POLYPHENOLS OF THE HERB Hypericum perforatum AND THE PREPARATION NOVOIMANIN

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Hypericum perforatum L. (common St. John's wort) is one of the most important medicinal plants with a broad-action spectrum. H. perforatum possesses diuretic, antiinflammatory, vulnerary, photosensitizing, and antimicrobial properties. From this plant are obtained extracts, infusions, decoctions, and the preparations imanin, imanin-A, novoimanin, and floristen, which are used in diseases of the respiratory and digestive tracts, gynecological diseases, and externally for burns, frostbite, and suppurating wounds [1-3]. In the herb Hypericum perforatum have also been found such compounds as hypericin, pseudohypericin, protopseudohypericin, hypericodehydrodianthrone, pseudohypericodehydrodianthrone, frangula emodin anthranol, hyperoside, rutin, quercitrin, isoquercitrin, quercetin, essential oil, terpenes and sesquiterpenes, tanning substances, carotene, ceryl alcohol, and a small amount of choline [1, 3-9].

In spite of the available papers on the biologically active substances present in H. perforatum, many active substances of this plant have not yet been isolated and studied. Thus, at the present time, although several authors have repeatedly attempted to establish the composition of novoimanin and imanin [10-17], the composition of the antibiotic preparation novoimanin has not been determined, and its active substances have not yet been isolated.

The present paper gives the results of an investigation of the polyphenols of the herb H. perforatum, of the preparation novoimanin, and of the waste from the raw material after the production of novoimanin, and also reports the development of a method for the quantitative determination of one of the main flavonoids of H. perforatum – hyperoside – in the raw material and the wastes from the production of novoimanin.

For the experiments we took the raw material of industrial preparations, the waste from the production of novoimanin at the Borshchagovsk chemical and pharmaceutical factory, a 1% ethanolic solution of novoimanin from several batches, and a preparation of hyperoside.

Analysis of the polyphenols of the herb H. perforatum before and after the extraction of the novoimanin was performed by the two-dimensional chromatography of acetone, aqueous acetone, and aqueous extracts in the systems: 1) butanol–acetic acid–water (4:1:2) and 2) 15% acetic acid.

The presence of polyphenolic compounds on the chromatograms was shown by their fluorescence in UV light before and after the treatment of the chromatograms with a 1% ethanolic solution of ferric chloride, a 1% ethanolic solution of aluminum chloride, diazotized sulfanilic acid, and 5% methanolic solution of caustic soda. The results obtained (Table 1) show that from H. perforatum acetone extracted 12 polyphenolic compounds, 20% acetone 14, and water three. The medicinal raw material after the extraction of the novoimanin with acetone still contained practically all the polyphenolic compounds, and the flavonoids, such as rutin and quercitrin were also scarcely extracted by the acetone. Hyperoside (see below) was extracted from the raw material by acetone to only a small extent. The results of these investigations permit the herb H. perforatum to be recommended for the production of quercetin, hyperoside, and other flavonoids after the extraction of the novoimanin with acetone.

To identify the polyphenols of the herb H. perforatum, the substances were isolated preparatively after two-dimensional paper chromatography.

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TABLE 1. Polyphenolic Compounds of the Herb H. perforatum and of Novoimanin

Substance	Color of the spots in UV light	R_f in systems		Relative content in the substances in the				Products of acid hydrolysis	
				extract			novoimanin	aglycone	sugar component
		1	2	acetone	aqueous acetone	aqu.			
1	Light-brown	0,98	0,008	++++	++	−	++++	C R_f 0,97	A biose and glucose
2	Light-brown	0,96	0,008	+++	+	−	+++	C R_f 0,97	A biose and glucose
3	Light-brown	0,94	0,008	+++	+	−	+++	C R_f 0,97	A biose and glucose
Hypericin	Pink	0,87	0,008	++	++	+	+−	—	—
Pseudohypericin	Pink	0,76	0,008	++	++	+	+−	—	—
Protopseudohypericin	Pink	0,66	0,01	++	+	+	+−	—	—
7	Light-brown	0,99	0,16	++	+	−	++	C R_f 0,84	Glucose
8	Light-brown	0,98	0,16	++	+	−	++	C R_f 0,84	Glucose
Hyperoside	Yellow	0,53	0,26	+	++++	−	+−	Quercetin	Galactose
Quercitrin	Yellow	0,61	0,22	+	++++	−	+−	Quercetin	Rhamnose
Isoquercitrin	Yellow	0,63	0,34	+−	++++	−	+−	Quercetin	Glucose
Rutin	Yellow	0,32	0,67	+−	++++	−	+−	Quercetin	Rutinose
Quercetin	Yellow	0,71	0,02	++	++++	−	−	—	
Hypericodehydrodianthrone	Pink	0,93	0,011	++	+	+−	+−	—	—

TABLE 2. Results of a Quantitative Determination of Hyperoside in H. perforatum Raw Material and the Wastes from the Production of Novoimanin

Material studied	Serial No.	Wt., g	Vol. of extract, ml	Amt. of extract used for chrom., ml	Found: optical dens. of the eluate	Found: hyperoside, %	No. of expts.	$J_{0,95}$	A(% ±)
H. perforatum	121 970	4,00	250	0,1	0,091	1,04	5	$5,20 \cdot 10^{-2}$	4,98
Wastes from the production of novoimanin	121 970	4,00	250	0,2	0,164	0,94	5	$2,95 \cdot 10^{-2}$	3,15
H. perforatum	101 970	4,00	250	0,1	0,153	1,76	4	$6,32 \cdot 10^{-2}$	3,59
Wastes from the production of novoimanin	101 970	4,00	250	0,2	0,301	1,73	7	$3,67 \cdot 10^{-2}$	2,12
H. perforatum	4 047 021	4,00	250	0,1	0,131	1,50	5	$4,90 \cdot 10^{-2}$	3,26
Wastes from the production of novoimanin	4 047 021	2,00	200	0,2	0,155	1,43	7	$2,46 \cdot 10^{-2}$	1,72
Flowers of H. per.	4 047 021	4,00	500	0,2	0,247	2,83	5	$11,07 \cdot 10^{-2}$	3,91
Stems of H. perforatum	4 047 021	4,00	250	0,2	0,023	0,13	5	$0,98 \cdot 10^{-2}$	7,30
The water-soluble resin	—	4,87	500	0,1	0,076	1,43	4	$8,41 \cdot 10^{-2}$	5,88

The substances obtained were analyzed by paper chromatography with a selection of reference samples from their IR absorption spectra and the products of alkaline hydrolysis. Nine substances were identified: hyperoside, quercitrin, isoquercitrin, quercetin, rutin, hypericin, pseudohypericin, protopseudohypericin, and hypericodehydrodianthrone. Five substances (1, 2, 3, 7, and 8) were characterized provisionally as glycosides of an undetermined group of phenols forming on hydrolysis phenolic aglycones of low polarity with R_f 0.97 and 0.84 in the benzene–ethyl acetate–acetic acid–formamide (74 : 23 : 2 : 1) system and also glucose and a biose.

The polyphenols of a 1% ethanolic solution of novoimanin were analyzed by two-dimensional chromatography on FN-15 paper. Figure 1 shows that novoimanin contains 12 polyphenolic compounds, eight of which we identified: hyperoside, quercitrin, isoquercitrin, rutin, hypericin, pseudohypericin, protopseudohypericin, and hypericodehydrodianthrone.

The amount of substances identified was small in comparison with that of a suitable amount of acetone extract taken as standard. Consequently, the purification of the acetone extract with activated carbon in the preparation of novoimanin leads to a reduction in the content of flavonoid and dianthrone compounds in the novoimanin preparation. The amounts of substances 1, 2, 3, 7, and 8 in novoimanin are higher than those of the other compounds, which permits the assumption of the presence among them of the main active substance that is responsible for the antimicrobial activity of the preparation novoimanin.

The amount of the main flavonoid of H. perforatum – hyperoside – in the raw material and also in the individual parts of the plant and in the wastes from the production of novoimanin was determined by a spectrophotometric method after preliminary chromatographic separation on FN-1 paper.

The hyperoside was extracted from the materials studied by 70% methanol for 5 h at the rate of 5-7 overflows per hour. Three series of H. perforatum raw material were studied. Two to three methanolic extracts were made from an average sample of each series, and also separately from the flowers and stems of the plant. Each extract was chromatographed repeatedly and eluted. The results, which are given in Table 2, are the averages of not less than four to seven determinations.

It follows from Table 2 that the maximum amount of hyperoside is present in the flowers of H. perforatum (about 3%) and that there is considerably less of it in the herbage (1.05-1.80%) and a negligibly small amount in the stems (0.13%). A determination of the percentage of hyperoside in the wastes from the raw material from the production of novoimanin showed that after treatment with acetone it contained only slightly less hyperoside than before extraction.

EXPERIMENTAL

Extraction of Polyphenols from the Herb Hypericum perforatum. The polyphenols were extracted from the plant successively with acetone, 20% aqueous acetone, and water. For analysis, three 10.0-g samples of the raw material were taken and were extracted twice with a 10-fold amount of solvent, and 0.1- ml portions of the extracts were deposited on chromatograms. The wastes from the production of novoimanin were extracted successively with 20% acetone and with water. Samples of the factory wastes and extracts with 20% acetone after the preliminary acetone extraction of the novoimanin fraction from the dry herb H. perforatum proved to be identical.

The preparative isolation of the substances was performed after two-dimensional chromatographic separation. The substances were eluted from the spots with 70% ethanol, the extracts were evaporated, and the residues were investigated. The IR spectra were taken on a UR-10 instrument (tablets of potassium bromide).

Hydrolysis of Micro Amounts of the Polyphenols. Approximately 1 mg of substance was dissolved in 0.5 ml of 2% hydrochloric acid and hydrolyzed in the water bath for 1 h. The hydrolysate was treated several times with 2-ml portions of ether, the ethereal extract was evaporated, and the amount of aglycones in the residue was determined with a set of reference samples in the benzene–ethyl acetate–acetic acid–formamide (74 : 23 : 2 : 1) system. After neutralization, the aqueous residue was analyzed for its sugar content.

Quantitative Determination of Hyperoside. The comminuted H. perforatum raw material 2.0-5.0 g) was extracted with 70% methanol for 5 h, and the extract was made up with methanol to a definite volume. Then it was filtered and 0.1-0.2 ml of the resulting extract was chromatographed on FN-1 paper by the ascending method in the butan-1-ol–acetic acid–water (4 : 1 : 5) (aqueous phase) system for 40 h. The position of the hyperoside spots was revealed in UV light with the aid of standard hyperoside. The R_f value of hyperoside was 0.65 ± 0.2.

The dynamic elution of the hyperoside was performed with dimethylformamide in a 5-ml measuring flask for 1.5-2 h. The optical density of the eluate was determined on an SF-4A spectrophotometer at a wavelength of 366 nm (absorption maximum of the dimethylformamide solution of hyperoside). The control solution was a dimethylformamide eluate of the same chromatographic paper. The concentration of hyperoside was calculated from a calibration curve constructed for pure hyperoside or from the specific absorption of hyperoside that we have found – 304.25 ± 6.538.

It was shown by repeated experiments that hyperoside is 89.5% extracted from FN-1 paper, and this must be taken into account in the calculations. The percentage of hyperoside (X) was calculated from the

formula

$$X = \frac{D \cdot Y \cdot 5 \cdot 100 \cdot 100}{304.25 \cdot p \cdot Y_1 \cdot 89.57 \cdot 100},$$

where D is the optical density of the dimethylformamide eluate at λ 366 nm; Y is the volume of the extract obtained from the raw material on extraction; p is the weight of the initial raw material, g; and Y_1 is the amount of extract taken for chromatography, ml.

The study of the active substances of novoimanin and the development of methods for the quantitative determination of other polyphenolic compounds of H. perforatum is continuing.

SUMMARY

1. The herb Hypericum perforatum L. has been shown by paper chromatography to contain 14 substances with a polyphenolic nature, five from the flavonoid group (identified as hyperoside, isoquercitrin, quercitrin, rutin, and quercetin), four from the dianthrone group, and five from the group of low-polarity phenolic glycosides of undetermined structure.

2. The composition of the polyphenols of novoimanin has been studied. This preparation has been found to contain five substances from the group of low-polarity phenolic glycosides, which are present in considerable amounts, and nine substances from the group of flavonoids and dianthrones which are present in small amounts.

3. It has been established that the H. perforatum raw material after the extraction of the novoimanin still contains a considerable amount of flavonoid compounds, and the raw material can be used for the preparation of quercetin and hyperoside.

4. A spectrophotometric method for the quantitative determination of hyperoside in the herbage, stems, and flowers of H. perforatum and in the wastes from the production of novoimanin has been developed.

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