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IDENTIFICATION AND ASSAY OF THE FLAVONOIDS IN MEDICINAL PLANTS WITH HEPATOPROTECTIVE ACTION

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Abstract. The article describes the identification and assay of the flavonoids in medicinal plants with hepatoprotective action, harvested as a culture at the Cultivation Center of the Medicinal Plants within State University of Medicine and Pharmacy „Nicolae Testemițanu" from the Republic of Moldova, using Pharmacopoeia methods. The flavonoids, found in the examined medicinal product, are responsible for hepatoprotective activity due to antioxidant activity, exhibited by neutralizing free radicals. The flavonoids were identified by using the Chinode method and thin layer chromatography, operating with 3 systems of solvents, and as biomarkers rutine, quercetine and luteolin ewere used. The results of the quantitative analysis point out that the content of the flavonoids determined by using spectrophotometric method is in the range of 0,620% to 1,204%, in studied vegetal products.

Key words: hepatoprotective medicinal plants, flavonoids, identification, assay, spectrophotometric method

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Introduction

At the beginning of the third millennium, acute and chronic hepatitis, especially with parenteral transmission continues to be a major public health problem because of the prevalence, endemicity, increased morbidity and mortality and high disability rate after chronicity of infection. It is astounding fact that one in 12 people worldwide are living with either chronic hepatitis B or C, which is much higher prevalence than the prevalence of HIV or any type of cancer. Thus, the problem remains acute and the need to assess medicinal plants and active principles responsible for hepatoprotective action is undeniable, both in terms of medical, social and economic points (Programul național... 2012-2016). Hepatoprotective medicinal plants are an excellent natural remedy for the treatment of hepatobiliary diseases by increasing bile secretion, stimulation of gallbladder contraction and bile ducts, lower cholesterol and blood glucose, increase the dry residue of bile, liver cell regeneration. At once, improve liver functions, playing the role of detoxification, including by normalizing cholesterol, increasing resistance to infection by liver antioxidant activity. However, against free radicals, the body builds antioxidant compounds, whichare recommended to be delivered to the body from the exogenous sources too, including the use of medicinal plants, these ones containing flavonoids in larger or smaller amounts. At the same time, flavonoids are able to potentiate the physiological effects of other antioxidants such as vitamins E, A, C. (Ciulei *et al.* 1995; Cojocaru-Toma 2014). The target of the study is the identification and the spectrophotometric determination of the flavonoids in medicinal plants with hepatoprotective activity, vegetal products harvested from the collection of the Cultivation Center of the Medicinal Plants within State University of Medicine and Pharmacy „NicolaeTestemițanu".

Material and methods

In the study were included vegetal products, harvested according to their provenience and pharmacopoeia recommendations: artichoke leaves – *Cynarae folium*, thistle fruits – *Sylibi fructus*, underground parts of astragalus – *Astragali radices*, aerial parts of chicory – *Cichorii herba*, marigold flowers – *Calendulae flores*, mint

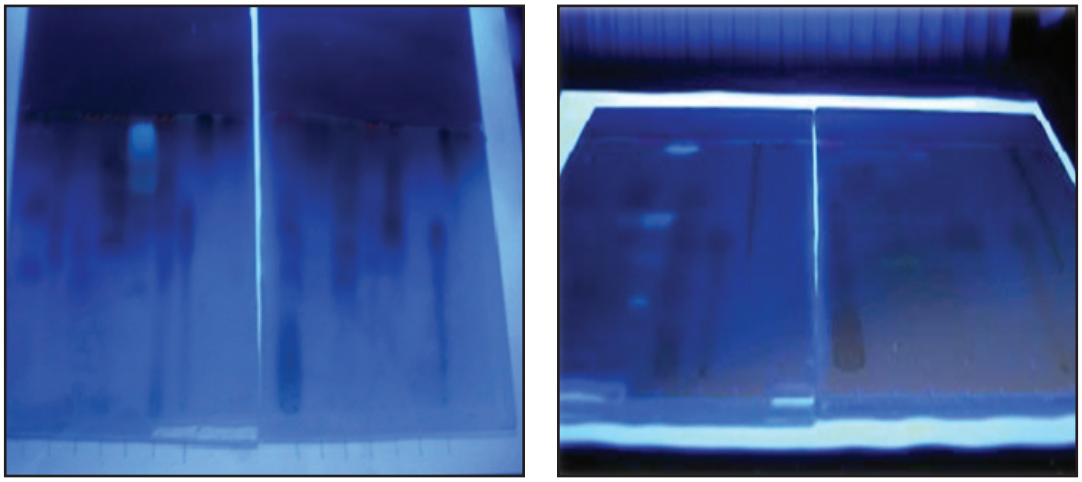

Fig. 1. Identification of flavonoids by thin-layer chromatografy, a and b systems.

leaves – *Menthae piperitae folia*, septfoil rhizomes – *Tormentillae rhizomata*, immortelle flowers – *Helichrysi italici flores*, aerial parts of sweet clover – *Meliloti officinalis herba*, comfrey roots – *Symphyti radices*, aerial parts of agrimony – *Agrimoniae herba*, tansy flowers – *Tanaceti flores*, immortelle flowers – *Helichrysi arenarii flores*.

Medicinal plants with hepatoprotective action were selected based on scientific publications, identified by determinators (Negru 2007) and assessed by reference Pharmacopoeias, according to Ministry of Health Order no. 113 of 17.02.2011, regarding the approval of reference Pharmacopoeias in Moldova, which establishes the European Pharmacopoeia as a reference in the elaboration, standardization and quality control of drugs in Moldova. As a result of examination the analysis methods of vegetal products and of active principles from the reference Pharmacopoeias mentioned above, we find out that the assay of flavonoids is presented for the following vegetal products: thistle fruits, immortelle flowers, chicory flowers, marigold flowers, aerial parts of rattles, artichoke leaves (European Pharmacopoeia 2014). Flavonoids, in turn, are related phenolic vegetal pigments with polyphenolic structure, widespread in the plant kingdom found most often in the form of heterosides whose aglycone/genin are derivatives of *phenyl-benzo-y-pirone C6-C3-C6*. The extraction of flavonoids is based on their solubility in hot water or alcohol. Sometimes crystalline forms are obtained by simply cooling the extraction solution. The extraction is performed in alcohol, the resulting solutions are subjected to evaporation, and the residue is taken up in hot water and exhausted with ethyl acetate and then with butanol. In preparing extracts for the qualitative reactions, exactly weighed samples of plant products, sprayed and treated with alcohol are maintained on the condenser water bath until exhausting the vegetal product. After cooling, the extracts are filtered and used in the identification reactions: the Chinode reaction and thin layer chromatography. In the determination of the flavonoids we used the spectrophotometric method, according to European Pharmacopoeia, based on the colored reaction with a solution of aluminum chloride (European Pharmacopoeia 2014).

Results

The principle of the Chinode reaction is based on the fact that the flavonols, flavones and flavanones by reducing with the magnesium, in the presence of hydrochloric acid, present the red or orange color, due to the formation of anthocyanidines, the reaction which is negative for aurones and chalcones, which at the addition of hydrochloric acid, in the absence of magnesium, give red color, with the formation of oxonium salts. The Chinode reaction

Tab. 1. Results of thin layer chromatography.

Nr.	Species	(Rf) in the 1st system *ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26)*	(Rf) in the 2nd system *n-butanol: glacial acetic acid: water (4:1:2)*
1.	*Cynara scolimus*	0,49; 0,55; 0,87	0,48; 0,62; 0,72; 0,82
2.	*Silybum marianum*	0,39	0,39
3.	*Astragalus glycyphyllos*	0,42; 0,81	0,68; 0,86
4.	*Cichorium intybus*	0,19; 0,33; 0,41; 0,51; 0,54; 0,87	0,67; 0,74; 0,83
5.	*Calendula officinale*	0,41	0,38; 0,68; 0,89
6.	*Mentha piperita*	0,41; 0,76	0,68
7.	*Potentilla erecta*	0,55; 0,76; 0,89	0,15; 0,54; 0,87
8.	*Helichrysum arenarium*	0,33; 0,41; 0,49; 0,82; 0,89	0,69; 0,83; 0,88
9.	*Helichrysum italicum*	0,33; 0,36; 0,41; 0,49; 0,65; 0,86; 0,89	0,69; 0,59; 0,46; 0, 83; 0,88
10.	*Melilotus officinalis*	0,41	0,66
11.	*Symphytum officinale*	0,41	0,68
12.	*Agrimonia eupatoria*	0,41; 0,87	0,66, 0,83
13.	*Tanacetum vulgare*	0,41; 0,86	0,67; 0,84
Rutine (etalon)		0,41	0,68
Quercitine (etalon)		0,89	0,87
Luteoline (etalon)		0,87	0,83

was positive for all samples studied, showing the presence of flavonoids in all vegetal products listed.

Flavonoids were identified also by thin layer chromatography, using three systems (Fig. 1):

a) ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26);

b) *n*-butanol: glacial acetic acid: water (4:1:2);

c) toluene: acetic acid: water (60:22:1,2).

The system toluene: acetic acid: water (60:22:1.2) gave less positive results, the spots being visible for the first two systems mentioned above and as etalon were used: *rutine, quercetine* and *luteoline*. For the identification of flavonoids, on the same chromatogram the color and the fluorescence of the spots are compared, the value of the retention coefficient (Rf) for the sample – solution and the etalon – solution. The detection of the spots was performed by the own fluorescence and in the UV – light, with aluminum chloride 2,5% (Tab. 1).

After conducting thin-layer chromatography in plants with hepatoprotective action the following components were identified:

Rutine – in: *Cynara scolimus, Sylibum marianum, Astragalus glycyphyllos, Cichorium intybus, Calendula officinale, Mentha piperita, Helichrysum arenarium, Helichrysum italicum, Melilotus officinalis, Symphytum officinale, Agrimonia eupatoria, Tancetum vulgare.*

Quercetine – in: *Cichorium intybus, Calendula officinale, Potentilla erecta, Helichrysum arenarium, Helichrysum italicum.*

Luteoline – in: *Agrimonia eupatoria, Cynara scolimus, Cichorium intybus, Helichrysum arenarium, Helichrysum italicum, Tanacetum vulgare.*

Our aim was to perform assay of total flavonoids using spectrophotometric method, which is also found in the European Pharmacopoeia (2014). Spectrophotometric method is based on the principle of color reaction of flavonoids with a solution of aluminum chloride. The flavonoid concentration is calculated using the standard graph constructed on the absorbance corresponding to rutozide solutions of different concentrations. In the working technique, the exactly weighed

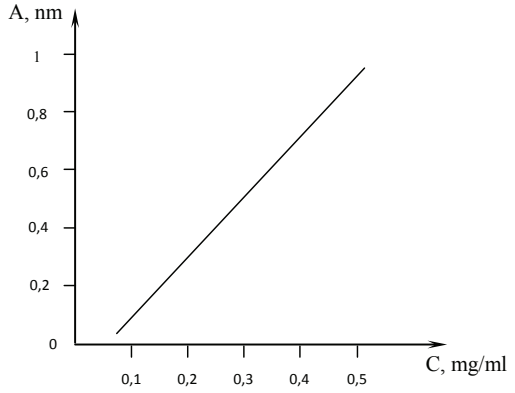

Fig. 2. Total flavonoids content calibration graph of rutine.

sample of the pulverized vegetal product, 50 % ethyl alcohol is added and the extraction is carried out by boiling in a water bath under reflux for 30 minutes. The hot solution was filtered and after cooling made up to volume specifically, by washing with the same solvent the residue thus obtaining the A solution.10 ml of solution A is diluted with methanol to 25 ml in a volumetric flask, then stirred for 2-3 minutes and allowed to stand for 10 minutes. The first portion was filtered and removed. At the 5 ml of the filtrate is added 5 ml of sodium acetate 100 g/l and 3 ml of aluminum chloride 25 g/l , the obtained solution is stirred and completed with methanol until 25 ml in a volumetric flask, thus obtaining the sample – solution. The absorbance of the solution is determined at 430 nm, using as the compensation liquid a solution obtained under the same conditions as the sample solution, 5 ml of the filtrate, 8 ml of water and 25 ml methanol in a volumetric flask. The flavonoids concentration of the analyzed sample is calculated using a standard curve established in parallel and in the same conditions as the sample solution taking into work: 1,0 ; 2,0 ; 3,0 ; 4,0 and 5,0 ml standard solution of rutozide 0,1 g/l in methanol, applying the formula (ONIGA *et al.* 2004).

The experimental results shows that the total content of flavonoids (Fig. 2) in vegetal products studied using the spectrophotometric method in recalculation on rutine are: 0,620% for *Silybi fructus*; 0,698% in *Cichorium intybus*; 0,646% in *Symphytum officinale*; 0,701% in *Calendula officinale*; 0,712% in *Astragalus glycyphyllos*; 0,717% in *Tanacetum vulgare*; 0,764% in *Helichrysum arenarium*; 0,803% in *Melilotus officinalis*; 0,816% in *Potentilla erecta*; 0,871% in *Agrimonia eupatoria*; 0,890% in *Helichrysum italicum*; 0,947% in *Mentha piperita* and the highest content of 1,204% for *Cynarae folium*, with a relative error of 0 to 0,796.

Discussion and conclusions

The vegetal products were harvested from the collection of the Cultivation Center of the Medicinal Plants within the State University of Medicine and Pharmacy „NicolaeTestemiţanu" according to the nature of the vegetal product and Pharmacopoeia recommendations. Identification of flavonoids was performed by identifying reactions and thin layer chromatography method using three systems, including system toluene: acetic acid: water (60:22:1,2), which gave negative results, the spots being visible in systems: ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) and n-butanol: glacial acetic acid: water (4:1:2) as the etalon using: rutine, quercetine and luteoline. The content of flavonoid in the medicinal plants with hepatoprotective activity was determined by spectrophotometry according to the Pharmacopoeia and deviates from 0,620 % for *Silybi fructus* to 1,204 % for *Cynarae folium*.

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