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ANTIMICROBIAL ACTIVITY OF SCUTELLARIA L. PLANT EXTRACTS

The work deals with the evaluating the antimicrobial activity of ethanolic extracts of different plant organs (roots, leaves, buds) of three species of *Scutellaria* genus: *S. albida* L., *S. altissima* L., *S. baicalensis* L., grown in M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, against gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens*) and gram-positive (*Staphylococcus aureus*) microorganisms. Analysis of the effect of ethanolic extracts of juvenile *Scutellaria* plants on bacterial cultures showed that *S. albida* had the highest inhibitory potential, as it inhibited the growth of all bacterial test objects. *S. creticola* extracts effected both gram-positive bacteria and gram-negative *Staphylococcus aureus*. The extracts of *S. altissima* and *S. baicalensis* juvenile plants were characterized by their activity against two gram-positive test objects – *Escherichia coli* and *Pseudomonas aeruginosa*. The analysis of the antibacterial activity of ethanolic fractions of the extracts of *Scutellaria* individual organs showed that the highest inhibitory effect was demonstrated by the extracts of leaves and roots of *S. baicalensis* against *Escherichia coli*, the leaves and roots of *S. albida* against *Staphylococcus aureus*, and *S. albida* of against *Pseudomonas aeruginosa*. The results confirmed the antimicrobial effect of different *Scutellaria* species, so that these plants could be used for the treatment of bacterial infections.

Key words: *Scutellaria* L., *S. albida*, *S. altissima*, *S. baicalensis*, *S. creticola*, ethanol extract, antimicrobial activity.

Introduction

Modern pharmaceutical industry offers a wide range of chemical-based medicines that are characterized by their rapid effect on human body but at the same time they are known by the side effects and contraindications. Since the development of microbial resistance to antibiotics being dangerous with the background of the reducing immune response of human body [20–23], it is becoming increasingly important to search for herbal medicines with antibacterial properties alongside to minimal side effects. So that, the *Scutellaria* plants are being actively studied. These species are used in both traditional and official medicine due to the high content of flavonoids (baicalin, baicalein, vogonin, hmizin, etc.) and tannins, moreover, they are characterized by antitumor, antiviral, hepatoprotective, anti-inflammatory, antioxidant, anticonvulsant, antimicrobial properties [5, 15, 17, 26].

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Scutellaria L. (skullcap) is a large polymorphic genus of *Lamiaceae* family [19]. The world flora includes from 350 to 425 species of this genus [25].

Nine skullcap species have been described for the flora of Ukraine – *S. albida* L., *S. altissima* L., *S. creticola* Juz., *S. dubia* Taliev et Sirj., *S. galericulata* L., *S. hastifolia* L., *S. orientalis* L., *S. verna* Bess., *S. woronowii* Juz, which is 2.1–2.5% of the world's genus fund. It should be mentioned that four species of the Ukrainian flora (*S. altissima*, *S. creticola*, *S. galericulata*, *S. orientalis*) are used only for traditional medicine [12]. And only the remedies based on Baikal skullcap (*S. baicalensis* Georgi) are used for official medicine. This species grows naturally in Mongolia, China, Japan, Eastern Siberia and Russian Far East, and it is cultivated in Ukraine.

S. edelbergii Rech. is known to inhibit the growth of gram-negative strains (*Escherichia coli* and *Klebsiella pneumoniae*) and the growth of gram-positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*) significantly [21]. The potential antimicrobial and antifungal activity of *Scutellaria baicalensis* is greatly important due to the growing number of the cases of rapidly developing bacterial and fungal infections [18]. Also, this species causes the significant inhibition of HIV-1 protease activity, and the aqueous extracts of *S. indica* and *S. barbata* demonstrate antiviral activity against human RSV virus [17].

Therefore, the representatives of *Scutellaria* genus have attracted the attention of scientists from all over the world in recent decades. The species of aboriginal flora that have not yet been included in the Pharmacopoeia are being especially actively studied [4, 10, 11].

This way, the investigation of natural effective antimicrobial substances of plant origin and the analysis of the antimicrobial activity of *Scutellaria* species are important nowadays. The aim of our study was to analyze the antimicrobial activity of ethanol plant extracts of four *Scutellaria* species grown in laboratory (juvenile plants) and openair (budding plants) against gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens*) and gram-positive (*Staphylococcus aureus*) microorganisms.

Escherichia coli is the representative of microflora of large intestine of healthy person and performs a number of functions beneficial for the macroorganism as part of the intestinal microbiocenosis. As the main aerobic form of the intestinal microflora some representatives of this bacterial species can cause escherichiosis [13].

Staphylococcus aureus colonizes the mucous membranes of the nasal cavity and skin. Most diseases caused by staphylococcus are endogenous. Since *Staphylococcus aureus* can affect almost any tissue of the human body, is a causative agent of such diseases such as pneumonia, staphylococcal bacteremia, osteomyelitis, arthritis, endocarditis, and can cause food poisoning, toxic shock syndromes, and scalded skin.

Pseudomonas aeruginosa is a major causative agent of local and systemic infections in humans. *P. aeruginosa* is known to cause abscesses, keratitis, otitis externa, meningitis, bacteremia, endocarditis, enteritis, pneumonia, osteomyelitis, and arthritis [27].

The bacteria *Serratia marcescens* can live in the subgingival teeth biofilm in human mouth. *S. marcescens* is a part of many associations of purulent and inflammatory infections in humans. In particular, they are the causative agents of such diseases as catheter-associated bacteremia, urinary tract infections and purulent wounds. It is usually located in the respiratory and urinary tracts of adults and in the gastrointestinal tract of children. Occasionally, *S. marcescens* can cause endocarditis and osteomyelitis, pneumonia and meningitis. A rare clinical form of infantile gastroenteritis is also caused by *S. marcescens* infection [24].

Material and methods

Plant Material. We suppose the juvenile 1–2 month-old plants (cultivated indoors despite of the season) to have antimicrobial activity. *Scutellaria* (*S. creticola*, *S. altissima*, *S. albida*, *S. baicalensis*) seeds were germinated in greenhouse in order to analyse the antimicrobial activity of these species plant material (Fig. 1).



Fig. 1. Germination of seeds of various *Scutellaria* species

S. altissima, *S. albida*, and *S. baicalensis* plants were grown openair in M.M. Gryshko National Botanical Garden of National Academy of Sciences of Ukraine (Fig. 2 and Fig. 3). *S. creticola* seedlings were not able to develop into plants in soil and soon died.

When flowering, the experimental plants were collected and subsequently used for the extract preparation (Fig. 4). Then the analysis of the antimicrobial activity of leaf, bud and root extracts of three skullcap species against gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens*) and gram-positive (*Staphylococcus aureus*) strains of microorganisms was carried out. The experiment was repeated six times.



Fig. 2. Cultivation of plants of various *Scutellaria* species:
A – in greenhouse conditions; B – on the collection area

Preparation of the Plant Extracts. Fresh leaves were washed, weighed and homogenized in 96% ethanol in order to obtain 10% ethanolic extracts (Fig. 5). *Scutellaria* plants were previously shown to have high content of flavonoids, which may have provided the antibacterial properties of the plants [8, 9]. As the flavonoids are extracted with alcohol, we used 70% ethanolic solution to the plant extracts for our study.

Antimicrobial activity. After the extraction procedure, we performed the disc-diffusion tests in order to analyse antibacterial activity with Whatman AA discs (13.0 mm; Whatman International Ltd, Maidstone, UK) with various concentrations of the extracts. The discs were placed on Mueller–Hinton agar inoculated with 105 colony-forming units/ml of MRSA (methicillin-resistant *Staphylococcus aureus*),

Escherichia coli, *Pseudomonas aeruginosa* or *Serratia marcescens*. Cell suspensions (1×10^6 colony-forming units/ml) of the examined bacteria were inoculated onto agar plates. The results were analysed after 20 h plate incubation at 37°C.



Fig. 3. *Scutellaria* plants grown on the plot of the Laboratory of Medical Botany of M.M. Hryshko National Botanical Garden of National Academy of Sciences of Ukraine:
A – *S. albida* L.; B – *S. altissima* L.; C – *S. baicalensis* Georgi



Fig. 4. Plants of *Scutellaria* genus at the phases of budding and flowering:
A – *S. altissima* L.; B – *S. albida* L.; C – *S. baicalensis* Georgi.

The presence of inhibition zones around each paper disk after incubation was considered as the antimicrobial effect, while the absence of any measurable zone of inhibition was interpreted as the absence of antimicrobial activity. Each time the disks impregnated with ethanol were used as the negative control.

The bacterial culture was considered as the one of low sensitivity in case of diameter of the growth zone up to 10 mm, the ones of medium sensitivity were considered in case of 11–14 mm, and the ones of more than 15 mm was considered the ones of high sensitivity [6, 14].



Fig. 5. Ethanolic extracts of skullcap leaves:
1 – *S. creticola*; 3 – *S. altissima*; 4 – *S. albida*; 5 – *S. baicalensis*.

Test microorganisms. Bacterial cultures were suspended in sterile solution of 0.9% NaCl, the turbidity corresponding to an equivalent value of 0.5 according to the McFarland standard. The culture was inoculated on Mueller–Hinton (MN) agar plates.

Statistical analysis. Statistical calculations were performed with STATISTICA 8.0 (StatSoft).

Results and discussion

Study of antimicrobial activity of juvenile plants. This study demonstrated the ethanolic extracts of *S. creticola* leaves to show an average level of antimicrobial activity against *E. coli*, *S. aureus* and *P. aeruginosa*. *S. altissima* extracts showed an average level against *E. coli* and *P. aeruginosa* strains. In our studies, *S. albida* extracts exhibited antibacterial activity against all test microorganisms: *E. coli*, *S. aureus*, *P. aeruginosa* and *S. marcescens*. The antibacterial activity was also observed for *S. baicalensis* plant extracts against two test objects: *E. coli* and *P. aeruginosa*.

Table 1
Antimicrobial activity of *Scutellaria* plant extracts, mm

Species	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Serratia marcescens</i>
<i>S. creticola</i>	13,6±2,16	13,5±1,00	12,4±1,52	5,25±0,38
<i>S. altissima</i>	13,8±1,84	–	12,8±2,16	–
<i>S. albida</i>	14,4±1,12	13,0±2,67	10,6±1,12	12,6±2,08
<i>S. baicalensis</i>	7,6±0,88	–	11,0±1,00	–

Basing the results of our research, we found out the antimicrobial activity of the extracts of *Scutellaria* plants (*S. creticola*, *S. altissima*, *S. albida* and *S. baicalensis*) against gram-negative strains (*P. aeruginosa*, *E. coli* and *S. marcescens*) and gram-positive (*S. aureus*) microorganisms (Fig. 6).

S. albida is characterized by the highest inhibitory potential and inhibited the growth of all microbial test objects. The lowest antimicrobial activity was observed

for *S. albida* extracts against *Pseudomonas aeruginosa* (10.6 ± 1.12 mm) and against *Escherichia coli* (14.4 ± 1.12 mm). *S. creticola* extracts also affected both gram-positive bacteria and gram-negative *Staphylococcus aureus*, while *Escherichia coli* (13.6 ± 2.16 mm), *Staphylococcus aureus* (13.5 ± 1.00 mm) and *Pseudomonas aeruginosa* (12.4 ± 1.52 mm). The extracts of juvenile plants *S. altissima* and *S. baicalensis* showed the activity against two gram-positive test objects – *Escherichia coli* and *Pseudomonas aeruginosa*.

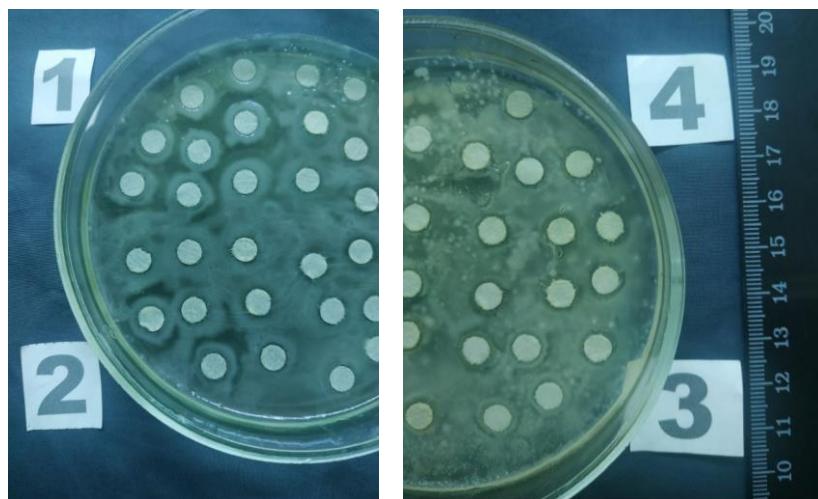


Fig. 6. Antimicrobial activity of ethanolic extracts of *Scutellaria* plants against *E. coli*:
1 – *S. creticola*; 2 – *S. altissima*; 3 – *S. albida*; 4 – *S. baicalensis*

Analysis of antimicrobial activity of the plants at the budding phase. Antimicrobial activity was determined for the extracts of various plant organs. Basing the results of this research, we report the antimicrobial activity of the ethanolic extracts of above-ground and underground parts of plants of skullcap species against the studied microorganisms.

As already mentioned above, *Scutellaria* plants owe antimicrobial effect against bacteria and viruses to a great range of phenolic compounds – flavonoids and phenylethanoid glycosides [11], as well as terpene compounds (iridoids, diterpenoids, triterpenoids) and other biologically active substances [17]. The data on separately isolated flavonoids apigenin and luteolin against Methicillin-resistant *Staphylococcus aureus* studied in *Scutellaria barbata* are also particularly noteworthy [10]. Since flavonoids have the most powerful therapeutic potential among the substances listed above, we conducted the preliminary studies to determine the total amount of flavonoids and observed a tendency towards the increased content of flavonoids in the culture of transgenic roots of *Scutellaria altissima* L. compared to the culture of intact roots [7].

Among the extracts of juvenile *Scutellaria* plants analyzed by us, the greatest inhibitory potential was demonstrated by the extracts of *S. albida*, which inhibited the growth of all microbial test objects. The lowest sensitivity of its extracts was observed in relation to *P. aeruginosa* (10.6 ± 1.12 mm), and the highest was in against *E. coli* (14.4 ± 1.12 mm). Also, *S. creticola* extracts affected both gram-positive bacteria and gram-negative *S. aureus*, but they were insensitive to *S. marcescens* (5.25 ± 0.38 mm). At the same time, *E. coli* (13.6 ± 2.16 mm), *S. aureus* (13.5 ± 1.00 mm) and *P. aeruginosa* (12.4 ± 1.52 mm) were moderately sensitive to the extracts of this species. The extracts of juvenile *S. altissima* and *S. baicalensis* plants demonstrated

activity against two gram-positive test objects - *E. coli* and *P. aeruginosa*. At the same time, we observed higher activity for *S. altissima* (13.8 ± 1.84 mm and 12.8 ± 2.16 mm, respectively) than for *S. baicalensis* (7.6 ± 0.88 mm and 11.0 ± 1.00 mm, respectively). It should be noted that we did not find similar data in scientific publications and can make an assumption that in the ontogeny of plants, the accumulation of flavonoid compounds in *S. baicalensis* begins later than in Ukraine flora.

Table 2
Antimicrobial activity of the extracts of above-ground and underground parts of *Scutellaria* species

Organs of plants	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Serratia marcescens</i>
<i>S. altissima</i>				
leaves	11.8 ± 3.90	12.5 ± 3.51	10.0 ± 0.71	10.0 ± 2.24
roots	10.5 ± 1.21	8.0 ± 2.00	6.4 ± 0.55	—
buds	11.2 ± 3.56	11.8 ± 2.59	11.0 ± 3.74	11.8 ± 2.59
<i>S. baicalensis</i>				
leaves	19.2 ± 0.84	8.71 ± 1.11	14.0 ± 3.74	11.75 ± 2.36
roots	18.9 ± 1.55	12.0 ± 2.12	—	6.67 ± 0.82
buds	12.8 ± 2.17	5.50 ± 0.58	8.33 ± 1.37	6.00 ± 0.82
<i>S. albida</i>				
leaves	—	18.6 ± 1.14	—	8.50 ± 1.04
roots	11.6 ± 2.70	19.0 ± 1.41	19.2 ± 1.30	6.33 ± 0.52
buds	—	—	—	10.43 ± 0.98

Basing our analysis of the antimicrobial activity of the ethanolic extracts of individual organs of the studied species, we should report the highest inhibitory effect of leaves (19.2 ± 0.84 mm) and roots (18.9 ± 1.55 mm) of *S. baicalensis* in relation to *E. coli*, as well as leaves (18.6 ± 1.14 mm) and roots (19.0 ± 1.41 mm) of *S. albida* in relation to *S. aureus*, and also roots (19.2 ± 1.30 mm) of *S. albida* in relation to *P. aeruginosa*. All four test objects showed average sensitivity to the action of the extracts of leaves and buds of *S. altissima*, the other sensitivity degree to the effect of the extracts of leaves and buds of *S. baicalensis*, as well as a different degree of sensitivity to the effect of the extracts obtained from the roots of *S. albida*.

Our results indicate a significant potential for the use these species of flora of Ukraine in solving the problem of treating bacterial and fungal infections. We believe this research should be continued in order to optimize the production of fractions based on different solvents, as well as to study their effect on a larger number of test objects [1–3].

Conclusions

Our research has demonstrated the usage of *Scutellaria* plants to be promising in medicine, in the production of effective and affordable medicines based on medicinal raw materials as a safe alternative to synthetic drugs for the treatment of resistant bacterial infections.

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