AMOUNTS OF FLAVONOIDS IN VARIOUS Rosa SPECIES GROWING IN THE WESTERN PAMIR

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A procedure has been developed for the spectrophotometric quantitative determination of flavonoids in rose hips after complex-formation with aluminum chloride. The amounts of flavonoids in the raw material of roses growing in the Western Pamir have been determined as a function of the height above sea level. It has been shown that the flavonoids are distributed nonuniformly in different parts of the rose hip: The greatest accumulation of flavonoids is found in the hairs and the smallest amount in the seeds.

According to the XIth edition of the State Pharmacopeia (GF XI) the quality control of rose raw material is made from the level of ascorbic acid [1]. The main active principles of the rose, in addition to ascorbic acid, are flavonoids; kaempferol, quercetin, isoquercetin, and tiliroside, which are present in an amount of up to 4% in the fresh raw material [5, 6]. A number of methods have been proposed for the quantitative determination of flavonoids in medicinal raw material: gravimetric after chromatographic isolation of the flavonoids on a column of polyamide sorbent, and spectrophotometric after the purification of the flavonoids on a column of polyamide sorbent [4].

The conditions for the extraction of the flavonoids, their freeing from ballast substances, and their quantitative determination in rose hips have not been developed, and it does not appear possible to apply the procedures described in the literature to our material. For the quantitative determination of flavonoids we have used a spectrophotometric method after the performance of complex-formation with aluminum chloride, which causes a bathochromic shift of the absorption band of flavonoids from 330/350 to 390/410 nm [2]. This permits the use of the solution under investigation without the reagent to be used as control and thereby the exclusion of the influence of colored accompanying substances. To obtain more reproducible results, the complex-forming reaction is performed in an acetic acid–hexamethylenetetramine buffer solution in 40-45% ethanol.

As the standard substance we selected rutin, the differential absorption spectrum of which with aluminum chloride under the conditions suggested coincides with the differential absorption spectrum of the rose flavonoids (Fig. 1). Furthermore, rutin is close in chemical structure to the flavonoids present in rose hips, is produced industrially in sufficient amount, and corresponds to the requirements of the Xth edition of the State Pharmacopeia (GF X).

A calibration graph has been plotted in the coordinates D–C, where D is the optical density and C the rutin concentration, %. The calibration curve of the relationship between

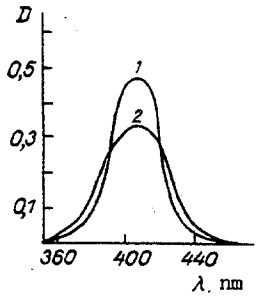

Fig. 1. Differential absorption spectra of rutin (1) and of the total rose flavonoids (2) with aluminum chloride in 40% ethanol, in acetic acid–hexamethylenetetramine buffer.

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TABLE 1. Results of the Quantitative Determination of the Flavonoids in Rose Raw Materials Using the Method of Additives with Rutin

Sample No.	Rutin taken, g	Flavonoids found, g/100 g of raw material	Flavonoids and rutin found g/100 g of raw material	Rutin found, g	Metrological characteristics
1	0,2500	0,3864	0,6136	0,2272	$\bar{X}$=0,2303
			0,6159	0,2295	$S_{\bar{x}}$=0,005
			0,6159	0,2295	$\Delta_{\bar{x}}$=0,013
			0,6417	0,2553	A=±5,61%
			0,6227	0,2363	
			0,6250	0,2384	
2	0,2500	0,1279	0,3930	0,2651	$\bar{X}$=0,2516
			0,3895	0,2616	$S_{\bar{x}}$=0,005
			0,3837	0,2558	$\Delta_{\bar{x}}$=0,614
			0,3663	0,2384	A=±5,41%
			0,3605	0,2326	
			0,3837	0,2558	
3	0,2500	0,2046	0,3920	0,1874	$\bar{X}$=0,1827
			0,3864	0,1818	$S_{\bar{x}}$=0,0012
			0,3841	0,1795	$\Delta_{\bar{x}}$=0,003
			0,3852	0,1806	A=∓1,69%
			0,3898	0,1852	
			0,3864	0,1818	
4	0,2500	0,5341	0,7500	0,2159	$\bar{X}$=0,2044
			0,7443	0,2102	$S_{\bar{x}}$=0,0024
			0,7421	0,2088	$\Delta_{\bar{x}}$=0,0062
			0,7307	0,1966	A=±3,04%
			0,7273	0,1932	
			0,7364	0,2023	
5	0,3000	0,1136	0,4091	0,2955	$\bar{X}$=0,2908
			0,4091	0,2955	$S_{\bar{x}}$=0,0006
			0,4068	0,2932	$\Delta_{\bar{x}}$=0,0016
			0,4000	0,2864	A=±0,55%
			0,3997	0,2841	
			0,4034	0,2898	

the optical density and the concentration of rutin solutions is linear within the range of concentrations from 0.00002 to 0.0020%. The sensitivity of the determination is 0.002 mg/ml of rutin.

In a study of the extraction conditions it was established that the optimum yield of flavonoids is achieved at a particle size of the material of 0.5-2 mm, with 70% ethanol as extractant, a ratio of raw material and extractant of 1:20, and an extraction time of 45 min. In a study of the methods of isolating and purifying the flavonoids from rose hips it was found that it is difficult to purify the crude product by extraction methods. Chromatography on a column of polyamide sorbent is used most frequently to isolate and purify the flavonoids from a plant extract [3]. The optimum amount of polyamide has been established experimentally: 1 g of polyamide (TU [Technical Specification] 26-90-10-822-73) proved to be sufficient for the purification of 10 ml of extract from rose hips. The flavonoids were eluted from the polyamides most completely by 96% ethanol.

The results obtained were used in the development of a procedure for the quantitative determination of the total flavonoids in rose hips. The procedure has been tested on five samples of rose raw material using the method of additives (Table 1). Satisfactory results were obtained, the relative error not exceeding ±6%.

We studied rose species growing in the Pamir at different heights above sea level. As control we took the cinnamon rose growing in Moscow province and included in the GF X. The

TABLE 2. Levels of Flavonoids as a Function of the Species of Rose and Its Growth Site

Collection site	Rose species	Height above sea level, m	Level of flavonoids, % on the absolutely dry raw material
Valley of the R. Shakhdara; Khushtapundara gorge	Shipovnik guntskii	3400	0,534
Northern slope of the Shugnanskii range; Bidzhundara gorge	Shipovnik guntskii	2000	0,386
Western slope of the Rushanskii range; Badzhu gorge	Shipovnik guntskii	2700	0,386
North-western slope of the Ishkashimskii range	Shipovnik guntskii	2700	0,295
Valley of the R. Shakhdara Bez-dara	Fedtchenko rose	2700	0,307
Ishkashimskii region of Garm-chas	Shipovnik guntskii	2600	0,207
Western slope of the Shugnanskii range; botanical garden	Dog rose	2300	0,114
Northern slope of the Yazgulemskii range; Vanch region	Begger rose	2300	0,205
Southern slope of the Darvaza range; Kalai-Khumb region	Corymb rose	1800	0,128
Moscow province	Cinnamon rose		0,057

TABLE 3. Amounts of Flavonoids in Various Parts of the Rose

Rose species	Amount of flavonoids, % on the absolutely dry raw material		
	hairs	pericarp	seeds
Shipovnik guntskii	0,682	0,477	0,040
Corymb	0,462	0,354	0,031
Begger	0,355	0,278	0,025

level of flavonoids depends on the height of growth above sea level. Thus, the greatest level of flavonoids (0.534%) was found in a rose (shipovnik guntskii*) growing in the valley of the R. Shakhdara at height of 3400 m above sea level, and the smallest amount (0.128%) in a corymb rose growing in the Kalai-Khumb region at a height of 1800 m above sea level [7] (Table 2).

The study of the amounts of flavonoids in various parts of the rose hips showed that the highest level of flavonoids was present in the hairs and the lowest level in the seeds (Table 3).

As a result of the investigations performed, a method has been developed for the quantitative determination of flavonoids in rose hips. With the aid of it we have determined the amounts of flavonoids in rose raw material from the Western Pamir as a function of the height of growth above sea level. It has been found that various rose species from the Western Pamir have a higher level of flavonoids than roses growing in the neighborhood of Moscow. It has been established that the flavonoids are distributed nonuniformly in different parts of rose hips. The greatest accumulation of flavonoids is found in the hairs and the smallest in the seeds.

EXPERIMENTAL

Constructing the Calibration Graph. With heating, 0.0500 g (accurately weighed) of rutin (GF X, p. 587) previously dried at 135°C for 3 h was dissolved in 70% ethanol in a 100-ml measuring flask and, after cooling, the volume of the solution was made up with the same ethanol to the mark and it was carefully mixed (solution A). A 100-ml measuring flask was charged with 10 ml of solution A and this was made up to the mark with 70% ethanol (solution B). Solution B in amounts of 1, 2, 3, 4, 5, 8, and 10 ml was transferred to 25-ml measuring flasks and to each was added 10 ml of 96% ethanol, 0.5 ml of a 33% solution of

*Species not identified – Translator.

acetic acid, 1.5 ml of a 10% solution of aluminum chloride, and 2 ml of a 5% solution of hexamethylenetetramine, and the volumes of the solutions in the flasks were made up to the marks with water.

After 30 min, the optical densities of the solutions were measured with the aid of SF-16 spectrophotometer at a wavelength of 407 nm in a cell with a layer thickness of 10 mm. As comparison solutions we used solutions consisting of 1, 2, 3, 4, 5, 8, and 10 ml of solution B, 10 ml of 96% ethanol, 0.5 ml of 33% acetic acid, and water to 25 ml.

Procedure. About 5 g (accurately weighed) of raw material that had been passed through a sieve with 2-mm-diameter apertures was added to a 10-ml round-bottomed flask and was covered with 60 ml of 70% ethanol, and the contents of the flask were boiled under reflux for 30 min. After cooling, the alcoholic extract was decanted through a paper filter into a 100-ml measuring flask. Another 40 ml of 70% ethanol was added to the flask with the raw material and the contents were boiled under reflux for 15 min. After cooling, this mixture was filtered into the same measuring flask, and the volume of the solution in the flask was brought up to the mark with the same alcohol; 10 ml of the filtrate obtained was evaporated on the boiling water bath to approximately 5 ml; this was diluted with water to the original volume and was transferred to a column 1 cm in diameter and 25 cm high containing 1 g of polyamide sorbent. The column was washed with 10 ml of water. The aqueous eluate was discarded.

The total flavonoids were eluted with 25 ml of 96% ethanol, which was added to the column gradually in 5-ml portions. The migration of the flavonoids was monitored in visible light. When the zone had reached the bottom of the sorbent, the eluent was collected in a 25-ml measuring flask. The volume of eluate in the flask was made up to the mark with 96% ethanol and the contents were carefully mixed.

A 5-ml aliquot of the resulting solution was transferred to a 25-ml measuring flask, and 10 ml of 96% ethanol, 0.5 ml of 33% acetic acid, 1.5 ml of 10% aluminum chloride solution, and 2 ml of hexamethylenetetramine solution were added and the volume of the solution was made up to the mark with water. After 30 min, the optical density of the solution was measured with the aid of a SF-16 spectrophotometer at a wavelength of 407 nm in a cell with a layer thickness of 10 mm. As the comparison solution we used a mixture consisting of 5 ml of the test solution, 10 ml of 96% ethanol, 0.5 ml of 33% acetic acid, and water to 25 ml.

The optical density of 1 ml of solution A that had been prepared for plotting the calibration graph was measured in a similar way to the test solution.

The total amount of flavonoids, calculated as rutin, on the absolutely dry weight of the raw material as a percentage (X) was deduced from the formula

$$X = \frac{D \cdot 0{,}00002 \cdot 100 \cdot 25 \cdot 25 \cdot 100 \cdot 100}{D_0 \cdot a \cdot 10 \cdot 5\,(100 - m)} = \frac{D \cdot 250}{D_0 \cdot a\,(100 - m)},$$

where D is the optical density of the test solution; D_0 is the optical density of the solution of the standard rutin sample being analyzed; 0.00002 is the amount of rutin in 1 ml of the solution of the standard sample being analyzed; a is the weight of raw material, g; and m is the moisture content of the raw material.

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