QUANTITATIVE DETERMINATION OF FLAVONOIDS IN *Spartium junceum*

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A procedure has been developed for the quantitative spectrophotometric determination of benzo-γ-pyrone derivatives in weavers' broom which is based on the hydrolysis of an ethanolic extract of the raw material and the chromatographic isolation of the hydrolysis products followed by the recalculation of the flavonoid content to the predominating components — luteolin and genistein. The fairly high flavonoid content (4.2%) permits this species to be recommended as a new source of this class of biologically active substances.

We have previously reported on the study of the qualitative flavonoid composition of weavers' broom *Spartium junceum* [1, 2]. For the qualitative estimation of the flavonoids of this type of raw material — compounds having intrinsic absorption in the ultraviolet and, especially, in the visible regions of the spectrum — we have used a spectrophotometric method of analysis. It does not require large amounts of raw material and is distinguished by a fairly high accuracy [3].

The method is based on the chromatospectrophotometric determination of the combined aglycones of the flavones and isoflavones when they are present together, using Vierordt's method [4]. A procedure for the quantitative determination of flavonoids in an infusion of *Sophora japonica* based on the hydrolysis of their combination has been described in the literature [5].

As the result of investigations on the isolation and identification of benzo-γ-pyrone derivatives in *S. junceum* it was found that the predominating components of the total flavonoids are luteolin and genistein and their glycosides. The quantitative analysis of the raw material included the hydrolysis of an ethanolic extract of the plant under investigation followed by the isolation of the aglycones by paper chromatography and their spectrophotometric determination. The concentration of total flavonoids was calculated as luteolin and that of the isoflavones as genistein. We selected these substances as standards and they corresponded in their physicochemical characteristics to compounds described in the literature [6].

In the extraction of the flavonoids we used the preliminary treatment of the raw material under investigation with liquid carbon dioxide or superhigh-frequency (SHF) heating. The investigations, which were performed in association with G. I. Molchanov, showed the absence of flavonoids in the CO_2 extracts but these compounds were extracted from the residual meal far more readily by ethanol and were less subject to oxidative processes. The use of the SHF treatment of the raw material for 150 sec enabled the time of extraction to be reduced to one third. The hydrolysis of the extent obtained was performed with 10% H_2SO_4. In view of the fact that the plant material contained, in addition to the flavonoids, a number of other substances affecting the quantitative determination of the benzo-γ-pyrone derivatives, the aglycones were separated by two-dimensional paper chromatography.

The UV absorption spectrum of a methanolic solution of the total flavonoids of *S. junceum* showed two maxima — at 268 and 343 nm. The absorption at a wavelength of 343 nm is composed of the absorption of luteolin and the aglycones of other flavones present in minor amounts, and the absorption at a wavelength of 268 nm relates to the total absorption of genistein with all the aglycones of the isoflavonoids.

Since the strongest absorption bands of the ethanolic solutions of aglycones of the flavonoids from *S. junceum* and the standards had close values, the 263 and 350 nm lines were

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used as the analytical wavelengths in the experiment. The absorption maximum of genistein in 95% ethanol is located at 263 nm and the absorption maximum of luteolin in 95% ethanol at 350 nm.

The concentration c of the flavonoids (%) was calculated from the formulas

$$c_1 = \frac{A_1 \cdot V}{E_1 \cdot l \cdot m} \cdot 100, \quad (1)$$

$$c_2 = \frac{(E_1 \cdot A_2 - E_2 \cdot A_1) \cdot V}{E_3 \cdot E_1 \cdot l \cdot m} \cdot 100, \quad (2)$$

where c_1 is the concentration of combined flavonoids calculated as luteolin; c_2 is the concentration of the total isoflavones, calculated as genistein; A_1 and A_2 are the optical densities of the solution under investigation at 350 and 263 nm, respectively; E_1 is the specific absorption index of luteolin in 95% ethanol at a wavelength of 350 nm, which is 962; E_2 is the specific absorption index of luteolin in 95% ethanol at a wavelength of 263 nm, which is 800; E_3 is the specific absorption index of genistein in 95% ethanol at a wavelength of 263, which is 888; l is the layer thickness of the cell, which was 10 mm; m is the weight of raw material, g; and V is the dilution.

The results of the quantitative determination of the flavonoids in *S. junceum* and the metrological characteristics of this method are given below:

Component Determined	Weight of raw material, g	No. of determinations, n	Concentration, c, %	Metrological Characteristics
Flavones	0.9876	6	2.76	$S = 9.98 \cdot 10^{-2}$
Isoflavones	0.9876	6	1.43	$S = 4.07 \cdot 10^{-2}$ $= \pm 0.1$
				$E_{rel.fl} = \pm 3.62\%$
				$E_{rel.isofl} = \pm 2.10\%$

The relative error of the method at the 95% confidence level when it is performed in sextuplicate does not exceed 3.7% for the flavones and 2.1% for the isoflavones, which indicates a satisfactory reproducibility of the method.

The absence of a systematic error of the method was shown by experiments with additions to a hydrolysate with a known concentration of flavonoids of definite amounts of standard solutions of luteolin and genistein:

Amount in the Hydrolysate, g		Added, g		Found, g		Relative error, %	
Flavone	Isoflavone	Luteolin	Genistein	Flavone	Isoflavone	Flavone	Isoflavone
0.0283	0.0137	0.0153	0.0112	0.0441	0.0249	+1.15	−2.82
0.0283	0.0137	0.0098	0.0076	0.0369	0.0217	−3.15	+1.88
0.0283	0.0137	0.0061	0.0047	0.0342	0.0179	−0.58	−2.72

EXPERIMENTAL

About 1 g (accurately weighted) of comminuted raw material (particle size 3-4 mm) after SHF heating (Slavyanka furnace, 150 sec) or about 1 g of the meal obtained after the separation of the CO_2 extract was placed in a 50-ml round-bottomed flask with a reflux condenser. Extraction was carried out with 10 ml of 60% ethanol for 3 h on the water bath. The extract was cooled and was filtered into a 50 ml measuring flask. It was made up to the mark with 60% ethanol. Of the resulting solution, 10 ml was transferred to a 50-ml round-bottomed flask and 10 ml of 10% H_2SO_4 was added, after which a reflux condenser was attached and the mixture was heated in the boiling water bath for 6 h. After cooling, the hydrolysate was neutralized with sodium hydroxide (pH 5.0-6.0). The resulting solution was chromatographed by two-dimensional paper chromatography in the BAW (4:1:5) and 15% AcOH systems. The flavonoids were extracted from the chromatograms with ethanol. The ethanolic extract was transferred to a 10-ml measuring flask and made up to the mark with 95% ethanol. The intensity of absorption of the solution was measured on a SF-4A spectrophotometer in cells with a thickness of 10 mm at wavelengths of 263 and 350 nm using 95% ethanol as the comparison solution. Calculation was

made by formulas (1) and (2) with allowance for dilution.

Preparation of Standard Solutions from Luteolin (mp 328-330°C) and Geinistein (mp 290-292°C). A chromatographically pure sample of the substance (0.05 g, accurately weighed) was dissolved in 80 ml of 95% ethanol with periodic stirring in a 100-ml measuring flask. The volume of the solution was made up to the mark with same ethanol and, after mixing, 1 ml of the resulting solution was transferred to a 50-ml measuring flask and the solution was made up to the mark with 95% ethanol and mixed.

SUMMARY

A procedure has been developed for the quantitative spectrophotometric determination of benzo-γ-pyrone derivatives in *Spartium junceum* which is based on the hydrolysis of an ethanolic extract of the raw material, the chromatographic separation of the hydrolysis products, and the subsequent calculation of the flavonoid contents to the predominating components — luteolin and genistein. The fairly high flavonoid content (4.2%) permits this species to be recommended as a new source of this class of biologically active substances.

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