ATLANTIS PRESS

Advances in Biological Sciences Research, volume 7

1st International Symposium Innovations in Life Sciences (ISILS 2019)

Phytochemical Studies and Standardization of *Scabiosa Succisa* L.

Lyudmila Efremencko
Belgorod State National Research University
Belgorod, Russia
liudmilaefremencko@yandex.ru

Anastasiya Malyutina
Belgorod State National Research University
Belgorod, Russia
malyutina_a@bsu.edu.ru

Dmitriy Pisarev
Peoples' Friendship University of Russia
Moscow, Russia
pisarev@bsu.edu.ru

Valentina Scorbach
Belgorod State National Research University
Belgorod, Russia
scorbach@bsu.edu.ru

Nataliya Shestopalova
Belgorod State National Research University
Belgorod, Russia
shestopalova@bsu.edu.ru

Valentina Kasakova
Belgorod State National Research University
Belgorod, Russia
kazakova@bsu.edu.ru

Dariya Fadeeva
Belgorod State National Research University
Belgorod, Russia
fadeeva@bsu.edu.ru

***Abstract*—The aim of this work is phytochemical studies and standardization of *Scabiosa succisa* L. By reverse phase high performance liquid chromatography of ethanol extract substances belonging to polyphenolic compounds were found, among which hydroxycinnamic acids predominate. Chlorogenic acid and flavonoid glycosides of apigenin and luteolin were identified. Microscopic analysis revealed the main diagnostic features of the *Scabiosa succisa* L. herb.**

As a result of research component composition of *Scabiosa succisa* L. polyphenols was partially installed, which substantiates its anti-inflammatory properties, and microdiagnostics signs were discovered, which can determine the plant authenticity.

Keywords*—*Scabiosa succisa* L., *Caprifoliaceae*, *standardization, high-performance liquid chromatography, microscopy, phenolic compounds

I. INTRODUCTION

According to information provided by the world health organization, most people in the world, choosing medicines over-the-counter, prefer drugs based on phytocomponents. The growing demand for herbal medicines is also typical for the Russian pharmaceutical market, while biologically active additives (herbal teas, fees, capsules, tablets with plant extracts) and parapharmaceutical products (creams, lotions, shampoos, etc.) are also very popular. It's connected with the population's centuries-old commitment to treatment by means of natural origin, which, in addition, have a number of advantages over synthetic ones: they are non-toxic, have a minimum of side effects and can be used for a long treatment course [1 – 6]. The herbal remedies market is updated, new combinations of plant components and medicines based on new plant sources appear, and the questions of ways to confirm the medicinal plant raw materials quality remain relevant.

Quality standards of medicinal plant raw materials are described in almost all pharmacopoeias, including International one. The State Pharmacopoeia of Russian Federation normalizes such parameters as authenticity testing (raw material external signs, microscopy, determination of the main groups of biologically active substances), numerical indicators (humidity, total ash, ash insoluble in hydrochloric acid, microbiological purity), impurities, quantitative content of the main active substances. The development of standards for these parameters is especially important for the first time-considered plants, which are promising sources of biologically active substances [4,7-9].

One such plant is a member of the *Caprifoliaceae* family - *Scabiosa succisa* L. It is a perennial herbaceous plant common in Western Europe and throughout the European part of Russia, which prefers moist, slightly acidic soils of meadows, forests, thickets and forest edges. *Scabiosa succisa* L. has a long erect stem, branched at the top, glabrous at the base and pubescent to the top. Leaves are ovate-lanceolate, glabrous, with petioles, upper leaves are sessile, linear-lanceolate. Flowers have a four-lobed purple corolla. They are collected in apical hemispherical inflorescences, surrounded by involucre of a double leaf row. Fruit is an achene [10,11].

In the middle ages a property of *Scabiosa succissa* L. to heal a toothache was described by Pope John XXI in the sourcebook of traditional medicine "Thesaurus pauperum". A *Scabiosa succisa* L. decoction was used as an antidote against the poisonous snakes bites. It was also used to treat headaches and stomach cramps. In Russia, the plant leaves were applied to ulcers, on the skin for scabies and eczema and were used as a wound healing agent. In Europe folk medicine expectorant and anthelmintic properties of *Scabiosa succisa* L. are valued. Researchers conducted by modern scientists have shown that the plant also has antifungal activity comparable to nystatin [11-13].

All these properties give reason to believe that this plant may be interesting for medicine and the pharmaceutical industry as a promising source of biologically active substances. However, the available data of *Scabiosa succisa* L. chemical composition is not enough, from the literary source it is known only that it contains tannins, saponins and flavonoids, without specifying their qualitative and quantitative composition [14]. And the presence of some similar species, which are difficult to distinguish from *Scabiosa succisa* L. only by external description, makes it necessary to search for a number of microdiagnostic signs

that can confirm the authenticity of *Scabiosa succissa* L. raw material.

Therefore, the aim of this research is phytochemical study and standardization of *Scabiosa succissa* L.

II. EXPERIMENTAL

The investigation object was a dried whole *Scabiosa succissa* L. herb, harvested in 2018 in the Ivanovo region in the flowering period.

The study of the herb component composition was carried out by high-performance liquid chromatography on the *"Agilent Technologies 1200 Infinity"* device. The device has an automatic sampler *Agilent 1200*, a vacuum microcapacitor, gradient pump and thermostat of the same series. Chromatographic column is *«Ascentis express C182,7μм × 100 mm × 4.6 mm».*

Ethyl alcohol and water of HPLC qualification were used as the mobile phase, formic acid was an acid modifier.

Chromatography was performed under the following conditions:

column thermostat temperature - +35 ° C;

mobile phase speed-0.5 ml / min;

the sample volume is 1 μl.

Gradient elution conditions are given in table I.

TABLE I. CONDITIONS FOR A GRADIENT ELUTION OF SCABIOSA SUCCISA L. POLYPHENOLS

Time, min	A,%	B,%
0	90	10
10	80	20
20	70	30
30	50	50
40	10	90

The absorption spectra were recorded using a diode-matrix detector of the *Agilent 1200* series (wavelength range from 190 to 950 nm), the scanning period was 2 nm. Detection was carried out using the following wavelengths: for flavones – 336 nm, for hydroxycinnamic acids – 310, 325 nm.

The spectra and chromatograms were processed using *Agilent Chem Station* software. Identification of separated polyphenolic compounds was performed by comparing the retention times of the peaks of the test sample with the retention time of the standards. The quantitative content of substances was assessed by internal normalization of the peak area.

For the purpose of chromatography, the extraction from the dried raw material was prepared as follows. 2.5 g of *Scabiosa succisa* L. herb, crushed to a particle size of 1 mm, was filled with 70% ethyl alcohol in an amount of 50 ml, placed in a 100 ml volumetric flask and extracted in a water bath with reflux condenser for 30 minutes.

The microscopic structure of the *Scabiosa succisa* L. herb was analyzed using the methods set out in the XIV State Pharmacopoeia of Russian Federation, using a laboratory microscope "Micromed-5" with a digital nozzle [9]. Processing of microphotographs was carried out by computer programs "Adobe Photoshop CS5 Extended" and "Microsoft Office Picture Manager".

III. RESULTS AND DISCUSSION

During chromatography 21 peaks of various substances were registered, which are typical for the complex of polyphenolic compounds (fig. 1). Among them hydroxy-cinnamic acid predominate, flavonoid glycosides are found in smaller amounts. C-glycosides of luteolin and apigenin were identified by characteristic retention times, among acids – chlorogenic acid (table II).

The UV profile of chlorogenic acid is shown in fig. 2.

The carrier of important anatomical and diagnostic signs of the plant is the stem. In its microscopic analysis, attention is paid to such characteristics as the primary and secondary structure, the thickness of the collenchyma, the presence of a starch sheath, the type of vascular bundle and the nature of the conductive tissues location [15].

As a result of the *Scabiosa succisa* L. stem cross section microscopic analysis, the following microdiagnostic signs were revealed.

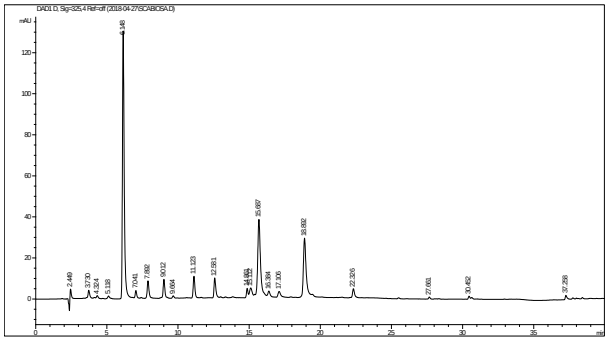

Fig. 1. Chromatogram of the separation of *Scabiosa succisa* L. polyphenolic complex (diode matrix detection, λ=325 nm).

TABLE II. CHARACTERISTICS OF SUBSTANCES ISOLATED FROM THE *SCABIOSA SUCCISA* L. HERB ALCOHOLIC EXTRACT

Substance	Retention time, min	Quantitative relation, %
Chlorogenic acid	6,146	42,17
C-glycoside of apigenin	11,123	3,26
C-glycoside of luteolin	9,012	3,10

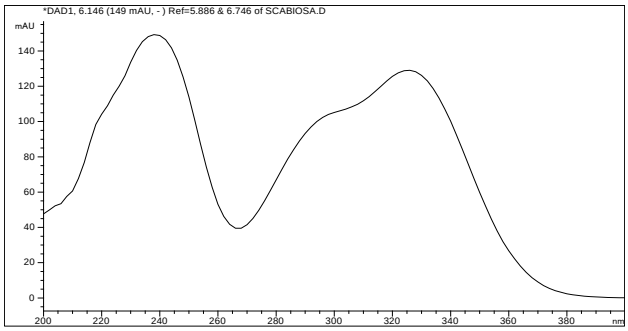

Fig. 2. The UV profile of chlorogenic acid.

The stem in the cross section is round, with protruding ribs, bundle-type structure (fig. 3). From the surface of the stem epidermis cells on the ribs are longitudinally elongated, straight-walled, sometimes narrowed at the ends or with oblique transverse walls. Stomata are usually absent. In the intercostal epidermal cells are of shorter length but greater width with sloping ends and frequent stomata. Stomatal apparatus is of stefanotenore type [16].

The difference between the epidermal cells on the ribs and on the intercostal is most pronounced in the lower and middle part of the stem and gradually fades to the top, where the cells are shaped closer to the epidermal cells of the rib (fig. 4).

The epidermis of the stem is pubescent with trichomes of three types:

- on the surface of the ribs, rarely – in the intercostal space there are rare long unicellular thick-walled trichomes with a blunt tip (fig.5);
- capitate trichomes on a unicellular stalk, expanding to the top, with a head consisting of two elongated rectangular cells (fig. 5) - are also located on the ribs;
- long unicellular trichomes with falling walls are typical for both ribs and intercostal space.

The stem is covered by the epidermis, behind which is a well-defined primary bark. It consists of a lamellar two-row collenchyma, behind which is a wide layer of chlorenchyma. The endoderm is represented by a starch sheath.

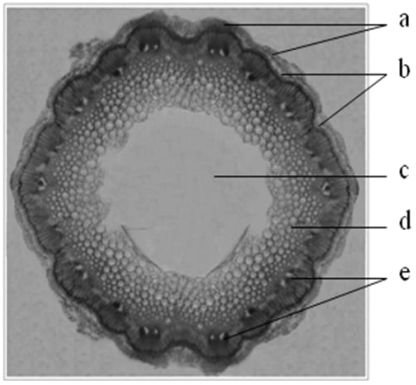

Fig. 3. The cross section of the stem (x180):
a - rib; b - intercostal space; c - aquifer cavity; d - parenchyma; e - conducting bundles

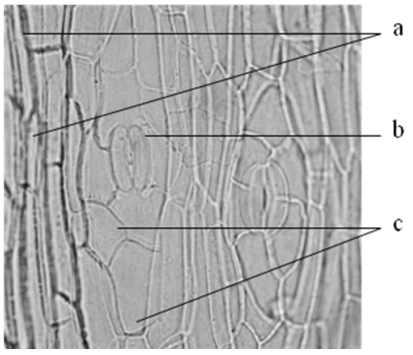

Fig. 4. A stem epidermis fragment (x720):
a - epidermis cells in the ribs; b - stomata; c - epidermis cells in the intercostal space

The central cylinder begins with a continuous broad rod of sclerenchyma, consisting of 6-15 rows of cells, adjacent to the starch sheath. The remaining part is filled with parenchyma, in which open collateral conducting bundles are arranged in one circle, doubled on protruding ribs, separated by sections of lignified inter-bundle parenchyma. The cells of the pith are partially destroyed, forming an air cavity in the center of the stem. Aquifer cavity is adjacent to each bundle from the center of the stem (fig. 6).

The next stage was the analysis of the main photosynthetic organ of the plant, which makes up the bulk of the above–ground plant part - the leaf. The leaf blade on both sides is shown in fig. 7 and 8. The epidermis cells of the *Scabiosa succissa* L. leaf are winding on both sides of the leaf blade, but not to the same extent: on the lower epidermis, the walls tortuosity is more pronounced than on the upper one. The upper epidermis cells on the root (old) leaves are closer in shape to the polygonal and have thickened walls. Both on the root and on the young apical *Scabiosa succisa* L. leaves, the cuticle folding and the porosity of the cell walls are clearly visible. Stomata are of anomocytic type, submerged, numerous, with scaphoid guard cells.

On the lower and upper epidermis, such important microdiagnostic signs as capitate trichomes with unicellular expanding to the top stalk and a 4-6-cell head also were found. On the upper side of the leaf blade of old leaves head cells are more elongated than in young ones, and at the base

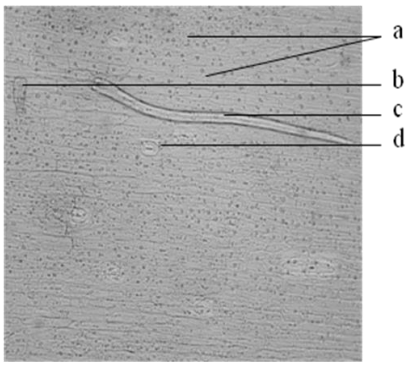

Fig. 5. A stem epidermis fragment (x180):
a - epidermis cells; b - capitate trichome with 2-cell head; c - single-celled trichome; d - stoma

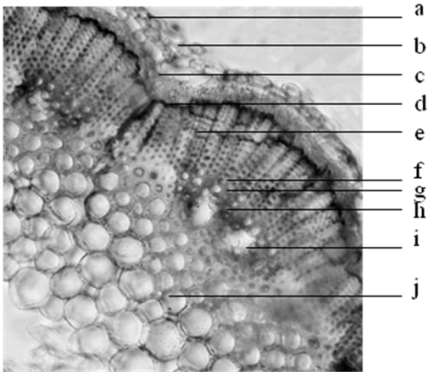

Fig. 6. Fragment of the stem cross section (x180):
a - epidermis; b - collenchyma; c - chlorenhima; d - starch sheath; e - sclerenchyma; f - phloem; g - bundle cambium; h - xylem; i - aquifer cavity; j - parenchyma of the pith

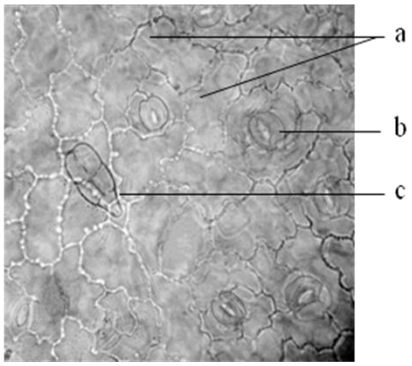

Fig. 7. Fragment of the leaf lower epidermis (x720):
a - epidermis cells; b - stomata; c - capitate trichome with a 4-cell head

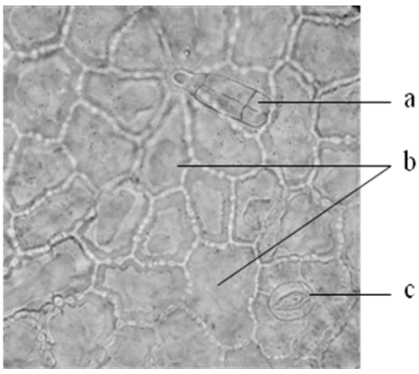

Fig. 8. Fragment of the root leaf upper epidermis (x720):
a - capitate trichome with a 4-cell head; b - epidermis cells; c - stomata

of the trichome stalk there is a ring of radially divergent epidermal cells [17, 18].

The *Scabiosa succisa* L. inflorescence is surrounded by a two-row involucre protecting many small purple flowers with a four-lobed corolla. Many related species have a similar inflorescence, such as *Knautia arvensis*, plants of the *Scabiosa*, *Cephalaria* genus. Therefore, all the components of the inflorescence deserve detailed consideration, both involucre leaves and corolla petals.

The outer involucre epidermis is polygonal on both sides, has porous walls, along the edge of the leaf involucre and along the vein cells are elongated, with sloping ends. Stomata have spheroidal guard cells, most numerous in the upper part of the leaf, stomatal apparatus is of anomocytic type. 6 types of hairs were found on the outer involucre epidermis:

- large single-celled trichomes with a pointed tip, immersed in the epidermis and located at 45° angle to the top of the involucre;
- single-celled cone-shaped trichomes with a pointed tip;
- capitate trichomes on a unicellular stalk extending to the top with a head consisting of four elongated cells;
- capitate trichomes on a unicellular stalk extending to the top with a four-eight-cell head;
- capitate trichomes on a unicellular stalk expanding to the top with a two-row head, where one row consists of two elongated cells, and the other one consists of three - five smaller cells;
- capitate trichomes on a unicellular stalk expanding to the top with a head of two cell rows forming a notch at the end, where one row consists of 2 elongated cells, and the other one consists of 3-5 cells. Microdiagnostic signs of the outer involucre are shown in fig. 9.

Epidermis cells of the inner involucre have the same structural features as the outer one: polygonal shape, which is elongated at the leaf edge and along the vein, the porosity of the walls. Stomata are found over the entire surface and have spheroidal guard cells. Stomatal apparatus is of anomocytic type. In total, 17 types of trichomes were identified on the inner involucre, among which the following are most common:

- single-celled, cone-shaped, thin-walled trichomes with a pointed tip;
- retort-shaped, single-celled, thin-walled trichomes;
- numerous large submerged unicellular trichomes with a pointed tip, which are directed at an angle to the tip of the leaf involucre;
- capitate trichomes on a unicellular stalk extending to the top with a two-row multicellular head of four - eight elongated cells;
- capitate trichomes on a unicellular stalk extending to the top with a two-row multicellular head, where one row is composed of 2-3 elongated cells, and the other one – of 3-5 smaller cells [19]. The epidermis structure of the inner involucre is shown in fig. 10.

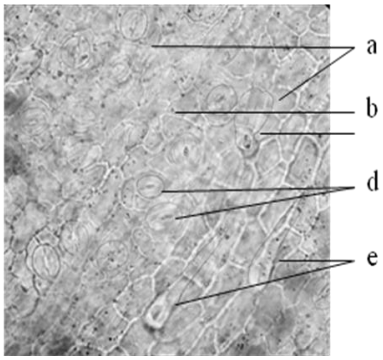

Fig. 9. Fragment of the lower epidermis of the outer involucre tip (x720):
a - epidermis cells; b - vein; c - capitate trichome with a 4-cell head; d - stomata; e - unicellular cone-shaped trichomes with a pointed tip

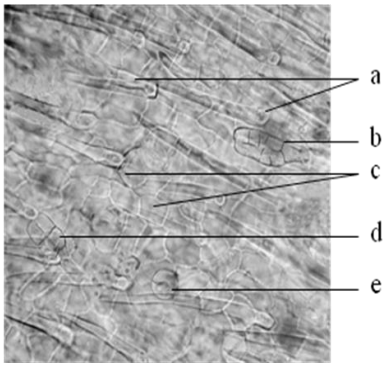

Fig. 10. Fragment of the upper epidermis of the inner involucre base (x720):
a - retort-shaped trichomes; b - capitate trichome with biseriate head; c - epidermis cells; d - capitate trichome with a 4-cell head; e - stoma

Corolla petals on the upper and lower epidermis at the tip and along the edge of the flower limb have polygonal cells with cone-shaped outgrowths. Towards the base of the flower growths gradually disappear, and the cell walls become more sinuous. Due to the smoothing of cone-shaped outgrowths, the epidermis at the petal tip looks like fish scales. Large stomata of the anomocytic type are located in the middle part and at the base of the petal and are absent at the tip and on the corolla limb. There are following types of trichomes on both epidermis sides of petals:

- simple single-celled trichomes with a pointed tip, which increase and become more sinuous towards the corolla base;
- unicellular, cone-shaped, thick-walled trichomes with a blunt tip;
- capitate trichomes on a unicellular stalk extending to the top with a four-cell head;
- capitate trichomes on a unicellular stalk extending to the top with a flattened two-row head, where each row consists of two or three cells;
- capitate trichomes on a unicellular stalk extending to the top with an elongated two-row head with eight square cells;
- capitate trichomes on a unicellular stalk extending to the top with a two-row elongated head, where one row consists of two cells, and the other one consists of two - four cells [20].

The upper and lower epidermis structure of the *Scabiosa succisa* L. petal is shown in fig. 11 and 12.

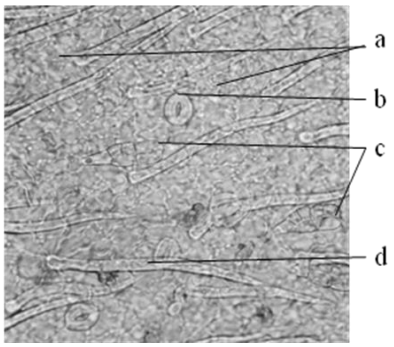

Fig. 11. Fragment of the corolla central part lower epidermis (x720): a - epidermal cells; b - stoma; c - capitate trichomes with biseriate head; d - unicellular trichomes with pointed tip

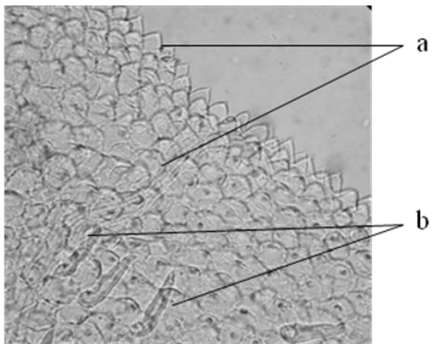

Fig. 12. Fragment of the edge of corolla limb upper epidermis (x720): a - epidermis cells with cone-shaped outgrowths; b - unicellular cone-shaped trichomes with a blunt tip

Thus, by high performance liquid chromatography it was found that herb is characterized by the presence of polyphenolic complex with a high hydroxy-cinnamic acids content, in particular chlorogenic acid (quantitative relation 42,17%). Flavonoid glycosides of apigenin and luteolin were also identified, but in smaller amounts (3.26% and 3.10%, respectively).

By microscopy the following microdiagnostics traits were revealed in *Scabiosa succisa* L. raw material:

- stem of bundle type structure has a well-defined sclerenchyma and air cavity;
- open collateral conductive bundles with adjacent aquifer cavity;
- stem stomatal apparatus of the stefanotenore type;
- cuticle folding of the epidermis;
- stomatal apparatus of leaves and inflorescences of anomocytic type;
- capitate trichomes with multicellular two-row heads on a unicellular stalk extending to the top;
- unicellular trichomes with a pointed tip;
- characteristic outgrowths of the petal epidermis, resembling "fish scales".

IV. Conclusion

The presence of polyphenolic complex in the *Scabiosa succisa* L. herb was confirmed, which includes a significant amount of hydroxycinnamic acids and flavonoid glycosides, most of which are of unidentified nature. The identified compounds are chlorogenic acid and apigenin and luteolin glycosides. A number of microscopic features have also been established, which can determine the *Scabiosa succissa* L. herb authenticity.

References

[1] S.N. Gontarev, I.S. Gontareva and A.V. Nikishaeva, “Usage of phytotherapy in pediatric dentistry”, Scientific result, № 2(8), pp. 17-21, 2016.

[2] N.F. Gusev et al., ‘Prospects for the use of medicinal plants in modern Russia”, Proceedings of the Orenburg state agrarian University, № 2, pp. 167-170, 2014.

[3] I.B. Ershova and T.F. Osipova, “General requirements for the preparation of infusions, decoctions. Dosage of herbal remedies”, Actual Infectology, № 3(12), pp. 123-127, 2016.

[4] A.N. Mironov, I.V. Sakaeva, E.I. Sakanyan, L.V. Korsun and O.A. Mochkina, “Current approaches to standardization of herbal substances”, Bulletin of Scientific Centre for Expert Evaluation of Medicinal Products, № 2, pp. 52-56, 2013.

[5] V.A. Kurkin and I.K. Petrukhina, “Actual aspects of the creation the domestic medicinal plant preparations”, № 11-2, pp. 366-371, 2014.

[6] S.Y. Pan, G. Litscher, K. Chan, Z.L. Yu, H.Q. Chen and K.M. Ko, “Traditional medicines in the world: where to go next?”, Evidence-based Complementary and Alternative Medicine, vol. 2014, pp. 1-4.

[7] O.A. Smyslova, T.M. Litvinova, N.N. Sapronova, I.U. Glazkova and D.A. Ermakov, “Study of morphological and anatomical signs of the new herbal medicine phytourol”, Journal of Pharmaceutical Sciences and Researh, vol. 10, № 4, pp. 782-785, 2018.

[8] A.P. Bogoyavlensky, P.G. Aleksyuk, A.S. Turmagambetova and V.E. Berezin, “Actual problems of standardization the herbal preparations

and substances for their production", Fundamental researches, № 6, pp. 1184-1187, 2013.

[9] State Pharmacopoeia of Russian Federation. XIV ed. T.IV. Moscow, 2018. Retrieved from: http://resource.rucml.ru/feml/pharmacopia/14_4/HTML/index.html.

[10] I.A. Gubanov et al., "Illustrated determinant of plants in Central Russia in 3 vols. Angiosperms (dicotyledons)", Moscow, 2004, 520 p.

[11] J. Kukuła and E. Witkowska-Banaszczak, "Medicinal plants of the dipsacaceae", Postępy Fitoterapii, № 4, pp. 232-238, 2014.

[12] A.L. Budantsev and E.E. Lesiovskaya, "Wild useful plants of Russia". Botanical Institute V.L. Komarova, St. Petersburg Academy of chemical and pharmaceutical sciences. SPb.: SPCPA, 2001, 663 p.

[13] A. Kowalczyk and J. Krzyzanowska, "Preliminary antifungal activity of some Dipsacaceae family plants", Herba Polonica, vol. 45, № 2, pp. 101-107, 1999.

[14] A.Yu. Malyutina, O.O. Novikov and A.V. Vasilchencko, "*Scabiosa succissa* L. - promising plant of folk medicine", Pharmaceutical Sciences: from theory to practice: conference proceedings, p. 228, 2016.

[15] Y.C. Cheryatova, "Basic anatomical and diagnostic features of medicinal plants stems", Science today: a collection of proceedings on the materials of VII international scientific-practical conference, in 4 parts, part 1, Vologda, pp. 125-126, October 28, 2015.

[16] M.A. Baranova, Principles of comparative-stomatographical study of flowering plants, 69 p., 1990.

[17] M. Lalitha Eswari, R. Vijaya Bharathi and N. Jayshree, "Pharmacognostical and physico-chemical standartization on the leaves of Ziziphus oenoplia", International Journal of Green Pharmacy, vol. 8, issue 4, pp. 267-270, 2014.

[18] L.A. Efremencko, A.Yu. Malyutina and N.N. Shestopalova, "The study of anatomical traits of the *Scabiosa succissa* L. leaf". Fundamental and applied research: topical issues, achievements and innovations: collection of proceedings of the XXII International scientific conference, Penza, pp. 258-260, 2019.

[19] L.A. Efremencko, A.Yu. Malyutina and N.N. Shestopalova, "Anatomical features of the *Scabiosa succissa* L. inflorescence involucres", European Scientific Conference: collection of proceedings of the XV International scientific conference, Penza, pp. 258-264, 2019.

[20] L.A. Efremencko, A.Yu. Malyutina and N.N. Shestopalova "Morphological and anatomical study of *Scabiosa succissa* L. flowers". Achievements of University science 2019: collection of proceedings of the IV International research competition, Penza, pp. 174-177, 2019.