

**STUDY OF CAROTENOIDS AND CHLOROPHYLLS CONTENT IN  
*THYMUS* L. ESSENTIAL OILS GENUS SPECIES AT THE REPRODUCTIVE  
PERIOD**

**Key words:** species of the genus *Thymus*, herb, essential oils, carotenoids, chlorophylls, quantitative content, methods of thin-layer chromatography and spectrophotometry, reproductive period

**ABSTRACT**

The genus *Thymus* (*Thymus* L., *Lamiaceae* L.) is polymorphic, containing various races and forms with minor morphological differences. Up to 200 species have been identified in the world, of which up to 50 are found in Ukraine.

Widespread wild essential oil species of the genus *Thymus* L. in Ukraine are: creeping thyme, Crimean thyme, Dnieper thyme, Marshall's thyme, flea thyme, Pallas's thyme. Successfully cultivated: common thyme, Spanish white thin thyme, Spanish white flowering thyme, creeping thyme, lemon thyme.

Thyme is known for its rich chemical composition. Carotenoids and chlorophylls contribute to the accumulation of essential oil, polyphenolic compounds, antioxidant, detoxification and radioprotective effects.

For the standardization of raw materials of *Thymus* L. species, it is advisable to determine the accumulation of carotenoids and chlorophylls during the reproductive period.

The purpose of the work is to identify and determine the quantitative content of carotenoids and chlorophylls during the reproductive period in the herb species of the genus *Thymus* L. of the flora of Ukraine.

Raw materials were collected in steppe biocenoses and specialized farms of southeastern Ukraine during the reproductive period (May–September 2018–2023) according to generally accepted methods. They were dried at 35 °C to a moisture content of no more than 10%. n-Hexane extracts (1:100) were used to identify substances and establish their quantitative content. The determination was made by thin-layer chromatography (TLC) followed by densitometry and spectrophotometry.

By the methods of thin-layer chromatography and spectrophotometry in n-hexane extracts from grass species of the genus *Thymus* L. of flora of Ukraine 4 carotenoids and 2 chlorophylls were identified. Cultivated species had the highest content: *Th. vulgaris* L. and *Th. x citriodorus* (Pers.) Schreb. var. «Silver Queen».

The accumulation of carotenoids and chlorophylls in the raw materials of the genus *Thymus* L. during the reproductive period was studied. The results indicate the expediency of standardization of the studied raw materials according to the accumulation of carotenoids and chlorophylls.

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## ДОСЛІДЖЕННЯ ВМІСТУ КАРОТИНОЇДІВ ТА ХЛОРОФІЛІВ У ТРАВІ ЕФІРООЛІЙНИХ ВИДІВ РОДУ *THYMUS* L. У РЕПРОДУКТИВНИЙ ПЕРІОД

**Ключові слова:** види роду Чебрець, трава, ефірні олії, каротиноїди, хлорофіли, кількісний вміст, методи тонкошарової хроматографії та спектрофотометрії, репродуктивний період

### АНОТАЦІЯ

Рід чебрець (*Thymus* L., *Lamiaceae* L.) є поліморфним, містить різноманітні раси та форми з дрібними морфологічними відмінностями. У світі ідентифіковано до 200 видів, з яких в Україні трапляються до 50.

Розповсюдженими дикорослими ефіроолійними видами роду *Thymus* L. в Україні є: чебрець повзучий, ч. кримський, ч. дніпровський, ч. Маршаллів, ч. блошиний, ч. Палласів. Успішно культивуються: чебрець звичайний, ч. іспанський білий тонкий, ч. іспанський білий квітучий, ч. повзучий, ч. лимонний.

Чебрець відомий багатим хімічним складом. Каротиноїди та хлорофіли сприяють накопиченню ефірної олії, поліфенольних сполук, антиоксидантній, детоксикаційній та радіопротекторній дії.

Для стандартизації сировини видів роду *Thymus* L. доцільним є визначення накопичення каротиноїдів і хлорофілів протягом репродуктивного періоду.

Мета роботи – ідентифікація та визначення кількісного вмісту каротиноїдів та хлорофілів у репродуктивний період у траві видів роду *Thymus* L. флори України.

Сировину збирали у степових біоценозах та спеціалізованих господарствах південно-східної України під час репродуктивного періоду (травень–вересень 2018–2023 рр.) за загальноприйнятими методиками. Висушували за температури 35 °С та вологості не більше 10%. Для ідентифікації речовин та встановлення їх кількісного вмісту використовували н-гексанові витяги (1:100). Визначення здійснювали методами тонкошарової хроматографії з наступною денситометрією та спектрофотометрії.

Методами тонкошарової хроматограми та спектрофотометрії в н-гексанових витягах із трави видів роду *Thymus* L. флори України ідентифіковано 4 каротиноїди та 2 хлорофіли. Найбільший вміст був притаманний культивованим видам: *Th. vulgaris* L. та *Th. x citriodorus* (Pers.) Schreb. var. «Silver Queen».

Досліджено накопичення каротиноїдів та хлорофілів у сировині роду *Thymus* L. у репродуктивний період. Результати свідчать про доцільність стандартизації досліджуваної сировини за накопиченням каротиноїдів та хлорофілів.

### Introduction

The study of medicinal plant raw materials of essential oil species of the polymorphic genus *Thymus* L. of the numerous St. Nettle family (*Lamiaceae* L.) has significant theoretical and practical significance for modern medicine, which is widespread in the plant world and unites about 7,900 species.

The genus *Thymus* L. is extremely polymorphic, containing a variety of races and forms with some minor morphological differences.

Wild species of the genus *Thymus* L. are perennial herbs or semi-shrubs that grow in natural biocenoses of countries with a temperate climate: Northern and Central Europe, North Africa, Asia, the Mediterranean. According to modern botanical studies, up to 200 autochthonous species have been identified in the world flora, of which up to 50 are found in Ukraine [1, 2].

In modern medicine, the medicinal plant raw materials of the species are a source of obtaining numerous complex extraction phytopreparations, medicines of plant origin, dietary supplements with pronounced expectorant, antimicrobial and anti-inflammatory activity [3, 4, 5].

At the same time, it was established that the pronounced biological effect of medicinal products from the herb *Thymus* L. was caused mainly by the accumulation during the reproductive period of essential oil and polyphenolic compounds, hydroxycinnamic compounds [6, 7].

Common wild essential oil species of the genus *Thymus* L. in Ukraine are: creeping thyme (*Th. serpyllum* L.), Crimean thyme (*Th. tauricus* Klok. et Shost.), Dnipro thyme (*Th. borysthenticus* Klok. et Shost.), Marshall's thyme (*Th. marchallianus* Willd.), flea thyme (*Th. pulegioides* L.), Pallas's thyme (*Th. pallasianus* H. Braun.). In many countries of the world, the following are successfully cultivated: common thyme (*Th. vulgaris* L.); thin Spanish white thyme (*Th. zygis* L. var. *gracilis* Bois.); Spanish white flowering thyme (*Th. zygis* L. var. *floribundus* Bois.), creeping thyme, lemon thyme (*Th. x citriodorus* (Pers.) Schreb. var. «Silver Queen») [8, 9].

To the State Pharmacopoeia of Ukraine 1 edition. (appendix 3) includes the herb *Thymus serpyllum* L. (creeping part) and a mixture of the herb *Th. vulgaris* L. (part creeping) with *Th. zygis* L. (part Spanish white) without distinguishing distinctive diagnostic features of the plant material of the species [10].

During the reproductive period in species of the genus *Thymus* L., the presence and accumulation of many classes of biologically active compounds of primary and secondary biosynthesis were established: essential and fatty oils, flavonoids, hydroxycinnamic acids, triterpene saponins, polysaccharides, vitamins, tannins, proteins, amino acids, organic acids, micro- and macroelements, etc. [11–20].

Natural pigments carotenoids and chlorophylls are of great importance in the life of plants. These are the most widespread classes of biological compounds in the plant world. In plants, carotenoids actively stimulate the growth of pollen tubes and the accumulation of pollen during plant photosynthesis and participate in the creation of peroxide compounds.

Chlorophyll is the main component in the formation of hemoglobin, improves the condition of blood vessels. Carotenoids and chlorophylls are responsible for the reception of light in animals, plants and microorganisms, perform photosynthetic, photoprotective and antioxidant functions. At the same time, they activate plant metabolism, contribute to the accumulation of essential oil, polyphenolic compounds during the reproductive period. In the composition of phytopreparations, medicinal products of plant origin and dietary supplements, pronounced wound-healing, anti-inflammatory, antioxidant, detoxifying, radioprotective effects are revealed. These compounds are vital for the human body, because they are not synthesized in it, they are replenished only with food or medicinal products of plant origin [21, 22, 23, 26, 27].

The analysis of scientific literature shows limited scientific research of medicinal plant raw materials and medicinal products from wild and cultivated species of the genus *Thymus* L. on the content and accumulation of carotenoids and chlorophylls.

Taking into account the sufficient raw material base of essential oil species of the genus *Thymus* L. in the conditions of Ukraine and the world, the pronounced antioxidant, detoxifying and radioprotective effect of carotenoids and chlorophylls, study of their content and accumulation during the reproductive period is promising and expedient.

**The purpose** of the work is to determine the presence and quantitative content of carotenoids and chlorophylls in the grass of the studied widespread essential oil species of the genus *Thymus* L. of the flora of Ukraine in the reproductive period by methods of thin-layer chromatography followed by densitometry and spectrophotometry.

## Materials and methods

The object of research was selected herb of wild species of the genus *Thymus* L.: *Th. serpyllum* L., *Th. tauricus* Klok. et Shost., *Th. borysthenicus* Klok. et Shost., *Th. marchallianus* Willd., *Th. pulegioides* L., *Th. pallasianus* H. Braun.

It was harvested in steppe biocenoses and specialized farms of southeastern Ukraine (Zaporizhia, Dnipropetrovsk, Poltava, Kherson, Mykolaiv, Odesa regions) during the reproductive period (May–September 2018–2023). Cultivated medicinal plant raw materials *Th. vulgaris* L., *Th. x citriodorus* (Pers.) Schreb. var. «Silver Queen» collected at the research site for growing medicinal plants of the Zaporizhia State Medical and Pharmaceutical University in accordance with the requirements of the State Pharmacopoeia of Ukraine [24].

Medicinal plant raw materials of species of the genus *Thymus* L. consisted of flowering apical shoots with inflorescences up to 15 cm long, individual leaves and parts of twigs (no more than 2%).

It was dried in a «Termolab SNOL 24/350» drying cabinet (Ukraine) at a temperature of 35 °C, a layer thickness of 1 cm, and a humidity of no more than 10%.

n-Hexane extracts (1:1 and 1:100) were used to identify substances and establish their quantitative content.

Extracts were obtained according to the method: 1.0 g of plant material was crushed ( $d = 0.3$  mm), 50 ml of n-hexane solvent was added, heated in a water bath «VB-4 micromed» ( $t = 100$  °C) in a conical flask with a reflux refrigerator for 15 minutes. The extraction process was carried out two more times with new portions of the solvent. The combined extracts were cooled, centrifuged on the «SM-3.01 micromed» device, filtered through a membrane filter ( $d = 0.45$  μm) for 30 minutes into a vial for analysis.

Solvent systems were used to identify carotenoids and chlorophylls, respectively: diethyl ether – benzene – anhydrous ethanol (10:10:80) and chloroform – methanol (9:1).

The research was carried out by thin-layer chromatography methods on Aluminum oxide plates 150 F 254 (0.20 mm) (Merck KGaA, Germany), with densitometric determination «Biostep» CD 60 (Germany).

As analytical standards, substances from Sigma-Aldrich, USA were used: α-Carotene (7488-99-5), β-Carotene (7235-40-7), γ-Carotene (472-93-5), Lutein (127-40-2), Chlorophyll a (95145), Chlorophyll b (00538).

To identify and determine the quantitative content of the studied compounds in n-hexane extracts (1:100) from the grass of the studied species of the *Thymus* L. genus, the method of UV spectrophotometry was also used.

It is the most convenient and suitable for the identification and quantification of carotenoids and chlorophylls in extracts from plant raw materials and allows the use of standard samples of substances.

The proposed research methods allow simultaneous identification of carotenoids and chlorophylls, determination of their quantitative content in plant raw materials. Important advantages of the methods include: the usage of small sample weights, relative speed of research, good reproducibility of results and small error of measurements.

*Methodology:* About 1.0 g (exact weight) of medicinal plant raw materials, previously crushed ( $d = 0.3$  mm), was introduced into a flask with a capacity of 100 ml, 30 ml of n-hexane was added, heated in a water bath at ( $t = 60$  °C) within 5 min. The resulting solution was filtered into a volumetric flask with a capacity of 100 ml. The extraction process was repeated two more times. The solutions were combined, cooled, filtered, and the volume was brought up to 100 ml. Then 10 ml of the resulting solution was placed in a 25 ml volumetric flask and brought up to the mark with the appropriate solvent.

The optical density was measured on a spectrophotometer «Lambda 365» (USA) in quartz cuvettes ( $l = 10$  mm). n-Hexane (Merck KGaA, Germany) was used as a comparison solution. The content of the sum of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -carotenes, which are similar in chemical structure, was converted to  $\beta$ -carotene. The content of chlorophyll a and chlorophyll b was determined separately using the corresponding standard samples of the compounds.

The proposed research methods allow simultaneous identification of carotenoids and chlorophylls, determination of their quantitative content in medicinal plant raw materials. Important advantages of the methods include: the use of small amounts of samples, relative speed of research, good reproducibility of results and small error of measurements.

Statistical processing of the results was carried out using the standard analysis package of Microsoft Office Excel statistical processing programs. The reliability of the obtained differences in values was assessed by Student's t-test ( $p > 95\%$ ) [25].

## The results and discussion

The obtained results are shown in Fig. 1 and in Tables 1, 2, 3.

During the comparison of the obtained spectra of n-hexane extracts from the herb of the studied essential oil species of the genus *Thymus* L. with the spectra of solutions of standard samples of  $\alpha$ -,  $\beta$ - and  $\gamma$ -carotene, lutein, chlorophyll a, and chlorophyll b, their identity was established.

The coincidence of the absorption maxima ( $\lambda$  nm) of n-hexane extracts with the used standard samples of substances at 420–430 nm, 445–460 nm, 470–480 nm, and 620–650 nm was established (Fig. 1).

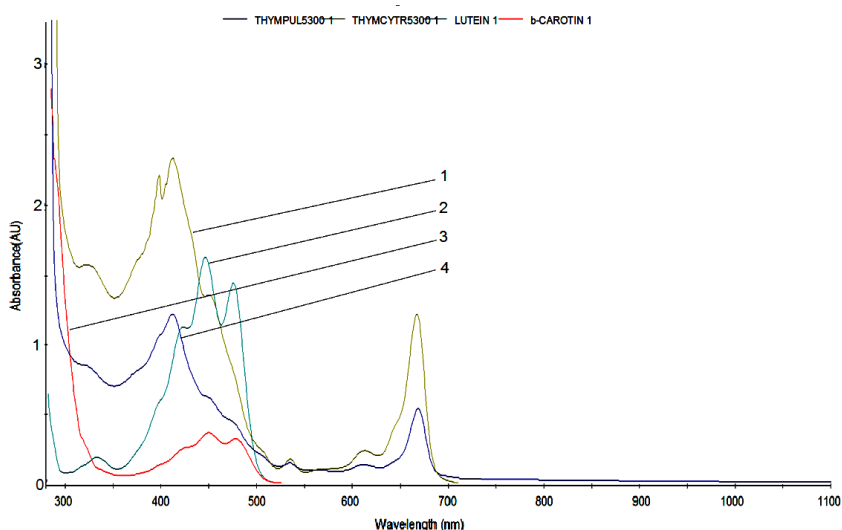


Fig. UV absorption spectra of n-hexane extracts:

- 1 – herb *Thymus x citriodorus* (Pers.) Schreb. var. «Silver Queen» (1:100);
- 2 – herb *Thymus pulegioides* L. (1:100); 3 – a solution of a standard sample of  $\beta$ -carotene (10  $\mu\text{g/ml}$ ); 4 – a solution of a standard sample of lutein (20  $\mu\text{g/ml}$ )

It was established that the highest content of carotenoids is characteristic of the herb *Thymus tauricus* Klok. et Shost. during the flowering period, which ranged from  $44.29 \pm 2.21$  mg% to  $53.21 \pm 2.66$  mg%. Several lower concentrations of these substances were determined for the herb *Thymus vulgaris* L., from  $36.19 \pm 1.89$  mg% to  $40.16 \pm 1.83$  mg%, respectively.

When comparing the obtained spectra with the spectra of  $\beta$ -carotene, lutein, chlorophylls a and b, their identity was established, which indicates the presence of the investigated compounds in medicinal plant raw materials of essential oil species of the genus *Thymus* L. during the reproductive period (Figure).

The quantitative content of carotenoids and chlorophylls in the studied grass did not differ, but their accumulation differed significantly (Tables 1, 2, 3).

Table 1

**The content of total carotenes ( $\alpha$ -,  $\beta$ -,  $\gamma$ -) and lutein in the herb of essential oil species of the genus *Thymus* L. during the reproductive period (May–August)**

Species name	The content of the sum of carotenes ( $\alpha$ -, $\beta$ -, $\gamma$ -) and lutein, ( $\bar{x} \pm \Delta\bar{x}$ ), mg%, $\mu = 6^*$		
	Budding	Flowering	Fruiting
<i>Th. vulgaris</i> L.	16,00 $\pm$ 1,11	44,01 $\pm$ 2,88	20,30 $\pm$ 1,84
<i>Th. tauricus</i> Klok. et Shost.	14,32 $\pm$ 1,29	27,09 $\pm$ 2,31	18,47 $\pm$ 1,57
<i>Th. borysthenticus</i> Klok. et Shost.	13,10 $\pm$ 1,28	20,20 $\pm$ 1,89	15,20 $\pm$ 1,32
<i>Th. marchallianus</i> Willd.	13,44 $\pm$ 1,55	19,16 $\pm$ 1,89	16,55 $\pm$ 1,73
<i>Th. pulegioides</i> L.	15,32 $\pm$ 1,49	30,20 $\pm$ 2,95	19,11 $\pm$ 1,77
<i>Th. pallasianus</i> H. Braun.	12,20 $\pm$ 1,19	22,19 $\pm$ 1,90	14,76 $\pm$ 1,42
<i>Th. x citriodorus</i> (Pers.) Schreb. var. «Silver Queen»	18,00 $\pm$ 1,75	48,77 $\pm$ 3,66	25,30 $\pm$ 1,99
<i>Th. serpyllum</i> L.	13,10 $\pm$ 1,23	18,20 $\pm$ 1,77	15,25 $\pm$ 1,38

Примітка: \* –  $p < 0,05$ .

Table 2

**The content of chlorophyll b in the herb of essential oil species of the genus *Thymus* L. during the reproductive period (May–August)**

Species name	Chlorophyll content b, ( $\bar{x} \pm \Delta\bar{x}$ ), mg%, $\mu = 6^*$		
	Budding	Flowering	Fruiting
<i>Th. vulgaris</i> L.	5,00 $\pm$ 0,49	10,33 $\pm$ 1,28	4,34 $\pm$ 0,41
<i>Th. tauricus</i> Klok. et Shost.	4,77 $\pm$ 0,48	6,03 $\pm$ 0,59	3,61 $\pm$ 0,39
<i>Th. borysthenticus</i> Klok. et Shost.	4,72 $\pm$ 0,50	5,31 $\pm$ 0,64	3,37 $\pm$ 0,44
<i>Th. marchallianus</i> Willd.	2,68 $\pm$ 0,24	3,13 $\pm$ 0,30	2,36 $\pm$ 0,22
<i>Th. pulegioides</i> L.	3,11 $\pm$ 0,32	5,22 $\pm$ 0,49	3,00 $\pm$ 0,28
<i>Th. pallasianus</i> H. Braun.	3,99 $\pm$ 0,50	5,41 $\pm$ 0,57	3,08 $\pm$ 0,32
<i>Th. x citriodorus</i> (Pers.) Schreb. var. «Silver Queen»	5,22 $\pm$ 0,49	7,78 $\pm$ 0,79	4,33 $\pm$ 0,46
<i>Th. serpyllum</i> L.	4,09 $\pm$ 0,43	5,06 $\pm$ 0,52	3,38 $\pm$ 0,36

Примітка: \* –  $p < 0,05$ .

Table 3

**The amount of chlorophyll a in the herb of essential oil species of the genus *Thymus* L. during the reproductive period (May–August)**

Species name	Chlorophyll content a, ( $\bar{x} \pm \Delta\bar{x}$ ), mg%, $\mu = 6^*$		
	Budding	Flowering	Fruiting
<i>Th. vulgaris</i> L.	1,67 $\pm$ 0,17	3,44 $\pm$ 0,30	1,45 $\pm$ 0,16
<i>Th. tauricus</i> Klok. et Shost.	1,59 $\pm$ 0,18	2,01 $\pm$ 0,22	1,21 $\pm$ 0,14
<i>Th. borysthenticus</i> Klok. et Shost.	1,57 $\pm$ 0,18	1,77 $\pm$ 0,21	1,12 $\pm$ 0,10
<i>Th. marchallianus</i> Willd.	0,89 $\pm$ 0,08	1,04 $\pm$ 0,10	0,77 $\pm$ 0,08
<i>Th. pulegioides</i> L.	1,04 $\pm$ 0,10	1,74 $\pm$ 0,16	1,00 $\pm$ 0,11
<i>Th. pallasianus</i> H. Braun.	1,33 $\pm$ 0,15	1,80 $\pm$ 0,20	1,02 $\pm$ 0,12
<i>Th. x citriodorus</i> (Pers.) Schreb. var. «Silver Queen»	1,74 $\pm$ 0,19	2,59 $\pm$ 0,27	1,44 $\pm$ 0,16
<i>Th. serpyllum</i> L.	1,36 $\pm$ 0,14	1,69 $\pm$ 0,18	1,13 $\pm$ 0,12

Примітка: \* –  $p < 0,05$ .

The obtained results indicate that the composition of carotenoids and chlorophylls in the grass of the studied 6 wild and 2 cultivated essential oil species of the genus *Thymus* L. did not differ, but their accumulation during the reproductive period was significantly different.

The greatest accumulation of carotenoids is observed in the herb of the studied species during flowering (May–July), respectively from  $18.20 \pm 1.77$  mg% (*Th. serpyllum* L.) to  $48.77 \pm 3.66$  mg% (*Th. x citriodorus* (Pers.) Schreb. var. «Silver Queen»). The highest content of chlorophyll b was determined during the flowering period (May–July), from  $3.13 \pm 0.30$  mg% (*Th. marchallianus* Willd.) to  $10.33 \pm 1.28$  mg% (*Th. vulgaris* L.), respectively. At the same time, the accumulation of chlorophyll a ranged from  $1.04 \pm 0.10$  mg% (*Th. marchallianus* Willd.) to  $3.44 \pm 0.30$  mg% (*Th. vulgaris* L.), respectively.

A greater quantity of carotenoids and chlorophylls was characteristic of cultivated species of the genus *Th. vulgaris* L. and *Th. x citriodorus* (Pers.) Schreb. var. «Silver Queen». At the same time, the qualitative composition and quantitative content of the studied compounds in the herb of species from different places of growth did not reveal significant differences during the reproductive period.

During the fruiting of the species, their content decreased significantly and amounted to  $14.76 \pm 1.42$  mg% for carotenoids (*Th. pallasianus* H. Braun), up to  $25.30 \pm 1.99$  mg% (*Th. x citriodorus* (Pers.) Schreb. var. «Silver Queen»); chlorophyll b from  $2.36 \pm 0.14$  mg% to  $18.20 \pm 1.02$  mg% (*Th. marchallianus* Willd.); of chlorophyll a from  $0.77 \pm 0.08$  mg% (*Th. marchallianus* Willd.) to  $1.45 \pm 0.16$  mg% (*Th. vulgaris* L.).

Control of the composition and quantity of carotenoids and chlorophylls during the reproductive period of species of the genus *Thymus* L. is an important indicator of their normal physiological development and the quality of the obtained medicinal plant raw materials.

In the composition of phytopreparations, medicinal products of plant origin and dietary supplements, these important natural pigments enhance their biological activity, exhibit pronounced antioxidant, detoxifying and radioprotective effects.

## Conclusions

1. By the methods of thin-layer chromatography and spectrophotometry in n-hexane extract (1:100) from 6 studied wild plants and 2 cultivated essential oil species of the genus *Thymus* L. flora of Ukraine 6 compounds were identified: 4 carotenoids and 2 chlorophylls.

2. It was established during the reproductive period that the greatest accumulation of carotenoids and chlorophylls is characteristic of cultivated species of the genus *Thymus* L.: *Th. vulgaris* L. and *Th. x citriodorus* (Pers.) Schreb. var. «Silver Queen».

3. In order to obtain high-quality medicinal plant raw materials of 6 wild and 2 cultivated species of the genus *Thymus* L., it is advisable to standardize it according to the content of natural pigments from the groups of carotenoids and chlorophylls.

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