

PHENOLIC COMPOUNDS OF *SERRATULA CENTAUROIDES* AND ANXIOLYTIC EFFECT

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Abstract

The paper presents the results of the study of a dry extract of *Serratula centauroides* (*Asteraceae*) using a HPLC method and estimation of his anxiolytic activity. 17 phenolic substances and 15 respectively were identified in the herb in the roots. In the herb of the plant, there were revealed hyperoside and luteolin. The aerial part contains twice as much ecdysterone as the roots of *S. centauroides*. The experiments conducted on animals have shown that the *S. centauroides* dry extract at the doses comprised between 50 - 150 mg/kg bw has a marked anxiolytic effect. *S. centauroides* extract increases the number of entries into the open branches of elevated plus maze (EPM) and prolongs a residence time in the open arms of EPM and light compartment of the dark/light chamber. The use of *S. centauroides* increases the number of punished water intakes in the Vogel conflict test.

Rezumat

Lucrarea prezintă rezultatele studiului efectuat asupra extractului uscat obținut de la *Serratula centauroides* (*Asteraceae*) prin metoda HPLC și estimarea activității anxiolitice a acestuia. Au fost identificate 17 substanțe fenolice în partea aeriană a plantei și 15 substanțe fenolice în rădăcini. În partea aeriană a plantei au fost descoperite hiperozida și luteolina. Partea aeriană conține de două ori mai mult ecdysteron decât rădăcinile de *S. centauroides*. Experimentele pe animale au arătat că extractul uscat administrat în doze cuprinse între 50 - 150 mg/kgc are un efect anxiolitic marcant în testul labirintului și testul Vogel.

Keywords: *Serratula centauroides* (L.), phenolic compounds, HPLC, anxiolytic effect

Introduction

Serratula centauroides (L.) of *Asteraceae* family is an ecdysteroid-containing plant commonly found in Siberia, Far East, Mongolia, Europe and Caucasus [8, 15]. The plant material contains a complex of following biologically active substances: ecdysteroids, flavonoids, polysaccharides, tannins, triterpenoid saponins, coumarins and amino acids [11]. The lipid composition of the plant has been studied and fat acids, phytosterols, alkanes and hydroxy acids have been identified [22]. The ether oil component composition of the plant has been determined [23]. In the plant material of *S. centauroides* there were identified B vitamins [21]. Among ecdysteroids there were identified 20-hydroxyecdysone (ecdysterone), 2-desoxy-20-hydroxyecdysone, integristerone A and viticosterone E. in the underground and aerial organs of *Serratula centauroides* there was studied the distribution of 20-hydroxyecdysone (ecdysterone), 2-desoxy-20-hydroxyecdysone and integristerone [26, 27]. The presence

of 20-hydroxyecdysone (ecdysterone) and 2-desoxy-20-hydroxyecdysone is characteristic for most species of *Serratula* [1, 25]. 20-hydroxyecdysone is prevalent among ecdysteroids extracted from the plant; the total content of ecdysteroids in the herb of *S. centauroides* amounts to 1.42% [12].

In the folk medicine, particularly, in the practice of Buryat lamas, the decoction of the plant was used as antihemorrhagic remedy [3]; the experiments have revealed that preparations from the plant have haemostatic and anabolic properties [15].

The dry extract from the *S. centauroides* herb has shown nootropic, antihypoxic, anticonvulsant and membrane stabilizing effects in the conducted studies [17-19].

The present research aimed the study of phenolic composition of *Serratula centauroides* by HPLC method and estimation of the anxiolytic activity of the dry extract obtained from *S. centauroides*.

Materials and Methods

The specimens of the *Serratula centauroides* aerial part were gathered in Ivolginsky district of Buryatia Republic (Russia) in July 2017 in the flowering period and roots were gathered in August 2017 in the wilting period.

The study of the phenolic composition in the aerial part and roots of *S. centauroides* was carried out on a HPLC ("Gilson", France), manual injector (Rheodyne 7125, USA) with the following computer processing of the results (Multichrom program for Windows). As a stationary phase there was used a metal column (4.6 x 250 mm) filled with Chromasil C18; the size of the particles – 5 microns. As a mobile phase there was used methanol-water-concentrated phosphoric acid (400:600:5). The analysis was carried out at room temperature. The rate of eluent feed was 0.8 mL/min. The duration of the analysis was 170 min. Detection was carried out with the use of an UV-detector ("Gilson" UV/VIS, 151) at 254 nm wave length. The following reliable samples of compounds were used for the identification of substances in the extracts: rutin (Sigma Aldrich, USA, cat. No R5143; ≥ 94%), quercetin (Sigma Aldrich, USA, cat. No Q4951; ≥ 95%), luteolin (Sigma Aldrich, USA, cat. No L9283; ≥ 98%), gallic acid (Sigma Aldrich, USA, cat. No G7384; ≥ 97.5%), caffeic acid (SigmaAldrich, USA, cat. No C0625; ≥ 98%), chlorogenic acid (Sigma Aldrich, USA, cat. No C3878; ≥ 95%), hyperoside (Sigma Aldrich, USA, cat. No 83388; ≥ 97%), ferulic acid (Sigma Aldrich, USA, cat. No 128708; ≥ 99%), neochlorogenic acid (SigmaAldrich, USA, cat. No 94419; ≥ 98%), chicoric acid (SigmaAldrich, USA, cat. No C7243; ≥ 95%), dicoumarin (Sigma Aldrich, USA, cat. No M1390; ≥ 94%), dihydroquercetin (Sigma Aldrich, USA, cat. No T4512; ≥ 90%), epicatechin (Sigma Aldrich, USA, cat. No E1753; ≥ 90%), ecdysterone (Sigma Aldrich, USA, cat. No H5142; ≥ 93%), o-methoxycoumarin (Sigma Aldrich, USA, cat. No W515809; ≥ 98%) and epigallocatechin gallate (Sigma Aldrich, USA, cat. No E4143; ≥ 95%). Identification of separated substances was carried out by comparing retention times of peaks in the chromatogram of the tested solution with those in the standard solution chromatogram. The quantitative ratio of identified substances in the tested samples was estimated according to the peaks square with the use of the internal normalization method.

The plant material was ground up till particles passing through cover with 2 mm sieve size. Precise test portion (≈ 2.0 g) of the comminuted plant material was put into the retort (V = 100 mL) and 20 mL of 70% ethyl alcohol was added; the retort was connected to a backflow condenser and heated on a water bath for 1 hour since boiling of the alcohol mixture. After cooling the mixture was filtered through a paper filter into the measuring bottle (V =

25 mL) and then ethyl alcohol was added till mark (solution A). At the same time the series of 0.05% solutions of comparison, namely, the solutions of rutin, quercetin, luteolin, gallic acid, caffeic acid, chlorogenic acid, hyperoside, ferulic acid, neochlorogenic acid, chicoric acid, dicoumarin, dihydroquercetin, epicatechin, ecdysterone, o-methoxycoumarin and epigallocatechin gallate were prepared in 70% ethyl alcohol. The tested solutions and the solutions of comparison in the volume of 50 μL each were put into the chromatograph and were chromatographed according to the above method.

To obtain the dry extract from *S. centauroides* the comminuted herb (the plant material was gathered in July 2017) was triply extracted with 70, 40 and 20% ethyl alcohol successively at a temperature of 60°C. Alcoholic extractions were boiled off and dried in the vacuum oven. The dry extract was the amorphous hygroscopic brown powder with specific odour; weight loss on drying is not more than 5%.

The pharmacological studies were carried out on 144 white male and female Wistar rats weighing 180 - 200 g. They were maintained in standard laboratory conditions of the vivarium at the Institute of General and Experimental Biology SB RAS. 10 rats were housed *per* plastic cage on wood shavings litter. The temperature in the vivarium was 20 - 22°C, indoor air humidity was not more than 50%, air exchange (air intake/outlet) was 8:10, light regime (day/night) was 1:1. The animals were fed twice a day. The animal care was compliant with the "Rules of Laboratory Practice" (GLP) and the Order of the Russian Health Ministry "On approval of Rules for Laboratory Practice" (No. 708N, 23.08.2010).

The experimental work followed the European Communities Council Directive of 24 November 1986 (86/609/EEC). The research report was approved by the Ethics Committee at IGEB SB RAS (Protocol N 1 dated 28.01.2014). The Ethics Committee protocol is valid till the project finish (2020).

Before the start of the experiments, the animals meeting the criteria (apparently healthy, mature, aged 10 - 11 months) were divided into groups with due account of sex, age, weight and randomization principle.

The dry extract from *S. centauroides* was administered to animals of experimental groups at the doses of 50, 100 and 150 mg/kgbw in the form of water solution for 7 days before study; the last dose was administered 1 hour before testing. The animals of the control group received the purified water in the equivalent volume according to the analogous scheme. Each group consisted of 36 animals. 12 animals from each group were used for one test.

Anxiolytic effect of *S. centauroides* was estimated according to methodical guidelines for the study of tranquilizing (anxiolytic) effect of pharmacological substances [28].

In the elevated plus maze (EPM) test there was used a device consisting of two open (10 cm × 10 cm × 50 cm) and two enclosed arms (10 cm × 10 cm × 50 cm), extending from a central platform at right angle to the centre (5 cm × 5 cm) and raised 80 cm above floor level. The given test was based on the natural preference of rats for dark areas and aversion for open and elevated areas. Just before the beginning of the experiment the animals stayed in dark cages for 3 minutes. Then the animals were placed in the centre of the maze, facing an open arm. The time spent in the open and closed arms, the number of entries into the open and closed arms, rearings and defecations were recorded during a 5 min observation period.

The light/dark test was also based on the natural aversion of rodents to brightly illuminated areas. In the given experiment, the animals were placed in the light compartment and the number of transfers from the dark compartment to the light compartment and the time spent in the light and dark compartments were recorded over 5 min.

A conflict situation according to the Vogel test was created colliding water-intake and self-defence motivations. In this test the rats were water-deprived for 48 h without limiting feed; on the 3rd day the rat was housed in the chamber with a waterer. After habituation to drinking from the waterer on the 4th day the rat was housed in the chamber for 3 minutes and every 10 seconds each water intake was punished by a mild electric shock (1 mA). As a result, to satisfy thirst the rat had to get over fear of punishment. The

anxiolytic effect of the *S. centauroides* extract was estimated according to the number of punished water intake during a three min observation period.

Experimental material was processed with the use of statistic programs Microsoft Excel 2010 and Statistica 13. The data were expressed as means (M) ± S.E.M (m). Validity of differences in means between groups was estimated with the use of the Student's t-test (for independent groups the differences were considered significant when $p < 0.05$).

Results and Discussion

The studies revealed 25 compounds, 17 of them are of phenolic nature (Table I, Figure 1). The data in the Table I have shown that the quantitative composition and qualitative content of substances in specimens have some differences: 17 substances were identified in the aerial part and 15 ones – in the roots of *S. centauroides*. Among identified substances, flavonoids prevail in the aerial part topping 31.28% of the total quantity of identified substances and phenol carbonic acids prevail in the roots (36.12%). The content of phenol carbonic acids in the aerial part is 24.52% and the content of flavonoids in the roots is 30.40% of the total quantity of the identified substances. The content of coumarins in the aerial part and roots of *S. centauroides* is roughly equal, 22.90 and 21.08% respectively. In the aerial part of the plant unlike the roots there were revealed hyperoside and luteolin. The content of ecdysterone in the aerial part (21.30%) is twice its content in the roots (12.40%).

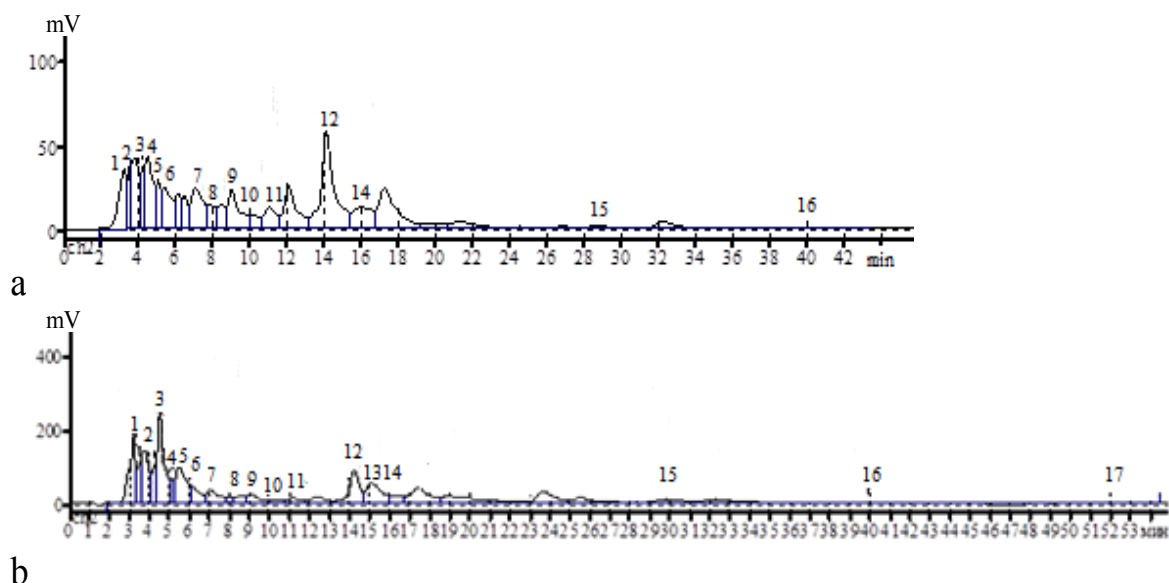


Figure 1.

(a) Chromatogram of extractions from *S. centauroides* roots; (b) Chromatogram of extractions from *S. centauroides* aerial part

Table IPhenolic composition of *S. centauroides*

	Name	Retention time, min	Aerial part	Roots
			The content, %, method of internal normalization	
1	Gallic acid	3.66	7.34	5.62
2	Epigallocatechin gallate	4.20	2.51	2.11
3	Dicoumarin	4.46	11.11	6.71
4	Chlorogenic acid	5.05	1.81	2.40
5	Epicatechin	5.43	7.43	4.20
6	Caffeic acid	6.08	2.78	2.26
7	Chicoric acid	6.97	3.26	5.43
8	Neochlorogenic acid	7.84	0.80	2.28
9	Dihydroquercetin	9.04	2.50	5.04
10	Ferulic acid	10.37	0.96	1.81
11	Coumarin	11.11	1.52	2.96
12	Ecdysterone	14.18	14.73	6.80
13	Hyperoside	15.05	5.84	-
14	Rutin	16.03	2.15	4.41
15	O-methoxycoumarin	29.76	3.20	1.89
16	Quercetin	39.85	0.99	0.91
17	Luteolin	52.43	0.21	-

The results of pharmacological studies have shown that the course administration of the *S. centauroides* extract using doses of 50, 100 and 150 mg/kgbw

relieved anxiety and fear increasing the punished water intake by 1.7, 2.3 and 1.8 times respectively as compared to the control group (Table II).

Table IIInfluence of the dry extract from *S. centauroides* on the behaviour of white rats in the elevated plus maze (EPM) test

Indices	Animal groups			
	Control (H ₂ O)	Experimental 1 (extract of <i>S. centauroides</i> , 50 mg/kgbw)	Experimental 2 (extract of <i>S. centauroidis</i> , 100 mg/kgbw)	Experimental 3 (extract of <i>S. centauroidis</i> , 150 mg/kgbw)
Number of entries				
Open arm	0.6 ± 0.2	1.1 ± 0.4	0.9 ± 0.3	0.8 ± 0.2
Closed arm	1.8 ± 0.2	2.5 ± 0.4	2.9 ± 0.3*	2.1 ± 0.3
Residence time				
Open arm	4.4 ± 1.6	13.5 ± 6.9	10.8 ± 3.6	9.3 ± 6.4
Closed arm	289.2 ± 2.6	274.9 ± 10.8	277.0 ± 5.2	279.3 ± 0.4
Central area	6.4 ± 1.0	11.4 ± 4.2	13.0 ± 3.9	11.4 ± 4.8
Rearing	3.9 ± 0.4*	9.8 ± 1.3	11.0 ± 1.4*	6.4 ± 0.9*
Defecation	1.5 ± 0.1*	0.6 ± 0.2	0.4 ± 0.1*	0.5 ± 0.2*

* - $p \leq 0.05$ vs. control

The testing of animals in the light/dark compartment and EPM has shown that the control animals preferably spent time in the dark compartment and closed arms of EPM (Table II, Table III).

The use of the *S. centauroides* extract relieved fear of the open area in the EPM (Table III). In the first and second experimental groups, the number of entries into the open arms was 1.8 and 1.6 times increased, the time spent in them was 3.1 and 2.5 times increased and the time spent in the central area was 1.8 and 2.0 times increased respectively as compared to the same indices in the control group animals. The extract of *S. centauroides* at the dose of 150 mg/kgbw demonstrated less pronounced influence in the given indices.

Besides, more active locomotion expressed by an increased number of closed arm entries and rearing was noted in all experimental groups in the EPM as compared to the control animals (Table III). The increase in the exploration activity and reduced fear and anxiety in animals of the experimental groups may be explained by emotional intensity decrease. In animals of the experimental groups the number of faecal boluses was 3.0 times lower than in the control animals.

The course administration of the *S. centauroides* to animals at the doses of 50 and 150 mg/kgbw increases the number of transitions between dark and light compartments by 48% and the residence time in the light compartment – by 49% and 30% as compared to these indices in control group animals (Table II). In

the given experiment, the *S. centauroides* extract at the dose of 100 mg/kgbw has more marked anxiolytic effect increasing the given indices by 76% and 52% as compared to the control.

The administration of the *S. centauroides* extract at the doses of 50, 100 and 150 mg/kgbw increased the number of punished water-intakes in the Vogel test by 2.9, 3.2 and 3.9 times respectively as compared to the control group (Table III).

Table III

The influence of the dry extract from *S. centauroides* on the behaviour of rats in the “dark/light” test and Vogel conflict test

Animal groups	Number of transfers in the dark/light chamber	Residence time in the light compartment	Number of punished water-intakes
Control (H ₂ O)	2.9 ± 0.3	40.1 ± 4.9	1.8 ± 0.3
Experimental 1 (extract of <i>S. centauroides</i> , 50 mg/kgbw)	4.3 ± 0.6*	59.9 ± 5.2*	5.3 ± 0.8*
Experimental 2 (extract of <i>S. centauroides</i> , 100 mg/kgbw)	5.1 ± 0.5*	60.8 ± 5.2*	5.8 ± 0.6*
Experimental 3 (extract of <i>S. centauroides</i> , 150 mg/kgbw)	4.3 ± 0.6*	52.3 ± 5.0	7.0 ± 1.3*

* - $p \leq 0.05$ vs. control

Psychic disorders are known to be common. Both synthetic and plant remedies are widely used for the treatment of anxio-depressive disorders. Studies conducted in animals have shown that the anxiolytic effect of plant remedies is due to the complex of bioactive substances such as terpenes, flavonoids, phenolic acids, lignans, cinnamates and saponins. Numerous structurally different classes of phytochemicals demonstrate high affinity for the benzodiazepine binding site of the GABA-A receptor complex. Specific plant-derived ligands (such as alkaloids, terpenes, flavonoids, phenolic acids, lignans, cinnamates, or saponins) were described to modulate the GABA-A receptor. These compounds cause membrane hyperpolarization by allowing chloride anion Cl⁻ influx, which is followed by inhibition of excitatory transmission and reduction of anxiety [2, 6, 9, 24].

Accumulating evidence showed that the consumption of flavonoid-rich foods is associated with lower rates of dementia and has some beneficial effects on memory and learning [29]. Phenolic hydroxyl groups of flavonoids contribute to their antioxidant, anti-radical and anti-inflammatory properties. According to Wang X *et al.* the phenolic group provides anxiolytic properties of compounds. The anxiolytic activities of seven simple phenols, including phloroglucinol, eugenol, protocatechuic aldehyde, vanillin, thymol, ferulic acid and caffeic acid were assayed with the elevated plus maze (EPM) test in mice [30]. Quercetin reportedly also has anxiolytic properties. The course administration of quercetin to white rats at the dose of 20 mg/kgbw significantly increased the number of entries in the open arms of the plus-maze and prolonged the time spent there, without a significant change in the locomotor activity of animals [7].

Quercetin and rutin isolated from *Tagetes erecta* L. have demonstrated anxiolytic properties [14]. Coleta M *et al.* have reported that 5 mg/kgbw luteolin increases the percentage of entries into the open arms of the EPM influencing no time spent in those areas [5].

Bouayed *et al.* revealed anxiolytic effect of chlorogenic acid with the use of the EPM and “light/dark box” tests [4]. Chlorogenic acid has neuroprotective effect *in vitro* and *in vivo* conditions [16]. Rosmarinic acid and its metabolite – caffeic acid isolated from the *Perilla* herb have a marked anxiolytic and anti-depressant properties [20]. Chemically pure caffeic acid, at the dose of 1 mg/kgbw increases the number of entries and time spent in the open arms of the EPM and at the doses of 0.5 - 2 mg/kgbw it influences the locomotor and exploratory activities of the animals in the open-field test [13]. Gallic acid demonstrates the properties of classic anxiolytic at the doses comprised between 50 - 300 mg/kgbw (single administration) [10], and at the dose of 20 mg/kgbw (long-term administration) [7].

Conclusions

Thus, the phenolic composition of the aerial part and roots of *Serratula centauroides* (*Asteraceae*) has been studied. There have been identified 17 substances of phenolic nature including flavonoids, phenol carbonic acids, coumarins, etc. In the aerial part, unlike the roots, there have been found hyperoside and luteolin. The ecdysterone content in the aerial part is twice as large as in the roots. The wealth of biologically active substances in the aerial part of *S. centauroides* contributes to the marked anxiolytic effect of the tested extract.

The *S. centauroides* extract at the doses comprised between 50 and 150 mg/kgbw has the marked anxiolytic effect in the conditions of punished behaviour, significantly increasing the number of punished water-intake in the Vogel conflict test and unpunished behaviour increasing the number of entries and time spent in open arms of the EPM and the number of transitions and time spent in the light compartment of the dark/light box.

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Conflict of interests

Authors declare no conflict of interests.

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