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STUDY OF *NEPETAE CATARIAE* HERBA FRUITS AS PROMISING MEDICINAL PLANT RAW MATERIAL

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Nepetae catariae herba is used in the Russian Federation as spice. The chemical compounds of herb have been studied. This plant has been introduced into the culture. There are its domestic varieties. The fruits of the plant accumulate up to 25% of fatty oil and contain specific laballenic acid, which has a wide range of antimicrobial and fungicidal actions. The yield of fruit crops is 5–6 c/ha.

The aim is to study some criteria for the standardization of the quality of *Nepetae catariae* herb as a new medicinal plant raw material.

Materials and methods. The studied fruits are from the biological collection of Federal State Budgetary Institution of All – Russia Research Institute of Medicinal and Aromatic Plants. Binocular magnifier brand MBS-10 and Axioplan 2 imaging microscope by Carl Zeiss were used. The sample preparation was carried out according to the State Pharmacopoeia of the Russian Federation (XIV edition). Anatomical diagnostic features were studied in powder and cross section with staining reagents for the presence of some biologically active substances. Qualitative reactions were carried out with water and alcohol-water extracts from the fruits. The content of the lipid complex according to pharmacopoeia monograph 2.5.0035.15 has been determined.

Results. A description of the external and diagnostic anatomical features is given. The main groups of biologically active substances and the content of the lipid complex in a possible new material – the fruits of *Nepeta cataria* – have been identified.

Conclusion. The description of the external features of the fruit has been specified. For the first time, crushed fruits have been characterized. It has been established that the morphology of endocarp cells and seed embryo cells are best preserved in a mellow fruit. Physico-optical properties of cellular structures and the ability for basic microchemical reactions are preserved in all zones of pericarp and seeds. Qualitative reactions showed the presence of the following components in the fruits: saponins, flavonoids and a lipid complex. A dispersion composition has been studied. The yield of the lipid complex and its appearance have been determined. Fruits can be used as promising fat-oil raw materials. The results of the study can be used in drafting Pharmacovigilance Reference Document considering a promising type of medicinal plant raw material on the basis of *Nepetae catariae* herba fruits.

Keywords: *Nepeta catariae*, fruits, external features, fatty oil, dispersion analysis

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ИЗУЧЕНИЕ ПЛОДОВ КОТОВНИКА КОШАЧЬЕГО (FRUCTUS NEPETAE CATARIAE) КАК ПЕРСПЕКТИВНОГО ЛЕКАРСТВЕННОГО РАСТИТЕЛЬНОГО СЫРЬЯ

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Траву котовника кошачьего (Herba *Nepetae catariae*) в РФ используют как пряность. Изучен ее химический состав. Растение введено в культуру, имеются отечественные сорта. Плоды растения накапливают до 25% жирного масла и содержат специфичную лабалленовую кислоту, обладающую широким спектром антимикробного и фунгицидного действия. Урожайность плодов 5–6 ц/га.

Цель исследования – изучение некоторых критериев для стандартизации качества плодов котовника кошачьего как перспективного лекарственного сырья.

Материалы и методы. Плоды биоколлекции ФГБНУ ВИЛАР. Использовали бинокулярную лупу марки МБС-10, микроскоп Axioplan 2 imaging Carl Zeiss. Пробоподготовка согласно ГФ РФ XIV издания. Анатомические диагностические признаки изучали в порошке и на поперечном срезе с окрашиванием реактивами на присутствие некоторых БАВ. Качественные реакции проводили с водным и спиртоводным извлечениями из плодов. Определение содержания липидного комплекса согласно ФС 2.5.0035.15.

Результаты. Дано описание внешних и диагностических анатомических признаков, определены основные группы БАВ и содержание липидного комплекса в возможном новом сырье – плоды котовника кошачьего.

Заключение. Уточнено описание внешних признаков плодов, впервые охарактеризованы измельченные плоды. Установлено, что в зрелом плоде лучше всего сохраняется морфология клеток эндокарпия и зародыша семени. Физико-оптические свойства клеточных структур и способность к основным микрохимическим реакциям сохраняются во всех зонах перикарпия и семени. Качественными реакциями показано наличие в плодах: сапонинов, флавоноидов и липидного комплекса. Изучен дисперсный состав. Определен выход липидного комплекса и его внешний вид. Плоды могут служить перспективным сырьем для получения жирного масла и препаратов на его основе. Результаты исследования могут быть использованы при составлении проекта НД на перспективный вид сырья «Fructus Nepetae catariae – Плоды котовника кошачьего».

Ключевые слова. *Nepeta cataria*, плоды, внешние признаки, липидный комплекс, микроскопия, дисперсный анализ

Список сокращений: ЛРС – лекарственное растительное сырье, НД – нормативная документация

INTRODUCTION

Nepeta cataria L. is a plant of the *Lamiaceae* family [1]. The Nepeta genus L. was referred to the Nepetoideae subfamily, the *Nepetae* tribe, by A.L. Budantsev [2]. *Nepeta cataria* occurs in the flora of the Commonwealth of Independent States [3, 4]. The plant is also registered in Central and Southern Europe up to Central Asia; North and South America [5].

The herb of *Nepeta cataria* contains essential oil, iridoids, which include nepetolactone; triterpene saponins, phenols and their derivatives, phenolcarboxylic, hydroxycinnamic acids and their derivatives, flavonoids [6–8]. *Herba Nepetae catariae* is found in teas with antipyretic and sedative effects [9].

Infusion of *Herba Nepetae catariae* reduces the temperature and decreases skin rashes with measles and chicken pox. It is considered an effective and harmless sedative remedy [10]. The essential oil and extracts from *Herba Nepetae catariae* obtained by non-polar and polar organic solvents, exhibit cytotoxic activity and affect the apoptosis of PC3, DU-145 and MCF-7 cell lines [11]. Researchers from Bulgaria showed that *Herba Nepetae catariae* extract in 70% ethanol exhibits antioxidant activity [12].

Herba *Nepetae catariae* used to be harvested from wild plants. However, the demand for essential oil and the herb for the production of spice has increased. This plant has been introduced into the culture. As a result of selection work, varieties of *Nepeta cataria* "Kentavr", "Basilio", "Goluboy ineyi", "Barkhatt" with a pronounced lemon scent are zoned in Russia [13].

According to the literature data, the fruits of *Nepeta cataria* are rich in fatty oil, the content of which reaches 25%. The characteristic feature of the fruits of the *Lamiaceae* family is the presence of fatty acids with an allenic group in them. Most often such acids are represented by labullenic acid (Fig. 1).

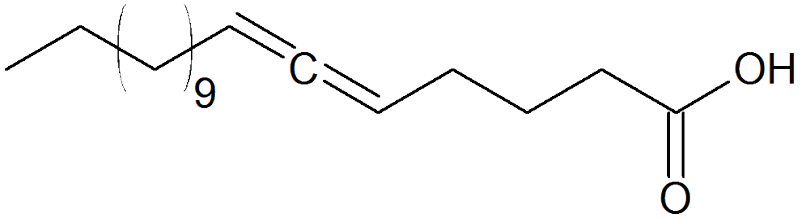

Figure 1. Labullenic acid

Allenic acids have a wide range of antimicrobial and fungicidal actions [15]. Labullenic acid is also founds in fatty oil of *Nepeta cataria* fruits [14]. The yield of *Nepeta cataria* fruit crops is 5–6 c/ha, and a standard quantity of seed per hectare in herb cultivated is 6–7 kg. [16]. The seed material is fruits (eremas). It is necessary to have a seed insurance fund when growing crops.

The fruits unused for sowing, in case of an excess of insurance material, or substandard low-germination fruits, can be recycled to obtain fatty oil. Thus, study of *Nepeta cataria* fruits as a promising kind of medicinal raw material, is relevant.

THE AIM of the work is to study some criteria for the standardization of the quality of *Nepeta cataria* fruit as a new promising type of medicinal plant raw material.

To achieve the aim of the work, the following tasks were set:

to establish diagnostic features in the morphology and anatomical structure of *Nepeta cataria* fruits;

to carry out qualitative reactions;

to determine the content of the lipid complex in *Nepeta cataria* fruits.

MATERIALS AND METHODS

Medicinal plant raw material

Nepeta cataria fruits were obtained from the plants raised in the All Russia Research Institute of Medicinal and Aromatic Plants Botanic Garden (Central district, nonchernozem belt), during the period of 2016–2018.

Microscopic study

A binocular magnifier brand MBS-10 and a Axioplan 2 imaging microscope by Carl Zeiss were use for conducting the research. The harvested fruits were prepared for the anatomical research, according to the methods described in the State Pharmacopoeia of the Russian Federation (XIV edition) and the reference book on botanical microtechnology [17–19].

Histochemical study

For carrying out an appropriate investigation of the optical properties of cell membranes and intracellular structures, the microchemical study of microscope slides was undertaken in transmitted polarized light with crossed axes of the polarizer and analyzer.

To evaluate the distribution of flobaphenes, the sections were not stained, since flobaphenes have their own brown color;

To determine the localization of lignin, the lignification reaction with a 1% solution of phloroglucinol and concentrated hydrochloric acid was carried out;

To elicit the presence of starch, the sample was processed with a solution of iodine in potassium iodide;

To identify the distribution of proteins, the xanthoproteic reaction was carried out;

To determine the localization of lipids and other non-polar substances the sample was processed with a Sudan III solution [17].

Qualitative reactions were carried out with water- and alcohol-water extracts [20]. Fruit crushing was produced using a mechanical mill. The used reagents were: 5% aqueous solution of NaOH, 2% alcohol solution of $AlCl_3$, HCl conc. plus powder Mg.

Quantitative determination

The study of the lipid complex level was carried out according to the method described in monograph 2.5.0035.15 (*"Sílybum mariánum fruits")* of the Russian State Pharmacopoeia (XIV edition). [21]. A precisely weighed quantity of the crushed medicinal plant raw material for the analysis was about 10.0 g. The extractant was removed from the extract using a vacuum rotary evaporator in the water bath at the temperature of +50° C and a residual pressure of 0.25 mm Hg.

RESULTS AND DISCUSSION

Morphological examination

A general review of the external features of the *Lamiaceae* family fruits is given in "Comparative Seeds Anatomy" [18]. The morphological features of *Nepeta cataria* fruits are listed in the directory by V. Brouwer and A. Shtelin [5]. The external features of the fruits

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have been adjusted in the article by A.L. Budantsev and T.A. Lobova: “Erem ovoid, 1.2–1.5 mm long, 0.8–1 mm wide, 0.7–0.9 mm thick”.

The abaxial side is convex, with a round edge, the adaxial side is obtuse roof-like. The hilum forms two white “eyes” in the shape of the letter V (areola) at the base of the adaxial side. The surface is slightly rough, matte. The color is from red-brown to black [19]. The fruits studied, were completely consistent with the descriptions in the mentioned above literature. The appearance of *Nepeta cataria* fruits from the adaxial side, where the areola is visible, is in fig. 2. The appearance of *Nepeta cataria* fruits from the abaxial side with the rounded edge is in Fig. 3.

The description of the fruit features, have been supplemented with characteristics of the areola. Each part of the white areola has a size of 0.25–0.30 mm, there is a dark brown gap of 0.15–0.20 mm between them.

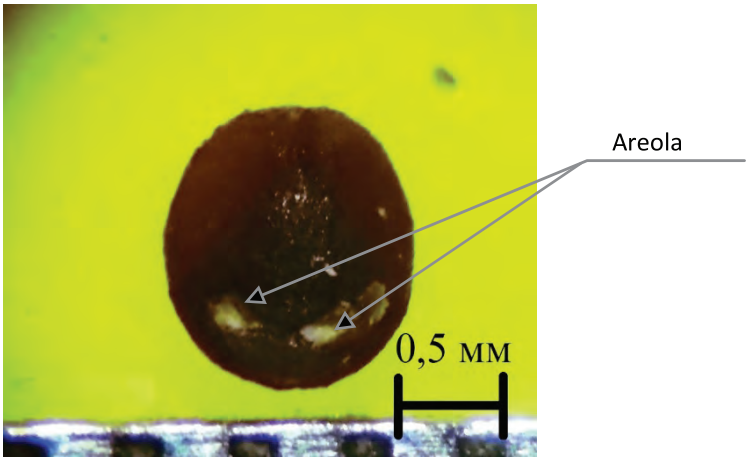

Figure 2. The adaxial side of Nepeta cataria fruit

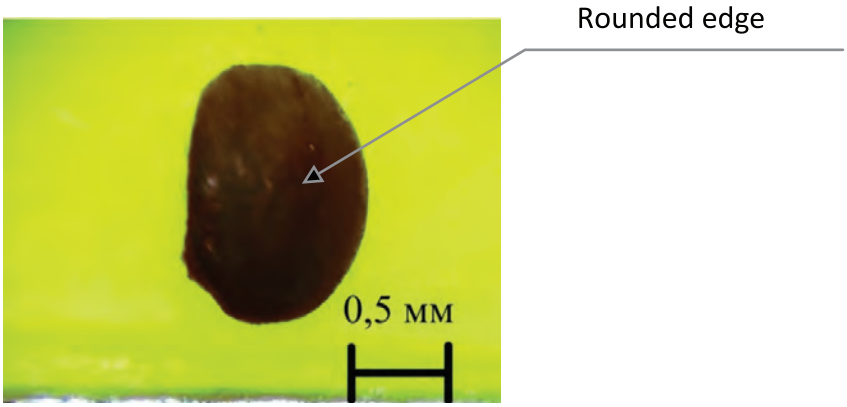

Figure 3. The abaxial side of Nepeta cataria fruit

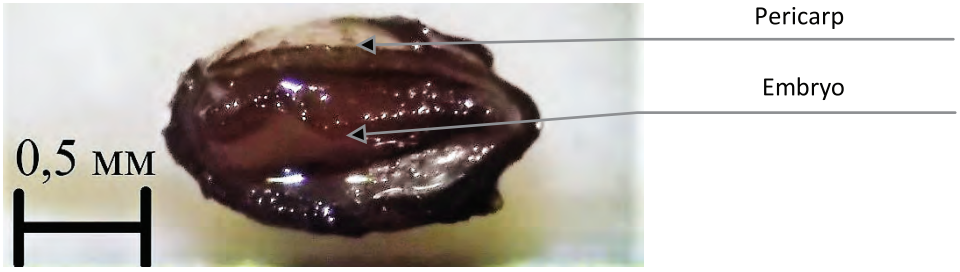

Figure 4. Lengthwise section of the erem

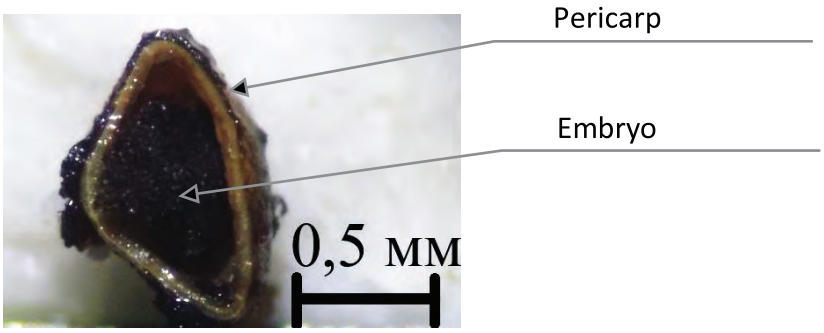

Figure 5. Cross section of the erem

The authors also suggest characterizing the shape of the cross and lengthwise sections of the fruit at half the length / width of the erem when describing the external features (Fig. 4, 5).

The cross section contour is a smoothed triangle, where the convex part corresponds to the rounded edge. The lengthwise section of the fruit is elliptical. In the middle, cotyledons of the embryo are visible. The shape of the contour of the sections can be used for diagnosis and in establishing the authenticity of the studied raw materials by the method of macroscopy.

The ultrastructure of the fruits exocarp has been studied by Romanian researchers using a scanning electron microscope. The results are presented in the work [24].

Microscopic examination. At the cross section of the fruit there is single-layer exocarp. It consists on radially flattened cells with thickened outer non-ligated walls (Fig. 6). The mesocarp is two- or three-layered with thin-walled obliterated cells, between which the boundaries are invisible. The cell shells of exo- and mesocarp are optically isotropic with a slightly alkaline reaction. The cell content is colored dark brown or black by flobaphenes.

The endocarp is two-layered. The outer layer consists of radially elongated cells with thickened walls and a small rounded cavity. Numerous pore channels are noticeable in the outer walls of the endocarp. The shells of endocarp cells are acidic, optically anisotropic, non-ligated and give a positive protein response. The cells contain a light-brown substance. The inner layer of the endocarp consists of isodiametric empty cells with unthickened, optically anisotropic shells.

An important diagnostic feature is the lack of lignin response of the outer layer membranes of the endocarp.

Spermoderma is two-layered. The outer spermoderma layer consists of obliterated cells with light brown content. The inner layer of the spermoderma consists of elongate in the periclinal direction cells with slightly thickened, optically anisotropic shells. Their reaction is slightly alkaline.

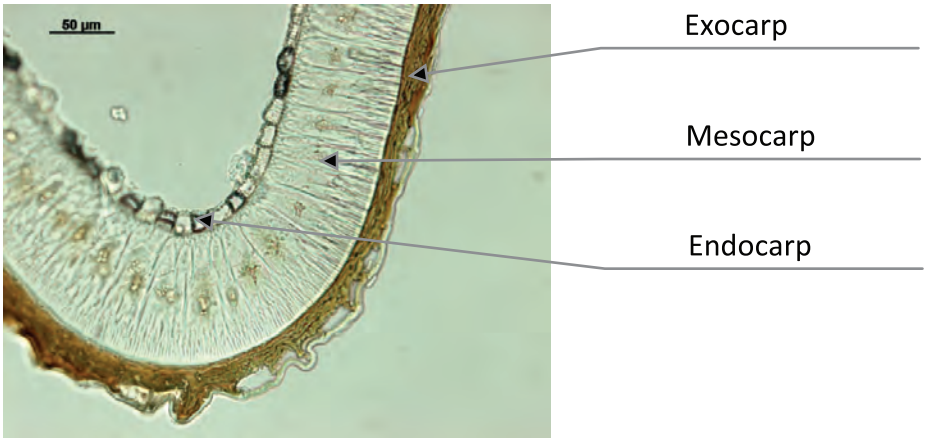

Figure 6. Fragment of the cross section of Nepeta cataria fruit

The embryo is composed of isodiametric thin-walled cells. The shells and walls of these cells are optically isotropic. There are structures with double refraction in the cell content of the embryo (Fig. 7).

The cell content gives a positive xanthoproteic reaction. The germ cells absorbed iodine. Their color became dark blue. This indicates the presence of starch. As a result of histochemical reaction with Sudan III, the vestige of the cuticle, the cell membrane of the outer layer of the mesocarp, numerous drops of fatty oil in the endosperm and the embryo turned orange.

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Figure 7. Fragment of cotyledon cross section of Nepeta cataria embryo

The fruits were crush before receiving the lipid complex. It was a mixture of pericarp fragments and cotyledons (Fig. 8).

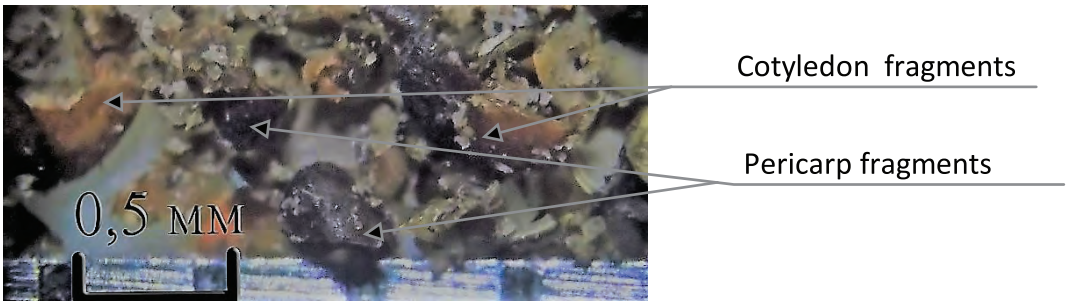

Figure 8. Crushed Nepeta cataria fruits

We noted the presence of numerous drops of fatty oil in the study of micropreparations of crushed fruits was noted (Fig. 9).

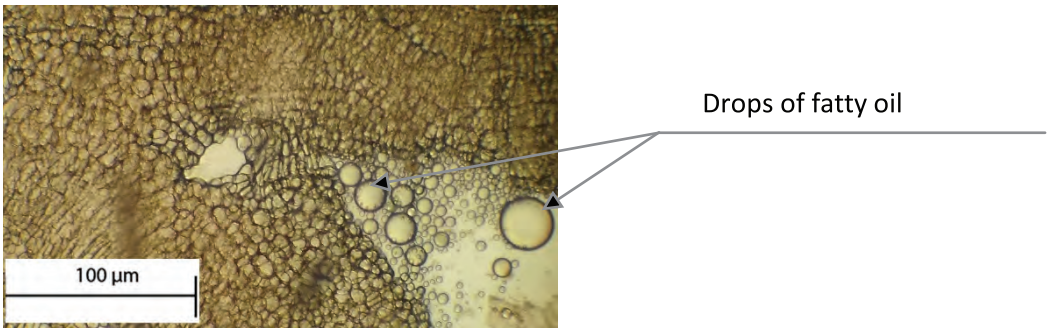

Figure 9. Fragment of cotyledon of *Nepeta cataria* embryo viewed from the surface

When adding reagent Sudan III, the drop of fatty oil turned orange (Fig. 10).

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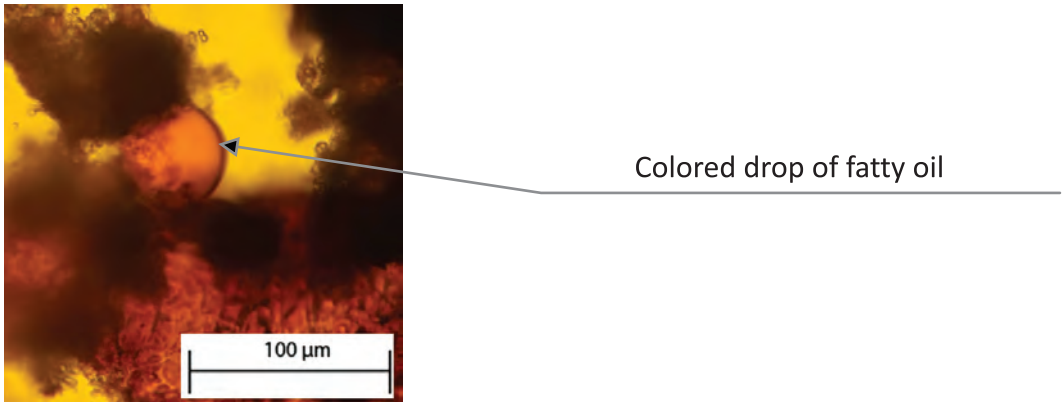

Figure 10. Fragment of Nepeta cataria fruit powder

Dispersed analysis

The dispersed analysis showed that the composition of whole fruits could be established using a set of sieves. The fruits passed through a 2 mm sieve, and the main fraction was localized on a sieve with 1 mm openings in diameter. After crushing, fruit powder was obtained, hereby, the main fraction of the particles was located on a sieve with 0.25 mm openings in diameter.

The micropreparations of the fruit powder were fragments of cotyledons and pericarp. On the exocarp fragment there are polygonal cells with evident thickenings of the walls (Fig. 11).

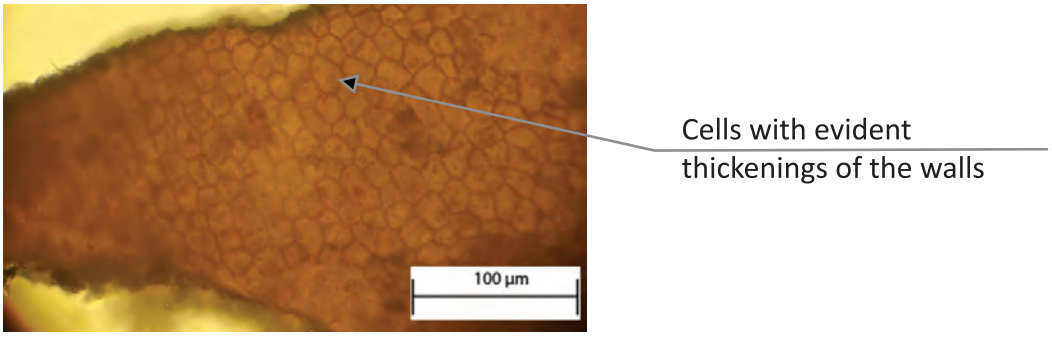

Figure 11. Fragment of Nepeta cataria fruit exocarp

Fragments of exocarp epidermis were observed the among the particles of the crushed fruits (Fig. 12). These are polygonal isodiametric cells. They do not contain pigment.

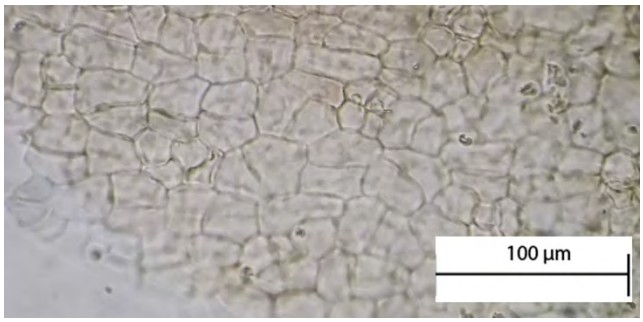

Figure 12. Fragment of exocarp epidermis of Nepeta cataria fruit

On the fruit epidermis the presence of 4-cell trichomes has been established. They are similar to the essential oil glands on the epidermis of *Nepeta cataria* leaf blade (Fig. 13) [25].

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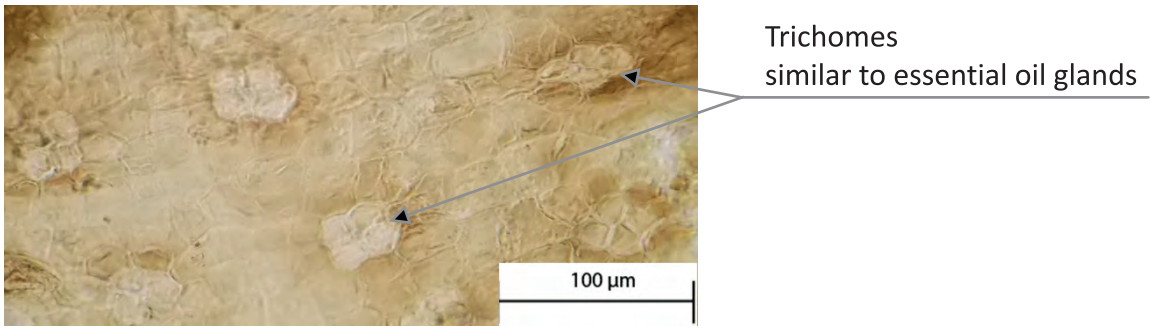

Figure 13. Fragment of exocarp epidermis of Nepeta cataria fruit with trichomes

Lipid complex was obtained from whole and crushed *Nepeta cataria* fruits. In terms of absolutely dry raw material from whole raw material, its content was 9.58%. From crushed raw material it was 26.72%. It corresponds to the literature data [14].The lipid complex looked a fluent, oily, turbid yellowish liquid.

Qualitative reactions. Qualitative reactions were conducted with the crushed fruits on the presence of some biologically active compounds. In *Nepeta cataria* fruits there are such types of biologically active substances as flavonoids and saponins. These data can be also used to establish the authenticity of *Nepeta cataria* fruits.

CONCLUSION

As a result of the investigation of whole and crushed *Nepeta cataria* fruits, a description external features and peculiarities of the anatomical structure has been given, and qualitative reactions have been carried out. It has established that morphology of endocarp and seed embryo cells are best preserved in mature fruits. Physico-optical properties of cellular structures and the ability for basic microchemical reactions are preserved in all zones of pericarp and semen. An important diagnostic feature is the lack of lignin response of the outer layer membranes of the endocarp.

The contour of the cross and lengthwise sections of the fruit has been characterized, a description of epidermis cells and trichomes on it has been given. The presence of saponins, flavonoids and lipid complex in fruits is shown by qualitative reactions. Dispersion composition has been studied. The yield of the lipid complex has been determined, characteristics of its external features have been given.

Due to the presence of laballenic acid in fatty oil of fruits and its wide antimicrobial and antifungal activity, it can be considered a valuable source of phytochemical drugs. The results of the study can be used in drafting Pharmacovigilance Reference Document considering a promising type of medicinal plant raw material on the basis of *Nepetae catariae* fruits.

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All authors had equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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