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Investigation of tannins in *Anchusa officinalis* L.

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Abstract
The investigation of tannins in *Anchusa officinalis* L. was conducted. As a result, qualitative and quantitative tannins content in the herba and the roots were determined. For this purpose we have used high-performance liquid chromatography, titrimetric and spectrophotometric methods. We have revealed gallocatechin, epigallocatechin, catechin, epicatechin, epicatechingallate, catechingallate and gallic acid, and determined their quantitative content by the high-performance liquid chromatography. The total tannins content established using titrimetric method is between 3,00 - 3,53%, spectrophotometric method - 1,13 - 1,74%, high-performance liquid chromatography - 4,22 - 4,29%. The highest content is recorded for epigallocatechin (51, 79 % and 69, 56 % relative to the total tannins content in *Anchusa officinalis* L. herba and roots respectively).

Keywords: *Anchusa officinalis* L., tannins, catechins, high-performance liquid chromatography

Introduction
The investigation of new sources of biologically active substances is a promising direction of the modern pharmaceutical science. Anchusa genus plants contain a wide range of active substances, including tannins [1-3].

Tannins are polyphenolic compounds of the plants with different molecular weights. They are divided into two large groups: hydrolyzable (the derivatives of tannin, gallic and ellagic acids) and condensed (the derivatives of catechin). They show tannic properties and are astringent in taste. Tannins are valuable biologically active substances with numerous pharmacological effects. Their main pharmacological effects are: anti-inflammatory, antioxidant, antimicrobial, antitumor. They are stable when they pass through the gastrointestinal tract, but they have low bioavailability due to active metabolism in the liver [4-8].

Gallic acid, gallocatechin, epigallocatechin, catechin, epicatechin, epicatechin gallate and catechin gallate are the main tannins which show high biological activity. In addition, these substances are able to eliminate methicillin resistance in bacteria, in particular in *Staphylococcus aureus*, to reduce the risk of sudden appearance of type II diabetes, preventing the development of Parkinson's disease. In general, they have a positive effect on a cardiovascular system and metabolic reactions in a body [7, 9, 10].

The purpose of this work is to establish the qualitative and quantitative content of tannins in *Anchusa officinalis* L.

Materials and Methods
The objects of this research are *Anchusa officinalis* L. herba and roots, that were harvested in Ivano-Frankivsk region in 2016. For our investigation we have used high-performance liquid chromatography (HPLC), titrimetric and spectrophotometric methods. The titrimetric determination was carried out by titrating of the plant water extract with a solution of potassium permanganate. We used the indigosulfonic acid as an indicator. Spectrophotometric determination was performed according to State Pharmacopoeia of Ukraine 2.0, Volume 1 [11].

The studies were carried out by measuring of the absorption in the investigated solutions at 760 nm. We used the Spectrophotometer Specord M 40 (Carl Zeiss, Germany) for this purpose. The chromatographic determination was performed by the high-performance liquid chromatograph Agilent Technoligies 1200 (USA) with UV Vis G1315C photometric diode detector, equipped with a flowing degasser G1322A, the autosampler G1329A, the thermostat of columns G1316A, in conjunction with a personal computer with the Agilent ChemStation software. The method is based on the chromatographic determination of tannins with the Discovery C_{18} 25x4.6 reversephase column and a diode-matrix UV detector.

Tannins recorded throughout the UV range wavelengths, which makes it possible to identify them not only in the retention time, but also in the nature of the spectrum of the analyzed component. The mass concentration of tannins was calculated according to the calibration characteristic (the dependence of the chromatographic peak area at 255 nm and 280 nm on the mass concentration of tannins in the solution of the prepared sample). The analysis was carried out in comparison with standard samples of especially pure chemical reagents of gallic acid, gallocatechin, epigallocatechin, catechin, epicatechin, epicatechin galatte and catechin gallate (Sigma-Aldrich).

Results and Discussion

As a result of the analysis of *Anchusa officinalis* L., the qualitative and quantitative content of tannins was determined. The comparative characteristics of the tannins quantitative content in *Anchusa officinalis* L. with different methods are presented in Table. 1

Table 1: Quantitative content of tannins in *Anchusa officinalis* L.

Plant raw material	Titrimetric method, %	Spectrophotometric method, %	HPLC, %
Anchusa officinalis L herba	3,53	1,74	4,22
Anchusa officinalis L roots	3,00	1,13	4,29

Data in Table 1 indicates a significant difference of tannins content, due to deficiencies in their determination with different methods. Titrimetric method defines not only tannins, but also other phenolic compounds which are the oxidation targets. Spectrophotometric method defines only those tannins that are capable of forming insoluble complexes with the skin powder. By HPLC it is possible to determine compounds that are poorly capable of reaction and those that are difficult to extract from plant material. However, the establishment of qualitative and quantitative content is possible only for tannins with the available standard samples.

The quantitative content of individual tannins determined by the HPLC and their chromatograms are shown in Fig. 1, 2 and in the Table 2.

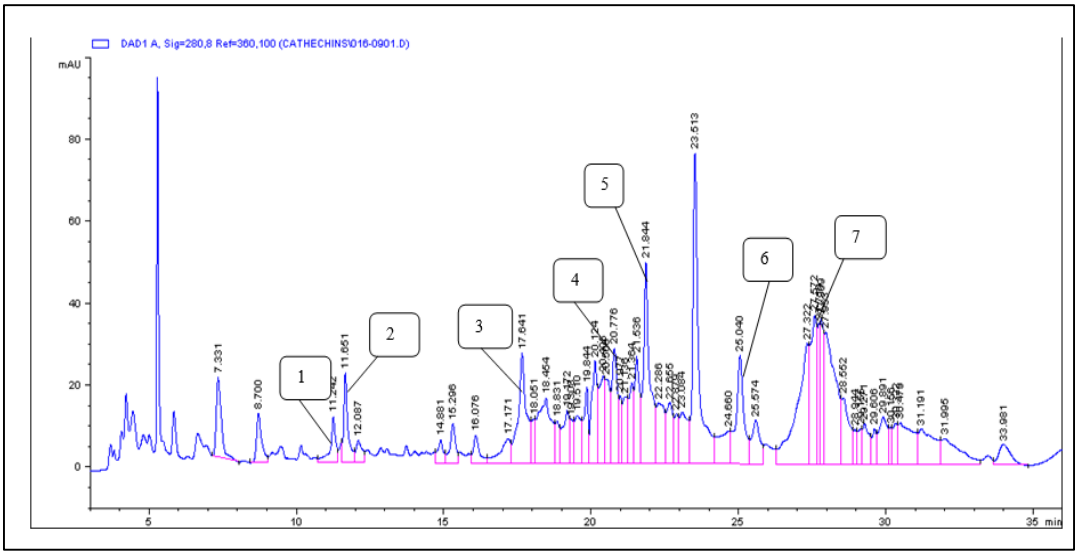


Fig 1: Chromatogram of tannins in *Anchusa officinalis* L. herba (1 - gallic acid, 2 - gallocatechin, 3 - epigallocatechin, 4 - catechin, 5 - epicatechin, 6 - epicatechin gallate, 7 - catechin gallate)

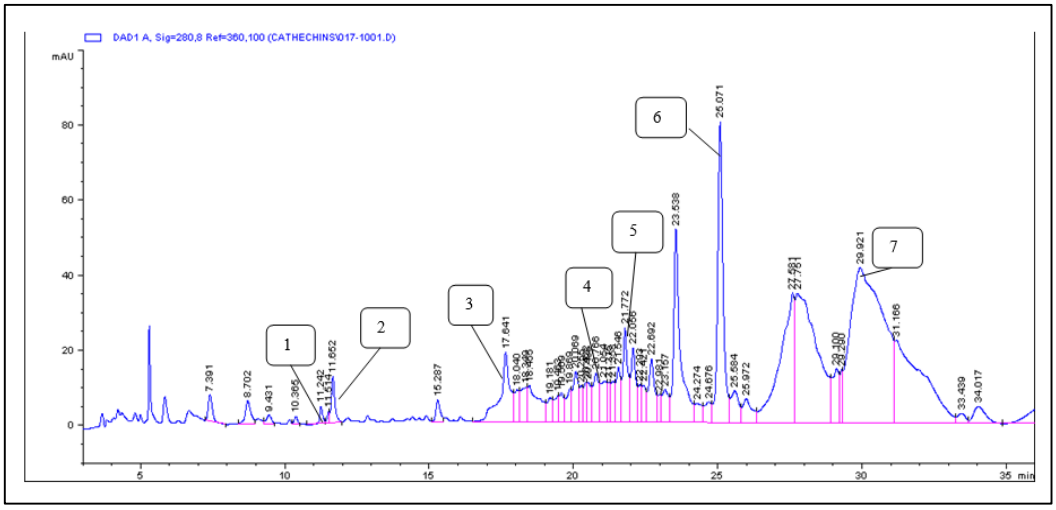
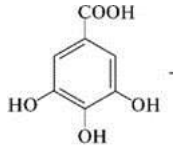
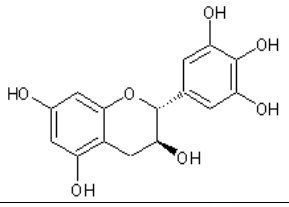
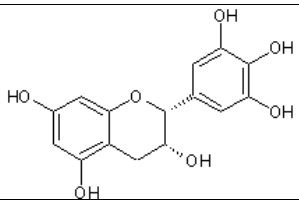
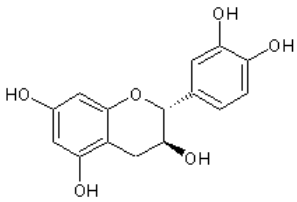
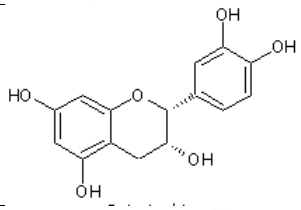
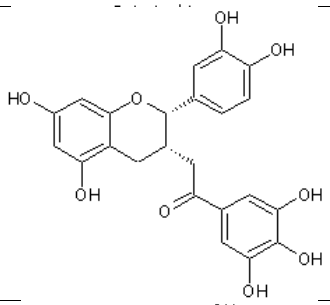
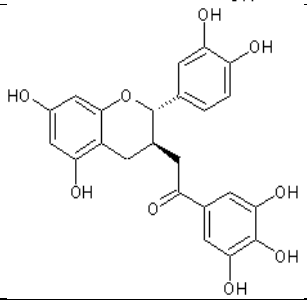


Fig 2: Chromatogram of tannins in *Anchusa officinalis* L. roots (1 - gallic acid, 2 - gallocatechin, 3 - epigallocatechin, 4 - catechin, 5 - epicatechin, 6 - epicatechin gallate, 7 - catechin gallate)

Table 2: Tannins content in *Anchusa officinalis* L.

S. No	Substance	Formula	Herba		Roots	
			mg/kg	%*	mg/kg	%*
Hydrolysable tannins (the derivatives of gallic acid)						
1.	Gallic acid		100,58	0,24	22,99	0,05
Condensed tannins (the derivatives of flavan-3-ol)						
2.	Gallocatechin		3811,20	9,03	1753,71	4,09
3.	Epigallocatechin		21864,28	51,79	29850,06	69,56
4.	Catechin		4355,38	10,32	1966,35	4,58
5.	Epicatechin		7092,19	16,80	3168,53	7,38
6.	Epicatechin gallate		1036,64	2,45	1673,1	3,90
7.	Catechin gallate		3954,41	9,37	4476,4	10,43
Total content			42214,68	100	42911,14	100

Note * - relative to the total tannins content in the plant

Data of Table 2 indicates that gallic acid, gallocatechin, epigallocatechin, catechin, epicatechin, epicatetechin gallate and catechin gallate are constituents of *Anchusa officinalis* L. raw material. The highest content is recorded for epigallocatechin (51,79 % and 69,56 % relative to the total tannins content in *Anchusa officinalis* L. herba and roots respectively).

Conclusions

1. Qualitative and quantitative content of tannins in *Anchusa officinalis* L. herba and roots were established.
2. The total tannins content established using titrimetric method is between 3,00 – 3,53%, spectrophotometric method – 1,13 – 1,74%, HPLC – 4,22 – 4,29%.
3. It was revealed gallocatechin, epigallocatechin, catechin, epicatechin, epicatechin gallate, catechin gallate and gallic acid and determined their quantitative content by the HPLC.
4. The highest content is recorded for epigallocatechin (51,79 % and 69,56 % relative to the total tannins content in *Anchusa officinalis* L. herba and roots respectively).

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