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Investigation of phenolic compounds of the leaves of *Crambe cordifolia* Steven and *Crambe koktebelica* (Junge) N.

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Abstract
An in-depth phytochemical study of *Crambe cordifolia* Steven and *Crambe koktebelica* (Junge) N. raw materials as sources of biologically active substances of phenolic nature is an urgent task of modern pharmacy. Therefore, the purpose of the research was to study the composition of phenolic compounds of the studied plant species. The qualitative composition and quantitative content of the phenolic compounds in *Crambe cordifolia* Steven and *Crambe koktebelica* (Junge) N. leaves were determined by HPLC analysis on Agilent 1200 chromatograph. The quantitative content of the following individual phenolic compounds was identified and established: chlorogenic, ferulic, caffeic, *p*-coumaric, syringic, sinapic, cinnamic, hydroxyphenylacetic and quinic acid, neohesperidin, rutin, quercetin-3-D-glucoside and luteolin. In *Crambe cordifolia* Steven leaves prevails among certain neohesperidin (1676.71 µg/g), серед hydroxycinnamic acids – caffeic (218.43 µg/g) and chlorogenic (144,11 µg/g). A significant content of chlorogenic acid (547.62 µg/g), ferulic acid (267.71 µg/g) and neohesperidin (1809.44 µg/g) was determined in *Crambe koktebelica* (Junge) N.

Keywords: *Crambe cordifolia* Steven, *Crambe koktebelica* (Junge) n., flavonoids, hydroxycinnamic acids, HPLC

1. Introduction
For centuries, plants have been used not only as a source of nutrition but also in the fight against ailments. The plants that have a complex effect, because it is possible to use a single agent for the treatment of a variety of comorbidities are of most interest. The advantage of herbal drugs over synthetic ones is that they rarely cause adverse reactions and are well tolerated by patients regardless of age [1]. Inefficient use of natural resources leads to an annual decline in the stock of wild plants and encourages the use of plants of cultivated flora. Such plants are *Crambe cordifolia* Steven and *Crambe koktebelica* (Junge) N., belonging to the genus *Crambe* L., *Brassicaceae* family. Plants of the genus *Crambe* L. are widely used for environmental, food, technical purposes and as a source of biofuels. In folk medicine, they are used when violation of digestive processes, as an anti-cingulate agent and a mustard plaster substitute. Methanol extract root of *Crambe cordifolia* Steven manifests antioxidant and antimicrobial activity against most of the tested microbial strains *Escherichia coli, Bacillus subtilis, Pasteurella multocida, Staphylococcus aureus, Aspergillus niger* та *Fusarium solani* [2, 3]. *Crambe cordifolia* Steven is a perennial herbaceous plant 182 cm tall. This plant is an endemic to the North Caucasus, which is one of the most valuable low-abundant forage crops. Root leaves of the plant up to 35 cm long, stem – small, 6-13 cm [4], inflorescence branched, spherical. *Crambe koktebelica* (Junge) N. – local endemic species, the plants of which grow singly or in small groups on limestone-gravelly slopes and sea breaks [5]. Local populations of *Crambe koktebelica* on the coast of Koktebel bay and the Karadah mountain range are known [4]. The chemical composition of *Crambe cordifolia* Steven and *Crambe koktebelica* (Junge) N. leaves has been poorly studied. An in-depth phytochemical study of the raw materials of these plant species is relevant. The aim of the study was to learn the composition of phenolic compounds of the studied plant species.

2. Materials and Methods
2.1 Plant material
The subjects for the study were the leaves of *Crambe cordifolia* Steven and *Crambe*

koktebelica (Junge) N. The raw material was harvested at the experimental sites of the Cultural Flora Department of M. Hryshko National Botanical Garden of the National Academy of Sciences of Ukraine in Kyiv in 2018 during flowering of plants.

2.2 Chemicals and Standards

Standards of flavonoids were of analytical grade (> 99 % purity). The chemicals were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). All other chemicals were of analytical grade (>95% purity). High performance liquid chromatography (HPLC) method was determined the qualitative composition and quantitative content of flavonoids.

2.3 HPLC-analysis of flavonoids

The qualitative composition and quantitative content of the individual flavonoids were determined by HPLC on Agilent 1200 chromatograph (*Agilent Technologies*, USA) [6]. Acetonitrile (solvent A) and 0.1 % formic acid solution in water (solvent B) were used as the mobile phase. The dilution was performed on Zorbax SB-C18 chromatographic column (150 mm x 4.6 mm x 3.5 µm) (Agilent Technologies, USA). Chromatographic mode: flow rate of carrier gas (helium) through column 0.25 ml/min, thermostat temperature 30 ^{0}C, injection volume 4 µL. The gradient elution mode is shown in Table 1. Detection was performed using a diode-matrix detector with a signal recording at a wavelength of 280, 365 nm (for flavonoids) and 250, 275 nm (for hydroxycoric acids) and fixation of the absorption spectra in the range 210–700 nm [7].

Table 1: The gradient elution parameters for flavonoids and hydroxycinnamic acids

Hydroxycinnamic acids				
Time, min	0	20	22	30
Solvent A, %	25	75	100	100
Solvent B, %	75	25	0	0
Flavonoids				
Time, min	0	20	22	30
Solvent A, %	30	70	100	100
Solvent B, %	70	30	0	0

Sample preparation. 1.00 g (accurate weight) of the raw material was extracted in 5 ml of 70% (for flavonoids) and 60% (for hydroxycinnamic acids) ethyl alcohol solution in an ultrasonic bath at 80 ^{0}C for 5 hours (for flavonoids) and 4 hours (for hydroxycinnamic acids) in glass sealed vials with teflon lid. The obtained extract was centrifuged at 3000 rpm and filtered through 0.22 µm disposable membrane filters.

Identification and quantitative analysis were performed using standard flavonoid and hydroxycinnamic acid solutions.

The content of compounds (X) was determined by the formula:

$$X = \frac{C \times V}{m},$$

Where: C – the concentration of the compound, determined chromatographically, µg/ml; V – extract volume, ml; m – mass of the studied raw material, g [7].

3. Results and Discussion

The HPLC chromatogram of extract of *Crambe cordifolia* Steven leaves showed the presence of chlorogenic, caffeic, syringic, ferulic, sinapic, cinnamic and quinic acids as depicted in Fig. 1. The analyzed extract of *Crambe koktebelica* (Junge) N. leaves revealed the presence of hydroxypheny lactic, chlorogenic, syringic, *p*-coumaric, ferulic, sinapic and cinnamic acids as presented in the HPLC chromatogram in Fig. 2. Hydroxycinnamic acids, common to the two Crambe species studied, can serve as markers for plants of this genus.

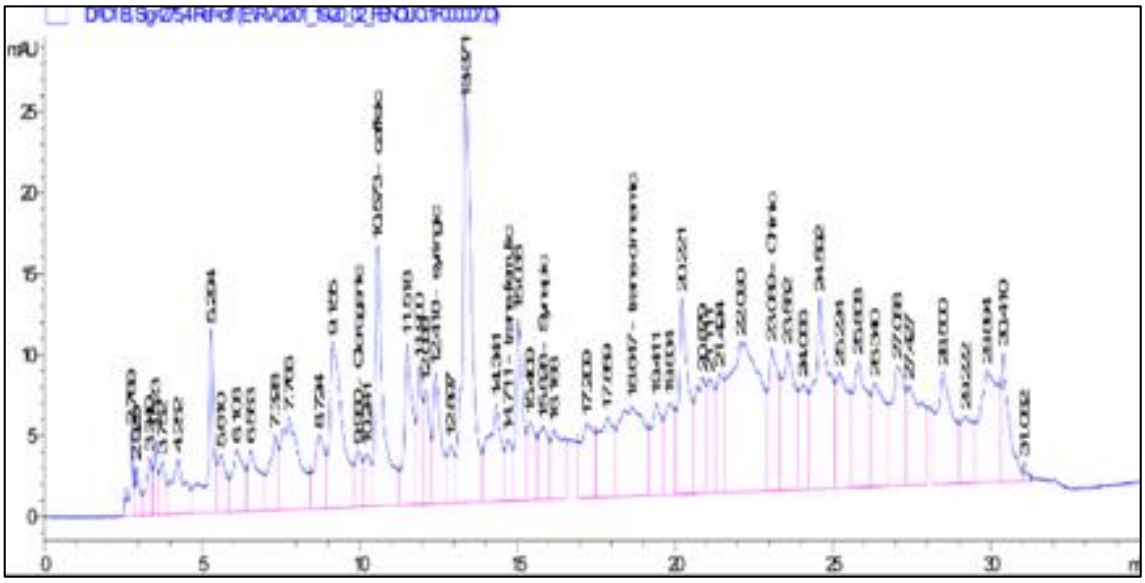

Fig 1: HPLC - chromatogram of hydroxycinnamic acids from *Crambe cordifolia* Steven leaves

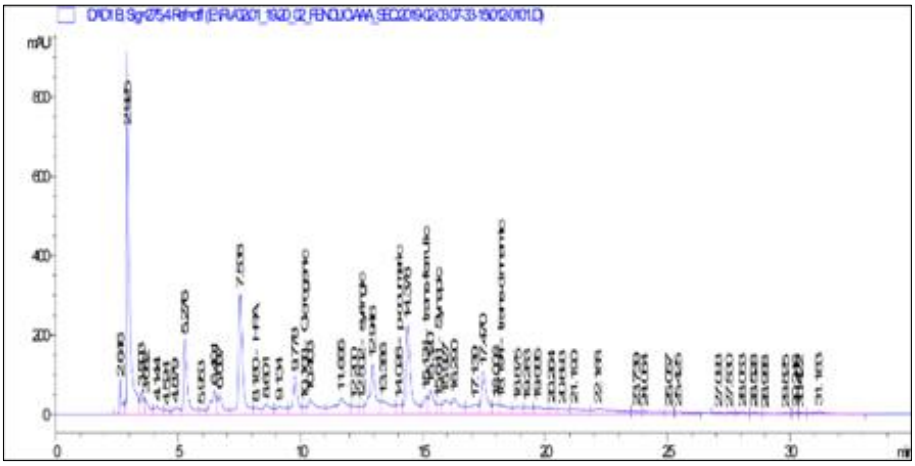

Fig 2: HPLC - chromatogram of hydroxycinnamic acids from *Crambe koktebelica* (Junge) N. leaves.

Quantitative content of hydroxycinnamic acids is presented in Table 2.

Table 2: Content of hydroxycinnamic acids in *Crambe cordifolia* Steven and *Crambe koktebelica* (Junge) N. Leaves

Compound	Content in dry raw material, µg/g	
	Crambe cordifolia Steven	Crambe koktebelica (Junge) N.
Hydroxyphenylacetic acid	–	177.0
Chlorogenic acid	144.11	547.62
Caffeic acid	218.43	–
Syringic acid	54.90	97.31
Ferulic acid	24.21	267.71
Sinapic acid	34.64	109.70
Cinnamic acid	49.61	96.83
p-coumaric acid	–	176.04
Quinic acid	54.90	–

Note: – not detected.

Chlorogenic, ferulic and caffeic acids are predominant among hydroxycinnamic acids. These acids have an antitumor effect. Chlorogenic and caffeic acids have a significant stimulating effect on the synthesis of IgG antibodies. Caffeic acid is also known as a selective leukotriene biosynthesis blocker. Ferulic acid manifests antimicrobial and antifungal properties, has inhibitory activity against virus growth and amplification.

Two objects (*Crambe cordifolia* Steven, *Crambe koktebelica* (Junge) N.) consists such flavonoids as neohesperidin and luteolin. Quercetin-3-D-glucoside conntaine in *Crambe cordifolia* Steven leaves, rutin - *Crambe koktebelica* (Junge) N. (Fig. 3, Fig. 4).

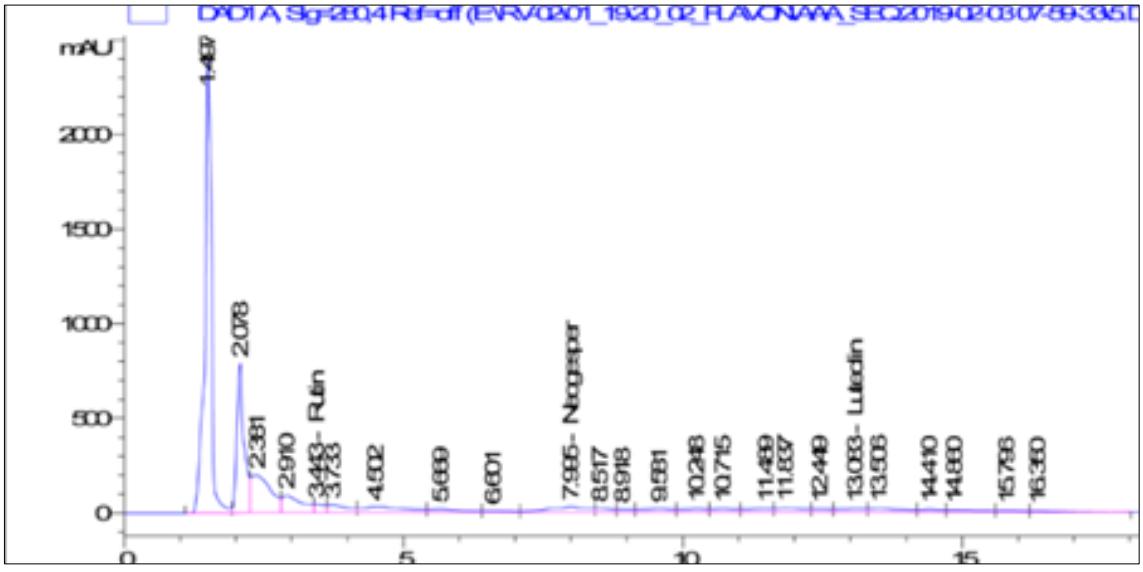

Fig 3: HPLC - chromatogram of flavonoids from *Crambe cordifolia* Steven leaves

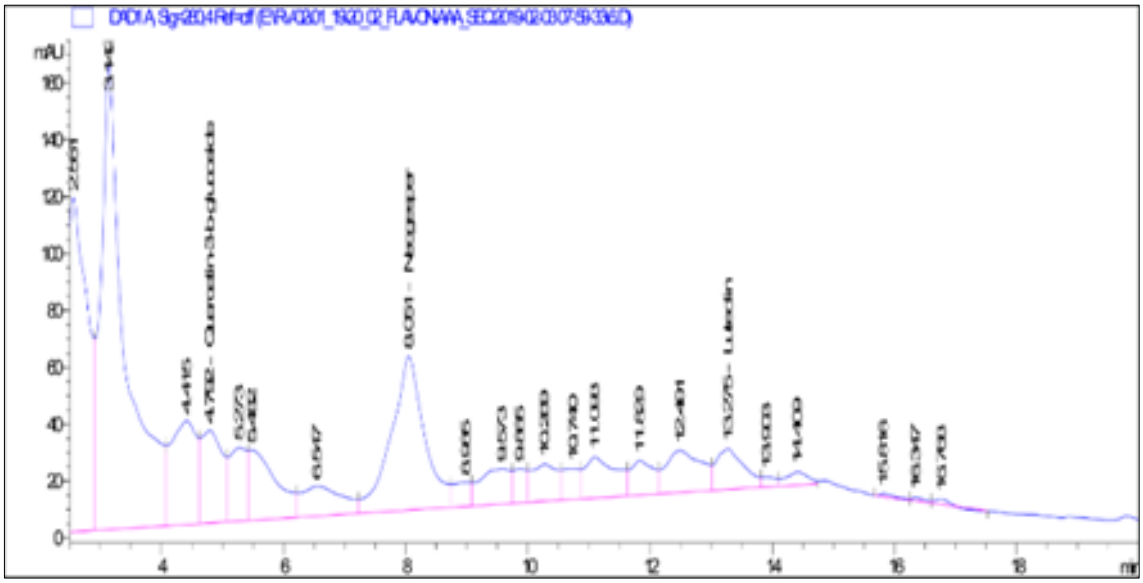

Fig 4: HPLC - chromatogram of flavonoids from *Crambe koktebelica* (Junge) N. leaves.

The results of determining the quantitative content of flavonoids are presented in Table 3.

Table 3: Content of flavonoids in *Crambe cordifolia* Steven and *Crambe koktebelica* (Junge) N. Leaves

Compound	Content in dry raw material, µg/g	
	Crambe cordifolia Steven	Crambe koktebelica (Junge) N.
Neohesperidin	1676.71	1809.44
Luteolin	543.10	349.69
Quercetin-3-D-glucoside	–	86.03
Rutin	570.95	–

Note: – not detected.

Among the flavonoids in the leaves of the studied species, Neohesperidin, which belongs to bitter glycosides of flavones and provides a bitter taste of raw materials, prevails. This flavonoid has a sedative effect in combination with diosmin but slightly less than rutin. Neohesperidin also exhibits hypolipidemic and antihypertensive activity.

4. Conclusions

For the first time, the qualitative composition was investigated and the quantitative content of phenolic compounds was determined in *Crambe cordifolia* Steven and *Crambe koktebelica* (Junge) N. leaves. It is established that these types of plants differ both in qualitative composition and quantitative content of the investigated species of biologically active substances. HPLC analysis of *Crambe cordifolia* Steven and *Crambe koktebelica* (Junge) N. leaves identified and quantified the content of the following individual phenolic compounds: neohesperidin, luteolin, quercetin-3-D-glucoside, rutin, hydroxyphenylacetic, chlorogenic, caffeic, syringic, ferulic, sinapic, cinnamic, *p*-coumaric and quinic acid. It was determined that in *Crambe cordifolia* Steven leaves caffeic acid prevails–218.43 µg/g (found only in *Crambe cordifolia* Steven), chlorogenic acid – 144.11 µg/g and neohesperidin – 1676.71 µg/g. In *Crambe koktebelica* (Junge) N. chlorogenic acid prevails – 547.62 µg/g, ferulic acid – 267.71 µg/g and neohesperidin – 1809.44 µg/g.

5. References

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