OnLine Journal of Biological Sciences

Research Notes

Morphometric Study of *Hyssopus Ambiguus* Growing in the Territory of Central Kazakhstan

[1]Yekaterina Victorovna Lakomkina, [2]Margarita Yulaevna Ishmuratova and [1]Gayane Abdulhakimovna Atazhanova

[1] Department of Pharmaceutical Disciplines and Chemistry, NC JSC "Karaganda Medical University", Karaganda, Kazakhstan
[2]Department of Botany, NS JSC "E.A. Buketov Karaganda University", Karaganda, Kazakhstan

Article history
Received: 13-10-2021
Revised: 10-01-2022
Accepted: 19-01-2022

Corresponding Author:
Yekaterina Victorovna Lakomkina
Department of Pharmaceutical Disciplines and Chemistry, NC JSC "Karaganda Medical University", Karaganda, Kazakhstan
Email: yekaterina.yankovkskaya@inbox.ru

Abstract: The article presents the results of the study of *Hyssopus ambiguus* herb, collected on the territory of Central Kazakhstan. We obtained data on diagnostic features of the studied species at morphological and anatomical levels. The purpose of the study is to determine the macro-and microscopic diagnostic features of the above-ground part of *Hyssopus ambiguus*. Materials and Methods: Raw material was collected on the territory of the Republic of Kazakhstan, Karaganda region. The microscopic and morphological studies were carried out according to the methods of the Republic of Kazakhstan State Pharmacopoeia (RKSP) using a Biomed-4 light microscope and a Levenhuk macroscope. Results: The main diagnostic features of *Hyssopus ambiguus* had been determined: Trichomas and essential oil glandules. Analysis of the anatomical and morphological features of leaves, shoots, and sepals was made; the characteristics of essential oil glandules were described. During the morphological and anatomical study of *Hyssopus ambiguus*, the diagnostically significant macro-and microscopic features of raw materials were identified and biometric characteristics were established. The experimental data allowed us to confirm the authenticity, identification, and standardization of the above-ground part of *Hyssopus ambiguus*.

Keywords: *Hyssopus Ambiguus*, Medical Plant, Macro-and Microscopic Diagnostic Features, Morphological and Anatomical Analysis

Introduction

The studies of unexplored medicinal plants aimed to identify their potential in traditional medicine are of great importance. Biologically active substances included in the compositions of medicinal plants determine their pharmacological activity. They affect more relative to the human body, the various biochemical processes occurring in it and, therefore, are better absorbing than their synthetic analogs.

In particular, it is of interest to study vegetable raw materials with antimicrobial properties as an alternative to synthetic antibacterial drugs. Several studies by scientists from different countries proved the antibacterial properties of the plants from the genus *Hyssopus.* Kukaniev *et al*. (2012) detected excellent antibacterial activity of *Hyssopus zeravshanicus* against *Bacillus cereus* bacteria, *Staphylococcus aureus*, but weak activity against gram-negative bacteria and fungi. Diego Sampietro *et al*. (2017) revealed strong antifungal activity of *Hyssopus ambiguus*, which grows in West Kazakhstan, with activity in *Fusarium* strains, due to the camphen content and other oxygen-containing monoterpenes. Moulodi *et al*. (2018) proved *Hyssopus officinalis* essential oil activity concerning *Escherichia coli*. Tomasz Baj *et al*. (2018) pointed to the difference between the antibacterial activity of *Hyssopus officinalis* essential oil obtained from plants with white flowers and with pink flowers: The first one exhibits antimicrobial activity against *Streptococcus pyogenes, S. pneumoniae, S. mutans, Candida albicans, C. parapsilosis*, whereas the second one-against such gram-positive bacteria as *Staphyllococcus aureus, S. epidermidis, Streptococcus pyogenes, S. pneumoniae, S. mutans, Bacillus subtilis*.

According to the research of Kovalenko *et al*. (2019), essential oil of *Hyssopus officinalis* obtained from a rose-color variety has antibacterial activity against *Salmonella alony*, *Clostridium sp.*, *Escherichia coli*, but essential oil obtained from Zeva's variety inhibits the growth of bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

As an object of the present study, we have chosen *Hyssopus ambiguus*, a plant, which widespread in the Karaganda region of the Republic of Kazakhstan. Essential oils, contained in *Hyssopus ambiguus*, represent perspective of the pharmaceutical industry as a source of antimicrobial and antifungal components. Previously, *Hissopus ambiguus*, growing on Kalbinsky Range (East Kazakhstan), has been studied and described by Kazakhstani scientists in several articles (Suleimen *et al*., 2015; Tursynova *et al*., 2015; Myshabaliyeva *et al*., 2016; Diego. Sampietroa *et al*., 2017; Ishmuratova *et al*., 2020). Medicinal preparations based on *Hyssopus ambiguus* do not yet exist.

According N.V. Pavlov (1964), *Hyssopus ambiguus* (Trautv.) Iljin ex Prochorov and Lebel. (family *Lamiaceae*) refers to perennial plants; 25-40 cm height, consisting of numerous four-pointed stalks, rustic at the base; leaves without omitting, with whole edges, narrow core, dorsal-ventral type; inflorescences of *Hyssopus ambiguus* are thin, length from 5 to 12 cm, with narrowing to the top; the flowers are numerous; sepals exceed the sizes of the whorls, in which there are 5-6 flowers; a sepal consists of 5 almost identical teeth and has a length of 4 to 6 mm; the length of teeth are 1/3 of the sizes of the cup; sepals are blue or violet color, from 0.8 to 1 cm long, consists of two almost equal lips: The top lip is two-blade, the lower-three-blade; 4 stamens, of which 2 are longer than the sepal and 2 are equal to its by length; the pistil column coincides in length with longer stamens; fruits are tetra-achenes, oblong ovoid shape, have 3-sided. *Hyssopus ambiguus* grows on the slopes of steppe hills, mountains, lowland, and pebbles. The species grows in East and Central Kazakhstan.

Objective: To determine macro-and microscopic diagnostic features of aboveground organs of *Hyssopus ambiguus*.

Materials and Methods

Plant Material

The vegetable raw material was collected in Karaganda region of the Republic of Kazakhstan: Karkaraly Mountains, June 2020; phase-flowering (Fig. 1). The plant material was determined by the professor of the Botany Department M.Yu. Ishmuratova and specimen deposited in the herbarium of the faculty of biology and geography. The study of macro and microscopic features was carried out according to the methods of the Republic of Kazakhstan State Pharmacopoeia (RKSP) using a Biomed-4 light microscope and a Levenhuk microscope (Musinov and Tulegenova, 2016).

Fig. 1: Hyssopus ambiguus in its flowering phase

Macroscopic Analysis

The study of macroscopic features of raw materials of *Hyssopus ambiguus* was carried out according to the methods of RKSP, Vol. I using a *Levenhuk* macroscope (Musinov and Tulegenova, 2016).

Microscopic Analysis

The study of microscopic features of raw materials was conducted according to the procedures of RKSP, Vol. 1 using a Biomed-4 microscope (Musinov and Tulegenova, 2016).

To investigate the above-ground part of *Hyssopus ambiguous*, dry plant material was softened in Straus-Fleming solution (a mixture of alcohol-glycerol-distilled water in a ratio of 1:1:1). Surface preparations and sections were prepared manually. Photos of micro preparations were made in Altami Studio and processed in Paint 10.0. To describe the anatomical structure, we relied on the works of, Vekhov and Filin (1980),; Nikitina *et al*. (2019), and Kovalev (2003).

Statistical Processing

The sample consisted of 10 measurements. The identified macro-and microscopic features of the studied raw materials were compared with existing data. Statistical data analysis was performed using the Statgraphics Centurion XVI software for Windows.

Results

Macroscopic Features

We used a Levenhuk microscope when examining the composite components of the analytical sample to identify the characteristic external features of the above-ground organs of *Hyssopus ambiguus* (Fig. 2).

The surface of the leaf on both sides is rough, and green, with numerous essential oil glands of orange-brown color. Trichomas are absent (Fig. 2A).

A leaf sheet is a narrow-lancing form, dorsal and ventral type, 10-20 mm long and 1-1.5 mm wide. The petiole is very short, 1-2 mm long. The edges of the leaf sheets are slightly bent on the lower side. The vein is

expressed weakly; the main vein on the bottom of the leaf is observed (Fig. 2B, C).

A stalk is reprehensive, along the entire length of the green, in the nodes painted in a lilac-purple color; on the cross-section 4-graded; white color. The diameter of a stem is 2-4 mm (Fig. 3A, C).

The surface of the stalk is smooth, and hardware and trichomas are not expressed (Fig. 3B).

A sepal is not clear two-lips, up to 5-8 mm long and up to 2-3 mm width; the shape is narrow-bell, and the surface is ribbed. The upper part of sepal is expanded and has short and pointed teeth. The Colour of teeth and veins is lilac-purple, and the lower part of a sepal is green-painted (Fig. 4A, B).

Longitudinal veins are noted on the surface of the sepa. Along the veins, large essential oil glandules, amber or light brown, are placed in rows. There are rare simple trichomas, white ones on the teeth of the sepal (Fig. 4C).

Microscopic Features

The upper and lower epidermis cells of *Hyssopus ambiguus* leaf, with thickened walls, 0.8 nm long and 1.0 mm wide (Fig. 5, 6). The stomata are diacytic type, 1.5-1.6 μm wide, and are located on both sides of the leaf sheet. Over the entire surface of the leaf, numerous essential-oil glandules of a rounded form with a diameter of 0.5-0.7 μm are scattered. Trichomas are simple, located along the edge of the leaf sheet, and have a length of 1-1.2 μm and a width of 0.2 μm.

The cross-section of a leaf of *Hyssopus ambiguus* is dorsal and ventral type (Fig. 7), with a clear-pronounced division of the mesophyll on the palisade and spongy tissues. The width of the leaf sheet is 3.6-5 μm, the length is 12.5-13.6 μm. Both sides of the leaf are surrounded by a single-layer epidermis, a width of 0.3-0.4 μm, whose cells are rounded rectangular shape with thickened walls. Palisade mesophyll consists of three cell layers and has a width of 1-1.5 μm; spongy mesophyll is with a thickness of 1.4-1.8 μm.

Conductive bundles are collateral type, closed, and consists of the site of xylem and phloem. The diameter of the central conductive bundle is 2-2.2 μm, and the diameter of the lateral conductive bundle is 1.2-1.3 μm. Rare simple trichomas are well observed, the length of which is 0.2-0.6 μm and the width of 0.2 μm. The essential-oil glandules are rounded in shape, are raised above the epidermis surface, and their diameter is from 0.2 to 0.5 μm.

A stalk of *Hyssopus ambiguus* on the cross-section is a four-sided, rectangular blade (Fig. 8). The perimeter of the stalk contains a single-layer epidermis with a width of 0.3-0.4 μm, with thickened outer cell walls. Rounded essential oil glandules have a diameter of 0.2-0.4 μm, and simple trichomes with a width of 0.2 to 0.4 μm and a length of 0.2-0.5 μm are detected on the epidermis of the stalk. Under the epidermis, the sites of the chlorenchima are located, 0.5-0.6 μm wide, interrupting the edges of the corner collenchymas with the thickness of 2 μm. There is core parenchyma with a thickness of 0.5-0.7 μm between the conductive zone and chlorenchima. The conductive zone is restrictive to single-layer endoderm, which width is from 0.7 to 1.5 μm. The conductive system is represented by phloem and xylem-oriented ring. The rows of the xylem of 2-3.5 μm width are well expressed. In the center of the stalk is the pith parenchyma representing the lying thin-walled cells, the diameter of the pith is from 9.5 to 12 μm.

The main cell epidermis cells have a prosenchymal shape, the walls are slightly winding, 0.3 x 1 μm (Fig. 9). The entire surface of the cup is covered with numerous essential oil glandules of a rounded shape, a diameter of glandular is 0.5-1 μm. Through the surface of the cup, veins with a thickness of 0.6 to 1.1 μm are running. Trichomas are simple, long 0.9-1.5 μm and a width of 0.2 μm, and are mainly located along the edge of the teeth.

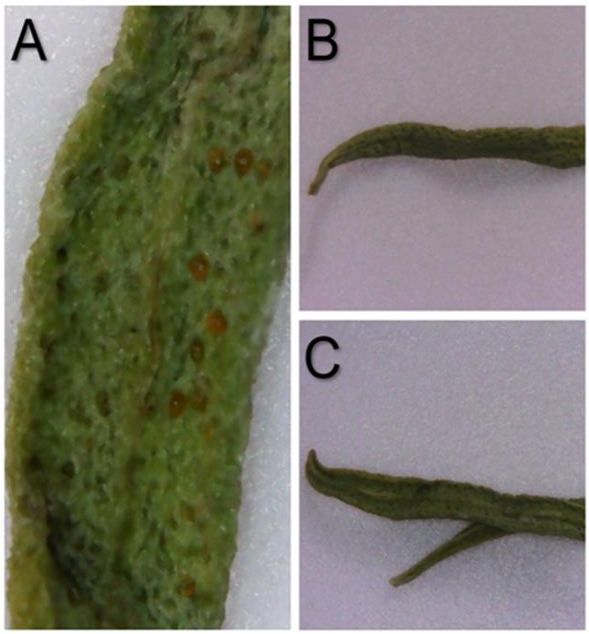


Fig. 2: The leaf of *Hyssopus ambiguus* under the macroscope: A-leaf surface structure close-up; A-the top side of the leaf; C-the bottom side of the leaf. Magnification x10-20\

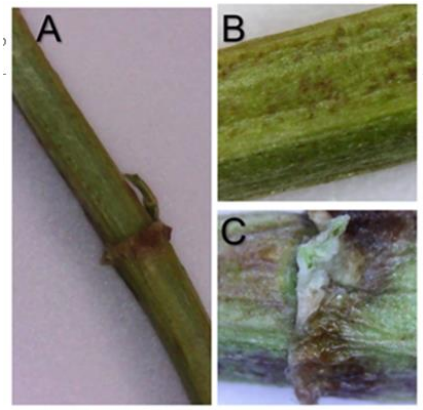


Fig. 3: The stalk of *Hyssopus ambiguus*. Magnification x 10-20.

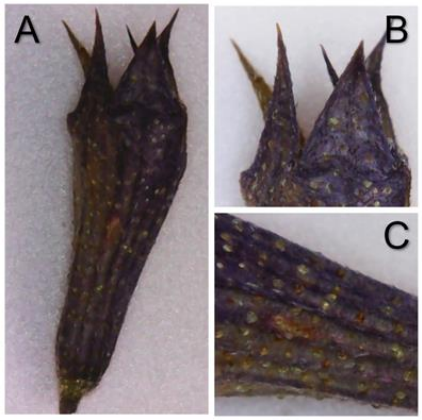


Fig. 4: The sepal of *Hyssopus ambiguusa*. Magnification x 10-20

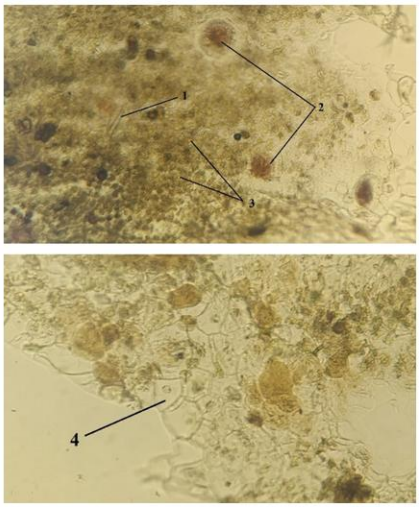


Fig: 5: Upper leaf epidermis of *Hyssopus ambiguus*. Preparation from the surface. Magnification 10 x 15. A fragment with essential-oil glandules and with simple trichomes, a fragment with main cells of the epidermis; 1-trichoma, 2-stoma, 3-essential oils, 4-main cells of the epidermis

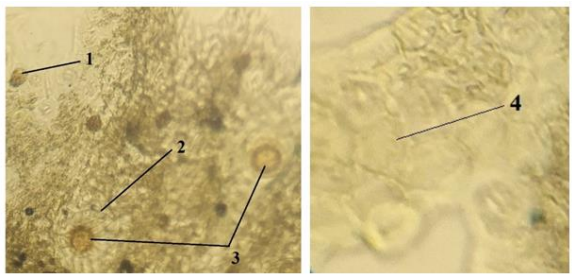
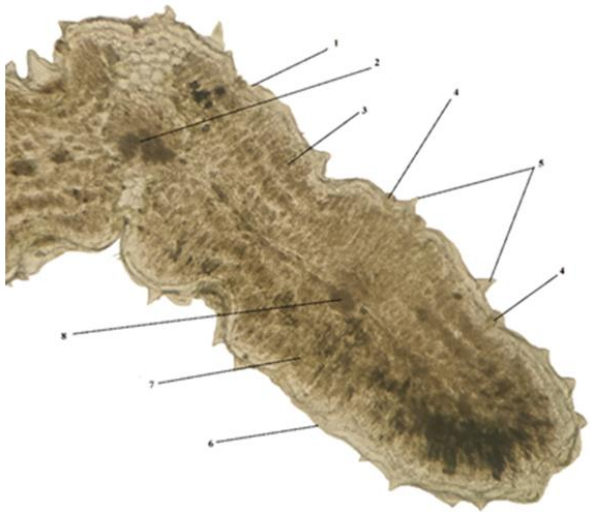
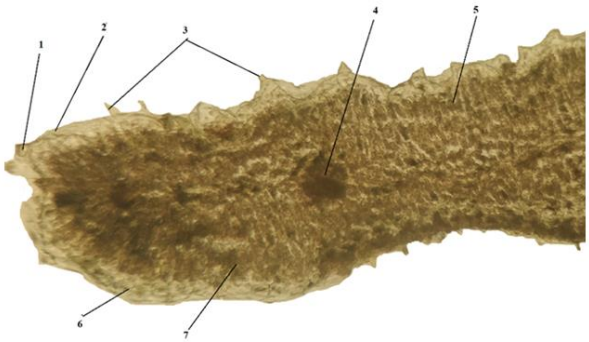


Fig. 6: Lower epidermis leaf of *Hyssopus ambiguus*. Preparation from the surface. Magnification 10 x 15. Afragment with; 1-trachoma, 2, 3-essential oil glandules, 4-main cells of the epidermis



(A)



(b)

Fig. 7: Cross-section of Hyssopus Ambiguus leaf. Magnification 10 x 15. A-a fragment with a central dwelling, B-lateral fragment of the leaf; (a) 1-lower epidermis, 2-central conductive bundle, 3-palisade mesophyll, 4-essential oil glandular, 5-trichoma, 6-upper epidermis, 7-sponge mesophyll, 8-alateral conductive fascicle; (b) 1-essential oil glandular, 2-lower epidermis, 3-trichoma, 4-conductive bundle, 5-paliside mesophyll, 6-sponge mesophyll, 7-upper epidermis

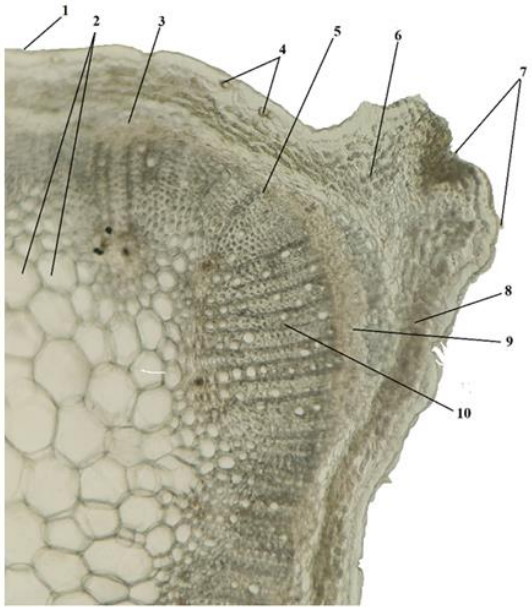


Fig. 8: Cross slice of the stalk of *Hyssopus ambiguus*. Nagnification 10 x 15; 1-epidermis, 2-pith parenchima, 3-cork parenchima, 4-trichomas, 5-endoderm, 6-corner collenchymas, 7-essential oil glandules, 8-chlorenchima, 9-floem, 10-xylem

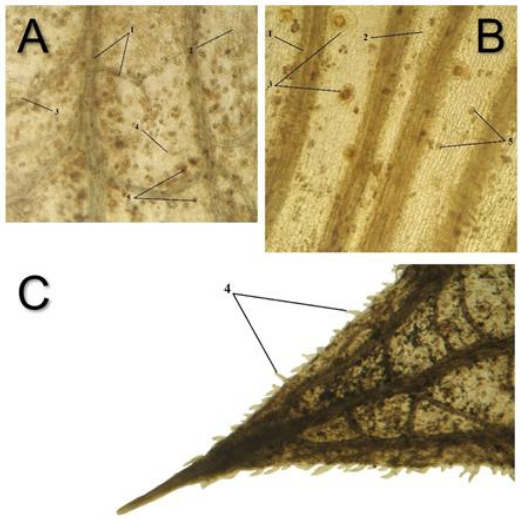


Fig: 9: The sepal of *Hyssopus ambiguus*. Preparation from the surface. Magnification 10 x 15. A, B-fragment of the middle part of the sepal, C-tooth of a cup; 1-veins, 2-the main cells of the epidermis, 3-essential oil glandules, 4-trichoma, 5-pollen grains

Discussion

The results of the microscopic diagnostic features study of *Hyssopus ambiguus* are the following:

1) A form of the leaf blade-narrowly lanceolate
2) A color of stalk-green over the entire length in the nodes dyed lilac-violet color, at the break-almost white
3) A sepal shape-narrowly bell-shaped, surface-ribbed
4) Trichomas location-sepal teeth covered with sparse simple white trichomas
5) The arrangement of essential oil glandules are abundantly coated surface of the blade on both sides, are completely absent on the stem, and arranged in rows along the veins of the sepal

The diagnostic features of *Hyssopus ambiguus* on the microscopic level are:

1) A leaf: Form of the main cells of the epidermis, location, type of essential oil glandular and trichomes
2) A stalk: Conductive system of closed type, shape, and location of glandules and trichomes
3) A sepal: The shape of the cells of the epidermis, the location of essential oil glandules over the surface of the epidermis and simple trichomes on the teeth of the flower cup

Conclusion

The diagnostic features of *Hyssopus ambiguus* are the form and the structure of the epidermis cells of a leaf and a stalk, the shape and the arrangement of essential oil glandules and trichomes, and the structure of a leaf and a stalk on the transverse cut.

The diagnostic features of *Hyssopus ambiguus* on the macroscopic level are The shape of a leaf, the coloration of a stalk, the absence of trichomas on stalks, the form of a flower cup; the location of trichomas and essential oil glandules.

The diagnostic features of *Hyssopus ambiguus* on the microscopic level are:

1. A leaf: Form of the main cells of the epidermis, location, type of essential oil glandular and trichomes
2. A stalk: Conductive system of closed type, shape, and location of glandules and trichomes
3. A sepal: The shape of the cells of the epidermis, the location of essential oil glandules over the surface of the epidermis and simple trichomes on the teeth of the flower cup

Acknowledgment

The authors are grateful to the authorities and staff of the Department of Botany, NS JSC “E.A. Buketov Karaganda University", for the opportunity for anatomical and morphological research.

Autor's Contributions

Yekaterina Victorovna Lakomkina: Concept, manuscript writing, critical revision of the manuscript, macro- and microscopical study, data collection and final approval.

Margarita Yulaevna Ishmuratova: Analysis, manuscript writing, data interpretation, identification of species and collecting of raw material.

Gayane Abdulhakimovna Atazhanova: Data interpretation, manuscript writing, critical revision of the manuscript and final approval.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all co-authors have read and approved the manuscript and that no ethical issues are involved.

References

Baj, T., Korona-Głowniak, I., Kowalski, R., & Malm, A. (2018). Chemical composition and microbiological evaluation of essential oil from *Hyssopus officinalis* L. with white and pink flowers. Open chemistry,

Diego, A., Sampietroa, A. de L. A., Gomeza, C. M. J., Emilio, F., Lizarragac, Z. A. I., Yerlan, M. S., & Cesar, A. C. (2017). Chemical composition and antifungal activity of essential oils from medicinal plants of Kazakhstan, Natural Product Research 31 (2017) 1464-1467. DOI.org/10.1080/14786419.2016.1258560

Ishmuratova, M. Y., Butumbayeva, M. K., Takhanova, D. A., Zholdybayeva, K. B., & Silant'eva, M. M. (2020). Analysis of representatives of the Lamiaceae family in the flora of Central Kazakhstan. Bulletin of Karaganda University. Series: Biology. The medicine. Geography, 98(2), 37-44. https://rep.ksu.kz/handle/data/9905?show=full.

Kovalenko, N. A., Supichenko, G. N., Ahramovich, T. I., Feskova, A. V., Leontiev, V. N., & Shutova, A. G. (2019). Antimicrobial properties of Pseudotsuga menziesii essential oil. Proceedings of the National Academy of Sciences of Belarus, Biological Series, 64(4), 431-439. DOI.org/10.14258/jcprm.2019014083

Kovalev, V. N. (2003) Workshop on pharmacognosia. Tutorial, Kharkov, 2003, 192. https://dspace.nuph.edu.ua/handle/123456789/9822

Kukaniev M.A., Jamshedov J.N., Sharopov F.S., The study of the chemical composition of the essential oil of Hyssop Zeravshanicus, Tajikistan medicinal plants, 2012. http://medacad.tj/medacad/uploads/2017/09/2012-3-11.pdf

Moulodi, F., Khezerlou, A., Zolfaghari, H., Mohamadzadeh, A., & Foad Alimoradi F., (2018). Chemical Composition and Antioxidant and Antimicrobial Properties of the Essential Oil of *Hyssopus officinalis* L., J Kermanshah Univ Med Sci. 2018 December; 22(4), e85256. DOI.org/10.5812/jkums.85256

Musinov, S. R., & Tulegenova, A. U. (2016). The State Pharmacopoeia-main standard of quality of medicines and medical devices in the Republic of Kazakhstan. The Bulletin of the Scientific Centre for Expert Evaluation of Medicinal Products. 2016 (2), 26-30. (In Russ.)

Myshabaliyeva, A. B., Medeubayeva, B. Z., Slyimen, E. M., Ibatayev, J. A. (2016). Investigation of the component composition of the essential oil of *Hyssopus Ambiguus* L. in the conditions of East Kazakhstan, a collection of scientific works of the International Scientific and Practical Conference "Enon" 1 7 (19) (2016), 9-11. https://www.elibrary.ru/download/elibrary_26584561_21010720.pdf

Nikitina, A. S., Logvinenko, L. A., Nikitina, N. V., & Nigaryan, S. A. (2019). Morphometric and histochemical research of melissa officinalis l. herb from the collection of nikitsky botanic garden. Pharmacy and Pharmacology, 6(6), 504-534. DOI.org/10.19163/2307-9266-2018-6-6-504-534

Pavlov, N. V. (1964). Flora of Kazakhstan, Alma-Ata, 1964, 7 Vol, 232. https://booksee.org/book/545279

Suleimen, E. M., Tursynova, N. K., Ibataev, Z. A. (2015). Constituent Composition and Biological Activity of Essential Oil from *Hyssopus ambiguus*. Chem Nat Compd, 51 (2015), 1186-1187. DOI.org/10.1007/s10600-015-1527-x

Tursynova, N. K., Iskakova, Zh. B., Suleimen, E. M., Ishmuratova, M. Yu. (2015). Hyssopus Ambiguus (Trautv.) Iljin (Lamiaceae) ocimdigisoshyndalarynyn biology belsendiligi jane osimdik Shikizatynyn Anatomiyalyk kurylysy, Topical Issues of Modernity 1(7), (2015), 238-246. https://www.elibrary.ru/item.asp?id=27360455.

Vekhov, L. I. L., & Filin, V. R. (1980). the workshop on the anatomy and the morphology of higher plants, Moscow, 1980, 560. https://msu-botany.ru/gallery/vekhov_1980_prac_po_anatomii.pdf