Phytochemical analysis and antibacterial activity of raspberry (*Rubus idaeus*) fruit extract against Gram-negative multi-drug resistant bacteria from clinical isolates

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The basic therapy for microbial infections involves the application of antibiotics. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains. The purpose of our work was to study the phytochemical composition of a thick extract of raspberry fruits, as well as to investigate *in vitro* and *in silico* antibacterial activity against clinical multidrug-resistant strains of *A. baumannii, P. aeruginosa, K. pneumoniae* and *E. cloacae*. The quantification of biologically active substances (BAS) was accomplished with spectrophotometric, titrimetric and HPLC methods of analysis; antimicrobial effects were evaluated by the well diffusion method, whereas minimum inhibition concentration was determined by well plate method. The total content of phenolic compounds was 0.60 and 10.10%, organic acids – 4.60 and 1.60% for raspberry fruit thick and green tea leaf extract. The total content of anthocyanins in the raspberry fruit thick extract was 110.0 mg/100 g, where cyanidin-3-O-sophoroside was dominated (52.14±1.04 mg/100 g). Theoretical studies have shown that neither a single antibiotic nor anthocyanins are highly effective in inhibiting all antimicrobial mechanisms of resistant Gram-negative bacteria. The thick raspberry fruit extract actively inhibits all resistant strains of *A. baumannii, K. pneumoniae, P. aeruginosa* and *E. cloacae*. These findings have shown that to inhibit resistant strains of bacteria, you need to use only a complex drug or dietary supplements of raspberry together, and in turn, herbal medicines are a “lifeline” for their creation and there is a chance of old antimicrobial drugs back in life.

Keywords: raspberry fruit; anthocyanins; multi-drug resistant; Gram-negative strains; molecular docking.

Таңқұрай (*Rubus idaeus*) жеміс сығындысының фитохимиялық талдауы және клиникалық изоляттардан алынған грам-теріс көп дәріге төзімді бактерияларға қарсы белсенділігі

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Микробтық инфекцияларға қарсы негізгі терапия – антибиотиктерді қолдану. Алайда антибиотиктерді шамадан тыс қолдану мульти-резистентті штамдардың пайда болуы мен таралуының негізгі факторына айналды. Біздің жұмысымыздың мақсаты – таңқұрай жемістері қою сығындысының фитохимиялық құрамын зерттеу, сондай-ақ клиникалық мульти-резистентті *A. baumannii, P. aeruginosa, K. pneumoniae* және *E. cloacae* штамдарына қарсы *in vitro* және *in silico* бактерияға қарсы белсенділігін зерттеу. Биологиялық белсенді заттарды (ББЗ) сандық анықтау спектрофотометриялық, титриметриялық және ЖЭСХ әдістерімен жүргізілді; микробқа қарсы әсерлері ұңғыма әдісі және ең төменгі ингибирлеуші концентрация арқылы бағаланды. Фенолдық қосылыстардың жалпы мөлшері таңқұрай жемісінің қою сығындысы мен жасыл шай жапырағының сығындысы үшін тиісінше 0,60 және 10,10%, органикалық қышқылдар – 4,60 және 1,60% құрады. Таңқұрай жемістерінің қою сығындысында антоциандардың жалпы мөлшері 110,0 мг/100 г болды, құрамында цианидин-3-О-софоросид басым (52,14±1,04 мг/100 г) екені анықталды. Теориялық зерттеулер көрсеткендей, ешбір антибиотик немесе антоциан төзімді грам-теріс бактериялардың барлық микробқа қарсы механизмдерін тиімді тежей алмайды. Таңқұрай жемісінің қою сығындысы *A. baumannii, K. pneumoniae, P. aeruginosa* және *E. cloacae* төзімді штамдарының барлығын белсенді ингибирлейді. Бұл нәтижелер бактериялардың төзімді штамдарын тежеу үшін тек кешенді препаратты қолдану немесе таңқұрай қоспаларын бірлесіп қолдану қажет екенін көрсетеді, ал дәрілік өсімдіктер оларды жасаудың «құтқару шеңбері» болып табылады және ескі микробқа қарсы препараттарды қайта қолдану мүмкіндігі бар.

Түйін сөздер: таңқұрай жемісі; антоциандар; мульти-резистентті; грам-теріс штамдар; молекулалық докинг.

Фитохимический анализ и антибактериальная активность экстракта плодов малины (*Rubus idaeus*) против грамотрицательных мульти-резистентных бактерий из клинических изолятов

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Основным методом терапии микробных инфекций является применение антибиотиков. Однако чрезмерное использование антибиотиков стало главным фактором возникновения и распространения многорезистентных штаммов. Целью нашей работы было изучение фитохимического состава густого экстракта плодов малины, а также исследование его *in vitro* и *in silico* антибактериальной активности против клинических многорезистентных штаммов *A. baumannii, P. aeruginosa, K. pneumoniae* и *E. cloacae*. Количественное определение биологически активных веществ (БАВ) проводилось с использованием спектрофотометрических, титриметрических и ВЭЖХ (высокоэффективная жидкостная хроматография) методов анализа. Антимикробные эффекты оценивали методом диффузии в агар, а минимальную ингибирующую концентрацию определяли методом планшетного титрования. Общее содержание фенольных соединений составило 0,60% и 10,10%, органических кислот – 4,60% и 1,60% для густого экстракта плодов малины и экстракта листьев зеленого чая соответственно. Общее содержание антоцианов в густом экстракте плодов малины составило 110,0 мг/100 г, причем доминирующим соединением был цианидин-3-О-софоросид (52,14±1,04 мг/100 г). Теоретические исследования показали, что ни один отдельный антибиотик, ни антоцианы не обладают высокой эффективностью в подавлении всех антимикробных механизмов резистентных грамотрицательных бактерий. Густой экстракт плодов малины активно ингибировал все устойчивые штаммы *A. baumannii, K. pneumoniae, P. aeruginosa* и *E. cloacae*. Эти результаты показали, что для подавления устойчивых бактериальных штаммов необходимо использовать только комплексные препараты или биологически активные добавки на основе малины. Таким образом, растительные препараты становятся «спасательным кругом» для создания новых средств, и есть шанс вернуть старые антимикробные препараты в медицинскую практику.

Ключевые слова: плоды малины; антоцианы; мульти-резистентные; грамотрицательные штаммы; молекулярный докинг.

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Article

Phytochemical analysis and antibacterial activity of raspberry (*Rubus idaeus*) fruit extract against Gram-negative multi-drug resistant bacteria from clinical isolates

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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 baumannii, Pseudomonas aeruginosa* and *Klebsiela pneumoniae* are predominant among all isolated pathogens. Among all Gram-negative bacteria (*A. baumannii, P. aeruginosa and K. pneumoniae*), 71.3% were resistant to the antibiotic carbapenem, which is the last "line of defense" 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. baumannii, K. pneumoniae*, 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 raspberries, blackberries, and lingonberries, to treat infectious diseases [6]. For example, the leaves and shoots of the above-mentioned plants are a rich source of tannins, flavonoids and hydroxycinnamic acids, while anthocyanins and organic acids dominate in the fruits [7]. In folk medicine, raspberries, blackberries and lingonberries are used to treat urinary tract infections, gastrointestinal disorders, respiratory diseases and skin infections. The antimicrobial action of raspberry fruit anthocyanins against Gram-negative bacteria was previously reported [8]. Raspberries (*Rubus idaeus* L.) are cultivated throughout America, Eastern Europe, Russia, Asian as well as raspberry are closely related to blackberries and other brambles or cranberries. A red and black raspberry is the most widespread throughout the world. *R. idaeus* fruits composition is represented by a variety of flavonoid derivatives, is represented by anthocyanin, quercetin derivatives as well as phenolicarboxylic acids, organic acids, vitamin C [8]

Thus, the **purpose** of our work is to study the phytochemical composition of raspberry fruit thick extract, as well as to investigate *in vitro* and *in silico* antibacterial activity against clinical multidrug-resistant strains of *A. baumannii, P. eruginosa, K. pneumoniae* and *E. cloacae*.

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2. Experiment

2.1 Plant material

The raspberry (*Rubus idaeus*) fruits were the object of the study, which were collected from places of its native cultivation. The material was collected in 2021 after the fruiting period at July in the vicinity of the village of Ternova, Kharkiv region (50°19′31″ N, 36°66′93″ E; the altitude above sea: 92 m).

The study focused on the leaf of *Camellia sinensis* from the Chun Myn variety, which was gathered as raw material in the Anhui province of China during the months of March through May (31°03′41′′ N, 116°33′25′′ E; the altitude above sea: 100 m).

2.2 Reagents

Acetonitrile (purchased from «Allchem», Kharkiv), acetic acid (purchased from «Allchem», Kharkiv), phosphoric acid (purchased from «Allchem», Kharkiv), cyanidine-3-O-glucoside (≥98.0%), cyanidin-3-O-sophoroside (≥98.0%), pelargonidin-3-O-sophoroside (≥98.0%), cyanidin-3-O-rutinoside (≥98.0%), cyaniding-3-rutinoside-5-glucoside (≥98.0%),were purchased in Sigma Aldrich Company, Lublin, Poland.

2.3 Phytochemical analysis

The total phenolic compounds were quantified using the Folin-Ciocalteu method, with absorbance readings taken at 760 nm [9]. The total catechins were assessed using the vanillin reagent assay, where absorbance was measured at 505 nm [10]. For the quantification of total anthocyanin content, molecular adsorption analysis was utilized, with measurements of absorbance at 546 nm [11]. The content of total organic acids was established through acid-base titration, using a potentiometric method to determine the end-point [12].

2.4 Equipment

Potentiometric measurements were performed on a HANNA 2550 pH meter (FRG) with a glass electrode HI11310 (FRG). The quantitative analysis of biologically active compounds was performed on a UV-1000 spectrophotometer (LabAnalyt, China) with matched 1 cm quartz cells

2.5 Extraction procedure

One hundred milligrams (exact mass) of *R. idaeus* berries were pressed. Subsequently, 96% ethanol was added threefold to the extraction. Afterwards, the mixture was filtered, and the resulting filtrate was concentrated using a vacuum evaporator at a temperature of 50-60°C until the extract's moisture content reached 25%.

The *C. sinensis* extract was obtained by the following way: a 100.0 g of raw material was extracted with 60% ethanol at 80°C within 1 h with a condenser, with 1/20 ratio of raw material/solvent. The extraction technique was repeated twice to provide totally extract all BAS, then the filtrates were combined and evaporated by vacuum rotary to give ratio of extract to raw material 1:2.

2.6 HPLC analysis of *R. idaeus* berry thick extract

For the analysis, a Prominence LC-20 Shimadzu liquid chromatography system with a Thermo Scientific Syncronis aQ C18 column (4.6 × 250) was utilized. All analyses were conducted at 40°C. The mobile phases consisted of a methanol aqueous solution (A) and a 1.0% solution of phosphoric acid (B). The gradient protocol started with 20-42% A over the first 15 minutes, shifted to 42-43% A from 15 to 25 min, changed to 43-90% A from 25 to 45 min, maintained 90% A from 45 to 55 min, decreased to 20% A from 55 to 60 min, and then held at 20% A from 60 to 70 min. Prior to use, the mobile phases were filtered using 25mm × 0.45 µm Supelco Iso-Disc Filters PTFE 25-4 and degassed. A flow rate of 0.5 mL/min was maintained, and the injection volume of the samples was 5 µL. Detection wavelengths were set at 255, 286, 350 and 530 nm. Chromatographic peaks of analytes were identified by the following similarity indexes, which were calculated between the test substance and the standard according to the formulas:

$$\mathrm{I_T} = 1 - |\mathrm{T_{st}} - \mathrm{T_u}|$$

$$\mathrm{I_{255}} = 1 - \left|\mathrm{h_{255_{st}}} - \mathrm{h_{255_u}}\right|$$

$$\mathrm{I_{286}} = 1 - \left|\mathrm{h_{286_{st}}} - \mathrm{h_{286_u}}\right|$$

$$\mathrm{I_{350}} = 1 - \left|\mathrm{h_{350_{st}}} - \mathrm{h_{350_u}}\right|$$

where, I_T – retention time similarity index (SI), T_{st} – retention time of standard (min), T_u – test substance retention time (min), I_{255}, I_{286} and I_{350} – spectral similarity indices, h_{255st}, h_{286st} and h_{350st} – spectral characteristics of the standard, h_{255u}, h_{286u} и h_{350u} – spectral characteristics of the test substance.

The least among the three similarity index values of spectral characteristics dictates the similarity level (SL) between substances and standards based on these traits. A higher SL value increases the probability of more precise identification of the substance. Substances whose similarity index with the catechin standard was at least 0.7, and whose peaks on the chromatogram appeared between the catechin peak and the earliest flavonoid peak, were classified as catechins [15].

2.7 Test organisms

A four clinical isolates of multidrug-resistant Gram-negative bacteria were chosen for research: *Acinetobacter baumannii, Klebsiella pneumonia, Pseudomonas aeruginosa, and Enterobacter cloacae.* 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.

2.8 Screening antimicrobial activity of extracts

The agar well diffusion test was carried out as described in [13]. Table 1 shows interpretation criteria for microbial sensitivity. As standards it was applied following antibiotics: 30 µg/disk of chloramphenicol, 1.86 mM; 5 µg/disk of levofloxacin, 0.28 mM, 30 µg/disk of gentamicin, 0.42 mM; 30 µg/disk of doxycycline, 1.35 mM; 30 µg/disk of ceftriaxone, 1.08 mM; 30 µg/disk of ceftazidime, 0.94 mM; 30 µg/disk of cefepime, 1.25 mM; 5 µg/disk of moxifloxacin, 0.23 mM; 30 µg/disk of netilmicin, 0.42 mM; 30 µg/disk of amoxicillin, 1.64 mM.

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

2.9 Assay of determination of minimum inhibitory concentration (MIC)

MIC is the lowest concentration of antibacterial agent that completely inhibits the bacterial growth. The MIC of the different extracts was assessed using the broth microdilution method [14].

3.0 Molecular docking

A molecular docking study was conducted using the tool known as AutoDockTools 1.5.6. 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 [15]. 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 cyanidin-3-O-glucoside (CID_12303220), cyanidin-3-O-rutinoside (CID_29231), cyanidin-3-O-malonyl glycoside (CID_443915), cyanidin-3-O-xyloside (CID_87948385) and cyanidin-3,3'-diglucoside (CID_44256727) were obtained from PubChem database [16]. The active site of the docking protein was identified utilizing the Computed Atlas for Surface Topography of Proteins (CASTp) [17].

3.1 Statistical analysis

For all the experiments, two samples were analyzed and all the assays were carried out in 5 times. The results were expressed as mean values with confident interval. The MS EXCEL 7.0 was used to provide statistical analysis.

3. Results and discussion

According to obtained results shown in Table 2, the *C. sinensis* leaf extract (10.10±0.25%) had higher content of phenolic compounds, than in *R. idaeus* thick fruit extract (0.60±0.02%).

Table 2 demonstrates that the total content of anthocyanin in *R. idaeus* thick fruit extract was 0.10±0.002%, whereas in *C. sinensis* leaf extract anthocyanin was not presence. The percentage of anthocyanin out of total polyphenols was 17% in *R. idaeus* extract.

The highest amount of organic acids was determined in *R. idaeus* thick fruits extract (4.60±0.50%), whereas in the *C. sinensis* leaf extract it was lower 65% (1.60±0.02%). In *R. idaeus* extract, the total organic acids were in 8.5 times higher than polyphenols, whereas in the *C. sinensis* leaf, the total organic acids were in 6.3 times lower than polyphenols (Table 2).

The HPLC method was used to carry out a qualitative and quantitative analysis of anthocyanins in the obtained extract of *R. idaeus* fruits extract. According to the results of the study, 6 anthocyanins were identified in *R. idaeus* (Figure 1).

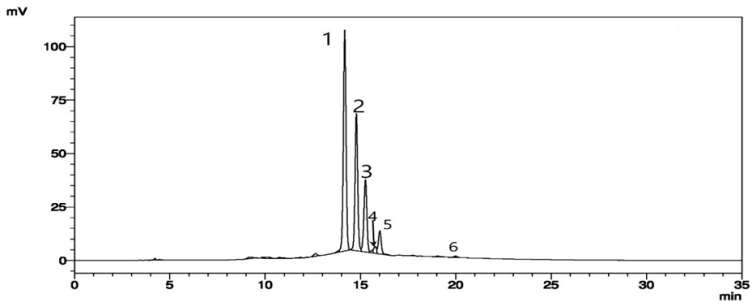

Figure 1 – HPLC fingerprint (530 nm) of the *R. idaeus* fruit thick extract

Table 2 – Quantitative content of total phenolic compounds, anthocyanin and organic acids in *R. fruticosus* fruit thick extract

Sample	Total phenolic compounds, %±SD	Total anthocyanin, %±SD	Total catechin, %±SD	Total of organic acids, %±SD
R. idaeus fruit thick extract	0.60±0.02	0.10±0.002	—	4.60±0.50
Green tea leaf extract	**10.10±0.25**	—	**10.47±0.25**	**1.60±0.10**

Notes: SD – standard deviation, n=5.

As shown in Table 3, cyanidin-3-O-sophoroside dominated among all anthocyanins (47.4% out of the total anthocyanins), cyanidin-3-rutinoside-5-glucoside (29.0% out of the total anthocyanins) was in second place, and the lowest content was pelargonidin-3-O-sophoroside (0.47% out of the total anthocyanins).

The next stage of our research was to conduct a theoretical investigation of the antimicrobial activity of the identified compounds using molecular docking for realsing 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 tetracyclines (Doxycycline), aminoglycosides (Gentamicin, Netilmicin), fluoroquinolones (Moxifloxacin, Levofloxacin), β-lactames (Cefepime, Ceftazidime, Ceftriaxone), penicillins (Amoxicillin), and amphenicols (Chloramphenicol).

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. In this work, we will use a conditional rating classification based on the value of binding energy. This rating is based purely on our results obtained during our theoretical research. We propose to divide the binding energy value into three levels: low (binding energy up to -4.5 kcal/mol), medium (binding energy from -4.5 to -9.5 kcal/mol) and high (binding energy > -9.5 kcal/mol).

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 cyanidin-3-O-sophoroside, pelargonidin-3-O-sophproside, doxycycline, cyanidin-3-O-rutinoside, moxifloxacin, cyanidin-3-O-glycoside had high affinity to the active site, whereas the lowest level had gentamicin (Table 4).

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. 5, the following compounds had high binding energies: cyanidin-3-O-sophoroside, pelargonidin-3-O-sophproside, doxycycline, cyanidin-3-O-glycoside, cyanidin-3-O-rutinoside, moxifloxacin, netilmicin, ceftazidime (Table 4).

Table 3 – Chemical composition of antocyanins in *R. idaeus* fruit thick extract by HPLC analysis

№	Antocyanins	Retention time, min	Content of antocyanins in extract, mg/100 g of extract ±SD	% out of total antocyanins
1	Cyanidin-3-O-sophoroside	14.170	52.14±1.04	47.4
2	Cyanidin-3-rutinoside-5-glucoside	14.780	31.90±0.64	29.0
3	Cyanidine-3-O-glucoside	15.255	18.15±0.36	16.5
4	Cyanidin-3-O-rutinoside	16.200	5.59±0.11	5.08
5	Cyanidin 3-O-xylosyl-rutinoside	16.393	1.61±0.03	1.46
6	Pelargonidin-3-O-sophoroside	19.977	0.47±0.01	0.43
	THE TOTAL ANTOCYANINS		110.0	

Notes: SD – standard deviation, n=5.

Table 4 – Results of molecular docking of the compounds identified by the HPLC in the *R. idaeus* thick extract and antimicrobials drug standards with the DNA-gyrase, DHFR and deacytelese structures (ΔGbind[a], kcal/mol)

№	Ligand	DNA-gyrase	Ligand	DHFR	Ligand	Dea-cytelese
1	2	3	4	5	6	7
1	Cyanidin-3-O-sophoroside	-12.92	Cyanidin-3-O-sophoroside	-11,66	Cyanidin-3-O-sophoroside	-13,39
2	Pelargonidin-3-O-sophoroside	-12.00	Pelargonidin-3-O-sophoroside	-11,10	Pelargonidin-3-O-sophoroside	-13,15
3	**Doxycycline**	**-11.59**	**Doxycycline**	**-11.59**	**Netilmicin**	**-11.09**
4	Cyanidin-3-O-rutinoside	-11.41	Cyanidin-3-O-glycoside	-11.42	**Doxycycline**	**-11.03**
5	Moxifloxacin	-10.29	Cyanidin-3-O-rutinoside	-11.16	**Ceftazidime**	**-10.37**

Table 4 *(continued)*

1	2	3	4	5	6	7
6	Cyanidin-3-O-glycoside	-9.69	**Moxifloxacin**	**-10.89**	**Moxifloxacin**	**-9.78**
7	**Netilmicin**	**-9.00**	**Netilmicin**	-10.70	Cyanidin-3-O-glycoside	-9.74
8	**Levofloxacin**	**-8.69**	**Ceftazidime**	-9.49	Cyanidin-3-O-rutinoside	-9.61
9	**Cefepime**	**-8.27**	**Levofloxacin**	-8.98	**Cefepime**	**-8.77**
10	Cyanidin-3-rutinoside-5-glucoside	-8.04	**Cefepime**	**-8.37**	**Levofloxacin**	**-8.34**
11	Cyanidin 3-O-xylosyl-rutinoside	-7.56	Cyanidin 3-O-xylosyl-rutinoside	-8.25	Cyanidin 3-O-xylosyl-rutinoside	-8.32
12	**Amoxicillin**	**-7.24**	**Chloramphenicol**	**-7.97**	**Gentamicin**	**-7.45**
13	**Ceftazidime**	**-6.48**	Cyanidin-3-rutinoside-5-glucoside	-7.90	**Chloramphenicol**	**-7.19**
14	**Chloramphenicol**	**-6.38**	**Amoxicillin**	**-7.87**	Cyanidin-3-rutinoside-5-glucoside	-6.80
15	**Ceftriaxone**	**-4.61**	**Gentamicin**	**-6.78**	**Amoxicillin**	**-6.64**
16	**Gentamicin**	**-4.08**	**Ceftriaxone**	**-6.36**	**Ceftriaxone**	**-6.09**

Notes: red colour – low level of affinity ΔG <4.5 kcal/mol; orange colour – medium level of affinity 4.5<ΔG<9.5 kcal/mol; green colour – high level of of affinity ΔG>9.5 kcal/mol.

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 rating, it was established that cyanidin-3-O-sophoroside, pelargonidin-3-O-sophproside, netilmicin, doxycycline, ceftazidime, moxifloxacin, cyanidin-3-O-glycoside, cyanidin-3-O-rutinoside had high affinity to the active site (Table 5).

Table 5 – Results of molecular docking of the compounds identified by the HPLC in the *R. idaeus* thick extract and antimicrobials drug standards with the AHS LasI, AHS RhI, diguanylate cyclase structures (ΔGbind[a], kcal/mol)

№	Ligand	AHS LasI	Ligand	AHS RhI	Ligand	Diguanyl-ate cyclase
1	**Chloramphenicol**	**-10.76**	Cyanidin-3-O-rutinoside	-12.94	**Doxycycline**	**-9.14**
2	**Ceftriaxone**	**-6.56**	**Doxycycline**	**-10.99**	**Ceftazidime**	**-8.06**
3	**Amoxicillin**	**-6.55**	Cyanidin 3-O-xylosyl-rutinoside	-10.85	Cyanidin-3-O-rutinoside	-7.66
4	**Moxifloxacin**	**-6.34**	Cyanidin-3-rutinoside-5-glucoside	-9.80	Cyanidin-3-O-glycoside	-7.63
5	**Doxycycline**	**-4.99**	**Netilmicin**	**-8.36**	Cyanidin-3-O-sophoroside	-7.09
6	**Levofloxacin**	**-4.11**	**Moxifloxacin**	**-8.27**	Pelargonidin-3-O-sophoroside	-7.00
7	Cyanidin-3-O-glycoside	—	**Amoxicillin**	**-7.41**	**Chloramphenicol**	**-6.59**
8	Cyanidin-3-O-rutinoside	—	**Levofloxacin**	**-6.62**	Cyanidin-3-rutinoside-5-glucoside	-6.54
9	Cyanidin 3-O-xylosyl-rutinoside	—	**Ceftazidime**	**-6.43**	Cyanidin 3-O-xylosyl-rutinoside	-6.47
10	Cyanidin-3-rutinoside-5-glucoside	—	**Chloramphenicol**	**-5.88**	**Moxifloxacin**	**-6.3**
11	Cyanidin-3-O-sophoroside	—	**Cefepime**	**-5.05**	**Netilmicin**	**-6.06**
12	Pelargonidin-3-O-sophoroside		**Ceftriaxone**	**-4.48**	**Amoxicillin**	**-5.89**
13	**Gentamicin**	—	Pelargonidin-3-O-sophoroside	-2.50	**Levofloxacin**	**-5.32**
14	**Ceftazidime**	—	Cyanidin-3-O-sophoroside	-2.46	**Ceftriaxone**	**-5.19**
15	**Cefepime**	—	Cyanidin-3-O-glycoside	—	**Gentamicin**	**-4.49**
16	**Netilmicin**	—	**Gentamicin**	—	**Cefepime**	**-4.40**

Notes: red colour – low level of affinity ΔG <4.5 kcal/mol; orange colour – medium level of affinity 4.5<ΔG<9.5 kcal/mol; green colour – high level of of affinity ΔG>9.5 kcal/mol.

The AHS LasI was 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. 6, the following compounds had high binding energy: chloramphenicol, whereas levofloxacin had the lowest level of free energy as well as cyanidin-3-O-glycoside, cyanidin-3-O-rutinoside, cyaniding-3-O-xylosyl-rutinoside, cyanidin-3-rutinoside-5-glucoside, cyanidin-3-O-sophoroside, pelargonidin-3-O-sophoroside gentamicin, ceftazidime, cefepime and netilmicin were not interact with active center of AHS LasI (Table 6).

Molecular modeling 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 rating, it was established that cyanidin-3-O-rutinoside, doxycycline, cyanidin-3-O-xylosyl-rutinoside, whereas cyanidin-3-O-sophoroside had the lowest level of binding as well as cyanidin-3-O-glycoside and gentamicin were 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 was any compound possessing high affinity energy. All compounds except gentamicin and cefepime had medium level of energy affinity (Table 5).

Table 6 – Schematic division of antimicrobial drug standards and identified anthocyanins in two categories

№	Compound	DNA-gyrase	DHFR	Deacytelese	AHS LasI	AHS RhI	Diguanylate cyclase	№ of inhibition enzymes of «First line of protection”	№ of inhibition enzymes of «Biofilm”
Antimicrobial drug standards									
1	**Chloramphenicol**							0	1
2	**Levofloxacin**							0	0
3	**Gentamicin**							0	0
4	**Doxycycline**							3	1
5	**Ceftriaxone**							0	0
6	**Ceftazidime**							1	0
7	**Cefepime**							0	0
8	**Moxifloxacin**							3	0
9	**Netilmicin**							2	0
10	**Amoxicillin**							0	0
Identified anthocyanins									
11	Cyanidin-3-O-glycoside							3	0
12	Cyanidin-3-O-rutinoside							3	1
13	Cyanidin-3-rutinoside-5-glucoside							0	0
14	Cyanidin-3-O-xylosyl-rutinoside							0	1
15	Pelargonidin-3-O-sophoroside							3	0
16	Cyanidin-3-O-sophoroside							3	0

Notes: green colour – high level of inhibition; red colour – lower and medium of inhibition.

Further, all antibiotics and anthocyanins were conditionally divided into two categories. The first category included compounds that had a high affinity for the active site, and the second category included compounds that had medium and low binding energies. 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 obtained shown in Table 7, it can be seen that among all antibiotics, doxycycline works best, which inhibits all enzymes of the “first line of defense”, and in the case of biofilm formation, only one mechanism was inhibited – AHS LasI. Amoxacillin, cefepime, ceftriaxone, gentamicin, levofloxacin were not highly effectively inhibit any of the mechanisms of antimicrobial action presented above. In the case of anthocyanins, among all the compounds presented, only cyanidin-3-O-rutinoside inhibits

the largest number of mechanisms, namely this compound actively inhibits the "first line of defense" of bacteria, and one mechanism of biofilm formation – AHS RhI. In turn, cyanidin-3-rutinoside-5-glucoside was not highly effectively inhibit any of the mechanisms of action.

In this research work, the antimicrobial and antifungal activity of the obtained *R. idaeus* thick fruit extract was investigated against the following antimicrobial resistance strains of *A. baumannii*, *K. pneumoniae, P. aeruginosa, E. cloacae*. According to the obtained results, extract obtained from the *R. idaeus* fruit had a potent antimicrobial effect.

Among all Gram-negative strains *R. idaeus* fruit extract was the most active against *K. pneumoniae*, whereas the lowest inhibition had against *E. cloacae*. However, the strains of *A. baumannii* and *P. aeruginosa* were also sensitive to the action of *R. idaeus* fruit extract. Comparing obtained results with reference standards of antimicrobial drugs, it was established that *A. baumannii*, *K. pneumoniae, P. aeruginosa* were absolutely resistant to the action of antimicrobial drugs. In the case of *E. cloacae* only 3 antibiotics inhibited the growth of resistant strains as well as other reference standards were not affect to the growth of *E. cloacae* strain at all (Table 7).

Table 7 – Inhibition diameter (mm) resulting from the screening of antimicrobial effect against resistance strains of *A. baumannii*, *K. pneumoniae, P. aeruginosa, E. cloacae* by well diffusion method with of raspberry thick extract and drug standards (Chloramphenicol, Levofloxacin, Gentamicin, Doxycycline, Ceftriaxone, Ceftazidime, Cefepime, Moxifloxacin, Netilmicin, Amoxicillin)

Sample	Concentration, mM	Diameter of the growth retardation zone, mm±SD			
		A. baumannii 150	*K. pneumoniae 18*	*P. aeruginosa 18*	*E. cloacae 17*
Raspberry thick extract	0.009[a]	18.0±0.4	20.0±0.30	19.5±0.5	17.5±0.5
Chloramphenicol	1.86	**Growth**	**Growth**	**Growth**	**Growth**
Levofloxacin	0.28	**Growth**	**Growth**	**Growth**	**Growth**
Gentamicin	0.42	**Growth**	**Growth**	**Growth**	**12.0±0.6**
Doxycycline	1.35	**Growth**	**Growth**	**Growth**	**12.0±0.6**
Ceftriaxone	1.08	**Growth**	**Growth**	**Growth**	**Growth**
Ceftazidime	0.94	**Growth**	**Growth**	**Growth**	**Growth**
Cefepime	1.25	**Growth**	**Growth**	**Growth**	**Growth**
Moxifloxacin	0.23	**Growth**	**Growth**	**Growth**	**Growth**
Netilmicin	0.42	**Growth**	**Growth**	**Growth**	**11.0±0.6**
Amoxicillin	1.64	**Growth**	**Growth**	**Growth**	**Growth**

Notes: SD – standard deviation, n=5, a – molar concertation of total phenolic compounds in terms of gallic acid.

The investigated extract *R. idaeus* significantly inhibit the antibiotic resistant strains with MIC. In the previously above conducted antimicrobial study, the extract of *R. idaeus* fruit extract was the most active independently of the tested strains. Table 7 shows, the *R. idaeus* fruits extract with MIC value of 1.13 µM was the most active against *A. baumannii*, *K. pneumoniae, P. aeruginosa,* whereas *E. cloacae* was the most resistant against *R. idaeus* thick extrac (Table 8).

The content of BAS in *R. idaeus* fruit extracts was quantified by spectrophotometric, titrimetric and HPLC methods of analysis. The organic acids were present in both extracts, where the highest content of organic acids was determined in *R. idaeus* fruits extract than in green tea leaf extract. In our view, it relates with different purpose of accumulation organic acids. The organic acids are precursor for biosynthesis of sugars in fruits, whereas in leaf, organic acids only play a role in photosynthesis as result there is no purpose of high accumulation organic acids in leaf [18]. Szymanowska U. *et al.* [19] investigated anthocyanin content of *R. idaeus* fruit 90% methanol extract by HPLC method. According to their

Table 8 – Minimal inhibitory concentration of the *R. idaeus* thick extract against the resistance strains of *A. baumannii*, *K. pneumoniae, P. aeruginosa, E. cloacae*

Sample	MIC, µM			
	A. baumannii	*K. pneumoniae*	*P. aeruginosa*	*E. cloacae*
Raspberry thick extract	1.13	1.13	1.13	2.25

results, it was detected following anthocyanins (mg/100 g per extract): cyanidin-3-O-glucoside (40 mg/100 g), cyanidin-3-O-rutinoside (40 mg/100 g), cyanidin-3-O-sophoroside (70 mg/100 g), cyanidin-3-rutinoside-5-glucoside (45 mg/100 g) and pelargonidin-3-O-sophoroside (1.0 mg/100 g) Compering with our results, the content of anthocyanins in our research was lower, but cyanidin-3-O-glucoside was dominated in both extracts. The chemical composition of fruit is changed during fruits development, its ripening, and different cultivars.

The search of new antimicrobial drugs has become a very important task for scientific community. One of the way to create effective antimicrobial drugs is focused on the application of natural compounds such as catechins and anthocyanins. 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. baumannii, K. pneumoniae, P. aeruginosa* and *E. cloacae* [20]. 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 [21]. 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 [22].

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 [23]. 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 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 [24]. 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 not a single antibiotic and not a single anthocyanin highly effectively inhibits all mechanisms of antimicrobial action, which suggests that in order to inhibit the growth of "superbacteria," a complex antimicrobial drug or jointly apply with dietary supplements of raspberry should be used. According to our results, chloramphenicol works highly effectively through only one mechanism - AHS LasI; doxycycline is effective against DNA gyrase, DHFR, deacetylase and AHS RhI; ceftazidime works well against deacetylase; Moxifloxacin is effective against DNA gyrase, DHFR and deacetylase, while netilmicin works only against DHFR and deacetylase.

A serious threat to human health is the emergence of "superbacteria". This issue is especially relevant in relation to *A. baumannii, K. pneumoniae, 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, fluoroquinolones, as well as to the action of the "last line of defense" - carbapenems [25]. 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 [26].

In our experimental studies showed that no antibiotic has an effect on *A. baumannii, K. pneumoniae* and *P. aeruginosa*, and in the case of *E. cloacae*, the antibiotics gentamicin, doxycycline and netilmicin work, but *E. cloacae* is low sensitive to these antibiotics. At the same time, the thick *R. idaeus* extract actively inhibits all of the above-mentioned resistant strains of bacteria. In theoretical studies, it was found that no single antibiotic highly effectively inhibits all antimicrobial mechanisms; in the case of the identified anthocyanins, it was also shown that anthocyanins also did not inhibit all mechanisms of action. But, we want to note that *R. idaeus* extract is a complex drug, therefore, in experimental studies, *R. idaeus* extract inhibited the growth of all bacteria, and in turn, antibiotics only inhibited the bacterial strain *E. cloacae*.

The next important point is that the dose of anthocyanins in the thick *R. idaeus* extract was an order of magnitude less than the dose of antibiotics, and the *R. idaeus* extract effectively inhibited resistant bacteria. But, this raises the question of how anthocyanins could so actively inhibit bacterial growth, despite the fact that in molecular docking studies they do not inhibit all mechanisms of antimicrobial action. Phytochemical analysis showed that the thick *R. idaeus* extract contains a high content of organic acids, which exceeds the content of phenolic compounds by 6 times. In our opinion, organic acids can inhibit those mechanisms that anthocyanins cannot or have a weak effect on. Organic acids are promising antimicrobial compounds, so in our previously published work we studied a lipophilic extract of green tea leaves, where the main group of compounds

were organic acids. As a result of our research, it was shown that it was organic acids that made the main contribution to the antimicrobial effect of the extract. Although, the main advantage of *R. idaeus* extract – minimal side effects. Pharmaceuticals based on natural compounds do not lead to liver cirrhosis, as with the use of tetracyclines; do not have nephrotoxicity and do not lead to deafness, as in the case of taking aminoglycosides. According to obtained theoretical and practical results, it was concluded that obtaining a single drug that will inhibit the growth of resistant strains of bacteria is impossible.

4. Conclusion

It has been established that thick raspberry extract contains phenolic compounds, anthocyanins and organic acids. Cyanidin-3-O-sophoroside is one of the main anthocyanins in raspberry fruit extract. Theoretical studies have found that no single antibiotic highly effectively inhibits all antimicrobial mechanisms of resistant Gram-negative bacteria. Thick raspberry fruit extract actively inhibits all resistant strains of *A. baumannii, K. pneumoniae, P. aeruginosa,* and *E. cloacae*. These studies show that to inhibit resistant strains of bacteria, you need to use only a complex drug or jointly apply with dietary supplements of raspberry, and in turn, herbal medicines are a "lifeline" for their creation and there is a chance to return old antimicrobial drugs in life.

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CRediT authorship contribution statement

Maslov O.Y.: Writing - Original draft, Methodology, Investigation; Komisarenko M.A.: Data curation, Investigation, Visualization; Ponomarenko S.V.: Data curation, Investigation, Visualization; Osolodchenko T.P.: Conceptualization, Supervision; Kolisnyk S.V.: Conceptualization, Supervision; Polishchuk S.I.: Writing- Review & Editing.

References

1 Mende K, Akers KS, Tyner SD, Bennett JW, Simons MP, et al. (2022) Mil Med 187(Supplement_2):42-51. *https://doi.org/10.1093/milmed/usab131*

2 Kondratiuk V, Jones BT, Kovalchuk V, Kovalenko I, Ganiuk V, et al. (2021) J Hosp Infect 112:69-76. *https://doi.org/10.1016/j.jhin.2021.03.020*

3 Petrosillo N, Petersen E, Antoniak S (2023) Lancet Infect Dis 1:1-10. *https://doi.org/10.1016/s1473-3099(23)00264-5*

4 World Health Organization Regional Office for Europe (WHO/Europe)/European Centre for Disease Prevention and Control (ECDC). Antimicrobial resistance surveillance in Europe 2022–2020 data. Copenhagen: WHO/Europe; 2022. Web-page: *https://www.ecdc.europa.eu/sites/default/files/documents/ECDC-WHO-AMR-report.pdf*

5 Schultze T, Hogardt M, Velázquez ES, Hack D, Besier S, et al. (2023) Eurosurveillance 28(1):1-10. *https://doi.org/10.2807/1560-7917.es.2023.28.1.2200850*

6 Khan R, Islam B, Akram M, Shakil S, Ahmad AA, et al. (2009) Molecules 14(2):586-597. *https://doi.org/10.3390/molecules14020586*

7 Maslov OY, Kolisnyk SV, Komisarenko MA, Kolisnyk OV, Ponomarenko SV (2021) Pharmacologyonline (3):291-298. *https://doi.org/10.5281/zenodo.7813115*

8 Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J (2015) Int J Mol Sci 16(10):24673-24706. *https://doi.org/10.3390/ijms161024673*

9 Maslov OY, Komisarenko MA, Golik MY, Kolisnyk SV, Altukhov AA, et al. (2023) Vitae 30(1):1-8. *https://doi.org/10.17533/udea.vitae.v30n1a351486*

10 Maslov O, Kolisnyk S, Komisarenko M, Golik M (2022) Herba Pol 68(1):1-9. *https://doi.org/10.2478/hepo-2022-0003*

11 Maslov OY, Komisarenko MA, Kolisnyk SV, Golik MY, Doroshenko SR, et al. (2023) News Pharm 106(2):5-12. *https://doi.org/10.24959/nphj.23.119*

12 Maslov O, Kolesnik S, Komisarenko M, Altukhov A, Dynnyk K, Kostina T (2021) Pharmakeftiki 33(4):304–311. *https://doi.org/10.5281/zenodo.7813135*

13 Maslov O, Komisarenko M, Ponomarenko S, Horopashna D, Osolodchenko T, et al. (2022) Curr Issues Pharm Med Sci 35(4):229-235. *https://doi.org/10.2478/cipms-2022-0040*

14 Mbarga MJ, Podoprigora IV, Volina EG, Ermolaev AV, Smolyakova LA (2021) J Pharm Res Int 158-167. *https://doi.org/10.9734/jpri/2021/v33i29b31601*

15 RCSB PDB: Homepage. RCSB PDB: Homepage; Web-page: *https://www.rcsb.org/.*

16 PubChem. PubChem; Web-page: *https://pubchem.ncbi.nlm.nih.gov/.*

17 Tian W, Chen C, Lei X, Zhao J, Liang J. (2018) Nucleic Acids Res 46(W1):W363-W367. *https://doi.org/10.1093/nar/gky473*

18 Arena ME, Povilonis IS, Borroni V, Pérez E, Pellegrino N, Cacciatore C, Radice S (2023) Horticulturae 9(3):314. *https://doi.org/10.3390/horticulturae9030314*

19 Szymanowska U, Baraniak B, Bogucka-Kocka A (2018) Molecules 23(7):1812. *https://doi.org/10.3390/molecules23071812*

20 Abinaya M, Gayathri M (2019) Bioorganic Chem 87:291-301. *https://doi.org/10.1016/j.bioorg.2019.03.050*
21 Jogula S, Krishna VS, Meda N, Balraju V, Sriram D (2020) Bioorganic Chem 100:103905. *https://doi.org/10.1016/j.bioorg.2020.103905*
22 Zuo K, Liang L, Du W, Sun X, Liu W, Gou X, et al. (2017) Int J Mol Sci 18(5):761. *https://doi.org/10.3390/ijms18050761*
23 Abinaya M, Gayathri M (2019) Bioorganic Chem 87:291-301. *https://doi.org/10.1016/j.bioorg.2019.03.050*
24 Valentini M, Filloux A (2016) J Biol Chem 291(24):12547-12555. *https://doi.org/10.1074/jbc.r115.711507*
25 Jean SS, Harnod D, Hsueh PR. (2022) Front Cell Infect Microbiol 12. *https://doi.org/10.3389/fcimb.2022.823684*
26 Aranaga C, Pantoja LD, Martínez EA, Falco A (2022) Int J Mol Sci 23(9):4577. *https://doi.org/10.3390/ijms23094577*