Agrimonia pilosa Ledeb. (Rosaceae) - Chemical Composition, Biological Effects and Anatomy

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Two fractions of polysaccharide have been extracted by the method of sequential extraction from the underground part of the plant; the monomeric composition of these fractions has been defined by the acid hydrolysis of these fractions and the following application of paper chromatography. The anatomy structure of *Agrimonia pilosa* Ledeb rootstocks and roots has been researched by the method of light microscopy. The fascicular rootstock structure has been defined; the primary and secondary roots structures have been described.

Key words: *Agrimonia pilosa*, anatomy, rootstock, roots, bioactive substances.

Agrimony pilose (*Agrimonia pilosa* Ledeb) is a plurannual herbage plant of the rose family (Rosaceae). This kind of plants is widely used in the folk medicine for treating liver diseases, malignant tumors, malaria, fever and rheumatism[1]. The plant is recognized as official in some European and Asian countries. In Bulgaria and Great Britain the overground part of the agrimony is used as an anti-inflammatory agent, in Korean medicine – as antiparasitic, in the medicine of China and Vietnam it is used as a hemostatic agent[2]. The plant is included in the British plant pharmacopoeia as an anti-inflammatory agent[3].

As results of the experimental researches, the hepatoprotective, antitumoral, antibacterial, antifungal, stimulating, diuretic, anticoagulant and cytotoxic activity of the *A. pilosa* has been defined[4, 5, 6]. It has been discovered that biologically active substances (BAS) of this species improve blood rheological properties, and the screening assays have showed the presence of their antitumoral activity[7]. In recent years in Siberian State Medical University (SSMU) the rhythm modeling effect of the *A.pilosa* extraxts [8] and the antioxidant activity of aqueous extracts of the plant underground part were found for the first time.

Such various nature of the *A.pilosa* biological effect must be caused by the presence of different BAS groups in the plant composition. According to scientific works, its overground part contains phenol compounds: agrimophol[9] and agrimols A, B, C, D, E[10], flavonoids and their glycosides: apigenin, kaempferol, kaempferol -3-O-(6''-coumaroyl)-glucoside, kaempferol 3-O-α-L-rhamnopyranoside, kaempferol 3-O-β-D–

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glucopyranoside, quercetin, quercetin 3-O-α-L-rhamnopyranoside, quercetin, isoquercitrin, rutin, luteolin and its 7-O-β-D-glucopyranoside[11, 12, 13], tanning agents: agrimoniin and potentillin[14]; coumarines (esculetin, esculin, umbelliferone, scopoletin); hydroxi-cinnamonic acids (caffeic, chlorogenic); polysaccharides, amino acids[15], macro- and microelements[16]. In the *A.pilosa* overground part there are phenol compounds: agrimophol, psudoaspidin[17]; isocoumarin agrimonolid and its 6-O-β-D-glucoside[18]; phenolcarbonic acids: ellagic, trioxybenzoic[19]. At the pharmatheutical chemistry department of the Siberian State Medical University the presence of flavonoids, tanning agents, water-soluble polysaccharides, pectic substances and their monomeric composition have been discovered by the classic phytochemical methods.

A. pilosa grows in forest and steppe zones, often in the areas with destroyed natural cover, off the roads, in gullies, on watersides, on the skirts of deciduous and coniferous forests. It is spread in many areas of Europe and Asia. In Russia it mainly grows in non-black soil zones of the European part and also in the south of the Western and Eastern Siberia, in the Amur region, the Khabarovsk territory, north of the Primorsky Krai. The subspecies of the agrimony grow in Mongolia, North-East China, Korea and Japan [20, 21].

Based on the above, this species is very interesting for scientific medicine. The variety of pharmacological effects as well as the wide spread allow using it as a source of medical plant raw material in the territory of the Russian Federation. At the same time the plant underground part is poorly known. That is why the aim of this work is the anatomic study of the *A. pilosa* rootstock and roots and finding out its chemical composition.

MATERIALS AND METHODS

The sampling material was collected in September 2012 in the neighborhood of Tomsk-city. The plant underground part was fixed with 95% ethanol. Anatomic-morphologic features of the roots and rootstock structure were researched with the help of the light microscopy and histochemical reactions. For the chemical composition study, the rootstocks with roots were dried by the airily-shadow method and disintegrated up to the size of the particles going through the sieve with the holes 2–4 mm in diameter. For the research, the raw material with 9,20 % ± 0,3% moister content was used. The qualitative composition of the natural compounds was studied by the modern methods of phytochemical analysis[22-24]. For the chromatograhy in the thin layer, the plates "Sorbfil" (Russia) 10´10 cm in size were used, and for the chromatography on paper the chromatography paper "Filtrak FN– 4" (Germany) was used. The solvents of high grade and reagent grade were applied.

The chemical composition of the BAS groups was defined with the help of qualitative reactions, paper and the thin layer chromatography. To discover the flavonoids, we used the special reaction – cyanidin test of G. Synod; the tanning agents were discovered with the gelatine water of 0,5%; the presence of coumarines was defined by lactonic test and their ability to interact with alkali with the following azoic dye creation. We defined anthraquinones according to Bortregger; we discovered sesquiterpene lactones by the method of the thin layer chorography using 0,5% permanganate potassium solution in 1 % sulphuric acid solution. Ecdysteroids were defined by the method of UV spectrophotometry at the wavelength λ– 242 nm.

The polysaccharides extracting was conducted by the sequential extraction method with the solutions of hydrochloric acid and ammonium oxalate, followed by fractionation, by precipitation with 95% ethanol and dialysis. Complete acid hydrolysis of the polysaccharide fractions was performed with 2 mol/L trifluoroacitic acid solution. The mixture was incubated for 5 hours at 100^0 C in a sealed ampula, the excess acid was removed by repeated evaporation of hydrolyzate with methanol to dryness. The monomer composition of the polysaccharide fractions was set by ascending chromatography in the system butanol-pyridine - water (6:4:3) and the following detection by anilinftalatom.

Results and discussion

The rootstock structure

The rootstock (10-20 cm long and 0.7-1.1 cm in diameter) is plagiotropic with the sympodial type of branching. Threadlike additional roots (4-7 cm long and 0.2-0.3 cm in diameter) with numerous

filamentous and capilliform lateral roots branch from it. The rootstock has the bundle type of the structure: open collateral bundles are arranged in a circle (eustela). They have a fusiform, elliptical or almost rectangular shape with two-three-layer bundle cambium. The xylem has numerous fibers and tracheids.

Large vessels are usually concentrated in the spring part of the xylem. Wood parenchyma is arranged in 1-2 line rays. The volume of the xylem and phloem are approximately equal. Soft bast cells are arranged in neat rows, flattened in the tangential direction. The phloem parenchyma is well developed and performs the function of stocking. Bast fibers often have the softwood cell wall. The primary cortex and core are made by the storage parenchyma. Its cells with simple starch grains are rounded or oval, 2-3 times larger than the phloem parenchyma, tangentially elongated. Intercellular spaces are small or absent. In the primary cortex some idioblast cells with druses are found.

The primary root structure

In the center of the primary roots structure the triarhny or tetrarhny radial bunch is situated. The phloem is presented only by the soft bast. The xylem has plenty of fiber and small vessels. The pericycle can be 1-3-layer. The endoderm consists of cells with U-shape thickened cell walls. The mesoderm consists of parenchyma, the intercellular spaces of which are almost equal to the diameter of the cells. The ekzoderma is perfomed in one or two rows of suberized cells. The epiblema is usually in the form of trichoblasts remains.

The secondary structure root

In the center of the root there is 3-4-ray protoxylem presented by small diameter vessels and numerous wood fibers. Next the secondary xylem with large vessels, numerous tracheids and fibers separated by 1-2 (3)-line medullar rays is situated. Closer to the periphery the xylem is surrounded by 2-4-layer cambium and a wide ring of the bark. The inner part of the cortex is made form the soft phloem and the outer is made of several layers of parenchyma and angular collenchyme. The cover tissue is multilayer periderm, cork of which is dark brown.

The presence of tanning agents was judged by the formation of heavy precipitation on the addition of 0.5% gelatin solution to an aqueous extract of the plant underground part. Defining flavonoids, coumarins and the gallic acid was carried out by the TSH method in the chloroform-ethanol system (3:1). The presence of the flavonoids and the gallic acid was found out by the BH method using 60% acetic acid solution as the mobile phase. The solutions of the corresponding AR grade compounds were used as tracking substances. The detection of the compounds was performed in UV light at the wavelength λ - 254 nm and by the processing of chromatograms with 10% NaOH solution followed by applying a diazoreagent. The result has revealed the absence of anthraquinone and sesquiterpene lactones in the underground parts of the *A. pilosa.* The monomer composition the polysaccharide fractions: water-soluble polysaccharide-L-rhamnose and D-galactose, pectins - galacturonic acid, arabinose and xylose has been found.

The chemical composition of the *A. pilosa.* underground part

During the phytochemical studies, the presence of hydrolysable tannins, flavonoids, coumarins and gallic acid in the underground part was revealed. The presence of tanning agents was judged according to the formation of heavy precipitation on the addition of 0.5% gelatin solution to an aqueous extract of the underground parts of the plant. The flavonoids, coumarins and gallic acid were defined by the TLC method in the chloroform-ethanol system (3:1). The presence of the flavonoids and gallic acid was defined by BH method using the 60% acetic acid solution as a mobile phase.

The solutions of the corresponding AR compounds were used as tracking substances. The detection of the compounds was performed with UV light of the wavelength λ- 254 nm and by the processing of chromatograms of 10% - M NaOH solution followed by applying the diazo reagent. The results have revealed the absence of anthraquinones and sesquiterpene lactones in the underground parts of A. pilosa.

The monomer composition of the polysaccharide fractions has been established: water-soluble polysaccharide-L-rhamnose and D-galactose, pectins - galacturonic acid, arabinose and xylose.

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