

RESEARCH ARTICLE

Rubus caesius L. leaves: Pharmacognostic analysis and the study of hypoglycemic activity

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Received: December 13, 2016; Accepted: January 24, 2017

ABSTRACT

Background: *Rubus caesius* L. is used in traditional medicine, but pharmacological activity data and standardization methods are lacking. **Aims and Objectives:** This study sought to conduct a pharmacognostic analysis of *R. caesius* L. leaves and assess these leaves' hypoglycemic activity. **Materials and Methods:** External, anatomical, and diagnostic features of the leaves were studied in accordance with the State Pharmacopoeia of the Republic of Belarus T1. Conventional qualitative thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) were used. The hypoglycemic activity of the aqueous extract of the leaves was investigated in an alloxan-induced diabetes model. **Results:** External, anatomical, and diagnostic features of the leaves were determined. The stomata were anomocytic; calcium oxalate crystals were observed in the mesophyll; stellate-arrayed hairs, star-arrayed hairs, capitate hairs, and simple unicellular hairs with a helical fold were identified. Qualitative results indicated that biologically active substances, such as flavonoids and tannins, were present in *R. caesius* L. leaves. HPLC and TLC revealed the presence of hyperoside in these leaves. Soundness indicators were defined: The weight loss on drying the *R. caesius* L. leaves varied from 8.61% to 9.52%, and the average weight loss was 9.1%; determinations of total ash varied from 5.40% to 6.50%; determinations of ash insoluble in hydrochloric acid varied from 0.12% to 0.13%. The hypoglycemic activity of an aqueous extract of *R. caesius* L. leaves in a 500 mg/kg draught was determined in a rat model of alloxan-induced diabetes. **Conclusion:** The data obtained here can be used to develop regulatory documentation for a new type of medicinal plant tissue: *R. caesius* L. leaves.

KEY WORDS: Pharmacognostic; Phytochemical; Hyperoside; Standardization; Hypoglycemic Activity

INTRODUCTION

Medicinal herbal remedies are widely used to treat various pathologies. Plant-based drugs exert fewer negative effects on the body than synthetic drugs; thus, they are better tolerated by patients, have low toxicity, and are significantly

less likely to cause adverse allergic reactions. Developing new pharmaceutical drugs, such as ones based on medicinal plants, are a high priority. The Earth's flora is rich and diverse and represents an inexhaustible source of new medicinal plants, many of which are used in traditional medicine and are not officinal. One such plant is *Rubus caesius* L. in folk medicine, the leaves and fruits of plants of the genus *Rubus* are used in various treatments.^[1-3]

As reported in the literature, plants of the genus *Rubus* contain tannins, flavonoids, and phenol carboxylic acids, which have a wide spectrum of pharmacological activities. Flavonoids and polyphenols tend to accumulate in large quantities, and thus, these plants can serve as a source of

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2017.7.1234224012017	

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these compounds. It should be noted that both the fruits and other parts of a plant (e.g., the leaves) may contain pharmacologically active compounds. Therefore, utilizing these other constituents of the plant can increase the yield of biologically active compounds. Because the harvested quantity of leaves tends to vary less than that of fruits, the additional utilization of leaves can also result in a more constant supply of raw plant material that is less dependent on seasonal variations.^[1,2,4-11]

R. caesius L. is a shrub that can reach heights of 50-150 cm. The shoots are usually of two types: Softwood annuals and vegetative woody perennials. The plant blooms from May to August, and the fruit ripens in June-October. Wild forms are widely distributed in Europe, North America, and Asia, where *R. caesius* L. grows in moist forests, forest ravines, clearings, meadows, and thickets. Bluish blackberry has been cultured in many countries worldwide (e.g., Russia, England, France, Holland, Italy, Japan, the United States, Bulgaria, and others). Gisteva studied the acute toxicity of *R. caesius* L. and concluded that this crop is safe and non-toxic and can be used in medical treatments.^[12,13]

Pharmacognostic studies of the genus *Rubus* have mainly focused on the fruits. This study aims to investigate the pharmacognostic properties of *R. caesius* L., which is widely cultured and available in Russia. After characterizing the chemical compounds extracted from the leaves, we investigated whether the extracts exhibited antidiabetic properties in an alloxan-induced diabetes rat model.^[1,4,7,8,10,14-16]

MATERIALS AND METHODS

Experimental

Study of external, anatomical and diagnostic features of R. caesius L. bluish blackberry leaves

The leaves from wild-growing *R. caesius* L. plants were harvested in late spring and summer of 2012 (May-September) from the Vitebsk region of Belarus. The botanical determination of the leaves was conducted at the Institute of Pharmacognosy of the Medical University of Vitebsk to confirm the identities of the plants. The raw material was dried at room temperature in a well-ventilated area out of direct sunlight using the air shadow method.^[2,3]

The anatomical and diagnostic features were examined using a 295 Leica microscope (Germany) with a magnification of $\times 40/0.65$.

When studying the external characteristics, attention was paid to the shape and hairiness of the leaves and the characters of the margins and venation. The length and width of the leaf blade were also measured.

The leaf blade margin and vein (upper and lower sides) were removed and placed in a vial. Then, a 25 g/L solution of sodium hydroxide was added, and the mixture was boiled for 2 min to determine the anatomical and diagnostic features. Then, the contents were poured into a Petri dish. The original liquid was poured out, and the working material was rewetted. The pieces of working material were then placed on a slide in a drop of chloral hydrate, covered with a coverslip, and observed under a Leica 295 microscope (Germany) with a magnification of $\times 40/0.65$.^[17]

Chemical Characterization

Qualitative chemical analysis

The flavonoid content in the aqueous extract was qualitatively analyzed by complexation with aluminum chloride. Two drops of aluminum chloride were added to 1 mL of the aqueous extract. This solution turned yellow when flavonoids were present.^[2,3,18]

Qualitative determination of biologically active compounds in the bluish blackberry leaves was performed using conventional qualitative reactions, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC).^[2,3,9,18,19]

TLC

About 1 g of crushed *R. caesius* L. leaves was added to 50 ml of 60% ethanol, and the solution was heated in a steam bath under reflux for 30 min to prepare the test solution. The substance was cooled and filtered through filter paper. The reference solution was prepared by dissolving 0.025 g (accurately weighed) of hyperoside in 96% ethanol.

About 30 μ l of the alcoholic extract and 5 μ l of the reference solution were added to a TLC system (Merck, Germany) that used cellulose plates as the stationary phase. A solution containing 2-propanolol, anhydrous formic acid, and water (2:5:5) was utilized as the mobile phase. The system was dried for 10 min at 100°C and cooled at room temperature. A solution of 20 g of aluminum chloride in 96% ethanol was sprayed onto the chromatogram. The cellulose plate was then subjected to 365 nm ultraviolet light, and the fluorescence was recorded.^[2,3,8,12,16,20,21]

HPLC

This analysis was performed on an Agilent HP 1100 liquid chromatograph with a G1311A quaternary pump system. This system employs a G1315V column oven and is connected to a G1315V diode-array detector. Data collection and the processing of chromatograms and absorption spectra were performed using the Agilent ChemStation for LC 3D. A Zorbax Eclipse XDS C-18 column 150 mm $\times$ 4.6 mm i.d. With a 5 μ m particle size (Agilent) was used as the stationary phase. The column was

maintained at 30°C. A potassium dihydrogen phosphate solution (pH = 3.0 ± 0.2) consisting of phosphoric acid and acetonitrile (80:20) (Agilent) was employed as the mobile phase. The mobile phase velocity was 1.0 ml/min, and the injected sample volume was 10 µl. The sample was detected in the ultraviolet range at a wavelength of 340 nm. The various solute compounds were identified by comparison of the retention times and absorption spectra with those obtained from a reference library constructed with different known standards.^[2,3,20,21]

Defining the Purity Indicators

The weight loss upon drying, total ash content, and hydrochloric acid-insoluble ash content were determined using the procedures described in the State Pharmacopoeia of the Republic of Belarus.^[2,3]

Assessments of the Hypoglycaemic Activity of *R. caesius* L. Leaves: Laboratory Animals and Treatments

Crushed *R. caesius* L. leaves were extracted in water according to the Belarus Pharmacopoeia to investigate the antidiabetic activity of *R. caesius* L. leaf extracts in an alloxan-induced diabetes rat model. For this experiment, wild-growing young leaves harvested in Vitebsk from May to September 2012 were infused in pure water at 100°C for 15 min. The infusion was filtered after cooling at room temperature for 45 min.^[17]

The leaves were then crushed and extracted in water and ethanol. Water extraction was performed by sifting 2.0 g of accurately weighed crushed leaves through a sieve with a pore size of 2 mm. The crushed leaves were stirred in 250 ml of boiling water for 30 min. The liquid was cooled to room temperature and filtered through cotton wool; then, the particles of the raw extract of 1 g of accurately weighed crushed leaves were added to 50 ml of 60% ethanol. The flask was attached to a reflux condenser and heated in a steam bath for 30 min with periodic shaking to wash the raw material particles from the flask walls. The extract was centrifuged for 5 min at 3,000 rpm, and the supernatant was collected.^[2,3]

The aqueous extract was then administered to rats daily via intragastric gavage for 10 days. Adult male rats, each weighing 200-250 g, were used and maintained as required by state regulations (approved by the order of the Rector of VSMU: No. 55-N from 09.09.2001; No. 65-NIR from 10.06.2009; No. 13-L from 04.03.2010; No. 31-NIR from 24.02.2011; and No. 67-NIR from 27.04.2011). The animals were housed in one-tier cells with a front wire mesh wall and were provided a drinker with fresh, sterilized water. The room temperature was set at 20-22°C, and the humidity was set to F 45-50%. The animals were subjected to natural lighting and all received a standard vivarium diet.^[5,9,20,22,23]

To the alloxan-induced diabetes model rats, 1 ml of a 5% alloxan monohydrate solution prepared in citrate

buffer, pH 4.5, corresponding to a dose of 125 mg/kg was administered by gavage once at the beginning of the experiment. This compound impairs the β-cells of the pancreatic islets, selectively leading to the development of diabetes. 7 days after the alloxan injection, the different treatment schedules were initiated and continued for 10 days in the control and intervention groups.^[20,22-24]

The rats were separated into four different groups, each consisting of 6 animals. Table 1 presents information on the absence/presence of diabetes and the detailed treatment schedules for the different groups. Treatment was initiated after an induction period, which lasted for 7 days after the alloxan injection. The rats in Group A were housed for the same duration without the administration of alloxan before the placebo treatment began.^[22,23]

Blood Sampling and Glucose Measurements

Fasting venous blood samples were collected from the tail vein and used to determine the glucose concentrations. The glucose concentrations were measured before beginning the experiment. Only rats with a baseline glucose level of 3.5-6.5 mmol/l were allowed to participate in the study. The rats in Groups B, C, and D were intraperitoneally injected once with 1 ml of a 5% alloxan monohydrate solution, corresponding to a dose of 125 mg/kg.^[20,22,23]

After the 7-day induction period, baseline samples were collected to measure the blood glucose levels immediately before the treatment protocol began. Subsequently, additional measurements were performed after 0, 3, 6 and 10 days of treatment to capture the potential treatment effect. Whole blood was immediately analyzed after drawing being drawn using a Satellite plus portable glucose express meter and test strips (Satellite, Russia).

Table 1: Glycemic status and treatment schedules for the different groups. In diabetic rats, alloxan diabetes was induced by intraperitoneally injecting 1 ml of 5% alloxan at the beginning of the study

Group	Glycaemic status	Treatment
A	Nondiabetic	1 ml distilled water (intraperitoneally) per day for 10 days
B	Diabetic	1 ml of a 5% solution of alloxan monohydrate at a dose of 125 mg/kg
C	Diabetic	Alloxan monohydrate+glibenclamide (10 mg/kg). Glibenclamide was injected (10 mg/kg) throughout the experiment (10 days)
D	Diabetic	Alloxan monohydrate+aqueous extract of <i>Rubus caesius</i> L. Leaves (500 mg/kg) The aqueous extract of <i>Rubus caesius</i> L. Leaves was injected (500 mg/kg) throughout the experiment (10 days)

Statistical Methods

Qualitative and quantitative descriptions of the data are provided. The glucose concentrations during the treatment phase were compared using the Friedman repeated measures on ranks test. The glucose concentrations among groups with different treatment schemes were compared using the Mann-Whitney U test for comparisons of two groups or the Kruskal-Wallis test for comparisons of three groups. When significant differences were observed among three or more groups, pairwise *post-hoc* testing was performed via the Conover method. Microsoft Excel (Microsoft, USA) and Medcalc (Medcalc software, Belgium) were employed. $P < 0.05$ were considered significant.

RESULTS

External Characteristics of *R. caesius* L. Leaves

The leaves are ternate-compound, and the terminal leaflet is occasionally tripartite. The leaves are greenish-grey, with a slightly white lower surface because of the thin trichomes. The leaves have sparse hair on the top side and unevenly incised-toothed margins; the terminal leaflet is ovate-rhombic on short stalks, and the sides are sessile. The venation consists of a pinnate-net. The leaf length and width are 7.76 ± 1.5 cm and 5.67 ± 2.3 cm, respectively.

Anatomical and Diagnostic Features of Bluish Blackberry Leaves

In the integral raw material, the meandering upper epidermis cells are visible. The stomata are oval and mostly surrounded by 5-6 cell-companions (anomocytic type); many more such structures exist on the lower surface. Numerous hairs are present on both surfaces of the leaves, with more hairs existing on the lower surface. The presence of several types of hair is typical, including simple, single-celled with a spiral fold, simple capitate hairs, and star-rayed hairs.

Many calcium oxalate druses are present in the leaf mesophyll.

As shown in Figures 1-4, the basic microscopic diagnostic features of *R. caesius* L. leaves are the presence of simple single-celled hairs with a spiral fold, star-arrayed hairs, capitate hairs, calcium oxalate crystals in the mesophyll, and anomocytic stomata.

Substance Characterization

The results of the qualitative chemical analysis of the alcoholic and aqueous extracts are shown in Table 2. Flavonoids and tannins were detected in both extracts. The TLC results of the alcoholic extract are shown in Figure 5 and reveal 6 spots under ultraviolet light. Three spots have

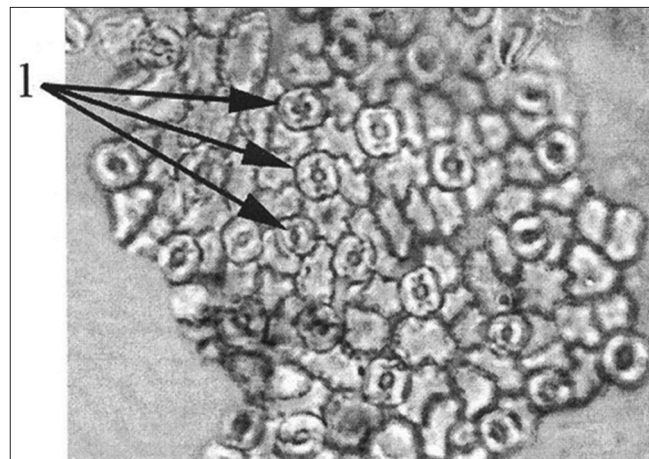


Figure 1: Epidermal cells. (1) The stomata of anomocytic type

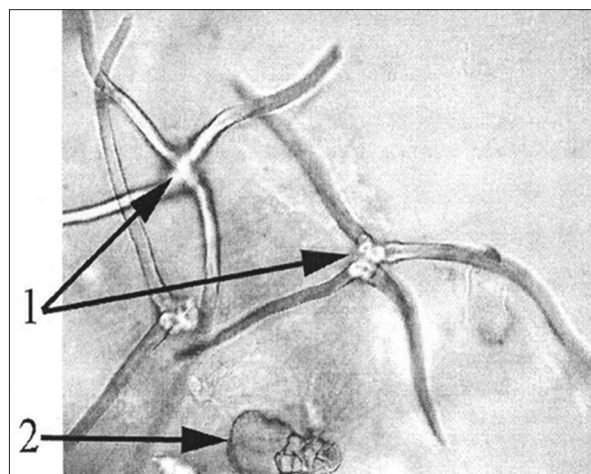


Figure 2: Types of hairs: (1) Star-rayed hairs; (2) capitate hair

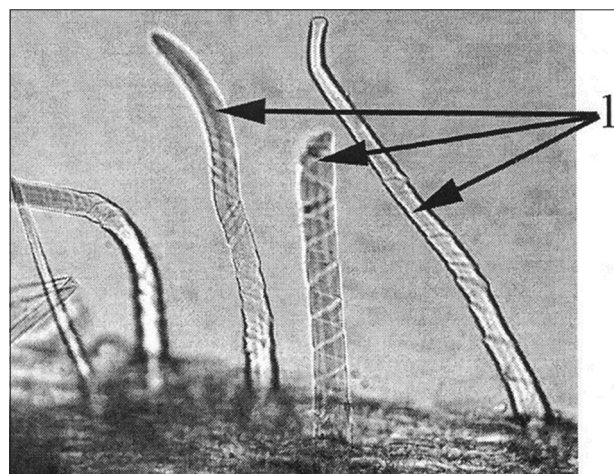


Figure 3: Simple single-celled hairs with helical fold

a yellow color and represent flavonoids. Comparing spot 4 from the extract to spot 6 from the reference solution indicates that spot 4 is consistent with hyperoside. Spots 2 and 3 could not be characterized by comparison to the reference solution. Based on their positions and colors, spot 2 likely represents the flavonoid rutin (diglucoside-quercetin), and spot 3 probably corresponds to the flavonoid

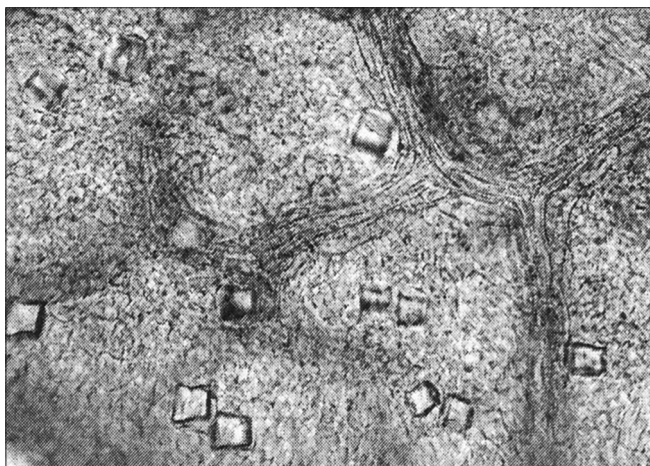


Figure 4: Calcium oxalate crystals in the mesophyll

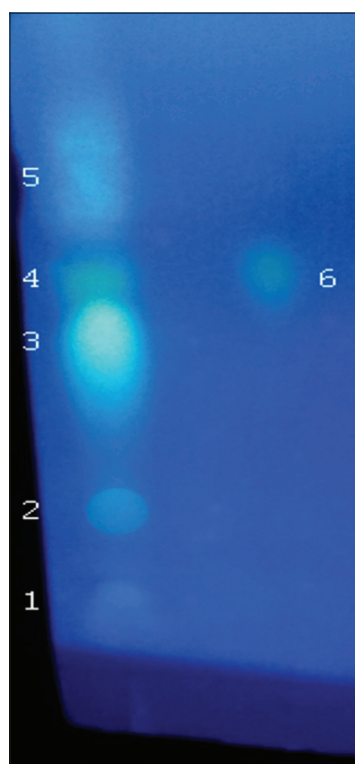


Figure 5: Thin layer chromatography of an alcoholic extract of *L. Rubus caesius* leaves. A reference solution containing hyperoside (spot 6, yellow) was applied on the right side of the gel, whereas the extract displaying spots 1-5 (1=purple; 2=yellow; 3=lemon yellow; 4= yellow; 5=blue) was applied to the left side of the gel. Discussion of results in the text. Spot 6 (yellow) is a standard probe of hyperoside. It matches spot 4 in the tested extract

kaempferol (isoquercitrin-glycoside). The characterization of spots 2 and 3 can be inferred from literature data, which attribute yellow color and shorter migration to quercetin-diglucoside and lemon-yellow color and longer migration to isoquercitrin-glycoside. Isoquercitrin-glycoside exhibits a shorter migration than monosides, such as hyperoside. The other spots are purple (spot 1) and blue (spot 5) and correspond to phenolic acids; no further specification is possible via TLC.^[2,3,8,10,16,17,21]

Identification of Biologically Active Substances in *R. caesius* L. Leaves using TLC

TLC of an alcoholic extract of *R. caesius* L. Leaves. A reference solution containing hyperoside (spot 6, yellow) was applied on the right side of the gel, whereas the extract containing spots 1-5 (1 = purple, 2 = yellow, 3 = lemon yellow, 4 = yellow, and 5 = blue) was applied to the left side of the gel. The results are discussed in the text. Spot 6 (yellow) is a standard probe for hyperoside and matches spot 4 in the tested extract.

Identification of Biologically Active Substances in *R. caesius* L. Leaves using HPLC

Phenolic compounds in *R. caesius* L. leaves were analyzed by HPLC using a mobile phase consisting of 0.01 M potassium dihydrogen phosphate and acetonitrile (80:20). The peak with a retention time of 9.9 min corresponds to hyperoside.

The chromatogram of the phenolic compounds contained in the *R. caesius* L. leaves was obtained at 280 nm.

Soundness indicators

The composition of the blue-grey *R. caesius* L. leaves, as determined using qualitative reactions, is presented in Tables 3-5.

As indicated in Table 3, the weight loss on drying the *R. caesius* L. leaves varied from 8.61 to 9.52%, and the average weight loss was 9.1%.

Hypoglycemic Activity of *R. caesius* L. Leaves

The baseline glucose concentrations of the three groups of diabetic rats were not significantly different ($P = 0.69$). It treatment with the biologically active substances clearly resulted in a highly significant effect on the glucose concentrations in both Groups C and D ($P < 0.001$). In both groups, this effect was progressive until day 10. Within these groups, *post-hoc* testing revealed that the differences observed on a specific day were significantly different from those on the other 3 days ($P < 0.05$). The glucose concentrations did not differ significantly between Groups C and D on days 6 ($P = 0.52$) and 10 ($P = 0.11$). However, glibenclamide exerted more prominent and significant treatment effect on day 3 ($P = 0.04$).

In Groups A, C and D, the animals were calm and exhibited normal urination with no acetone smell. None of the animals died during the experiment. In contrast, the animals in Group B were very active and aggressive and exhibited frequent urination with the smell of acetone.

After observing the experimental animals, the following results were obtained:

In Group A, the animals were calm and exhibited normal urination with no smell of acetone and a slight increase in their body weights (5-7 g).

In Group B, the animals were very active and aggressive and exhibited frequent urination with the smell of acetone and a significant decrease in their body weights (20-25 g).

In Group C, the animals were calm and exhibited normal urination with no smell of acetone and a slight increase in their body weights (5-7 g).

In Group D, the animals were calm and exhibited normal urination with no smell of acetone and a slight decrease in their body weights (5-7 g).

External diagnostic features of *R. caesius* L. leaves were established: The leaves are ternate-compound, and the terminal leaflet is occasionally tripartite. The leaves are greenish-gray, and their lower surface is slightly white because of the thin trichomes. The leaves have sparse hairs on the top side and unevenly incised-toothed margins; the terminal leaflet is ovate-rhombic on short stalks, and the sides are sessile. The venation consists of a pinnate-net (Table 6).

DISCUSSION

Thus, *R. caesius* L. leaves may be used as follows: (1) To determine their authenticity and distinguish such leaves from contaminants from other similar plants of the *Rubus* genus, (2) to develop regulatory documentation for *R. caesius* L. leaves, a new type of medicinal plant tissue, (3) to treat diabetes and thereby reduce the frequency of synthetic drug use, and (4) to generate substances that may be used to create synthetic drugs and new drugs of plant origin (such as filter bags, granules, and tablets).

Further experiments will focus on determining the optimal dosage, identifying markers for the detection of other biologically active substances in bluish blackberry leaves, and compiling the collection.

To the best of our knowledge, our work is the first to describe a hypoglycemic effect of *R. caesius* L. extracts on diabetic rats. Similar to the study by Jouad et al. In *Rubus fruticosus*, we investigated leaf extracts for their potential hypoglycemic activity. Jouad et al. found that extracts of *R. fruticosus* leaves reduced the glucose levels in normal and diabetic rats. Sharma and Kumar manufactured extracts from *Rubus ellipticus* fruits and reported significant reductions in the glucose levels of diabetic rats on administration of aqueous solutions of alcohol, water, and petroleum ether extracts. Kanegusuku et al. reported that methanolic extracts of *Rubus imperialis* exert a hypoglycemic effect on normoglycemic rats. Taken together, these results indicate that *Rubus* species fruit and leaf extracts exhibit antidiabetic activities.

Table 2: Qualitative chemical determinations of biologically active substances in alcoholic and aqueous extracts of *Rubus caesius* L. leaves

Substances	Alcoholic extract	Aqueous extract
Coumarins	-	-
Flavonoids	+	+
Tanning agents	+	+
Anthracene derivatives	-	-
Alkaloids	-	-

+: Substances present, -: Substances absent

Table 3: Weight loss on drying (%)

Sample	W ₁ %	W ₂ %	W ₃ %	W _{avg} %
1	8.85	8.61	8.79	8.75
2	9.15	9.27	9.52	9.31
3	9.17	9.24	9.28	9.23
4	9.02	9.11	9.09	9.07

Table 4: Determinations of total ash (%)

Sample	X ₁ %	X ₂ %	X ₃ %	X _{avg} %
1	6.57	6.46	6.46	6.50
2	6.31	6.00	6.11	6.14
3	5.26	5.26	5.22	5.25
4	5.34	5.42	5.44	5.40

Table 5: Determinations of ash insoluble in hydrochloric acid (%)

Sample	X ₁ %	X ₂ %	X ₃ %	X _{avg} %
1	0.11	0.11	0.13	0.12
2	0.14	0.16	0.12	0.12
3	0.13	0.15	0.10	0.13
4	0.11	0.12	0.12	0.12

Table 6: Hypoglycemic activity of the aqueous extract of *Rubus caesius* L. leaves at a dose of 500 mg/kg in a rat model of alloxan-induced diabetes

Group	The glucose level in blood, mmol/l			
	Day			
	Onset	3	6	10
A. Normal control	4.3±0.5	4.9±0.4	4.7±0.6	4.4±0.3
B. Diabetic control	15.2±1.5	15.8±1.1	16.4±1.4	17.6±1.3
C. Standard	14.8±1.2	10.5±1.4*	8.8±1.2*	6.9±0.7*
D. Aqueous extract	15.3±1.3	11.8±0.9	9.2±1.5*	7.6±0.8*

*Differences are significant with respect to the alloxan control group of animals, based on a Mann-Whitney test ($P < 0.05$)

The absolute mean reduction in the glucose levels in normal rats was approximately 0.7 mmol/L after 15 days of treatment, as reported by Kanegusuku et al. and approximately 1.0 mmol/L after 9 days of treatment according to Jouad

et al. Indeed, Jouad et al. reported a decrease in the mean blood glucose levels in diabetic rats from approximately 18 mmol/L to approximately 5.8 mmol/L after 9 days of treatment, whereas Sharma and Kumar Indicated a mean decrease from 14.35 mmol/l to 5.3 mmol/l after 10 days of treatment with an aqueous *R. ellipticus* extract. Our findings were somewhat less pronounced. However, they were comparable to the effect exerted by a frequently used antidiabetic agent, glibenclamide. The antidiabetic effect observed here occurred after 6 days of extract administration, and in other studies, this effect became apparent after 3-9 days. Regarding the timing and amount of the decreases, the glucose concentrations observed on the administration of the *Rubus* species extracts in our study are comparable to those reported in other studies.^[11,24,25]

Other studies proposed mechanisms for the antidiabetic activity exerted by *Rubus* species extracts. Jouad et al. measured insulin levels on the administration of *R. fruticosus* extract and showed that insulin secretion was not affected in normal or diabetic mice. Thus, they concluded that at the employed doses, the *Rubus* species extract exerts its effects via extrapancreatic mechanisms. Investigations of extracts from plants other than *Rubus* species suggested that hypoglycemic activity may be mediated by the inhibition of corticoid secretion. Our study did not investigate the mechanisms by which the *R. caesius* L. extract exerted its anti-diabetic effect. Accordingly, we cannot speculate whether the identified substances are responsible.^[22,26,27]

The application of a biologically active substance to an organism always raises the question of toxicity. Gisteva investigated the acute toxicity of *R. caesius* L. and concluded that this crop is safe and non-toxic class and can be used medicinally. The toxicities of other *Rubus* species extracts were determined in terms of their LD50 values. In mice receiving an aqueous extract of *R. fruticosus* L. leaves, the LD50 was 8.1 g/kg body weight, and doses of up to 6 g/kg body weight were well tolerated. Sharma and Kumar observed no behavioral changes or mortality at doses up to an LD50 of 2 g/kg body weight. Our study applied a daily dose of 0.5 g/kg body weight. At this dose, we observed no gross behavioral changes in the investigated rats. This observation is in accord with the results of the toxicity studies by other groups and suggests that *R. caesius* L. leaves may be safely used at higher doses than those employed in this work, which could increase the treatment effect while minimizing the side effects.^[11,23,25]

There are several limitations of our study. First, we were unable to perform a mass spectrometric analysis to achieve a more precise characterization of the extract components. However, we were able to identify several biologically active compounds that are also found in extracts from other plants. Second, we did not perform a dose-effect escalation in the rat model, instead using only a fixed dose of 0.5 g/kg

body weight. We assume that higher doses would not lead to toxicity and may increase the hypoglycemic effect. Third, we did not assess the influence of the harvesting season on the hypoglycemic effects. Kellogg et al. reported the seasonal dependencies of bioactivity in plants in Alaska. The extent to which seasonal variability will modulate the hypoglycemic effects of *R. caesius* L. leaf extracts was not determined. Finally, whether our findings in this alloxan-induced diabetes rat model can be extrapolated to human beings suffering from diabetes remains unknown. In summary, however, we believe that these limitations do not invalidate our findings.^[15,21,28]

CONCLUSION

In conclusion, this study characterized, for the first time, the chemical composition of *R. caesius* L. leaves. Furthermore, a considerable hypoglycemic effect of the aqueous extract was observed in an established alloxan-induced diabetes rat model. A topic that could be addressed in future research is whether the identified substances are responsible for the observed antidiabetic effect.

REFERENCES

1. Gao J, Sun CR, Yang JH, Shi JM, Du YG, Zhang YY, et al. Evaluation of the hepatoprotective and antioxidant activities of *Rubus parvifolius* L. J Zhejiang Univ Sci B. 2011;12(2):135-2.
2. Godovalnikova GV. General methods for quality control of medicines. Tom. 1. State Pharmacopoeia of the Republic of Belarus. Minsk: Min. Reg. PTC Printing; 2006.
3. Sheryakova AA. Quality control of auxiliaries and drugs. Grow. Tom. 2. State Pharmacopoeia of the Republic of Belarus. Maladziechna: Pobeda; 2008.
4. Ferreira de Araujo PR, da Silva Santos V, Rodrigues Machado A, Gevehr Fernandes C, Silva JA, da Silva Rodrigues R. Benefits of blackberry nectar (*Rubus* spp.) Relative to hypercholesterolemia and lipid peroxidation. Nutr Hosp. 2011;26(5):984-90.
5. Ding M, Feng R, Wang SY, Bowman L, Lu Y, Qian Y, et al. Cyanidin-3-glucoside, a natural product derived from blackberry, exhibits chemopreventive and chemotherapeutic activity. J Biol Chem. 2006;281(25):17359-68.
6. Ni W, Zhang X, Bi H, Iteku J, Ji L, Sun C, et al. Preparation of a glucan from the roots of *Rubus crataegifolius* bge and its immunological activity. Carbohydr Res. 2009;344(18):2512-8.
7. Noda Y, Kaneyuki T, Mori A, Packer L. Antioxidant activities of pomegranate fruit extract and its anthocyanidins: Delphinidin, cyanidin, and pelargonidin. J Agric Food Chem. 2002;50(1):166-71.
8. Yuan W, Jiuming H, Hui C, Disheng Z, Hua C, Huibo S. Analysis of flavones in *Rubus parvifolius* Linn by high performance liquid chromatography combined with electrospray ionization-mass spectrometry and thin-layer chromatography combined with Fourier transform surface enhanced Raman spectroscopy. Chin J Anal Chem. 2006;34(8):1073-7.
9. Muzafarova MH. Pharmacology of Antidiabetic Collection. Dushanbe: DisserCat; 2012.

10. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr*. 2005;45(4):287-306.
11. Sharma U, Kumar A. Anti-diabetic effect of *Rubus ellipticus* fruits extracts in alloxan-induced diabetic rats. *J Diabetol*. 2011;2(4):1-6.
12. Medić-Šarić M, Jasprica I, Smolčić-Bubalo A, Mornar A. Optimization of chromatographic conditions in thin layer chromatography of flavonoids and phenolic acids. *Croat Chem Acta*. 2004;77(1-2):361-6.
13. Gisceva OA. Pharmacological Study of *Rubus caesius* L. Shoots and Creation on Their Basis Medicinal Herbs Collection. M.Sc. Thesis. Kharkov.
14. Bhandary B, Lee GH, Marahatta A, Lee HY, Kim SY, So BO, et al. Water extracts of immature *Rubus coreanus* regulate lipid metabolism in liver cells. *Biol Pharm Bull*. 2012;35(4):1907-13.
15. Kellogg J, Wang J, Flint C, Ribnicky D, Kuhn P, De Mejia EG, et al. Alaskan wild berry resources and human health under the cloud of climate change. *J Agric Food Chem*. 2010;58(7):3884-900.
16. Wang SY, Lin HS. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J Agric Food Chem*. 2000;48(2):140-6.
17. Godovalnikova GV, editor. State pharmacopoeia of the Republic of Belarus. Under total. General Methods for Quality Control of Medicines. Vol. 1. Minsk: Min. Reg. PTC Printing; 2006.
18. Gudej J, Tomczyk M. Determination of flavonoids, tannins and ellagic acid in leaves from *Rubus* L. species. *Arch Pharm Res*. 2004;27(11):1114-9.
19. Jennings DL, Carmichael E. Anthocyanin variation in the genus *Rubus*. *New Phytol*. 1980;84(3):505-13.
20. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*. 2001;50(6):537-46.
21. Vasco C, Riihinen K, Ruales J, Kamal-Eldin A. Phenolic compounds in *Rosaceae* fruits from Ecuador. *J Agric Food Chem*. 2009;57(4):1204-12.
22. Jouad H, Maghrani M, Eddouks M. Hypoglycaemic effect of *Rubus fruticosus* L. and *Globularia alypum* L. In normal and streptozotocin-induced diabetic rats. *J Ethnopharmacol*. 2002;81(3):351-6.
23. Seeram NP, Adams LS, Zhang Y, Lee R, Sand D, Scheuller HS, et al. Bluish blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells *in vitro*. *J Agric Food Chem*. 2006;54(25):9329-39.
24. Kanegusuku M, Benassi JC, Pedrosa RC, Yunes RA, Filho VC, Maia AA, et al. Cytotoxic, hypoglycemic activity and phytochemical analysis of *Rubus imperialis* (*Rosaceae*). *Z Naturforsch C*. 2002;57(3-4):272-6.
25. Kaume L, Howard LR, Devareddy L. The blackberry fruit: A review on its composition and chemistry, metabolism and bioavailability, and health benefits. *J Agric Food Chem*. 2012;60(23):5716-27.
26. Gholap S, Kar A. Effects of *Inula racemosa* root and *Gymnema sylvestre* leaf extracts in the regulation of corticosteroid induced diabetes mellitus: Involvement of thyroid hormones. *Pharmazie*. 2003;58(6):413-5.
27. Gholap S, Kar A. Hypoglycaemic effects of some plant extracts are possibly mediated through inhibition in corticosteroid concentration. *Pharmazie*. 2004;59(11):876-8.
28. Jain DK, Arya RK. Anomalies in alloxan-induced diabetic model: It is better to standardize it first. *Indian J Pharmacol*. 2011;43(1):91.

How to cite this article: Schädler V, Dergatschewa S. *Rubus caesius* L. leaves: Pharmacognostic analysis and the study of hypoglycemic activity. *Natl J Physiol Pharm Pharmacol* 2017;7(5):501-508.

Source of Support: Nil, **Conflict of Interest:** None declared.