SYNERGISTIC ACTION OF CHEMICAL COMPOUNDS AND SPIRULINA EXTRACTS

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Keywords: *chemical compounds, biological compounds, synergistic action, antimicrobial action.*

Introduction. *Antimicrobial resistance is a critical global issue, and the demand for new drugs is urgent. The current model of antimicrobial discovery is not providing sufficient new agents to address current levels of antimicrobial resistance. In this study, we aim to assess the antimicrobial activity of recently synthesized chemical compounds and spirulina extracts, along with their associative effects.*

Material and methods. *It is an experimental study, which includes seven newly synthesized chemical compounds and three extracts from Spirulina platensis.*

Results. *The in vitro results of the present study determined the MIC values of chemical compounds and spirulina extracts against gram-negative, gram-positive microorganisms, and fungi of the genus Candida. The optimal combination of two compounds can enhance benefits over time and minimize side effects. The synergistic or partially synergistic effects of biological compounds in conjunction with chemical compounds strongly corroborated this assertion. Time-kill curves and FICI scores confirmed the ability of the compounds to synergistically reduce the microbial count below the lowest detectable limit within 24 hours.*

Conclusions. *Our study offers a potential therapeutic option for antibiotic-resistant microbial agents by combining natural extracts with a variety of chemical compounds from different classes. The results of the present study are promising, and this knowledge holds potential utility for the development of future therapeutic strategies.*

Keywords: *chemical compounds, biological compounds, synergistic action, antimicrobial action.*

Cuvinte-cheie: *compuși chimici, compuși biologici, acțiune sinergică, acțiune antimicrobiană.*

ACȚIUNEA SINERGICĂ A COMPUȘILOR CHIMICI ȘI A EXTRACTELOR DIN SPIRULINĂ

Introducere. *Rezistența la antimicrobiene reprezintă o problemă globală gravă, de aceea obținerea unor noi remedii cu acțiune antimicrobiană este o urgență. Modelul actual de descoperire a antimicrobienelor nu furnizează agenți noi, suficienți pentru combaterea nivelurilor actualre de rezistență la antimicrobiene. În acest studiu ne-am propunem să evaluăm activitatea antimicrobiană a unor compuși chimici noi sintetizați și a extractelor din spirulină, precum și efectele asociative ale acestora.*

Material și metode. *În prezentul studiu experimental au fost testați șapte compuși chimici noi sintetizați și trei extracte din Spirulina platensis.*

Rezultate. *In vitro au fost determinate valorile CMI ale celor șapte compuși chimici noi sintetizați și a trei extracte din spirulină împotriva microorganismelor gramnegative, grampozitive și a micetelor din genul Candida. Combinația adecvată a doi compuși poate mări beneficiile în timp și minimaliza reacțiile adverse. Efectele sinergice sau parțial sinergice ale compușilor biologici în combinație cu compușii chimici au susținut acest efect. Curbele de omorâre în timp și scorurile FICI au confirmat capacitatea compușilor testași de a reduce sinergic numărul de microorganisme sub cea mai mică limită detectabilă în 24 de ore.*

Concluzii. *Studiul oferă o opțiune terapeutică potențială pentru agenții microbieni rezistenți la antibiotice prin combinarea extractelor naturale și a compușilor chimici din diferite clase. Rezultatele obținute sunt promițătoare și pot fi utile pentru dezvoltarea viitoarelor strategii terapeutice.*

INTRODUCTION

Bacterial resistance to conventional antimicrobials has become a significant and life-threatening issue worldwide, imposing a substantial economic burden on healthcare systems.

Over the recent decades, the number of antimicrobial-resistant bacteria has increased dramatically due to the widespread use of antibiotics (1, 2). Despite the emergence of new antibiotics with different mechanisms of action, the process of drug discovery and development typically spans between 10 and 17 years, with a success rate below 10% (3). Furthermore, while new single-target antimicrobials are widely used, new antibiotic-resistant strains will inevitably arise.

This ever-growing challenge makes the discovery of new antibiotics inevitable, as well as the development of new alternative approaches. Increased attention among these alternative approaches has been given to antimicrobials combined with plant extracts. The latter approach, namely, the combination therapy or synergistic therapy used in combating resistant microorganisms can lead to new ways of treating infectious diseases, which is likely to outline a potential area for future investigations. Combination therapy is also efficient in patients with severe infections caused by antibiotic-resistant pathogens. Synergism consists of intensifying the pharmacodynamic effects of two or more associated compounds (4).

Combination therapy is the most common empirical treatment recommended in cases of bacterial infections within intensive care units, where monotherapy may not be effective against all the potential disease-causing pathogens. Moreover, combination therapy may reduce the drug dosage and thus minimize the side effects, which might result in overcoming the toxicity problem and the spread of resistant microbial strains (5).

MATERIAL AND METHODS

Chemical compounds

The study included the following chemical compounds, which were synthesized at the Department of Inorganic Chemistry, Department of Chemistry, at State University of Moldova: C1 ($C_{14}H_{19}CuN_7O_4S$), C2 ($C_{13}H_{16}Br_2CuN_4S$), C3 ($C_{10}H_{14}CuN_4O_5S_2$), C4 ($C_{13}H_{17}ClCuN_4S$), C5 ($C_{18}H_{20}CuN_4O_2S$), C6 ($C_{14}H_{20}N_4S$) and C7 ($C_{14}H_{19}ClCuN_4S$).

ES1, ES2 and ES3 Spirulina extracts

The biologically active complexes *ES1, ES2* and *ES3* extracts were obtained through a biotechnological process from the cyanobacterial strain of *Spirulina platensis* CNMN CB-02 (Spirulina), stored within the National Collection of Non-Pathogenic Microorganisms, at the Institute of Microbiology and Biotechnology.

Spirulina biomass was obtained from a cyanobacterial growth culture and through controlled synthesis of its biologically active compounds. Biologically active complexes, encompassing free amino acids, oligopeptides, proteins, sulphated polysaccharides, and phospholipids, were extracted from the Spirulina biomass. These extracts were successively fractionated and purified by using benign solvents and techniques. Relevant formulas were developed and standardized for complex compositions of extracts, based on biologically active complexes derived from Spirulina biomass. All extracts were natural and devoid of herbicides, toxins, or preservatives.

ES1 spirulina extract is an amino acid/oligopeptide complex, which contains non-essential (glycine, alanine, serine, cysteine, tyrosine, aspartic acid, glutamic acid, and proline) and essential amino acids (arginine, phenylalanine, histidine, isoleucine, leucine, lysine, threonine, tryptophan, and valine), being in their free state or combined in oligopeptides (up to 10kDa), as well as biologically functionalized macro- and microelements. *In vitro* tests were used for *ES1 form*, which is an alcoholic solution, having 10mg/ml of extract concentration and 50% of alcohol concentration. *ES2 spirulina extract* is a synergistic combination of amino acid/oligopeptide complex, sulfated polysaccharides, proteins, and biologically functionalized macro- and microelements derived from spirulina. For the *ES2 form*, *in vitro* tests were conducted using an alcoholic solution with a concentration of 20 mg/ml of extract and 45% alcohol concentration.

ES3 spirulina extract is a glycosidic carotenoid. *In vitro* tests were conducted using an 80% aqueous-ethanol solution.

Microbial strains

Microbial strains of *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC*

27853, Acinetobacter baumannii ATCC BAA-747, and Candida *albicans ATCC 10231* were used in the study. The microbial strains were cultured on appropriate nutrient media at their optimum growth temperature. Overnight cultures of the bacterial strains were used for further investigations.

Antimicrobial activity

Serial culture dilutions were used to determine the antibacterial activity of the plant, allowing for the assessment of the minimum inhibitory concentration (MIC) and the minimum bactericidal/fungicidal concentration (MBC/MFC). MIC and MBC/MFC were determined using a discontinuous gradient of extract concentrations tested in Muller-Hinton broth, followed by the addition of 100µL of bacterial suspension according to the 0.5 McFarland turbidity standard. The tubes were then incubated at 35-37°C for 18-24 hours, and the MIC value was determined by macroscopic analysis of the tubes, based on the presence or absence of bacterial growth. MBC/MFC was determined by replicates of Muller-Hinton agar dilution. The MBC/MFC value represented the lowest concentration of extract that reduced the number of microbial colonies by up to 99.9% (6, 7).

The negative control sample consisted of Muller-Hinton broth containing the studied extracts, while the positive control sample involved Muller-Hinton broth inoculated with the studied microorganisms. All experiments were conducted in triplicate.

Methods of synergy testing

The combined antibacterial effects of two preparations were assessed using the checkerboard method, followed by the determination of the FIC index (Fractional Inhibitory Concentration). Stock solutions and double dilutions of each compound were prepared in accordance with EUCAST recommendations immediately before testing (6). The assays were conducted in multiwell plates, with 50µL of Mueller-Hinton broth added per well. The first combined compound was serially diluted along the y-axis (y), while the second compound was diluted along the abscissa axis (x). A suspension corresponding to the McFarland 0.5 turbidity standard was prepared from the tested strain, and subsequently, 100 µL of microbial suspension ($5x10^5$CFU / ml) was added to each well. The plates were incubated at 35°C for 24 hours aerobically.

Fractional inhibitory concentration was calculated using the MIC of the combined compounds, as well as the MIC of each compound obtained through parallel testing, according to the following formula:

where,

$$\Sigma FIC = FIC\ (A) + FIC\ (B)$$

$$FIC\ (A) = \frac{MIC\ (A)\ combination}{MIC\ (A)\ alone}$$

and

$$FIC\ (B) = \frac{MIC\ (B)\ combination}{MIC\ (B)\ alone}$$

The ΣFICI values were interpreted as follows: ∑FIC ≤ 0,5 = Synergistic; 0,5 < ∑FICI ≤ 1 = Additive; 1 < ∑FICI ≤ 4 = Indifferent; ∑FIC > 4,0 = Antagonistic.

Time-Kill Assay

Time-Kill Assay enables the measurement of changes in the population of aerobic microorganisms over a defined period in the presence of antimicrobial agents. The assay was conducted on *S.aureus, E.coli* and *C.albicans* strains, based on the aforementioned method with minor modifications (8). A saline suspension was prepared from an 18-24 hour microbial culture, by finally obtaining an inoculum of 1×10^6 CFU/mL. This suspension was evenly distributed into four tubes: I tube (culture control sample) - Mueller Hinton broth; II tube - Mueller Hinton broth + 0.5×CMI extract; III tube - Mueller Hinton broth + 1xCMI extract; IV tube - Mueller Hinton broth + 2×CMI extract. The tubes were incubated at 35°C for 24 hours. 100µL from each tube was replicated on the plate medium at specific intervals. Afterwards, the plates were incubated at 37°C for 24 hours and the CFU/plate was measured, followed by CFU/mL (the mean number of colonies multiplied by dilution). The tests were performed in triplicate (three independent experiments).

Time-Kill curves were graphically represented by $\log_{10}$CFU mL^{-1} versus the 24-hour time period. Bactericidal activity (99.9% of killing) was defined as a ≥3-$\log_{10}$CFU mL^{-1} reduction in the number of colonies from the initial inoculum.

Statistical analysis

Data are expressed as mean standard deviation. Statistical analysis involved a one-way analysis of variance (ANOVA). $P<0.001$ was considered to indicate a statistically significant difference.

RESULTS

The antimicrobial effect of chemical compounds and biologically active complex extracts derived from the cyanobacterium *Spirulina platensis*, namely *ES1, ES2,* and *ES3,* were observed during the initial stage of the present research.

Based on this, we conducted in vitro assays to assess the bactericidal and antifungal effects of seven new chemical compounds on gram-positive bacteria, gram-negative bacteria, and fungi of the genus Candida. The tests demonstrated that all chemical compounds exhibited superior bactericidal and bacteriostatic actions against gram-positive bacteria. In comparison to control drug preparations (Furacillin and Miconazole), most compounds were effective at lower doses (tab. 1).

Table 1. The antibacterial activity of chemical compounds against some microbial strains (µg/mL).

Chemical compounds		*S. aureus*	*B. subtilis*	*A. baumannii*	*E. coli*	*P. aeruginosa*	*C. albicans*
C1	**MIC**	0.976	0.976	0.976	7.812	250	15.62
	MBC	1.953	0.976	1.953	15.62	500	31.25
C2	**MIC**	0.976	0.976	15.62	15.62	125	3.906
	MBC	1.953	1.953	31.25	31.25	250	7.812
C3	**MIC**	0.122	0.122	1.953	3.906	15.62	31.25
	MBC	0.244	0.244	3.906	7.812	31.25	62.5
C4	**MIC**	0.488	0.122	7.812	15.62	>500	1.953
	MBC	1.953	0.122	15.62	31.25	>500	31.25
C5	**MIC**	0.488	0.244	3.906	7.81	125	7.81
	MBC	0.976	0.488	7.812	15.62	250	15.62
C6	**MIC**	0.061	1.953	0.25	>500	>500	0.976
	MBC	0.244	3.906	0.50	>500	>500	1.953
C7	**MIC**	0.976	0.488	62.50	31.25	>500	7.812
	MBC	1.953	0.976	125	62.50	>500	15.62
Furacillinum	**CMI**	4.67	4.67	4.67	4.67	4.67	-
	MBC	4.67	4.67	9.35	4.67	9.35	-
Miconazole	**MIC**	-	-	-	-	-	16.0
	MFC	-	-	-	-	-	32.0

Note: *S. aureus* (*Staphylococcus aureus* ATCC 25923); *B. subtilis* (*Bacillus subtilis* ATCC 6633); *A. baumannii* (*Acinetobacter baumannii* BAA-747); *E. coli* (*Escherichia coli* ATCC 25922); *P. aeruginosa* (*Pseudomonas aeruginosa* ATCC 27853); *C. albicans* (*Candida albicans* ATCC 10231). MIC – minimum inhibitory concentration; MBC – minimum bactericidal concentration; MFC – minimum fungicidal concentration.

C6 (*S. aureus* MIC 0.061 µg/mL), C4 and C5 (*S. aureus* MIC 0.488 µg/mL) were identified as the most active compounds against gram-positive strains. Compounds C6 (*A. baumannii* MIC 0.25 µg/mL) and C1 (*A. baumannii* MIC 0.976) exhibited a higher activity on gram-negative strains. Only four compounds were active against *P. aeruginosa* strains, the most active being C3 (MIC 15.62).

C. albicans strain demonstrated sensitivity to all tested compounds, whereas C6 (MIC 0.976) and C4 (MIC 1.953) compounds showed a higher low-dose activity.

The experimental testing of the biological compounds showed that all extracts of *S. platensis* exhibited promising antimicrobial activity against both gram-positive and gram-negative bacterial strains used in the present research (tab. 2).

The *ES3* extract exhibited a high antibacterial and antifungal activity compared to *ES1* and *ES2* extracts. The highest activity of *ES3* extract was recorded against *B. subtilis* strains (MIC 0.004 mg/mL) and *C. albicans* strains (0.004 mg/mL), as well as against gram-negative bacillus strains, being active at MIC 0.009 mg/mL. The *ES2* extract displayed bacteriostatic activity against all tested species at higher concentrations, compared to *ES1* and *ES3* extracts.

Table 2. The action of biologically active complex extracts of *Spirulina platensis* against some bacterial strains.

Species	*ES1*		*ES2*		*ES3*		Ampicillin
	MIC mg/mL	MBC mg/mL	MIC mg/mL	MBC mg/mL	MIC mg/mL	MBC mg/mL	MIC µg/mL
Gram-positive bacteria							
***S. aureus* ATCC 25923**	0.625	1.25	2.5	5.0	0.018	0.037	0.08
***B. subtilis* ATCC 6633**	0.156	0.156	2.5	5.0	0.004	0.004	0.078
Gram-negative bacteria							
P. aeruginosa* ATCC *27853	0.625	1.25	1.25	2.5	0.009	0.018	0.012
***E. coli* ATCC 25922**	0.625	1.25	2.5	5.0	0.009	0.018	0.025
***A. baumanni* BAA-747**	0,625	1.25	1.25	2.5	0.009	0.018	0.25
Yeast	MIC mg/mL	MFC mg/mL	MIC mg/mL	MFC mg/mL	MIC mg/mL	MFC mg/mL	Miconasole MIC µg/ml
***C. albicans* ATCC 10231**	0.625	1.25	1.25	2.5	0.004	0.009	16.0

Subsequently, the synergistic action of the chemical compounds combined with the biological compounds was assayed on six reference strains. The determination of the Fractional Inhibitory Concentration Index revealed synergistic actions in 87.2% of cases, additive actions in 6.8%, and indifferent actions in 6.0%. The tested compounds did not show any antagonistic relationship (FICI> 4) (ta. 3).

The testing of the chemical compound C1 combined with three biological compounds revealed the synergism phenomenon in most cases, except for *P. aeruginosa* bacteria, where the additive and indifferent phenomenon were registered in combinations with *ES1* and *ES2*, respectively.

The C2 compound showed synergism against the tested bacteria in all combinations, with the exception of its combination with the ES2 compound. In this particular case, an indifferent action (FICI-1.56) was observed against the *P. aeruginosa* strain.

The C3 compound, in combination with biological compounds, exhibited synergistic action in 72.2% of cases, additive action in 22.2% of cases, and indifferent action in 5.6% of cases against the tested strains, whereas the C4 compound showed predominantly synergistic action (94.4%) and additive action in only 5.6% of cases.

The C5 compound, when combined with *ES2*, showed indifferent action against *E. coli* and *P. aeruginosa* strains. Additionally, when combined with *ES1*, it showed indifference against *P. aeruginosa* strains only.

C6 exhibited indifferent action against *A. baumannii* strain when combined with *ES2* and showed additive action in combination with *ES1*. On the other hand, the C7 compound, when combined with *ES2*, revealed an additive effect against *E. coli* strains.

The chemical compounds combined with the biological compound *ES3* showed a synergistic action against all tested bacterial species, except for C3 that showed additive effects (FICI 0.56) against *P. aeruginosa* strains.

The minimum inhibition concentrations for the combined chemical and biological compounds resulted in a 4 to 32-fold decrease in the MIC of the parent compound (tab. 4). The additive and indifferent actions were particularly recorded against gram-negative bacilli and fungi of the *Candida* genus.

To determine the synergistic activity of the chemical compounds in combination with the biological compounds, along with the treatment duration and efficiency on the viability of microbial cells, the time-kill behavior was assessed.

Table 3. Interaction between chemical compounds and biological compounds expressed by FICI.

Tested compounds		*S. aureus* ATCC 25923	*B. subtilis* ATCC 6633	*A. baumannii* BAA-747	*E. coli* ATCC 25922	*P. aeruginosa* ATCC27853	*C. albicans* ATCC 10231
C1	***ES1***	0.35 (S)	0.12 (S)	0.5 (S)	0.32 (S)	0.61 (Ad)	0.32 (S)
	ES2	0.32 (S)	0.35 (S)	0.5 (S)	0.37 (S)	1.37 (I)	0.35 (S)
	ES3	0.35 (S)	0.12 (S)	0.40 (S)	0.32 (S)	0.5 (S)	0.32 (S)
C2	***ES1***	0.32 (S)	0.35 (S)	0.30 (S)	0.35 (S)	0.5 (S)	0.44 (S)
	ES2	0.35 (S)	0.35 (S)	0.30 (S)	0.35 (S)	1.56 (I)	0.5 (S)
	ES3	0.12 (S)	0.12 (S)	0.32 (S)	0.35 (S)	0.44 (S)	0.37 (S)
C3	***ES1***	0.37 (S)	0.35 (S)	0.32 (S)	0.32 (S)	0.75 (Ad)	0.5 (S)
	ES2	0.44 (S)	0.32 (S)	0.44 (S)	0.56 (Ad)	1.23 (I)	0.61 (Ad)
	ES3	0.32 (S)	0.12 (S)	0.25 (S)	0.32 (S)	0.56 (Ad)	0.44 (S)
C4	***ES1***	0.32 (S)	0.35 (S)	0.5 (S)	0.44 (S)	0.5 (S)	0.37 (S)
	ES2	0.37 (S)	0.32 (S)	0.5 (S)	0.5 (S)	0.84 (Ad)	0.44 (S)
	ES3	0.32 (S)	0.32 (S)	0.44 (S)	0.44 (S)	0.44 (S)	0.32 (S)
C5	***ES1***	0.32 (S)	0.25 (S)	0.32 (S)	0.5 (S)	1.23 (I)	0.44 (S)
	ES2	0.32 (S)	0.32 (S)	0.37 (S)	1.24 (I)	1.42 (I)	0.5 (S)
	ES3	0.30 (S)	0.35 (S)	0.25 (S)	0.44 (S)	0.5 (S)	0.37 (S)
C6	***ES1***	0.35 (S)	0.37 (S)	0.56 (Ad)	NT	NT	0.32 (S)
	ES2	0.32 (S)	0.44 (S)	1.07 (I)	NT	NT	0.37 (S)
	ES3	0.12 (S)	0.32 (S)	0.5 (S)	NT	NT	0.35 (S)
C7	***ES1***	0.35 (S)	0.25 (S)	0.44 (S)	0.5 (S)	NT	0.39 (S)
	ES2	0.32 (S)	0.32 (S)	0.5 (S)	0.84 (Ad)	NT	0.44 (S)
	ES3	0.32 (S)	0.25 (S)	0.37 (S)	0.44 (S)	NT	0.35 (S)

Note: Synergistic (S) actions- FICI≤0.5; Additive (Ad) actions - 0.5<FICI≤1; Indifferent actions (I) - 1<FICI≤4; Antagonistic actions (An) FICI>4; NT – not tested.

Distinct time-kill profiles were recorded for each bacterial strain within 24 hours after inoculation. The results indicated no increase in the microbial strains tested in the first 30 minutes after inoculation. However, variations in antimicrobial activity among the tested compounds or bacterial strains were observed over the subsequent 90 minutes of incubation. No decrease in the number of CFUs was noted in the control tubes, and the use of chemical or biological compounds alone at a concentration of 0.5MIC did not induce bacterial death unless observed over 24 hours.

The combined chemical and biological compounds showed a significant reduction in the number of microorganisms. The combination of chemical compounds with *ES3* exhibited the best results on the tested species, as the microorganisms were killed over 8, 12, 16 and 20 hours. The combinations of 0.25MIC C1 + 0.25×MIC *ES3* and 0.25×MIC C2 + 0.25×MIC *ES3* completely inhibited the growth of *S. aureus* strains over 8 hours.

The combinations of chemical compounds with the *ES2* biological compound showed bactericidal action for 16 and 20 hours. Notably, only the combination of *ES3* with C2 displayed a bactericidal action against *S. aureus* strains over 8 hours.

DISCUSSIONS

Arthrospira platensis (also called *Spirulina platensis*), one of the most well-known cyanobacteria produced on an industrial scale, has attracted the attention of researchers as a natural compound with therapeutic properties and potential antimicrobial effect (antibacterial, antifungal, and antiviral). During the investigation of *Arthrospira platensis* as a source of proteins, vitamins (such as vitamin B12 and provitamin A), and essential fatty acids such as γ-linolenic acid, biologically active compounds with antimicrobial activity against some species of microorganisms were obtained. Therefore, commercial production of spirulina has gained importance worldwide due to

its multiple benefits. Spirulina can suppress the growth of several microorganisms due to its rich content of bioactive ingredients with antimicrobial activity. The effectiveness of spirulina extracts on microorganisms with multiple resistance to antimicrobials was experimentally demonstrated. Their antimicrobial activity is attributed to the presence of carbohydrates, phenolic compounds, flavonoids, and tannins in their composition. One objective of this study was to investigate the antimicrobial activity of spirulina, and the results indicated that spirulina extracts have a greater potential to inhibit the growth of gram-positive bacteria compared to gram-negative bacteria. This effect can be attributed to the complicated structure of the cell wall (outer membrane) of gram-negative bacteria (9, 10, 11).

Table 4. The synergistic antimicrobial effects of chemical and biological compounds against the reference strains.

Tested compounds		*S. aureus* ATCC 25923	*B. subtilis* ATCC 6633	*A. baumannii* BAA-747	*E. coli* ATCC 25922	*P. aeruginosa* ATCC27853	*C. albicans* ATCC 10231
C1	*ES1*	$1/32_C + 1/16_B$	$1/32_C + 1/32_B$	$1/4_C + 1/4_B$	$1/16_C + 1/8_B$	-	$1/16_C + 1/8_B$
	ES2	$1/16_C + 1/8_B$	$1/32_C + 1/16_B$	$1/4_C + 1/4_B$	$1/8_C + 1/8_B$	-	$1/16_C + 1/32_B$
	ES3	$1/32_C + 1/16_B$	$1/32_C + /32_B$	$1/16_C + 1/4_B$	$1/8_C + 1/16_B$	$1/4_C + 1/4_B$	$1/16_C + 1/8_B$
C2	*ES1*	$1/8_C + 1/16_B$	$1/32_C + 1/16_B$	$1/8_C + 1/32_B$	$1/32_C + 1/16_B$	$1/4_C + 1/4_B$	$1/8_C + 1/4_B$
	ES2	$1/16_C + 1/32_B$	$1/32_C + 1/16_B$	$1/8_C + 1/32_B$	$1/16_C + 1/32_B$	-	$1/4_C + 1/8_B$
	ES3	$1/32_C + 1/32_B$	$1/32_C + 1/32_B$	$1/16_C + 1/8_B$	$1/32_C + 1/16_B$	$1/4_C + 1/4_B$	$1/8_C + 1/8_B$
C3	*ES1*	$1/8_C + 1/8_B$	$1/32_C + 1/16_B$	$1/16_C + 1/8_B$	$1/16_C + 1/8_B$	-	$1/4_C + 1/4_B$
	ES2	$1/8_C + 1/4_B$	$1/16_C + 1/8_B$	$1/4_C + 1/8_B$	-	-	-
	ES3	$1/16_C + 1/8_B$	$1/32_C + 1/32_B$	$1/16_C + 1/16_B$	$1/16_C + 1/8_B$	-	$1/4_C + 1/8_B$
C4	*ES1*	$1/16_C + 1/8_B$	$1/32_C + 1/16_B$	$1/4_C + 1/4_B$	$1/4_C + 1/8_B$	$1/4_C + 1/4_B$	$1/8_C + 1/8_B$
	ES2	$1/8_C + 1/8_B$	$1/16_C + 1/8_B$	$1/4_C + 1/4_B$	$1/4_C + 1/4_B$	-	$1/8_C + 1/4_B$
	ES3	$1/8_C + 1/16_B$	$1/8_C + 1/16_B$	$1/8_C + 1/4_B$	$1/8_C + 1/4_B$	$1/8_C + 1/4_B$	$1/16_C + 1/8_B$
C5	*ES1*	$1/16_C + 1/8_B$	$1/16_C + 1/16_B$	$1/16_C + 1/8_B$	$1/4_C + 1/4_B$	-	$1/8_C + 1/4_B$
	ES2	$1/8_C + 1/16_B$	$1/8_C + 1/16_B$	$1/8_C + 1/8_B$	-	-	$1/4_C + 1/4_B$
	ES3	$1/32_C + 1/8_B$	$1/32_C + 1/16_B$	$1/16_C + 1/8_B$	$1/8_C + 1/4_B$	$1/4_C + 1/4_B$	$1/8_C + 1/8_B$
C6	*ES1*	$1/32_C + 1/16_B$	$1/8_C + 1/8_B$	-	NT	NT	$1/16_C + 1/8_B$
	ES2	$1/16_C + 1/8_B$	$1/8_C + 1/4_B$	-	NT	NT	$1/8_C + 1/8_B$
	ES3	$1/32_C + 1/32_B$	$1/16_C + 1/8_B$	$1/4_C + 1/4_B$	NT	NT	$1/32_C + 1/16_B$
C7	*ES1*	$1/32_C + 1/16_B$	$1/16_C + 1/16_B$	$1/8_C + 1/4_B$	$1/4_C + 1/4_B$	NT	$1/4_C + 1/16_B$
	ES2	$1/8_C + 1/16_B$	$1/8_C + 1/16_B$	$1/4_C + 1/4_B$	-	NT	$1/4_C + 1/8_B$
	ES3	$1/8_C + 1/16_B$	$1/16_C + 1/16_B$	$1/8_C + 1/8_B$	$1/8_C + 1/4_B$	NT	$1/16_C + 1/32_B$

In recent years, the number of works dedicated to the research of coordination complexes has increased significantly, which indicates the increased interest of researchers in these chemical compounds. The main advantages of these compounds are their homogeneity, stability, the possibility of exact dosing, the ease of assessing metabolic processes, as well as the strict observance of technological operations. Most of the studies emphasize the potential for controlling the properties and biological effects of these compounds (12, 13).

Coordination chemistry remains one of the most important and current areas of inorganic chemistry. Of particular interest is the synthesis and study of the physico-chemical properties of coordination complexes formed by transition metals with organic ligands. Ligands with a high coordination tendency form compounds with diverse compositions, structures, and properties. Due to their valuable biological activity, these compounds are used in medical practice. Among them are heterocyclic thiosemicarbazones with antibacterial, antifungal, antimalarial, and antiviral properties (14, 15).

In recent decades, the development of new antimicrobial drugs has been based on combining traditional antimicrobials with various coordinating

compounds. The remarkable results of these studies have been confirmed by the intensification of activity and broadening of the spectrum of action when using compounds with both synergistic and cumulative effects, as well as the prevention of antimicrobial resistance. This approach also allows for the reduction of treatment doses, costs, and toxic side effects (16).

The research of recent years has not only highlighted a new alternative in combating multiresistant microorganisms but has also suggested the possibility of restoring the antimicrobial effect of some antimicrobials previously categorized as ineffective. Promising results have also been observed when natural compounds with antimicrobial action were combined with synthetic antimicrobials. The combined use of natural and synthetic antimicrobial compounds in the treatment of infectious diseases potentiates the antimicrobial spectrum, reduces the toxicity of some antimicrobials, and prevents the development of antimicrobial resistance (17, 18).

Combination therapy is more effective in polymicrobial infections compared to monotherapy. Antimicrobial remedies of natural origin, when used in combination with synthetic medicines, have shown a series of effective interactions, including the synergistic amplification of antimicrobial potential and reduction of the adverse effects of synthetic medicines. These synergistic effects of combined medicines reduce therapeutic failures, increase efficacy, and shorten hospital stays (19, 20).

The synergy of natural medicines combined with antimicrobials, particularly against microorganisms prone to developing resistance, has been investigated by several researchers. Some have observed that Berberis aetnensis leaf extracts significantly reduce the minimum inhibitory concentration of ciprofloxacin, thereby restoring its efficacy in the therapy of *S. aureus, E. coli* and *P. aeruginosa* infections. In another study, a significant increase in antimicrobial activity against multiresistant *P. aeruginosa* strains was demonstrated when antibiotics were combined with clove, jambolan, pomegranate, and thyme extracts. The use of clove-ampicillin and clove-tetracycline combinations resulted in increased antimicrobial activity against *K. pneumoniae* and *Proteus* spp. strains (20).

CONCLUSIONS

1. All chemical and biological compounds included in the study have exhibited antibacterial and antifungal activity at various concentrations.
2. 87.2% of the combinations of chemical compounds with biological ones exhibited synergistic actions, while only 6.8% showed additive actions, and 6.0% were indifferent. Additive and indifferent effects were particularly observed on gram-negative bacilli and yeast-like fungi. No antagonistic actions were recorded when combining chemical compounds with biological ones. In combinations, the MIC for chemical and biological compounds decreased from four to 32 times compared to the MIC of individual compounds.
3. When using chemical and biological compounds at a concentration of 0.25 times the MIC, in most cases, there was no recorded reduction in the number of microbial cells. However, when these compounds were combined, the microorganisms were killed within 8-24 hours. The shortest time for microbial destruction (8-20 hours) was observed when combining chemical compounds with the biological compound ES3.
4. This study presents a potential therapeutic option for antibiotic-resistant microorganisms by combining natural extracts with a range of different classes of chemical compounds. The most effective approach to developing antimicrobials with minimal toxic or adverse side effects is through the use of natural products.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ETHICAL APPROVAL

The study was conducted and approved by the Ethics Committee no. 3/14.04.2023 of *Nicolae Testemitanu* State University of Medicine and Pharmacy of the Republic of Moldova.

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