, and confirm the content of oxygen-containing heterocyclic compounds. Proof of this is expressed intense broad band in the absorption spectrums of the studying fraction, which is consistent with the data obtained in the hexane extraction.

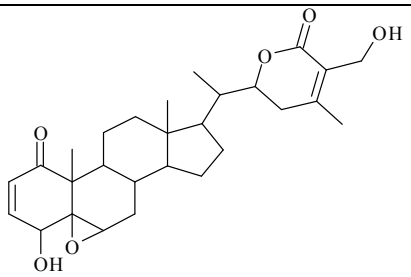
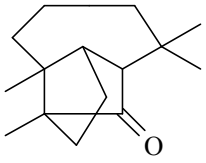
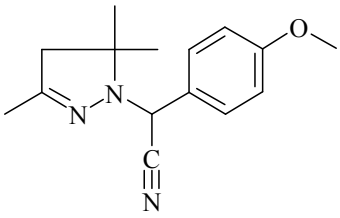
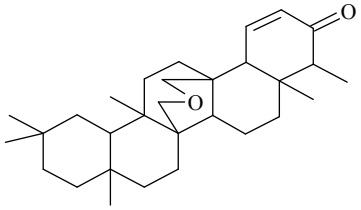
Table 1 – Component composition of CO_2-Calamus extract, displaying separation of ingredients according to the retention time

№	Name of component	Retention time, min	structural formula	content in the mixture, %
1	guanidine	0.681		0.212
2	Pivalil chloride	0.944		1.482
3	acid diethylacetic	1.192		0.513
4	1,3-dioxolane, 2-methyl-2-(4-methyl-3-methylenpentil)	1.510		0.121
5	Oxatin 1,4, 2,3-dihydro-6-methyl	1.685		0.018
6	Methyl ester of (±) 2 - methyl-butyric acid	1.921		0.104
7	Hexanal	2.155		0.268
8	3 Decin	4.180		0.322
9	1 - (1,2,3-Trimethyl-cyclopent-2-enyl)-ethanone	4.359		1.688
10	Cyclopentyl 3 - (2-methylpropyl)	4.564		7.612

11	1S-α-pinene	5.029		0.329
12	Camphora	5.760		0.416
13	2-methyl-bicyclo [4.3.0] non-1 (6)-ene	5.932		0.699
14	β-pinene	6.684		0.111
15	Tret-butylbenzene	7.129		0.598
16	1-Isopropenyl-3-propenyl cyclopentane	7.318		0.129
17	Lilac alcohol A	7.701		1.403
18	Cis-linalool oxide	12.677		0.731
19	4-methyl-hexadien-1, 4	12.783		0.369
20	1,6-Dimethyl-cyclohexen	13.053		0.253

21	(3R-trans)-3-methyl-6-(1-methylethenyl)-cyclohexene	13.263		1.001
22	(Z) -3,7-Dimethyl-1,3,6-octatriene	13.616		0.201
23	α-phellandrene	13.774		0.203
24	Isobornyl formiate	13.920		0.488
25	Methyl ester of 3,7-dimethyl-2, 6 -octadiene acid	14.305		0.598
26	(1S) -1,2,3,5,6,8 a-hexa hydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	14.505		0.242
27	α-farnesene	15.156		0.338
28	Cedrene	15.761		0.169
29	4-methoxyphenyl ester of cyclopentane carboxylic acid	16.478		0.257

30	Bicyclo [3.1.1] heptane, 6-methyl-2-methylene-6-(4-methyl-3-pentenyl)	18.029		0.553
31	Cycloisolongifolen	18.364		1.145
32	[(1-Methyl-2-propenyl) oxy]-benzol	18.419		0.489
33	1,2,3,4,4 a, 5,6,8 a-octahydro-4a,8-dimethyl-2-(1-methyl ethenyl)-naphthalene	19.056		5.753
34	5,5,8a-trimethyl-3, 5,6,7,8,8 a-hexahydro-2H-chromene	19.116		3.250
35	Ethanone, 1 - (5,6,7,8-tetrahydro-2,8,8-trimethyl-4H-cyclohepta [b] furan-5	19.170		9.544
36	α-Kalakoren	19.347		3.886

37	Vitaferin A	19.450		0.944
38	2,6,6,9-Tetramethyltricyclo [5.4.0.02,9] undecan-8-one	19.553		2.434
39	(4-methoxyphenyl) - (3,5,5-trimethyl-4,5-dihydro-1-yl) acetonitrile	19.603		6.437
40	D: A Friedoolean-1-en-3-on, 25,26-epoxy	19.736		2.013

4. Discussions

Thus, on the basis of experimental data obtained in different fractions of CO_2-calamus rhizome extract were found substances of terpenoid, steroid nature, aromatic compounds and oxygen-containing heterocycles.

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