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Anatomical, Morphological and Biochemical Analysis of Medicinal Species *Agrimonia* L. Growing at South-east of Kazakhstan

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The paper presents morphological and anatomical data on medicinal plant *Agrimonia* L. growing in the south-east of Kazakhstan. Here you can find the data on peroxidase activity of leaves, stems and roots, as well as analysis of total proteins concentrations in the stems of different populations of medicinal plants *Agrimonia asiatica* Juz., *Agrimonia eupatoria* L., *Agrimonia pilosa* Ldb. The spectrum of cathode isoforms of all organs of plants *Agrimonia pilosa* Ldb. from the 4th population shows specificity by activity and heterogeneity of the spectrum.

Key words: *Agrimonia asiatica* Juz, *Agrimonia pilosa* Ldb, *Agrimonia eupatoria* L., Population, phloem, xylem, epidermis, isoform, peroxidase, esterase, cytoplasmic proteins.

The role of medicinal plants

Conservation and sustainable use of plant resources of our planet is now a global issue of interstate level (Proskuriakov M.A., 2012). Many years of experience in the study of medicinal plants shows that their extractions have low toxicity and possess the necessary medicinal properties. The variety of biologically active substances provides a wide range of pharmacological effects of herbal medicines (Goldberg E.D. and Zueva E.P., 2000). The flora of Kazakhstan is an inexhaustible source of vegetable raw materials in particular in the mountain areas. Medicinal plants serve as a valuable raw material for a wide range of herbal remedies and therapeutic pharmacological actions that are fast-acting, do not have cumulative effect and cause adverse effects to a lesser extent. Medicinal plants of Kazakhstan contain most of the known classes of biologically active substances. According to the phytochemical composition medicinal plants possess a very broad spectrum of pharmacological actions (Grudzinskaya L.M. *et al*., 2014). Plant *Agrimonia* L. of the wild flora of Kazakhstan is an issue of the particular interest for its pharmacologically promising species. Due to this fact the study of the direction of change in genetic and biochemical traits in populations of valuable

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species of plants growing in different environmental conditions has a great theoretical and practical interest.

Therapeutic properties and medical use

Biochemistry of plants represents rich and diverse source materials and tools that provide healthy, happy and full-fledged lives of people. *A.pilosa* Ldb. may be a good source of natural antioxidants and alpha-glucosidaseinhibitors exhibiting remarkable potential value for the therapy of T2DM (By Liu. *et al*., 2014). Nine compounds were isolated from the active section with lowering blood sugar of agrimony, and their structures were identified as oleanoic acid, ursolic acid, 19-alpha-hydroxy ursolic acid, tormentic acid, apigenin, luteolin, kaempferol, 3,3'-di-O-methyl ellagic acid, and kaempferol-7-O-alpha-L-rhamnoside (Cehn YS. *et al*., 2010). Few previous reports have investigated the chemical components of *A.pilosa* Ldb. Chemical studies on *A.pilosa* L. have shown the presence of polyphenols such as flavonoids (Kato H. *et al*., 2010). In a bioassay-guided search for acetylcholinesterase (AChE) inhibitors from 180 medicinal plants, an ethyl acetate extract of whole plants of *Agrimonia pilosa* ledeb yielded tiliroside, 3-methoxy quercetin, quercitrin and quercetin. We report herein for the first time that all four flavonol compounds showed significant inhibitory effects on AChE, particularly quercetin. This showed the activity of dehydroevodiamine (DHED) (Jung M. and Park M., 2007). *A.pilosa* Ldb. has potent estrogenic activity and may have beneficial effects for postmenopausal women requiring ERT (Lee YM. *et al*., 2012). *A. pilosa* Ldb. extract shows an antinociceptive property in various pain models. Furthermore, this antinociceptive effect of A. pilosa Ldb. extract may be mediated by α 2- adrenergic receptor, but not opioidergic and serotonergic receptors (Soo-Hyun Park. *et al*., 2012). Essential oils, tannins, vitamin K, flavonoids (quercetin and others), choline, bitterness, silicic acid, catechins, triterpenes, and organic acids were found in *A.eupatoria* L. Medical drugs based on sarcocolla were used as anti-inflammatory, antispasmodic, expectorant, diaphoretic, choleretic and diuretic agents.

The purpose of the current study is to reveal morphological and anatomical structure of vegetative organs, activity of proteins, esterase and peroxidase in the aboveground and underground parts of plants of the genus *Agrimonia* L. collected in different populations.

METHODS

Objects of the study were the following: *Agrimonia asiatica* Juz. - asian agrimony, *Agrimonia pilosa* Ldb.- hairy agrimony, *Agrimonia eupatoria* L. - ordinary agrimony from the family Rosaceae Juss growing at the southeast of Kazakhstan.

Materials and methods of anatomical and morphological study

The materials for the study were samples of plants of *A.asiatica* Juz. (1-2) population growing in the village of Koldi of the Main Botanical Garden of Almaty. *A.eupatoria* L. was collected at the territory of the Main Botanical Garden of Almaty. Whereas samples of the plant *A.pilosa* Ldb. were collected from the populations growing at the mountain areas at the territory of the National Park (NP) called Kolsai lakes. Specific definition of the studied plants was carried out according to the Flora of Kazakhstan and illustrated determinant of the plants of Kazakhstan (Kazakhstan Flora.1961, Illustrated determinant of the plants of Kazakhstan, 1969). Based on the material collected in 2012-2013 and preserved in the ratio of alcohol, glycerol, and water as 1:1:1 respectively for Strasburger-Flemming, anatomical and morphological studies were conducted according to the procedure of M.N. Prozina (Prozina M.N., 1960). Anatomical sections of plants were made using a microtome MZP-01 “technom”, Ekaterinburg. For the quantitative analysis we measured the morphometric parameters using a microscope MCX 100 micros Austria equipped with photo attachment and lenses x4/0.10, magnification EW 10x/20.

Materials and methods of biochemical study

Objects of the research for biochemical analysis were 3 types of plants of the genus *Agrimonia* L. Samples of plants *A.asiatica* Juz. were collected from four different populations[1-4] growing at the territory of the village of Koldi at the Main Botanical Garden of Almaty and near to the hole Butakovka on the territory of health-

improving and recreation complex Akbulak. Samples of plants *A.eupatoria* L. were collected at the territory of the Main Botanical Garden of Almaty. Whereas samples of plants of the species *A.pilosa* Ldb. were collected from the mountain populations[1-4] at the territory of the village of Sata, Tomarsaz; Karasai; National Park – NP Kolsay.

Method for the determination of peroxidase activity

Peroxidase isolated from leaves, stems and roots by grinding in liquid nitrogen and exposure to tris-glycine buffer with pH 8.3. Total peroxidase activity was measured by the photo-calorimetry FEC 56 by the velocity of oxidation of benzidine by Boyarkin (Boyarkin A.N., 1951). Translation of the activity into nanokatals was conducted by Lebedeva O.V. (Lebedeva O.V. *et al*., 1977). Electrophoretic separation of cathodic peroxidases was performed in tris-glycine buffer at pH 8.3 (Kabzhanova S.B. and Peruasky Y.V., 1975). We used Thermoscientific kits (Lithuania) as a molecular weight marker comprising a mixture of purified protein with a molecular mass from 10 to 200 kDa.

Methods for detection of cytoplasmic protein

Extraction of cytoplasmic proteins was performed in phosphate buffer pH 7.5. An extract was centrifuged at 10 000 rpm. A chilled acetone was added to the supernatant. The precipitate was re-dissolved in 0.0618 M tris-HCl buffer by the solution containing SDS Na - 3%, glycerol 10%, merkaptetonol 4% and bromophenol blue dye. The sample was alkylated, heated for two minutes in a boiling water bath. 24 µl of the sample was added into the pockets of 10% polyacrylamide gel. Preparation of the gel and electrophoresis was carried out using the Laemmli (1970) method modified by Bulatova K.M. (Bulatova K.M., 1985). Thermoscientific kit, Lithuania was used as molecular weight marker (200 kDa, 150 kDa, 120 kDa, 100 kDa, 85 kDa, 70 kDa, 60 kDa, 50 kDa, 40 kDa, 30 kDa, 25 kDa, 15 kDa, 10 kDa).

Method of determining the esterase activity

Tissue extract from various types of *Agrimonia* L. was used for the analysis of peroxidase enzyme. It was also used for fractionation of esterases. In order to display isoforms esterase, we used alpha- and beta-naphtylacetate as a substrate. This substrate is the most preferred one for the imaging of nonspecific esterase (Peskan T.*et al*., 1996).

RESULTS AND DISCUSSION

Botanical characteristics of the genus *Agrimonia* L.

Asian agrimony *(A.asiatica* Juz. belongs to the family *Rosaceae* Juss. It is perennial, rather tall plant (up to 80-117 cm) with a straight stem and long branches. Number of stems per plant is 10-14. Rhizome is branching with a length of 15-20 cm. Leaves are green on the top, densely adjacent and piliferous, gray and green on the bottom, densely and velvety-hairy, with small yellow glands. Plant has 70-157 leaflets, with a length of 2-8 cm, and a width of 1-4 cm, elliptic, oblong-ovate, large and sharp-toothed. Inflorescence is a spicate acervulus with straddling at the bottom, and closely spaced flowers and fruits at the top. Flowers have short pedicels, 5 calyx lobes and 5 golden yellow petals. Fruits have spines (Kaliyeva A.N. and Dyuskaliyeva G.U., 2014).

Hairy agrimony *(Agrimonia pilosa* Ldb.) is a perennial plant that belongs to the family Rosaceae Juss. It reaches 63 to 90 cm in height. The stem is cylindrical, branched, covered with hairs. Leaves are straddling, broken-pinnate, naked or sparsely hairy, green on both sides, darker on top. The number of leaves on one plant was 96 ± 2.01. Inflorescence is a spicate acervulus, 6-8 mm in diameter. Fruits are erect and droop only after full maturation. Petals are oblong, pale yellow, branching rhizome (Kaliyeva A.N. *et al.*, 2015).

Ordinary agrimony *(Agrimónia eupatória* L.*)* is a perennial plant from the rose family. Plants have 60-150 cm in height, straight stem covered by greenish-gray, fluffy hair as well as leaves. Leaves are intermittent pinnate, elliptic, toothed. Flowers have five yellow petals, 6-12 mm in sizes, inflorescence is a spicate acervulus. Flowering is in June - August. The fruit is a single coccus enclosed in a dry hypanthium with hooked spines.

Anatomical features of medicinal species of the genus *Agrimonia* L.

Anatomical structure of *A. asiatica* Juz. vegetative organs.

In the internal structure of the limb there are well-developed simple trichomes on the lower epidermis. On the main vein there is a large conductive fascicle with well-developed xylem vessels. On cross-section of the stem *A.asiatica* Juz. xylem vessels have developed twice more than phloem structures and friable parenchyma cells with the round shape. On the cross-section of root there are xylem fascicles of different sizes.

Anatomical structure of *A. eupatoria* L. vegetative organs

In cross-section of the leaf on both sides of epidermis there are trichomes, columnar and spongy mesophyll in a single row. In the center of a leaf there is collanterial, larger conductive fascicle with well-developed xylem vessels. On cross-section of the stem there is a single row epidermis, consisting of small cells and vascular bundles. The core consists of a friable, rounded parenchyma cells. Root on the cross section has a rounded shaped. Its internal regions occupy the central cylinder. Phloem is located between the xylem fascicles. Xylem is composed of vessels of different sizes.

Anatomical structure of *A. pilosa* Ldb. vegetative organs.

Cross section of the leaf reveals epidermal cells, columnar and spongy mesophyll from the external surface. Leaf has a dorsoventral structure. On the main vein there are two

Table 1. Total peroxidase activity of leaves, stems and roots of species *Agrimonia* L. (*A. asiatica* Juz., *A.eupatoria* L., *A.pilosa* Ldb.)

Species, population	E_1	Nanokatales	E_2	Nanokatales	E_3	Nanokatales	E_{mean}	Nanokatales
Leaf								
A.asiatica Juz, *p.1*	0.109	484	0.111	493	118	524	0.113	502
A.asiatica Juz., *p.2*	0.107	475	0.109	484	0.104	462	0.106	471
A.asiatica Juz. *p.3*	0.122	542	0.118	524	0.125	555	0.121	537
A.asiatica Juz. *p.4*	0.072	320	0.071	315	0.068	302	0.070	311
A.eupatoria L.	0.085	377	0.090	400	0.092	408	0.089	395
A.pilosa Ldb. *p.1*	0.122	542	0.132	586	0.127	564	0.127	564
A.pilosa Ldb. *p.2*	0.071	315	0.086	382	0.075	333	0.077	343
A.pilosa Ldb. *p.3*	0.105	466	0.112	497	0.108	481	0.108	481
A.pilosa Ldb. *p.4*	0.295	1311	0.286	1271	0.290	1290	0.290	1290
Stem								
A.asiatica Juz, *p.1*	0.242	1075	0.250	1111	0.244	1084	0.245	1089
A.asiatica Juz., *p.2*	0.158	702	0.154	684	0.155	689	0.156	693
A.asiatica Juz. *p.3*	0.251	1115	0.268	1191	0.262	1164	0.260	1155
A.asiatica Juz. *p.4*	0.490	2178	0.480	2133	0.482	2142	0.484	2151
A.eupatoria L	0.375	1667	0.388	1724	0.377	1675	0.380	1689
A.pilosa Ldb. *p.1*	0.120	533	0.109	484	0.117	520	0.115	511
A. pilosa Ldb. *p.2*	0.102	453	0.105	466	0.104	462	0.103	458
A.pilosa Ldb. *p.3*	0.115	511	0.118	524	0.119	529	0.117	520
A. pilosa Ldb. *p.4*	0.152	675	0.155	689	0.152	675	0.153	680
Root								
A. asiatica Juz, *p.1*	0.105	466	0.101	449	0.102	453	0.103	457
A. asiatica Juz., *p.2*	0.110	488	0.101	449	0.107	475	0.106	471
A. asiatica Juz. *p.3*	0.109	484	0.106	472	0.105	466	0.107	475
A. asiatica Juz *p.4*	0.118	524	0.107	475	0.112	497	0.117	520
A.eupatoria L.	0.121	537	0.119	529	0.118	524	0.119	528
A. pilosa Ldb. *p.1*	0.115	511	0.113	502	0.111	493	0.114	506
A.pilosa Ldb. *p.2*	0.111	493	0.112	497	0.111	493	0.111	493
A.pilosa Ldb. *p.3*	0.119	529	0.115	512	0.122	542	0.118	524
A.pilosa Ldb. *p.4*	0.158	702	0.167	742	0.162	720	0.162	720

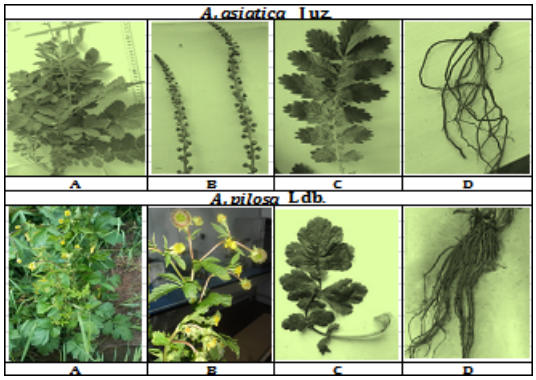
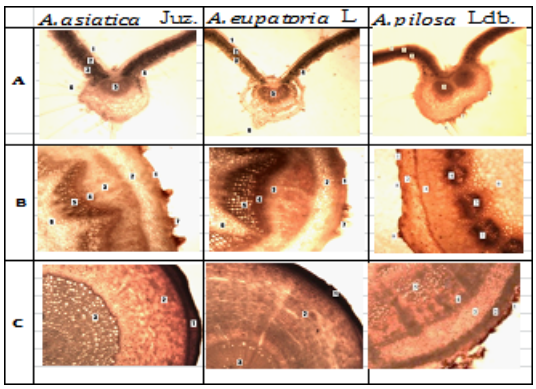


Fig. 1. Morphology of medicinal species of the genus *Agrimonia* L.*:* A-stem, B - fruit, C - leaves, D-root



A - leaves: 1 - upper epidermis, 2 - lower epidermis, 3- spongy mesophyll, 4-columnar mesophyll, 5- xylem, 6- phloem, 7- conductive fascicle; 8 - trichomes; B - stem: 1 - epidermis, 2 - primary bark, 3 - sclerenchyma, 4 - phloem, 5 - xylem, 6 - parenchyma core, 7- conductive fascicle, 8 - trichomes; C - root: 1 - periderm, 2-primary bark, 3 - secondary bark, 4 - phloem, 5 - cambium, 6 - xylem.

Fig. 2. Anatomical structure of vegetative organs of plants

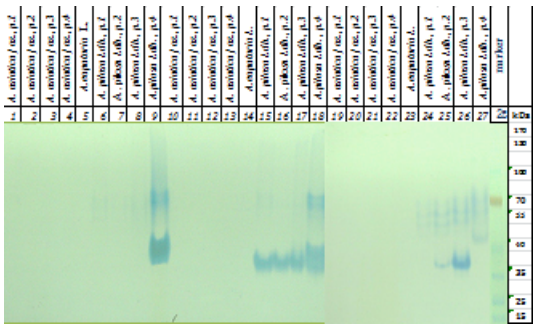


Fig. 3. Spectrum of cathode peroxidase isoform of leaves (1-9), stems (10 -18) and roots (19-27) of the species *Agrimonia* L., 28-marker

collateral, closed-type vascular bundles. Xylem elements are well developed. Simple and edgeless cone-shaped trichomes are located on both sides of the leaf. Collateral vascular bundles of the stem have a close localization on the surface of the stem, evenly in a single row. In the stem xylem vessels evolved twice more than the elements of the phloem. By the way there was bass lining of the fascicles. On the cross section of the root xylem, fascicles were more developed than the phloem.

Biochemical analysis of the species of the genus *Agrimonia* L.

Electrophoretic analysis of proteins and isozymes remains to be one of the easiest and most affordable biochemical methods allowing clear identification of not only individual species but also subspecies, populations or biotypes of plants for the minimal cost (Peterson A.N. *et al.*, 1991; Khavkin E.E., 1997). Enzymes are substances of protein nature which are able to accelerate chemical reactions in the body and serve as organic catalysts. They also play an important role in metabolism. Peroxidase enzyme belongs to the class of oxidoreductases. By its chemical composition it is a heme-containing glycoprotein that catalyzes the oxidation of several substrates by hydrogen peroxide (Baranova T.V. *et al.*, 2001). Active groups of peroxidase contain iron, hydrogen peroxide. It is activated and acts as a hydrogen acceptor. Hydrolases catalyze the hydrolysis and sometimes synthesis of organic compounds with water bonding to it. The most important are esterases which accelerate the hydrolysis reaction and synthesis of esters. Oxidoreductases are enzymes that accelerate the oxidation-reduction reactions. These include oxidase, peroxidase and catalase involved in the breathing process. Hydrolases are enzymes which catalyze cleavage of complex organic compounds with participation of water. Esterase catalyzes the cleavage and synthesis of esters.

Total peroxidase activity of medicinal species of the genus *Agrimonia* L.

Total peroxidase activity of leaves in *Agrimonia* L. species varies from 311 to 1,290 of nanokatales. It should be noted that total peroxidase activity is the highest in *A.pilosa* Ldb. from population 4. Total enzyme activity of stems

in the species *A.asiatica* Juz. and *A.eupatoria* L. is higher than that in the stem of *A.pilosa* Ldb. Peroxidase activity in roots is the highest in species *A.pilosa* Ldb. (Table 1).

Results of peroxidases electrophoresis conducted under alkaline conditions

Peroxidases electrophoresis is conducted under alkaline conditions. This procedure showed virtually no signs of the activity of all isoforms of the cathode in species *A.asiatica* Juz. and *A.eupatoria* L. After fractionation of peroxidases of the species *A.pilosa* Ldb. it was noted that the highest activity is attributed to the isoforms with molecular weight of 35-70 kDa. Moreover, the most active isoforms are those with a molecular weight of 37-40 kDa. The spectrum of cathode isoforms of all organs of plants of the 4th population of this species shows specificity both in activity and heterogeneity of the spectrum (Figure 3).

An absence of activity signs in the spectrum of peroxidase isoforms of the species

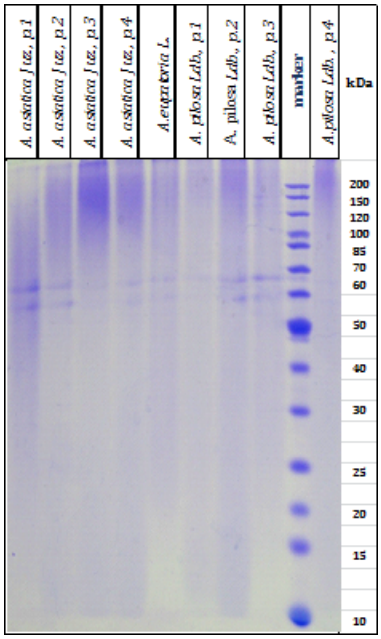


Fig. 4. The spectrum of cytoplasmic proteins of the stem in species *Agrimonia* L

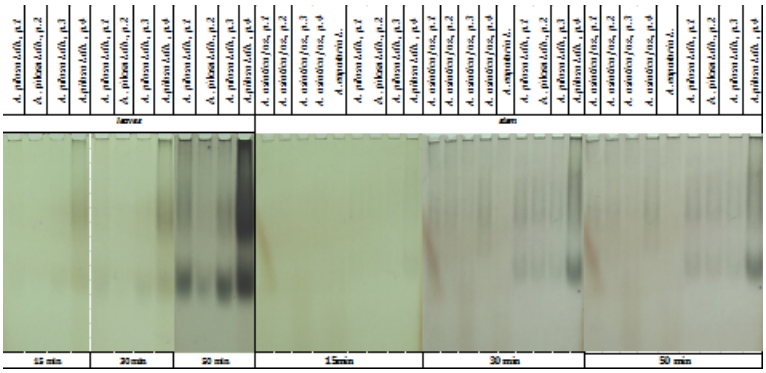


Fig. 5. Character of manifestation of esterase isoforms after 15min, 30 min and 50 min exposure in the solution

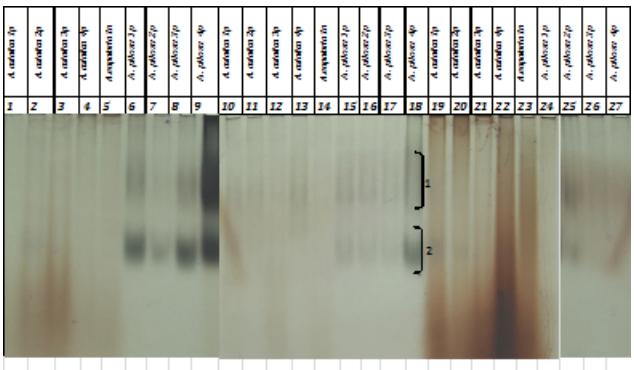


Fig. 6. Spectrum of esterase isoforms in leaves (1 to 9), stems (10 - 18) and roots (19 -27) of the species *Agrimonia* L

A.asiatica Juz. and *A.eupatoria* L. is apparently caused by their inhibition by phenolic compounds. Basic compound of water-alcoholic extract of *A.eupatoria* L. are flavonoids. Extract also contains large amounts of tanines and has a high content of phenolic compounds which have an inhibitory effect on the enzymes. Antioxidant activity of *A. eupatoria* L. might be due to chemical structure of polyphenols and herb ability to activate the endogenous antioxidant defence systems (Ivanova D. *et al*., 2011). Polyphenol profile of *Agrimonia* herbs, antioxidant activity and inhibition of ±-glucosidase support the traditional use of these plants as anti-diabetic and anti-inflammatory drug (Kubinova R. *et al*., 2012). Enzymes that are capable to neutralize superoxide radicals and peroxyl species in the cells play the main role in the protection from reactive oxygen intermediates. Superoxide dismutase (SOD) destroys superoxide anion radicals to hydrogen peroxide. Catalase reduces hydrogen peroxide to water and oxygen. Peroxidase reduces hydrogen peroxide to water but with participation of organic reducing agents (Menytsikova E.B. and Zenkov N.K., 1993).

The results of electrophoresis of cytoplasmic proteins of the stems of species *Agrimonia* L.

Proteins in all organisms are produced on the matrices - molecules of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). These molecules are able to preserve and carry hereditary characteristics of organisms through thousands of generations. All other products formed by proteins serve as a base to form proteins, which are identical or very similar in all living beings.

Electrophoresis of cytoplasmic proteins isolated from the stems of plants *Agrimonia asiatica* Juz., *Agrimonia eupatoria* L., *Agrimonia pilosa* Ldb. presented by different populations did not reveal any diversity between species and between populations (Figure 4).

Proteins were presented by two intense and several minor bands. They were expressed most clearly in the electrophoretic spectrum of the molecular weight from 60 to 70 kDa.

Character of manifestation of esterase isoforms in the species of *Agrimonia* L

In order to display isoforms, we performed electrophoresis in 10% polyacrylamide gel. Then in a 100 mL glass plate we poured 20 ml of tris-HCl buffer and 80 ml of distilled water. 5 minutes before staining the gel 50 mg naphtylacetate was dissolved in 5 ml of acetone and then was slowly poured in the buffer with stirring. The gel was transferred to this solution. Pre-filtered and dissolved BB salt (50 mg in 10 ml distilled water) was added to gel-containing cuvette (Figure 5).

First bands were thin and weak. After coloring they became wider and more intense. In the spectrum of esterase in species *Agrimonia* L. there are 2 groups of isoforms. Isoforms are present most intensively in the spectrum related to stems and leaves of *A.pilosa* Ldb. (Figure 6).

It should be noted that the activity of isoforms in populations selected in NP Kolsay - *(A.pilosa* Ldb., 4p.) was the highest in both cases.

CONCLUSION

The study revealed particular features of anatomical and morphological structure of medicinal species of the genus *Agrimonia* L. growing in the south-eastern region of Kazakhstan. Xylem vessels can be clearly visualized in the cross-section of the stem of *A.asiatica* Juz., *A.eupatoria* L. In the lower epidermis of leaf there are simple trichomes. Xylem fascicles can be clearly visualized in the cross-section of the root. Vascular bundles of the stem of *A.pilosa* Ldb. are collateral, xylem vessels are more expressed in the stem. There was developed bast-fiber sheath. Analysis of the data showed that there are no apparent differences in the anatomy of the species *A.asiatica* Juz., *A.eupatoria* L. According to its therapeutic action, *A.pilosa* Ldb. is similar to *A.asiatica* Juz. It differs from it only in small morphological features. In addition to the main vein there are two vascular bundles of the collateral type. Total peroxidase activity in the leaves and roots of *A.pilosa* Ldb. from the 4th population is the highest. Total enzyme activity of stems is higher in *A. asiatica* Juz. *and A.eupatoria* L. species than in *A.pilosa* Ldb. species. The result of peroxidases electrophoresis conducted under alkaline conditions revealed no signs of the activity of the cathode isoforms of all organs in *A. asiatica* Juz. and *A.eupatoria* L. species. All

plant organs in *A.pilosa* Ldb. species of the population 4 are active. Esterase activity of the stem and leaf is high in *A.pilosa* Ldb. species, 4th population. In roots an isoform activity is manifested in trace amounts.

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