Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(5); 812-819

ISSN: 0975-4873

Research Article

Macroscopic and Morpho-Anatomical Diagnostic Features of *Ziziphora bungeana* Juz. From Kazakhstan

Zhaparkulova A. Karlygash[1]*, Sakipova B. Zuriydda[1], Ternynko I. Inna[2], Raman Vijayasankar[3], Kurbatova V. Natalya[4], Ross A. Samir[3], Khan Ikhlas.[5]

[1]*Department of Pharmacy, Asfendiyarov Kazakh National Medical University, Republic of Kazakhstan.*
[2]*Department of Pharmaceutical Chemistry, Saint-Petersburg Chemical-Pharmaceutical Academy, Russian Federation.*
[3]*National Center for Natural Product Research, School of Pharmacy, University of Mississippi, USA.*
[4]*Institute of Botany and Phytointroduction, Science committee-Ministry of Education and Science of the Republic of Kazakhstan.*
[5]*Department of BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, USA.*

Available Online: 1st May, 2016

ABSTRACT

Studies have been conducted on organographic and anatomical structures of Ziziphora bungeana Juz. Diagnostic features which have been defined are as follows: aromatic plant with a specific pleasant smell. Stems are numerous, hardishly ribbed. Leaves are narrow-lanceolate or ovate. The plant has very thin hairs pointing downward. Anatomical diagnostic features include oil glands with eight radially arranged cells, glandular hairs with unicellular stalk and unicellular head, simple unicellular triangular-shaped hairs with roughly verrucose surface, and two- or three-celled thin-walled occasionally geniculate hairs. Cell walls of the leaf and stem epidermis have clearly visible thickenings. The stomatal complex is diacytic and has two phloem cells located transversely to the stomatic cleft with one of the subsidiary cells is less than the other. The stem is tetraquetrous. Under the epidermis there are several layers of the angular collenchyma arranged in four sides and consisting of the closely compressed, elongated and curved cells; two-three layers of the chlorenchyma cells are between the chlorenchyma bundles. The data obtained will be used in the future for prepation the Identification section of normative documents for medicinal plant raw materials of Ziziphora bungeana Juz.

Key words: Ziziphora bungeana Juz, macroscopy, morphological and anatomical features, glandular hairs, essential oil glands, stomata, diagnostic features.

INTRODUCTION

The Ziziphora L. genus belongs to Lamiaceae Lindl. family and includes about 30 species mainly distributed in the Mediterranean, Middle East, Central Asia, western China, Mongolia, Eastern Siberia, and in the Caucasus region. Plants of this genus are perennial sub-shrubs or annual herbs with woody and thick rootstocks and grow in meadows, on rubbly and stony slopes, coiffed sections of river banks, and mostly in mountainous areas[1,2].

Six species of Ziziphora grow in Kazakhstan of which only Z. bungeana Juz. is endemic to the domestic flora. It is mainly distributed in southern and central regions, as well as on the Tien Shan mountain ranges[3].

Chemical composition of various species of Ziziphora is well studied; phytochemistry refer it to essential oil plants. According to the literature, essential oil from various species of Ziziphora ranges from 0.7% to 1.5%. Component composition of essential oil is variable, but researchers[4-13] point out a number of dominant components – menthone, pulegone, limonene, menthol, thymol, pinene, carvacrol which are accumulated in various ratio and this characterizes the habitats and places of collected raw materials and may be accepted as a basis for the chemotaxonomic systematization of the plant. Thus, Iranian scientists[14] identified 29 components in the essential oil of Ziziphora clinopodidoides Lam. growing in the territory of Iran and the major compounds were pulegone, menthone, and thymol. In 2002, Korolyuket alias[13] studied composition of the essential oil of Ziziphora clinopodioides Lam. growing in the Altai Territory, Russian Federation, and found that its principal components were limonene (4–16%), menthone (1–11%), isomenthone (2–21%), isomenthol (0.1–9%), and pulegone (36–82%). A. D. Dembiytski et al is found at least 50 compounds in the essential oil of Ziziphora bungeana Juz. growing in the Almaty Region of Kazakhstan and identified 41 compounds. In this context it was revealed that principal components of the essential oils of Ziziphora bungeana Juz. originated from Kazakhstan were menthone (6.7%), isomenthone (28%), pulegone (55.2%), and thymol (2.5%)[10].

Ziziphora analysis also revealed glycosides15, phenolic compounds, including flavonoids16. Chinese scientists[17]

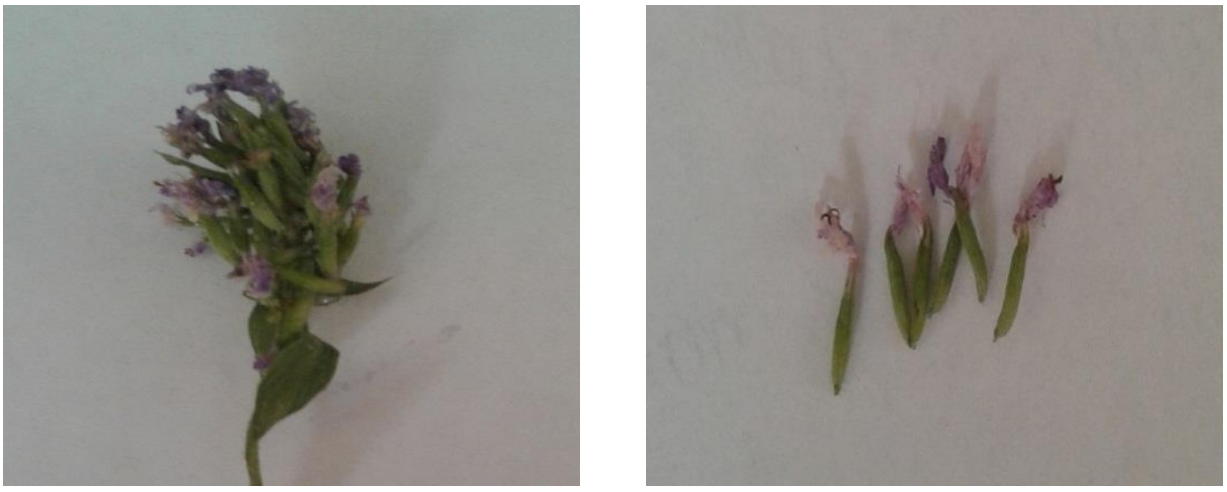
**Author for Correspondence*

A B

Figure 1: Appearance of flowering shoots (A), herbarium specimen (B) of *Ziziphora bungeana* Juz.

A B

Figure 2: Leaf of *Ziziphora bungeana* Juz.: abaxial (A) and adaxial (B) surfaces.



A B

Figure 3 Inflorescence (A) and florets (B) of *Ziziphora bungeana* Juz.

derived ursolic and oleanolic acids from Ziziphora clinopodidoides and investigated polyphenol compounds. In 1985, A.A. Bimurzayev derived previously unknown isomere of ursolic acid (bungeolic acid) from Ziziphora bungeana Juz. raw materials and its identification and

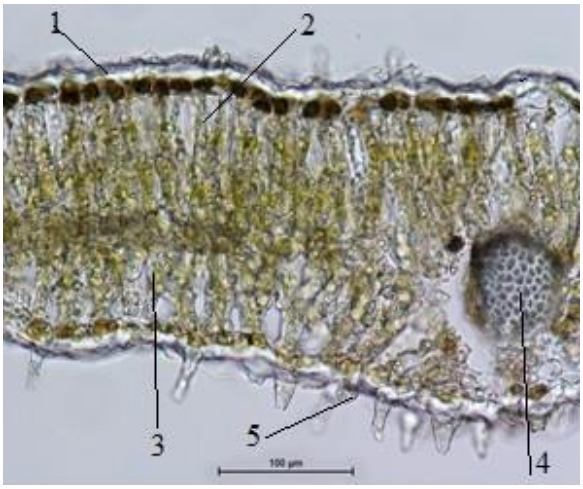
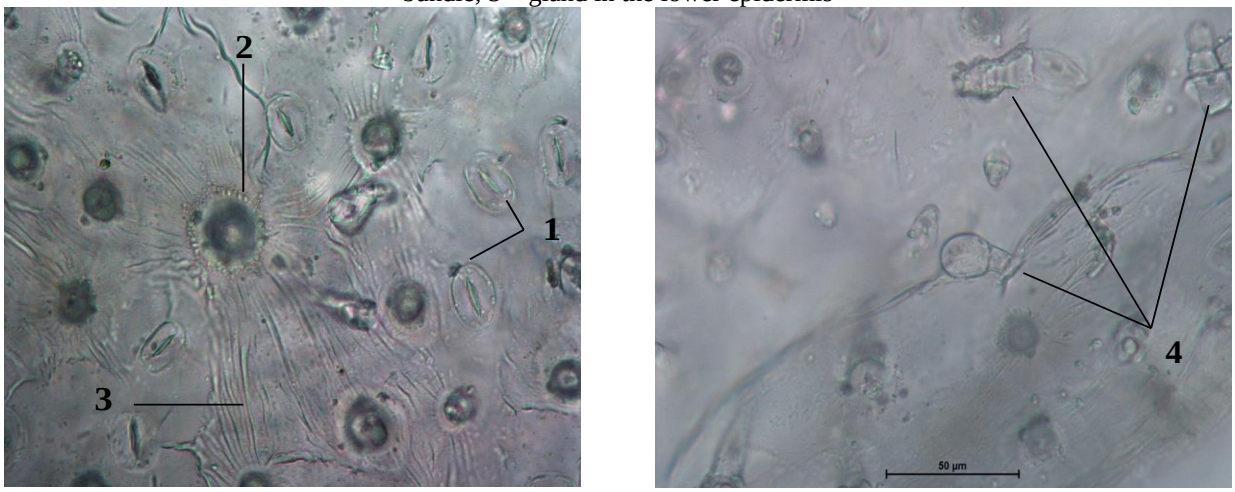


Figure 4: Cross section of the leaf of *Ziziphora bungeana* Juz.
1 – upper epidermis, 2 – columnar mesophyll, 3 – spongy mesophyll, 4 – sclerenchyma sheath of the conducting bundle, 5 – gland in the lower epidermis



A B

Figure 5: The upper epidermis of the leaf of *Ziziphora bungeana* Juz.: appearance (A) and trichomes (B) (surface preparations): 1-stomata; 2-place of hair attachment; 3-cuticle striations; 4-trichomes.

Figure 6: An essential oil gland of the *Lamiaceae* family

quantitative analysis were determined as the herb standardization parameter[4]. Various species of Ziziphora are successfully used in traditional medicine of Southeast and Central Asian countries. It is used in treating fever, neurasthenia, insomnia, congestion, as well as for the treatment of cardiovascular pathologies and hypertension[18,19]. Some species of Ziziphora are used as a demulcent, expectorant, and restorative in Iranian traditional medicine. It is recommended as a remedy against gastric, cardiac, catarrhal, and diarrhoeal diseases[20,21]. Ziziphora is used as wound healing, antiseptic, and antifoaming agent[22,23] in Turkey. Essential oil of Ziziphora is applied in food manufacturing industry to aromatize non-alcoholic beverages and in cookery. Also Ziziphora species are nectariferous[24]. Since olden times Ziziphora has been used as a medicine from gastrointestinal disorders and cardiovascular diseases in the Kazakh traditional medicine4. All Ziziphora species show antimicrobial activity, inhibit growth of Staphylococci and Streptococci[5-9,12,25]. The flavonoid fraction of Ziziphora is used as a remedy against hypertension. The pharmaceutical market has a combined medication drug of Ziziphora bungeana Juz. in the granules and capsules

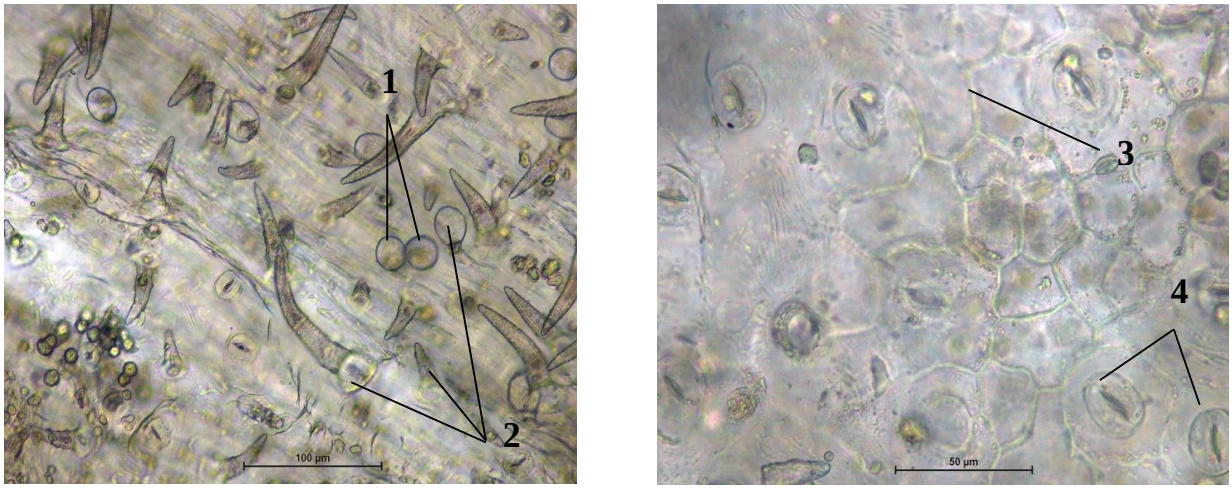


Figure 7: Lower epidermis of the leaf of *Ziziphora bungeana* Juz. (surface preparations): 1-glands; 2- trichomes of various types; 3-clearly seen thickenings of cell walls; 4-stomata.

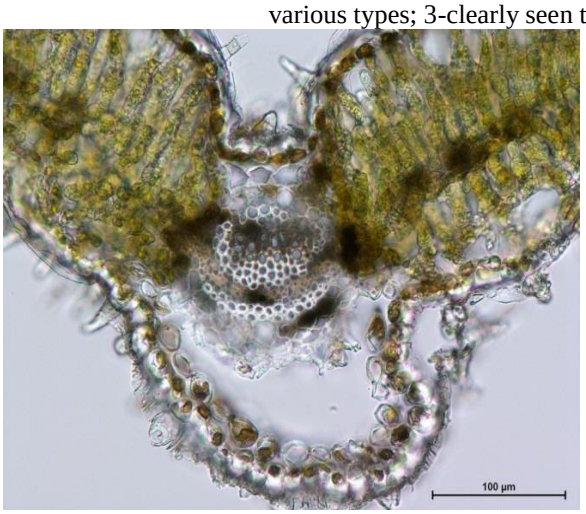


Figure 8: Midrib of vein of *Ziziphora bungeana* Juz. leaf (cross section)

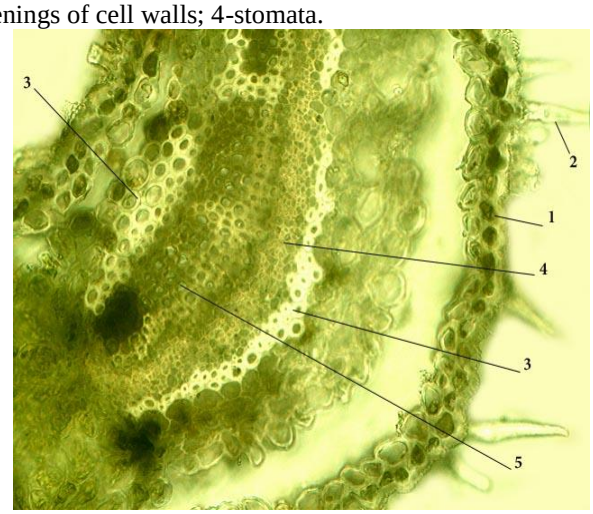
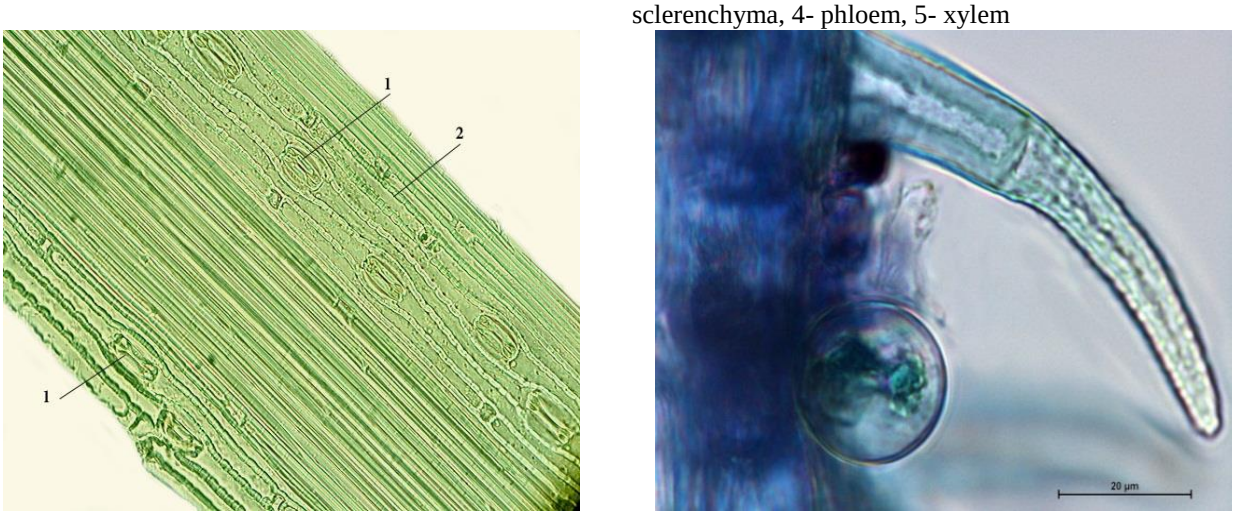


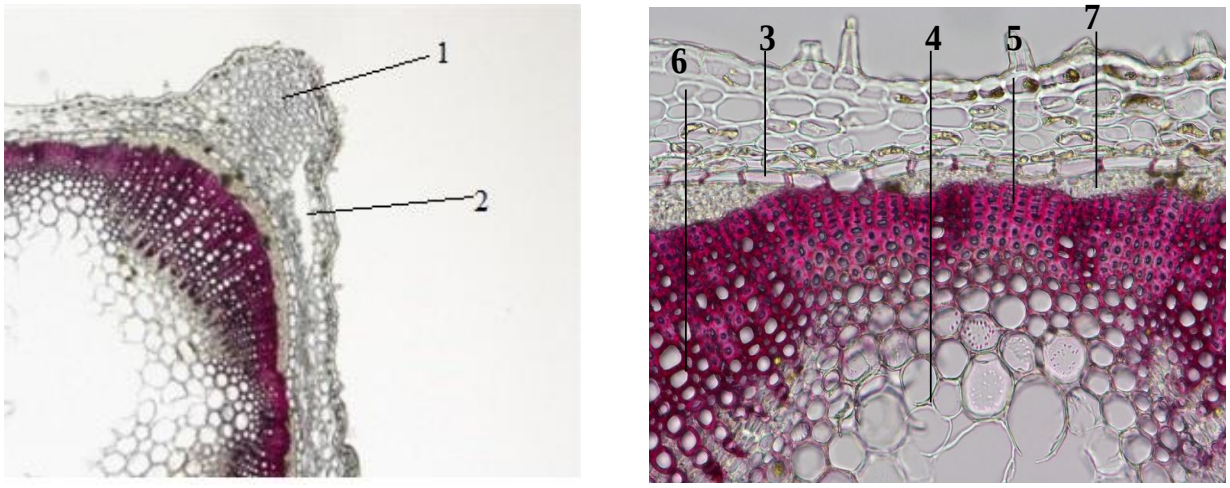
Figure 9: Crosssection of the lamina vein of *Ziziphora bungeana* Juz. (x720): 1-epidermis, 2-trichome, 3-sclerenchyma, 4- phloem, 5- xylem



A B

Figure 10: The stem epidermis of *Ziziphora bungeana* Juz. (surface preparations) (x720) A – epidermis appearance, B – a trichome and gland of the stem epidermis: 1-stomata; 2-epidermal cells with thick cuticle.

dosage form which are prescribed as a remedy against ischemic heart disease. An oral health spray was developed on the basis of the essential oil of Ziziphora clinopodidoides Lam. Essential oils obtained from Z. clinopodioides are also used for prophylaxis and for the treatment of phytophathological conditions such as sclerotinia. Ziziphora is included as a component of biologically active additives and phyto-teas[26].



A B

Figure 11: Cross section of the stem of *Ziziphora bungeana* Juz.: A- fragment of the stem part (cross section); B – fragment of conducting elements stained withphloroglucinol: 1-angular collenchyma; 2-aerenchyma; 3-endodermis; 4-pith cells; 5- phloem; 6-xylem vessels; 7 – sclerenchyma cells.

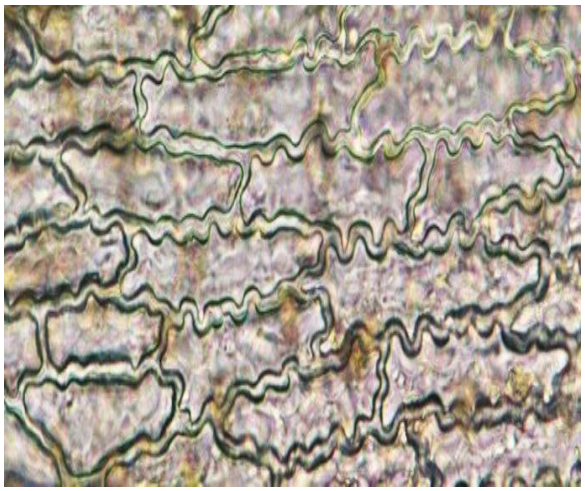


Figure 12: Epidermis cells of the calyx of *Ziziphora bungeana* Juz. (surface preparations)

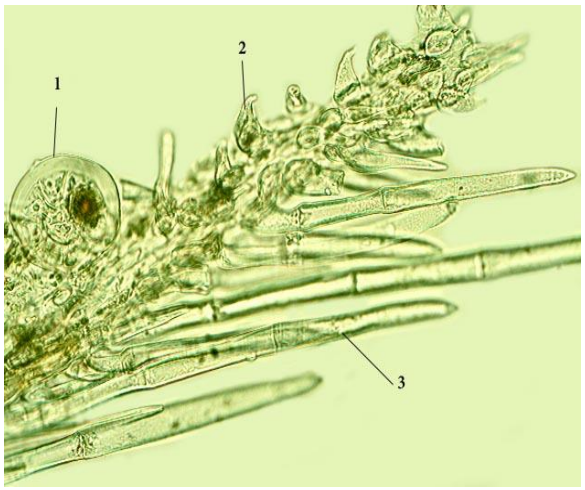


Figure 13: Calyx epidermis trichomes of *Ziziphora bungeana* Juz.: 1-essential oil gland, 2-unicellular hair, 3-simple multicellular hair

There are considerable reserves of Ziziphora in the Republic of Kazakhstan. So, according to the literature data[4], total biological reserves of various species of Ziziphora in Kazakhstan are over 100 tonnes of air-dry materials. As reported by I. I. Losev[27], approximate reserves of Ziziphora bungeana Juz. are three tonnes of air-dry materials per year. Almaty, Zhambyl, South-Kazakhstan, and East-Kazakhstan regions are appropriate areas for collection of Ziziphora materials. In the 1980s, efforts were made to introduce Ziziphora bungeana Juz. in the Shymkent Region[4].

However, regardless its considerable reserves and experience of its use in medicine, Ziziphora bungeana Juz. is not a pharmacopoeial raw material and as a consequence there are no approaches to its standardization which leads to suppression of development and pharmaceutical manufacturing application of associated herbal medicinal products.

Therefore, taking into account the represented data and the government program oriented to the domestic pharmaceutical industry stimulation in order to reduce import dependency, development of quality control criteria of Ziziphora bungeana Juz. Is a timely and relevant task.

The objectives of our research were to study diagnostic, macroscopic, morphological and anatomic features of Ziziphora bungeana Juz. to develop Introduction section of normative documents (analytic-normative documentation, monographs, etc.) for medicinal plant raw materials.

MATERIALS AND METHODS

Ziziphora bungeana Juz. was collected from the wild plants during their blossom season in May-July, 2014 in areas of Ile Alatau foothills, Almaty Region, Republic of Kazakhstan, and were air-dried in shade. Identified by Dr. Gemejiyeva N.G. (Institute of Botany and Phytointroduction, Science committee-Ministry of Education and Science of the Republic of Kazakhstan). A voucher sample (No. 01-04/257) has been deposited in the herbarium of the Institute of Botany and

Phytointroduction, Science committee-Ministry of Education and Science of the Republic of Kazakhstan.

Organographic (macroscopic) characters of Ziziphora raw materials which were softened in a moist environment were analyzed by the unaided eye and also with use of magnifying lens (×6-10). The leaves, stems, and flowers of Ziziphora were fixed in FAA and used to prepare microspecimen for anatomical studies. Surface preparations, longitudinal-radial, longitudinal-tangential, and cross sections were studied using Nikon DS-Fivlight microscope. Findings were captured by digital cameras Nikon E600 and Nikon E600 POL. Graphic images were processed with Nikon Elements software[28-30].

Samples of air-dried materials were softened by immersion in boiling water for 2 minutes and fixed in formalin - acetic acid - ethyl alcohol mixture for two days. Samples were washed thoroughly in water and kept in 30% ethyl alcohol and after that in 50% ethyl alcohol.

Prepared microspecimens were placed in solution of chloral hydrate (for clarification) and stained with 1% phloroglucinol solution in muriatic acid. When the epidermis was analyzed it was clarified by its fixation in 5% sodium hypochlorite solution.

RESULTS AND CONCLUSION

Organographic (macroscopic) characters of Ziziphora bungeana Juz. The stems are simple or slightly branched, hardish, thin, upright (curved in some places), their very thin and turned-back hairs pointing downward especially in upper parts of the stems. The stem is tetraquetrous. Lower parts of stems are woody what is typical for the Lamiáceae family (Fig.1)

The leaves are 0.5 to 1.5 cm long and 1.5 to 6 mm wide, narrow-lanceolate or ovate, tapering at ends, cuspidate at apex, entire along margins, slightly hairy on the both sides, gland-dotted, 2-3 veins on the each side which usually are raised on the undersides. Petioles are up to 4 mm long, have very thin hairs pointing downwards. Bracts are smaller than leaves; they are often linear-lanceolate, turned upward or horizontal, without ciliae margins (Fig. 2).

Flowers are small and collected into false whorls (capitate, hemispherical, loose inflorescences) at the apices of stems and branches, pedicels are short 1.5 – 3 mm. Flowers are composite, have calyxes and corollas. The calyx is short and narrow, up to 5 mm long, greyish due to thin hairs. The calyx lobes are sharp, often squarrose during blooming. The corolla is small with a barely exserted, widened upward tube, pink or lavender colored. The plant has a pleasant, aromatic smell (Fig. 3).

Microscopic characters of ZiziphorabungeanaJuz

Leaf: The lamina is dorsoventral, amphistomatic. Both sides of the lamina have the stomata showing its amphistomatic structure. The palisade tissue consists of elongated cells arranged in 3-4 rows. The columnar mesophyll is made of 2 rows of chlorophyll-containing cells. The spongy mesophyll has isodiametric cells with large intercellular spaces. The conducting bundles collateral about which the sclerenchyma sheath is arranged (Fig. 4).

Upper epidermis: Upper epidermis cells of the leaf are tabular, often with thickened walls, compactly arranged, of parenchymal form. Stomata are of diacytic type with two subsidiary cells occurring perpendicularly to the stomatic cleft (Fig. 5 A). Large oil glands consisting of eight radial cells with single cuticle (specific feature of the Lamiaceae family) often occur on the epidermis surface (Fig. 6). Epidermal cells surrounding the oil glands are radially arranged and rugose (Fig. 5 A). There are glandular trichomes with the unicellular stalk and unicellular oval head (Fig. 5 B) and simple trichomes of two types (Fig. 7):

1. Simple two- or three-celled hairs with roughly verrucose surface and thickening where cells contact. In some places hairs are geniculate (the upper cell is at an angle to others).
2. Simple unicellular hairs are triangular-shaped (papilliform), thin-walled with roughly verrucose surface.

Lower epidermis is notable for a long-range sinuation of the epidermal cells membranes and numerous stomata similar to the structure of the upper epidermis stomata of the diacytic type. Structure of the glands, glandular and simple trichomes is analogous to the upper epidermis. The trichomes are numerous (Fig. 7).

Midrib vein. The epidermis above the vein does not contain the stomata, with unicellular trichomes similar to those of the upper epidermis. The midrib vein is single-bundled, slightly convex above and elongated beneath (Fig.8). The vascular bundle is located in the median region of midrib, closed and collateral. It is distinguished by formation of the strengthening tissues represented by the sclerenchyma which surrounds the xylem and phloem. Parenchyma cells of the bundle are large and thin-walled (Fig. 9). There are two lateral veins significantly smaller than the midrib vein.

Stem: The stem epidermis consists of elongated cells sometimes with clearly visible thickenings of the cell walls. Types of stomata are diacytic and anisocytic (as compared to the lamina epidermis) (Fig. 10 A). Trichomes are uni- or bi-cellular hairs with roughly verrucose surface, similar to the lamina trichomes. There are essential oil glands (Fig. 10 B).

Under the epidermis there several layers of the collenchyma arranged in four sides and consisting of the closely compressed, elongated and curved cells; layers of the chlorenchyma cells are between four chlorenchyma bundles, 2-3 chlorenchyma layers are subepidermal. Further is a one-row layer of the endodermis cells. The cortex has aerenchyma cells of various forms. Single-layered endodermis is well defined. The vascular bundles form a complete ring. The phloem consists of thin-walled cells. The xylem lies in the internal part and is made up of large radially arranged vessels which form a complete ring with diameter 2-3 times greater than that of the phloem. The pith is spongy and consists of the large parenchyma cells. Part of the pith is destroyed in the center (Fig. 11 A). Xylem elements show thickened and lignified walls. Staining with 0.1% alcoholic solution of phloroglucinol shows evidence of excessive lignification of the conduction system elements by giving a deep pink staining. (Fig. 11B).

Calyx: Epidermal cells of the calyx are thick-walled, with sinuate anticlinal walls (Fig. 12). There are simple hairs with roughly verrucose surface that are similar in their structures to lamina hairs: unicellular, papilliform; and two-celled, straight or geniculate (Fig. 13). Trichomes are numerous on the calyx surface. There are oil glands made up of 8 cells.

CONCLUSIONS

Studies were conducted on the macroscopic and morphological and anatomical diagnostic features of Ziziphora bungeana Juz. Organographical diagnostic properties which have been defined are as follows: aromatic plant with a specific pleasant smell. Stems are numerous, hardish, ribbed, woody in their lower parts. Leaves are narrow-lanceolate or ovate. The plant has numerous very thin hairs pointing downwards. Anatomical diagnostic elements may include presence of the essential oil glands with eight secretory, radially arranged cells, glandular hairs with unicellular stalk and unicellular oval head, simple unicellular triangular-shaped hairs with roughly verrucose surface and two- or three-celled thin-walled hairs which occasionally may be geniculate. Cell-walls of the leaf and stem epidermis are covered with thick cuticles. Stomata type is diacytic. The stem is tetraquetrous and has a continuous ring of vascular tissue enclosing a wide. Conducting elements of the phloem and xylem have continuous rings. The angular collenchyma is seen in the sides.

The data obtained will be useful in preparing the Identification section of normative documents (monographs) for the widely used traditional medicinal plant Ziziphora bungeana Juz.

ACKNOWLEDGMENT

We thank Kazakhstan government for the financial support, and National Center for Natural Product Research, University of Mississippi. We would like to acknowledge the Institute of Botany and Phytointroduction, Science committee - Ministry of Education and Science of the Republic of Kazakhstan.

REFERENCES

1. Flora of Kazakhstan: Edited by Pavlov N.V., Alma-Ata V. №7: 1964. 434
2. Flora of USSR. Edited by Komarov V.L., 1954. V №21 386-404
3. Bimurzaev A.A. (1985) Phytochemical studies Ziziphora bungeanae Juz. Dissertation, Kazakh Medical University.1985. 25-28.
4. Pirbalouti AG., Arezo A, Firozeh B, Behzad H. Diversity in the chemical composition of essential oils of Ziziphora tenuioras a potential source of pulegone. Chemija. 2013; 24 (3).:234–9.
5. Verdian–Rizi M. Essential oil Compozition and Biologocal activity Ziziphora clinopodidoides Lam. from Iran. Research Jurnal of Pharmacology.2008;2 (2).: 17-19.
6. Ozturk S, Sezai E. Antibacterial activity and chemical constitutions of Ziziphora clinopodioides. Food Control.2007; 18: 535–540.
7. Aghajani Z., Assadian F., Masoudi Sh., Chalabian F., Esmaeili A., Tabatabaei- Anaraki M. et all. Chemical composition and in vitro antibacterial activities of the oil of Ziziphora clinopodioides and Z. capitata subsp. Capitata from Iran. Chemistry of Natural Compounds. 2008; 44 (3): 387-89
8. Sonbolia A, Mohammad HM, Javad H, Samad NE, Morteza Y. Antibacterial Activity and Composition of the Essential Oil of Ziziphora clinopodioides subsp. bungeana(Juz.) Rech. f. from Iran. Z. Naturforsch. 2006: 61: 677-80.
9. Shahla SN. Chemical composition and in vitro antibacterial activity of Ziziphoraclinopodioides Lam. essential oil against some pathogenic bacteria. African Journal of Microbiology Research. 2012; 6 (7): 1504-08.
10. Dembitskii AD, Bergaliev ES, Kyazimov IM. Chemical Composition of the essential oils of Ziziphora growing under various ecological conditions.Chemistry of Natural compounds.1994; 30 (6), 6: 673-75.
11. Darbandia T, Bizhan H, Mojtaba SN, Ardeshir R. Extraction of Ziziphora tenuior essential oil using supercritical CO2. European Journal of Experimental Biology. 2013; 3 (3): 687-95.
12. Salehi P, Sonboli A, Eftekhar F, Samad N, Yousefzadi M. Essential Oil Composition, Antibacterial and Antioxidant Activity of the Oil and Various Extracts of Ziziphora clinopodioides subsp. rigida(BOISS.) RECH. f. from Iran. Biol. Pharm. Bull. 2005; 28 (10):1892—96.
13. Korolyuk EA, Koenig W, Tkachev AV. The composition of the essential oil Ziziphora clinopodioides Lam of Altai region and Altai Republic (Ziziphora clinopodioides Lam.).Chemistry of plant raw materials. 2002; 1: 49–52
14. Amiri H. Composition and Antioxidant Activity of the Essential Oil and Methanolic Extract of Ziziphora clinopodioides Lam in Preflowering Stage. Journal of Kerman University of medical sciences. 2009; 16 (1): 79-86
15. Bahang YY., Liu Y, Zou G, Aisa HA. Chemical constituents of Ziziphora clinopodioides. Chemistry of Natural Compounds. 2012; 48(4): 681—82
16. Oganesvan GB, Galstyan AM, Mnatsakanyan VA, Paronikyan RV, TerZakharyan YZ. Phenolic and flavonoid compounds of Ziziphora clinopodioides. Chemistry of Natural Compounds. 1991; 27(2): 247
17. Tian S, Shi Y, Zhou X, Ge L, Upur H. Total polyphenolic (flavonoids) content and antioxidant capacity of different Ziziphora clinopodioides Lam. Extracts. Pharmacogn Mag. 2011; 7(25): 65-68.
18. Senejoux F, Demougeot C, Kerram P, Aisa HA, Berthelot A, Bévalot F, Girard-Thernier C. Bioassay-guided isolation of vasorelaxant compounds from Ziziphoraclinopodioides Lam. (Lamiaceae). Fitoterapia. 2012; 83: 377—82.

19. SenejouxF, Girard C, Kerram P, Aisa HA, Berthelot A, Bevalot Fet all. Mechanisms of vasorelaxation induced by Ziziphora clinopodioides Lam. (Lamiaceae) extract in rat thoracic aorta. Journal of Ethnopharmacology. 2010;132: 268–73.
20. Zargari A. Medicinal Plants. Tehran University Press. Tehran. 1995.;4: 103-104.
21. Naghibi F, Mossaddaegh M, Motamed SM, Ghorbani A. Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. Iranian J. Pharm. Res. 2005; 2 :63-79.
22. Beikmohammadi M. The Evaluation of Medicinal Properties of Ziziphoraclinopodioides.World Applied Sciences Journal. 2011;12 (9): 1635-38.
23. Gluchov M.M. Honey plant. Publish - The Academy of Sciences of the USSR. 1955; 82
24. Aliakbarlu J,Shameli F. In vitro antioxidant and antibacterial properties and total phenolic contents of essential oils from Thymus vulgaris, T. kotschyanus, Ziziphora tenuior and Z. clinopodioides. Turk. J. Biochem. 2013; 38 (4): 425–31.
25. Zhaparkulova KA, Sakipova ZB, editors. Analysis of the use of products from plants of the genus Ziziphora. International Scientific Conference of students and graduate students "Young Pharmacy - the potential of the future": materials of the Conference, Saint Petersburg, 2015, 20- 21 April.458-461.
26. Loseva IV. The raw material base of medicinal plants of Kazakhstan and its rational use. Book. 2008: 110 p.
27. Barykina RP. Reference botanical microtechnology. Basics and Methods. 2004: 312p.
28. Basic of microtechnology researcher in Botany: reference guide. 2000: 127 p.
29. Dashek WV. Methods in Plant Electron Microscopy and Phytochemistry. N.Y. Humana Press, 2000: 301p.