ORIGINAL ARTICLE

The study of microbiological activity of medicinal plants of flora of Prycarpathia

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Abstract

Objectives: The investigation of antimicrobial activity of water-alcohol plant extracts *Ruta graveolens* L., *Nepeta cataria* L., and *Stellaria media* (L.) Vill. and the detection of the synergism of their antimicrobial action with erythromycin against the skin isolates of *Staphylococcus aureus* with different mechanisms of acrolides, lincosamides, and streptogramins (MLS)-resistance. **Materials and Methods:** It has been carried out by the micromethod of diffusion into agar and the method of diffusion of active substances into agar using paper disks. **Results:** The direct antimicrobial effect of the investigated extracts to the test strains was established. The synergistic antimicrobial effect of 1/4 and 1/64 minimum decreasing concentration of erythromycin to all strains of staphylococcus was demonstrated by extracts from *R. graveolens* L. The biologically active substances of medicinal plants more effectively restore the sensitivity to erythromycin for staphylococcus with low MLS-resistance through blocking reflex mechanisms. High-level MLS-resistance undergoes to a modification influenced considerably less. **Conclusion:** The investigated plant extracts of *R. graveolens* L. show antimicrobial activity against all tested strains of microorganisms: Gram-positive and Gram-negative bacteria, *Candida tropicalis*. *N. cataria* L. extract influenced some of the tested cultures of microorganisms. Extract of *S. media* (L.) Vill. inhibited the growth of microorganisms, mostly to streams of *Streptococcus aureus* ATCC 25923 and *S. aureus* ATCC 6538 in a dose of 200 mcg/ml.

Key words: Antimicrobial effect, erythromycin, plant extracts, synergism of antimicrobial action

INTRODUCTION

Pathogenic microorganisms cause infectious diseases that are the companions of human history. Making use of synthetic drugs for the treatment of diseases caused by microorganisms generally leads to the development of pathogenic microflora resistance to them, persistent side effects on the human body, various allergic reactions. These negative aspects can be avoided using herbal medicine. Natural biologically active substances with antimicrobial effect include plant antibiotics, phytoncides, essential oils, balsams, resins, tannins, organic acids, alkaloids, and glycosides.[1,2]

Anthropogenic changes in vegetation and inappropriate store process of medicinal raw materials have led to a decline in the supply of many medicinal plant species (such as *Adonis vernalis, Valerian, Centaurium, Gentiana lutea,* and *Arnica montana*), that has contributed to the search for new types of medicinal plants with antimicrobial properties.[3-5] For this reason, it is important to study the antibacterial properties of some representatives of the plant world such as *Ruta graveolens* L., *Nepeta cataria* L., and *Stellaria media* (L.) Vill.

The multicomponent chemical composition of plants of the genus *Ruta* predetermines a wide range of their pharmacological activity. Galen drugs of the genus *Ruta* show antispasmodic, anti-inflammatory, antibacterial, fungicidal, antihypertensive, sedative, diuretic, choleretic, and angioprotective effects. It has been established that furocoumarins of plants of the genus *Ruta* increase the sensitivity of the skin to ultraviolet rays; acridine compound acronicin exhibits antitumor activity. Herb *R. graveolens* L.

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is used in medicine to treat spasms of smooth muscles of the stomach, intestine, bile and urinary tract, and dizziness.[6-10]

Valuable sources of biologically active substances are the species of the family Lamiacaeae *(Labiatae)*, i.e., *N. cataria* L. In folk medicine, *N. cataria* L. has long been used as a sedative, for diseases of the stomach and liver, bronchitis, and colds. Aboveground parts of plants of the genus *Nepeta* accumulate various chemical compounds. The main active ingredients are essential oil (0.4–0.6%), tannins and bitter substances, flavonoids, glycosides, saponins, in the leaves are vitamin C (up to 190 mg %), and carotene.[11-15]

S. media (L.) Vill. is a well-known plant in folk medicine in the world. The herb contains flavonoids, tannins, saponins, organic acids, and Vitamins C, E, and K. The fresh juice and herb's infusion contribute to the improvement of cardiac activity, have a positive effect on the nervous system, stop bleeding, show analgesic and anti-inflammatory actions, stimulate healing of wounds.[16-19]

To study the influence of active substances of medicinal plants on various types of microorganisms, their water-alcohol extracts, which are received from different types of extracting, are used predominantly.[20]

The usage of natural substances that possess antimicrobial properties is essential. It is evidenced by the following factors: Microorganisms have lack of resistance to these compounds, and therefore their long-term use is possible; do not cause harmful (negative) action on the human body; due to its inhibitory effect on unwanted microflora can be used in medicine.

The purpose of the work was to conduct a study of the microbiological activity of the plant extracts and to identify their synergistic antimicrobial action with erythromycin against skin isolates of *S. aureus* with different mechanisms of acrolides, lincosamides, and streptogramines (MLS)-resistance.

MATERIALES AND METHODS

Water-alcohol extracts of *R. graveolens* L. herb, *N. cataria* L. herb, and *S. media* (L.) Vill. herb were used for conducting research.

Screening of antimicrobial activity of the plant extracts was carried out with the help of the micromethod of diffusion into agar; the method was produced by the Department of Microbiology, Virology, and Immunology of the Ivano-Frankivsk National Medical University. The study of antimicrobial activity of an extract of *Stellaria* was carried out on the basis of Mechnikov Institute of Microbiology and Immunology in Kharkov.

The exploration of the antimicrobial activity of plant extracts had been done on clinical isolates of antibiotic-sensitive and antibiotic-resistant microorganisms. Bacterial cultures were identified based on the biochemical Microtest "STAPHYtest 16," "ENTEROtest 24," "NEFERMENT test 24" (Lachema, the Czech Republic), as well as taking into account the complex of morphological and cultural features, in accordance with the recommendations of the 9th edition of the "Bergey's Manual of Determinative Bacteriology." Yeast-fungal cultures were identified based on 40 biochemical tests using the VITEK 2 system with the VITEK 2 YST ID card (biomerieux, France).[21]

Petri dishes were filled with 30 ml of agar. After the medium was congealed, holes 4.0 mm in diameter were made by a special tool with even edges. Agar was uniformly seeded with a suspension of test culture (concentration 1 × 10^7 CFU/ml). 20 µL of the plant extracts were put into the experimental holes, and 20 microliters of extractants (40%, 70%, and 90% aqueous ethanol) were added to the test holes. After cultivating for 24 h, the diameters of the growth retardation zones (GRZ) of bacterial test cultures were determined.

To assess the synergism of antimicrobial activity of erythromycin extracts into nutrient agar, an antibiotic was added at a final concentration of 1/4 or 1/64 MBsC for each test strain. After 24 h of incubation, the diameters of GRZ of microorganisms under the influence of plant extracts on a medium without antibiotics and on mediums with sub-bacteriostatic concentrations of erythromycin were compared. Taking into account the results of control experiments, to exclude the effect of extractant (90% of ethanol) on the growth of test cultures, the diameters GRZ <7.00 mm were not beared in mind.

The survey of the antimicrobial activity of *S. media* L. Vill. was conducted by diffusion of active substances into agar with the use of paper disks. Concentration of the active substance on the disks was 5 mg. It had been used 5% of bloody agar and daily broths of cultures on the basis of 1% sugar soup as universal nutrient mediums, in a suspension of 1 billion microbial bodies. 1 ml of a bacterial suspension was applied to a surface of 5% of blood agar and evenly spread into it. Bacterial fields were incubated at 37°C for 24–72 h depending on the characteristics of the culture under study. Antimicrobial activity was evaluated by measuring the zone of growth retardation of microorganisms (in mm) around the study drug. The following cultures were used: *S. aureus* ATCC 25923, *S. aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Pseudomonas vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* ATCC 9027, *Candida albicans* ATCC 855/653, and *Staphylococcus pyogenes* Dick-1.

For statistical processing of the results, computer programs UTHSCSA ImageTool 2.0 (UTHSCSA Image Tool 2.0, The University of Texas Health Science Center in San Antonio, © 1995-1996) and Microsoft Office Excel 2003 were used.

RESULTS AND DISCUSSION

Evaluation of the activity of the investigated herb extracts of *R. graveolens* L. (RG-4, extracts of 40% ethanol, RG-7, and extractant 70% ethanol), herb extract of *N. cataria* L. (NC, 40% ethanol extractant), and *S. media* (L.) Vill. herb extract (extractant 40% ethanol) was carried out on standard strains of microorganisms recommended by the WHO. The results of the studies are presented in Table 1 and Figure 1.

The results of the conducted studies indicate that the samples of extracts have the ability to inhibit the growth of microorganisms. Extracts RG-4, RG-7, and NC showed a more pronounced bacteriostatic action against a rod-shaped and coccus microflora. The NC extract does not exhibit antifungal activity against *A. niger*. It has been discovered that the RG-7 extract also shows a pronounced antifungal effect on *C. tropicalis* and *A. niger*.

The synergism of antimicrobial action of erythromycin plant extracts was investigated using *S. aureus* clinical isolate with a non-inductive MLS-resistant mechanism (resistant to erythromycin for MBsC 125 mcg/ml and MBdC 250 mcg/ml without induction of resistance to clindamycin). To assess the synergism of the antimicrobial activity of erythromycin extracts into nutrient agar, an antibiotic was added at a final concentration of 1/4 or 1/64 MBsC. After 24 h of incubation, the diameters of the staphylococcal delayed regions under the influence of the plant extracts on a medium without antibiotics and on a media with sub bacteriostatic concentrations of erythromycin were compared. Synergic interaction of the plant extracts with erythromycin in comparison with MLS-resistant *S. aureus* is presented in Table 2.

As a result of the performed research, it was found that extracts of RG-4 and RG-7 exhibit antimicrobial activity against *S. aureus* in comparison with erythromycin, and extract NC do not detect this kind of activity.

Table 1: The results of study of antimicrobial activity of the plant extracts to certain microorganisms

Test culture of microorganisms	Zone of growth retardation of microorganisms, mm		
	RG-7	RG-4	NC
1	2	3	4
Streptococcus aureus MS	7.39±1.13*	8.05±0.18*	5.09±0.24
Streptococcus aureus MR	16.74±0.51*	15.10±0.23*	-
Streptococcus aureus MLS	3.38±0.17	9.20±0.47*	-
Streptococcus epidermidis MS	7.11±0.67	9.33±0.55*	5.12±0.24
Streptococcus hemolyticus MR	8.08±0.36*	9.70±0.56*	-
β-hemolytic (group A) *Streptococcus pyogenes*	8.59±0.41*	6.07±0.33*	-
β-hemolytic (group G) *Streptococcus sp.*	11.83±1.36*	8.55±0.68*	-
α-hemolytic *Streptococcus gordonii*	5.10±0.24	6.58±0.07*	-
β-hemolytic (group B) *Streptococcus agalacticae*	10.31±0.11*	7.07±0.40*	-
Propionibacterium acnes MLS	14.31±0.26*	9.92±0.50*	-
Enterococcus faecalis	6.50±0.40	10.92±0.38*	-
Klebsiella ozaenae	5.21±0.49	8.04±0.97*	-
Escherichia coli	7.79±0.57*	8.07±0.42*	6.78±0.63
Citrobacter freundii	7.40±0.28*	9.38±0.55*	-
Pseudomonas aeruginosa	6.14±0.19	6.80±0.63	4.12±0.71
Candida albicans	4.59±0.71	9.86±0.23*	-
Candida tropicalis	6.84±0.52*	5.05±0.46	-
Aspergillus niger sprouting spores	12.14±1.80*	12.95±1.21*	-
Aspergillus niger mycelial growth	-	-	-
Aspergillus niger formation of conidia	[4.72±0.29]	[5.71±0.61]	-

MS: Methicillin-susceptible, MR: Methicillin-resistant *Staphylococcus*, MLS: Resistant to *Staphylococcal macrolides* and propionic bacteria. In square brackets, there are zones of partial suppression of growth of microorganisms (bacteriostatic/fungistatic action); *P<0.01 when compared with control

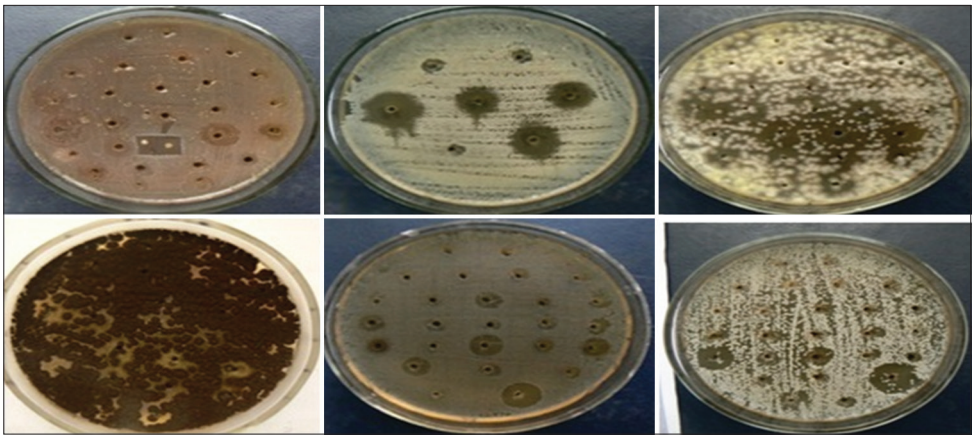

Figure 1: The determination of the minimum bactericidal/fungicidal concentration of *Ruta graveolens* L., *Nepeta cataria* L. extracts to test cultures of microorganisms: (a) *Escherichia coli*, (b) *Staphylococcus epidermidis*, (c) *Candida tropicalis*, (d) *Aspergillus niger*, (e) *Enterococcus faecalis*, (f) *Staphylococcus aureus*

Table 2: The synergistic interaction of the erythromycin plant extracts in comparison with MLS-resistant *Streptococcus aureus*

Extract symbol	Medium without ERY	$^1/_{64}$ MDC	$^1/_4$ MDC
NC	5.64±0.44	6.84±0.26	-
RG-7	9.55±0.32	11.83±0.88	18.27±1.33
RG-4	6.52±0.29	6.17±0.43	7.12±0.33

-: There is no zone of growth retardation of microorganisms. MLS: Mechanisms of acrolides, lincosamides, and streptogramines

Table 3: Antimicrobial activity of *Stellaria media* (L.) Vill. herb extract to standard test strains of the microorganisms by the method of diffusion into agar

Test culture of microorganisms	Zone of growth retardation of microorganisms, mm			
	200 mcg/ml	100 mcg/ml	50 mcg/ml	25 mcg/ml
1	2	3	4	5
Streptococcus aureus ATCC 25923	22.4±1.4	20.0±0.9	11.8±0.6	11.5±0.6
Streptococcus aureus ATCC 6538	20.8±1.0	19.6±0.6	11.6±0.7	10.8±0.70
Escherichia coli ATCC 25922	-	-	-	-
Pseudomonas vulgaris ATCC 4636	-	-	-	-
Bacillus subtilis ATCC 6633	18±0.7	-	-	-
Pseudomonas aeruginosa ATCC 27853	17.8±1.0	12.0±0.9	-	-
Pseudomonas aeruginosa ATCC 9027	21.4±1.4	16±0.9	-	-
Candida albicans ATCC 855/653	15.0±0.9	-	-	-
Streptococcus pyogenes Dick-1	18.6±0.6	-	-	-

-: There is no zone of growth retardation of microorganisms

The obtained results of our study may be the basis for expanding the usage of extracts in medicine. Experimental data indicate the appropriateness of extract combinations with antibiotics in therapeutic schemes, as well as the promising development of combined medicine on their basis, i.e., for dermatology. The introduction of combined chemotherapy into clinical practice can really help to solve two actual problems of modern medicine: To slow the process of obtaining resistance to antibiotics by microorganisms (i.e. Staphylococcus) and to improve the effectiveness of treatment for infections caused by resistant strains.

S. media (L.) Vill. herb extract was investigated on 6 strains of the microorganisms. The investigated extract did not detect activity against *E. coli* ATCC 25922, *P. vulgaris* ATCC 4636 [Table 3].

According to the results of the study *S. media* (L.) Vill. extract had suppresses microorganism growth in a dose of 200 mcg/ml, a more pronounced activity of the extract in this dose to strains *S. aureus* ATCC 25923 and *S. aureus* ATCC 6538.

In addition, the extract in a dose of 200 mcg/ml reduced the growth diameter of *B. subtillis* ATCC 6633, *C. alibicans* ATCC 885/653, and *S. pyogenes* Dick-1.

CONCLUSIONS

It was found that the investigated plant extracts of *R. graveolens* L. exhibit antimicrobial activity against all used test strains of the microorganisms: Gram-positive bacteria, Gram-negative bacteria, and *C. tropicalis* fungus. Extracts of *N. cataria* L. influenced only some of the test culture of microorganisms. The plant extracts (RG-7, RG-4, and NC) did not exhibit antimicrobial activity against *A. niger*. Extract of *S. media* (L.) Vill. inhibited the growth of microorganisms, most pronounced against *S. aureus* strain ATCC 25923 and *S. aureus* ATCC 6538 in a dose of 200 mcg/ml.

The further experimental pharmacological study of *R. graveolens* L., *N. cataria* L., and *S. media* (L.) Vill. as a medicinal plant material showing bactericidal, choleretic, hepatoprotective, anti-inflammatory, and diuretic effects has been promising.

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