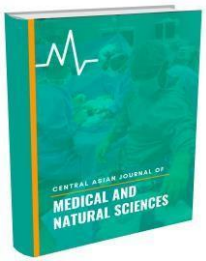

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Article

Urtica Dioica, Melissa Officinalis Ba Leonurus Cardiaca, Optimization of the Process of Extraction of Flavonoids from Collective RAW Materials with a Sedative Effect Formed from the Surface Part of Plants

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Abstract: Factors influencing the extraction process of flavonoids with 70% ethyl alcohol from medicinal nettle, a mixture of medicinal ratios, were studied by mathematical planning using the Box-Wilson method, and the following optimal conditions were selected: Process time: 6 hours; degree of grinding of raw materials: 6 mm; process temperature: 30 °C; raw material and solvent ratio: 1:4.5. By researching the dynamics of the extraction process, it was determined that extraction should be done five times. It was found that these conditions allow to extract 95.25% of flavonoids compared to the storage in raw materials.

Keywords: Medicinal nettle (Urtica dioica L.), medicinal lemon balm (Melissa officinalis L.), Leonurus turkestanicus V. Kreez et Kupr), flavonoid, extraction, optimization, mathematical planning, Boxu-Wilson method.

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1. Introduction

In recent years, it has been observed that the number of nervous diseases is constantly increasing. Complex treatment methods play an important role in the treatment of nervous diseases and prevention of their complications. In this regard, the use of sedative drugs is effective. Because sedative drugs are able to improve the patient's condition and prevent more serious changes in the psychological and physiological health of a person in the complex treatment of diseases of the central nervous system [1, 2]. Therefore, creation of sedative drugs based on biologically active substances of medicinal plants remains one of the urgent tasks of the pharmaceutical industry. In this regard, medicinal nettle, medicinal lemon, and Turkestan lion's ear, which have sedative properties, are interesting objects. Because these medicinal plants can be grown in any quantity in Uzbekistan by agrarian means.

Medicinal nettle or Scorpion (Urtica dioica L.) is a plant belonging to the Urticaceae family (Urticaceae Juss.) and is rich in A, S, K, E, and H drugs, as well as macro- and microelements, phenolic acids. In the Pharmacopoeia of the Russian Federation, nettle is standardized by the amount of oxychoric acids compared to chlorogenic acid [3]. Oxycoric

acids belong to the phenol compounds of the C6-C3 series, which have a calming and neuroprotective effect. The sedative effect of nettle extract is explained by a decrease in locomotor activity in mice and rats [4, 5].

Medicinal lemon balm (Melissa officinalis L.) belongs to the Labiatae (Lamiaceae) family and has been used in folk medicine for the treatment of nervous diseases. Essential oil, S, V1, V2 drugs, flavonoids, phenolic acids (rosemary, chlorogenic, caffeic), coumarins, flavoring substances, oleanol, ursolic, and other acids are found in the composition of the above-ground part of lemon [6]. A possible mechanism for the sedative activity of lemon balm has been shown to be related to the inhibition of acetylcholinesterase of the central nervous system and the reduction of the activity of nicotinic and muscarinic receptors in the cerebral cortex [7, 8]. In the British and Russian pharmacopoeias, lemon balm is standardized by the amount of phenolic compounds compared to rosmarinic acid [9, 10].

Lion's ear species belong to the Lamiaceae (Labiatae) family; two types of it are used in medicine: the five-lobed lion's ear, Leonurus Quinquelobatus L., and the ordinary lion's ear, Leonurus cardiaca L.Turkestan lionear (Leonurus turkestanicus V Krees. et. Kupr.) is widespread in Uzbekistan. In medicine, the above-ground part of the lion's ear species is used. Medicinal products derived from lion's ear have sedative, sleep quality improvement, nighttime awakenings, and antidepressant activities [11]. Lion's ear contains flavonoids, astringents, iridoids, alkaloids, bitter glycosides, terpenoids, essential oils, ascorbic acid, macro- and micronutrients [12]. Scientists have proven that the soothing properties of dandelion depend on stachydrin, leonurin, iridoids and flavonoids [13, 14]. In this case, the valerian herb was standardized by the spectrophotometric method for the sum of flavonoids (not less than 0.2% compared to rutin) and extractive substances (not less than 15% in 70% ethyl alcohol) [15].

Taking into account the above, a complex tincture with a calming effect obtained with 70% ethyl alcohol has been developed at the Tashkent Institute of Pharmaceutical Sciences, which consists of a 1:1:1 mixture of the above-ground parts of medicinal nettle, medicinal lemon balm, and Turkestan lion's ear plants. It has been found that this complex tincture is equivalent to the drug "Novopassit" of the Czech company "TEVA Czech Industries, s.r.o" in terms of sedative properties [16]. However, due to the presence of alcohol in the composition of tinctures, caution is required in some cases, including children and kidney and liver diseases. This shortcoming can be overcome by switching to the form of solid drugs, especially tablets and capsules. Therefore, we continued our scientific research to create a solid dosage form of the proposed sedative tincture. For this, it became necessary to obtain a dry extract based on tincture. When obtaining dry extracts, effective extraction of active substances from raw materials is required first. Therefore, we continued our scientific research to determine the optimal conditions for extracting flavonoids from a 1:1:1 mixture of above-ground parts of medicinal nettle, medicinal lemon balm, and Turkestan lion's ear plants.

The research of technological processes requires numerous and time-consuming studies. Therefore, it is important to obtain valid results with a small number of experiments. In this case, the optimization of the processes based on the obtained results allows to increase the efficiency of scientific research by conducting experiments in determining the optimal conditions of the factors affecting the efficiency of all stages of the technology through mathematical modeling. In this regard, Box-Wilson, Box-Behnken, 3x3 or 4x4 models of Latin squares, and other mathematical planning methods are increasingly used [17-21].

2. Materials and Methods

Optimizing the extraction process of flavonoids with 70% ethyl alcohol from a 1:1:1 mixture of above-ground parts of nettle, lemon balm, and Turkestan lion's ear plants in a 1:1:1 ratio by mathematical planning using the Box-Wilson method.

The objects and methods of research.

Medicinal nettle, medicinal lemon balm, and medicinal plants of Turkestan lion's ear were prepared in 2023. The prepared raw materials were dried in a well-ventilated room protected from direct sunlight, ground in a mill, and mixed in a 1:1:1 ratio (hereafter referred to as bulk raw materials in the text).

According to the information presented above, our soothing dry extract was standardized by the sum of flavonoids compared to rutin. The prepared aggregate raw material was analyzed, and the following results were obtained in the tests: moisture: 8.52%, total ash: 6.25%, ash insoluble in 10% hydrochloric acid solution: 1.16%, organic additives: 1.48%, mineral additives: 0.73%, and the amount of flavonoids: 0.77%.

The process of extraction of flavonoids from sedating raw materials was carried out on the basis of mathematical planning, Box-Wilson method optimization, and statistical analysis, as well as the dynamics of the process [17, 18, 22] based on the methods presented in the literature.

During the experiments, the sum of flavonoids was determined spectrometrically against rutin [23]: For this purpose, 0.1 g (exact draw) sample volume is placed in a 100-ml flask, and 50 ml of hot 50% ethyl alcohol is added and mixed. After cooling to room temperature, the same solvent is added until the volume of the solution reaches the mark of the flask (Solution A). 3 ml of solution A is placed in a 25 ml volumetric flask, 3 ml of 2% aluminum chloride, 1 drop of dilute acetic acid is added, and 96% ethyl alcohol is added until the volume of the solution reaches the mark of the flask. The solution is mixed and placed in a dark place. After 40 minutes, the solution is filtered through a "white strip" paper filter and the optical density of the resulting solution is immediately measured in a spectrophotometer at a wavelength of 400 nm in a cuvette with a layer thickness of 10 mm. As a reference solution, a solution prepared in the same way but without the addition of aluminum chloride solution is used.

In parallel, the optical density of the standard sample of the porous substance rutin solution (WSSS) is measured.

The amount of total flavonoids in percent relative to rutin (X) is calculated by the following Formula:

$$\tilde{O} = \frac{D_1 \cdot 100 \cdot 25 \cdot \grave{a}_0 \cdot 1 \cdot 100 \cdot Đ}{D_0 \cdot a_1 \cdot 3 \cdot 100 \cdot 25 \cdot 100}; \qquad (1)$$

where:

D_1 – *optical density of the test solution;*

D_0 – *optical density of the standard sample of the porous substance rutin solution (WSSS);*

a_1 – *weight of drug sample, g;*

a_0 – *weight of standard sample (WSSS) of porous substance rutin solution, g*;

P – *Amount of rutin in WSSS, %.*

Preparation of routine standard sample solution (SSS). 0.05 g (exact draw) of rutin (FS

42 Uz-0137-2013), dried at 130-135 °C for 3 hours, is placed in a 100 ml volumetric flask and dissolved in 85 ml of 96% ethyl alcohol by heating in a water bath. After the alcoholic solution of rutin is cooled to room temperature, the same solvent is added until the volume of the solution reaches the mark of the flask (Solution A) and mixed. 1 ml of solution A is placed in a 25 ml volumetric flask, and 3 ml of 2% aluminum chloride, 1 drop of dilute acetic acid, and 96% ethyl alcohol are added until the volume of the solution

reaches the mark of the flask. The solution is mixed and placed in a dark place. After 40 minutes, the solution is filtered through a "white strip" paper filter, and the optical density of the resulting solution is immediately measured in a spectrophotometer at a wavelength of 400 nm in a cuvette with a layer thickness of 10 mm.

3. Result

As a studied indicator of optimization, the yield of flavonoids was taken in relation to the retention of the phases in the raw material during the first exposure. The experiments were carried out by taking 1.0 kg of raw material and extracting it once with 70% ethyl alcohol, changing the factors affecting the extraction process.

The step limits (upper and lower) and the difference between them were defined as follows (Table No.1).

Table No.1

Indicators of influencing factors

Level limits of influencing factors	Influencing factors			
	X_1 process travel time, hours	X_2 raw material grinding level, mm	X_3 process temperature, ° C	X_4 raw material to solvent ratio
The top step	6	6	30	1:4,5
Middle stage	5	4	20	1:4,0
The bottom step	4	2	10	1:3,5
Difference of borders	1	2	10	0,5

Mathematical planning of the obtained results was carried out on the basis of four-factorial, twice-replicated experiments. The fourth planning factor (X4) was calculated depending on the other two factors (X1 and X2) (X4=X1X2). Thus, the total number of experiments is 16 and we got total experiments in the form of Y=24-1 of complete factors.

A root of planning for conducting the experiments was drawn up and based on that it was carried out. The plan of the experiments and the value of the obtained results are presented in Table No.2.

Table No.2

The root of the planning and the results of the experiments

Experimental procedure	Process coding					The yield of flavonoids in relation to the storage in raw materials, %		
	X_0	X_1	X_2	X_3	X_4	Y_1	Y_2	$Y_{ўpr.}$
1	+	+	+	+	+	48,9	50,9	49,9
2	+	+	-	+	-	41,3	38,3	39,8
3	+	-	+	+	-	39,6	37,6	38,6
4	+	-	-	+	+	32,0	33,4	32,7
5	+	+	+	-	+	47,8	46,4	47,1
6	+	+	-	-	-	33,2	32,6	32,9
7	+	-	+	-	-	31,3	31,9	31,6
8	+	-	-	-	+	29,7	30,1	29,9

The regression equation of the process was constructed by Formula No.2.

$$Y=B_0+B_1X_1+B_2X_2+B_3X_3+B_4X_4 \quad (2)$$

where: B_0, B_1, B_2, B_3, B_4,– the coefficients of the regression equation

For this, first, the coefficients of the regression equation were calculated using Formula No.3.

$$b_j = \frac{\sum_{j=1}^{N} B_{ij} Y_j}{N}, \quad (3)$$

where: i- ordinal number of experiments (i=1,2,…,8); j- ordinal number of the factor (j=1,2,…,5); N – number of experiments in root; X_{ij} – coding value of factors.

Found: $b_0 = 37,81$; $b_1 = 4,61$; $b_2 = 3,98$; $b_3 = 2,43$; $b_4 = 2,08$

The following regression equation was created by putting the values of the "B" coefficient into formula 2.

$$Y = 37,81 + 4,61 X_1 + 3,98 X_2 + 2,43 X_3 + 2,08 X_4$$

In order to verify the validity of the conducted experiments and the proportionality of the obtained model, a statistical analysis was performed and the results were recorded in Table No.3.

Table No.3

Statistical analysis

ΔY_i	ΔY_i^2	S_i^2	$Y_{хсб}$	$\Delta Y'_i$	$(\Delta Y_i')^2$
- 1,0	1,0	2,0	50,93	-1,04	1,076
1,5	2,25	4,5	38,78	1,01	1,025
1,0	1,0	2,0	37,53	1,06	1,129
-0,7	0,49	0,98	33,73	-1,04	0,076
0,7	0,49	0,98	46,06	1,04	1,076
0,3	0,09	0,18	33,91	-1,01	1,025
-0,3	0,09	0,18	32,66	-1,06	1,129
-0,2	0,04	0,08	28,86	1,04	1,076
		$\sum S_i^2 = 10,90$			$\sum (\Delta Y_i^l)^2 = 8,61$

The variance of experiments was calculated using formula 4 (Table No. 3).

$$S_i^2 = \frac{2\Delta Y^2}{1} \quad (4)$$

The primary value of the variance was checked according to the condition of the Cochrane criterion (Formula 5). According to this, if the condition is fulfilled, it is proved that the variance is primary.

$$G_{экс} = \frac{S_{max}^2}{\sum_{i=1}^{N} S_i^2} \le G_{кр} \quad (5)$$

$G_{кр} = 0,6798$ [17]

Топилди: $G_{экс} = 0,4128$

It can be seen that the obtained variance satisfied the condition of the Cochrane criterion, which means that the variance is primary.

Model adequacy was found by formula No. 6.

$$S_{ад}^2 = \frac{\sum_{i=1}^{N} \left(\Delta Y_i^1\right)^2}{f}, \quad (6)$$

For this, the value of ΔY^1 was first determined using formula 7 (Table No. 3).

$$\Delta Y^1 = Y_{ўр} - Y_{хсб} \quad (7)$$

The value of variance generated (for two replicate experiments) was found by Formula No. 8:

$F_{экс} < F_{таб}$; (10) $F_{экс} = \frac{S^2_{ад}}{S^2_y}$ (11)

$$S_y^2 = \frac{2\sum_{i=1}^{N}(Y_{iq} - Y)^2}{N} = \frac{\sum_{i=1}^{N} S_i^2}{N}, \quad (8)$$ where: i =1,2, …, N

Found: $S_y^2 = 1,3625$

Using the obtained results, the dispersion adequacy was found by formula 9.

$$S^2_{ад} = \frac{n\sum(Y_{ср} - Y_{рас})^2}{N\text{-}q} \quad (9)$$ where: q = k + 1; k is the number of regression coefficients.

Model adequacy was checked by Fisher's criterion (10 formulas). If the condition is met, the found model is considered adequate. For this, $F_{экс}$ was first determined by Formula No. 11.

In our experiment, $F_{ж.д}$ was found in the table for the value f1=2; f2=8. Accordingly, it was found that $F_{0,05}^{(2,8)} = 4.5$ for our indicators [17].

According to the obtained results, the condition was fulfilled ($F_{экс} = 4,2146 < F_{таб} = 4,5$) and the adequacy of the model was proved.

The confidence interval (Δb_i) to determine the value of the coefficients found was found by formula 12.

$\Delta b_i = \pm t S_{bi}$ (12) Where: t-the result of the Student's criterion selected at the level of 0.05 is presented in the table; S_{bi} is the squared error of the regression coefficients.

Taking into account that $S_{bi} = \pm\sqrt{S_{bi}^2}$, the variance of regression coefficients S_{bi}^2 was determined by formula 13:

$$S^2_{bi} = \frac{S^2_y}{N}; \quad (13)$$

Found: $S_{bi}^2 = 0,17$

$S_{bi} = 0,41$

Table No. 4

Value check results

b_i–value	The sign	Δb_i–value	Summary results
$b_0 = 37,81$	>		The coefficient is valuable
$b_1 = 4,61$	>		The coefficient is valuable
$b_2 = 3,98$	>	1,76	The coefficient is valuable
$b_3 = 2,43$	>		The coefficient is valuable
$b_4 = 2,08$	>		The coefficient is valuable

Table No. 4 shows that all the coefficients of the regression equation are significant.

In addition, all selected factors have an influence on the efficiency of the extraction process according to the value of the coefficients as follows: $X_1 > X_2 > X_3 > X_4$

The best result was obtained in the 1st experiment (49.9%), and accordingly, the factors affecting the extraction process of aggregate raw materials with 70% ethyl alcohol were selected as follows: process time - 6 hours; fineness of raw materials - 6 mm; process temperature – 30 °C; raw material and solvent ratio - 1:4.5.

The dynamics of extraction of flavonoids from bulk raw materials under the

influence of the determined factors was studied. Based on the results of the experiment, it was concluded that it is necessary to extract the aggregated raw material five times in 70% ethyl alcohol. In this case, the duration of pouring the first extract should not be less than 6 hours, the second and third - 4, the fourth and fifth - 2 hours.

4. Conclusion

Based on the selected conditions, the extraction process was carried out five times, and it was found that the yield of flavonoids was not less than 95.45%. This is considered an acceptable indicator for the extraction process.

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