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Isolation and Analysis of Extractives from Potentilla Alba (*Potentilla Alba L.*) Grown Under Different Conditions

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Abstract—*Potentilla alba L.* is used as a medicinal plant that contains a variety of biologically active substances. Natural resources are not meeting the needs of the pharmaceutical industry. We obtained the first biomass of *P. alba* plant-regenerants using hydroponics technique with clonal micropropagation. We have conducted a comprehensive study of the chemical composition of a vegetable raw materials *P. alba*, obtained in the Biotechnology Department (plant-regenerants) in comparison with raw material obtained from plants grown under field conditions (intact plants). We have established the quantitative content of the structural components in the samples: cellulose in roots and rhizomes of intact plants—15.4%, in the roots of plant-regenerants—4.3%, in the leaves of plant-regenerants—2.5%; lignin in roots and rhizomes of intact plants—40.7%, in the roots of plant-regenerants—37.0%, and in the leaves of plant-regenerants—36.8%. We determined the content of extractives in *P. alba* samples, recovered by sequential treatment with hexane, 96% and 40% ethanol solution, water and 1% aqueous sodium hydroxide solution. We identified the total content of extractives in the roots and rhizomes of intact plants at 15.3%, in the roots of plants-regenerants—11.2%, and in the leaves of plant-regenerants—5.1%. We characterized the chemical composition of extractives by UV-spectroscopy. Phenolic substances (λ = 279.5–280.0 nm) were dominant in the composition of the extracts. We have established the authenticity of the samples by the TLC chromatography based on the presence of flavonoids and tannins. We showed the identity of the phytochemical composition of vegetable raw materials produced by micropropagation and grown hydroponically for two months.

Keywords: medicinal herbs, bloodroot white, *Potentilla alba* L., intact plants, plant-regenerants, extractives, chemical composition, flavonoids, TLC-chromatography, UV-spectroscopy

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INTRODUCTION

Potentilla alba L. started to be used as an official medicine more than 30 years ago [1]. It is known that the *P. alba* plant contains carbohydrates (starch), iridoids, saponins, phenolcarboxylic acids, flavonoids, tannins [2–4]. Drugs based on *P. alba* affect the thyroid gland [5, 6]. Even though the plant is most widely used in the treatment of hypertension of the gland (thyrotoxicosis), a positive effect also was noted in the treatment of hypofunction [7, 8]. The thyrotropic activity of the *P. alba* roots explains the fact that in Belorussian Poles'e, where the use of *P. alba* as decoction instead of tea is common, after the Chernobyl accident very few cases of endemic goiter in comparison with other regions adjacent to the place of tragedy were detected [3]. In addition, phytotherapists recommend the use of *P. alba* for the prevention and therapy of liver, cardiovascular and gastrointestinal diseases, as well as an antiseptic and wound-healing agent [9]. Despite its wide distribution, the natural reserves of *P. alba* are rather limited and cannot satisfy the current demands of the pharmaceutical industry [10].

Scientists of the Altai State University developed a method to produce medicinal plant *P. alba* raw material grown using a hydroponics technique with clonal micropropagation [11, 12].

The goal of this study was a comparative phytochemical characteristic of the biomass of *P. alba* plant material grown in the field and using a hydroponics technique with clonal micropropagation.

MATERIALS AND METHODS

Roots and rhizomes samples of intact *P. alba* plants, grown and used in the production of ZAO Evalar (Biysk, Russia), and roots and leaves of regenerated plants, grown using hydroponics technique with clonal micropropagation in the Plant Biotechnology Department of Altai State University (Barnaul, Russia), were used as the object of the study.

Drying and grinding of plant raw materials. Before drying, the plant raw material was sorted and purified from foreign impurities and decayed parts.

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For drying, the plant raw materials, immediately after collection, were dispersed in a thin layer in accordance with the recommendations [13]. Dried medicinal raw materials should contain no more than 12–15% of hygroscopic moisture.

Air-dried samples of leaves and roots, rhizomes of *P. alba* were ground on a hand grinder to a particle size of 2–4 mm.

Samples prepared by this method were analyzed for moisture, ash, cellulose and lignin content by standard methods [14].

Extraction of plant raw material. The scheme described by V.M. Kosman et al. was used as a basis for phytochemical research [15]. According to this scheme, the extractives were extracted from the plant material by sequential treatment of the samples with various solvents: hexane, ethyl alcohol of descending concentration (96 and 40%), water and 1% sodium hydroxide solution. Extraction with hexane and alcohol solutions was carried out in the Soxhlet apparatus by the treatment of the samples with a raw material-extractant ratio of 1 : 50. The treatment with water and 1% alkali solution was carried out by holding the samples in solution at a temperature of 50–70°C, the ratio with a feed-extractant with a raw material-extractant ratio of 1 : 50.

The content of extractives extracted by different solvents from the same sample by sequential treatment was quantitatively determined after distillation of the solvent, the calculation was made taking into account the moisture of the dry extract obtained after removal of the solvent.

Identification of the main biologically active components of the tinder as P. alba was performed in accordance with conventional methods [16]. Flavonoids were identified by a qualitative cyanide reaction (reduction by zinc dust in an acid medium). The reduction of flavonoids in the presence of concentrated hydrochloric acid produces a red color.

Polysaccharides, interacting with alkali, were discolored to yellow.

Black-green coloration was observed when tanning substances of extracts interacted with iron salts.

Polyphenolic substances in the extract with ferrous sulfate produced a dark blue stain color even after the reaction with lead acetate.

Anthocyanidins in an acidic medium immediately form colored oxonium salts. The Briand test allows the detection of aglycones and/or glycosides in extracts.

Characterization of the group chemical composition of the extracts was carried out by UV spectroscopy using UV-Vis Cary 60 (Agilent Technologies, the United States) in the spectral region 210–600 nm based on the optical densities of various solutions.

Authentication of the P. alba specimens by the presence of flavonoids was carried out by TLC chromatography according to the methods implemented in pharmaceuticals [17].

The elution of the analyzed mixture was carried out using ascending technique with a mixture of solvents. The stationary phase was silica gel on an aluminum substrate. The analyzed substance was applied in an amount of 30 μL to the plate start line and elution was carried out with a mixture of solvents ethyl acetate-methanol-water-formic acid (50 : 2 : 3 : 6 by volume, respectively). The chromatogram was developed using aminoethyl diphenylborinate solution and macrogol 400, then examined in UV light.

Authentication of P. alba samples by the presence of tannins. Tannins from the samples were extracted with water and ethyl acetate, the extracts were combined and filtered through anhydrous sodium sulfate, evaporated to dryness and dissolved in 2 mL of ethyl acetate. The analysis was done by TLC using a comparison with a tracking substance (1.0 mg catechin in 1.0 mL methanol solution), the development was done with a freshly prepared 5 g/L fast blue B salt R (3,3'-dimethoxybiphenyl-4,4'-bis (diazonium) hexachlorocintate) dye solution in daylight.

All measurements were performed at least in triplicate. All calculations for the content of various substances are presented per absolutely dry substance.

DISCUSSION

In the Department of Plant Biotechnology of the South-Siberian Botanical Garden of Altai State University (ASU), the biomass of *P. alba* regenerated plants was for the first time obtained under hydroponics conditions associated with clonal micropropagation. A comprehensive study of the chemical composition of the medicinal raw material of *P. alba* (intact plants) grown in the field and used in the production of ZAO Evalar (Biysk, Russia) as well as raw materials obtained by the alternative method in the Department of Plant Biotechnology (regenerated plants) was performed in Department of Organic Chemistry of the Chemical Faculty of ASU.

Air-dried samples of the above-ground biomass, roots and rhizomes of *P. alba* were analyzed for the content of ash, moisture and high-molecular weight substances (Table 1).

The moisture values were within the permissible limits, since for most types of medicinal plant raw material the maximum moisture is usually not more than 12–15%. The moisture content of the roots of regenerated plants and intact plants was insignificantly different, while the leaves of regenerated plants were characterized by a lower moisture value, due to the peculiarities of the morphological structure of various parts of plants.

The presence of macro- and microelements in medicinal plant raw material characterizes its quality. The presence and accumulation of mineral substances

Table 1. Macrocomponent composition of *P. alba* (on absolute dry substance, a.d.s.), %

Raw material	Ash content	Moisture	Cellulose	Lignin
Roots and rhizomes (intact plants)	5.1 ± 0.2	8.5 ± 0.1	15.4 ± 0.4	40.7 ± 0.3
Roots (regenerated plants)	3.2 ± 0.1	8.1 ± 0.1	4.3 ± 0.2	37.0 ± 0.3
Leaves (regenerated plants)	2.3 ± 0.1	5.1 ± 0.2	2.5 ± 0.3	36.8 ± 0.1

Table 2. The content of extractives of *P. alba* during sequential extraction

Solvent	In the roots and rhizomes of intact plants	In roots of regenerated plants	In leaves of regenerated plants
Hexane	1.7 ± 0.1	0.9 ± 0.1	0.3 ± 0.2
96% ethanol solution	5.6 ± 0.2	4.4 ± 0.4	2.5 ± 0.2
40% ethanol solution	5.2 ± 0.2	4.2 ± 0.2	1.7 ± 0.3
Water	1.9 ± 0.3	1.0 ± 0.2	0.3 ± 0.2
1% NaOH solution	0.9 ± 0.2	0.7 ± 0.1	0.3 ± 0.1
Total content	15.3	11.2	5.1

depends on the soil and the growth conditions of medicinal plants and determined by the amount of ash. The amount of ash in the medicinal raw material usually varies widely and depends on many factors, including the method of collection and drying conditions. As was shown by the authors of the study [15], the content of total ash in the roots and rhizomes of three-year old *P. alba* samples is higher (5.8%) than in the four-year old (5.0%) samples. Samples of intact plants we studied were insignificantly different by content of ash from those described in the literature. *P. alba* samples, obtained using the clonal micropropagation method, differ significantly in the content of ash-forming substances. We also found that individual parts of *P. alba* samples grown under clonal micropropagation conditions had different ash content. The author [18] points out that greater amounts of mineral substances were detected in the subterranean part of the plants than in the aboveground, in addition, the accumulation of certain mineral substances is associated with the growing season and it was maximal during the flowering phase. Thus, low values of ash content in samples of regenerated plants were associated with insignificant age of these plants (two months) and vegetative development before flowering.

An interesting aspect is the ratio of high-molecular-weight components in the compared plant raw material. Thus, the content of cellulose in the roots and rhizomes of intact plants was 15.4%, in the roots of regenerated plants it was much lower, 4.3%. The lower content of cellulose in regenerated plants is probably because their age was only two months, and intact plants were about four years old. Therefore, the biomass of young plants prior to the harvesting of plant raw materials consisted of a larger number of living tissues (parenchymal cells with a thin primary wall, where complex chemical processes and metabolism took place), and as the age of the plant increased, the accumulation of prosenchym cells containing mainly cellulose fiber occurred. The content of cellulose in the leaves of regenerated plants was even lower, 2.5%, since the green parts of plants have various functions of living plants and very diverse composition of substances and a low content of high-molecular-weight polysaccharides. The content of lignin (as a sum of condensed phenolic substances) in the samples was not significantly different: in the roots and rhizomes of intact plants it was 40.7%, in the roots and leaves of regenerative plants, 37.0 and 36.8%, respectively, which most likely, indicates the completion of the process of lignin biosynthesis.

IR spectroscopic analysis of lignins isolated from the investigated samples of plant raw materials (Fig. 1) demonstrates the identity of their structure.

Lignin is synthesized in the formed plant cells during the early stages. In some plants, on the 2nd–3rd day, the cell walls begin to give a qualitative reaction to lignin, a red staining with a hydrochloric acid solution of floroglucinol. Regardless of the growing conditions of *P. alba*, the lignin structure of the cell wall remains unchanged and a hereditarily fixed trait.

The most important components of plant raw materials are extractives extracted by various solvents. According to the study [18], the content of extractives in *P. alba* can reach 17% in the underground part (during the flowering phase), 6% in the aerial part of the plant.

The quantitative content of extractives (in the form of a dry extract obtained after removal of the solvent) recovered by different solvents from the same sample during sequential treatment is presented in Table 2.

Because of consecutive continuous treatment of samples by solvents, a more complete extraction of

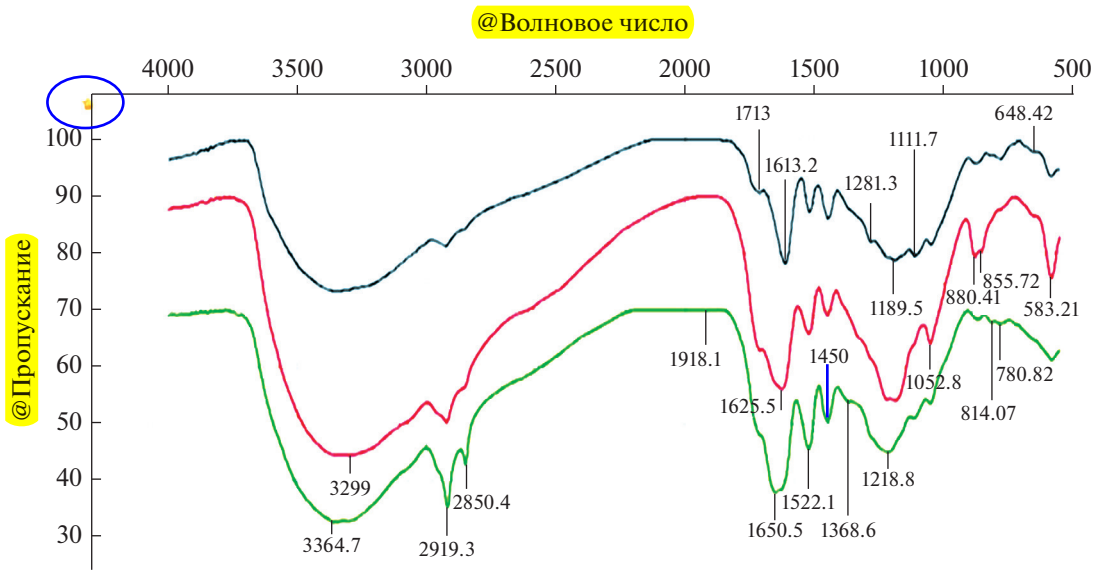

Fig. 1. IR spectra of lignin from *P. alba*: *1*–roots and rhizomes of intact plants; *2*–roots of regenerated plants; *3*–leaves of regenerated plants.

extractives was achieved. The total content of extractives in the roots and rhizomes of intact plants was 15.3%, in the roots of regenerated plants, 11.2%, and in the leaves of regenerated plants, 5.1%, which does not contradict the known data [18].

The content of extractives extracted by hexane (lipids) was minimal, from 0.3 to 1.7%, the extract had a light yellow color.

The highest amount of extractives (yield from 2.5 to 5.6%) was extracted with 96% ethanol.

The content of extractives extracted with 40% ethanol was from 1.7 to 5.2%. Alcohol extracts of roots and rhizomes had a dark yellow color in extracts of leaves had green color. Polyphenol substances, phenolic acids, flavonoids, anthocyanidins were extracted with ethanol.

The content of extractives extracted by water (polysaccharides, amino acids and tannins) was from 0.3 to 1.9%, the color of the extracts was dark brown.

The content of extractives extracted with 1% solution of NaOH (tannins) was from 0.3 to 0.9%, the color of extracts was dark red [15].

Despite the lower total content of extractives in the roots and leaves of regenerated plants, the ratio of different groups of substances in extracts differs slightly from that of intact plants (Fig. 2).

In extracts from samples of regenerated plants, lower amounts of substances soluble in hexane (lipids) and water (amino acids and polysaccharides) were detected. In a 1% aqueous solution of NaOH, a comparable amount of substances (tannins) was dissolved. This was due to a decrease in the number of conducting tissues in the phloem of 4-year-old intact plants and an increase in the biomass of the storage cells, accumulating lipids, amino acids and tannins. At the same time, extracts from the roots of regenerated plants contained more substances soluble in 96% and 40% ethanol (polyphenolic substances, flavonoids, anthocyanins). Extracts from the leaves of regenerated plants were superior to all other samples by the content of substances soluble in 96% ethanol (polyphenolic substances). The authors of the study [19] noted an increase in the total amount of phenolic substances in calli in comparison with intact plants and an uneven distribution of these substances in the callus of leaf and root origin. Thus, extracts from regenerated plants were slightly enriched with biologically active phenolic substances.

The main groups of biologically active substances were qualitatively identified in the extracts (Table 3).

Flavonoids, polysaccharides, tannins, anthocyanidins, glycosides and aglycons were detected in all studied *P. alba* samples regardless of the preparation method by qualitative reactions. The identity of the group composition of biologically active substances of intact plants and regenerated plants was established.

The extractives from the roots and roots of *P. alba* intact plants, the roots and leaves of regenerated plants extracted by various solvents were analyzed by UV spectroscopy. The following solvents were used: 96% and 40% aqueous solutions of ethanol, water, and 1% NaOH solution (Table 4). The extracts of leaves, roots and rhizomes of *P. alba* contained mainly flavonones and flavonols [20], which was confirmed by the position of the band in the 275–280 nm region and the absence of bands at higher wavelengths in the UV spectra (see the electronic appendix , Fig. 1).

The analysis of spectra showed that aqueous and hydroalcoholic extracts contain substances of a similar

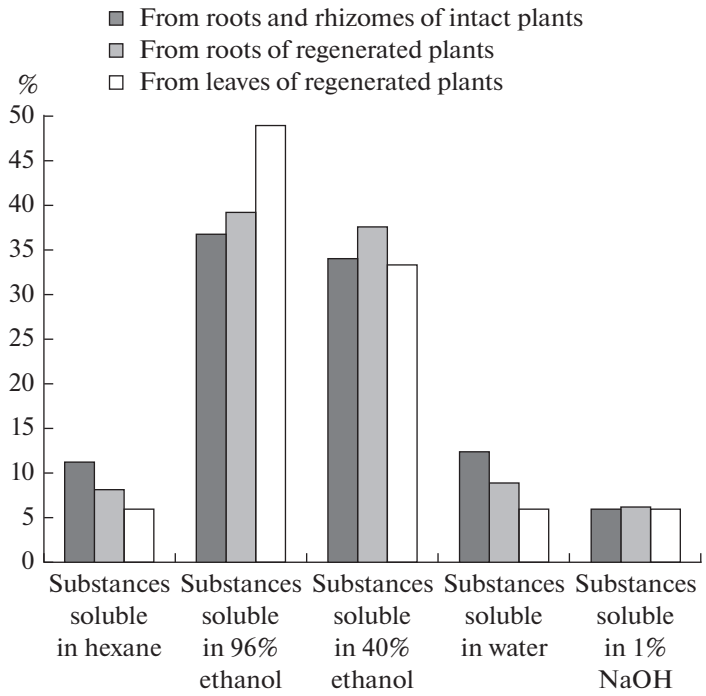

Fig. 2. Accumulation of the main groups of biologically active substances in intact and regenerated plants.

nature with absorption maxima in the 280 nm region, which is characteristic for phenolic substances (flavonoids) [20].

The study [21] describes the UV spectrum of apigenin, which is part of the flavonoids of *P. alba* (Fig. 3A). According to the position of the maximum of this spectrum, the authors proposed to standardize medicinal raw materials for the content of flavonoids. The UV spectrum of the water–alcohol (40%) extract from the roots of the regenerated *P. alba* plants (Fig. 3B) was identical to the position of the maximum described in the literature. Thus, we can conclude that regenerated plants, even with an insignificant introduction periods (2 months), contain biologically active substances from the flavonoid group.

Authentication of P. alba samples

Flavonoids are the main components that have therapeutic activity. They are not toxic to humans via any form of administration. Many flavonoids have P-vitamin activity, reduce the fragility of the blood capillaries (rutin), enhance the effect of ascorbic acid, and have a sedative effect. This group of substances attracts attention of scientists due to diverse biological activity and extremely low toxicity. In recent years, reports about the antitumor effects of flavonoids appeared. Therefore, one of the important tasks in the production of drugs is authentication of the presence of flavonoids in medicinal raw materials.

Recently, chromatography on paper and in a thin layer of sorbent were widely used for the detection of

Table 3. Characteristics of the group composition of *P. alba*

Group of substances	In the roots and rhizomes of intact plants	In roots of regenerated plants	In leaves of regenerated plants
Flavonoids (flavones, flavonols and flavonones) – cyanidin test	+	+	+
Polysaccharides	+	+	+
Tannins	+	+	+
Polyphenol substances	+	+	+
Anthocyanidins	+	+	+
Glycosides	+	+	+
Agclycons	+	+	+

Table 4. Absorption bands in UV spectra of biologically active substances of *P. alba*

Extracting agent	Sample	Adsorption maximum
96% aqueous ethanol solution	Roots and rhizomes of intact plants	279.5 nm, weakly expressed, unintensive
	Roots of regenerated plants	279.5 nm, weakly expressed, unintensive
	Leaves of regenerated plants	Absent
40% aqueous ethanol solution	Roots and rhizomes of intact plants	280.0 nm, expressed, intensive
	Roots of regenerated plants	280.0 nm, expressed, intensive
	Leaves of regenerated plants	280.0 nm, expressed, intensive
Water	Roots and rhizomes of intact plants	280.0 nm, weakly expressed, unintensive (in the form of shoulder)
	Roots of regenerated plants	277.5 nm, expressed, intensive
	Leaves of regenerated plants	279.5 nm, weakly expressed, unintensive
1% NaOH solution	Roots and rhizomes of intact plants	279.5 nm, weakly expressed, unintensive (in the form of shoulder)
	Roots of regenerated plants	280.0 nm, weakly expressed, unintensive (in the form of shoulder)
	Leaves of regenerated plants	280.0 nm, weakly expressed, unintensive (in the form of shoulder)

flavonoids. Authentication of the studied samples of *P. alba* was carried out by TLC chromatography according to the methods implemented in pharmaceutical industries.

The results of TLC (see the electronic appendix of Fig. 2) showed that in extracts obtained from the roots and rhizomes of intact plants, roots and leaves of regenerated plants using a 96% solution of ethanol, there are four identical groups of substances with Rf equal to 0.58, which corresponds to cinaroside, 0.61 to rutin, 0.93 to apigenin, and 0.97 to quercetin.

Roots of regenerated plants contain flavonoids identical in composition to flavonoids of roots and rhizomes of intact plants. Based on the results of TLC, it is shown that the leaves of regenerated plants contain the composition of flavonoids identical to the roots and rhizomes. Similar results were obtained by the author [18] studying the aerial part of *P. alba*, cultivated on the territory of the Central Botanical Garden of the NAS of Belarus. The aerial part (leaves) of *P. alba* can also be used for the manufacture of medicinal products, since it contains similar groups of biologically active substances.

Thus, the authenticity of the *P. alba* samples was confirmed by TLC chromatography: biomass of *P. alba* samples grown by the clonal micropropagation corresponded to the biomass of *P. alba* samples grown under natural conditions by qualitative composition.

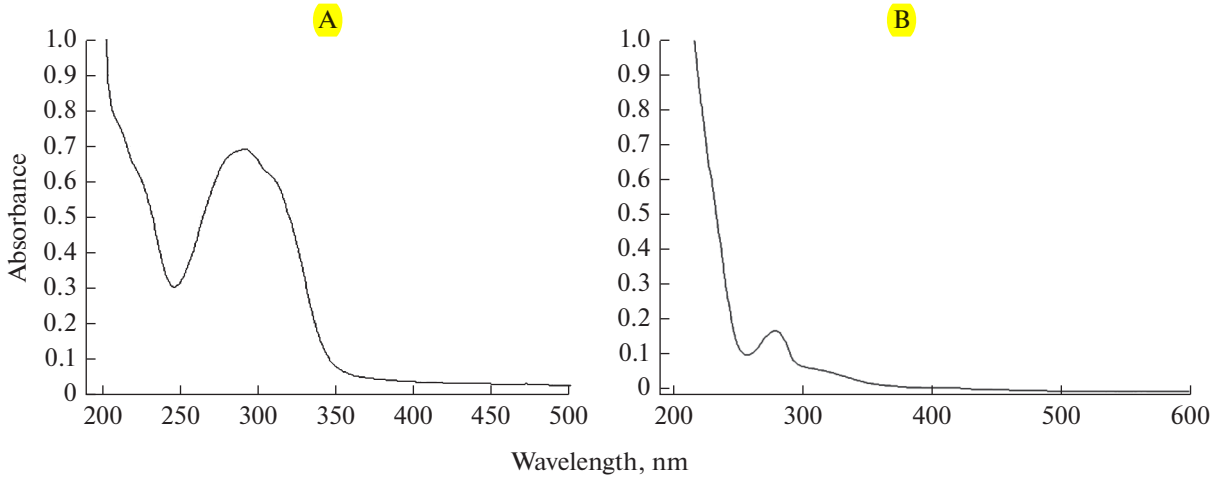

Fig. 3. UV spectra of apigenin (A) according to [21] and water-alcohol extracts from the roots of *P. alba* regenerated plants (B).

Tannins are a group of phenolic substances of plant origin containing a large number of OH groups. In medicine, tannins are used as astringent drugs, antidotes (for poisoning with lead salts, mercury, etc.), antidiarrheal, hemostatic and antihemorrhoidal drugs; therefore, their presence is necessary in medicinal raw materials. Therefore, the establishment of authenticity for tannins is one of the mandatory procedures.

Tannins from the studied samples were extracted with water and ethyl acetate, and then analyzed by TLC (see the electronic appendix of Fig. 3).

An insignificant quantity of tannins was present in roots and in leaves of regenerated plants, in comparison with the standard and intact plants.

CONCLUSIONS

The analysis of the macrocomponent composition and extractive substances of *P. alba* intact plants (the introduction period was four years) and the regenerated plants obtained by the clonal micropropagation method and grown under hydroponics conditions in the Department of Biotechnology of the Altai State University (the introduction period was two months) was performed. The investigated samples of regenerated plants contained all groups of biologically active substances characteristic of this type of plant raw material. It was shown that the ratio of BAS groups in the extracts of the samples were not significantly different, and in extracts of regenerated plants, a greater amount of substances soluble in 96% and 40% ethanol was found. The method of UV spectroscopy revealed the presence of flavonoids, and TLC revealed the presence of cinaroside, rutin, apigenin, quercetin in ethanol extracts from roots and rhizomes of intact plants, roots and leaves of regenerated plants. Thus, the identity of the phytochemical composition of plant raw materials obtained by the method of clonal micropropagation and grown under hydroponic conditions for two months was shown.

Further study of the quantitative ratio of individual biologically active substances in extracts of regenerated plants will allow us to evaluate the application potential of this type of medicinal plant raw material as one of the most important sources of medicinal and prophylactic means of modern medicine.

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