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**О.А. Kyslychenko****FLAVONOIDS DETERMINATION IN THE ABOVEGROUND PART OF  
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**Кисличенко А.А.** Определение флавоноидов в надземной части травы тысячелистника обыкновенного // Украинський медичний альманах. – 2014. – Том 17, № 3. – С. 46-48.

В результате исследования качественного состава и количественного содержания флавоноидов в надземной части травы тысячелистника обыкновенного обнаружено 26 флавоноидов, 12 из которых идентифицировано. Установлено, наибольшее количество флавоноидов накапливается в цветках, а наименьшее – в стеблях. Основываясь на выходе спиртовых экстрактов и содержании биологически активных веществ, целесообразным является использовать обмолоченную траву тысячелистника обыкновенного.

**Ключевые слова:** флавоноиды, качественный состав, количественное содержание, тысячелистник обыкновенный, лист, стебли, цветки.

**Кисличенко О.А.** Визначення флавоноїдів у надземній частині дерев'яного звичайного // Український медичний альманах. – 2014. – Том 17, № 3. – С. 46-48.

В результаті дослідження якісного складу та кількісного вмісту флавоноїдів у надземній частині дерев'яного звичайного виявлено 26 флавоноїдів, 12 з яких ідентифіковано. Встановлено, що найбільша кількість флавоноїдів накопичується у квітках, а найменше – у стеблах. Спираючись на вихід спиртових екстрактів та вміст біологічно активних сполук, доцільно використовувати обмолочену траву дерев'яного звичайного.

**Ключові слова:** флавоноїди, якісний склад, кількісний вміст, дерев'яний звичайний, листя, стебла, квітки.

**Kyslychenko O.A.** Flavonoids determination in the aboveground part of *Achillea millefolium* // Український медичний альманах. – 2014. – Том 17, № 3. – С. 46-48.

As a result of the studies of qualitative composition and quantitative content of flavonoids in *Achillea millefolium* aboveground part 26 flavonoids were determined, 12 of which were identified. It was found out that the highest amount of flavonoids is accumulated in flowers, the least – in stems. Due to the yield of ethanol extracts as well as to the content of biologically active compounds it is reasonable to use threshed herb of *Achillea millefolium*.

**Key words:** flavonoids, qualitative composition, quantitative content, *Achillea millefolium*, leaves, flowers, stems.

**Formulation of the problem.** *Achillea* genus joins 150 species spread in Europe, Asia, North Africa and North America. 19 *Achillea* species grow in Ukraine. *Achillea millefolium* is used in official medicine [4]. There are 20 remedies at Ukrainian market and market of Russian Federation (for example, Rotokan, Wundehil) which contain biologically active compounds of yarrow herb. These remedies show coagulant, antimicrobial and anti-inflammatory effect [3].

Yarrow herb contains essential oil, flavonoids, tannins, bitters, vitamin K, alkaloids and organic acids [4], [6]. Qualitative and quantitative content of the essential oil is studied the most. It is found that yarrow herb essential oil contains sesquiterpenoids (proazulene – up to 25-30 %) and monoterpenoids [5].

**Setting goals.** Still, all the data is regarding the whole aboveground part. No data presenting the chemical content of separate part of herb (leaves, stems, flowers) can be found in the literature with an open access to it. That is why the aim of our work was the study of qualitative and quantitative content of flavonoids in aboveground organs of *Achillea millefolium*.

This work is dedicated to the qualitative and

quantitative determination of flavonoids in yarrow herb, allowing the further development of the new plant raw material standardization criteria. The work was conducted according to the plan of the complex scientific and research work at National University of Pharmacy “Pharmacognostical study of biologically active compounds, development of remedies of plant origin” (State registration number № 0103U000476).

**Experimental part.** Yarrow herb, leaves, stems and flowers collected in Kharkiv region in summer 2010 were objects of study. Analysis of the plant raw material was carried out according to the requirements of the State Pharmacopoeia of Ukraine [1]. Extraction of biologically active compounds (BAC) was made by 70 % ethanol.

The preliminary chemical analysis of the obtained extracts was carried out by the common applied methods, with the help of qualitative reactions, paper chromatography (PC), high performance liquid chromatography (HPLC). Ethylacetate-ethanol fraction (8:2) of the extracts was studied by two dimensional paper chromatography (Filtrak № 4): I direction – 2 % acetic acid, II direction – n-butanol – acetic acid – water (4:1:2). It was detected 10 compounds of flavonoid origin.

Apigenin, luteolin and quercetin were identified in comparison to reliable aglycone samples via PC in the system of solvents chloroform – acetic acid – water (13:6:2). Chromatographic analysis was performed after the total hydrolysis of the analyzed fraction by 5 % sulfuric acid.

Qualitative and quantitative content of flavonoids in yarrow herb and aboveground organs of yarrow is represented in the table. The study was made with the help of HPLC, chromatograph Agilent Technologies 1100 with vacuum degasser flow G1379A, four valve pump with low pressure gradient G1311A, automatic injector G1313A, column oven G13116A and diode-matrix detector G1316A [1]. 0,5 g of the extract (precise weight) was transferred into 5 ml vial and added 90 % methanol till the mark. Afterwards, the vial was closed, stored at the ultrasound bath for 30 min and at room temperature during 3-4 hours. Then a specimen was transferred to an ultrasound bath for 15 min. The vial content was centrifuged and filtrated via membrane teflon filter with sieves size of 0.45  $\mu\text{m}$  in to the vial for analysis. A column of a size of 2.1 $\times$ 150 mm filled with octade-

cylsilyl sorbent and graininess of 3.5  $\mu\text{m}$ , "ZORBAX-SB C-18". Solution A (0.6 % trifluoroacetic acid), solution B (70 % MeOH and 0,6 % trifluoroacetic acid) and solution C (100 % MeOH) were used as a mobile phase. Mobile phase flow rate was 0.25 ml/min, work pressure of eluent 240-300 kPa, temperature of column oven – 35  $^{\circ}\text{C}$ , probe volume – 2  $\mu\text{l}$ . The following parameters of detection were set: detection rate – 1.0, scanning time – 0.5 sec, every peak – 190-600 nm. Identification of flavonoids was carried out by retention time of standards (Sigma Chemical Company, USA) and due to spectral characteristics.

It was studied via weight method that yarrow herb consists of leaves (10.2 $\pm$ 6.7%), flowers (54.9 $\pm$ 14.7%) and stems (34.8 $\pm$ 9.7%). As a result of preliminary chemical analysis of phenolic compounds content derivatives of hydroxycinnamic acids, coumarines, flavonoids and other polyphenol compounds were determined [2]. Qualitative and quantitative content of yarrow herb and organs of its aboveground part is presented in the table.

**Table.** Flavonoids in *Achillea millefolium* herb

№	Retention time, min	Compound	Quantitative content (mg/kg)			
			Herb	Leaves	Flowers	Stems
1.	15.66	Vicenin-2	676.2	1075.5	103.9	62.6
2.	16.38	Flavonoid 1	181.5	18.5	13.5	
3.	17.03	Luteolin-3',7-O-diglucoside	298.4	97.0	87.9	27.6
4.	17.23	Flavonoid 2	36.2	338.8		88.5
5.	17.37	Apigenin glycoside	177.2	918.4	170.6	
6.	19.33	Luteolin-7-O-glucoside	2797.1	2728.9	3711.5	163.5
7.	19.64	Rutin	625.6	1894.8		410.6
8.	20.00	Flavonoid 3	1663.1	1020.8	592.8	357.8
9.	20.57	Apigenin-7-O-rutinoside	552.7	183.1	533.8	94.5
10.	20.87	Apigenin-7-O-glucoside	666.1	163.6	1249.6	0.0
11.	21.04	Flavonoid 4	2814.7	2475.6	867.4	747.7
12.	21.26	Flavonoid 5	1177.6	1222.6	1612.1	146.8
13.	22.42	Apigenin derivative 1	321.7	246.2	120.5	81.7
14.	22.80	Apigenin derivative 2	582.6	186.4	1144.5	
15.	24.23	Luteolin	1335.7	241.8	5799.7	12.6
16.	25.84	Apigenin	153.9	41.8	1263.9	4.5
17.	26.21	Flavonoid 6	236.7	95.1	769.6	23.5
18.	26.65	Chrysoeriol	159.4	1246.6		42.7
19.	27.48	Flavonoid 7	298.0	123.6	38.3	0.0
20.	27.88	Diosmetin	272.4	404.1	532.7	20.4
21.	28.95	Flavonoid 8	315.1	550.8	790.0	32.0
22.	29.45	Flavonoid 9	175.6	97.1		
23.	29.57	Flavonoid 10	337.0	101.1	63.4	4.3
24.	30.22	Genkwanin	58.1	54.4		
25.	30.39	Flavonoid 11	522.4	317.0	369.7	9.2
26.	30.91	Acacetin	108.9	33.9		
Flavonoid content, %			1.65	1.59	1.98	0.23

26 flavonoids were found in *Achillea millefolium* grass. 12 of 26 flavonoids found were identified. In flowers and leaves the highest

amount of luteolin-7-O-glucoside, in flowers – of luteolin, in stems – of rutin was determined. According to the data shown in the table, the highest

amount of flavonoids is stored in flowers, less – in leaves. The minimal amount of flavonoids was determined in stems.

**Conclusions:**

1. The study of qualitative and quantitative content of phenolic compounds in yarrow herb as well as its aboveground organs was conducted.
2. On the whole, 26 flavonoids were found in yarrow herb, 12 of them were identified.
3. According to the results of the study it

was found that the highest amount of flavonoids is stored in flowers, less – in leaves.

4. To take into consideration the yield of extracts obtained from different organs of the aboveground part of *Achillea millefolium*, it is reasonable to use threshed yarrow herb. Still, it's needed the extended studies.

5. The obtained results can be further used for plant raw material standardization criteria development.

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