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QUANTITATIVE DETERMINATION OF THE TOTAL FLAVONOLS IN *Alhagi pseudoalhagi*

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The optimum conditions for extracting flavonols from Alhagi pseudoalhagi *has been determined. A method for the UV-spectrometric identification of the total alkaloids has been developed which permits reliable reproducible results to be obtained. The relative error of the determination does not exceed ±1.7%.*

Camel thorn *Alhagi pseudoalhagi* has long been used in folk medicine for the treatment of ulcers of the stomach and duodenum, hemorrhoids, etc. [1]. The main active substances of this plant are flavonol glycosides (mainly glycosides of isorhamnetin), catechins, and proanthocyanidins [2, 3]. In view of this, the development of a method for the quantitative determination of isorhamnetin glycosides in the herbage of camel thorn based on their hydrolysis followed by the spectrophotometric determination of the aglycon formed, quercetin, is a matter of urgency [4].

In the UV spectrum of an acid hydrolysate of the herbage of camel thorn an absorption maximum is found in the 370 ± 1 nm region which is characteristic for quercetin [4, 5], and therefore for the quantitative determination of the total flavonols in the raw material we used the absorption maximum at 370 nm. The spectrophotometric determination of the total flavonols was carried out in relation to a solution of a standard sample of quercetin.

The raw materials for the analyses were the herbage of camel thorn collected in the period of mass flowering and fruit formation that had been comminuted and dried at room temperature. The level of total flavonols in the period of mass flowering was 1.25%, and in the period of fruit formation 0.98%. As extractants we used hot water (90-95°C) and aqueous solutions of ethanol with various concentrations, and we studied the dependence of the extraction of the total flavonols on the time of extraction (Table 1). The best extractant proved to be a 48% solution of ethanol, with an extraction time of 1 h.

More complete extraction of the total flavonols was achieved when the herbage was ground to a particle size of 2 mm (Table 2).

The extracts were subjected to hydrolysis in solutions of hydrochloric acid at 95-100°C, and the dependence of the total yield of flavonols on the concentration of acid and the time of hydrolysis was studied (Table 3). The maximum yield was achieved on hydrolysis with 10% hydrochloric acid for 3 hours.

The flavonols were extracted from the hydrolysate with ethyl acetate. The experimental results showed that extraction had to be performed 6 times. After the ethyl acetate had been driven off and the dry residue had been dissolved in 95% ethanol, the total amount of flavonols was determined spectrophotometrically at a wavelength of 370 nm.

EXPERIMENTAL

Preparation of a Solution of a Standard Sample of Quercetin. A standard sample of quercetin [PS (Pharmaceutical Standard) 42-1296-79] that had been dried to constant weight at 60-65°C in vacuum (0.01 g, accurately weighed) was dissolved in 95% ethanol in a 50-ml measuring flask, and the volume of the solution was brought up to the mark with 95% ethanol (solution A). After the transfer of 10 ml of solution A into a 50-ml measuring flask the volume was made up to the mark with 95% ethanol (solution B). In another 50-ml measuring flask, 7.5 ml of solution B was made up to the mark with 95% ethanol and the resulting solution was used for quantitative determination. This solution of the standard sample of quercetin contained 0.000006 g of quercetin per ml.

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TABLE 1. Dependence of the Degree of Extraction of the Total Flavonoids from Camel Thorn Herbage (in the period of fruit formation) on the Extractant and the Time

Extractant	Time of extraction, h	Total amount of flavonols, %
Water	1	0.42
40% solution of ethanol	1	0.78
70% solution of ethanol	1	0.70
48% solution of ethanol	0.5	0.60
"	1	0.98
"	2	0.97
"	3	0.98

TABLE 2. Influence of the Degree of Comminution of the Herbage on the Total Yield of Flavonols

Degree of comminution of the raw material (in the period of fruit formation), mm	Total amount of flavonols, %
2	0.98
2-5	0.88
5	0.85
10	0.76

TABLE 3. Dependence of the Yield of Aglycon on the Concentration of Hydrochloric Acid during Hydrolysis

Concentration of hydrochloric acid, %	Time of hydrolysis, h	Aglycon content, %
1	1	0.47
2	1	0.58
5	1	0.62
10	0.5	0.68
"	1	0.76
"	2	0.80
"	3	0.97

Procedure. About 1 g (accurately weighed) of the comminuted herbage (particle size 2 mm) was placed in a 100 ml conical flask and was covered with 50 ml of the 48% solution of methanol. The flask was attached to a a reflux condenser and the contents were boiled on the water bath for 1 h. The extract was filtered through absorbent cotton into a 50-ml measuring flask and the volume of the solution was made up to the mark with a 48% solution of ethanol (solution A). After the transfer of 20 ml of solution A to a 50-ml flask, 10 ml of a 10% solution of hydrochloric acid was added, and the flask was attached

TABLE 4. Results of the Quantitative Determination of the Total Flavonoids in the Herbage of Camel Thorn Calculated as Quercetin

Raw material	Moisture content, %	Flavonols found, %	Metrological characteristics
Mass flowering (1989 crop)	7.80	1.26 1.27 1.25 1.23 1.26	$\bar{X}$=1.25 $S\bar{X}$=0.00677 0.95=±0.018 A_{rel} =±1.50%
Mass flowering (1987 crop)	8.20	1.25 1.23 1.27 1.26 1.25	$\bar{X}$=1.25 $S\bar{X}$=0.00661 0.95=±0.018 A_{rel} =±1.46.%
Fruit formation (1989 crop)	9.30	0.99 0.97 0.98 0.96 0.97	$\bar{X}$=0.97 $S\bar{X}$=0.00509 0.98=±0.014 A_{rel} =±1.45 %
Fruit formation (1987 crop)	8.20	0.98 0.96 0.97 0.95 0.98	$\bar{X}$=0.97 $S\bar{X}$=0.00581 0.95=±0.016 A_{rel} =±1.65 %

to a reflux condenser and heated in boiling water bath for 3 h. Flask contents were cooled to room temperature and trans-ferred quantitatively to a separatory funnel, after which 20 ml of ethyl acetate was added and the mixture was shaken for 3 min.

The ethyl acetate extract was transferred to a 100-ml measuring flask. Extraction was performed five more times (20, 20, 10, 10, 10 ml). The ethyl acetate extracts were combined, transferred to the measuring flask, and the volume was made up to the mark with ethyl acetate (solution B). In a porcelain dish, 4 ml of solution B was evaporataed to dryness on the water bath. The dry residue was transferred with the aid of 95% ethanol (in 10-, 10-, 10-, 10-, and 5-ml portions) quantitatively into a 50-ml measuring flask and the volume of the solution was made up to the mark with 95% ethanol (solution C). The optical density of solution C so obtained was measured at a wavelength of 370 nm in a spectrophotometer. The control used was 95% ethanol.

The flavonol content was determined with the aid of a solution of a standard sample of quercetin from the formula

$$X = \frac{D_1 \cdot 0{,}000006 \cdot 50 \cdot 100 \cdot 50 \cdot 100}{D_0 \cdot 20 \cdot 4 \cdot a},$$

where D_1 and D_0 are the optical densities of solution C and the solution of the standard sample of quercetin at 370 nm and a is the weight of the sample, g.

The procedure has been tested on four samples of raw material. The comparative results are given in Table 4, from which it can be seen that in the quantitative determination of the total flavonols in the herbage of camel thorn by the proposed procedure reproducible results are obtained.

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