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Oleksandr MASLOV
PhD, Assistant at the Department of General Chemistry, National University of Pharmacy, Grigory Skovorody Street, 53, Kharkiv, Ukraine, 61002 (alexmaslov392@gmail.com)
ORCID: *0000-0001-9256-0934*
SCOPUS: *57226660079*

Mykola KOMISARENKO
PhD, Assistant at the Department of Pharmacognosy and Nutriciology, National University of Pharmacy, Grigory Skovorody Street, 53, Kharkiv, Ukraine, 61002 (a0503012358@gmail.com)
ORCID: *0000-0002-1161-8151*
SCOPUS: *57212146273*

Valerii MINUKHIN
DcS, Professor, Director of the I.I. Mechnikov Institute of Microbiology and Immunology of the National Academy of Sciences of Ukraine, Grigory Skovorody Street, 12, Kharkiv, Ukraine, 61002 (chemistry29@meta.ua)
ORCID: *0000-0002-9682-9686*
SCOPUS: *6603854139*

Tetiana OSOLODCHENKO
PhD, Head of Laboratory of Biochemistry and Biotechnology, I.I. Mechnikov Institute of Microbiology and Immunology of the National Academy of Sciences of Ukraine, Grigory Skovorody Street, 12, Kharkiv, Ukraine, 61002 (imi_lbb@ukr.net)
ORCID: *0000-0002-5064-6550*
SCOPUS: *57199560169*

Sergii KOLISNYK
Doctor of Pharmaceutical Sciences, Professor, Head of Department of General Chemistry, National University of Pharmacy, Grigory Skovorody Street, 53, Kharkiv, Ukraine, 61002 (s_kolesnik@nuph.edu.ua)
ORCID: *0000-0002-4920-6064*
SCOPUS: *57217102532*

Andrey KOMISARENKO
Doctor of Pharmaceutical Sciences, Professor, Pharmaceutical Company "ZDRAVOPHARM", Uspenskaya Street, 6, Sorokivka, Kharkiv region, 62430 (nik25spider@gmail.com)
ORCID: *0000-0002-2238-5138*
SCOPUS: *6602821633*

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THEORETICAL AND PRACTICAL JUSTIFICATION OF THE COMPOSITION OF ANHYDROUS GEL "HYPERICUM-DERM" USED FOR THE TREATMENT OF PURULENT WOUNDS CAUSED BY MULTIDRUG-RESISTANT BACTERIAL AND FUNGAL STRAINS

Actuality. *Antimicrobial resistance is a huge problem for health care worldwide. One of the first mentions of the emergence of antibiotic-resistant bacterial strains in humans was received during the military conflicts in Iraq and Afghanistan 20 years ago. In addition, according to the latest data, Acinetobacter baumani, Pseudomonas aeruginosa, Klebsiela pneumonia and Enterobacter cloacae predominate among all isolated resistant pathogens. Therefore, the development a new antibacterial drug capable of combating resistance bacteria is the topical task.*

The aim of the study. Conduct theoretical and practical research to substantiate the composition of the anhydrous gel "Hypericum-Derm" with antimicrobial action against multiresistant strains of P. aeruginosa, A. baumanii, K. pneumonia, E. cloaceae and Candida albicans, and also study the profile of phenolic compounds of the obtained extracts of St. John's wort and hawthorn leaves and flowers.

Material and methods. *The objects of the study were anhydrous gel "Hypericum-Derm": α-arbutin, clotrimazole, lidocaine hydrochloride, Hypericum perforatum herb extract, Crataegus monogyna leaf and flower extract. Molecular docking was performed using AutoDockTools 1.5.6; the quantification of polyphenols was analyzed by HPLC; antibacterial effects were assessed by the well method. Isolates were obtained from clinical samples, including tracheal aspirate and bronchoalveolar lavage.*

Research results. *The 10 phenolic compounds were identified in the H. perforatum herb extract and 7 compounds were found in the C. monogyna leaves and flowers extract. In H. perforatum herb extract was dominated hyperoside (1 341,00 ± 2,68 mg/100 g), rutin (754,86 mg/100 g) and quercetin (46,00 ± 0,92 mg/100 g), whereas in C. monogyna was prevailed also hyperoside*

(353,40 ± 7,00 mg/100 g), chlorogenic acid (376,20 ± 7,57 mg/100 g), and (±)-catechin (79,80 ± 1,60 mg/100 g). The combination compounds of "Hypericum-Derm" anhydrous gel was high selective inhibited all targets of "first line of defense" of gram-negative bacteria: DNA-gyrase, dihydrofolate reductase (DHFR), deacetylase, and targets of biofilm formation: AHS LasI, AHS RhI, and diguanylate cyclase as well as key mechanisms of fungi growth were blocked: 14α-demethylase, 1,3-β-glucagon synthetase, thymidylate synthetase and squalene epoxidase. Experimental research was demonstrated that anhydrous gel "Hypericum-Derm" was active against resistant strain of P. aeruginosa (24,0 mm), A. baumanii (25,0 mm), K. pneumonia (24,0 mm), E. cloacea (25,0 mm) and C. albicans (25,0 mm).

Conclusion. *In the course of the theoretical study, the composition of the anhydrous gel "Hypericum-Derm" was developed, consisting of α-arbutin, clotrimazole, lidocaine hydrochloride, H. perforatum herb extract and C. monogyna leaves and flowers. Experimental studies have shown that the developed gel "Hypericum-Derm" is able to actively suppress the growth of resistant strains of P. aeruginosa, A. baumanii, K. pneumonia, E. cloaceae and C. albicans. In addition, an approach to the development of a drug with antimicrobial action based on the results of molecular docking prediction was described for the first time.*

Key words: *anhydrous gel, molecular docking, infectious wounds, HPLC, antimicrobials.*

Олександр МАСЛОВ
доктор філософії, асистент кафедри загальної хімії, Національний фармацевтичний університет, вул. Григорія Сковороди, 53, м. Харків, Україна, 61002 (alexmaslov392@gmail.com)
ORCID: *0000-0001-9256-0934*
SCOPUS: *57226660079*

Микола КОМІСАРЕНКО
кандидат фармацевтичних наук, асистент кафедри фармакогнозії та нутриціології, Національний фармацевтичний університет, вул. Григорія Сковороди, 53, м. Харків, Україна, 61002 (a0503012358@gmail.com)
ORCID: *0000-0002-1161-8151*
SCOPUS: *57212146273*

Валерій МІНУХІН
доктор медичних наук, професор, директор Інституту мікробіології та імунології імені І.І. Мечникова, Національна академія наук України, вул. Григорія Сковороди, 12, м. Харків, Україна, 61002 (chemistry29@meta.ua)
ORCID: *0000-0002-9682-9686*
SCOPUS: *6603854139*

Тетяна ОСОЛОДЧЕНКО
кандидат біологічних наук, завідувачка лабораторії біохімії та біотехнології, Інститут мікробіології та імунології імені І.І. Мечникова, Національна академія наук України, вул. Григорія Сковороди, 12, м. Харків, Україна, 61002 (imi_lbb@ukr.net)
ORCID: *0000-0002-5064-6550*
SCOPUS: *57199560169*

Сергій КОЛІСНИК
доктор фармацевтичних наук, професор, завідувач кафедри загальної хімії, Національний фармацевтичний університет, вул. Григорія Сковороди, 53, м. Харків, Україна, 61002 (s_kolesnik@nuph.edu.ua)
ORCID: *0000-0002-4920-6064*
SCOPUS: *57217102532*

Андрій КОМІСАРЕНКО
доктор фармацевтичних наук, професор, фармацевтична компанія «ЗДРАВОФАРМ», вул. Успеканська, 6, м. Сороковка, Харківська обл., 62430 (nik25spider@gmail.com)
ORCID: *0000-0002-2238-5138*
SCOPUS: *6602821633*

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ТЕОРЕТИЧНЕ І ПРАКТИЧНЕ ОБҐРУНТУВАННЯ СКЛАДУ БЕЗВОДНОГО ГЕЛЮ «ГІПЕРІКУМ-ДЕРМ», ВИКОРИСТАНОГО ДЛЯ ЛІКУВАННЯ ГНІЙНИХ РАН, ВИКЛИКАНИХ МУЛЬТИРЕЗИСТЕНТНИМИ ШТАМАМИ БАКТЕРІЙ І ГРИБІВ

Актуальність. *Резистентність до антимікробних препаратів є величезною проблемою для охорони здоров'я в усьому світі. Одна з перших згадок про появу штамів бактерій, стійких до антибіотиків, у людей була отримана під час воєнних конфліктів в Іраку й Афганістані, 20 років тому. Окрім того, за останніми даними, серед усіх ізольованих резистентних патогенів переважають Acinetobacter baumani, Pseudomonas aeruginosa, Klebsiela pneumonia та Enterobacter cloacae.*

Тому розроблення нового антибактеріального препарату, здатного боротися з резистентними бактеріями, є актуальним завданням.

Мета дослідження. *Об'єктами дослідження були безводний гель "Hypericum-Derm": α-арбутин, клотримазол, лідокаїну гідрохлорид, екстракт трави звіробою звичайного, екстракт листя та квіток глоду одноклітинного. Молекулярний докінг проводили за допомогою AutoDockTools 1.5.6; кількісне визначення поліфенолів аналізували за допомогою ВЕРХ, антибактеріальну дію оцінювали методом лунок. Ізоляти отримували із клінічних зразків, включаючи трахеальний аспірат і бронхоальвеолярний лаваж.*

Матеріал і методи. *Об'єктами дослідження були безводний гель «Гіперікум-Дерм», α-арбутин, клотримазол, лідокаїн гідрохлорид, екстракт трави Hypericum perforatum, екстракт листя та квіток Crataegus monogyna. Молекулярний докінг проводили за допомогою AutoDockTools 1.5.6; антибактеріальні ефекти оцінювали методом свердловин. Ізоляти отримували із клінічних зразків, включаючи трахеальний аспірат і бронхоальвеолярний лаваж.*

Результати дослідження. *В екстракті трави H. perforatum було ідентифіковано 10 фенольних сполук, а в екстракті листя та квітів C. monogyna – 7 сполук. В екстракті трави H. perforatum переважали гіперозид (1 341,00 ± 2,68 мг/100 г), рутин (754,86 мг/100 г) та кверцетин (46,00 ± 0,92 мг/100 г), тоді як у C. monogyna також переважали гіперозид (353,40 ± 7,00 мг/100 г), хлорогенова кислота (376,20 ± 7,57 мг/100 г) та (±)-катехін (79,80 ± 1,60 мг/100 г). Комбіновані сполуки безводного гелю "Hypericum-Derm" високоселективно пригнічували всі мішені «першої лінії захисту» грамнегативних бактерій: ДНК-гіразу, дигідрофолатредуктазу (ДГФР), деацетилазу, а також мішені утворення біоплівки: AHS LasI, AHS RhI та дигуанілатциклазу, а також блокували ключові механізми росту грибів: 14α-деметилазу, 1,3-β-глюкагонсинтетазу, тимідилатсинтетазу та сквален епоксидазу. Експериментальні дослідження показали, що безводний гель "Hypericum-Derm" активний проти резистентних штамів P. aeruginosa (24,0 мм), A. baumanii (25,0 мм), K. pneumonia (24,0 мм), E. cloacea (25,0 мм) та C. albicans (25,0 мм).*

Висновок. *Результати дослідження показали, що панівною сполукою серед фенольних сполук екстракту трави H. perforatum є гіперозид і рутин, а щодо листя та квіток C. monogyna – гіперозид і хлорогенова кислота. У процесі теоретичного дослідження було розроблено склад безводного гелю "Hypericum-Derm", що складається з α-арбутину, клотримазолу, лідокаїну гідрохлориду, екстракту трави H. perforatum і листя та квітів C. monogyna. Експериментальні дослідження показали, що розроблений гель "Hypericum-Derm" здатний активно пригнічувати ріст резистентних штамів P. aeruginosa, A. baumanii, K. pneumonia, E. cloaceae та C. albicans. Окрім того, уперше описано підхід до розроблення препарату з антимікробною дією, заснований на результатах молекулярного докінгового прогнозування.*

Ключові слова: *безводний гель, молекулярний докінг, інфекційні рани, ВЕРХ, протимікробні препарати.*

Introduction. Actuality. The armed confrontation in the area the anti-terrorist operation/Joint Forces Operation, which began in 2014 and transformed into a war on February 24, 2022, continues to this day. According to data provided by the Office of the UN High Commissioner for Human Rights, more than 40 thousand people became victims of the military conflict from April 2014 to the end of 2020. people, more than 4 thousand military personnel were killed, more than 12 thousand were injured (Hodgetts, 2023, p. e002435). There is a clear gradation in the types of injuries: up to 60% are mine-explosive, 20–22% are combined, 10-13% are burns. These data indicate the relevance of the problem of wound and burn treatment for the healthcare system, both in the civil and military spheres, as general state tasks (Gumeniuk, 2023, p. 1–15).

Antibiotic resistance is a modern problem in combat wound surgery. The first mention of resistant strains was detected 21 years ago in people who received combat wounds during US military operations against Iraq and Afghanistan (Murray, 2010, p. S102 – S108). By the beginning of 2022, according to official statistics, strains of *K. pneumonia* and *P. aeruginosa* had already been isolated in Ukrainian hospitals, which were 80% resistant to the action of combined broad-spectrum antibiotics (Ljungquist, 2022, p. 784–786). At this time, in May 2025, a paper was published (Pallett, 2025, p. 101274), where screening studies of the resistance of isolated isolates from wound infections obtained from regional hospitals in Kharkov, Krivoy Rog and Kyiv were conducted. Based on the results of the study, three resistant gram-negative strains were identified: *A. baumanii*, *P. aeruginosa* and *E. cloacea*. All tested strains were insensitive to the action of ceftriaxones, ceftazidime, co-amoxiclav and ciprofloxacin, while in the case of gentamicin and amikacin, the sensitivity ranged from 8% for *P. aeruginosa* and *A. baumanii* to 30% for *E. cloacea*.

Another important problem of infected wounds, especially burns, is the addition of fungal infections C. albicans, after the use of local broad-spectrum antimicrobials. Infection with several strains of bacteria and fungi is a fairly common problem, which leads to an increase in wound healing time. The key reason for the delay in wound healing is the formation of a polymicrobial biofilm between, for example, *P. aeruginosa* or *Staphylococcus aureus* and *C. albicans*. In this case, bacteria are in symbiosis and, when forming a biofilm, contribute to the development of resistance to broad-spectrum antimicrobials (Kamath, 2024, p. 1–5). Thus, the drug should not only suppress the growth of only gram-positive or gram-negative bacterial strains, but also inhibit the growth of the fungus.

In modern literature, a wound is defined as a violation of the integrity of the skin or mucous membranes caused by mechanical action and usually accompanied by

damage to deeper tissues or organs (Wang, 2018, p. 94–101). During the wound healing process, three phases are usually distinguished: Phase I is the inflammatory phase, characterized by the release of the wound from necrotic tissue and foreign bodies; Phase II is the proliferation or regeneration phase; Phase III is the maturation or remodeling phase, characterized by wound closure and final scar formation (Song, 2012, p. 141–157). The developed anhydrous gel "Hypericum-Derm" will solve the problems of phase I wounds. The main task for the treatment of phase I wounds is to suppress infections, cleanse the wound from necrotic tissue and reduce inflammatory reactions. Therefore, the anhydrous gel "Hypericum-Derm" should have an antimicrobial effect, have a high osmotic force, and also suppress inflammation.

Thus, the aim of our study was to conduct theoretical and practical research to substantiate the composition of the anhydrous gel "Hypericum-Derm" with antimicrobial action against multiresistant strains of *P. aeruginosa, A. baumanii, K. pneumonia, E. cloaceae* and *C. albicans*, as well as to study the profile of phenolic compounds of the obtained extracts of St. John's wort and hawthorn leaves and flowers.

Materials and research methods. ***Plant material.*** The *Hypericum perforatum* (*H. perforatum*) herb and Crataegus monogyna (C. monogyna) leaves and flowers was the object of the study, which were collected in the places of its cultivation. The *H. perforatum* herb was collected in 2022 during the flowering period at July, whereas *C. monogyna* leaves and flowers was collected in 2023 during the flowering period at May in the vicinity of the village of Ternova, Kharkiv region (50°19′31″ N, 36°66′93″ E; the altitude above sea: 92 m).

Extraction procedure

A 25,0 g of *H. perforatum* herb and *C. monogyna* leaves and flowers were ground to 1–2 mm in size. The extraction was carried out by 96% ethanol two times at the ratio raw material/solvent 1/10 (*m/v*) in a water bath at 80 °C with reflux for 1 hour. After cooling, the solutions were filtrated and concentrated to a raw material mass ratio of 1 to 1 by a rotary evaporator at 40 °C under a vacuum.

Reagents

α-arbutin (≥98,0%); clotrimazole (≥98,0%); lidocaine hydrochloride (≥98,0%); clarithromycin (≥98,0%); azithromycin (≥98,0%); gentamycin (≥98,0%); ciprofloxacin (≥98,0%); levofloxacin (≥98,0%); ceftriaxone (≥98,0%); meropenem (≥98,0%); ceftazidime (≥98,0%); doxycycline (≥98,0%); chloramphenicol (≥98,0%) were provided by pharmaceutical company "Astrapharm", Kyiv, Ukraine; and by pharmaceutical company "Zdravopharm", Kharkiv, Ukraine. Hypericin (≥98,0%), hyperoside (≥98,0%), rutin (≥98,0%), quercetin (≥98,0%), vitexin (≥98,0%), isovitexin (≥98,0%), (+)-catechin (≥98,0%), chlorogenic acid (≥98,0%), caffeic acid (≥98,0%), gallic acid (≥98,0%), ferulic acid (≥98,0%), naringenin (≥98,0%), naringin (≥98.0%) were purchased in Sigma Aldrich Company, Lublin, Poland, Acetonitrile (purchased from "Allchem", Kharkiv), acetic acid (purchased from "Allchem", Kharkiv), phosphoric acid (purchased from "Allchem", Kharkiv); "Levomekol" (PrAT Pharmaceutical Factory "Viola") – series number LMK-03-250722-01-UA.

Test organisms

Resistant strains of *P. aeruginosa 18, A. baumani 150, K. pneumonia 18, E. cloacea 17* were chosen for research. A one clinical isolates of multidrug-resistant fungi: *Candida albicans* 69.

HPLC analysis of H. perforatum herb and C. monogyna leaves and flowers extracts

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 a temperature of 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 minutes, changed to 43–90% A from 25 to 45 minutes, maintained 90% A from 45 to 55 minutes, decreased to 20% A from 55 to 60 minutes, and then held at 20% A from 60 to 70 minutes. Prior to use, the mobile phases were filtered using 25 mm × 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. 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:

$$I_T = 1 - \left|T_{st} - T_u\right|,$$

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

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

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

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 (Khodakov, 2012, p. 132–142).

Antimicrobial activity

The method of diffusion of the drug into agar carried out using the method of "wells" (Osolodchenko, 2024, p. 19–23). Table 1 shows interpretation criteria for microbial sensitivity (Volianskyi, 2004, p. 38).

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

Molecular docking

A molecular docking study was conducted using the tool known as AutoDockTools 1.5.6 (Morris, 2008).

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. In the case of fungal strains, the following were selected: 14α-demethylase (PDB ID: 6ayb), 1,3-β-glucagon synthetase (PDB ID: 1eqp), thymidylate synthetase (PDB ID: 5uiv), squalene epoxidase (PDB ID: 6c6r). 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 rutin (CID_5280805); α-arbutin (CID_158637); clotrimazole (CID_2812); hypericin (CID_3663); hyperoside (CID_5281643); lidocaine hydrochloride (CID_6314), vitexin (CID_5280441), isovitexin (CID_162350); quercetin (CID_5280343); gentamycin (CID_3467); chloramphenicol (CID_5959); meropenem (CID_441130); ceftazidime (CID_5481173), doxycycline (CID_54671203); clarithromycin (CID_84029); azithromycin (CID_447043); ciprofloxacin (CID_2764); levofloxacin (CID_149096); ceftriaxone (CID_5479530) 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 3.0).

Statistical analysis

To obtain statistical results, the Statistica 10 program was used, the results were analyzed using one-way ANOVA with Tukey's criterion. Differences were considered significant at $p < 0,05$.

Research results and their discussion. One of the key tasks of the gel for the treatment of wounds in phase I is the cleansing of wounds from necrotic tissue and secondary metabolites of inflammation. To solve this problem, bases with high osmotic action are used, which provide an active outflow of purulent-wound contents, eliminate tissue hypertension, limit the absorption of decay products, relieve intoxication, and create conditions for the healing of damaged tissues. In the research (Pertsev, 2002, p. 1–10) work of the luminaries I.M. Pertsov it was shown that the alloy of polyethyleneglycol (PEG) 400 and PEG-1500 (8:2) has the maximum osmotic effect (336,5 ± 1,4%). It was also noted in the work that this base is most effective in the first phase of the wound, and in the second phase, where the stages of granulation and proliferation already occur, the base will only slow down wound healing. Thus, based on the above facts, we chose the alloy of PEG 400 and PEG 1500 (8:2) as the basis for the anhydrous gel.

The next key issue is the composition of combinations of active pharmaceutical ingredients that will have an antimicrobial effect against resistant gram-negative strains of bacteria and fungi, as well as help suppress inflammation and inactivate free radicals. To solve this problem, we turned to the experience of Soviet pharmacists; in the 60s of the 20th century, the drug "Novoimanin" was developed and introduced in the USSR (Drobotko, 1966, p. 1–5). This drug was used in the form of a solution for the treatment of burns, purulent-inflammatory diseases of wounds infected with gram-positive strains. The active component of "Novoimanin" is an extract of St. John's wort, the main biologically active substances of the extract are flavonoid derivatives (rutin, hyperoside, quercetin), and anthracene derivatives (hypericin). Many studies have described that St. John's wort extract has anti-inflammatory, antimicrobial, antioxidant, anticancer and analgesic effects (Nobakht, 2022, p. 1045–1058), therefore, St. John's wort extract is a suitable component for creating anhydrous gel. Since we have a task to obtain anhydrous gel that can inhibit "superbugs", we selected an important component such as α-arbutin to solve this problem. In our earlier works (Maslov, 2024a; Maslov, 2024b) it was shown that α-ar-

butin promotes active inhibition of the biofilm formation mechanism in resistant bacteria, such as AHS LasI, which is responsible for the biofilm formation signaling system.

To prevent the formation of a polymicrobial biofilm between bacteria and fungi, we selected an antifungal drug – clotrimazole. Polymicrobial biofilm is the main reason for non-healing in chronic wounds, especially in burns, to prevent the occurrence of symbiosis between bacteria and fungi, an antifungal agent should be used with the prescribed therapy of broad-spectrum antibacterial drugs.

To enhance anti-inflammatory and antioxidant activity, we included hawthorn leaf and flower extract in the composition, since the extract contains such active compounds as vitexin and isovitexin. In available studies, journals indexed in Scopus and Web of Science, it was shown that these compounds have high cardioprotective, antimicrobial, wound-healing, anti-inflammatory, antioxidant, and neuroprotective effects (He, 2016, p. 74–85).

The last and most important component of our anhydrous gel is lidocaine hydrochloride. This component plays not only the role of a local anesthetic, but primarily as a compound that will suppress and prevent the formation of bacterial film (Razavi, 2019, p. 991–1002).

Before starting the theoretical studies, we conducted HPLC analysis of phenolic compounds of the extract of St. John's wort, extract of leaves and flowers of hawthorn. Since extracts are a complex drug and for a theoretical assessment of its pharmacological capabilities, it is necessary to take into account which compounds are present in the extract and in what quantities.

The HPLC method was used to carry out a qualitative and quantitative analysis of phenolic compounds in the obtained extract of *H. perforatum* herb. According to the results of the study, 10 phenolic compounds were identified in extract. The total content of phenolic compounds in the extract was 2 300,00 mg/100 g of extract (fig. 1, table 2).

As shown in table 2, hyperoside dominated among all phenolic compounds (58,32% out of the total phenolic compounds), rutin (32,82% out of the total phenolic compounds) was in second place, whereas the third place was quercetin (2,00% out of the total phenolic compounds) and the lowest content was caffeic and ferulic acid (0,10 out of the total phenolic compounds).

In the case of *C. monogyna* leaves and flowers extract was identified 7 compounds (fig. 2). The total content of phenolic compounds in the obtained extract was 1 140,00 mg/100 g of which flavonoids – 491,11 mg/100 g (43,0% out of the total phenolic compounds), phenol carboxylic acids – 456,00 mg/100 g (40,0% out of the total phenolic compounds) (table 3).

Among flavonoids, hyperoside dominates – 353,40 ± 7,00 mg/100 g (31,0% out of the total phenolic compounds), and – (+)-catechin 79,80 ± 0,60 mg/100 g (7,00% out of the total phenolic compounds). Among phenol carboxylic acids, 2 compounds were identified: chlorogenic acid – 376,20 ± 7,52 mg/100 g (33,00% out of the total phenolic compounds), and caffeic acid – 5,70 ± 0,11 mg/100 g (0,50% out of the total phenolic compounds).

As shown in table 3, chlorogenic acid dominates among all phenolic compounds, hyperoside is in the second place, and (+)-catechin is in third place.

To support our arguments, we conducted a theoretical *in silico* study of the main bioactive compounds of St. John's wort extract, hawthorn leaves and flowers, as well as α-arbutin, clotrimazole and lidocaine hydrochloride against six key enzymes of Gram-negative bacterial strains, namely three "first line defense" targets: DNA gyrase, DHFR and deacetylase, as well as three biofilm formation targets: AHS LasI, AHS RhI and diguany-

Table 2

Chemical composition of phenolic profile in *H. perforatum* herb extract by HPLC analysis

№	Phenolic compound	Retention time, min	Content of phenolic compound in extract, mg/100 g of extract ±SD	% out of total phenolic compound
1	Gallic acid	8,887	0,92 ± 0,18	0,04
2	(+)-catechin	13,914	41,40 ± 0,83	1,80
3	Chlorogenic acid	16,526	77,05 ± 1,54	3,35
4	Caffeic acid	19,371	2,30 ± 0,20	0,10
5	Ferulic acid	25,484	2,30 ± 0,20	0,10
6	Hyperoside	29,683	1 341,00 ± 2,68	58,32
7	Rutin	30,259	754,86 ± 1,51	32,82
8	Quercetin	40,873	46,00 ± 0,92	2,00
9	Apigenin	45,469	1,15 ± 0,23	0,05
10	Hyperecin	53,600	32,66 ± 0,65	1,42
	the total phenolic compounds		2 300,00	

Notes: SD – standard deviation, n = 5.

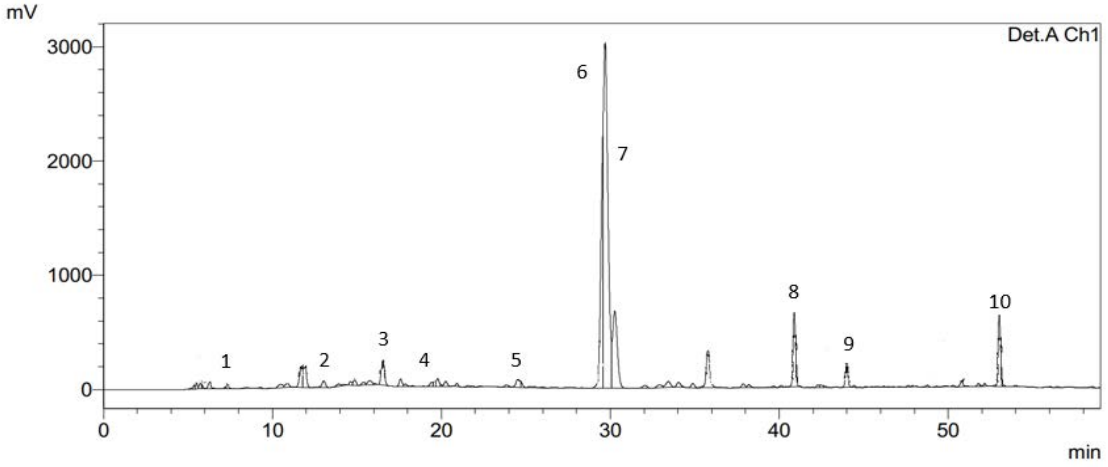

Fig 1. HPLC fingerprint (255 nm) of the *H. perforatum* herb extract

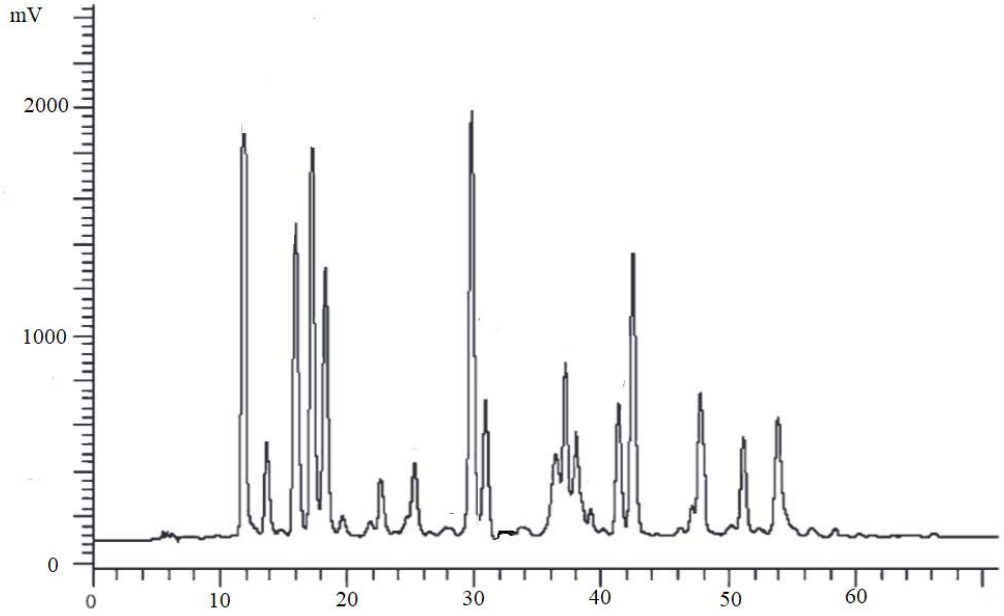

Fig. 2. HPLC fingerprint (255 nm) of the *C. monogyna* leaves and flowers extract

Table 3

Chemical composition of phenolic profile in *C. monogyna* leaves and flowers extract by HPLC analysis

№	Phenolic compound	Retention time, min	Content of phenolic compound in extract, mg/100 g of extract ±SD	% out of total phenolic compound
1.	(+)-catechin	13,920	79,80 ± 1,60	7,00
2.	Chlorogenic acid	16,550	376,20 ± 7,52	33,00
3.	Caffeic acid	19,360	5,70 ± 0,11	0,50
4.	Hyperoside	29,700	353,40 ± 7,00	31,00
5.	Rutin	30,600	15,96 ± 0,32	1,40
6.	Isovitexin	37,110	39,90 ± 0,60	3,50
7.	Vitexin	37,800	2,05 ± 0,04	0,18
8.	Unidentified compounds		266,99 ± 5,00	23,42
	the total phenolic compounds		1 140,00	

Notes: SD – standard deviation, n = 5.

late cyclase. In the case of antifungal activity, four key enzymes were studied: 14α-demethylase, 1,3-β-glucagon synthetase, thymidylate synthetase and squalene epoxidase. As standards of comparison we took broad-spectrum antimicrobial drugs, namely: the group of tetracyclines (doxycycline), aminoglycosides (gentamicin), fluoroquinolones (ciprofloxacin, levofloxacin), cephalosporins (cefepime, ceftazidime, ceftriaxone), amphenicol (chloramphenicol), macrolides (azithromycin, clarithromycin) and carbapenems (meropenem). When assessing antifungal activity, we selected the following drugs as standards of comparison: fluconazole, nystatin, ketoconazole, terbinafine.To understand the level of selectivity of inhibition of the studied substances to the active centers of bacterial enzymes, we applied the following classification of selectivity (Kondza, 2024, p. 1–15): IC50 < 0,001 mM (high selective); 0,05 > IC50 > 0,01 (medium selective); IC50 > 0,05 mM (low selective).

DNA gyrase is an enzyme responsible for the temporary division of bacterial DNA into two strands, subsequently the replication stage begins (Jogula, 2020, p. 103905). Active center of DNA-gyrase was consisted by amino acids: Arg75, Lys102, Arg135, Asp80. Trp387, Lys109, Asp72, Thr166. Table 4 shows that clarithromycin, hypericin, azithromycin, doxycycline, vitexin, hyperozide, isovitexin, clotrimazole, rutin, levofloxacin, cefepime and α-arbutin had high selectivity, in the case of ciprofloxacin, lidocaine hydrochloride, meropenem, (+)-catechin, apigenin, ceftazidime and chloramphenicol had medium selectivity, whereas quercetin, ferulic acid, ceftriaxone, gentamycin, chlorogenic acid, caffeic acid and gallic acid – low selectivity. The top three compounds that possesses highest binding energy are clarithromycin, hypericin and azithromycin.

The next crucial enzyme that was assessed by molecular docking was DHFR, this enzyme is responsible for the formation of folic acid, which is necessary for the existence of bacteria (Jogula, 2020, p. 103905). The active center of enzyme was containing of NADP, Tyr110, Asp30, Ile8, Phe34, Ile104, Arg55, Arg60. Table 4 shows that clarithromycin, azithromycin, isovitexin, hypericin, doxycycline, clotrimazole, hyperozide, rutin, vitexin, ceftazidime, α-arbutin, lefloxacin, (+)-catechin, apigenin, ciprofloxacin and cefepime had high selective, in the case of lidocaine hydrochloride, chloramphenicol, meropenem, gentamicin, ceftriaxone, quercetin, ferulic acid had medium selectivity, whereas chlorogenic acid, caffeic acid and gallic acid – low selectivity.

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 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 (Mbarga, 2021, p. 158–167). The active center of enzyme was containing of Thr190, Lys238, Gly92. Phe191, Leu18, Ala206. Table 4 shows that azithromycin, clarithromycin, hypericin, hyperozide, vitexin, doxycycline, rutin, ceftazidime, isovitexin, clotrimazole, cefepime, α-arbutin, levofloxacin had high selectivity, in the case of (+)-catechin, ferulic acid, ciprofloxacin, gentamicin, lidocaine hydrochloride, chloramphenicol, meropenem and ceftriaxone had medium selectivity, whereas quercetin, apigenin, gallic acid, chlorogenic acid and caffeic acid had low selectivity.

The biofilm is a structure of bacteria colony that prevent penetration antimicrobial drugs into the bacteria. The main mechanism of formation biofilm is activation 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 is required 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 (Mbarga, 2021, p. 158–167). 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 4 shows that α-arbutin, chlorogenic acid, chloramphenicol, (+)-catechin, lidocaine hydrochloride, apigenin had high selectivity, ferulic acid, quercetin, ceftriaxone, caffeic acid and clotrimazole had medium selectivity, whereas gallic acid, doxycycline, levofloxacin, meropenem and ciprofloxacin had low selectivity.

The active center of AHS RhI was represented by following amino acids: Glu254, Asp48, Tyr54, Met42. Leu63, Leu56. Table 4 shows that clarithromycin, hyperozide, hyperecin, vitexin, clotrimazole, doxycycline, rutin, azithromycin, isovitexin had high selectivity, (+)-catechin, α-arbutin, ciprofloxacin, lidocaine hydrochloride, apigenin, levofloxacin, ceftazidime, quercetin, chlorogenic acid, chloramphenicol had medium selectivity, whereas cefepime, meropenem, ferulic acid, ceftriaxone, caffeic acid, gallic acid, gentamicin had low selectivity.

The next crucial enzyme that was evaluated by molecular docking was diguanylate cyclase. The active

Table 4

Molecular docking of the identified compounds and antibacterial drug standards with the DNA-gyrase, DHFR, deacytelese, AHS LasI and RhI, diguanylate cyclase structures of Gram-negative strain

№	DNA-gyrase			
	Ligand	**ΔGbind (kcal/mol)**	**Ki (mmol)**	**Level of selectivity**
1.	Clarithromycin	−14,96	0,00000001087	High selective
2.	Hypericin	−13,34	0,00000016593	High selective
3.	Azithromycin	−12,57	0,00000061435	High selective
4.	Doxycycline	−11,59	0,0000032	High selective
5.	Vitexin	−11,24	0,00000575	High selective
6.	Hyperozide	−11,23	0,0000059	High selective
7.	Isovitexin	−10,51	0,00001993	High selective
8.	Clotrimazole	−10,46	0,00002153	High selective
9.	Rutin	−10,45	0,00002184	High selective
10.	Levofloxacin	−8,69	0,00042853	High selective
11.	Cefepime	−8,27	0,00086484	High selective
12.	α-arbutin	−8,10	0,00093344	High selective
13.	Ciprofloxacin	−8,06	0,00123	Medium selective
14.	Lidocaine hydrochloride	−7,49	0.00324	Medium selective
15.	Meropenem	−7,13	0,00589	Medium selective
16.	(+)-catechin	−6,60	0,01464	Medium selective
17.	Apigenin	−6,50	0,01480	Medium selective
18.	Ceftazidime	−6,48	0,01776	Medium selective
19.	Chloramphenicol	−6.38	0,02114	Medium selective
20.	Quercetin	−5.00	0,21618	Low selective
21.	Ferulic acid	−4,70	0,31214	Low selective
22.	Ceftriaxone	−4,61	0,41631	Low selective
23.	Gentamycin	−4,08	1,03	Low selective
24.	Chlorogenic acid	−4,00	1,20	Low selective
25.	Caffeic acid	−3,95	1,30	Low selective
26.	Gallic acid	−3,90	1,38	Low selective
№	DHFR			
1.	Clarithromycin	−16,78	0,00000000050408	High selective
2.	Azithromycin	−14,50	0,00000002336	High selective
3.	Isovitexin	−12,87	0,00000036574	High selective
4.	Hypericin	−11,70	0,00000265	High selective
5.	Doxycycline	−11,59	0,00000319	High selective
6.	Clotrimazole	−11,51	0,00000369	High selective
7.	Hyperozide	−11,45	0,00000404	High selective
8.	Rutin	−10,72	0,00001379	High selective
9.	Vitexin	−10,72	0,00001382	High selective
10.	Ceftazidime	−9,49	0,00011119	High selective
11.	α-arbutin	−9,10	0,00019023	High selective
12.	Lefloxacin	−8,98	0,00026376	High selective
13.	(+)-catechin	−8,52	0,00056708	High selective
14.	Apigenin	−8,50	0,00060213	High selective
15.	Ciprofloxacin	−8,44	0,00064808	High selective
16.	Cefepime	−8,37	0,00072803	High selective
17.	Lidocaine hydrochloride	−8,12	0,00113	Medium selective
18.	Chloramphenicol	−7,97	0,00143	Medium selective
19.	Meropenem	−7,65	0,00249	Medium selective
20.	Gentamicin	−6,78	0,01073	Medium selective
21.	Ceftriaxone	−6,36	0,02164	Medium selective
22.	Quercetin	−6,32	0,02329	Medium selective
23.	Ferulic acid	−6,10	0,02412	Medium selective
24.	Chlorogenic acid	−4,55	0,35023	Low selective
25.	Caffeic acid	−4,30	0,72522	Low selective
26.	Gallic acid	−3,76	1,77	Low selective

№	DNA-gyrase			
	Ligand	**ΔGbind (kcal/mol)**	**Ki (mmol)**	**Level of selectivity**
№	Deacytelese			
1.	Azithromycin	−14,04	0,000000051	High selective
2.	Clarithromycin	−13,98	0,000000057	High selective
3.	Hypericin	−12,89	0,00000036	High selective
4.	Hyperozide	−12,14	0,00000126	High selective
5.	Vitexin	−11,49	0,00000377	High selective
6.	Doxycycline	−11,03	0,00000816	High selective
7.	Rutin	−10,47	0,00002101	High selective
8.	Ceftazidime	−10,37	0,00002509	High selective
9.	Isovitexin	−10,24	0,00003114	High selective
10.	Clotrimazole	−9,45	0,00011882	High selective
11.	Cefepime	−8,77	0,00037448	High selective
12.	α-arbutin	−8,35	0,00070090	High selective
13.	Levofloxacin	−8,34	0,00077565	High selective
14.	(+)-catechin	−7,57	0,00284	Medium selective
15.	Ferulic acid	−7,53	0,00296	Medium selective
16.	Ciprofloxacin	−7,51	0,00313	Medium selective
17.	Gentamicin	−7,45	0,00346	Medium selective
18.	Lidocaine hydrochloride	−7,32	0,00433	Medium selective
19.	Chloramphenicol	−7,19	0,00536	Medium selective
20.	Meropenem	−6,73	0,01163	Medium selective
21.	Ceftriaxone	−6,09	0,03444	Medium selective
22.	Quercetin	−5,81	0,05541	Low selective
23.	Apigenin	−5,72	0,05600	Low selective
24.	Gallic acid	−5,05	0,19945	Low selective
25.	Chlorogenic acid	−5,04	0,20211	Low selective
26.	Caffeic acid	−4,69	0,36756	Low selective
№	AHS LasI			
1.	α-arbutin	−12,11	0,0000010	High selective
2.	Chlorogenic acid	−11,24	0,00000573	High selective
3.	Chloramphenicol	−10,76	0,00001304	High selective
4.	(+)-catechin	−9,11	0,0002103	High selective
5.	Lidocaine hydrochloride	−9,03	0,00023918	High selective
6.	Apigenin	−8,94	0,00028195	High selective
7.	Ferulic acid	−6,88	0,00905	Medium selective
8.	Quercetin	−6,70	0,01223	Medium selective
9.	Ceftriaxone	−6,56	0,01561	Medium selective
10.	Caffeic acid	−6,55	0,01575	Medium selective
11.	Clotrimazole	−6,32	0,02328	Medium selective
12.	Gallic acid	−5,51	0,09116	Low selective
13.	Doxycycline	−4,99	0,22146	Low selective
14.	Levofloxacin	−4,11	0,97221	Low selective
15.	Meropenem	−3,9	1,40	Low selective
16.	Ciprofloxacin	−2,41	16,98	Low selective
17.	Azithromycin	–	–	Inactive
18.	Clarithromycin	–	–	Inactive
19.	Cefepime	–	–	Inactive
20.	Gentamicin	–	–	Inactive
21.	Isovitexin	–	–	Inactive
22.	Vitexin	–	–	Inactive
23.	Ceftazidime	–	–	Inactive
24.	Rutin	–	–	Inactive
25.	Hyperecin	–	–	Inactive
26.	Hyperozide	–	–	Inactive
№	AHS RhI			
1.	Clarithromycin	−18,54	0,00000000002535	High selective

№	DNA-gyrase			
	Ligand	**ΔGbind (kcal/mol)**	**Ki (mmol)**	**Level of selectivity**
2.	Hyperozide	−13,93	0,00000006167	High selective
3.	Hyperecin	−13,79	0,00000007750	High selective
4.	Vitexin	−12,02	0,00000155	High selective
5.	Clotrimazole	−11,77	0,00000237	High selective
6.	Doxycycline	−10,99	0,00000881	High selective
7.	Rutin	−10,88	0,00001053	High selective
8.	Azithromycin	−10,16	0,00003572	High selective
9.	Isovitexin	−8,51	0,00058	High selective
10.	(+)-catechin	−8,01	0,00133	Medium selective
11.	α-arbutin	−7,95	0,0015	Medium selective
12.	Ciprofloxacin	−7,84	0,00178	Medium selective
13.	Lidocaine hydrochloride	−7,54	0,00296	Medium selective
14.	Apigenin	−7,01	0,00240	Medium selective
15.	Levofloxacin	−6,62	0,01408	Medium selective
16.	Ceftazidime	−6,43	0,0193	Medium selective
17.	Quercetin	−6,20	0,02877	Medium selective
18.	Chlorogenic acid	−6,00	0,03012	Medium selective
19.	Chloramphenicol	−5,88	0,04912	Medium selective
20.	Cefepime	−5,05	0,19772	Low selective
21.	Meropenem	−4,80	0,30528	Low selective
22.	Ferulic acid	−4,68	0,36930	Low selective
23.	Ceftriaxone	−4,48	0,51643	Low selective
24.	Caffeic acid	−4,34	0,65716	Low selective
25.	Gallic acid	−4,21	0,82000	Low selective
26.	Gentamicin	–	–	Inactive
№	Diguanylate cyclase			
1.	Doxycycline	−9,14	0,00019913	High selective
2.	Vitexin	−8,81	0,000346	High selective
3.	Isovitexin	−8,46	0,000629	High selective
4.	Hyperozide	−8,07	0,001220	Medium selective
5.	Arbutin	−8,05	0,001240	Medium selective
6.	Ceftazidime	−8,05	0,001240	Medium selective
7.	Clarithromycin	−8,03	0,00131	Medium selective
8.	(+)-catechin	−7,24	0,00491	Medium selective
9.	Clotrimazole	−6,79	0,01061	Medium selective
10.	Chloramphenicol	−6,59	0,01488	Medium selective
11.	Chlorogenic acid	−6,50	0,0172	Medium selective
12.	Meropenem	−6,35	0,02203	Medium selective
13.	Ciprofloxacin	−6,31	0,02356	Medium selective
14.	Caffeic acid	−5,96	0,043040	Medium selective
15.	Hyperecin	−5,83	0,053710	Low selective
16.	Apigenin	−5,80	0,055640	Low selective
17.	Gallic acid	−5,60	0,0781	Low selective
18.	Levofloxacin	−5,32	0,12516	Low selective
19.	Ceftriaxone	−5,19	0,15567	Low selective
20.	Lidocaine	−4,96	0,23053	Low selective
21.	Ferulic acid	−4,90	0,2562	Low selective
22.	Rutin	−4,88	0,2660	Low selective
23.	Quercetin	−4,73	0,34204	Low selective
24.	Gentamicin	−4,49	0,51373	Low selective
25.	Cefepime	−4,40	0,593	Low selective
26.	Azithromycin	−2,79	8,97	Low selective

Notes: ΔGbind – free-binding energy, Ki – concentration inhibited 50% of enzyme activity, green colour – high selective, yellow colour – medium selective, red colour – low selective.

center of enzyme was consisted of amino acids such as Glu254, Glu253, Glu252, Lys327, Arg331, Thr262, Arg198, Arg194. Table 4 demonstrates that only doxycycline, vitexin and isovitexin had high selectivity, hyperozide, α-arbutin, ceftazidime, clarithromycin, (+)-catechin, clotrimazole, chloramphenicol, chlorogenic acid, meropenem, ciprofloxacin and caffeic acid had medium selectivity, whereas hyperecin, apigenin, gallic acid, levofloxacin, ceftriaxone, lidocaine hydrochloride, ferulic acid, rutin, quercetin, gentamicin, cefepime, azithromycin had low selectivity.

14α-demethylase is an enzyme that involved in the conversion of lanosterol to 4,4-dimethylcholesta-8(9),14,24-trien-3β-ol. 14α-demethylase is responsible for an essential step in the biosynthesis of sterols, this protein removes of the C-14α-methyl group from lanosterol (Prajapati, 2022, p. 106237). The active center of 14α-demethylase was represented by following amino acids: Ala182, Ser183. Leu291, He288. Val239, Leu200, His296, His205. Table 5 demonstrates that the first place (−16,30 kcal/mol) of selectivity was taken by ketoconazole, the second place –

Table 5

Molecular docking of the identified compounds and antifungal drug standards with the 14α-demethylase, 1,3-β-glucagon synthetase, thymidylate synthetase and squalene epoxidase structures of fungi

№	14α-demethylase			
	Ligand	**ΔGbind (kcal/mol)**	**Ki (mmol)**	**Level of selectivity**
1.	Ketoconazole	−16,30	0,00000000113	High selective
2.	Hypericin	−14,15	0,00000004214	High selective
3.	Vitexin	−13,51	0,000000125	High selective
4.	Clotrimazole	−12,96	0,00000031589	High selective
5.	Nystatin	−12,76	0,00000044056	High selective
6.	Isovitexin	−12,65	0,00000052996	High selective
7.	Rutin	−11,85	0,00000206	High selective
8.	Hyperozide	−11,61	0,00000309	High selective
9.	Terbinafine	−11,26	0,00000556	High selective
10.	α-arbutin	−9,40	0,000125	High selective
11.	Lidocaine hydrochloride	−8,59	0,00050139	High selective
12.	Fluconazole	−8,53	0,00056181	High selective
13.	Apigenin	−8,52	0,000567	High selective
14.	(+)-catechin	−7,99	0,00139	Medium selective
15.	Chlorogenic acid	−7,00	0,00738	Medium selective
16.	Quercetin	−4,84	0,28223	Low selective
17.	Ferulic acid	−4,06	1,05	Low selective
18.	Caffeic acid	−3,98	1,22	Low selective
19.	Gallic acid	−3,23	4,29	Low selective
№	1,3-β-glucagon synthetase			
1.	Hypericin	−13,68	0,000000093290	High selective
2.	Isovitexin	−12,92	0,000000340780	High selective
3.	Hyperozide	−12,73	0,000000466120	High selective
4.	Ketoconazole	−12,61	0,000000569480	High selective
5.	Nystatin	−11,94	0,00000176	High selective
6.	Vitexin	−11,68	0,00000274	High selective
7.	Clotrimazole	−9,44	0,00012116	High selective
8.	α-arbutin	−9,17	0,000189	High selective
9.	(+)-catechin	−8,14	0,00108	Medium selective
10.	Fluconazole	−7,71	0,00554	Medium selective
11.	Apigenin	−7,60	0,0512	Low selective
12.	Lidocaine hydrochloride	−7,35	0,00412	Low selective
13.	Chlorogenic acid	−7,00	0,03054	Low selective
14.	Quercetin	−6,20	0,02863	Low selective
15.	Ferulic acid	−6,00	0,02945	Low selective
16.	Caffeic acid	−5,12	0,17781	Low selective

17.	Gallic acid	−2,75	9,65	Low selective
18.	Terbinafine	–	–	Inactive
19.	Rutin	–	–	Inactive
№	thymidylate synthetase			
1.	Ketoconazole	−13,60	0,000000107	High selective
2.	Isovitexin	−13,27	0,000000186	High selective
3.	Vitexin	−12,86	0,000000375	High selective
4.	Hyperozide	−12,81	0,000000407	High selective
5.	Clotrimazole	−11,71	0,00000262	High selective
6.	Rutin	−10,85	0,00001097	High selective
7.	Hypericin	−10,55	0,0000185	High selective
8.	Nystatin	−10,46	0,00002152	High selective
9.	Terbinafine	−9,41	0,00012669	High selective
10.	α-arbutin	−9,10	0,00020504	High selective
11.	Fluconazole	−9,10	0,00020504	High selective
12.	Lidocaine hydrochloride	−7,86	0,00174	Medium selective
13.	Apigenin	−7,70	0,00205	Medium selective
14.	(+)-catechin	−7,64	0,00250	Medium selective
15.	Chlorogenic acid	−6,00	0,04304	Medium selective
16.	Caffeic acid	−5,73	0,06348	Low selective
17.	Quercetin	−5,42	0,10636	Low selective
18.	Ferulic acid	−5,40	0,10700	Low selective
19.	Gallic acid	−5,06	0,19594	Low selective
№	squalene epoxidase			
1.	Clotrimazole	−10,79	0,0000124	High selective
2.	Hypericin	−10,46	0,00002163	High selective
3.	Hyperozide	−8,74	0,00039351	High selective
4.	Vitexin	−8,33	0,00077848	High selective
5.	Ketoconazole	−8,15	0,00107	Medium selective
6.	Isovitexin	−7,74	0,00213	Medium selective
7.	Fluconazole	−7,06	0,00665	Medium selective
8.	Rutin	−6,25	0,0257	Medium selective
9.	Terbinafine	−6,12	0,03286	Medium selective
10.	α-arbutin	−5,80	0,0565	Low selective
11.	Lidocaine hydrochloride	−5,76	0,05998	Low selective
12.	(+)-catechin	−5,52	0,09046	Low selective
13.	Apigenin	−5,51	0,09107	Low selective
14.	Chlorogenic acid	−4,94	0,23825	Low selective
15.	Quercetin	−3,72	1,87	Low selective
16.	Ferulic acid	−3,65	2,11	Low selective
17.	Caffeic acid	−2,93	7,09	Low selective
18.	Gallic acid	−2,44	16,20	Low selective
19.	Nystatin	–	–	Inactive

Notes: ΔGbind – free-binding energy, Ki – concentration inhibited 50% of enzyme activity, green colour – high selective, yellow colour – medium selective, red colour – low selective.

hyperecin (−14,15 kcal/mol), and third one – vitexin (−13,51 kcal/mol).

1,3-β-glucagon synthetase is an enzyme primarily found in fungi and is crucial for synthesizing β-1,3-glucan, a major component of the fungal cell wall (da Nobrega, 2020, p. 5969). The active center of 1,3-β-glucagon synthetase was represented by following amino acids: Tyr29, Tyr255, Asn146, Trp373, Leu304, Glu292, Rhe258, Asp145, Glu27, Trp373, Glu27, Trp373, Glu92, Phe144. Table 5 shown top three high selective compounds: hyperecin, vitexin and hyperozide as well as the worst selective compounds were terbinafine and rutin, they were not blocked enzyme at all.

Thymidylate synthetase is an enzyme that catalyzes the formation of thymidine monophosphate from deoxyuridine monophosphate. thymidine monophosphate is

one of the four main building blocks of DNA of fungi (Karunaratne, 2017, p. 11–19). Active center was represented by following aminoacids: Arg92, Tyr100, Glu159, Pro37, Leu51, Tyr161, Tyr93, Tyr100, Phe36, Phe67, Ser96, Gly97, Arg71, Lys35. Table 5 demonstrates that the first place (−13,60 kcal/mol) of selectivity was taken by ketoconazole, the second place – isovitexin (−13,27 kcal/mol), and third one – vitexin (−12,86 kcal/mol).

Squalene epoxidase also known as squalene monooxygenase, is a crucial enzyme in the ergosterol biosynthesis pathways. It catalyzes the epoxidation of squalene to 2,3-(S)-oxidosqualene, a key step in the synthesis of ergosterol in fungi (Sukmawaty, 2022, p. 359–367) The active center of squalene epoxidase was represented by following amino acids: Tyr494, Tyr532, Ile528, His522, Ala525, Cys501. Table 5 shown top three high selective compounds: clotrimazole, hyperecin and hyperozide as well as the worst selective compounds was nystatin, which was not not blocked enzyme at all.

The next step of our research was to sum up obtained data mentioned before. All antimicrobial drugs and compounds of anhydrous gel "Hypericum-Derm" were conditionally divided into two categories (table 6). 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. Table 6 demonstrates that there was not present any of antimicrobial drugs or biological active compounds that inhibited highly selective all six

Table 6

Schematic classification of antimicrobial drug standards alongside main compounds of anhydrous gel "Hypericum-Derm" into 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.	Clarithromycin							3	1
2.	Chloramphenicol							0	1
3.	Ciprofloxacin							1	0
4.	Levofloxacin							3	0
5.	Ceftriaxone							0	0
6.	Gentamycin							0	0
7.	Azithromycin							3	1
8.	Meropenem							0	0
9.	Cefepime							3	0
10.	Ceftazidime							2	0
11.	Doxycycline							3	2
Compounds of anhydrous gel "Hypericum-Derm"									
1.	Vitexin							3	2
2.	Isovitexin							3	2
3.	Hyperoside							3	1
4.	Rutin							3	2
5.	Quercetin							0	0
6.	Hyperecin							3	1
7.	(±)-catechin							1	1
8.	Caffeic acid							0	0
9.	Ferulic acid							0	0
10.	Chlorogenic acid							0	1
11.	Lidocaine hydrochloride							0	1
12.	Clotrimazole							3	0
13.	α-arbutin							3	1
14.	Apigenin							1	1
15.	Gallic acid							0	0

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

mechanisms. The results of the study showed that only doxycycline was highly selective in inhibiting the maximum number of five out of six enzymes of the "first line of defense" and biofilm formation, the next highly selective antimicrobial drugs were clarithromycin and azithromycin, which inhibited four out of six possible target targets. The levofloxacine actively inhibited all enzymes of "first line of defense" such as DNA-gyrase, DHFR, deacytelese. It is also worth noting that only 4 out of 11 widely used antimicrobial agents are highly selective inhibitors of biofilm formation enzymes, which indicates the importance of finding substances that will act highly selectively on target targets of biofilm formation.

Next, we evaluated the theoretical possibilities of antimicrobial activity of the components of the developed anhydrous gel "Hypericu-Derm". The results showed that, as in the evaluation of synthetic antimicrobial drugs, all targets cannot be inhibited by one compound; it is necessary to use the concept of an integrated approach. The leaders among the presented compounds, which highly selectively inhibited most targets, were vitexin and isovitexin. The second place in the number of inhibited targets was taken by hyperoside, rutin and hypericin. Quercetin, caffeic, ferulic and gallic acid are not highly selective inhibitors of key targets of the "first line of defense" and biofilm formation, only chlorogenic acid showed itself to be a highly selective inhibitor in relation to the targets of AHS LasI. Also, we would like to note that the enzymes AHS LasI and diguanylate cyclase are the least sensitive targets to the action of the main biologically active substances of the herb extracts of St. John's wort and hawthorn: hyperoside, rutin, vitexin and isovitexin. To enhance the action of the combinations of hypericin, rutin, vitexin and isovitexin, lidocaine hydrochloride and α-arbutin were added. According to the results of the study shown in table 4, α-arbutin is the most highly selective inhibitor with respect to AHS LasI (−12,11 kcal/mol).

Table 7 shows the summarized results of molecular docking of antifungal standards and components of the anhydrous gel "Hypericum-Derm" against the target targets of the fungus. In contrast to the results of the inhibition of the targets of gram-negative strains, we found a compound that blocks highly selectively all key targets of the fungus for development and growth, and it turned out to be clotrimazole. In the case of the natural components of the gel "Hypericum-Derm", we found that vitexin and isovitexin are the only highly selective inhibitors of all targets. Also, we noted that in the case

Table 7

Schematic classification of antifungal drug standards alongside main compounds of anhydrous gel "Hypericum-Derm" into two categories

№	Compound	14α-demethylase	1,3-β-glucagon synthetase	Thymidylate synthetase	Squalene epoxidase	№ of inhibition enzymes
Antifungal drug standards						
1.	Nystatin					3
2.	Ketoconazole					3
3.	Clotrimazole					4
4.	Terbinafine					2
5.	Fluconazole					2
Compounds of anhydrous gel "Hypericum-Derm"						
1.	Vitexin					4
2.	Isovitexin					4
3.	Hyperoside					3
4.	Rutin					2
5.	Quercetin					0
6.	Hyperecin					2
7.	(±)-catechin					1
8.	Caffeic acid					0
9.	Ferulic acid					0
10.	Chlorogenic acid					0
11.	Lidocaine hydrochloride					3
12.	Clotrimazole					4
13.	α-arbutin					3
14.	Apigenin					1
15.	Gallic acid					0

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

of a theoretical assessment of the antibacterial action, quercetin, caffeic, ferulic, gallic and chlorogenic acids are not highly selective inhibitors of any of the key mechanisms. Therefore, we have proposed a gel composition that will not only be able to inhibit the growth of resistant gram-negative bacterial strains, but will also actively cope with resistant fungal strains, which is relevant in the treatment of chronic wounds and burns.

Following from the above theoretical results, we concluded that it is necessary to develop and implement the concept of complex use of combinations of synthetic antimicrobial, antifungal agents and extracts of medicinal plants of their isolated individual components. According to the modern protocol for the treatment of infectious diseases (Protokoly likuvannia) only monotherapy with one group of antimicrobial drugs is used, for example, a group of cephalosporins in the treatment of pyelonephritis, but this "treatment" only aggravates the course of the disease, since bacteria are able to adapt and become resistant, due to the fact that cephalosporins do not inhibit all key targets of bacteria, and leave the colonies of bacteria "path" for survival and growth. When using the concepts of complex application of combinations of broad-spectrum antimicrobial or antifungal agents and extracts, we are able to deprive bacterial colonies of the ability to "survive" and adapt, since the complex of drugs will actively affect all key targets of bacterial and fungal activity at once. Also, do not forget that most antimicrobial drugs are hepatoxic and nephrotoxic, and when using combinations with extracts, it is possible to reduce the toxicity of broad-spectrum antimicrobial drugs.

The next part of our work was to confirm the antimicrobial action of our developed composition of the anhydrous gel "Hypericum-Derm" in relation to resistant strains of bacteria *P. aeruginosa, A. baumanii, K. pneumonia, E. cloaceae* and *fungus C. albicans*. For this purpose, we obtained extracts of *H. perforatum* herb, *C. monogyna* leaves and flowers, prepared solutions of α-arbutin, clotrimazole in concentrations obtained from the results of molecular docking, and lidocaine hydrochloride in concentrations of 2%, for the gel to have an anesthetic effect, and also made a ready-made gel consisting of all the above components.

To calculate the theoretical dose, we used the method we developed, which is described in the following works on the study of the anti-inflammatory and antioxidant activity of raspberry leaves (Maslov, 2024, p. 143–155). The essence of this method is that we summed up the molar concentrations of IC50 of those enzymes, where the selected compounds were highly selective, and also calculated the dose IC100. Based on the results obtained, it was found that the concentration for suppressing the activity of bacteria should be 0,00365112 mmol/L or 0,10%, while in the case of clotrimazole, the concentration for complete suppression of fungal growth was 0,00027299178 mmol/L or 0,01% (table 8, 9).

For the standards of comparison we took the drug "Novoimanin", we received it according to the patent (Drobotko, 1966, p. 1–5), after this solution was diluted with water 10 times and we used this ready solution for comparison, also broad-spectrum antimicrobial drugs were used: clarithromycin, azithromycin, chloramphenicol, ciprofloxacin, levofloxacin, ceftriaxone, gentamicin, meropenem, doxycycline in concentrations of 0,01 M, and in the case of antifungal drugs: fluconazole in concentrations of 0,01 M (table 10). According to results most of antimicrobial drugs were not effect resistant strains.

The greatest antibacterial effect was exerted by gel of "Hypericum-Derm". It demonstrated the following inhibition actions: gel towards *P. aeruginosa* (24,0 mm), *A. baumanii* (25,0 mm), *K. pneumonia* (24,0 mm), *E. cloacea* (25,0 mm). Antibacterial effect of gel "Hypericum-Derm" was much higher than its own compounds: α-arbutin, clotrimazole, lidocaine hydrochloride, *H. perforatum* herb and *C. monogyna* leaves, flowers. Moreover, gel "Hypericum-Derm" showed stronger antibacterial effects than drug standards: "Novoimanin"

Table 8

Results of calculation the total theoretical dose of α-arbutin for inhibition bacteria growth

Ligand	DNA-gyrase, mmol/L	DHFR, mmol/L	deacytelese, mmol/L	AHS LasI, mmol/L	AHS RhI, mmol/L	diguanylate cyclase, mmol/L	TOTAL IC50, mmol/L	TOTAL IC100, mmol/L
α-arbutin	0,00093344	0,00019023	0,0007009	0,0000010	Not high selective	Not high selective	0,00182556	0,00365112

Table 9

Results of calculation the total theoretical dose of clotrimazole for inhibition fungi growth

Ligand	14α-demethylase, mmol/L	1,3-β-glucagon synthetase, mmol/L	thymidylate synthetase, mmol/L	squalene epoxidase, mmol/L	TOTAL IC50, mmol/L	TOTAL IC100, mmol/L
Clotrimazole	0,00000031589	0,00012116	0,00000262	0,0000124	0,00013649589	0,00027299178

and “Levomekol”. Inhibition zones of growth of gel “Hypericum-Derm” was observed in 29% higher than inhibition zones of “Novoimanin” and in 29 to 15% higher than drug “Levomekol” (table 9).

No literature review revealed any previous studies on antibacterial effect of clotrimazole against resistant Gramm-negative strains. Clotrimazole caused antibacterial effects on *P. aeruginosa*, *A. baumanii*, *K. pneumonia*, *E. cloacea*, with inhibition zones of 17,0, 21,0, 17,0, 18,5 and 18,0 mm, respectively.

H. perforatum herb and *C. monogyna* leaves and flowers extracts displayed inhibition effect on growth of the resistant colonies against *P. aeruginosa* (20,0 and 18,5 mm), *A. baumanii* (17,0 and 18,0 mm), *K. pneumonia* (20,5 and 19,0 mm), *E. cloacea* (19,0 and 21,0 mm), and *C. albicans* (17,0 and 20,0 mm).

Lidocaine hydrochloride and α-arbutine displayed the same antibacterial effects against resistant strains, except *C. albicans*. Inhibition zone of growth of the colony by lidocaine hydrochloride was observed 16,0 mm, whereas α-arbutine – 19,5 mm.

Based on the above experimental results, we can see that we have managed to obtain a conditional antimicrobial “panacea” for resistant gram-negative bacteria

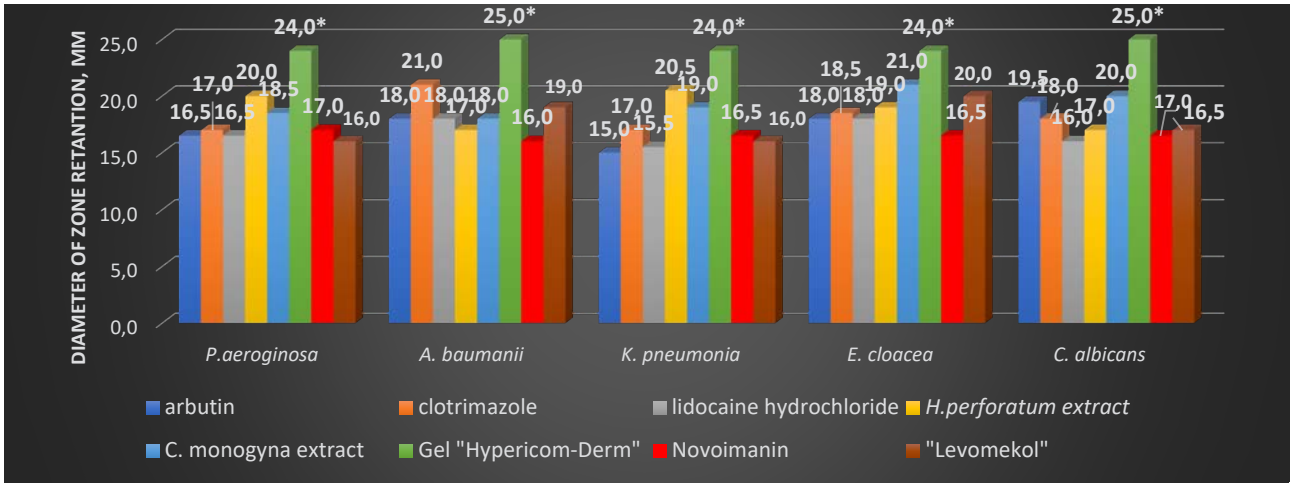

Fig. 3. Antimicrobial activity of α-arbutin, clotrimazole, lidocaine hydrochloride, *H. perforatum* herb extract, *C. monogyna* leaves and flowers extract, gel “Hypericum-Derm”, “Novoimanin” and drug “Levomekol” against multiresistant strains of P. *aeruginosa, A. baumanii, K. pneumonia, E. cloaceae* и *C. albicans.* (The values are presented as the mean ± standard deviation. A one-way ANOVA with Tukey post-hoc test was applied. * – means p < 0,05)

Table 10

Antibacterial effect of gel “Hypericum-Derm” and antibacterial, antifungal drugs standards against multiresistant strains of *P. aeruginosa, A. baumanii, K. pneumonia, E. cloaceae* и *C. albicans*

Sample	Retardation zone, mm ± SD				
	P. aeruginosa 18	*E. cloacae 17*	*A. baumani 150*	*K. pneumoniae 18*	*C. albicans 69*
Gel “Hypericum-Derm”	24,0 ± 0,2*	24,0 ± 0,2*	25,0 ± 0,2*	24,0 ± 0,1*	25,0 ± 0,1*
Clarithromycin	#	#	#	#	–
Azithromycin	#	#	#	#	–
Chloramphenicol	12,5 ± 0,5	19,5 ± 0,5	#	#	–
Ciprofloxacin	#	#	#	15,5 ± 0,5	–
Levofloxacin	#	23,5 ± 0,5	#	20,5 ± 0,5	–
Ceftriaxone	#	23,0 ± 0,2	#	19,5 ± 0,5	–
Doxycycline	#	#	#	17,0 ± 0,2	–
Meropenem	#	#	#	16,0 ± 0,2	–
Gentamycin	#	22,0 ± 0,2	#	17,5 ± 0,5	–
Fluconazol	–	–	–	–	18,5
Clotrimazole	–	–	–	–	18,0

Notes: The values are presented as the mean ± standard deviation. A one-way ANOVA with Tukey post-hoc test was applied. * – means p < 0,05, # – growth.

and fungi. This approach to developing a drug composition is based on a theoretical result and is a modern approach for the 21st century. In our subjective opinion, this approach is pioneering and this approach to developing a drug has not previously been described in any available journal indexed in Scopus and Web of Science.

The drug "Novoimanin" for its time is a good drug for the treatment of infected wounds, but modern problems require modern solutions and approaches. Thanks to the use of molecular docking and predicting the pharmacological action of individual substances, we are able to create a conditional "panacea" that can cure at least one disease associated with resistant bacteria and fungi in the treatment of wounds and burns.

We would also like to note that the concept of the complex use of combinations of synthetic antimicrobial, antifungal agents and extracts of medicinal raw materials lies behind the future victory over resistant strains of bacteria and fungi, not only in the case of external use, but also for internal use.

Conclusions. Theoretical and experimental studies were conducted to substantiate the composition of the anhydrous gel "Hypericum-Derm" with antimicrobial action against multiresistant strains of *P. aeruginosa, A. baumanii, K. pneumonia, E. cloaceae* and *C. albicans*. According to theoretical studies, it has been shown that no synthetic antimicrobial drug or natural compound is capable of inhibiting all target targets of gram-negative bacterial strains. Clotrimazole, vitexin and isovitexin are theoretically capable of inhibiting all key targets of fungal growth and development. In the course of theoretical research, the composition of the anhydrous gel "Hypericum-Derm" was developed, consisting of α-arbutin, clotrimazole, lidocaine hydrochloride, extract of *H. perforatum* herb and *C. monogyna* leaves and flowers. Experimental studies have shown that the developed gel "Hypericum-Derm" is able to actively suppress the growth of resistant strains of *P. aeruginosa, A. baumanii, K. pneumonia, E. cloaceae* and *C. albicans*. Also, for the first time, an approach to the development of a drug with antimicrobial action based on the results of molecular docking prediction was described.

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Osolodchenko T.P. – experimental research, idea, conceptualization, methodology;
Kolisnyk S.V. – participation in the writing and editing of the article;
Komisarenko A.M. – methodology, conclusions.

Email address for correspondence with the authors: ***alexmaslov392@gmail.com***