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THE NEW COMPOUNDS ACTING ON MICROORGANISMS ISOLATED FROM TROPHIC ULCERS

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ABBREVIATIONS

ALA	Anti-lysozyme activity
TAA	Total antioxidant activity
DNA	Deoxyribonucleic acid
ATCC	American Type Culture Collection
GNB	Gram-negative bacilli
BSBL	Broad-spectrum β-lactamase
CECT	Spanish Type Culture Collection
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
MFC	Minimum Fungicide Concentration
CoNS	Coagulase-negative staphylococci
Ct	Catalase
CV	Curriculum vitae
MDA	Malondialdehyde
OD	Optical density
FIC	Fraction inhibitory concentration
IL	Interleukin
PHCI	Public Healthcare Institution
GPx	Glutathione peroxidase
GR	Glutathione reductase
GST	Glutathione S-transferase
LDH	Lactate dehydrogenase
MDR	Multidrug resistant strains
MLS_B	Macrolide-lincosamide-streptogramin B
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSB	Macrolide-streptogramin B
MSSA	Methicillin-susceptible *Staphylococcus aureus*
WHO	World Health Organization
PPOA	Advanced oxidation protein products
AMR	Antimicrobial resistance
SOD	Superoxide dismutase
TNF	Tumor necrosis factor
XDR	Extensively drug-resistant strains

CONCEPTUAL RESEARCH FRAMEWORK

The relevance and significance of the research study

The discovery and production of penicillin was one of the greatest achievements in the recent history of mankind, which helped to change the quality of life, reduce mortality rate and increase life expectancy. This milestone discovery provided the basis for further researches to develop new antimicrobials. Unfortunately, antimicrobial molecules with new mechanisms of action were no longer developed since late 1960s. The last class of antimicrobials was discovered in 1968, meaning that most of the antimicrobials used over this period were adapted versions of the previously discovered ones [26, 28].

The dramatic decline in the number of novel antimicrobial drugs on the pharmaceutical market is due to an increase in the costs of research and development of these drugs, as well as their cost-effectiveness [4].

Despite recent advances in modern medicine, microorganisms continue to be one of the major health challenges. One of the reasons is the development of microorganisms resistant to antimicrobial drugs, thus not allowing them to show their effect. Antimicrobial resistance (AMR) is a natural phenomenon caused by mutations in bacterial genes, while overuse and misuse of antimicrobials accelerate the emergence and spread of antimicrobial-resistant bacteria. As it becomes a major public health problem worldwide, RAM imposes a significant economic burden on the healthcare sector [6,41].

A problem-solving strategy is required both in discovering new antimicrobials and developing new alternative approaches. The development of alternative methods of treatment is considered by the World Health Organization (WHO) as a challenge that medical community is facing today [3, 44].

Studies on synergism of combined compounds show potential in the fight against antimicrobial-resistant pathogens. Recently, researchers have experimented on combining synthetic antimicrobials with biological compounds. Studies have shown that in patients with severe infections, combination or synergistic therapy against resistant microorganisms is a new way to treat infectious diseases and is likely to have perspectives for further researches [40].

According to literature data, there has been a significant increase in interest on studying the synergistic interaction of plant extracts and antimicrobials with the potential for the resistance development. Several investigators have found that crude extracts of various plants, when used in combination with certain antimicrobials, significantly reduce minimum inhibitory concentration (MIC) values for multidrug-resistant strains [25].

Trophic ulcer is a major health problem due to its high incidence and significant socio-economic impact, resulting from an impaired patient's ability to perform daily activities (social and occupational tasks), low quality of life and financial constraints [2]. The dramatic worldwide impact of trophic ulcers is driven by rising healthcare costs as a result of frequent and long-term hospital stays, disabilities and early retirement. In Western Europe countries, 1-2.6% of the healthcare budget is billed annually for this pathology [18, 34].

Literature data on the incidence of trophic ulcers of the lower extremities show that about 70% occurs due to venous circulation pathology, i.e. varicose veins, which affects about 1% of the overall population, 3% of the elderly over 60 and 5% of those aged over 80 [27, 33]. Chinese studies showed that traumas or infected traumatic wounds are the primary etiological factor in the development of trophic ulcers, which were recorded in approximately 67% of patients [1]. Another factor is atherosclerosis obliterans, which is the main cause of peripheral arterial disease in the lower limbs among patients over 30, registered in about 8% of cases [17]. Diabetes is the cause of about 3% of skin disorders. Other causes include thrombosis, various injuries, innervation disorders, etc. [12, 13].

Specialized literature data show that the prevalence of trophic ulcers in society ranges from 1.9% to 13.1% [21]. Sasanka S. Chatterjee points out that both population aging and various risk factors, such as smoking, obesity, diabetes mellitus, etc., are the major reasons for an increase in the incidence of ulcers [32]. It is estimated that up to 25% of diabetic patients develop trophic

ulcers of the lower extremities, most of which become infected, and one in five patients require limb amputation [36]. Studies have shown that approximately 10% of the population develop a trophic ulcer, of which 2.5% die from this condition [23].

One of the most common complications of trophic ulcer are the associated microbial infections, which are formed by a mixed bacterial flora in 40-75% of cases. The etiological spectrum of microorganisms involved in the infection of trophic ulcers is quite diverse, being predominated by *Staphylococcus aureus* and followed by *Enterobacteriaceae* family and *Pseudomonas aeruginosa* species. α - or β-hemolytic streptococci and anaerobic bacteria are less commonly isolated, especially from the genera of *Clostridium* and *Bacteroides* [16]. Although some of these bacteria are not pathogenic, their multiple associations may lead to critical colonization of trophic ulcers and a delayed healing [43].

Chronic trophic ulcers are prone to bacterial infections, especially to venous ones, as a result of tissue hypoxia affecting the leukocyte activity on bacteria by reducing the "oxidative stress" [36]. Compared to conventional wounds, the healing process may stall in the inflammatory phase and does not progress due to certain factors, such as age, associated infections, ischemia, diabetes mellitus, tumours, malnutrition, etc.

Microorganisms influence the progression of trophic ulcers in different ways. One of the most unfavourable phenomenon is the persistence of the inflammatory response with the release of toxins and enzymes that destroy the regenerative factors, thus delaying healing due to the development of pseudosquamous tissue [38].

The infected trophic wound healing is also dependent on the development of the microbial biofilm, which causes resistance to the host's immune response and the action of antimicrobial drugs, thus triggering an inflammatory response. As a result, an infected trophic ulcer leads to a long-term inflammation, which in turn results in the release of cytokines and proteases, followed by tissue degradation.

Microorganisms isolated from trophic ulcers exhibit both antimicrobial-resistant mechanisms and pathogenicity factors, responsible for initiating complications and leading to therapeutic failures. Thus, most of the existing antimicrobials do not inhibit some pathogenic factors, such as biofilm formation, especially in the resistant strains. In order to overcome this problem, many researches recommend to study the antibiophilic effect of natural compounds (e.g., of animal, plant, fungal, bacterial origin, etc.) or synthetic ones. Thus, the issues of antimicrobial resistance and pathogenicity factors of microorganisms involved in trophic ulcers has led to the development of new therapeutic strategies [31, 35].

Over the recent decades, new alternative therapies have emerged as a new area of pharmaceutical research, by using preparations containing active ingredients of natural origin, combined with synthetic ones [37].

The original and comprehensive study conducted on the antimicrobial activity of combined drugs and their use in order to develop new dosage forms highlights the importance, novelty and relevance of this present research.

Purpose of the study. To assess the antimicrobial activity of novel compounds in order to develop the principles of designing effective multicomponent drugs used in the treatment of infected trophic ulcers.

Research objectives:

1. To determine the wide microbial spectrum isolated from infected trophic ulcers.
2. To highlight the antimicrobial resistance phenotypes and pathogenicity factors of microbial strains isolated from trophic ulcers.
3. To determine the antimicrobial activity of new chemical and biological entities against the reference and clinical microbial strains.
4. To study the synergistic action of chemical and biological substances, as well as of the possibilities of obtaining multicomponent antimicrobial compounds.
5. To determine the effects of new single-component and combined compounds on the expression of bacterial pathogenicity factors.

6. To assess the changes in biochemical parameters of bacterial cultures under exposure to both separate and combined compounds.
7. To study the effect of biologically active compounds on oxidative stress markers, the antioxidant system and the inflammatory patterns to obtain potential drugs.

Research hypothesis. The results of recent researches in the field of microbiology increasingly have proven therapeutic failures regarding the use of modern antimicrobials. New treatment options are required to counter this life-threatening condition in patients, including those with infected trophic ulcers. Therefore, it is crucial to conduct studies of the antimicrobial activity of new compounds of biological and chemical origin, followed by an assessment of the synergistic effect of effective multicomponent drugs used against microorganisms isolated from an infected trophic ulcer. Antimicrobials are essential in modern medicine and the emergence of resistant strains is an inevitable phenomenon that will lead to a decrease in their effectiveness and thus their further withdrawal. New antimicrobials should be developed to prevent these side effects. Hitherto, new natural or synthetic antimicrobials were used, whereas, recently, the emphasis has been placed on combined antimicrobial drugs of different origins. These combinations provide a synergistic interaction between biological and chemical compounds, which has proven to be the most effective method of combating resistance to antibacterial drugs.

Thus, the ability of biological compounds to "reuse" the existing antimicrobials in the treatment of infectious diseases may have a significant impact on fighting resistant microorganisms. Screening and identification of new chemical and biological compounds having antimicrobial properties can become the basis for obtaining new drugs that would be active against resistant strains. Despite the available data, further studies are required to reveal the antimicrobial effects of combined drugs, as well as to establish the synergistic action and develop principles for the designing effective multicomponent drugs in the treatment of trophic ulcers.

Synthesis of the scientific research methodology and justification of the chosen research methods. The study was conducted at the Department of Preventive Medicine, disciplines of Microbiology and Immunology, in the Biochemistry Laboratory of the "Nicolae Testemitanu" State University of Medicine and Pharmacy, at the Phycobiotechnology Laboratory of the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova and at the Microbiological Laboratory of the National Public Health Agency.

To prove the research hypothesis, specific methods were used for each objective, being nationally and internationally validated.

The present research included several stages in order to determine the microbial spectrum, viz. the assessment of the antimicrobial resistance phenotypes and pathogenicity factors of strains isolated from infected trophic ulcers; study of the antimicrobial activity (MIC and MBC) of chemical and biological compounds on reference and clinical strains isolated from trophic ulcers; establishing the synergistic action of chemical and biological single-component, as well as possibly obtaining the multicomponent antimicrobial compounds; determining the activity of the new compounds on the expression of pathogenicity factors of microorganisms; identifying the changes in the biochemical parameters of microorganisms on exposure to chemical and biological compounds; emphasizing the immunomodulatory action of the isolated compounds.

Based on the stated research purpose and objectives, classical and modern methods were applied within the present study, including: microbiological methods for determining the etiological spectrum of infected trophic ulcers, antimicrobial resistance phenotypes and pathogenicity factors of the studied strains; determination of the action of new compounds on microorganisms and the expression of pathogenic factors; biochemical and immunochemical methods for determining the effect of new compounds on the spontaneous production of biochemical and immunochemical parameters, as well as of the changes in the biochemical parameters of microorganisms on exposure to chemical and biological compounds; statistical quantitative and qualitative data analysis methods.

Novelty and scientific originality of the obtained results. For the first time, research was conducted on the assessment of antimicrobial, antioxidant and immunomodulatory effects of new

compounds, followed by determining the spectrum of microorganisms involved in the infection of trophic ulcers, as well as of the new compounds action on the pathogenic factors of microbial agents.

The obtained results made it possible to develop and implement guidelines for the management of infected trophic ulcers, which will allow standardizing the diagnostic methodology and timely identifying the etiological agent, by indicating the target treatment and avoiding both the therapeutic failure and the development of antimicrobial resistance.

The values of the antimicrobial, antipathogenic, immunomodulatory and antioxidant potential of the studied compounds indicate the need for further research on the development of alternative anti-infective strategies, as well as of new multicomponent compounds that might generate not only empirical, but also evidence-based scientific evidence for their therapeutic use.

The data obtained in this research were used in the development of nine patents.

The scientific research paper implemented some of the findings into the following national and international research projects:

20.80009.8007.09 Study of the resistance of gram-negative bacilli to antimicrobials in order to strengthen the national system for surveillance and control of communicable diseases (2020-2023).

AUF-MECR 2020-2021 Synthese et caracterisations de nouveaux complexes de coordination pour des applications biologiques (2020-2021).

18.51.07.01A / PS Reduction of contamination of raw materials and food products with pathogenic microorganisms (2018-2019).

09.816.09.05A Compounds with concomitant antimicrobial and antifungal properties (2009-2010).

The research problem solved within this paper is to scientifically substantiate the action of some new chemical and biological compounds on microorganisms isolated from infected trophic ulcers. The problem solution contributes to the formulation of principles for the development of multicomponent effective drugs used in the treatment of infected trophic ulcers, as well as to the elaboration of alternative anti-infective strategies to avoid therapeutic failures and the development of drug-resistant pathogens.

Theoretical significance and practical value of the study. The study results complement previous studies on the action of chemical and biological compounds on both reference and clinical strains isolated from trophic ulcers and the synergistic effect of these compounds by identifying combinations of compounds that potentiate the antimicrobial action.

During the study, data were collected on the effect of new compounds on the expression of enzymatic factors of microbial pathogenicity and persistence.

The obtained results allowed highlighting the immunomodulatory effect of chemical and biological compounds, both separately and combined, by determining their action on the spontaneous production of biochemical and immunochemical indices.

Based on the data collected during the study, a standardized algorithm was developed for the diagnosis of the etiological agent, mechanisms of antimicrobial resistance, and pathogenicity factors of microorganisms associated with trophic ulcers.

The study results into the particular action of compounds of chemical and biological origin and the synergistic action of compound compounds are scientific evidence that can be used as structural models to obtain new polycomponent antimicrobial therapeutic agents without major adverse effects, and in other research initiated in within the discipline.

The research results on the specific action of certain chemical and biological compounds, as well as on the synergistic action of combined compounds have been scientifically proved, thus may be used as structural models to obtain new multicomponent antimicrobial and therapeutic agents with no serious side effects, as well as in other studies initiated within the discipline.

Major scientific results submitted for the defence:

1. Diversity of microbial species associated with infected trophic ulcers.

2. Pathogenicity factors associated with the persistence of microbial strains isolated from infected trophic ulcers.
3. Characteristics of antimicrobial sensitivity of isolates by highlighting the mechanisms of resistance to multidrug-resistant pathogens.
4. Evaluation of the antimicrobial activity of some chemical and biological compounds on reference and clinical strains.
5. Assessment of the synergistic effect of chemical and biological compounds and the kill-time of microorganisms.
6. Scientific validation of the action of single-component and combined compounds on the expression of pathogenicity factors of microorganisms involved in trophic ulcers.
7. Assessment of the value of biochemical parameters of microorganisms on exposure to single-component and combined compounds.
8. Providing relevant evidence on the action of biologically active compounds on oxidative stress markers, antioxidant system, and inflammatory patterns in order to formulate the principles of designing effective antimicrobial drugs used in the treatment of trophic ulcers.

Implementation of scientific results. The research results were applied within the activity of the Microbiology Laboratory of IMPH "Timofei Moşneaga" Republican Clinical Hospital, at the Bacteriology Laboratory of IMPH "Valentin Ignatenco" Municipal Clinical Hospital for Children, at the Microbiology Laboratory of IMPH "Toma Ciorba" Clinical Hospital for Infectious Diseases, within the healthcare practice of IMPH Health Centre from Călărași, at the Surgery Department of IMPH District Hospital from Călărași, as well as in teaching the Microbiology and Immunology disciplines, at the Preventive Medicine Department of the "Nicolae Testemitanu" State University of Medicine and Pharmacy from the Republic of Moldova.

Approval of scientific results. The study results were presented and discussed within the following national and international scientific forums: the VIIth National Conference on Microbiology and Epidemiology on "Current challenges in the diagnosis and epidemiology of communicable and non-communicable diseases with an impact on public health" (Bucharest, Romania, 2014) ; the International Conference on "Socio-psycho-medical lifestyle changes of the modern family" (Bucharest, Romania, 2015); the International Scientific Conference on Microbial Biotechnology (3rd edition) (Chisinau, Republic of Moldova, 2016); the National Conference on Otorhinolaryngology and Cervical and Facial Surgery (Sibiu, Romania, 2017); the VIIth Annual International Scientific and Practical Conference on " Recent Issues in Medicine" (Baku, Azerbaijan, 2018); First Balkan Conference of Medical Mycology and Mycotoxicology "Balkan Fungus 2018" (Timişoara, Romania, 2018); the XXXVth National Conference of Chemistry Călimăneşti - Căciulata (Vâlcea, Romania, 2018); the International Scientific Conference on Microbial Biotechnology (4th edition) (Chisinau, 2018); within the Days of the University of Medicine and Pharmacy of Craiova XLIXth edition (Craiova, Romania, 2019); the International Conference Achievements and Perspectives of Modern Chemistry dedicated to the 60th anniversary from the foundation of the Institute of Chemistry (Chisinau, Republic of Moldova, 2019); the National Congress of Specialists in the Field of Public Health and Health Management from the Republic of Moldova with International Participation "One Health" (Chisinau, Republic of Moldova, 2019); the Congress dedicated to the 75th anniversary since the foundation of "Nicolae Testemitanu" SUMPh (Chisinau, Republic of Moldova, 2020); the National Scientific Conference with International Participation "Advanced Materials in Biopharmaceuticals and Technology" (Chisinau, Republic of Moldova, 2021); the National Conference with International participation "One Health Approach in a Changing World" (Chisinau, Republic of Moldova, 2021).

Moreover, the study results were submitted to the following invention salons, being awarded with distinctions: The World Exhibition on Inventions, Research and New Technologies (Valencia, Spain, 2018 - gold medal); 46E Salon International des Inventions de Genéve (Geneva, 2018 – gold medal); Euroinvent 2018 (Iasi, Romania, 2018 - gold medal, bronze medal); Inventica 2018 (Iasi, Romania, 2018 - 2 gold medals); International Exhibition of Inventions Innovations

"Traian Vuia" (Timisoara, Romania, 2018 - 2 gold medals); PRO INVENT 17th Edition (Cluj-Napoca, Romania, 2019 - diploma of excellence); 47E Salon International des Inventions de Genéve (Geneva, Switzerland, 2019 - silver medal); Euroinvent 2019 (Iasi, Romania, 2019 - gold medal); International Exhibition of Inventions Innovations "Traian Vuia" (Timisoara, Romania, 2019 - gold medal); Inventica 2019 (Iasi, Romania, 2019 - gold medal); Infoinvent 16th Edition (Chisinau, Republic of Moldova, 2019 - silver medal); Euroinvent 2020 (Iasi, Romania, 2020 - gold medal); Inventica 2020 (Iasi, Romania, 2020 - gold medal); Innovation and Creative Education Fair for Youth ICE-USV - IVth Edition (Suceava, Romania, 2020 - silver medal, gold medal); "Traian Vuia" International Salon of Inventions and Innovations, the 6th edition (Timişoara, Romania, 2020 - gold medal, silver medal); International Exhibition Inventcor (Deva, Romania, 2020 - 2 gold medals); Euroinvent 2021 (Iasi, Romania, 2021 - gold medal); Innovation and Creative Education Fair for Youth ICE-USV - Vth Edition (Suceava, Romania, 2021 - gold medal, silver medal); Inventica 2021 (Iasi, Romania, 2021 - 2 silver medals); PRO INVENT, 19th Edition (Cluj-Napoca, Romania, 2021 - gold medal); Infoinvent , 17th Edition (Chisinau, Republic of Moldova, 2021 - gold medal); International Exhibition Inventcor, IInd edition (Deva, Romania, 2021 - gold medal).

Publications on the research topic.

The study results were published in 77 scientific papers, including a single-author monograph on "Resistance of microorganisms to antibiotics" (240 pages), five articles were submitted to the journals from international databases, six articles in the internationally recognized scientific journals, as well as 16 articles were submitted to the scientific journals of the National Register of specialized journals, scientific congresses and conferences, of which eight included single-author publications, 22 abstracts in paper collections of international conferences (abroad), 18 abstracts at international conferences in the republic, two guidebooks, three methodological and didactic works, nine patents, and three innovations. The scientific results of the thesis were presented at 30 international scientific forums and 5 national scientific forums with international participation.

Summary of thesis compartments

The thesis manuscript is written in Romanian on 191 computer-edited pages. It has been structured in accordance with the requirements on Habilitation Thesis submission, consisting of a title page, a copyright page, a table of contents, annotations in Romanian, English and Russian, introduction, six chapters, general conclusions, recommendations and a bibliography list of 350 references, 8 annexes, the statement on the responsibility assumption and the author's CV. The research work includes 40 tables, 47 figures and 12 formulas.

Keywords: infected trophic ulcer, pathogenicity factors, new compounds, antimicrobial activity.

THESIS CONTENT

1. THE CURRENT STATE OF RESEARCH IN THE DEVELOPMENT OF ANTIMICROBIALS AND NEW APPROACHES IN TROPHIC ULCER THERAPY

The literature study on antibiotic resistance phenomenon, on diagnostic possibilities, as well as on the new treatment strategies allowed to highlight the shortcomings in the treatment of trophic ulcer infected with antimicrobial-resistant microorganisms. Thus, therapeutic failures, which lead to an increased morbidity, disability and mortality, are mainly the consequences of multiple antimicrobial resistance of these microorganisms.

Both the accuracy and speed of reporting the laboratory test results on the etiological agent, mechanisms of resistance and the proper antimicrobial drug used in the antibiogram-based treatment are essential for infectious disease therapy.

Over the last three decades, the infectious disease therapy has become increasingly challenging due to the lack of new antimicrobials resulting from exacerbated development and implementation costs, relatively short patent life, introduction of the reserve drugs, and, finally, small profits for pharmaceutical companies.

Modern medicine faces a number of problems associated with trophic ulcers, such as the clinical course, severe complications leading to death, scientific challenges to validate the new therapeutic and surgical strategies.

Trophic ulcers are prone to microbial invasion, and one of the most frequent complications is the association of a predominantly mixed microbial infection. Microbiological diagnostics that determines the microbial pathogens and their resistance mechanisms might prevent the development of complications.

The microbial adaptation and long-term survival in trophic ulcers have been explained by the presence of associated pathogenicity and persistence factors that inactivate the mechanisms of antibacterial resistance of the immune system, such as the anti-lysozyme, anti-complementary, anti-interferon and other activities. The potential persistence of the microorganisms, including the resistance level, determines the duration of their survival within the macroorganism. Hence, the need to develop antimicrobial drugs that can reduce the infectious potential of the microorganisms.

Over the recent years, in attempt to search new therapeutic alternatives in the treatment of infectious diseases, scientists have focused on the development of combined antimicrobial drugs. The synergy of biological and synthetic antimicrobial compounds is one of the current areas of medical research, opening up new perspectives in the development of active molecules against antimicrobial multidrug-resistant microorganisms, which will reduce the healing time, including infected trophic ulcers.

2. RESEARCH MATERIALS AND METHODS

This scientific research was conducted during 2013-2021 within the Department of Microbiology and Immunology, at the Biochemistry Laboratory of the "Nicolae Testemitanu" State University of Medicine and Pharmacy, at the Phycobiotechnology Laboratory of the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova and at the Microbiology Laboratory of the National Public Health Agency. Scientific consultants: Rudic Valeriu, Dr. habil. in Biology, univ. prof., academician, Emeritus of the Republic of Moldova (specialty 313.02 Microbiology, Medical Virology) and Gulea Aurelian, Dr. habil. in Chemistry, univ. prof., academician, Emeritus of the Republic of Moldova (specialty 141.01. Inorganic Chemistry; 141.02; Coordinative Chemistry).

The research methodology was approved by the Research Ethics Committee of "Nicolae Testemitanu" SUMPh, opinion no. 65 of April 12, 2017.

The main purpose of this study is to evaluate the antimicrobial activity of new compounds and to develop principles for designing new effective multicomponent preparations in the treatment of infected trophic ulcers.

The importance and need of this research have aroused due to the significant impact of antimicrobial resistance phenomenon on global public health. Infections caused by antimicrobial-resistant microorganisms, as they do not respond to therapy, have a longer course of progression, requiring longer hospital stays and leading to an increased mortality. [20, 39]. Screening and identification of new chemical and biological compounds with antimicrobial properties opens up opportunities for the development of new active preparations against resistant strains. Therefore, intensive care, transplantation, cancer chemotherapy, premature newborn care, and even conventional surgery are impossible without these drugs [42].

The reference strains from the international collections served as the study objects for th present research, including *Staphylococcus aureus* ATCC 25923, ATCC 29213, *Streptococcus pyogenes* ATCC 12344, *Enterococcus faecalis* ATCC 19433, *Enterococcus faecium* ATCC 6569, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Cryptococcus neoformans* CECT 1043, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter baumannii* ATCC® BAA-747, *Proteus mirabilis* ATCC 25933, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae* ATCC 13047 and clinical strains isolated from trophic ulcers. The clinical strains were provided by the Microbiology Laboratory of the “Timofei Moşneaga" Republican Clinical Hospital.

The study included new chemical compounds synthesized at the Department of Inorganic Chemistry, at the Department of Chemistry of the State University of Moldova, whereas the biological remedies were provided by the Phycobiotechnology Laboratory of the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova and "Phycobiotechnology" scientific research laboratory of the State University of Moldova.

To achieve the target purpose and objectives, the following standardized methods were used, being adapted to the studies conducted in the present research:

- ✓ *historical, descriptive, bibliographic-analytical, logical* methods were used in the theoretical study of bibliographic sources, concepts and theoretical approaches
- ✓ *epidemiological* – in the analysis of epidemiological indicators
- ✓ *microbiological* - to isolate the microbial agents associated with trophic ulcers; in determining resistance patterns; in the study of the pathogenicity factors of isolated strains; in determining the antimicrobial activity of chemical and biological compounds on separate and combined reference strains and clinical isolates
- ✓ *biochemical, immunochemical* – in determining the action of chemical and biological compounds on the spontaneous production of biochemical and immunochemical indices
- ✓ *observation* – in the analysis of the antimicrobial and immunomodulatory effects by analysing cytokines and pro- and anti-inflammatory cytokines
- ✓ *systematic analysis* - to identify the object of study, to characterize accurately, precisely and sensitively the subject of the study, by minimizing deviations
- ✓ *comparative analysis* - used at all research stages, when comparing the antimicrobial activity of separate and combined chemical and biological compounds on the reference strains and clinical isolates, thus highlighting the synergistic particularities
- ✓ *experimental analysis* - to confirm the research hypothesis and to validate the structural model for obtaining new therapeutic agents with no serious adverse effects
- ✓ *synthesis* – used for verifying the correctness of in each step, keeping only what was revealed with maximum clarity and complete reliability
- ✓ *statistics* - for the quantitative and qualitative analysis of the data obtained in the study
- ✓ *mathematics* - for the numerical data processing, analysis and simulation of the obtained data
- ✓ *verification and critical evaluation of the results* – to exclude other ways leading to the same results or to identify the possibilities of obtaining other results in this way.

The studies envisaged by the study objectives were organized and carried out according to the following stages:

Stage 1. The general research plan was designed, the problem was finalized, and the theoretical and practical documentation on the phenomena was planned for observation. The research hypothesis was formulated, as well as the means and methods of investigation and data collection were traced out.

Stage 2. The microorganisms involved in the etiology of the infected trophic ulcer were determined and studied. Data on antimicrobial susceptibility / resistance of isolated strains were analysed. There have been studied the pathogenicity factors of microorganisms isolated from trophic ulcers, which contribute to the persistence of the infectious disease. Pathogenicity factors were selected for research, depending on their involvement in the microbial persistence phenomenon within the host organism and chronicity of the infectious process.

Stage 3. There was established the qualitative and quantitative effects of the new chemical and biological compounds on the reference strains by diffusimetric methods and successive dilutions in liquid media. The minimum inhibitory and minimum bactericidal concentration of these compounds was determined and, based on the results obtained, there were selected only those that exhibited antimicrobial activity confirmed by further studies.

Step 4. There was studied the synergistic action of chemical and biological compounds on the reference strains by identifying combinations that increased the antimicrobial effect. The microbial time-kill rate was determined under the action of the separate and combined compounds.

The action of the new compounds on the expression of enzymatic factors of pathogenicity (gelatinase, DNA -ase, lecithinase, amylase, lipase, hemolysin) and the microbial persistence (anti-complementary and anti-lysozyme action) was studied. The effect of the new compounds on the bacterial adherence to inert substrates, as well as their anti-biofilm action were also determined.

Stage 5. The immunomodulatory effects of the separate and in combined chemical and biological compounds were determined. Their effects on the spontaneous production of biochemical and immunochemical parameters, as well as the changes they cause in some biochemical parameters of microorganisms, have been established.

Stage 6. The obtained results were systematically analysed based on the suggested objectives and hypotheses. Practical conclusions and recommendations were formulated based on the results obtained.

The study results were used to develop two guidelines: "Guidelines for identifying mechanisms of antimicrobial resistance, interpretation and clinical application of the results" and "Guidelines for the management of infected trophic ulcers."

3. SPECIES DIVERSITY, ANTIMICROBIAL RESISTANCE AND VIRULENT FACTORS OF STRAINS ISOLATED FROM TROPHIC ULCERS

3.1. Evaluation of spectrum and occurrence of the microorganisms associated with trophic ulcers

The etiological spectrum of the infected trophic ulcer greatly varies by involving several species of microorganisms, predominantly of *S. aureus* strains, representatives of the family *Enterobacteriaceae* or *P. aeruginosa* strains.

Limited antimicrobial therapeutic options for infected trophic ulcers are among the major challenges in medicine, thus requiring, first of all, highlighting the species diversity involved in this pathological process. For this purpose, this study determined the spectrum and occurrence of microorganisms in the samples retrieved from trophic ulcers. 387 biological samples were analysed. In 176 (41.5%; CI 5% 44.8-45.4) cases, only one microbial species was isolated, in 172 (40.6%; CI 95% 40.3-40.9) - two species and in 37 (8.7%; 95% 8.5-8.9) - three species. In 39 (9.2%; 95% CI 38.7-39.3) samples, the microorganisms were not isolated as a diagnosis. A total of 631 microbial strains were isolated.

Microorganisms often associated with trophic ulcers belonged to the genus *Staphylococcus* (predominantly *Staphylococcus aureus*), followed in a decreasing order of occurrence by streptococci (*Enterococcus spp., Streptococcus pyogenes*), enterobacteria (*Klebsiella spp., Escherichia, Proteus spp., Citrobacter spp.*), non-fermenting bacilli *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and yeast fungi of the *Candida* genus.

Besides *Staphylococcus aureus*, the most common species involved in the infections caused by a single microbial species (19.3%; 95% CI 18.8-19.8), *Pseudomonas aeruginosa* (17.6% 95% CI 17.1-18.1), *Enterococcus spp.* (13.6%; 95% CI 13.2-14.0), *Proteus spp.* (12.5%; 95% CI 12.1-12, 9), *Klebsiella pneumoniae* (11.4%; 95% CI 11.0-11.8), *Acinetobacter baumannii* (10.2%; 95% CI 9.8-10.6), *Streptococcus pyogenes* (8.5%; 95% CI 8.2-8.8%) and *Escherichia coli* (6.8%; 95% CI 6.5-7.1%) were also isolated.

In mixed infections, 62.8% of cases were dominated by *S. aureus* strains, associated in 47.7% of cases with *K. pneumoniae* and *P. aeruginosa* species.

Gram-negative bacilli were more commonly isolated from trophic ulcers (56.3%; 95% CI 53.2-54.0) than gram-positive cocci (41.5%; 95% CI 41.2-41.8). The most common isolate was *S. aureus* species (21.9%; 95% CI 21.7-22.1), followed by a decreasing incidence of *P. aeruginosa* (15.2%; 95% CI 15.0-15, 4), *K. pneumoniae* (13.3%; 95% CI 13.1-13.5) and *Enterococcus spp.* (11.9%; 95% CI 11.7-12.1).

3.2. Antibiotic resistance of strains isolated from trophic ulcers

Of the 158 strains of *Staphylococcus* spp. isolated from trophic ulcers, 108 (68.4%; 95% CI 67.4-69.4) cases were multidrug-resistant strains, 69 (43.7%; 95% CI 42.9- 44.5) were MRSA

(Methicillin-Resistant Staphylococcus Aureus), 31 (19.6%; 95% CI 19.0-20.2) tested positive for test D and only 20 (12.6%; 95 CI 12,2-13.0) were sensitive to all the tested antimicrobials.

The highest sensitivity level of *Staphylococcus* spp. strains was recorded to glycopeptides (100%; 95% CI 99.5-100.5), followed by tetracyclines (91.8%; 95% CI 91.3-92.3), oxazolidone (89.9%; 95% CI 89.4-90.4) and amphenicols (87.9%; 95% CI 87.4-88.4).

Of the 158 *Staphylococcus* spp. strains tested, 31 (19.6%; 95% CI 19.0-20.2) showed inducible MLSB (macrolide-lincosamide-streptogramins B) resistance, 13 (8.2%; 95% CI 7.8-8.6) - constitutive MLSB resistance and 44 (27.8%; 95% CI 27.1-28.5) - macrolide-streptogramins B (MSB) resistance. The inducible MLSB resistance was higher among MRSA compared to MSSA ($p = 0.037$) (Table 1).

Table 1. **Clindamycin resistance phenotypes in *Staphylococcus* spp. strains isolated from trophic ulcers**

Phenotypes	**E**	**Cm**	**Test D**	**MRSA (n=69)**	**MSSA (n=89)**	***Staphylococcus spp.* in total (n=158)**
				n (%; CI_{95})	**n (%; CI_{95})**	**n (%; CI_{95})**
inducible MLSB	R	S	+	25 (36,2; 35,7-36,7)	6 (6,7; 6,4-7,0)	31 (19,6; 19,0-20,2)
constitutive MLSB	R	R	-	7 (10,1; 9,8-10,4)	6 (6,7; 6,4-7,0)	13 (8,2; 7,8-8,6)
MSB	R	S	-	14 (20,3; 19,9-20,7)	30 (33,7; 33,1-34,3)	44 (27,8; 27,1-28,5)
Sensitive	S	S	-	23 (33,3; 32,8-33,8)	47 (52,8; 52,2-53,4)	70 (44,3; 43,4-45,2)

Note: MLSB - macrolides-lincosamides-streptogramins B; MSB - macrolide-streptogramins B; E - erythromycin; Cm - clindamycin

The *Enterococcus* spp., strains isolated from trophic ulcers showed a high resistance to fluoroquinolones (ciprofloxacin - (84.0%; 95% CI 83.5-84.5); levofloxacin - (78.7%; 95% CI 9578.2-79.2), sulphonamides (54.7%; 95% CI 54.3-55.1), penicillins and tetracyclines (45.3%; 95% CI 45.0-45.6). Higher sensitivity was reported to carbapenems and glycopeptides (57.3%).

Of the total enterococcus strains, 35 (46.7%; 95% CI 47.8–51.6) were multidrug resistant and 32 (54.7%; 95% CI 52.7–56.7) were resistant to vancomycin (VRE). Of the multidrug-resistant strains, 100% (95% CI 97.3–102.7) were non-susceptible to ciprofloxacin, 57.1% (95% CI 55.1–59.1) to trimethoprim/sulfamethoxazole, and 54.3% (95% CI 52.3–56.3) to tigecycline.

Enterobacteriaceae strains isolated from trophic ulcers showed a pronounced resistance to aminopenicillins (ampicillin - 93.2%; 95% CI 90.4-96.0), penicillins with beta-lactamase inhibitors (amoxiclav - 89.3%; 95% CI 86.5-92.1) and cephalosporins (ceftazidime - 85.9%; 95% CI 83.2-88.6, cefepime - 84.0%; 95% CI 81.3-86.7, cefotaxime - 78.2%; 95% CI 75.6 -80.8). The most effective drugs, to which enterobacteria showed a lower resistance, were monobactams (aztreonam - 14.6%; 95% CI 13.5-15.7), sulfonamides (trimethoprim/sulfamethoxazole - 18.4%; 95% CI 17 .1-19.7), carbapenems (imipenem - 21.4%; 95% CI 20.0-22.8), meropenem - 24.8%; 95% 23.3-26.3, ertapenem - 10.2%; 95% CI 10.5-9.9) and aminoglycosides (amikacin - 25.7%; 95% CI 24.2-27.2, gentamicin - 32.5%; 95% CI 30.8-34.2, tobramycin - 20.9%, 95% CI 19.6-22.2). Enterobacteria strains showed moderate resistance to fluoroquinolones (ciprofloxacin - 68.0%; 95% CI 65.6-70.4; levofloxacin - 38.3%; 95% CI 36.5-40.1).

P. aeruginosa strains showed the highest sensitivity to carbapenems (ertapenem - 91.7% (95% CI 88.9-94.5), imipenem - 59.4% (95% CI 57.1-61, 7), meropenem - 55.2% (95% CI 53.0-57.4)) and aminoglycosides (amicacin - 59.4% (95% CI 57.1-61.7), tobramycin - 57.3% (CI 95%

55.1-59.5)). The remaining groups of drugs showed a higher resistance, namely, penicillins (ticarcillin - 96.9% (95% CI 94.9-98.9), piperacillin-tazobactam - 90.6% (95% CI 88.6-92.6 %), cephalosporins (cefepime - 84.4 (95% CI 82.5-86.3), ceftazidime - 80.2% (95% CI 78.3-82.1)) monobactam (aztreonam 83.3% (95% CI 81.4-85.2)) and fluoroquinolones (ciprofloxacin - 75.0% (95% CI 73.2) -76.8), levofloxacin - 71.9% (95%) 70.1-73 .7%).

The overall resistance rate of gram-negative bacilli to three or more antimicrobials was 71.3%, and only one strain (0.28%) was sensitive to all antimicrobial preparations tested.

When analysing the multidrug resistance patterns of gram-negative bacilli, *P. aeruginosa* (88.5%; 95% CI 86.5-90.5) strains had the highest level, followed by *K. pneumoniae* (81.0%; 95% CI 78.9–83.1), *A. baumannii* (71.7%; 95% CI 68.5–74.9) and *E. coli* (69.8%; 95% 66.7–72.9). The overall resistance rate of gram-negative bacilli to three or more antimicrobials was 71.3%, and only one strain (0.28%) was susceptible to all antimicrobials tested.

Gram-negative bacilli were screened for broad-spectrum beta-lactamases and phenotypic tests to confirm BLSE production. In the present study, 97 (27.3 ± 0.29%; 95% CI 27.0-27.6) of gram-negative bacilli strains producing broad-spectrum beta-lactamases were identified by phenotypic testing. The gram-negative bacilli species, showing a higher rate of BLSE production, included *P. aeruginosa* (40.6 ± 1.32%; 95% CI 39.3-41.9), followed by *K. pneumoniae* (36.9 ± 1.44%; 95% CI 35.7-38.1) and *E. coli* (33.9 ± 2.19%; 95% CI 32.7-35.1). However, 15 (28.3 ± 2.00%; 95% CI 27.0-29.6) strains of *A. baumannii*, 7 (8.3 ± 0.68%; 95% CI 7.6-9, 0) of *K. pneumoniae* and 4 (4.2 ± 0.42%; 95% CI 3.47-4.93) of *P. aeruginosa* produced AmpC β-lactamases.

Of the gram-negative bacillus strains isolated from trophic ulcers, 50 (16.6 ± 0.27%; 95% CI 16.4-16.8) were positive for carbapenemases at phenotypic testing and only 9 (3.0% ; 95% CI 2.9-3.1) were confirmed by genotypic tests.

To identify resistance determinants viz. genes that encode carbapenemase production, molecular characterization of carbapenemase-producing strains revealed 5.9±0.58% (95% CI 5.3-6.5) isolates from *K. pneumoniae*, 3.8 ±0.73% (95% CI 3.1–4.5) - *E. coli* and 2.1 ± 0.30% (95% CI 1.8–2.4) of *P. aeruginosa* strains. The most common carbapenemase type found was OXA-48 (2.6 ± 0.10%, 95% CI 2.5–2.7), followed by NDM (0,3 ± 0.26%, 95% CI 0.26-0.34). Class A carbapenemases (KPC) were not found in strains isolated from trophic ulcers.

Carbapenem resistance of *A. baumannii* strains is mainly mediated by bla_{OXA} genes (such as $bla_{\text{OXA-51}}$, $bla_{\text{OXA-58}}$, $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-40}}$), which encode hydrolyzed carbapenem class D β-lactamases. Tests revealed that *A. baumanii* strains isolated from trophic ulcers in 9.5 ± 1.15% (95% CI 8.4-10.7) cases showed carbapenem resistance genes, of which 5 .7 ± 0.90% (95% CI 4.8-6.6) were $bla_{\text{OXA-23}}$ genes and 3.8 ± 0.73% (95% CI 3.1-4.5) were $bla_{\text{OXA-58}}$ genes.

3.3. Assessment of pathogenicity factors of microbial strains isolated from trophic ulcers

Microbial pathogenicity involves all biochemical mechanisms of the microorganisms to cause diseases. Not all pathogenic or potentially pathogenic microorganisms exhibit this ability in the same way. For these reasons, it is important to determine and know the entire “arsenal” of virulence factors , which enable the microbial strains to get involved in the infectious process.

This present research work studied some enzymatic virulence factors of bacterial strains isolated from trophic ulcers, which allow microorganisms to persist in the infectious outbreak.

One of these factors is hemolysin, a cytolytic protein that has the ability to lyse human and animal erythrocytes. Hemolysin is usually produced by strains of bacteria that have shown high virulence in experimental studies, being associated with even a more severe infectious process.

Among the strains isolated from trophic ulcers, 356 (57.7%; 95% CI 51.6–52.0) showed hemolytic activity, of which 184 (51.8%; 95% CI 51.4–52.2) gram-negative bacilli and 172 (65.6%; 95% CI 65.2-66.1) gram-positive cocci strains. This pathogenicity factor was found to be more pronounced among gram-positive cocci (65.6%; 95% CI 64.7-66.5), followed by non-fermenting gram-negative bacilli (59.1%; 95% CI 58.2-60.0) and enterobacteria (46.6%; 95% CI 45.8–47.9).

The enzyme lecithinase was detected in 154 (58.8%) gram-positive coccus strains and in 177 (49.9%) gram-negative bacillus strains. Among the gram-negative bacilli, the most lecithinase-producing strains were recorded among *Proteus* spp. (70.4 ± 1.60%; 95% CI 68.8-72.3), *P. aeruginosa* (65.6 ± 0.49%; 95% CI 65.1-66.1) and *K. pneumoniae* (57.1 ± 0.45%; 95% CI 56.7-57.6). Lecithinase production was higher in gram-negative glucose-nonfermenting bacilli (52.3 ± 1.02%; 95% CI 51.3-53.3) compared to gram-negative glucose-fermenting bacilli strains (48.1 ± 0.98%; 95% CI 47.1-49.1). The synthesis of this enzyme was found in 58.8 ± 0.99% (95% CI 57.8-59.8) of gram-positive cocci, predominantly among *S. aureus* (100%).

Lipase producing microorganisms turned out to be a large number of both gram-negative (77.2 ± 1.41%; 95% CI 71.1–73.8) and gram-positive (70.2 ± 0.36%) strains (95% 69.8– 70.6). The expression rate of this enzyme was recorded in over 70% of species isolated from trophic ulcers, except for *E. coli* (58.5%) and CoNS (25.0%).

DNA-ase is an enzyme that cleaves chromosomal and plasmid nucleic acids. It reduces the secretion viscosity, which accumulates DNA from damaged cells, providing them with mononucleotides for their own synthesis, thus allowing their dissemination in the host body [7]. This pathogenicity enzyme was more commonly recorded in gram-positive coccus strains (43.9 ± 0.50%; 95% CI 43.4-44.4) compared to gram-negative bacilli (42.2 ± 0.4%). 49%; CI 95% 41.7-42.7). Among gram-negative bacilli, this pathogenicity factor was more in strains of enterobacteria (61.7 ± 0.59%; 95% CI 61.1-62.3), compared with non-fermenting gram-negative bacilli (15%), 4 ± 0.29 %; 95% CI 15.1-15.7).

Hydrolases, such as amylase, are important for microbial metabolism, being responsible for the breakdown of proteins, carbohydrates and lipids. This pathogenicity factor was recorded in 43.9% of tested strains, being more expressed in enterobacteria (57.8%) and non-fermenting gram-negative bacilli (53.0%). Gram-positive cocci strains showed enzyme expression in 27.9% of cases.

The secretion of proteolytic enzymes (gelatinase and caseinase) is extremely important in the bacterial survival and invasion into the host tissues. Numerous studies have shown that proteases synthesized by microorganisms involved in the infectious process contribute to the development of severe infections. According to the study results, the protease production was more expressed in gram-positive cocci strains (caseinase - 75.6 ± 0.74% (95% CI 74.9-76.3), gelatinase - 65.6 ± 0, 37% (95% CI 65.2-66.0), especially in *S. aureus* and *Enterococcus* spp. strains - 77.3 ± 0.41% (95% CI 76.9-77.7).

When comparing the degrees of expression of enzymatic pathogenicity factors in single-culture and associated strains, it was found that it is lower in those isolated in monocultures, compared to those in microbial associations ($p = 0.026$) (Figure 1).

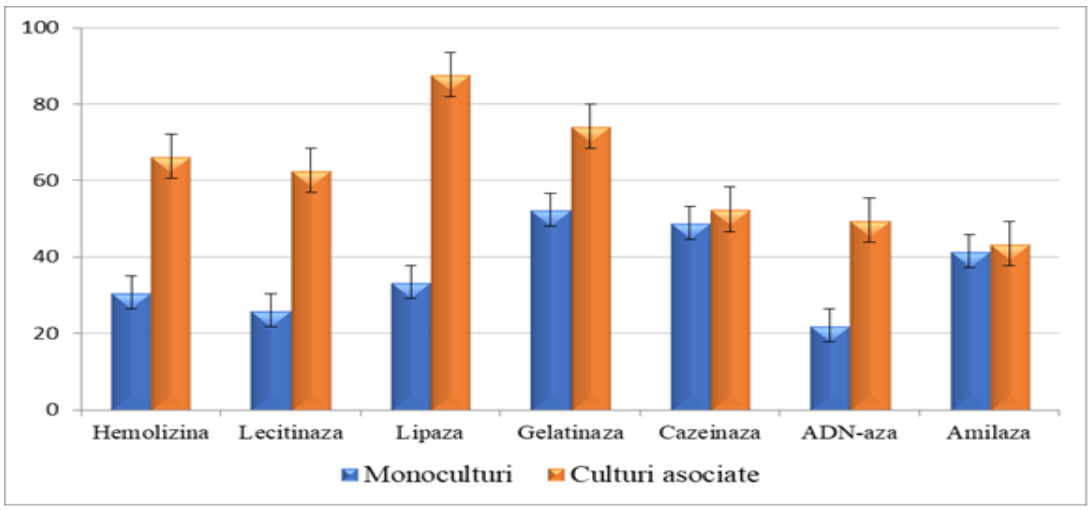


Figure 1. **Expression of enzymatic pathogenicity factors in strains isolated in monocultures and associated strains (%)**

When determining and comparing the expression rate of enzymatic pathogenicity factors in antimicrobial and susceptible gram-negative and gram-positive coccus strains, it showed a higher level in multidrug-resistant strains, both in gram-negative bacilli (GNB) and in gram-positive cocci, compared to sensitive ones (p = 0.036).

GNB multidrug-resistant strains showed a higher rate of expression of lipase, gelatinase, hemolysin and lecithinase by 58.8%, 57.9%, 49.3% and 41.1%, compared to susceptible strains, respectively. Minor differences were observed in the production of DNA-ase and amylase, the multidrug-resistant strains exceeding by 35.9% and sensitive strains by 26.0%.

Among the multi-resistant and sensitive gram-positive coccus strains, greater differences in the production of pathogenic enzymes were found in the case of lipase (73.2%), lecithinase (65.8%), hemolysin (57.7%) and caseinase. (56.3%).

Another pathogenicity factor studied in the present research was the anti-lysozyme activity of isolated microorganisms. Of the 631 strains isolated from trophic ulcers, 589 (93.3 ± 0.29%; 95% CI 93.6-94.2) and only 42 (6.7 ± 0.08%; 95% (6.6-6.8%) were inactive. Anti-lysozyme activity was exhibited among 98.8 ± 0.79% (95% CI 98.0-99.6) of gram-positive coccus strains, among non-fermenting gram-negative bacillus strains - 97.3 ± 1.35% (95% CI 95.9-98.7), among the yeast fungi of the *Candida* genus - 92.9 ± 14.95% (95% CI 78.1-107.7) and among the strains of gram-negative fermenting bacilli - 83 .1 ± 1.25% (95% 81.8-84.4). Most of the clinical strains - 278 (47.2 ± 0.23%; CI 95% 47.0-47.4) showed a moderate anti-lysozyme expression (K0.5-2.49), 174 (29.5 ± 0.18%; 95% CI 29.3-29.7) - high expression (K> 2.5) and 137 (23.3 ± 0.16%; 95% CI 23.1- 23.5) - low expression level (K <0.49) (Table 2).

Table 2. **Lysozyme inactivation capacity of strains isolated from trophic ulcers**

Degree of ALA expression	**Gram-positive cocci (n = 259)**		**Non-fermenting gram-negative bacilli (n = 145)**		**Fermenting gram-negative bacilli (n = 172)**		**Fungi of genus *Candida* (n = 13)**		**Total (n = 589)**	
	abs	**%**	**abs**	**%**	**abs**	**%**	**abs**	**%**	**abs**	**%**
High (K>2,5)	73	28,2	46	31,7	51	29,6	4	30,8	174	29,5
Moderate (K0,5-2,49)	118	45,6	69	47,6	84	48,8	7	53,8	278	47,2
Low (K<0,49)	68	26,2	30	20,7	37	21,5	2	15,4	137	23,3

The study also compared the lysozyme inactivation capacity of methicillin-resistant and sensitive *Staphylococcus* spp. strains, as well as of multidrug-resistant and susceptible GNB to antimicrobial drugs. All 100% MRSA strains (95% CI 97.1-100.0) inactivated lysozyme, 45.0% (95% CI 43.1-46.9) of which showing a high expression of anti- lysozyme activity, 53 .7% (95% CI 51.6-55.8) – moderate and 1.3% (95% CI 1.0-1.6) - low expression level. MSSA strains inactivated lysozyme in 97.1% (95% CI 94.9-99.3) of cases, of which 1.0% (95% CI 0.8-1.1) showed a high anti-lysozyme expression, 32.3% (95% CI 31.0-3306) - medium and 66.8% (95% CI 65.0-68.6) – low expression.

Analysis of the anti-lysozyme activity of multidrug-resistant and antimicrobial-sensitive GNB strains found that 98.4 ± 1.36% (95% CI 97.0-99.8) of multidrug-resistant strains exhibited this capacity, of which 37.4 ± 0.84% (95% CI 36.6-38.2) showed a high level of expression, 59.4 ± 1.06% (95% CI 58.3-60.5) – moderate level and 3.2 ± 0.25% (95% CI 3.0-3.4) - low expression. The susceptible GNB inactivated lysozyme in 66.7 ± 1.12% (95% CI 65.6-67.8) of cases, of which 5.9 ± 0.33% (95% CI 5.6-6.2) showed a high expression of anti-lysozyme activity, 7.3 ± 0.37% (95% CI 6.9-7.7) - moderate and 86.8 ± 1.28% (95% CI 85.5-88.1) - low level of expression.

Another important factor contributing to the persistence of microorganisms in the outbreak, is the ability of bacterial cells to inactivate the complement system of the host organism. Of the 631 strains studied, 601 (95.2 ± 0.31%; 95% CI 94.9-95.5) showed anticomplementary activity, of which 480 (79.9 ± 0.28%; 95% CI 79.6-80.2) inactivated the complement system at a concentration higher than 15CH50 / ml, 68 (11.3 ± 0.11%; 95% CI 11.2-11.4) - at a concentration of 5CH50 / ml up to 15CH50 / ml and 53 (8.8 ± 0.0.09%; 95% CI 8.7-8.9%) - at a concentration of 5CH50 / ml. Only 30 strains showed no anticomplementary activity (4.8 ± 0.07%; 95% CI 4.73-4.87%).

The analysis of the complement inactivation of methicillin-resistant and susceptible strains of *Staphylococcus* spp. and of multidrug-resistant and antimicrobial- susceptible GNB strains showed the following results (Figure 2).

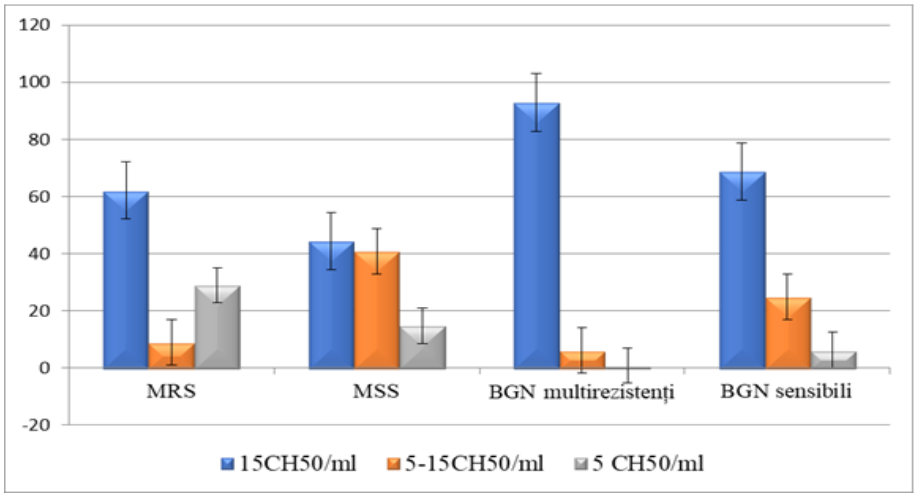


Figure 2. **Complement inactivation capacity of multidrug-resistant and antimicrobial-susceptible strains (%)**

According to the analysis results, it was found that 77.5±0.27% (95% CI 77.2-77.8) of MRSA strains inactivated complement, of which 62.1±0.24% (95% CI 61), 9- 62.3) at a concentration of 15CH50/ml, 8.9±0.09% (95% CI 8.8-9.0) - at a concentration of 5CH50/ml to 15CH50/ml and 29.0±0.16% (95% CI 28.8-29.2) - at a concentration of 5CH50. Of the MSSA strains, only 26.5±0.15% (95% CI 26.1-26.4) showed this ability, of which 44.4±0.20% (95% CI

44.2-44, 6) inactivated complement at a concentration of 15CH50/ml, 40.7±0.19% (95% CI 40.5-40.9) at a concentration of 5-15CH50/ml and 14.8±0.12% (95% CI 14), 7-14.9) - at a concentration of 5CH50.

100% antimicrobial multidrug-resistant GNB strains and 78.4 ± 1.22% sensitive strains (95% CI 77.2-79.6) inactivated the complement. Most multidrug-resistant and susceptible strains inactivated complement at a concentration of 15CH50 / ml, 92.9 ± 1.32% (95% CI 91.6-94.2) and 68.7 ± 1.14% (95% CI 67.6-69.8), respectively.

When comparing data on anti-lysozyme and anti-complementary activity of monocultures and their associations, it was found that associated bacterial strains more often exhibited moderate and high anti-lysozyme and anti-complementary activity ($p = 0.028$).

The present paper also studied the biofilm-producing ability of strains isolated from trophic ulcers. Therefore, of the 631 strains isolated from trophic ulcers, 462 (73.2 ± 0.27%; 95% CI 72.9-73.5) produced detectable biofilms (OD> 0.056) and 169 (26 .8 ± 0.16%; 95% CI (26.6-27.0) did not produce biofilms (DO≤0.056). Regarding the biofilm status, 193 (41.8 ± 0.20%; 95% CI 41.6-42.0) of isolates produced strong biofilms (OD> 0.220), 202 (43.7 ± 0, 21%; 95% CI 43.5-43.9) - moderate biofilms (OD 0.112-0.220) and 67 (14.5 ± 0.12%; 95% CI 14.4-14.6) - weak biofilms (0.056 <DO≤0.112).

The highest level of adherence and biofilm formation was recorded in gram-negative bacillus strains (78.0 ± 0.50%; 95% CI 77.5-78.5), especially in *Proteus* spp. 87.0 ± 3.34%; 95% CI 83.6-90.4), *Klebsiella* spp. (86.9 ± 2.21%; 95% CI 84.5-88.9), *Citrobacter* spp. (80.0 ± 35.6%; 95% CI 44.5-115.6), *P. aeruginosa* (73.9 ± 1.78%; 95% CI 72.1-75.7) and *A. baumannii* (73.6 ± 3.22%; CI 95% 70.4-76.8). Strong adherent biofilms were formed by 34.9 ± 0.42% (95% CI 34.4-35.3) of the strains, moderately adherent biofilms in 42.9 ± 0.47% (95% CI 42.4-43.4), and weakly adherent biofilms by 12.2 ± 0.25% (95% CI 11.9-12.5) of strains.

The analysis of the relationship between biofilm-forming ability and antimicrobial resistance of gram-negative bacillus strains showed that among biofilm isolates 52.3 ± 0.52% (95% CI 51.8-52.8) were MDR strains 31.4 ± 0.40%. (95% CI 31.0–31.8) are XDR and only 16.2 ± 0.25% (95% CI 15.9–16.5) are non-MDR. MDR and XDR strains formed mainly strong adherent biofilms - 41.4 ± 0.29% (95% CI 41.11-41.69) and 70.1 ± 1.20% (95% CI 68.90-71.30), as well as moderately adherent ones- 57.2 ± 1.20% (95% CI 56.0-58.40) and 25.3 ± 1.00% (95% CI 24.30-26.30), respectively. Non-MDR strains were involved in the formation of particularly weakly adherent biofilms 60.0 ± 3.70% (95% CI 56.3-63.7), whereas 55.9 ± 2.20% (95% CI) 53.7-58.1) of strains produced no biofilms.

A detectable biofilm was formed in 67.6±0.62% (95% CI 67.0-68.2) of Gram-positive coccus strains. The highest biofilm-forming ability was recorded in strains of *Enterococcus* spp. (86.7 ± 2.47% (95% CI 84.2-89.2)), followed by the decreasing incidence of CoNS (65, 0 ± 8.02% (95% CI 57.0-73.0)), *S. pyogenes* (62.1 ± 5.41% (95% CI 56.7-67.5)) and *S. aureus* (58.7 ± 1.10% (95% CI 57.6-59.8)). Strongly adherent biofilm was formed by 65 (36.7 ± 1.85% (95% CI 34.85-38.55)), moderately adherent biofilms - 80 (45.2 ± 1.67% (95% CI 43.53-46.87)) and weakly adherent biofilms - 32 (18.1 ± 2.65% (95% CI 15.45-20.75)) of the gram-positive coccus strains.

When studying the relationship between the biofilm-forming capacity and the methicillin-resistant staphylococcal strains, a significantly higher biofilm-forming capacity was found in MRSA strains (94.2 ± 2.80%; 95% CI 91.4-97. 0), compared to MSSA strains (32.6 ± 1.28%; 95% CI 31.3-33.9) ($p = 0.036$). Methicillin-resistant staphylococcal strains predominantly formed strongly adherent (50.8 ± 2.06%; 95% CI 48.7-52.9) and moderately adherent biofilms (44.6 ±

1.93%; 95% CI 42.7-46.5), while the methicillin-sensitive staphylococci revealed prevailingly weakly adherent biofilms (62.1 ± 1.76%; 95% CI 59.8-64.4). Of the MRSA strains, only 5.8 ± 0.69% (95% CI 5.1–6.5) did not form a detectable biofilm, and of the MSSA strains - 67.4 ± 1.87% (95% CI 65.6) -69.2).

According to the analysis results of the relationship between multidrug-resistant *Enterococcus* spp. strains isolated from trophic ulcers and the biofilm-forming ability, MDR strains formed detectable biofilms in 100% of cases, while sensitive strains - in 75.0 ± 1.94% 95% 73.1-76.9) of cases (Figure 3). The MDR *Enterococcus* spp. strains formed mainly moderately adherent (57.1 ± 1.69% (95% CI 55.4-58.8)) and strongly adherent (40.0 ± 1.41% (95% CI) 38.6-41.4%) biofilms, while the sensitive strains formed moderately adherent biofilms in 42.5 ± 1.46% (95% CI 41.0-44.0) of cases and weakly adherent biofilms in 17.5 ± 0 94% (95% CI 16.6-18.4) of cases.

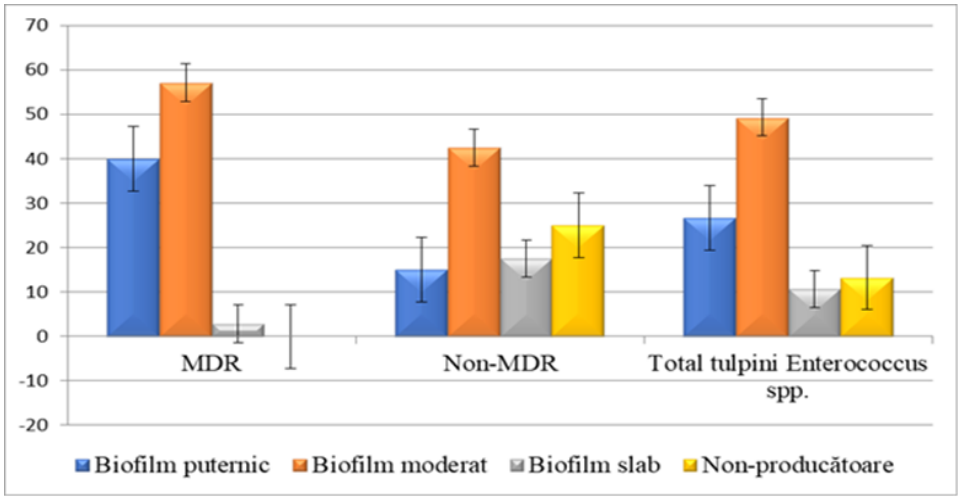


Figure 3. **Biofilm-forming ability of *Enterococcus* spp. strains isolated from trophic ulcers (%)**

All the microbial strains isolated from trophic ulcers showed a high biofilm-producing capacity (> 70%).

The study of virulence factors of microorganisms isolated from trophic ulcers showed a higher expression level in strains isolated from mixed and multidrug-resistant infections (1.0-1.5 times), compared to strains isolated from mono-infections and those sensitive to antimicrobials (p = 0.348).

4. ANTIMICROBIAL ACTIVITY OF NEW CHEMICAL AND BIOLOGICAL COMPOUNDS

4.1. The assessment results of antimicrobial activity of new chemical and biological substances

The studied chemical compounds were synthesized in hot ethanol solutions (50-55 ° C) of imidazole or 3,4-dimethylpyridine (in case II) with copper nitrate trihydrate (2+) and N- (prop- 2-en-1-yl) -2- [1- (pyridin-2-yl) ethylidene] hydrazincarbothioamide (4-allylthiosemicarbazone 2-acetylpyridine), taken in a 2: 1: 1 molar ratio. The reaction mechanism involved adding two amine molecules to the copper ion (2+) with the formation of the adduct, which interacted with 4-allylthosemicarbazone 2-acetylpyridine, which is a mono-deprotonated NNS tridentate ligand. Subsequently, an amine molecule was substituted with polydentate azomethine, resulting in imidazolium nitrate or 3,4-dimethylpyridinium by deprotonated thiosemicarbazone. The fourth

and fifth places, in the coordination sphere of this central atom, are taken by 10 nitrate-ions and the remaining amine molecule.

The highest bacteriostatic and bactericidal activity on *S. aureus* ATCC 25923 bacteria was proved by the $C_{14}H_{20}ClCuN_3O_6S$ (MIC - 0.03 µg / ml; MBC - 0.98 µg / ml) and $C_{18}H_{22}CuN_6O_3S$ (MIC - 0.06 µg / ml MBC - 0.24 µg / ml) compounds.

The highest activity upon the strains of *B. cereus* ATCC 11778 was proved by the chemical compound $C_{10}H_{12}CuN_6O_6S$, showing a bacteriostatic action at a concentration of 0.007 µg / ml and bactericidal action at a concentration of 0.03 µg / ml. The compound $C_{11}H_{13}BrCuN_4S$ (MIC - 0.06 µg / ml; MBC - 0.12µg / ml) showed high activity on *B. cereus* ATCC 11778 strains as well.

B. subtilis ATCC 6633 strains proved to be more sensitive to the tested compounds compared *B. cereus* ATCC 11778. $C_{10}H_{12}CuN_6O_6S$ compound (MIC - 0.007 µg / ml; MBC - 0, 03µg / ml) exhibited the highest activity.

Of all the tested gram-positive bacteria, *S. pyogenes* ATCC 12344 was the most sensitive to the chemical compounds. Both compounds $C_{24}H_{32}Cu_2N_8O_6S_2$ and $C_{11}H_{14}CuN_6O_6S$, MIC - 0.002 µg / ml and MBC - 0.003 µg / ml showed the highest activity on these bacteria.

Most of the tested chemical compounds had a bacteriostatic and bactericidal effect at higher concentrations on enterococcus strains compared to other gram-positive species. The compound $C_{11}H_{13}BrCuN_4S$ (MIC - 0.06 µg / ml and MBC - 0.12 µg / ml) showed the highest activity on *E. faecalis* ATCC 19433, and $C_{11}H_{14}CuN_6O_6S$ (MIC -0. 12 µg / ml and MBC - 0.24 µg / ml) on *E. faecium* ATCC 6569.

According to the results obtained, the compounds $C_{11}H_{14}CuN_6O_6S$ and $C_{11}H_{13}BrCuN_4S$ showed bacteriostatic and bactericidal action on gram-positive bacteria at low concentrations.

MICs and MBCs of the studied compounds were also determined for fermenting gram-negative bacteria (*E. coli* ATCC 25922, *P. mirabilis* ATCC 25933, *K. pneumoniae* ATCC 13883, *E. cloacae* ATCC 13047) and non-fermenting bacteria (*P. aeruginosa* ATCC 27853, *A. baumannii* BAA-747).

The analysis of the obtained results showed that the chemical compound $C_{11}H_{14}CuN_6O_6S$, MIC - 0.24 µg / ml and MBC - 0.49 µg / ml showed a higher activity on *E. coli* strains ATCC 25922. $C_{10}H_{12}CuN_6O_6S$ and $C_{24}H_{32}Cu_2N_8O_6S_2$ also showed activity at concentrations of 1.95 µg / ml (MIC) and 3.91 µg / ml (MBC).

Other species of *Enterobacteriaceae* (*K. pneumoniae* ATCC 13883, *P. mirabilis* ATCC 25933 and *E. cloacae* ATCC 13047) were more resistant to all the studied chemical compounds compared to *E. coli* ATCC 13047.

K. pneumoniae ATCC 13883 strains were more sensitive to $C_{10}H_{12}CuN_6O_6S$, $C_{10}H_{11}BrCuN_4S$ and $C_{24}H_{32}Cu_2N_8O_6S_2$, MIC and MBC being of 1.95 µg / ml and 3.91 µg / ml, respectively.

The compounds $C_{10}H_{12}CuN_6O_6S$ and $C_{11}H_{14}CuN_6O_6S$, were more active on *P. mirabilis* bacteria ATCC 25933, showing bactericidal and bacteriostatic effects at concentrations of 3.91 µg / ml and 1.95 µg / ml, respectively.

E. cloacae ATCC 13047 strains showed a higher sensitivity to $C_{10}H_{11}BrCuN_4S$ and $C_{11}H_{14}CuN_6O_6S$, MIC being of 0.98 µg / ml and MBC - 1.95 µg / ml.

The tested chemical compounds showed antimicrobial activity on non-fermenting gram-negative bacillus strains at higher concentrations. Thus, the most active compound on *A. baumannii* BAA-747 strains was found to be $C_{11}H_{14}CuN_6O_6S$, with MIC - 0.98 µg / ml and MBC - 1.95 µg / ml. *P. aeruginosa* ATCC 27853 strains showed higher sensitivity to $C_{10}H_{11}BrCuN_4S$,

$C_{11}H_{14}CuN_6O_6S$, and $C_{11}H_{13}BrCuN_4S$, MIC and MBC being of 3.91 µg / ml and 7.81 µg / ml respectively.

All reference gram-negative bacteria showed resistance to the four tested chemical compounds at concentrations of 500 /g / ml, namely $C_{14}H_{20}ClCuN_3O_6S$, $C_{15}H_{20}N_4NiO_2S$, $C_{11}H_{11}Br_2CuN_3O_2S$ and $C_{16}H_{14}Br_2CuN_4OS$. The $C_{18}H_{22}CuN_6O_3S$ compound showed an activity only on *A. baumanii* BAA-747 strains, being at high concentrations (MIC - 333.3 µg / ml and MBC - 500 µg / ml).

The next stage involved the study of the chemical compound's activity on yeast fungi (*C. albicans* ATCC 10231 and *C. neoformans* CECT 1043). According to the results obtained, the highest antifungal activity on *C. albicans* fungi ATCC 10231 was demonstrated by the $C_{24}H_{23}CuN_7O_7S$ and C18H22CuN6O3S compounds at MIC -0.49 µg / ml and 0.98 µg / ml, and MFC 15.6 µg / ml and 1.96 µg / ml, respectively. $C_{18}H_{20}CuN_4O_2S$ (MIC - 0.03 µg / ml, MFC - 0.06 µg / ml), $C_{14}H_{20}N_4S$ (CMI - 0.12 µg / ml, MFC - 0,24 µg/ml) and $C_{14}H_{19}CuN_7O_4S$ (MIC - 0.24 µg / ml, MFC - 0.24 µg / ml) demonstrated a marked antifungal activity on *C. neoformans* CECT 1043 fungi. The other compounds tested showed antifungal activity at higher concentrations, with the exception of compound $C_{11}H_{11}Br_2CuN_3O_2S$, which was not active on *C. neoformans* CECT 1043 fungi at a concentration of 500 /g / ml. *C. neoformans* CECT 1043 yeast fungi were inhibited and killed at lower concentrations of chemical compounds compared to *C. albicans* ATCC 10231 fungi.

The biological compounds used in the research (*ES, ES1, ES2, MX1* and *MX2*) are extracts of the *Spirulina platensis* CNMN CB-02 cyanobacterium. When testing the antimicrobial action of these extracts on gram-positive reference strains, the *MX1, MX2* and *ES* compounds showed high activity. The highest antimicrobial activity on gram-positive bacteria was demonstrated by the *MX1* compound (MIC - 0.004-0.02 mg / ml; MBC - 0.009-0.04 mg / ml), followed by a decrease in *MX2* activity (MIC - 0.01-0.03 mg / ml; MBC - 0.01-0.05 mg / ml) and *ES* (CMI - 0.16-0.62 mg / ml; MBC - 0.16-1.25 mg / ml). The *ES1* and *ES2* compounds showed antimicrobial activity at higher concentrations (MIC-1.25-2.5 mg / ml; MBC -1.25-5.0 mg / ml).

This study also assessed the antimicrobial action of the biological compounds on gram-negative bacteria. According to the data obtained, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *A. baumanii* BAA-747 were more sensitive to the action of the *MX1* compound, MIC - 0.009 mg / ml, and MBC - 0.02 mg / ml. The *MX2* compound (MIC - 0.02-0.03 mg / ml; MBC-0.03 mg / ml) also showed high activity on gram-negative bacteria, followed by *ES* (MIC - 0.625-1.25 mg / ml MBC - 1.25-2.5 mg / ml). *ES1* and *ES2* extracts showed antimicrobial effect on all gram-negative bacilli, however at higher concentrations (MIC - 1.25-5.0 mg / ml; MBC - 2.5-5.0 mg / ml).

The antifungal action of spirulina extracts was studied on two yeast fungi, namely *C. albicans* ATCC 10231 and *C. neoformans* CECT 1043. A higher antifungal activity was demonstrated by *MX2* (MIC - 0.007 mg / ml; MFC - 0.01 mg / ml), followed in a decreasing order by extracts *MX 1* (MIC - 0.05 mg / ml; MFC - 0.09 mg / ml) and *ES* (MIC - 0.31-0.62 mg / ml; MFC- 0.62-1.25 mg / ml). Similar to the antibacterial effect, *ES1* and *ES2* extracts showed an antifungal activity at higher concentrations (MIC - 0.62-1.25 mg / ml; MFC - 1.25-2.5 mg / ml).

Daphnia magna crustacean was used to determine the toxicity of the new chemical compounds, by performing an immobilization test. Analysing the obtained results, it was found that both the ligand (LC50 5.53 ± 0.90) and the coordination copper compounds of (Cu L Br -

LC50 4.4 ± 0.96; Cu L Cl - LC50 3.5 ± 0. 91) showed lower toxicity compared to doxorubicin hydrochloride (LC50 3.27 ± 0.30).

4.2. Antimicrobial activity of new chemical and biological compounds on strains isolated from trophic ulcers

The present study, tested seven chemical compounds on 120 clinical strains of *S. aureus*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*, isolated from trophic ulcers. The lowest minimum inhibitory concentration on clinical strains of *S. aureus* was recorded for the following chemical compounds $C_{14}H_{20}N_4S$ (MIC: 0.17 ± 0.098) and $C_{10}H_{14}CuN_4O_5S_2$ (MIC: 0.20 ± 0.113). The $C_{10}H_{14}CuN_4O_5S_2$ (MIC: 3.19 ± 2.018 - 18.8 ± 6.36) and $C_{14}H_{19}CuN_7O_4S$ compounds showed inhibitory activity on *A. baumannii* strains at minimal inhibitory concentrations (MIC: 0.17 ± 0.098).

The MIC and MBC of the biological *ES*, *ES1*, *ES2*, *MX1* and *MX2* compounds were studied on 120 gram-positive and gram-negative microbial strains isolated from trophic ulcers.

The biological *MX1* (MIC: 0.01 ± 0.001 - 0.02 ± 0.003) and *MX2* (MIC: 0.015 ± 0.003 - 0.03 ± 0.007) compounds showed an inhibitory and bactericidal activity on clinical strains at lower concentrations. These biological compounds exerted an inhibitory activity at lower concentrations on gram-negative bacilli compared to *S. aureus* strains. When comparing the minimum inhibitory concentrations of the chemical compounds on the reference and clinical strains in both gram-positive and gram-negative bacteria, it was found that some compounds had an inhibitory effect on the clinical strains at higher concentrations compared to the reference strains. (Figure 4).

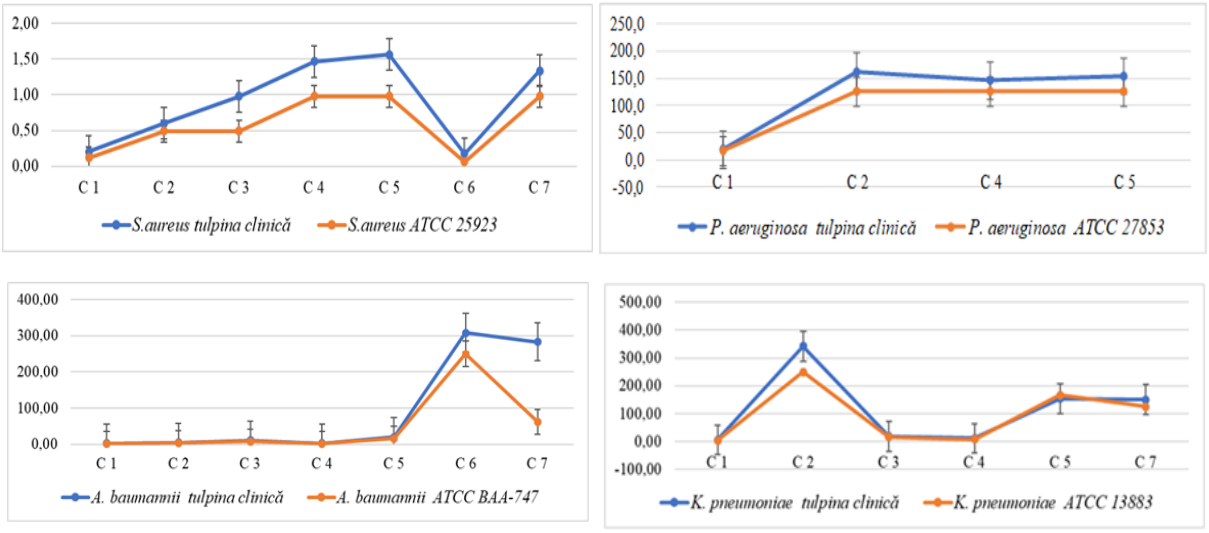


Figure 4. **The effects of chemical compounds on the reference strains and those isolated from trophic ulcers**

The minimum bactericidal concentration was higher for *S. aureus* clinical strains than for the reference strains, especially in $C_{13}H_{17}ClCuN_4S$, $C_{18}H_{22}CuN_6O_3S$ and $C_{13}H_{16}Br_2CuN_4S$ chemical compounds (MIC for reference strains - 0.49 µg / mL, 0.98 m, 0.98 .98 µg / mL; MIC for clinical strains - 0.98 µg / mL, 1.46 µg / mL and 1.56 µg / mL, respectively).

The MIC values for $C_{14}H_{19}ClCuN_4S$ and $C_{14}H_{20}N_4S$ increased significantly from 62.5 µg / mL to 283.3 µg / mL and from 250 µg / mL to at 308.3 µg / mL for *A. baumannii* clinical strains compared to the reference strains, respectively. For the remaining tested compounds, the MIC did not increase significantly for *A. baumannii* clinical strains compared to that of the reference strains.

When comparing the studied chemical compounds action upon both the clinical and reference strains of *K. pneumoniae* and *P. aeruginosa*, it was found that the MIC for the

$C_{18}H_{20}CuN_4O_2S$ compound considerably increased. The MIC values increased twice, from 250 g / mL to 500.0 µg / mL for *K. pneumoniae* strains, and for *P. aeruginosa* strains from 125 µg / mL to 266.7 µg / mL.

The comparison study on the action of biological compounds on reference and clinical strains showed the following (Figure 5):

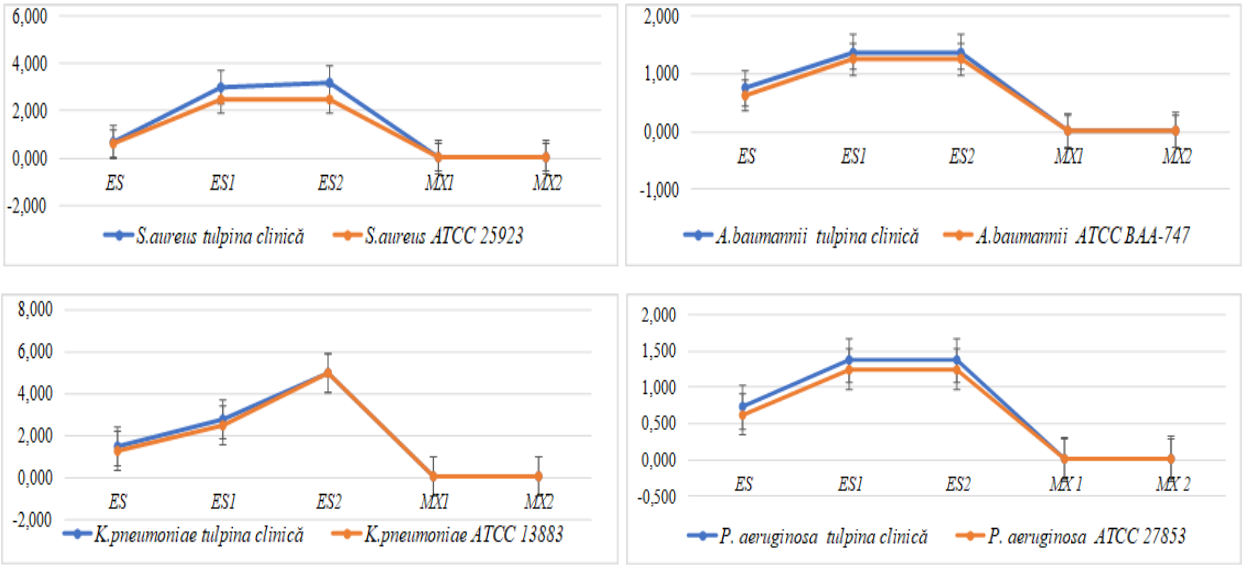


Figure 5. **The effects of biological compounds on both the reference strains and those isolated from trophic ulcers**

Analysis of the obtained results showed that *MX1* and *MX2* biological compounds showed antibacterial activity on both the reference and clinical strains at similar concentrations.

4.3. Synergistic effects of new chemical and biological compounds

To determine the type of interaction between the new chemical and biological compounds, seven chemical compounds ($C_{18}H_{20}CuN_4O_2S$, $C_{14}H_{20}N_4S$, $C_{14}H_{19}ClCuN_4S$, $C_{10}H_{14}CuN_4O_5S_2$, $C_{13}H_{17}ClCuN_4S$, $C_{14}H_{19}CuN_7O_4S$, and $C_{13}H_{16}Br_2CuN_4S$) were tested on six reference strains, in combination with three biological compounds (*ES, ES1, MX1*)

The values of the FIC index, which showed the inhibitory concentration of fractions, determined in the present study, indicated synergistic action in 87.2% of cases, additive action in 6.8%, and indifferent action in 6.0% of cases. Antagonistic actions were not reported among the tested compounds (FIC> 4).

The tested $C_{14}H_{19}CuN_7O_4S$ chemical compound in combination with the three biological compounds under study showed synergy in most cases, except for *P. aeruginosa* strains, which showed additive and indifferent actions when combined with the *ES* and *ES1* biological compounds

The $C_{13}H_{16}Br_2CuN_4S$ chemical compound showed synergy on the reference microorganisms in all its combinations, except for being indifferent (FIC = 1.56) when combined with the *ES1* biological compound upon the *P. aeruginosa* strains.

$C_{10}H_{14}CuN_4O_5S_2$ in combination with the tested biological compounds, showed synergistic action in 72.2% of cases, additivity - in 22.2% and indifference - in 5.6% on the reference strains.

The $C_{13}H_{17}ClCuN_4S$ compound in combination with all the studied biological compounds showed mainly a synergistic action (94.4%) and additive action only in 5.6% of cases.

$C_{18}H_{20}CuN_4O_2S$ in combination with *ES1* showed indifferent action on *E. coli* and *P. aeruginosa* strains, whereas combined with *ES*, it was indifferent only on *P. aeruginosa* strains.

$C_{14}H_{20}N_4S$ showed indifference to *A. baumannii* strains in combination with *ES1* and additivity in combination with *ES*.

$C_{14}H_{19}ClCuN_4S$ in combination with *ES1* had an additive effect on *E. coli* strains.

All the tested chemical compounds combined with the *MX* biological compound showed synergistic action on all reference strains. The combinations between chemical and biological compounds showed additive and indifferent effect mainly on gram-negative bacilli and yeast fungi of the genus *Candida*.

The minimum inhibition concentrations of the combined chemical and biological compounds were much lower than those of in separate compounds, being reduced by 4-32 times.

Each reference strain showed different profiles of the time-kill over a 24-hour period after inoculation. According to the results obtained, there was no evidence of an increase in the reference strains over the first 30 minutes after inoculation. The differences in antimicrobial activity of the tested compounds on the reference strains were recorded over 90 minutes after incubation. There was no reduction in the number of CFUs in the studied tubes. The individual use of chemical and biological compounds at a concentration of MIC 0.25 did not induce the death of microorganisms until for 24 hours. On the contrary, a significant reduction in the microbial count was recorded when testing the combined chemical and biological compounds.

The chemical compound MIC 0.25 $C_{14}H_{19}CuN_7O_4S$ in combination with MIC 0.25 *MX* showed a bactericidal action on *S. aureus* strains over 8 hours of action. The combination of MIC 0.25 $C_{14}H_{19}CuN_7O_4S$ + MIC 0.25 *ES* completely destroyed *S. aureus* strains after 20 hours of action, whereas MIC 0.25 $C_{14}H_{19}CuN_7O_4S$ + MIC 0.25 *ES1* - after 24 hours. Gram-negative bacillus strains *E. coli* and *A. baumannii* were killed after 12 hours of action in combination of MIC 0.25 $C_{14}H_{19}CuN_7O_4S$ + MIC 0.25 *MX* and over 20 and 24 hours in combinations of MIC 0.25 $C_{14}H_{19}CuN_7O_4S$ + MIC 025 *ES* and MIC 0.25 $C_{14}H_{19}CuN_7O_4S$ + MIC 0.25 *ES1*, respectively (Figure 6).

The combination of MIC 0.25 $C_{14}H_{19}CuN_7O_4S$ + MIC 0.25 *MX* demonstrated an antifungal effect on *C. albicans* yeast fungi over 20 hours, whereas the combinations of MIC 0.25 $C_{14}H_{19}CuN_7O_4S$ + MIC 0.25 *ES* and MIC 0.25 $C_{14}H_{19}CuN_7O_4S$ + MIC 0.25 *ES* after 24 hours of action.

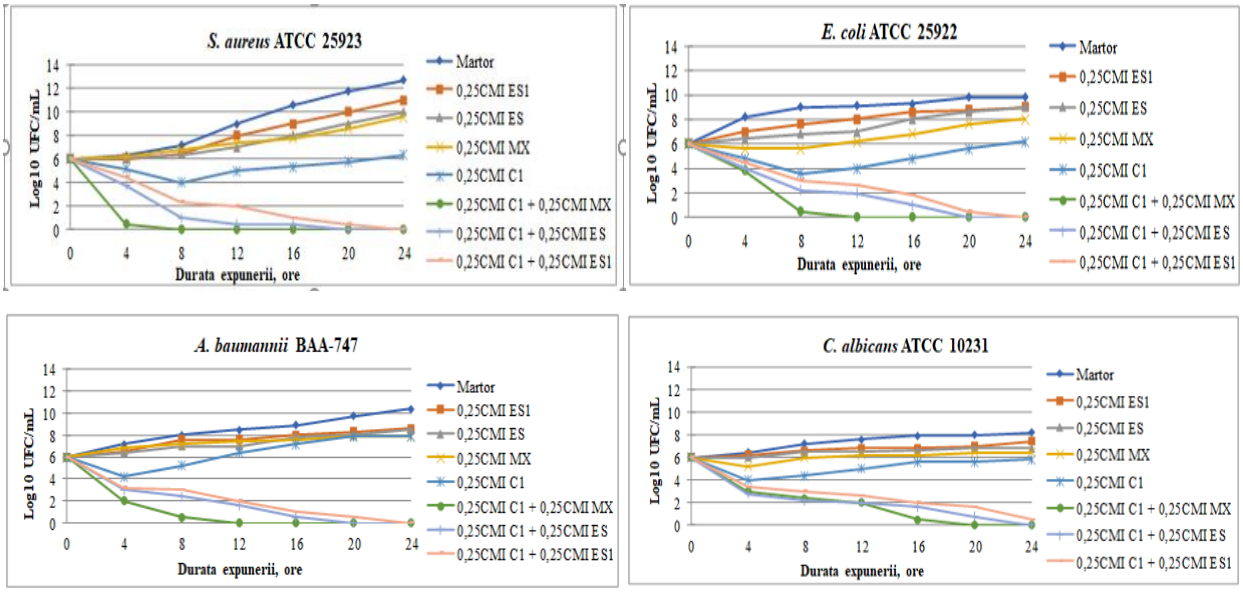


Figure 6. **Kinetics of antimicrobial activity of the $C_{14}H_{19}CuN_7O_4S$ chemical compound and biological compounds (*ES, ES1, MX*) upon the reference strains**

Note. the values are expressed as the mean ± SD of three independent values, the results ranging between the 95% - 99% confidence intervals

The $C_{13}H_{16}Br_2CuN_4S$ chemical compound in combination with the *MX* biological compound (MIC 0.25 $C_{13}H_{16}Br_2CuN_4S$ + MIC 0.25 *MX*) showed a bactericidal effect on *S. aureus* strains after 8 hours of action. The combination of MIC 0.25 $C_{13}H_{16}Br_2CuN_4S$ + MIC 0.25 *ES* killed *S. aureus* strains after 20 hours, and the combination of MIC 0.25 $C_{13}H_{16}Br_2CuN_4S$ + MIC 0.25 *ES1* - after 16 hours (Figure 7).

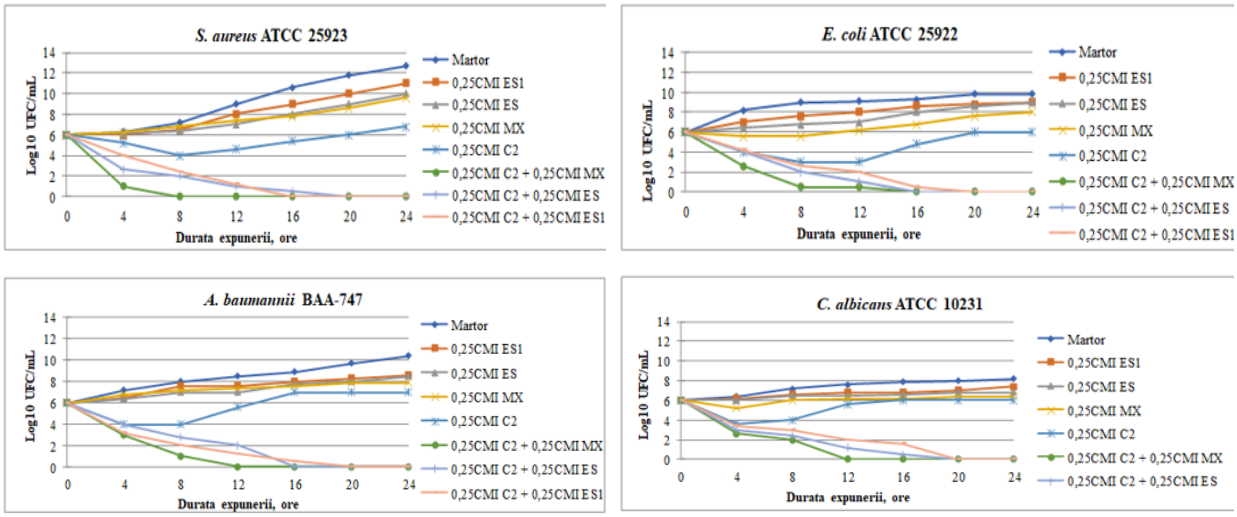


Figure 7. **Kinetics of antimicrobial activity of the $C_{13}H_{16}Br_2CuN_4S$ chemical compound and biological compounds (*ES, ES1, MX*) on the reference strains**

Note. the values are expressed as the mean ± SD of three independent values, the results ranging between the 95% - 99% confidence intervals

E. coli strains were killed after 16 hours of exposure to combined 0.25 MIC $C_{13}H_{16}Br_2CuN_4S$ + 0.25 MIC *MX* and 0.25 MIC $C_{13}H_{16}Br_2CuN_4S$ + 0.25 MIC *ES*, and after 20 hours upon exposure to combined 0.25MIC $C_{13}H_{16}Br_2CuN_4S$ + 0.25MIC *ES1*.

A. baumannii strains were killed after 12 hours of action by the combined 0.25MIC $C_{13}H_{16}Br_2CuN_4S$ + 0.25MIC *MX*, as well as after 16 and 20 hours by combinations of 0.25MIC $C_{13}H_{16}Br_2CuN_4S$ + 0.25MIC *ES* and 0.25MIC $C_{13}H_{16}Br_2CuN_4S$ + 0.25MIC *ES1*, respectively (Figure 7).

The combination of 0.25MIC $C_{13}H_{16}Br_2CuN_4S$ + 0.25CMI *MX* demonstrated fungicidal effects on *C. albicans* yeast fungi after a 12-hour exposure, whereas the combinations of 0.25MIC $C_{13}H_{16}Br_2CuN_4S$ + 0.25MIC *ES* and 0.25MIC $C_{13}H_{16}Br_2CuN_4S$ + 0.25 MIC *ES1* over 20 hours of action (Figure 4.4).

The combination between the $C_{10}H_{14}CuN_4O_5S_2$ chemical compound and the biological compound *MX* (0.25MIC $C_{10}H_{14}CuN_4O_5S_2$ + 0.25MIC *MX*) showed a bactericidal effect on *S. aureus* strains after a 12-hour exposure, and the combinations of 0.25MIC $C_{10}H_{14}CuN_4O_5S_2$ +25 MIC *ES* and 0,25CMI $C_{10}H_{14}CuN_4O_5S_2$ + 0.25CMI after 16 and 24 hours, respectively (Figure 8).

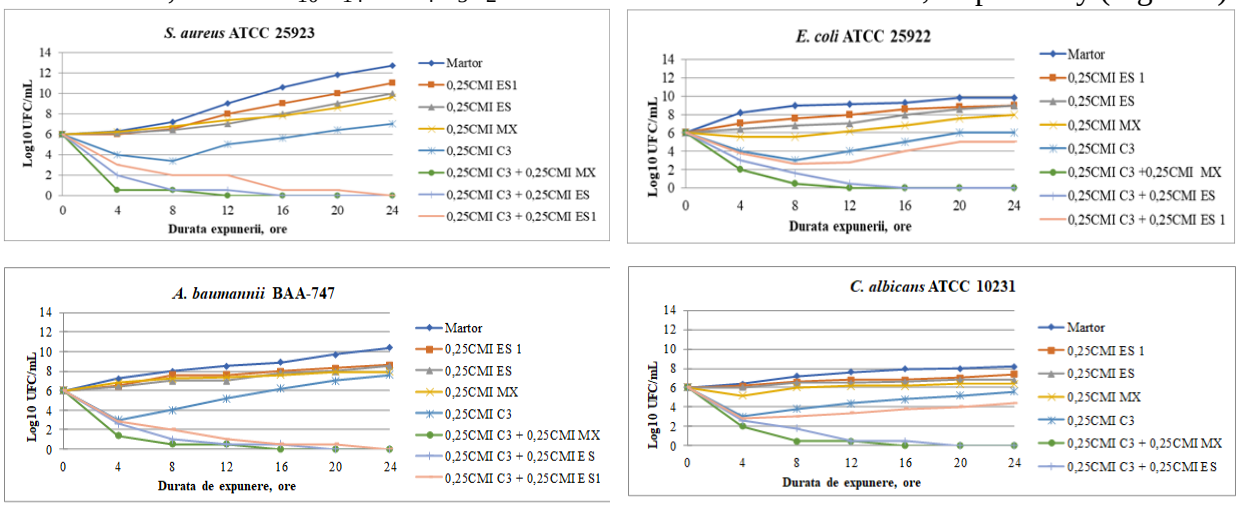


Figure 8. **Kinetics of antimicrobial activity of the $C_{10}H_{14}CuN_4O_5S_2$ chemical compound and biological compounds (*ES, ES1, MX*) on the reference strains**

Note. the values are expressed as the mean ± SD of three independent values, the results ranging between the 95% -99% confidence intervals

The combinations of 0.25MIC $C_{10}H_{14}CuN_4O_5S_2$ + 0.25MIC *MX* and 0.25MIC $C_{10}H_{14}CuN_4O_5S_2$ + 0.25MIC *ES* demonstrated a bactericidal action on *E. coli* strains after a 12-hour and 16-hour exposure, respectively. The combination of 0.25MIC $C_{10}H_{14}CuN_4O_5S_2$ + 0.25MIC *ES1* did not show a bactericidal effect on *E. coli* strains (Figure 8).

A. baumannii strains were killed after 16 hours of action by the combination of 0.25MIC $C_{10}H_{14}CuN_4O_5S_2$ + 0.25MIC *MX1* and after 20-hour and 24-hour exposure of 0.25MIC $C_{10}H_{14}CuN_4O_5S_2$ + 0.25MIC *ES* and 0.25MIC $C_{10}H_{14}CuN_4O_5S_2$ + 0.25CMI *ES1*, respectively.

The combinations of 0.25MIC $C_{10}H_{14}CuN_4O_5S_2$ + 0.25MIC *MX* and 0.25MIC $C_{10}H_{14}CuN_4O_5S$ + 0.25MIC *ES* exhibited a fungicidal effect on *C. albicans* yeast like fungi over a 16-hour and 20-hour action, respectively, whereas the combination of 0.25MIC $C_{10}H_{14}CuN_4O_5S_2$+ 25MIC *ES1* showed no fungicidal effect (Figure 8).

When analysing the bactericidal action of the combinations of the $C_{13}H_{17}ClCuN_4S$ chemical compound with the studied biological compounds, it was found that the combination 0.25MIC $C_{13}H_{17}ClCuN_4S$ + 0.25MIC *MX* showed a bactericidal effect on *S. aureus* strains after 12 hours of action, whereas the combinations 0.25MIC $C_{13}H_{17}ClCuN_4S$ + 0.25MIC *ES* and 0.25MIC $C_{13}H_{17}ClCuN_4S$ + 0.25MIC *ES1* after a 16 -hour and 20-hour exposure, respectively (Figure 9).

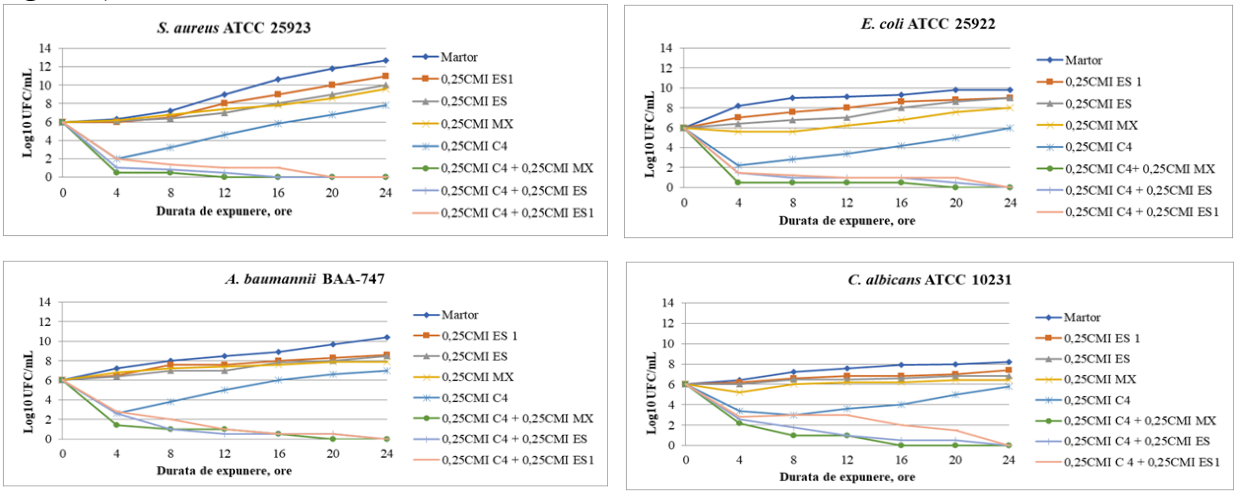


Figure 9. **Kinetics of antimicrobial activity of the $C_{13}H_{17}ClCuN_4S$ chemical compound and biological compounds (*ES, ES1, MX*) on the reference strains**

Note. the values are expressed as the mean ± SD of three independent values, the results ranging between the 95% -99% confidence intervals

The combination of 0.25MIC $C_{13}H_{17}ClCuN_4S$ + 0.25MIC *MX* exhibited a bactericidal action on *E. coli* and *A. baumannii* strains over a 20-hour exposure, whereas the combinations of 0.25MIC $C_{13}H_{17}ClCuN_4S$ + 0.25MIC *ES* and 0.25MIC $C_{13}H_{17}ClCuN_4S$ + 0, 25MIC *ES1* killed the gram-negative bacilli after 24 hours of action (Figure 9).

The combination of 0.25MIC $C_{13}H_{17}ClCuN_4S$ C + 0.25MIC *MX* showed a fungicidal effect on *C. albicans* yeastlike fungi after 16 hours of exposure, whereas the combinations 0.25MIC $C_{13}H_{17}ClCuN_4S$ + 0.25MIC *ES* and 0.25MIC $C_{13}H_{17}ClCuN_4S$ + 0.25MIC *ES1* after 24 hours (Figure 9). The chemical compounds combined with the biological compound *MX* were the most effective ones, killing the reference strains over 8, 12, 16 and 20 hours upon exposure.

4.4. The effects of new compounds on pathogenicity factors of the microorganisms

Besides the various mechanisms to resist against the antimicrobial drugs, microorganisms are also endowed with versatile pathogenic factors, which are responsible for initiating major lethal effects.

The present study was conducted on the effects of the chemical and biological compounds tested at different concentrations on the microbial anticomplementary activity on 80 gram-positive and gram-negative microbial strains, which initially inactivated the complement at concentrations of 15CH50 / mL (Figure 10).

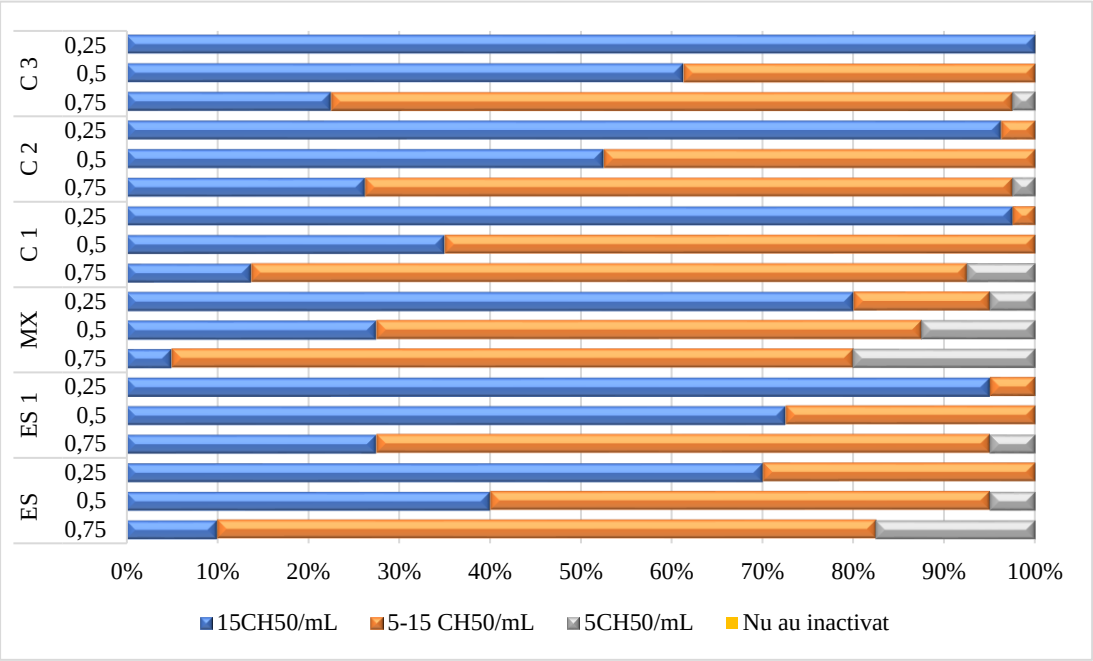


Figure 10. **The microbial anticomplementary activity under the action of chemical and biological compounds**

Note. C1 - $C_{14}H_{19}CuN_7O_4S$, C2 - $C_{13}H_{16}Br_2CuN_4S$, C3 - $C_{13}H_{17}ClCuN_4S$

The analysis of the obtained results showed that the tested compounds reduced the microbial anticomplementary activity mainly at a concentration of 75%. The biological compounds, namely *MX1* and *ES*, showed the highest-level action on the anticomplementary activity of the tested strains. Most strains (75.0% of cases) inactivated the complement at a concentration of 5-15CH50/mL and 20.0% of strains- at a concentration of 5CH50 / mL under the action of the biological compound 75% *MX*. The biological compound 75% *ES* also decreased the anticomplementary activity of microbial strains in 72.5% of cases at a concentration of 5-15CH50 / mL, and in 17.5% of cases at a concentration of 5CH50/mL.

Among the chemical compounds, the $C_{14}H_{19}CuN_7O_4S$ compound (at a 75% concentration) revealed a more pronounced anticomplementary activity. Upon its exposure, 78.8% of the tested strains inhibited the complement at a concentration of 5-15CH50/mL, 7.5% of cases - at 5-CH50/mL, and 13.8% of strains - at 15CH5/mL.

The anticomplementary action of microbial strains showed slight or no reduction when both chemical and biological compounds were used at a 25% concentration. It should be noted that neither compound completely inhibited the complement inactivating property of the strains.

When testing the anticomplementary activity of the combined chemical and biological compounds, a more pronounced effect was found in the combination of the biological compounds *MX* and *ES* with the chemical compounds (Figure 11).

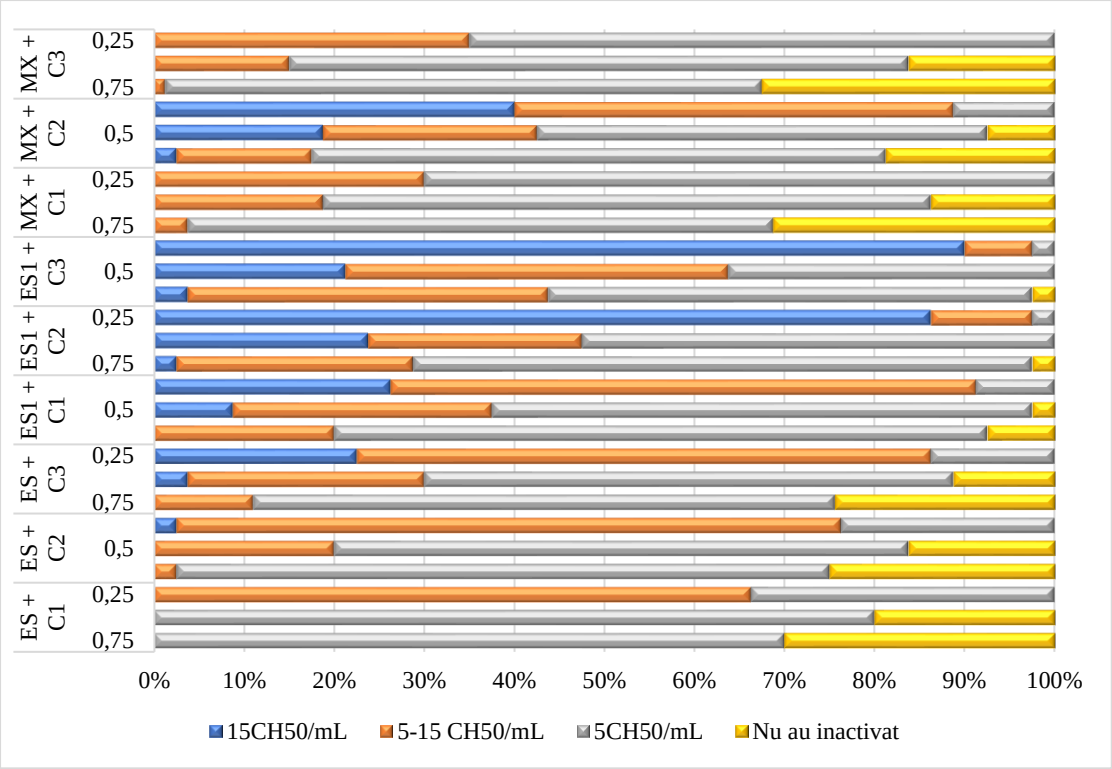


Figure 11. **The microbial anticomplementary activity under the action of combined chemical and biological compounds (%)**

Note. C1 - $C_{14}H_{19}CuN_7O_4S$, C2 - $C_{13}H_{16}Br_2CuN_4S$, C3 - $C_{13}H_{17}ClCuN_4S$

The *MX* biological compound combined with the $C_{13}H_{17}ClCuN_4S$ and $C_{14}H_{19}CuN_7O_4S$ chemical compounds at concentrations of 75% completely inactivated the anticomplementary activity in 32.5% and 31.3% of the tested strains, respectively.

The biological compound *ES* combined with the chemical compound $C_{14}H_{19}CuN_7O_4S$ monohydrate revealed that at a concentration of 75%, 70.0% of strains inactivated the complement at a concentration of 5CH50 / mL, and 30.0% did not show any anticomplementary action. When the same combination of compounds was tested at a 50% concentration, 80.0% of the strains inactivated the complement at a concentration of 5CH50 / mL, and 20.0% did not inactivate the complement.

The study also determined the anti-lysozyme activity of *S. aureus* and *E. coli* strains when exposed to separate chemical and biological compounds under study (Figure 12).

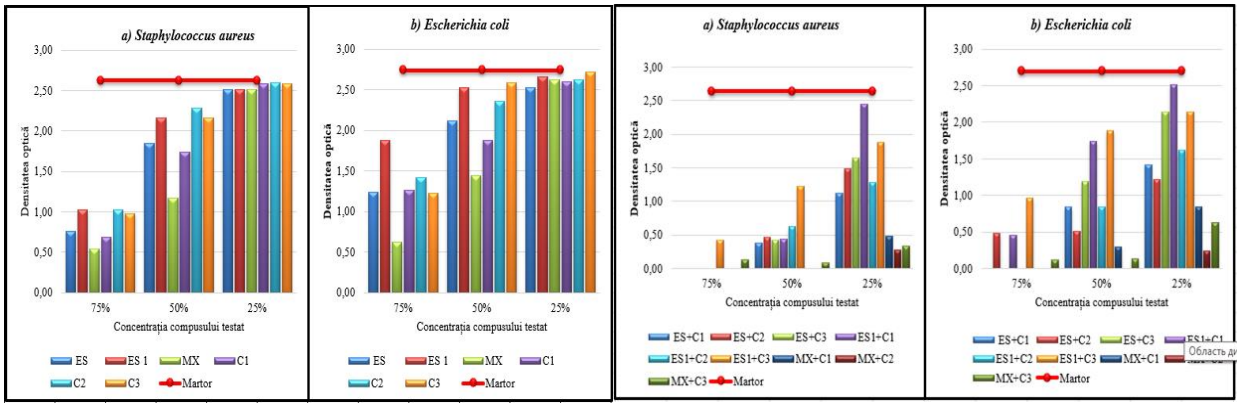


Figure 12. **The anti-lysozyme activity of microorganisms upon exposure to separate and combined chemical and biological compounds**

Note. C1 - $C_{14}H_{19}CuN_7O_4S$, C2 - $C_{13}H_{16}Br_2CuN_4S$, C3 - $C_{13}H_{17}ClCuN_4S$

The analysis of the obtained results found that both the chemical and the biological compounds inactivated the anti-lysozyme activity of the tested strains, compared to the control strains, which showed a high anti-lysozyme activity (K 2,62-2,74). The anti-lysozyme activity of *S. aureus* and *E. coli* strains was maintained high under the action of biological and chemical compounds at a concentration of 25%. The compounds tested in concentrates of 50% and 70% resulted in a decreased anti-lysozyme activity expression (medium expression) in both microbial species, except for the *ES1* compound (50%) on *E. coli*, which showed a high level of anti-lysozyme activity.

The use of combined chemical and biological compounds (at concentrations of 50% and 70%) showed a low expression of anti-lysozyme activity in *S. aureus* strains, except for the combination of 50% *ES1* and 50% $C_{13}H_{17}ClCuN_4S$, which revealed a medium of anti-lysozyme activity expression. *E. coli* strains showed a low anti-lysozyme activity upon exposure to a concentration of 75%, except for the combination of *ES1* and $C_{13}H_{17}ClCuN_4S$, which showed a medium degree of expression.

Combinations of the chemical compound with the biological compound *MX* showed the best effect on the anti-lysozyme activity expression of the microbial strains under study. The combinations of *MX* + $C_{14}H_{19}CuN_7O_4S$ and *MX* + $C_{14}H_{19}CuN_7O_4S$ at concentrations of 50% and 75% decreased to zero the anti-lysozyme activity of the microbial strains.

The microbial strains showed a low anti-lysozyme activity expression upon exposure to combined chemical compounds with the biological compound *MX* tested at a concentration of 25%, except for the combination *MX* + $C_{18}H_{22}CuN_6O_3S$, in which *E. coli* strains showed a medium anti-lysosome activity expression (K = 0.63).

The study also determined the effects of chemical and biological compounds on the microbial biofilm formation (Figure 13). According to the results obtained, the microorganisms produced a strong biofilm (OD> 0.220) under exposure to chemical and biological compounds at a 25% concentration, as well as to the compounds *ES1* and $C_{13}H_{17}ClCuN_4S$ at concentration of 50%. These compounds showed a better anti-biofilm action at a concentration of 75%, the microorganisms producing predominantly moderate biofilms (0.220 <OD <0.112), except for the *MX* biological compound, when strains produced weak biofilms (0.112≤DO <0.056). *S. aureus* strains also produced weak biofilms under the action of the *ES* biological compound and the $C_{13}H_{16}Br_2CuN_4S$ chemical compound.

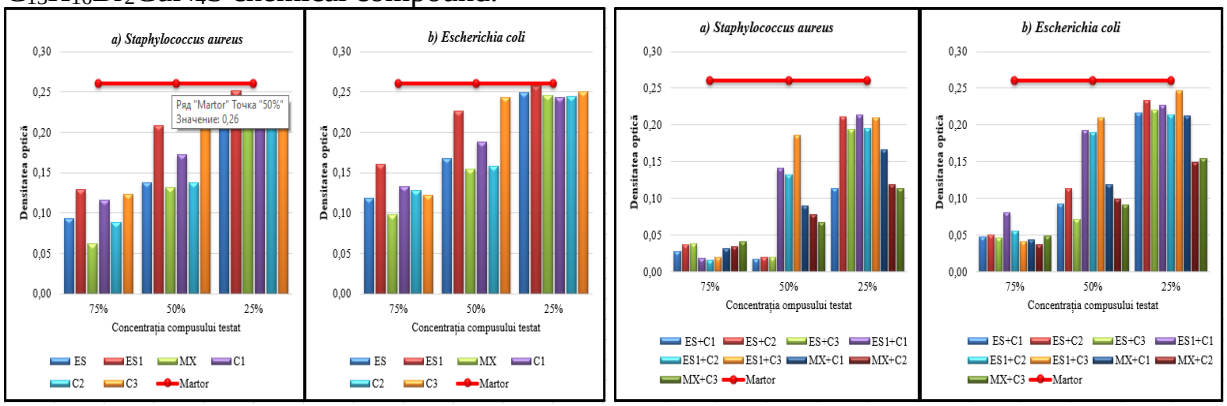


Figure 13. **The microbial biofilm formation levels under exposure to separate and combined chemical and biological compounds.**

Note. C1 - $C_{14}H_{19}CuN_7O_4S$, C2 - $C_{13}H_{16}Br_2CuN_4S$, C3 - $C_{13}H_{17}ClCuN_4S$

A higher microbial anti-biofilm activity was demonstrated by the *ES* and *MX* biological compounds combined with the chemical compounds at concentrations of 50% and 75%. Under the action of these combinations, the microbial strains lost their biofilm-forming ability or formed

weak biofilms, except for the 50% *ES* + $C_{13}H_{16}Br_2CuN_4S$ combination; *MX* + $C_{14}H_{19}CuN_7O_4S$, as well as combinations of chemical compounds with the *ES1* biological compound, to which bacteria formed a moderate biofilm.

The strains mostly produced strong biofilms under exposure to 25% concentration combinations, except for the combinations between the biological compound *MX* and the chemical compounds as in *ES* + $C_{14}H_{19}CuN_7O_4S$, *ES* + $C_{13}H_{17}ClCuN_4S$ and *ES1* + $C_{13}H_{16}Br_2CuN_4S$, to which bacteria formed moderate biofilms.

The results of testing the chemical and biological compounds on the expression of the lecithinase enzyme pathogenicity are shown in Figure 14. Higher anti-lecithinase activity in both gram-positive and gram-negative microorganisms has been demonstrated by chemical compounds at a concentration of 75%. $C_{13}H_{16}Br_2CuN_4S$ was the most active, followed by $C_{13}H_{17}ClCuN_4S$ and $C_{14}H_{19}CuN_7O_4S$.

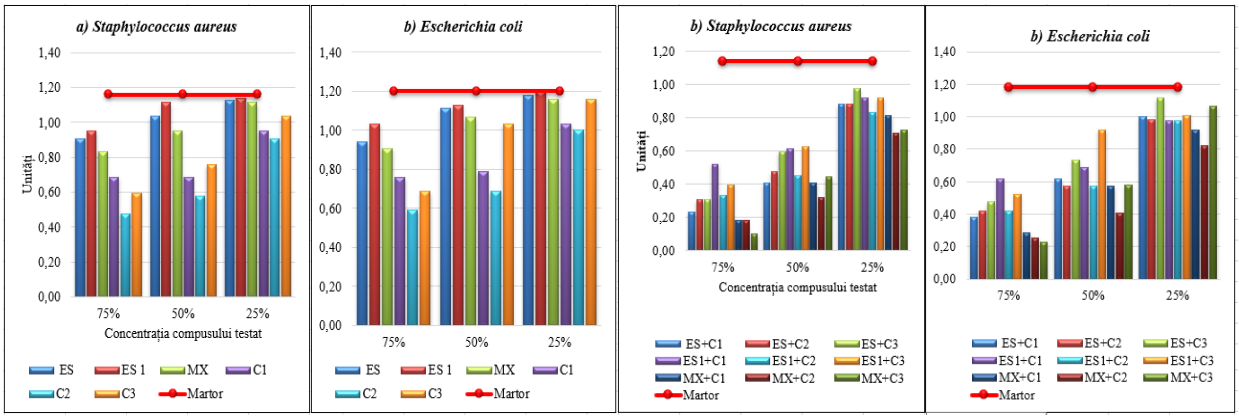


Figure 14. **The microbial lecithinase expression levels under exposure to separate and combined chemical and biological compounds**

Note. C1 - $C_{14}H_{19}CuN_7O_4S$, C2 - $C_{13}H_{16}Br_2CuN_4S$, C3 - $C_{13}H_{17}ClCuN_4S$

The inhibitory action of the lecithinase enzyme by chemical compounds was higher than that of the biological compounds. The highest activity was exhibited by the compounds at a concentration of 75%, especially by $C_{13}H_{16}Br_2CuN_4S$, thus decreasing the lecithinase activity from 1.16 ± 0.027 units to 0.48 ± 0.031 units for *S. aureus* strains, and from 1, 2 ± 0.0 to 0.48 units ± 0.013 units for *E. coli* strains.

MX was the most active biological compound, which reduced lecithinase activity from 1.6 ± 0.027 units to 0.83 ± 0.036 units in *S. aureus* strains and from 1.2 ± 0.00 to at 0.91 ± 0.031 units, for *E. coli* strains. The combined use of chemical and biological compounds has proved to increase the lecithinase-reducing effect. The highest effect was recorded in both gram-positive and gram-negative bacteria, when combining the biological compound *MX* with the chemical compounds at concentrations of 75%.

MX combined with the chemical compound $C_{13}H_{17}ClCuN_4S$ reduced the lecithinase activity mostly in *S. aureus* strains from 1.14 ± 0.00 to 0.10 ± 0.024 units and in *E. coli* strains from 1.18 ± 0.027 units to 0.23 ± 0.033 units. The biological compound *MX* combined with the chemical compound $C_{13}H_{16}Br_2CuN_4S$ at a concentration of 50% showed a decrease in the lecithinase activity for *S. aureus* strains from 1.14 ± 0.00 to 0.32 ± 0.027 units, and for *E. coli* strains from 1.18 ± 0.027 to 0.41 ± 0.013 units.

The test results on the effects of chemical and biological compounds on the expression level of the hemolysin enzyme by microbial strains are shown in Figure 15.

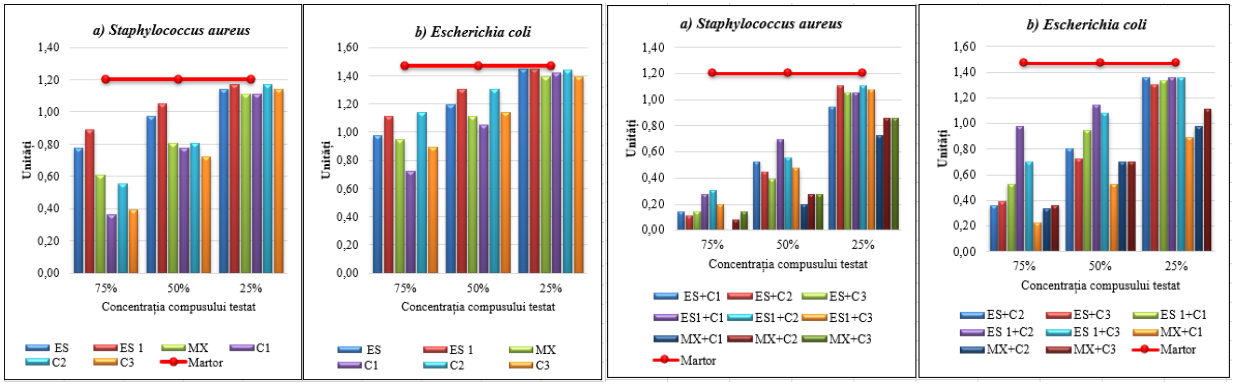


Figure 15. **The expression level of bacterial hemolysin under exposure to separate and combined chemical and biological compounds**

Note. C1 - $C_{14}H_{19}CuN_7O_4S$, C2 - $C_{13}H_{16}Br_2CuN_4S$, C3 - $C_{13}H_{17}ClCuN_4S$

A greater reducing effect of the hemolysin activity was recorded on exposure to $C_{14}H_{19}CuN_7O_4S$ and $C_{13}H_{17}ClCuN_4S$ chemical compounds at a concentration of 75%. In *S. aureus* strains, hemolysin activity decreased from 1.20 ± 0.036 to 0.36 ± 0.04 and from 1.20 ± 0.036 to 0.39 ± 0.04, whereas in *E. coli* strains from 1.47 ± 0.036 to 0.72 ± 0.036 and from 1.47 ± 0.036 to 0.89 ± 0.04. Both chemical and biological compounds at a concentration of 25% almost did not affect the hemolysin expression level.

The assessment on the effects of the chemical compounds combined with the biological ones, a major decrease in hemolysin expression was observed when using the combination of the biological compound *MX* and the chemical compound $C_{14}H_{19}CuN_7O_4S$ at all concentrations. This combination of compounds reduced the enzyme hemolysin expression for *S. aureus* strains from 1.20 ± 0.036 to 0.00 ± 0.00 - at a concentration of 75%, from 1.20 ± 0.036 to 0.20 ± 0.036 - at a concentration of 50% and from 1.20 ± 0.036 to 0.72 ± 0.036 - at a concentration of 25%.

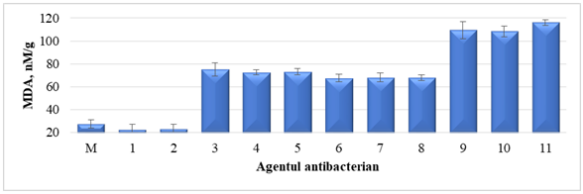
In the case of *E. coli* strains, this combination of compounds showed a lesser inhibitory hemolysin expression: 1.47 ± 0.036 units for control strains and 0.22 ± 0.071 units at 75%, 0.53 ± 0.036 units at a concentration of 50% and 0.89 ± 0.04 units at a concentration of 25%.

5. CHANGES IN BIOCHEMICAL PARAMETERS OF MICROORGANISMS CAUSED BY CHEMICAL AND BIOLOGICAL COMPOUNDS

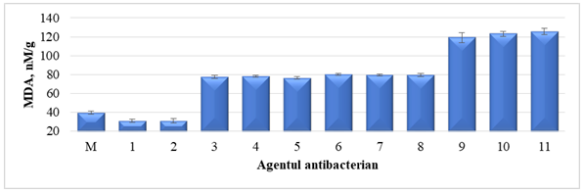
5.1. Modification of oxidative stress indices in microbial cultures upon exposure to chemical and biological compounds

The toxicity level of separate and combined chemical and biological compounds was assessed by the amount of malondialdehyde in the tested microbial biomass. The results obtained are shown in Figure 16. All the three studied types of microorganisms showed the same reactive patterns to the tested agents: natural compounds did not change the MDA content, while the chemical compounds both tested separately and combined with natural compounds, caused a significant increase in the MDA content, depending on the compound and type of bacteria.

A)



B)



C)

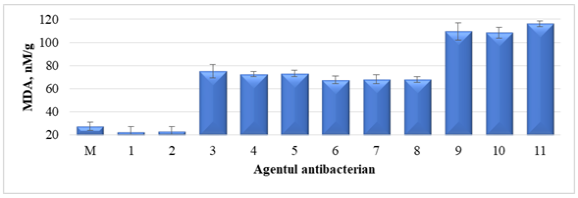


Figure 16. **Effects of the tested compounds on the malondialdehyde content in the cell mass of (A) *P. aeruginosa* ATCC 27853, (B) *E. coli* ATCC 25922 and (C) *S. aureus* ATCC 25923**

Note. C– control samples, 1 – mixoxanthophyll, (MX), 2 –extract of spirulina (ES), 3 – C14H19CuN7O4S; 4 – C14H19CuN7O4S +ES; 5 - C14H19CuN7O4S+MX; 6 – C10H14CuN4O5S2; 7 – C10H14CuN4O5S2+ES; 8 – C10H14CuN4O5S2+MX; 9 – C13H16Br2CuN4S; 10 – C13H16Br2CuN4S+ES; 11- C13H16Br2CuN4S+MX

Thus, in the case of *P. aeruginosa* strains, the natural compounds (mixoxanthophyll and *ES* spirulina extract, having 2 experimental variants 1 and 2) did not change the level of MDA in biomass (Figure 5.1A). Although the obtained mean values for these experimental variants were lower than in the control samples, viz. 13.8 and 17.3%, respectively, the statistical analysis showed a weak significance level ($p = 0.054$ and $p = 0.078$ respectively). The three separate chemical compounds (experimental variants 3, 6, 9), as well as combined with mixoxanthophyll (variants 5, 8, 11) or with the spirulina extract *ES* (variants 4,7,10), produced a significant increase in MDA (for all cases $p<0.001$). The MDA values in the experimental samples were quite homogeneous and showed an increase of 2.2 -2.5 times compared to the control samples.

In the case of *E. coli* strains, the natural compounds produced a decrease in the MDA amount by more than 20% compared to the control samples. The difference was statistically true ($p = 0.003$ for mixoxanthophyll and $p = 0.008$ for *ES*) for both compounds. The chemical compounds $C_{14}H_{19}CuN_7O_4S$ and $C_{10}H_{14}CuN_4O_5S_2$, both used separately and in combination with *MX* and *ES*, doubled the MDA amount. $C_{13}H_{16}Br_2CuN_4S$ seemed to be more toxic, producing a threefold increase in the MDA amount, both when used alone and in combination with *ES* spirulina extract.

S. aureus strains reacted to natural compounds similarly as in *E. coli*, however lacking the reducing effect of the MDA level. The MDA values upon action of myxoxanthophyll and spirulina extract were the same as in the control group. The toxic effect in this strain was also more pronounced when tested by $C_{13}H_{16}Br_2CuN_4S$.

According to the results obtained, the increase in the MDA content was greater in *S. aureus* strains. Thus, the compounds $C_{14}H_{19}CuN_7O_4S$ and $C_{10}H_{14}CuN_4O_5S_2$ produced a 2.5-2.7-time increase in the DAM content compared to the control samples (compared to 1.9-2.0 times in *E. coli*), whereas the compound $C_{13}H_{16}Br_2CuN_4S$ showed a 3.9- 4.2-time increase (compared to 3.0-3.2 in *E. coli*).

The obtained test results on the lactate dehydrogenase (LDH) activity released by the three bacterial species are shown in Figure 17.

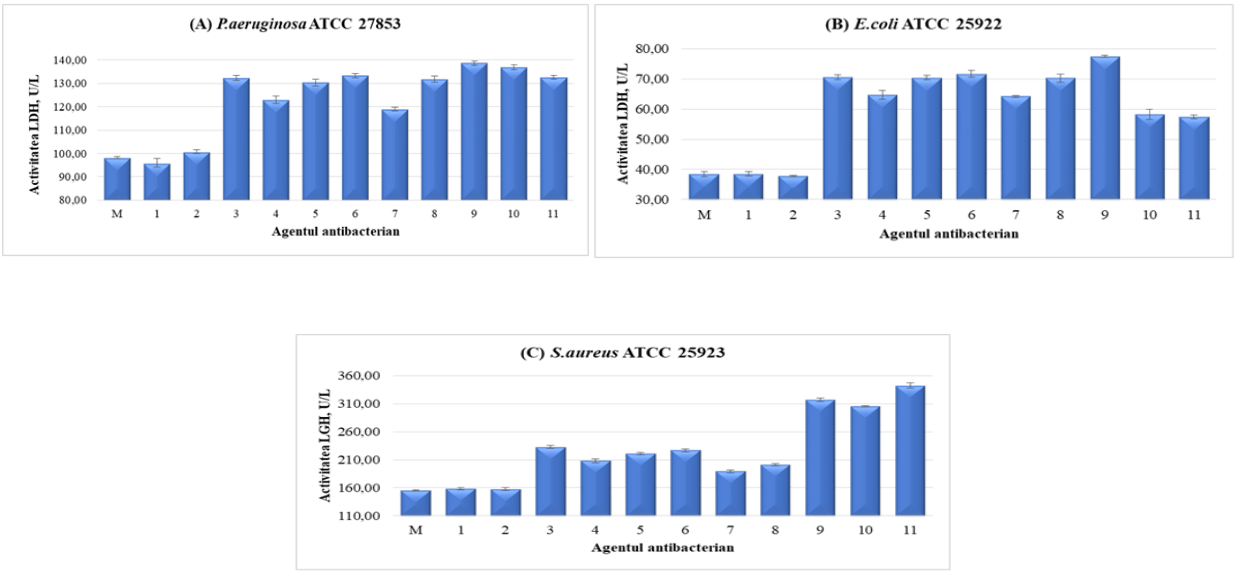


Figure 17. **The effects of the tested compounds on lactate dehydrogenase activity released by (A) *P. aeruginosa* ATCC 27853, (B) by *E. coli* ATCC 25922 and (C) by *S. aureus* ATCC 25923**

Note. C – control sample, 1 – mixoxanthophyll, (MX), 2 –extract of spirulina (ES), 3 – C14H19CuN7O4S; 4 – C14H19CuN7O4S+ES; 5 – C14H19CuN7O4S+MX; 6 – C10H14CuN4O5S2; 7 – C10H14CuN4O5S2 +ES; 8 – C10H14CuN4O5S2+MX; 9 – C13H16Br2CuN4S; 10 – C13H16Br2CuN4S +ES; 11 – C13H16Br2CuN4S +MX

In the case of control samples, this enzyme activity ranged from 38 units / litre for *E. coli* to 155 units / litre for *S. aureus*. This difference was also observed for malondialdehyde, which can be caused by the natural differences between these two species, as well as by different sensitivity to DMSO, a solubilizing agent used to dissolve the chemical compounds under the study.

All the three tested bacterial species responded differently to the action of chemical compounds, used separately or combined with natural ones, however, as in the case of malonidialdehyde, both myxoxanthophyll and spirulina extract *ES* did not alter the LDH activity released in the culture fluid. The lactate dehydrogenase activity, released by *P. aeruginosa* strains was significantly higher when exposed to chemical compounds and their combinations with mixoxanthophyll and *ES*, if compared to the control samples (1.21-1.39 times, $p<0.001$).

It is noteworthy that the three chemical compounds used in combination with *ES* revealed a decrease in toxicity compared to the action of the separate compounds. Although these differences were insignificant, their statistical significance suggests an alleviation of the adverse effects observed in the experimental variants. Thus, $C_{14}H_{19}CuN_7O_4S$ caused a 34.7% increase in LGH activity released by *P. aeruginosa* strains, while in combination between the chemical compound $C_{14}H_{19}CuN_7O_4S$ with *ES* this increase made up 25.2% (P = 0, 0005). The same phenomenon was recorded in C10H14CuN4O5S2 which increased by 35.7% the LDH activity, whereas in combination with *ES* the increase was 21.0%. In case of C13H16Br2CuN4S use, mixoxanthophyll showed a reducing effect on LDH activity released by *P. aeruginosa* strains (a decrease from an increase by 41.0% to 35.0%). In both cases, the differences were statistically true. It should be noted that the amount of separate chemical compounds used in the experimental variants is four times lower compared to those variants using combinations with natural compounds. Currently, the present research cannot draw any conclusions on the individual role of

each component regarding their antibacterial activity. Undoubtedly, these interactions are of theoretical and applied interest.

Under the influence of chemical and natural compounds (except for separate extracts), the increase of LDH activity in the extracellular environment was 1.5-2.0 times higher in *E. coli* strains compared to the experimental variant. The natural extracts in this bacterial species also seemed to have an alleviating effect on the separate chemical compounds, especially in $C_{13}H_{16}Br_2CuN_4S$, where a quarter of the toxic effect of the compound was removed in mixoxanthophyll and *ES* variants.

S. aureus strains reacted similarly to *E. coli* strains, both under the action of antimicrobial agents used separately and in the corresponding combinations. Thus, $C_{13}H_{16}Br_2CuN_4S$ revealed the most pronounced toxic effect, both used separately and in combination, except for myxoxanthophyll which intensified the effect generated by the chemical compound. According to the results obtained, under the influence of the separate chemical compound $C_{13}H_{16}Br_2CuN_4S$, the released LDH activity increased 2.0 times, and when combined with *MX*, it showed a 2.2 - time increase, the difference being statistically true ($p = 0.0007$).

5.2. Modification of the microbial antioxidant capacity under the influence of new chemical and biological compounds

In case of pronounced stress, the enzyme antioxidant activity decreases, resulting in cell inability to protect themselves against harmful free radicals, formed by the interaction with toxic xenobiotics. In the studied bacterial cultures, the first-line antioxidant protection is formed by the primary antioxidant enzymes. They defeat the superoxide radicals (superoxide dismutase) and hydrogen peroxide, formed as a product of the dismutation reaction (catalase and glutathione peroxidase).

The test results for antimicrobial action on *P. aeruginosa* strains are shown in Figure 18. Mixoxanthophyll and *ES* spirulina extract did not significantly alter the SOD activity level, either the intracellular SOD or that secreted in the culture medium. However, the SOD activity lowered in all experimental variants, viz. it showed an almost 2-time decrease in the biomass (by 46.7-54.9%), and by 59.2-78.8% in the culture fluid.

$C_{10}H_{14}CuN_4O_5S_2$ and $C_{13}H_{16}Br_2CuN_4S$ sulphate compounds most strongly reduced the intracellular SOD activity. SOD activity was similarly reduced in the culture fluid by $C_{18}H_{22}CuN_6O_3S$ and $C_{10}H_{14}CuN_4O_5S_2$. The chemical compound $C_{13}H_{16}Br_2CuN_4S$ was more active compared to the first two ones. There were no significant differences found in the superoxide dismutase activity between the experimental variants, where the chemical compounds were used separately and those combined with spirulina extracts.

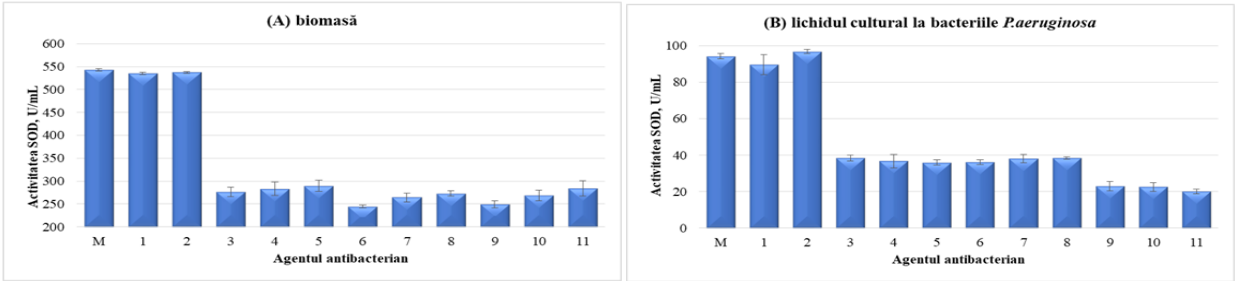


Figure 18. **The effects of the tested compounds on superoxide dismutase activity in (A) biomass and (B) in culture fluid of *P. aeruginosa* bacteria.**

Note. C – control sample, 1 – mixoxanthophyll, (MX), 2 –extract of spirulina (ES), 3 – $C_{14}H_{19}CuN_7O_4S$; 4 – $C_{14}H_{19}CuN_7O_4S$+ES; 5 – $C_{14}H_{19}CuN_7O_4S$+MX; 6 – $C_{10}H_{14}CuN_4O_5S_2$; 7 – $C_{10}H_{14}CuN_4O_5S_2$+ES; 8 – $C_{10}H_{14}CuN_4O_5S_2$+MX; 9 – $C_{13}H_{16}Br_2CuN_4S$; 10 – $C_{13}H_{16}Br_2CuN_4S$+ES; 11 – $C_{13}H_{16}Br_2CuN_4S$+MX

The physiological level of SOD activity was lower in *E. coli* strains compared to *P. aeruginosa*, but the effects of the tested compounds on this parameter were very similar (Figure 19). However, the decrease in SOD activity in terms of its values was even more pronounced, especially in the culture fluid, where the compound $C_{13}H_{16}Br_2CuN_4S$ and its combinations nearly reduced to zero the enzyme activity. The SOD activity decreased by 53.8-79.9% in the biomass upon the action of antibacterial agents and by 56.8-97.7% in the culture fluid. Combinations of chemical and natural compounds proved to be more effective in blocking the SOD activity both inside the cells and of the released enzyme. Given that the concentrations of the combined compounds were 2-4 times lower compared to the separate variants, a synergistic effect of the combined compounds can be assumed, which requires further detailed studies.

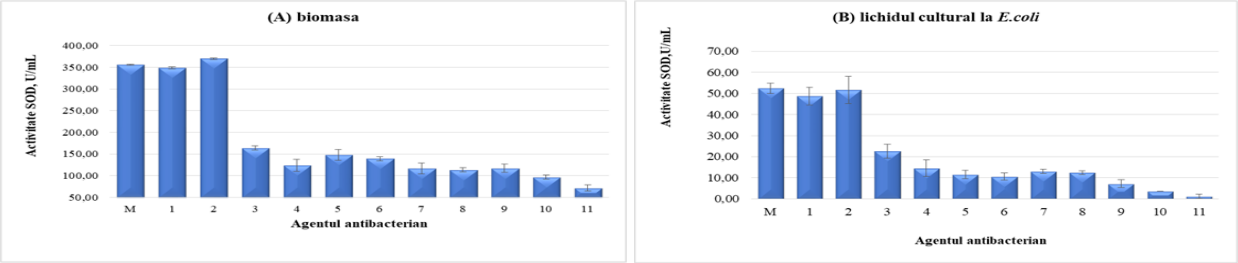


Figure 19. **The effects of tested compounds on superoxide dismutase activity in (A) biomass and (B) in culture fluid of *E. coli***

Note. C – control sample, 1 – mixoxanthophyll, (MX), 2 –extract of spirulina (ES), 3 – $C_{14}H_{19}CuN_7O_4S$; 4 – $C_{14}H_{19}CuN_7O_4S$+ES; 5 – $C_{14}H_{19}CuN_7O_4S$+MX; 6 – $C_{10}H_{14}CuN_4O_5S_2$; 7 – $C_{10}H_{14}CuN_4O_5S_2$+ES; 8 – $C_{10}H_{14}CuN_4O_5S_2$+MX; 9 – $C_{13}H_{16}Br_2CuN_4S$; 10 – $C_{13}H_{16}Br_2CuN_4S$+ES; 11 – $C_{13}H_{16}Br_2CuN_4S$ +MX

Based on the physiological intra- and extracellular SOD activity level, *S. aureus* was closer to *P. aeruginosa* (Figure 20). The antibacterial response also resembled to these two strains. Furthermore, the intracellular SOD activity was reduced to a lesser extent compared to extracellular SOD one in case of *S. aureus.* Thus, the SOD activity decreased by 45-47% in biomasses and by 69.8-77.9% in the culture fluids of all experimental variants, except for those in which special natural compounds were used. The obtained values were very close, the differences being insignificant. When the myxoxanthophyll and the *ES* spirulina extract were used, both the intra- and extracellular superoxide dismutase activity was maintained at the level of the control sample.

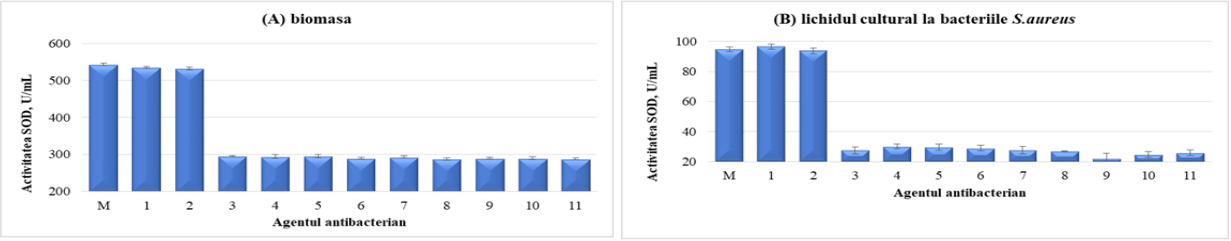


Figure 20. **The effects of the tested compounds on superoxide dismutase activity in (A) biomass and (B) in culture fluid of *S. aureus* bacteria**

Note. C – control sample, 1 – mixoxanthophyll, (MX), 2 –extract of spirulina (ES), 3 – $C_{14}H_{19}CuN_7O_4S$; 4 – $C_{14}H_{19}CuN_7O_4S$+ES; 5 – $C_{14}H_{19}CuN_7O_4S$+MX; 6 – $C_{10}H_{14}CuN_4O_5S_2$; 7 – $C_{10}H_{14}CuN_4O_5S_2$ +ES; 8 – $C_{10}H_{14}CuN_4O_5S_2$ +MX; 9 – $C_{13}H_{16}Br_2CuN_4S$; 10 – $C_{13}H_{16}Br_2CuN_4S$ +ES; 11 – $C_{13}H_{16}Br_2CuN_4S$ +MX

SOD inactivation is a harmful effect for aerobic microorganisms, which become unable to perform at least normal physiological processes, resulting in the formation of superoxide radicals, not to mention the pathological ones generated by the presence of toxic substances in the environment.

Oxygen peroxide is a reactive molecule with a pronounced harmful effect due to its relatively long lifespan, compared to other free radicals. In living cells, several enzymes are responsible for the removal of this substance, the main of which are catalase and peroxidase.

Catalase activity in bacterial cultures treated with the studied antibacterial agents is shown in Figure 21. As in the case of other parameters assessed above, the separate use of myxoxanthophyll and *ES* spirulina extract did not statistically alter the catalase (Ct) activity in the three reference bacterial cultures. The separate use of chemical compounds significantly decreased the Ct activity, while combined with mixoxanthophyll or *ES* spirulina extract, this effect was partially annihilated and, in some cases, even completely. In the case of *P. aeruginosa* strains, the $C_{14}H_{19}CuN_7O_4S$ chemical compound reduced the Ct activity by 34%, while combined with *ES* by 6.4% (P = 0.0021), and with myxoxanthophyll by 14.0% (p = 0.0006). In *E. coli* strains, the exposure to $C_{14}H_{19}CuN_7O_4S$ decreased the Ct activity by 49%, while in combination with *ES* only by 9.8%, and in combination with *MX* it was somewhat more by 34.2%. In *S. aureus* strains, this compound alone reduced Ct activity by 52.9%, in combination with *ES* - by 25.6% and in combination with *MX* - by 30.7%. Almost the same effect on the Ct activity of the reference strains was exerted by two other studied chemical compounds used both separately and in combination with two biological compounds (*MX* and *ES*). Thus, $C_{10}H_{14}CuN_4O_5S_2$ reduced Ct activity in *P. aeruginosa* strains by 55.23%, in *E. coli* strains 58.3%, and in *S. aureus* by 67.17%. When using this compound in combination with *ES*, the decrease in Ct activity was at a lower level, ranging between 10.6-26.4%. Results very close to those reported in combination with *ES* were also obtained in combination with *MX*.

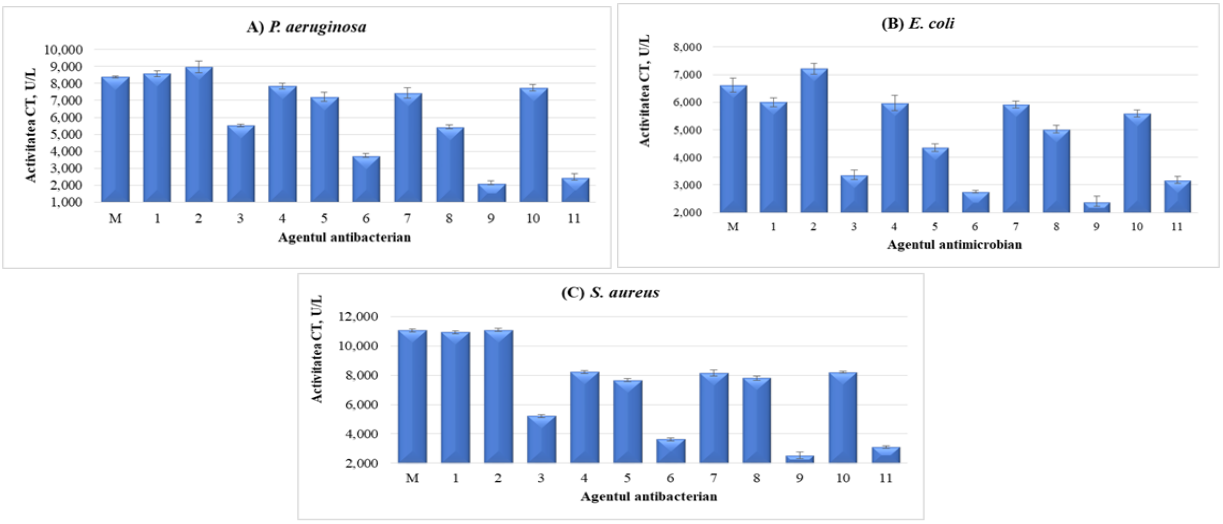


Figure 21. **The effects of the tested compounds on catalase activity in (A) *P. aeruginosa*, (B) in *E. coli* and (C) in *S. aureus***

Note. C – control sample, 1 – mixoxanthophyll, (MX), 2 –extract of spirulina (ES), 3 – $C_{14}H_{19}CuN_7O_4S$; 4 – $C_{14}H_{19}CuN_7O_4S$+ES; 5 – $C_{14}H_{19}CuN_7O_4S$+MX; 6 – $C_{10}H_{14}CuN_4O_5S_2$; 7 – $C_{10}H_{14}CuN_4O_5S_2$ +ES; 8 – $C_{10}H_{14}CuN_4O_5S_2$ +MX; 9 – $C_{13}H_{16}Br_2CuN_4S$; 10 – $C_{13}H_{16}Br_2CuN_4S$ +ES; 11 – $C_{13}H_{16}Br_2CuN_4S$ +MX

Only in the case of *P. aeruginosa* strains, the difference between the effect of $C_{10}H_{14}CuN_4O_5S_2$ alone and in combination with *MX* on Ct activity remained high - 35%.

The most significant reduction in Ct activity (63.6-77.4%) was caused by $C_{13}H_{16}Br_2CuN_4S$. In the case of *P. aeruginosa* strains, the combination of $C_{13}H_{16}Br_2CuN_4S$ + *ES* brought the Ct activity back to normal, being only 7.72% lower compared to the control samples. When combining this chemical compound with MX, although its concentration was 4 times lower than when applied alone, Ct activity decreased by 70.2% compared to the control. The results reported for *E. coli* strains were very similar. So, when used alone, the chemical compound caused a

decrease in the activity of Ct by 63.6%, in combination with *MX* - by 51.8%, and with *ES* - only by 15.5%. For strains of *S. aureus*, the following results were obtained: a decrease in Ct activity by 77.1% when the compound was used alone; by 72.0% in combination with *MX* and by 25.7% in combination with *ES*.

Another enzyme involved in the degradation of hydrogen peroxide is glutathione peroxidase (GPx). The results of testing the effects of the studied chemical and biological compounds on the activity of GPx are shown in Figure 22. Natural compounds, as in the case of Ct, did not significantly change the activity of GPx, and only in the case of *ES*, *P. aeruginosa* strains showed a statistically significant 10.3% (p=0.004) increase in the activity of this enzyme. Otherwise, the response pattern of the studied bacteria was very close to that observed for Ct. In *P. aeruginosa* strains, $C_{14}H_{19}CuN_7O_4S$ caused a decrease in GPx activity by 51.1%, and in combination with *ES* and *MX*, the difference in enzyme activity compared to the control one was 6.9% and 10.8%, respectively. Compound $C_{10}H_{14}CuN_4O_5S_2$ reduced GPx activity in *P. aeruginosa* strains by 47.9%, whereas in combinations- by 25.5% (*ES)* and by 37.7% (*MX*). The most significant decrease in GPx activity in *P. aeruginosa* strains was reported in $C_{13}H_{16}Br_2CuN_4S$ viz. by 64%. In combinations, the decrease was much more modest, ranging between 17.5-19.9%.

GPx activity in *E. coli* strains was reduced by 52.6-64.7% on exposure to three separate chemical compounds, whereas the combinations caused a lesser but significant decrease by 14.1-28.1%.

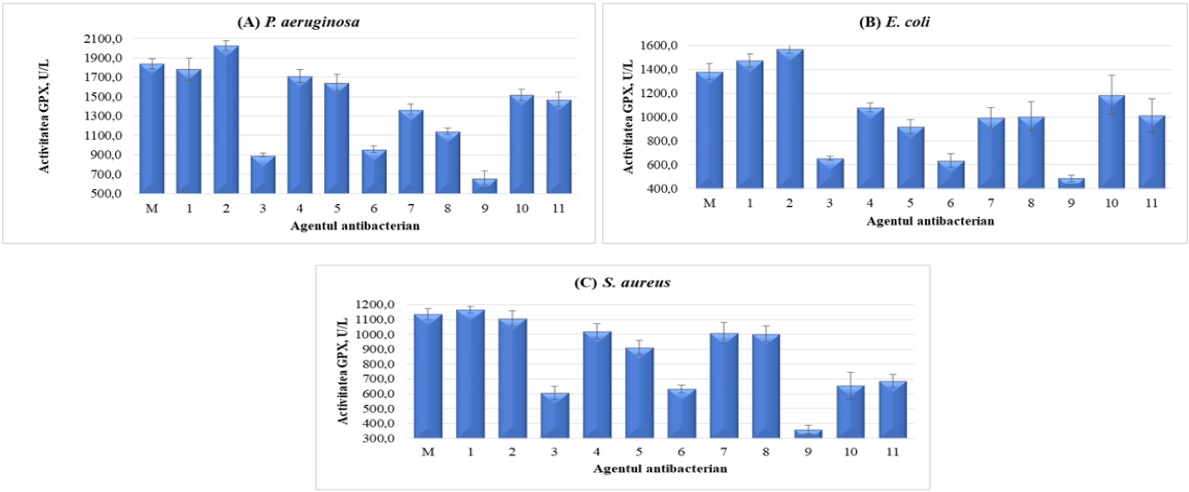


Figure 22. **The effects of tested compounds on glutathione peroxidase activity in (A) P. *aeruginosa*, (B) in *E. coli and* (C) in *S. aureus***

Note. C – control sample, 1 – mixoxanthophyll, (MX), 2 –extract of spirulina (ES), 3 – $C_{14}H_{19}CuN_7O_4S$; 4 – $C_{14}H_{19}CuN_7O_4S$+ES; 5 – $C_{14}H_{19}CuN_7O_4S$+MX; 6 – $C_{10}H_{14}CuN_4O_5S_2$; 7 – $C_{10}H_{14}CuN_4O_5S_2$ +ES; 8 – $C_{10}H_{14}CuN_4O_5S_2$ +MX; 9 – $C_{13}H_{16}Br_2CuN_4S$; 10 – $C_{13}H_{16}Br_2CuN_4S$ +ES; 11 – $C_{13}H_{16}Br_2CuN_4S$ +MX

The effects of the tested compounds on GPx activity in *S. aureus* strains was found to be similar to that reported in the other two bacterial species. Thus, $C_{14}H_{19}CuN_7O_4S$ used alone showed a decrease in GPx activity by 46.6%, in combination with *ES* - by 10.2%, and in combination with *MX* - by 20%. $C_{10}H_{14}CuN_4O_5S_2$used separately, reduced the activity of glutathione peroxidase by 44.39%, while in combination with *ES* and *MX* - by 11.22 and 11.96% respectively. $C_{13}H_{16}Br_2CuN_4S$ caused the most dramatic decrease in GPx activity - by 68.43%. When combined with spirulina extract *ES* and mixoxanthophyll, a strong level of GPx inhibition was reported- by 42.54 and 39.7%, respectively.

According to the obtained results, the activity of these two antioxidant enzymes, involved in detoxifying cells by removing hydrogen peroxide, was strongly affected by the chemical compounds used both separately and combined with *ES* spirulina extract and mixoxanthophyll, obtained from the same species of cyanobacteria. The significant decrease in the intracellular

superoxide dismutase activity and that released in the culture fluid showed that the effects of the studied antibacterial agents resulted in the blockage of the first-line antioxidant defence of microbial cells, which made them vulnerable to the protection factors of the host organism. This finding may be useful to develop a future antimicrobial therapeutic agent.

6. THE INFLUENCE OF BIOLOGICALLY ACTIVE COMPOUNDS ON OXIDATIVE STRESS MARKERS, ANTIOXIDANT SYSTEM AND INFLAMMATORY PATTERNS

In order to assess the effects of the separate and combined biologically active compounds on the oxidative stress markers, antioxidant system and inflammatory pattern, *in vitro* tests were performed, being slightly adapted by the procedure described by Râjcova S. et al. Summarizing the results obtained from the comparative evaluation of lots, as well as by conducting *post-hoc* tests, allowed us to conclude that the studied substances have a certain effect on *in vitro* physiological parameters (Table 3).

The *ES* biological compound exhibited positive effects on all the studied parameters, decreasing, on the one hand, the MDA values, µM / L (p = 0.008), OAPP, µM / L (p = 0.018) and the proinflammatory system indices (IL-1β, pg / ml (p = 0.02), IL-6, pg / ml (p = 0.005)), and increasing, on the other hand, the antioxidant potential (SOD, u / c (p = 0.003), Ct, µM / L (p = 0.002), TAA by ABTS, µM / L (p = 0.005), GST, nM / s.L (p = 0.024), Gpo, nM / s.L (p = 0.004), GR, nM / s.L = 0.006)) and the anti-inflammatory indices (IL-10, pg / ml (p = 0.008)).

Table 3. **Heatmap illustrating the comparative analysis of the studied parameters and the control sample for separate and combined compounds**

Lots	B1	B2	C1	C1+B1	C1+B2	C2	C2+B1	C2+B2
Control, MDA, µM/L	0,0085	0,0085	0,0085	0,0166	0,0085	0,4908	0,0113	0,0085
Contol, OAPP, µM/L	0,018	0,012	0,080	0,695	0,409	0,027	0,073	0,012
Control, SOD, u/c	0,0035	0,0096	0,0035	0,0035	0,0035	0,0035	0,0035	0,0035
Control, Ct, µM/L	0,0029	0,0029	0,0248	0,0029	0,0029	0,0309	0,0073	0,0029
Control, TAA with ABTS, µM/L	0,0059	0,0059	0,0173	0,0059	0,0059	0,0059	0,0125	0,0059
Control, GST, nM/s.L	0,024	0,014	0,023	0,024	0,014	0,014	0,014	0,024
Control, Gpo, nM/s.L	0,0041	0,0041	0,0365	0,0070	0,0070	0,0975	0,0041	0,0041
Control, GR, nM/s.L	0,0064	0,0064	0,0076	0,0064	0,0064	0,0064	0,0064	0,0064
Control, IL-1β, pg/ml	0,020	0,015	0,010	0,822	0,196	0,020	0,207	0,033
Control, TNF-α, pg/ml	0,7362	0,0579	0,0088	1,0000	0,3541	0,0579	0,5716	0,2062
Control, IL-6, pg/ml	0,0054	0,0088	0,0224	0,1043	0,0293	0,0054	0,0859	0,0112
Control, IL-10, pg/ml	0,0082	0,0035	0,0204	0,0035	0,0035	0,0082	0,0035	0,0035
	statistically significant difference, showing lower value in the control sample							
	statistically significant difference, showing higher value in the control sample							
	statistically insignificant difference.							

Note: *B1 - S. platensis extract (ES); B2 - mixoxanthophyll carotenoid pigment (MX); C1 - chemical compound $C_{10}H_{14}CuN_4O_5S_2$; C2 - chemical compound $C_{14}H_{19}CuN_7O_4S$, MDA - malonic dialdehyde, OAPP - advanced oxidation protein products, SOD - superoxide dismutase, TAA - total antioxidant activity, GST - glutadione S-transferase, Gpo - glutadione peroxidase, GR – glutathione reductase, IL - interleukin, TNF - tumor necrosis factor.*

The biological compound *MX* showed similar effects on the *ES* compound; on the one hand, it reduced the values of MDA, µM / L (p = 0.008), OAPP, µM / L (p = 0.012) and of the pro-inflammatory system (IL-1β, pg / ml (p = 0.015), IL-6, pg / ml (p = 0.008)), and, on the other hand, it increased the antioxidant potential (SOD, u / c (p = 0.009), Ct, µM / L (p = 0.002), TAA by ABTS, µM / L (p = 0.005), GST, nM / s.L (p = 0.014), Gpo, nM / s.L (p = 0.004), GR, nM / s.L (p = 0.006))) and the anti-inflammatory system (IL-10, pg / ml (p = 0.003)).

$C_{10}H_{14}CuN_4O_5S_2$ showed negative effects, causing elevated MDA values, µM / L (p = 0.008), as well as of the pro-inflammatory system components IL-1β, pg / ml (p = 0.010), TNF-α, pg / ml (p = 0.008), IL-6, pg / ml (p = 0.022). At the same time, this compound favoured the antioxidant system (SOD, u / c (p = 0.003), Ct, µM / L (p = 0.024), TAA by ABTS, µM / L (p = 0.017), GST, nM / s.L (p = 0.023), Gpo, nM / s.L (p = 0.036), GR, nM / s.L (p = 0.007)) and the anti-inflammatory system (IL-10, pg / ml (p = 0.020)).

The $C_{14}H_{19}CuN_7O_4S$ compound had similar effects as $C_{10}H_{14}CuN_4O_5S_2$, by increasing the values of all the studied parameters, compared to the control group: OAPP, µM / L (p = 0.027), SOD, u / c (p = 0.003), Ct, µM / L (p = 0.030), TAA with ABTS, µM / L (p = 0.005), GST, nM / s.L (p = 0.014), GR, nM / s.L (p = 0.006), IL-1β, pg / ml = 0.020), IL-6, pg / ml (p = 0.005) and IL-10, pg / ml (p = 0.008).

Positive effects were registered mainly on the anti-inflammatory and the MDA-OAPP systems by the biological compounds *ES* and *MX*, optimal characteristics being registered in the combination of $C_{14}H_{19}CuN_7O_4S$ + *MX*. Biological compounds *ES* and *MX*, due to their optimal characteristics regarding the effects on the studied systems, showed a potential for further studies.

GENERAL CONCLUSIONS

1. Solving the scientific problem by confirming the effects of new chemical and biological compounds on microorganisms isolated from infected trophic ulcers, made it possible to formulate the principles for the development of effective multicomponent drugs in the treatment of infected trophic ulcers, as well as to develop alternative anti-infective strategies to prevent both therapeutic failures and the development of antimicrobial resistance.
2. The results obtained in the present study demonstrated that all the infected trophic ulcers involved a microbial species in 50.6% of cases and several species in 49.4% of cases, which may explain the flare-ups or delayed healing of the trophic ulcers. The diversity of microorganisms associated with trophic ulcer was represented mainly by *Staphylococcus aureus* (21.9%; 95% CI 21.7-22.1), followed in order of a decreasing frequency by *Pseudomonas aeruginosa* (15.2%; 95% CI 15.0-15 ,4), *Klebsiella. pneumoniae* (13.3%; 95% 13.1-13.5) and *Enterococcus* spp. species (11.9%; 95% 11.7-12.1).
3. The antimicrobial resistance profiles of isolates from infected trophic ulcers showed a pronounced resistance to these drugs. *Staphylococcus* spp. strains exhibited multiple antimicrobial resistance in 68.4% of cases, including 43.7% of methicillin-resistant strains and 19.6% of induced clindamycin-resistant strains. Enterobacteria showed strong resistance to aminopenicillins, penicillins with beta-lactamase inhibitors, and cephalosporins. *P. aeruginosa* and *A. baumannii* strains were highly resistant to most of the drugs used in treatment.
4. A significant number of gram-negative bacillus strains was found to produce broad-spectrum β-lactamases, predominantly by the *P. aeruginosa* strains - 40.6% (95% CI 39.3-

41.9), followed by *K. pneumoniae* strains - 36.9% (95% CI 35.7-38.1) and *E. coli* - 33.9% (95% CI 32.7-35.1). AmpC-type β-lactamases-producing strains were found to be *A. baumannii* - 28.3% (95% CI 27.0-29.6), *K. pneumoniae* - 4.2% (95% CI 3.47-4.93) *and P. aeruginosa* - 4.2% (95% CI 3.47-4.93). The most common types of carbapenemases involved OXA-48 (2.6%; 95% CI 2.5-2.7) and NDM (0.3%; 95% CI 0.26-0.34), whereas in the *A. baumanii* strains - OXA-23 (5.7%; 95% CI 4.8-6.6) and OXA-58 (3.8%; 95% CI 3.1-4.5).

5. The vpenotypic expression of virulence factors in the tested microbial agents varied, depending on the reference strains. Most strains expressed pore-forming toxins, especially lipase (74.2%), lecithinase (53.6%) and hemolysins (57.7%), which were involved in both invasion and host immune response. Lysozyme was inactivated in 93.3% of strains, while most of species (47.2%) showed an average anti-lysozyme expression level. 95.2% of the tested strains showed anticomplementary activity. Biofilm-forming ability was recorded in 73.2% of strains isolated from trophic ulcers. All the determined pathogenicity factors were recorded at a higher rate in strains isolated from polymicrobial infections, exhibiting multiple antimicrobial resistance.
6. The study of the antimicrobial activity of some chemical compounds, which have copper (II) nitrate as a synthetic precursor, showed a high antimicrobial action on the studied gram-positive microorganisms. Thiosemicarbazones from transition metal class proved a more pronounced activity, as well as the copper chloride- synthesized compounds -on gram-negative bacilli. Biological compounds showed higher antimicrobial activity against gram-positive bacteria, namely MX1, with a subsequent decrease in the action of *MX2* and *ES* compounds. While determining the acute toxicity of new chemical compounds to daphnia, it has been demonstrated that both the ligand (LC50 5.53 ± 0.90) and the coordinating copper compounds (Cu L Br - LC50 4.4 ± 0.96; Cu L Cl - LC50 3.5 ± 0.91) showed a lower toxicity level compared to doxorubicin hydrochloride (LC50 3.27 ± 0.30).
7. The studied chemical and biological compounds showed synergistic effect in 87.2% of cases, additive effects in 6.8% and indifferent action in 6.0%. Additive and indifferent effects have been observed especially for gram-negative bacilli and yeast fungi. No antagonistic effects were reported in the combined chemical and biological compounds under study. The MIC values for combined chemical and biological compounds showed a 4-32-time decrease compared to MICs of separate compounds.
8. The separate use of chemical and biological compounds at a concentration of 0.25MIC showed no reduction in the number of microbial cells, while combining these compounds, the microorganisms being killed over 8-24 hours. The shortest microbial kill-time (8-20 hours) was recorded when the chemical compounds were combined with the biological compound *MX1*.
9. The present study showed that some microbial pathogenicity factors such as anticomplementary activity, anti-lysosyme, lecithinase, hemolytic activity and the microbial biofilm-forming ability were further reduced by the use of both separate and combined chemical and biological compounds at concentrations of 75%. A more prominent inhibition of pathogenicity factors was exhibited by the biological compound *MX1* combined with the chemical compounds.
10. Spirulina extracts did not show a direct toxic effect on the reference strains, compared to chemical compounds that, at minimal inhibitory concentrations, had a pronounced toxic

effect on reference strains. The joint effect of the compounds on the studied parameters was more effective, since the minimally invasive concentration of the compounds was 2-4 times lower than that of the compounds used separately. The chemical and natural compounds acted synergistically through mechanisms, which were not identified in this study, but which hold great promise as antimicrobial agents with low toxicity and low potential for the resistance development.

11. As regarding the effects of biologically active compounds on physiological parameters *in vitro*, positive effects were recorded on all the studied parameters, manifested by a decrease in oxidative stress values, pro-inflammatory system, as well as an elevated antioxidant and anti-inflammatory potential. The chemical compounds showed negative effects with high values for oxidative stress indices and pro-inflammatory system components being registered, thus, favouring the antioxidant and anti-inflammatory systems. The combined effects of the studied compounds showed positive effects, mainly on both the anti-inflammatory system and the oxidative stress indices.

PRACTICAL RECOMMENDATIONS

1. Procedures have been suggested to highlight the timely expression of the microbial pathogenicity factors, which allows developing practical recommendations for administering targeted antimicrobial treatments that will help reduce antimicrobial resistance.
2. Biologically active compounds with pronounced antimicrobial activity should be recommended as substances with antibacterial action against strains involved in infected trophic ulcers, as well as further clinical studies are proposed to develop combined synthetic and natural antimicrobial drugs.
3. The applied research methods and the results obtained in this study can be further used within the university and postgraduate training at the disciplines of clinical microbiology and microbiological laboratory.

Suggestions for Future Research

1. The obtained study results require further *in vivo* research studies to be confirmed by developing preclinical tests on antimicrobial effects of biologically active compounds.
2. Further studies are needed to highlight the mechanisms of action of bioactive compounds with pronounced antimicrobial activity on microorganisms associated with trophic ulcers.

SELECTIVE BIBLIOGRAPHY

1. AGALE, S. Chronic Leg Ulcers: Epidemiology, Aetiopathogenesis and Management. In: *Hindawi Publ Corp Ulcers.* 2013, p. 9-23. ISSN 2314-6133.
2. ALRUB, A. HYASSAT, D., KHADER, Y., BANI-MUSTAFA, R., YOUNES, N., AJLOUNI, K. Factors Associated with Health-Related Quality of Life among Jordanian Patients with Diabetic Foot Ulcer. In: *Journal of Diabetes Research*. 2019, pp. 1-8. ISSN 23146745 (IF: 2.965).
3. ANDERSSON, D., BALABAN, N., BAQUERO, F., COURVALIN, P., GLASER, P., GOPHNA, U., et al. Antibiotic resistance: turning evolutionary principles into clinical reality. In: *FEMS Microbiol. Rev*. 2020, nr. 43, pp. 341-361. ISSN 0168-6445 (IF: 16.408).
4. ASTRID, L., UNNI, G., KARSTEN, G. et. al. Antimicrobial Resistance in a One Health and One World Perspective – Mechanisms and Solutions. In: INTERNATIONAL ENCYCLOPEDIA OF PUBLIC HEALTH. Second Edition Academic Press, 2016, vol. 1, chapter 22, pp. 140-153. ISBN 9780128037089.

5. AYED, M., ABABNEH, M., ROBERT, A., ALZAID, A., AHMED, R., SALMAN, A., et al. Common pathogens and antibiotic sensitivity profiles of infected diabetic foot ulcers in Saudi Arabia. In: *Int J Lower Extrem Wounds*. 2018, pp. 1-8. ISSN 1534-7346 (IF: 0.570).
6. BONDAREVA, G., STADNYUK, G., GREBENNIKOVA, T., SHASHLOVA, M., DONINA, M. Antimicrobial Properties of Some Zinc Compounds. In: *Sys Rev Pharm*. 2020, 11(12), pp. 2065-2066. ISSN 0975-8453 (IF: 5.373).
7. BUIUC, D. NEGUŢ, M. *Tratat de microbiologie clinică.* Bucureşti: Ed. Medicală, 2017. 1250 p. ISBN 978-973-39-0593-6.
8. CZAPLEWSKI, L., BAX, R., CLOKIE, M., DAWSON, M., FAIRHEAD, H., FISCHETTI, VA., et al. Alternatives to antibiotics-a pipeline portfolio review. In: *Lancet Infect Dis*. 2016, nr. 16, pp. 239-251. ISSN 14733099. (IF:19.864).
9. DATTA, P, CHANDER, J, GUPTA, V, MOHI, G., ATTRI, A. Evaluarea diverșilor factori de risc asociați cu organisme rezistente la medicamente, izolate de la pacienții cu ulcer diabetic. In : *J Medici de laborator*. 2019, 11 (1), pp. 58-62. ISSN 1841-6624. (IF:0.945).
10. DEFOIRDT, T. Quorum-sensing systems as targets for antivirulence therapy. In: *Trends Microbiol.* 2017, nr. 26, pp. 313-328. ISSN 18784380.
11. DINGUES, M., ORWIN, P., SCHLIEVERT, M**.** Exotoxins of *Staphylococcus aureus*. In: *Clin Microb Reviews.* 2000, 13(1), pp. 16-34. ISSN1098-6618. (IF:13.484).
12. EIKMEYER, F., HADIATI, A., SZCZEPANOWSKI, R., et al. The complete genome sequences of four new IncN plasmids from wastewater treatment plant effluent provide new insights into IncN plasmid diversity and evolution. In*: Plasmid.* 2012, vol. 68, nr.1., pp. 13-24. ISSN 0147-619X.
13. ELBARSHA, A., HAMEDH, M. AI.. ELSAEITI, M. Prevalence and risk factors of diabetic peripheral neuropathy in patients with type 2 diabetes mellitus. In: *Ibnosina Journal of Medicine and Biomedical Sciences.* 2019, 11(1), pp. 25-28. ISSN: 1947489X.
14. FONG , J., ZHANG, C., YANG, R., BOO, Z., TAN, S., NIELSEN, T., et al. Combination therapy strategy of quorum quenching enzyme and quorum sensing inhibitor in suppressing multiple quorum sensing pathways of *P. aeruginosa*. In: *Sci. Rep*. 2018, nr. 8, p. 1155-1166. ISSN 2045-2322.
15. FOTHERGILL, A. Antifungal Susceptibility Testing: Clinical Laboratory and Standards Institute (CLSI) Methods. In: H*all GS, editor. Interactions of Yeasts, Moulds, and Antifungal Agents.* Totowa, NJ: Humana Press. 2012, pp. 65-74.
16. GARCIA, T., BORGES E., JUNHO T., SPIRA J. Microbiological profile of leg ulcer infections: review study. In: *Rev Bras Enferm.* 2021, nr. 74(3):e20190763, pp. 923-930. ISSN 347167.
17. GLADUN, N. et al. Ateroscleroza obliterantă a membrelor inerioare. În: Arta Medica. 2007, 3 (24), p. 44-46. ISSN 1810-1852.
18. GREEN, J., JESTER, R., MCKINLEY, R. , POOLER, A. The impact of chronic venous leg ulcers: asystematic review. In: *J Wound Care.* 2014, nr. 23, pp. 601-612. ISSN: 0969-0700. (IF:1.069).
19. GUAN, H., DONG, W., LU, Y., et al. Distribution and Antibiotic Resistance Patterns of Pathogenic Bacteria in Patients With Chronic Cutaneous Wounds in China. In: *Frontiers in Medicine*. 2021, vol. 8, p. 274-287. ISSN 2296-858X. (IF: 5.091).
20. HAY, S., RAO, P., DOLECEK, C., DAY, N., STERGACHIS, A., LOPEZ, A., et al**.** Measuring and mapping the global burden of antimicrobial resistance. In: *BMC Med*. 2018, 16(1), pp. 78-81. ISSN 1741-7015. (IF: 8.285).
21. KULESZA, T, MITRUT T, JOJCZUK M, JAHOŁKOWSKI L, NODALSKI A, PRYSTUPA A. Treatment of lower limb trophic ulcers using hyperbaric oxygenation. In: *J Pre Clin Clin Res.* 2014, 8(1), pp. 44-47. ISSN 1898-7516.
22. LINDSAY, S., OATES, A., BOURDILLON, K. The detrimental impact of extracellular bacterial proteases on wound healing. In: *International Wound Journal*. 2017, nr. 14, pp. 1237-1247. ISSN 1742-4801.

23. MARKEVICH, P., MARKEVICH, L., PLEKHANOV, A. The pharmacotherapeutic efficiency of cytoflavin in patients with diabetic foot syndrome: immediate and long-term results. *Klin Med.* 2015, vol.33, nr. 6, pp. 67-72. ISSN 2077-0383. (IF: 5.583).
24. NAYLOR, NR, ATUN R, ZHU N, KULASABANATHAN K, SILVA S, CHATTERJEE A, et al. Estimating the burden of antimicrobial resistance: a systematic literature review. In: *Antimicrob Resist Infect Control*. 2018, nr. 7, pp. 58.
25. PIROG, T., KLIUCHKA, I., KLIUCHKA, L. Synergistic action on microorganisms of complex of essential oiles with the biocides. In: *Biotechnologia Acta*. 2019, vol. 12, pp. 5-17.
26. PODOLSKY, S. The evolving response to antibiotic resistance (1945–2018). *Palgrave Commun.* 2018, nr. 4, pp. 214-132.
27. PUGLIESE, D. Infection in Venous Leg Ulcers: Considerations for Optimal Management in the Elderly. In: *Drugs Aging*. 2016, nr. 33, pp. 87-96. ISSN 1170-229X.
28. RAHMAN, M., SARKER S. D. Antimicrobial natural products. In: Anual reports in Medicinal Chemistry. 2020, vol. 55, pp. 77-113. ISSN 0065-7743, ISBN 9780128210192.
29. RAJPUT A., THAKUR, A., SHARMA, S., AND KUMAR, M. aBiofilm: a resource of anti-biofilm agents and their potential implications in targeting antibiotic drug resistance. In: *Nucleic Acids Res.* 2017, nr. 46, pp. 894–900.
30. RIBEIRO, S.., FELICIO, M., BOAS, E. V., GONÇALVES, S., COSTA, F. F., SAMY, R. P., et al. New frontiers for anti-biofilm drug developmen. In: *Pharmacol. Ther*. 2016, nr. 160, pp. 133-144.
31. SAHA, R, SARKAR S, MAJUMDER M, BANERJEE G. Bacteriological Profile of Aerobic and Anaerobic Isolates of Trophic Ulcer in Leprosy: A Study from Eastern India. In: *Indian J Dermatol.* 2019, 64(5), pp. 372-376.
32. SASANKA, S. Venous ulcers of the lower limb: where do we stand? In: *Indian Journal of Plastic Surgery*. 2012, vol. 45, nr. 2, pp. 266-274. ISSN 0970-0358.
33. SELIVESTROV, E.I., AVAKYANTS, I.P., NIKISHKOV, A.S., & ZOLOTUKHIN, I. A. Epidemiology of Chronic Venous Disease. In: *Journal of Venous Disorders (Flebologiya).* 2016, nr. 1, pp. 35-40.
34. SERMSATHANASAWADI, N., PRUEKPRASERT, K., PITAKSANTAYOTHIN, W., CHINSAKCHAI, K., WONGWANIT, C., RUANGSETAKIT, C., MUTIRANGURA, P. Prevalence, risk factors, and evaluation of iliocaval obstruction in advanced chronic venous insufficiency. In: *J Vasc Surg Venous Lymphat Disord*. 2019, nr. 7, pp. 441-447.
35. SHAHI, S., KUMAR, A. Isolation and Genetic Analysis of Multidrug Resistant Bacteria from Diabetic Foot Ulcers. In: *Frontiers in Microbiology*. 2016.Vol. 6, pp. 59-64. ISSN1664-302X. (IF:4.076).
36. SHIMANKO, A., DIBIROV, M., ZUBRITSKY, V., et.al. The Combined Treatment of Trophic Ulcers of Venous Etiology. In: *Journal of Venous Disorders (Flebologiya).* 2017, nr. 2, pp. 91-95.
37. SILLANKORV, S. , PEREIRA, M., HENRIQUES, M. *Antibiotic Alternatives and Combinational Therapies for Bacterial Infections*. In: *Front Microbiol.* 2019, vol. 9, pp. 3359-3361.
38. SORG, H., TILKORN, D. J., HAGER, S., HAUSER, J., MIRASIJSKI, U. Skin Wound Healing: An Update on the Current Knowledge and Concepts. In: *Eur Surg Res*. 2016, nr. 58, pp. 81-94.
39. TROTTER, A. J., AYDIN, A., STRINDEN, M. J. & O'GRADY, J. Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance. In: *Curr. Opin. Microbiol.* 2019, nr. 51, pp. 39-45. ISSN 1369-5274 (IF: 8.134).
40. WORLD HEALTH ORGANIZATION (WHO) [online]. Lack of new antibiotics threatens global efforts to contain drug-resistant infections. 2020 [citat 09.06.21]. Disponibil: *https://www.who.int.*

41. WHO. Antibiotic Resistance [online]. 2020. [citat 09.06.21].Disponibil: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance.
42. WHO. Ten threats to global health in 2019 [online], 2019 [citat 13.02.2020]. Disponibil: https://www.who.int/emergencies/ten-threats-to-global-health-in-2019.
43. ZAVARUEV, A.V., BREGADZE A.A., & KOZKA A.A. The Experience with the Surgical Treatment of Venous Trophic Ulcers. In: *Flebologiya.* 2016, nr. 2, pp. 103–105. ISSN: 2309-5601.
44. ZHARKOVA, M. S., ORLOV, D. S., GOLUBEVA, O. Y., CHAKCHIR, O. B., ELISEEV, I. E., GRINCHUK, T. M., et al. Application of antimicrobial peptides of the innate immune system in combination with conventional antibiotics-a novel way to combat antibiotic resistance? In: *Front. Cell. Infect. Microbiol*. 2019, nr. 9, pp. 128. ISSN: 2235-2988 (IF: 4.123).
45. МИКИТИН, И.Л., КАРАПЕТЯН, Г.Э., КОЧЕТОВА, Л.В., ЯКИМОВ, С.В., ПАХОМОВА, Р.А. Современный взгляд на лечение трофических язв. В: *Креативная хирургия и онкология.* 2013, №4, с. 108-112. ISSN: 2307-0501.

LIST OF PUBLICATIONS ON THESIS

1. Monographs

1.1. Single author monographs

1.1.1 **BĂLAN, G.** *Rezistenţa microorganismelor la antibiotice.* Chişinău: Tipografia „Print-Caro", 2018. 240 p. ISBN 978-9975-56-597-4.

2. Articles submitted to various scientific journals

2.1. to the Web of Science and SCOPUS databases

2.1.1. GULEA, A., GRAUR, V., CHUMAKOV, YU., PETRENKO, P., **BALAN, G.** et al. Synthesis, Structure, and Biological Activity of Copper and Cobalt Coordination Compounds with Substituted 2-(2-Hydroxybenzylidene)-N-(prop-2-en-1-yl)hydrazinecarbothioamides. In: *Russian Journal of General Chemistry*. 2019, 89(5), 953-964. Doi: 10.1134/S1070363219050153 **(IF: 0,761).**

2.1.2. GULEA, A., MITKEVICH, N., CHUMAKOV, YU., PETRENKO, P**., BALAN, G.** et al. Synthesis, Structure, and Biological Activity of Coordination Compounds of Cobalt(II), Nickel(II), and Copper(II) with N-(Methoxyphenyl)-2-[(5-nitrofuryl)methylene]hydrazine Carbothioamides. In: *Russian Journal of General Chemistry.* 2019, 89(7), 1415-1423. Doi: 10.1134/S1070363219070119 **(IF: 0,761).**

2.1.3. **BALAN, G.,** BURDUNIUC, O., USATAIA, I. et al. Novel 2-formylpyridine 4-allyl-Smethylisothiosemicarbazone and Zn(II), Cu(II), Ni(II) and Co(III) complexes: Synthesis, characterization, crystal structure, antioxidant, antimicrobial and antiproliferative activity. In: *Applied Organometallic Chemistry*. 2020, 34: e5423. Doi: org/10.1002/aoc.5423 **(IF: 4,105).**

2.1.4. GULEA, A., USATAIA, I., GRAUR, V., CHUMAKOV, YU., PETRENKO, P., **BALAN, G**. et al. Synthesis, Structure and Biological Activity of Coordination Compounds of Copper, Nickel, Cobalt, and Iron with Ethyl N'-(2-Hydroxybenzylidene)-N-prop-2-en-1-ylcarbamohydrazonothioate. In: *Russian Journal of General Chemistry*. 2020, 90(4), pp. 630–639. ISSN 1070-3632. Doi: 10.1134/S107036322004012X. **(IF: 0,767).**

2.1.5. GULEA, A., GRAUR, V., DIURICI, E., ULCHINA, IA., BOUROSH, P., **BALAN, G.** et al. Synthesis, Structure, and Biological Activity of Copper(II), Nickel(II), Cobalt(III), and Iron(III) Coordination Compounds with 2-{2-[(Prop-2-en-1-yl)carbamothioyl]hydrazinylidene}propanoic Acid. In: *Russ J Gen Chem.* 2020, 90(11), 2120-2127. Doi: 10.1134/S107036322011016X **(IF: 0,767).**

2.2. to international recognized journals

2.2.1. **BALAN, G.** et al. Frequency of methicillin-resistant *Stapylococcus aureus* strains in healthcare associated infections in Republic of Moldova. In: *Romanian Archives of Microbiology and Immunology*. 2017, vol. 76 (2), pp.79-84. ISSN 1222-3891.

2.2.2. **БЭЛАН, Г.,** БЕХТА, Е. Биопленкообразующая способность штаммов *Pseudomonas aeruginosa* выделенных из трофических язв и их ассоциация с антимикробной резистентностью. В: *Международный научный журнал „Научные горизонты”.* 2020, 9(37), с. 100-109. ISSN 2587-618X.
2.2.3. **BALAN, G.,** BURDUNIUC, O. Antibiotic resistance and biofilm formation of *S. aureus* and *C. albicans* strains isolated from trophic ulcers. В: *Международный научный журнал „Научные горизонты”*. 2020, 9(37), с. 110-118. ISSN 2587-618X.
2.2.4. BURDUNIUC, O., DJUR, S., CHIRIAC, T., RUDIC, V., **BALAN, G.** *In vitro* evaluation of antimicrobial and biofilm inhibitory activity of *Spirulina platensis* extracts. В: *Здоров'я суспільства*. 2020, том 9, № 3; 118-123. ISSN 2306-2436.
2.2.5. **BALAN, G.** et al. Antibiotic susceptibility of clinical *Acinetobacter baumannii* strains. В: *Международный научный журнал „Научные горизонты”*. 2020, 11(39), с. 161-167. ISSN 2587-618X.
2.2.6. **БЭЛАН, Г.**Чувствительность к антибиотикам и некоторые факторы патогенности штаммов *Klebsiella* spp., выделенных из трофических язв. В: *Здоров'я суспільства*. 2020, том 9, № 4; 150-154. ISSN 2306-2436.

2.3. within the National Register of specialized journals (category indicated)

- B category

2.3.1. GHENDOV-MOȘANU, A., COJOCARI, D., **BALAN, G.**, STURZA, R. Antimicrobial activity of rose hip and hawthorn powders on pathogenic bacteria. In: *Journal of Engineering Science*. Chişinău, 2018,Vol. XXV (3), p. 100-107. ISSN 2587-3474.
2.3.2. **BALAN, G.** Antibiotic susceptibility and some persistence factors of Gram-negative bacilli isolated from trophic ulcers. In: *The Moldovan Medical Journal*. 2019, 62(3): 13-17. ISSN 2537-6373.
2.3.3. **BALAN, G.** The bacterial strains isolated from trophic ulcers their persistence factors. In: *The Moldovan Medical Journal.* 2019, 62(4): 36-18. ISSN 2537-6373.
2.3.4. **BALAN, G.,** BURDUNIUC O. Antimicrobial susceptibility and biofilm production among *Staphylococcus* and *Candida* species. In: *The Moldovan Medical Journal.* 2020, 63(1): 3-7. ISSN 2537-6373.
2.3.5. **BALAN, G.** Antibiotic susceptibility and factors involved in virulence and persistence of *Acinetobacter baumannii* strains. In: The *Moldovan Medical Journal.* 2021, 64(1):4. ISSN 2537-6373.
2.3.6. **BĂLAN, G.,** RUDIC V. Spectrul etiologic şi dificultăţile terapeutice în infecţiile de plagă. În: *Sănătate Publică, Economie şi Management în Medicină.* 2014, 3(54): 147-149. ISSN 1729-8687.
2.3.7. BURDUNIUC, O., **BĂLAN, G.** Conştientizarea populaţiei privind consumul de antimicrobiene în Republica Moldova. În: *Sănătate Publică, Economie şi Management în Medicină.* 2015, 3(60): 160-163. ISSN 1729-8687.
2.3.8. **BĂLAN, G.** Formarea biofilmelor *in vitro* de către tulpinile de *Pseudomonas aeruginosa* şi asocierea acestora cu rezistenţa antimicrobiană. În: *Sănătate Publică, Economie şi Management în Medicină*. 2019, 4(82): 276-280. ISSN 1729-8687.
2.3.9. **BĂLAN, G.** Rezistenţa la antibiotice şi formarea biofilmelor de către tulpinile de *Staphylococcus aureus* izolate din ulcere trofice. În: *Sănătate Publică, Economie şi Management în Medicină.* 2020, 1(83): 48-52. ISSN 1729-8687.
2.3.10. RUSNAC, R., BÎRCĂ, M., ȘOVA, S., COTOVAIA, A., **BALAN, G.** et al. Sinteza și proprietățile antibacteriene și antifungice ale compușilor coordinativi ai Fe(III) Cu 4-ciclohexiltiosemicarbazona 4-benzoil-3-metil-1-fenil-2-pirazolin-5-onă. În: *Studia Universitatis Moldaviae. Chişinău*. 2020, nr.1(131) Seria “Ştiinţe Reale şi ale Naturii”, pp. 32-37. ISSN 1814-3237.
2.3.11. BEHTA, E., BURDUNIUC, O., BUCOVA, V., CRACIUN, O., BIVOL, M, BURDUNIUC, A., BRÎNZĂ, O. GRUMEZA, M., **BALAN, G.** Antimicrobial discovery – impact

of the natural sources. În: *Studia Universitatis Moldaviae. Chişinău*. 2020, nr.6(136) Seria "Ştiinţe Reale şi ale Naturii", p. 39-48. ISSN 1814-3237.

2.3.12. BUCOV, V., BURDUNIUC, O., **BALAN, G.** ş. a. Rezistența la antimicrobiene. Caracteristica rezistenței la preparate antimicrobiene a bacteriilor Gram-negative. În: *Sănătate Publică, Economie şi Management în Medicină.* 2021, 1(88): 50-56. ISSN 1729-8687.

- C category

2.3.13. BURDUNIUC, O., COJOCARU, R., **BĂLAN, G.** ş. a. Determinarea unor markeri ai rezistenţei enterobacteriilor la preparatele antimicrobiene. În: *Buletinul Academiei de Stiinţe a Moldovei*. *Ştiinţele Vieţii.* 2012, 3(318): 151-158. ISSN 1857-064X.

3. Compilations of scientific research

3.1. in the proceedings of international scientific conferences (the Republic of Moldova)

3.1.1. **BĂLAN, G.** Incidence and antibiotic susceptibility of bacterial strains isolates from wound infections. In: *Scientific Conference on Microbial Biotechnology (3rd edition).* Chişinău; 2016, pp. 24-27. ISBN 978-9975-3129-3-6.

3.2. în lucrările conferinţelor ştiinţifice internaţionale (Republica Moldova)

3.2.1. **BALAN, G.** et al. Synergistic Action of Some Chemical and Biological Compounds. În: *Materialele Conferinței stiințifice naționale cu participare internațională „Materiale avansate în biofarmaceutică și tehnică".* Chişinău, 2021, pp. 314-325. ISBN 978-9975-89-216-2.

3.2.2. LOZAN-TIRSU, C., RUDIC, V., **BALAN, G.,** GULEA, A. Activitatea enzimelor antioxidante în culturile de referinţă la acţiunea compuşilor chimici noi. În: *Materialele Conferinței stiințifice naționale cu participare internațională „Materiale avansate în biofarmaceutică și tehnică*". Chişinău, 2021, pp. 154-162. ISBN 978-9975-89-216-2.

4. Theses in scientific collections

4.1. within international scientific conferences database (abroad)

4.1.1. **BĂLAN, G.** et al. Antifungal activity of 2-acetylpyridine{n-(4-aminophenyl)-acetamid}thiosemicarbazone and salicylaldehyde { n-(4-amino-phenyl)-acetamid} thiosemicarbazone. În: *Supliment la Revista Română de Medicină de Laborator*. Timişoara, România, 2018, nr.3, vol. 26, p. 50. ISSN 18416624.

4.1.2. **BĂLAN, G.** et al. Antifungal activity of new copper (II) complexes with 4-benzoyl-5-methyl-2-phenyl-2,4-dihygro-3h-pirazol-3-one n(4)-ciclohexylthiosemicarbazone. În: *Supliment la Revista Română de Medicină de Laborator.* Timişoara, România, 2018, nr.3, vol. 26, p. 50. ISSN 18416624.

4.1.3. BURDUNIUC, O., **BĂLAN, G.** et al. Antifungal activity of some 3d metal coordination compounds with 2-[2-(prop-2-en-1-ylcarbamothioy)-hydrazinylidene]-propanoic acid. În: *Supliment la Revista Română de Medicină de Laborator.* Timişoara, România, 2018, nr.3, vol. 26, p. 50. ISSN 18416624.

4.1.4. BURDUNIUC, O., **BĂLAN, G.** et al. Antifungal activity of iron, cobalt, nickel and zinc coordination with 2-[1-(2,4-dihydroxypheny)ethylidene]-n-(prop-2-en-1-yl)-hydrazinnecarbothioamide. În: *Supliment la Revista Română de Medicină de Laborator.*Timişoara, România, 2018, nr.3, vol. 26, p. 50. ISSN 18416624.

4.1.5. **БАЛАН, Г.** и др. Видовая структура и резистентность к антибиотикам микроорганизмов, выделенных из инфицированных ран. В: *Материалы XII Межгосударственной научно-практической конференции „Вклад государств-участников содружества независимых государств в обеспечение санитарно-эпидемиологического благополучия населения в современных условиях"*. Саратов, Россия, 2014, с. 22-23. ISBN 978-5-4253-0773-6.

4.1.6. **BĂLAN, G.** et al. Staph wound intectlons and methicillin resistant *Staphylococcus aureus.* În: *Materealele celei de-a VII-a Conferinţă Naţională de Microbiologie şi Epidemiologie.* Bucureşti, România, 2014, vol. 59, p. 41. ISSN: 1220 – 3696.

4.1.7. **БАЛАН, Г.** и др. Антибиотикорезистентность *Staphylococcus aureus*, выделенных из гнойных ран. В: *Епідеміологічні дослідження в клінічній та профілактичній медицині: досягнення та перспективи: матеріали науково-практичної конференції з міжнародною*

участю, присвяченої 210-й річниці Харківського національного медичного університету та 85-річчю кафедри епідеміології. Харків, Україна, 2015, с. 35-37. ISBN 978-617-7225-50-7.

4.1.8. **BĂLAN, G.** et al. Prevalence and antibiotic sensitivity of bacteria isolated from nosocomial infections. In: *International Conference titled "Socio-psycho-medical changes in the lifestyles of the contemporary family". Antropological Research and Studies.* Bucharest, Romania, 2015, nr. 5, p. 12. ISSN-1 0039 – 3886.

4.1.9. **BĂLAN, G.** et al. Multidrug – resistent bacteria isolates in infected wounds. In: *International Conference titled "Socio-psycho-medical changes in the lifestyles of the contemporary family". Antropological Research and Studies.* Bucharest, Romania, 2015, nr. 5, p. 11. ISSN-1 0039 – 3886.

4.1.10. GULEA, A., CEBOTARI, D., **BĂLAN, G.** et al. Synthesis, structure and antimicrobial activity of some 3d-metal coordination compounds with 2-hydroxy-3-methoxybenzaldehyde 4-(dimethylphenyl)thiosemicarbazones. In: *Abstract book 4ème Colloque Franco-Roumain de Chimie Médicinale.* Iaşi, România, 2017, p. 72.

4.1.11. DIACOVA, S., **BALAN, G.** et al. Antimicrobial susceptibility of *Pseudomonas aeruginosa*. In: *Abstract book National ENT, Head and Neck Surgery Conference*. Sibiu, România, 2017, p. 122.

4.1.12. ЯСЫБАШ, О., **БАЛІАН, Г.** и др. Внебольничные инфекции, обусловленные метициллинрезистентным стафилококком. В: VI *Ежегодная Международная Научно-Практическая Конференция „Актуальные вопросы медицины"*. Баку, Азербайджан, 2017, с. 13-14. ISSN 978-81-942709-5-9.

4.1.13. **БАЛІАН, Г.** Спектр антибиотикоустойчивости и персистентные свойства штамов *Staphylococcus aureus* выделенных из содержимого трофических язв. В: VII Ежегодная Международная Научно-Практическая Конференция „Актуальные вопросы медицины ". Баку, Азербайджан, 2018, с. 15-17. ISBN 978-9952-8276-0-6.

4.1.14. **БАЛІАН, Г.** и др. Влияние препарата BioR на персистентные свойства микроорганизмов. В: *VII Ежегодная Международная Научно- Практическая Конференция „Актуальные вопросы медицины* ". Баку, Азербайджан, 2018, с. 12-13. ISBN 978-9952-8276-0-6.

4.1.15. GULEA, A., **BĂLAN, G.** et al. Synthesis, structure and biological activity of some 3d-metal coordination compounds with N'-[3-(methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)(phenyl)methylidene] benzohydra- zide. In: *A XXXV-a Conferinţă Naţională de Chimie. Călimăneşti-Căciulata,* Vâlcea, România, 2018, p. 24.

4.1.16. **BALAN, G.** Bacterial etiology and antibiotic susceptibility pattern of bacteria isolated from diabetic foot ulcers. In: *Materials of the Conference "Contemporary Issues In Preventive Medicine".* Yerevan, Republic of Armenia, 2018, p. 48-49. ISBN 978-9939-65-204-7.

4.1.16. **БЭЛІАН, Г.** и др. Исследование антибактериальных свойств координационных соединений меди(II) на основе этилового эфира 4-({[2-(2-гидроксибензилиден) гидразинил]карбонотиоил}-амино)бензоата. В: *VIII Ежегодная Международная Научно-Практическая Конференция „Актуальные вопросы медицины "*. Баку, Азербайджан, 2019, с. 4. ISSN 978-9952-8279-6-4.

4.1.17. **BALAN, G.** et al. A study on biofilm production among *Candida* species. In: *VIII Annual International Scientific-Practical Conference „Medicine pressing questions".* Baku, Azerbaijan, 2019, c. 60. ISSN 978-9952-8279-6-4.

4.1.18. **BALAN, G.** Biofilm formation by *Pseudomonas aeruginosa* isolated from trophic ulcers and their association with antimicrobial resistance. În: *Zilele U.M.F. din Craiova a XLIX-a. Volum de rezumate*. Craiova, România, 2019, p. 65. ISSN: 1843-2441.

4.1.19. **BALAN, G.** ş. a. Rezistenţa la antibiotice a tulpnilor de *Staphylococcus* spp. izolate din ulcere trofice a membrelor inferioare. În: *Zilele U.M.F. din Craiova a XLIX-a. Volum de rezumate.* Craiova, România, 2019, p. 67. ISSN: 1843-2441.

4.1.20. **BALAN, G.,** BURDUNIUC, O. Synergistic antimicrobial activity of some chemical compounds combined with spirulina extracts. In: *Scientific Collection «InterConf», (44): with the*

Proceedings of the 8 th International Scientific and Practical Conference «Scientific Research in XXI Century». Ottawa, Canada, 2021, pp. 437-439. ISBN 978-0-458-20903-3.

4.1.21. **BALAN, G.** Association between antibiotic resistance and virulence factors of Klebsiella pneumoniae strains. *Scientific Collection «InterConf», (45): with the Proceedings of the 3 th International Scientific and Practical Conference «Scientific Community: Interdisciplinary Research»*. Hamburg, Germany, 2021, pp. 324-329. ISBN 978-3-512-31217-5.

4.2. within international scientific conference database (in the Republic of Moldova)

4.2.1. **BĂLAN, G.,** RUDIC, V. Optimizing antimicrobial prescribing: meticillin resistant Staphylococcus aureus in staph wound infections. In: *Scientific international conference on microbial biotechnology (2nd edition).* Chişinău, 2014, p. 99. ISBN 978-9975-4432-8-9.

4.2.2. ORLIOGLO, A., **BĂLAN, G.** Antibiotic resistance of *Pseudomonas aeruginosa* strains. In: *The international conference - Life sciences in the dialogue of generations: „Connections between Universities, Academia and Business Community".* Chişinău, 2016, p.92.

4.2.2. RUDIC, V., **BĂLAN, G.** et al. Antibacterial and antifungal properties of algae product BioR. In: *International Scientific Conference on Microbial Biotechnology (3rd edition).* Chişinău, 2016, p. 81. ISBN 978-9975-3129-3-6.

4.2.3. **BĂLAN, G.** et al. Bacterial profile and antibiotic resistance in patients with trophic ulcers. In: *International Scientific Conference on Microbial Biotechnology (4nd edition).* Chişinău, 2018, p. 27. ISBN 978-9975-3178-8-7.

4.2.4. BURDUNIUC, O., **BĂLAN, G.** et al. Antimicrobial activity of some copper (II) coordination compounds with N-(4-(2-((3-methyl-5-oxo-1-phenyl)-4,5-dihydro-1H-pyrazol)methylene) hidrazincarbotioamido)phenyl)acetamide. In: *International Scientific Conference on Microbial Biotechnology (4nd edition).* Chişinău, 2018, p. 36. ISBN 978-9975-3178-8-7.

4.2.5. BURDUNIUC, O., **BĂLAN, G.** et al. Antifungal activity of some heterocyclic compounds. In: *International Scientific Conference on Microbial Biotechnology (4nd edition).* Chişinău, 2018, p. 37. ISBN 978-9975-3178-8-7.

4.2.6. RUSNAC, R., BOTNARU, M., ŢAPCOV, V., BURDUNIUC, O., **BĂLAN, G.**, GULEA, A. Azachalcone derivatives and their antifungal activity. In: *International Scientific Conference on Microbial Biotechnology (4nd edition).* Chişinău, 2018, p. 39. ISBN 978-9975-3178-8-7.

4.2.7. GRAUR, V., ŢAPCOV, V., MOLDOVAN, E., **BĂLAN, G.** et al. Antimicrobial and antifungal activities of iron (III), cobalt (III), nickel (II) and zinc (II) coordination compounds with 2,4-dihydroxyacetophenone 4-allylthiosemicarbazone. In: *International Scientific Conference on Microbial Biotechnology (4nd edition).* Chişinău, 2018, pp. 51-52. ISBN 978-9975-3178-8-7.

4.2.8. ŢAPCOV, V., COTOVAIA, A., MILENTIEV, A., BURDUNIUC, O., **BĂLAN, G.,** et al. Antimicrobial and antifungal effect of some biometal coordination compounds with 2-[(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)-(phenyl) methylidene]hydrazin-ecarboximidamide. In: *International Scientific Conference on Microbial Biotechnology (4nd edition).* Chişinău, 2018, pp. 55-56. ISBN 978-9975-3178-8-7.

4.2.9. USATAIA, I., GRAUR, V., ŢAPCOV, V., VASÎLCA, M., **BĂLAN, G.** et al. Antibacterial and antifungal activities of iron (III), cobalt (III), nickel (II) and copper (II) coordination compounds with 3,5-dibromosalicylaldehyde 4-allyl-S-methylisothiosemicarbazone. In: *International Scientific Conference on Microbial Biotechnology (4nd edition).* Chişinău, 2018, p. 57-58. ISBN 978-9975-3178-8-7.

4.2.10. GRAUR, V., ŢAPCOV, V., DIURICI, E., **BĂLAN, G.** et al. Bacteriostatic and bactericide activities of some 3D metal complexes with 2-[2-(prop-2-en-1-ylcarbamothioyl)-hydrazinylidene]propanoic acid. In: *International Scientific Conference on Microbial Biotechnology (4nd edition).* Chişinău, 2018, pp. 65-66. ISBN 978-9975-3178-8-7.

4.2.11. BIRCA, M., RUSNAC, R., BIRSANU, I., **BALAN, G.** et al. Synthesis, characterization, crystal structure and antimicrobial activity of copper(II) complexes. In: *International Conference Achievements and Perspectives of Modern Chemistry dedicated to the 60th anniversary from the foundation of the Institute of Chemistry.* Chişinău, 2019, p.78. ISBN 978-9975-62-428-2.

4.2.12. JOVMIR, T., POPA, T., **BALAN, G.** et al. Water-soluble dicopper complex having antimicrobial activity. In: *International Conference Achievements and Perspectives of Modern Chemistry dedicated to the 60th anniversary from the foundation of the Institute of Chemistry*. Chişinău, 2019, p. 117. ISBN 978-9975-62-428-2.
4.2.13. RUSNAC, A., **BALAN, G.** et al. Synthesis and antimicrobial properties of new copper(II) complexes with benzoate of ethyl 4-({2-[(pyridin-2yl)methylidene]hydrazinecarbothiolyl}amino). In: *International Conference Achievements and Perspectives of Modern Chemistry dedicated to the 60th anniversary from the foundation of the Institute of Chemistry*. Chişinău, 2019, p.140. ISBN 978-9975-62-428-2.
4.2.14. GULEA, A., ULCHINA, I., GRAUR, V., TSAPCOV, V., GHUMAKOV, YU., PETRENKO, P., **BALAN, G.,** BURDUNIUC, O. Synthesis, antimicrobial and antifungal properties of copper(II) coordination compounds with 2,4-dihydroxybenzaldehyde 4-allylthiosemicarbazone containing amines. In: *International Conference Achievements and Perspectives of Modern Chemistry dedicated to the 60th anniversary from the foundation of the Institute of Chemistry*. Chişinău, 2019, p. 149. ISBN 978-9975-62-428-2.
4.2.15. RUSNAC, A., **BĂLAN, G.** et al. Cercetarea relaţiei structură-activitate a compuşilor coordinativi ai Cu(II) cu benzoat de etil-4-tiosemicarbazone-2-formil(acetil şi benzoil)piridinei asupra microorganismelor *Acinetobacter baumannii* şi *Escherichia coli*. În: *Conferinţa ştiinţifică naţională cu participare internaţională „Integrare prin cercetare şi inovare”.* Chişinău, 2019, pp. 223-225. ISBN 978-9975-149-47-1.
4.2.16. IUNAC, D., **BALAN, G.** Rezistenţa la antibiotice şi factorii de persistenţă a tulpinilor de *Staphylococcus aureus* izolat din ulcere trofice. În: *Materialele Congresului consacrat aniversării a 75-a de la formarea USMF „Nicolae Testemiţanu”.* Chişinău, 2020, p. 174.
4.2.17. **BALAN, G.** Antimicrobial susceptibility and biofilm formation of *Klebsiella pneumoniae* strains. În: *Materialele Congresului consacrat aniversării a 75-a de la formarea USMF „Nicolae Testemiţanu”.* Chişinău, 2020, p. 165.
4.3. în lucrările conferinţilor ştiinţifice naţionale cu participare internaţională
4.3.1. **BALAN, G.** et al. Antibiotic resistance of *Pseudomonas aeruginosa* strains isolated from patients. In: *Materials of the National Scientific Conference with international participation "One Health" approach in a changing world.* One Health&Risk Management, Supplement, 2021, vol. 2, nr. 4, p. 28.
4.3.2. **BALAN, G.** The influence of biological active compounds on markers of oxidative stress and antioxidant system. In: *Materials of the National Scientific Conference with international participation "One Health" approach in a changing world.* One Health&Risk Management, Supplement, 2021, vol. 2, nr. 4, p. 92.
4.3.3. BURDUNIUC, O., BIVOL, M., BRINZA, M., CRACIUN, O., **BALAN, G.** Emergence of carbapenem-resistant enterobacteriaceae: overview of a major public health challenge. In: *Materials of the National Scientific Conference with international participation "One Health" approach in a changing world.* One Health&Risk Management, Supplement, 2021, vol. 2, nr. 4, p. 29.
4.3.4. CRECIUN, L., **BALAN G.** Antibiotic resistance and biofilm formation of strains isolated from trophic ulcers. In: *Materials of the National Scientific Conference with international participation "One Health" approach in a changing world.* One Health&Risk Management, Supplement, 2021, vol. 2, nr. 4, p. 17.

6. Patents and other intellectual property (IPO)

- Patents

6.1. BURDUNIUC, O., **BĂLAN, G.** ş. a. *Compuşi ai cuprului (II) cu 4-aliltiosemicarbazona 2-acetilpiridinei în calitate de inhibitori ai proliferării fungilor din specia Cryptococcus neoformans.* //Brevet de invenție MD 4621 C1 2019.09.30. BOPI nr. 2/2019 din 28.02.2019.
6.2. GULEA, A., BURDUNIUC, O., **BĂLAN, G.** ş. a. *Utilizare a N-ciclohexil-2-[1-(piridin-2-il)]-hidrazincarbotioamidei în calitate de inhibitor al proliferării microorganismelor gram-*

pozitive și fungilor Candida albicans. // Brevet de invenție MD 4648 C1 2020.04.30. BOPI nr. 9/2019 din 30.09.2019.

6.3. **BĂLAN, G.** ş. a. *Complecşii sulfatului de cupru(II) cu 2-(2-hidroxibenziliden)-N-(metoxifenil)hidrazincarbotioamide, care manifestă activitate antimicrobiană faţă de microorganismele gram-pozitive.* // Brevet de invenție MD 4652. C1 2020.04.30. BOPI nr. 9/2019 din 30.09.2019.

4.4. GULEA, A., ȚAPCOV, V., **BĂLAN, G.** ş. a. *Tetrachis{[m3-2-(1-oxibutan-2-il) iminometil]-fenolato(2-)-O,N,O-Oalc}-tetracupru(II), care manifestă activitate antimicrobiană și antimicotică.* // Brevet de invenție MD 4667 C1 2020.07.31. BOPI nr. 12/2019 din 31.12.2019.

4.5. GULEA, A., ȚAPCOV, V., CEBOTARI, D., **BĂLAN, G.** ş. a. *Inhibitor al proliferării fungilor din specia Cryptococcus neoformans în baza hidratului de cloro-{[4-(2,4-dimetilfenil)-2-(oxo-3-metoxibenziliden)hidrazincarbotioamido(1-)]-O,N,S}-{[4-(2,4-dimetilfenil)-5-(2-hidroxi-3-metoxifenil)-2,4-dihidro-3H-1,2,4-triazol-3-tion]-S}cupru.* // Brevet de invenție MD 4675 C1 2020.11.30. BOPI nr. 2/2020 din 29.02.2020.

4.6. GULEA, A., **BĂLAN, G.** ş. a. *[N'-(3,5-dibromo-2-oxidobenziliden)-N-prop-2-en-1-ilcarbamo-hidrazontioato] piridincupru, care manifestă activitate bacteriostatică faţă de bacteriile din speciile Bacillus cereus și Bacillus subtilis.* // Brevet de invenție MD 4707 B1 2020.08.31. BOPI nr. 8/2020 din 31.08.2020.

4.7. GORINCIOI, V., LOZAN, V., BURDUNIUC, O., **BĂLAN G.,** ş. a. *Utilizarea tetrasalicilatului de bariu-cupru în calitate de inhibitor al proliferării fungilor Cryptococcus neoformans.* // Brevet de invenție MD 4712 B1 2020.09.30. BOPI nr. 9/2020 din 30.09.2020.

4.8. JOVMIR, T., **BĂLAN, G.** ş. a. *Compus hidrosolubil 2,6-bis(S-metilizotiosemicarbazidometiliden)-4-metilfenolato-tricloro-dicupru-hidrat pentru utilizare în calitate de remediu farmaceutic selectiv contra bacteriiilor Gram-pozitive.* // Brevet de invenție MD 4687 C1 2021.01.31. BOPI nr. 4/2020 din 30.04.2020.

4.9. GORINCIOI, V., LOZAN, V., BURDUNIUC, O., **BĂLAN, G.** ş. a. *Utilizarea clusterului de oxohepta(salicilat)trifier(III)-polisolvat în calitate de inhibitor al proliferării fungilor din specia Cryptococcus neoformans.* // Brevet de invenție MD 4742 B1 2021.02.28. BOPI nr. 2/2021 din 28.02.2021.

Scientifically methodical and didactic works

10. Methodical indications / guidelines

10.1. BURDUNIUC, O., COJOCARU, R., **BĂLAN, G.** ş. a. *Detectarea de laborator a producerii de betalactamaze la microorganismele familiei Enterobacteriaceae.* Chişinău: Tipografia „Print-Caro". 2013, 49 p. ISBN 978-9975-113-84-7.

10.2. BURDUNIUC, O., **BĂLAN, G.** *Antimicrobial susceptibility testing.* Chişinău: Tipografia „Print-Caro". 2014, 37 p. ISBN 978-9975-56-169-3.

10.3. **BĂLAN, G.** ş. a. *Factorii de patogenitate a microorganismelor – semnificaţia lor în patologia infecţioasă.* Indicaţie metodică. Chişinău: „Print-Caro", 2018, 72 p. ISBN 978-9975-56-615-5.

10,3. **BĂLAN, G.,** RUDIC, V. *Ghid privind managementul ulcerului trofic infectat.* Chişinău: „Print-Caro SRL", 2021, 12 p. ISBN 978-9975-56-905-7.

10.4. BURDUNIUC, O., **BĂLAN, G** ş. a. *Ghid: Detectarea mecanismelor de rezistenţă la antimicrobiene, interpretarea şi aplicarea clinică a rezultatelor.* Spre publicare.

ADNOTARE
BĂLAN Greta
COMPUŞI NOI CU ACŢIUNE ASUPRA MICROORGANISMELOR IZOLATE DIN ULCERE TROFICE
Teza de doctor habilitat în ştiinţe medicale, Chişinău, 2022

Structura: Introducere, reviul literaturii, materiale şi metode de cercetare, patru capitole explorative, concluzii generale și recomandări, bibliografia din 350 de titluri, 15 anexe, 191 pagini de text de bază, 47 de figuri și 40 de tabele. Rezultatele obţinute sunt publicate în 77 de lucrări ştiinţifice.

Cuvinte cheie: ulcer trofic infectat, factori de patogenitate, compuşi noi, activitate antimicrobiană.

Domeniul de studiu: Microbiologie, Virusologie medicală.

Scopul: Evaluarea activităţii antimicrobiene a unor compuşi noi în vederea formulării principiilor de elaborare a preparatelor policomponente eficiente în tratamentul ulcerului trofic infectat.

Obiectivele lucrării: Determinarea spectrului etiologic al microorganismelor izolate din ulcere trofice infectate; elucidarea fenotipurilor de rezistenţă la antimicrobiene şi a factorilor de patogenitate ai tulpinilor izolate din ulcere trofice; studiul calitativ şi cantitativ al activităţii antimicrobiene a unor entităţi chimice şi biologice noi faţă de tulpinile microbiene de referinţă şi clinice; studierea acţiunii sinergice a compușilor monocomponenți și a posibilităţilor de obţinere a compuşilor policomponenţi cu acţiune antimicrobiană; determinarea influenţei compuşilor noi monocomponenţi şi în combinaţie asupra expresiei unor factori de patogenitate a microorganismelor; evaluarea modificărilor unor parametri biochimici ai culturilor bacteriene sub influența substanţelor monocomponente şi în combinaţie; influenţa compuşilor biologic activi asupra markerilor stresului oxidativ, sistemului antioxidant și pattern-ului inflamator pentru obţinerea potenţialelor preparate medicamentoase, eficiente în tratamentul ulcerului trofic infectat.

Noutatea şi originalitatea ştiinţifică: În premieră, pe baza unui studiu complex, a fost evaluată acţiunea antimicrobiană, antioxidantă şi imunomodulatoare a unor compuşi noi, determinat spectrul microorganismelor implicate în ulcere trofice infectate şi stabilită acţiunea compușilor noi asupra factorilor de patogenitate ai agenților microbieni.

Problema ştiinţifică soluționată: fundamentarea științifică a acțiunii unor compuși chimici și biologici noi asupra microorganismelor izolate din ulcere trofice infectate. Aceasta contribuie la formularea principiilor de dezvoltare a preparatelor policomponente eficiente în tratamentul ulcerului trofic infectat și la elaborarea unor strategii antiinfecţioase alternative, în scopul evitării eșecurilor terapeutice și dezvoltării rezistenței la antimicrobiene.

Semnificaţia teoretică şi valoarea aplicativă a studiului: Rezultatele prezentei cercetări completează studiile anterioare despre efectul compușilor chimici și biologici asupra tulpinilor de referință și a tulpinilor clinice, izolate din ulcerele trofice, despre acțiunea sinergică a acestor compuși și despre combinaţiile de compuşi care potenţiază efectul antimicrobian. Totodată au fost acumulate date despre influenţa compuşilor noi asupra expresiei factorilor enzimatici de patogenitate şi a factorilor de persistenţă ai microorganismelor. Datele obținute pot fi utilizate ca dovezi la elaborarea unor strategii antiinfecţioase alternative şi la obținerea altor compuşi policomponenţi, generând astfel nu doar o utilizare empirică ci şi una bazată pe dovezi științifice privind proprietăţile lor terapeutice. Rezultatele obținute au permis evidențierea efectului imunomodulator al compuşilor chimici şi biologici noi aparte şi în combinaţie, prin determinarea acţiunii lor asupra producerii spontane a indicilor biochimici și imunochimici. Rezultatele privind pattern-urile de rezistenţă la antibiotice vor servi drept argument pentru reactualizarea listei de antimicrobiene și procurarea argumentată a acestora, pentru elaborarea măsurilor coerente de control.

Implementarea rezultatelor ştiinţifice: Rezultatele studiului au fost implementate în activitatea laboratoarelor microbiologice, a medicilor din cadrul Centrelor de Sănătate, a secțiilor de chirurgie, în procesul didactic la Disciplina de microbiologie şi imunologie, Departamentul Medicină Preventivă, USMF „Nicolae Testemiţanu”.

SUMMARY

BALAN Greta

THE NEW COMPOUNDS ACTING ON MICROORGANISMS ISOLATED FROM TROPHIC ULCERS

The Habilitation Thesis in Medical Sciences, Chisinau, 2022

Structure: This research paper includes introduction, literature review, research materials and methods, 4 investigative chapters, general conclusions and recommendations, a bibliography list of 363 references, 15 annexes, 191 pages of basic text, 47 figures, and 40 tables. The obtained results have been published in 77 scientific papers.

Keywords: trophic ulcer, pathogenic factors, new compounds, antimicrobial activity.

Field of study: Microbiology and Medical Virology.

The purpose of the research: To assess the antimicrobial activity of new compounds in order to develop the principles for obtaining multicomponent drug products effective in the treatment of infected trophic ulcers.

The research objectives: To determine the etiological spectrum of microorganisms isolated from infected trophic ulcers; to highlight the antimicrobial resistance phenotypes and the pathogenicity factors of microbial strains isolated from trophic ulcers; to perform a qualitative and quantitative study of the new chemical and biological antimicrobial entities compared to the reference and clinical microbial strains; to study the synergistic action of single-component substances and the possibility to obtain multicomponent antimicrobial compounds; to determine the effects of new single-component compounds and their combination on the manifestations of microbial pathogenicity factors; to assess the biochemical changes of bacterial cultures under the action of single-component and combined substances; to study the impact of biologically active compounds on oxidative stress markers, antioxidant system and inflammatory patterns for the development of potential drugs.

Scientific novelty and originality of the research: We report for the first time a comprehensive study assessing the antimicrobial, antioxidant and immunomodulatory activity of new compounds, as well as determining the microbial spectrum associated with the infected trophic ulcers and activity of new compounds on microbial pathogenicity factors.

The scientific problem solved: The present research scientifically validated the activity of new chemical and biological compounds on microorganisms isolated from infected trophic ulcers. It contributes to developing the principles for the improvement of effective multicomponent drug products in the treatment of infected trophic ulcers and for development of alternative anti-infective strategies to prevent therapeutic failures and the emergence of antimicrobial resistance.

Theoretical significance and applicative value of the study: The research findings complement the previous studies on the effect of chemical and biological compounds on the reference and clinical strains isolated from trophic ulcers, as well as on the synergistic action of these compounds by identifying combinations of compounds that might enhance the antimicrobial effect. At the same time, new data were collected regarding the influence of new compounds on the manifestations of enzymatic pathogenicity and microbial persistence factors. The obtained evidence-based data can be used to develop alternative, anti-infective strategies and other multicomponent drug compounds, thus providing both an empirical approach and scientific evidence of their therapeutic properties. The obtained results allowed highlighting the immunomodulatory effects of single and combined chemical and biological compounds by determining their action on the spontaneous production of biochemical and immunochemical indices. The study results regarding the antibiotic resistance patterns will enable updating of the list of antimicrobials and organizing their justified procurement, as well as developing rational control measures.

Implementation of scientific results. The results of the study were introduced within the microbiological laboratories, the medical practice of the Health Centers, at surgery departments, in teaching the Discipline of Microbiology and Immunology at the Department of Preventive Medicine of "Nicolae Testemitanu" SUMPh.

АННОТАЦИЯ
БЭЛАН Грета
НОВЫЕ СОЕДИНЕНИЯ С ВОЗДЕЙСТВИЕМ НА МИКРООРГАНИЗМЫ ИЗОЛИРОВАННЫЕ ИЗ ТРОФИЧЕСКИХ ЯЗВ
Диссертация доктора медицинских наук. Кишинев, 2022.

Структура: Введение, обзор литературы, материалы и методы исследования, 4 ознакомительных глав, общие выводы и рекомендации, библиография из 350 наименований, 15 приложений, 191 страниц основного текста, 47 рисунков, 40 таблиц. Полученные результаты опубликованы в 77 научных статьях.
Ключевые слова: трофическая язва, патогенные факторы, новые соединения, антимикробное действие.
Специализация: Микробиология, медицинская вирусология.
Цель: оценка антимикробной активности новых соединений с целью формулировки принципов разработки поликомпонентных препаратов, эффективных при лечении инфицированных трофических язв.
Задачи работы: Определение этиологического спектра микроорганизмов, выделенных из инфицированных трофических язв; выявление фенотипов устойчивости к противомикробным препаратам и факторов патогенности штаммов микроорганизмов, выделенных из трофических язв; качественное и количественное изучение антимикробной активности новых химических и биологических соединений по отношению к эталонным и клиническим штаммам микробов; изучение синергетического действия однокомпонентных веществ и возможности получения поликомпонентных соединений с антимикробным действием; определение влияния новых однокомпонентных соединений и их комбинаци на проявление факторов патогенности микроорганизмов; оценка изменения биохимических показателей бактериальных культур под влиянием однокомпонентных веществ и их комбинаций; изучение влияния биологически активных соединений на маркеры окислительного стресса, антиоксидантную систему.
Научная новизна и оригинальность: Впервые, на основе комплексного исследования, было оценено антимикробное, антиоксидантное и иммуномодулирующее действие новых соединений, определен спектр микроорганизмов, вовлеченных в этиопатогенез инфицированных трофических язв, и действие новых соединений на факторы микробной патогенности.
Решенная научная задача: Научное обоснование действия новых химических и биологических соединений на микроорганизмы, выделенных из инфицированных трофических язв. Это способствует формулированию принципов разработки эффективных поликомпонентных препаратов для лечения инфицированных трофических язв и выработке альтернативных противоинфекционных стратегий, позволяющих избежать терапевтических неудач и развития устойчивости к противомикробным препаратам.
Теоретическая значимость и прикладная ценность исследования: результаты исследования дополняют предыдущие исследования влияния химических и биологических соединений на эталонные штаммы, клинические штаммы, выделенные из трофических язв, и синергетическое действие этих соединений путем выявления комбинаций соединений, которые усиливали противомикробный эффект. Одновременно накапливались новые данные о влиянии новых соединений на экспрессию ферментативных факторов патогенности и факторов персистенции микроорганизмов. Полученные данные могут быть использованы в качестве доказательства для получения альтернативных противоинфекционных стратегий и других поликомпонентных соединений, таким образом обеспечивая их не только эмпирическое использование, но и основанное на научных данных их терапевтические свойства. Полученные результаты позволили выделить иммуномодулирующее действие химических и биологических соединений по отдельности и в комбинации, определив действие этих соединений на спонтанную выработку биохимических и иммунохимических показателей. Результаты, касающиеся паттернов устойчивости к антибиотикам, послужат доказательством для обновления списка противомикробных препаратов и организации их обоснованной закупки, разработки последовательных мер контроля.
Внедрение научных результатов. Результаты исследования были внедрены в деятельность микробиологических лабораторий, врачей медицинских центров, хирургических отделений, в учебный процесс по Дисциплине микробиология и иммунология Департамента профилактической медицины, КГУМиФ «Николае Тестемицану».

BALAN Greta

THE NEW COMPOUNDS ACTING ON MICROORGANISMS ISOLATED FROM TROPHIC ULCERS

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