https://doi.org/10.31925/farmacia.2026.4.5 **ORIGINAL ARTICLE**

THE POLYPHENOLIC PROFILE, DIURETIC, ANTI-INFLAMMATORY, AND ANALGESIC EFFECTS OF THE RESIN FROM *BETULA PENDULA* ROTH. BUDS

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Abstract

This study investigated the polyphenolic profile, as well as the diuretic, anti-inflammatory, and analgesic activities of the resin secreted by the buds of Romanian *Betula pendula* Roth. (silver birch). LC-MS/MS analysis revealed the predominance of ellagic acid (109.761 µg/100 mg), accompanied by three major flavonoids: hyperoside (88.596 µg/100 mg), acacetin (52.516 µg/100 mg), and genkwanin (48.132 µg/100 mg). The resin produced a moderate and gradually installed diuretic effect with an increased urinary excretion of Na^+ and K^+ ions, in saline loaded Wistar rats. Although inferior to furosemide, the diuretic effect of the resin was significant during the final phase of the 24-hour monitoring period, with a urine output of 6.20 ± 0.25 mL. Additionally, the resin demonstrated a significant and dose-dependent anti-inflammatory effect in λ-carrageenan-induced rat paw edema model, the effect being slightly inferior to diclofenac. Also, the resin (500 mg/kg) produced a moderate analgesic effect in Randall-Selitto test in rats, with a maximum tolerated amount of pressure of 51 g compared to 70 g for diclofenac. These findings highlight the therapeutic potential of resinous buds of silver birch.

Rezumat

Acest studiu a investigat profilul polifenolic, precum și activitățile diuretice, antiinflamatoare și analgezice ale rășinii secretate de mugurii de *Betula pendula* Roth. (mesteacăn) proveniți din România. Analiza LC-MS/MS a relevat predominanța acidului elagic (109,761 µg/100 mg), însoțit de trei flavonoide principale: hiperozidă (88,596 µg/100 mg), acacetină (52,516 µg/100 mg) și genkwanină (48,132 µg/100 mg). Rășina a produs un efect diuretic moderat și gradual la șobolani Wistar hidratați cu soluție salină izotonă, cu o excreție urinară crescută a ionilor de Na^+ și K^+. Deși inferior furosemidului, efectul diuretic al rășinei a fost semnificativ în faza finală a perioadei de monitorizare de 24 de ore, cu o valoare de 6,20 ± 0,25 mL. În plus, rășina a demonstrat un efect antiinflamator semnificativ, doză-dependent, în modelul experimental al edemului labei de șobolan indus de λ-caragenan, având o eficiență ușor inferioară diclofenacului. De asemenea, rășina la doza de 500 mg/kg a produs un efect analgezic moderat în testul Randall-Selitto, cu o presiune maximă tolerată de 51 g, comparativ cu 70 g pentru diclofenac. Aceste rezultate evidențiază potențialul terapeutic al rezinei mugurilor de mesteacăn.

Keywords: *Betula pendula* Roth., polyphenols, diuretic, anti-inflammatory, analgesic

Introduction

Betula pendula Roth. (syn. *B. verrucosa* Ehrh.), commonly named silver birch, belongs to the Betulaceae family. This species is widely distributed in Europe and the northern regions of Asia, showing a preference for moist and acidic soils. It is also the most widespread birch species in the wild flora of Romania alongside *Betula pubescens* Ehrh. (downy birch) (1-3). *Betula pendula* is a monoecious tree that reaches heights of 15 - 25 m. The bark of the juvenile tree is brown, but as it ages, it turns silvery-white with dark gray horizontal lenticels that deepen and break. It features natural peeling layers typical of birch trees, contributing to its unique pattern. The buds are ovoid, conical, appressed to the stem, and grey brown in color. The deciduous leaves are small and simple, rhomboidal in shape, with a doubly serrate margin. The flowers are unisexual and arranged in catkins; the long, loose male catkins hanging down in clusters of 2 - 3 at the ends of twigs, whereas the female catkins are small, stiff and stand erect of the branches when they appear in spring alongside new leaves (1, 2, 4).

The pharmaceutical, cosmetics and food industries use birch leaves, bark and sap to produce herbal medicines, antioxidants, cosmetics, dietary supplements and drinks (5). Silver birch belongs to medicinal plants. The various parts of the birch are widely used in both traditional and officinal medicine: bark tar and essential oils are used to cure skin diseases, while decoctions and extracts from dried leaves and resinous buds are applied for the treatment of inflammation and as diuretics (1, 6, 7).

The resinous buds of birch are widely used in Russian and Chinese traditional medicine mainly as a diuretic and diaphoretic agent but also as an antiseptic, anti-inflammatory and analgesic (7, 8). Possessing anti-inflammatory and antiseptic properties, birch buds are traditionally utilized in dermatology for wound epithelialization, skin cleansing, and the reduction of cutaneous hyperpigmentation. Furthermore, birch bud infusions and decoctions are widely applied within dentistry and otolaryngology to manage mucosal and respiratory inflammations, including stomatitis, gingivitis, periodontitis, glossitis, and acute upper respiratory tract infections (9). The scientifically validated therapeutic efficacy of bud extracts is largely attributed to their potent diuretic action, alongside their antimicrobial and antioxidant activities. Additionally, birch bud extracts provide a promising pool of bioactive compounds with broad-spectrum anticancer activity (7). The pharmacological effects are attributed to their chemical composition, mainly to their high content of polyphenolic compounds (such as flavonoids, phenolic acids, tannins) and terpenic compounds present in different structural types (pentacyclic triterpenes of lupeolic type such as betulin and its derivatives), sesquiterpenic compounds (from essential oil), sterolic compounds (2).

Only a few works have addressed the chemical composition of birch bud extracts and there is practically not much information about the compounds contained in birch bud exudates (resin), which may form the first line of plant protection against microorganisms and herbivores and also serve as the main source of propolis in temperate areas (6, 10, 11). The propolis produced in Russia from birch buds consists mainly of flavones, flavonols, flavonones and sesquiterpenes (11).

The chemical composition of bud exudate from *B. pendula* species has been characterized by Isidorov and colleagues using gas chromatography–mass spectrometry (GC-MS). Their results demonstrated that silver birch exudate is a rich, complex mixture containing numerous organic compounds, primarily triterpenoids (most predominant), sesquiterpenes and flavonoids (6).

This study aimed to determine the polyphenolic profile of silver birch bud resin and evaluate its diuretic, anti-inflammatory and analgesic effects. Polyphenols have been reported to have anti-inflammatory, antimicrobial, antioxidant, immunomodulatory, antimutagenic, and anti-allergic properties (12).

Materials and Methods

Plant material and sample preparation

Silver birch buds were collected in the appropriate vegetative period of the plant before opening, in the early spring from Ciucea, Cluj County, Romania. The plant was taxonomically authenticated by Professor Mircea Tămaș and a voucher specimen of *B. pendula* Roth. (No. 37.1.2.1) was deposited for reference in the Department of Pharmaceutical Botany, University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca, Romania.

After collection, the buds were air-dried until a constant mass, and then the extract was achieved. Subsequently, 10 g of buds were grinded, and the resulting plant material underwent two cold maceration cycles for one hour each, with 50 ml of absolute ethyl alcohol. The filtered solutions from both cycles were pooled and adjusted to volume in a 100 cm^3 volumetric flask. To obtain the sticky resin, 10 mL of the extract was evaporated to dryness within a controlled-temperature environment (at 35ºC) (13).

Phytochemical analysis of bud resin samples

Reagents and solvents

For the LC-MS/MS analysis, 74 reference standards of phenolic compounds were purchased from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Schnelldorf, Germany), Carl Roth (Karlsruhe, Germany), Merck (Darmstadt, Germany), Supelco (Bellefonte, PA, USA), and Extrasynthese (Genay, France). HPLC-grade methanol and analytical-grade acetic acid were purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water was obtained using a Milli-Q Direct 8 water purification system (Millipore, Bedford, MA, USA).

Instrumentation

Liquid chromatography was performed on a Shimadzu Nexera X3 HPLC system (Kyoto, Japan), equipped with dual LC-40D X3 pumps, an integrated degasser, an SIL-40C X3 autosampler, and a CTO-40S column oven. This system was interfaced with a Shimadzu QTOF 9030 mass spectrometer. Data acquisition and processing were handled by LabSolution software (version 5.137, Shimadzu Corporation).

LC-MS/MS conditions and method validation

The analytical methodology for phenolic compounds was adapted from our previously described protocols (14-20). Chromatographic separation was achieved using a Zorbax SB-C18 column (100 × 3.0 mm i.d., 3.5 µm particle size; Agilent Technologies) maintained at 48°C. The mobile phase consisted in 0.2% (v/v) aqueous acetic acid (Solvent A) and 0.2% (v/v) acetic acid in methanol (Solvent B). A linear gradient elution was applied as follows: starting at 5% B and increasing to 70% B over 30 minutes, followed by a 4-minute re-equilibration at 5% B. The flow rate was

maintained at 1 mL/min with an injection volume of 1 µL.

Regarding the mass spectrometry parameters, detection was conducted in negative electrospray ionization (ESI) mode, utilizing either high-resolution MS (HR-MS) or HR-MS/MS, both operating with a 15 ppm mass tolerance. The ESI source parameters were optimized as follows: source voltage - 2000 V, interface temperature 300°C, nebulizing gas flow 3 L/min, heating gas (air) flow 15 L/min, drying gas (nitrogen) flow 15 L/min, desolvation line temperature 300°C, and heat block temperature 400°C.

The QTOF mass spectrometer utilized multiple-scheduled time events for the analysis. For qualitative identification, an HR-MS scan and a data-dependent analysis (DDA) were scheduled concurrently between 0.8 and 30 minutes. DDA spectral data provided qualitative confirmation, requiring a spectral match score > 90 and an ion ratio calculation for two qualifier ions within a ± 20% tolerance of library spectra. Upon a positive library match, the peak area was determined from the HR-MS data, and the relative concentration was expressed as the closest related active moiety.

Quantitative analysis employed specific time-scheduled events for each available standard, spanning 0.8 minutes before and after its respective retention time in either HR-MS or HR-MS/MS mode. Compound quantification was strictly contingent upon prior qualitative confirmation *via* DDA spectral data.

The quantitative method was fully validated for linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision. Linearity was established across a 0.05 - 50 µg/mL concentration range. LOD and LOQ thresholds were defined by signal-to-noise (S/N) ratios of ≥ 10 and ≥ 20, respectively (Supplementary Material).

To ensure optimal mass accuracy (< 3 ppm error), an internal standard calibration event was executed early in each run (0.1 - 0.3 minutes, prior to the chromatographic dead-time). This calibration utilized a 400 mg/L sodium iodide standard solution (P/N 220-91239-90, Shimadzu Corporation), with mass corrections automatically applied to the spectral data by the LabSolution software.

Evaluation of pharmacological activities of the bud resin

Animals

Seventy-two male Wistar rats with a medium weight of 213 ± 19 g were purchased from the Practical Skills and Experimental Medicine Centre of the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. The animals were maintained in standard conditions (22 ± 2°C, a relative humidity of 45 ± 10%, 12:12-h light:dark cycle), with free access to standard pelleted food ("Cantacuzino" Institute, Bucharest, Romania) and filtered water throughout the experiment, except for the day when the test substances were administered.

Diuretic effect and electrolyte excretion

The evaluation of the diuretic effect of the resin from *Betula pendula* (BPR) was performed according to a method which used isotonic saline solution as hydrating fluid (21). Twenty-four Wistar rats were randomized in four groups (n = 6). The rats from the control group were treated orally with 25 mL/kg isotonic saline solution (Braun, Germany), while the rats from the reference group were treated orally with 10 mg/kg furosemide (Zentiva, Romania), dissolved in 25 mL/kg isotonic saline solution. Other two groups of rats were treated orally with 250 and 500 mg/kg BPR also dispersed in 25 mL/kg isotonic saline solution.

Afterwards, the animals were individually placed in metabolic cages, the environmental temperature being maintained at 22ºC. The cumulative urine output (mL) was recorded for each animal at two different time intervals: 5 h and 24 h after the administration of a single dose from the tested substances (22).

In order to evaluate electrolyte excretion, urinary concentration of sodium and potassium ions (U_{Na} and U_K) was determined in the collected urine samples, 5 h and 24 h after the substance administration, by a potentiometric method with selective electrodes, using a VITROS 250 Chemistry System automatic analyzer (Johnson and Johnson Clinical Diagnostic), being expressed in mEq/kg (23).

Anti-inflammatory effect

The anti-inflammatory effect of the resin from *Betula pendula* (BPR) was tested in rats using the experimental model of rat paw edema test induced by λ-carrageenan (24). Twenty-four rats were randomized in four groups (n = 6). Rats from the control group were treated orally with normal saline solution (vehicle) while the animals from the reference group received orally a dose of 20 mg/kg diclofenac. BPR was administered to the other two groups of rats by oral route in doses of 250 and 500 mg/kg. All substances were administered 60 minutes prior to a subplantar injection of λ-carrageenan (1% w/v, 0.1 mL). The paw edema was evaluated with a digital plethysmometer (model 7140, Ugo Basile, Italy) initially, and at 1, 2, 3 and 4 hours after the administration of λ-carrageenan. Edema was calculated as the difference between inflamed paw volume and the initial paw volume, for all time intervals.

Analgesic effect

The analgesic effect of the resin from *Betula pendula* (BPR) was assessed by the Randall Selitto test in rodents (25). In this model, the injection of the phlogistic agent λ-carrageenan into the footpads of rats produces a persistent pain, closely mimicking the time course of some pain types in humans. For this method, twenty-four rats were randomized in four groups (n = 6). Initially, the animals received a

subplantar injection of λ-carrageenan (1% w/v, 0.1 mL). Thirty minutes after the induction of inflammation, the substances were orally administered to the animal groups: BPR 250 and 500 mg/kg for the test groups, normal saline solution to the control group and diclofenac 20 mg/kg to the reference group. At multiple time points (1 h, 2 h, 3 h and 4 h) the inflamed posterior member was mechanically compressed with an analgesimeter (Ugo Basile, Italy), the specific reaction of each animal being considered sign of hyperalgesia. The analgesimeter indicated the intensity of pressure (g) tolerated by the animals at the mentioned time points.

Statistical analysis

Data were expressed as mean values ± standard deviation (SD). Initially, Shapiro-Wilk test was used to evaluate data distribution, which showed a normal pattern. Afterwards, parametric tests were employed (one way ANOVA method followed by Dunnett's test), using GraphPad Prism 7 software (GraphPad Software, San Diego, USA). The differences between the BPR-treated groups and the saline-treated control groups which were considered statistically significant were marked with "*'' (p values < 0.05).

Results and Discussion

The extraction process yield of resin

The extraction yield of the resin of the dry buds of *B. pendula*, was 27.72%. Ethanol dissolves buds' resins and their less polar components, such as flavonoid aglycones and hydroxycinnamic acids esters (26).

Polyphenolic profile of bud resin

During the polyphenolic profiling of *B. pendula* bud resin by LC-MS/MS, a total of 164 compounds were tested, of which 45 were identified and quantified (Table I).

Table I

Polyphenolic compounds identified in *B. pendula* bud resin

Phenolic group	Components	Content, µg /100 mg resin	Quantified
Phenolic acids	3,5-Dicaffeoylquinic acid	0.162 ± 0.011	As Gallic acid
	Gallic acid	5.082 ± 0.355	Itself
	Protocatechuic acid	0.484 ± 0.029	Itself
	3-Hydroxybenzoic acid	0.108 ± 0.002	Itself
	Vanillic acid	0.896 ± 0.062	Itself
	Chlorogenic acid	4.761 ± 0.381	Itself
	Caffeic acid	0.228 ± 0.012	Itself
	4-*O*-Caffeoylquinic acid	0.356 ± 0.013	Itself
	Syringic acid	0.191 ± 0.012	Itself
	p-Coumaric acid	0.538 ± 0.016	Itself
	Salicylic acid	0.072 ± 0.005	Itself
	Ferulic acid	3.446 ± 0.068	Itself
	Ellagic acid	109.761 ± 5.488	Itself
Glycosylated phenolic acids	Protocatechuic acid-4-O-glucoside	0.0978 ± 0.005	As Gallic acid
	Vanillic acid-4-O-glucoside	0.138 ± 0.002	As Gallic acid
Flavonoids			
Flavones	Apigenin	7.729 ± 0.232	Itself
	Hispidulin	5.618 ± 0.449	Itself
	Eupatilin	4.127 ± 0.206	Itself
	Chrysin	0.005 ± 0.001	Itself
	Acacetin	52.516 ± 0.421	Itself
	Genkwanin	48.132 ± 3.851	Itself
	Vitexin	0.022 ± 0.001	Itself
	Apigenin 7-glucoside	0.624 ± 0.043	Itself
Flavans	Catechin-O-glucoside	0.173 ± 0.003	As Catechin
	Gallocatechin	0.757 ± 0.031	Itself
Flavonols	Isoquercitrin	8.856 ± 0.265	Itself
	Chrysosplenetin	11.483 ± 0.802	As Quercetin
	Quercetin-3-O-arabinoside	7.896 ± 0.477	As Quercetin
	Quercitrin	0.715 ± 0.004	Itself
	Quercetin	0.779 ± 0.016	Itself
	Kaempferol	10.492 ± 0.733	Itself
	Tamarixetin	3.497 ± 0.245	As Quercetin
	Quercetin-7,3'-dimethyl ether	20.851 ± 0.417	As Quercetin
	Isorhamnetin-3-O-glucoside	0.482 ± 0.009	As Quercetin
	Kaempferol-3-O-galactoside	0.613 ± 0.024	As Quercetin
	Hyperoside	88.596 ± 3.546	Itself

Phenolic group	Components	Content, µg /100 mg resin	Quantified
Flavanols	Catechin	13.669 ± 0.683	Itself
	Epicatechin	0.321 ± 0.022	Itself
	Procyanidin B1	0.435 ± 0.017	Itself
	Procyanidin B2	0.401 ± 0.028	Itself
	Procyanidin B3	3.828 ± 0.306	Itself
Flavanonols	Aromadendrin-O-Glucoside	0.144 ± 0.001	As Quercetin
Phenylpropanoids	1-O-Caffeoyl-beta-D-glucose	0.109 ± 0.008	As Gallic acid
Phenolic aldehydes	Coniferyl aldehyde	0.032 ± 0.001	Itself
	Protocatechuic aldehyde	0.219 ± 0.017	As Gallic acid

The term "polyphenols" encompasses various compounds, including flavonoids, phenolic acids, lignans, stilbenes, and anthocyanins, each with a unique chemical structure and biological activity (27).

The primary constituent of silver birch bud resin was ellagic acid (109.761 µg/100 mg), a phenolic acid known for its antioxidant, analgesic, anti-edematous, and anti-inflammatory efficacy, especially against allergic conditions (28, 29). *B. pendula* bud resin is rich in flavonoids, mainly flavones and flavanols. In the last decade, there has been a high interest in the involvement of polyphenolic compounds, especially flavonoids, in anti-oxidative, anti-inflammatory and analgesic effects. Flavonoids are credited with a significant anti-inflammatory activity, comparable to the drugs from NSAID class (nonsteroidal anti-inflammatory drugs). Along with this, flavonoids also restrict the action of other proinflammatory mediators different from those synthesized by COX (cyclooxygenase). In addition, flavonoids have enormous modulation potential toward transcriptional factors in inflammatory and antioxidant pathways such as NF-κB and Nrf-2 (30). Three major flavonoids were found in high concentration in the tested silver birch bud resin: hyperoside (88.596 µg/100 mg), acacetin (52.516 µg/100 mg), and genkwanin (48.132 µg/mg). Hyperoside (quercetin-3-O-galactoside), a flavonol glycoside, demonstrated anti-inflammatory activity by inhibiting both arachidonic acid-induced and croton oil-induced edema. It also inhibited COX-2 and hyaluronidase enzymes (30). Acacetin (5,7-dihydroxy-4'-methoxyflavone) has demonstrated significant anti-inflammatory properties in multiple animal models of inflammation. It was reported that systemic administration of acacetin decreased visceral and inflammatory nociception and prevented the formalin-induced oedema (31, 32). Genkwanin (4′,5-Dihydroxy-7-methoxyflavone) is one of the major non-glycosylated flavonoids in many herbs which possess a potent anti-inflammatory effect by inhibiting inflammatory mediators and pathways. It acts largely by regulating oxidative stress and reducing pro-inflammatory cytokines (33).

Birch buds have been used in patients with edema of cardiac origin as a diuretic. A significant increase in diuresis (urine production) and a sharp decrease in edema were reported by previous studies (8). Many flavonoids exert diuretic and saluretic effects by increasing urine production and electrolyte output (34, 35). In the *B. pendula* bud resin, we identified and quantified two flavonols with proven diuretic activity include: kaempferol (10.491 µg/100 mg) and isoquercitrin (8.856 µg/100 mg). Kaempferol acted as a mild diuretic and saluretic (36) while isoquercitrin demonstrated a strong saluretic and diuretic effect, not associated with osmotic mechanisms (37).

Evaluation of pharmacological activities of the bud resin

Diuretic effect and electrolyte excretion

The resin from *B. pendula* (BPR) produced a statistically significant and dose-dependent increase in urine output in saline loaded Wistar rats. The effect was progressive, being more intense at 24 h. The dose of 500 mg/kg BPR produced the most intense diuretic effect, with a urine output of 6.20 ± 0.25 mL at 24 h. The reference diuretic, furosemide, increased sharply the urine output in the first 5 hours after the oral administration (7.18 ± 0.28 mL), then reaching a plateau (7.61 ± 0.26 mL) at 24 h, as expected for a high ceiling diuretic drug. The diuretic effect of BPR, although inferior to furosemide, was more gradually installed, being significant in the last part of the 24 h time interval, as seen in Figure 1.

The gradual profile of the diuretic effect of BPR can offer an advantage in the treatment of a series of chronic cardiovascular diseases, where a rapid effect could generate adverse reactions. Also, the resin from *B. pendula* (BPR) increased the urinary excretion of Na^+ and K^+ ions (U_{Na}, U_K), the main cationic electrolytes from the urine.

The urinary excretion of Na^+ and K^+ ions produced by BPR followed the same pattern as for the diuretic effect, being superior at 24 h. The most significant excretion of the Na^+ and K^+ ions was produced by the 500 mg/kg dose of BPR, with U_{Na} and U_K values of 2.14 ± 0.48 and 1.73 ± 0.44 mEq/kg, 24 h after the substance administration. The calculated Na^+/K^+ ratio for BPR treated groups did not show values above 10 at any moment of determination, thus indicating a lack of a potassium-sparing effect. The results are detailed in Table II.

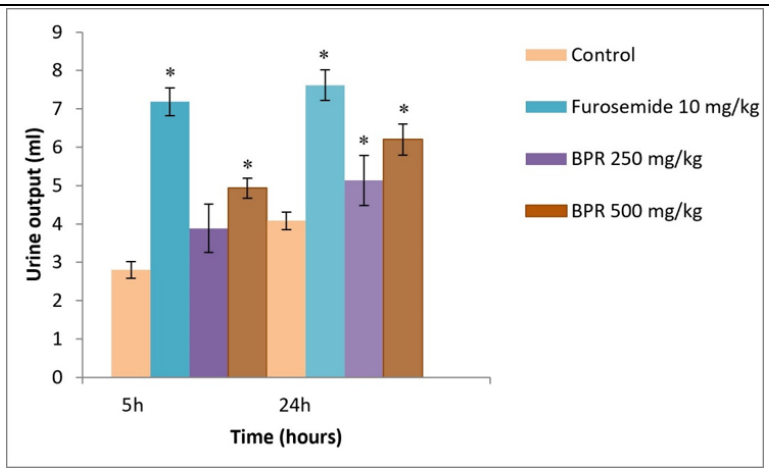

Figure 1.
Effect of the resin from *Betula pendula* (BPR) on urine output recorded at 5 h and 24 h in saline loaded Wistar rats (* $p < 0.05$ *vs.* saline control)

Table II
Effect of the resin from *B. pendula* (BPR) on urinary excretion of sodium (U_{Na}) and potassium (U_K) 5 h and 24 h after the substance administration, and the ratio Na/K in saline loaded Crl:WI rats. Values of $U_{Na}V$ and U_KV are expressed as Mean ± SD (n = 6); (* $p < 0.05$ *vs.* saline control)

Group (Dose)	U_{Na} at 5 h (mEq/kg)	U_K at 5 h (mEq/kg)	U_{Na} at 24 h (mEq/kg)	U_K at 24 h (mEq/kg)	Na/K at 24 h
Control (saline)	1.34 ± 0.17	1.13 ± 0.11	1.52 ± 0.18	1.35 ± 0.20	1.12
Furosemide (10 mg/kg)	4.89 ± 0.44*	2.93 ± 0.31*	5.28 ± 0.39*	3.36 ± 0.24*	1.57
BPR (250 mg/kg)	1.45 ± 0.13	1.22 ± 0.23	1.75 ± 0.27*	1.52 ± 0.41*	1.15
BPR (500 mg/kg)	1.87 ± 0.39*	1.45 ± 0.25*	2.14 ± 0.48*	1.73 ± 0.44*	1.23

The classical diuretic drugs like furosemide cause an inhibition of key co-transporters in the nephron with a subsequent reduction of electrolyte and water reabsorption with potent diuretic and saluretic effects. Natural remedies like the tested BPR cause a mild diuretic and saluretic effect probably due to significant content in flavonoids (23), but other chemical constituents may also be responsible for the effect. Moreover, two flavonoids identified in the chemical composition of BPR (kaempferol and isoquercitrin) have shown diuretic and saluretic effects in previously published studies (36, 37). Our study confirmed these results but did not prove potassium-sparing effects, due to a different chemical composition of BPR compared with the extract tested by Gasparatto and colleagues (37).

Although the diuretic effect of the leaves from *B. pendula* was researched before in laboratory animals and humans, our study investigated for the first time the diuretic effect of the resin obtained from the plants' buds (BPR) in saline loaded Wistar rats. Additional studies are needed to clarify the mechanism of the diuretic effect of BPR.

Anti-inflammatory effect

The results of the assessment of anti-inflammatory effect of BPR in the experimental model of rat paw edema test induced by λ-carrageenan are presented in Table III.

Table III
Anti-inflammatory effect of the resin from *B. pendula* (BPR) in rat paw edema induced by λ-carrageenan

Group	Dose	Edema 1 h (mL)	Edema 2 h (mL)	Edema 3 h (mL)	Edema 4 h (mL)
Control (vehicle)	-	0.87 ± 0.31	2.04 ± 0.67	3.13 ± 0.62	3.23 ± 0.59
BPR	500 mg/kg	0.57 ± 0.28	1.52 ± 0.38	1.61 ± 0.44*	1.74 ± 0.39*
BPR	250 mg/kg	0.64 ± 0.14	1.98 ± 0.41	2.03 ± 0.35*	2.69 ± 0.43*
Diclofenac	20 mg/kg	0.73 ± 0.21	1.24 ± 0.29*	1.35 ± 0.36*	1.62 ± 0.58*

*Statistically significant, $p \leq 0.05$; Data are expressed as Mean ± SD

The analysis of the obtained data for the animal groups treated with BPR showed a statistically significant and dose-dependent anti-inflammatory effect with the reduction of edema progression, at 3 h and 4 h after the induction of inflammation. The results were slightly inferior to diclofenac, a reference non-steroidal anti-inflammatory agent for the group treated with BPR 500 mg/kg.

In the experimental model of carrageenan-induced rat paw oedema, two distinct phases are present. Initially, in the first hour, the inflammatory reaction is caused by histamine, serotonin and bradykinin

release, but later in the time interval of 2 - 4 h, an increased synthesis of prostaglandins in the affected tissues is considered to be the key pathological phenomenon (38, 39). The administration of BPR caused a statistically significant anti-inflammatory effect in the 2 - 4 h time-interval which may suggest that COX inhibition with a reduction of pro-inflammatory prostaglandins could be the main mechanism responsible for this pharmacological activity. Previously published studies proved the antiinflammatory effect of flavonoids like hyperoside which were able to reduce the concentration of pro-inflammatory prostaglandins by inhibiting COX (40). Additionally, acacetin and genkwanin, main constituents in the chemical composition of BPR proved anti-inflammatory effects in prior studies (31, 33). Nevertheless, other chemical constituents could be responsible for the anti-inflammatory effect, hence further research is needed to clarify the molecular aspects of BPR effects.

Analgesic effect

The results of the assessment of analgesic effect of BPR in the experimental Randall Selitto test in rats are presented in Figure 2.

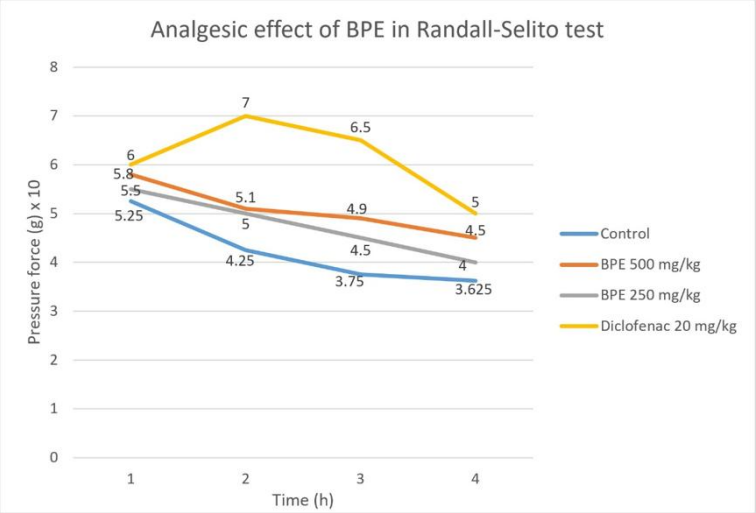

Figure 2.

Analgesic effect of the resin from *B. pendula* (BPR) in Randall-Selito test

The oral administration of BPR at the dose of 500 mg/kg produced a moderate analgesic effect, with a maximal amount of pressure supported by the animals of 51 g, at 2 h, compared to 70 g in case of diclofenac, the reference analgesic drug.

In Randall-Selitto experimental model, the intraplantar administration of a noxious substance triggers the activation of COX2 in the affected tissues with accelerated synthesis of pro-inflammatory prostaglandins which stimulate local nociceptors, causing pain. In this type of experimental model, the administration of BPR caused a moderate analgesic effect, probably by a peripheral mechanism represented by the reduction of prostaglandin synthesis.

The analgesic effect of acacetin, hyperoside and other polyphenolic compounds was proved by previously published studies (32, 39, 40). However, in case of BPR, other chemical constituents could be responsible for the analgesic effect, hence, additional studies are needed to explain the molecular aspects of this effect.

The present study has several limitations. The pharmacological effects tested in laboratory animals were caused by the administration of silver birch bud resin (BPR), a natural product with a complex chemical composition. Moreover, the evaluation of safety profile of the resin was not performed in this study. Therefore, additional research is needed to assess the pharmacological effects and toxicity of the individual chemical constituents present in the tested resin.

Conclusions

Betula pendula Roth. (silver birch) bud resin from Romania showed a rich polyphenolic profile, characterized by high levels of ellagic acid and flavonoids (hyperoside, acacetin and genkwanin). The pharmacological evaluation of the resin proved statistically significant and dose-dependent diuretic, anti-inflammatory and analgesic effects. Further research is needed to elucidate the molecular mechanism of action of the active compounds identified in silver birch bud resin.

The obtained results complement the limited literature data on silver birch bud exudates, given that most current studies are focused on gemmotherapy or propolis.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

All experimental procedures were approved by Ethics Committee of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania and ANSVSA (approval No. 150/2018).

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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