*Pak. J. Bot.*, 51(1): 213-219, 2019. DOI: 10.30848/PJB2019-1(47)

# COMPARATIVE MORPHOLOGICAL AND ANATOMICAL CHARACTERISTICS OF *SAUSSUREA AMARA* (L.) DC. AND *S. SALSA* PALL. SPRENG.

**AIDOSOVA S.S.[1], AKHTAEVA N.Z.[1], TULEUOVA G.K.H.[2], AHMETOVA A.B.[1], TAZHIBAY A.M.[2], SHAUSHEKOV Z.K.[2], SHURUPOVA M.N.[3] AND ADEKENOV S.M.[2]**

*[1]Al-Farabi Kazakh National University, Almaty*
*[2]JSC International Research and Production Holding "Phytochemistry" Karaganda,*
*[3]National Research Tomsk State University, Tomsk*
*[*]Corresponding author's email: aidosova_s@mail.ru*

**Abstract**

In this paper, the authors conducted research of the comparative morphological and anatomical characteristics of two species of Saussurea, which grow in the steppes of Central Kazakhstan - *S. amara* L. (*S. bitter*) and *S. salsa* Pall. Spreng. (*S. solonchak*). Researchers revealed a number of clear distinctive morphological and anatomical signs of the over ground organs of *S. amara* and *S. salsa*, which make it possible to distinguish these species both as at the stage of collection of plants and also in the process of laboratory examination of the raw materials. The distinctive features of the anatomical stems of *S. amara* and *S. salsa* are the degree of furrows, strongly marked sclerenchyma of the conducting beams, and the size of parenchymatous cells of the primary cortex and the core. *S. amara* compared with *S. salsa* is characterized by more furrowed stems, less sclerenchyma in the conducting vascular bundles and smaller parenchymatous cells.

**Key words:** *Saussurea salsa* Pall. Spreng., *S. amara* (L.) DC, medicinal plant, morphology, anatomy, pharmacognosy.

## Introduction

Genus *Saussurea* DC. of the family *Asteraceae* includes a large number of promising species for medicine (Malyshev *et al*., 2005; Pogodin *et al.*, 2012; Zheng & Chao, 2013; Shurupova *et al.*, 2016; Avdeeva *et al.*, 2017). Many of them are widely used in Siberian, Chinese, Indian, Mongolian, Tibetan folk medicine and are characterized by a high content of biologically active substances: primarily terpenoids and flavonoids.

The genus is complex in a systematic way. According to Lipshitz (1979), it includes 350-400 species. The latest edition of the Flora of China indicates *Saussurea* as no longer a single genus, but a group of 15 genera that includes about 700 species (Shi & von Raab, 2011); however, the systematic processing of S.Yu. Lipshitz still remains relevant since there is no new monograph on this group of plants yet. The genus is one of the characteristic East Asian genera with the centers of species formation in the Himalayan mountainous country and mountain systems of China, where 264 species of *Saussurea* grow (Shi *et al*., 2011). Fifty-four species and 2 subspecies of this genus were recorded in Siberia (Shurupova & Zverev, 2017), 35 of which can possibly be observed in the Altai mountainous country in the territory of the Russian Federation (Gray, 1997; Smirnov, 2007). In Kazakhstan, there are 41 species (Filatova, 1972), 11 of which inhabit the steppe zone (Vasilieva *et al*., 1966).

This study deals with the comparative morphological and anatomical characteristics of two species of *Saussurea*, which grow in the steppes of Central Kazakhstan - *S. amara* L. and *S. salsa* Pall. Spreng. *S. amara* is the Eurasian species. The area covers the eastern regions of the European part of Russia, Central Asia, Siberia, the Far East, Mongolia and North-Eastern China (Serykh, 1997, Anon., 2013). In the aerial part of *S. amara*, cinaropicrin (Konovalova *et al*., 1979) and 7-O-glucoside apigenin (Glasl *et al*., 2007) have been identified. Tincture and extract with hemostatic, choleretic and immunomodulating properties were tested as medicinal forms (Shishkina *et al*., 1975; Khasenbekova, 2006; Glasl *et al*., 2007; Khasenbekova *et al*., 2008). *S. salsa* also belongs to the Eurasian species and grows in the Caucasus, in the European part of Russia, in Central Asia, Siberia, Iran, Mongolia and China. In the above-ground part we can find *S. salsa*, cinaropicrin, where the presence is related to the antiparasitic activity of the plant extract (Drab *et al*., 2005a, b; 2006). At the moment, based on *S. salsa*, a new antiparasitic drug Sausalin (Anon., 2016) is developed and produced by Karaganda Pharmaceutical Plant.

*S. amara* and *S. salsa* prefer similar habitats: saline meadows, solonetzes, solonchaks, river banks and salt lake banks. Often, these species grow together, which makes it difficult to differentiate them when collecting raw materials. Species are not closely related: *S. amara* belongs to the section *Theodorea* (Cass.) DC. of subgenus *Theodorea* (Cass.) Lipsch., *S. salsa* is in the section *Laguranthera* (C.A. Mey. ex Endl.) Lipsch. of the subgenus *Saussurea* (Lipschitz, 1979). However, representatives of the genus *Saussurea* are characterized by phenotypic plasticity, instability and polymorphism of vegetative and generative organs, and therefore it is necessary to use not one feature but their complex to identify. Previously, the anatomical structure of the leaf, stem and inflorescence of *S. salsa* (Lebedeva *et al*., 2014) was investigated. Nevertheless, a comparative study of the raw materials of *S. amara* and *S. salsa* have not been conducted by the researchers. The purpose of this work is to reveal the morphological features of *S. amara* and *S. salsa*, which will help distinguish them at the stage of raw material procurement, and also to describe their anatomical features that will form the basis for the development of the pharmacopial use.

## Materials and Methods

A study of the morphological features of *S. amara* and *S. salsa* we carried out using dried samples and materials of the herbarium stock of JSC International Research and Production Holding "Phytochemistry". Samples of *S. amara* and *S. salsa* for the anatomical study were collected approximately the Trudovoe settlement of the Osakarovsky district of Karaganda region in the flowering phase (Fig. 1).

Morphological and anatomical features of *S. amara* and *S. salsa* were described according to generally accepted classifications (Vekhov *et al*., 1980; Esau, 1969; Lotova, 2007; Akhmetova *et al.*, 2015; Atabayeva *et al.*, 2016). Photographs of anthodium involúcrum were obtained using a stereoscopic microscope MBS-10 (magnification 8 × 1, 8 × 2, 8 × 4).

Anatomic study of *S. amara* and *S. salsa* samples was carried out according to the method of G.G. Furst (1979). Air-dry raw material was soaked in a mixture of glycerin: water: alcohol 96% (1: 1: 1). Surface and pressure preparations and transverse sections were prepared. Fragments of the leaves were boiled in 5 % sodium hydroxide solution for 5-10 minutes, then washed with water, the epidermis was separated and examined from the surface. From the residual tissue material, the preparation was prepared by crushing the object with a scalpel on a slide in glycerine solution. Cross-sectional preparations were done using a microtome with a TOS-2 freezing device (INMEDPROM, Russia). The thickness of the sections was 10-15 mkm.

The preparations were covered with a cover slip, examined from both sides with magnifications × 180-720 using a microscope MC-300 (MICROS, Austria) with a color 21.1 pixel Canon 3D photo/video camera for microscopic and microchemical study of medicinal plant raw material (Barykina & Veselova, 2004) . Over 30 temporary preparations have been prepared.


Fig. 1. Individual plants of *Saussurea amara* (a) and *S. salsa* (b) in the natural population.


Fig. 2. Herbarium specimens of *Saussurea amara* (a) and *S. salsa* (b).

**Table 1. Comparative morphological characteristics of *S. amara* and *S. salsa*.**

| Part of the sprout | Feature | Characteristics of the aboveground organs | |
|---|---|---|---|
| | | ***S. amara*** | ***S. salsa*** |
| Stem | branching | simple or branched | simple or branched |
| | form in section | **Wingless** furrowed | **with wings** furrowed |
| | location of leaves | uniform over the entire length | uniform over the entire length |
| | height, cm | 15–60 | 15–50 |
| Leaves: basal and lower cauline; | form of leave plate | **ovoid-oblong**, oblong-lancet, **whole** | oblong-lancet, **lyre -pinnate dissected** |
| | form of base | gradually narrowed, turning into a long petiole | |
| | edge shape | sinuate-notched, unequal notch | coarse-sinuate-notched |
| | length, cm | 5–20 | 5–35 |
| | width, cm | 1,5–10 | 2–12 |
| middle and upper cauline | form of leave plate | oblong-lancet | oblong-lancet |
| | edge shape | **whole edge** | **fine-notched** |
| | epidermal formations | not thick pubescence, **shiny yellow sessile glands** | short hard hairs |
| | attachment to the stalk | **short-petioles**, usually descending | **sessile**, slightly descending |
| Inflorescence | specific | anthodium | anthodium |
| | common | dense corymba | thick corymba |
| | involúcrum pubescence | sparsely corymba pubescence | **bare** or slightly cobweb pubescent |
| | number of rows of anthodium involúcrum | **5** | **4** |
| | form of involúcrum leaves | external - **unclear-lancet**; middle and inner oblong-linear | obtuse, external-**ovoid;** internal- oblong |
| | the presence (and signs) of appendages on involúcrum leaves | **available** (on the outer - dark green, oblong-ovoid, notch; on medium-rounded, filmy, notched | **no** |
| | Character of anthodium receptáculum | thick-membrane | thick-membrane |
| | form of receptáculum membrane | unequal, linear-pricker like | unequal, linear-pricker like |

Note - Distinguished features are shown in bold

## Results and Discussion

**Morphological features of *S. amara* and *S. salsa*:** Morphological analysis of *S. amara* and *S. salsa* samples revealed clear distinctive features of the aerial shoot part of plants: stem, leaves and inflorescences (Table 1).

At the stem level, the diagnostic feature is the presence of "wings" (narrow mesophyll strips extending along the grooves of the stem down from the leaf base). A wingless stem characterizes *S. amara*, *S. salsa* has stem with wings (Fig. 2).

At the leaf level, distinctive features are the nature of the attachment of the leaf to the stem, the shape of the leaf plate and its edge, and the degree of dissection of the leaf plate. In *S. amara* basal and lower cauline leaves are whole, sometimes they are ovoid-oblong, in *S. salsa* they are lyre like the one pinnate dissected and have only oblong-lancet form. The middle and upper cauline leaves of *S. amara* have short-petioles form, always have whole edges and covered with shiny glands, in S. *salsa* - sessile and have fine notched, without glands.

At the inflorescence level, the distinctive features are the number of rows of anthodium involúcrum, the shape of the outer involúcrum leaves, and the presence of an appendage on them. *S. amara* characterized by 5-row anthodium involúcrum, on all involúcrum segments there are appendages, while the outer leaves are unclear-lancet. Anthodium of *S. salsa* has 4-row involúcrum, there are no appendages on the involúcrum leaves, while the outer leaves of the involúcrum are ovoid (Fig. 3).

**Comparative anatomical characteristics of *Saussurea amara* and *S. Salsa*:** The anatomical structure of *S. amara and S. salsa* leaves testifies to xerophytism. Leaves of both species are characterized by a strong branching of the veins, pubescence of the lower side of the leaf, small size of stomata, thickened outer walls of the epidermal cells, the presence of a thick cuticle layer, and weakly expressed intercellular spaces.

According to the type of assimilation tissue, the *S. amara* leaf is isolateral. Under the epidermis in the area of the central vein, sections of collenchyma are located. In the central vein there are 3 collateral conductive bundles of a closed type. Conductive beams on both sides are surrounded by a mechanical tissue - sclerenchyma (Figs. 4, 5).

*S. salsa* also has an isolateral leaves with an undifferentiated mesophyll. Epidermal cells on the cut are rounded, with a thickened outer wall. In the area of large veins, a collenchyma is developed on both sides of the leaf under the epidermis. The main vein contains only one collateral conductive bundle of a closed type. On both sides it is strengthened by sclerenchyma. Xylem vessels are arranged in even rows. Inside the leaf plate, small conducting beams are located on both sides of the central vein (Figs. 6, 7).

The upper epidermis of *S. amara* consists of polygonal straight-wall cells covered with a radiant-wrinkled cuticle. Cells of the lower epidermis also have a polygonal shape; the folding of the cuticle is less shown (Figs. 6 and 8). Leaf stomata is of anomocyte type, located mainly on the underside of the leaf. The covering and glandular trichomes are located, mainly, on the lower epidermis. There are two types of covering trichomes: filamentary, straight or curved, with a blunt apex, consisting of 2-6 cells; with an enlarged cell base, narrowed upward, and consisting of 2-3 cells. The glandular capitate trichomes have 1-2 cells in the base and multicellular oval heads. On the lower epidermis, there are sedentary "domed" glands (Fig. 5).

The cells of the upper and lower epidermis of the *S. salsa* leaf are polygonal with straight walls with a wavy-folded cuticle (Fig. 9). External walls of cells of leaf epidermis form trichomes of two types: covering filamentary, consisting of 3-10 cells; glandular glands, consisting of 2-3 cells in the base and a multicellular oval head. On the underside of the leaf, there are sessile glandular hairs, with oval surface and transverse septum, located in the epidermis and filled with yellowish brown contents (Fig. 9).

*S. amara* and *S. salsa* have many common features of anatomical structure of the leaf, but there are also differences - the number of conducting starts in the central vein, the type and structure of trichomes.

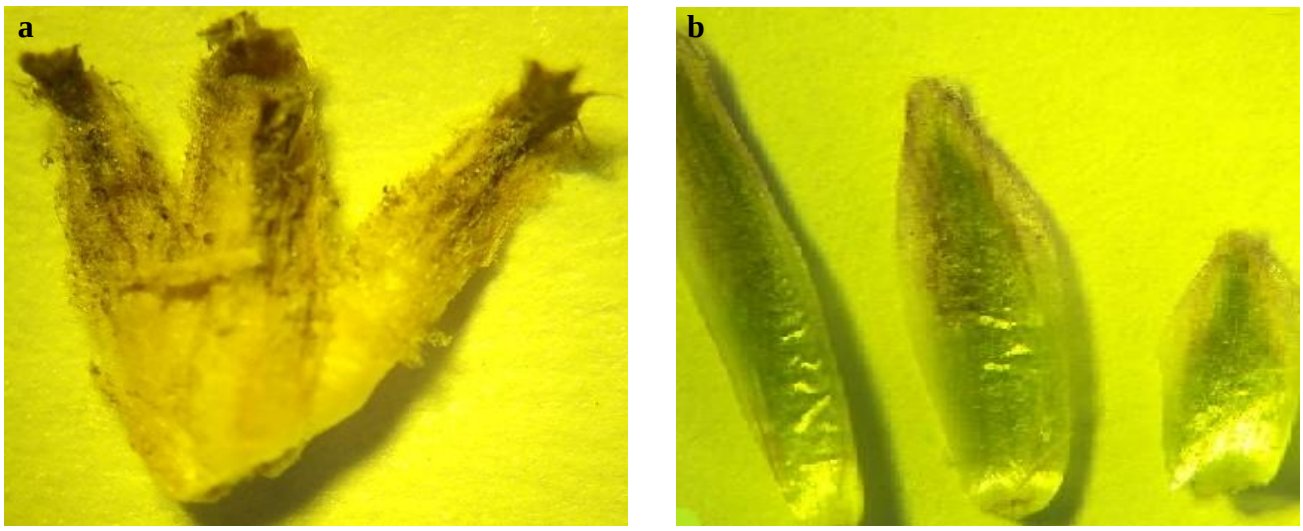


Fig. 3. Involúcrum leaves of *Saussurea amara* (a) and *S. salsa* (b).

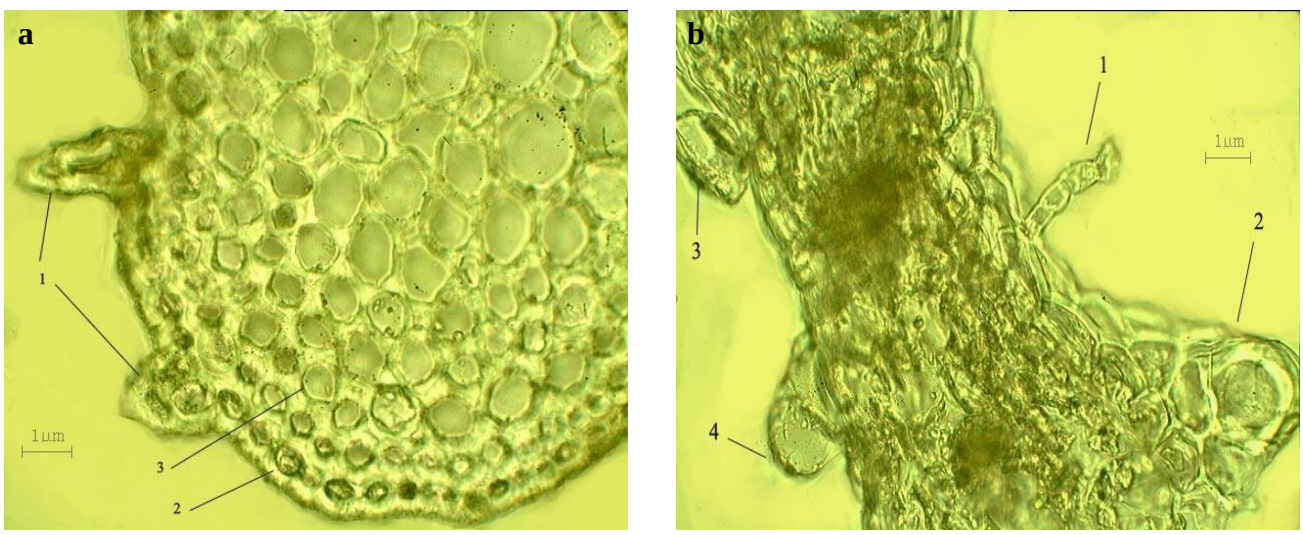


Fig. 5. Cross sections of the *Saussurea amara* leaf (× 720):
A: 1- trichomes, 2- vein epidermis, 3- collenchyma; B: 1- filamentous multicellular trichomes, 2- multicellular trichomes with an enlarged base cell, 3- "dome-like" glands, 4- "clavate" glandular hairs

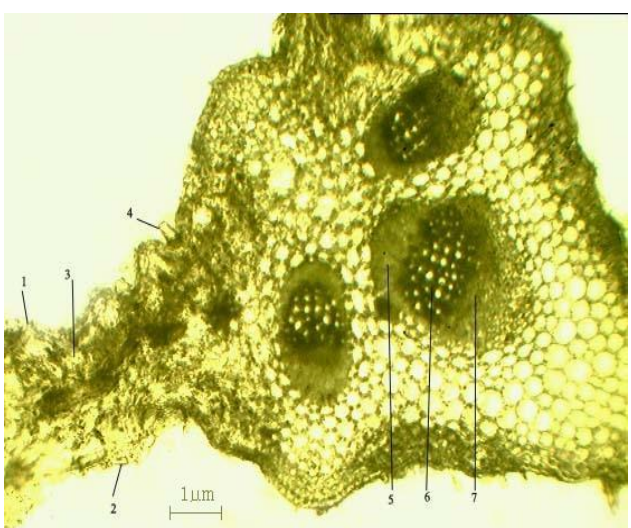


Fig. 4. Cross section of the *Saussurea amara* leaf (× 180):
1- upper epidermis, 2- lower epidermis, 3- mesophyll, 4- trichomes, 5- sclerenchyma, 6- xylem, 7- phloem

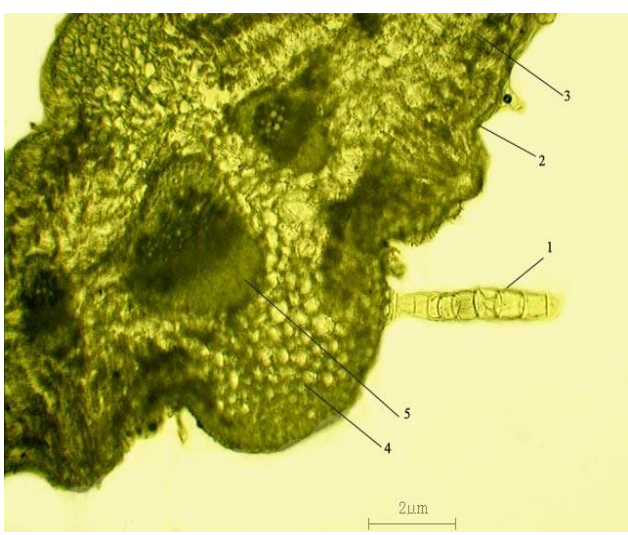


Fig. 6. Cross section of the Saussurea salsa leaf (× 180):
1- multicellular covering trichome, 2- lower epidermis, 3- mesophyll, 4-cololenchyma, 5- conductive beam

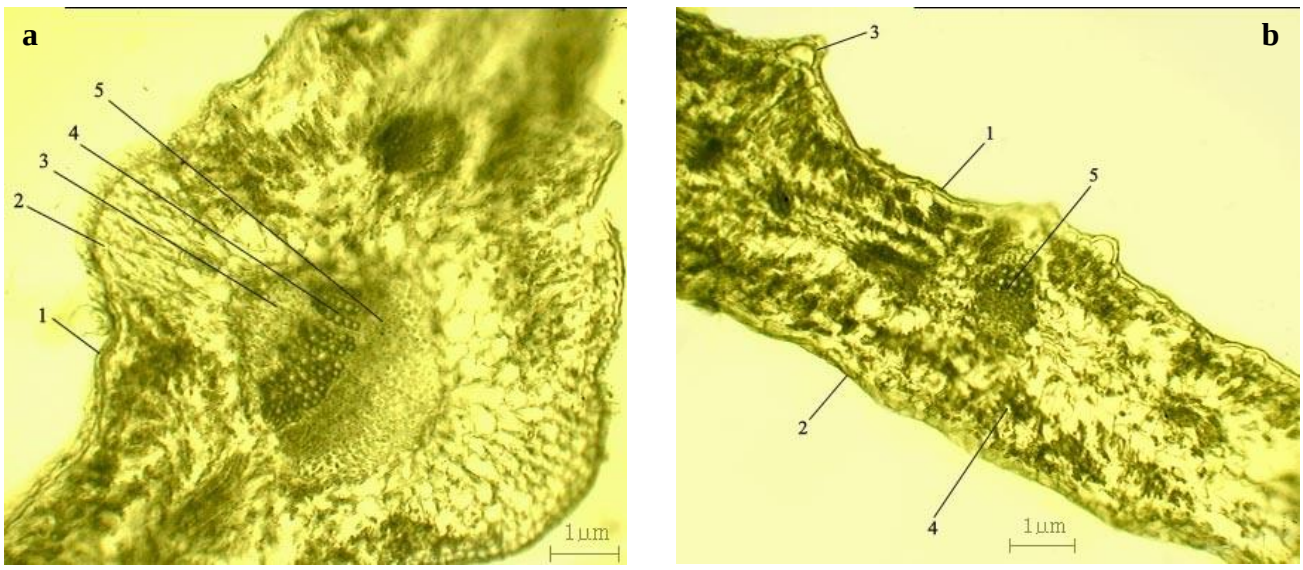


Fig. 7. Cross section of the *Saussurea salsa* leaf (× 720): A: 1- upper epidermis, 2- collenchyma, 3- sclerenchyma, 4- xylem, 5- phloem; B: 1- upper epidermis, 2- lower epidermis, 3- enlarged cell of trichome base, 5- conductive beam

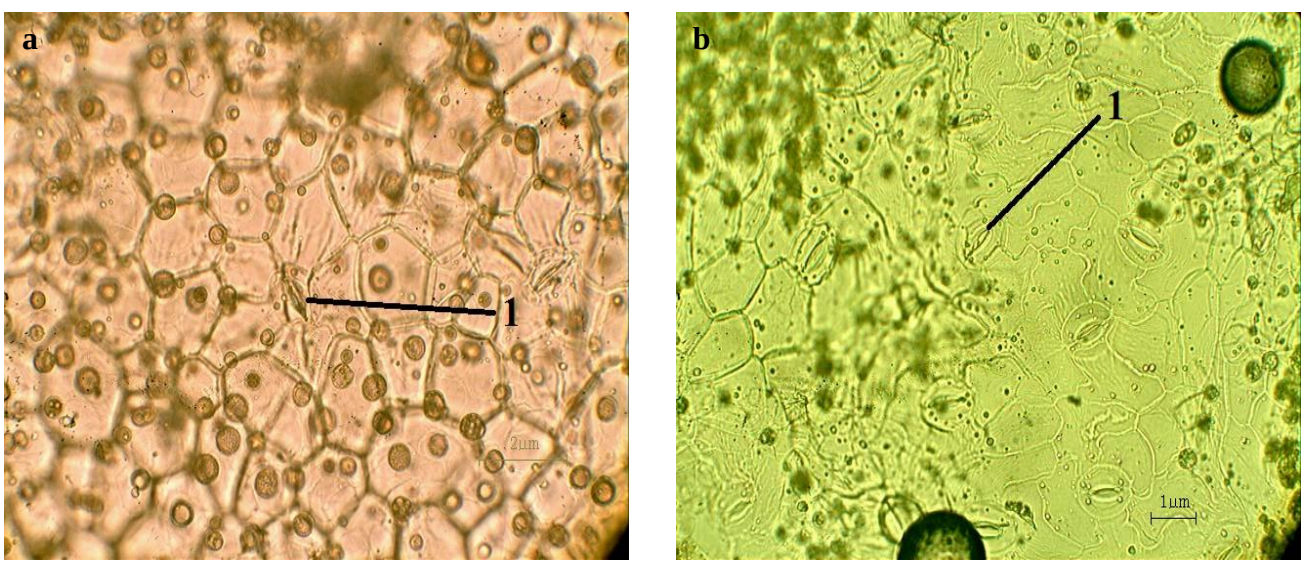


Fig. 8. Upper (A) and lower (B) leaf epidermis (× 720): *Saussurea amara* (×720): 1- stomata

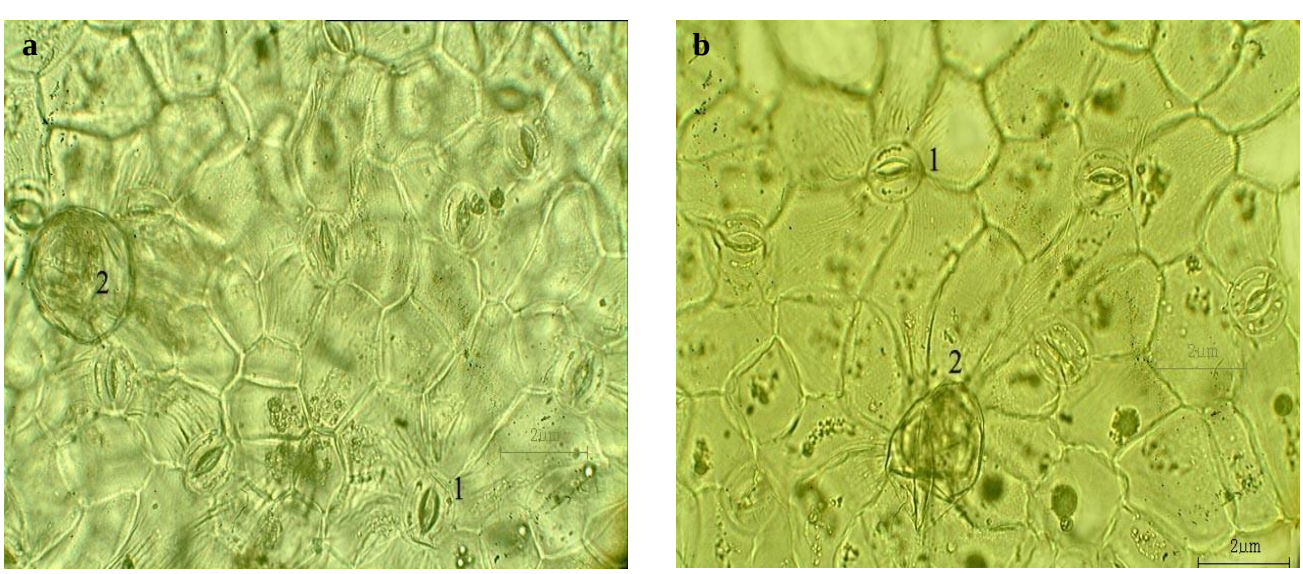


Fig. 9. Upper (A) and lower (B) epidermis of *Saussurea salsa* leaf (× 720): 1- stomata, 2- glandular trichome.

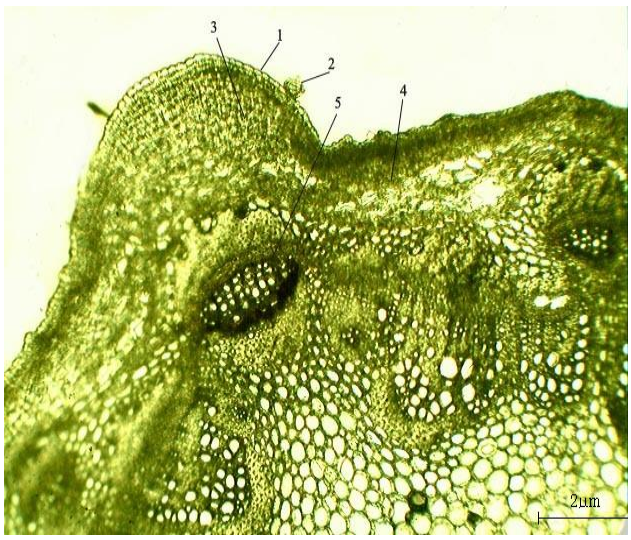


Fig. 10. Transverse section of the Saussurea amara stalk (× 180): 1- epidermis, 2- expanded cell of trichome base, 3- collenchyma, 4- chlorenchyma of the primary cortex, 5- conductive beam

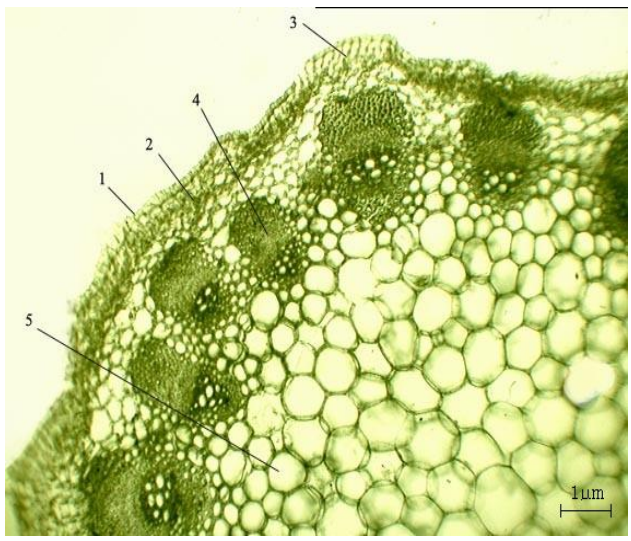


Fig. 11. Transverse section of the *S. salsa* stem (×180): 1- epidermis, 2- chlorenchyma, 3- collenchyma, 4- conductive bundle, 5- parenchyma of the core

Stem of *S. amara* in cross-section is rounded and furrowed. In the ribs above the grooves, the collenchyma is well developed (Fig. 10). The main part of the primary cortex consists of chlorenchyme. Vascular tissues are large, collateral, open, located along the periphery of the central cylinder. Conductive beams are strengthened on both sides by the sclerenchyma. The core is loose, consisting of large parenchymatous cells without intercellular spaces.

Stem of *S. salsa* in cross-section is rounded, furrowed, but the furrows are weaker than in *S. amara* (Fig. 11). Collenchyma is well developed in the ribs. Primary cortex under the epidermis is represented by a 2-3-row chlorenchyme. On the periphery of the central cylinder there are collateral conductive bundles, surrounded on both sides by a sclerenchyma. The loose core occupies most of the transverse section of the stem and consists of rounded cells without intercellular spaces.

The distinctive anatomic features of *S. amara* and *S. salsa* stalks are the degree of fissuration, the prominence of sclerenchyma of conducting bundles and the size of parenchyma cells of primary cortex and a core. More furrowed stalks characterize S. amara in comparison with *S. salsa*, a smaller presence of sclerenchyma in the conducting bundles and tinier parenchyma.

## Conclusions

We revealed a number of clear distinctive morphological and anatomical signs of the aerial parts of *S. amara* and *S. salsa*, which make it possible to distinguish these species both at the stage of collection of plants and in the process of laboratory examination of raw materials.

In the field conditions, raw material suppliers should pay attention to the following plant characteristics. A wingless stalk, whole root and lower cauline leaves, short-petioles whole-edge middle and upper stem leaves, 5-row anthodium involucre with appendages on leaflets characterize *S. amara*. *S. salsa* has a winged stem, lyre-pinnate dissected root and lower stem leaves, sessile fine-notched middle and upper stem leaves, 4-row anthodium involucres have no appendage on leaflets.

To differentiate already collected and chopped raw materials of these species in the laboratory, it is necessary to analyze the transverse sections of leaves and stems. *S. amara* is characterized by the presence of three conducting bundles in the central vein of the leaf, as well as glandular trichomes of two types - capitate and sessile "domed", which are occasionally found on the upper epidermis and in a considerable amount on the lower epidermis. The stem of this species has well-defined grooves, thin strands of sclerenchyma of conducting bundles and small cells of parenchyma of the primary cortex and central cylinder. *S. salsa* is characterized by the presence of a single conducting bundle in the central vein of the leaf and two parallel to it on both sides outside the central vein, a small number on the lower epidermis of the capitate trichomes. The stem of this species is weakly grooved, with strongly expressed sclerenchyma of conduction beams and very large parenchymal cells of the primary cortex and the core.

The obtained data make it possible to prepare pharmacopoeial articles on *S. amara* and *S. salsa* for further use of these species in official medicine.

### References

Akhmetova, A., N. Mukhitdinov and A. Ydyrys. 2015. Anatomical indicators of the leaf structure of Ferula iliensis, growing in the eastern part of Zailiyskiy Alatau (Big Boguty Mountains). *Pak. J. Bot*., 47(2): 511-515.

Anonymous. 2013. Plant resources of Russia: Wild-growing flowering plants, their component analysis and biological activity. Vol. 5. Family *Asteraceae* (*Compositae*). Part 2. Genera *Echinops-Youngia*. (Ed.): A.A. Budantsev. Association of scientific publications KMK, 312 p.

Anonymous. 2016. Eurasian patent No. 023377 dated May 31, 2016. Adekenov S.M. / Method of preparation of anti-lamblia and antiepisthorchiasis means from *Saussurea salsa* (Pall.) Spreng.

Atabayeva, S., T. Lee, A. Nurmahanova, A. Akhmetova, M. Narmuratova, M. Asrandina, A. Beisenova and R. Alybayeva. 2016. Anatomical peculiarities in wheat (*Triticum aestivum* L.) varieties under copper stress. *Pak. J. Bot*., 48(4): 1399-1405.

Avdeeva, E., Y. Reshetov, M. Shurupova, L. Zibareva, E. Borisova and M. Belousov. 2017. Chemical analysis of bioactive substances in seven Siberian *Saussurea* species. *AIP Conference Proceedings*. Vol. 1899. P. 050001–7.

Barykina, R.P. and T.A. Veselova. 2004. *Reference book on botanical microtechnology*. Moscow, PH MSU, 322 p.

Drab, A.I., E.N. Martynova, R.N. Pak, V.S. Tritek, A.T. Kuliyasov and S.M. Adekenov. 2006. Investigation of contraceptive properties of the extract from *Saussurea salsa* (Pall.) Spreng. *Chem. & Pharma. Bull.,* 40(4): 25-28.

Drab, A.I., K.A. Nurmukhametova, R.N. Pak, S.M. Adekenov. 2005b. Anti-opisthorchiasis effect of *Saussurea salsa* extract. *Chem. & Pharma. Bull.*, 39(8): 30-32.

Drab, A.I., R.N. Pak, R.B. Seidakhmetova and S.M. Adekenov. 2005a. Influence of extract from *Saussurea salsa* (*Asteraceae*) on the course of experimental acute purulent endometritis. *Rastitelnye Resursy*. 41(4): 92-98.

Filatova, N.S. 1972. Saussurea (gorkusha) *Saussurea* DC. Illustrated determinant of plants of Kazakhstan. *Science*, 2: 397-404.

Furst, G.G. 1979. Methods of anatomic, histochemical study of plant tissues. *Nauka*, 154 p.

Glasl, S., D. Tsendayush, U. Batchimeg, N. Holec, E. Wurm, C. Kletter and D. Godevac. 2007. Choleretic effects of the Mongolian medicinal plant *Saussurea amara* in the isolated perfused rat liver. *Planta Med*., 73(1): 59-66.

Khasenbekova, Z.R. 2006. Immunotropic properties of *Saussurea amara* tincture. New achievements in development of natural medicines. Proceedings of the All-Russian scientific practical conference dedicated to the 100th anniversary of Professor L.N. Bereznegovskaya birth. Tomsk, 352-357.

Konovalova, O.A., K.S. Rybalko and M.G. Pimenov. 1979. On a sesquiterpene lactone from *Saussurea amara. Chem. Nat. Comp.*, 6: 865-866.

Lebedeva, M.S., E.M. Gabdullin, S.S. Aidosova, N.A. Uly and S.M. Adekenov. 2014. Features of the anatomical structure of vegetative organs of some plant species of the genus *Saussurea* DC. *Izvestiya NAS RK, Biol. & Med. Series*, 3: 67-73.

Lipshitz, S.Y. 1979. Genus *Saussurea* DC. L . Nauka, 282 p.

Malyshev, L.I., G.A. Peshkova, K.S. Baikov, O.D. Nikiforova, N.V. Vlasova, V.M. Doronkin, V.V. Zuev, N.K. Kovtonyuk and S.V. Ovchinikova. 2005. *Synopsis of the flora of Siberia: vascular plants* Novosibirsk: Nauka, 326 p.

Pogodin, I.S., E.I. Grishina and E.A. Luksha. 2012. Morphological and anatomical study of *Saussurea amara* (L.) DC. *Med. & Edu. Siberia*, 6: 38-40.

Serykh, G.I. 1997. Genus *Saussurea* DC. *Saussurea amara*. In: *Flora of Siberia Asteraceae* (*Compositae*). Novosibirsk, Nauka, 13: 180-209.

Shi, Z. and E. von Raab. 2011. Straube *Saussurea* DC. In: *Flora of China*. Vol. 20-21. pp. 56-149.

Shishkina, E.S., Y.P. Nikitin, K.S. Sobolevskaya, K.G. Potekhin, V.G. Minayeva and V.F. Izrailson. 1975. On the study of coagulating and anti-coagulating properties of some plants of Siberian flora. *Res. Natural & Syn. Med*., 90-92.

Shurupova, M., P. Shchetinin, T. Kukushkina and A. Petruk. 2016. Content of biologically active substances in the raw materials of some Siberian *Saussurea* species. *AIP Conference Proceedings*. Vol. 172. pp. 050008-1–6.

Shurupova, M.N. and A.A. Zverev. 2017. Conservation categories and rarity types of Siberian *Saussurea* species, *Int. J. Environ. Stud.*, 74(5): 724-731.

Smirnov, S.V. 2007. A synopsis of the genus *Saussurea* DC. (Asteraceae) in the Altai mountainous country. *Turczaninowia*, 10: 3-4. Pp 5-35.

Vasilieva, A.N., A.P. Gamayunova, V.P. Goloskokov and D.C. Gorkusha. 1966. *Flora of Kazakhstan*. Vol. 9. (Ed.): N.V. Pavlov. Alma-Ata: Nauka Publishing House of the Kazakh SSR, pp. 248-281.

Vekhov, V.N., L.I. Lotova and V.R. Filin. 1980. *Practical work on anatomy and morphology of higher plants*. M., PH MSU, 196 p.

Zheng, G.U. and Z.G. Chao. 2013. *Saussurea involucrate*.: Xinyi Science and Technology Press, 213 p.

(Received for publication 15 February 2018)