

ISOLATION OF THE TOTAL FLAVONOIDS FROM THE EPIGEAL PART OF *Thermopsis alterniflora*

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UDC 615.322.543.42.66.011

Plants of the genus *Thermopsis* are a rich source of alkaloids and flavonoids [1, 2] possessing a broad spectrum of pharmacological action.

The drug cytosine is obtained from the epigeal part of *Thermopsis alterniflora* Regel et Schmalh., fam. Fabaceae.

With the aim of utilizing the wastes from the preparation of cytosine, we have investigated the extraction of the total flavonoids from a meal of the herbage of this plant after the isolation of the total alkaloids.

In the choice of extractant we used various alcohols and their aqueous solutions. Extraction was carried out by steeping at room temperature with an accuracy of $\pm 2^\circ\text{C}$. The alkaloids were first extracted with water.

A single extraction did not permit an unambiguous evaluation of the results of the experiment. When fivefold extraction was used, it was found that methanol and 70-80% ethanol and isopropanol possessed the greatest extractant activities (Table 1). Samples of each extract were analyzed spectrophotometrically [3].

To find the optimum conditions for extracting the flavonoids we used the Box-Wilson method for the mathematical planning of an experiment [4] on the first contact of the phases.

The following factors affecting this process to the greatest degree were varied: X_1 — the time of extraction, h; X_2 — the concentration of the extractant, %; X_3 — the temperature of extraction, $^\circ\text{C}$; and X_4 — the degree of comminution of the raw material, mm. As the optimization parameter (Y) we chose the total yield of flavonoids at the first phase contact as a percentage of their amount in the raw material.

In the planning of the experiment we used a 1/2 replica of a total factorial experiment of the $Y = 2^4$ type. The following initial levels of the factors and ranges of their variation were selected: X_1 — 6 ± 2 h; X_2 — $70 \pm 10\%$; X_3 — $45 \pm 20^\circ\text{C}$; X_4 — 10 ± 5 mm.

TABLE 1. Influence of the Extractant on the Passage of the Total Flavonoids into the Extract

Extractant,	%	Extracted, % of the amount in the raw material	
		single extraction	five extractions
Methanol	99.5	54.9	94.2
	90	51.2	92.4
	80	44.3	90.3
	70	44.0	89.6
	60	32.1	71.4
Ethanol	95	43.7	89.2
	90	47.2	90.3
	80	55.2	95.2
	70	54.8	94.3
	60	31.7	70.1
Isopropanol	100	43.8	87.1
	90	45.1	89.4
	80	53.7	94.0
	70	52.9	93.5
	60	32.0	70.8

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. Translated from *Khimiya Prirodnykh Soedinenii*, No. 6, pp. 911-912, November-December, 1995. Original article submitted March 27, 1995.

After treatment of the results obtained, we arrived at the following mathematical model of the process:

$$Y = 42.21 + 1.55X_1 + 8.03X_2 + 3.44X_3$$

The regression coefficients after the calculation of the confidence interval ($b = \pm 1.12$) revealed the main factors affecting the extraction process: time of extraction > temperature of extraction > concentration of the extractant. Statistical analysis of the results obtained ($F_{\text{calc}} = 1.99 < F_{\text{tab}} = 4,50$) showed that the model adequately describes an approach to the optimum. No steepest ascent was carried out, since the total yield of flavonoids obtained was 56.87%, which is quite acceptable at first phase contact.

Thus, the method of mathematical planning of experimental work has revealed the optimum conditions for extraction from the raw material at first contact of the phases, namely: 80% alcohol at 65°C, with a degree of comminution of the raw material such that 70% passes through a sieve with an aperture diameter of 5 mm, and a process time of 8 h.

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