Study of the polyphenolic composition of Serpukha tinctoria (Serratula tinctoria L.)

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Research of the saw-wort (Serratula tinctoria L.) polyphenol composition

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RESUME

In the alcoholic extract of Serratula tinctoria L. herb presence of 22 substances of polyphenolic nature with help of high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC) has been established. Quantitative content of total flavonoids in the Serratula tinctoria L. herb 3.12 ± 0.05% using a technique of differential spectrophotometry has been determined.

Keywords: Serratula tinctoria L., phenolic compounds, HPLC, TLC, spectrophotometry.

SUMMARY

High performance liquid chromatography (HPLC) and thin layer chromatography (TLC) in the alcohol-water extract of the herb Serratula tinctoria L. revealed the presence of 22 polyphenolic substances. Using the method of differential spectrophotometry, the quantitative content of the sum of flavonoids in the herb of serrata dye was determined as 3.12 ± 0.05%.

Keywords: Serratula tinctoria L., phenolic compounds, HPLC, TLC, spectrophotometry.

Introduction

Currently, much attention is paid to the issue of introducing herbal medicinal substances into medical practice. This is due, on the one hand, to a wide range of therapeutic effects of biological complexes that make up medicinal plants, on the other hand, with small side effects. Therefore, the search for new sources of raw materials is an urgent task for domestic pharmacy.

This work is a continuation of the previously begun studies of the chemical composition of the dye serpukha, which is widely represented in the flora of the Central Black Earth region [6, 8].

Materials and research methods

As an object of study, we used the aboveground organs of Serratula tinctoria L., collected during the growing season: “the end of flowering - the beginning of fruiting” in the Kursk and Belgorod regions. The collected raw materials were subjected to air drying in natural conditions at a temperature of 25 ° C, without access to direct sunlight.

For the study of polyphenolic compounds by the method of extraction with 70% ethyl alcohol when heated for 60 minutes in the process of fractionation of natural compounds from the meal remaining after the isolation of the amount of lipophilic compounds, an alcohol-water fraction was obtained. The chemical composition of the alcohol-water fraction was investigated using qualitative reactions to the groups of the studied biologically active substances, TLC

in various solvent systems, spectrophotometry, HPLC.

To establish the flavonoid composition, the method of ascending thin layer chromatography on Sorbfil plates was used. Solvent systems were used as the mobile phase: n-butanol - glacial acetic acid - water (4: 1: 2), 15% acetic acid. Chromatographic analysis of coumarins in a thin layer was carried out using as solvent systems: chloroform - benzene (1: 2), ethyl acetate - benzene (1: 2), phenol carboxylic acids: chloroform - ethyl acetate - formic acid (5: 4: 1) , chloroform - methanol - water (61: 32: 7). The spots were identified in UV light before and after processing chromatograms with chromogenic reagents [2, 7].

For the quantitative determination of flavonoid compounds with their own absorption in the UV and visible spectral regions, contained in the samples under study, we used the spectrophotometric method of analysis, as it does not require large portions of raw materials, is characterized by a sufficiently high accuracy and is widely used for the analysis of biologically active substances. To determine the content of the sum of flavonoids in the raw serpukha dyeing, the dependence of the extraction completeness on the following technological factors was studied: raw material fineness, extraction ratio, extraction time, type of extractant, hydromodule [1, 4, 5].

Determination of the chemical composition of the alcohol-water fraction was also carried out using HPLC on a Gilston high performance liquid chromatograph (manual injector, model Rheodyne 7125 USA), followed by computer processing of the research results using the Multichrome program for Windows. The stationary phase was a 4.6 x 250 mm Kromasil C 18 metal column with a particle size of 5 microns. A system of solvents methanol - water - concentrated phosphoric acid in a ratio of 400: 600: 5 was used as a mobile phase. The analysis was carried out at room temperature. The flow rate of the eluent is 0.8 ml / min. Analysis duration 70 min. Detection was carried out using a Gilston UV / VIS UV detector at a wavelength of 254 nm [3].

The raw material in the process of sample preparation was crushed to a particle size passing through a sieve with a hole diameter of 1 mm. 7.0 g of raw materials were placed in flasks with a capacity of 200.0 ml, 60 ml of 70% ethyl alcohol were added, connected to reflux condensers and heated in a boiling water bath for 60 minutes from the moment the alcohol-water mixture boiled in the flasks. After cooling, the resulting mixtures were filtered through paper filters into volumetric flasks with a volume of 100.0 ml and brought up to the mark with 70% ethyl alcohol (test solutions).

In parallel, a series of 0.05% solutions of standard samples (GSO) in 70% ethyl alcohol was prepared: rutin, tannin, quercetin, luteolin, luteolin-7-glycoside, gisperidin, apigenin, hyperoside, dihydroquercetin, kaempferol, vitexin, isovitexin, baicaringin, na , isoramnetin, gallic, coffee, chlogenic, chicory, cinnamic, ferulic, ellagic, o-coumaric acids, umbelliferone, esculetin, coumarin, 3-methoxycoumarin, epigallocatechin gallate, epicatechin. 50 μL of the test solution and GSO were injected into the chromatograph and chromatographed as described above.

Research results and their discussion

As a result of chromatographic analysis (TLC), it was found that the grass of the dye herb contains at least 4 substances, derivatives of 2-phenyl-benzo-γ-pyrone, including rutin, 2 substances of derivatives of 5,6-benzo-α-pyrone, including identified - coumarin and dihydrocoumarin, at least 3 phenolcarboxylic acids were also found, one substance was identified as chlorogenic acid.

The carried out research on choice optimal conditions extraction indicate that the maximum extraction of the sum of flavonoids from the herb of serpukha dyeing is achieved with a double extraction for 30 minutes with 70% ethyl alcohol with a particle size of 1 mm, with a hydromodule of 1:40. The quantitative content of the sum of flavonoids in the raw serpukha dye is in the range from 3.07 to 3.17%. To prove the reproducibility of the method, the metrological characteristics of the fivefold determination of the content of the sum of flavonoids in the raw material of the dyeing sickle were carried out. The relative error of a single determination with a confidence level of 95% does not exceed 5%, which indicates a satisfactory reproducibility of the method.

The data obtained using HPLC indicate the content of 22 phenolic compounds in the herb of serrata dye: derivatives of 2-phenyl-benzo-γ-pyrone (flavonoids), benzo-α-pyrone (coumarins), as well as phenol carboxylic acids, of which 14 were identified. (Table 1).

The quantitative ratio of substances of polyphenolic nature in the studied extracts was established by the method of normalization by the areas of chromatographic peaks. Based on the data presented in table. 1, of the 2-phenyl-benzo-γ-pyrone derivatives identified in the herb of serrata dye, hyperoside and lutein-7-glycoside predominate, of phenol carboxylic acids, chlorogenic and chicory ones, tannin is present in a significant amount.

As a result of the studies, it was found that the chemical composition of the alcohol-water fraction of Serratula tinctoria L. is represented by a wide range of biologically active polyphenolic compounds, which determine the relevance of their further study.

conclusions

1. Serpukha dye contains a wide range of biologically active compounds, not less than 22 substances of polyphenolic nature.

2. Polyphenols of serrata dye are represented by flavonoids, coumarins, phenol carboxylic acids.

3. The quantitative content of the sum of 2-phenyl-benzo-γ-pyrone derivatives - 3.12 ± 0.05%.

Table 1

Identified polyphenolic compounds of the alcohol-water fraction of the herb dyestuff

P / p No.	Retention time	Identified substances	Quantitative ratio,%
one	186.8	tannin	22.57
2	188.7	gallic acid	8.23
3	299.0	chicory acid	8.51
4	384.2	caffeic acid	5.02
5	889.0	hesperidin	4.82
6	451.0	ferulic acid	4.02
7	550.9	dihydrocoumarin	3.02
eight	620.1	coumarin	2.68
9	805.5	luteolin-7-glycoside	7.66
10	977.5	hyperoside	11.25
eleven	1094.3	routine	4.25
12	1200.1	dihydroquercetin	5.03
thirteen	1205.7	vitexin	5.16
14	2917.5	quercetin	3.06

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Study of the polyphenolic composition of Serpukha tinctoria L. / V.Ya. Yatsyuk, L.E. Siplaya, G.V. Siplivy, A.V. Kukureka // Traditional medicine. - 2015. - No. 2 (41). - P.22-24.