



Study of the ascorbic acid accumulation in *Thymus L.* genus species of Ukraine flora

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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 *Thymus L.* genus species are extremely widespread in temperate countries and number up to 200 main wild plants, of which up to 50 have been identified in the modern Ukraine flora. 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 presence and accumulation of biologically active ascorbic acid in the herbs of widespread wild and cultivated species of the *Thymus L.* genus.

The aim of the work is to determine the presence and accumulation of biologically active L-ascorbic acid in the herbs of four widespread wild and cultivated species of the *Thymus L.* genus of Ukraine flora using thin layer chromatography (TLC) and spectrophotometry methods during vegetation season.

Materials and methods. The research used herbs of four widespread wild and cultivated species of the *Thymus L.* genus of Ukraine flora during the growing season (June – August 2023). The presence and quantitative content of ascorbic acid were determined by TLC on a “Biostep” CD 60 densitometer (Germany) and spectrophotometry on a “Lambda 365” device (USA).

Results. TLC and spectrophotometry methods were used to determine the presence and quantitative content of ascorbic acid in the herbs of four widespread *Thymus L.* genus species of Ukraine flora during the growing season. Accumulation of the compound was higher during flowering of cultivated species than wild plants. For the herbs *Thymus vulgaris L.* up to 39.10 ± 3.88 mg%; *Th. x citriodorus* (Pers.) Schreb. var. “Silver Queen” up to 36.19 ± 3.59 mg%.

Conclusions. Considering the results obtained by TLC and spectrophotometry methods, it can be concluded that the determination of ascorbic acid presence and content in the *Thymus L.* species genus herbs is appropriate for obtaining cosmetology preparations with pronounced anti-inflammatory, antioxidant, and regenerating activity for normal and problem skin.

Keywords: *Thymus L.* genus species, herb, thin layer chromatography, spectrophotometry, anti-inflammatory, antioxidant, regenerating activity.

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Визначення накопичення аскорбінової кислоти у видах роду *Thymus L.* флори України

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Види роду Чебрець (*Thymus L.*) надзвичайно поширені у країнах світу з помірним кліматом. Розрізняють до 200 основних дикорослих видів, з котрих у сучасній флорі України ідентифіковано до 50. Екстракти з фармакопейних видів роду входять до складу ефективних косметологічних лікарських засобів із вираженою антимікробною, протизапальною та антиоксидантною дією для нормальної та проблемної шкіри. Перспективним напрямом сучасних фітохімічних досліджень є визначення наявності та накопичення аскорбінової кислоти у траві поширених дикорослих і культивованих видів роду *Thymus L.*

Мета роботи – за допомогою методів тонкошарової хроматографії (ТШХ) та спектрофотометрії визначити наявність і накопичення аскорбінової кислоти у траві чотирьох поширених дикорослих і культивованих видів роду *Thymus L.* флори України.

Матеріали і методи. Для досліджень використали траву чотирьох поширених дикорослих і культивованих видів роду *Thymus L.* флори України під час вегетаційного періоду (червень – серпень 2023 р.). Наявність і кількісний вміст аскорбінової кислоти визначали методом ТШХ на денситометрі «Biostep» CD 60 (Німеччина) та спектрофотометрії на приладі «Lambda 365» (США).

Результати. Методами ТШХ і спектрофотометрії встановлено наявність і кількісний вміст аскорбінової кислоти у траві чотирьох поширених видів роду *Thymus L.* флори України під час вегетації. Виявили, що накопичення сполуки найбільше під час цвітіння

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Keywords: *Thymus L.* genus species, herb, thin layer chromatography, spectrophotometry, anti-inflammatory, antioxidant, regenerating activity.

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культивованих видів, ніж дикорослих. Для трави *Th. vulgaris* L. – до $39,10 \pm 3,88$ мг%; трави *Th. x citriodorus* (Pers.) Schreb. var. «Silver Queen» – до $36,19 \pm 3,59$ мг%.

Висновки. Враховуючи дані ТШХ і спектрофотометрії, зробили висновок, що визначення вмісту аскорбінової кислоти у траві видів роду *Thymus* L. доцільне для отримання косметологічних лікарських засобів із вираженою протизапальною, антиоксидантною та регенеруючою активністю для нормальної та проблемної шкіри.

Ключові слова: види роду чебрець, трава, тонкошарова хроматографія, спектрофотометрія, протизапальна, антиоксидантна, регенеруюча активність.

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In modern medical cosmetology, significant attention is given to the use of products derived from medicinal plants, known for their pronounced antimicrobial, anti-inflammatory, and antioxidant activities. The World Health Organization forecasts that in the coming years, up to 60 % of modern drugs in European Union countries will be sourced from natural raw materials.

Modern medical cosmetology recognizes the tonic and antioxidant effects of products obtained from medicinal plant raw materials. These products, which contain natural vitamins, are essential for correcting disorders of collagen formation in normal, problem, and aging skin [1].

The well-known compound water-soluble ascorbic acid (vitamin C), common in plants, participates in many redox reactions in the body. It inhibits the peroxidation processes of lipids, proteins, and other cell components, protecting them from damage.

Ascorbic acid has an antioxidant effect; when applied to the skin, it ensures the functioning of enzyme systems that synthesize collagen protein, normalizes the condition of endothelial cells of blood vessels and tissues, reduces the negative effects of aging and photoaging, and neutralizes the influence of free radicals [2].

Until now, there have been no studies on the presence and quantitative content of the biologically active form of L-ascorbic acid in herbal raw materials with pronounced anti-inflammatory, antioxidant, and regenerating activity [3].

According to its chemical structure, L-ascorbic acid ($C_6H_8O_6$) is a natural compound derivative of L-acid (γ -lactone of 2,3-dehydro-L-gulonic acid).

In modern medical cosmetology, especially for microbial and inflammatory skin diseases, significant attention is paid to plant-origin products with *Thymus* L. genus species extracts. The extremely polymorphic *Thymus* L. genus is part of the *Lamiaceae* family and includes up to 200 main wild plant species distributed in Europe, Asia, and Africa, primarily in temperate climates. According to external morphological features, these are mainly shrubs or semi-shrubs [3,4].

Up to 50 of the most widespread species of this genus are known in the modern flora of Ukraine [5,6,7,8,9,10,11].

The best known in modern cosmetology are *Thymus vulgaris* L., *Th. zygis* L. (*Th. zygis* L. var. *gracilis* Bois.; *Th. zygis* L. var. *floribundus* Bois., *Th. serpyllum* L., *Th. x citriodorus* (Pers.) Schreb. var. «Silver Queen» [12,13,14].

The accumulation of essential and fatty oils, flavonoids, hydroxycinnamic acids, polysaccharides, tannins, triterpenoid saponins, organic and amino acids, inorganic elements has been established in the *Thymus* L. genus species [15,16,17].

The essential oils and extracts from herbal raw materials of *Thymus* L. genus species for external use are prescribed in ointments and gels for treating microbial and inflammatory diseases of normal, problem, and aging skin [18,19,20].

When applied to the skin in medical cosmetics, *Thymus* L. genus species extracts show pronounced anti-inflammatory and regenerating activity. Notably, these extracts do not cause side effects such as irritation and toxicity [21].

At the same time, it was established that the pronounced normalizing and restoring collagen synthesis action of these drugs is largely due to the presence of vitamins, primarily ascorbic acid [22,23,24].

However, until now, studies of the presence and accumulation of ascorbic acid during the growing season in herbal raw materials of *Thymus* L. genus species have not been conducted. Scientific publications on this line of research are very limited.

The sufficient prevalence of cultivated and wild *Thymus* L. genus species in the Ukrainian flora, along with the pronounced regenerating and antioxidant activity of medicinal drugs derived from these plants, makes it reasonable to determine the presence and accumulation of ascorbic acid during the vegetation period in the herbal raw materials.

Aim

The aim of this work is to determine the presence and accumulation of biologically active L-ascorbic acid in the herbs of four widespread wild and cultivated species of the *Thymus* L. genus of Ukraine flora using TLC and spectrophotometry methods during vegetation season.

Materials and methods

The research used herbs of four widespread wild and cultivated species of the *Thymus* L. genus of Ukraine flora from different places of growth during the vegetation season (June – August 2023). The harvesting of herbal raw materials was carried out during the vegetation season in various regions of Ukraine (Zaporizhzhia, Kharkiv, Poltava, Dnipropetrovsk regions) to the article of the State Pharmacopoeia of Ukraine. The herbal raw materials were flowering upper shoots with inflorescences up to 15 cm long, individual leaves, and parts of twigs (no more than 2 %) [25].

The process of drying was carried out for 24 hours in the drying device «Termolab SNOL 24/350» at a temperature of 35 °C, a layer thickness of 1 cm, to the last moisture in the composition no more than 10 %.

The identify and quantitative content of ascorbic acid in herbal raw materials were determined by TLC method on plates with a glass base of the brand “Merkieselguhr F₂₅₄” 20 × 20 (Merck KGaA, Germany) in the systems acetone-glacial acetic acid-methanol-toluene (5:5:20:70) and n-butanol-formic acid-water (30:5:10) on the “Biostep” CD 60 densitometer device (Germany) and spectrophotometry on the “Lambda 365” device (USA).

Methodology: about 5.0 g (exact weight) of herbal raw material were crushed to a particle diameter ($d = 2$ mm), placed in a flask with a capacity of 100 ml, 25 ml of 96 % ethanol was added, shaken for 30 min. and filtered through a membrane filter ($d = 0.45$ μm).

Comparison solution: 10 mg of ascorbic acid Merck standard (Supelco 47863) in 5.0 ml of 60 % ethanol was dissolved.

The 20 μl of tested solution and 2 μl of comparison solution to the starting line of the chromatographic plate were applied.

The moving phase must pass a distance of 15 cm from the starting line. Drying is carried out in the air. Detection of the presence of ascorbic acid is carried out in UV light at a wavelength of 254 nm.

The chromatogram of the tested solution shows an absorption zone at the level of the main zone of the standard sample. They are sprayed with a solution of 0.2 g/l dichlorophenolindophenol sodium in 96 % ethanol and viewed in daylight.

The chromatogram of the tested solution reveals a white zone on a pink background (ascorbic acid) at the level of the main zone on the comparison.

Simultaneous chromatographic determination of the presence of ascorbic acid was carried out with a standard sample of the Merck standard compound (Supelco 47863) on plates in selected chromatographic systems on a “Biostep” CD 60 device (Germany).

Method of quantitative determination: about 5.0 g (exact weight) of plant material was crushed to a particle diameter ($d = 2$ mm), introduced into a round-bottom flask with a capacity of 100 ml, a solution of 1.0 g of oxalic acid in 50.0 ml of methanol was added, boiled refluxed for 10 min., cooled in an ice bath to a temperature of 15–20 °C and filtered through a membrane filter ($d = 0.45$ μm).

The 2.0 ml of the filtrate were transferred to a 50 ml conical flask, 2.0 ml of the dichlorophenolindophenol sodium standard solution successively were added, shaking gently after each addition, then, after exactly 60 s, 0.5 ml of a solution of 100 g/l thiourea in ethanol (50 %) and 0.7 ml of dinitrophenylhydrazine-sulfuric acid solution, was heated under reflux at a temperature of 50 °C for 75 min and immediately placed in an ice bath for 5 min.

Drops of 5.0 ml of a mixture of 12 ml of purified water and 50 ml of sulfuric acid, added for a period of not less than 90 s and not more than 120 s, vigorously shaking the flask in an ice bath.

The solution was kept for 30 minutes. at room temperature and measured the optical density at a wavelength of 520 nm using solution A as a compensating solution.

Solution A. 2.0 ml of the filtrate obtained during the preparation of the test solution is processed according to the

method, adding dinitrophenylhydrazine-sulfuric acid solution immediately before measuring the optical density.

Solution for comparison. 40.0 mg of L-ascorbic acid standard is dissolved in a freshly prepared solution of 20 g/l oxalic acid in methanol, the volume of the solution is brought to 100.0 ml with the same solvent.

The 5.0 ml of the obtained solution was brought up to 100.0 ml with a freshly prepared solution of 20 g/l oxalic acid in methanol.

The 2.0 ml of the resulting solutions were processed according to the method. The optical density is measured at a wavelength of 520 nm using solution B as a compensating solution.

Solution B. The 2.0 ml of the comparison solution was processed similarly to solution A.

The optical density of the obtained stabilized complex of ascorbic acid with a standard solution of dichlorophenolindophenol sodium was determined at room temperature at a wavelength of 520 nm, relative to the compensation solution [26].

The obtained results were processed by the mathematical statistics under the license program Statistica for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J).

The reliability of the obtained differences in values according to the State Pharmacopoeia of Ukraine (version 1) was assessed by the Student’s t-test ($p > 95$ %) [27].

Results

By TLC and spectrophotometry methods the presence and quantitative content of L-ascorbic acid in the herbal raw materials of four widespread *Thymus* L. genus species of the Ukraine flora from different places of growth during the vegetation season (June – August 2023) were used.

The obtained research results are shown in *Table 1* and *Fig. 1*.

The accumulation of L-ascorbic acid was identified and determined by the TLC and spectrophotometry methods during the vegetation season in the herbal raw materials of four widespread *Thymus* L. genus species.

It was established that the highest presence of the compound was inherent during the flowering season of the species, from 20.20 ± 2.00 mg% in the herbal raw material of *Thymus serpyllum* L. to 39.10 ± 3.88 mg% in *Th. vulgaris* L.

The smallest accumulation of the compound is characteristic of the fruiting season of the species, from 9.14 ± 0.89 mg% in the herbal raw material of *Thymus serpyllum* L. to 15.49 ± 1.51 mg% in *Th. vulgaris* L.

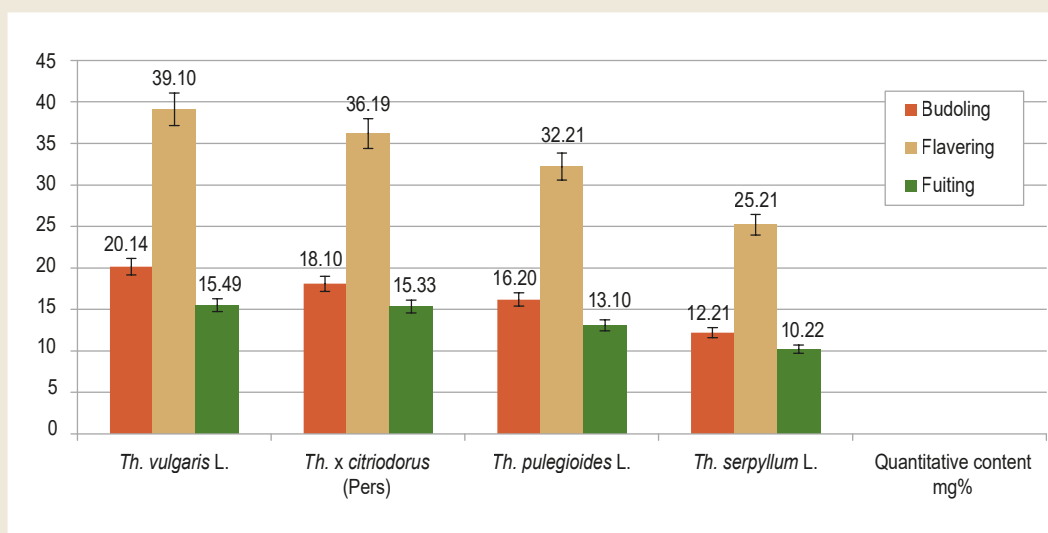
During the budding season, the content of L-ascorbic acid relatively small and were ranged from 10.13 ± 1.04 mg% in herbal raw material of *Thymus serpyllum* L. to 18.10 ± 1.79 mg% y *Th. vulgaris* L.

For cultivated species of the *Thymus* genus L., the accumulation of this compound was at a higher level than in the wild.

For the herb *Thymus vulgaris* L. from different places of growth quantitative content ranged from 37.22 ± 3.70 mg% to 39.10 ± 3.88 mg%; for *Th. x citriodorus* (Pers.) Schreb. var. “Silver Queen” from 34.21 ± 3.39 mg% to 36.19 ± 3.59 mg%.

Table 1. The results of determining the quantitative content of L-ascorbic acid in the herbal raw materials of *Thymus* L. genus species (June – August 2023), mg%, $\mu = 6$

Species name	Place of collection	Quantitative content ($\bar{x} \pm \Delta\bar{x}$), mg%		
		Budding	Flowering	Fruiting
<i>Th. serpyllum</i> L.	Vilniansk, Zaporizhzhia reg.	10.11 \pm 1.11	23.22 \pm 2.20	9.14 \pm 0.89
<i>Th. serpyllum</i> L.	Lozova, Kharkiv reg.	12.21 \pm 1.22	25.21 \pm 2.49	10.22 \pm 1.07
<i>Th. serpyllum</i> L.	Berezotocha, Poltava reg.	10.13 \pm 1.04	20.20 \pm 2.00	9.17 \pm 0.89
<i>Th. vulgaris</i> L.	Beryslav, Kherson reg.	20.14 \pm 2.12	39.10 \pm 3.88	15.49 \pm 1.51
<i>Th. vulgaris</i> L.	Pavlohrad, Dnipropetrovsk reg.	19.10 \pm 1.90	38.19 \pm 3.79	16.11 \pm 1.59
<i>Th. vulgaris</i> L.	Pidstepne, Kherson reg.	19.14 \pm 1.89	37.22 \pm 3.70	17.20 \pm 1.68
<i>Th. pulegioides</i> L.	Baburka, Zaporizhzhia reg.	16.20 \pm 1.65	32.21 \pm 3.20	13.10 \pm 1.33
<i>Th. pulegioides</i> L.	Solone, Dnipropetrovsk reg.	16.10 \pm 1.63	32.20 \pm 3.19	12.25 \pm 1.22
<i>Th. pulegioides</i> L.	Orikhiv, Zaporizhzhia reg.	16.13 \pm 1.59	32.10 \pm 3.22	12.10 \pm 1.20
<i>Th. x citriodorus</i> (Pers.) Schreb. var. "Silver Queen"	Volodymyrivka, Zaporizhzhia reg.	18.10 \pm 1.79	36.19 \pm 3.59	15.33 \pm 1.50
<i>Th. x citriodorus</i> (Pers.) Schreb. var. "Silver Queen"	Dnipreistan, Dnipropetrovsk reg.	17.11 \pm 1.69	35.22 \pm 3.46	16.14 \pm 1.59
<i>Th. x citriodorus</i> (Pers.) Schreb. var. Silver Queen"	Synelnikove, Dnipropetrovsk reg.	17.23 \pm 1.71	34.21 \pm 3.39	15.28 \pm 1.50

**Fig. 1.** The maximum accumulation of L-ascorbic acid in the herbal raw materials of *Thymus* L. genus species during the vegetation season (June – August 2023), ($\bar{x} \pm \Delta\bar{x}$) mg%, $\mu = 6$.

Discussion

It was established that the accumulation of L-ascorbic acid during the growing season in the herbal raw materials of four studied widespread wild and cultivated species of the *Thymus* L. genus ranges from 9.14 \pm 0.89 mg% to 39.10 \pm 3.88 mg%.

The compound is an important factor that determines the passage of all stages of plant ontogenesis. Its maximum accumulation was characteristic during the flowering season for cultivated species of the *Thymus* L. genus.

In the herbal raw materials of *Thymus x citriodorus* (Pers.) Schreb. var. "Silver Queen" from 15.28 \pm 1.50 mg% to 36.19 \pm 3.59 mg%; *Th. vulgaris* L. from 15.49 \pm 1.51 mg% to 39.10 \pm 3.88 mg%.

The transition of ascorbic acid to the composition of extracts from species of the genus *Thymus* L. contributes to the increase of collagen biosynthesis, anti-inflammatory and antioxidant activity of drugs during dermal application.

Conclusions

1. The accumulation of L-ascorbic acid, which contributes to the increase of collagen biosynthesis, anti-inflammatory and antioxidant effects, was identified and determined by TLC and spectrophotometry methods during the vegetation season in the herbal raw materials of four widespread *Thymus* L. genus species.

2. The highest accumulation of L-ascorbic acid during the vegetation season is characteristic of the herbal raw materials of the studied species during flowering.

3. Taking into account the sufficient raw material base of the researched promising species of the *Thymus* L. genus, it should be considered appropriate to cultivate them in the conditions of Ukraine to obtain effective complex medicinal drugs for dermal application.

Prospects for further research. The methods of identification and quantitative analysis of L-ascorbic acid can be used for further research on *Thymus* L. genus species as well as for inclusion in the draft monographies for the standardization of these species.

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