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Phytochemical and pharmacological study of biologically active compounds and dry extracts of *Populus rubrinervis* Hort. Alb. buds of various polarities

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The aim of the work was a phytochemical and pharmacological study of biologically active compounds (BACs) and *Populus rubrinervis* Hort. Alb. buds preparations of various polarities.

Materials and methods. The object of the study was dry extracts of *P. rubrinervis* Hort. Alb. buds the samples of which were prepared in January–March 2023 in the Botanical Garden of Samara University (Samara, Russia). The separation of the amount of current substances was carried consecutively by the method of circulating extraction (chloroform), then, by the method of fractional percolation, a tincture was received on 70% ethyl alcohol (1:5). Pinostrobin was used as the standard sample (SS). The analysis of the substances was carried out by the TLC method. The electronic spectra registration was carried out with a spectrophotometer "Specord 40" (Analytik Jena, Germany). The study of the pharmacological (diuretic) activity of *P. rubrinervis* Hort. Alb. buds dried extracts was carried out on 60 white outbred rats of both sexes weighing 200–220 g in the experiments with aqueous diuresis.

Results. *P. rubrinervis* Hort. Alb. buds dried extracts of various polarities (extract No. 1 (chloroform) and extract No. 2 (70% ethanol) were received. By the method of thin-layer chromatography, it was determined that the dominant complexes of the lipophilic nature with pinostrobin are isolated in extract No. 1, phenolic substances of the glycoside nature prevail in extract No. 2. Despite various polarities of the extragents, spectral characteristics of extract No. 2 have significant similarities with extract No. 1. When studying the diuretic activity, it was established that when SS pinostrobin was injected at a dose of 1 mg/kg, for 4 h of the experiment, an isolated increase in diuresis was noted (from 1.72±0.11 to 1.97±0.03 ml, p <0.05); at the same time, an isolated increase in creatininuresis (from 1.50±0.29 to 2.39±0.15 mg, p <0.05) was observed during 24 h of the experiment. When extract No. 2 was injected at a dose of 10 mg/kg, there was a moderate significant increase in diuresis (from 1.82±0.02 to 2.07±0.04 ml and from 2.38±0.39 to 3.02±0.11 ml, p <0.05) and a significant increase in creatininuresis (from 0.14±0.01 to 0.19±0.03 mg and from 2.31±0.42 to 2.79±0.51 mg, p <0.05) for 4 and 24 h of the experiment, respectively.

Conclusion. The extraction separation of the amount of *P. rubrinervis* Hort. Alb. buds by the polarity degree was carried out. Pinostrobin SS at a dose of 1 mg/kg and extract No. 2 at a dose of 10 mg/kg had a diuretic activity, in connection with which they are promising in terms of the development of effective drugs.

Keywords: *Populus rubrinervis* Hort. Alb.; dry extract; pinostrobin; UV spectrophotometry; diuretic activity

Abbreviations: HM – herbal medicine; SPh RF – State Pharmacopoeia of the Russian Federation; BAC – biologically active compounds; SS – standard sample; DSA – diazobenzenesulfonic acid; PhM – pharmacopoeial monograph.

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Фитохимическое и фармакологическое исследование биологически активных веществ и сухих экстрактов почек тополя краснонервного (*Populus rubrinervis* Hort. Alb.) различной полярности

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Цель. Проведение фитохимического и фармакологического исследования биологически активных веществ (БАВ) и препаратов на основе почек тополя краснонервного (*Populus rubrinervis* Hort. Alb.) различной полярности.

Материалы и методы. Объектом исследования стали сухие экстракты почек тополя краснонервного, образцы которых были заготовлены в январе–марте 2023 года на территории Ботанического сада Самарского университета (г. Самара, Россия). Разделение суммы действующих веществ проводили последовательно методом циркуляционной экстракции (хлороформ), а затем методом дробной перколяции получали настойку на спирте этиловом 70% (1:5). В качестве стандартного образца (СО) использовали пиностробин. Анализ веществ проводили методом тонкослойной хроматографии. Регистрацию УФ-спектров осуществляли с помощью спектрофотометра «Specord®40» (Analytik Jena, Германия). Исследование фармакологической (диуретической) активности сухих экстрактов почек тополя краснонервного проводили на 60 белых аутбредных крысах обоего пола массой 200–220 г в экспериментах с водным диурезом.

Результаты. Получены сухие экстракты на основе почек тополя краснонервного различной полярности (экстракт № 1 (хлороформ) и экстракт № 2 (спирт этиловый 70%). Методом тонкослойной хроматографии определено, что доминирующие комплексы липофильной природы с пиностробином изолированы в экстракт № 1, в экстракте № 2 превалируют фенольные вещества гликозидной природы. Несмотря на разную полярность экстрагентов, спектральные характеристики экстракта № 2 имели значительное сходство с экстрактом № 1. При изучении диуретической активности определено, что при введении СО пиностробина в дозе 1 мг/кг за 4 ч опыта было отмечено изолированное повышение диуреза (с 1,72±0,11 до 1,97±0,03 мл, p <0,05), в тоже время за 24 ч опыта отмечено изолированное повышение креатининуреза (с 1,50±0,29 до 2,39±0,15 мг, p <0,05). При введении экстракта № 2 в дозе 10 мг/кг отмечено умеренное достоверное повышение диуреза (с 1,82±0,02 до 2,07±0,04 мл и с 2,38±0,39 до 3,02±0,11 мл, p <0,05) и значительное увеличение креатининуреза (с 0,14±0,01 до 0,19±0,03 мг и с 2,31±0,42 до 2,79±0,51 мг, p <0,05) за 4 и 24 ч опыта соответственно.

Заключение. Проведено экстракционное разделение суммы веществ почек тополя краснонервного по степени полярности. СО пиностробина в дозе 1 мг/кг и экстракт № 2 в дозе 10 мг/кг проявляли диуретическую активность, в связи с чем и являются перспективными в плане разработки эффективных лекарственных средств.

Ключевые слова: тополь краснонервный; *Populus rubrinervis* Hort. Alb.; почки; сухой экстракт; пиностробин; УФ-спектрофотометрия; диуретическая активность

Список сокращений: ЛРП – лекарственный растительный препарат; ГФ РФ – Государственная фармакопея Российской Федерации; БАВ – биологически активные вещества; СО – стандартный образец; ДСК – диазобензолсульфокислота; ФС – фармакопейная статья.

INTRODUCTION

At present, herbal medicines (HMs) are actively used in medical practice for the treatment and prevention of various diseases [1–4]. These drugs have a number of advantages over synthetic ones: they are relatively safe, less toxic, and have a broader spectrum of action [4, 5].

One of the interesting and perspective sources of medicines are plants of the Willow family (*Salicaceae*) of the Poplar genus (*Populus* L.), which grow widely in temperate countries: Western and Eastern Siberia, Europe, Eastern Kazakhstan, Central Asia, and Western China [5–8]. About 20 species of poplar grow in central Russia, the most common of which (including the Samara region) are: *P. nigra* L., *P. deltoides* Marsh; *P. suaveolens* Fisch; *P. laurifolia* Ledeb; *P. balsamifera* L.; *P. alba* L.; *P. tremula* L.; *P. rubrinervis* Hort. Alb. [6, 9, 10]. Now State Pharmacopoeia of the Russian Federation XV edition (SP RF XV ed.) includes a pharmacopoeial monograph – PhM.2.5.0042.15 “Poplar buds”[1]. The following species

[1] State Pharmacopoeia of the Russian Federation. XV ed. Vol. 4. Moscow; 2018. 1832 p. Available from: https://docs.rucml.ru/feml/pharma/v14/vol4/. Russian

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are pharmacopoeial: *P. nigra* L., *P. balsamifera* L., *P. deltoides* Marsh., *P. suaveolens* Fisch., *P. laurifolia* Ledeb.

Drugs based on medicinal plant raw materials of these genus have anti-inflammatory, antipyretic, analgesic, wound healing, antimicrobial, anti-ulcer, antioxidant activities and are widely used in official and folk medicine [7, 11–13]. In current scientific studies, there is also a mention of the investigation of *Populus balsamifera* L. buds dihydrochalcones as an effective agent for a topical treatment of psoriasis along with anti-inflammatory and antioxidant effects [14–17].

A chemical composition of a pharmacopoeial raw material – Poplar buds – is rich and quite diverse. The literature sources describe the *Populus* L. buds as mainly containing compounds of phenolic nature: simple phenols (salicin, salicylic alcohol); phenolic acids, phenylpropanoids; terpenoids (mono- and sesquiterpenoids); flavanones (pinocembrin, pinostrobin); flavones (apigenin, chrysin, tectochrysin); flavonols (galangin, kaempferol, quercetin); chalcones and dihydrochalcones; caffeic and ferulic acids, as well as their derivatives [6, 14, 15, 18]. It should be noted that phenolic compounds (flavonoids, phenylpropanoids and simple phenols) make a significant contribution to the manifestation of the pharmacological activity of the HMs based on poplar raw materials [6, 19–21]. So, the leading group of BACs of the pharmacopoeial Poplar species buds are flavonoids, among which flavanones pinostrobin (5-hydroxy-7-methoxyflavanone) and pinocembrin (5,7-dihydroxyflavanone) dominate; this fact determines the presence of the drugs antimicrobial activity (Fig. 1) [22–25].

However, this group has other pharmacological properties as well [19, 26–28]. For example, a recently described study compared the anti-inflammatory activity of *Populus* L. bud extracts and flavanones (pinocembrin and pinostrobin) against pro-inflammatory human gingival fibroblasts (HGF-1), which revealed the potential protective role of both BACs and *Populus* L. bud extracts and demonstrated antioxidant properties [29]. The hepatoprotective and cardiovascular activities of the alcoholic *Populus* L. bud extract were also evaluated along with antioxidant and anti-inflammatory activities. In this study, the alcoholic extract showed significant anti-inflammatory, hepatoprotective, and vasodilatory activities, the latter being endothelium-independent and believed to be mediated mainly by the ability of the individual components to exhibit antioxidant properties, probably, related to the inhibition of Ca^{2+} ion influx [30].

The analysis of literature sources has shown the presence of a diuretic activity, i.e., an increase in the renal excretion of water, electrolytes, creatinine preparations and individual BACs from the raw materials of the genus *Poplar* plants: decoction of the aspen bark (*Populus tremula* L.) (100 µl/kg), the infusion of aspen buds (100 µl/kg), tincture of aspen buds (100 µl/kg), tincture of the aspen bark (100 µl/kg), tincture of poplar buds (50 and 100 µl/kg), an increase in the renal excretion of water, electrolytes, creatinine [5]. Tremuloidin isolated from the aspen bark at doses of 25 and 50 mg/kg stimulated diuresis, saluresis, and creatininuresis, and at a dose of 100 mg/kg, it stimulated only the excretion of sodium, potassium, and creatinine [5]. The diuretic effect was also found for the dry extract of *P. simonii* Carrier buds [31, 32].

So, the research of the family *Salicaceae* of the genus *Populus* representatives is currently relevant for the scientists all over the world. The annually updated and summarized data on the chemical composition of buds of the genus *Poplar* plants, as well as on the pharmacological activity of the active substances and developed dosage forms based on the buds of the genus Poplar plants [19, 22–24]. Currently, it is known that *P. rubrinervis* Hort. Alb. is actively used for landscaping of populated areas [10]. It has its own differences and advantages over other species: all plants are male specimens, it does not form fluffy seeds, it is winter-hardy, gas tolerant, propagates well by unwooded stem cuttings, and it is distinguished from other species by the highest growth vigor [9, 10, 33]. The significant phytomass of the bud allows us to talk about a greater economic efficiency in the processing of this raw material relative to the classical species – black poplar [33]. However, there is very little information in the literature on the chemical composition and pharmacological activity of preparations and BACs of *P. rubrinervis* Hort. Alb. buds, which contributed to the authors' scientific interest in this object.

THE AIM of the work was a comparative phytochemical and pharmacological study of biologically active compounds (BACs) and *Populus rubrinervis* Hort. Alb. buds preparations of various polarities.

MATERIALS AND METHODS

The object of the study was dry extracts of *P. rubrinervis* Hort. Alb. buds, harvested in the period from the end of January to March 2023 before swelling. The collection plantings of the Botanical Garden of Samara University were the main base for collecting raw materials (Samara, Russia). Drying was carried out naturally without heating. The dried raw materials were subjected to a classical sample preparation in accordance with the requirements of SP RF XV edition of the pharmacopoeial monograph "Buds" (GPhM.1.5.1.0009.15)[2]. The SS of pinostrobin (5-hydroxy-7-methoxy-2-phenylchroman-4-one),

[2] GPhM.1.5.1.0009.15 "Buds". State Pharmacopoeia of the Russian Federation. XV ed. Moscow, 2023. Available from: https://pharmacopoeia.regmed.ru/pharmacopoeia/izdanie-15/1/1-5/1-5-2/pochki/. Russian

obtained earlier from *P. balsamifera* L. buds according to the requirements of PhM 42-0073-01 "Pinostrobin as a standard sample" was also analyzed.

Preparation of work solutions for analysis by thin-layer chromatography and UV-spectrophotometry methods

In order to separate the amount of current substances contained in the buds of *P. rubrinervis* Hort. Alb., the forextraction of raw materials by the method of circulating extraction in the Soxhlet apparatus, was carried out. During the extraction, 30 complete cycles were counted, the end of the extraction was determined by the absence of staining of the extractant in the working Soxhlet flask. Chloroform was used as an extractant. The resulting lipophilic chloroform extract of *Populus rubrinervis* Hort. Alb. buds (extract No. 1) had been evaporated in a rotary evaporator under vacuum at 45°C until a dry extract was obtained. After the forextraction, the defatted raw material was dried under traction without heating. Then, a tincture on a 70% ethyl alcohol in the ratio of raw material and extractant is 1:5 was obtained on the basis of this raw material by the method of a fractional percolation. Then, to obtain a dry extract of *P. rubrinervis* Hort. Alb. buds (extract No. 2), the tincture, similarly to the lipophilic extract, was evaporated in a rotary evaporator under vacuum at 65°C t.

Sample processing for dry extract of *P. rubrinervis* Hort. Alb. buds (extract No. 1)

The aliquot of 0.3 g of *P. rubrinervis* Hort. Alb. buds dry extract (chloroform) (accurately weighed quantity) was placed in a 50 ml capacity measuring flask, dissolved in 25 ml of 96% ethyl alcohol by heating in a boiling water bath and the volume of the solution was adjusted by the same solvent (sample solution A_1). The aliquot of 1 ml of the sample solution A_1 was placed in a 50 ml capacity measuring flask, 2 ml of 3% ethanolic solutions of aluminum (III) chloride was added, and the volume of the solution was adjusted by 96% ethanolic solutions (sample solution B_1). The reference solution was prepared in the following way: 1 ml of the sample solution A was placed in a 50 ml capacity measuring flask and the volume of the solution was adjusted by 96 % ethanolic solutions.

Sample processing for dry extract of *P. rubrinervis* Hort. Alb. buds (extract No. 2)

The aliquot of 0.3 g of *P. rubrinervis* Hort. Alb. buds dry extract (ethanol) (accurately weighed quantity) was placed in a 50 ml capacity measuring flask, dissolved in 25 ml of 96% ethyl alcohol by heating in a boiling water bath and the volume of the solution was adjusted by the same solvent (sample solution A_2). The aliquot of 2 ml of the sample solution A_2 was placed in a 25 ml capacity measuring flask, 2 ml of 3% ethanolic solutions of aluminum (III) chloride was added, and the volume of the solution was adjusted by 96% ethanolic solutions (sample solution B_2). The reference solution was prepared in the following way: 2 ml of the sample solution A_2 was placed in a 25 ml capacity measuring flask and the volume of the solution was adjusted by 96% ethanolic solutions.

Methods of qualitative analysis of *P. rubrinervis* Hort. Alb. buds preparations by thin-layer chromatography and UV spectrophotometry.

To study dry extracts from *P. rubrinervis* Hort. Alb. buds in the phytochemical analysis, the thin-layer chromatography method was used.

The chromatographic plates used in the study were types «Sorbfil-PTSH-AF-A-UV» and «Sorbfil-PTSH-P-A-UV». The plates were placed in a thermostat at 100–105°C before chromatographic analysis. The solvent system tested in the study was chloroform – 96% ethanol (19:1).

The extraction samples were applied with a capillary to the start line and then the plate was immersed in a chromatography chamber pre-saturated with the vapors of the solvent system for 24 h. Chromatography was carried out in an ascending technique. The analysis was considered complete when the solvent front reached 7–8 cm.

After the removal from the system, the plates were dried and evaluated visually in daylight. Additionally, the plates were viewed in the UV light at a wavelength of 254 and 366 nm, then treated with an alkaline solution of diazobenzenesulfonic acid (DSA).

As physical and chemical methods, the method of direct and differential spectrometry was used.

The analysis was performed on a spectrophotometer "Specord 40" (Analytik Jena) at wavelengths in the range of 190–600 nm in cuvettes with a layer thickness of 10 mm. The results of the spectrophotometric determination were processed using the program "WinAspect Excel".

Methodology of BACs pharmacologic study and P. *rubrinervis* Hort. Alb. Buds preparations

The pharmacological study was conducted in accordance with the plan of research works of Samara State Medical University (Samara, Russia) on the theme: "Chemical-pharmaceutical, biotechnological, pharmacological and organizational and economic research on the development, analysis and application of pharmaceutical substances and drugs", State Registration No. AAAA-A19-119051490148-7, dated 14.05.2019. The study was approved by the Bioethics Committee of Samara State Medical University (Report No. 222 dated 07.04.2021).

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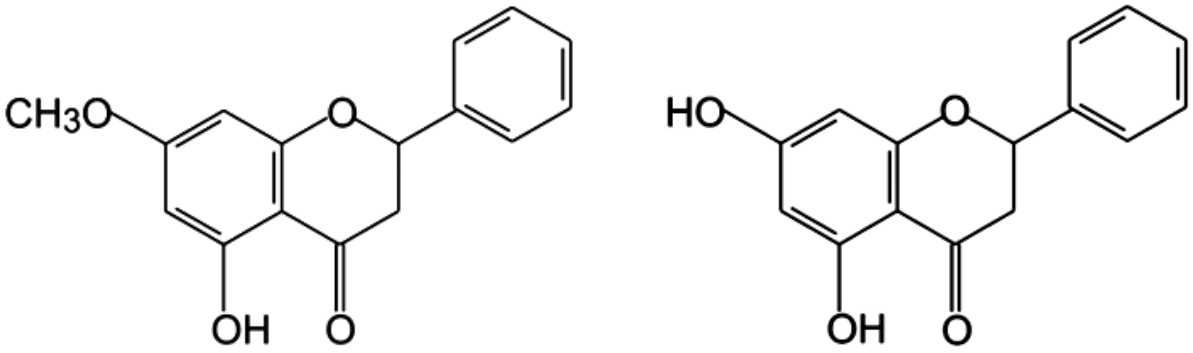
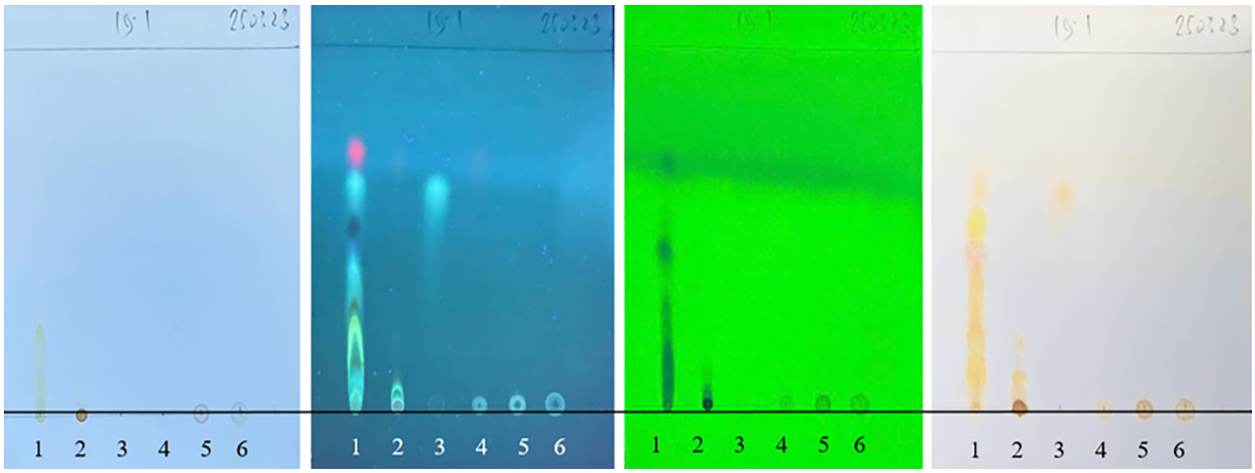


1 2

Figure 1 – Structural formulas of dominant buds flavonoids of pharmacopoeia species of *Populus* genus

Note: 1 – pinostrobin; 2 – pinocembrin.



A B C D

Figure 2 – Chromatogram analysis of extracts and meal from *P. rubrinervis* Hort. Alb. buds in chloroform: ethanol (19:1) solvent system

Note: A – in daylight; B – UV detection in UV light at a wavelength of 366 nm; C – UV detection in UV light at a wavelength of 254 nm; D – DSA treatment; 1 – dry extract No. 1 of *P. rubrinervis* Hort. Alb. buds; 2 – dry extract No. 2 of *P. rubrinervis* Hort. Alb. buds; 3 – pinostrobin; 4 – meal (ethanol 96%); 5 – meal (ethanol 70%); 6 – meal (ethanol 40%).

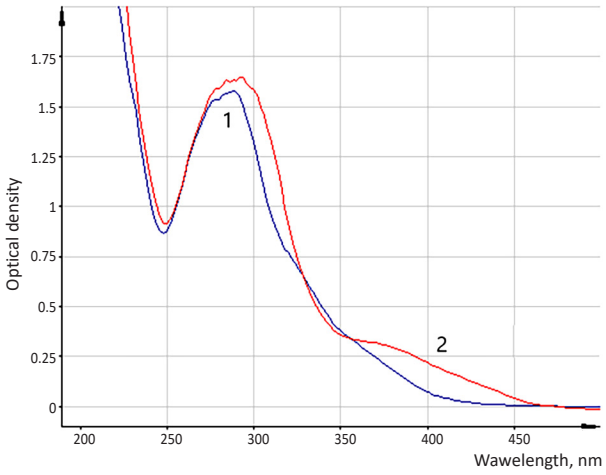


Figure 3 – Electronic spectra of *P. rubrinervis* Hort. Alb. buds chloroform extract (extract No. 1)

Note (here and for Fig. 5): 1 – electronic spectrum of the extract; 2 – electronic spectrum of the extract in the presence of aluminum (III) chloride.

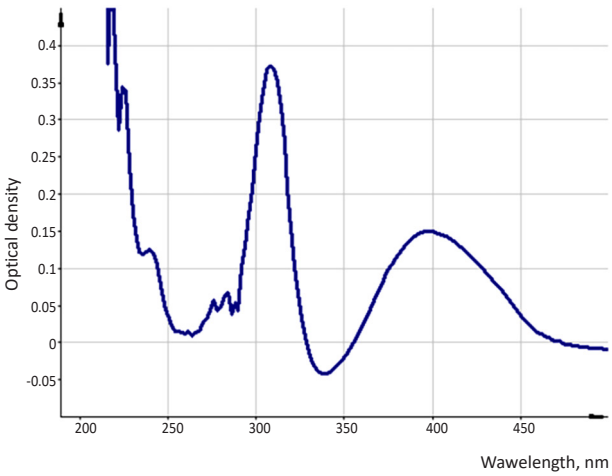


Figure 4 – Differential spectrum of *P. rubrinervis* Hort. Alb. buds chloroform extract (extract No. 1)

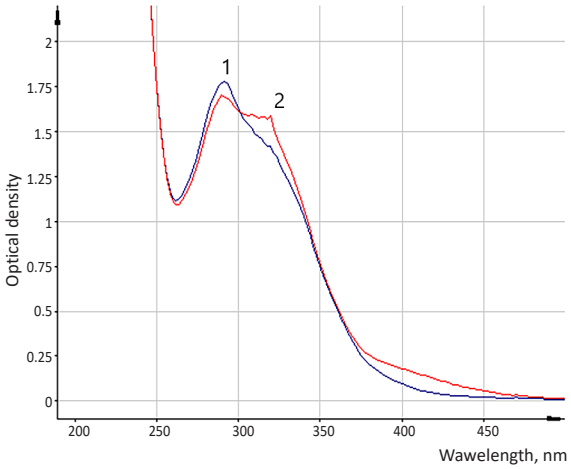


Figure 5 – Electronic spectra of 70% alcohol extract of *P. rubrinervis* Hort. Alb. buds (extract No. 2)

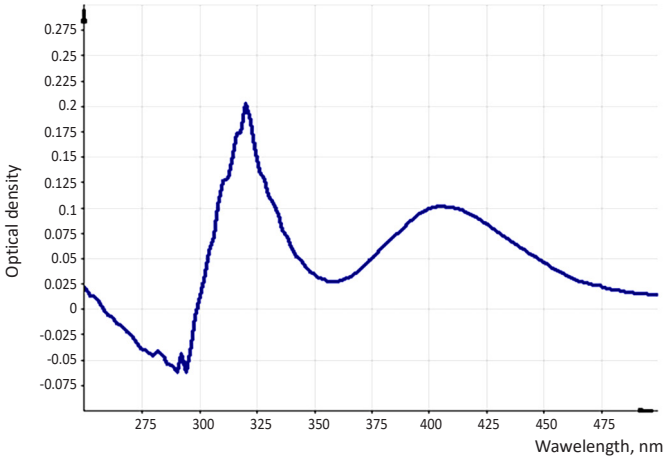


Figure 6 – Differential spectrum of 70% alcohol extract of *P. rubrinervis* Hort. Alb. buds (extract No. 2)

Table 1 – Effect of intragastric injection of SS pinostrobin at a dose of 1 mg/kg and dry extracts of *P. rubrinervis* Hort. Alb. buds No. 1 and No. 2 at a dose of 10 mg/kg on excretory function of intact rats' kidneys of (M±m, *n*=10)

Time, h	Indicators	Control	Experiment No. 1: SS of pinostrobin, 1 mg/kg	Experiment No. 2: Extract No. 1 (chloroform), 10 mg/kg	Experiment No. 3: Extract No. 2 (70% ethanol), 10 mg/kg
4	Diuresis, ml	1.72±0.11	1.97±0.03*	1.82±0.02	2.07±0.04*
	Diuresis, %	100	115	106	120
	Reliability	–	*p*=0.047	*p*=0.698	*p*=0.011
	Creatinine excretion, mg	0.10±0.02	0.13±0.01	0.14±0.01	0.19±0.03*
	Creatinine excretion, %	100	130	140	190
	Reliability	–	*p*=0.341	*p*=0.249	*p*=0.038
24	Diuresis, ml	2.58±0.10	2.52±0.25	2.38±0.39	3.02±0.11*
	Diuresis, %	100	98	92	117
	Reliability	–	*p*=0.844	*p*=0.629	*p*=0.011
	Creatinine excretion, mg	1.50±0.29	2.39±0.15 *	2.31±0.42	2.79±0.51*
	Creatinine excretion, %	100	159	147	186
	Reliability	–	*p*=0.019	*p*=0.229	*p*=0.047

Note: here and further * – $p < 0.05$ – reliability of differences between the data of the experimental group and the control group.

Table 2 – Effect of intragastric injection of comparative drug furosemide at a threshold dose of 1 mg/kg on intact rats' renal excretory function of (M±m, *n*=10)

Time, h	Indicators	Control	Furosemide 1 mg/kg
4	Diuresis, ml	1.97±0.13	3.81±0.22*
	Diuresis, %	100	193
	Reliability	–	*p*=0.001
	Creatinine excretion, mg	0.07±0.01	0.09±0.02
	Creatinine excretion, %	100	129
	Reliability	–	*p*=0.361
24	Diuresis, ml	2.98±0.22	5.42±0.34*
	Diuresis, %	100	182
	Reliability	–	*p*=0.001
	Creatinine excretion, mg	1.19±0.11	1.58±0.13
	Creatinine excretion, %	100	133
	Reliability	–	*p*=0.052

Note: here and further * – $p < 0.05$ – reliability of differences between the data of the experimental group and the control group

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The diuretic activity was studied on 60 white outbred rats of both sexes weighing 200–220 g in chronic experiments with aqueous diuresis. The animals were obtained from the vivarium at the Research Institute of Biotechnology "BioTech", Samara State Medical University of the Ministry of Health of Russia (Samara, Russia). The rats were kept in cages of 4 individuals of the same sex in the vivarium conditions on the standard food ration and with a free access to water. Four experimental and two control groups of animals participated in the experiment. The distribution of animals into groups was performed by drawing lots. Each group consisted of ten animals (n=10). Control and experimental animals received an intragastric 3% water load using a special probe. The experimental animals were additionally administered intragastrically with dried extracts of *P. rubrinervis* Hort. Alb. buds No. 1 and No. 2 at a dose of 10 mg/kg, SS pinostrobin at a dose of 1 mg/kg. Classical diuretics – furosemide at a threshold dose of 1 mg/kg (4 h experiments) and hypothiazide at an effective mean therapeutic dose of 20 mg/kg (24 h experiments) were taken as drugs. After all manipulations, the animals were placed in exchange cages for a urine collection with an access to food and water. After 4 and 24 h, the obtained urine portions were collected. Their volume (diuresis) was determined and creatininuresis was recorded by a calorimetry method on KFK-3 (Zagorsky Optical and Mechanical Plant, Russia).

Statistical processing

In accordance with the general recommendations for the preclinical study of drugs, a comprehensive statistical processing of the data obtained from the pharmacological experiments was carried out using adequate methods of the statistical analysis, the required volume of statistical samples, in the presence of reference drugs. The statistical processing of the results obtained was carried out using the Statistica 10.0 program using the Mann–Whitney test with Bonferroni and Kruskal–Wallis correction. The nonparametric test was chosen because the sample was small, and the distribution in the sample was non-normal (Shapiro-Wilk W test; if p <0.05, the analyzed distribution was considered to be different from normal). The significance level was accepted at p <0.05.

RESULTS AND DISCUSSION

The obtained dry extracts based on the buds of the *P. rubrinervis* Hort. Alb. were in the form of dry powder of golden yellow (extract No. 1) and brick red (extract No. 2) with a specific odor.

The analysis of chromatographic profiles of dry extracts from *P. rubrinervis* Hort. Alb. buds compared with SS of pinostrobin in the solvent system chloroform: ethanol (19:1) allowed us to reliably determine its presence in the studied samples and to assume the presence and dominance of phenolic compounds in the studied object. The TLC analysis of the obtained dry extracts allowed us to arrive at the conclusion about the separation of the initial amount of substances (Fig. 2).

The dominant phenolic complexes of a lipophilic nature with pinostrobin were isolated into extract No. 1 obtained on chloroform. In the hydrophilic extract (extract No. 2), phenolic substances of a glycosidic nature prevail. Specifically, catechins and a number of other phenolic compounds not identified at this stage (Fig. 2B and 2D).

After a double extraction, the chromatographic profiles of the aqueous-alcohol extractions from the raw meal on alcohols of different concentrations (40, 70, 96% ethyl alcohol) showed a maximally depleted composition (Fig. 2).

Thus, using different types of extraction and solvents of a different polarity, it was possible to separate the initial amount of metabolites into lipophilic and hydrophilic complexes. Two dry extracts based on the *P. rubrinervis* Hort. Alb. buds differing in chemical composition, were received. It should be noted that the dominant component of pharmacopoeial poplar buds, pinostrobin, is localized in the first lipophilic (hydrophobic) extract.

The dry extracts were further studied by spectrophotometry. Based on the known previously developed methods for the analysis of pharmacopoeial poplar species buds [1, 2, 4], the direct spectra of *P. rubrinervis* Hort. Alb. buds extracts were analyzed (Fig. 3 and 4).

The analysis of spectral curves showed that extract No. 1 is characterized by the presence of one pronounced maximum in the spectral curve in the area of 288±2 nm and a small "shoulder" in the area of 320±2 nm, which coincides with the absorption maximum of the extracts from the pharmacopoeia poplars buds (289±2 nm) (Fig. 3). Such coincidence can be explained by the presence of the main dominant flavonoids of the buds of pharmacopoeial poplar species, particularly pinostrobin, the content of which had been proven by TLC before.

The differential absorption curve has two expressed analytical maxima: 308±2 nm, characteristic for the phenylpropanoids, and 400±2 nm, characteristic for the amount of flavonoid substances, indicating the presence of these groups of substances in the composition of *P. rubrinervis* Hort. Alb. buds, as in pharmacopoeial species (Fig. 4).

Despite the different polarity of the extractants, the spectral characteristics of hydrophilic extract No. 2 have significant similarities with extract No. 1 obtained using chloroform (Fig. 5 and 6).

Based on the data of chromatographic studies of the extracts (Fig. 2), it can be assumed that the characteristics of the spectra of the compared extracts are largely determined by the presence of simple phenolic compounds that differ in polarity due to

the presence or absence of glycosidic bonds. For a more accurate separation in the future, the study and isolation of biologically active substances from the buds of *P. rubrinervis* Hort. Alb. by column chromatography is planned to be carried out.

The samples of the dry extracts of *P. rubrinervis* Hort. Alb. buds No. 1 and No. 2 and SS pinostrobin were used to study the excretory function of the kidneys in preclinical studies on white mongrel laboratory rats at a dose of 10 mg/kg for the extracts and 1 mg/kg for the SS pinostrobin (Table 1).

When studying the effect of SS pinostrobin on the excretory function of the kidneys it was revealed that in a 4-hour chronic experiment with a single intragastric injection of BAC at a dose of 1 mg/kg against the background of a 3% water load, there was a significant isolated increase in diuresis (by 15%). There was also a significant isolated reliable increase in creatininuresis (by 59%) during 24 h of the experiment (Table 1).

Consequently, SS pinostrobin at a dose of 1 mg/kg against the background of a 3% water load caused an accelerated diuretic response mainly due to an increase in the tubular reabsorption of water, as evidenced by the increase in diuresis in the first 4 h of the experiment, as well as by a delayed increase in glomerular filtration, which was confirmed by an increase in creatininuresis over 24 h.

At the same time, when analyzing the effect of dry extract No. 2 (70% ethanol) of *P. rubrinervis* Hort. Alb. buds it was found that in a 4-hour chronic experiment at a single intragastric injection of the dry extract at a dose of 10 mg/kg against the background of a 3% water load in the animals of the experimental group relative to the indicators of a water control, there was a significant increase in diuresis (by 20%) and a significant increase in creatininuresis (by 90%); at the same time, for 24 hours of the experiment there was a significant increase in diuresis (by 17%) and a significant increase in creatininuresis (by 86%).

Thus, dry extract No. 2 of *P. rubrinervis* Hort. Alb. buds (ethanol) at a dose of 10 mg/kg in the 4-h and daily experiments induced an accelerated and prolonged diuretic response, both by increasing a tubular reabsorption of water (an increase in the renal excretion of water) and by increasing a tubular filtration (an increase in the renal excretion of creatinine).

However, when studying the effect of dry extract No. 1 of *P. rubrinervis* Hort. Alb. buds (chloroform) on the excretory function of kidneys in 4 and 24-hour chronic experiments at a single intragastric injection at a dose of 10 mg/kg against a background of a 3% water load in the animals of the experimental group relative to the indicators of the water control, no significant differences were found.

Presumably, this is due to the fact that this extractant contributed to the release of associated lipophilic compounds from the medicinal plant raw materials, which did not have a proper stimulating effect on the tubular and tubule apparatus of the kidneys.

In its turn, the comparative drug furosemide at a threshold dose of 1 mg/kg in a 4-hour experiment against the background of a 3% water load significantly increased diuresis (by 93%) in the experimental group of animals relative to the water control (Table 2).

It follows that SS pinostrobin and dry extract No. 2 moderately stimulated the renal excretion of water, significantly inferior to the comparative drug furosemide (causing maximal diuresis). It is noteworthy that SS pinostrobin and dry extract No. 2 have the ability to stimulate the tubular filtration, in contrast to furosemide, which has an exclusively tubular mechanism of a diuretic action. Based on all of the above, these drugs are promising in terms of the development of drugs with nephroprotective properties.

CONCLUSION

The presence of a promising understudied representative of the genus Poplar (*Populus* L.) – *P. rubrinervis* Hort. Alb., favorably differing from pharmacopoeial species of poplars by a much larger phytomass of buds, has been revealed. The literature review has also made it possible to confirm the existing significant habitat of this representative in the Samara region, which indicates the prospect of harvesting this type of raw materials.

The extraction separation of the amount of *P. rubrinervis* Hort. Alb. buds substances by polarity has been conducted using technological methods – a circulation extraction and percolation.

The presence of pinostrobin as the main flavonoid of pharmacopoeial poplar species was proven chromatographically in the chloroform extract of *P. rubrinervis* Hort. Alb. buds and its absence in the alcoholic extract. The similarity of spectral absorption curves of lipophilic and hydrophilic amounts of phenolic compounds of *P. rubrinervis* Hort. Alb. buds extracts of various polarities has been revealed, i.e., the presence of a pronounced absorption maximum at 289 nm±2 nm and a “shoulder” at 320±2 nm.

At a single intragastric injection of the BAC pinostrobin at a dose of 1 mg/kg against the background a 3% water load in the animals of the experimental group against the indicators of the water control, an isolated increase in diuresis was observed for 4 hours of the experiment, at the same time, for 24 hours of the experiment there was an increase in creatininuresis.

Consequently, pinostrobin at a dose of 1 mg/kg caused an accelerated diuretic response, inferior to the comparative drug furosemide at a threshold dose of 1 mg/kg. The SS Pinostrobin at the indicated dose exhibited a delayed creatininuretic response, which compares it favorably with comparative drugs.

At the same time, at a single intragastric injection of

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dried extract of *P. rubrinervis* Hort. Alb. buds No. 2 on 70% ethanol at a dose of 10 mg/kg against the background of a 3% water load in the animals of the experimental group against the indicators of a water control, a moderate reliable increase in diuresis and a significant increase in creatininuresis for 4 and 24 hours of the experiment was observed due to an increase in glomerular filtration. The action of the study drug is inferior in the strength of diuresis to furosemide at a threshold dose of 1 mg/kg (4 h experience) and hypothiazide at an effective threshold dose of 20 mg/kg (a 24 h experience), but superior to comparative drugs in stimulation of the glomerular filtration, contributing to the increase in creatininuresis. When studying the effect of *P. rubrinervis* Hort. Alb. buds dry extract on chloroform No. 1 on the excretory function of the kidneys for 4 and 24 h, no differences between the experimental group and the control have been found, therefore, further studies of the dose-dependent effect of this preparation are required.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Elena A. Urbanchik – data collection, conducting an experiment, writing a text and compiling a list of references; Vladimir A. Kurkin – concept and design of the study, editing, final approval for the publication of the manuscript; Elena N. Zaitseva – conception and design of pharmacological experiments, participation in the description and analysis of the results obtained, statistical processing of measurement results, participation in manuscript writing and final approval for publication; Vitaly M. Ryzhov – collection of plant material for analysis, participation in the study; Alexey V. Dubishchev – critical study analysis; Anastasia S. Tsybina – participation in the research, literature analysis; Anastasia I. Altareva – participation in the research; Yulia D. Sirotkina – participation in the research.
All the authors confirm that their authorship meets the ICMJE international criteria (all the authors contributed substantially to the conceptualization, research and preparation of the article, read and approved of the final version before publication).

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