ANTIBACTERIAL ACTIVITY OF PHYTOSUBSTANTS FROM VACCINIUM VITIS-IDAEA LEAVES

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Urinary system infections are currently a very important problem in medicine and the health system as a whole. Significant economic losses due to the loss of working capacity of patients at a young age, the disability of the children and adults, and the expensive treatment of patients at all stages of the pathological process determine the social significance of diseases of the organs of the urinary system [1, 2, 3, 4]. Urinary tract infections are one of the most common diseases of women of reproductive age. The most common manifestation of uncomplicated urinary tract infection is acute cystitis. For last years cystitis is one of the leading places among urological diseases. As for the population of Ukraine, according to the data for 2012, the incidence of kidney infections was at 1666.5 per 100 thousand population, prevalence and incidence of cystitis - 439.9 cases.

According to WHO in 2012, acute cystitis in women in Europe is ranked second with ARI. Pyelonephritis is also a frequent disease of the urinary tract, severe in the course and unfavorable in prognosis, which may endanger the life of the patient and, often, the loss of a vital organ. According to the literature, acute pyelonephritis accounts for 14% of all kidney diseases, in 33% of patients develop purulent-destructive forms, mainly in women. [5, 6].

Pyelonephritis is the most common urological disease in all age groups, which determines its relevance and clinical significance in modern conditions and is associated with a range of both social and economic factors [7]. The incidence of acute pyelonephritis annually in the US is 250,000 cases [8, 9]. In Ukraine the incidence of acute pyelonephritis is 0.9 million cases per year. [6, 7].

Etiopathogenetic treatment of patients with an ICS consists of antibacterial therapy, respectively, susceptibility to pathogenic microflora, anesthetic, diuretic, analgesic and anti-inflammatory therapy. But antibacterial drugs have a number of side effects, among which the resistance of uropathogens is important, therefore the creation of new herbal remedies for the treatment of ICS has a rather promising direction in pharmacy as a whole.

The aim of the work was to study the antibacterial activity of phytosochnids from Vaccinium vitis-idaea leaves.

Materials and methods.

Substances were obtained at the Department of Pharmacognosy of the National Pharmaceutical University under the direction of Professor O.M. Koshovoy from Vaccinium vitis-idaea leaves. The halogenated dry extracts were obtained using solvents of different polarity: water, 50% ethanol solution and 96% ethyl ether, which received the phytobacterial names №. 1, № 2 and № 3, respectively [6].

Phytosubstance № 1 is a powder of brown color, which 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 is a powder of brown color, which 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 No. 3 is a brown powder containing 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%.

To establish the relationship between the chemical composition of the extracts and their diuretic activity on the basis of phytosubstantions No. 1, No. 2 and No. 3, another 10 modified novaginal substances were obtained.

A polysaccharide complex was obtained from phytosubstance №1 by planting (phytosubstance № 4) and phytosubstance №5 containing phenolic compounds glycosides from supernatant. Since phytosubstantions №2 and №3 dont contain polysaccharides, phytosubstance №4 was added to these substances in a ratio of 1: 1, and corresponding phytosubstituted plants № 6 and №7 were obtained, respectively.

A complex of triterpene saponins derived from ursolic acid (phytosubstance №8) was obtained by planting arom phytosubstace №2. Since the content of different groups of phenolic compounds was the largest in phytosubstance №2, on its basis were obtained by planting a complex of tannins (phytosubstance №9) and from supernatant phytosubstace №10. From a phytosubstance №2, after the hydrolysis, was received a complex of phenol aggliphos aggliphos (phytosubstance № 11), which contains hydroquinone, coffee acid, quercetin, campherol, and etc.

Aminoacids affect solubility, bioavailability and general pharmacotherapeutic effect of extracts, therefore arginine in triple equimolar quantity in relation to the total amount of phenolic compounds was added to phytosubstance №2 and phytosubstance №12 was obtained. Also, from phytosubstance №2, aminoacids were isolated by passing it through cationite and phytosubstaтce №13 was received.The resulting dry complexes from the leaves were dissolved in distilled water, 50% alcohol, 96% alcohol. Concentration of drugs was 1.0%.

During the experiment alcoholic and aqueous extracts of Vaccinium vitis-idaea leaves were used to establish antibacterial activity. The study of antibacterial activity of the extracts obtained was carried out by the method of diffusion in agar 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.

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In accordance with WHO recommendations for the evaluation of the activity of the drugs used reference strains of Staphylococcus aureus ATCC 25923, Staphylococcus aureus 6538 ATCC, Escherichia coli ATCC 25922, Proteus vulgaris NSTS 4636, Pseudomonas aeruginosa ATCC 27853, Pseudomonas aeruginosa 9027 ATCC and Candida albicans 885/653 ATCC. 1% solution of extracts was used for the study, physiological solution and 50% and 96% alcohol were used as solvents. The method of diffusion of the drug into agar was carried out using the method of "wells". For the study, pure cultures of microorganisms that were previously grown at 37 ° C for 24 hours on meat-pepper agar were used. The bacterial suspension was prepared on a sterile 0.9% sodium chloride solution. On the agar, which was frozen, with the help of a pipette under sterile conditions, Petri dishes were put in 1 ml of suspension of microorganisms. After uniform distribution of microorganisms throughout the agar surface, the cups were incubated at room temperature for 15-20 min. Further in the cups were made wells with a diameter of 6 mm, which contained solutions of the substances studied. Samples were incubated at 37 ° C. for 16 hours. After the incubation, the cups were placed upside down on a dark matte surface so that the light fell on them at an angle of 45 ° (reflected in reflected light). The diameter of the growth retardation zones was measured with an accuracy of 1 mm using a caliper. When measuring zones of growth retardation, they focused on the zone of complete suppression of visible growth. The evaluation of the results was carried out in the presence or absence of microorganism growth around the well, calculating by measuring the diameter around the well in millimeters. Antimicrobial activity was evaluated according to the criteria [6]:

- zones of growth retardation of microorganisms up to 10 mm indicate the insensitivity of the microorganism to the drug introduced into the well;
- growth retardation zones with a diameter of 10-15 mm indicate the low sensitivity of the culture of microorganisms to the tested concentration of antimicrobial substance;
- growth retardation zones with a diameter of 15-25 mm are considered as an indicator of the microorganism sensitivity to the tested means;
- growth retardation zones greater than 25 mm testify to the high sensitivity of microorganisms to the drug.

Determination of the statistical validity of the results of experiments was carried out for the state control system (1 st spec., Supplement 1, p. 187). The processing of experimental data was performed using mathematical statistics methods using the applied computer programs STATISTIKA 6.0 and MS EXCEL 7.0.

The comparison drug was the drug Inurek, the manufacturer of Pharmaceuticals Manufacchurin S.L., Spain for Brufarmexport s.p.r.l., Belgium, which contains concentrated extract of American cranberries 150 mg. This drug was not accidental, since it is the only drug in the Ukrainian market standardized by proanthocyanidin (60 mg), and they provide an antibacterial effect in the treatment of urological diseases and recommended by the European Association of Urologists for the prevention of urinary system infections in a dose of 36 mg.

Results and discussion

The results of the study of the diuretic activity of the phytosanitary substances obtained in the course of the study are presented in the table**.**

Table. Antibacterial activity of phytosubstances from Vaccinium vitis-idaea leaves

Phytosubstance	Solvent	Diameter of zones of growth retardation of microorganisms, mm				
		E.coli	S.aureus	Proteus vulgaris	P. aeruginosa	Candida albicans
1	water	15±0,08	16±0,06	15±0,06	13±0,07	15±0,09
2	50% ethanol	15±0,08	11±0,06	17±0,06	9±0,05	10±0,05
3	96% ethanol	11±0,08	17±0,07	22±0,08	11±0,08	18±0,08
4	water	growth	growth	growth	growth	growth
	50% ethanol	growth	14±0,07	13±0,07	12±0,09	14±0,09
	96% ethanol	growth	12±0,07	11±0,07	12±0,07	12±0,07
5	water	growth	growth	growth	growth	growth
	50% ethanol	13±0,05	growth	14±0,06	12±0,08	14±0,08
	96% ethanol	11±0,09	11±0,05	12±0,08	13±0,08	13±0,08
6	water	growth	growth	growth	growth	growth
	50% ethanol	12±0,09	growth	14±0,09	12±0,09	15±0,09
	96% ethanol	12±0,09	11±0,09	13	12±0,09	13±0,08
7	water	12±0,09	growth	growth	growth	growth
	50% ethanol	13±0,08	growth	14±0,09	12±0,09	14±0,09
	96% ethanol	11±0,09	12±0,09	14±0,09	14±0,09	13±0,08
8	water	growth	growth	growth	growth	growth
	50% ethanol	13	17	13	15	16
	96% ethanol	13	15	15	14	16
9	water	growth	growth	growth	growth	growth

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	50% ethanol	13	12	growth	12	15
	96% ethanol	13	13	12	14	13
10	water	12	13	growth	14	15
	50% ethanol	12	14	14	14	growth
	96% ethanol	12	13	12	12	11
11	water	growth	growth	growth	growth	growth
	50% ethanol	16	16	15	14	15
	96% ethanol	12	14	14	12	13
12	water	12	growth	growth	growth	growth
	50% ethanol	18	16	16	16	17
	96% ethanol	17	15	14	14	14
13	water	growth	15	growth	14	15
	50% ethanol	12	13	growth	12	14
	96% ethanol	13	12	13	11	15
Inurec	water	14	15	14	16	15
	50% ethanol	16	13	17	16	16
	96% ethanol	16	17	16	16	15

It is evident from the table that the most pronounced antibacterial effect on the main uropathogens characteristic for phytosubstance № 12, with the most potent antibacterial effect of the extract, dissolved in 50% alcohol.

The emergence of the maximal antibacterial effect of phytosubstance №12 can be explained by a combination of phenolic compounds with arginine, which has an antibacterial effect on the major uropathogens with an average activity index and exhibits a maximum antibacterial effect against E.coli, the diameter of the growth retardation zone was 18 mm. At the same time removal of amino acids from phytosubstance № 2 leads to a decrease in antibacterial activity. Thus it was established that aminoacids in combination with phenolic compounds of Vaccinium vitis-idaea influence the solubility and bioavailability of the extract by enhancing the antibacterial effect.

Phytosubstance №1 had a mean antibacterial effect on such pathogenic strains as E.coli, S.aureus, Proteus vulgaris and Candida albicans.

Phytosubstance №2 to uropathogens E. coli (diameter of the growth retardation zone -15 mm) and Proteus vulgaris (diameter of the growth retardation zone-17 mm) showed an average antibacterial effect, or other antibiotic effect was absent from other uropathogens.

Phytosubstance №3 for S. aureus (diameter of the growth retardation region of 17 mm) and Ptoteus vulgaris (diameter of the growth retardation zone-19 mm) showed an average antibacterial effect; to other uropathogens, this phytopathogen had a low antibacterial effect.

Polysaccharide complex of Vaccinium vitis-idaea leaves (phytosubstance №4), where the physiological solution acts as a solvent, showed no antibacterial effect, while the alcoholic solutions of this extract had a low antibacterial effect. The addition of polysaccharides to phytosubstances № 2 and № 3, that is, phytosubstances №6 and №7 did not lead to an increase in antibacterial effect or had no significant effect. Thus, it has been established that polysaccharides of Vaccinium vitis-idaea leaves have a low antibacterial effect, and in combination with phenolic compounds do not significantly affect the antibacterial effect.

Phytosubstance №8, which is a complex of saponins of triterpene nature, almost did not manifest itself as an antibacterial agent. Tannins' substances of Vaccinium vitis-idaea leaves (phytosubstance № 9) also expressed antibacterial effect did not possess.

Phytosubstance №11 is a complex of phenol compounds aglycones, which dissolved in 50% alcohol, which had a medium antibacterial effect, but the antibacterial effect was less pronounced than phytosubstance №12, which contains glycosides of phenolic compounds. This indicates that the phenolic compounds of the Vaccinium vitis-idaea leaves exhibit a greater antibacterial effect in the form of glycosides.

The drug, Inurek, proved to be an antibacterial agent to which the major uropathogens were sensitive. From the table it is seen that Inurek, dissolved in alcohols, had a greater antibacterial activity than an aqueous solution.

Conclusions

1. In the work the study of antibacterial activity of 13 phytosubstances from Vaccinium vitis-idaea leaves was conducted and the most promising ones were found out. Phytosubstance № 12, which is a complex of glycosides of phenolic compounds with arginine, has the maximum antibacterial effect.
2. Polysaccharides of Vaccinium vitis-idaea leaves have a low antibacterial effect, the addition of phenolic compounds did not significantly affect the increase of the antibacterial effect.
3. Glycosides of phenolic compounds of Vaccinium vitis-idaea leaves have more pronounced antibacterial activity than their aglycones.

Prospects for further research. Obtained results are a prerequisite for the creation of effective domestic phytosanitary plants from the Vaccinium vitis-idaea leaves, and medicinal forms on its basis, in the future.

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

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The purpose of the work is to study the antibacterial activity of phytosubstances from *Vaccinium vitis-idaea* leaves. **Materials and methods**. The objects of the study were 13 phytosubstances, obtained from Vaccinium vitis-idaea leaves. The study of antibacterial activity of the extracts was performed by the method of diffusion in agar 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 research and analysis of experimental data was carried out in comparison with the standard drug Inurec containing the concentrated extract of American cranberries 150 mg (manufactured by PHARMACEERY MANUFEKCHURIN SL SPAIN) **Results**. The most pronounced antibacterial effect was phytosubstance from Vaccinium vitis-idaea leaves, which contains a complex of phenolic compounds with arginine, dissolved in 50% alcohol. It was found that amino acids in combination with phenolic compounds of Vaccinium vitis-idaea have an antibacterial effect on the main uropathogens. The polysaccharide complex of Vaccinium vitis-idaea leaves manifested itself as an agent with a low level of antibacterial effect. The aglycones of the phenolic compounds of Vaccinium vitis-idaea leaf had less pronounced antibacterial effect than their glycosides. **Conclusions**. The study of antibacterial activity of 13 phytosubstantions from Vaccinium vitis-idaea leaves was conducted. The most promising substance was a complex of glycosides of phenolic compounds from Vaccinium vitis-idaea leaves with arginine. It has found that aminoacids potentiate the antibacterial effect of phenolic compounds of Vaccinium vitis-idaea, and polysaccharides, on the contrary, were inverted. Glycosides of phenolic compounds of Vaccinium vitis-idaea usually have a more pronounced antibacterial effect than their aglycones.

Key words: phytosubstantion, leaves, Vaccinium vitis-idaea, antibacterial activity

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