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Polyphenolic Compounds Composition Study in *Ocimum basilicum* L. Herb and the Development of Their Quantification Assessment.

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ABSTRACT

The article demonstrates the study results of the garden basil chemical composition - Ocimum basilicum L. It is established that the basic polyphenols of a plant are hydroxycinnamic acids - rosemary, caftaric, chlorogenic. Flavonoids, the dominant of which is routine, are presented in smaller quantities. An optimal method for the quantitative determination of O. basilicum L. polyphenols is offered. It consists in a dominant component determination i.e. rosmarinic acid by an internal standard method and routine by the method of absolute calibration. The performed calculations showed that the concentration of rosmarinic acid made 3.1%, and the concentration of routine acid made 0.51.

Keywords: polyphenolic compounds, garden basil, high performance of liquid chromatography, internal standard.

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INTRODUCTION

Traditionally the expansion of medicinal preparations assortment based on vegetable raw materials occurs primarily as a result of perspective plants borrowing from traditional medicine [1,2]. One of these plants is a well-known garden basil - Ocimum basilicum L. [3].

Therefore, the aim of this study was the development of qualitative and quantitative analysis methods for O. Basilicum L. to ensure its proper quality control.

METHODS

The liquid extract (1:1) is used to study the composition of the herb O. Basilicum L.

A reverse phase high-performance liquid chromatography (RP-HPLC) was used for the analysis of biological active substances of O. Basilicum L. liquid extract (1:1).

Chromatographic studies were performed by the chromatographic device of «Agilent Technologies 1200 Infinity» company manufactured by USA with the autosampler "Agilent 1200", a vacuum microdegasifier, a gradient pump and a thermostat of the same series. The electronic absorption spectra were recorded using a spectrophotometric diode matrix detector of the series "Agilent 1200" (the wavelength range made from 190 to 950 nm, the cell with a path length of 10 mm; the volume made 13 mcl), the scanning step made 2 nm.

The software «Agilent Chem Station» was used for the registration and the processing of spectral data and chromatograms.

A steel chromatography column "Ascentis express" C_{18} 2,7µm × 100 mm × 4,6 mm was used for the tests.

The effectiveness of the column was determined by the calculation of theoretical plates number. The value of at least 5000 was used as an optimal criterion for the column efficiency.

An optimal criterion of the peaks partition ratio Rs should be at least 1.5 according to the European Pharmacopoeia.

The optimal value of the asymmetry coefficient Tf is the value less than 2 [4,5].

MAIN PART

The amount of O. Basilicum L. polyphenol compounds was chromatographed under the following conditions: mobile phase: (A) - 1.0% of formic acid aqueous solution (B) - ethyl alcohol in a gradient elution mode; the rate of the mobile phase makes 0.5 mL / min; the column temperature makes + 35 °C, the volume of injected sample made 1 µl.

Diode matrix detection was carried out for flavones and flavonols of 350 nm, for hydroxycinnamic acids of 325 nm [6,7,8].

The obtained results of chromatography are shown on Figure 1.

Table 1: Indicators of chromatographic system suitability for the determination of O. Basilicum L. polyphenols.

t_R	S	N	R_s	T_f	W_b	Identified component
7.423	3248	13243.4	3.87	0.7	0.129	Chlorogenic acid
22.339	12279	67600	2.7	0.578	0.1716	Caftaric acid
27.462	1734	93727.82	2.83	1.557	0.1794	Rutin
29.634	17916	16080	8.75	0.559	0.1478	Rosmarinic acid

t_R- absolute retention time, S - peak area, N - the number of theoretical plates, HETP - the height equivalent to a theoretical plate, R_s- peak partition coefficient, T_f - skewness ratio, W_b - peak width at the baseline

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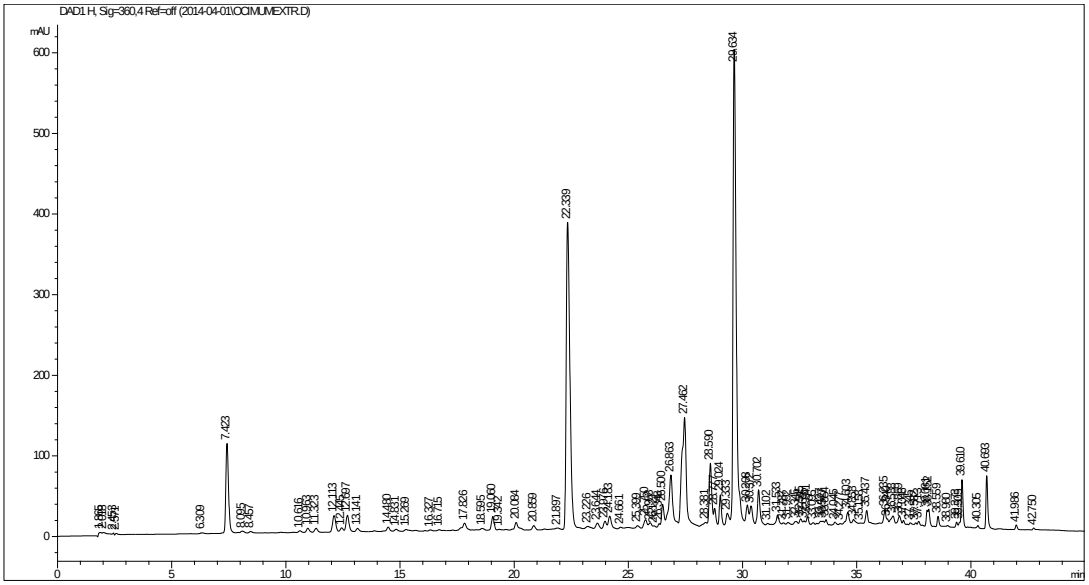
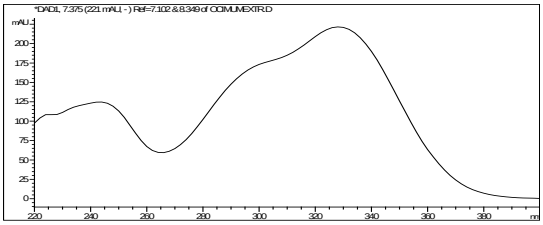
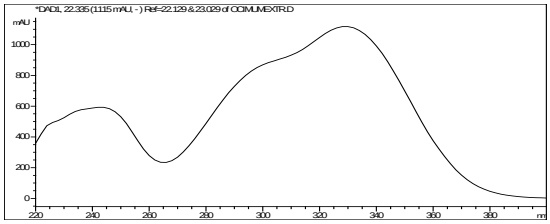
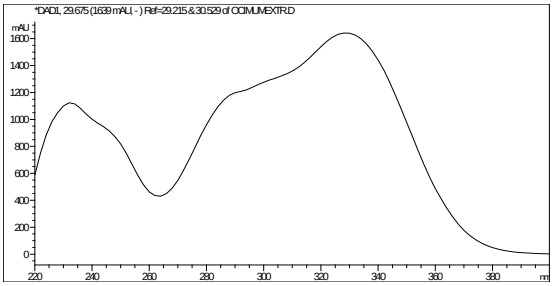


Figure 1: Chromatogram of separation of polyphenol complex of liquid extract herbs *O. basilicum* L.

The results of the criteria calculation for the validation of the used chromatographic system are presented in Table 1.

Table 2: Component composition of O. Basilicum L. polyphenol complex

Retention time, min	UV-spectrum	Total relative content, %	Identified component
7,37		6,61	Chlorogenic acid
22,33		25,02	Caftaric acid
29,675		36,5	Rosmarinic acid

According to the Table 1, the basic criteria (N>5000, R_s>1,5, T_f<2) correspond to the normalized values.

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Thus, the used chromatographic system may be considered suitable for the determination of O. Basilicum L polyphenols.

The obtained results indicate the presence of 118 components in O. Basilicum L. extract. The main components are shown in Table 2.

The identification of the components was carried out by the coincidence of witness samples retention time and the results of diode-matrix detection [9,10].

Table 2 data indicate that the bulk of all polyphenolic compounds of O. Basilicum L. extract are presented by hydroxycinnamic acids, namely chlorogenic, caftaric and rosmarinic one.

In addition to the presented components in the basil extract, the other polyphenolic compounds are presented, namely flavonoids, represented by apigenin, quercetin (rutin, isoquercetin) glycosides.

The next stage of this research was the development of the liquid extract standards for the herb O. Basilicum L. in order to ensure a proper quality control.

Previously we found that the dominant components of O. Basilicum L. herb are rosmarinic acid and routines. Therefore, we offered the option of of a liquid extract standardization on the basis of a mentioned plant in terms of these two components.

Due to the lack of a standard image for rosemary acid, for the quantitative determination of this component, we used an internal standard method. Caffeic acid was used as an internal standard, since the latter is similar to rosmarinic acid by structure.

In order to assess the linearity of the internal standard concentration dependence on an analytical signal (the ratio of the internal standard and analyte intensities) a calibration curve is developed within the coordinates of $C_{standard}$,% - I_{st} / $I_{an.}$ (Figure 2).

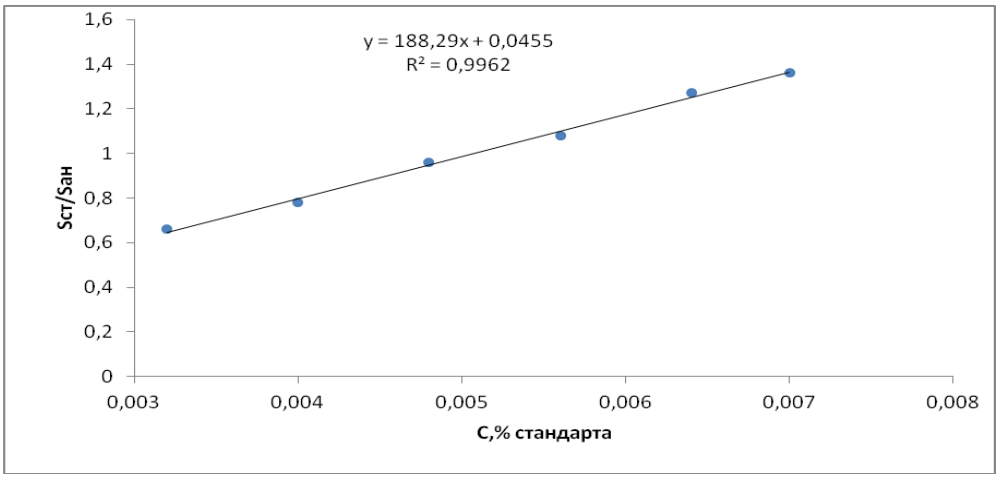

Figure 2: The calibration graph of the relationship of the peak area of rosmarinic acid and caffeic acid concentration of caffeic acid

The presented graph shows that the ratio Ist/Ian of the standard solution concentration has a straight relationship. This indicates that the ratio of the analyte peak areas and the internal standard remain a constant one.

The content of the tested substance was found by the formula 1 [4]:

$$C_x = K_x \times \left(\frac{C_{st}}{S_{st}}\right) \times S_{an}, \quad (1)$$

where K_x – response factor;

C_{st} – the amount of internal standard;

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S_{st}– peak area of a standard sample;
S_{an}– peak area of analyte;

The carried out calculations showed that the concentration of rosmarinic acid in the extract made 3.1%.

Another important component of O.basilicum L. extract is rutin. Its content was determined by an absolute calibration method, by the way of peak areas comparison concerning the object under study and a standard sample of the routine witness material.

The calibration curve is shown by Figure 3.

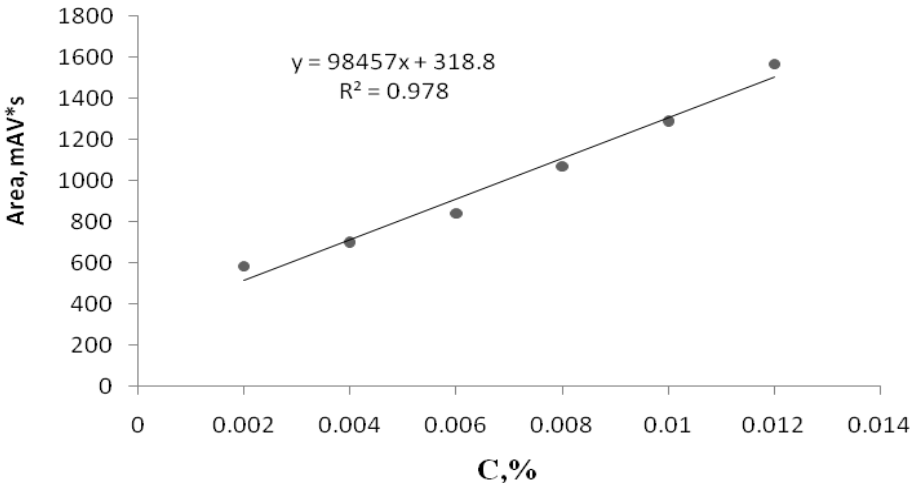

Figure 3: The calibration plot of the peak area of the concentration of standard sample routine.

Then, a peak area was measured in the test sample and their amount was calculated according to the calibration curve and the formula 2 [4]:

$$x = \frac{C_{CT} \times W \times 100}{m \times (100 - B)}, \quad (2)$$

where C_{CT} – CO rutin concentration, found by calibration graph, %
m – sample weight;
W – sample separation;
B – weight loss at sample drying.
The rutin content in *O.basilicum* L. structure made 0,51%.

CONCLUSION

Thus, in the course of the performed surveys the chemical composition of O.basilicum L. herb was studied. It was found that the main polyphenols of the plant are hydroxycinnamic acids. An optimal standardization method of O. basilicum L. liquid extract (1:1) is proposed, consisting in the dominant component determination - rosmarinic acid by internal standard method and routine - by the method of absolute calibration.

SUMMARY

The studied composition of O.basilicum L. polyphenols allows to determine its rich polyphenolic composition. It certainly opens the prospects for the creation of drugs based on it with diverse pharmacological effects. It is planned to provide a compact dosage form based on O.basilicum L. and perform its pharmacological tests.

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