

Comparative Morphological and Anatomical Studies of Two Herbal Drugs: *Nepeta Cataria* L. and *Melissa Officinalis* L.

Nguyen Thi Hai Yen^{1*}, Yakovleva O. V.², Terninko I. I.¹

¹ Saint Petersburg State Chemical-Pharmaceutical Academy, 197376, Russia, Saint-Petersburg, Professora Popova street, 14.

² Komarov Botanical Institute of the RAS, 197376, Russia, Saint-Petersburg, Professora Popova street, 2.

Abstract.

This article describes the vegetative morphology and anatomy (leaves, petioles, stems, and flowers) of *Nepeta cataria* L. and *Melissa officinalis* L. which was added for comparison.

The diagnostic and distinctive characteristics of two herbal drugs are discussed. For the morphological features, the shape of leaves, type of leaf margins; petioles long; leaf and flower arrangement; the color of ribbed, corolla, calyx teeth, and mature nutlets; the shape of nutlets were observed for the differentiation. In the anatomical studies, epidermal cell anticlinal walls; epidermal cell of calyx and corolla; the presence and types of trichomes; the number and the distribution of vascular bundles, chlorenchyma, collenchyma, sclerenchyma, palisade layers of cells; intercellular spaces of spongy parenchyma and phloem properties are distinctive features.

The information presented here should aid in the identification authentically of *Nepeta cataria* L. and will be further used in the development of regulatory documents on this type of herbal medicinal raw materials.

Keywords: anatomical features, cross-section, epidermis, *Melissa officinalis* L., morphological features, *Nepeta cataria* L., trichome.

INTRODUCTION

The family *Lamiaceae* is the important source of medicinal and aromatic plants of the pharmaceutical industry. The genus *Nepeta* which is one of the largest genera within the family *Lamiaceae*, comprises about 200 species of herbaceous occurring in various climatic-geographical zones: central and south of Western Europe, European part of Commonwealth of Independent States (CIS) countries, Western Siberia, Far East and North Asia. This plant cultivated in Western Europe, America, CIS countries as an aromatic plant [5, 8].

Although *Nepeta* genus is widely distributed, including *Nepeta cataria* Linn but this plant is relatively little studied in aspects of botanical knowledge and phytochemistry. *Nepeta cataria* L., commonly known as Catnip, is a perennial herbaceous plant that has various biological activities: antimicrobial activity against 11 bacteria, 12 fungi, and 1 yeast species; immunomodulatory activity and antioxidant activity [1, 4, 6]. With the medical purpose, the aerial parts of *Nepeta cataria* L. were collected in the flowering time which used as an antispasmodic, antidepressant, sedative. Moreover, traditionally, the tea made of *Nepeta cataria* L. also used to relieve gastrointestinal and respiratory disorders such as colic, diarrhea, cough, chronic bronchitis, to improve the function of the hepatobiliary system and the human female reproductive system [5].

Nepeta cataria L. with typical characteristics of the family *Lamiaceae* often replacing *Melissa officinalis* L. in the market. In view of the fact that one of the main elements of standardization of herbal medicinal raw materials is their macroscopic and microscopic descriptions. It is, therefore, necessary to establish diagnostic and distinctive characteristics to differentiate these the plants.

The purpose of this study was to make the morphological

and anatomical comparison between *Nepeta cataria* L. and *Melissa officinalis* L.

MATERIALS AND METHODS

This study was based upon examination of plants of *Nepeta cataria* L. and *Melissa officinalis* L., which cultivated at the Saint Petersburg State Chemical-Pharmaceutical Academy's Arboretum (188695, Vsevolozhsky District of Leningrad Oblast, Russia) in the flowering season. Materials were air-dried under shade.

The macroscopic and microscopic evaluation was carried out according to general pharmacopoeial monograph of State Pharmacopoeia of Russian Federation XIII ed., Vol. 2, "Herba" (GPM.1.5.1.0002.15); "A technique of microscopic and microchemical study of herbal medicinal raw materials and herbal medicinal products" (GPM.1.5.3.0003.15) [7]. The material was separated into leaves, petioles, stems, and flowers, then the slides for microscopic examination were prepared using the usual procedures [2, 3]. Cross-sections and surface sections were investigated. Anatomical study by using binocular microscope Carl Zeiss Axio Lab.A1 (10× eyepiece and lenses: 5×, 10×, 20×, 40×) with phototube AxioCam MRCS 5 and ZEN lite 2011 software. To improve the resulting image by Helicon focus software.

RESULTS AND DISCUSSION

Morphological analysis:

The morphological characteristics of both the plants have shown resemblance which is typical for the family *Lamiaceae*: herbaceous plant, perennial with erect leafy stems; the stems are green, strong, branched, square stalk and 4-ribbed, single-furrowed along their sides and glabrous to softly white tomentose, fracture of stem is white in colour; leaves petiolate, internodes 3–9 cm long,

light green or green, glandular and eglandular trichomes is present on both upper and lower epidermis of leaves; flowers are hermaphrodite, pentamerous hypogynous, corolla about 1,5-2 times longer than the calyx and visibly comes out from among the teeth of the latter; nutles smooth surface.

The two herbal drugs of *Lamiaceae* family are similar in structure but they also exhibit differences. Detailed morphological differences between the two plants are listed in Table 1.

Based on the morphological analysis, significant differences were detected in regard of the shape of leaves, type of leaf margins, leaf base; petioles long; leaf and flower arrangement; the color of ribbed, corolla, calyx teeth and mature nutlets; the shape of nutlets.

Anatomical analysis

Investigations into the epidermal features (leaves, petioles, stems, corolla, and calyx) and cross section (stems, leaves, petioles) of *Nepeta cataria* L. and *Melissa officinalis* L. was carried out.

The epidermis in surface view composed of cells with straight to wavy, slightly sinuous to sinuous anticlinal walls (Fig. 2-3). The stomata of the diacytic type are present on the epidermis of stem, petiole, on both surfaces leaf (amphistomatic leaves) and calyx. The epidermis in both the plants possess an indumentum constituted by sessile glands, glandular and non-glandular trichomes (Fig. 1).

Sessile glands:

- capitate-sessile trichomes made up of a short stalk cell and a large globular-shaped head, which is made up of 4-6 cells (C1a) or 8 cells (C1b) separated by vertical walls.

The non-glandular trichomes were of five types:

- thick-walled, simple 1-3 celled trichome, short, uniseriate, straight to curved with the verrucose surface, arrow indicate cells organized in a rosette (E1).
- thin-walled, simple multicellular trichome, long, uniseriate, hooked, straight or cranked with pointed terminal cell and verrucose surface, the base of the trichomes is surrounded by 3-7 adjacent subsidiary cells which organized in a rosette (E2).
- fingerlike trichome, 1-3 celled with the verrucose surface (E3).
- thin-walled, simple 1 celled (rarely 2-3 celled) papillate or conical trichome, straight to curved with the verrucose surface, trichome attached directly to a small epidermis cell (basal cell) (E4).
- thin-walled, simple multicellular, hose-like, very long, smooth surface, arrow indicate cells organized in a rosette, the cell flat between the joints and the first or second distal cells usually collapsed (E5).

The glandular trichomes were of two types:

- glandular trichomes made up of a short stalk cell with 1-2 celled head (G1).
- glandular trichomes made up of stalk long, several cells with a sub-spheroidal head (G2).

Based on the anatomical analysis of the epidermis in surface view, some important characters about the authenticity of *Nepeta cataria* L. and *Melissa officinalis* L. are explored which might be helpful in identification between these plants. Epidermal cell anticlinal walls; epidermis properties of calyx and corolla; the presence and types of trichomes, especially in type C1, E1, E4, E5, and G2 are distinctive features.

Table 1. The comparative characteristic of the revealed morphological signs of *Nepeta cataria* L. and *Melissa officinalis* L.

	<i>Nepeta cataria</i> L.	<i>Melissa officinalis</i> L.
Colour of ribbed	dark-purple	green
Leaf arrangement	opposite or whorled	opposite
Leaves	leaves 2,5-7,5 cm, blade triangular-cordate, cordate at base, acuminate at apex, margin coarsely dentate	leaves 3-8 cm, blade ovate in shape, broadly wedge-shaped to rounded at base, obtuse or acuminate at apex, margin crenate or crenate-serrate
Petioles long	up to 7 mm	up to 3 cm
Flower arrangement	an inflorescence consisting of verticillasters at the end of stem ramifications, raceme shaped, verticillasters 2-15-flowered in opposite cymes	verticillasters of 2-12-flowered develop from the axils of the upper leaves
Corolla	4-12 mm long, petals typical bilabiate, pale-violet	up to 15 mm long, pale yellow, rarely pink tinted or pale-violet
Calyx	2-6 mm long with 5 light purple or dark-purple teeth	5-7 mm long with 5 green teeth
Nutles	ellipsoid, brownish-black	lanceoloid, light brown

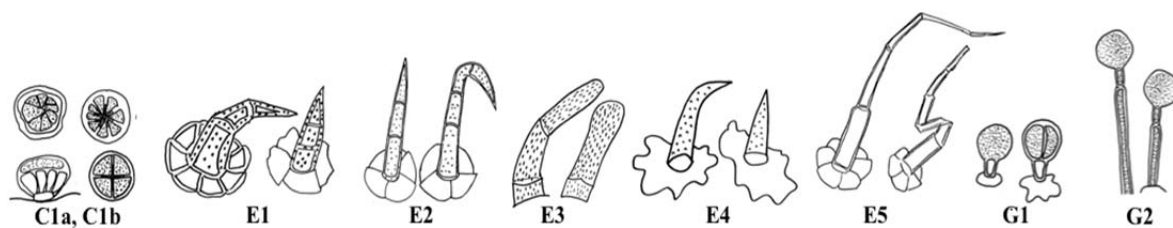


Figure 1. Types of trichomes.

Table 2. The epidermis in surface view of the plants *Nepeta cataria* L. and *Melissa officinalis* L.

Various organs	<i>Nepeta cataria</i> L.	<i>Melissa officinalis</i> L.
Stem	longitudinally elongated epidermal cells, rectangular to polygonal shaped with straight anticlinal walls (Fig. 2a, f, g). Trichomes: E1; G1; C1a (Fig. 1, 2a)	Trichomes: E2; E4; E5; G1; G2; C1b (Fig. 1, 2f, g)
Petiole	polygonal shaped epidermal cells with slightly wavy anticlinal walls (Fig. 2b). Trichomes: E1; G1; C1a (Fig. 1, 2c)	elongated epidermal cells, polygonal shaped with straight anticlinal walls. Trichomes: E4; E5; G1 (Fig. 1, 2h).
Leaf: upper surface	polygonal shaped epidermal cells with thick, straight anticlinal walls. Trichomes: E1; G1 (Fig. 1, 2d)	polygonal shaped epidermal cells with sinuous anticlinal walls on both surfaces. Trichomes: E4; E5; G1; C1b (Fig. 1, 2i, j).
lower surface	epidermal cells with slightly sinuous anticlinal walls. Trichomes: E1; G1; C1a (Fig. 1, 2e).	
Calyx	longitudinally elongated epidermal cells with sinuous anticlinal walls (Fig. 3a). Trichomes: E1; E2; E5; G1; C1a (Fig. 1, 3a).	epidermal cells with strongly sinuous anticlinal walls (Fig. 3f). Trichomes: E2; E4; E5; G1; G2; C1b (Fig. 1, 3f, g)
Corolla	in the corolla tube, epidermal cells present no differences with regard to calyx epidermal cells, while the epidermis of the petal lobe polygonal cells on both surfaces with slightly wavy anticlinal walls (Fig. 3b, c, d). Trichomes: E1; E2; E3; E5; G1; C1a (Fig. 1, 3d, e).	in the corolla tube and inside petal lobe epidermal cells with strongly sinuous anticlinal walls (Fig. 3i), outside petal lobe epidermal cells small, rounded , tightly adjacent to each other (Fig. 3h). Trichomes: E2; E3; E4; E5; G1; C1b (Fig. 1, 3i-k).

Table 3. The main anatomical features in cross section of differentiation between *Nepeta cataria* L. and *Melissa officinalis* L.

Anatomical characteristics	STEM (Fig. 4a, d)		PETIOLE (Fig. 4b, e)		LEAF (Fig. 4c, f)	
	<i>Nepeta cataria</i> L.	<i>Melissa officinalis</i> L.	<i>Nepeta cataria</i> L.	<i>Melissa officinalis</i> L.	<i>Nepeta cataria</i> L.	<i>Melissa officinalis</i> L.
Quantify of vascular bundles	at the corners		small subsidiary vascular bundles in each wing		single large vascular bundle in the center	
	2	1				
	between the corners					
	1 small	poorly developed or absent	2	1		
Parenchyma	2-3 layers of closely pack chlorenchyma cells	1-2 layers of closely pack chlorenchyma cells	Several parenchyma cell layers		palisade parenchyma	
					2- layers	1- layer
					spongy parenchyma	
					with large intercellular spaces	with small intercellular spaces
Collenchyma	angular collenchyma as a continuous ring		angular collenchyma adjoining the epidermis		adjoining the upper epidermis in the midrib region	
	1 layers	1-2 layers	1 layer	1-2 layers	1-3 layers	1-2 layers
	at the corners		in each wing		adjoining the lower epidermis in the midrib region	
	5-7 layers	1-3 layers	5-8 layers	3-5 layers	1-2 layers	1 layers
Sclerenchyma	present between the corners	absent between the corners	abaxial sclerenchyma patches adjoining the phloem	on the side of large vascular bundles 1-3 layers adjoining the phloem	sporadic sclerenchyma cells adjoining the phloem	on the side of large vascular bundles 1-2 layers
Phloem	non-compressed	closely compressed	slightly compressed	compressed	slightly compressed	compressed

Our anatomical results of the epidermis in surface view of both the plants are observed in Table 2.

Anatomical characters in cross-section revealed between *Nepeta cataria* L. and *Melissa officinalis* L.:

Stem (Fig. 4a, d).

The stem is clearly quadrangular with 4 corners. The cuticle is thin. The epidermis consists of a single layer of oval or rectangular cells. Beneath the epidermis, the number of angular collenchyma layers is present at the corners and between the corners of stem as a continuous ring. Thin-walled, irregular in shape parenchymatous cells

are present, between the corners of stem several cells parenchyma replaced by chlorenchyma cells, the presence of chlorenchyma in *Melissa officinalis* L. is less than in *Nepeta cataria* L. The phloem is surrounded by sclerenchyma layers. The phloem is non-compressed (*Nepeta cataria* L.) or closely compressed (*Melissa officinalis* L.). Cambium is absent. The pith consists of the large thin-walled parenchyma cells and may be destroyed in the center.

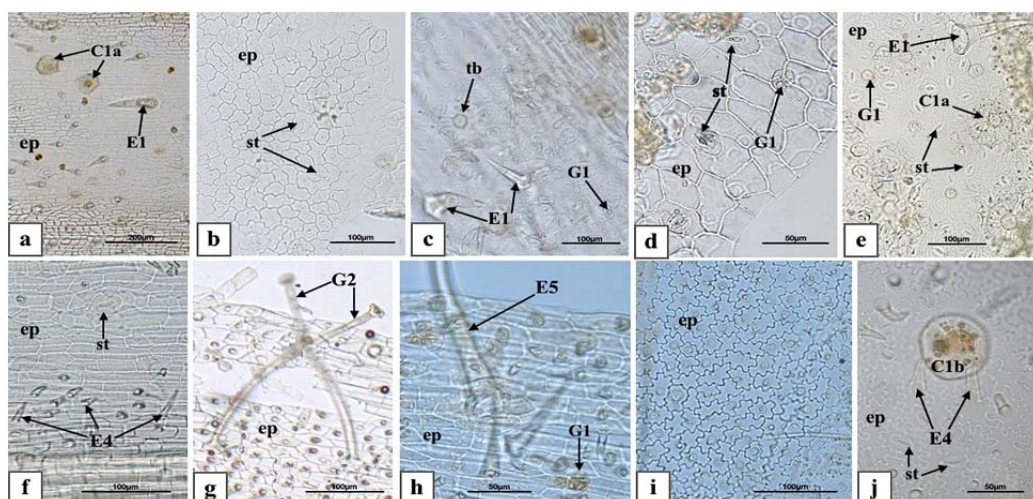


Figure 2. The epidermis of stem (a, f, g), petiole (b, c, h), upper surface of leaf (d, i), lower surface of leaf (e, j): *Nepeta cataria* L. (a-e) and *Melissa officinalis* L. (f-j). ep: epidermis, st: stomata, tb: trichome based.

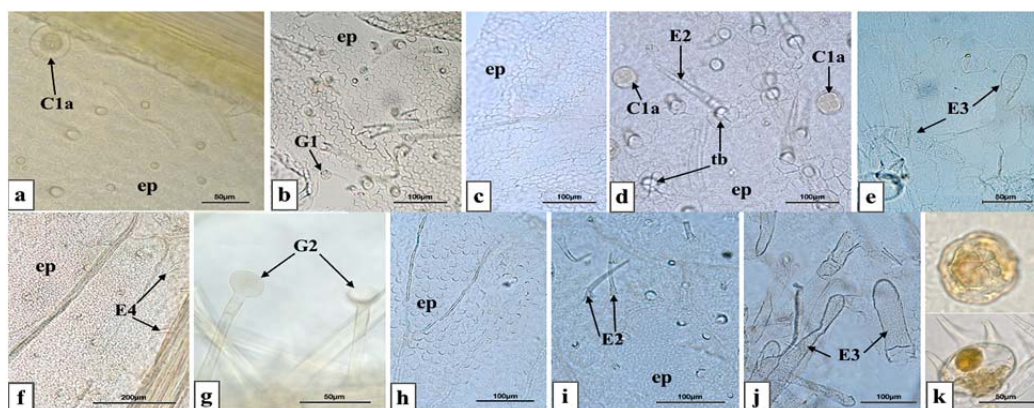


Figure 3. The epidermis of calyx (a, f), corolla tube (b), petal lobe (c, d, h, i); trichome type E3 (e, j), type G2 (g) and type C1b (k): *Nepeta cataria* L. (a-e) and *Melissa officinalis* L. (f-k). ep: epidermis, st: stomata.

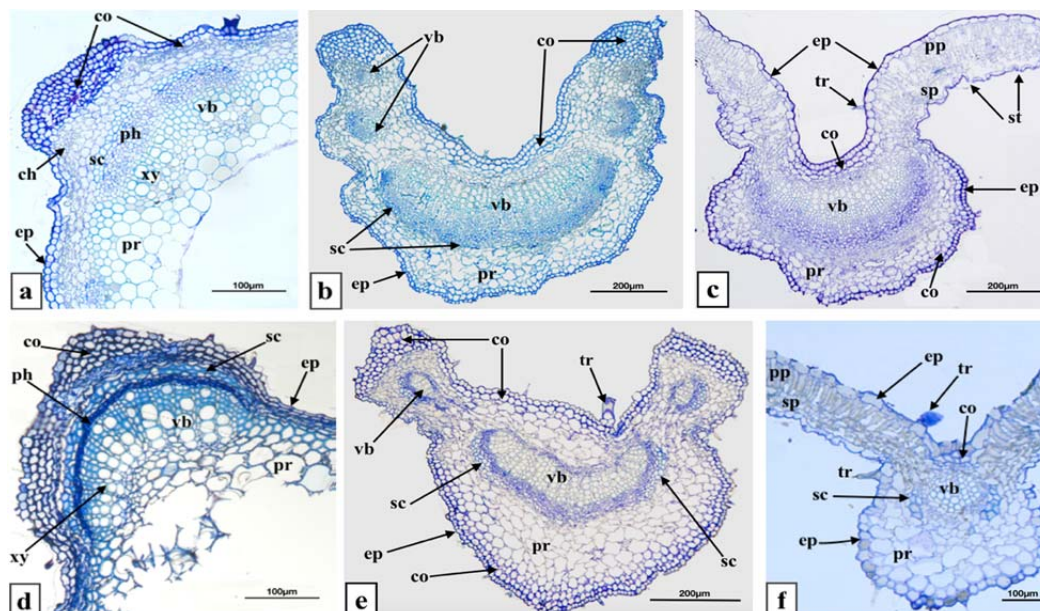


Figure 4. Cross-sections of stem (a, d), petiole (b, e) and leaf (c, f): *Nepeta cataria* L. (a-c) and *Melissa officinalis* L. (d-f). ch: chlorenchyma, co: collenchyma, ep: epidermis, ph: phloem, pp: palisade parenchyma, pr: parenchyma, sc: sclerenchyma, sp: spongy parenchyma, st: stomata, tb: trichome based, tr: trichome, vb: vascular bundle, xy: xylem.

Petiole (Fig. 4b, e).

Petiole is a shallow arc-shaped (*Nepeta cataria* L.) or arc-shaped (*Melissa officinalis* L.) with 2 wings. The epidermal cells are squarish and nearly rectangular with thin cuticle. The number of angular collenchyma layers are present in each wing and adjoining the single layer epidermal cell in the large vascular bundle region. Vascular bundle in conjoint is collateral, one large arc-shaped is present in center and smaller, circular bundles in each wing which surrounded by the large, irregular parenchyma cells. The parenchyma cell is larger in *Melissa officinalis* L. than those of *Nepeta cataria* L.. The distribution of associated sclerenchyma varies in different both the plants: sclerenchyma patches (*Nepeta cataria* L.) or layers (*Melissa officinalis* L.) adjoin to a phloem part. The phloem is slightly compressed (*Nepeta cataria* L.) or compressed (*Melissa officinalis* L.). Cambium is absent.

Leaf (Fig. 4c, f).

Leaf: dorsiventral. The epidermis consists of a single layer of uniseriate oval or rectangular cells which is covered by thin cuticles. Beneath epidermis, elongated palisade cells with chlorophyll and isodiametric spongy parenchymatic cells with intercellular spaces.

In the midrib region presents no differences with regard to petiole: leaf with a single large vascular bundle in the center presents a convexity on the abaxial side and a concavity on the adaxial side, the angular collenchyma layers are present adjoining the upper and lower epidermis. Sclerenchyma cells or layers adjoin to a phloem. The phloem is slightly compressed (*Nepeta cataria* L.) or compressed (*Melissa officinalis* L.). Cambium is absent.

Significant differences occur concerning the number and the distribution of vascular bundles, chlorenchyma, collenchyma, sclerenchyma, palisade layers of cells; intercellular spaces of spongy parenchyma and phloem properties. The main different characters are observed and shown in Table 3.

Anatomical results demonstrated the differences in the epidermis in surface view of stem, petiole, leaf, calyx, corolla and in cross-section of stem, petiole, leaf. In the epidermis in surface view were found to be anatomically different, especially in regard to epidermal cell anticlinal walls; epidermal cell of calyx and corolla; types of trichomes. In cross-section, the number and the distribution

of vascular bundles, chlorenchyma, collenchyma, sclerenchyma, palisade layers of cells; intercellular spaces of spongy parenchyma and phloem properties are distinctive anatomical features in both studied plants.

CONCLUSION

Investigations into the vegetative morphology and anatomy of two herbal drugs *Nepeta cataria* L. and *Melissa officinalis* L. was carried out. Identification key based on the characters of morphological features, anatomical features of the epidermis in surface view and cross-section is described.

In this paper, we report the results of a morphological and anatomical study of *Nepeta cataria* L. so as to improve the present knowledge for identification purposes and might be further used in the development of regulatory documents on this type of herbal medicinal raw materials.

REFERENCES

- [1] Adiguzel A. et al., Antimicrobial and antioxidant activity of the essential oil and methanol extract of *Nepeta cataria*. *Pol J Microbiol*, 2009, 58 (1), 69-76.
- [2] Barykina, R.P. et al., Spravochnik po botanicheskoi mikrotekhnike. Osnovy i metody (*Handbook for Botanic Microtechnique. Basics and Methods*), Moscow: Mosk. Gos. Univ., 2004.
- [3] Dashek W.V., *Methods in Plant Electron Microscopy and Cytochemistry*, N.Y. Humana Press, 2000.
- [4] Duda S.C. et al., Changes in major bioactive compounds with antioxidant activity of *Agastache foeniculum*, *Lavandula angustifolia*, *Melissa officinalis* and *Nepeta cataria*: Effect of harvest time and plant species, *Industrial Crops and Products*, 2015, 77, 499-507.
- [5] Paliy I.N., Fiziologicheskie osobennosti *Agastache foeniculum* Pursh. i *Nepeta cataria* var. *citriodora* Veck. v usloviyah yuzhnogo berega Kryima: dissertatsiya ... kandidata biologicheskikh nauk: 03.01.05. (Physiological characteristics of *Agastache foeniculum* Pursh. i *Nepeta cataria* var. *citriodora* Beck. in conditions of southern coast of Crimea: dissertation ... of candidate of biological Sciences). Voronezh. Gos. Univ., Yalta, 2015. 181 p.
- [6] Prescott T.A.K. et al., Direct inhibition of calcineurin by caffeoyl phenylethanoid glycosides from *Teucrium chamaedrys* and *Nepeta cataria*. *Journal of ethnopharmacology*. 2011. 137 (3), 1306-1310.
- [7] State Pharmacopoeia of the XIII edition, Moscow: Medicine, 2016. (<http://femb.ru/feml>)
- [8] Tzakou, O. et al., Essential oil composition of *Nepeta argolica* Bory et Chaub. subsp. *argolica*. *Flavour and Fragrance Journal*. 2000. 15, 115-118.