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THE STUDY OF THE CHEMICAL COMPOSITION AND THE PHARMACOLOGICAL ACTIVITY OF THE POLYSACCHARIDE COMPLEX OBTAINED FROM *LEDUM PALUSTRE*

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Key words: Ericaceae; Labrador tea shoots; polysaccharide complex; anti-inflammatory activity; antimicrobial activity

*Ledum palustre shoots (Cormus Ledi palustri) have long been used as an antispasmodic, diuretic, diaphoretic, disinfectant, anti-inflammatory, sedative and antitussive remedy in folk medicine. Terpene compounds are the most studied, they are a component of essential oil, and «Ledin» medicine with the antitussive effect has been developed on their basis. At the same time the polysaccharide complex (PSC) of this plant has not been practically studied. The aim of our research was to study the polysaccharide complex obtained from *Ledum palustre* shoots and determine its pharmacological activity. PSC was prepared by adding 96% ethyl alcohol to the concentrated aqueous extract of the plant raw material. The yield was $2.60 \pm 0.07\%$ calculated with reference to the absolutely dried raw material. The dynamic of PSC yield was studied. D-glucose, D-galactose, L-rhamnose and L-arabinose were identified in the hydrolyzate of PSC. The amount of monosaccharides in PSC after hydrolysis calculated with reference to glucose was $43.25 \pm 0.80\%$. The anti-inflammatory activity of PSC was studied on the model of carrageenan-induced paw edema in rats. PSC showed the highest anti-inflammatory activity in the dose of 50 mg/kg, and edema decreased by 84% compared to the control. The study of the antibacterial activity of PSC was performed using the agar diffusion method. PSC showed the antimicrobial activity against all microorganisms, except *Candida albicans*. The maximum growth inhibition was observed against *Escherichia coli* – 36 mm. The studies conducted indicate the prospects of the substance and confirm the possibility of creating new drugs with the anti-inflammatory and antimicrobial activity on its basis.*

Herbal medicines have more advantages than their synthetic analogues. Among them there is low toxicity, a gradual achievement of the pharmacological effect, and the complex action. Thus, isolation and study of biologically active substances from plants are of great current interest in modern pharmacy.

One of the promising plant raw material is *Ledum palustre* shoots (*Cormus Ledi palustri*), which have long been used as an antispasmodic, diuretic, diaphoretic, disinfectant, anti-inflammatory, sedative and antitussive remedy in folk medicine. The wide spectrum of the biological activity is due to the presence of terpene compounds, flavonoids, tannins, organic acids, polysaccharides and other substances in the plant raw material [6].

Terpene compounds are the most studied, they are a component of essential oil, and «Ledin» medicine with the antitussive effect has been developed on their basis. At the same time the polysaccharide complex (PSC) of this plant has not been practically studied [2, 6].

Therefore, the aim of our research was to study the polysaccharide complex obtained from *Ledum palustre* shoots and determine its pharmacological activity.

Materials and Methods

The object of our study was PSC obtained from *Ledum palustre* shoots. To prepare PSC 200 g of the powdered raw material with the particle size less than 2 mm

was placed in a 2 L volumetric flask with a thin section, 1000 mL of water was added, and the flask was heated on water bath for 30 min at reflux. Extraction was repeated three times with a new portion of the extractant. Water extracts were collected separately in containers, centrifuged, decanted in 1000 ml volumetric flasks and diluted with water to the volume. After that 25 ml of each water extracts was taken for analysis. The extracts were combined and evaporated to the volume of 100 ml in a vacuum-circulation apparatus at 100°C. To the concentrated solution 300 mL of ethyl alcohol was added. The precipitate of polysaccharides was formed; it was allowed to stand for one hour, centrifuged, washed with 30 mL of 96% ethyl alcohol and dried at room temperature to dryness [1, 4].

The phytochemical study of PSC was carried out by paper chromatography, gravimetry and spectrophotometry.

The dynamics of PSC extraction was determined by the gravimetric method. For this purpose 20 ml of the first, second and third extracts were placed in centrifuge tubes, 60 ml of 95% ethanol was added in each tube, stirred and heated on the water bath at 30°C for 5 min. In one hour the contents of the tubes were centrifuged with the rotation speed of 5000 rpm for 30 min. Supernatants were filtered under vacuum at 13-16 kPa through POR-16 glass filters with the diameter of 40 mm, dried

to the constant weight at a temperature of 100-105°C. Precipitates were quantitatively transferred to filters and successively washed with 15 mL of the mixture of 95% ethanol: water (3:1) and 10 mL of ethyl acetate. Filters with precipitates were dried first on the air and then at 100-105°C to the constant weight [4, 6]. The yield of polysaccharides calculated with reference to the absolutely dried raw material was calculated by the formula in percentage (X):

$$X = \frac{(m_2 - m_1) \cdot 1000 \cdot 100 \cdot 100}{m \cdot 20 \cdot (100 - w)},$$

where: m_1 – is the weight of the filter, g; m_2 – is the weight of the filter with the precipitate, g; m – is the weight of the raw material, g; w – is the loss on drying, %.

The qualitative composition of monosaccharides in PSC was determined by the paper chromatography method after acid hydrolysis.

In a flask with a thin section 0.2 g of PSC was dissolved in the minimum volume of water (5 mL), the same volume of 20% sulphuric acid was added and hydrolyzed by heating on a water bath for 2.5 h. The hydrolyzate was neutralized with barium carbonate to the neutral reaction with the universal indicator, the solution was filtered. The filtrate was evaporated under vacuum to dryness, which was dissolved in 0.5 mL of ethanol. The resulting solution was applied on chromatographic paper and chromatographed in the solvent system of *n*-butanol-cetic acid-water (4:1:2) by the top-down method in the presence of reference solutions of monosaccharides. After chromatography the chromatogram was air dried, treated with anilinphthalate reagent and heated in a drying cabinet for 10 min at 100-105°C [3].

The total amount of monosaccharides after hydrolysis was determined by spectrophotometry calculated with reference to glucose.

0.1 g of PSC was placed into a 10 ml volumetric flask, dissolved in water by heating to 30°C, diluted to the volume. Then 4.0 ml of the resulting solution was placed into a 50 ml round-bottomed flask, 6 ml of purified water and 10.0 mL of diluted sulphuric acid were added and heated for 2.5 h at reflux. The solution was cooled and quantitatively transferred with purified water into a 25.0 mL volumetric flask, diluted to the volume with the same solvent and mixed [3]. Initially 5 ml of the solution was neutralized by the universal indicator paper with 30% sodium hydroxide solution and diluted sulphuric acid [3].

The neutralized solution was filtered through a paper filter, quantitatively transferred to a 25.0 mL volumetric flask, diluted to the volume with water and mixed. Then 1.0 ml of the solution obtained was placed into a 25.0 mL volumetric flask and 1.0 mL of 1% picric acid, 3.0 ml of 20% sodium carbonate were added and heated at 100°C for 20 min. After cooling the volume was diluted with water and mixed. Simultaneously, under the same conditions, the experiment with 2.0 ml of glucose standard sample was conducted [3].

The optical density of the test solution and the solution of glucose standard sample (SS) were measured at the wavelength of 463 nm in the cell with the layer thickness of 10 mm on a Specol 1500 spectrophotometer (Switzerland). The mixture of 1.0 mL of 1% picric acid solution, 3 ml 20% sodium carbonate solution and 1.0 ml of water was used as a blank solution [3].

The total content of monosaccharides in the polysaccharide complex calculated with reference to glucose was calculated by the formula (X):

$$X = \frac{D_1 \cdot a_0 \cdot 10 \cdot 25 \cdot 25 \cdot 4 \cdot 5 \cdot 1 \cdot 100 \cdot 100}{D_0 \cdot a_1 \cdot 4 \cdot 5 \cdot 1 \cdot 50 \cdot 25 \cdot (100 - w)},$$

where: D_1 – is the optical density of the test solution; D_0 – is the optical density of glucose SS; a_1 – is the weight of PSC, g; a_0 – is the weight of glucose SS, g; w – is the loss on drying, %.

Note: *Preparation of the solution of glucose standard sample (SS)*. Approximately 0.02 g (accurate weight) of glucose (PhS 42-2419-86) dried at 105°C to the constant weight was placed in a 50 ml volumetric flask, dissolved in water, diluted with water to the volume and mixed. The expiration date of the resulting solution was 3 days [3].

The anti-inflammatory and antimicrobial activities of PSC from *Ledum palustre* were analyzed. The anti-inflammatory activity of PSC was studied on the model of carrageenan-induced paw edema in rats at the Pharmacotherapy Department of the NUPh under supervision of Prof. I.V.Kireyev.

The experiment consisted of two stages. At the first stage the screening in the doses of 10 mg/kg, 20 mg/kg, 50 mg/kg, and 100 mg/kg was carried out. It was found that the anti-inflammatory activity was the highest in the doses of 50 mg/kg and 100 mg/kg; therefore, further studies were conducted exactly in these doses.

At the second stage the study was conducted using the control and the reference drug. Before the experiment the initial volume of the rat's paw was measured. Then 1 hour prior carrageenan introduction the test extract (PSC) was injected intragastrically. After that 0.1 ml of 1% carrageenan solution was injected subplantarily. The volume of the animal's paw was measured every hour for 4 h after induction of inflammation. The normal saline solution was used as the control, and diclofenac sodium was used as the reference drug [7].

The study of the antibacterial activity of PSC was performed at the State Institution "Institute of Microbiology and Immunology named after I.I.Mechnikov of the National Academy of Medical Sciences of Ukraine" in the Laboratory of Biochemistry of Microorganisms and Nutrient Media under the supervision of Candidate of Biology T.P. Osolodchenko using the agar diffusion method with five reference strains: *Staphylococcus aureus* 6538 ATCC, *Escherichia coli* ATCC 25922, *Proteus vulgaris* NSTS 4636, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* 885/653 ATCC. For analysis 1% aqueous solution was used [5, 7].

Table 1

The anti-inflammatory activity of PSC from *Ledum palustre* shoots

The group of animals	Dose, mg/kg	The antiexudative activity, %			
		1 h	2 h	3 h	4 h
Diclofenac sodium	8	19%	63%	38%	96%
Control		–	–	–	–
PSC from <i>Ledum palustre</i> shoots	50	37%	63%	73%	84%
PSC from <i>Ledum palustre</i> shoots	100	32%	34%	56%	67%

Table 2

The antimicrobial activity of PSC from *Ledum palustre* shoots

Microorganisms	Inhibition zone, mm
<i>E. coli</i> 25922 ATCC	36±0.5
<i>S. aureus</i> 6538 ATCC	16±0.7
<i>P. vulgaris</i> 4636 NCTC	18±0.4
<i>P. aeruginosa</i> 27853 ATCC	21±0.8
<i>C. albicans</i> 885/653 ATCC	no

Results and Discussion

PSC was obtained from *Ledum palustre* shoots, its yield was 2.60±0.07% calculated with reference to the absolutely dried raw material. The dynamic of PSC yield from *Ledum palustre* shoots was determined by gravimetry, it was 0.97±0.03% during the first extraction, 1.24±0.03% during the second extraction, and 0.39±0.01% during the third extraction. D-glucose, D-galactose, L-rhamnose and L-arabinose were identified in the hydrolyzate of PSC by the method of paper chromatography. The amount of monosaccharides in PSC calculated with reference to glucose was determined by spectrophotometry. After statistical analysis of the results the content was found to be 43.25±0.80%.

The results of studying the anti-inflammatory activity of PSC and the reference drugs are given in Tab. 1.

Tab. 1 shows that PSC demonstrated the highest anti-inflammatory activity in the dose of 50 mg/kg. Edema decreased by 84% compared to the control, it was only

12% less than the activity of the standardized drug – diclofenac sodium. The antiexudative effect was 67% in the dose of 100 mg/kg.

The study of the antimicrobial activity of PSC is shown in Tab. 2.

PSC from *Ledum palustre* shoots showed the antimicrobial activity against all microorganisms, except *Candida albicans*. The maximum growth inhibition was observed against *Escherichia coli* – 36 mm. The inhibition zone for *Pseudomonas aeruginosa* was 21 mm. The small areas of the growth inhibition were against *Staphylococcus aureus* (16 mm) and *Pseudomonas aeruginosa* (18 mm).

CONCLUSIONS

The phytochemical and pharmacological studies of PSC from *Ledum palustre* shoots indicate the prospects of the substance and confirm the possibility of creating new drugs with the anti-inflammatory and antimicrobial activity on its basis.

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ДОСЛІДЖЕННЯ ХІМІЧНОГО СКЛАДУ ТА ФАРМАКОЛОГІЧНОЇ АКТИВНОСТІ ПОЛІСАХАРИДНОГО КОМПЛЕКСУ БАГНА ЗВИЧАЙНОГО

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Ключові слова: Вересові; пагони багна звичайного; полісахаридний комплекс; протизапальна активність; антимікробна активність

Багно звичайне здавна використовується в народній медицині як спазмолітичний, сечогінний, протизапальний та протикашльовий засіб. Найбільш дослідженою є ефірна олія, з якої

виготовлявся препарат «Ледин» з протикашльовим ефектом, в той час як полісахаридний комплекс (ПСК) цієї рослини майже не вивчений. Метою даної роботи було дослідження ПСК пагонів багна звичайного та встановлення його фармакологічної активності. ПСК одержували шляхом додавання 96% спирту етилового до водної витяжки з сировини. Вихід складав $2,60 \pm 0,07\%$ в перерахунку на абсолютно суху сировину. Досліджено динаміку виходу ПСК, в гідролізаті якого було ідентифіковано моноцукри: D-глюкозу, D-галактозу, L-рамнозу та L-арабінозу. Вміст моноцукрів у ПСК в перерахунку на глюкозу складав $43,25 \pm 0,80\%$. Протизапальну активність ПСК вивчали на моделі карагенінового набряку лапи щурів. ПСК проявив найбільшу протизапальну активність у дозі 50 мг/кг та зменшував набряк на 84% в порівнянні з контролем. Вивчення антибактеріальної активності проводили методом дифузії в агар. ПСК проявляє високу антимікробну активність по відношенню до всіх досліджуваних штамів мікроорганізмів, крім *Candida albicans*. Максимальна затримка росту спостерігалася по відношенню до *Escherichia coli* 36 мм. Проведені дослідження свідчать про перспективність даної субстанції та підтверджують можливість створення на її основі нових лікарських засобів з протизапальною та антимікробною активністю.

ИССЛЕДОВАНИЕ ХИМИЧЕСКОГО СОСТАВА И ФАРМАКОЛОГИЧЕСКОЙ АКТИВНОСТИ ПОЛИСАХАРИДНОГО КОМПЛЕКСА БАГУЛЬНИКА БОЛОТНОГО

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Ключевые слова: Вересковые; побеги багульника болотного; полисахаридный комплекс; противовоспалительная активность; антимикробная активность

Багульник болотный издавна используется в народной медицине как спазмолитическое, мочегонное, противовоспалительное и противокашлевое средство. Наиболее исследованным является эфирное масло растения, из которого изготавливался препарат «Ледин» с противокашлевым эффектом, в то время как полисахаридный комплекс (ПСК) этого растения почти не изучен. Целью данной работы было исследование ПСК побегов багульника болотного и установление его фармакологической активности. ПСК получали путем добавления 96% спирта этилового к водному экстракту сырья. Его выход составлял $2,60 \pm 0,07\%$ в пересчете на абсолютно сухое сырье. Была исследована динамика выхода ПСК, в гидролизате которого были идентифицированы моносахара D-глюкоза, D-галактоза, L-рамноза и L-арабиноза. Содержание моносахаров в ПСК после гидролиза в пересчете на глюкозу составляет $43,25 \pm 0,80\%$. Противовоспалительную активность ПСК изучали на модели карагенинового отека лапы крыс. ПСК проявил наибольшую противовоспалительную активность в дозе 50 мг / кг и уменьшал отек на 84% по сравнению с контролем. Изучение антибактериальной активности проводили методом диффузии в агар. ПСК проявлял высокую антимикробную активность в отношении всех исследуемых штаммов микроорганизмов, кроме *Candida albicans*. Максимальная задержка роста наблюдалась по отношению к *Escherichia coli* 36 мм. Проведенные исследования свидетельствуют о перспективности данной субстанции и подтверждают возможность создания на ее основе новых лекарственных средств с противовоспалительной и антимикробной активностью.