

MEDICINAL PLANTS

EVALUATION OF THE ANTIOXIDANT ACTIVITY AND ANTI-INFLAMMATORY EFFECT OF ROOT EXTRACTS OF ASTRAGALUS MEMBRANACEUS, SCUTELLARIA BAICALENSIS, AND PHLOJODICARPUS SIBIRICUS

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The goal of the study was to determine quantitatively the content of phenolic compounds in the extract from roots of *Astragalus membranaceus*, *Scutellaria baicalensis*, and *Phlojodicarpus sibiricus* and to evaluate the antioxidant activity and anti-inflammatory effect of the extract. The quantitative content of the flavonoid complex (baicalin, rutin, quercetin, epicatechin) was determined. The antioxidant activity of the dry EtOH extract was evaluated using the DPPH test (*in vitro*). The antioxidant activity was shown to be directly dependent on the concentration of the extract. The half-maximal inhibitory concentration IC_{50} was 210.99 $\mu\text{g/mL}$. The anti-inflammatory effect of the tested agent was studied in the model of formalin edema in white rats. Administration of the extract to rats at doses of 50, 100, and 200 mg/kg gave edema values of 31.4, 19.2, and 16.6% of the parameters (foot thickness) before administration of the formalin solution, which was 2 – 4 times less than the control values ($p < 0.05$) and were comparable to those obtained with indomethacin.

Keywords: *Astragalus membranaceus*, *Scutellaria baicalensis*, *Phlojodicarpus sibiricus*, flavonoids, antioxidant activity, anti-inflammatory effect.

The development of inflammatory processes is associated with oxidative stress and accompanies the course of many pathologies [1]. The use of synthetic drugs often causes adverse side effects, despite the achievements of pharmaceutical sciences and the existing pharmacological arsenal [2]. Therefore, the search for safe anti-inflammatory agents, particularly from plant sources, is timely. Many medicinal plants possess mild pharmacotherapeutic action and are typified by low toxicity. Plant phenolic compounds and fatty acids were shown to exhibit antioxidant and/or anti-inflammatory properties [3 – 5]. Recently, plant phenolic compounds were ascribed a regulatory/adaptive role in the development of inflammatory reactions [6]. For example, the plant *Astragalus membranaceus*, which is popular in traditional

Eastern medicine, is used as an anti-inflammatory agent for various diseases [7, 8]. Extracts from *Scutellaria baicalensis* roots were shown to possess an anti-inflammatory effect [9]. An agent from *Phlojodicarpus sibiricus* roots improved microcirculation in peripheral tissues and could manifest anti-inflammatory activity [10]. Many phenolic compounds have been shown to possess antioxidant properties [11 – 13].

Hence, it can be assumed that the native plant complex of flavonoids, coumarins, and fatty acids with trace elements can have anti-inflammatory and antioxidant effects via various metabolic pathways and/or regulatory mechanisms without a harmful xenobiotic load on the body with prolonged use. Considering the above, it can be assumed that the complex of biologically active compounds from *A. membranaceus*, *S. baicalensis*, and *P. sibiricus* will have a polymodal and/or synergistic pharmacotherapeutic effect for many pathologies occurring with inflammatory outbreaks.

The aim of the present research was to determine the content of total phenolic compounds in extracts from roots of

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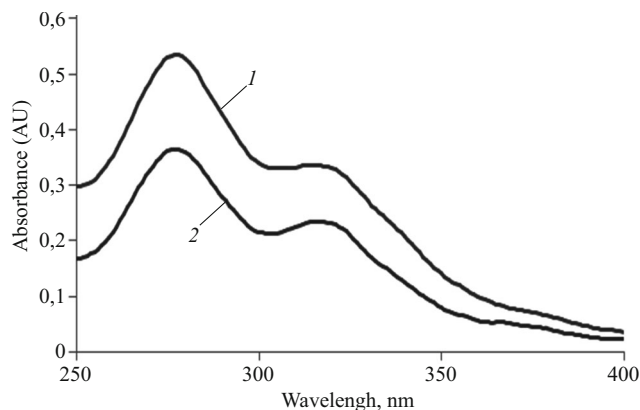


Fig. 1. Absorption spectra: Extract (1); baicalin (RS) (2).

A. membranaceus, *S. baicalensis*, and *P. sibiricus* and to evaluate the antioxidant activity and anti-inflammatory action of the extracts.

EXPERIMENTAL CHEMICAL PART

Plant raw material (roots of *A. membranaceus*, *S. baicalensis*, and *P. sibiricus*) was collected in the Republic of Buryatia and Zabaikal'skii Krai. The extract was prepared according to requirements of the SP, XIVth Ed., Vol. II. Biologically active compounds were extracted and chromatography systems were prepared using solvents (purified H₂O, EtOH, MeOH, pure and chemically pure grades). HPLC analysis of phenolic compounds used a Gilson model 305 chromatograph (France) with a Rheodyne 7125 manual injector (USA). The stationary phase was a metallic column with Kromasil C18 (4.6 × 250 mm, 5 μm; Altech OA-1000 Organic Acids, 6.5 × 300 mm). The mobile phase consisted of MeOH(H₂O)(H₃PO₄ (40:60:0.5) and H₂SO₄ (0.05 M). Detection was made at 254 nm (for phenolic compounds) using a Gilson model 131 Refractive Index UV detector. The number of flavonoids in the extract was determined by direct spectrophotometry. The reference standard (RS) was baicalin, the absorption maximum of which corresponded to that of the EtOH extract (Fig. 1).

Results were statistically processed using variational statistics methods and Excel software. The statistical significance of parameters was evaluated using the Student *t*-criterion for $p < 0.05$.

EXPERIMENTAL BIOLOGICAL PART

Determination of the antioxidant activity of the extract in the DPPH test

The extract (at increasing concentrations in the range 80–400 μg/mL) was dissolved in EtOH (95%) and mixed with DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (0.004%) in EtOH (95%). The mixture was incubated for

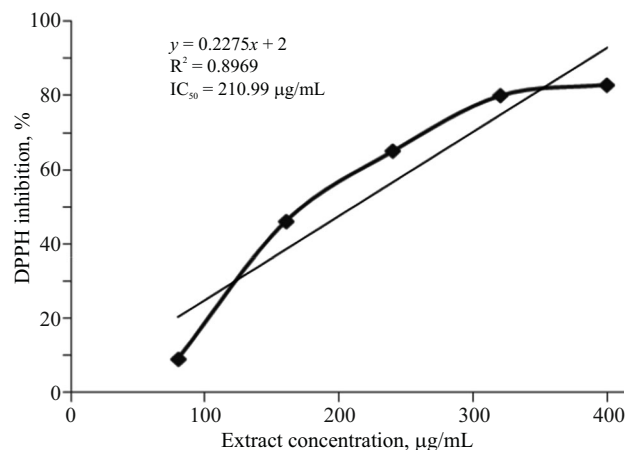


Fig. 2. Antioxidant activity of extract in DPPH test.

30 min in the dark at room temperature. The antioxidant activity of the extract was determined spectrophotometrically at $\lambda = 517$ nm [14]. The positive control was ascorbic acid. The antioxidant activity parameter was expressed in % inhibition of DPPH-radicals and was calculated using the formula:

$$\% \text{ inhibition of DPPH-radicals} = (A_0 - A_1/A_0) \times 100,$$

where A_0 is the absorption of the control and A_1 , the absorption of the extract. The IC₅₀ value of the extract antioxidant activity was determined by regression analysis.

Determination of the anti-inflammatory activity of the extract

The anti-inflammatory activity was determined using the formalin edema model in rat feet. The experiments used Wistar rats (180–200 g) that were distributed into five groups ($n = 6$ in each). The extract was administered at doses of 50, 100, and 200 mg/kg for 7 d and 1 h before conducting the experiments. The reference drug was indomethacin tablets (OOO Ozon, Russia), which were administered intragastrically to the animals at a dose of 10 mg/kg 1 h before the experiments. Acute inflammatory edema was induced by subplantar injection of formalin solution (2%, 0.1 mL) into the right hind foot of anesthetized rats (sodium thiopental, 40 mg/kg). Control animals received purified H₂O of an equivalent volume by an analogous regime. The amount of edema was determined by measuring the foot thickness using a digital caliper.

RESULTS AND DISCUSSION

The flavonoids baicalin, dihydroquercetin, luteolin-7-glycoside, rutin, quercetin, epigallocatechin gallate, and epicatechin were identified in the plant collection (roots of *A. membranaceus*, *S. baicalensis*, and *P. sibiricus*) using HPLC [15]. The experimental conditions for the analysis were de-

TABLE 1. Metrological Characteristics of Quantitative Determination Method for Total Flavonoids Recalculated as Baicalin in Extract

Number of degrees of freedom, f	Mean \bar{x}	Dispersion S^2	Standard deviation S	Confidence probability P , %	Student criterion $t(P, f)$	Absolute error of separate determination Δx	Relative uncertainty of mean result E , %
4	7.67	0.01683	0.12972	95	2.78	0.36062	4.78

TABLE 2. Effect of Extract on Inflammatory Edema of Rat Foot After Administration of Formalin Solution (After 2 h)

Group	Foot thickness, mm		Edema, %	Inhibition of edema, %
	before admin.	after admin.		
Control (normal saline), $n = 6$	4.80 ± 0.06	8.00 ± 0.06 [#]	66.6	–
Extract, 50 mg/kg, $n = 6$	4.77 ± 0.03	6.27 ± 0.29 [#]	31.4	52.8
Extract, 100 mg/kg, $n = 6$	4.83 ± 0.03	5.70 ± 0.18 ^{#*}	19.2	71.2*
Extract, 200 mg/kg, $n = 6$	4.80 ± 0.06	5.60 ± 0.49*	16.6	75.1*
Indomethacin, 10 mg/kg, $n = 6$	4.83 ± 0.03	5.37 ± 0.22*	10.9	83.5*

[#] differences statistically significant vs. value before administration; * significance of differences vs. control, $p < 0.05$.

gree of raw-material grinding, 1 mm; extractant, EtOH (60%); raw-material(extractant ratio, 1:100; multiple, 1; analysis time, 1 h; $t = 100^\circ\text{C}$. The flavonoid content recalculated as RS baicalin was $\geq 6.0\%$ of the total raw material (Table 1). The reproducibility of the results was checked by five-fold repetitions of the experiment. The relative error of the method was $\pm 4.78\%$. Test results with additions were indicative of the lack of systematic error.

The DPPH test found that the extract possessed antioxidant activity that was directly dependent on its concentration. The IC_{50} value was 210.99 $\mu\text{g/mL}$ (Fig. 2). The antioxidant properties of the extract were probably due to the phenolic compounds, particularly flavonoids. The IC_{50} value of ascorbic acid was 112.0 $\mu\text{g/mL}$. The antioxidant activity of the extract was less than that of the reference drug possibly because of interference and/or slower reaction rates of the active constituents with DPPH-radicals during the fixed time of the experiment.

Inflammatory edema of the foot developed 2 h after subplantar injection of formalin solution to rats. The thickness increased by 66.6% ($p < 0.05$). Administration of the extract at doses of 50, 100, and 200 mg/kg to the rats gave edema values 31.4, 19.2, and 16.6% of those (foot thickness) before formalin solution injection, which was 2–4 times less than the control ($p < 0.05$) and comparable to the values for indomethacin (Table 2). The anti-inflammatory activity of the extract was directly dependent on the dose.

The investigations found that the tested extract possessed pronounced antioxidant and anti-inflammatory properties. The observed effects of the extract were due primarily to the contents of phenolic compounds, particularly flavonoids. For example, the flavonoids baicalin, quercetin, dihydroquer-

etin, and rutin were secondary metabolites of these plants and are known to possess pronounced therapeutic activity for inflammations [3]. Previously, the lipophilic fraction of the tested extract was found to contain a complex of unsaturated fatty acids, including essential ones (linolenic and linoleic) [16]. The complex of phenolic compounds and essential fatty acids was shown to possess systemic anti-inflammatory activity [17]. Results of the present investigations and literature data led to the conclusion that phenolic compounds and fatty acids in the total extract from roots of *A. membranaceus*, *S. baicalensis*, and *P. sibiricus* were responsible for the anti-inflammatory effect. The antioxidant activity of the flavonoids additionally contributed to the anti-inflammatory activity of the extract.

Thus, the anti-inflammatory and antioxidant effects of the tested extract were due primarily to the phenolic compounds quercetin, baicalin, and coumarins. The presence of fatty acids could diminish the inflammatory action of inflammation mediators.

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