



Study of the antimicrobial and fungicidal activity of the essential oil *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen"

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The main problem of modern phytotherapy is the medicinal plants that have a sufficient raw material base and contain a large number of biologically active substances. Treatment of diseases with synthetic drugs leads to the development of resistance to pathogenic microflora and the appearance of allergic reactions. Therefore, to solve this problem, it is necessary to find new plant-based antimicrobials that are safe for long-term use and have a wide spectrum of action.

The aim of the work was to study the antibacterial and fungicidal activity of the essential oil of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen".

Materials and methods. The essential oil of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" was used for the experimental part. To test the antimicrobial and antifungal action of the essential oil, reference test strains, gram-positive and gram-negative bacteria, as well as yeast-like fungi of the genus *Candida* were selected. The studies were performed *in vitro* using the disco-diffusion method.

Results. Studies have shown that the essential oil of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" has a significant antibacterial effect against *S. aureus* ATCC 25923 (diameters of growth inhibition were 14.60 ± 1.52 mm) and fungicidal effect *Candida albicans* ATCC 885-6530 (29.30 ± 2.82 mm). Antibacterial activity of the essential oil was detected in relation to *E. coli* (19.60 ± 1.85 mm). It was experimentally proven that the essential oil had no bactericidal effect on the test strain of *P. aeruginosa*.

Conclusions. It was found that the essential oil of the studied hybrid species *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" shows a significant antibacterial activity and is promising for further research.

Key words: *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen", antibacterial activity, essential oil, herb, resistance, fungicidal activity.

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Дослідження антибактеріальної та фунгіцидної активності ефірної олії *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen»

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Основна проблема сучасної фітотерапії – пошук лікарських рослин, які мали б достатню сировинну базу та містили б велику кількість біологічно активних речовин. Лікування захворювань синтетичними лікарськими засобами призводить до розвитку резистентності патогенної мікрофлори та появи алергічних реакцій. Отже, для вирішення цього завдання необхідний пошук нових антимікробних засобів на рослинній основі, які були б безпечними під час тривалого використання та мали широкий спектр дії.

Мета роботи – дослідження антибактеріальної та фунгіцидної активності ефірної олії чебрецю лимоннозапашного (*Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen»).

Матеріали та методи. Для експериментальної частини використали ефірну олію з трави чебрецю лимоннозапашного (*Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen»). Для вивчення протимікробної та протигрибкової дії ефірної олії взяли еталонні тест-штами грам позитивних і грам негативних бактерії, а також дріжджоподібні гриби роду *Candida*. Дослідження виконали *in vitro* за допомогою диско-дифузійного методу.

Результати. Під час досліджень встановили, що ефірна олія чебрецю лимоннозапашного (*Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen») має виражену антибактеріальну дію щодо *S. aureus* ATCC 25923 (діаметри затримки росту становили $14,60 \pm 1,52$ мм) і фунгіцидну дію *Candida albicans* ATCC 885–6530 ($29,30 \pm 2,82$ мм). Антибактеріальну активність ефірної олії чебрецю виявили щодо *E. coli* ($19,60 \pm 1,85$ мм). Експериментально довели, що ефірна олія не мала бактерицидного впливу на тест-штам *P. aeruginosa*.

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Key words: *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen", antibacterial activity, essential oil, herb, resistance, fungicidal activity.

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Висновки. Встановили, що ефірна олія гібридного виду *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen» має виражену антибактеріальну активність і перспективна для наступних досліджень.

Ключові слова: *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen», антибактеріальна активність, ефірна олія, трава, резистентність, фунгіцидна активність.

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Исследования антибактериальной и фунгицидной активности эфирного масла *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen»

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Основная проблема современной фитотерапии – поиск лекарственных растений, которые обладали бы достаточной сырьевой базой и содержали большое количество биологически активных веществ. Лечение заболеваний синтетическими лекарственными средствами приводит к развитию резистентности патогенной микрофлоры и появлению аллергических реакций. Поэтому для решения этой задачи необходим поиск новых антимикробных средств на растительной основе, которые были бы безопасны при длительном использовании и имели широкий спектр действия.

Цель работы – исследование антибактериальной и фунгицидной активности эфирного масла *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen».

Материалы и методы. Для экспериментальной части использовали эфирное масло чабреца лимоннопахнущего (*Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen»). Для изучения противомикробного и противогрибкового действия эфирного масла отобрали эталонные тест-штаммы грамположительных и грамотрицательных бактерий, а также дрожжеподобные грибы рода *Candida*. Исследования проведены *in vitro* с помощью диско-диффузионного метода.

Результаты. В ходе исследований установлено, что эфирное масло чабреца лимоннопахнущего (*Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen») обладает выраженным антибактериальным действием по отношению к *S. aureus* ATCC 25923 (диаметры задержки роста составили $14,60 \pm 1,52$ мм) и *Candida albicans* ATCC 88–6530 ($29,30 \pm 2,82$ мм). Установили антибактериальную активность эфирного масла по отношению к *E. coli* ($19,60 \pm 1,85$ мм). Экспериментально доказано, что эфирное масло не имеет бактерицидного влияния на тест-штамм *P. aeruginosa*.

Выводы. Установили, что эфирное масло гибридного вида *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen» проявляет антибактериальную активность и перспективно для дальнейших исследований.

Ключевые слова: *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen», антибактериальная активность, эфирное масло, трава, резистентность.

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The study of antibacterial activity is relevant to the scientific community. Resistance to antibiotics affects the disease incidence, as well as the development of allergic reactions. Today, the main goal of the pharmaceutical industry is to find herbal medicines that would inhibit the growth and spread of antibiotic-resistant microorganisms to the maximum possible degree. Scientific data analysis indicates that essential oils of plants and their components have an antibacterial effect. They are low-toxic, mild, and safe to use. The essential oil can be a highly effective supplement to the treatment of infectious diseases. It should be noted that the production of essential oil does not require massive expenses and is safe for people. The little-studied species with high concentrations of essential oil and thymol have viable research potential.

The family *Lamiaceae* is one of the most numerous and widespread in the modern flora. It has up to 200 genera and 7.000 species of grasses, shrubs, and semi-shrubs, cultivated hybrids of various species. Plants are used in modern medicine, as ornamental plants in horticulture, and as industrial crops.

The genus *Thymus* L. (Thyme), one of the most famous in this family, contains about 400 species, of which up to 50 are identified in the modern flora of Ukraine. Essential oils and extracts are part of phytopreparations with pronounced antimicrobial, anti-inflammatory, and antioxidant effects.

Comprehensive phytochemical study of species of the genus *Thymus* L. for further study and development of phytopreparations based on them is of great practical importance in medicine. *Thymus x citriodorus* (Pers.) Schreb. var. “Silver Queen” is a natural interspecific hybrid of broad-leaved *Thymus pulegoides* L. and common *Thymus vulgaris* L., which are widespread in the wild nature. Information on the accumulation of biologically active substances for this species in the scientific literature is limited. The genus has a large raw material base, a long growing season, which contributes to the accumulation of essential oil and thymol in its composition [1–5].

Aim

The aim of the work was to study the antibacterial activity of the essential oil of *Thymus x citriodorus* (Pers.) Schreb. var. “Silver Queen” by the method of *in vitro* against opportunistic pathogens of bacterial and fungal microorganisms cultures.

Materials and methods

The first step of our work was to obtain the essential oil of *Thymus x citriodorus* (Pers.) Schreb. var. “Silver Queen”, the herb of the plant was used. Plant raw materials were har-

Table 1. Studies of the antimicrobial and fungicidal activity of the oil according to the size of the zones of growth inhibition (mm) of microorganisms cultures ($x \pm \Delta x$), % $\mu = 3$

Object	Strains and the size of the zones of growth inhibition (mm) of microorganisms cultures			
	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 259220	<i>P. aeruginosa</i> ATCC 90270	<i>C. albicans</i> ATCC 885/6530
Essential oil of <i>Thymus x citriodorus</i> (Pers.) Schreb. var. "Silver Queen"	14.60 \pm 1.52	19.60 \pm 1.85	0	29.30 \pm 2.82

vested during flowering in the central and south-eastern part of Ukraine in 2017–2018 (June–October) in accordance with the requirements of the SPU (2.3) subsection (2.3.2). Studies of the antibacterial activity essential oils of a series of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" were performed on the basis of the microbiological laboratory of ZSMU. The studies were performed in vitro using the disco-diffusion method using reference test strains belonging to different groups of microorganisms: *Escherichia coli* ATCC 25922 (gram-negative enterobacteria), *Pseudomonas aeruginosa* ATCC 27853 (non-fermenting gram-negative microorganisms), *Candida albicans* ATCC 885-653 (yeast-like fungi of the genus *Candida*).

The experiments used 24h cultures of bacteria, from which NS suspensions were prepared with a density of 0.5 according to McFarland, which corresponded to 5×10^6 CFU/ml for *Candida albicans* and 1.5×10^8 CFU/ml for all other microorganisms. Also, disks soaked with essential oil were used for experiments. For this purpose, ready-made paper disks with a diameter of 6 mm were used, which in laboratory practice were normally used for impregnation with antibiotics.

The disk was immersed in essential oil for a few seconds, after which it was dried and used in research. The day-old culture of the test strain was seeded on the surface of Mueller–Hinton agar, dried for 5–10 min, then impregnated discs were placed on the agar surface. Plates were incubated at $35 \pm 1^\circ\text{C}$ for 18 h in studies with *Escherichia coli*, *Pseudomonas*, and *Staphylococcus* and for 48 h in experiments with *Candida*. Sensitivity/ resistance to the essential oil was determined by the presence/absence of growth inhibition zones around the oil disk. The diameter of the growth inhibition was measured in millimeters to an accuracy of 1 mm. The study was performed in triplicate.

It was calculated using the standard statistical package of the licensed program Statistica for Windows 13 (StatSoft Inc., № JPZ804I382130ARCN10-J), as well as SPSS 16.0 and Microsoft Office Excel 2003).

Results

Microbiological studies have shown that the essential oil has a significant antibacterial effect against *S. aureus* ATCC 25923, *E. coli* ATCC 259220 and fungicidal activity *Candida albicans* ATCC 885/6530 (Table 1).

The essential oil has been represented by a wider range of bactericidal action. The activity against *S. aureus* was analyzed (growth retardation diameters amounted to 14.60 ± 1.52), as well as for *E. coli* (19.60 ± 1.85), and *Candida albicans* (29.30 ± 2.82). It was experimentally proven that the essential oil had no bactericidal effect on the test strain of *P. aeruginosa*.

Discussion

Upon analysis of the study results, we can state that in our in vitro antibacterial studies we obtained data confirming that the essential oil has an antibacterial effect against the reference test strains of *S. aureus*, *C. albicans* and *E. coli*. The obtained results were indicated the prospect of further research and studied of the essential oil of the little-studied hybrid species of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" as a main antibacterial component for phytopreparations [6–11].

Conclusions

1. Upon analysis of the study results, it was found that the essential oil of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" has a pronounced antibacterial effect against the reference test strains of *S. aureus* ATCC 25923, *C. albicans* and *E. coli* ATCC 259220.

2. The data obtained were indicated that further study of the hybrid species had a promising medicinal perspective as a source of herbal antibacterial drugs.

Prospects for further research. The data obtained indicate positive further prospects for the study of essential oil of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" as an active ingredient in herbal antibacterials.

Conflicts of interest: authors have no conflict of interest to declare
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