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Research Article

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Study of lipophilic and hydrophilic fractions of CO₂ calamus rhizome extract

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ABSTRACT

Herbal medicinal products are essential in medical practice due to component composition that determines their maximum effect. Peculiarities of the determined substances action depends on the technology of plant extracts. The aim of our work was studyoflipophilicandhydrophilicfractionsofCO₂calamusrhizomeextract. Obtained results indicate the presence of terpenoid, steroid nature substances, aromatic and oxygenated heteroaromatic compounds in a rhizomes Calamus CO₂ extract.

Key words: Acorus calamus, CO₂ extract, spectrophotometrical analysis, chemical compose.

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INTRODUCTION

Creation of new herbal drugs for today remains relevant. Kazakhstan's natural resources include a large number of plant material, which at additional phytochemical research can be used in medical practice.

In the official medicine use rhizome of calamus (*RhizomataCalami*) in the form of decoctions and species for improvement of digestion, and as part of complex products "Vikalin", "Vikair" for the treatment of peptic ulcer and gastritis [1,2,3].

Recently the method plant extraction by liquefied CO₂ has attracted attention of researchers.

The aim of our work was to study the phytochemical composition of CO_2 -extract of rhizomes Calamus for the further implementation of the obtained results in pharmaceutical practice.

EXPERIMENTAL SECTION

Studies were performed on a spectrophotometer Evolutions 60 S.

At subcritical CO_2 extraction into extract pass non-polar lipophilic and relatively polar substances . In order to study the composition of the Calamus rhizomes CO_2 extract, we have attempted to divide the extract into fractions. For this precisely weighed 1.0 g of extract fractional treated by hexane, as a result part of the sample dissolved and part deposited on the wall in the form of a solidified resinous substance. After drying, the weight of the hexane-insoluble residue was 0.6996 g (69.96 %). Thus, into hexane extract passes about 30% extractives. The combined hexane extract was placed into a volumetric flask and add hexane up to 50 ml (solution A). 1.0 ml of the hexane extract was adjusted with the same solvent to 50.0 ml.

RESULTS AND DISCUSSION

The absorption spectrum of the obtained solution in the range from 220 nm to 400 nm is the sum of absorption of a number extractive substances and like the majority of similar spectra has specific peaks, and is characterized by

number of adsorption curve bends limiting absorption of functional fragments of the lipophilic fraction individual components (Fig. 1).

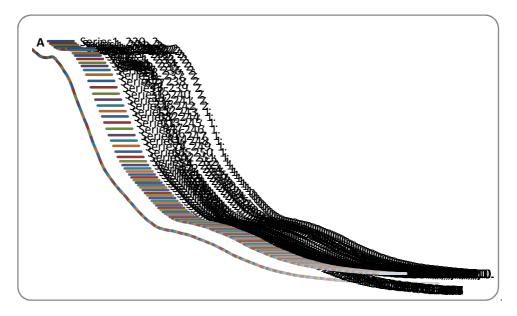


Fig. 1. The absorption spectrum of a hexane extract of Calamus rhizomes CO2 extract

In the 229 nm peak is negligible, corresponding to the absorption of π -electron of unsaturated bonds . The strip limited by inflections at 232 nm and 242 nm may indicate the presence of triterpenoid and steroid compounds. The spectral curve containing the inflections at 242 nm, 253 nm, 278 nm and 313 nm may indicate the presence in the extract of aromatic and oxygen containing heteroaromatic compounds of different degrees of conjugation. In the near- UV and visible light (from 313 nm to 400 nm) is observed red function absorption with minor inflections at 328 nm and 340 nm due to the presence of compounds with unexpressed chromophores.

The resinous residue obtained after processing of the extract with hexane, dissolved in 96% alcohol and adjusted by the same solvent up to 50.0 ml (solution B). 1.0 ml of the solution was adjusted with 96% alcohol up to 50.0 ml. The absorption spectrum of the obtained solution characterized by three major absorption bands which are superimposed and overlapped with each other. The strip limited by inflections at 232 - 258 nm may indicate the presence of triterpenoid and steroid compounds. In the area 267 - 292 nm is located a plateau corresponding to the sum of aromatic compounds with various auks chromium. Area 310 - 340 nm, characterized by one more plateau which suggests the presence of oxygen-containing heteroaromatic compounds. Than observed a gradual fall in absorption caused by the presence of the sum of weakly dyed in yellow and yellow-brown compounds (Fig. 2).

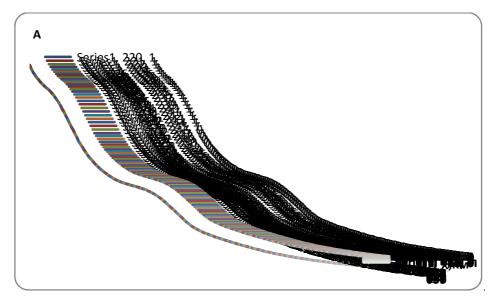


Fig. 2. The absorption spectrum of an alcoholic solution of the polar fraction of the Calamus rhizomes CO2 extract

In order to clarify the nature of the compounds contained in the CO_2 calamus rhizome extract, have been recorded absorption spectra in concentrated sulfuric acid of summary solutions, obtained at the fractionation with hexane and alcohol. For this purpose 1.0 ml of solution A was evaporated to dryness in evaporating cup. The residue was dissolved in concentrated sulfuric acid and was adjusted to 10.0 ml. 1.0 ml of this solution was adjusted to 25.0 ml by concentrated sulfuric acid. The absorption spectrum of the resulting solution is characterized by expressed band with a maximum at 309 nm and an inflection point at 338 nm, which can be classified to the absorption of the sum of oftriterpenoids and phytosterols (Fig. 3) [4]. The wide, intense band in visible light with a peak at 417 nm may indicate the formation of oxonium salts of ethers and oxygen-heteroaromatic compounds, which is consistent with literature data [5].

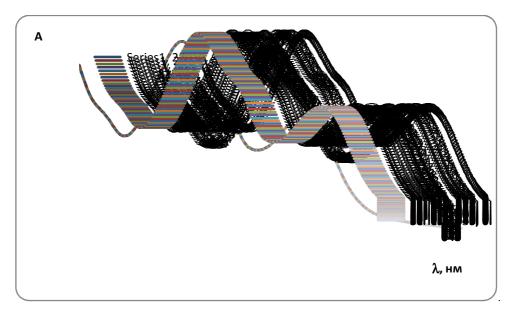


Fig. 3. The absorption spectrum of a hexane extraction solution of Calamus rhizomes CO2 extractin concentrated sulfuricacid

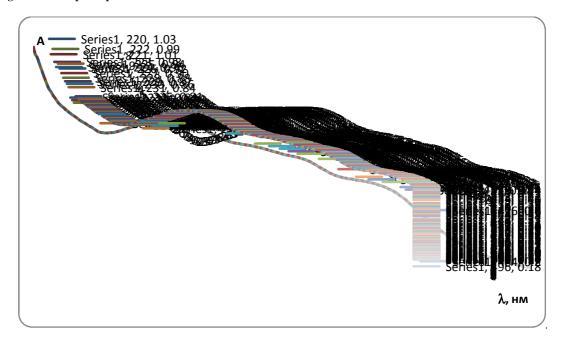


Fig. 4. The absorption spectrum of the alcoholic solution of the polar fraction of the Calamus rhizomes CO_2 extract in concentrated sulfuric acid

1.0 ml of solution B were evaporated to dryness in evaporating cup. The residue was dissolved in concentrated sulfuric acid and was adjusted to 10.0 ml. 1.0 ml of the solution was adjusted to 10.0 ml by concentrated sulfuric acid. The absorption spectrum of the resulting solution is characterized by the presence of four absorption bands. A broad band with a maximum at 297 nm corresponds to the sum of the absorption $\pi \rightarrow \pi^*$ crossings of aromatic and heteroaromatic compounds. Two inexpressive, limited by inflections stripes in the 333 - 343nm and 359 - 376nm

may indicate the presence in the extract of oxygen hetero-compounds of different nature, and a wide plateau in the 411 - 430 nm may indicate the halocromnic effect which occurs after the protonation of these compounds in the concentrated sulfuric acid (Fig. 4).

For studying the qualitative composition of the test calamus CO₂-extract has also been used chromatography method in thin layer sorbent (TLC) in such solvent systems:

- 1) Toluene ethyl acetate 93:7;
- 2) benzene acetone 25:1;
- 3) hexane acetone 7:3;
- 4) hexane chloroform 2:1.

At the starting line of the chromatographic plate Sorbfilv applied as a strip of 10 mcl of a hexane extraction solution of the investigated Calamus CO₂-extract and chromatographed by ascending. Obtained chromatograms viewed under UV light and were treated with solutions of chromogenic reagents. The best results were obtained for the system number 1 at displaying by 1% alcoholic vanillin solution and at adding an equal amount of 10% sulfuric acid alcoholic solution and at heating for 5-10 min. at 110°C. In this case on the chromatogram were found 8 strips, which in color and RF value can be tentatively identified as borneol (Rf 0.24), linalool (Rf 0.28), an unidentified substance of sesquiterpenoids nature (Rf 0.40), carvone (Rf 0. 49), bornyl acetate (Rf 0.67), nerol derivative (Rf 0.70), menthyl (Rf 0.72), anethol (Rf 0.86) [6].

At displaying of similar chromatograms by 5% alcoholic solution of phosphomolybdic acid followed by heating for 5 min. at 105 ° C were detected 11 strips of varying intensity that can presumably indicate the presence of steroid nature substances in the sample, carotenoids, iridoids...

Chromatography in system 1 of solution B showed the presence of an intense strip on a starting line and up to 4 strips, one of which appears only in iodine that may indicate the presence of aromatic and heteroaromatic substances.

CONCLUSION

Thus obtained preliminary data indicate the presence of terpenoid, steroid nature substances, aromatic and oxygenated heteroaromatic compounds in a rhizomes Calamus CO₂-extract. For a more complete understanding of extract composition is needed additional research using such methods of analysis as a gas, liquid chromatography and mass spectrometry.

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