Original research

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

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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 *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 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 ± 1.42 %), 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 %).

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.

Current issues in pharmacy and medicine: science and practice 2022; 15 (1), 46–51

Триметичний рід *Thymus* L. (Чебрець) дуже поширений 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 перспективним з погляду достатньої сировинної бази для заготівлі рослинної сировини. Тому визначення методом ГХ-МС (газової хроматографії-мас-спектрометрії) компонентного складу екстракту з трави рослин 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 заготувані в різних регіонах України. Компонентний склад досліджено методом ГХ-МС на пристрої Agilent 7890B із мас-спектрометричним детектором 5977B.

Результати. За допомогою методу ГХ-МС ідентифікували 63 сполук. Не ідентифікували 2 сполуки. Відзначено, що 7 біологічно активних сполук навіяно в концентрації понад 5 %: олеїнова кислота (10,35 ± 1,42 %), каріофілен оксид (9,62 ± 0,97 %), матрикарин (7,59 ± 0,86 %), транс-цитраль (6,35 ± 0,75 %), β-бісаболен (6,15 ± 0,75 %), тимол (5,46 ± 0,70 %).

Висновки. На підставі даних ГХ-МС методу встановлено, 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
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 Labiatae contains many valuable medicinal plants. For example, essential oils of Thyme have many different active compounds: aldehydes, ketones, esters, phenols, terpenes (monoterpenes 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 (phenol monoterpene). Thymol has anti-inflammatory properties, antibacterial properties, which is very important for 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 monoterpane). Thymol has anti-inflammatory, antibacterial properties, which is very important for the preparation of phytopreparations [10].
Genus *Thymus x citriodorus* is widely used for research. It is known that hybrid 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*. *Th. tauricus* 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 ± 1.42 %), caryophyllene-oxide (9.62 ± 0.97 %), matricarin (7.59 ± 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, $(x \pm \Delta x)\%$, $\mu = 6$

| 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-dimethyl(2-methylene,[1S(1.alpha.,3.alpha.,5.alpha.)]- | 6.46     | 0.09 ± 0.01|
| 14  | Camphora                                                                       | 6.56     | 1.96 ± 0.24|
| 15  | Bicyclo[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  | a-Kubinene                                                                      | 13.56    | 0.24 ± 0.01|
| 38  | (E)-4-(3-Hydroxypropan-1-en-1-ii)-2- methoxyphenol                              | 13.97    | 0.24 ± 0.01|
| 39  | Chamazulene                                                                     | 14.02    | 0.36 ± 0.02|
| 40  | a-Humulene                                                                      | 14.11    | 0.39 ± 0.02|
(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.

**Discussion**

The obtained data was 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

| No. | Compound label | RT (min) | Area Sum % |
|-----|----------------|----------|------------|
| 41  | Tetradecanoic acid | 14.16    | 0.27 ± 0.01 |
| 42  | 7-hexyldocosane | 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  | Linalolpropionate | 14.77 | 0.29 ± 0.02 |
| 46  | β-βisabolene | 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-tetramethyl- | 15.40 | 1.95 ± 0.22 |
| 49  | 5-(3-Hydroxypropyl)-2,3dimethoxyphenol | 15.53 | 0.26 ± 0.01 |
| 50  | 5-Pentadien-7-ll | 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(acetyloxy)1,1a,1b,4a,4,7a,7b,8,9,9a-decachydro-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  | Heptacosan | 22.87 | 2.11 ± 0.26 |
(9.53 ± 1.23 %), Caryophyllene oxide (9.62 ± 0.97 %), matricar (7.59 ± 0.86 %), trans-citral (6.35 ± 0.75 %), β-bisabolene (6.15 ± 0.75 %), thymol (5.46 ± 0.70 %).

2. The accumulation of 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 on 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.

Funding
The work was carried out within the framework of the scientific research program of the Zaporizhzhia State Medical University: “Pharmacognostic and ecological research of promising species of flora of Ukraine with standardization of plant products and production of medicines”, state registration No. 0117U006960.

Acknowledgements
The team of authors thanks the rector of Zaporizhzhia State Medical University for the opportunity to conduct experiments.

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

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Original research

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