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QUANTITATIVE ANALYSIS OF FLAVONOIDS IN THE TOTAL PREPARATION OF *Thermopsis alterniflora*

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A chromatographic-spectrophotometric method for determining the dominant components of the total preparation has been developed. It includes TLC separation of all substances and elution of flavonoids with subsequent spectrophotometric determination.

Key words: total flavonoids, analysis.

We previously determined the content of the total flavones and isoflavones in the aerial part of *Thermopsis alterniflora* (Fabaceae) [1].

In order to utilize waste from producing cytisine, the total preparation from grist that contains the flavonoids was obtained [2].

Experiments in various solvent systems under various conditions were performed in order to study the qualitative composition of the total preparation. The substances were separated best on Silufol UV-254 plates (Czech Rep.) using a mobile phase of $CHCl_3$—CH_3OH—HCO_2H (31:1:0.15). Nine spots were found on the plate.

The following flavonoids were identified by using certified standards and comparing UV spectra of eluted bands located at the level of the standards: luteolin, chrysoeriol, formononetin, apigenin, and cynaroside. A semi-quantitative method used the sizes and intensities of the spots to establish that the dominant components were luteolin, chrysoeriol, and formononetin.

The pharmacological properties of the flavones luteolin and chrysoeriol and the isoflavone formononetin were investigated in the pharmacology laboratory of the institute. Their hypolipidemic and antiatherosclerotic activities were measured [3].

The present article contains results of the quantitative analysis of total flavonoids and the dominant components in the total preparation.

The total content of flavones and isoflavones was found by the Vierordt method [1, 4] to be 83-85%. The metrological characteristics of the method for determining flavones and isoflavones are listed in Table 1.

The uncertainty of a single measurement at the 95% confidence level and $n = 6$ is less than ±2.26% for I and ±1.91% for II; for a three-fold determination, ±1.31 and 1.11%, respectively.

The substances were separated using $CHCl_3$—CH_3OH—HCO_2H (32:1:0.15) on Silufol UV-254 plates (Czech Rep.) with UV detection during the development of the chromatographic-spectrophotometric determination of the dominant components in the total preparation. The flavonoids were detected visually and by viewing in 254-nm UV light. The R_f values of luteolin, formononetin, and chrysoeriol are 0.09, 0.58, and 0.35, respectively.

The optimal elution conditions for the flavonoids were found by studying the desorption of pure samples of luteolin and formononetin. The results showed that dynamic equilibrium between the solutions and sorbents is attained after 2 h with shaking on a vibrational shaker and using 95% ethanol as eluent.

Spectrophotometry of the luteolin and chrysoeriol eluates was performed at 353 nm; of the formononetin eluate, at 249 nm in 10-mm cuvettes. The reference solution was the eluate from the control track.

The metrological characteristics of the chromatographic-spectrophotometric determination method for flavones and isoflavones are listed in Table 1.

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TABLE 1. Metrologic Characteristics

N	$\bar{x}$	S^2	S	±Δx	±E, %	$\pm E_3$, %
Determination of flavones (I) and isoflavones (II) by the Vierordt method						
I	45.25	0.945	0.972	1.02	2.26	1.31
II	39.87	0.521	0.722	0.76	1.91	1.11
Determination of total luteolin, chrysoeriol (I), and formononetin (II) by chromatography-spectrophotometry						
I	25.36	0.351	0.593	0.622	2.45	1.43
II	11.00	0.085	0.292	0.301	2.73	1.59

f = 5, P = 95%, $t_{p,f}$ = 2.57.

Thus, the content of the dominant components of the total preparation are luteolin and chrysoeriol, 24.7-26.0%; formononetin, 10.7-11.3%. The uncertainty of a single determination at the 95% confidence level and n = 6 is less than ±2.45% for I and ±2.73% for II; for a three-fold analysis, ±1.43 and 1.59%, respectively.

EXPERIMENTAL

Determination of Flavones and Isoflavones in Total Preparation. Preparation that was ground in a mortar and dried to constant mass (~0.1 g, accurate weight) was placed in a 100-mL volumetric flask, treated with alcohol (95%, 70 mL), and dissolved with heating on a water bath at 60°C. The solution was cooled, adjusted with the same 95% alcohol to the mark, and mixed (solution A). The solution was filtered through "Blue-ribbon" filter paper, discarding the first 10 mL of filtrate. The resulting solution (0.25 mL) was transfered to a 25-mL volumetric flask, adjusted with the same 95% alcohol to the mark, and mixed (solution B). The optical density of solution B was measured on a spectrophotometer in a 10-mm cuvette relative to 95% alcohol at 260 and 353 nm.

Calculations were performed using equations from the literature [4].

Chromatographic-Spectrophotometric Method for Determining Luteolin, Chrysoeriol, and Formononetin. Preparation that was ground in a mortar and dried to constant weight (~0.25 g, accurate weight) was placed in a 25-mL volumetric flask, treated with alcohol (95%, 15 mL), and dissolved with heating on a water bath at 60°C. The solution was cooled, adjusted to the mark with the same 95% alcohol, and mixed. Seven tracks were developed on a 20×20-cm Silufol UV-254 plate with UV-indicator. The resulting solution was placed as a 2-cm band of 0.01 mL at the origin of the first four tracks. The fifth track had luteolin solution (0.01 mL, 100 µg); sixth, formononetin (0.01 mL, 100 µg). The seventh track was a control. The plate with the samples was dried in air for 30 min and chromatographed in ascending mode in a chamber with $CHCl_3$—CH_3OH—HCO_2H (32:1:0.15). When the solvent front reached the edge of the plate, the plate was removed from the chamber and dried in air for 15-20 min. The contents of luteolin, chrysoeriol, and formononetin were marked in UV light.

The sorbent bands containing luteolin, chrysoeriol, and formononetin and the control band were scraped off, placed in 100-mL flasks with stopcocks, treated with 95% alcohol (10 mL), and eluted by shaking on a vibrational shaker for 2 h (luteolin and chrysoeriol were eluted together). The eluates were filtered through glass filters (pore size 16). The optical densities of the solutions were determined at 353 nm (for luteolin and chrysoeriol) and 249 nm (for formononetin) in 10-mm cuvettes.

The contents of luteolin, chrysoeriol (X_1) and formononetin (X_2) were calculated using the equation:

$$X_{1,2} = D \cdot a_0 \cdot 100/(D_0 \cdot a),$$

where D_0 is the optical density of the luteolin-reference or formononetin-reference band at 353 and 249 nm, respectively; D is the optical density of the luteolin and chrysoeriol elutate or the formononetin eluate at these same wavelengths; a_0 is the weight of luteolin-reference or formononetin-reference in g; and a is the weight of the total preparation in g.

Preparation of Luteolin and Formononetin Solutions. Luteolin or formononetin that was previously dried to constant mass at 105ºC (~0.25 g, accurate weight) was placed in a 25-mL volumetric flask, treated with 95% alcohol (15 mL), and dissolved. The solution was adjusted to the mark with the same 95% alcohol and mixed.

Thus, the developed methods can with sufficient accuracy and reproducibility evaluate the content of flavones and isoflavones and the dominant components in the total preparation from *Thermopsis alterniflora*.

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