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FLAVONOIDS QUANTIFICATION OF YARROW HERB | DETERMINAREA CANTITATIVĂ A FLAVONOIDELOR DIN PĂRȚI AERIENE DE COADA ȘORICELULUI

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Rezumat. În acest articol sunt prezentate rezultatele elaborării tehnicii de determinare cantitativă a flavonoidelor din părți aeriene de coada șoricelului. În urma realizării studiului, s-a constatat că optimal este adăugarea la extractul cercetat a unei soluții apoase de 4% de clorură de aluminiu într-un volum de 200 µl pentru prepararea soluției probă și a acidului acetic glacial într-un volum de 10 µl pentru prepararea atât a soluției probă, cât și a soluției de compensare. Timpul optim de reacție între flavonoide și clorura de aluminiu este de 60 de minute.

Cuvinte cheie: flavonoide, spectrofotometrie, Achillea millefolium, luteolin-7-O-glucozid, determinare cantitativă.

Abstract. This article presents the results of the technique choice for the quantitative determination of herb yarrow flavonoids. As a result of the study, it was found that the optimal is the addition to investigate extract of a 4% aqueous solution of aluminum chloride in a volume of 200 µl for the preparation of the test solution and glacial acetic acid in a volume of 10 µl for the preparation of both the test solution and the compensation solution. The optimal reaction time between flavonoids and aluminum chloride is 60 minutes.

Keywords: flavonoids, spectrophotometry, *Achillea millefolium*, luteolin-7-O-glucoside, quantitation.

INTRODUCTION

A number of pharmacological effects of the herb yarrow are due to the presence of flavonoids, the predominant among which are the 7-O-glucosides of apigenin and luteolin [1]. However, in a number of national (Belarus, Ukraine, Kazakhstan, Great Britain) and the European Pharmacopoeias, it is proposed to standardize it only by the content of essential oil. Based on this, the data obtained can be used in the development of a technique for the quantitative determination of flavonoids in this plant raw material.

MATERIALS AND METHODS

The object of the study was a yarrow herb harvested during the period of mass flowering in places of natural growth in 2020 in the vicinity of Baranovichi. Prior to research, the plant raw materials were stored in paper bags.

For the extraction of flavonoids, we took a weighed portion of 0,05 ± 0,003 g of yarrow herb, preliminarily crushed and sifted through a sieve with aperture size of 180 µm. For work, we took a fraction that passed through a sieve. For extraction, 80% methanol was used in an amount of 5,00 ± 0,250 ml. Extraction was carried out in a water bath for 60 min at 80 °C in tightly sealed vials with a screw cap. The above presented extraction parameters were selected experimentally in previous studies [2, 3, 4]. After extraction, the obtained extract was filtered, the resulting filtrate was used for the quantitative determination of flavonoids.

To prepare the test solution, 200±10,0-1400±70,0 ml (with a step of 200±10,0 ml) 1-5% (with a step of 1%) aqueous aluminum chloride solution, as well as 10,0±0,500; 25,0±1,25; 50,0±2,50; 75,0±3,75 and 100±5,00 µl of glacial acetic acid were added to 100±5,00 µl of the extract and made up to 5,00±0,250 ml with water.

To prepare a compensation solution, 10,0±0,500; 25,0±1,25; 50,0±2,50; 75,0±3,75 and 100±5,00 µl of glacial acetic acid were added to 100±5,00 µl of the extract, after which the resulting mixture was brought to 5,00±0,250 ml with water. After 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 min, the optical density of the test solution was measured at 400 nm against the compensation solution.

The content of the sum of flavonoids (X, %) in terms of luteolin-7-O-glucoside was calculated using the formula 1.1:

$$X,\% = \left(\frac{A+0,0174}{0,3474}\right) \times 0,5/m \qquad (1)$$

where A – is the optical density of the test solution;
m – is the mass of the sample of crushed raw materials, g.

The obtained experimental data were processed using Microsoft Excel 2016.

Tables 1 and 2 show the results of selecting the optimal concentration and volume of an aqueous solution of $AlCl_3$, respectively.

Based on the data obtained in tables 1 and 2, it can be said that with an increase in the concentration of the $AlCl_3$ solution, the content of flavonoids increases and, starting from a concentration of 4%, reaches a plateau. No significant differences in the content of flavonoids when using different volumes of $AlCl_3$ are observed, which is confirmed by the value of Student's t-criterion between the content of flavonoids when adding 200 µl of aluminum chloride solution and 1400 µl, which is equal to 0,41> 0,05.

Table 1. Dependence of the content of flavonoids (%) on the concentration of the $AlCl_3$ solution used in the quantitative determination of flavonoids

Concentration of an aqueous solution of $AlCl_3$, %	Flavonoid content, %
1	3,59±0,180
2	3,59±0,180
3	3,57±0,179
4	3,78±0,189
5	3,76±0,188

Table 2. Dependence of the content of flavonoids (%) on the volume of the $AlCl_3$ solution used in the quantitative determination of flavonoids

Volume of added $AlCl_3$, solution, µl	Flavonoid content, %
200	3,82±0,191
400	3,78±0,189
600	3,87±0,194
800	3,73±0,187
1000	3,77±0,189
1200	3,61±0,181
1400	3,79±0,190

Thus, according to the results of this experiment, we can say that the optimal use for the quantitative determination of flavonoids is the use of a 4% aqueous solution of $AlCl_3$ in a volume of 200 ± 10,0 µl.

Table 3 shows the results of the selection of the optimal volume of glacial acetic acid.

Table 3. Dependence of the content of flavonoids (%) on the volume of a solution of glacial acetic acid used in the quantitative determination of flavonoids

Glacial acetic acid volume, µl	Flavonoid content, %
200	4,85±0,243
400	4,39±0,220
600	3,82±0,191
800	3,89±0,195
1000	3,69±0,185

As can be seen from the data obtained, with an increase in the amount of added glacial acetic acid, the content of flavonoids decreases, because acid is a stop agent in the reaction between flavonoids and aluminum chloride. Its use is optimal in a volume of 10,0±0,500 µl.

Table 4 shows the results of the selection of the optimal reaction time between flavonoids and aluminum chloride.

Table 4. Dependence of the content of flavonoids (%) on the reaction time between flavonoids and aluminum chloride

Reaction time, min	Flavonoid content, %	Reaction time, min	Flavonoid content, %
5	4,44±0,222	50	4,97±0,249
10	4,60±0,230	60	5,00±0,250

15	4,70±0,235	70	5,02±0,251
20	4,76±0,238	80	5,05±0,253
30	4,86±0,243	90	5,06±0,253
40	4,93±0,247	100	5,07±0,254

Based on the results obtained, it can be seen that with an increase in the reaction time, the content of flavonoids increases due to the formation of a larger amount of complexes between flavonoids and aluminum chloride. The peak in the percentage of flavonoids is observed at 60 minutes (5,00 ± 0,250%), then it reaches a plateau. Student's t-test between the content of flavonoids at a reaction time of 60 and 100 minutes is 0,30> 0,05, which indicates that there is no statistically significant change in the amount of flavonoids from 60 minutes to 100 minutes.

CONCLUSIONS

For the quantitative determination of yarrow flavonoids, it is optimal to add, for the preparation of the test solution, a 4% aqueous solution of aluminum chloride in a volume of 200 ± 10,0 µl and glacial acetic acid in a volume of 10,0 ± 0,500 µl for the preparation of both the test solution and the compensation solution, the optimal reaction time is between flavonoids and aluminum chloride is 60 minutes.

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