Study of the chemical composition and biological activity of the lipophilic fraction from herbs and flowers of Serpukha dyer Serratula tinctoria L.

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A study of the chemical composition and biological activity of the lipophilic fraction of Serratula tinctoria L. herb and flowers

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SUMMARY

Chromatomass spectrometric analysis of the lipophilic fraction of the raw material of the herb and flowers of Serratula tinctoria L. 55 compounds were identified in the flowers, and 51 compounds in the herb belonging to various classes of biologically active substances (fatty acids, sterols, alcohols, heavy terpenoids). At the same time, 48 compounds were found and identified in both morphological groups of raw materials. Impurities of sugars, amino acids and parts of artifacts are excluded.

Pharmacological studies have shown that the lipophilic complex isolated from the raw materials of Serratula tinctoria L. exhibits analgesic and anti-inflammatory activity, enhances the antimicrobial activity of gentamicin in toxic kidney damage in combination with staphylococcal infection.

Key words: lipophilic fraction, chromato-mass spectrometry, serpu dyer, Serratula tinctoria L.

RESUME

A chromatographicspectrometric analysis of the lipophilic fraction of the raw material of grass and flowers of Serratula tinctoria L. 55 flowers were identified in the flowers, 51 compounds belonging to different classes of biologically active substances (fatty acids, sterols, alcohols, heavy terpenoids) in the grass. At the same time 48 compounds were detected and identified in both morphological groups of the raw material. The impurities of sugars, amino acids and parts of artifacts were excluded.

Pharmacological studies have shown that the lipophilic complex isolated from Serratula tinctoria L. raw material shows analgesic, antiinflammatory activity, enhances the antimicrobial activity of gentamicin in toxic kidney damage in combination with staphylococcal infection.

Keywords: Lipophilic fraction, chromatographymass spectrometry, sawwort (sawwort), Serratula tinctoria L.

INTRODUCTION

The search for promising domestic sources of medicinal plant materials, the development and implementation of new herbal medicines are among the priority areas of domestic medical and pharmaceutical science.

The need to expand the range of domestic herbal preparations is due, among other things, to their lower toxicity with a sufficiently high efficiency [3]. This work is a logical continuation of a series of studies of the chemical composition of the dyer's serpu, Serratula tinctoria L. (family Asteraceae), which forms sufficient reserves for industrial harvesting in the Central Black Earth region [6].

Previously, we studied some representatives of the genus Serratula L. for the presence of substances of primary and secondary synthesis and found that water-soluble

the polysaccharide complex and the complex of pectin substances (PV) of the leaves and flowers of Serratula tinctoria L. have a similar composition, but differ in the quantitative content of individual components. The monosaccharide composition of the water-soluble polysaccharide complex and the HP complex is represented by glucose, galactose, xylose, arabinose, rhamnose and fructose. At the same time, the maximum amount of glucose and the minimum amount of rhamnose were found in the water-soluble polysaccharide complex of leaves and inflorescences of the serpentine dye. In the HP complex, on the contrary, rhamnose dominates, and galactose is detected in a smaller amount [10].

Using the methods of high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) in an alcohol-water extract of the herb Serratula tinctoria L., we previously revealed the presence of 22 substances of a polyphenolic nature (flavonoids, coumarins, phenolcarboxylic acids). Using the technique of differential spectrophotometry, the content of the sum of flavonoids was quantified - 3.1 ± 0.5% [9].

The purpose of this work is to study the chemical composition and some types of pharmacological activity of the native complex of substances of primary biosynthesis, passing into the lipophilic fraction from the flowers and grass of Serratula tinctoria L.

MATERIALS AND RESEARCH METHODS

The objects of the study were the above-ground organs (grass, flowers) of the dye serpu, collected in the vegetation phase "end of flowering - beginning of fruiting" (maximum yield of raw materials) 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.

The lipophilic fraction for determining the chemical composition using the chromato-mass-spectrometric method of analysis was obtained by maceration for 24 hours (the extractant was diethyl ether, the hydromodulus was 1:10). The resulting extracts were filtered and evaporated to dryness under vacuum. Dry extracts (3–5 mg, accurately weighed) were treated with 0.4 ml of 1 M hydrochloric acid in methanol at 80°C for 180 minutes (acid methanolysis). Fatty acid methyl esters and other lipid components formed during methanolysis were extracted with hexane. Hexane was evaporated, and the dry residue was silylated in 20 μl of BSTFA (bis(trimethylsilyl)trifluoroacetamide) for 15 min. at 80°C and diluted with hexane to 10 μl. For analysis, 1 μl of the mixture was injected into the injector of the chromato-mass spectrometer system operating in the automatic mode [7].

Chromatography-mass-spectrometric analysis was carried out on an AT-5850/5973 Agillent Technologies chromato-mass spectrometer (USA). Quadrupole mass spectrometer with a mass range of 2–950 amu. has a resolution of 0.5 a.m.u. throughout the entire operating range. Ionization by electrons 70 eV. The sensitivity of the instrument is 0.01 ng for methyl stearate.

For chromatographic separation of samples, a fused silica column 25 m long and 0.25 mm in inner diameter was used. Stationary phase HP-5ms Hewlett-Packard with a layer thickness of 0.2 μs. Chromatography was carried out in the temperature programming mode from 135 to 320°C at a rate of 7 deg/min. The temperature of the injector and interface is 280 °C. Data processing was carried out using standard programs of the device. Substances in the chromatographic peaks were identified using library programs with the NIST mass spectra database.

For pharmacological studies and dosing optimization, the obtained lipophilic extracts were evaporated to the consistency of thick extracts. Standardization of the lipophilic extract was carried out according to the content of pigments (carotenoids and chlorophylls) by a spectrophotometric method, which allows them to be determined by joint

presence [10].

The study of biological activity was carried out on rats and mice, 6 animals in each series, according to the objectives of the experiment.

The acute toxicity of the lipophilic complex was determined on healthy animals according to the method of B.M. Shtabsky [8]. The study of analgesic activity was carried out on mice using the "vinegar writhing" model and the formalin pain response model [2]. A model of microbial compromise of the body with involvement of the kidneys in the pathological process was created by intragastric administration of mercury dichloride at a dose of 2 mg/kg and intraperitoneal injection of pre-titrated doses of a daily agar culture of Staphylococcus aureus containing $1 \times 10_{eight}$microbial bodies in 0.5 ml of solution [1, 2, 4].

To obtain reliable data, the rats were divided into 4 groups of 9 animals: the control group (intact healthy rats), the second group of animals with impaired renal excretory activity under conditions of staphylococcal infection (experimental group), the third group of experimental rats treated only with gentamicin, and the fourth a group of experimental animals treated with gentamicin and a lipophilic complex. The introduction of gentamicin and biologically active complex was carried out intramuscularly, once for 5 days, gentamicin at a dose of 4 mg/kg and lipophilic extract - 3 mg/kg. Gentamicin at a dose of 4 mg/kg was used as a reference drug. The excretory function of the kidneys was assessed by the amount of urea and creatinine in the blood [5].

RESULTS OF THE STUDY AND THEIR DISCUSSION

As a result of the chromato-mass-spectrometric analysis, the presence of at least 59 components was found in the composition of the lipophilic fraction of the serpukha dyer grass (Table 1). It was possible to identify 55 compounds in the flowers of serpuhi tinnitus, and 51 compounds belonging to the classes of fatty acids, sterols, alcohols, and heavy terpenoids in the herb. At the same time, only 48 compounds are present in both studied morphological groups of raw materials (Table 1).

Table 1

The results of the study of the composition of the lipophilic fraction of the grass and flowers of Serratula tinctoria L. by chromato-mass spectrometry

Number peak	Time holding, min.	Substance	Composition from the amount, %	
			herbs	flowers
one	6.698	Lauric acid	0.580	0.666
2	7.093	azealic	-	0.232
3	7.169	Elemicin (trimethoxyallylbenzene)	-	0.523
4	9.576	Myristic	1,300	1.944
5	10.445	Iso-pentadecanic	0.072	0.221
6	10.520	Anteiso-pentadecanic	1.230	1.130
7	11.006	Pentadecanoic	0.507	0.422
eight	11,230	Hexahydrofarnesol-acetone	0.727	0.917
9	11,802	Iso-hexadecanoic	0.306	0.127
10	12.154	Hexadecenoic acid	1.782	2.380
eleven	12.257	Hexadecane	8.875	10.621
12	12.359	3-hydroxy-myristic	1.230	0.178
thirteen	13.215	Iso-heptadecanic	0.587	0.356
14	13.496	Azeite-heptadecanic	0.366	0.385

15	13.621	Cyclopropane-heptadecane	1.253	1.192
sixteen	13.687	Heptradecanoic	1.652	0.809
17	14.689	Linoleic	7.043	13.216
eighteen	14.738	Oleic	6,980	6.957
nineteen	15.023	Stearic	3.714	4.121
twenty	15.182	2-hydroxy palmitic	0.084	1.203
21	15.495	Octadecanol	0.309	0.295
22	16.933	Octadecatriene, conjugated	-	0.279
23	17.031	Octadecatriene, conjugated	-	0.387
24	17.266	N-trieicosan	1.798	0.954
25	17.265	Eicosenoic acid	-	0.248
26	17.634	Eicosanoic acid	1.669	2.955
27	17.925	Eicosanol	1.833	1.785
28	18.462	N-pentacosane	0.508	0.486
29	18.710	Heneicosanoic acid	0.578	0.446
thirty	18.901	Geneicosanol	-	0.258
31	19.532	N-hexacosan	4.233	3.095
32	19.974	Docosanoic acid	2.907	1.532
33	20.295	Docosanol	2.808	3.013
34	20.710	N-heptacosan	0.469	0.896
35	20.970	Tricosanoic acid	0.990	0.700
36	21.272	Tricosanol	1,580	0.505
37	21.704	N-octacosan	5.727	5.333
38	21.827	Tetracosenoic acid	-	1.292
39	21.907	2-hydroxy-docosanoic acid	2.279	2.257
40	22.152	Tetracosanoic acid	6.205	4.752
41	22.215	Tetracosanol	6.965	4.007
42	22.828	N-nonacosan	0.740	0.750
43	22.929	2-hydroxy-tricosanoic acid	0.742	0.680
44	23.175	Pentacosanoic acid	0.705	0.629
45	23.303	Pentacosanol	0.620	0.408
46	23,850	N-triacontane	9.336	7,800
47	23.918	2-hydroxy-tetracosanoic acid	5.287	-
48	24.077	Hexacosanoic acid	2.481	2.983
49	24.279	Hexacosanol	2.495	1.875
50	24.705	N-gentriacontane	3.009	-
51	24.945	2-hydroxy-pentacosanoic acid	1.454	1.458
52	25.042	Heptacosanoic acid	-	1.520
53	25.665	N-dotriacontane	9.074	6.360
54	25.823	2-hydroxy-hexacosanoic acid	1,840	-
55	25.976	Octacosanoic acid	2.516	2,930
56	27.776	Ergostenol	1.072	0.740
57	27.898	Stigmasterol	2.933	4.210
58	28.709	sitosterol	8.975	9.416
59	29.503	Lanostadienol	2.909	3.451

When determining the toxicity of the lipophilic extract from the flowers and the grass of the serpukha dyer, it was possible to establish that the extract is not toxic in the studied doses. The study of pharmacological activity showed that the lipophilic extract has

high analgesic activity in kinin pain response suppresses pain response by 55% ($p < 0.01$); antinociceptive activity against the central component of pain formation and reduces the sensitivity of pain receptors to the action of inflammatory mediators by 40% ($p < 0.01$); anti-inflammatory effect on the model of acute aseptic inflammation, reducing the intensity of edema by 41.3%.

It has been established that the lipophilic extract administered with gentamicin accelerates the normalization of the excretory activity of the kidneys when staphylococcus is administered (Table 2).

table 2

Changes in the excretory activity of the kidneys in rats with the introduction of gentamicin and the lipophilic fraction of the grass and flowers of Serratula tinctoria L.

Group	Creatinine mmol/l	Urea, mmol/l
1. Control (healthy rats)	10.9±1.1	141.2 ± 14.8
2. Rats with impaired renal excretory function and administration of staphylococcus aureus	19.8 ± 2.4*one	237.4 ± 21.9*one
3. Rats with impaired renal excretory function under conditions of staphylococcus treated with gentamicin	15.2 ± 1.4*1.2	201.2 ± 20.3*1.2
4. Rats with impaired renal excretory function treated with gentamicin and lipophilic fraction	12.3 ± 1.0*2.2	156.8 ± 15.6*2.3

Note. * and the number next to it indicate significant differences between the groups ($p \leq 0.05$).

CONCLUSIONS

1. The chemical composition of the lipophilic fractions of grass and flowers of serpukha dyer. 55 compounds were identified in flowers, 51 compounds belonging to different classes of biologically active substances were identified in grass. At the same time, 48 compounds were found and identified in both morphological groups of raw materials.

2. It has been experimentally proven that the lipophilic extract from the raw material of the dye significantly increases the anti-inflammatory effect of gentamicin, as evidenced by a decrease in the levels of creatinine and urea in the blood of experimental animals.

3. The results obtained allow us to consider it expedient to further study lipophilic fraction of serpukha dyer with the aim of creating domestic herbal preparations with a given pharmacotherapeutic activity.

LITERATURE

1. Afanasiev V.A., Laskova I.L. On the mechanism of development of immunosuppression in acute renal insufficiency // Pathological physiology and experimental therapy. - 1997. - No. 3. – P.31–35.

2. Gatsura V.V. Methods of primary pharmacological research biologically active substances. - M.: Medicine, 1974. - 143 p.

3. Golyshenko P.P. Medicinal plants and their use. - Saransk: Mordovian book publishing house, 1996. - P.291

4. Esilevsky Yu.M. The pathogenesis of pyelonephritis. - M.: MEDpress-inform, 2007. - 368 p.

5. Menshikov V.V. Laboratory research methods in the clinic: a reference book. – M.:

Medicine, 1987. - 365 p.

6. Poluyanov A.V., Prudnikov N.A. Vascular plants of the Kursk region: educational allowance. - Kursk: KSU, 2005. - P.67.

7. Khmelnitsky R.A., Brodsky E.S. Chromato-mass spectrometry. - M.: Chemistry, 1984. - pp.150–178.

8. Shtabsky B.M. On the method for determining average lethal doses and concentrations chemicals // Hygiene and sanitation. - 1980. - No. 10. – P.49–61.

9. Yatsyuk V.Ya., Siplivy G.V., Siplivaya L.E. et al. Study of polyphenol composition Serpukha dye (Serratula tinctoria L.) // Traditional medicine. - 2015. - No. 2, V.41. – P.22–24.

10. Yatsyuk V.Ya., Siplivy G.V., Siplivaya L.E. etc. Prospects for the use of serpuha crowned (Serratula tinctoria L.) as a raw material for herbal preparations // Traditional Medicine. - 2014. - No. 3, V.38. – P.18–21.

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