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.
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.

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**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 ℃, a layer thickness of 1 cm, to the last moisture in the composition no more than 10 %.

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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 F34”, 20 × 20 (Merck KGaA, Germany) in the systems acetone-glacial acetic acid-methanol-toluene (5:25:20:70) 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 dichlophenolin dophenol 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 dichlorophenoldophonol 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 dinitropheynylhydrazine-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 dichlorophenoldophonol 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. JPZ804182130ARCN10-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%.
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 ± 0.89 mg% to 39.10 ± 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 ± 1.50 mg% to 36.19 ± 3.59 mg%; *Th. vulgaris* L. from 15.49 ± 1.51 mg% to 39.10 ± 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.

**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%, µ = 6

<table>
<thead>
<tr>
<th>Species name</th>
<th>Place of collection</th>
<th>Quantitative content (x ± Δx), mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Budding</strong></td>
<td></td>
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<tr>
<td><em>Th. serpyllum</em> L.</td>
<td>Vilniask, Zaporizhzhia reg.</td>
<td>10.11 ± 1.11</td>
</tr>
<tr>
<td><em>Th. serpyllum</em> L.</td>
<td>Lozova, Kharkiv reg.</td>
<td>12.21 ± 1.22</td>
</tr>
<tr>
<td><em>Th. serpyllum</em> L.</td>
<td>Berezotocha, Poltava reg.</td>
<td>10.13 ± 1.04</td>
</tr>
<tr>
<td><em>Th. vulgaris</em> L.</td>
<td>Beryslav, Kherson reg.</td>
<td>20.14 ± 2.12</td>
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<tr>
<td><em>Th. vulgaris</em> L.</td>
<td>Pavlohrad, Dnipropetrovsk reg.</td>
<td>19.10 ± 1.90</td>
</tr>
<tr>
<td><em>Th. vulgaris</em> L.</td>
<td>Pidstipne, Kherson reg.</td>
<td>19.14 ± 1.89</td>
</tr>
<tr>
<td><em>Th. pulegioides</em> L.</td>
<td>Baburka, Zaporizhzhia reg.</td>
<td>16.20 ± 1.65</td>
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<tr>
<td><em>Th. pulegioides</em> L.</td>
<td>Solone, Dnipropetrovsk reg.</td>
<td>16.10 ± 1.63</td>
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<tr>
<td><em>Th. pulegioides</em> L.</td>
<td>Orkhiv, Zaporizhzhia reg.</td>
<td>16.13 ± 1.59</td>
</tr>
<tr>
<td><em>Th. x citriodorus</em> (Pers.) Schreb. var. “Silver Queen”</td>
<td>Volodymyrivka, Zaporizhzhia reg.</td>
<td>18.10 ± 1.79</td>
</tr>
<tr>
<td><em>Th. x citriodorus</em> (Pers.) Schreb. var. “Silver Queen”</td>
<td>Dnipreistan, Dnipropetrovsk reg.</td>
<td>17.11 ± 1.69</td>
</tr>
<tr>
<td><em>Th. x citriodorus</em> (Pers.) Schreb. var. Silver Queen”</td>
<td>Synelnikove, Dnipropetrovsk reg.</td>
<td>17.23 ± 1.71</td>
</tr>
<tr>
<td><strong>Flowering</strong></td>
<td></td>
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</tr>
<tr>
<td><em>Th. serpyllum</em> L.</td>
<td>Vilniask, Zaporizhzhia reg.</td>
<td>23.22 ± 2.20</td>
</tr>
<tr>
<td><em>Th. serpyllum</em> L.</td>
<td>Lozova, Kharkiv reg.</td>
<td>25.21 ± 2.49</td>
</tr>
<tr>
<td><em>Th. serpyllum</em> L.</td>
<td>Berezotocha, Poltava reg.</td>
<td>20.20 ± 2.00</td>
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<td><em>Th. vulgaris</em> L.</td>
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<td><em>Th. x citriodorus</em> (Pers.) Schreb. var. “Silver Queen”</td>
<td>Dnipreistan, Dnipropetrovsk reg.</td>
<td>35.22 ± 3.46</td>
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<td><em>Th. x citriodorus</em> (Pers.) Schreb. var. Silver Queen”</td>
<td>Synelnikove, Dnipropetrovsk reg.</td>
<td>34.21 ± 3.39</td>
</tr>
<tr>
<td><strong>Fruiting</strong></td>
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<tr>
<td><em>Th. serpyllum</em> L.</td>
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<td>9.14 ± 0.89</td>
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<tr>
<td><em>Th. serpyllum</em> L.</td>
<td>Lozova, Kharkiv reg.</td>
<td>10.22 ± 1.07</td>
</tr>
<tr>
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<td>Berezotocha, Poltava reg.</td>
<td>9.17 ± 0.89</td>
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<td><em>Th. vulgaris</em> L.</td>
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<td>17.20 ± 1.68</td>
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</table>

**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 ± 0.89 mg% to 39.10 ± 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.

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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.
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 monographs for the standardization of these species.

**Funding**

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

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