STUDY OF THE ANTIBACTERIAL ACTIVITY OF NEW MODIFIED GALENIC AND NOVOGALENIC PHYTOSUBSTANCES FROM VACCINIUM VITIS-IDAEA LEAVES

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Urinary tract infections are the most common infectious diseases that require significant financial costs. Nosocomial UTI are the largest reservoir of antibiotic-resistant microorganisms in hospitals [1]. In addition to the fact that infectious and inflammatory diseases of the urinary system are a common pathology, the chronicity of the infectious process, recurrence and progress with the formation of nephrosclerosis and the development of chronic renal failure, leading to disability and requiring extraordinary medical interventions, make this problem especially relevant [2].

According to epidemiological data, the most common UTI (Urinary tract infections) develop in women: almost 50% of women have at least UTI carried once in a lifetime. In men, the incidence of UTI is much less and is 5-8 cases Urinary tract infections for 10,000 people. In elderly and senile people, UTI rank second in frequency of occurrence and make up about 25% of all infectious diseases [2]. Data presented emphasize the importance of timely diagnosis and treatment of UTI, development preventive measures and dynamic observation of patients.

Uncomplicated UTI caused in more than 95% of cases by gram-negative microorganisms from the Enterobacteriaceae family, the main causative agent being Escherichia coli, a permanent inhabitant of the large intestine of humans and animals, causing uncomplicated UTI in 80–90% of cases. 3–5%), Klebsiella spp., Proteus mirabilis, etc [3]. With a sudden outbreak of UTI, the shedding rate reduced to 60% and E. coli to 30%. At the same time, other pathogens are more common - Proteus spp., Pseudomonas spp., Klebsiella spp., Candida albicans. Cortical abscess in 90% caused by Staphylococcus aureus, which causes apostematous nephritis, kidney abscess localized in the medullary substance are E. coli, Klebsiella spp., Proteus spp [4].

As with other bacterial infections, the sensitivity of pathogens to antibiotics is critical when choosing a drug for empiric treatment of UTI. Moreover, the structure of pathogens causing uncomplicated UTI is quite predictable, since E. coli causes infection in 75–90%. Antibiotic resistance, which has long been considered a problem in the treatment of nosocomial and complicated UTI, is now becoming relevant in the treatment of uncomplicated UTI. Today, plant substances with an antibacterial effect are of increasing interest in the world of science [5,6].

The aim of the work was to study the antibacterial activity of new modified galenic and novogalenic phytosubstances from Vaccinium vitis-idaea leaves.

Materials and methods

The investigated phytosubstances were obtained at the Department of Pharmacognosy of the National University of Pharmacy under the leadership of the head of the department Koshovoy O.M [5]. Phytosubstances № 1, № 2, № 3 - are dry galenic extracts using solvents of different polarity (water, 50% ethanol, 96% ethanol).

Phytosubstance № 1 contains in its composition a complex of phenolic compounds (hydroxycinnamic acids - 1,71 ± 0,02%, flavonoids - 0,13 ± 0,01%, hydroquinone derivatives - 10,66 ± 0,03 %, the amount of phenolic compounds - 13.5 ± 0.02%) and polysaccharides.

Phytosubstance № 2 contains in its composition a complex of phenolic compounds: hydroxycinnamic acids - 2,17 ± 0,02%, flavonoids - 3,36 ± 0,01%, hydroquinone derivatives - 7,28 ± 0,02 %, the amount of phenolic compounds is 20.48 ± 0.01%. The highest content of almost all groups of phenolic compounds was precisely in this extract.

Phytosubstance № 3 is a complex of phenolic compounds: hydroxycinnamic acids - 2,19 ± 0,02%, flavonoids - 1,79 ± 0,01%, hydroquinone derivatives - 3,98 ± 0,02 %, the amount of phenolic compounds is 14.75 ± 0.02%.

From the phytosubstance № 1 was obtained by planting a polysaccharide complex (phytosubstance № 4). Phytosubstance № 4 is a brown substance that contains a complex of phenolic compounds (hydroxycinnamic acids, p-coumaric, caffeic, rosemary, chlorogenic acids - 1.10%, flavonoids (catechins, quercetin, hyperoside, rutin, kaempferol 3-O-glycoside and apigenin) - 1.13%, hydroquinone derivatives (hydroquinone, arbutin) - 2.01, the amount of phenolic compounds (gallic and ellagic acids, tannins that are hydrolyzed) - 7.37%) with the addition of polysaccharides (components: glucose, rhamnose, ribose, arabinose), the content of which is 50%.

From the phytosubstance №1 from the supernatant was obtained the phytosubstance № 5, which contains glycosides of phenolic compounds. Phytosubstance №5 is a light brown substance that contains a complex of low-chain polysaccharides of cranberry leaves (components: glucose, rhamnose, ribose, arabinose), soluble in 20% ethyl alcohol.

Since phytosubstances № 2 and № 3 do not contain polysaccharides, phytosubstance № 4 in the ratio 1: 1 added to these substances, and the corresponding phytosubstances № 6 and 7, respectively, obtained. A complex of triterpene saponins derived from ursolic acid (phytosubstance № 8) obtained from the phytosubstance № 2 by planting. Phytosubstance № 8 is a light brown substance that contains a complex of saponins of triterpene nature (derivatives of ursolic acid). Phytosubstance № 9 is a brown substance that contains a complex of tannins of lingonberry leaves with zinc and polysaccharides of lingonberry leaves. Phytocomplex № 10 is a brown substance that contains a complex of phenolic compounds (hydroxycinnamic acids, flavonoids, hydroquinone derivatives, organic acids, amino acids), which are soluble in 50% alcohol, additionally purified from tannins. Phytosubstance № 11 is a light brown substance and contains a complex of aglycones of phenolic compounds from Vaccinium vitis-idaea leaves. Phytosubstance № 12 is a brown substance that contains a complex of phenolic compounds (hydroxycinnamic acids 1.30%, flavonoids 4.01%, hydroquinone derivatives - 8.61, the

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amount of phenolic compounds - 22.03%) in combination with arginine - 39%. Among hydroxyroic acids: chlorogenic - 121.43 mg / 100 g, caffeic acid derivatives - 400.87 mg / 100 g). Among the flavonoids: rutin - 554.72 mg / 100 g, quercetin glycoside - 855.22 mg / 100 g, quercetin - 27.08 mg / 100 g, kaempferol glycoside - 13.35 mg / 100 g. Phytosubstance № 13, which is a brown substance that contains a complex of phenolic compounds (hydroxycinnamic acids - 2.14%, flavonoids - 3.31%, hydroquinone derivatives - 7.22%, the sum of phenolic compounds - 20.08 %), soluble in 50% alcohol, which are further purified from metal cations and amino acids.

In our research alcoholic and aqueous extracts of Vaccinium vitis-idaea leaves were used to establish antibacterial activity in the next series of experiments. The study of antibacterial activity of the phytosubstances was in the laboratory of biochemistry of microorganisms and nutrient medium of the Mechnikov Institute of Microbiology and Immunology under the direction of candidate of biological sciences Osolodchenko T.P.

Reference strains of Staphylococcus aureus ATCC 25923, Staphylococcus aureus 6538 ATCC, Escherichia coli ATCC 25922, Proteus vulgaris NTCS 4636, Pseudomonas aeruginosa ATCC 27853 and Candida albicans 885/653 ATCC were used in accordance with the recommendations for the assessment of antimicrobial activity of drugs. In our study, we used 1% solutions of extracts, the solvents of which were water and 50% and 96% alcohol. The method of diffusion of the drug into agar carried out using the method of "wells". Studies of antibacterial activity performed using the method of wells. On solidified agar, using a pipette under sterile conditions in Petri dishes made 1 ml of a suspension of microorganisms. After uniform distribution of microorganisms over the entire surface of the agar, the plates were incubated at room temperature for 15-20 minutes Next, wells with a diameter of 6 mm were made in the cups, into which solutions of the test substances were introduced. The samples incubated at 37 ° C for 16-24 hours. After incubation, the plates placed upside down on a dark matte surface so that light fell on them at an angle of 45 ° (accounting in reflected light). The diameter of the growth retardation zones measured using a caliper.

Determination of the statistical validity of the results of experiments carried out for the state control system. The processing of experimental data performed using mathematical statistics methods using the applied computer programs STATISTIKA 6.0 and MS EXCEL 7.0.

The comparison drug was Inurek, which contains American cranberries (150 mg) and is standardized for proanthocyanidins (60 mg).

Results and discussion

The results of the study of the of new modified galenic and novogalenic phytosubstances from Vaccinium vitis-idaea leaves obtained in the course of the study are presented in the table**.**

Table. Antibacterial activity of new modified galenic and novogalenic phytosubstances from Vaccinium vitis-idaea leaves

Phyto-substance	Solvent	Diameter of a zone of a growth delay, mm				
		E.coli	S.aureus	Proteus vulgaris	P.aeruginosa	Candida albicans
1	H_2O	15±0,8*	16±0,5	15±0,8*	13±0,8*	15±0,8
2	50% C_2H_5OH	15±0,8	11±0,6*	17±0,6	9±0,5*	10±0,5*
3	96% C_2H_5OH	11±0,8*	17±0,8*	19±0, 8*	11±0,8*	18±0,8*
4	H_2O	рістр	рістр	рістр	рістр	Рістр
	50% C_2H_5OH	рістр	14,5±0,7	13±0,7*	12±0,9*	14±0,9*
	96% C_2H_5OH	рістр	12±0,8	11±0,8*	12±0,8*	12±0,8*
5	H_2O	рістр	рістр	рістр	рістр	Рістр
	50% C_2H_5OH	13±0,5*	рістр	14±0,6*	12±0,8*	14±0,8*
	96% C_2H_5OH	11±0,8*	11±0,8*	12±0,8*	13±0,8*	13±0,8*
6	H_2O	рістр	рістр	рістр	рістр	Рістр
	50% C_2H_5OH	12±0,8*	рістр	14±0,8*	12±0,8*	15±0,8
	96% C_2H_5OH	12±0,8*	11±0,8*	13±0,5*	12±0,8*	13±0,8*
7	H_2O	12±0,9*	рістр	рістр	рістр	Рістр
	50% C_2H_5OH	13±0,8*	рістр	14±0,9*	12±0,9*	14±0,9*
	96% C_2H_5OH	11±0,9*	12±0,9	14±0,9*	14±0,9*	13±0,8*
8	H_2O	рістр	рістр	рістр	рістр	Рістр
	50% C_2H_5OH	13±0,8*	17±0,5*	13±1,0*	15±0,5	16±0,5
	96% C_2H_5OH	13±0,8*	15±1,4*	15±0,8*	14±0,8*	16±1,3
9	H_2O	рістр	рістр	рістр	рістр	Рістр
	50% C_2H_5OH	13±0,8*	12±1,0	рістр	12±0,8*	15±0,8

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	96% C_2H_5OH	13±0,8*	13±0,8	12±1,41*	14±0,8*	13±0,8*
10	H_2O	12±0,5*	13±0,5	ріст	14±0,5*	15±0,5
	50% C_2H_5OH	12±0,8*	14±0,8	14±0,8*	14±0,5*	Ріст
	96% C_2H_5OH	12±0,8*	13±0,5	12±0,8*	12±0,8*	11±0,8*
11	H_2O	ріст	ріст	ріст	Ріст	Ріст
	50% C_2H_5OH	16±0,8	16±0,8*	15±0,8*	14,25±0,5*	15±0,8
	96% C_2H_5OH	12±0,8*	14±0,8	14±0,8*	12±0,8*	13±0,8*
12	H_2O	12±0,5*	ріст	Ріст	Ріст	Ріст
	50% C_2H_5OH	18±0,8*	16±0,8*	16±0,8	15,08±0,5	17,08±0,8*
	96% C_2H_5OH	16,8±0,5*	14,8±0,5*	13,8±1.0*	14±0,8*	13,8±0,5*
13	H_2O	ріст	15±0,5	Ріст	14±0,5*	15±0,5
	50% C_2H_5OH	12±0,8*	13±0,8	Ріст	12±0,8*	14±0,8*
	96% C_2H_5OH	13±0,8*	12±0,8	13±0,8*	11±0,8*	15±0,8
Інурек	H_2O	13,5±0,58	14,5±1,29	13,75±0,5	15,75±0,5	15±0,82
	50% C_2H_5OH	15,8±0,5	12,8±1,0	16,8±0,5	15,8±0,5	15,8±0,5
	96%C_2H_5OH	15,8±0,5	12,8±1,0	16,8±1,0	15,8±0,5	15,8±0,5

Notes: * - p≤0.05 in relation to Inurek**.**

According to the obtained results, the most pronounced antibacterial effect against the main uropathogens had phytosubstance № 12 dissolved in 50% alcohol, which is a complex of glycosides of phenolic compounds with arginine.

Phytosubstance №12 has an antibacterial effect on the main uropathogen E. coli with an average activity, the diameter of the growth retardation zone was 18 mm (P <0,05). To the following pathogenic microorganisms such as S.aureus, Proteus vulgaris, the studied phytosubstance led to zones of growth retardation at the mark of 16 mm, to P.aeruginosa 15 mm, to Candida albicans the growth retardation zone was 17 mm (P <0.05). This indicates that this phytosubstance can be recommended as a therapy for nosocomial infections, where the etiological factors are microbial association. Removal of amino acids from the phytosubstance № 13 leads to a decrease in antibacterial activity in the form of growth retardation zones at the level of 12 mm (P <0.05) to E. coli, to S. aureus at the level of 13 mm, to P. vulgaris growth was observed, and to Candida albicans 14mm (P <0.05). These results regarded as an indicator of low sensitivity of test microorganisms to this phytosubstance. The results were similar, where 96% ethanol used as a solvent

To phytosubstance №1 pathogenic microorganisms had low sensitivity, in particular, S.aureus, which had a medium sensitivity in the form of a 16 mm zone of growth retardation. Phytosubstance №2 to uropathogens E.coli (diameter of the growth retardation-15 mm) had a low antibacterial effect, to Proteus vulgaris (diameter of the growth retardation-17 mm) - showed a medium antibacterial effect, to other uropathogens low antibacterial effect or was absent. Phytosubstance №3 in relation to S. aureus (diameter of growth retardation zone 17 mm) and Ptoteus vulgaris (diameter of growth retardation zone-19 mm) (P <0.05) showed a medium antibacterial effect, to other uropathogens this phytosubstance had a low antibacterial effect (including E.coli).

The main uropathogens had low sensitivity to the polysaccharide complex (phytosubstance №4). Removal of polysaccharides also did not increase the anibacterial effect. Addition of low-chain polysaccharides to phytosubstances №2 (phytosubstance №6) to increase the antimicrobial action. Phytosubstance № 8 (triterpene saponins) did not actually prove to be an antibacterial agent, only S.aureus, C.albicans were sensitive to this phytosubstance (17 mm and 16 mm) (P <0.05), respectively. The complex of tannins from the leaves of lingonberry (phytosubstance № 9) also did not have a pronounced antibacterial effect.

Complex of aglycones of phenolic compounds (№11), when dissolved in 50% alcohol, had a medium antibacterial effect against all uropathogens (P <0.05). It can be concluded that phenolic compounds from the leaves of lingonberry have a more pronounced antibacterial effect in the form of glycosides.

Despite the fact that the drug Inurek is a standardized on the cranberry extract, it had an antibacterial effect lower than the phytosubstance №12.

Conclusions

1. A study of antibacterial properties technologically new modified galenic and new galenic phytosubstances from Vaccinium vitis-idaea leaves. The antibacterial effect of the phytosubstine №12 was confirmed
2. It is proved that the polysaccharide complex itself, its addition and removal did not increase the antibacterial effect
3. It has been proved that glycosides of phenolic compounds have a more pronounced antibacterial effect than aglycones of phenolic compounds.

Prospects for further research.

The obtained results prompt more in-depth studies of phytosubstance №12 and create conditions for the creation of a domestic drug with antibacterial activity in order to prevent recurrence of urinary tract infections.

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

Study of the antibacterial activity of new modified galenic and novogalenic phytosubstances from Vaccinium vitis-idaea leaves

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The purpose of the work is to study the antibacterial activity of new modified galenic and novogalenic phytosubstances

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from *Vaccinium vitis-idaea* leaves. **Materials and methods**. The object of our research new modified galenic and novogalenic phytosubstances from Vaccinium vitis-idaea leaves. Pharmacopoeia agar diffusion method biological method for determining the activity of antibiotics, based on the ability of molecules antibiotic substances diffuse in agar media and form zones of inhibition, in which the test microorganisms used do not develop sensitive to the antibiotic under study. The study of antibacterial activity of the phytosubstances was in the laboratory of biochemistry of microorganisms and nutrient medium of the Mechnikov Institute of Microbiology and Immunology under the direction of candidate of biological sciences Osolodchenko T.P. The reference drug was Inurek, recommended by the European Association of Urology for the prophylactic treatment of urinary tract infections. **Results**. We have identified the most active phytosubstance, which is the glycosides of phenolic compounds in combination with the arginine, dissolved in 50% alcohol. It has been established that polysaccharides from the leaves of Vaccinium vitis-idaea leaves may have a low antibacterial effect, and in the complex with phenolics, they do not infuse an antibacterial effect on the antibacterial effect. It has been established that phenolics from half of the leaves of lingonberry extraordinarily exhibit a greater antibacterial effect in the presence of glucosides. **Conclusions**. A screening study of the antibacterial activity of 13 new modified galenic and novogalenic phyto-substances from Vaccinium vitis-idaea leaves carried out. The most active was phytosubstance 12, which is a complex of glycosides of phenolic compounds with arginine. It has been proven that it is phenolic compounds in the form of glycosides that have a more pronounced effect in the main relations of uropathogens than their aglycones..

Keywords: new modified galenic and novogalenic phytosubstances, leaves, Vaccinium vitis-idaea, antibacterial activity

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