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ORIGINAL PAPER

DEVELOPMENT OF OPTIMAL TECHNOLOGY OF ALCOHOL EXTRACT *CENTAURIUM ERYTHRAEA* RAFN. HERB

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Abstract

Introduction. Throughout the centuries, plants have been used not only as a source of nutrition, but also in the fight against diseases. The most interesting are medicinal plants being well tolerated by patients regardless of age. One of these plants is *Centaurium erythraea* Rafn.
The aim of the study was to develop an optimal technology for obtaining an alcoholic extract with the highest content of biologically active substances: hydroxycinnamic acids, phenolic compounds.
Materials and methods. We have performed a study of the concentration of ethanol and multiplicity of dilution with the help of a symmetric rotary composite plan of the second order.
Results and discussion. The regression equations were adequate – $F_{exp.} < F_{tab.}$. The nature of the influence of the studied factors was determined by the magnitude and signs of the coefficients from regression. To obtain the alcohol extract with the highest content of hydroxycinnamic acids and phenolic compounds, the concentration of ethanol should be 69%, and the ratio of raw material to extractant should be 1 to 5.
Conclusions. Further pharmacological studies showed the extract obtained reliability of its application for an increase in gastric acid secretion.

Résumé

Développement de la technologie optimale de l'extrait d'alcool de l'herbe *Centaurium erythraea* Rafn.

Introduction. Au cours des siècles, les plantes ont été utilisées non seulement comme source de nutrition, mais également dans la lutte contre les maladies. Les plus intéressantes sont les plantes médicinales bien tolérées par les patients, quel que soit leur âge. Une de ces plantes est *Centaurium erythraea* Rafn. Le but de l'étude était de développer une technologie optimale pour obtenir un extrait alcoolique avec la plus forte teneur en substances biologiquement actives: acides hydroxyconiques, composés phénoliques.
Matériels et méthodes. L'étude de la concentration en éthanol et la multiplicité de dilution ont été réalisées à l'aide d'un plan composite rotatif symétrique de second ordre.
Résultats et discussion. Les équations de régression étaient adéquates – Fexp. < Ftab. La nature de l'influence des facteurs étudiés a été déterminée par les amplitudes et les signes des coefficients de régression. Pour obtenir l'extrait alcoolique avec la teneur la plus élevée en acides hydroxycinnamiques et en composés phénoliques, la concentration en éthanol doit être de

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Keywords: hydroxycinnamic acids, phenolic compounds, ethanol concentration.

69% et le rapport matière première / agent d'extraction doit être compris entre 1 et 5.
Conclusion. D'autres études pharmacologiques ont montré que l'extrait obtenu était fiable pour son application dans le but de l'augmentation de la sécrétion d'acide gastrique.

Mots-clés: acides hydroxycinnamiques, composés phénoliques, concentration en éthanol.

Introduction

During many centuries, plants have been used not only as a source of nutrition, but also in the struggle with diseases[1]. The importance of medicinal plants did not reduce the annual increase in the number of synthetic medicines, which often model biologically active substances of plants and are their chemical equivalents[2].

The most interesting are medicinal plants that have a long history of use and have been shown to possess low side effects and are well tolerated by patients, regardless of age[3]. These plants include *Centaurium erythraea* Rafn, an official plant that is part of the European Pharmacopoeia[4], the Ukrainian Pharmacopoeia[5], the British Herbal Pharmacopoeia[6] and many other pharmacopoeias.

Centaurium erythraea Rafn. stimulates the secretion of the glands of the digestive tract, and have choleretic, antitumor, sedative[7], antidiabetic[8], anti-inflammatory, antipyretic, analgetic[9], antimalarial, tonic[10], anticholinesterase and antimutagenic properties[11], due to the presence of phenolic compounds[12], polysaccharides[7], volatile compounds[13], chlorophylls and carotenoids[14]. Centaurium erythraea Rafn. ordinary grass is part of the following preparations and nutritional supplements: „Canephron® N" ("Bionorica SE", Germany)[15], „Original Bigger Bittner Balsam" („Richard Bittner AG", Austria)[16], „Löwenzahn, Edelweiss, Tausendgüldenkraut und Cholin Saft" ("Dr. Dünner", Switzerland)[17], „Diacure" ("Lehning Laboratoires", France)[18], Stomaran" ("Leros", Czech Republic)[19].

The aim of the study was to develop an optimal technology for obtaining the alcoholic extract with the highest content of biologically active substances: hydroxycinnamic acids, phenolic compounds.

When planning an experiment, mathematical methods were used not only at the stage of processing the results, but also at the very first stage of experimentation, called the creation of an experiment plan[20]. The plan is designed to reduce the total number of experiments, but at the same time to be able to thoroughly analyze the data of the experiment and obtain reliable results, which cannot always be achieved with the help of a subjective approach[21]. The stage of the planning of the experiment is especially crucial, when the influence of quantitative factors is studied. Quantitative factors are variables that can be quantified: measure, weight, etc[22,23].

Material and methods

Centaurium erythraea Rafn. herb was collected in Ukraine, on the outskirts of Zboriv (Ternopil region) during the flowering period in July 2013. The raw material was authenticated by Professor Dr. Svitlana Marchyshyn. A voucher specimen no. 133 is kept at the Department of Pharmacognosy and Medical Botany, I. Horbachevsky Ternopil State Medical University, Ukraine. The plant material was dried using conventional methods and then stored in paper bags in dry place[7].

When developing the optimal technology of alcohol extract of *Centaurium erythraea* Rafn. herb, the concentration of ethanol and the multiplicity of dilution were studied at five levels[24,25].

The study of these two factors or two independent variables[26] (ethanol concentration, the multiplicity of dilution) was carried out with the help of a symmetrical rotatable composite plan of the second order. The magnitude of „star shoulder" for this plan is 1.414[27,28]. The „star point" was found by multiplying the variation interval by the value of „star shoulder". These values were added to the basic level, receiving the upper „star point" ("+α") and subtracted to obtain the lower „star point" ("−α")[29].

The results of the research were obtained from the regression analysis. We found the relationship between the studied factors and the content of hydroxycinnamic acids and phenolic compounds using the second order regression equation.

The second order model for two factors has the following form[30,31]:

$$y = b_0x_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{22}x_2^2$$

Table I. Quantitative factors and their levels, examined in the study of alcohol extracts derived from *Centaurium erythraea* Rafn. herb

Factor	The interval of variation	Factor level				
		Lower star point «–α»	Bottom «–»	Main «0»	Top «+»	Top star point «+α»
X_1 – concentration of ethanol, %	10	41	45	55	65	69
X_2 – the ratio of raw materials and extractant	2	5	6	8	10	11

Table II. The matrix of experiment planning and the results of the study of alcohol extracts from *Centaurium erythraea* Rafn. herbs

Series No.	x_1	x_2	y_1	y_2	*Comment*
1	+	+	9.57	13.98	Full factorial experiment
2	–	+	9.13	15.92	
3	+	–	9.99	15.74	
4	–	–	9.98	12.47	
5	+ 1.414	0	9.97	15.57	Star points
6	– 1.414	0	9.89	14.43	
7	0	+ 1.414	8.73	14.13	
8	0	– 1.414	9.56	12.99	
9	0	0	8.59	12.13	Main level
10	0	0	8.62	12.31	
11	0	0	8.57	12.14	
12	0	0	8.49	12.15	
13	0	0	8.52	12.30	
14	0	0	8.54	12.21	
15	0	0	8.51	12.25	
16	0	0	8.55	12.17	

Comment: y_1 – content of hydroxycinnamic acids, mg/ml;˝y_2 – content of phenolic compounds, mg/ml.

Using the F-criterion, the statistical significance of the coefficients and the adequacy of the models were testified[7].

Among the indicated values of the concentration of ethanol (41% *v/v*, 45% *v/v*, 55% *v/v*, 65% *v/v*, 69% *v/v*) and the ratio of raw material: extractant (1:5, 1:6, 1:8, 1:10, 1:11), their optimal value was defined for obtaining the alcohol extract of *Centaurium erythraea* Rafn. herb with the highest content of hydroxycinnamic acids and phenolic compounds. The list of quantitative factors and five levels is given in Table I[32,33].

To process the experimental data obtained, we used the method of mathematical planning of the experiment used by Visio and Statistica 6.0.

Results and discussion

The regression equations were adequate – $F_{exp.} < F_{tab}$. The nature of the influence of the studied factors was determined by the magnitudes and signs of the coefficients from regression.

The matrix of experiment planning and the results of the study of alcohol extracts from *Centaurium erythraea* Rafn. herbs are presented in Table II.

The relationship between the investigated factors and the content of hydroxycinnamic acids is described by the following regression equation:

$$y_1 = 8.55 + 0.07x_1 - 0.31x_2 + 0.11x_1x_2 + 0.72x_1^2 + 0.33x_2^2.$$

We analyzed the magnitude of the coefficients and signs in front of them from the regression equation. However, in cases where the values of the coefficient of pair interaction ($0.11x_1x_2$) and quadratic coefficients ($0.72x_1^2$, $0.33x_2^2$) are greater than linear coefficients, it is appropriate to consider the analysis of the regression equation on the basis of graphic one-factor figures.

The influence of ethanol concentration and the ratio of raw material to extractant on the content of hydroxycinnamic acids of the alcohol extract of *Centaurium erythraea* Rafn. herb is depicted in Figures 1 and 2.

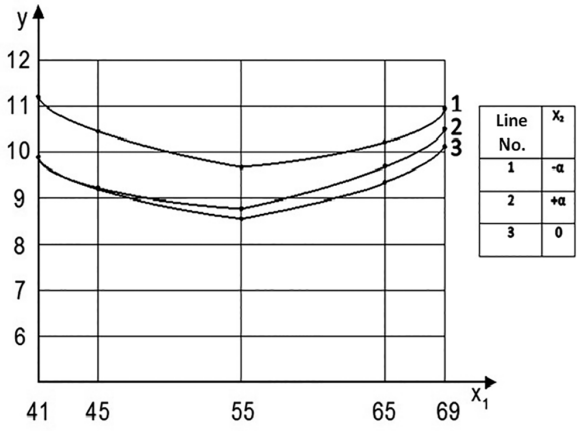

Figure 1. The influence of ethanol concentration on the extraction of hydroxycinnamic acids

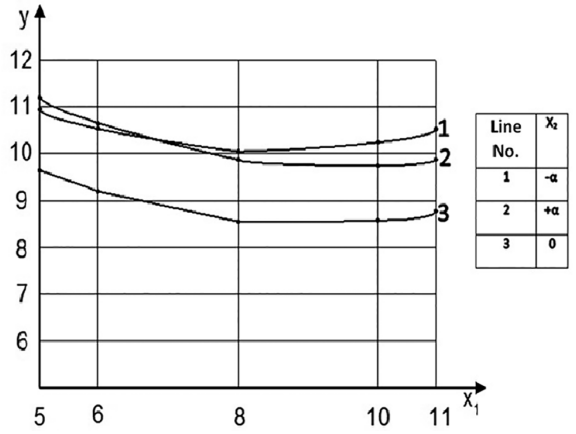

Figure 2. The effect of multiplicity of dilution on the extraction of hydroxycinnamic acids

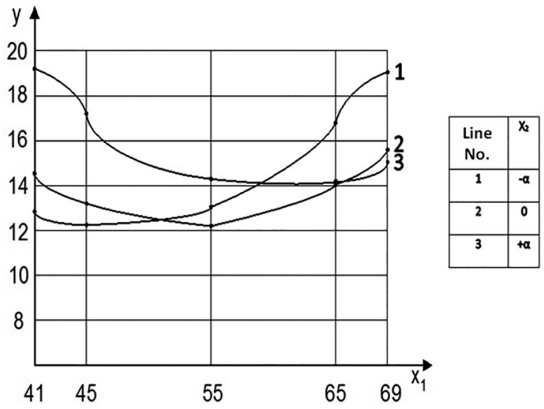

Figure 3. Effect of ethanol concentration on the extraction of phenolic compounds

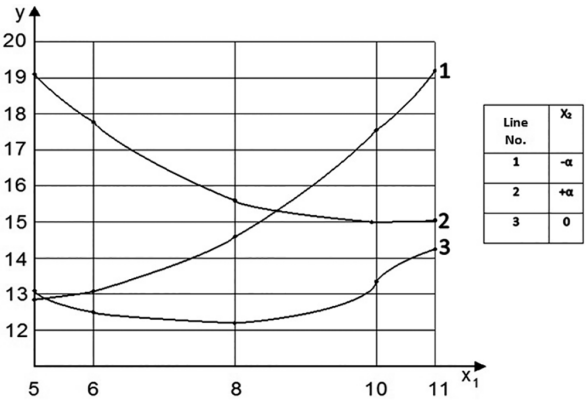

Figure 4. The impact of multiplicity of dilution on extraction of phenolic compounds

Line no. 1 ($x_2 = -\alpha$) is the most promising for consideration, when the factor x_2 is studied at the lower star point. As we can see from Figure 1, the content of hydroxycinnamic acids in alcoholic extract initially decreases from 11.21 mg/mL to 9.65 mg/mL with an increase in the concentration of ethanol from 41% to 55%, and it increases to 10.97 mg/mL with further increase of ethanol concentration from 55% to 69%.

Line no. 1 ($x_2 = -\alpha$) is the most promising for consideration, when the factor x_2 is studied at the lower star point. As we can see from Figure 2, the content of hydroxycinnamic acids in alcoholic extract initially decreases from 10.97 mg/mL to 10.09 mg/mL with an increase in the ratio of raw materials to the extractant from 1:5 to 1:8 and it rises to 10.53 mg/mL with a further increase in the ratio of raw material to extractant from 1:8 to 1:11.

The relationship between the investigated factors and the content of phenolic compounds is described by the following regression equation:

$$y_2 = 12.21 + 0.37x_1 + 0.41x_2 - 1.30x_1x_2 + 1.45x_1^2 + 0.73x_2^2;$$

The impact of the values of ethanol concentration and multiplicity of dilution on the production of alcoholic extract from *Centaurium erythraea* Rafn. herbs with the highest content of phenolic compounds is presented in Figures 3 and 4.

Line no. 1 ($x_2 = -\alpha$) is the most promising for consideration, when the factor x_2 is studied at the lower star point. As we can see from Figure 3, the content of phenolic compounds in alcoholic extract initially decreases from 12.87 mg/mL to 12.33 mg/mL, with an increase in the concentration of ethanol from 41% to 45%, and it increases to 19.11 mg/mL with a further increase in the concentration of ethanol from 45% to 69%.

Line no. 1 ($x_2 = -\alpha$) is the most promising for consideration, when the factor x_2 is studied at the lower star point. As we can see from Figure 4, the content of hydroxycinnamic acids in alcoholic extract increases from 12.87 mg/ml to 19.22 mg/mL with an increase in the ratio of raw materials to the extractant from 1:5 to 1:11. The nature of the lines in the

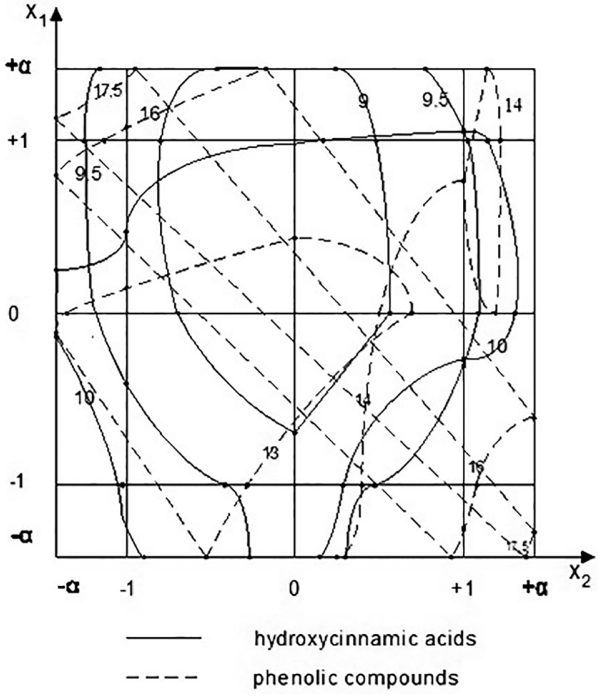

Figure 5. Lines of equal output of hydroxycinnamic acids and phenolic compounds in the coordinate system x_1x_2 according to the results of transformed regression equations

figure is determined not only by the magnitude of the linear coefficients but also by the pair and quadratic coefficients.

At the next stage of the analysis of second order regression equations, the optimal value of the factors (ethanol concentration, the ratio of raw materials to extractant) was found for obtaining the alcohol extract from *Centaurium erythraea* Rafn. herbs with the highest content of hydroxycinnamic acids and phenolic compounds. For this purpose, the regression equation is transformed into a canonical form. The canonical transformation consists of choosing a coordinate system, which greatly facilitates the geometric analysis of the equation.

In the study of two factors, we constructed the lines of equal yield of hydroxycinnamic acids and phenolic compounds in the coordinate system x_1x_2 (Figure 5).

As a result of the study, two optimizations were obtained:

- the first one is the content of hydroxycinnamic acids (y_1 = 9.5), the factor x_1 is studied on the upper star point, the factor x_2 – on the lower star point; the content of phenolic compounds (y_2 = 17.5), the factor x_1 is studied on the upper star point, the factor x_2 – on the lower star point.
- the second one is the content of hydroxycinnamic acids (y_1 = 10), the factor x_1 is studied at the basic level, the factor x_2 – on the upper star point; the content of phenolic compounds (y_2 = 14), the factor x_1 is studied on the main level, the factor x_2 – on the upper star point.

The best option is the first one, when the factor x_1 for y_1 and y_2 is studied at the top star point, and the factor x_2 is at the lower star point.

Conclusions

To obtain the alcohol extract of *Centaurium erythraea* Rafn. herbs with the highest content of hydroxycinnamic acids and phenolic compounds, the concentration of ethanol should be 69%, and the ratio of raw material to extractant should be 1 to 5. Further pharmacological studies of the resulting extract showed an evidence of the relevance of its use in order to increase the secretion of gastric juice.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

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