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Implementation Chemical Research of the Woolly Erva Plants Growing in the Republic of Uzbekistan

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Abstract. This research paper presents the results of studies of flavonoids isorhamnetin–3–O–β–D - glycoside, narcissin (isorhamnetin-3-O-rutinoside) with a strong diuretic effect, as well as derivatives of hydroxycinnamic acid — vanillic, ferulic, lilac acids. For the first time, isorhamnetin –3–O–β–D – glycoside and ferulic acid were isolated from the herb Aerva lanata. And also the polysaccharide composition of the herva woolly herb was studied using BH and GLC methods.
Keywords: Erva woolly, heptaacetate, isorhamnetin –3–O–β–D – glycoside, sitosterol.

Introduction

In the search for harmless diuretics that could be used when the appointment of synthetic diuretics is contraindicated, medicinal plants with a diuretic effect deserve special attention in folk medicine. Medicinal plants in folk medicine are a valuable and inexhaustible source for obtaining new effective medicinal preparations.

Erva woolly (half-floor) - Aerva lanata Juss. belongs to the amaranth family - Amaranthaceae. In the traditional medicine of India and Ceylon, the herb Aerva lanata is used as a sedative, diuretic and antihelminthic. Information on chemical studies of plants of this genus is scarce. The literature provides data on the study of flavonoids of three Aerva species: four kaempferol glycosides were isolated from the herb Aerva tomentosa. From the herb Aerva javanica-kaempferol-3-galactoside and kaempferol-3-rhamnogalactoside, and from the roots of Aerva persica-chrysin-7-O-galactoside and 7,4[1]-dioxyflavanone-8-C-galactoside. It was also reported about the composition of amino acids in Aerva pseudotomentosa, the release of ecdysone from the roots of Aerva tomentosa, saturated fatty acids and triterpenes from the herb of this plant; A number of higher saturated hydrocarbons, triterpenes and sterols, have been isolated from the herb Aerva javanica.

Earlier, α - sitosterol, β-amyrin, β-sitosteryl-palmitate were obtained from the essential extract of the herb Erva woolly, and it was shown that the alcoholic extract contains mainly inorganic salts and free sugars.

Methods of research

Research the qualitative composition, the dried and crushed aerial part (1.3 kg) of the woolly erva, harvested during the flowering period in August 1999 (Middle-Chirchik region, Tashkent region, Republic of Uzbekistan), was extracted 3 times with purified water at a ratio of 1:20 (raw material-extractant) by heating at a temperature of 100 ºC for 20 minutes. The aqueous extract was

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concentrated in a vacuum (to 1.0 L), treated with n-butanol 10 times, 500 ml each, the solvent was separated by distilling off the solvent, 14.0 g of a butanol thick fraction was obtained, which was chromatographed on a column (110 × 2.6 cm) with silica gel (1:20, 280 g), eluting successively with chloroform and systems 1-3. Fractions were collected in 300 ml. 136 factions were collected. Of these, 85-92 and 114-125 fractions gave positive reactions to flavonoids. At the same time, the well-known reactions of the Synod (Mg + HCl) and with an alcohol solution of $FeCl_3$.

System 2 isolated 0.085 g of isorhamnetin –3–O–β–D –glycoside from the butanol fraction 85–92. When the column was eluted with system 3, 0.15 g of narcissin was isolated from fraction 114–125.

Methods of research

Crystalline substance of the composition C_{22} H_{22} O_{12}, so 162-164 ^{0}C UV spectrum: λ max (ethanol): 257, 267, 360 nm typical of flavonol derivatives.

The IR spectrum of V_{max} (KBr) contains absorption bands of hydroxyl groups (3394 cm^{-1}), methoxyl group (2935 cm^{-1}), γ–-pyrone carbonyl (1665 cm^{-1}), aromatic C = C bonds (1611, 1578 , 1513 cm^{-1}) and C-O vibrations of glycosides (1076, 1025 cm^{-1}) (Fig. 1.).

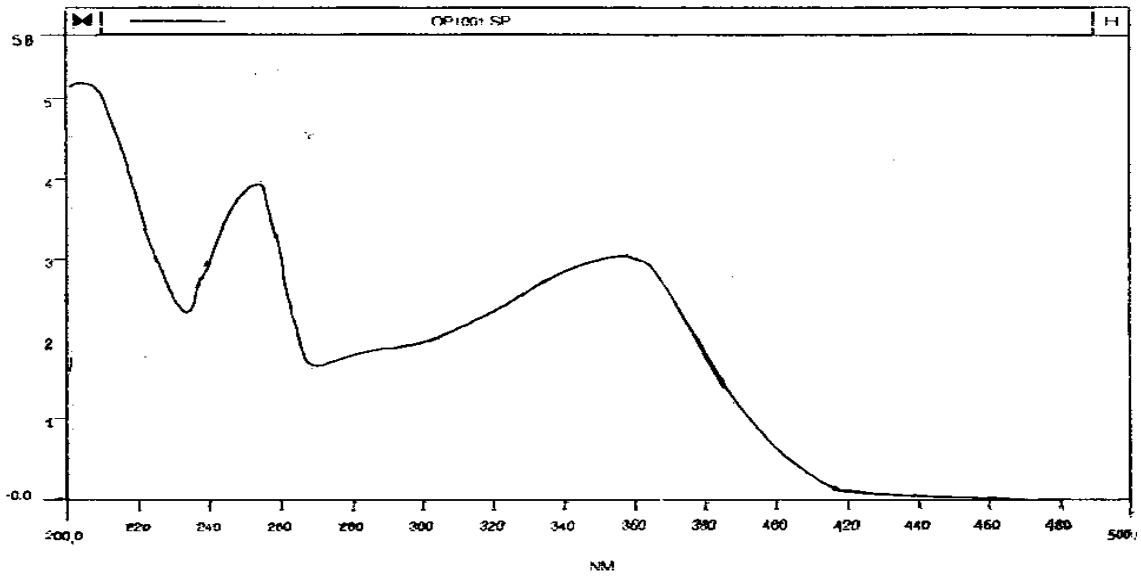

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Fig. 1. UV spectrum of isorhamnetin–3–O–β–D–glycoside

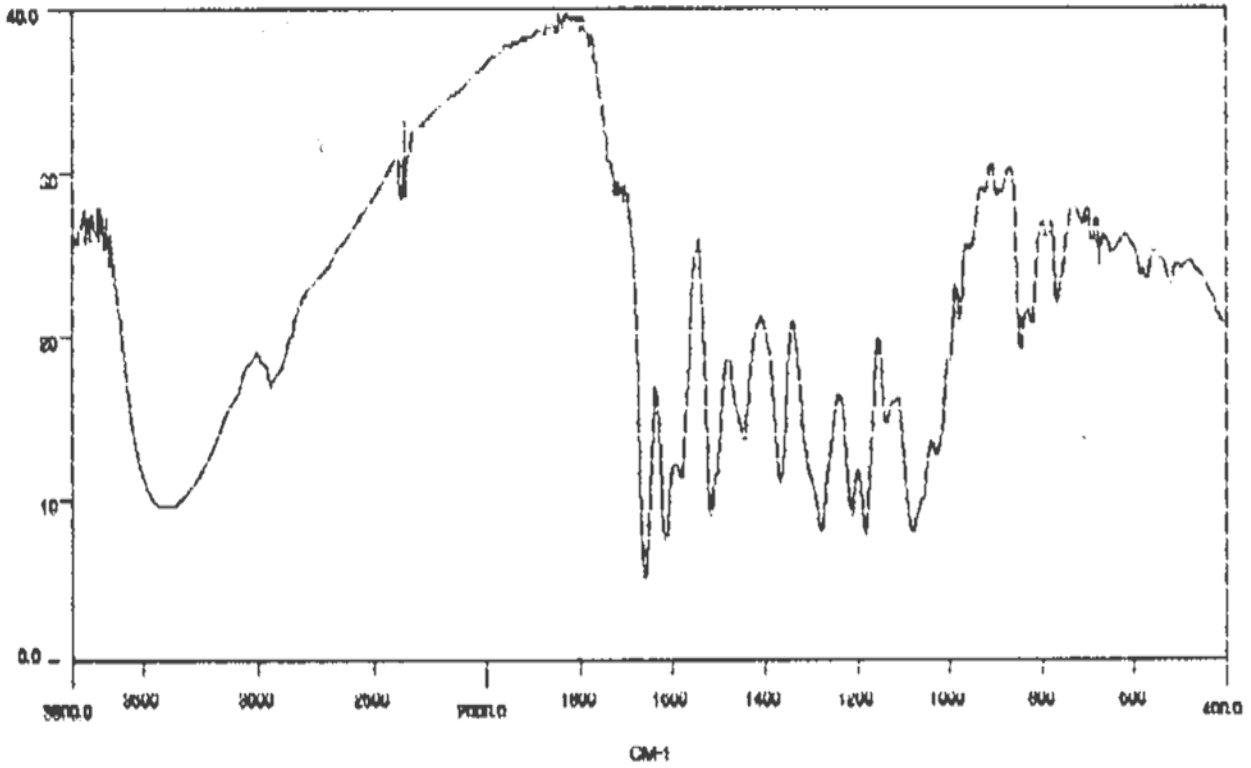

Fig. 2. IR spectrum of isorhamnetin–3–O–β–D– glycoside

In the PMR spectrum of compound 4, the signals of the anomeric proton (6.00 ppm 1N.f J = 6.5 Hz) and other protons of the carbohydrate residue (3.50-4.52 ppm) of the methoxyl group of the flavonol nucleus (3.82 ppm 3N. s.), as well as signals of five aromatic protons H-6 (6.48 ppm 1H, d, J = 2.0 Hz), H-8 (6.65 ppm, 1H d, J = 2.0Hz), H- 51 (7.12 ppm, 1N.f J = 8.0 Hz), H-61 (7.65 ppm, 1H, dd, J = 2.0 and J = 8.0 Hz) and H-21 (8.15 ppm .1N.d, J = 2.0 Hz).

Therefore, the substance in question is a glycoside. To study the aglycone and sugar parts of compound 4, acid hydrolysis of the chemical transformation of isorhamnetin3–O–β–D–glycoside was carried out.

Glycoside (18 mg) was hydrolyzed in 15 ml of 5% hydrochloric acid solution for 3 hours in a boiling water bath. The precipitated aglycone precipitate was filtered off after cooling the mixture and recrystallized from ethanol. Received 7 mg of isorhamnetin (5) [3,5,7,41 - tetrahydroxy-31-methoxyflavone (λ max 255, 266, 372 nm)] so pl. 305 - 307 0C composition C16H12O7, (M + 316). D-glucose was found in the hydrolyzate by the BH method in system 4. The identification of D-glucose was carried out in comparison with a standard substance and a characteristic reaction with acidic aniline phthalate when spraying a chromatogram. To detect hydroxyl groups, an acetylation reaction was carried out. As a result, isorhamnetin 3–O–β–D–glycoside heptaacetate was obtained.

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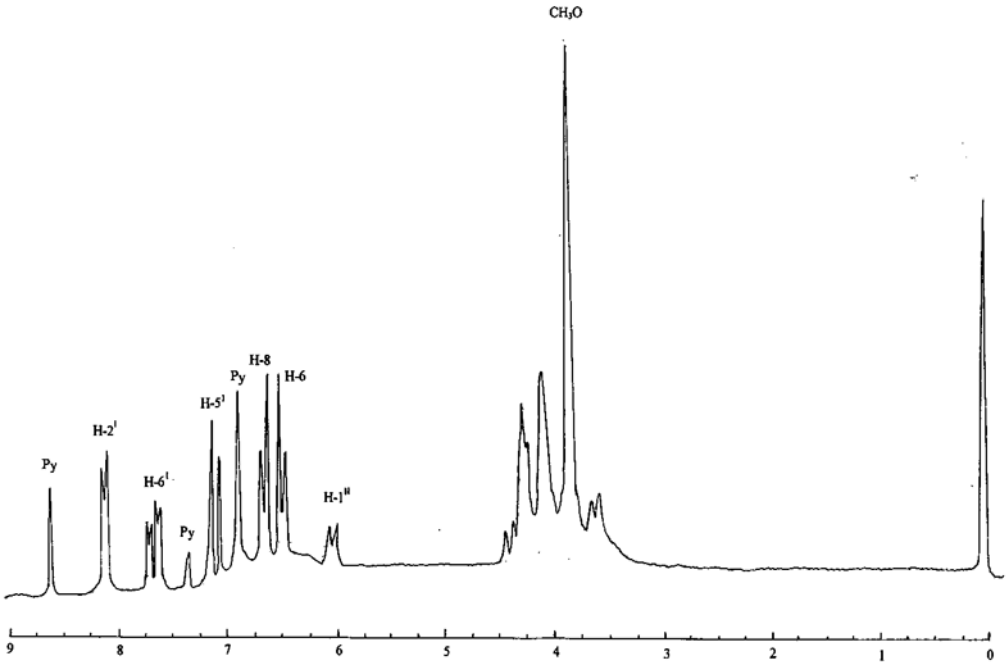

Fig. 3. PMR spectrum of isorhamnetin-3-O-□-D-glycoside

The place of attachment of the carbohydrate residue to the 3-OH group of the aglycone was established by studying the UV spectra of the glycosides and its aglycone.

In the UV spectrum of aglycone 5 (max, 372 nm), in contrast to glycoside 4 (max, 360 nm), in band I, a hypsochromic shift of absorption maxima by 12 nm is observed, which indicates glycation of the 3-OH group of aglycone.

In the PMR spectrum of compound 4, the signal of the anomeric proton of D-glucose appears at 6.00 ppm. in the form of a doublet with a CSCR of 6.5 Hz. This indicates the glycosidic bond of the carbohydrate residue with aglycone.

The glycoside (15 mg) was dissolved in a mixture of 1 ml of pyridine with 3 ml of acetic anhydride and left for 4 hours at room temperature. On adding ice water to the mixture, a precipitate formed, which was sucked off and recrystallized from ethanol. We obtained 10 mg of heptaacetate (6) with the composition C36H36O19; along with the peak of the molecular ion m / z 772, there are intense peaks of fragment ions of the teraacetylhexose residue with m / z M + 331, 329, 271, 169. Therefore, compound 4 has the structure isoramnetin 3–O–β–D – glucopyronoside.

To identify narcissin, we also studied the melting point, UV–, IR–, and PMR spectra, carried out acid hydrolysis, as well as acetylation and methylation of compound 7 with the composition C28 H32 O16 with mp. 177-179 0C. UV spectrum of substance 7: max (ethanol): 254, 265 PL, 305 PL, 356 nm. characteristic of flavonol derivatives, and the V max (KBr) IR spectrum contains absorption bands of hydroxyl groups (3370 cm^1), methoxyl group (2928 cm^1), карбон-pyrone carbonyl (1656 cm^1), aromatic C = C bonds (1608, 1508 cm^1) and CO vibrations of glycosides (1075, 1017 cm^1).

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Chromatographic mobility, PMR spectrum data, as well as the presence of absorption bands of C – O glycosides in the IR spectrum indicate the glycosidic nature of the compound under study.

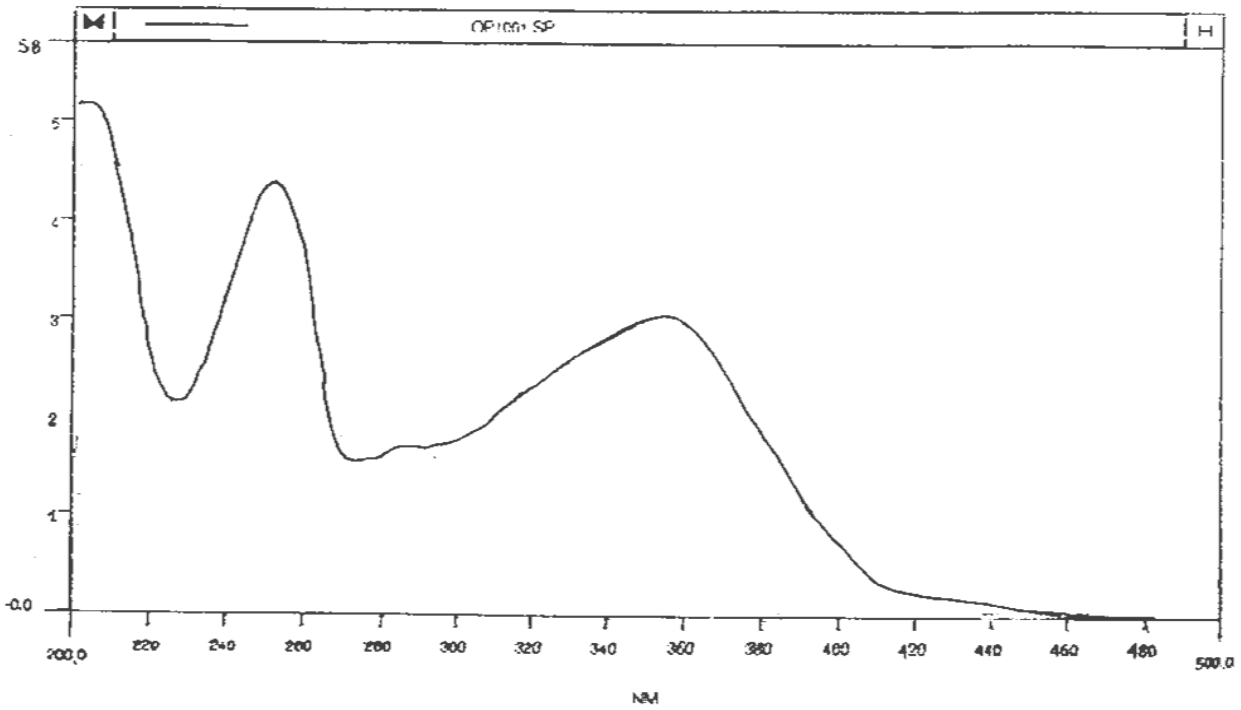

Fig. 4. UV spectrum of narcissin (isorhamnetin-7-O-rutinoside)

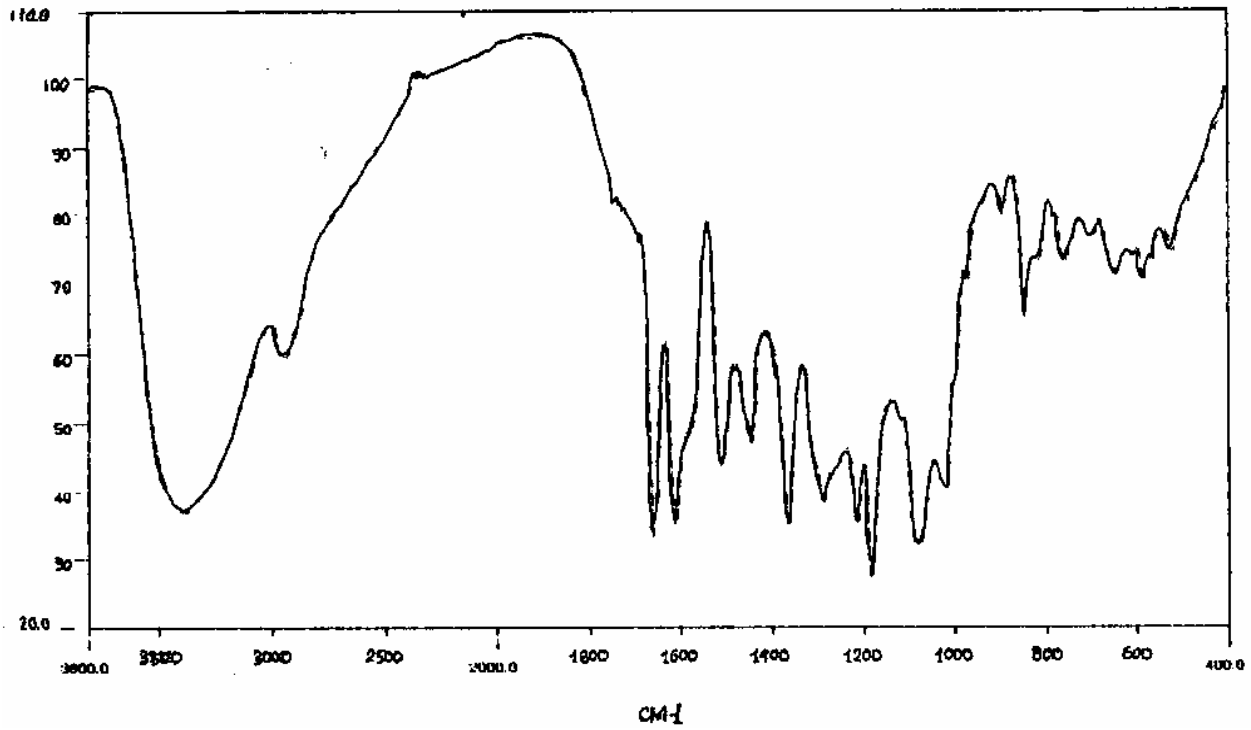

Fig.5. IR spectrum of narcissin (isorhamnetin-7-O-rutinazide)

In the PMR spectrum of compound 7, the signals of anomeric protons of D-glucose (5.78 ppm, 1H d, J = 7.0, Hz), L-rhamnose (4.80 ppm, 1 N. br .c) the methyl group of L-rhamnose (1.35

ppm 3H J = 6.0 Hz) and other protons of the carbohydrate residue (3.50-4.50 ppm), the methoxyl group of the flavonol nucleus (3, 80 ppm, 3 Hs), as well as signals of five aromatic protons H-6 (6.56 ppm, 1 ppm J = 2.5 Hz), H-8 (6.73 ppm ., 1 N. d. J = 2.5 Hz), H-51 (7.25 ppm 1 N. d. J = 8.0 Hz), H-61 (1 N. dd. J = 2 , 5 and 8.0 Hz) and H-21 (1 n.d. J = 2.5 Hz)

The processes of hydrolysis with hydrochloric and acetic acid, acetylation and methylation were carried out to study the aglyconic and sugar parts of the isolated substance, to detect hydroxyl groups and the place of attachment of the sugar part (Scheme 3.4).

Narcissin (25 mg) was hydrolyzed in 20 ml of 5% aqueous methanol solution of hydrochloric acid for 4 hours in a boiling water bath. The precipitated aglycone precipitate was filtered off and recrystallized from ethanol. Complete acidic hydrolysis of the substance under consideration led to the preparation of aglycone (5) with the composition C16H12O7 M + 316 identified by its physicochemical properties and UV-, PMR-, and mass spectrum spectra with known 3, 5, 7, 41-terahydroxy -31-methoxy flavone (isorhamnetin) (5). In the hydrolyzate of the oligosaccharide moiety of glycoside 7 by the HD method, D-glucose and L-rhamnose were found in system 4.

Narcissin (7) (30 mg) was hydrolyzed with 10% acetic acid solution for 3 hours in a water bath. The reaction mixture was diluted with water and exhaustively extracted with ethyl acetate.

The ethyl acetate extract was washed with water and evaporated. The residue was chromatographed on a silica gel column in system 2. 6 mg of isorhamnetin 3–O–β–D – glycoside (4) of composition C22 H22 O12 with mp 161–163 0C was isolated. UV spectrum: max 257, 267, 360 nm.

Narcissin (7) (20 mg) was dissolved in a mixture of 1 ml of pyridine with 3 ml of acetic anhydride and left for 4 hours at room temperature. On adding ice water, a precipitate formed, which was filtered off and recrystallized from ethanol. This gave 15 mg of nonaacetate (8) with the composition C46 H50 O25 m.p. 117-119 0C, mass spectrum m / z: M + 1002, 561, 273, 213, 153, etc.

To a solution prepared from 20 mg of glycoside in 10 ml of dry dimethyl sulfoxide, 20 mg of sodium hydride was added in small portions, the mixture was stirred for 1 hour at room temperature. Then to the reaction mixture was added dropwise 4 ml of methyl iodide and stirred for another 3 hours. The reaction mixture was poured into 100 ml of 2% sodium hyposulfite solution, and the formed permitylate was recovered with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate. After distilling off the solvent and drying, 12 mg of the methylation product was obtained, in the IR spectrum of which there are no absorption bands from hydroxyl groups.

The methylation product (12 mg) was dissolved in 6 ml of a 6% methanol solution of hydrogen chloride and boiled in a water bath for 4 hours. The reaction mixture was neutralized with silver carbonate, the formed precipitate was filtered off, the filtrate was evaporated to dryness. In the residue by TLC, 2,3,4-tri-O-methyl-D-glucose and 2,3,4-tri-O-methyl-L-rhamnose were identified.

Acetylation of glycoside 7 with acetic anhydride in pyridine gave a non-acetyl derivative 8 with the composition C46H50O25, in the mass spectrum of which, along with the peak of the molecular ion with m / z 1002, there are intense biosis with m / z 561 and terminal rhamnose with m / z 273, 213 and 153.

Consequently, compound 7 is a bioside of isorhamnetin. The sequence of attachment of monosaccharide residues in glycoside 7 was confirmed by partial acid hydrolysis. As a result, we obtained progenin 4, which decomposes upon hydrolysis into isorhamnetin and glucose.

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Therefore, glucose in compound 7 is directly related to aglycone.

The obtained progenin 4 was identified with isorhamnetin 3–O–β–D glycoside by physicochemical constants and by comparison with a known sample. The place of attachment and the size of oxide rings, monosaccharides were determined by the method of methylation according to Hakomori followed by TLC in the presence of notorious witnesses, 2, 3, 4-tri-O-methyl-L-rhamnopyranoside was identified. Thus, in glycoside 7, rhamnose is attached to glucose by a 1 and 6 bond.

In the UV spectrum of aglycone 5 (max, 372 nm), in contrast to glycoside 7, (max, 356 nm), in band I, a hypsochromic shift of absorption maxima by 16 nm is observed, which indicates glycation of the 3 – OH group of aglycone.

In the PMR spectrum of glycoside 7, the signal of the anomeric protons of D-glucose and L-rhamnose resonates in the form of a doublet with a coupling constant of 7.0 Hz and a broadened singlet, respectively, from which follows the configuration of the anomeric center of the D-glucose residue and configuration of the L-rhamnose residue.

Thus, flavonoglycoside 7 has the structure 3 – O– [L – rhamnopyranosyl– (1 and 6) 3–O–β–D – glycopyranoside] –5,7,41 – trihydroxy – 31-methoxyflavone or isorhamnetin – 3 –O – rutinoside.

Thus, as a result of the studies carried out to study the flavonoid composition of woolly erva, the following were isolated: isorhamnetin 3–O–β–D -glycoside and narcissin (isorhamnetin-7-O-rutinoside), the latter of which has a strong diuretic effect.

Isorhamnetin-3–O–β–D -glycoside from the aerial chatsi of the Herva woolly herb was isolated for the first time.

Along with flavonoids, according to literature data, woolly erva also contains oxycinnamic acids. Therefore, further we carried out research on the study of oxycinnamic acids of the woolly erva.

When the column was eluted with system 1, 0.09 g of vanillic acid was isolated from fraction 45-50, and 0.1 g of lilac acid from fraction 56-61. When the column was eluted with system 2, 0.07 g of ferulic acid was isolated from fraction 72-79, which were subsequently identified by physicochemical methods.

Crystalline substance of the composition C8H8O4 (M + 168) with so pl. 212-213 ºC, UV - spectrum max (ethanol) 218, 234 pl, 260, 280 pl. 291 nm. UV spectrum is typical for aromatic acids.

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Fig. 6. UV - spectrum of vanillic acid.

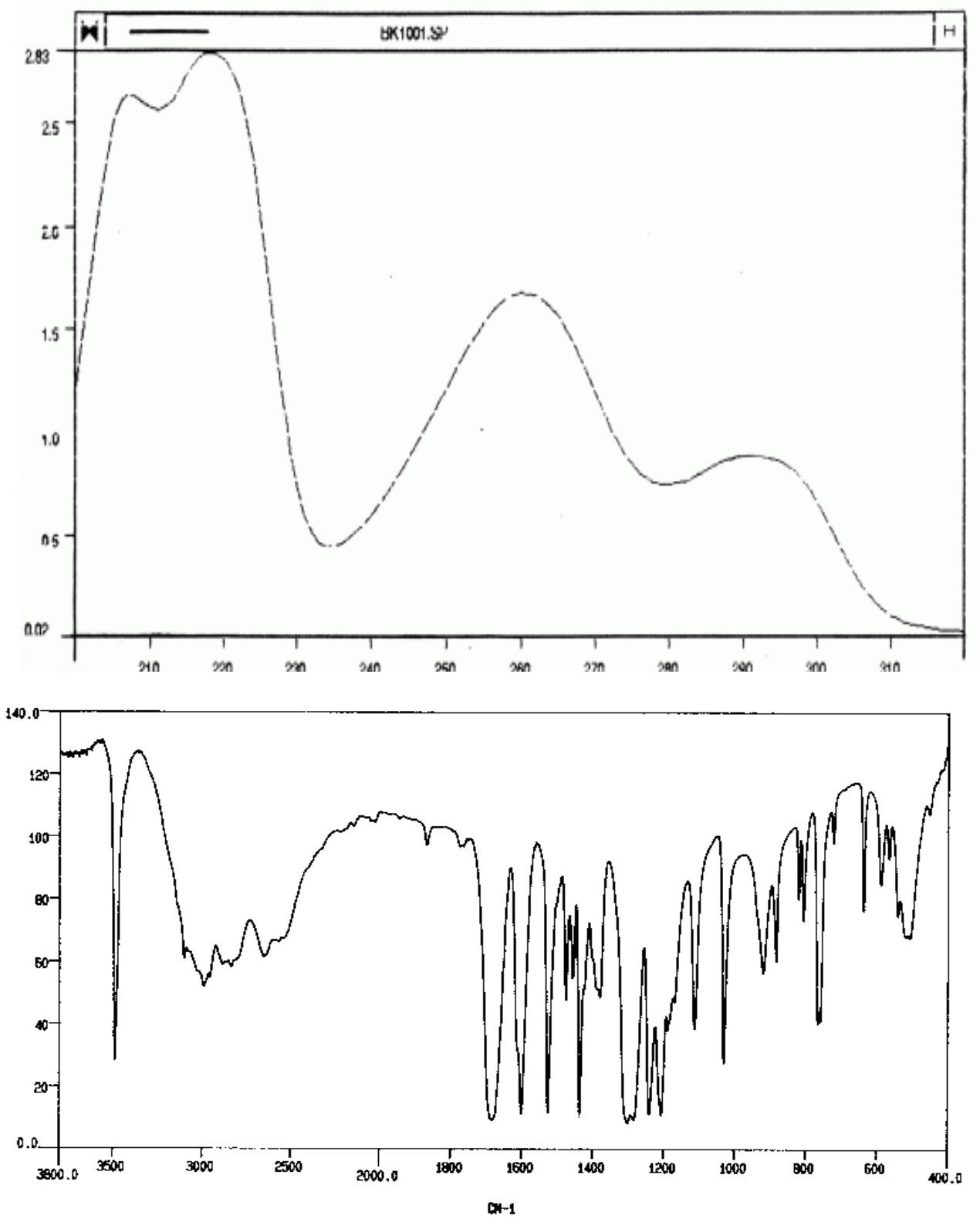

Fig. 7. IR spectrum of vanillic acid.

The V max (KBr) IR spectrum of compound 1 contains absorption bands of hydroxyl groups (3486-3000 cm^{-1}), a methoxyl group (2836 cm^{-1}), a carboxyl group (1682 cm^{-1}), as well as aromatic C = C bonds (1599, 1524cm^{1}).

In the PMR spectrum of compound 1 (Py – d5), the signals of the proton of the methoxyl group (3.63 ppm in the form of a 3H singlet), H– 5 (7.19 ppm in the form of a 1H doublet with a coupling constant J = 8.5 Hz), H -2 (7.97 ppm as a 1H doublet with an SSCR J = 2.0 Hz) H-6 (8.07

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ppm as a 1H doublet of doublets with an SSCR J = 2.0 Hz and J = 8.5 Hz) and 4-OH (10.30 ppm as a 1H broadened singlet).

Results

Crystalline substance of composition C9 H10 O5 m.p. 202 - 203 0C. UV spectrum max (ethanol) 217, 239 pl., 273 nm., Typical for aromatic acids.

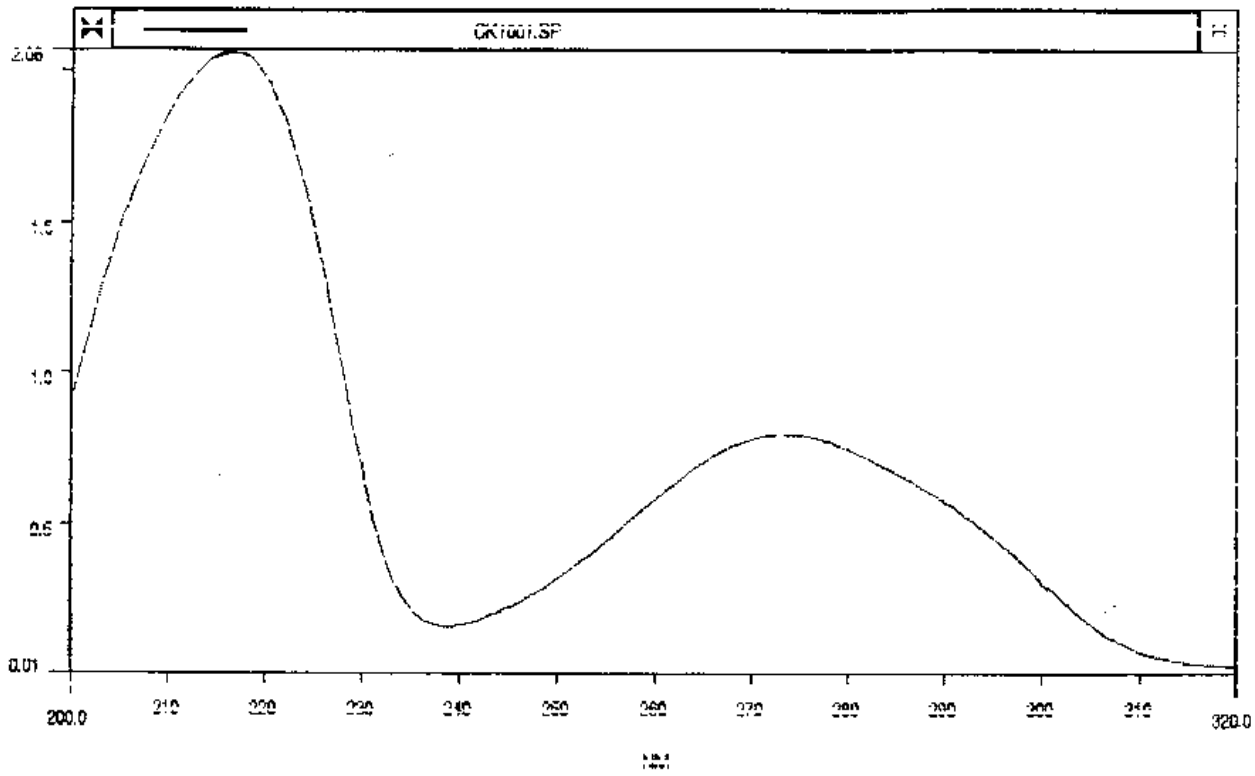

Fig. 8. UV spectrum of lilac acid

The V max (KBr) IR spectrum of compound 2 contains absorption bands of hydroxyl groups (3475-3140 cm-1), a methoxyl group (2835 cm-1) and a COOH group (1680 cm-1), as well as aromatic C = C bonds (1593, 1520 cm-1).

In the PMR spectrum of compound 2 (Py – d5), the signals of the proton of two methoxyl groups (3.70 ppm in the form of a 6H singlet) H-2, H-6 (7.82 ppm in the form of a 2H singlet) and 4-OH (9.75 ppm as a 1H broadened singlet).

Mass spectrum 2 contains the peaks of ions with m / z 198 (M +, 100%), 183 (83), 181 (27), 155 (27), 154 (8), 153 (16), 137 (23), 127 (79).

Therefore, compound 2 contains two methoxy, one hydroxyl and one carboxyl group and is a lilac acid.

Crystalline substance of composition C10 H10 O4 m.p. 166 - 1680C. UV spectrum: max (ethanol) 218, 226 PL, 235, 261 PL, 322 nm, typical for aromatic unsaturated acids.

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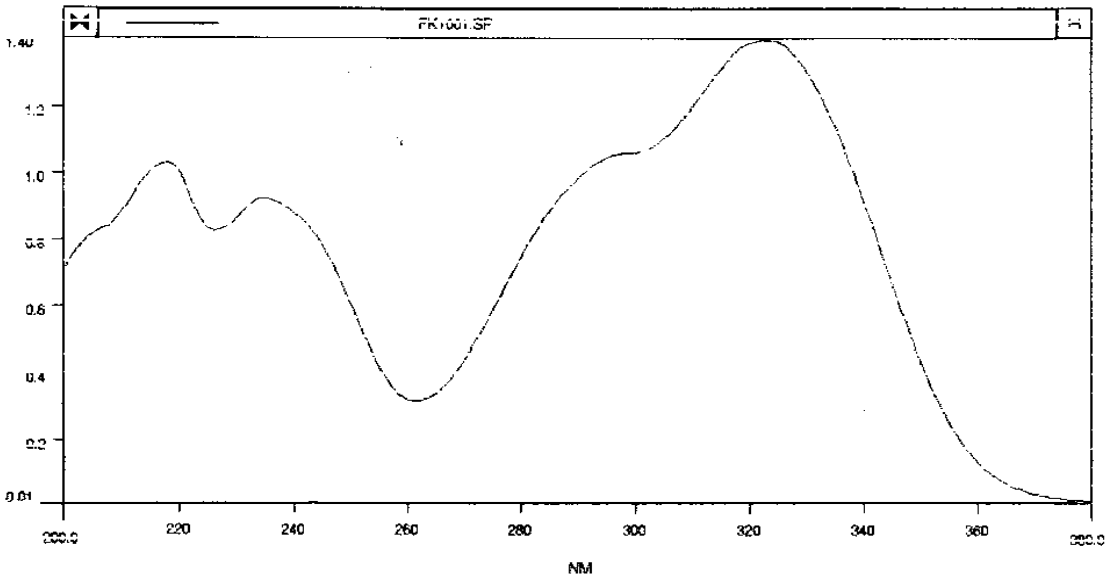

Fig. 9. UV spectrum of ferulic acid

The V max (KBr) IR spectrum of compound 3 contains absorption bands of hydroxyl groups (3437-3018 cm^1), a methoxyl group (2943 cm^1), C = O, unsaturated (1692 cm^1), COOH group (1666 cm^1) and aromatic C = C bonds (1620, 1601, 1519 cm^1).

In the PMR spectrum of compound 3 (Py – d5), the signals of the proton of the methoxyl group (3.63 ppm in the form of a 3H singlet), H-(6.75 ppm in the form of a 1H. Doublet with a constant spin- spin interaction J = 15.8, Hz) H-5 (7.06 ppm, in the form of a 1 H doublet with SSCC J = 8.5 Hz), H-2 (7.12 ppm in the form 1 H doublet with a CSCR J = 2.0 Hz), H-6 (7.23 ppm as a 1H doublet of doublets with a CSCR J = 2.0 and J = 8.5 Hz), H-6 (8 , 00 ppm as a 1H doublet of SSCC J = 15.8 Hz) and 4-OH (9.90 ppm, as a 1 H broadened singlet).

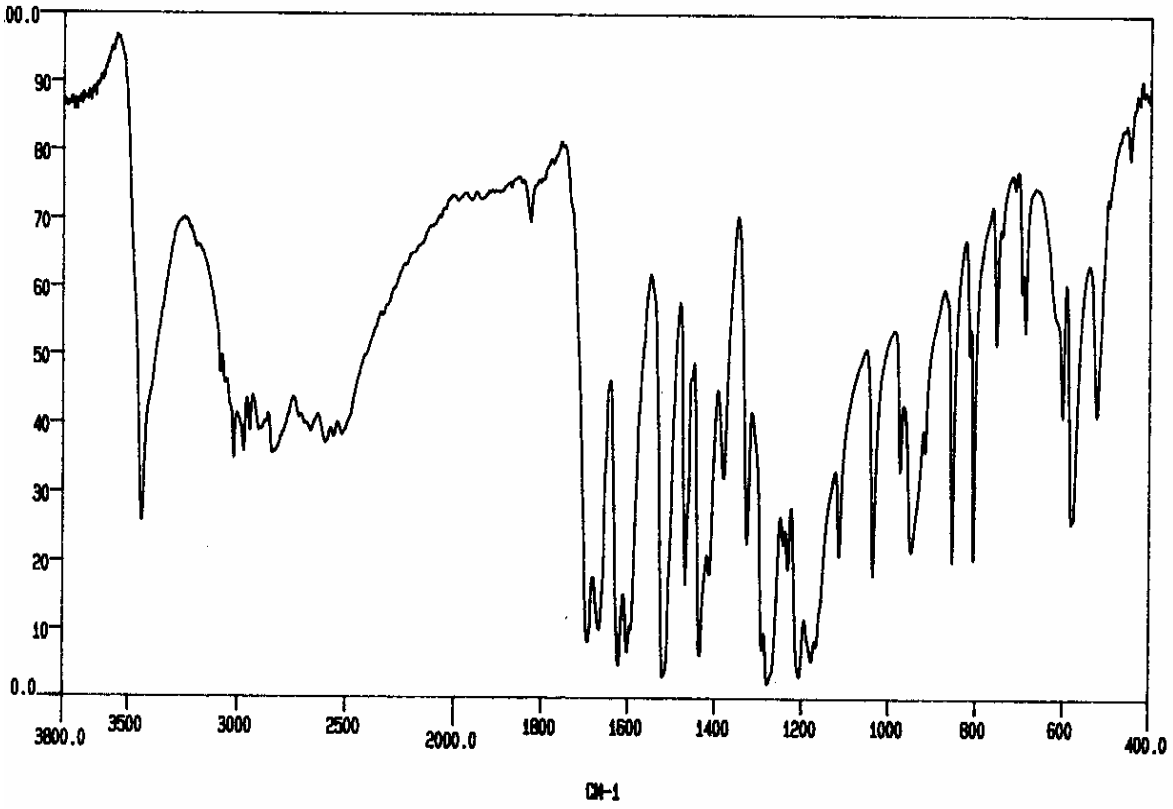

Fig. 10. IR - spectrum of ferulic acid

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Thus, the results of the studies carried out to study the hydroxycinnamic acids of the woolly erva cultivated in the territory of Uzbekistan showed the content of vanillic, lilac and ferulic acids. Ferulic acid was isolated for the first time from erva woolly

The dried and crushed aerial part of the woolly erva (40 g) was extracted with purified water in a round-bottom flask at a temperature of 100 ° C once at a ratio of 1:35 for 20 minutes. Then the extract was cooled, filtered through a coarse calico filter, the filtrate was evaporated on a rotary evaporator at 40 ° C to a thick solution. From a thick solution, a water-soluble polysaccharide was precipitated with 1.0 l of alcohol. The precipitate of the WSP was separated by centrifugation, washed sequentially with 96 ° alcohol and acetone, and dried in a desiccator over P2O5. The output of the water-soluble polysaccharide (WSPC) was 3.01 g – 7.52%.

After the separation of the WSPC, the alcoholic mother liquor was distilled on a rotary evaporator at 40 ° C to a volume of 0.5 l. They were treated with activated carbon, then with cation and anion exchangers. The aqueous solution was evaporated to dryness under vacuum on a rotary evaporator at a temperature of 40 ° C. Then the dry product was dried in a vacuum over P2O5 in a desiccator. The yield of free sugars was 2.72 g - 6.8%.

The qualitative composition of the polysaccharide stock solution was analyzed by paper chromatography. Glucose, galactose, xylose and rhamnose were detected by paper chromatography. The quantitative determination of monosaccharides was carried out by the GLC method, for which derivatives of these monosaccharides were obtained. GLC data showed that in the composition of free sugars, glucose is 53.0%, galactose 35.0%, xylose 7.7%, rhamnose 4.3%.

To determine the monosaccharide composition, the water-soluble polysaccharide was subjected to complete acid hydrolysis. After appropriate treatment, the hydrolyzate was analyzed by paper chromatography and detected galactose, glucose, apiose, arabinose. In the same way as in the case of monosaccharides, derivatives of monosaccharides VRPS for GLC were obtained from the mother liquor. According to GLC data, it was determined that in the amount of water-soluble polysaccharides (WSPP) galactose is 25.9%, glucose - 44.7%, apiosis - 0.7%, arabinose - 11.8%. The results of the GLC analysis are shown in Table 1.

Table 1. Monosaccharide composition and its ratio in water-soluble polysaccharides and free sugars

Carbohydrates	Output, %	Monosaccharide composition and their ratios						
		Gal	Glk	Ara	Xyl	Api	Rha	Gal UA
VRPS	7,52	2,2	3,8	1,0	1,5	сл.	–	+
St. C	6,80	12,4	8,2	–	1,8	–	1,0	–

As can be seen from Table 1.1, the predominant monosaccharides are galactose and glucose in the composition of the VRPS, and apiosis was first found in the composition of the VRPS. This monasaccharide is rarely found in plants. In addition, a small amount of an acidic monosaccharide was found in the composition of the VRPS along with neutral monosaccharides, i.e. galacturonic acid.

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Thus, as a result of the study of the carbohydrate composition of woolly erva, water-soluble polysaccharides and free sugars were isolated and characterized. For the first time in the composition of polysaccharides discovered a rare monosaccharide - apiosis.

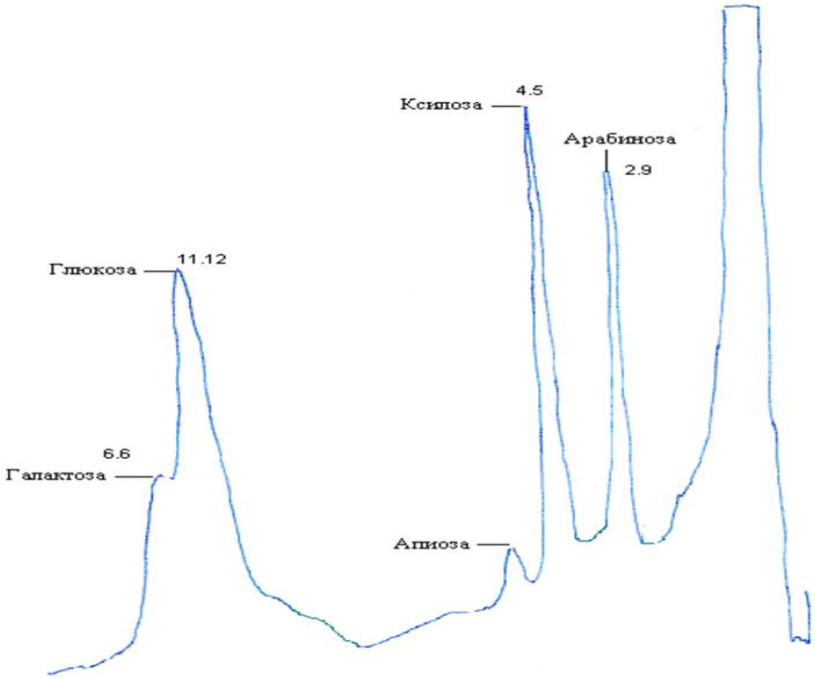

Fig. 11. Chromatogram of the CPPS of the Herva woolly herb

For the first time, the chemical composition of the Herva woolly herb cultivated in the territory of Uzbekistan has been studied by the method of column chromatography.

To identify the isolated substances, we used the data of UV–, IR–, PMR–, mass spectrometry, as well as the results of chemical transformations and their direct comparison with reliable samples.

Conclusion

Thus, studies have shown the presence of such flavonoids as: isorhamnetin 3–O–β–D - glycoside, narcissin (isorhamnetin – 3 – O – rutinoside) with a strong diuretic effect, as well as derivatives of hydroxycinnamic acid – vanillic, ferulic, lilac acids.

For the first time, isorhamnetin 3–O–β–D -glycoside and ferulic acid were isolated from the herb Aerva lanata.

The polysaccharide composition of erva woolly herb was studied by BH and GLC methods. Water-soluble polysaccharides and free sugars have been isolated and characterized. For the first time, a rare monosaccharide-apiosis was found in the composition of polysaccharides.

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