IDENTIFICATION AND QUANTITATIVE CONTENT OF CHLOROGENIC AND ROSMARINIC ACIDS IN *CENTAUREA CYANUS* L. AND *CENTAUREA MONTANA* L. HERBS

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Introduction

Genus *Centaurea* L. of *Asteraceae* L. family includes more than 700 plant species. They mostly grow in Europe, North and South America, Australia, Africa, Asia [1-3].

Certain *Centaurea* L. species are used in medicine as anti-inflammatory, antimicrobial, analgesic, reparative, diuretic, hypoglycemic, antipyretic, antitumor agents [1, 2, 4, 5].

Centaurea L. genus herbs contain sesquiterpene lactones, phenolic compounds, including flavonoids, phenolic acids, procyanidins, anthocyans, lignans, as well as triterpenes, steroids, carotenoids, polysaccharides, polyacetylenes [1, 3, 5-9].

Earlier we have studied fatty acids of *Centaurea cyanus* L. [10].

Phenolic compounds are secondary metabolites of plants which are widely represented in them and are capable of accumulation at substantial amounts. Phenolic compounds may account for antioxidant, anti-inflammatory, antimicrobial, diuretic, hypoglycemic action of plants [5, 11-14].

Chlorogenic acid is known to take part in antioxidant, anti-inflammatory, antibacterial, antiviral, hypolipidemic, hypotensive, hypoglycemic activities [15-17].

Rosmarinic acid is responsible for antimicrobial, anti-inflammatory, hypoglycemic, antiallergic, hepatoprotective and nephroprotective activities [18-21].[18-21].

The purpose of the present work was the identification and quantitative content determination of chlorogenic and rosmarinic acids in herb of *Centaurea cyanus* L. and *Centaurea montana* L., which are widely cultivated in Ukraine as decorative plants.

Materials and methods

Centaurea cyanus L. and *Centaurea montana* L. herb was collected during blossoming in Kharkiv and Odessa regions of Ukraine in June-July 2020/2021.

The powdered materials of plant (0.5 g) were weighed into a volumetric flask, added with 50 % ethanol (100 ml) and boiled for 40 min. The solutions were filtered through a membrane filter (0.45 μm) prior to use. The volume of the solutions was brought to 100 ml.

Chromatographic study was performed at a Shimadzu HPLC-system, ser.20 liquid chromatograph equipped with a diode matrix detector. Column ACE C18 (2) (250 mm × 4.6 mm, 5.0 μm). Elution was performed at a flow rate of 1 ml/min. The mobile phase consisted of 0.1% trifluoroacetic acid in water (eluent A) and 0.1% trifluoroacetic acid in acetonitrile (eluent B). The mobile phase is shown in table 1. The column temperature was constant 35 °C. The injection volume of the sample solution was 5 μL. The chromatograms were recorded at different wavelengths according to substances. Detector wavelength 330 nm.

Table 1. Mobile phase

Chromatography time, min	Eluent A, %	Eluent B, %
0–5	95	5
5–35	95 → 75	5 → 25
35–40	75	25
40–60	75 → 50	25 → 50
60–65	50 → 20	50 → 80
65–70	20	80
70–85	95	5

The components were identified by their retention time and conformity of their UV spectra to standard substance. The calculations were performed by the equation:

$$X, \% = \frac{A_{pr} \times m_{st} \times V_{pr} \times P \times 100}{A_{st} \times V_{st} \times m_{pr} \times 100}$$

where:
A_{pr} – substance peak area in tested solution chromatogram;
A_{st} – substance peak area in reference solution chromatogram;
m_{st} –mass of substance standard sample, mg;
m_{pr} – mass of tested herb sample, mg;
V_{pr} – dilution of tested solution, ml;
V_{st} – dilution of reference solution, ml;
P – activity of standard, %.

Results and discussions

HPLC chromatograms of chlorogenic acid and rosmarinic acid standard are shown in Fig. 1 and Fig. 2.

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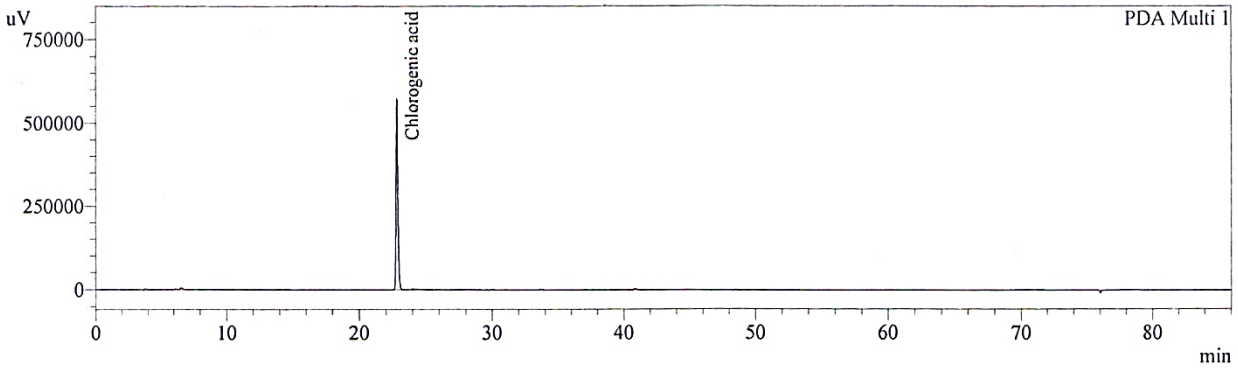

Fig. 1. HPLC chromatogram of chlorogenic acid standard

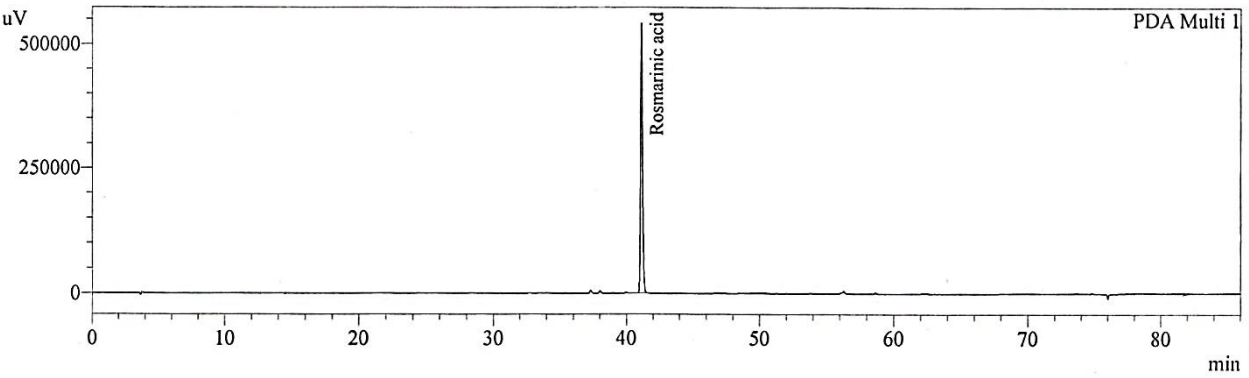

Fig. 2. HPLC chromatogram of rosmarinic acid standard

Chromatographic parameters of chlorogenic acid and rosmarinic acid standard substances are specified in Table 2.

Table 2. Chromatographic parameters of chlorogenic acid and rosmarinic acid standard substances

Title	Retention time	Area	Tailing factor	Theoretical plate	Resolution
Chlorogenic acid	22.831 ± 0.064	5522891 ± 10302	1.286 ± 0.006	121254.428 ± 1154.469	0.000
Rosmarinic acid	41.126 ± 0.156	5477366 ± 11241	1.307 ± 0.007	359101.236 ± 3784.912	26.791 ± 0.056

HPLC chromatograms of *Centaurea cyanus* L. and *Centaurea montana* L. herb are specified in Figs. 3 and 4.

Chromatographic parameters of identified acids in *Centaurea cyanus* L. herb are specified in Table 3. Chromatographic parameters of identified acids in *Centaurea montana* L. herb are specified in Table 4.

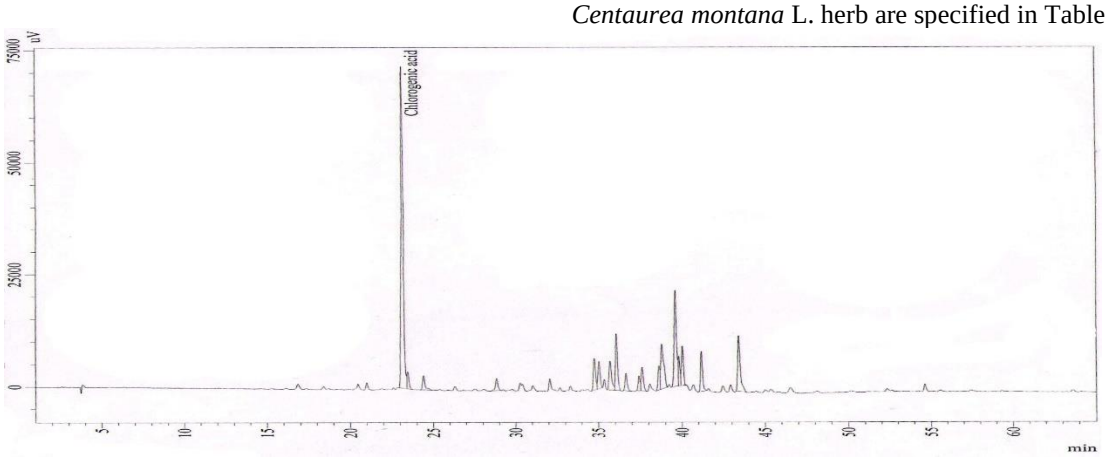

Fig. 3. HPLC chromatogram of *Centaurea cyanus* L. herb

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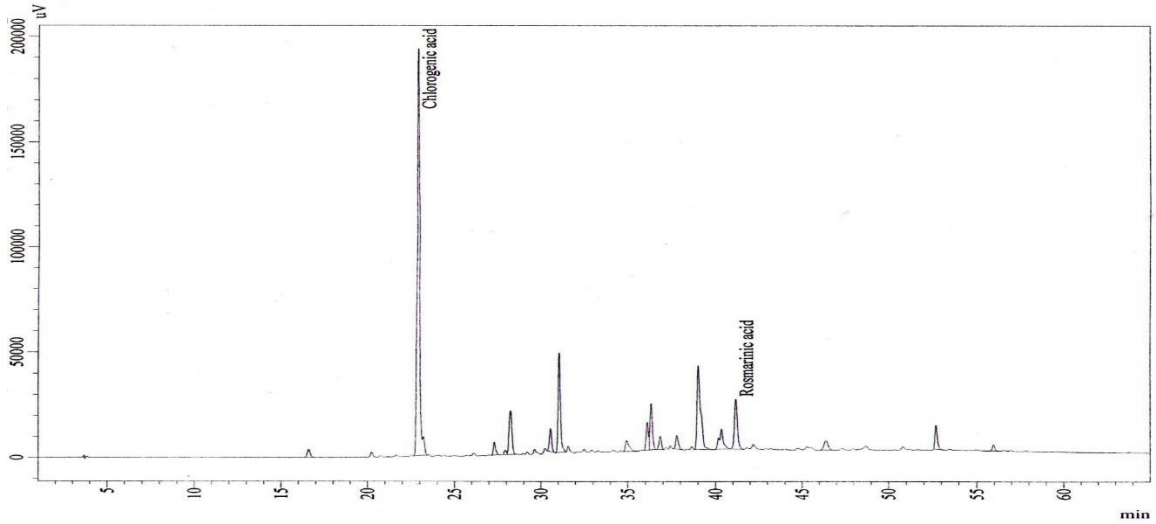

Fig. 4. HPLC chromatogram of *Centaurea montana* L. herb

Table 3. Chromatographic parameters of identified chlorogenic acid in *Centaurea cyanus* L. herb

Title	Retention time	Area	Tailing factor	Theoretical plate	Resolution
Chlorogenic acid	23.152 ± 0,071	669554 ± 1279	1.290 ± 0.006	132344.285 ± 1349.924	0.000

Table 4. Chromatographic parameters of identified chlorogenic acid in *Centaurea montana* L. herb

Title	Retention time	Area	Tailing factor	Theoretical plate	Resolution
Chlorogenic acid	22.901 ± 0.059	1916572 ± 3640	1.309 ± 0.007	124867.586 ± 1262.521	21.876 ± 0.039
Rosmarinic acid	41.179 ± 0.132	288359 ± 695	1.205 ± 0.006	262353.716 ± 2701.325	2.616 ± 0.006

In *Centaurea montana* L. herb chlorogenic acid and rosmarinic acid were identified to the amount of 4.24 ± 0.08 mg/100 g and 7.47 ± 0.15 mg/100 g respectively. In *Centaurea cyanus* L. herb only chlorogenic acid was identified to the amount of 1.40 ± 0.02 mg/100 g. According to data published by Al-Snafi A. E., *Centaurea cyanus* L. flowers contain aromatic acids, such as caffeic, protocatechic, chlorogenic, ferulic, *n*-coumaric, *n*-hydroxybenzoic, vanillic, syringic, salicylic, sinapic and hydroxyphenylacetic acid [4]. Litvinenko V. I. and Bubenchikova V. N. extracted and identified from *Centaurea cyanus* L. flowers 4 hydroxycinnamic acids: caffeic, chlorogenic, neochlorogenic and isochlorogenic acids [22]. Joint study by Portuguese and German researchers revealed in *Centaurea cyanus* L. flowers chlorogenic, caffeic and syringic acid at total amount of 0.134 ± 0.0003 mg/g [23]. In *Centaurea cyanus* L. collected in Brazil chlorogenic acid dominated among all identified acids (3897 ± 54 mg/100 g) [24]. Polish scientists found total content of phenolic acids in *Centaurea cyanus* L. flowers to be 47.85 ± 1.06 mg/100 g [25]. In blossoming part of *Centaurea solstitialis* L. such acids were determined as gallic, 3,4-dihydroxybenzoic, chlorogenic, vanillic, caffeic, *n*-coumaric, ferulic. The highest accumulated amount in *Centaurea solstitialis* L. related to caffeic acid [26]. Rosmarinic acid was identified in *Centaurea amaena* L. to the amount of 191.40 mg/ml.

Comparing our results with the data of other researchers, we may conclude that chlorogenic acid is most amply represented in *Centaurea* L. herbs, whereas rosmarinic acid is rarely met.

Conclusions

For the first time in *Centaurea cyanus* L. herb and *Centaurea montana* L. herb collected in Ukraine chlorogenic acid was identified by HPLC method, whereas in *Centaurea montana* L. herb additionally rosmarinic acid was found. *Centaurea montana* L. herb accumulated higher quantity of identified acids: 7.47 ± 0.15 mg/100 g rosmarinic acid and 4.24 ± 0.08 mg/100 g chlorogenic acid.

Our research can explain the antimicrobial, anti-inflammatory and antioxidant activities of *Centaurea cyanus* L. and *Centaurea montana* L. herb and predict these types of drug activities based on them. The obtained results may be used in the development of medicines and standardization of tested herb.

Identification and quantitative content of chlorogenic and rosmarinic acids in *Centaurea cyanus* l. and *Centaurea montana* l. Herbs

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Introduction. Plants of *Centaurea* L. genus are used in complex therapy of many diseases, in particular, as anti-inflammatory, antimicrobial, analgesic, reparative, diuretic, hypoglycemic agents. These activities are partially caused by availability of phenolic compounds, in particular, phenolic acids. For example, chlorogenic and rosmarinic acids are known to take part in antioxidant,

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antimicrobial, anti-inflammatory, hypolipidemic, hypotensive, hepatoprotective, hypoglycemic activities. Purpose of the present work was identification and quantitative content determination of chlorogenic and rosmarinic acids in herb of *Centaurea cyanus* L. and *Centaurea montana* L., which are widely cultivated in Ukraine as decorative plants. **Material & methods.** The objects of the study were herb of *Centaurea cyanus* L. and *Centaurea montana* L. The raw materials were collected during blossoming in Kharkiv and Odessa regions of Ukraine in June-July 2020/2021. Identification and quantitative content of chlorogenic and rosmarinic acids were carried out using the HPLC method. **Results & discussion.** In *Centaurea montana* L. herb chlorogenic and rosmarinic acids were identified to the amount of 4.24 ± 0.08 mg/100 g and 7.47 ± 0.15 mg/100 g respectively, in *Centaurea cyanus* L. only chlorogenic acid was identified to the amount of 1.40 ± 0.02 mg/100 g. **Conclusion.** For the first time in *Centaurea cyanus* L. herb and *Centaurea montana* L. herb collected in Ukraine chlorogenic acid was identified by HPLC method, whereas in *Centaurea montana* L. herb additionally rosmarinic acid was found. *Centaurea montana* L. herb accumulated higher quantity of identified acids. The obtained results may be used in the development of medicines and standardization of tested herb.
Keywords: *Centaurea cyanus* L., *Centaurea montana* L., chlorogenic acid, rosmarinic acid, HPLC method.

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