Antimicrobial activity of some berries' anthocyanin complexes

Filimonova N.I., Gliebova K.V., Bosenko O.L., Morozenko D.V.
National University of Pharmacy,
Department of microbiology, virology and immunology
(Kharkiv, Ukraine)
microbiology@nuph.edu.ua
Domarev A.P.
National Technical University "Kharkiv Polytechnic Institute",
Laboratory of Biomedical Electronics
(Kharkiv, Ukraine)
dap4806@ukr.net

Introduction. Plants of Ukrainian flora today are widely used in the treatment of various diseases, due to the high biological activity of their active substances, the absence of side effects and contraindications, which makes them irreplaceable in the treatment of cardiovascular, endocrine, cancer and other human pathologies [1–3]. Researchers are significantly interested in antimicrobial properties of flavonoids. Today mechanisms of influence of quercetin on gram-positive bacteria, flavonoids – on bacteria of the genus *Staphylococcus*, the antimicrobial action also have anthocyanins complexes (ACC) are well known [4].

However, it should be noted that the antimicrobial properties of berry extracts (aronia, currants and elderberry) and their influence on specific microorganisms – human pathogens have not been sufficiently studied today. In addition, the issues of the influence of antimicrobial substances, in particular, the anthocyanins of individual berries on the microflora of the gastrointestinal tract, on the growth and development of the organism, antioxidant properties, as well as the possibility of their use in the treatment of inflammatory processes, which determines the relevance of our research, remain unclear.

The aim of the study was to determine the degree of antimicrobial activity of the anthocyanin complexes of Aronia melanocarpa (*Aronia melanocarpa*), black currant (*Ribes nigrum*), elderberry *(Sambucus nigra*) with reference culture *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* NCTC 885-653.

Materials and Methods. The research was conducted at the Department of Microbiology, Virology and Immunology at the National University of Pharmacy (Kharkiv) in 2017. The object of the research was three experimental samples of the preparations of anthocyanin complexes, obtained from *Aronia melanocarpa*, black currant (*Ribes nigrum*), elderberry black (*Sambucus nigra*). Anthocyanin complexes (ACC) containing anthocyanin glycosides were obtained by extraction (Rudakov et al., 2004). The determination of antimicrobial activity was carried out by diffusion method in the agar gel, according to **the State Pharmacopoeia of Ukraine**, in five replicates with each sample of ACC. According to World Health Organization recommendations, for the evaluation of the activity of the drugs, reference cultures of *Staphylococcus aureus ATCC 25923*, *Escherichia coli ATCC 25922*, *Bacillus subtilis ATCC 6633*, *Pseudomonas aeruginosa ATCC 27853*, *Candida albicans NSTC 885-653* were used. The purity of each culture of the microorganism was confirmed by typical morphological, tinctorial, cultural and biochemical properties.

Results and discussion. When examining antibacterial properties of anthocyanin complexes derived from black currants, black elder and aronia, it was found that in the native form ACC from black currant had the best antibacterial properties – the highest sensitivity was observed in all tested microorganisms. *Escherichia coli* and *Bacillus subtilis* had a high sensitivity to the ACC of the elderberry of the black and blue-green aronia, and *Staphylococcus aureus* and *Pseudomonas aeruginosa* – a moderate sensitivity. In the dilution of 1:1, a high sensitivity of black currant to ACC was observed only in *Bacillus subtilis*, the rest microorganisms had a moderate sensitivity.

Staphylococcus aureus, *Pseudomonas aeruginosa* and *Bacillus subtilis* had a medium sensitivity to ACC from elderberry black, *E. coli* had low-sensitivity. *Bacillus subtilis* infectious culture had an average sensitivity to the ACC from aronia; a culture of *Staphylococcus aureus* was insensitive. In the dilution 1:2, the average sensitivity to ACC of black currant and elderberry black was observed only in B. subtilis. The mechanism of action of ACC on microorganisms, obviously, is aimed at the destruction of cell walls and inhibition of nutrition of microbial cells. When investigating sensitivity to ACC from *Candida albicans* fungi, their antimycotic effect in vitro was not established. The mechanisms underlying the anthocyanin activity include both the membrane and intracellular interactions of these compounds.

The antimicrobial activity of anthocyanins contained in fruits is most likely due to multiple mechanisms and synergies, since they contain various compounds, including anthocyanins, weak organic acids, phenolic acids and mixtures of different chemical forms. Today the antimicrobial activity of crude extracts of phenolic compounds of various fruits (mainly berries) against human pathogens is extensively studied; however, there is lack of information about the antimicrobial activity of pure anthocyanins. As a rule, anthocyanins are active against different microbes; however, gram-positive bacteria are usually more susceptible to the action of anthocyanin than gram-negative bacteria.

A general study demonstrates the potential of anthocyanin extracts as natural alternative effective antimicrobial agents. In addition, the ability of the extract to reduce adhesion without reducing bacterial growth declines the probability of developing resistance while reducing the likelihood of infection. In assessing the antimicrobial properties of anthocyanins contained in the extract of Viburnum opulus fruits, there has been a significant inhibition of the growth of a wide range of human pathogenic bacteria, both gram-negative (*Salmonella typhimurium* and *Salmonella agona*) and Gram-positive (*Staphylococcus aureus*, *Lysteria monocytogenes* and *Enterococcus faecalis*).

Conclusions. The highest degree of microbiological sensitivity of bacterial cultures *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, derived from black currant (*Ribes nigrum*), was observed to anthocyanin complexes in the native form; *Escherichia coli* and *Bacillus subtilis*, obtained from elder black (*Sambucus nigra*) to the anthocyanin complexes in native form. It indicates their high antimicrobial activity against the microorganisms mentioned above. The decrease in the concentration of anthocyanin complexes in the alcohol extract at dilution in the ratio of 1:1 and 1:2 significantly reduced microbiological sensitivity to these microorganisms of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis and Pseudomonas aeruginosa.* In the study of the effects of anthocyanin complexes from *Aronia melanocarpa*, *Ribes nigrum*, Sambucus nigra on *Candida albicans* fungi culture, no sensitivity was established, which indicates the absence of antimycotic activity of the above complexes in laboratory conditions in vitro. Anthocyanin complexes obtained from *Aronia melanocarpa*, *Ribes nigrum*, *Sambucus nigra* are complex according to the chemical composition of the compound for which the integral value of antioxidant activity extracts of the samples under study is a quantitative estimate of the total content of the bioflavonoids that determines their antimicrobial activity in vitro.

References

1. Dietary antioxidants, peroxidation and cardiovascular risks / Barbaste M., Berké B., Dumas M. [et al.] // The Journal of Nutrition, Health & Aging. – 2002. – № 6(3). – P. 209–222.

2. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications / Brewer M. S. // Comprehensive Reviews in Food Science and Food Safety. – 2011. – № 10. – P. 221–246.

3. Potential Mechanisms of Cancer Chemoprevention by Anthocyanins Current / De-Xing Hou // Molecular Medicine. – 2003. – № 3. – P. 149–159.

4. RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity / C. Proestos, N. Chorianopoulos, G.J. Nychas [et al.]. // Journal of Agricultural and Food Chemistry. – 2005. – № 53(4). – P. 1190–1195.