



Study by GC-MS method of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" extracts component composition

Ya. M. Steshenko^{ID*1,B,C,D}, O. V. Mazulin^{ID1,A,D,E,F}

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;
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The *Thyme* L. genus is quite common in Ukraine, herbal extracts are part of many effective herbal medicinal products that are widely used in modern phytocosmetology. Today, genus *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" is poorly studied but promising in terms of sufficient raw material base and BAS. Therefore, the GC-MS method of designating the component of the extract from herb splint is relevant.

The aim of the work is to determine by the GC-MS method of the component alcohol extracts (1:10) compositions of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" herbs from Ukrainian flora.

Materials and methods. For the experimental studies, we used alcoholic extracts of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" from herbs (1:10) harvested from Ukrainian flora. The component composition of extracts was analyzed using an Agilent 7890B gas chromatography with 5977B a mass – spectrometric detector.

Results. By the GC-MS method were revealed up to 63 compounds. Two compounds were not identified. It is important to note, that seven biologically active compounds were revealed in a concentration over 5 %: oleic acid ($10.35 \pm 1.42\%$), caryophyllene oxide ($9.62 \pm 0.97\%$), matricarin ($7.59 \pm 0.86\%$), trans-citral ($6.35 \pm 0.75\%$), β -bisabolene ($6.15 \pm 0.75\%$), thymol ($5.46 \pm 0.70\%$).

Conclusions. Taking into account by GC-MS method, it can be concluded that the studied species is relevant for further phytochemical study.

Key words: *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen", gas-chromatography with mass-spectrometric detector, herb, pharmacological activity.

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Дослідження методом ГХ-МС компонентного складу екстракту *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen»

Я. М. Стешенко, О. В. Мазулін

Рід *Thymus* L. (Чебрець) доволі поширеній в Україні, екстракти з цієї трави входять до складу багатьох ефективних фітопрепаратів, що широко використовують в сучасній медицині. Нині відомий вид роду *Thymus* L. чебрець лимоннозапашний (*Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen») є недостатньо досліджений, але перспективний з погляду достатньою сировинною базою для заготівлі рослинної сировини. Тому визначення методом ГХ-МС (газової хроматографії-мас-спектрометрії) компонентного складу екстракту з трави рослини є актуальним і доцільним.

Мета роботи – методом ГХ-МС визначити компонентний склад і кількісний вміст летких сполук у спиртових екстрактах (1:10) із трави чебрецю лимоннозапашного флори України.

Матеріали та методи. Для експериментальних досліджень використовували спиртові екстракти трави чебрецю лимоннозапашного (1:10), що заготували в різних регіонах України. Компонентний склад досліджено методом ГХ-МС на пристрії Agilent 7890B із мас-спектрометричним детектором 5977B.

Результати. За допомогою методу ГХ-МС ідентифікували 63 сполуки. Не ідентифікували 2 сполуки. Відзначимо, що 5 біологічно активних сполук наявні в концентраціях понад 5 %: олеїнова кислота ($10.35 \pm 1.42\%$), каріофілен оксид ($9.62 \pm 0.97\%$), матрикарин ($7.59 \pm 0.86\%$), транс-цитраль ($6.35 \pm 0.75\%$), β -бісаболен ($6.15 \pm 0.75\%$), тимол ($5.46 \pm 0.70\%$),

Висновки. На підставі даних ГХ-МС методу встановили, що трава чебрецю лимоннозапашного – перспективний об'єкт для подальшого фітохімічного дослідження.

Ключові слова: чебрець лимоннозапашний, ГХ-МС метод, трава, фармакологічна активність.

Актуальні питання фармацевтичної і медичної науки та практики. 2022. Т. 15, № 1(38). С. 46–51

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*E-mail: anastesenko07@gmail.com

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Исследование ГХ-МС методом компонентного состава экстракта *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen»

Я. Н. Стешенко, А. В. Мазулин

Род *Thymus* L. (Чабрец) достаточно распространён в Украине, экстракты из травы которого входят в состав многих эффективных фитопрепаратов, широко используемых в современной медицине. В настоящее время известный вид рода *Thymus* L. тимьян лимоннапахнущий (сорт *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen») недостаточно исследован, но перспективен с точки зрения достаточной сырьевой базы для заготовки растительного сырья. Поэтому ГХ-МС метод определения компонентного состава экстракта из травы растения является актуальным и целесообразным.

Цель работы – методом ГХ-МС определить компонентный состав и количественное содержание летучих соединений в спиртовых экстрактах (1:10) из травы чабреца лимоннапахнущего флоры Украины.

Материалы и методы. Для экспериментальных исследований использовали спиртовые экстракты травы чабреца лимоннапахнущего (1:10), заготовленные в разных регионах Украины. Компонентный состав исследован методом ГХ-МС на устройстве Agilent 7890B с масс-спектрометрическим детектором 5977B.

Результаты. С помощью метода ГХ-МС идентифицированы 63 соединения. Не идентифицированы 2 соединения. Следует обозначить, что 5 биологически активных соединений присутствуют в концентрации больше 5 %: олеиновая кислота ($10,35 \pm 1,42$ %), карифиллен оксид ($9,62 \pm 0,97$ %), матрикарин ($7,59 \pm 0,86$ %), транс-цитраль ($6,35 \pm 0,75$ %), β -бисаболен ($6,15 \pm 0,75$ %), тимол ($5,46 \pm 0,70$ %).

Выводы. На основании полученных данных ГХ-МС метода установили, что трава чабреца лимоннапахнущего является перспективным объектом для дальнейшего фитохимического исследования.

Ключевые слова: чабрец лимоннапахнущий, ГХ-МС метод, трава, фармакологическая активность.

Актуальные вопросы фармацевтической и медицинской науки и практики. 2022. Т. 15, № 1(38). С. 46–51

At present, in modern phytotherapy, there is a question of finding new medicinal plants that have a sufficient raw material base as a source for obtaining biologically active substances (BAS). The main task in modern pharmacy is to create drugs that exhibit pronounced biological activity, have no side effects, are affordable and easy to use. Species of the genus *Thymus* L. are one of the most famous in the family Lamiaceae and have about 400 members, of which only up to 50 have been identified in the flora of Ukraine. The family Lamiaceae or Labiate contains many valuable medicinal plants. For example, essential oils of Thyme have many different active compounds: aldehydes, ketones, esters, phenols, terpenes (monoterpene and sesquiterpenes). Essential oil of *Thymus pulegioides* L. have compounds: thymol (26.0 %), carvacrol (21.0 %), γ -Terpinene (8.8 %), p-Cymene (7.8 %), octan-3-one (3.9 %), camphor (3.9 %), β -Bisabolene (3.0 %), borneol (2.9 %), oct-1-en-3-ol (2.0 %). Sesquiterpene β -caryophyllene is an important antifungal component in the Lamiaceae family [1]. In the scientific literature, there is a lot of information about the specifics of research on Thyme. Many different compounds: vitamin E, flavonoids, catechin, and phenolic acids. Ethanolic extract of *Thymus vulgaris* leaves is rich in phenols and has antioxidant activities, according to S. U. Wisam et al. [2].

It should be noted that this is a polymorphic genus. Phytochemical studies in the grass of species of the genus *Thymus* L. revealed the presence of BAS: essential oil, flavonoids (derivatives of apigenin, luteolin), hydroxycinnamic acids, polysaccharides, tannins, essential oil, vitamins, triterpene saponins [3]. In Italian, some species (*T. vulgaris* "Silver Poise", *T. vulgaris* "Erectus", *T. vulgaris* "Faustini", *T. x citriodorus* "Anderson's Gold", and *T. x citriodorus* "Silver Queen") cultivated and have some phytochemical differences the essential oil of the *T. x citriodorus* have more while geraniol (61–67 %). In all the analyzed essential oils,

non-oxygenated (16–79 %) [4]. GC-MS analysis is part of modern phytochemistry. Thymol, geraniol, and carvacrol are specific compounds the accumulation of which determines the chemotype of the species and the growing period of plants [5].

Antimicrobial, anti-inflammatory, antioxidant, and wound-healing effect is a promising *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen". It was widespread as a cultivated and wild plant almost in Ukraine. Considering the results of previous research and a sufficient raw material base in Ukraine, it is relevant and appropriate to conduct an in-depth study of this promising species. These are hybrid species that are promising for research on the chemical composition of essential oil and use for determining the biological activity of herbal extracts. For example, antifungal activity of the essential oil *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" is due to the presence of polyphenolic compounds and thymol, which inhibits growth of *Candida albicans* (29.30 ± 2.82 mm) [6]. Many extractions of the volatile constituents of the different plant materials were carried out by hydrodistillation (HD) processes. Nowadays use GC/MS analytical method in modern research [7].

Lamiaceae family is a widespread aroma plant cultivated in the Mediterranean region. Essential oil of *Thymus citriodorus* L. has geraniol (60 %), geranyl acetate (1.0 %), geranyl butyrate (0.8 %), nerol (2.8 %), according to Suleyman Kizil, Ozlem Toncer [8]. Some essential oils and their single compounds use in cosmetology. Since the composition of Thyme oil consists of thymol and carvacrol, it is used in the component composition of phytopreparations in the treatment of dermatitis [9]. Component composition of raw materials has a different structure. For example, thymol is a natural component (phenol monoterpene). Thymol has anti-inflammatory, antibacterial properties, which is very important for the preparation of phytopreparations [10].

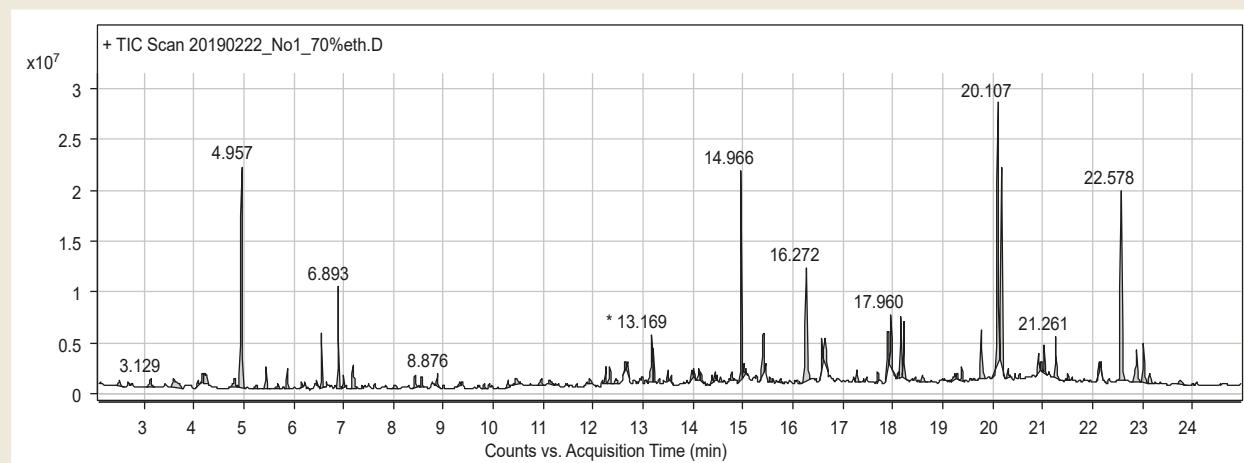


Fig. 1. Chromatogram of alcohol extract of *Thymus x citriodorus* (Pers.) Schreb var. “Silver Queen”.

Genus *Thymus x citriodorus* is widely used for research. It is known that hydrided species are better adapted to climatic conditions. Raw materials are rich in the accumulation of organic acids and polyphenolic compounds. Scientists of Zaporizhzhia State Medical University actively research species of the genus *Thymus* L. *Th. tauricus* L. is a specific species that grows in Ukraine. Characterized by a very strong aromatic plant. Phytochemical studies of this species are relevant for scientific research. Phenolic composition, amino acid composition, the study of the accumulation of ascorbic acid basis for pharmacological research [11]. So, after reading the literature, we can say that *Thymus x citriodorus* (Pers.) Schreb. var. “Silver Queen” is an excellent resource for studying the chemical composition of raw materials and further pharmacological experiments [12]. Despite the significant diversity of flora of Ukraine, resources medicinal plant raw materials are not limitless. Cultivation is considered as a promising strategy for the conservation of natural resources and as a reliable way of production of a sufficient number of phytopreparations on the basis of species with proper raw material base. *Th. x citriodorus* “Silver Queen” tincture is used for pneumonia, bronchitis. Compresses and baths from the stems and leaves of the plant reduce pain in arthritis. A wide range of research is currently underway, affecting various aspects of phytochemical and pharmacological study of aromatic plants.

Aim

The aim of this work is to study the component composition and quantitative content of volatile compounds in alcohol extracts (1:10) from *Thymus x citriodorus* (Pers.) Schreb. var. “Silver Queen” flora of Ukraine. To establish the possible presence of thymol-containing substances.

Materials and methods

For the experimental part we used alcohol extract (1:10) of *Thymus x citriodorus* (Pers.) Schreb. var. “Silver Queen”. Plant raw materials (herb) were harvested during flowering

in the central and south-eastern part of Ukraine in 2017–2021 (June–October) in accordance with the requirements of the SPU (2.3) subsection (2.3.2). Studies of the chemical composition of *Thymus x citriodorus* (Pers.) Schreb. var. “Silver Queen” were on the basis of the laboratory of ZSMU. The identity of alcohol extract (1:10) has been confirmed by gas chromatography via Agilent 7890B GC system connected with Agilent 5977B mass spectrometry detector (USA). The column used for separation was DB-5ms with the following dimensions: 30 m × 250 μm × 0.25 μm. Carrier gas (helium) flow rate was 2.0 mL/min. Injection volume: 0.5 μL. Flow split was 1:5. The temperature of the injection system was programmed as follows: 200 °C → 12 °C/s → 265 °C. Oven temperature: programmable, with the initial temperature of 70 °C (1 minute delay) → 10 °C/min → 270 °C (4 min delay). The total time of the chromatographic run was 25 min. The temperature of the GC-MS interface was maintained at 275 °C; the ion source temperature was 230 °C; the temperature of the quadrupole mass analyzer was 150 °C. Type of ionization: electron impact (EI) with electron energy of 70 eV. Range of scanned mass ratios: 30–700 m/z. The NIST14 mass spectrum library was used to identify the components.

Results

The qualitative composition and quantitative content of compounds in the alcoholic extract of *Thymus x citriodorus* (Pers.) Schreb. var. “Silver Queen” were identified by the GC-MS method. The results of the study are presented in Table 1 and Fig. 1.

The obtained data indicate the presence of volatile compounds from the grass *Thymus x citriodorus* (Pers.) Schreb. var. “Silver Queen” up to 63 components from classes: organic acids, esters of organic acids, alcohols, phenols, terpenoids, unsaturated hydrocarbons. Two compounds have not been identified. It should be noted that 7 compounds were present in the alcohol extract in concentrations above 5 %: oleic acid ($10.35 \pm 1.42\%$), caryophyllene-oxide ($9.62 \pm 0.97\%$), matricarin ($7.59 \pm 0.86\%$), trans-citral

Table 1. The results of studying the chemical composition composition of raw materials of species *Thymus L.* by GC-MS method, ($x + \Delta x$) %, $\mu = 6$

No.	Compound label	RT (min)	Area Sum %
1	Camphene	3.13	0.46 ± 0.05
2	Thujene	3.59	1.05 ± 0.13
3	β-Pinene	4.08	0.35 ± 0.04
4	Skvalen	4.18	0.54 ± 0.07
5	γ-Pinene	4.19	0.70 ± 0.08
6	γ-Terpinene	4.82	0.60 ± 0.08
7	Eucalyptol	4.96	9.53 ± 1.23
8	α-Terpinene	5.25	0.25 ± 0.03
9	Sabinene	5.45	0.72 ± 0.08
10	3,5-Heptadienal,2-ethylidene-6-methyl-	5.68	0.23 ± 0.01
11	1-octen-3-ol	5.87	0.63 ± 0.08
12	Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7trimethyl-	6.16	0.20 ± 0.01
13	Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl2-methylene,[1S(1.alpha.,3.alpha.,5.alpha.)]-	6.46	0.09 ± 0.01
14	Camphora	6.56	1.96 ± 0.24
15	Bicycl[2.2.1]hentan-2-ol,1,7,7,3- methylethyl -, (1S-endo)-	6.89	3.09 ± 0.40
16	Terpinen-4-ol	6.99	0.33 ± 0.14
17	α -Terpineol	7.19	1.08 ± 0.02
18	Unidentified compound	8.02	0.28 ± 0.01
19	Phenol, 2-methyl-5-(1-methylethyl)	8.43	0.65 ± 0.08
20	Thymol	8.56	5.46 ± 0.70
21	Carvacrol	8.88	0.53 ± 0.12
22	3-Allyl-6-methoxyphenol	9.32	0.22 ± 0.03
23	β-Bourbonene	9.82	0.28 ± 0.03
24	β-Caryophyllene	10.30	0.33 ± 0.03
25	Inozine	10.45	0.41 ± 0.16
26	Neral	10.96	0.43 ± 0.16
27	Geranyl phormate	11.13	0.25 ± 0.02
28	3-tert-Butyl-4-hydroxyanisole	11.93	0.30 ± 0.03
29	18-Nonadecenoic acid	12.25	0.57 ± 0.03
30	Geranyl acetate	12.33	0.67 ± 0.08
31	Germacrene D	12.45	0.33 ± 0.03
32	Dodecanoic acid	12.65	0.49 ± 0.03
33	Ocimen	12.68	0.25 ± 0.02
34	Linalool	13.17	2.16 ± 0.28
35	Neointermediol	13.21	0.69 ± 0.08
36	1,6-octadien-3-ol-3,7-dimethyl	13.49	0.31 ± 0.03
37	α-Kubinene	13.56	0.24 ± 0.01
38	(E)-4-(3-Hydroxypropan-1-en-1-il)-2- methoxyphenol	13.97	0.24 ± 0.01
39	Chamazulene	14.02	0.36 ± 0.02
40	α-Humulene	14.11	0.39 ± 0.02

Cont. of Table 1.

No.	Compound label	RT (min)	Area Sum %
41	Tetradecanoic acid	14.16	0,27 ± 0,01
42	7-hexylacosane	14.38	0.24 ± 0,01
43	2-Hydroxy-4,4,8trimethyltricyclo[6.3.1.0(1,5)]dodecan-9-on	14.44	0.24 ± 0,01
44	Tricosane	14.55	0.23 ± 0,01
45	Linaloilpropionate	14.77	0.29 ± 0,02
46	β-Bisabolene	14.97	6.15 ± 0.75
47	Heptacosan	15.01	0.26 ± 0,01
48	Tricyclo[5.1.0.0(2,4)]oct-5-ene-5propanoic acid, 3,3,8,8-tetramethyl-	15.40	1.95 ± 0.22
49	5-(3-Hydroxypropyl)-2,3dimethoxyphenol	15.53	0.26 ± 0,01
50	5-Pentadien-7-il	15.76	0.21 ± 0,02
51	Trans-citral	16.27	6.35 ± 0.75
52	Hexadecanoic acid ethyl ester	16.59	0.95 ± 0.12
53	Azulene	16.65	1.45 ± 0.12
54	Corymbolone	17.28	0.59 ± 0,04
55	Phytol	17.69	0.34 ± 0,01
56	9,12-Octadecadienoic acid (Z,Z)-	17.91	1.55 ± 0.17
57	Oleic acid methyl ester	17.96	1.60 ± 0.18
58	Linoleic acid ethyl ester	18.16	1.83 ± 0.22
59	Ethyl Oleate	18.22	1.82 ± 0.20
60	Unidentified compound	18.60	0.11 ± 0,01
61	5H-Cyclopropa[3,4]benz[1,2-e]azulen5-one,9a(acetoxy)1,1a,1b,4,4a,7a,7b,8,9,9a-decahydro-4a,7b,9trihydroxy-3(hydroxymethyl)-1,1,6,8tetramethyl-, [1aR(1a.alpha.,1b.beta.,4a.beta.,7a.alpha.,7b.alpha.,8.alpha.,9.beta.,9a.alpha.)]	19.24	0.20 ± 0,01
62	Oleic acid	20.11	10.35 ± 1.42
63	Matricarin	20.18	7.59 ± 0.86
64	Caryophyllene-oxide	22.58	9.62 ± 0.97
65	Heptacosane	22.87	2.11 ± 0.26

(6.35 ± 0.75 %), β-bisabolene (6.15 ± 0.75 %), thymol (5.46 ± 0.70 %). During the GC-MS study, the component composition was identified and the quantitative content of compounds in the alcohol extract (1:10) from the herb *Thymus x citriodorus* (Pers.) Schreb var. "Silver Queen" was determined. The 63 compounds were presented from the classes: organic acids, esters of organic acids, alcohols, phenols, terpenoids, unsaturated hydrocarbons. 2 compounds were not identified. 7 compounds with a content of more than 5 % were present to the greatest extent: oleic acid (10.35 ± 1.42 %), eucalyptol (9.53 ± 1.23 %), caryophyllene oxide (9.62 ± 0.97 %), matricarin (7.59 ± 0.86 %), trans-citral (6.35 ± 0.75 %), β-bisabolene (6.15 ± 0.75 %), thymol (5.46 ± 0.70 %). The obtained data indicated the prospects of phytochemical study of thyme for the creation of new promising drugs. The plant has a sufficient raw material base during cultivation and contains up to 63 components from classes of compounds with pronounced antimicrobial, anti-inflammatory, antioxidant activity. In view of all the above, *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" can be recommended for further research in phytochemistry and phytopharmacology.

Discussion

The obtained data indicated the prospects of phytochemical study of thyme for the creation of new promising drugs. The plant has a sufficient raw material base during cultivation and contains up to 63 components from classes of compounds with pronounced antimicrobial, anti-inflammatory, antioxidant activity. In view of all the above, *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" can be recommended for further research in phytochemistry and phytopharmacology.

Conclusions

1. We used GC-MS was an alcoholic extract of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" identified and quantified up to 63 compounds. Among them, was presented substances with pronounced antimicrobial, anti-inflammatory, antioxidant activity. There were 7 compounds in the greatest with pronounced biological activity and accumulation of more than 5 %: oleic acid (10.35 ± 1.42 %), eucalyptol

($9.53 \pm 1.23\%$), caryophyllene oxide ($9.62 \pm 0.97\%$), matricarin ($7.59 \pm 0.86\%$), trans-citral ($6.35 \pm 0.75\%$), β -bisabolene ($6.15 \pm 0.75\%$), thymol ($5.46 \pm 0.70\%$).

2. The accumulation of components with a pronounced biological effect in the plant extract provides an opportunity for the cultivation of thyme in Ukraine and the subsequent introduction of AND on the grass species as a draft official monograph to the SFU of Ukraine. The detected components of the alcoholic extract of the thyme herb can be used for further study of other morphological parts, as well as for the drafting of a monograph on a new species of LRS.

3. The analysis of the scientific literature showed that the chemical composition of the raw materials of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" had not been sufficiently studied. Nowadays this species *Thymus* L. is very popular for pharmaceutical research, but the biological properties of this hybrid species have not been fully studied. The study of the chemical composition of the plant is very important for the study of pharmacological properties *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen".

Prospects for further research. The data obtained indicate positive further prospects for the study of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" as an antimicrobial, anti-inflammatory, antioxidant activity in plants.

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Information about authors:

Steshenko Ya. M., Postgraduate student of the Department of Clinical Pharmacy, Pharmacotherapy, Pharmacognosy and Pharmaceutical Chemistry, Zaporizhzhia State Medical University, Ukraine.

ORCID ID: [0000-0002-7538-6740](https://orcid.org/0000-0002-7538-6740)

Mazulin O. V., PhD, DSc, Professor of the Department of Clinical Pharmacy, Pharmacotherapy, Pharmacognosy and Pharmaceutical Chemistry, Zaporizhzhia State Medical University, Ukraine.

ORCID ID: [0000-0003-0628-4457](https://orcid.org/0000-0003-0628-4457)

Відомості про авторів:

Стешенко Я. М., PhD-аспірантка каф. клінічної фармації, фармакотерапії, фармакогнозі та фармацевтичної хімії, Запорізький державний медичний університет, Україна.

Мазулін О. В., д-р фарм. наук, професор каф. клінічної фармації, фармакотерапії, фармакогнозі та фармацевтичної хімії, Запорізький державний медичний університет, Україна.

Сведения об авторах:

Стешенко Я. Н., PhD-аспирант каф. клинической фармации, фармакотерапии, фармакогнозии и фармацевтической химии, Запорожский государственный медицинский университет, Украина.

Мазулін А. В., д-р фарм. наук, профессор каф. клинической фармации, фармакотерапии, фармакогнозии и фармацевтической химии, Запорожский государственный медицинский университет, Украина.

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