ANTIBACTERIAL ACTION OF THE PHYTOSUBSTANCES FROM *LEDUM PALUSTRE* SHOOTS

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Annually, infectious diseases take hundreds of thousands of lives. The data of the world health statistics for 2013 show that in Ukraine the prevalence of infectious diseases was 94 cases per 100 000 people [1]. At the same time, according to the World Health Organization, lower respiratory tract infections remain the most common cause of death, only in 2015 in the world 3.2 million people died [2]. The European respiratory society has conducted epidemiological studies, which results show that 25 % of patients annually consult a doctor about respiratory infections [3].

Meanwhile, each year the treatment of bacterial infections is increasingly complicated due to the resistance of pathogens to antibiotics. Currently, antibiotic resistance is one of the most serious threats to human health [4]. According to the WHO data it is expected that in the next 35 years approximately 300 million people will die prematurely due to antibiotic resistance [5]. In January of 2018 the WHO published the first surveillance data on high antibiotic resistance of a number of serious bacterial infections. The results of the report of the Global Antimicrobial Resistance Surveillance System (GLASS) of the WHO approximately 500,000 people with suspected bacterial infection in 22 countries faced with resistance to antibiotics. The most common resistant bacteria were *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*, as well as *Salmonella spp*. [6].

The GLASS data confirm the relevance of the topic since among five resistant pathogens three of them cause respiratory infection.

Therefore, the topical issue is the search for phytosubstants with the antibacterial action.

In this regard, drugs of plant origin, which successfully combine a high pharmacological activity with minimal side effects, are promising.

Thus, our attention was drawn to the medicinal plant – *Ledum palustre* (wild rosemary), which has long been widely used in folk medicine as an anti-cough and anti-inflammatory agent.

In their composition *Ledum palustre* shoots have essential oil, glycoside, arbutin, tannins and flavanoids, etc. [7].

According to the literature data *Ledum palustre* exhibits the antibacterial effect against *Staphylococcus aureus* and *E. coli* [8]. But there is no evidence of the antimicrobial action on pathogens that cause respiratory tract infections; although due to the chemical structure of *Ledum palustre*, it can be assumed that the medicinal plant, in addition to the anti-cough action, has the antibacterial effect in relation to *Moraxella catarrhalis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus, Klebsiella pneumoniae*.

The aim of the research is to study the antibacterial activity phytosubstants from *Ledum palustre*.

Materials and methods

The study objects were 8 phytosubstants from *Ledum palustre* shoots obtained at the Pharmacognosy Department of the National University of Pharmacy (NUPh):

Phytosubstance 1 (*Ledum palustre* dry extract obtained by water). Phytosubstance 1 is a substance of a dark brown color, which contains the following components in its composition: polysaccharides – 22.12 ± 0.82 %; monosaccharides: D-glucose, D-galactose, L-rhamnose and L-arabinose; carboxylic acids – 3.30 % with such dominant acids as levulinic, malic and 4-hydroxybenzoic; amino acids – 0.33 % with such dominant acids as asparagine, proline, γ-aminobutyric acid, methionine and phenylalanine; hydroxycinnamic acids – 0.73 % with dominant chlorogenic acid; flavonoids – 0.16 %, including rutin, (-)-epicatechin, quercetin, (+)-D-catechin. The content of the amount of phenolic compounds is 0.81 %.

Phytosubstance 2 (*Ledum palustre* polysaccharide complex obtained from phytosubstance 1). Phytosubstance 2 is a substance of a light brown color, which contains a complex of polysaccharides of *Ledum palustre* shoots in its composition (components: D-glucose, D-galactose, L-rhamnose and L-arabinose). The content of monosaccharides calculated with reference to glucose is 44.13 %.

Phytosubstance 3 (Phytosubstance 1, with the exception of the polysaccharide complex). Phytosubstance 3 is a substance of a dark brown color, which contains carboxylic acids – 4.24 %, amino acids – 0.42 %, hydroxycinnamic acids – 0.94 %, flavonoids – 0.21 % in its composition.

Phytosubstance 4 (Phytosubstance 1, with the exception of saponins). Phytosubstance 4 is a substance of a light brown color, which contains the following components in its composition: monosaccharides – 35.13 %, among them D-glucose, D-galactose, L-rhamnose and L-arabinose; carboxylic acids – 3.36 %; amino acids – 0.34 %; hydroxycinnamic acids – 0.74 %; flavonoids – 0.16 % in its composition.

Phytosubstance 5 (Extraction of *Ledum palustre* shoots with 50 % ethyl alcohol). Phytosubstance 5 is a substance of a light brown color which contains the following components in its composition: amino acids – 0.31 %; hydroxycinnamic acids –
1.78 % with the highest content of chlorogenic acid – 8790.32 mg/kg; flavonoids – 12.34 % with the highest content of (+)-D-catechin – 30203.17 mg/kg, (-)-epicatechin – 34852.49 mg/kg and rutin – 9613.77 mg/kg; polyphenolic compounds – 13.47 %; carboxylic acids – 2.96 % with dominant levulinic acid – 9624.89 mg/kg, malic acid– 4478.13 mg/kg and citric acid – 8461.96 mg/kg; terpene compounds – 1.33 % with

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dominating ledol – 972.14 mg/kg, corimbolone – 617.92 mg/kg and *p*-cymene – 404.42 mg/kg.

Phytosubstance 6 (Extraction of *Ledum palustre* shoots with 50 % ethyl alcohol purified by ethyl acetate). Phytosubstance 6 is a substance of a light brown color, which contains the following components in its composition: hydroxycinnamic acids – 9.00 %, with the highest content of chlorogenic acid – 44487.61 mg/kg; 13 flavonoids – 17.25 % with dominating (-)-epicatechin – 48720.05 mg/kg, (+)-D-catechin – 42220.80 mg/kg and rutin – 13439.02 mg/kg; terpene compounds with dominating ledol – 4929.72 mg/kg, corimbolone – 3133.47 and *p*-cymene – 2050.81 mg/kg. The content of the amount of polyphenolic compounds in phytosubstance 6 is 58.14 %.

Phytosubstance 7 (1 % solution of the *Ledum palustre* extract extracted with 96 % ethyl alcohol). Phytosubstance 7 is a liquid substance of a dark green color, which contains essential oil – 0.06 % with dominating ledol, corimbolone and *p*-cymene; chlorophylls; organic acids, flavonoids – 0.04 % in its composition.

Phytosubstance 8 (Extraction of *Ledum palustre* shoots with 50 % ethyl alcohol further purified from metal cations, amino acids and organic acids by passing the extract through the cation resin). Phytosubstance 8 is a brown substance, which contains the complex of phenolic and terpene compounds with dominating ledol, corimbolone and *p*-cymene in its composition.

The study of the antibacterial activity of phytosubstance with *Ledum palustre* was performed at the premises of Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine in the Laboratory of Biochemistry and Biotechnology under the chief of laboratory, Ph. D. in biol., senior scientist Osolodchenko T. P.

The activity of the phytosubstance was studied on museum strains of microorganisms, namely *Moraxella catarrhalis*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*.

The suspension from microorganisms was prepared using a Densi-La-Meter device (manufactured by PLIVA-Lachema, Czech Republic; the wavelength of 540 nmTo determine the antibacterial effect the microbial load was 10^7 microbial cells per 1 ml (according to the McFarland standard) [9].

The activity of the substances was studied by the agar diffusion. The 18-24-hour culture of microorganisms and Müller-Hinton agar were used in the study. The method was performed on two layers of the solid nutrient medium in Petri dishes. In the lower layer the "starvation" uninoculated medium was used; it was a substrate having the height of 10 mm with horizontally mounted cylinders of stainless steel with the diameter of 8 mm and the height of 10 mm. Around the cylinders the top layer was filled with the nutrient agar (melted and cooled to 40°C), in it the corresponding standard of the daily culture of the test microbe was introduced. The volume of the medium for the upper layer ranged from 14 to 16 ml. After solidification of the agar the cylinders were pulled out with sterile forceps, and diluted phytosubstances were poured into the cells. The dry phytosubstances obtained from *Ledum palustre* shoots were dissolved in distilled water, 50 % alcohol, 96 % alcohol. The concentration of the drugs was 1.0 %. As a control, pure solvents in concentrations corresponding to their content in the drugs were used. After that, Petri dishes were transferred to the thermostat for 24 h.

The antimicrobial activity was assessed according to the following criteria [10]:

– the growth inhibition zones of microorganisms up to 10 mm indicated the microorganism insensitivity to the drug introduced in the cell;

– the growth inhibition zones of 10-15 mm in diameter indicated the low sensitivity of the microorganism culture to the test concentration of the antimicrobial substance;

– the growth inhibition zones of 15-25 mm in diameter were regarded as an indicator of the microorganism sensitivity to the drug tested;

– the growth inhibition zones of more than 25 mm indicated a high sensitivity of microorganisms to the drug.

The statistical reliability of the experimental results was determined according to the SPhU (edition 1, appendix. 1, p. 187). Processing of the experimental data was performed by the methods of mathematical statistics using such computer application programs as STATISTIKA 6.0 and MS EXEL 7.0

Results and discussion

Using the agar diffusion method it has been found that the phytosubstances obtained from *Ledum palustre* exhibit the antibacterial action against all test strains of microorganisms (Tab.1). The diameters of the growth inhibition zones of microorganisms are within 12-20 mm, and it indicates the sensitivity of the microorganism to the phytosubstances under research.

The data of the microbiological study of *Ledum palustre* compounds are presented in Tab. 1.

The data in Tab.1 show that phytosubstance 1 has an insignificant activity against

H. influenzae; however, when isolating the polysaccharide complex and the complex of saponins (Phytosubstance 3 and 4) the growth inhibition zone significantly increased up to 20 mm, indicating the sensitivity of *H. influenzae* to the phytosubstance. This can be explained by an increase in the concentration of phenolic compounds of *Ledum palustre*. S. pneumoniae showed a low sensitivity to phytosubstantia 1, but a polysaccharide complex of 22% resulted in an increase in the grow the retardation zone of up to 15 mm.

This is confirmed by the fact that phytosubstance 6 has the greatest inhibition zone of 20 mm when dissolving with both water and 50% ethyl alcohol, and it is a complex of glycosides and aglycones of phenolic compounds.

H. influenzae and *S. pneumoniae* showed a pronounced sensitivity to phytosubstance 6 due to the content of phenolic compounds.

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All investigated phytosubstances from *Ledum palustre* shoots have an average antibacterial effect in of *Moraxella catarrhalis.*

Table 1. The antibacterial action of the phytosubstances from Ledum palustre shoots.

		Diameter of the growth inhibition zones of microorganisms, mm				
Phyto-sub-stance	Dissolution	*H. influenzae*	*M. catarrhalis*	*K. pneumoniae*	*S. pneumoniae*	*S. aureus*
1	H_2O	12±0.5*	15±0.96*	15±0.50*	12±0.50*	12±0.50*
	50%C_2H_5OH	15±0.50**	12±0.82**	14±0.50	14±0.82	14±0.82
	96%C_2H_5OH	"-"	"-"	"-"	"-"	"-"
2	H_2O	12±0.82*	15±0.50*	growth	growth	growth
	50% C_2H_5OH	12±0.50**	12±0.50**	growth	14±0.50	14±0.58**
	96% C_2H_5OH	"-"	"-"	"-"	"-"	"-"
3	H_2O	15±0.50*	15±0.58*	growth	14±0.50*	growth
	50% C_2H_5OH	20±0.82**	12±0.82**	growth	15±0.50**	growth
	96% C_2H_5OH	15±0.82#	growth	growth	growth	12±0.82
4	H_2O	13±0.82*	15±0.50*	12±0.82*	growth	14±0.50*
	50% C_2H_5OH	17±0.82**	12±0.82	14±0.82	15±0.50**	14±0.50**
	96% C_2H_5OH	15±0.82#	13±0.50#	13±0.50	15±0.82	16±0.50#
5	H_2O	14±0.50*	14±0.50*	14±0.96*	14±0.50*	12±0.82*
	50% C_2H_5OH	15±0.50**	18±0.82**	16±0.96**	14±0.96	12±0.50
	96% C_2H_5OH	17±0.50#	20±0.82#	18±0.50#	18±0.50#	12±0.82
6	H_2O	20±0.82*	15±0.58*	15±0.82*	20±0.82*	16±0.82*
	50% C_2H_5OH	20±0.82**	15±0.82**	14±0.50	20±0.82**	15±0.82**
	96% C_2H_5OH	15±0.82#	16±0.82#	14±0.82	17±0.96	16±0.82#
7	Resulting solution	17±0.82*	13±0.82*	18±0.82*	growth	growth
8	H_2O	growth	15±0.82*	growth	14±0.50*	growth
	50% C_2H_5OH	growth	12±0.82**	18±0.82**	14±0.96	14±0.82**
	96% C_2H_5OH	12±0.50#	growth	14±0.82	15±0.82	15±0.82#
Control	H_2O	growth	growth	growth	growth	growth
	50% C_2H_5OH	growth	growth	12±1.41**	growth	growth
	96% C_2H_5OH	12±0.50#	growth	13±1.00#	17±0.58#	13±1.15#
Refe-rence drug	Pectoral tea	20±0.50*	17±0.50*	growth	growth	15±0.50*

*- p<0.05 compared to water; **- p<0.05 compared to 50% ethyl alcohol; # - p<0.05 compared to 96% ethyl alcohol; "-" – the study was not conducted because of the physical properties of the phytosubstance.

Staphylococcus aureus is sensitive to the test extracts obtained with 50 % alcohol (phytosubstances 5, 6, 8). This can be due to their chemical composition – the extracts additionally contain terpene compounds of *Ledum palustre*.

Phytosubstances 7 obtained in 96 % alcohol revealed the sensitivity index of *Klebsiella pneumoniae*. The growth inhibition zone was 18 mm. As can be seen from Tab.1, this fact occurs due to the content of terpene alcohols phytosubstance 7. Thus, the microorganism is insensitive to aqueous phytosubstances.

Conclusions

1. The antibacterial activity of 8 phytosubstances from *Ledum palustre* shoots has been studied in relation to microorganisms that cause respiratory tract infections.
2. The antibacterial effect of phytosubstances obtained for the first time from *Ledum palustre* shoots for the treatment of respiratory diseases has been experimentally proven.
3. It has been proven that the further study of the phytosubstances obtained from *Ledum palustre* is promising.
4. The phenolic complex with *Ledum palustre* has a pronounced bactericidal effect against *Haemophilus influenzae* and *Streptococcus pneumoniae*, as well as the bacteriostatic effect against *Moraxella catarrhalis*.
5. Due to the content of terpene compounds in phytosubstances 5, 6, 7, 8 there is the antibacterial action in relation to *K. pneumoniae* and *S. aureus*.

Prospects for further research. According to the results of microbiological the study the

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phytosubstances obtained from *Ledum palustre* shoots are a promising phytosubstance for creating a polytherapeutic drug for the treatment of respiratory diseases.

Conflicts of Interest: authors have no conflict of interest to declare.

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The aim of the research is to study the antibacterial activity of phytosubstances from *Ledum palustre* shoots. **Materials and Methods**. The study objects were 8 phytosubstances from *Ledum palustre* shoots. The study of the antibacterial activity of phytosubstances with *Ledum palustre* was performed at the premises of Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine in the Laboratory of Biochemistry and Biotechnology under the supervision of the head of the Laboratory, Candidate of Biology (Ph.D.) Osolodchenko T. P. **Results**. The study has shown that phytosubstances from *Ledum palustre* shoots exhibit the antibacterial action against pathogens of respiratory tract diseases, namely *Moraxella catarrhalis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*. According to the results of the study, phytosubstances, which in their composition have terpene compounds, have antibacterial effects in relation to *Klebsiella pneumoniae* and *Staphylococcus aureus.* Meanwhile, the Ledum palustre phenol complex exhibits a pronounced antibacterial effect to *Haemophilus influenzae* and *Streptococcus pneumoniae*, also medium antibacterial effectin relation to M*oraxella catarrhalis*.

Key words: phytosubstances from *Ledum palustre* shoots, antibacterial action, respiratory tract infections.

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