Study the polyphenolic compounds composition of *Thymus vulgaris* L. herb

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A – концепція та дизайн дослідження; B – збір даних; C – аналіз та інтерпретація даних; D – написання статті; E – редагування статті; F – остаточне затвердження статті

The *Thymus* L. genus is extremely widespread on the territory of Ukraine and includes up to 50 main species. Extracts from the official species of the *Thymus* L. genus are part of effective herbal preparations with pronounced anti-inflammatory, antimicrobial and antioxidant activities. A promising direction of modern phytochemical research is the determination of the accumulation of biologically active polyphenolic compounds in the *Thymus vulgaris* L. herb, which is widely cultivated in Ukraine and other countries.

The aim of the work is to determine the accumulation of polyphenolic compounds in *Thymus vulgaris* L. herb at the end of flowering using TLC and HPLC methods.

Materials and methods. For experimental studies, alcohol extracts from *Thymus vulgaris* L. herb (1:100) were used. The plants were cultivated in Volodymyrivka, Zaporizhzhia region during the period of maximum polyphenolic compounds accumulation (June – August 2022). The substances' component composition was studied by TLC method Biostep CD 60 densitometer (Germany) and HPLC Agilent 1260 Infinity HPLC System Open LABCDS Software (Japan).

Results. TLC and HPLC methods revealed up to 29 polyphenolic compounds. Experimentally determined flavonoids and hydroxycinnamic acids are known in phytotherapy for their pronounced anti-inflammatory, antimicrobial, and antioxidant activities.

Conclusions. Considering the results obtained by TLC and HPLC methods, it can be concluded that *Thymus vulgaris* L. is a promising species for further phytochemical research.

Key words: *Thymus vulgaris* L., TLC, HPLC, medicinal herb, anti-inflammatory activity, antimicrobial activity, antioxidant activity.

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Визначення накопичення поліфенольних сполук у траві чебрецю звичайного (*Thymus vulgaris* L.)

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Рід Чебрець (*Thymus* L.) надзвичайно поширений на території України, нараховує до 50 основних видів. Екстракти з фармакопейних видів роду входять до складу ефективних фітопрепаратів з вираженою протизапальною, протимікробною та антиоксидантною діями. Перспективний напрям сучасних фітохімічних досліджень – визначення накопичення біологічно активних поліфенольних сполук у траві широко культивованого в Україні та світі чебрецю звичайного (*Thymus vulgaris* L.).

Мета роботи – за допомогою методів тонкошарової хроматографії (ТШХ) та високоефективної рідинної хроматографії (ВЕРХ) визначити накопичення поліфенольних сполук у траві чебрецю звичайного під час цвітіння.

Матеріали та методи. Для експериментальних досліджень використали спиртові витяги з трави чебрецю звичайного (1:100), культивованого в с. Володимирівка Запорізької області (Україна) під час максимального накопичення поліфенольних сполук (червень – серпень 2022 року). Компонентний склад речовин досліджували методом ТШХ на денситометрі Biostep CD 60 (Німеччина) та ВЕРХ на хроматографі Agilent 1260 Infinity HPLC System Open LABCDS Software (Японія).

Результати. Методом ТШХ і ВЕРХ ідентифіковано 29 сполук поліфенольної природи. Експериментально визначено вміст біологічно активних речовин (флавоноїди, гідроксикоричні кислоти), що відомі в фітотерапії вираженою протизапальною, протимікробною, антиоксидантною діями.

Висновки. Враховуючи результати дослідження методами ТШХ і ВЕРХ, зробили висновок, що чебрець звичайний – перспективний вид для наступних поглиблених фітохімічних досліджень поліфенольних сполук.

Ключові слова: чебрець звичайний, ТШХ, ВЕРХ, лікарська рослина, протизапальна активність, протимікробна активність, антиоксидантна активність.

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Key words: *Thymus vulgaris* L., TLC, HPLC, medicinal herb, anti-inflammatory activity, antimicrobial activity, antioxidant activity.

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At the current stage of the development of medicine, much attention is paid to the appointment of medicinal plants and herbal preparations based on them. In our time, in the developed countries of the world, up to 35 % of drugs on the market are of plant origin, with a trend of constant growth.

Special attention is paid to well-known cultivated medicinal plants, which are distinguished by a sufficient herbal raw material base, a constant composition of compounds and a source of obtaining effective herbal preparations. At the same time, they have practically no toxic side effects with long-term use. Species of the polymorphic genus *Thymus* L. (*Thyme*) have long been known in various countries of the world as sources of effective anti-inflammatory, antimicrobial, and antioxidant herbal preparations. They include up to 400 species, of which only up to 50 have been identified in the modern flora of Ukraine [1,2].

The well-known species *Thymus vulgaris* L. is promising for growing in the conditions of the countries of Central Europe, the Mediterranean and Ukraine. In European countries, the best-known related species of the genus are *Thymus vulgaris* L. (*Common thyme*), two subspecies of *Thymus zygis* L. (*Thymus zygis* L. var. *gracilis* Bois. – *Thymus spanish white thin*; *Thymus zygis* L. var. *floribundus* Bois. – *Thymus spanish white flowering* [3,4].

The State Pharmacopoeia of Ukraine 1 edition (vol. 3) includes the herb *Thymus serpyllum* L. and a mixture of the herbs *Thymus vulgaris* L. with *Thymus zygis* L. without distinguishing distinctive diagnostic features of the plant material of the species [5].

During the research conducted in different countries of the world, the presence and accumulation of essential oil, polysaccharides, tannins, fatty oil, vitamins, triterpene saponins, and inorganic elements were established in *Thymus* L. species herb [6–10].

Essential oils and liquid alcohol extracts are part of herbal preparations with pronounced antimicrobial, anti-inflammatory, and antioxidant activity [10–14]. It was established that the pronounced biological activity of the infusion (1:10), tincture, and extracts from the herb of the plant is largely due to the presence of polyphenolic compounds, primarily flavonoids and hydroxycinnamic acids [16–19].

But at the same time, until now, there have been almost no studies of the presence and content of biologically active polyphenolic compounds in *Thymus vulgaris* L. herb. This is evidenced by the limited scientific data on this problem. Considering the sufficient raw material base of this cultivated plant in the conditions of Ukraine and the pronounced anti-inflammatory, antimicrobial, and antioxidant activity of medicines based on it, it was promising and expedient to determine the accumulation of polyphenolic compounds in the herb of this species.

Aim

The aim of the work is to determine the qualitative composition and quantitative content of biologically active polyphenolic compounds in *Thymus vulgaris L.* herb during flowering by TLC and HPLC methods.

Materials and methods

The object of research was *Thymus vulgaris* L., which was obtained from plants cultivated in Volodymyrivka, Zaporizhzhia region (June – August 2022). It consisted of flowering upper shoots with inflorescences up to 15 cm long, individual leaves, and parts of twigs (no more than 2 %) and fully met the requirements of the SPhU [20].

The herbal raw material consisted of flowering upper shoots with inflorescences up to 15 cm long, individual leaves and parts of twigs (no more than 2 %) and fully met the requirements of the SPhU [20].

The collection of herbal raw materials was carried out according to generally accepted methods. The drying process was carried out for 24 hours in the Termolab SNOL 24/350 at a temperature of 35 °C, up to the last water content no more than 12 %.

The compounds were identified by TLC on Sorbfil plates (TU 4215-002-43636866-2007) in chloroform-methanol-acetic acid-water systems (6:2:0.1:0.1); n-butanol-acetic acid-water (4:1:5) on the Biostep CD 60 densitometer device (Germany) and HPLC on the Agilent 1260 Infinity HPLC System Open LABCDS Software chromatograph (Japan).

The applied methods allow simultaneous separation of the studied components, their identification, and quantitative content determination. Their important advantages include the use of small samples, speed of research, good reproducibility of results, and small relative error of measurement results.

Methodology: about 1.0 g (exact weight) of herbal raw material was crushed to a particle diameter (d = 0.3 mm), placed in a flask with a capacity of 100 ml, 30 ml of ethanol was added and heated in boiling water heater VB-4 micromed (t = 100 °C) for 30 min. with thorough mixing. The process was repeated two more times with new portions of the ethanol. Extracts were combined, cooled for 30 min., centrifuged on the device SM-3.01. micromed, filtered into a flask with a capacity of 100 ml, and brought to the mark. Samples were filtered through a Teflon membrane filter (d = 0.45 μm) into an analysis vial. Chromatographic separation and determination of individual components were carried out in selected solvent systems. The standard sample solutions of flavonoids and hydroxycinnamic acids were used.

For HPLC research, a chromatographic column ZORBAX-SB C-18 (d = 2.1 mm, l = 150 mm) filled with octadecylsilyl sorbent (d = 3.5 μm) was used.

The mobile phase was: trifluoroacetic acid 0.2 %, anhydrous methanol and a mixture of trifluoroacetic acid 0.2 % with anhydrous methanol and a mixture of trifluoroacetic acid 0.2 % with methanol 70 %. The rate of supply of the mobile phase was 0.25 ml/min.; the working pressure of the eluent was from 240 kPa to 300 kPa; the temperature of the column thermostat is 32 °C; the sample volume was 5 μl. Definition parameters: measurement scale 1.0; scanning time 0.5 sec.; λ = 190–600 nm.

The identification of the components by the HPLC method was determined by the following parameters: the retention time of the standard sample and the spectral characteristics of the substances under study. The methods of standard additions and internal normalization were used.

Results

The qualitative component composition and quantitative content of compounds in the ethanol extract (1:100) from *Thymus vulgaris* L. herb were determined by TLC and HPLC methods. Up to 29 components of flavonoid derivates and hydroxycinnamic acids were identified and quantified. The obtained results are shown in *Table 1* and *Fig. 1*.

To the greatest degree from the total amount in the ethanol extract (1:100) from the herb of the studied species were present: rosmarinic acid (15.27 ± 1.48 %), luteolin-7-O-*β*-D-glucopyranoside (9.60 ± 0.91 %), chlorogenic acid (7.11 ± 0.67 %), luteolin-7-O-glucoside (5.00 ± 0.48 %), quercetin (4.61 ± 0.43 %), apigenin-7-O-*β*-D-glucopyranoside (4.05 ± 0.13 %), saponarin (3.93 ± 0.35 %), cirsilineol (3.71 ± 0.35 %), protocatechuic acid (3.46 ± 0.30 %), rutin (3.00 ± 0.29 %).

Discussion

The compounds of a polyphenolic nature were identified in *Thymus vulgaris* L. herb by TLC and HPLC methods, of which 13 were attributed to flavonoids and 16 to hydroxycinnamic acids. The obtained data indicate the perspective of further research of polyphenolic compounds of the *Thymus vulgaris* L. herb in the composition of extracts from plant raw materials and complex herbal preparations based on them. The pronounced anti-inflammatory, antimicrobial, and antioxidant activity of the *Thymus vulgaris* L. herbal raw material serve as a basis for the new complex herbal preparations introduction.

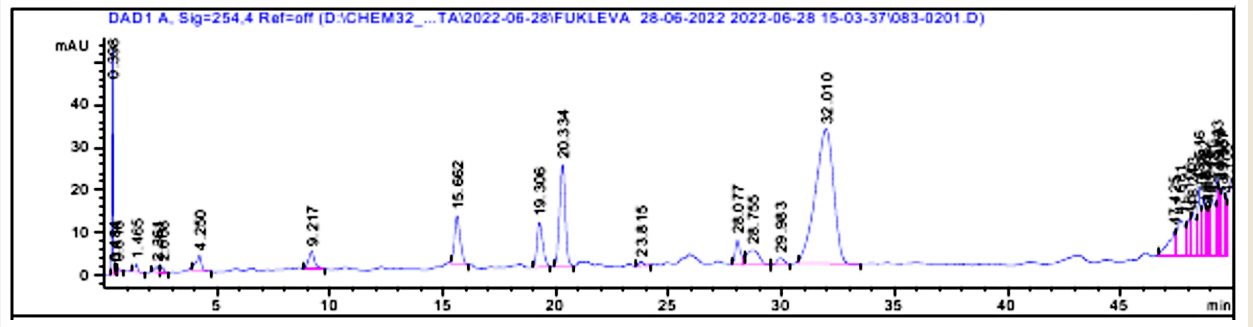

Fig. 1. HPLC of an ethanol extract (1:100) from *Thymus vulgaris* L. herb, Volodymyrivka, Zaporizhzhia region (June – August 2022), (x ± Δx) %, μ = 6.

Table 1. The results of determining the content of polyphenolic compounds in the ethanol extract (1:100) from *Thymus vulgaris* L. herb, Volodymyrivka, Zaporizhzhia region (June – August 2022), (x ± Δx) %, μ = 6

The name of the compounds	Quantitative content (%)	Holding time (min.)	$\lambda_{max.}$ (nm)
Protocatechuic acid	3.46 ± 0.30	2.361	208; 218; 260; 294
Caftric acid	1.13 ± 0.15	4.250	290
p-Coumaric acid	1.22 ± 0.14	5.39	210; 226; 295; 310
Tannic acid	1.24 ± 0.11	9.217	220; 275
Caffeic acid	1.39 ± 0.11	15.662	218; 240; 324; 298
Isochlorogenic acid	1.32 ± 0.11	19.306	219; 235; 245; 300; 329
Chlorogenic acid	7.11 ± 0.67	20.334	218; 242; 326; 297
Neochlorogenic acid	1.56 ± 0.17	23.815	218; 245; 300; 326
Trans-*β*-phenylacrylic acid	1.34 ± 0.12	28.077	204; 216; 278
Diosmin	0.88 ± 0.079	28.755	252; 268; 343
Luteolin-6-C-glucoside	2.56 ± 0.23	29.983	256; 265; 346
Rosmarinic acid	15.27 ± 1.48	32.010	215; 275; 325
Saponarin	3.93 ± 0.35	47.425	271; 336
Luteolin-7,3'-diglucoside	2.79 ± 0.25	47.681	255; 266; 349
Quercetin-3-O-rutinoside	1.70 ± 0.18	48.106	259; 369
Rutin	3.00 ± 0.29	48.243	259; 362.5
Luteolin-7-O-*β*-D-glucopyranoside	9.60 ± 0.91	48.732	255; 267; 348
Cirsilineol	3.71 ± 0.35	48.825	275; 346

Cont. of table 1.

The name of the compounds	Quantitative content (%)	Holding time (min.)	$\lambda_{max.}$ (nm)
Apigenin-7,4'-diglucoside	2.31 ± 0.22	48.940	267; 339
Luteolin-7-O-glucoside	5.00 ± 0.48	49.121	257; 268; 348
3,4-O-dicavoil-chinne acid	2.83 ± 0.27	49.333	220; 245; 300; 326
3,5-O-dicavoil-chinne acid	2.88 ± 0.26	28.12	222; 247; 302; 327
4,5-O-dicavoil-chinne acid	1.11 ± 0.10	29.50	222; 248; 303; 328
Dihydroquercetin	1.42 ± 0.12	32.33	289; 331
Apigenin-7-O-*β*-D-glucopyranoside	4.05 ± 0.13	49.413	268; 339
Luteolin	7.50 ± 0.71	49.557	242; 254; 266; 291; 350
Apigenin	3.81 ± 0.36	49.705	267; 296; 338
Quercetin	4.61 ± 0.43	47.25	255; 374
Hrizeriol	1.26 ± 0.11	47.83	207; 258; 271; 350
Total amount of flavonoids	58.13 ± 5.61		
Total amount of hydroxycinnamic acids	41.86 ± 4.09		

Conclusions

1. Up to 29 compounds were identified by TLC and HPLC in an alcoholic extract (1:100) of the herb *Thymus vulgaris* L., which are classified as flavonoids and hydroxycinnamic acids. Most of them are known for their pronounced anti-inflammatory, antimicrobial, and antioxidant activity.

2. Given the sufficient raw material base of this species, it should be considered promising for cultivation in Ukraine for further production of complex herbal preparations.

3. Simultaneous application of TLC and HPLC methods is promising for the determination of polyphenolic compounds in medicinal plant raw materials.

Prospects for further research. The identified organic compounds can be used for further research on common thyme, as well as for inclusion in the draft monograph for the standardization of the herb of this species.

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References

[1] Nachychko, V. O, & Honcharenko, V. I. (2016). Hybrydy rodu *Thymus* L. (Lamiaceae) u flori zakidnykh rehioniv Ukrainy: taksonomichnyi sklad i poshyrennia. [Hybrids of *Thymus* L. (Lamiaceae) genus in flora of the western regions of Ukraine: taxonomic composition and distribution]. *Visnik Lvivskoho Universitetu. Seriia biolohichna, 10*(1), 163-186. [in Ukrainian]. https://dx.doi.org/10.30970/sbi.1001.442

[2] Nachychko, V. O, & Honcharenko, V. I. (2017). Botaniko-heohrafichna kharakterystyka vydiv rodu *Thymus* L. (Lamiaceae) u flori zakhidnykh rehioniv Ukrainy [Botanical-geographical characterization of *Thymus* L. (*Lamiaceae*) species in the flora of western regions of Ukraine]. *Visnik Lvivskoho Universitetu. Seriia biolohichna, 75*, 35-47. [in Ukrainian]. https://dx.doi.org/10.30970/vlubs.2017.75.04

[3] Afonso, A. F., Pereira, O. R., Válega, M., Silva, A. M. S., & Cardoso, S. M. (2018). Metabolites and Biological Activities of *Thymus zygis*, *Thymus pulegioides*, and *Thymus fragrantissimus* Grown under Organic Cultivation. *Molecules*, *23*(7), 1514. https://doi.org/10.3390/molecules23071514

[4] Medicinal and Functional Values of Thyme (Thymus vulgaris L.) Herb. (2017). *Journal of Applied Biology & Biotechnology*, *5*(2), 017-022. https://doi.org/10.7324/jabb.2017.50203

[5] State Enterprise Ukrainian Scientific Pharmacopoeial Center of Medicines Quality. (2009). *Derzhavna Farmakopeia Ukrainy. Dopovnennia 3* [The State Pharmacopoeia of Ukraine] (1st ed., Suppl 3). Kharkiv: Naukovo-ekspertnyi farmakopeinyi tsentr. [in Ukrainian].

[6] Tohidi, B., Rahimmalek, M., & Arzani, A. (2017). Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran. *Food chemistry*, *220*, 153-161. https://doi.org/10.1016/j.foodchem.2016.09.203

[7] Zarivna, N. O. (2021). Rozrobka metodyky identyfikatsii monosakcharydiv u ridkomu ekstrakti chebretsiu povzuchoho [Development of monosaccharides identification methods in Thyme creeping extracts]. *Medychna ta klinichna khimiia*, *23*(1), 80-83. [in Ukrainian]. https://dx.doi.org/10.11603/mcch.2410-681x.2021.i1.12112

[8] Zarivna, N. O., & Logoyda, L. S. (2020). Rozrobka metodyky identyfikatsii flavonoydiv ta hidroksykorychnykh kyslot v ekstraktakh chebretsiu povzuchoho [Development of flavonoid and hydroxycinnamic acid identification methods in Thyme creeping extracts]. *Medychna ta klinichna khimiia, 22*(1), 107-111. [in Ukrainian]. https://doi.org/10.11603/mcch.2410-681X.2020.v.i1.11061

[9] Toncer, O., Karaman, S., Diraz, E., Sogut, T., & Kizil, S. (2017). Essential oil composition of Thymus × citriodorus (Pers.) Schreb. at different harvest stages. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *45*(1), 185-189. https://doi.org/10.15835/nbha45110672

[10] Vetvicka, V., & Vetvickova, J. (2016). Essential Oils from Thyme (Thymus vulgaris): Chemical Composition and Biological Effects in Mouse Model. *Journal of medicinal food*, *19*(12), 1180-1187. https://doi.org/10.1089/jmf.2016.0029

[11] Svydenko, L. V., & Hlushchenko, L. A. (2016). Komponentnyi sklad efirnoi olii u formakh vydiv chebretsiu povzuchoho (Thymus serpillum L.) i bloshynoho (Thymus pulegioides L.) v umovakh Khersonskoi oblasti [Component composition of essential oil in the forms of thymus serpyllum (Thymus serpillum L.) and thyme flea (Thymus pulegioides L.) species in conditions of Kherson region]. *Ahroekolohichnyi zhurnal*, (2), 129-134. [in Ukrainian]. https://doi.org/10.33730/2077-4893.2.2016.249116

[12] 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

[13] Aouam, I., Elatki, Y., Taroq, A., Elkamari, F., Elyoussi, B., & Abdellaoui, A. (2018). Chemical Composition and Antibacterial Activity of the Essential oil of *Thymus* citriodorus L. Growing Wild in Morocco: Preventive Approach against Nosocomial Infections. *International Journal of Pharmaceutical Sciences Review and Research*, *51*(1), 72-75.

[14] Kryvtsova, M. V., Salamon, I., Koscova, J., Bucko, D., & Spivak, M. (2019). Antimicrobial, antibiofilm and biochemichal properties of Thymus vulgaris essential oil against clinical isolates of opportunistic infections. *Biosystems Diversity*, *27*(3), 270-275. https://doi.org/10.15421/011936

[15] Beicu, R., Alexa, E., Obiștioiu, D., Cocan, I., Imbrea, F., Pop, G., Circioban, D., Moisa, C., Lupitu, A., Copolovici, L., Copolovici, D. M., & Imbrea, I. M. (2021). Antimicrobial Potential and Phytochemical Profile of Wild and Cultivated Populations of Thyme (*Thymus* sp.) Growing in Western Romania. *Plants (Basel, Switzerland)*, *10*(9), 1833. https://doi.org/10.3390/plants10091833

[16] Wisam, S. U., Nahla, T. K., & Tariq, N. M. (2017). Antioxidant activities of thyme extracts. *Pakistan Journal of Nutrition*, *17*(1), 46-50. https://doi.org/10.3923/pjn.2018.46.50

[17] Komaki, A., Hoseini, F., Shahidi, S., & Baharlouei, N. (2015). Study of the effect of extract of Thymus vulgaris on anxiety in male rats. *Journal of traditional and complementary medicine*, *6*(3), 257-261. https://doi.org/10.1016/j.jtcme.2015.01.001

[18] Mazandarani, M., & Ghafourian, M. (2017). Influence of extraction methods on total phenolic, flavonoids and antioxidant activity of Thymus kotchyanus L. extract in Semnan Province, Iran. *Trends in Phytochemical Research, 1*(1), 33-38.

[19] Niculae, M., Hanganu, D., Oniga, I., Benedec, D., Ielciu, I., Giupana, R., Sandru, C. D., Ciocârlan, N., & Spinu, M. (2019). Phytochemical Profile and Antimicrobial Potential of Extracts Obtained from *Thymus marschallianus* Willd. *Molecules*, *24*(17), 3101. https://doi.org/10.3390/molecules24173101

[20] State Enterprise Ukrainian Scientific Pharmacopoeial Center of Medicines Quality. (2008, February 1). *Derzhavna Farmakopeia Ukrainy. Dopovnennia 2* [The State Pharmacopoeia of Ukraine] (1st ed., Suppl 2). Kharkiv: Naukovo-ekspertnyi farmakopeinyi tsentr. [in Ukrainian].