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

Y. M. Steshenko, O. V. Mazulin, N. M. Polishchuk

Zaporizhzhia State Medical University, Ukraine

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.

Current issues in pharmacy and medicine: science and practice 2021; 14 (2), 211–214
Висновки. Встановлено, що ефірна олія гібридного виду Thymus x citriodorus (Pers.) Schreb. var. «Silver Queen» має виражену антибактеріальну активність і перспективу для наступних досліджень.

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

Актуальні питання фармацевтичної і медичної науки та практики. 2021. Т. 14, № 2(36). С. 211–214

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

Я. Н. Стешенко, А. В. Мазулин, Н. Н. Полящук

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 антибактериальных средств на растительной основе, которые были бы безопасны при длительном использовании и имели широкий спектр действия.

Цель работы — исследование антибактериальной и фунгцидной 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.). 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 зон задержки роста составили 14,60 ± 1,52 мм) и Candida albicans ATCC 88–6530 (29,30 ± 2,82 мм). Установлены антибактериальную активность эфирного масла по отношению к E. coli (19,60 ± 1,85 мм). Экспериментально доказано, что эфирное масло не имеет бактерицидного влияния на тест-штамм P. aeruginosa.

Выводы. Установлено, 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.

Актуальные вопросы фармацевтической и медицинской науки и практики. 2021. Т. 14, № 2(36). С. 211–214

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 interspecífic 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-
venerated 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 × 106 CFU/ml for *Candida albicans* and 1.5 × 108 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 ± 1 °C for 18 h in studies with *Escherichia coli*, *Pseudomonad*, 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., № JPZ8041382130ARCN10-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

**Information about authors:**

Steshenko Ya. M., Postgraduate student of the Department of Clinical Pharmacy, Pharmacotherapy, Pharmacognosy and Pharmaceutical Chemistry, Zaporozhzhia State Medical University, Ukraine. ORCID ID: 0000-0002-7558-6740

Mazulin O. V., PhD, DSc, Professor of the Department of Clinical Pharmacy, Pharmacotherapy, Pharmacognosy and Pharmaceutical Chemistry, Zaporozhzhia State Medical University, Ukraine. ORCID ID: 0000-0003-0628-4457

Polischuk N. M., MD, PhD, Associate Professor, Head of the Department of Microbiology, Virology and Immunology, Zaporozhzhia State Medical University, Ukraine. ORCID ID: 0000-0002-9791-5818

**Відомості про авторів:**

Стешенко Я. М., аспірант каф. клінічної фармації, фармацопеїї, фармахімії та фармацевтичної хімії, Запорізький державний медичний університет, Україна. ORCID ID: 0000-0002-7558-6740

Мазулин О. В., д-р фарм. наук, професор каф. клінічної фармації, фармацопеїї, фармахімії та фармацевтичної хімії, Запорізький державний медичний університет, Україна. ORCID ID: 0000-0003-0628-4457

Поліщук Н. М., канд. мед. наук, доцент, зав. каф. мікробіології, вірусології та імунології, Запорізький державний медичний університет, Україна.

### 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

| Object | Strains and the size of the zones of growth inhibition (mm) of microorganisms cultures |
|--------|-----------------------------------------------------------------------------------------|
| Essential oil of *Thymus x citriodorus* (Pers.) Schreb. var. “Silver Queen” | | |
| S. aureus ATCC 25923 | 14.60 ± 1.52 |
| E. coli ATCC 259220 | 19.60 ± 1.85 |
| P. aeruginosa ATCC 90270 | 0 |
| C. albicans ATCC 885/6530 | 29.30 ± 2.82 |
Сведения об авторах:
Стешенко Я. Н., PhD-аспирант каф. клинической фармации, фармакотерапии, фармакогнозии и фармацевтической химии, Запорожский государственный медицинский университет, Украина.
Мазулин А. В., д-р фарм. наук, профессор каф. клинической фармации, фармакотерапии, фармакогнозии и фармацевтической химии, Запорожский государственный медицинский университет, Украина.
Полищук Н. Н., канд. мед. наук, доцент, зав. каф. микробиологии, вирусологии и иммунологии, Запорожский государственный медицинский университет, Украина.

References
[1] Pavel, M., Ristic, M., & Stevic, T. (2010). Essential oils of Thymus pulegioides and Thymus glabrescens from Romania: chemical composition and antimicrobial activity. Journal of the Serbian Chemical Society, 75(1), 27-34. https://doi.org/10.2298/jsc1001027p
[2] Nabavi, S. M., Marchese, A., Izadi, M., Curti, V., Daglia, M., & Nabavi, S. F. (2015). Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy. Food Chemistry, 173, 339-347. https://doi.org/10.1016/j.foodchem.2014.10.042
[3] Wong, W. F., & Santiago, M. (2017). Microbial approaches for targeting antibiotic-resistant bacteria. Microbial Biotechnology, 10(5), 1047-1053. https://doi.org/10.1111/1751-7915.12783
[4] El Amri, J., Elbadaoui, K., Zair, T., Bouharb, H., Chakir, S., & Alaoui, T. (2014). Étude de l'activité antibactérienne des huiles essentielles de Teucrium capitatum L et l'extrait de Silène vulgaris sur différentes souches testées [Study the antibacterial activity of essential oils Teucrium capitatum L and Silene vulgaris extract on different strains tested]. Journal of Applied Biosciences, 82, 7481-7492. https://doi.org/10.4314/jab.v82i16
[5] Oubihi, A., Ouryemchi, I., Nounah, I., Tarfaoui, K., Harhar, H., Ouhssine, M., & Guessous, Z. (2020). Chemical composition, antibacterial and antifungal activities of Thymus leptobotrys Murb essential oil. Advances in Traditional Medicine, 20(4), 673-679. https://doi.org/10.1007/s13596-020-00488-w
[6] Nikolic, M., Glamoclija, J., Ciric, A., Peric, T., Markovic, D., Stevic, T., & Sokovic, M. (2012). Antimicrobial activity of ozone gas and colloidal silver against oral microorganisms. Digest Journal of Nanomaterials and Biostuctures, 7(4), 1093-1099.
[7] Reis, F. S., Martins, A., Barros, L., & Ferreira, I. C. (2012). Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: a comparative study between in vivo and in vitro samples. Food and chemical toxicology, 50(5), 1201-1207. https://doi.org/10.1016/j.fct.2012.02.013
[8] Venkateshappa, S. M., & Sreenath, K. P. (2013). Potential Medicinal Plants of Lamiaceae. American International Journal of Research in Formular, 3(1), 82-87.
[9] Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. Journal of applied microbiology, 86(6), 985-990. https://doi.org/10.1046/j.1365-2672.1999.00780.x