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THE APPLICATION OF ARBUTIN IN ELIMINATION RESISTANCE GRAMM-NEGATIVE MULTIDRUG RESISTANCE BACTERIA OF *PSEUDOMONAS AERUGINOSA* AND *ENTEROBACTER CLOACEA*

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The basic therapy against microbial infections is the application of antibiotics. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multidrug-resistant strains.
The aim. *The purpose of our work was to investigate the in vitro and in silico elimination resistance of antibiotics (clarithromycin, azithromycin, gentamycin, ciprofloxacin, levofloxacin, ceftriaxone, and chloramphenicol) against clinical multidrug-resistant strains of P. aeruginosa and E. cloacae by arbutin.*
Materials and methods. *The molecular docking was performed using AutoDockTools 1.5.6; antimicrobial effects were evaluated by the well method. Isolates were obtained from clinical samples including tracheal aspirate and broncoalveolar lavage.*
Results. *Theoretical studies have found that none of the investigated antibiotics and arbutin highly selectively inhibit all «targets» mechanisms of antimicrobial action. In experimental studies, it was observed that adding arbutin to the antibiotic led to the emergence of sensitivity on the part of resistant strains. All Gramm-negative resistance strains of bacteria were sensitive to the action of arbutin. Moreover, arbutin increased the antimicrobial effect of antibiotics from 8 to 55 %. It was estimated exceptions such as clarithromycin and azithromycin when assessing antimicrobial activity against P. aeruginosa.*
Conclusions. *These studies have shown that inhibiting resistant strains of bacteria requires the use of combinations of "classical" antimicrobials and herbal drugs or dietary supplements based on extracts obtained from arbutin-containing medicinal plants such as lingonberry, bearberry, and cranberry. This approach is a "lifeline" for the development of antimicrobial agents against resistant bacteria and gives "a second chance to return to life" for outdated antibiotics*
Keywords: *arbutin, multi-drug resistant, Gram-negative strains, molecular docking, removal resistance, antibiotics*

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1. Introduction

Today, antimicrobial resistance is the number one problem worldwide. One of the first mentions of the emergence of antibiotic-resistant strains of bacteria in humans was obtained during military conflicts in Iraq and Afghanistan 20 years ago. [1].

To date, no statistics have been officially published on the resistant strains of bacteria that have been isolated from combat wounds during the current conflict in Ukraine. However, between 2014 and 2020, statistics have shown that the detection rate of multi-resistant strains of bacteria in combat wounds was significantly higher than in civilian hospitals [2]. In addition, according to the latest data, it has found that *Acinetobacter baumani, Pseudomonas aeruginosa* and *Klebsiela pneumonia* are predominant among all isolated pathogens. Among all gram-negative bacteria (*A. baumani, P. aeruginosa and K. pneumonia*), 71.3 % were resistant to the antibiotic carbapenem, which is the last "line of defence" against resistant strains [3]. In March 2022, the European Center for Disease Prevention and Control reported that Ukrainian refugees with traumatic wounds may have resistant strains of *A. baumani* and *K. pneumonia* and made recommendations for isolating isolates and conducting screening studies [4]. At a German clinic in Frankfurt am Main, staff reported treating traumatic wounds in 103 Ukrainian patients between March and June 2022. Among all admitted patients, 17 % had resistant gram-negative strains of bacteria. [5] Thus, in light of data on the rapid spread of resistant strains of bacteria, it is necessary to search for new antimicrobial compounds.

Before the creation and use of antibiotics, people have applied drugs based on medicinal plants, such as lingonberry (*Vaccinium vitis-idaea* L.), bearberry (*Arctostaphylos uva-ursi* L.) and cranberry (*Vaccinium macrocarpon* L.) to treat infectious diseases [5]. The plants mentioned above are a rich source of tannins, flavonoids, hydroxycinnamic acids and hydroquinone derivatives [6]. Arbutin is a main constituent among hydroquinone derivatives; it is a β-D-glucopyranoside of hydroquinone presented in the medicinal plants of the *Ericaceous* family [7]. (Fig. 1) The leaves of the mentioned medicinal plants have been applied in folk medicine for the treatment of urinary infection diseases such as cystitis, pyelonephritis and glomerulonephritis. The antimicrobial

mechanism of arbutin still has not been investigated in all detail for today. However, recent research has shown that arbutin could destroy the bacterial membrane, and the influence of intracellular substances affects the synthesis of proteins and inhibits DNA-gyrase [8].

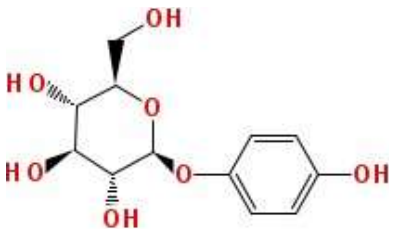

Fig. 1. The structural formula of arbutin

Thus, the purpose of our work was investigate *in vitro* and *in silico* elimination resistance of antibiotics (clarithromycin, azithromycin, gentamycin, ciprofloxacin, levofloxacin, ceftriaxone and chloramphenicol) against clinical multidrug-resistant strains of *P. aeruginosa, E. cloacae* by arbutin**.**

2. Planning of the research

The methodology of investigation of the application of arbutin in elimination resistance of antibiotics against gramm-negative multidrug resistance bacteria of *Pseudomonas aeruginosa* and *Enterobacter cloacea* includes:

1. Evaluation of the scientific sources.

2. Establishing antibacterial and anti-biofilm «targets» against Gramm-negative strains.

3. The molecular docking of arbutine and antibacterial drug standards against crucial antibacterial and anti-biofilm «targets».

4. The investigation antibacterial activity *in vitro* of arbutine and antibacterial drug standards as well as their combination against multidrug resistance bacteria of *Pseudomonas aeruginosa* and *Enterobacter cloacea.*

5. A conclusion about the perspective of the possibility of applying arbutin in the elimination of resistance to multidrug bacteria is made.

3. Materials and Methods

The scientific research was provided by National University of Pharmacy in collaboration with Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine during 2024 year.

3. 1. Reagents

Arbutin (≥98.0 %) was purchased in Sigma Aldrich Company, Lublin, Poland; clarithromycin (≥98.0 %); azithromycin (≥98.0 %); gentamycin (≥98.0 %); ciprofloxacin (≥98.0 %); levofloxacin (≥98.0 %); ceftriaxone (≥98.0 %); chloramphenicol (≥98.0 %) were provided by pharmaceutical company «Astrapharm» Kyiv, Ukraine; and by pharmaceutical company «Zdravopharm», Kharkiv, Ukraine.

3. 2. Test organisms

Two clinical isolates of multidrug-resistant Gram-negative bacteria were chosen for research: *Pseudomonas aeruginosa 18* and *Enterobacter cloacae 17.* Isolates from clinical samples, including tracheal aspirate and bronchoalveolar lavage, were provided by the Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine, Kharkiv. All strains are stored and accepted by the Head of Museum of strains – O.G. Peretyatko. *Pseudomonas aeruginosa 18* and *E. cloacea 17* were accepted on 01 November 2022.

3. 3. Screening antimicrobial activity

The method of diffusion of the drug into agar carried out using the method of "wells" [9, 10]. Table 1 shows the interpretation criteria for microbial sensitivity.

Table 1

Interpretation criteria for microbial sensitivity

Microbial sensitivity	Diameter of the growth retardation zone, mm
High sensitivity	>25
Sensitive	15–25
Low sensitivity	10–15
Not sensitivity	<10

3. 4. Molecular docking

A molecular docking study was conducted using the tool AutoDockTools 1.5.6 [11]. The preparation of the protein involved an optimization process, which included removing water and other atoms and adding a polar hydrogen group. Autogrid was used to configure the grid coordinates (*X*, *Y*, and *Z*) on the binding site. Genetic algorithm parameters were applied for ligand interaction, with 10 runs of this criterion.

DNA-gyrase (PDB ID: 1KIJ), DHFR (PDB ID: 1RX3), deacytelese (PDB ID: 3UHM), acyl-homoserinelactone synthase (AHS) LasI (PDB ID: 1RO5), acyl-homoserinelactone synthase (AHS) RhI (PDB ID: 1KZF), diguanylate cyclase (PDB ID: 3BRE) structures were obtained from PDB database [12]. The resolution of 1KIJ was 2.30 Å, 1RX3 – 2.20 Å, 3UHM – 2.20 Å, 1RO5 – 2.30 Å, 1KZF – 2.20 Å, 3BRE – 2.40 Å. For the docking experiment, protein structure is selected if the resolution is above 2 Å. So, all mentioned proteins can be used for the experiment. The ligand structures of arbutin (CID_12303220), clarithromycin (CID_84029); azithromycin (CID_447043); gentamycin (CID_3467); ciprofloxacin (CID_2764); levofloxacin (CID_149096); ceftriaxone (CID_5479530); chloramphenicol (CID_5959) were obtained from PubChem database [13]. The active site of the docking protein was identified utilizing the Computed Atlas for Surface Topography of Proteins (CASTp) [14].

3. 5. Statistical analysis

The measurements were conducted in five replicates. The results were expressed as mean values accompanied by standard deviation, reflecting the level of certainty in the measurements. Statistical analysis was performed using MS Excel 7.0 and Statistica 6.0 software, enabling thorough data evaluation and interpretation.

4. Results

4. 1. Molecular docking

A theoretical investigation of the antimicrobial activity of the arbutin and antibiotics were carried out using molecular docking, in order to understand their promising capabilities for suppressing the growth of gram-negative strains of bacteria. The assessment the antimicrobial effect was conducted with six key enzymes: DNA-gyrase, DHFR, Deacytelese, AHS LasI, AHS RhI and Diguanylate cyclase. A six groups of the most applied antimicrobial drugs were chosen as standards of comparison in theoretical study such as a group of aminoglycosides (Gentamycin), fluoroquinolones (Ciprofloxacin, Levofloxacin), β-lactames (Ceftriaxone), amphenicols (Chloramphenicol), macrolides (Clarithromycin, Azithromycin) and 5-nitroimidazole drugs (Metronidazole, Ornidazole).

In the indexed scientific journals Scopus and Web of Science, there are a large number of works with molecular docking on the study of the pharmacological activity of different groups of compounds. But, the main problem of these studies is the lack of rating assessment of the efficiency of binding of the ligand to the active site. A number of scientific works have used comparison standards, but in our view, this method is not promising as since more than one standard may be used for the enzyme protein being studied. Thus, this method of assessment will lead to confusion in the data among scientists. To understand the level of selectivity of inhibition of the studied substances to the active centers of bacterial enzymes, we applied the following classification of selectivity [15]: IC50<0.001 mM (high selective); 0.05>IC50>0.01 (medium selective); IC50>0.05 mM (low selective).

Molecular modelling of the identified compounds was carried out with the active site of DNA-gyrase. The active site was represented by the following amino acids: Arg75, Lys102, Arg135, Asp80. Trp387, Lys109, Asp72 and Thr166. According to the results of the study and conditional rating, it was established that clarithromycin, azithromycin, levofloxacine and arbutin were highly selective to the active site. In contrast, ciprofloxacin and chloramphenicol were medium selective and ornidazole, ceftrioxane, metronidazole, gentamycine were low selective (Table 2).

The next enzyme that was studied was DHFR. The active center of this enzyme was represented by the following amino acids: NADP, Tyr110, Asp30, Ile8, Phe34, Ile104, Arg55, Arg60. According to the results shown in Table 3, the following compounds had high selectivity: clarithromycin, azithromycin, levofloxacine and arbutin, whereas ornidazole and metronidazole were low selective.

Molecular modelling of the studied compounds was carried out with the active site of Deacytelese. The active center was represented by the following amino acids: Thr190, Lys238, Gly92. Phe191, Leu18, Ala206. According to the results of the study and conditional classification, it was established that clarithromycin, azithromycin, levofloxacine and arbutin had the highest selectivity, whereas ornidazole and metronidazole had the lowest selectivity (Table 4).

The AHS LasI was the next enzyme that was studied by molecular docking. The active center of this enzyme was represented by the following amino acids: Thr142, Thr144, Val143, Phe27, Arg30, Arg104, Met79, Leu102, Phe106, Ser103. According to the results shown in Table 5, the following compounds had the highest level of selectivity: arbutin, chloramphenicol, whereas ornidazole, metronidazole, levofloxacine, ciprofloxacine had the lowest level of selectivity as well as gentamycine, azithromycin and clarithromycin were not interact with active center of AHS LasI (Table 5).

Molecular modelling of the studied compounds was carried out with the active site of AHS RhI. The active center was represented by the following amino acids: Asp48, Tyr54, Met42. Leu63, Leu56. According to the results of the study and conditional classification, it was established that only clarithromycin and azithromycin had high selectivity, whereas ornidazole, metronidazole, and ceftrioxane had the lowest level of binding as well and gentamycin did not interact with protein (Table 6).

The diguanylate cyclase was the last protein enzyme that was assessed by molecular docking. The active center was represented by the following amino acids: Glu254, Glu253, Glu252, Lys327, Arg331, Thr262, Arg198, Arg194. The obtained results showed that there were any high selective inhibitors, in this case arbutin, clarithromycin, chloramphenicol, ciprofloxacin had medium selectivity, whereas levofloxacin, ceftriaxone, metronidazole, ornidazole, gentamycine and azithromycin had the lowest level of selectivity to the active site. (Table 7)

Table 2

Results of molecular docking of the arbutin and antimicrobials drug standards with the DNA-gyrase structure

No.	Ligand	DNA-gyrase		
		$\Delta Gbind^a$ (kcal/mol)	Ki^b (mmol)	Level of selectivity
1	Clarithromycin	–11.59	0.00000001087	High selective
2	Azithromycin	–10.29	0.00000061435	High selective
3	Levofloxacin	–8.69	0.00042853	High selective
4	Arbutin	–8.23	0.00093344	High selective
5	Ciprofloxacin	–8.06	0.00123	Medium selective
6	Chloramphenicol	–6.38	0.02114	Medium selective
7	Ornidazole	–5.07	0.19214	Low selective
8	Ceftriaxone	–4.61	0.41631	Low selective
9	Metronidazole	–4.54	0.46734	Low selective
10	Gentamycin	–4.08	1.03	Low selective

Note: [a] – free-binding energy; [b] – inhibition constant, IC50, mmol.

Further, all antibiotics and arbutin were conditionally divided into two categories. The first category included compounds that had a high selectivity for the active site, and the second category included compounds that had medium and low selectivity. This compound separation approach was necessary to clearly identify compounds that interact highly effectively with antimicrobial mechanisms and which compounds work below this level. According to the results shown in Table 8, there were no compounds that inhibit highly selectively all antibacterial mechanisms. Clarithromycin was the best antibiotic that inhibited approximately all mechanisms of the "first line of defence" and biofilm formation, except AHS LasI and diguanylate cyclase. The next antibiotic that inhibit high selectively antibacterial mechanisms was levofloxacine. The levofloxacine actively inhibited all enzymes of "first line of defense" such as DNA-gyrase, DHFR, Deacytelese. The last antibiotic that highly selectively inhibited antibacterial enzyme was chloramphenicol, an antimicrobial drug actively binding only with one enzyme of biofilm formation – AHS LasI, whereas other mechanisms were inhibited at the lower level (Table 8).

Table 3

Results of molecular docking of the arbutin and antimicrobials drug standards with the DHFR structure

No.	Ligand	DHFR		
		Δ*Gbind*[a] (kcal/mol)	*Ki*[b] (mmol)	Level of selectivity
1	Clarithromycin	–16.78	0.000000000504	High selective
2	Azithromycin	–14.5	0.00000002336	High selective
3	Levofloxacin	–8.98	0.00026376	High selective
4	Arbutin	–9.17	0.00019023	High selective
5	Ciprofloxacin	–8.44	0.00064808	Medium selective
6	Chloramphenicol	–7.97	0.00143	Medium selective
7	Gentamycin	–6.78	0.01073	Medium selective
8	Ceftriaxone	–6.36	0.02164	Medium selective
9	Ornidazol	–4.95	0.23625	Low selective
10	Metronidazole	–4.28	0.72416	Low selective

Note: [a] – free-binding energy; [b] – inhibition constant, IC50, mmol.

Table 4

Results of molecular docking of the arbutin and antimicrobials drug standards with the deacytelese structure

No.	Ligand	Deacytelese		
		Δ*Gbind*[a] (kcal/mol)	*Ki*[b] (mmol)	Level of selectivity
1	Azithromycin	–14.04	0.000000051	High selective
2	Clarithromycin	–13.98	0.000000057	High selective
3	Levofloxacin	–8.34	0.00077565	High selective
4	Arbutin	–8.40	0.00070067	High selective
5	Ciprofloxacin	–7.51	0.00313	Medium selective
6	Chloramphenicol	–7.19	0.00536	Medium selective
7	Gentamycin	–7.45	0.00346	Medium selective
8	Ceftriaxone	–6.09	0.03444	Medium selective
9	Ornidazole	–5.32	0.12638	Low selective
10	Metronidazole	–5.20	0.15555	Low selective

Note: [a] – free-binding energy; [b] – inhibition constant, IC50, mmol.

Table 5

Results of molecular docking of the arbutin and antimicrobials drug standards with the AHS LasI structure

No.	Ligand	AHS LasI		
		Δ*Gbind*[a] (kcal/mol)	*Ki*[b] (mmol)	Level of selectivity
1	Arbutin	–12.21	0.0000012	High selective
2	Chloramphenicol	–10.76	0.00001304	High selective
3	Ceftriaxone	–6.56	0.01561	Medium selective
4	Ornidazole	–5.83	0.05331	Low selective
5	Metronidazole	–5.38	0.113	Low selective
6	Levofloxacin	–4.11	0.97221	Low selective
7	Ciprofloxacin	–2.41	16.98	Low selective
8	Gentamycin	–	–	Inactive
9	Azithromycin	–	–	Inactive
10	Clarithromycin	–	–	Inactive

Note: [a] – free-binding energy; [b] – inhibition constant, IC50, mmol.

Table 6

Results of molecular docking of the arbutin and antimicrobials drug standards with the AHS RhI structure

No.	Ligand	AHS RhI		
		ΔGbind[a] (kcal/mol)	Ki[b] (mmol)	Level of selectivity
1	Clarithromycin	–18.54	0.0000000000253	High selective
2	Azithromycin	–10.16	0.00003572	High selective
3	Ciprofloxacin	–7.84	0.00178	Medium selective
4	Arbutin	–7.54	0.00298	Medium selective
5	Levofloxacin	–6.62	0.01408	Medium selective
6	Chloramphenicol	–5.88	0.04912	Medium selective
7	Ornidazole	–5.20	0.15405	Low selective
8	Metronidazole	–5.11	0.18023	Low selective
9	Ceftriaxone	–4.48	0.51643	Low selective
10	Gentamycin	–	–	Inactive

Note: [a] – free-binding energy; [b] – inhibition constant, IC50, mmol.

Table 7

Results of molecular docking of the arbutin and antimicrobials drug standards with the diguanylate cyclase structure

No.	Ligand	Diguanylate cyclase		
		ΔGbind[a] (kcal/mol)	Ki[b] (mmol)	Level of selectivity
1	Arbutin	–8.06	0.001230	Medium selective
2	Clarithromycin	–8.03	0.00131	Medium selective
3	Chloramphenicol	–6.59	0.01488	Medium selective
4	Ciprofloxacin	–6.31	0.02356	Medium selective
5	Levofloxacin	–5.32	0.12516	Low selective
6	Ceftriaxone	–5.19	0.15567	Low selective
7	Metronidazole	–4.94	0.23835	Low selective
8	Ornidazole	–4.72	0.34569	Low selective
9	Gentamycin	–4.49	0.51373	Low selective
10	Azithromycin	–2.79	8.97	Low selective

Note: [a] – free-binding energy; [b] – inhibition constant, IC50, mmol.

Table 8

Schematic division of antimicrobial drug standards and arbutin in two categories

No.	Compound	DNA-gyrase	DHFR	Deacytelese	AHS LasI	AHS RhI	Diguanylate cyclase	No. of inhibition enzymes of «First line of protection»	No. of inhibition enzymes of «Biofilm»
Antimicrobial drug standards									
1	Clarithromycin							3	1
2	Chloramphenicol							0	1
3	Ciprofloxacin							1	0
4	Levofloxacin							3	0
5	Ceftriaxone							0	0
6	Metronidazole							0	0
7	Ornidazole							0	0
8	Gentamycin							0	0
9	Azithromycin							3	1
Biological active compounds									
11	Arbutin							3	1

Note: green colour – high level of selectivity; red colour – lower and medium of selectivity.

Compared with antibiotics standards, arbutin hit all «targets» antibacterial mechanisms of the "first line of defence" of bacteria and one mechanism of biofilm formation, AHS LasI.

3. 2. Antimicrobial activity

In this research work, the antimicrobial activity of the arbutin, antibiotics and their combination at one concentration was investigated against the following antimicrobial resistance strains of *P. aeruginosa, E. cloacae*. According to the obtained results, all Gramm-negative resistance strains of bacteria were sensitive to the action of arbutin.

The bacterial strain *P. aeruginosa* was completely resistant to the action of the studied antibiotics, except for chloramphenicol, which had a low inhibitory effect on the strain (12.50±0.50 mm). In combination with arbutin, resistance to ciprofloxacin, levofloxacin, gentamicin, metro-

nidazole and ornidazole was eliminated, and in the case of chloramphenicol, the inhibitory activity increased by 29 %. However, *P. aeruginosa* remained resistant to clarithromycin, azithromycin and ceftriaxone (Table 8).

When studying the antimicrobial effect of antibiotics against the *E. cloacea* strain, it was found that clarithromycin, azithromycin, ciprofloxacin, metronidazole and ornidazole did not inhibit the growth of the *E. cloacea* strain. Meanwhile, in the case of chloramphenicol, levofloxacin, ceftriaxone, and gentamicin, moderate inhibition of strain growth was observed. When arbutin was added to antibiotics, it was found that clarithromycin, azithromycin, ciprofloxacin, metronidazole and ornidazole began to actively inhibit the resistant strain of *E. cloacea*. Moreover, it was found that after the addition of arbutin, the antimicrobial effect of chloramphenicol increased by 13 %, levofloxacin by 4 %, and gentamicin by 6 % (Table 9).

Table 9

Inhibition diameter (mm) resulting from the screening of antimicrobial effect against resistance strains of *P. aeruginosa* and *E. cloacae* by well diffusion method with arbutin and antibiotic standards

Sample	Concentration, mM	Diameter of the growth retardation zone, mm±SD			
		P. aeruginosa 18	*Difference, %*	*E. cloacae 17*	*Difference, %*
Arbutin	0.003	12.00±0.20	–	12.00±0.20	–
Clarithromycin	0.003	Growth	–	Growth	–
Arbutin+Clarithromycin	0.003+0.003	Growth	0	23.50±0.50	+100 %
Arbutin	0.003	12.00±0.20	–	12.00±0.20	–
Azithromycin	0.003	Growth	–	Growth	–
Arbutin+Azithromycin	0.003+0.003	Growth	0	23.00±0.20	+100 %
Arbutin	0.003	12.00±0.20	–	12.00±0.20	–
Chloramphenicol	0.003	12.50±0.50	–	19.50±0.50	–
Arbutin+Chloramphenicol	0.003+0.003	17.50±0.50	+29 %	22.50±0.50	+13 %
Arbutin	0.003	12.00±0.20	–	12.00±0.20	–
Ciprofloxacin	0.003	Growth	–	Growth	–
Arbutin+Ciprofloxacin	0.003+0.003	22.50±0.50	+100 %	23.00±0.20	+100 %
Arbutin	0.003	12.00±0.20	–	12.00±0.20	–
Levofloxacin	0.003	Growth	–	23.50±0.50	–
Arbutin+Levofloxacin	0.003+0.003	24.50±0.50	+100 %	24.50±0.50	+4 %
Arbutin	0.003	12.00±0.20	–	12.00±0.20	–
Ceftriaxone	0.003	Growth	–	23.00±0.20	–
Arbutin+Ceftriaxone	0.003+0.003	Growth	0	25.00±0.20	8 %
Arbutin	0.003	12.00±0.20	–	12.00±	–
Metronidazole	0.003	Growth	–	Growth	–
Arbutin+Metronidazole	0.003+0.003	16.50±0.50	+100 %	21.00±0.50	+100 %
Arbutin	0.003	12.00±0.20	–	12.00±0.20	–
Ornidazole	0.003	Growth	–	Growth	–
Arbutin+Ornidazole	0.003+0.003	15.50±0.50	+100 %	20.00±0.50	+100 %
Arbutin	0.003	12.00±0.20	–	12.00±0.20	–
Gentamycin	0.003	Growth	–	22.00±0.20	–
Arbutin+Gentamycin	0.003+0.003	21.00±0.20	+100 %	23.50±0.50	+6 %

Note: SD – standard deviation, n=3.

5. Discussion

5. 1. Molecular docking

The mechanisms of resistance of pathogens are achieved through the mode the antibacterial drug has affected. Above all, the resistance mostly depends on the chemical structure of the drug and the target site. Generally, antimicrobial resistance usually depends on biochemical and genetic aspects. Moreover, the high application of antimicrobial drugs in agriculture and the low level of infection control in health care caused further acceleration of the crisis [16]. Clarithromycin, azithromycin, chloramphenicol, ciprofloxacin, levofloxacin, ceftriaxone, metronidazole, ornidazole, gentamycin were chosen for research as according to WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) these mentioned antibiotics are the most susceptible to resistance from Gram-negative and Gram-positive strains.

Nowadays, a large number of multidrug-resistant bacteria, also called "superbacteria," have been reported worldwide. Most of the "superbacteria" are represented by gram-negative bacteria such as *A. baumanni, K. pneumonia, P. aeruginosa* and *E. cloacae* [17]. In order to inhibit the growth of any bacteria, you need to effectively influence 3 main mechanisms: DNA gyrase, DHFR and inhibition of membrane formation. DNA gyrase is an enzyme responsible for the temporary division of bacterial DNA into two strands, subsequently the replication stage begins. The next important enzyme is DHFR; this enzyme is responsible for forming folic acid, which is necessary for the existence of bacteria [18]. One of the main defence mechanisms of any bacteria is its membrane, and gram-negative strains are no exception to the rule. The membrane of gram-negative bacteria contains a special liposaccharide that causes an immune system response and fever. The enzyme UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase is responsible for the synthesis of liposaccharide; this enzyme has no homologs in humans and mammals and is present only in bacteria [19].

But, the main problem of multi-resistant strains of bacteria is that they can form biofilms, thereby preventing the bacteria from penetrating antibiotics into the bacterial cell itself. The mechanism of biofilm formation in gram-negative bacteria is the formation of a quorum system. The quorum system is a type of cellular

signalling that relies on the production and perception of chemical signalling molecules called autodoctors. For the formation of these signal molecules, the protein acyl-homoserine lactone synthetase LasI and RhI is responsible [20]. Also, one of the main stages of biofilm formation is the cell adhesion of bacteria to the surface. Adhesions require a signalling molecule, cyclic di-guanylate monophosphate (c-di-GMP). This molecule coordinates "the transition of the bacterial lifestyle from motile to immobile." c-di-GMP is synthesized from two molecules of guanylate triphosphate by the enzyme guanylate cyclase [21]. Thus, in order to inhibit the growth and development of "superbugs", it is necessary to act on six mechanisms: DNA gyrase, DHFR, deacetylases (membrane synthesis), AHS Las and RhI (biofilm formation), and diguanyl cyclase (cell adhesion).

According to the results obtained, it was found that none of the investigated antibiotics highly selectively inhibits all «targets» mechanisms of antimicrobial action. But, arbutin was shown excellent binding energy values against all the above-mentioned «targets». We suggest that a complex of antimicrobial drugs and arbutin or a complex of natural compounds should be used to inhibit the growth of "superbacteria". According to our results, chloramphenicol works highly effectively through only one mechanism – AHS LasI; clarytromycine was effective against DNA gyrase, DHFR, deacetylase, AHS RhI and diguanylate cyclase; levofloxacine works well against DNA gyrase, DHFR, deacetylase and AHS RhI; ciprofloxacin was a high inhibitor against DHFR mechanism only.

5. 2. Antimicrobial activity

A serious threat to human health is the emergence of "superbacteria". This issue is especially relevant to *P. aeruginosa* and *E. cloacae*. These bacterial strains are capable of causing nosocomial infections and respiratory-associated pneumonia. The above-mentioned bacteria have been isolated that are resistant to aminoglycosides and fluoroquinolones, as well as to the action of the "last line of defense" – carbapenems [22]. The scientific community has identified 3 main mechanisms of resistance to antibiotics: internal, acquired and adaptive resistance. Internal resistance consists of low membrane permeability, as well as the expression of genes responsible for the production of enzymes, which are inactivated by antibiotics. Acquired resistance is based on mutational changes or horizontal gene transfer. Adaptive resistance of bacteria is expressed in the formation of biofilms, which prevent the penetration of antibiotics into the bacterial cell [23].

Our studies showed that no antibiotic had an antimicrobial effect against *P. aeruginosa*, except for chloramphenicol. The bacterial strain *E. cloacea* was resistant to clarithromycin, azithromycin, metronidazole and ornidazole. The studied bacterial strains were not resistant to the antimicrobial action of arbutin.

Theoretical studies have shown that arbutin is a highly selective inhibitor of all targeted mechanisms of "first line of defence" and one biofilm mechanism – AHS LasI. In experimental studies, it was found that the addition of arbutin to the antibiotic led to the emergence of sensitivity on the part of resistant strains. Moreover, arbutin increased the antimicrobial effect of antibiotics from 8 to 55 %. We hypothesize that arbutin actively affects antimicrobial mechanisms that are resistant to antibiotics, thereby eliminating the resistance of bacterial strains. In research work, it was found exceptions such as clarithromycin and azithromycin when assessing antimicrobial activity against *P. aeruginosa*. This fact may be due to the fact that arbutin could be low-selective with respect to inhibition of the 5OS-ribosomal subunit. This high resistance to the group of macrolides can be explained by the fact that macrolides are used uncontrolled in any treatment of various infectious diseases.

This method of eliminating resistance can be used to "bring back to life" outdated antimicrobial drugs. Because the creation and development of new antibiotics are time-consuming and expensive. In addition to the above, we would like to note that arbutin, when compared with other antibiotics such as metronidazole, ornidazole, gentamicin, ceftriaxone, has minimal side effects. High doses of arbutin are not possessed nephrotoxicity, ototoxicity and hepatotoxicity as antibiotics from the group of aminoglycosides, cephalosporins and 5-nitroimidazoles.

Based on the above results, we can conclude that in order to obtain a highly effective antimicrobial drug against resistant strains, a complex of "classical" antimicrobial drugs and herbal drugs or dietary supplements based on extracts from arbutin-containing medicinal plants such as lingonberry, bearberry and cranberry should be used in treatment therapies.

Practical relevance. The application of arbutin in elimination resistance of antibiotics against multidrug resistance bacteria of *P. aeruginosa*, *E. cloacae.*

Research limitations. The research did not study antibiotics from a group of carbapenems.

Prospects for further research. Investigation influence of arbutin on biofilm-formation of *P. aeruginosa, E. cloacae*.

6. Conclusion

Theoretical studies have shown that no "classical" antibiotic is a highly selective inhibitor of all "targeted" antimicrobial mechanisms of gram-negative bacteria, unlike arbutin, which showed excellent selectivity for all mechanisms. Experimental studies have found that arbutin helps eliminate antibiotic resistance against bacterial strains *P. aeruginosa* and *E. cloacae*. These studies show that inhibiting resistant strains of bacteria requires the use of combinations of "classical" antimicrobial drugs and herbal drugs or dietary supplements based on extracts obtained from arbutin-containing medicinal plants such as lingonberry, bearberry, and cranberry. This approach is a "lifeline" for the development of antimicrobial agents against resistant bacteria and gives "a second chance to return to life" for outdated antibiotics.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be made available at a reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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