Prospects for the use of Crowned Serpukha (Serratula tinctoria L.) as a raw material for herbal preparations V.Ya. Yatsyuk, L.E. Siplaya, G.V. Siplivy, A.V. Kukureka

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Prospects for the use of Serratula tinctoria L. as a raw material for herbal medicines

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SUMMARY

Chromatographic and spectrophotometric methods carried out qualitative and quantitative analysis of pigments and amino acids in the raw material of Serratula tinctoria L. The acetone extraction obtained by maceration showed the presence of at least five carotenoids, one of which was identified as β-carotene. The optimal mode of extraction of lipophilic substances has been determined. In the aqueous extract, the content of nine nonessential and irreplaceable amino acids was determined.

Key words: carotenoids, chlorophylls, amino acids, Serratula tinctoria L., chromatography, spectrophotometry.

RESUME

The qualitative and quantitative analysis of pigments and amino acids in raw material of Serratula tinctoria L has been conducted with the use of chromatographic and spectrophotometric methods contained. We have revealed the presence of at least five carotenoids in the acetone extract which was obtained by maceration. One of them was identified by us as β-carotene. The optimal mode of lipophilic substances extraction has been also determined. The content of nine amino acids has been revealed in water extract and their quantitative contents has been established.

Keywords: carotenoids, chlorophylls, amino acids, Serratula tinctoria L., chromatography, spectrophotometry.

Introduction

Currently, modern medicine is increasingly turning to the centuries-old experience of traditional medicine, in particular to herbal medicine. However, as a result of anthropogenic human activity, the reserves of many types of medicinal plant materials have sharply decreased. In this regard, phytochemical and pharmacological research of plant objects that form significant reserves in order to identify the possibility of creating new domestic phytopreparations on their basis is an urgent task [8].

Plants of the genus Serpukha are a promising source of biologically active substances. The genus Serratula L. (Serpukha) has more than 20 species growing in the countries of Europe, Asia and America [11]. According to the literature, 4 species of the genus Serratula (S. coronata L., S. lycopifolia A. Kern, S. radiate (Waldst. Et Kit.) Bieb, S. tinctoria L.) [10, 12].

An analytical review of the literature data showed that the chemical composition of S. coronata L. has been studied most fully. A number of domestic and foreign authors have established

the presence of a wide range of biologically active substances in the raw material of the genus Serratula L., represented mainly by phytoecdysteroids, sesquiterpenoids, flavonoids, higher fatty acids; arbutin was found in some species [11].

The experiment established that alcoholic extracts of fruits, aerial parts, containing phytoecdysteroids and flavonoids, have adaptogenic, membranotropic, radioprotective and other properties. Aqueous-alcoholic and alcoholic extracts of the herb of the Crowned Herb (S. coronata L.) exhibit antitumor, antibacterial, antifidant activity [11].

Plants of the genus Serratula L. are not widely used in folk medicine and are not used in official medicine. The reason for this is the poorly studied composition.

This work presents the results of studies of the chemical composition of biologically active substances of primary metabolism that are part of the lipophilic and hydrophilic fractions (pigments, amino acids, etc.) of one of the plant species of the genus Serratula L. The identification and quantitative determination of these groups of substances is of undoubted interest for assessing the prospects for the use of plant raw materials in order to create on their basis drugs of various pharmacological activity.

Materials and research methods

The objects of scientific research were the aerial organs of the crowned serratus Serratula tinctoria L., collected during the growing season: the beginning of flowering, the end of flowering - the beginning of fruiting in the Kursk region. Chlorophylls were qualitatively detected in acetone extraction obtained by maceration, using their ability to produce red fluorescence under the influence of ultraviolet radiation. Carotenoids were determined in purified acetone extract by ascending TLC and direct comparison with reliable samples in solvent systems: hexane, hexane-diethyl ether (3: 5), and hexane-acetone (9: 1). The number of spots on chromatograms and their color were monitored visually.

To obtain individual substances, a solution of carotenoids in ether was chromatographed on a column (4 x 30 cm) using alumina as a sorbent. The column was developed with a mixture of hexane - diethyl ether (3: 7), benzene, and fractions of 10 ml were collected. The eluates were analyzed by chromatography in a thin layer of sorbent on Silufol plates in a solvent system: petroleum ether-benzene-methanol (81: 15: 4), acetone-petroleum ether (9: 1), hexane-diethyl ether (3: 5). Fractions having the same composition were combined, evaporated in vacuo and analyzed. The identification of carotenoids was carried out spectrophotometrically by absorption spectra, by color on an adsorbent and in solutions, by the location of zones on chromatograms, by chromatography of mixed samples of isolated carotenoids with reliable samples [2].

For quantitative definitions pigments used a spectrophotometric technique that allows the determination of carotenoids and chlorophylls in their joint presence [9]. The analysis of pigments was carried out at room temperature in diffused light, since chlorophyll photooxidation can occur under strong illumination. To determine the content of pigments in the raw material of canadian small petals, the dependence of the completeness of extraction on the following technological factors was studied: the fineness of the raw material, the frequency of extraction, the extractant, the extraction method.

For quality detecting amino acids v in one retrieving

canadian small petals used the ninhydrin reaction. Next, the chromatographic determination of amino acids was carried out by means of ascending TLC in a butanolacetic acid-water solvent system (3: 1: 1) in comparison with reliable samples, followed by their quantitative determination by densitometry. For this purpose, on a chromatographic plate 150 × 120 mm in size, "Silufol" was applied at a distance of 1 cm from the edge, 0.2 μg of the extract and the same amount of 0.5% aqueous solutions of standard samples of amino acids. After the solvent front passed a distance of 10 cm, the plate was removed from the chamber and dried in air. Then the chromatogram was treated with a 0.25% solution of ninhydrin in acetone and heated in an oven at 105 ° C for 2–3 minutes [6, 7, 13].

Determination of the quantitative content of amino acids in the test samples was carried out on an automatic analyzer "Amino Acid Analyzer T 339 M". Amino acid analysis was performed on a 3.9 x 150 mm Wasers AccQ Tag column using a stepwise elution method after hydrolysis with 6 M hydrochloric acid at 110 ° C for 24 hours.

Research results and their discussion

The method of ascending thin-layer chromatography was used to determine the presence of at least five substances classified by color as carotenoids (Table 1). Further identification was carried out by the spectrophotometric method. For this, solutions were prepared in benzene, hexane, and spectra were recorded. After analyzing the absorption spectra of all fractions, it was possible to identify β-carotene at the following maxima: 437, 475, 4492 nm and 427, 463, 474 nm for benzene and hexane, respectively. Further, a comparative analysis of the obtained compound by absorption spectra with a reliable sample of β-carotene was carried out, which made it possible to determine its presence in the sample. The remaining servings are a mixture of various carotenoids and require further study. The technological process of obtaining phytopreparations includes the stage of extracting biologically active substances from medicinal plant materials. During the extraction process, a mass exchange takes place between the extractant and the solution of substances in the plant cell until the concentrations equalize. The quantitative and qualitative composition of the extraction largely depends not only on the chemical composition of biologically active substances, but also on the chemical nature of the extractant, technological preparation of raw materials and the method of extraction [1, 4].

During the experiment, it was found that the highest yield of pigments is achieved when a mixture of hexane-acetone is used as an extractant in a ratio of 1: 1 (Table 2). Of the numerous methods of obtaining the extract, we have chosen the method of fractional maceration, since it provides the highest yield of pigments and, thus, allows the most complete extraction of biologically active substances, especially thermolabile ones (Table 3). In the extraction process, the degree of grinding of the raw material plays an important role. It is known that the rate of extraction is ideally directly proportional to the degree of grinding of the raw material. In fact, practice has shown that powders with a high degree of grinding are not used for extraction. In this regard, in each specific case, it is necessary to determine the optimal degree of grinding of raw materials [3, 5]. The research results showed

To assess the effect of the extraction rate on the content of pigments and extractives, a 4-stage extraction was carried out. The results obtained indicate that it is advisable to carry out threefold

extraction, since with the continuation of the process there is no increase in the yield of biologically active substances (Table 5). The amino acid composition was studied in the hydrophilic fraction. The presence of at least 18 amino acids was found in the sample, while 9 were identified. It was found that the largest amount is: alanine, histidine, arginine and aspartic acid (Table 6).

Таблица 1

Результаты ТСХ анализа каротиноидов в Serratula tinctoria L.

Номер зоны абсорбции на хроматограмме	Величина R_f в системах растворителей		
	гексан-диэтиловый эфир 3:5	гексан	гексан-ацетон 9:1
1	0,09	0,12	0,21
2	0,12	0,30	0,25
3	0,21	0,41	0,39
4	0,81	0,60	0,41
5 (β-каротин)	0,96	–	0,90

Таблица 2

Влияние экстрагента на содержание пигментов (каротиноидов и хлорофиллов) в Serratula tinctoria L.

Экстрагент	Содержание пигментов* в мг/100 г	
	1	2
Гексан	4,46	3,05
Ацетон	8,05	15,61
Гексан-ацетон (1:1)	14,02	22,12

* – среднее значение пяти определений; 1 – содержание каротиноидов; 2 – содержание хлорофиллов

Таблица 3

Влияние метода экстракции на содержание пигментов (каротиноидов и хлорофиллов) в Serratula tinctoria L.

Метод экстракции	Содержание пигментов* в мг/100 г	
	1	2
Экстракция при нагревании	14,02	22,12
Дробная мацерация	15,06	23,10

* – среднее значение пяти определений; 1 – содержание каротиноидов; 2 – содержание хлорофиллов

Таблица 4

Влияние степени измельчения сырья на содержание пигментов и экстрактивных веществ в Serratula tinctoria L.

Степень измельчения, мм	Содержание пигментов* в мг/100 г		Содержание экстрактивных веществ* в %
	1	2	
1	17,02	22,81	5,96
3	10,01	17,46	4,60
5	5,02	12,11	3,21

* – среднее значение пяти определений; 1 – содержание каротиноидов; 2 – содержание хлорофиллов.

In the course of the analysis, the substances of the primary biosynthesis of the raw material of the dye serpukha were studied: pigments and amino acids. The information obtained on the qualitative composition and quantitative content of primary metabolic substances suggests that further study of plants of the genus Serratula L. is promising for the creation of domestic phytopreparations based on them.

Таблица 5

Влияние кратности экстракции на содержание пигментов и экстрактивных веществ в Serratula tinctoria L.

Ступень экстракции	Содержание пигментов*, г/100 г		% извлечения		Содержание экстрактивных веществ*, %
	1	2	1	2	
1	17,01	22,05	41	44	5,57
2	12,01	15,08	32	31	3,82
3	10,08	12,34	22	23	3,21
4	2,05	3,01	7	8	2,08

* – среднее значение пяти определений; 1 – содержание каротиноидов; 2 – содержание хлорофиллов

Таблица 6

Результаты ТСХ анализа свободных аминокислот водного извлечения Serratula tinctoria L. в системе растворителей бутанол – уксусная кислота ледяная – вода (3:1:1)

Номер зоны абсорбции	R_f	Аминокислота	Количественное содержание в сырье*, %
1	0,08	аргинин	0,26
2	0,09	гистидин	0,32
3	0,38	аспарагиновая кислота	0,20
4	0,44	серин	0,12
5	0,51	аланин	0,50
6	0,52	треонин	0,10
7	0,55	глутаминовая кислота	0,8
8	0,60	валин	0,6
9	0,62	метионин	0,8

* – среднее значение пяти определений

conclusions

1. In the lipophilic extract from the raw material of Serratula tinctoria L., the presence of not less than five carotenoids, one of which has been identified as β-carotene.

2. In the hydrophilic fraction from the raw material of Serratula tinctoria L., the content of nine essential and nonessential amino acids.

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