Structure and antibacterial activity relationship of quercetin and rutin against test and clinical resistant gramm-negative strains of bacteria

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Today, antimicrobial resistance is the number one problem worldwide. According to the latest data, it has found that *Acinetobacter baumani, Pseudomonas aeruginosa*, *Klebsiela pneumonia* and *Enterobacter cloacae* are predominant among all isolated resistant pathogens. So, the search of a new antibacterial drug that can deal with antimicrobial resistance is task number one.

Aim. The study aimed to investigate theoretical and practical relationship of structure and antibacterial activity of quercetin and rutin against test Gram-negative strains: *Escherichia coli, Pseudomonas aeruginosa*, *Proteus vulgaris*, and clinical resistant strains such as *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae*.

Materials and methods. The research subjects were quercetin and rutin. The theoretical research was carried out using AutoDockTools 1.5.6; antibacterial effects were evaluated by the well method. Clinical strain of *P. aeruginosa, A. baumani, K. pneumonia, E. cloacea* were taken from tracheal aspirate and bronchoalveolar lavage.

Results. Theoretical studies showed that none of the investigated antimicrobial drugs inhibit all "targets" mechanism of antibacterial action. Rutin revealed high selectivity to DNA-gyrase, dihydrofolate reductase (DHFR), deacetylase, AHS RhI, Diguanylate cyclase, unlike quercetin which revealed low selectivity. Experimental research demonstrated that against *P. aeruginosa* rutin and quercetin inhibited growth – 17.0 ± 0.4 mm and 18.0 ± 0.4 mm, against *P. vulgaris* – 14.0 ± 0.5 mm and 16.0 ± 0.5 mm, against *E. coli* – 16.0 ± 0.5 mm and 20.0 ± 0.4 mm, respectively. Resistant strain of *P. aeruginosa, E. cloacea, A. baumani, K. pneumonia* were sensitive to the action of rutin – 23.0 ± 0.3 mm, 25.0 ± 0.2 mm, 24.0 ± 0.3 mm, 23.0 ± 0.3 mm, respectively, while to the action of quercetin resistant strain were low sensitive – 12.0 ± 0.6 mm, 14.0 ± 0.5 mm, 12.0 ± 0.6 mm, 12.0 ± 0.6 mm, respectively.

Conclusions. Theoretical studies of "standard" antimicrobial drugs used in infectious disease treatment protocols are not highly selective inhibitors of "target" antibacterial mechanisms of gram-negative bacteria, unlike rutin, which turned out to be a highly selective inhibitor. According to the results of the theoretical study, it was found that the potential antibacterial activity of rutin exceeds the effect of quercetin by two times. This pattern is fully confirmed by *in vitro* studies, where the antibacterial effect of rutin against resistant strains was also two times higher.

Keywords: rutin, quercetin, multi-drug resistant, Gram-negative strains, molecular docking.

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Зв'язок будови й антибактеріальної активності кверцетину та рутину проти тестових і клінічно резистентних грамнегативних штамів бактерій

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Резистентність до антибіотиків є однією з провідних проблем у всьому світі. За останніми даними, серед усіх виділених резистентних патогенів переважають *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* та *Enterobacter cloacae*. Відтак, пошук нових антибактеріальних препаратів, здатних подолати резистентність до антибіотиків, є першочерговим завданням.

Мета роботи – вивчення теоретичних і практичних аспектів зв'язку між структурою та антибактеріальною активністю кверцетину і рутину щодо тестових грамнегативних штамів *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, а також клінічних резистентних штамів *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae*.

Матеріали і методи. Об'єкти дослідження – кверцетин і рутин. Молекулярний докінг виконали за допомогою AutoDockTools 1.5.6, а антибактеріальні властивості оцінювали методом лунок. Клінічний штам *P. aeruginosa, A. baumannii, K. pneumoniae, E. cloacae* взято з аспірату трахеї та бронхоальвеолярного лаважу.

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Результати. Теоретичні дослідження дали змогу встановити, що жоден зі стандартних антимікробних препаратів не пригнічує всі «мішені» антибактеріальної дії. Рутин мав високу селективність до ДНК-гірази, дигідрофолатредуктази, деацетилази, AHS RhI та дигуанілатциклази, на відміну від кверцетину, який мав низьку селективність. Експериментальні дослідження показали: рутин і кверцетин пригнічували ріст *P. aeruginosa* – 17,0 ± 0,4 мм та 18,0 ± 0,4 мм, *P. vulgaris* – 14,0 ± 0,5 мм та 16,0 ± 0,5 мм, *E. coli* – 16,0 ± 0,5 мм та 20,0 ± 0,4 мм відповідно. Резистентні штами *P. aeruginosa*, *E. cloacae*, *A. baumannii*, *K. pneumoniae* чутливі до рутину, що показав зони пригнічення 23,0 ± 0,3 мм, 25,0 ± 0,2 мм, 24,0 ± 0,3 мм, 23,0 ± 0,3 мм відповідно. Кверцетин мав значно меншу активність проти резистентних штамів: 12,0 ± 0,3 мм, 14,0 ± 0,5 мм, 12,0 ± 0,3 мм, 12,0 ± 0,3 мм відповідно.

Висновки. Теоретичні дослідження «стандартних» антимікробних препаратів, які використовують у протоколах лікування інфекційних захворювань, дали змогу зробити висновок, що вони не є високоселективними інгібіторами антибактеріальних механізмів грамнегативних бактерій. На відміну від них, рутин характеризувався високою селективністю. За результатами теоретичного дослідження, потенційна антибактеріальна активність рутину вдвічі перевищує активність кверцетину. Ця закономірність підтверджена під час *in vitro* досліджень, де антибактеріальний ефект рутину проти резистентних штамів також удвічі вищий.

Ключові слова: рутин, кверцетин, мультирезистентність, грамнегативні штами, молекулярний докінг.

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Antimicrobial resistance is one of the greatest hazard in 21st century. The most sensitive to this threat are less economically developed countries. Based on statistical studies, infections caused by antimicrobial resistance led to a staggering 4.95 million deaths worldwide in 2019. This mortality rate from antibiotic-resistant bacteria significantly surpasses the annual global deaths from tuberculosis (1.5 million), malaria (643,000), and HIV/AIDS (864,000) [1]. The World Health Organization (WHO) has a prognosis that without any intervention in this problem the global deaths of antibiotic resistance could reach 10 million annually by 2050 [2]. The WHO was marked six main multidrug resistant pathogens that could be a great threat for health care: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Enterobacter faecalis* (ESKAPE) [3].

Nowadays the main strategy to avoid antimicrobial resistance is based on restriction of antibiotics application with a wide spectrum of action in treatment of influenza, sore throat, second one, decrease application of antibiotics in plant agriculture, and in food-producing animals [4]. As animals' bacteria resistance can be transferred to humans through the consumption of food [5]. The other way to inhibit antibiotic resistance spreading is to use a new substance or their combinations as antimicrobial agents to inhibit the growth of resistance bacteria. In our view, the most perspective substances could be derivatives of flavonols such as quercetin and its glycosides (rutin, hyperoside).

Quercetin is a 3,3′,4′,5,7-pentahydroxyflvanone that belongs to a flavonol. The name has been applied since 1857, and is derived from quercetum (Oak forest) [6]. Quercetin is an aglycone, it has a yellow color, crystal structure and entirely insoluble in water, but quite well soluble in alcohol, for pharmacy solubility of quercetin in water is a challenge number one [7]. Whereas, rutin is a quercetin glucoside that is formed by attaching a glycosylic group (glucose and rhamnose) as a replacement for OH group at third position. The glycosylic group at third position can change the solubility adsorption and pharmacological effects [8] *(Fig. 1)*.

Quercetin is the most widespread flavonoid, it was isolated from onions, grapes, tomatoes, and so on. Moreover, quercetin and rutin are highly dominated in medicinal herbs as *Ginkgo biloba*, *Hypericum perforatum*, *Sophora japonica*, *Fagopyrum esculentum* [9]. Quercetin and rutin possesses a variety of pharmacological activity such as anti-inflammatory, antiallergic, antiviral, hypolipidemic, antiplatelet, and antihypertension effects [10,11,12,13].

Aim

The study aimed to investigate theoretical and practical relationship of structure and antibacterial activity of quercetin and rutin against test Gram-negative strains: *Escherichia coli, Pseudomonas aeruginosa*, *Proteus vulgaris*, and clinical resistant strains such as *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae.*

Materials and methods

Compounds: rutin (≥98.0 %, Sigma Aldrich), quercetin (≥98.0 %, Sigma Aldrich); gentamycin (≥98.0 %, Sigma Aldrich); chloramphenicol (≥98.0 %, Sigma Aldrich).

Preparation solution of quercetin. A 0.0755 g (exact mass) of quercetin was gradually dissolved in 20 mL of aqueous solution of dimethyl sulfoxide (5 % out of total volume) with constant stirring on magnetic stirrer, after that solution was transferring in a measuring flask with volume 25.0 mL, and solution was made up to the mark with the same solvent.

Preparation solution of rutin. A 0.150 g (exact mass) of quercetin was gradually dissolved in 20 mL of aqueous solution of dimethyl sulfoxide (5 % out of total volume) with constant stirring on magnetic stirrer, after that solution was transferring in a measuring flask with volume 25.0 mL, and solution was made up to the mark with the same solvent.

Pseudomonas aeruginosa 18, *Acinetobacter baumannii* 150, *Klebsiella pneumoniae* 18, *Enterobacter cloacea* 17 were provided by the State Institution "I. Mechnikov Institute of Microbiology and Immunology National Academy of Medical Sciences of Ukraine". All strains are stored and accepted by the Head of Museum of strains – O. G. Peretyatko.

Test strains of *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *P. vulgaris* NTCS 4636 were chosen for research.

The method of diffusion of the drug into agar was carried out using the method of "wells" [14,15]. *Table 1* shows interpretation criteria for microbial sensitivity.

A molecular docking study was conducted using the tool known as AutoDockTools 1.5.6 [16].

The structures of DNA-gyrase (PDB ID: 1KIJ), DHFR (PDB ID: 1RX3), deacetylase (PDB ID: 3UHM), acyl-homoserine lactone synthase (AHS) LasI (PDB ID: 1RO5), acyl-homoserine lactone synthase (AHS) RhI (PDB ID: 1KZF), and diguanylate cyclase (PDB ID: 3BRE) were obtained from the PDB database [17]. The resolution of 1KIJ was 2.30 Å, 1RX3 – 2.20 Å, 3UHM – 2.20 Å, 1RO5 – 2.30 Å, 1KZF – 2.20 Å, and 3BRE – 2.40 Å. For docking experiments, protein structures are selected if their resolution is above 2 Å. Therefore, all the mentioned proteins are suitable for the experiment. The ligand structures of rutin (CID_5280805), quercetin (CID_5280343), gentamycin (CID_3467), and chloramphenicol (CID_5959) were retrieved from the PubChem database [18]. The binding site of the docked proteins was determined utilizing the Computed Atlas for Surface Topography of Proteins (CASTp) [19].

Results

Theoretical assessment of antibacterial activity of quercetin and rutin was provided by method of molecular docking of the crucial enzymes of gram-negative bacteria such as DNA-gyrase, DHFR, Deacetylase, AHS LasI, AHS RhI and Diguanylate cyclase. For comparison analysis it was taken following antimicrobial drugs: gentamycin, ciprofloxacin, levofloxacin, ceftriaxone, chloramphenicol, clarithromycin, azithromycin and metronidazole, ornidazole. It was applied following classification of selectivity to active site of enzyme [20]: IC50 < 0.001 mM (high selective); 0.05 > IC50 > 0.01 (medium selective); IC50 > 0.05 mM (low selective).

Active center of DNA-gyrase was consisted by amino acids: Arg75, Lys102, Arg135, Asp80. Trp387, Lys109, Asp72, Thr166. *Table 2* shows that clarithromycin, azithromycin, rutin and levofloxacin had high selectivity, in the case of ciprofloxacin, chloramphenicol – medium selectivity, whereas ornidazole, quercetin, ceftriaxone, metronidazole and gentamycin – low selectivity. Comparing the level of affinity of rutin and quercetin it was observed that binding energy with active center of DNA-gyrase of rutin was in two times higher than quercetin.

The next crucial enzyme that was assessed by molecular docking was DHFR. The active center of enzyme was containing of NADP, Tyr110, Asp30, Ile8, Phe34, Ile104, Arg55, Arg60. *Table 3* shows that clarithromycin, azithromycin, rutin and levofloxacin had high selectivity, in the case of ciprofloxacin, chloramphenicol, gentamycin, ceftriaxone, quercetin – medium selectivity, whereas ornidazole, metronidazole – low selectivity. Comparing the level of affinity of rutin and quercetin it was observed that binding energy with active center of DHFR of rutin was in two times higher than quercetin.

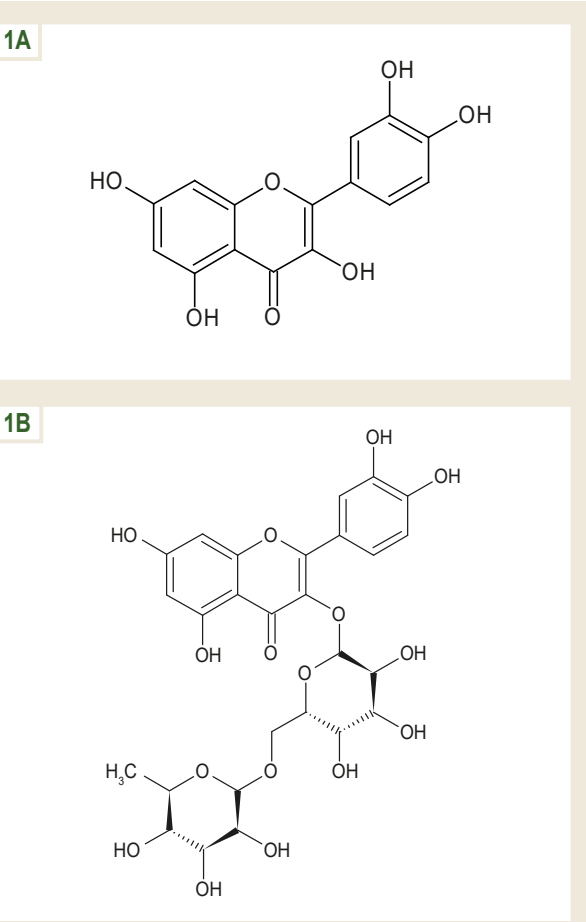

Fig. 1. Chemical structure of quercetin (**A**) and rutin (**B**).

The active center of deacetylase was represented by the following amino acid: Thr190, Lys238, Gly92. Phe191, Leu18, Ala206. According to the obtained results of the study it was established that clarithromycin, azithromycin, rutin and levofloxacin had high selectivity, in the case of ciprofloxacin, gentamycin, chloramphenicol, ceftriaxone – medium selectivity, whereas quercetin, ornidazole, metronidazole – low selectivity. Comparing the level of affinity of rutin and quercetin it was observed that binding energy with active center of DHFR of rutin was in two times higher than quercetin *(Table 4)*.

Table 1. Microbial sensitivity

Sensitivity	Retardation zone, mm
High sensitivity	>25
Sensitive	15–25
Low sensitivity	10–15
Not sensitivity	<10

Table 2. Affinity of quercetin, rutin and antimicrobials drug with the DNA-gyrase

Ligand	DNA-gyrase		
	ΔGbind, kcal/mol*	Ki, mmol#	Selectivity
Clarithromycin	-11.59	0.00000001087	High selective
Azithromycin	-10.29	0.00000061435	High selective
Rutin	-10.45	0.00002184	High selective
Levofloxacin	-8.69	0.00042853	High selective
Ciprofloxacin	-8.06	0.00123	Medium selective
Chloramphenicol	-6.38	0.02114	Medium selective
Ornidazole	-5.0	0.19214	Low selective
Quercetin	-5.00	0.21618	Low selective
Ceftriaxone	-4.61	0.41631	Low selective
Metronidazole	-4.54	0.46734	Low selective
Gentamycin	-4.08	1.03	Low selective

***:** free-binding energy; **#:** inhibition constant, IC50, mmol.

Table 3. Affinity of quercetin, rutin and antimicrobials drug standards with the DHFR

Ligand	DHFR		
	ΔGbind, kcal/mol*	Ki, mmol#	Selectivity
Clarithromycin	-16.78	0.000000000504	High selective
Azithromycin	-14.50	0.00000002336	High selective
Rutin	-10.72	0.00001379	High selective
Levofloxacin	-8.98	0.00026376	High selective
Ciprofloxacin	-8.44	0.00064808	Medium selective
Chloramphenicol	-7.97	0.00143	Medium selective
Gentamycin	-6.78	0.01073	Medium selective
Ceftriaxone	-6.36	0.02164	Medium selective
Quercetin	-6.32	0.02329	Medium selective
Ornidazole	-4.95	0.23625	Low selective
Metronidazole	-4.28	0.72416	Low selective

***:** free-binding energy; **#:** inhibition constant, IC50, mmol.

The next crucial enzyme that was evaluated by molecular docking was AHS LasI. The active center of enzyme consisted of amino acids: Thr190, Lys238, Gly92. Phe191, Leu18, Ala206. *Table 5* shows that chloramphenicol had high selectivity, in the case of quercetin, ceftriaxone – medium selectivity, whereas ornidazole, metronidazole, levofloxacin, ciprofloxacin – low selectivity. While gentamycin, rutin, azithromycin and clarithromycin were inactive. Comparing the level of affinity of rutin and quercetin it was observed that only quercetin was active against this enzyme while its glycoside was not binding.

The active center of AHS RhI was represented by following amino acids: Glu254, Asp48, Tyr54, Met42. Leu63, Leu56. *Table 6* shows that clarithromycin, rutin, azithromycin had high selectivity, ciprofloxacin, levofloxacin, quercetin, chloramphenicol – medium selectivity, while ornidazole, metronidazole, ceftriaxone – low selectivity as well as gentamycin had inactive. Comparing affinity of quercetin and rutin to active center it was observed that rutin in two times higher active than its aglycone form.

The next crucial enzyme that was evaluated by molecular docking was diguanylate cyclase. The active center of enzyme consisted of amino acids such as Glu254, Glu253, Glu252, Lys327, Arg331, Thr262, Arg198, Arg194. *Table 7* demonstrates that clarithromycin, chloramphenicol, ciprofloxacin had medium selectivity, levofloxacin, ceftriaxone, metronidazole, rutin, quercetin, ornidazole, gentamycin, azithromycine – low selectivity. Comparing affinity of quercetin and rutin to active center it was observed that rutin and quercetin had approximately the same value of binding energy.

Table 4. Affinity of molecular docking of the quercetin, rutin and antimicrobials drug standards with the deacetylase

Ligand	Deacetylase		
	ΔGbind, kcal/mol*	Ki, mmol#	Selectivity
Azithromycin	-14.04	0.000000051	High selective
Clarithromycin	-13.98	0.000000057	High selective
Rutin	-10.47	0.000021	High selective
Levofloxacin	-8.34	0.00077565	High selective
Ciprofloxacin	-7.51	0.00313	Medium selective
Gentamycin	-7.45	0.00346	Medium selective
Chloramphenicol	-7.19	0.00536	Medium selective
Ceftriaxone	-6.09	0.03444	Medium selective
Quercetin	-5.81	0.05541	Low selective
Ornidazole	-5.32	0.12638	Low selective
Metronidazole	-5.20	0.15555	Low selective

*: free-binding energy; #: inhibition constant, IC50, mmol.

Table 5. Affinity of quercetin, rutin and antimicrobials drug standards with the AHS LasI

Ligand	AHS LasI		
	ΔGbind, kcal/mol*	Ki, mmol#	Selectivity
Chloramphenicol	-10.76	0.00001304	High selective
Quercetin	-6.70	0.01223	Medium selective
Ceftriaxone	-6.56	0.01561	Medium selective
Ornidazole	-5.83	0.05331	Low selective
Metronidazole	-5.38	0.113	Low selective
Levofloxacin	-4.11	0.97221	Low selective
Ciprofloxacin	-2.41	16.98	Low selective
Gentamycin	–	–	Inactive
Rutin	–	–	Inactive
Azithromycin	–	–	Inactive
Clarithromycin	–	–	Inactive

*: free-binding energy; #: inhibition constant, IC50, mmol.

The next step of our research was to sum up the obtained data mentioned before. All antimicrobial drugs and rutin, quercetin were divided into two categories. To the first category belong compounds that had high selectivity, whereas to the second one belong compounds that had medium and low selectivity.

Table 8 demonstrates that there was not present any of antimicrobial drugs or biological active compounds that inhibited highly selective all six mechanisms. Results show that clarithromycin and azithromycin inhibited four out of six enzymes of the "first line of defense" and biofilm formation, except AHS LasI and diguanylate cyclase. Levofloxacin inhibited only enzymes of "first line of defense", whereas targets of biofilm formation were not sensitive to action of levofloxacin. Comparing the selectivity of quercetin and rutin it was found that quercetin cannot effectively inhibit any of target enzyme, whereas rutin inhibited five out of six enzymes, except diguanylate cyclase.

Rutin, quercetin and antimicrobial drugs were used to evaluate bacterial activity against test gram-negative strains such as *E. coli*, *P. vulgaris* and *P. aeruginosa (Table 9)*. Against *E. coli* quercetin was more active (20.0 ± 0.4 mm) than its glycoside form (16.0 ± 0.5 mm). The highest value of retardation zone had ciprofloxacin (29.0 ± 0.2 mm), levofloxacin (31.0 ± 0.2 mm), ceftriaxone (27.0 ± 0.2 mm), gentamycin (27.0 ± 0.3 mm).

We found that quercetin inhibited the growth of *P. vulgaris* stronger (16.0 ± 0.3 mm) than rutin (14.0 ± 0.5 mm). Comparing with antimicrobial drugs it was established that the *P. vulgaris* was the most sensitive to action of gen-

Table 6. Affinity of quercetin, rutin and antimicrobials drug standards with the AHS RhI

Ligand	AHS RhI		
	ΔGbind, kcal/mol*	Ki, mmol#	Selectivity
Clarithromycin	-18.54	0.0000000000253	High selective
Rutin	-10.90	0.00001053	High selective
Azithromycin	-10.16	0.00003572	High selective
Ciprofloxacin	-7.84	0.00178	Medium selective
Levofloxacin	-6.62	0.01408	Medium selective
Quercetin	-6.20	0.02877	Medium selective
Chloramphenicol	-5.88	0.04912	Medium selective
Ornidazole	-5.20	0.15405	Low selective
Metronidazole	-5.11	0.18023	Low selective
Ceftriaxone	-4.48	0.51643	Low selective
Gentamycin	–	–	Inactive

***:** free-binding energy; **#:** inhibition constant, IC50, mmol.

Table 7. Results of molecular docking of the quercetin, rutin and antimicrobials drug standards with the diguanylate cyclase structure

Ligand	Diguanylate cyclase		
	ΔGbind, kcal/mol*	Ki, mmol#	Selectivity
Clarithromycin	-8.03	0.00131	Medium selective
Chloramphenicol	-6.59	0.01488	Medium selective
Ciprofloxacin	-6.31	0.02356	Medium selective
Levofloxacin	-5.32	0.12516	Low selective
Ceftriaxone	-5.19	0.15567	Low selective
Metronidazole	-4.94	0.23835	Low selective
Rutin	-4.88	0.2660	Low selective
Quercetin	-4.73	0.3420	Low selective
Ornidazole	-4.72	0.34569	Low selective
Gentamycin	-4.49	0.51373	Low selective
Azithromycin	-2.79	8.97	Low selective

a: free-binding energy; **b:** inhibition constant, IC50, mmol.

tamycin (26.0 ± 0.3 mm), levofloxacin (30.0 ± 0.2 mm), whereas the lowest one to metronidazole and ornidazole (growth).

Against *P. aeruginosa* the zone of retardation of quercetin and rutin were 18.0 ± 0.4 mm and 17.0 ± 0.4 mm. The high sensitivity had ciprofloxacin (30.0 ± 0.2 mm), ceftriaxone (30.0 ± 0.2 mm), levofloxacin (29.0 ± 0.2 mm) and gentamycin (26.0 ± 0.3 mm), while the low sensitive were clarithromycin (14.0 ± 0.3 mm), metronidazole and ornidazole (12.0 ± 0.3 mm).

We evaluated antibacterial activity of rutin, quercetin and some of antimicrobial drugs against resistant strains of *P. aeruginosa*, *E. cloacae*, *A. baumannii* and *K. pneumoniae*. Resistant strain of *P. aeruginosa* was sensitive to rutin (23.0 ± 0.3 mm) and low sensitive to quercetin (12.0 ± 0.6 mm) and chloramphenicol (12.5 ± 0.3 mm). Against *E. cloacae* rutin had the strongest inhibition effect as well as antimicrobial drugs such as chloramphenicol (19.5 ± 0.5 mm), levofloxacin (23.5 ± 0.5 mm), ceftriaxone (23.0 ± 0.2 mm), and gentamycin (22.0 ± 0.2 mm) had medium antibacterial activity. In the case of *A. baumannii*, resistant strain was only sensitive to the action of rutin (24.0 ± 0.3 mm) and quercetin (12.0 ± 0.3 mm). Against *K. pneumoniae* rutin had the highest value of retardation zone, while quercetin had lower inhibition property (12.0 ± 0.3 mm), in the case of antimicrobial drugs levofloxacin (20.5 ± 0.5 mm), ceftriaxone (19.5 ± 0.5 mm), gentamycin (17.5 ± 0.5 mm), metronidazole and ornidazole (16.0 ± 0.2 mm) as well as ciprofloxacin (15.5 ± 0.5 mm) had the medium inhibition property *(Table 10)*.

Table 8. Schematic classification of antimicrobial drug standards alongside quercetin and rutin into two categories

Compound		DNA-gyrase	DHFR	Deace-tylase	AHS LasI	AHS RhI	Digua-nylate cyclase	No. of inhibition enzymes of "First line of protection"	No. of inhibition enzymes of "Biofilm"
Antibacterial drug standards	Clarithromycin	✓	✓	✓	#	✓	#	3	1
	Chloramphenicol[1]	#	#	#	✓	#	#	0	1
	Ciprofloxacin	#	✓	#	#	#	#	1	0
	Levofloxacin	✓	✓	✓	#	#	#	3	0
	Ceftriaxone	#	#	#	#	#	#	0	0
	Metronidazole	#	#	#	#	#	#	0	0
	Ornidazole	#	#	#	#	#	#	0	0
	Gentamycin[2]	#	#	#	#	#	#	0	0
	Azithromycin	✓	✓	✓	#	✓	#	3	1
Biological active compounds	Quercetin[3]	#	#	#	#	#	#	0	0
	Rutin[4]	✓	✓	✓	✓	✓	#	3	2

✓**:** high level of selectivity; **#:** lower and medium of selectivity.

Table 9. Antibacterial effect of quercetin and rutin against test strains of *E. coli, P. vulgaris* and *P. aeruginosa*

Sample	Concentration, mM	Diameter of the growth retardation zone, mm ± SD		
		E. coli ATCC 25922	*P. vulgaris* ATCC 4636	*P. aeruginosa* ATCC 27853
Rutin	0.003	16.0 ± 0.5	14.0 ± 0.5	17.0 ± 0.4
Quercetin	0.003	20.0 ± 0.4	16.0 ± 0.5	18.0 ± 0.4
Clarithromycin	0.003	22.0 ± 0.3	14.0 ± 0.3	14.0 ± 0.3
Azithromycin	0.003	25.0 ± 0.3	21.0 ± 0.4	21.0 ± 0.3
Chloramphenicol	0.003	16.0 ± 0.3	15.0 ± 0.3	16.0 ± 0.3
Ciprofloxacin	0.003	29.0 ± 0.2	29.0 ± 0.3	30.0 ± 0.2
Levofloxacin	0.003	31.0 ± 0.2	30.0 ± 0.2	29.0 ± 0.2
Ceftriaxone	0.003	27.0 ± 0.2	24.0 ± 0.3	30.0 ± 0.2
Metronidazole	0.003	15.0 ± 0.5	Growth	12.0 ± 0.6
Ornidazole	0.003	15.0 ± 0.5	Growth	12.0 ± 0.6
Gentamycin	0.003	27.0 ± 0.3	26.0 ± 0.3	26.0 ± 0.3
Aqueous solution with 5 % of dimethyl sulfoxide	–	Growth	Growth	Growth

SD: standard deviation, n = 3.

Discussion

The high antimicrobial resistance of bacteria is caused by widespread application of antibiotics in the treatment diseases with non-bacteria origin as well as uncontrolled application of antibiotics in agriculture, above all, in livestock breeding [21].

The multidrug resistant bacteria are also called superbacteria. According to WHO the suberbacteria are represented by *A. baumannii*, *K. pneumoniae, P. aeruginosa* and *E. cloacae* [22]. According to temporary aspects to inhibit the growth of any resistant bacteria, antibacterial drug has to inhibit six main mechanisms: above all, "the first line of defense", there are DNA-gyrase, DHFR and inhibition of membrane formation, the second group of enzymes is responsible for biofilm formation: acyl-homoserine-lactone synthetize LasI and RhI, cyclic di-guanylate monophosphate (c-di-GMP) [23].

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 [23]. A key protective mechanism in bacteria is their membrane, and gram-negative strains are no exception. The membrane of these bacteria contains a unique lipopolysaccharide that triggers an immune response and fever. The enzyme UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase

Table 10. Antibacterial effect of quercetin and rutin against resistance strains of *P. aeruginosa, E. cloacae, A. baumannii* and *K. pneumonia*

Sample	Concentration, mM	Diameter of the growth retardation zone, mm ± SD			
		P. aeruginosa 18	*E. cloacae* 17	*A. baumannii* 150	*K. pneumoniae* 18
Rutin	0.003	23.0 ± 0.3	25.0 ± 0.2	24.0 ± 0.3	23.0 ± 0.3
Quercetin	0.003	12.0 ± 0.3	14.0 ± 0.5	12.0 ± 0.3	12.0 ± 0.3
Clarithromycin	0.003	Growth	Growth	Growth	Growth
Azithromycin	0.003	Growth	Growth	Growth	Growth
Chloramphenicol	0.003	12.5 ± 0.5	19.5 ± 0.5	Growth	Growth
Ciprofloxacin	0.003	Growth	Growth	Growth	15.5 ± 0.5
Levofloxacin	0.003	Growth	23.5 ± 0.5	Growth	20.5 ± 0.5
Ceftriaxone	0.003	Growth	23.0 ± 0.2	Growth	19.5 ± 0.5
Metronidazole	0.003	Growth	Growth	Growth	16.0 ± 0.2
Ornidazole	0.003	Growth	Growth	Growth	16.0 ± 0.2
Gentamycin	0.003	Growth	22.0 ± 0.2	Growth	17.5 ± 0.5
Aqueous solution with 5 % of dimethyl sulfoxide	–	Growth	Growth	Growth	Growth

SD: standard deviation, n = 3.

plays a crucial role in the biosynthesis of this lipopolysaccharide. Notably, this enzyme is exclusive to bacteria, as it lacks homologs in both humans and other mammals.

However, the problem of resistant bacteria is their ability to form biofilm. The biofilm is a structure of bacteria colony that prevent penetration antimicrobial drugs into the bacteria. The main mechanism of formation biofilm is the activation 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. The signal molecules in resistant bacteria are acyl-homoserine-lactone synthetize LasI and RhI. Moreover, the formation of biofilm requires a stage of cell adhesion of resistant bacteria to the surface, a protein c-di-GMP is responsible for this stage of formation biofilm. c-di-GMP is coordinated the transition of the bacterial lifestyle from motile to immobile [22].

Results of research showed that none of the antimicrobial drugs highly selective inhibit all "targets" mechanisms. However, rutin showed high value of selectivity of DNA-gyrase, DHFR, deacytelase (formation membrane), AHS LasI, and AHS RhI, whereas quercetin was not highly selective against all six "targets" mechanisms. We can make a conclusion that glucoside of quercetin has higher antibacterial potential than its aglycone. In our view, it can be related with the presence of glucose and rhamnose at third position that radically change an antibacterial potential of quercetin.

In the research of A. Ragunathan et al. [24] antibacterial theoretical potential of rutin and quercetin by molecular docking was studied. Comparing with our results binding energy of quercetin in our research was lower 10.8 % of DNA-gyrase, and DHFR, respectively. Whereas, binding energy of rutin in our research was higher 50,30 % of DNA-gyrase and DHFR. Ragunathan A. et al. [24] showed that binding energy of quercetin and rutin were approximately at the same level. In our opinion such difference can be related with the different model of enzymes, or as well as with the fact, that another active center was chosen.

To evaluate the antibacterial activity, we prepared solutions of rutin, quercetin, and antimicrobial drugs in one molar concentration. Why did we give the concentration in moles, and not, for example, as usual, in percent or mg/mL? According to the textbook "Analytical Chemistry" [25], mole is the amount of a substance containing the same number of structural units (atoms, molecules) as atoms contained in 12 g of carbon-12, thus 1 mole contains 6.022×10^{23} particles (Avogadro's number). Therefore, when we compare substances in one molar dose, the number of particles in all studied solutions of the substance will be the same, and then we can freely take and compare their antibacterial action. In the case when we take in one concentration in percent, or mg/mL, the number of particles of substances will be different everywhere, since each substance has a different molecular weight. Thus, comparison of their antibacterial effects is impossible.

Comparing theoretical studies with practical results, we can state a certain pattern of the connection and structure of the action of rutin and quercetin. According to the results of molecular docking, it was shown that the binding energy of rutin in 5 of 6 mechanisms exceeded the binding energy of quercetin by two times. Experimental results of the antibacterial activity of rutin and quercetin against resistant strains showed the same pattern, that rutin has an antibacterial effect two times higher than quercetin. However, we cannot establish such a pattern in the case of gram-negative test strains. The results showed that the action of rutin and quercetin are almost the same. In our opinion, this may be due to the fact that the test strains lack a number of proteins or enzymes that would be highly sensitive to the action of rutin, unlike resistant strains, for example, proteins or enzymes responsible for the formation of biofilm.

We can also note that no antibiotic inhibited all resistant strains of bacteria. This fact indicates the need for mandatory studies of combinations of antimicrobial drugs and rutin, quercetin or extracts (which contain a high concentration of rutin and quercetin). An antimicrobial drug is not able to inhibit all 6 key enzymes, but only with the addition of, for example, rutin, they will have the ability to actively suppress the growth of resistant strains. Because all "target" targets of bacteria responsible for the vital activity of bacteria will be suppressed.

Conclusions

1. Theoretical studies of "standard" antimicrobial drugs used in infectious disease treatment protocols are not highly selective inhibitors of "target" antibacterial mechanisms of gram-negative bacteria, unlike rutin, which turned out to be a highly selective inhibitor of the following enzymes: DNA-gyrase, DHFR, deacetylase, AHS RhI, Diguanylate cyclase.

2. Theoretical research demonstrated the potential antibacterial activity of rutin that exceeds effect of quercetin by two times. This pattern is fully confirmed by in vitro studies, where the antibacterial effect of rutin against resistant strains was also 2 times higher.

3. It was shown that none of the "classic" antimicrobial drugs inhibited all resistant gram-negative strains. Based on the results obtained, we came to the conclusion that the next necessary stage of our research is to study a combination of antimicrobial drugs and rutin, quercetin or herbal extracts that contain a high content of these individual compounds.

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References

1. Walsh TR, Gales AC, Laxminarayan R, Dodd PC. Antimicrobial Resistance: Addressing a Global Threat to Humanity. PLOS Med. 2023;20(7):e1004264. doi: 10.1371/journal.pmed.1004264
2. Mende K, Akers KS, Tyner SD, Bennett JW, Simons MP, Blyth DM, et al. Multidrug-Resistant and Virulent Organisms Trauma Infections: Trauma Infectious Disease Outcomes Study Initiative. Mil Med. 2022;187(Suppl 2):42-51. doi: 10.1093/milmed/usab131
3. Kalpana S, Lin WY, Wang YC, Fu Y, Lakshmi A, Wang HY. Antibiotic Resistance Diagnosis in ESKAPE Pathogens-A Review on Proteomic Perspective. Diagnostics. 2023;13(6):1014. doi: 10.3390/diagnostics13061014
4. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrob Resist Amp Infect Control. 2017;6(1). doi: 10.1186/s13756-017-0208-x
5. Vidaver AK. Uses of antimicrobials in plant agriculture. Clin Infect Dis. 2002;34 Suppl 3:S107-10. doi: 10.1086/340247
6. Li Y, Yao J, Han C, Yang J, Chaudhry M, Wang S, et al. Quercetin, Inflammation and Immunity. Nutrients. 2016;8(3):167. doi: 10.3390/nu8030167
7. Ghanbari-Movahed M, Mondal A, Farzaei MH, Bishayee A. Quercetin- and rutin-based nano-formulations for cancer treatment: A systematic review of improved efficacy and molecular mechanisms. Phytomedicine. 2022;97:153909. doi: 10.1016/j.phymed.2021.153909
8. Kumar R, Vijayalakshmi S, Nadanasabapathi S. Health Benefits of Quercetin. Def Life Sci J. 2017;2(2):142. doi: 10.14429/dlsj.2.11359
9. Wach A, Pyrzyńska K, Biesaga M. Quercetin content in some food and herbal samples. Food Chem. 2007;100(2):699-704. doi: 10.1016/j.foodchem.2005.10.028
10. Aghababaei F, Hadidi M. Recent Advances in Potential Health Benefits of Quercetin. Pharmaceuticals. 2023;16(7):1020. doi: 10.3390/ph16071020
11. Shabir I, Kumar Pandey V, Shams R, Dar AH, Dash KK, Khan SA, et al. Promising bioactive properties of quercetin for potential food applications and health benefits: A review. Front Nutr. 2022;9:999752. doi: 10.3389/fnut.2022.999752

12. Semwal R, Joshi SK, Semwal RB, Semwal DK. Health benefits and limitations of rutin – A natural flavonoid with high nutraceutical value. Phytochem Lett. 2021;46:119-28. doi: 10.1016/j.phytol.2021.10.006
13. Iriti M, Varoni EM, Vitalini S. Bioactive Compounds in Health and Disease – Focus on Rutin. Bioact Compd Health Dis. 2023;6(10):235. doi: 10.31989/bchd.v6i10.1145
14. Maslov O, Komisarenko M, Ponomarenko S, Horopashna D, Osolodchenko T, Kolisnyk S, et al. 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. 2022;35(4): 229-35. doi: 10.2478/cipms-2022-0040
15. Osolodchenko T, Andreeva I, Martynov A, Zavada N, Batrak O, Ryabova I. [Monitoring the stability over time of the antimicrobial effect of pharmaceutical compositions based on nisin with resistance inhibitors]. Annals of Mechnikov Institute. 2024;(3):40-6. Ukrainian. doi: 10.5281/zenodo.13819951
16. Morris GM, Huey R, Olson AJ. Using AutoDock for ligand-receptor docking. Curr Protoc Bioinformatics. 2008;Chapter 8:Unit 8.14. doi: 10.1002/0471250953.bi0814s24
17. RCSB Protein Data Bank. RCSB PDB: Homepage [Internet]. Rcsb.org. [cited 2025 Feb 3]. Available from: https://www.rcsb.org
18. PubChem. PubChem [Internet]. Nih.gov. [cited 2025 Feb 3]. Available from: https://pubchem.ncbi.nlm.nih.gov/
19. CASTp 3.0: Computed Atlas of Surface Topography of proteins [Internet]. Uic.edu. [cited 2025 Feb 3]. Available from: http://sts.bioe.uic.edu/castp/index.html?201l
20. Kondža M, Brizić I, Jokić S. Flavonoids as CYP3A4 Inhibitors In Vitro. Biomedicines. 2024;12(3):644. doi: 10.3390/biomedicines12030644
21. Pulingam T, Parumasivam T, Gazzali AM, Sulaiman AM, Chee JY, Lakshmanan M, et al. Antimicrobial resistance: Prevalence, economic burden, mechanisms of resistance and strategies to overcome. Eur J Pharm Sci. 2022;170:106103. doi: 10.1016/j.ejps.2021.106103
22. Abinaya M, Gayathri M. Inhibition of biofilm formation, quorum sensing activity and molecular docking study of isolated 3, 5, 7-Trihydroxyflavone from Alstonia scholaris leaf against P.aeruginosa. Bioorg Chem. 2019;87:291-301. doi: 10.1016/j.bioorg.2019.03.050
23. Jogula S, Krishna VS, Meda N, Balraju V, Sriram D. Design, synthesis and biological evaluation of novel Pseudomonas aeruginosa DNA gyrase B inhibitors. Bioorg Chem. 2020;100:103905. doi: 10.1016/j.bioorg.2020.103905
24. Ragunathan A, Ravi L. Potential antibacterial drug targets for Quercetin and Rutin: An in silico study using AutoDock. Pharm Lett. 2015;7(11):68-72.
25. Gritsenko IS, Bolotov VV, Klimenko LY, Kostina TA, Mykytenko OY, Kolisnyk S. [Analytical Chemistry]. Kharkiv, Ukraine: NUPh: Golden Page; 2019. Ukrainian.