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Determination of flavonoids and hydroxycinnamic acids in the herb of common agrimony by HPLC method

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Abstract
The creation of new herbal products and the improvement of their production technologies is an important area of pharmaceutical science. A valuable source of biologically active substances is a representative of the Rose family (*Rosaceae*) common agrimony (*Agrimonia eupatoria* L.). Remedies on the basis of common agrimony are used to increase the secretion of the digestive glands, as appetizing, choleretic, hemostatic, astringent, anti-diuretic and anti-inflammatory agent. The chemical composition of the common agrimony herb grown on the territory of Ukraine is poorly studied, so conducting an in-depth phytochemical study of the raw material of this type of plant is relevant. The purpose of the work was the determination of individual flavonoids and hydroxycinnamic acids by high performance liquid chromatography (HPLC). The determination of the qualitative composition and the quantitative content of the individual flavonoids and hydroxycinnamic acids in the common agrimony herb were performed by HPLC on an *Agilent 1200* chromatograph.
As a result of the conducted studies, the quantitative content of 4 flavonoids – isoquercitrin (916.73 µg/g), neohesperidin (3850.93 µg/g), naringenin (308.28 µg/g) and luteolin (332.13 µg/g) was found and determined in the common agrimony herb. Among the hydroxycinnamic acids, hydroxyphenylacetate (1145.25 µg/g), caffeic (614.17 µg/g), syringic (215.86 µg/g), *p*-coumaric (827.54 µg/g), ferulic (267.72 µg/g), sinapic (381.35 µg/g), cinnamic (251.81 µg/g) and quinic (77.28 µg/g) acids were detected in the studied raw material. Neohesperidin, prevails among flavonoids in the common agrimony herb, hydroxyphenylacetate and *p*-coumaric – among the hydroxycinnamic acids.

Keywords: Common agrimony, flavonoids, hydroxycinnamic acids, high performance liquid chromatography

1. Introduction
The creation of new herbal remedies and the improvement of their production technologies is an important area of pharmaceutical science. A valuable source of biologically active substances is a representative of the Rose family (*Rosaceae*), in particular the Agrimony genus (*Agrimonia* L.) – a common agrimony (*Agrimonia eupatoria* L.) [1]. It is a perennial herb up to 90 cm tall with creeping simple or branched rhizome. Stem is erect, simple or branched at the top, 30–85 cm tall, densely covered with long rigid hairs [2, 3]. The leaves are alternate, interrupted by the papillae. The flowers are regular, five-petal, bisexual, clustered in a spiky tassel at the apex of the stem; petals are orange-yellow in color; blooms usual in June and August. The plant is spread throughout the territory of Ukraine on the slopes, in the forests, among the bushes, on the forest and road edges. The plant is unofficinal, part of the European, British and US pharmacopoeia [2].
The common agrimony herb contains tannins, saponins, coumarins, polysaccharides, volatile compounds, organic acids, vitamin C, amino acids, flavonoids, hydroxycinnamic acids, silica [1]. *Heilerova* and co-authors found that due to the presence of polyphenols aqueous extract from the common agrimony exhibits antioxidant properties [4].
Remedies on the basis of the common agrimony are used to increase the secretion of the digestive glands, as appetizing, choleretic and hemostatic, astringent, anti-diuretic, anti-inflammatory agent. It is also used for gallstone disease, liver disease, inflammation of the oral mucosa [1, 5].
The chemical composition of the common agrimony herb grown on the territory of Ukraine is poorly studied, so conducting an in-depth phytochemical study of the raw material of this type of plant is relevant.
The purpose of this work was to determine the individual flavonoids and hydroxycinnamic acids by high performance liquid chromatography (HPLC).

2. Materials and Methods

2.1 Plant material

For experimental research, we used the common agrimony herb. Raw materials were harvested in the Ivano-Frankivsk region during the flowering of the plant in July 2010.

2.2 Chemicals and Standards

Standards of flavonoids and hydroxycinnamic acids 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). HPLC method was determined the qualitative composition and quantitative content of flavonoids and hydroxycinnamic acids.

2.3 HPLC-analysis of flavonoids and hydroxycinnamic acids

The qualitative composition and quantitative content of individual flavonoids and hydroxycinnamic acids were determined by HPLC on an *Agilent 1200* chromatograph (*Agilent Technologies*, USA) [6].

Acetonitrile (eluent A) and 0.1 % formic acid solution in water (eluent B) were used as mobile phases. Dilution was performed on a Zorbax SB-C18 chromatographic column (for flavonoids), Zorbax SB-Aq (for hydroxycinnamic acids) (3.5 µm, 150 mm x 4.6 mm) (*Agilent Technologies*, USA). Chromatographic mode: flow rate of the carrier gas through the column 0.25 ml/min, thermostat temperature 30 ^{0}C, injection volume 4 µl. Elution was performed in gradient mode (Table 1).

Table 1: Parameters of gradient mode of elution of hydroxycinnamic acids and flavonoids

Hydroxycinnamic acids				
Time, min	0	20	27	35
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

Detection was performed using a diode array detector with a signal recording at a wavelength of 250, 275 nm (for hydroxycinnamic acids) and 280, 365 nm (for flavonoids) and fixation of the absorption spectra in the range 210–700 nm.

Identification and quantitative analysis were performed using standard solutions of phenolic compounds: hydroxyphenylacetate, chlorogenic, caffeic, syringic, *p*-coumaric, ferulic, sinapic, cinnamic and quinic acids and rutin, quercetin-3-D-glycoside, naringenin, kaempferol, luteolin.

Sample preparation: 0.425 g (exact sample) of the raw material was extracted in 5 ml of 60 % (for hydroxycinnamic acids) 70% (for flavonoids) of an ethyl alcohol solution in an ultrasonic bath at 80 ^{0}C for 4 h (for hydroxycinnamic acids) and 5h (for flavonoids) in glass sealed vials with teflon lid. The obtained extract was centrifuged at 3000 rpm. and filtered through 0.22 µm disposable membrane filters.

The content of compounds (X), in µg/g, was determined by the formula:

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

Where:

C – The concentration of the compound, determined chromatographically, µg/ml;

V – Volume of extract, ml;

m – The mass of the studied raw material, g [7].

3. Results and Discussion

HPLC identified the following hydroxycinnamic acids in the common agrimony herb: hydroxyphenylacetate, caffeic, syringic, *p*-coumaric, ferulic, sinapic, cinnamic and quinic (Fig. 1).

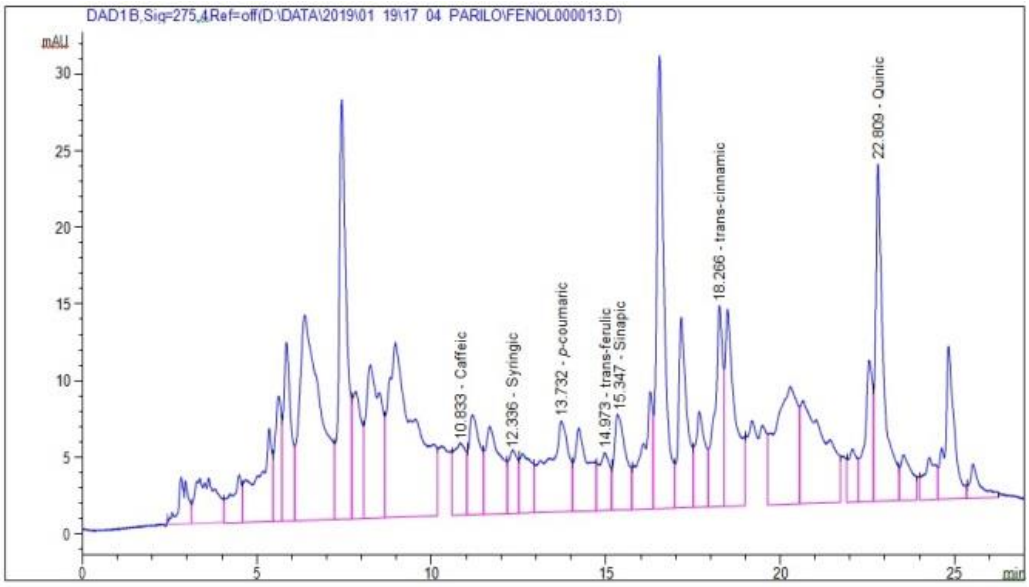

Fig 1: HPLC chromatogram of hydroxycinnamic acids of the common agrimony herb.

Quercetin-3-D-glycoside (iso-quercitrin), neohesperidine, naringenin, and luteolin were identified in the herb of the common agrimony among flavonoids by HPLC method (Fig. 2).

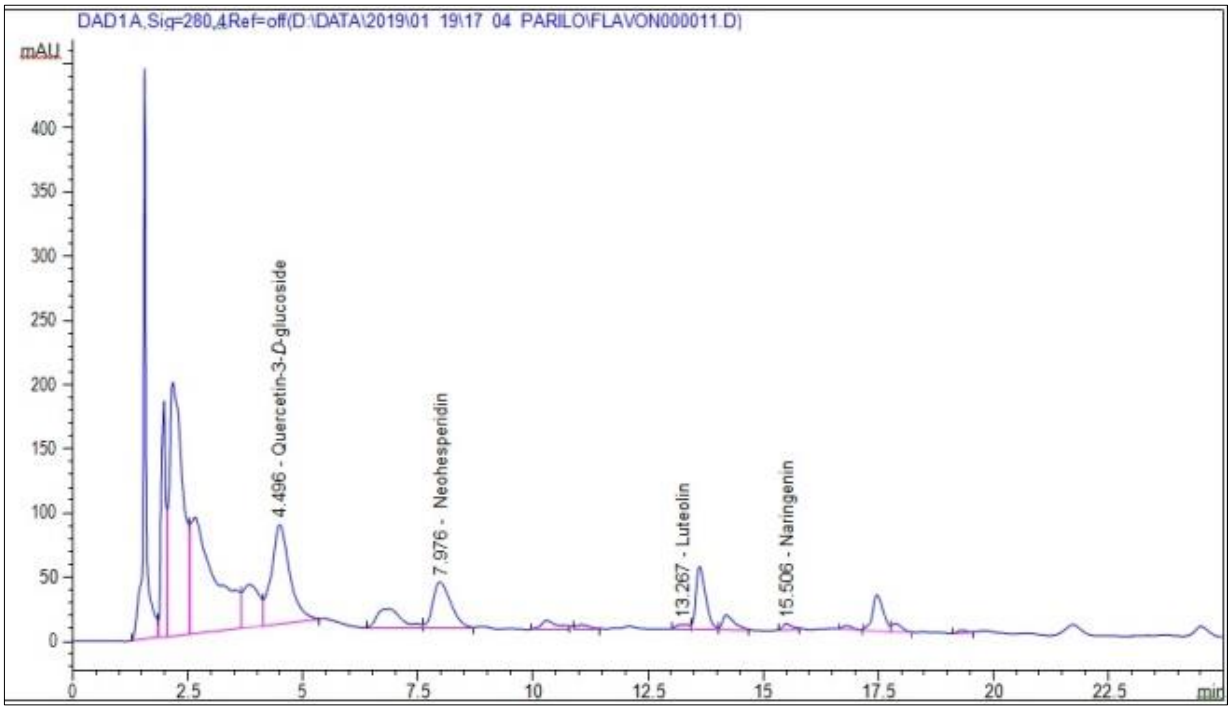

Fig 2: HPLC chromatogram of flavonoids of the common agrimony herb.

The results of determining the quantitative content of individual acids of hydroxycinnamic acids and individual flavonoids in the common agrimony herb are shown in Table 2.

Table 2: Quantitative content of individual hydroxycinnamic acids and individual flavonoids in the common agrimony herb (HPLC method)

Compound	Content in the dried plant raw material, µg/g
Hydroxycinnamic acids	
Hydroxyphenylacetate	1145.25
Caffeic	614.17
Syringic	215.86
p-coumaric	827.54
Ferulic	267.72
Sinapic	381.35
Cinnamic	251.81
Quinic	77.28
Flavonoids	
Isoquercitrin	916.73
Neohesperidin	3850.93
Naringenin	308.28
Luteolin	332.13

Among the flavonoids, neohesperidin, which belongs to bitter glycosides of flavanones and provides a bitter taste of raw materials, prevails in the common agrimony herb. This flavonoid has a sedative effect in combination with diosmin but slightly less than rutin. Neohesperidin also exhibits hypolipidemic and antihypertensive activity [8].

Among hydroxycinnamic acids, hydroxyphenylacetate and *p*-coumaric prevail in the common agrominy herb. *P*-coumaric acid exhibits antioxidant properties that reduce the risk of gastric cancer by reducing the formation of carcinogenic nitrosamines. *In vitro* experiments *p*-coumaric acid showed pronounced anti-inflammatory activity [9].

4. Conclusions

1. By HPLC analysis, the quantitative content of the following individual compounds of phenolic nature was identified and determined: hydroxyphenylacetate, caffeic, syringic, *p*-coumaric, ferulic, sinapic, cinnamic and quinic acids, isoquercitrin, neohesperidin, naringenin, luteolin.
2. Neohesperidin, 3850.93 µg/g prevails among flavonoids in the common agrimony herb, hydroxyphenylacetate (1145.25 µg/g) and *p*-coumaric (827.54 µg/g) – among the hydroxycinnamic acids.

5. References

1. Гузьо НМ. Хромато-мас-спектрометричне дослідження летких сполук парила звичайного (*Agriminia eupatoria* L.). Фармацевтичний журнал. 2013; 6:78-83.
2. Фармацевтична енциклопедія/гол. ред. Ради та автор передмови В. П. Черних. – 3-тє вид., пере-робл. і

доповн. К. : Моріон, 2016, 1952с.
3. Кароматов ИД, Кайимова ДИ. Репейничек обыкновенный, репешок. Биология и интегративная медицина. 2017; 2:237-247.
4. Heilerova L, Buckova M, Tarapcik P *et al*. Comparison of antioxidativ activity data for aqueous extracts of lemon balm (*Melissa officinalis* L.), oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.), and agrimony (*Agrimonia eupatoria* L.) obtained by conventional methods and the DNA-based biosensor. Czech Journal of Food Sciences. 2003; 21(2):78-84.
5. Ivanova D, Gerova D, Chervenkov T, Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. Journal of Ethnopharmacology. 2005; 96:145-150.
6. Стойко ЛІ. Фармакогностичне дослідження золототисячника звичайного (*Centaurium erythraea* Rafn.) і тирлича хрещатого (*Gentiana cruciata* L.) родини Gentianaceae : дис. на здобуття наукового ступеня к.фарм.н.: 15.00.02 / Національний фармацевтичний університет. Х, 2018, 167с.
7. Сініченко АВ, Марчишин СМ, Слободянюк ЛВ, Будняк ЛІ. Дослідження фенольних сполук у кореневищах з коренями *Primula denticulate* Smith, *Primula Juliae* Kusn., *Primula saxatilis* Kom. Фітотерапія. Часопис. 2019; 3:26-31.
8. Фармакогнозія: базовий підруч. для студ. вищ. фармац. навч. закл. (фармац. ф-тів) IV рівня акредитації / за ред. В. С. Кисличенко. Х.: Золоті сторінки, 2015, 736с.
9. Федосов АІ, Добровольний ОО, Шаламай АС. та ін. Порівняльний аналіз гідроксикоричних кислот артишоку, що вирощений в Україні та Франції. Актуальні питання фармацевтичної і медичної науки та практики. 2017; 1(10):49-53.