Indonesian Journal of Pharmacy

VOL 36 (2) 2025: 236–245 | RESEARCH ARTICLE

The Application Arbutin in Elimination Resistance of Antibiotics Against Gramm-Negative Multi-Drug Resistance Bacteria of *Acinetobacter baumanni* and *Klebsiella pneumoniae*

Oleksandr Maslov[1*], Mykola Komisarenko[2], Svitlana Ponomarenko[3], Tetiana Osolodchenko[3], Artem Marchenko[2], Sergii Kolisnyk[1], Oleh Koshovyi[4] and Andrey Komissarenko[5]

1. Department of General Chemistry, National University of Pharmacy, Kharkiv, Ukraine.
2. Department of Pharmacognosy and Nutricology, National University of Pharmacy, Kharkiv, Ukraine.
3. Laboratory of Biochemistry and Biotechnology, Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine, Kharkiv, Ukraine.
4. Faculty of Medicine, University of Tartu, Tartu, Estonia.
5. Pharmaceutical Company "ZDRAVOPHARM", Kharkiv, Ukraine.

Article Info

Submitted: 12-07-2024
Revised: 22-08-2024
Accepted: 26-08-2024

*Corresponding author
Oleksandr Maslov

Email:
alexmaslov392@gmail.com

ABSTRACT

The purpose of our work was investigating *in vitro* and *in silico* elimination resistance of antibiotics against clinical multidrug-resistant strains of *Acinetobacter baumanni, Klebsiella pneumoniae* by arbutin. The molecular docking was performed using AutoDockTools 1.5.6; antimicrobial effects were evaluated by the well method. Theoretical studies have found that none of the investigated antibiotics and arbutin highly selectively inhibits all "targets" mechanisms of antimicrobial action. In experimental studies, it was observed that the addition of 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 *A. baumani* and *P. aeruginosa*. These studies haves shown that to inhibit resistant strains of bacteria, require 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the development of antimicrobial agents against resistant bacteria and gives “a second chance to return to life” for outdated antibiotics.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

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 (Mende et al., 2022). 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 (Kondratiuk *et al.*, 2021). In addition, according to the latest data, it has found that *Acinetobacter baumanni, Pseudomonas aeruginosa* and *Klebsiela pneumoniae* are predominant among all isolated pathogens. Among all gram-negative bacteria (*A. baumanni, P. aeruginosa and K. pneumoniae*), 71.3% were resistant to the antibiotic carbapenem, which is the last “line of defense” against resistant strains (Petrosillo et al., 2023). In March 2022, the European Center for Disease Prevention and Control reported that Ukrainian refugees with traumatic wounds may have resistant strains of *A. baumanni, K. pneumoniae*, and made recommendations for isolating isolates and

conducting screening studies (World Health Organization, 2022). At 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 (Schultze *et al.*, 2023). Thus, in light of data on the rapid spread of resistant strains of bacteria, it is necessary to search for new antimicrobial compounds.

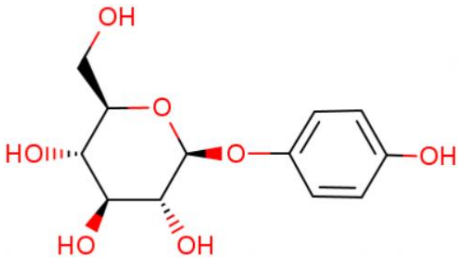

Figure 1. Structure of phenyl β-D-glucopyranoside

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 (Schultze *et al.*, 2023). The above-mentioned plants are a rich source of tannins, flavonoids, hydroxycinnamic acids and hydroquinone derivatives (Maslov *et al.*, 2023). Arbutin is a main constituent among hydroquinone derivatives, it a β-D-glucopyranoside of hydroquinone presented in the medicinal plants of *Ericaceous* family (Zhou *et al.*, 2019). (Figure 1) The leaves of the mentioned medicinal plants have been applied in folk medicine for treatment urinary infection diseases such as cystitis, pyelonephritis and glomerulonephritis. The antimicrobial mechanism of arbutin still have not investigated in all details for today. However, recent research has shown that arbutin could destroy the bacterial membrane, influence of intracellular substances affects synthesis of proteins and inhibit DNA-gyrase (Ma *et al.*, 2019). Include a clear description of materials, equipment, and methods in sufficient detail to allow repetition of the work elsewhere. Also, describe all safety considerations including any procedures requiring special precautions in sufficient detail so that those repeating the experiments can take appropriate safety measures. Published procedures should be cited, but not described, except for substantial modifications. Ethical clearance must be obtained for any study involving animal or human subjects.

MATERIALS AND METHODS

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" Kiev, Ukraine; and by pharmaceutical company "Zdravopharm", Kharkiv, Ukraine.

Test organisms

A three clinical isolates of multidrug-resistant Gram-negative bacteria were chosen for research: *Acinetobacter baumanni 150, Klebsiella pneumonic 18, Pseudomonas aeruginosa 18* and *E. cloacea 17.* Isolates from clinical samples including tracheal aspirate and broncoalveolar lavage, were provided by 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. *Acinetobacter baumanni 150, Klebsiella pneumonic 18, Pseudomonas aeruginosa 18* and *E. cloacea 17* were accepted at 01 November 2022.

Screening antimicrobial activity

The method of diffusion of the drug into agar carried out using the method of "wells" (Maslov *et al.*, 2022; Volyanskiy *et al.*, 2004) (Table I).

Table I. 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

Molecular docking

A molecular docking study was conducted using the tool known as AutoDockTools 1.5.6 (Morris *et al.*, 2008). The preparation of the protein involved an optimization process, which included the removal of water and other atoms, followed by the addition of 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 (RCSB PDB). 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 docking experiment protein structure is selected if resolution 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 (PubChem). The active site of the docking protein was identified utilizing the Computed Atlas for Surface Topography of Proteins (CASTp) (CASTp 3.0).

RESULTS AND DISCUSSION

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 of the antimicrobial effect was conducted with six key enzymes: DNA-gyrase, DHFR, Deacytelese, AHS LasI, AHS RhI and Diguanylate cyclase. 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 many 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. Several scientific works have used comparison standards, but in our view, this method is not promising as 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 the inhibition of the studied substances to the active centers of bacterial enzymes, we applied the following classification of selectivity [16]: IC50 < 0.001 mM (high selective); 0.05 > IC50 > 0.01 (medium selective); IC50 >0.05 mM (low selective).

Molecular modeling 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 high selective to the active site, whereas ciprofloxacin, chloramphenicol was medium selective and ornidazole, ceftrioxane, metronidazole, gentamycine were low selective (Table II).

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. The following compounds had high selectivity: clarithromycin, azithromycin, levofloxacine and arbutin, whereas ornidazole and metronidazole were low selective (Table II),.

Molecular modeling 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 II).

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. 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 II).

Table II. Results of molecular docking of the arbutin and antibacterial drug standards with the DNA-gyrase, DHFR, Deacytelese and AHS LasI structure

No	Ligand	Structure	ΔGbind[a] (kcal/mol)	Ki[b] (mmol)	Level of selectivity
1	**Clarithromycin**	**DNA-gyrase**	-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
11	**Clarithromycin**	**DHFR**	-16.78	0.000000000504	High selective
12	**Azithromycin**		-14.5	0.00000002336	High selective
13	**Levofloxacin**		-8.98	0.00026376	High selective
14	**Arbutin**		-9.17	0.00019023	High selective
15	**Ciprofloxacin**		-8.44	0.00064808	Medium selective
16	**Chloramphenicol**		-7.97	0.00143	Medium selective
17	**Gentamycin**		-6.78	0.01073	Medium selective
18	**Ceftriaxone**		-6.36	0.02164	Medium selective
19	**Ornidazol**		-4.95	0.23625	Low selective
20	**Metronidazole**		-4.28	0.72416	Low selective
21	**Azithromycin**	**Deacytelese**	-14.04	0.000000051	High selective
22	**Clarithromycin**		-13.98	0.000000057	High selective
23	**Levofloxacin**		-8.34	0.00077565	High selective
24	**Arbutin**		-8.40	0.00070067	High selective
25	**Ciprofloxacin**		-7.51	0.00313	Medium selective
26	**Chloramphenicol**		-7.19	0.00536	Medium selective
27	**Gentamycin**		-7.45	0.00346	Medium selective
28	**Ceftriaxone**		-6.09	0.03444	Medium selective
29	**Ornidazole**		-5.32	0.12638	Low selective
30	**Metronidazole**		-5.20	0.15555	Low selective
31	**Arbutin**	**AHS LasI**	-12.21	0.0000012	High selective
32	**Chloramphenicol**		-10.76	0.00001304	High selective
33	**Ceftriaxone**		-6.56	0.01561	Medium selective
34	**Ornidazole**		-5.83	0.05331	Low selective
35	**Metronidazole**		-5.38	0.113	Low selective
36	**Levofloxacin**		-4.11	0.97221	Low selective
37	**Ciprofloxacin**		-2.41	16.98	Low selective
38	**Gentamycin**		-	-	Inactive
39	**Azithromycin**		-	-	Inactive
40	**Clarithromycin**		-	-	Inactive

a – free-binding energy; b – inhibition constant, IC50, mmol, green color – high selective inhibitor, yellow color – medium selective inhibitor, red color – low selective inhibitor or inactive

Table III. Results of molecular docking of the arbutin and antibacterial drug standards with the AHS RhI and Diguanylate cyclase structure

No	Ligand	Structure	ΔGbind[a] (kcal/mol)	Ki[b] (mmol)	Level of selectivity
1	**Clarithromycin**	AHS RhI	-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
11	**Arbutin**	Diguanylate cyclase	-8.06	0.001230	Medium selective
12	**Clarithromycin**		-8.03	0.00131	Medium selective
13	**Chloramphenicol**		-6.59	0.01488	Medium selective
14	**Ciprofloxacin**		-6.31	0.02356	Medium selective
15	**Levofloxacin**		-5.32	0.12516	Low selective
16	**Ceftriaxone**		-5.19	0.15567	Low selective
17	**Metronidazole**		-4.94	0.23835	Low selective
18	**Ornidazole**		-4.72	0.34569	Low selective
19	**Gentamycin**		-4.49	0.51373	Low selective
20	**Azithromycin**		-2.79	8.97	Low selective

a – free-binding energy; b – inhibition constant, IC50, mmol, green color – high selective inhibitor, yellow color – medium selective inhibitor, red color – low selective inhibitor or inactive

Molecular modeling of the compounds studied 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, azithromycin, had high selectivity, whereas ornidazole, metronidazole, ceftrioxane had the lowest level of binding as well as gentamycin were not interacted with protein (Table III). 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 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 II).

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 VIII, there was no any compounds that inhibit high selectively all antibacterial mechanisms. The clarithromycin was the best antibiotic that inhibit approximately all mechanisms of the "first line of defense" 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 such antimicrobial drug actively binding only with one enzyme of biofilm formation – AHS LasI, whereas other mechanisms were inhibited at the lower level (Table IV).

Table IV. Schematic division of antibacterial 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"
	Antibacterial 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								
10	**Arbutin**							3	1

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

The mechanisms of resistance of pathogens are achieved through the mode the antibacterial drug has affected. Above all, the resistance mostly depends on chemical structure of drug and target site. Generally, antimicrobial resistance usually depends on biochemical and genetic aspects. Moreover, the high application of antimicrobial drugs in agriculture and low level of infection control in health care caused further acceleration of crisis (Pulingam *et al.*, 2022). Clarithromycin, azithromycin, chloramphenicol, ciprofloxacin, levofloxacin, ceftriaxone, metronidazole, ornidazole, gentamycin was 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, many 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. pneumoniae, P. aeruginosa* and *E. cloacae* (Abinaya *et al.*, 2019). 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 the formation of folic acid, which is necessary for the existence of bacteria (Jogula *et al.*, 2020). One of the main defense 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 (Mbarga *et al.*, 2021).

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 signaling that relies on the production and perception of chemical signaling molecules called autodoctors. For the formation of these signal molecules, the protein acyl-homoserine lactone synthetase LasI and RhI is responsible (Zuo *et al.*, 2017). Also, one of the main stages of biofilm formation is the cell adhesion of bacteria to the surface. Adhesions require a signaling molecule, cyclic di-guanylate mono-phosphate (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 (Abinaya *et al.*, 2019).

Table V. Inhibition diameter (mm) resulting from the screening of antibacterial effect against resistance strains of *A. baumani* and *K. pneumonia* by well diffusion method with arbutin and antibiotic standards.

Sample	**Concentration, mM**	**Diameter of the growth retardation zone, mm±SD**			
		A. baumani 150	*Difference, %*	*K. pneumonia 18*	*Difference, %*
Arbutin	0.003	12.00±0.70		12.00±0.70	
Clarithromycin	0.003	**Growth**		**Growth**	
Arbutin+ **Clarithromycin**	0.003+0.003	**Growth**	**0**	21.00±0.20	**+100%**
Arbutin	0.003	12.00±0.70		12.00±0.70	
Azithromycin	0.003	**Growth**		**Growth**	
Arbutin+**Azithromycin**	0.003+0.003	**Growth**	**0**	24.50±0.50	**+100%**
Arbutin	0.003	12.00±0.70		12.00±0.70	
Chloramphenicol	0.003	**Growth**		**Growth**	
Arbutin+ **Chloramphenicol**	0.003+0.003	17.50±0.50	**+100%**	18.50±0.50	**+100%**
Arbutin	0.003	12.00±0.70		12.00±0.20	
Ciprofloxacin	0.003	**Growth**		15.50±0.50	
Arbutin+ **Ciprofloxacin**	0.003+0.003	19.50±0.50	**+100%**	24.50±0.50	**+58%**
Arbutin	0.003	12.00±0.70		12.00±0.70	
Levofloxacin	0.003	**Growth**		20.50±0.40	
Arbutin+ **Levofloxacin**	0.003+0.003	24.50±	**+100%**	26.00±0.20	**+21%**
Arbutin	0.003	12.00±0.70		12.00±0.20	
Ceftriaxone	0.003	**Growth**		19.50±0.50	
Arbutin + **Ceftriaxone**	0.003+0.003	23.50±0.50	**+100%**	25.50±0.50	**+24%**
Arbutin	0.003	12.00±0.70		12.00±0.70	
Metronidazole	0.003	**Growth**		16.00±0.50	
Arbutin+ **Metronidazole**	0.003+0.003	20.00±0.20	**+100%**	18.50±0.40	**+14%**
Arbutin	0.003	12.00±0.70		12.00±0.70	
Ornidazol	0.003	**Growth**		16.00±0.50	
Arbutin+ **Ornidazole**	0.003+0.003	19.00±0.30	**+100%**	17.50±0.40	**+9%**
Arbutin	0.003	12.00±0.70		12.00±0.70	
Gentamycin	0.003	**Growth**		17.50±0.50	
Arbutin+ **Gentamycin**	0.003+0.003	18.50±0.50	**+100%**	20.50±0.20	**+15%**

Thus, 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 antibacterial action. But, arbutin was shown excellent values of binding energy against all mentioned above "targets". We suggest that a complex of antibacterial drug and arbutin or a complex of natural compounds should be used in order 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.

Antibacterial activity

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

In Table 8 was shown that *A. baumanni* was resistant to all tested antibiotics. When studying the antibacterial activity of combinations of antibiotics and arbutin at concentrations of 0.003 mM, it was found that in the case of chloramphenicol, ciprofloxacin, levofloxacin, ceftriaxone, metronidazole and gentamicin, antimicrobial resistance was removed, while

clarithromcin and azithromycin remained resistant to the bacterial strain *A. baumanni* (Table V). According to the results of the study, it was found that the following antibiotics were resistant to *K. pneumoniae*: clarithromycin, azithromycin and
chloramphenicol, while ciprofloxacin, levofloxacin, ceftriaxone, metronidazole, ornidazole, and gentamicin were sensitive. When arbutin was added to each antibiotic, it was found that clarithromycin, azithromycin and chloramphenicol began to actively inhibit the growth of the bacterial strain *K. pneumoniae*. In the case of non-resistant antibiotics, after the addition of arbutin, the antibacterial effect of ciprofloxacin increased by 58%, levofloxacin by 21%, ceftriaxone by 24%, metronidazole by 14%, ornidazole by 9% and gentamicin by 15% (Table V).

A serious threat to human health is the emergence of "superbacteria". This issue is especially relevant in relation to *A. baumanni, K. pneumoniae, P. aeruginosa* and *E. cloacae*. These bacterial strains are capable of causing nosocomial infections and respiratory associated pneumoniae. The above-mentioned bacteria have been isolated that are resistant to aminoglycosides, fluoroquinolones, as well as to the action of the "last line of defense" - carbapenems (Jean *et al.*, 2022). 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 (Aranage *et al.*, 2022).

Our studies showed that no antibiotic had an antibacterial effect against *A. baumanni*, except for chloramphenicol. The antibiotics clarithromycin, azithromycin and chloramphenicol were insensitive to *K. pneumoniae*, The studied bacterial strains were not resistant to the antibacterial action of arbutin.

Theoretical studies have shown that arbutin is a highly selective inhibitor of all targeted mechanisms of "first line of defense" 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 9 to 58%. We hypothesize that arbutin actively affects antibacterial 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 antibacterial activity against *A. baumanni*. This fact may be due to the fact that arbutin could 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 is a time-consuming and expensive investment. 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" antibacterial drugs and herbal drug or dietary supplements based on extracts from arbutin-containing medicinal plants such as lingonberry, bearberry and cranberry should be used in treatment therapies.

CONCLUSION

Theoretical studies have shown that no "classical" antibiotic is a highly selective inhibitor of all "targeted" antibacterial 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 *A. baumanni*, *K. pneumoniae*, *P. aeruginosa*, *E. cloacae*. These studies show that to inhibit resistant strains of bacteria, requires the use of combinations of "classical" antibacterial drugs and herbal drug 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 antibacterial agents against

resistant bacteria and gives “a second chance to return to life” for outdated antibiotics.

ACKNOWLEDGMENTS

The research was carried out within the framework of the topic “Development of anhydrous gel based on phenolic compounds for the treatment of purulent wounds caused by antibiotic-resistant *Pseudomonas aeruginosa*” of the list of scientific studies of the Ministry of Health of Ukraine, carried out at the expense of the state budget of Ukraine No. 0124U002080 (Order of the Ministry of Health of Ukraine No. 17 of 27.02.2024). Authors are grateful for the provided scientific and material help of pharmaceutical company "Astrapharm" Kiev, Ukraine; and pharmaceutical company "Zdravopharm", Kharkiv, Ukraine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abinaya, M., & Gayathri, M. (2019a). Inhibition of biofilm formation, quorum sensing activity and molecular docking study of isolated 3, 5, 7-Trihydroxyflavone from Alstonia scholaris leaf against P.aeruginosa. *Bioorganic Chemistry*, *87*, 291–301. https://doi.org/10.1016/j.bioorg.2019.03.050

Abinaya, M., & Gayathri, M. (2019b). Inhibition of biofilm formation, quorum sensing activity and molecular docking study of isolated 3, 5, 7-Trihydroxyflavone from Alstonia scholaris leaf against P.aeruginosa. *Bioorganic Chemistry*, *87*, 291–301. https://doi.org/10.1016/j.bioorg.2019.03.050

Aranaga, C., Pantoja, L. D., Martínez, E. A., & Falco, A. (2022). Phage therapy in the era of multidrug resistance in bacteria: a systematic review. *International Journal of Molecular Sciences*, *23*(9), 4577. https://doi.org/10.3390/ijms23094577

CASTp 3.0: Computed Atlas of Surface Topography of proteins. (n.d.). http://sts.bioe.uic.edu/castp/index.html?201l

Jean, S.-S., Harnod, D., & Hsueh, P.-R. (2022). Global threat of carbapenem-resistant gram-negative bacteria. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.823684

Jogula, S., Krishna, V. S., Meda, N., Balraju, V., & Sriram, D. (2020). Design, synthesis and biological evaluation of novel Pseudomonas aeruginosa DNA gyrase B inhibitors. *Bioorganic Chemistry*, *100*, 103905. https://doi.org/10.1016/j.bioorg.2020.103905

Kondratiuk, V., Jones, B. T., Kovalchuk, V., Kovalenko, I., Ganiuk, V., Kondratiuk, O., & Frantsishko, A. (2021). Phenotypic and genotypic characterization of antibiotic resistance in military hospital-associated bacteria from war injuries in the Eastern Ukraine conflict between 2014 and 2020. *Journal of Hospital Infection*, *112*, 69–76. https://doi.org/10.1016/j.jhin.2021.03.020

Kondža, M., Brizić, I., & Jokić, S. (2024). Flavonoids as CYP3A4 Inhibitors In Vitro. *Biomedicines*, *12*(3), 644. https://doi.org/10.3390/biomedicines12030644

Ma, C., He, N., Zhao, Y., Xia, D., Wei, J., & Kang, W. (2019). Antimicrobial mechanism of hydroquinone. *Applied Biochemistry and Biotechnology*, *189*(4), 1291–1303. https://doi.org/10.1007/s12010-019-03067-1

Maslov, O., Komisarenko, M., Kolisnyk, S., Tkachenko, O., Akhmedov, E., Poluain, S., Kostina, T., & Kolisnyk, O. (2023). Study of qualitative composition and quantitative content of free organic acids in lingberry leaves. *Fitoterapia*, (1), 77–82. https://doi.org/10.32782/2522-9680-2023-1-77

Maslov, O., Komisarenko, M., Ponomarenko, S., Horopashna, D., Osolodchenko, T., Kolisnyk, S., Derymedvid, L., Shovkova, Z., & Akhmedov, E. (2022). Investigation the influence of biologically active compounds on the antioxidant, antibacterial and anti-inflammatory activities of red raspberry (*Rubus idaeous* l.) leaf extract. *Current Issues in Pharmacy and Medical Sciences*, *35*(4), 229–235. https://doi.org/10.2478/cipms-2022-0040

Mbarga, M. J. A., Podoprigora, I. V., Volina, E. G., Ermolaev, A. V., & Smolyakova, L. A. (2021). Evaluation of changes induced in the probiotic Escherichia coli M17 following recurrent exposure to

antimicrobials. *Journal of Pharmaceutical Research International*, *9*,158–167. https://doi.org/10.9734/jpri/2021/v33i29b31601

Mende, K., Akers, K. S., Tyner, S. D., Bennett, J. W., Simons, M. P., Blyth, D. M., Li, P., Stewart, L., & Tribble, D. R. (2022). Multidrug-resistant and virulent organisms trauma infections: trauma infectious disease outcomes study initiative. *Military Medicine*, *187*(Supplement_2), 42–51. https://doi.org/10.1093/milmed/usab131

Morris, G. M., Huey, R., & Olson, A. J. (2008). Using autodock for ligand-receptor docking. *Current Protocols in Bioinformatics*, *24*(1). https://doi.org/10.1002/0471250953.bi0814s24

Petrosillo, N., Petersen, E., & Antoniak, S. (2023). Ukraine war and antimicrobial resistance. *The Lancet Infectious Diseases*. https://doi.org/10.1016/s1473-3099(23)00264-5

PubChem. (n.d.). PubChem. https://pubchem.ncbi.nlm.nih.gov/

Pulingam, T., Parumasivam, T., Gazzali, A. M., Sulaiman, A. M., Chee, J. Y., Lakshmanan, M., Chin, C. F., & Sudesh, K. (2022). Antimicrobial resistance: prevalence, economic burden, mechanisms of resistance and strategies to overcome. *European Journal of Pharmaceutical Sciences*, *170*, 106103. https://doi.org/10.1016/j.ejps.2021.106103

RCSB PDB: Homepage. (n.d.). RCSB PDB: Homepage. https://www.rcsb.org/

Schultze, T., Hogardt, M., Velázquez, E. S., Hack, D., Besier, S., Wichelhaus, T. A., Rochwalsky, U., Kempf, V. A., & Reinheimer, C. (2023a). Molecular surveillance of multidrug-resistant Gram-negative bacteria in Ukrainian patients, Germany, March to June 2022. *Eurosurveillance*, *28*(1). https://doi.org/10.2807/1560-7917.es.2023.28.1.2200850

Schultze, T., Hogardt, M., Velázquez, E. S., Hack, D., Besier, S., Wichelhaus, T. A., Rochwalsky, U., Kempf, V. A., & Reinheimer, C. (2023b). Molecular surveillance of multidrug-resistant Gram-negative bacteria in Ukrainian patients, Germany, March to June 2022. *Eurosurveillance*, *28*(1). https://doi.org/10.2807/1560-7917.es.2023.28.1.2200850

Volyanskiy, Y., Gritsenko, I., & Shyrokobokov, V. (2004). *The study of the specific activity of antimicrobial drugs: a method recommendation*. StEntScPhC Ministry of Helthcare of Ukraine: Kiev.

Zhou, H., Zhao, J., Li, A., & Reetz, M. T. (2019). Chemical and biocatalytic routes to arbutin. *Molecules*, *24*(18), 3303. https://doi.org/10.3390/molecules24183303

Zuo, K., Liang, L., Du, W., Sun, X., Liu, W., Gou, X., Wan, H., & Hu, J. (2017). 3D-QSAR, molecular docking and molecular dynamics simulation of Pseudomonas aeruginosa LpxC inhibitors. *International Journal of Molecular Sciences*, *18*(5), 761. https://doi.org/10.3390/ijms18050761