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STUDY OF THE POLYPHENOLIC COMPOUNDS ACCUMULATION IN *THYMUS SERPYLLUM* L. HERB DURING THE FLOWERING PERIOD

Key words: *Thymus serpyllum* L., herb, polyphenolic compounds, accumulation, TLC and HPLC methods, flowering period

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ВИВЧЕННЯ НАКОПИЧЕННЯ ПОЛІФЕНОЛЬНИХ СПОЛУК У ТРАВІ ЧЕБРЕЦЮ ПОВЗУЧОГО (*THYMUS SERPYLLUM* L.) ПІД ЧАС ЦВІТІННЯ

Ключові слова: чебрець повзучий, трава, поліфенольні сполуки, накопичення, методи ТЛХ та ВЕРХ, час цвітіння

Herbal raw materials, phytopreparations and biologically active food additives of them are widely used in modern medicine for the treatment and prevention of many common human diseases. At our time, according to the message of World Health Organization, in Europe and the USA up to 35% of drugs are obtained from medicinal plants. In the next twenty years, there will be a tendency for their increase up to 60% [1, 2, 3].

It is important to note, that wild species of the *Thymus* L. genus, growing in natural steppe and forest-steppe biocenoses of Ukraine flora, have a powerful plant raw material base with high cultivation potential for obtaining modern highly effective phytopreparations. At the same time, side toxic effects are not observed during long-term use in modern phytotherapy. They are number up to 200 species, of which only up to 50 have been found in the modern Ukraine flora [4, 5].

The well-known wild species of the *Thymus* L. genus (*Thyme*) are well distributed and constantly found in the natural biocenoses of many world countries with moderate climatic conditions as a source for obtaining highly effective medicinal plant raw materials, phytopreparations and biologically active additives with pronounced antimicrobial, anti-inflammatory and antioxidant activity [6, 7, 8].

The most widespread wild species in steppe and forest-steppe biocenoses of European countries and Ukraine is *Thymus serpyllum* L. This is promising for the procurement of herbal raw materials, cultivation in the conditions of specialized farms. Morphologically and phylogenetically closely related to it are the widespread species *Thymus marschallianus* Willd., *Th. pallasianus* H. Braun., *Th. ovatus* Mill., hybrids that have a similar chemical composition and are also widely used in modern folk medicine [1–8].

In European countries, the best-known related species of the genus are: *Thymus vulgaris* L. (Common thyme), two subspecies of *Thymus zygis* L. (*Th. zygis* L. var. *gracilis* Bois. – *Th. spanish white thin*; *Th. zygis* L. var. *floribundus* Bois. – *Thymus spanish white flowering* [9, 10, 11, 12].

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The State Pharmacopoeia of Ukraine includes the *Thymus serpyllum* L. herb and a mixture of the *Thymus vulgaris* L. with *Th. zygis* L. herbs without distinctive diagnostic features of these species herbal raw material [13].

The study of the chemical composition of *Thymus serpyllum* L. herbs from different countries of the world during the flowering period the presence and accumulation of essential oils, polysaccharides, tannins, fatty oils, vitamins, triterpene saponins, inorganic elements were established [14, 15, 16].

It is generally known that essential oils and liquid alcohol extracts from *Thymus serpyllum* L. herb are part of modern phytopreparations with pronounced antimicrobial, anti-inflammatory and antioxidant activity [17, 18].

Pharmacological studies have established that the pronounced biological activity of the aqueous infusion (1:10) and extractable phytopreparations obtained from *Thymus serpyllum* L. herb is largely determined by the presence of flavonoids, hydroxycinnamic acids and tannins [19, 20, 21, 22, 23].

But at the same time, almost no studies have been conducted on the presence and content of biologically active polyphenolic compounds from the groups of flavonoids and hydroxycinnamic acids in the *Thymus serpyllum* L. herb during the flowering period.

This is evidenced by the limited scientific information on studies of polyphenolic compounds presence in plant raw materials of *Thymus* L. genus species by Thin Layer Chromatography (TLC) and High Pressure Liquid Chromatography (HPLC) methods.

The distribution of the wild species *Thymus serpyllum* L. in Ukraine, the possibility of cultivation and obtaining phytopreparations with pronounced anti-inflammatory, antimicrobial and antioxidant activity provides the prospect of standardization of herbal raw materials according to the content of polyphenolic compounds.

Aim are determination the qualitative composition and quantitative content of biologically active polyphenolic compounds in *Thymus serpyllum* L. herbal raw material during the flowering period by Thin Layer Chromatography (TLC) and High Pressure Liquid Chromatography (HPLC) methods.

Materials and methods

The object of research was the *Thymus serpyllum* L. herbal raw material, which obtained from wild plants in v. Baburka, 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 State Pharmacopoeia of Ukraine (SPhU) [24].

The harvesting of the *Thymus serpyllum* L. herbal raw material was carried out according to generally accepted methods. The drying process was carried out for 24 hours in the Termolab SNOL 24/350 device at a temperature of 35 °C, up to the final water content no more than 10%.

The compounds were identified by TLC on plates (Merck KGaA, Germany) in chloroform–methanol–acetic acid–water (6:2:0.1:0.1) and n-butanol–acetic acid–water (4:1:5) systems on the Biostep CD 60 densitometer device (Germany) and HPLC Agilent 1260 Infinity HPLC System Open LABCDs Software chromatograph device (Japan).

The combination of TLC and HPLC methods allows simultaneous separation of the studied components, their identification and quantitative content determination. At the same time, the essential advantages are the use of small samples, speed of research, good reproducibility of results and small relative error of analyses.

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 96% ethanol was added, and heated on the VB-4 Micromed device ($t = 100$ °C) for 30 min with thorough mixing. The process was repeated two more times with new volume portions of the 96% ethanol. Extracts were combined, cooled for 30 min, centrifuged on the SM-3.01. Micromed device, filtered into a flask with a capacity of 100 ml and brought to the mark. Samples were filtered through a teflon membrane filter ($d = 0.45$ μ m) into an analysis vials. The chromatographic separation and determination of individual polyphenolic compounds were carried out in selected solvent systems.

The glass capillary chromatographic column ZORBAX-SB C-18 ($d = 2.1$ mm, $l = 150$ mm) filled with octadecylsilyl sorbent ($d = 3.5$ μ m) was used for HPLC determination.

The mobile phase was: trifluoroacetic acid 0.2%, 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 to 300 kPa; the temperature of the column thermostat is 32 °C; sample volume 5 μ l. Definition parameters: measurement scale 1.0; scanning time 0.5 sec; $\lambda = 190$ –600 nm.

The methods of standard additions samples 96 ethanol solutions of flavonoids and hydroxycinnamic acids were used.

The identification and quantitative content of polyphenolic compounds by the HPLC method were determined by the following parameters: retention time, spectral characteristics, peaks areas of compounds in comparison with standard samples.

Statistical processing of the results was carried out using the standard statistical package of the license program «STATISTICA® for Windows 6.1» (Stat Soft Inc., № AXXR712D833214FAN5), as well as «SPSS 16.0» [25].

Results and discussion

The qualitative component composition and quantitative content of compounds in the 96% ethanol extract (1:100) from *Thymus serpyllum* L. herb were determined by TLC and HPLC methods. The presence of 25 compounds of the polyphenolic structure were established.

Up to 13 components of flavonoids derivatives and 12 hydroxycinnamic acids were identified and quantified. The obtained results are shown in Table and Figure.

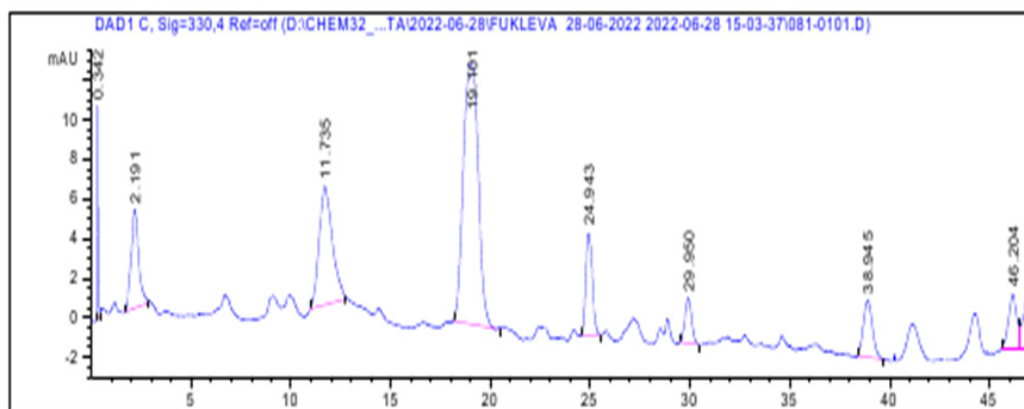


Fig. HPLC of an ethanol extract (1:100) from *Thymus serpyllum* L. herb,
v. Baburka, Zaporizhzhia region (June–August 2022), ($\bar{x} \pm \Delta \bar{x}$)%, $\mu = 6$

To the greatest degree of the total amount in the ethanol extract (1:100) from *Thymus serpyllum* L. herb were present: rosmarinic acid ($12.77 \pm 1.19\%$), chlorogenic acid ($8.55 \pm 0.79\%$), rutin ($8.45 \pm 0.79\%$), p-coumaric acid ($7.41 \pm 0.69\%$), luteolin ($3.87 \pm 0.37\%$), luteolin-7-O- β -D-glucopyranoside ($3.86 \pm 0.33\%$), apigenin-7,4'-diglucoside ($3.86 \pm 0.32\%$), quercetin ($3.85 \pm 0.34\%$), apigenin ($3.81 \pm 0.36\%$).

Table

The results of determining the content of polyphenolic compounds in the ethanol extract (1:100) from *Thymus serpyllum* L. herb,

v. Baburka, Zaporizhzhia region (June–August 2022), ($\bar{x} \pm \Delta \bar{x}\%$, $\mu = 6^*$

N	The name of the compounds	Quantitative content, %	Holding time, min	λ_{max}, nm
1	Protocatechuic acid	0.91 ± 0.08	0.342	208; 218; 260; 294
2	Cafric acid	1.26 ± 0.10	1.391	290
3	p-Coumaric acid	7.41 ± 0.69	2.191	210; 226; 295;310
4	Tannic acid	3.85 ± 0.33	6.781	220; 275
5	Caffeic acid	2.50 ± 0.22	9.243	218; 240; 324; 298
6	Isochlorogenic acid	1.98 ± 0.17	10.550	219;235;245;300;329
7	Chlorogenic acid	8.55 ± 0.79	11.735	218; 242; 326; 297
8	Neochlorogenic acid	4.02 ± 0.39	16.704	218; 245; 300; 326
9	Rosmarinic acid	12.77 ± 1.19	19.101	215; 275; 325
10	Diosmin	3.72 ± 0.33	22.520	252; 268; 343
11	Luteolin-6-C-glucoside	0.97 ± 0.08	24.252	256; 265; 346
12	Rutin	$8,45 \pm 0,79$	24,943	259; 362,5
13	Luteolin-7,3'-diglucoside	3.48 ± 0.30	27.223	255; 266; 349
14	Quercetin-3-O-rutinoside	2.48 ± 0.23	28.213	259; 369
15	Luteolin-7-O- β -D-glucopyranoside	3.86 ± 0.33	28.640	255; 267; 348
16	Apigenin-7-O- β -D-glucopyranoside	3.35 ± 0.30	29.950	268; 339
17	Apigenin-7,4'-diglucoside	3.86 ± 0.32	32.635	267; 339
18	Luteolin-7-O-glucoside	3.24 ± 0.30	32.837	257; 268; 348
19	3,4-O-dicavoil-chinne acid	2.30 ± 0.22	33.538	220; 245; 300; 326
20	3,5-O-dicavoil-chinne acid	2.88 ± 0.27	34.520	222; 247; 302; 327
21	4,5-O-dicavoil-chinne acid	3.11 ± 0.31	36.212	222; 248; 303; 328
22	Dihydroquercetin	3.42 ± 0.30	38.845	289; 331
23	Luteolin	3.87 ± 0.37	44.235	242; 254; 266; 291; 350
24	Apigenin	3.81 ± 0.36	46.110	267; 296; 338
25	Quercetin	3.85 ± 0.34	47.204	255; 374
Total amount of flavonoids		48.46 ± 4.77	–	–
Total amount of hydroxycinnamic acids		51.54 ± 5.19	–	–

Note: * – $p<0,05$.

Up to 25 polyphenolic compounds during the flowering period were identified in the *Thymus serpyllum* L. herb by TLC and HPLC methods, of which 13 were assigned to flavonoids and 12 to hydroxycinnamic acids.

The obtained results indicate for perspective of further research of *Thymus serpyllum* L. herbal raw materialare able is the pronounced effective anti-inflammatory, antimicrobial and antioxidant activities.

Conclusions

1. The *Thymus serpyllum* L. herb was studied for the polyphenolic compounds content during flowering period. Up to 25 polyphenolic compounds, classified as 13 flavonoids and 12 hydroxycinnamic acids, were identified in alcoholic extracts (1:100) by TLC and HPLC methods. Most of them are well known for their pronounced anti-inflammatory, antimicrobial and antioxidant activities.

2. The simultaneous use of TLC and HPLC methods is promising for the identifications and determinations of the polyphenolic compounds quantitative content in herbal raw materials of *Thymus* L. species.

3. According to the content of biologically active compounds, the *Thymus serpyllum* L. species is promising for cultivation in Ukraine for the purpose of obtaining herbal raw materials and new complex phytopreparations.

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STUDY OF THE POLYPHENOLIC COMPOUNDS ACCUMULATION IN *THYMUS SERPYLLUM* L. HERB DURING THE FLOWERING PERIOD

Key words: *Thymus serpyllum* L., herb, polyphenolic compounds, accumulation, TLC and HPLC methods, flowering period

ABSTRACT

The *Thymus serpyllum* L. is a widespread in the steppe biocenoses of southern and eastern Ukraine. It is well cultivated in specialized farms. A promising direction of modern phytochemical research of wild and cultivated *Thymus* L. species is the determination of the accumulation of biologically active polyphenolic compounds that exhibit pronounced biological activity.

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

The *Thymus serpyllum* L. herb was harvested in steppe biocenoses of southern and eastern Ukraine at the flowering, during the period of biologically active polyphenolic compounds maximum accumulation. For experimental studies, alcohol extracts from *Thymus serpyllum* L. herb (1:100) were used. 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).

TLC and HPLC methods revealed up to 25 polyphenolic compounds. Experimentally were determined 13 flavonoids and 12 hydroxycinnamic acids. All these biologically active compounds are well known in modern phytotherapy for their pronounced antimicrobial, anti-inflammatory and antioxidant activity. During the flowering period up to 25 polyphenolic compounds were identified and quantified in the ethanolic extract (1:100) obtained from *Thymus serpyllum* L. herbal raw material. Of these, 13 compounds were attributed to flavonoids with a defined total content (0.3 mg/100 mg) and 12 to hydroxycinnamic acids (0.9 mg/100 mg). The highest accumulation of hydroxycinnamic acids in the *Thymus serpyllum* L. herbal raw material were characterized by rosmarinic acid ($12.77 \pm 1.19\%$), chlorogenic acid ($8.55 \pm 0.79\%$) and p-coumaric acid ($7.41 \pm 0.69\%$). Of flavonoids, the highest content were established for rutin ($8.45 \pm 0.79\%$), luteolin ($3.87 \pm 0.37\%$), luteolin-7-O- β -D-glucopyranoside ($3.86 \pm 0.33\%$), apigenin-7,4'-diglucoside ($3.86 \pm 0.32\%$), quercetin ($3.85 \pm 0.34\%$) and apigenin ($3.81 \pm 0.36\%$).

The results obtained by TLC and HPLC methods indicate the perspective of polyphenolic compounds research for the purpose of determining the biological activity of extracts and carrying out the standardization of *Thymus serpyllum* L. herb. The study of the polyphenolic compounds accumulation in the *Thymus serpyllum* L. plant raw materials is important for this standardization and obtaining effective phytopreparations.

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ВИВЧЕННЯ НАКОПИЧЕННЯ ПОЛІФЕНОЛЬНИХ СПОЛУК У ТРАВІ ЧЕБРЕЦЮ ПОВЗУЧОГО (*THYMUS SERPYLLUM* L.) ПІД ЧАС ЦВІТІННЯ

Ключові слова: чебрець повзучий, трава, поліфенольні сполуки, накопичення, методи ТШХ та ВЕРХ, час цвітіння

АНОТАЦІЯ

Чебрець повзучий (*Thymus serpyllum* L.) є розповсюдженим дикорослим видом у степових біоценозах півдня та сходу України. Успішно культивується у спеціалізованих господарствах. Перспективним напрямом сучасних фітохімічних досліджень дикорослих та культивованих видів роду *Thymus* L. є визначення накопичення біологічно активних поліфенольних сполук, які виявляють виражену біологічну активність.

Мета роботи – методами ТШХ та ВЕРХ визначити накопичення поліфенольних сполук у траві чебрецю повзучого під час цвітіння.

Траву чебрецю повзучого заготовляли у степових біоценозах півдня та сходу України в кінці цвітіння під час максимального накопичення біологічно активних речовин. Для визначення присутності поліфенольних сполук та їх кількісного вмісту використовували спиртові витяги з трави виду (1:100).

Компонентний склад досліджували методом ТПХ на денситометрі Biostep CD 60 (Німеччина) та ВЕРХ на хроматографі Agilent 1260 Infinity HPLC System Open LABCDs Software (Японія).

Методом ТПХ та ВЕРХ було ідентифіковано до 25 речовин поліфенольної природи. Встановлено кількісний вміст 13 флавоноїдів та 12 гідроксикоричних кислот, для яких за фітотерапевтичного призначення притаманна виражена протизапальна, антимікробна та антиоксидантна активність. Під час цвітіння в етанольному екстракті (1:100), одержаному з трави *Thymus serpyllum* L., було ідентифіковано та визначено кількісний вміст до 25 сполук поліфенольної природи. Із них було 13 віднесено до флавоноїдів із визначеним загальним вмістом (0,5 мг/100 мг) та 12 – до гідроксикоричних кислот (1,5 мг/100 мг). Найбільше накопичення з гідроксикоричних кислот у траві досліджуваного виду було притаманне для розмаринової кислоти ($12,77 \pm 1,19\%$), хлорогенової ($8,55 \pm 0,79\%$) та п-кумарової ($7,41 \pm 0,69\%$). Із флавоноїдів найбільший вміст встановлено для рутину ($8,45 \pm 0,79\%$), лютеоліну ($3,87 \pm 0,37\%$), лютеолін-7-О- β -D-глюкопіранозиду ($3,86 \pm 0,33\%$), апігенін-7,4'-диглюкозиду ($3,86 \pm 0,32\%$), кверцетину ($3,85 \pm 0,34\%$) та апігеніну ($3,81 \pm 0,36\%$).

Результати, одержані методами ТПХ та ВЕРХ, свідчать про перспективність дослідження біологічної активності екстрактів та проведення стандартизації трави *Thymus serpyllum* L. за накопиченням поліфенольних сполук. Дослідження накопичення поліфенольних сполук у траві видів роду *Thymus* L. має важливе значення для заготівлі рослинної сировини та одержання фітопрепаратів на її основі.

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