The Pharma Innovation

ISSN: 2277- 7695
CODEN Code: PIHNBQ
ZDB-Number: 2663038-2
IC Journal No: 7725

Received: 26-03-2014
Accepted: 10-05-2014

Vol. 3 No. 4. 2014
Online Available at www.thepharmajournal.com

THE PHARMA INNOVATION - JOURNAL

Research phenolic compounds Malva sylvestris by high performance liquid chromatography

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Component composition and quantitative content of anthocyanins, hydroxycinnamic acids and flavonoids in the herb *Malva sylvestris* have been studied with usage high performance liquid chromatography (HPLC) method. Components of tanning agents, in particular ellagic acid, epicatechin, flavonoid glycosides – rutin, hyperoside and quercetin-3-D-glucoside flavonoid aglycones – luteolin, apigenin and quercetin; coumarin and 5 hydroxycinnamic acids (chlorogenic, rosmarinic, p-Coumaric, ferulic and caffeic) have been identified. The total content of anthocyanins is 36.80 mg/100 g. Luteolin and chlorogenic acid dominated among all phenolic compounds in the herb Malva sylvestris, the contents of which are 96.12 and 51.04 mg/100 g, respectively.

Keyword: Malva sylvestris, phenolic compounds, high performance liquid chromatography, standardization.

1. Introduction

The introduction of the native medical practice new types of medicinal plant materials, its processed products, expanding the range of phyto medications requires improved approaches to standardization and quality control. Medicines, including medicinal vegetative raw material as applied to medical practice must comply with the criteria of efficiency, secure, and therefore to the standardization of high demands are placed, as it should ensure the quality phyto preparations at all stages of production.

Standardization requires the use of modern physical methods of analysis, allows the determination of the qualitative and quantitative composition of the plant materials rapidity and with sufficient accuracy. Such methods include high-performance liquid chromatography, which is the most used in the study of the quantitative content of biologically active substances in medicinal plant material in Pharmacopoeias of different countries. Therefore, the introduction of modern alternative methods for determining qualitative and quantitative composition of biologically active substances promising types of vegetation the medicinal raw materials by HPLC is a relevant.

Malva sylvestris – a plant family of Malvaceae (Malvaceae), is well known in national medicine expectorant, anti-inflammatory, enveloping properties and other.

Phenolic compounds – is one of the most ubiquitous groups of biologically active

substances in the plant organism, which is known for a wide range of pharmacological activity, primarily as a substance capable of regulating the prooxidant-antioxidant homeostasis.

2. Objective and Methods

The aim was to study the component composition and quantitative content of phenolic compounds in the herb Malva sylvestris using the HPLC.

The object of investigation was used grass of Malva sylvestris which was harvested in June 2013 in the Lugansk region in Ukraine.

Analysis of phenolic compounds was performed by liquid chromatography system AT 1200 (company Agilent Technologies, USA), which is equipped with a flowing degasser, auto sampler, column oven and photometric diode array detector. Chromatographic separation hydroxycinnamic acids and flavonoids were performed with using columns «Discovery-C18» size 4,6 × 250 mm; anthocyanins – «Hypersil ODS-C18» size 4,0 × 250 mm. Installed the following mode chromatography: mobile phase flow rate of 1 ml/min; eluent operating pressure 240-300 kPa and the temperature of the column oven 25° C and 5 microliters of sample volume for determining the hydroxycinnamic acids and flavonoids, anthocyanins when determining – column oven temperature of 40° C and a sample volume of 10 microliters. As the eluents used the following solvents (qualification HPLC grade): for anthocyanins – formic acid, water and acetonitrile, for hydroxycinnamic acids and flavonoids – orthophosphoric acid and acetonitrile, which was supplied in a gradient mode.

Research was conducted in compliance with the conditions of detection parameters: scale measuring 1.0, scan time of 0.5 s, the wavelength of 518 nm (for anthocyanins), 255 nm, 320 nm and 330 nm (for other phenolic compounds).

Identification of phenolic compounds was carried out by comparing the retention time of the main peak and the external standard as well as by the character of analyzed component spectrum. As standards used aqueous and alcohol solutions, reference samples of phenolic compounds firm Sigma-Aldrich.

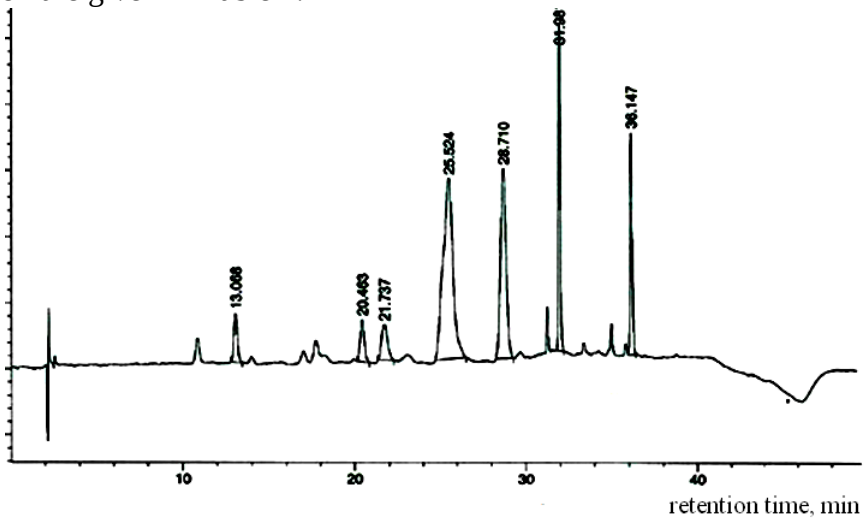
In the investigation of anthocyanins in order to increase the reliability of the qualitative determination of anthocyanins composition of raw materials used internal standard method, which have used as a kalistefin chloride – compound on the structure and physicochemical properties similar to anthocyanins. The peak areas and retention time of anthocyanin compounds were compared with the retention time and the area of the internal standard.

Chromatography of solutions of standard substances and test solutions were performed at least three times until they fulfilled the requirements of the system suitability.

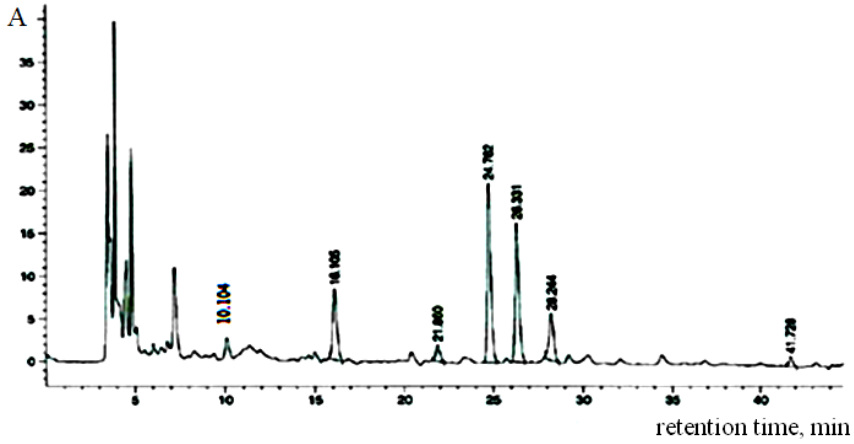
Sample preparation for studying of hydroxycinnamic acids and flavonoids were conducted as follows: weighed portion minced raw sample mass (2,00 ± 0,01) g was transferred to a 100 ml flask and 30 ml of methanol was poured (with different concentrations for each group BAS). The flask was heated under reflux for 30 min. Further, it was sonicated, cooled in a thermostat and the contents were quantitatively transferred to a volumetric flask with 50 ml. The volume of the flask was adjusted to the mark with methanol and mixed thoroughly. The supernatant was decanted carefully and filtered through a syringe membrane filter in a container for chromatography. Sample preparation for determination of anthocyanins was performed as follows: weighed portion the sample mass (2,00 ± 0,01) g was transferred to a 100 ml flask of 30 ml and poured with bidistilled water which was acidified with phosphoric acid solution. The flask was heated under reflux for 30 min. It was cooled in a thermostat and the contents were quantitatively transferred into a volumetric flask of 50 ml. The volume was diluted with bidistilled water which was acidified. Thoroughly mixed and filtered through a filter paper ("red" strip). 1 ml from the filtrate in container chromatography (light glass) was taken.

Control chromatography system, obtaining chromatograms and calculation results were performed with using the software Agilent Chemstation. Chromatograms of phenolic compounds of Malva herb are shown in Fig. 1.

tudied results of phenolic compounds and their parameters retention are given in Table 1.



A



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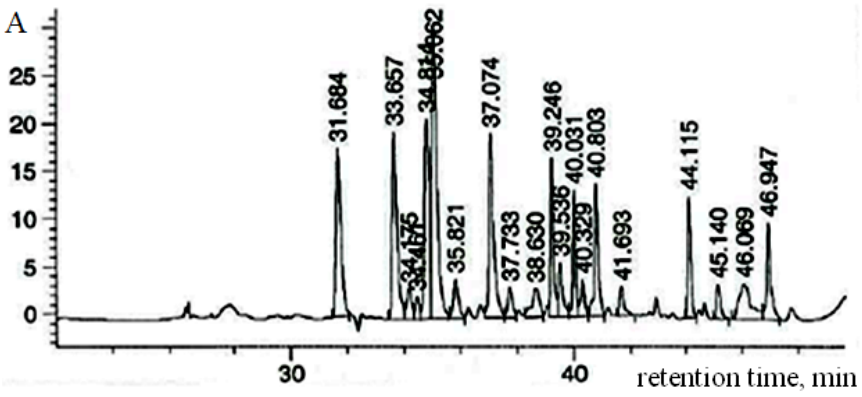

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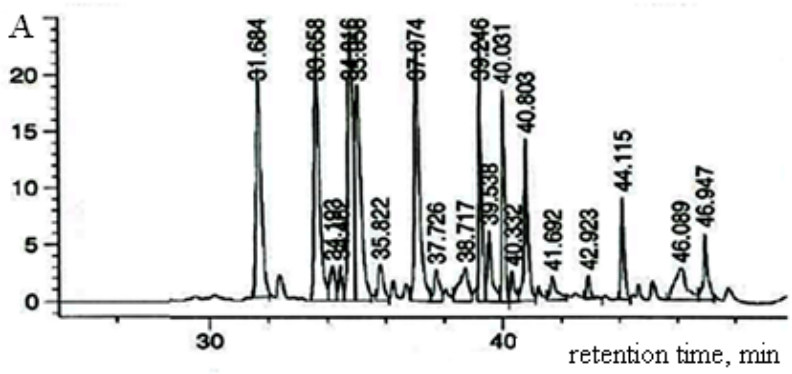

D

Fig. 1: HPLC determination of phenolic compounds in the herb Malva: A – anthocyanins at λ = 518 nm; B – phenolic compounds at λ = 255 nm; C – hydroxycinnamic acids at λ = 320 nm; D – hydroxycinnamic acids at λ = 330 nm

Table 1: Chemical composition of phenolic compounds in herb of Malva sylvestris

Retention time, min	Name of the compound	Contents mg/100 g
10,10	Ellagic acid	7,55
16,11	Rutin	19,08
20,08	Hyperoside	4,22
21,86	Quercetin-3-D-glucoside	8,03
24,76	Luteolin	96,12
26,33	Epicatechine	81,28
28,24	Coumarin	23,20
31,68	Chlorogenic acid	51,04
35,82	*n*-Coumaric acid	1,71
37,73	Ferulic acid	1,64
38,63	Caffeic acid	2,20
39,53	Rosmarinic acid	4,13
40,08	Apigenin	8,16
41,72	Quercetin	4,80

3. Conclusion

According to Figure 1 and Table 1, we can make the following conclusions: components of tanning agents, particularly ellagic acid, epicatechin, flavonoid glycosides – rutin, hyperoside and quercetin-3-D-glucoside, flavonoid aglycones – luteolin and quercetin and coumarin have been identified at a wavelength of 255 nm in the herb of Malva sylvestris. 5 hydroxycinnamic acids (chlorogenic acid and rosmarinic at 330 nm and p-coumaric, ferulic and caffeic at 320 nm) were identified and flavonoid aglycone – apigenin has been identified at a wavelength of 330 nm. The total content of anthocyanins is 36.80 mg/100 g. Among all the phenolic compounds in the herb of Malva sylvestris by majority components are luteolin content of which is 96.12 mg/100 g, and chlorogenic acid (51.04 mg/100 g).

There by component composition and quantitative content of phenolic compounds were defined in herb Malva sylvestris established dominant compounds, that enables use the results as a criterion standardization herb Malva sylvestris.

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