USE OF A CHROMATO-PHOTOCOLORIMETRIC ANALYSIS OF KAPLI LANDYSHEVO—PUSTYRNIKOVYE

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A method has been developed for the chromato-photocolorimetric determination of glycosides and the chromato-spectrophotometric determination of flavonoids in Kapli landyshevo-pustyrinkovye [convallaria—water fennel drops].

The preparation Kapli landyshevo-pustyrinkovye [convallaria—water fennel drops] contains a tincture of lily of the valley (Convallaria) and a tincture of water fennel in equal amounts. No quantitative determination of the flavonoids in the preparation is made. We set ourselves the task of developing a method for the quantitative determination of glycosides and flavonoids in Kapli landyshevo-pustyrnikovye using chromato-optical methods of analysis.

Alcoholic extracts from the plant raw material contain large amounts of chlorophyll, polyphenolic compounds, and other substances which interfere with quantitative determination. In view of this, the extracts were first purified. To separate the glycosides and flavonoids we used a column of polyamide sorbent [3]. Purification of the extract was achieved in the passage of the glycosides from an aqueous phase into an organic solvent [ethanol—chloroform (2:8)]. Additional purification of the extract was carried out on a column of alumina [4].

For quantitative determination we used a photocolorimetric method based on the reaction of the glycosides with sodium picrate. As the reference sample we used a standard extract of lily-of-the-valley produced by the experimental factory of VINIKhTLS [All-Union Scientific-Research Institute of Drug Chemistry and Technology].

EXPERIMENTAL

To elute the flavonoids from the column of polyamide sorbent we used 50% ethanol. The quantitative determination of the total flavonoids was made with the aid of a spectrophotometric method based on complex-formation with aluminum chloride. Rutin was used as the comparison substance [1, 2].

Procedure for Determining Glycosides in Kapli Landyshevo-pustyrnikovye. A 20-ml (accurately measured) sample of the preparation was evaporated in the water bath to a volume of approximately 5 ml. The residue was diluted with water to its initial volume (about 20 ml) and was filtered through a paper filter into a column (1 × 25 cm) containing 1.0 g of polyamide sorbent.

The filter was washed with 10 ml of water. Then the column was washed with 20 ml of water. The aqueous eluate was evaporated on the water bath to a volume of 10-12 ml and was placed in a separatory funnel to which 30 ml of ethanol—chloroform (2:8) was added, and the mixture was shaken for 5 min. After the separation of the layers, the organic solvent fraction was filtered through a paper filter containing 3.0 g of anhydrous sodium sulfate into an evaporating dish. Extraction with the mixture of alcohol and chloroform was repeated twice more using 30 ml of mixture of the same composition each time, and the chloroform extract was filtered into the same evaporating dish. The filter with the anhydrous sodium sulfate was washed with 10 ml of the alcohol—chloroform mixture. The resulting alcohol—chloroform extract was evaporated on the water bath to 2 ml. The last traces of solvent were eliminated by blowing with air.

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TABLE 1. Correlation of the Results of the Chromato-photocolorimetric and Biological Methods of Analyzing Kapli Landyshevo-pustyrnikovye and Tincture of Lily-of-the-Valley

Sample No.	Glycoside content					
	Kapli landyshevo-pusturkovye					
	Chromato-phostocolorimetric method (mg/ml)					
1	0,050	0,087	0,034	0,037	0,035	0,037
2	0,082	0,085	0,084	0,082	0,082	0,082
3	0,072	0,076	0,075	0,072	0,077	0,074
	Biological method (FAU/ml)					
1	6,6	5,8	5,8	6,6	6,6	5,8
2	5,9	5,3	5,9	5,3	5,9	5,9
3	5,7	5,7	6,6	5,7	6,6	6,6
	Tincture of lily of the valley					
	Chromato-photocolorimetric method (mg/ml)					
1	0,140	0,142	0,140	0,145	0,140	0,145
2	0,200	0,185	0,145	0,190	0,190	0,200
3	0,150	0,150	0,145	0,145	0,145	0,146
	Biological method (FAU/ml)					
1	13,3	11,7	13,3	15,5	13,3	11,7
2	12,9	12,9	11,9	12,9	15,2	11,9
3	12,9	11,9	12,9	12,9	11,9	15,2

$r = 0.891$.
$t_{obs} = 11.43$.
$t_{cr} = 2.73$.

The dry residue was transferred quantitatively from the evaporating dish with 70% ethanol into a 50-ml measuring flask. The volume of the solution in the flask was made up with ethanol to the mark, and the contents were mixed.

A 15-ml portion of the resulting solution was passed through a column (1 × 25 cm) containing 3.0 g of alumina of chromatography (activity grade II).

To 3 ml of the eluate were added 1.5 ml of a 1% solution of picric acid and 0.5 ml of a 2% solution of sodium hydroxide and the mixture was carefully stirred. After 10 min, the optical density of the resulting solution was measured on a FÉK-56-F photoelectric colorimeter at a wavelength of 495±10 nm in a cell with a cell thickness of 10 mm. As the comparison solution we used a mixture consisting of 6 ml of 70% ethanol, 3 ml of a 1% solution of picric acid, and 1 ml of a 2% solution of sodium hydroxide. The amount of glycosides in 1 ml of the preparation in mg (X) was calculated from the formula:

$$X = \frac{D \cdot 0.17}{D_0},$$

where D is the optical density of the solution under investigation;

D_0 is the optical density of the lily-of-the-valley standard; and

0.17 is the average amount of glycosides in 1 ml of preparation calculated as convallatoxin.

The method developed has been tested on industrial samples of Kapli landyshevo-pustyrnikovye and tincture of lily-of-the valley. Determinations of the biological activities of the preparation on frogs, Rana temporaria, with subcutaneous injection was made in parallel. The results of the determination are given in Table 1.

We have investigated the correlation of the results of the chromato-photocolorimetric and biological methods. The calculation of the calculation coefficient was made on a DVK-3c personal computer [5].

To check the hypothesis of the significance of the correlation efficient on the assumption of a normal two-dimensional distribution of the values of X (the amount of glycosides found by the chromato-photocolorimetric method) and Y (the amount of glycosides found by the biological method) we calculated the observed value of Student's criterion:

$$t_{obs} = \sqrt{\frac{n-1}{1-r^2}},$$

where n is the volume of the sample; and

r is the correlation coefficient.

For our measurements we have the following values

$$\overline{X} = 0{,}121 \quad \overline{XY} = 1{,}29 \quad \sigma_x = 0{,}0429$$
$$\overline{Y} = 9{,}51 \quad n = 36 \quad \sigma_y = 3{,}61$$

The correlation coefficient and Student's criterion are: r = 0.891; t_{obs} = 11.43.

For a significance level p = 0.01 (which corresponds to a level of probability of 0.99) and a number of degrees of freedom f = n − 2 we found the critical value of Student's criterion:

$$t_{cr}(0{,}01; 34) = 2{,}73.$$

Since $t_{obs} > t_{cr}$ the correlation coefficient r = 0.891 obtained is significant and, as its value differs little from unity, we may conclude that there is an extremely close, almost functional link between the results of the chromato-photocolorimetric and biological analyses.

We found the regression of Y on X by the method of least squares: Y = 75X + 0.5.

This formula permits the calculation of the glycoside content (FAU/ml) from the value measured by the chromato-photocolorimetric method (mg/ml).

Procedure for Determining Flavonoids in Kapli Landyshevo-pustyrnikovye. After the separation of the cardenolide fraction, the flavonoids were eluted with 25 ml of 50% ethanol, which was added in 5-ml portions. The migration of the flavonoids was monitored in UV light, in which they appeared as a yellow-brown zone. When the zone had reached the lower part of the sorbent the eluate was collected in a 25-ml measuring flask, made up to the mark with 50% ethanol, and carefully mixed. Of the resulting solution, 2 ml was transferred to a 25-ml measuring flask, and 10 ml of 95% ethanol, 0.5 ml of a 33% solution of acetic acid, 1.5 ml of a 10% solution of aluminum chloride, 2 ml of a solution of hexamethylenetetramine* were added.

As the comparison solution we used a mixture consisting of 2 ml of the solution to be analyzed, 10 ml of 95% ethanol, 0.5 ml of 33% acetic acid, and water to 2 ml. After 40 min, the optical densities of the solutions were measured on a SF-26 spectrophotometer at a wavelength of 407 nm in a cell with a layer thickness of 10 mm. The optical density of 1 ml of a 0.05% solution of rutin was measured in parallel, the complex-forming reaction being performed in a similar way to that of the solution under investigation. The sum of the flavonoids calculated as rutin as a percentage (X) was found from the formula:

$$X = \frac{D \cdot 0{,}00002 \cdot W \cdot 100}{D_0 \cdot V},$$

where 0.00002 is the amount of rutin in 1 ml of standard solution, g;

W is the dilution;

V is the volume taken for the determination; and

D and D_0 are the optical densities of the solution under investigation and the standard solution, respectively.

The procedure described was tested on three series of preparations. The results obtained are given in Table 2.

Thus, on the basis of the investigations performed we have studied the optimum conditions for extraction and purification of the activation substances of Kapli landyshevo-pustyrnikovye. Procedures have been developed for the chromato-photocolorimetric

*Concentration not given – Translator.

TABLE 2. Results of the Quantitative Determination of Flavonoids in Kapli Landyshevo-pustyrnikovye by a Chromatomass Spectrophotometric Method

Sample No.	Flavonoids found, calculated as rutin, mg/100 g, $\bar{x}$	Metrological characteristics		
		$S_{\bar{x}}$	E	A, %
1	4,89	0,0001	0,0002	4,76
2	5,48	0,0001	0,0001	2,07
3	5,23	0,0001	0,0001	1,71

determination of the flavonoids in the preparations studied. The procedures are simple and readily reproducible and permit the determination of the main active substances with adequate accuracy. Comparable results were obtained by the photocolorimetric and biological methods of analysis of the glycosides. The adequacy of the results of these methods have been confirmed by their correlation coefficient.

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SYNTHESIS OF 4α-ALKYLTHIOCARANE-3β-THIOLS

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Addition reactions of thiols to β-3,4-epithiocarane under conditions of base catalysis have been studied. The reaction takes place regio- and stereospecifically with the formation of 4α-alkylthiocarane-3β-thiols.

One of the most promising directions in the chemistry of terpenoids is the synthesis of sulfur-containing derivatives of this series, since it is known that the terpene sulfides present in Nature in trace amounts possess valuable practical properties [1, 2].

We propose a method for synthesizing 4α-thiocarane-3β-thiols according to the following scheme: 3-carene→α-3,4-epoxycarane→β-3,4-epithiocarane→4-alkylthiocarane-3β-thiol. With this aim, we have developed a convenient method for obtaining β-3,4-epithiocarane (II) by the reaction of α-3,4-epoxycarane (I) with thiourea in the presence of equimolar amounts of sulfuric acid (by analogy with the procedure of [3]). The spectral characteristics (PMR) and physicochemical constants (bp and n_D^{20}) of compound (II) agreed with those described

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