

The investigation of antimicrobial and antifungal activity of some *Artemisia* L. species

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Abstract

Background: Throughout history pathogenic microorganisms cause infectious diseases. Medicinal herbs play an important role in human life because they are used for the therapy and production of herbal remedies. Therefore, it is relevant to study the antibacterial activity of medicinal herbs including *Artemisia* L. species.

Aim: The purpose of this study was to investigate the antimicrobial and antifungal activity of *Artemisia* L. species extracts and to identify their synergistic antimicrobial effects with erythromycin against skin isolates of *Staphylococcus aureus* with different mechanisms of MLS-resistance.

Materials and methods: *Artemisia absinthium* L. herb extracts (AAs-4, AAs-7, AAs-9), *Artemisia vulgaris* L. herb extracts (AV-4, AV-7, AV-9) and *Artemisia abrotanum* L. herb extracts (AAr-4, AAr-7, AAr-9) were used in this study (solvents – 40%, 70% and 90% ethyl alcohol respectively).

The determination of antimicrobial activity of extracts was performed using clinical isolates of antibiotic susceptible and antibiotic resistant microorganisms. Bacterial cultures were identified on the basis of the biochemical microtests “STAPHYtest 16”, “ENTEROtest 24”, “NEFERMENTtest 24” taking into account the complex of their morphological and cultural properties in accordance with the recommendations of the 9th edition of “Bergey’s Manual of Systematic Bacteriology”. Yeast-like fungi cultures were identified on the basis of 40 biochemical tests with the help of the VITEK 2 system using the VITEK 2 YST ID card.

The synergism of the antimicrobial activity of extracts with erythromycin was investigated using the clinical isolate of *Staphylococcus aureus* with the non-inductive MLS-resistance mechanism (resistant to erythromycin at minimum bacteriostatic concentration of 125 µg/ml and minimum bactericidal concentration of 250 µg/ml without induction of resistance to clindamycin).

Research results: The evaluation of *Artemisia* L. herb extracts activity was carried out using standard strains of microorganisms which are recommended by the WHO.

The results of the conducted studies indicate that *Artemisia* L. herb extracts are able to reduce the growth of microorganisms. The investigated extracts showed the potent bacteriostatic action against the cocci or rod-shaped microflora. The antimicrobial activity of *Artemisia* L. herb extracts directly depends on the concentration of ethanol as the solvent. *Artemisia vulgaris* L. herb extracts (solvents – 70% and 90% ethanol) and *Artemisia abrotanum* L. herb extract (solvent – 90% ethanol) show synergism of antimicrobial activity with erythromycin in relation to *Staphylococcus aureus* with efflux mechanism of MLS-resistance. The obtained *Artemisia* L. herb extracts can be used to create antifungal drugs, as well as antimicrobial drugs (against gram-positive and gram-negative bacteria).

Keywords

Artemisia absinthium L., *Artemisia vulgaris* L., *Artemisia abrotanum* L., erythromycin, antimicrobial effect

Introduction

Throughout history pathogenic microorganisms cause infectious diseases. The treatment of diseases caused by microorganisms using synthetic drugs mainly leads to the resistance of pathogenic microflora, produces side effects and allergic reactions. These negative aspects can be avoided using herbal remedies. Natural biologically active substances with antimicrobial activity include plant antibiotics, phytoncides, essential oils, resins, tannins, organic acids, alkaloids, glycosides (Mykhailenko et al. 2017).

Medicinal herbs play an important role in human life because they are used for the therapy and production of herbal remedies. Anthropogenic changes in vegetation and inappropriate harvesting of medicinal raw materials led to the decrease in the stocks of many species (in particular *Adonis vernalis* L., *Valeriana officinalis* L., *Centaureum erythraea* Rafn., *Gentiana lutea* L., *Arnica montana* L.) and to the search for new types of medicinal herbs with potent antimicrobial properties as a result (Hrytsyk et al. 2006; Kutsyk et al. 2008; Kovalov et al. 2012). Therefore, it is relevant to study the antibacterial activity of medicinal herbs including *Artemisia* L. species (Hrodzinskii 1990; Hrechana et al. 2006; Derwich et al. 2009; Ochkur et al. 2011; Baykan et al. 2012). *Artemisia* L. consists of more than 500 species that are widespread throughout the world. About 30 species are found in Ukraine.

Artemisia absinthium L. herb contains the essential oil (0.5–2%), flavonoids, tannins, lignans, organic acids, carotene and vitamin C. The essential oil includes bicyclic monoterpenoids: pinene, cadinene; ketone thujone and thujyl alcohol; sesquiterpenoids: phellandrene, β -caryophyllene, γ -sepinene, sesquiterpene alcohols – absinthin, anabsinthin and artabsinthin, sesquiterpene lactones and monocyclic lactones. The main flavonoids of species are isoquercitrin, artemisetin, artemisin, isorhamnetin, narcissin (Hrechana et al. 2006). *Artemisia absinthium* L. in moderate doses shows sedative effect but in high doses the increase of excitation with subsequent inhibition is observed. In addition, it has anti-inflammatory, antiseptic, anti-ulcer and anthelmintic properties (Wasim et al. 2010).

Artemisia vulgaris L. herb contains essential oil (0.3–0.4%), alkaloids, carotene, ascorbic acid (up to 175 mg% in the leaves), vitamins of B group, rutin, tannins, flavonoids, bitter sesquiterpene lactones (tauremizin and others). The composition of the essential oil of the herb includes cineole, thujone, borneole, camphor. Roots contain mucus, resins, tannins, inulin and essential oil, which includes dihydro-matricaric ether and ketone (Hrechana et al. 2006). Galenicals of *Artemisia vulgaris* L. show sedative effect, suppress seizures, have mild hypnotics and diaphoretic activities, stimulate the appetite and regulate the functional activity of the digestive canal, normalize the menstrual cycle. The decoction of *Artemisia vulgaris* L. herb is recommended for the appetite increase, stimulation of digestion, treatment of epilepsy, neurasthenia and other nervous diseases (Wasim et al. 2010).

Artemisia abrotanum L. contains essential oil, alkaloid abrotine, tannins and bitterness (Hrodzinskii 1990). It is used in folk medicine for the treatment of many diseases, in particular toothache, cold, rheumatic, gastrointestinal, infectious and other diseases. It should be taken as a tea: infuse 1 tablespoon of crushed twigs in 1–2 cups of boiling water and take 2–3 cups per day. It is also used in mixtures with other herbs for the treatment of renal diseases and as a remedy that contributes to the increase in the formation of bile, including so-called “liver” herbal mixtures (Hrodzinskii 1990; Derwich et al. 2009).

A diverse chemical composition of *Artemisia* L. species causes a wide range of their pharmacological activity that predetermines the study of the antimicrobial activity of their extracts.

This investigation is a part of the complex research work “Research of cultivated and wild medicinal plants of the Western region of Ukraine and development of technologies for their use for medical purposes” (Pharmacy Department of Ivano-Frankivsk National Medical University, state registration number 0118U003809).

The purpose of this study was to investigate the antimicrobial activity of *Artemisia* L. species extracts and to identify their synergistic antimicrobial effects with erythromycin against skin isolates of *Staphylococcus aureus* with different mechanisms of MLS-resistance.

Materials and methods

Artemisia absinthium L. herb extracts (AAs-4, AAs-7, AAs-9), *Artemisia vulgaris* L. herb extracts (AV-4, AV-7, AV-9) and *Artemisia abrotanum* L. herb extracts (AAr-4, AAr-7, AAr-9) were used in this study (solvents – 40%, 70% and 90% ethyl alcohol respectively).

In order to obtain *Artemisia absinthium* L., *Artemisia vulgaris* L., *Artemisia abrotanum* L. herb extracts we have chosen the method of fractional maceration. It means that the plant raw material was re-extracted with separate portions of fresh solvent.

The preparation of laboratory samples of the *Artemisia* L. species liquid extracts was carried out by maceration method with the division of the solvent into parts. The total amount of solvent was divided into 3 parts and the plant raw material was gradually infused with the first, second and then with the third part of the solvent. The maceration of raw materials was carried out using shaking or stirring for the complete extraction of biologically active substances. The time of infusion was 24 hours for the first part of the solvent and 3 hours for other parts.

Dried and crushed raw material (diameter of particles was 1–3 mm) was put into the laboratory percolator and covered with the filtering material and the perforated disk.

The raw material was poured with the solvent to make a “mirror” with thickness of 20–30 mm and left for swelling for 4 hours. Then the rest of the solvent in the ratio of 1:5 was added and infused with periodic shaking for 18 hours. After a day the extract was poured out and the

raw material was poured with the fresh solvent in the ratio of 1:3. After the infusion for 3 hours the second part of extract was received. The third part was obtained in the ratio of 1:2 in similar conditions. After the extraction the raw material was pressed and all parts of extract were mixed. The overall ratio of raw material and extracts was 1:10. The extracts were infused at a temperature not over 10 °C for 48 hours in order to eliminate intermixtures, after which they were filtered.

The purified extracts were evaporated with the rotary evaporator of the laboratory type IR-1M2 in the ratio of raw material and the finished product 1:1. It ensures the compliance of the active substances content in the raw material and in the finished product in accordance with the requirements of the State Pharmacopoeia of Ukraine.

The determination of antimicrobial activity of extracts was performed using clinical isolates of antibiotic susceptible and antibiotic resistant microorganisms. Bacterial cultures were identified on the basis of the biochemical microtests “STAPHYtest 16”, “ENTEROtest 24”, “NEFERMENTtest 24” (Lachema, Czech Republic) taking into account the complex of morphological and cultural properties in accordance with the recommendations of the 9th edition of “Bergey’s Manual of Systematic Bacteriology”. Yeast-like fungi cultures were identified on the basis of 40 biochemical tests with the help of the VITEK 2 system using the VITEK 2 YST ID card (Biomérieux, France).

The screening of antimicrobial activity of herb extracts was carried out using the micromethod of diffusion in agar. It was designed at the Department of Microbiology, Virology and Immunology of Ivano-Frankivsk National Medical University (Kutsyk 2004).

This method is characterized by the high sensitivity and discriminating ability so that it is possible to differentiate active extracts from inactive ones definitely (Kutsyk 2004). 30 ml of agar was poured in Petri dishes, which were placed on a strictly horizontal surface. After gelation the holes with the diameter of 4.0 mm were made. The agar was uniformly seeded with a suspension of test culture (concentration 1×10^7 colony-forming unit / ml). 20 µl of each herb extract were introduced into the experimental holes and 20 µl of solvents (40%, 70% and 90% ethanol) – into the test holes. The diameters of the growth inhibition zones of the bacterial test cultures were measured after the culturing for 24 hours. The registration of fungistatic activity was carried out after 2 days and fungicidal – after 4 days of cultivation.

The synergism of the antimicrobial activity of extracts combinations with erythromycin was investigated using the clinical isolate of *Staphylococcus aureus* with the non-inductive mechanism of MLS-resistance (resistant to erythromycin at minimum bacteriostatic concentration of 125 µg/ml and minimum bactericidal concentration of 250 µg/ml without induction of resistance to clindamycin) (Iurchyshyn 2014). To assess the synergism of the antimicrobial action of extracts combinations with erythromycin the antibiotic was added to the agar at the final concentration of 1/4 or 1/64 of minimum bacteriostatic

concentration. The diameters of growth inhibition zones of *Staphylococcus aureus* under the influence of plant extracts on the medium without antibiotics and on the medium with subbacteriostatic concentrations of erythromycin were compared after 24 hours of incubation.

The actual material was processed using the method of variation statistics with the calculation of the average arithmetic and its standard error, the reliability of the comparable values was estimated according to Student, Wilkinson, Mann Whitney criteria; the probability level was determined as $p \leq 0.05$ using the Image Tool 2.0 (UTHSCSA ImageTool 2.0, The University of Texas Health Science Center in San Antonio, 1995–1996) and MS Excel (State Pharmacopoeia 2015).

Results

The evaluation of *Artemisia* L. herb extracts activity was carried out using clinical strains of microorganisms characterized by different degrees of resistance to modern antimicrobial drugs.

The results of the research are represented in Fig. 1 and in Table 1.

We found out that there are compounds in *Artemisia* L. species herb extracts that can inhibit the growth of microorganisms. The presence of compounds with antimicrobial properties in the extracts depends on the concentration of ethanol as a solvent. Extracts prepared with 40% ethanol showed minimal activity against all test cultures of microorganisms. Extracts prepared with 70% and 90% ethanol inhibited the growth of gram-positive staphylococci, streptococci (*Enterococcus faecalis*, *Propionibacterium acnes*) and gram-negative bacteria (*Esherichia coli*, *Klebsiella ozaenae*, *Citrobacter freundii*, *Pseudomonas aeruginosa*) (Tab. 1). Growth inhibition zones ranged from 3.79 mm to 7.33 mm for gram-positive bacteria and from 3.79 mm to 9.47 mm for gram-negative bacteria depending on the test culture and the type of the extract.

The highest activity in relation to staphylococci showed the extracts of *Artemisia abrotanum* L. herb (especially AAr-9) and *Artemisia absinthium* L. (especially AAs-7). They affected on antibiotic susceptible and antibiotic resistant strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*.

Along with staphylococci *Propionibacterium acnes* is the topical etiologic factor of pyoderma. The clinical strain of *Propionibacterium acnes* used in this study had high resistance to amoxicillin, cephalosporins of I and II generations, tetracyclines and fluoroquinolones. Therefore, the ability of *Artemisia abrotanum* L. (AAs-7) and *Artemisia absinthium* L. (AAs-7) herb extracts to suppress the growth of this strain is essential. These extracts can be used to create new therapeutic and cosmetic products for the treatment of acne and for the skin care.

Only *Artemisia* L. herb extracts prepared with 90% ethanol showed the significant antimicrobial activity against streptococci. The best suppression of the growth of

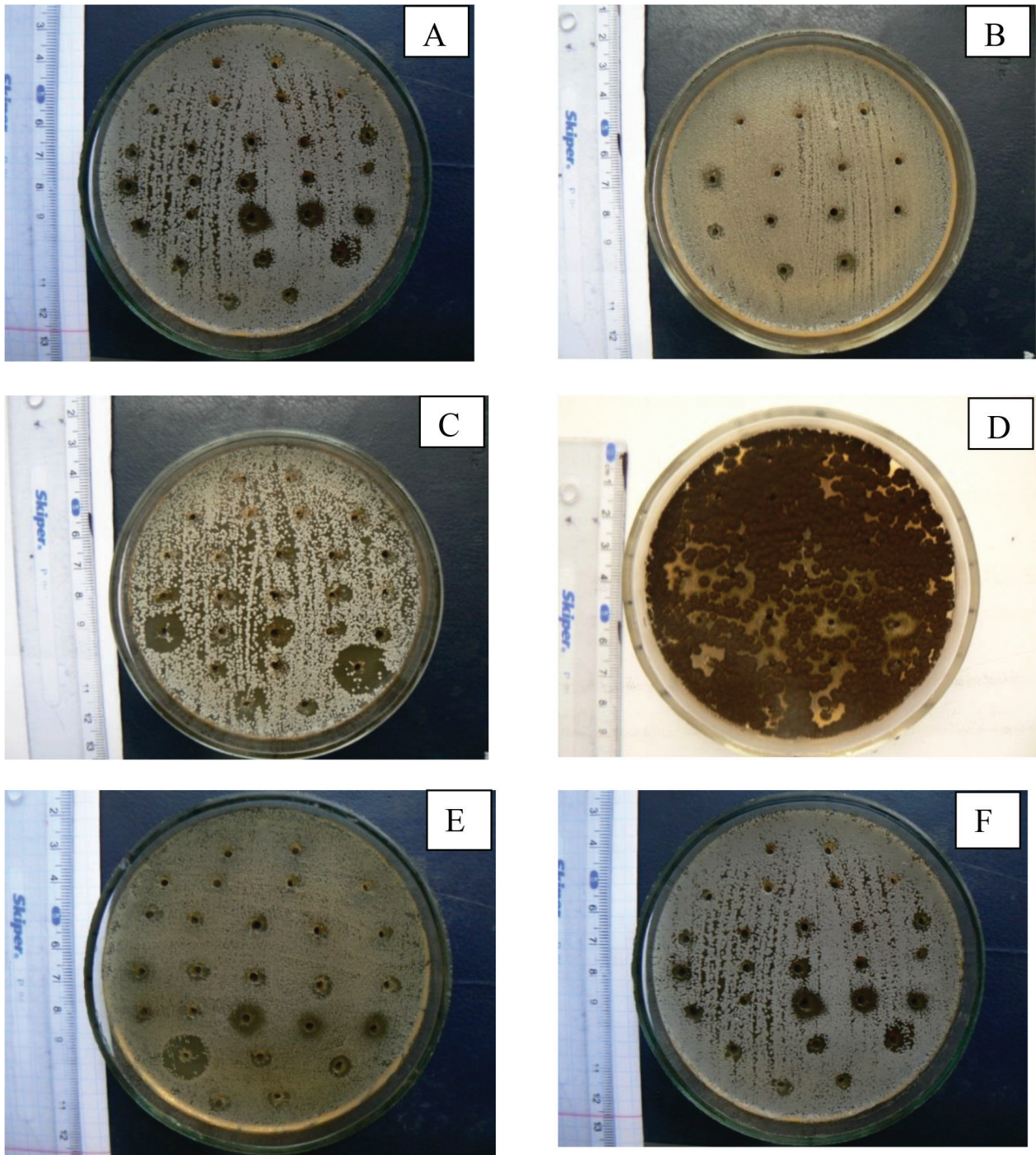


Figure 1. Antimicrobial activity of *Artemisia absinthium* L., *Artemisia vulgaris* L., *Artemisia abrotanum* L. extracts in relation to the test cultures of microorganisms: **A** *Escherichia coli*, **B** *Staphylococcus epidermidis*, **C** *Candida tropicalis*, **D** *Aspergillus niger*, **E** *Enterococcus faecalis*, **F** *Staphylococcus aureus*

all streptococci test strains was stated for *Artemisia absinthium* L. herb extract (AAs-9). Unlike streptococci *Enterococcus faecalis* was significantly more susceptible to all *Artemisia* L. herb extracts (especially to AAs-7, AAs-9, AAr-7, AV-9). The antibiotic-resistant enterococcus strain used in the study was among the few test cultures that were susceptible to *Artemisia abrotanum* L. herb extract (AAr-4). It is also of great practical interest because of the very common antibiotic-resistance of enterococci in clinical practice.

Artemisia abrotanum L. herb extracts (AAr-7, AAr-9), *Artemisia absinthium* L. herb extracts (AAs-7, AAs-9) and *Artemisia vulgaris* L. herb extract (AV-9) showed a significant antimicrobial activity against the gram-negative bacteria (enterobacteria and pseudomonads). The antifungal activity of *Artemisia* L. herb extracts was studied using clinical strains of *Candida albicans* and *Candida tropicalis* which are resistant to polyene antibiotics, imidazoles and triazoles. The significant fungici-

Table 1. Screening of the antimicrobial activity of *Artemisia L.* species extracts against some microorganisms.

Strain	Control (40% ethanol)	Solvent – 40% ethanol			Control (70% ethanol)	Solvent – 70% ethanol			Control (90% ethanol)	Solvent – 90% ethanol		
		AAs-4	AV-4	AAR-4		AAs-7	AV-7	AAR-7		AAs-9	AV-9	AAR-9
1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Staphylococcus aureus</i> MS	0	0	4.19 ± 0.15	4.66 ± 0.33	3.79 ± 0.19	9.37 ± 0.85*	4.23 ± 0.61	4.88 ± 0.88	5.02 ± 0.92	5.63 ± 0.42	5.88 ± 0.34	6.34 ± 0.63
<i>Staphylococcus aureus</i> MR	0	0	0	0	4.08 ± 0.34	6.35 ± 0.26*	0	5.38 ± 0.39*	5.17 ± 0.08	5.99 ± 0.38	5.62 ± 0.21	7.20 ± 0.31*
<i>Staphylococcus aureus</i> MLS	0	0	0	0	6.22 ± 0.46	4.65 ± 0.25	4.34 ± 0.25	4.40 ± 0.25	7.33 ± 0.16	4.45 ± 0.08	4.22 ± 0.12	4.52 ± 0.23
<i>Staphylococcus epidermidis</i> MS	4.47 ± 0.43	0	0	4.65 ± 0.40	4.14 ± 0.53	4.96 ± 0.29	5.08 ± 0.33	6.27 ± 0.30*	5.95 ± 0.33	6.02 ± 0.36	7.16 ± 0.42*	6.38 ± 0.67
<i>Staphylococcus haemolyticus</i> MR	4.45 ± 0.26	0	0	4.16 ± 0.45	3.94 ± 0.44	6.12 ± 0.39*	4.22 ± 0.19	5.82 ± 0.11*	5.20 ± 0.40	4.38 ± 0.43	4.85 ± 0.34	6.85 ± 0.80*
β-hemolytic (group A) <i>Streptococcus pyogenes</i>	0	0	0	0	0	0	[5.31 ± 0.25]	[5.29 ± 0.16]	0	5.11 ± 0.06	5.16 ± 0.23	4.45 ± 0.27
β-hemolytic <i>Streptococcus</i> group G	0	0	0	0	0	0	0	0	0	6.33 ± 0.84*	0	0
β-hemolytic (group B) <i>Streptococcus agalactiae</i>	0	0	0	0	0	4.55 ± 0.22	5.79 ± 0.43*	5.19 ± 0.28	0	6.40 ± 0.49*	4.34 ± 0.15	5.00 ± 0.54
α-hemolytic <i>Streptococcus gordonii</i>	0	0	4.73 ± 0.42	4.79 ± 0.32	4.82 ± 0.40	4.75 ± 0.33	5.41 ± 0.48	5.05 ± 0.21	4.48 ± 0.28	5.27 ± 0.52	5.24 ± 0.38	5.89 ± 0.51*
<i>Propionibacterium acnes</i> MLS	0	0	0	[4.92 ± 0.20]	0	7.26 ± 0.36*	4.78 ± 0.29	8.71 ± 0.52*	7.67 ± 0.59	7.23 ± 0.69	7.32 ± 0.63	6.29 ± 0.30
<i>Enterococcus faecalis</i>	3.80 ± 0.15	0	0	6.42 ± 0.29*	5.32 ± 0.36	7.06 ± 0.14*	5.14 ± 0.19	6.65 ± 0.06*	6.15 ± 0.73	7.66 ± 1.05	7.45 ± 0.70	5.70 ± 0.24
<i>Klebsiella ozaenae</i>	0	0	0	0	3.79 ± 0.33	0	0	4.80 ± 0.65	4.54 ± 0.47	4.30 ± 0.36	4.18 ± 0.27	4.76 ± 0.52
<i>Escherichia coli</i>	4.29 ± 0.66	0	4.86 ± 0.51	0	0	5.16 ± 0.40	5.98 ± 0.51	6.44 ± 0.20*	4.67 ± 0.58	5.69 ± 0.56	5.04 ± 0.36	5.86 ± 0.26*
<i>Citrobacter freundii</i>	4.29 ± 0.22	4.43 ± 0.50	5.03 ± 0.41	6.23 ± 0.96*	5.09 ± 0.35	6.45 ± 0.62*	5.90 ± 0.21	8.81 ± 1.01*	4.65 ± 0.86	9.47 ± 0.45*	8.38 ± 0.68*	6.10 ± 0.87*
<i>Pseudomonas aeruginosa</i>	4.29 ± 0.56	0	0	0	3.50 ± 0.27	5.33 ± 0.54*	5.54 ± 0.48*	8.52 ± 0.30*	4.50 ± 0.29	7.22 ± 0.79*	5.66 ± 0.92	4.26 ± 0.71
<i>Candida albicans</i>	0	0	5.43 ± 0.64	0	4.41 ± 0.26	4.99 ± 0.40	0	5.79 ± 0.65*	0	0	6.01 ± 0.14*	0
<i>Candida tropicalis</i>	4.50 ± 0.24	0	0	0	0	5.19 ± 0.23	0	5.69 ± 0.81*	4.92 ± 0.26	0	9.36 ± 1.19*	7.09 ± 0.77*
<i>Aspergillus niger</i> spore germination	0	0	0	0	0	0	0	0	0	0	[9.92 ± 0.98]	[13.32 ± 1.05]
<i>Aspergillus niger</i> mycelial growth	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aspergillus niger</i> formation of conidia	0	0	0	0	0	0	0	0	0	0	0	0

Notes: 1. MS – methicillin-susceptible, MR – methicillin-resistant staphylococci, MLS – staphylococci and propionic bacteria, which are resistant to macrolides; 2. in the square brackets there are zones of partial suppression of microorganisms growth (bacteriostatic / fungistatic action); 3. * – $p < 0.01$ when compared with control.

dal activity of *Artemisia vulgaris* L. herb extract (AV-9) against both test strains of yeast-like fungi and *Artemisia abrotanum* L. herb extract (AAR-9) against *Candida tropicalis* is essential. Besides, the mentioned extracts (AV-9 and AAR-9) were able to inhibit germination of *Aspergillus niger* spores (clinical isolate from the ear canal). The growth inhibition zones were 9.92 ± 0.98 mm and 13.32 ± 1.05 mm respectively. But the extracts did not completely affect the mycelial growth of the *Aspergillus niger* culture and the formation of conidia.

The results of studying of the synergistic interaction of *Artemisia L.* herb extracts with erythromycin in relation to MLS-resistant *Staphylococcus aureus* are represented in Table 2.

For this study we used the clinical isolate of *Staphylococcus aureus* with the non-inductive mechanism of MLS-resistance (resistant to erythromycin at minimum bacteriostatic concentration of 125 µg/ml and minimum bactericidal concentration of 250 µg/ml without induction of resistance to clindamycin). Resistance to macrolides in this strain is mediated by the efflux mechanism – the functioning of the membrane ATP-dependent pump (apparently MsrA). In the presence of sodium arsenate and dinitrophenol (known inhibitors of ATP synthesis) the 4- and 512-fold reduction of minimum bacteriostatic concentration of erythromycin was detected in this strain. In control trials (in the medium without erythromycin) all tested extracts in a dose

Table 2. The synergistic interaction of *Artemisia* L. herb extracts with erythromycin in relation to *Staphylococcus aureus* with noninductive phenotype of MLS-resistance.

Extracts	Medium without ERY	$1/_{64}$ MIC ERY (1.95 µg / ml)	$1/_{8}$ MIC ERY (31.25 µg / ml)
40% ethanol (control)	0	0	0
AAs-4	0	0	0
AV-4	0	0	0
AAr-4	0	0	0
70% ethanol (control)	0	0	0
AAs-7	0	0	0
AV-7	0	[11.18±0.95]*	12.33±0.61*
AAr-7	0	0	0
90% ethanol (control)	0	0	0
AAs-9	0	0	0
AV-9	0	6.43±0.54*	9.16±0.38*
AAr-9	0	6.83±0.31*	7.48±0.54*

Notes: 1. the zones of partial inhibition of microorganisms growth are given in square brackets (bacteriostatic action); 2. * - $p < 0.01$ when compared with control.

of 20 µL demonstrated the lack of direct antimicrobial activity against this strain of staphylococci.

In media with subbacteriostatic concentrations of erythromycin ($1/_{8}$ MIC – 31.25 µg / ml and $1/_{64}$ MIC – 1.95 µg/ml) distinct zones of inhibition of test culture growth around the holes with *Artemisia vulgaris* L. herb extracts (AV-7 and AV-9) and *Artemisia abrotanum* L. herb extract (AAr-9) were noted. In all cases the detected antibiotic potentiating activity had a distinct dose-dependent nature (in relation to the concentration of erythromycin in the nutrient medium).

Thus, the results of the experiments allow us to conclude that *Artemisia vulgaris* L. herb extracts (solvents – 70% and 90% ethanol) and *Artemisia abrotanum* L. herb extract (solvent – 90% ethanol) increase the susceptibility of *Staphylococcus aureus* with a non-inductive phenotype of MLS-resistance to macrolides. But the extracts of *Artemisia absinthium* L. have not got this property.

Discussion

The variety of *Artemisia* L. species, which are common in the temperate and semi-arid climatic zones of both hemispheres, their long-term use in folk medicine and gastronomy caused the undeniable interest of scientific medicine. In the second half of the twentieth century the intensive research of the chemical composition of *Artemisia* L. species and various aspects of the pharmacological action of the their galenic preparations and individual chemical compounds was carried out. The study of antimicrobial properties has always been the priority in the scientific research of *Artemisia* L. species. At the same time, the core attention of researchers was focused on the study of essential oils and alcohol extracts of *Artemisia* L. A broad spectrum of their antibacterial and antifungal activity has been established, which includes the great number of microorganisms of different taxonomic affiliations as well as antiviral, antiprotozoal, insecticidal and antihelminthic effects (Lopes-Lutz et al. 2008; Ho et al. 2013).

The considerable practical achievement in this regard was the creation of the new effective antimalarial agent – artemisinin (The Nobel Prize in Physiology or Medicine, 2015, Tu Youyou (China). Artemisinin, which was isolated from *Artemisia annua* L. – the plant of Chinese folk medicine, has established the family of endoperoxide (trioxane) sesquiterpene lactones, which also includes the new semisynthetic compounds (artemether, artesunate). They have the fundamentally new mechanism of action on the malarial plasmodia – the multivector free radical damage to cells (Brodin et al. 2007). But low biological digestibility, poor pharmacokinetic characteristics and development of resistance of the pathogen are serious barriers for using them as the monotherapy. However, the use of artemisinin-based combination therapy is the standard of treatment for tropical malaria around the world according to WHO recommendations.

Taking into account the growing demand for new remedies to overcome various infections caused by antibiotic resistant microorganisms, *Artemisia* L. species can act as the promising raw material for their creation. In addition, biologically active compounds of *Artemisia* L. have the ability to neutralize individual determinants of antibiotic resistance and thereby restore the susceptibility of resistant strains to the corresponding drugs (Liu et al. 1992; Li et al. 2011; Cremer et al. 2015). inhibit the process of obtaining of the antibiotic resistance (Dülger et al. 1999), inhibit the formation of microbial biofilms (Liu et al. 1992; Pandey et al. 2017), prevent the process of adhesion of microorganisms on the surface of the cells of the body (Dülger et al. 1999), reduce the antilysoytic activity of microorganisms (Dülger et al. 1999).

We have studied the spectrum of anti-fungal activity of *Artemisia* L. herb extracts which are common in the Carpathian and Precarpathian flora – *Artemisia absinthium* L., *Artemisia vulgaris* L. and *Artemisia abrotanum* L. The obtained results show that the antimicrobial activity of extracts directly depends on the concentration of ethanol as a solvent. These data are consistent with the results of Kashpur N.V. (Dülger et al. 1999) on the most pronounced antimicrobial properties of lipophilic extracts (in particular chloroform and ethyl acetate fractions) of *Artemisia absinthium* L., *Artemisia vulgaris* L. and *Artemisia austriaca* Jacq.

In this study we used modern clinical strains of bacteria and fungi that have different profiles of resistance to antimicrobial drugs as the test strains (unlike the number of other works that were performed using collectable strains (Stermitz et al. 2002; Fiamegos et al. 2011; Baykan et al. 2012). Our observations indicate that *Artemisia* L. herb extracts obtained by fractional maceration method show the low antimicrobial activity in relation to most of the examined strains. But the noticeable activity of *Artemisia* L. herb extracts against staphylococci, enterococci, propionic bacteria and yeast-like fungi may be interesting for further research. In particular the antimicrobial activity of *Artemisia* L. herb extracts covers both antibiotic susceptible and antibiotic resistant *Staphylococcus aureus* strains and coagulase-negative staphylococci that is consistent with the research of

other scientists (Dülger et al. 1999). It is interesting that *Candida tropicalis* – clinical strain that is high-resistant to antifungal antibiotics and synthetic antimycotics showed the high susceptibility to *Artemisia vulgaris* L. and *Artemisia abrotanum* L. extracts (solvent – 90% ethanol). Both of these extracts definitely inhibited the germination of spores of the *Aspergillus niger* clinical strain. Antifungal activity of the *Artemisia abrotanum* L. is associated with the presence of sesquiterpenoid davanone (Fiamegos et al. 2011).

However, the most promising area for further microbiological study of *Artemisia* L. species is their research as the sources of compounds with a new type of pharmacological activity – to modify the resistance to antibiotics (resistance modifying agents (RMAs)). One of the universal mechanisms of antibiotic resistance ability of microorganisms is the functioning of membrane efflux pumps (proton – MFS type and ATP-dependent – ABC-type) which provide active removal of the antibiotic outside the cell. Inhibitors of efflux pumps are identified in many plant species including *Artemisia* L. species. Thus, 4,5'-Dicofeilchinic acid isolated from *Artemisia absinthium* L. blocks the efflux pump of NorA *Staphylococcus aureus* and *E. faecalis*. It increases the susceptibility of the strains to berberine and fluoroquinolones in 4–8 times (Liu et al. 1992). On the basis of molecular docking to the molecules of potential biomixers and determination of the binding energy of the doped ligands it has been shown that 4,5'-Dicofeilchinic acid blocks the functional site of the membrane MFS-conveyors (which include the proton pump of NorA) more effectively than the ATP-dependent ABC type conveyors. The inhibitors of efflux pump of NorA *Staphylococcus aureus* are also flavonols – chrysofenol-D and chrysofenetin that were isolated from *Artemisia annua* L. extract (Cremer et al. 2015). The same flavonols increase the antimicrobial activity of artemisinin in relation to *Plasmodium falciparum* (Li et al. 2011). In our case such a synergistic effect is not discovered. But it is known that *Plasmodium falciparum* has the efflux pump of ABC type and at the same time plant flavones and flavonolignans are the inhibitors of such pumps.

The artemisinins also show the properties to modify the resistance of microorganisms. Artesunate increases the susceptibility of *Escherichia coli* to β -lactam antibiotics, but this property is not related to the effect on the production or activity of β -lactamase. It is associated with an increase of the accumulation of antibiotics in the cell due to the blockage of the AcrAB-TolC efflux pump system (Wondrack et al. 1996). Artesunate, artemether, artemisinin and dihydroartemisinin increase the ability of miconazole (but not fluconazole, caspofungin or amphotericin B) to suppress the formation of the *Candida albicans* biofilm (Pandey et al. 2017). The NorA 4,5'-Dicofeilchinic acid from *Artemisia absinthium* L. significantly inhibits the effect of berberine, etidium bromide and moxifloxacin (which are substrates of this multidrug resistance efflux pump) on *Staphylococcus aureus* and *Enterococcus faecalis* biofilm formation (Liu et al. 1992).

That is why we have investigated the synergism of antimicrobial action of *Artemisia* L. extracts and erythromycin. The clinical strain of *Staphylococcus aureus* with non-in-

ductive MLS-resistance mechanism was used for this study. It possesses a low level of MLS-resistance, which applies only to 14- and 15-membered macrolides without inducing resistance to other MLS-antibiotics. According to the literature data such a MLS-resistant phenotype is provided by the active efflux of macrolides from microbial cells mediated by the ATP-dependent ABC type membrane pump (Wondrack 1996). The additional confirmation of the efflux nature of the resistance to macrolides in this strain is the fact that there are 4- and 512-fold decrease in minimum bacteriostatic concentration of erythromycin together with known ATP synthesis inhibitors – 0.5 μ M/ml sodium arsenate and 0.5 μ M/ml dinitrophenol. These results allow us to make the preliminary conclusion about the possible presence of efflux pump inhibitors of macrolides MrsA *Staphylococcus aureus* in *Artemisia vulgaris* L. extracts (solvents – 70% and 90% ethanol) and *Artemisia abrotanum* L. extract (solvent – 90% ethanol). The phenomenon of synergism of the antimicrobial action of *Artemisia* L. biologically active substances with macrolides was previously unknown and it needs to be deeply investigated.

The results of our studies may be the basis for expanding the indications for the use of *Artemisia* L. extracts in medicine. They indicate the expediency of the combination of *Artemisia vulgaris* L. and *Artemisia abrotanum* L. extracts with antibiotics in therapeutic schemes as well as the prospects of creation of combined drugs on their basis, in particular for dermatology. The introduction of combined chemotherapy in clinical practice can really solve two actual problems of modern medicine: to slow down the process of gaining the resistance to antibiotics by microorganisms (in particular by staphylococci) and to improve the effectiveness of treatment of infections caused by resistant strains.

Conclusions

1. *Artemisia vulgaris* L. and *Artemisia abrotanum* L. herb extracts prepared by the method of fractional maceration show moderate antimicrobial activity against gram-positive microorganisms (staphylococci, enterococci, propionic bacteria) and yeast-like fungi.
2. The antimicrobial activity of *Artemisia* L. herb extracts directly depends on the concentration of ethanol as the solvent.
3. *Artemisia vulgaris* L. herb extracts (solvents – 70% and 90% ethanol) and *Artemisia abrotanum* L. herb extract (solvent – 90% ethanol) show synergism of antimicrobial activity with erythromycin in relation to *Staphylococcus aureus* with efflux mechanism of MLS-resistance.

The further study of the antimicrobial activity of *Artemisia* L. extracts of the Precarpathians flora, the study of synergism of their antimicrobial action with macrolides and antibiotics of other groups, the identification of individual active substances of these extracts and the development of new combinations of drugs on their basis are promising.

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