

## Study of Biologically Active Substances of Dry Extract from the Leaves of Ordinary Horse Chestnut with High-Performance Liquid Chromatography

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**Abstract:** The phenolic compounds, organic acids and sugars in the dry extract from the leaves of ordinary horse chestnut were studied with HPLC method. The qualitative composition has been determined and the content of certain groups of biologically active substances in the test object has been evaluated. Based on the obtained experimental data the researchers developed and unified the methodology of HPLC analysis of the dry extract from the leaves of ordinary horse chestnut. The development of qualitative and quantitative analysis was preceded by analytical work on the selection of the conditions of the study. Using the HPLC method with standard samples the authors have identified escin, 12 compounds related to phenolic compounds. The obtained results allowed offering reliable replicable methods of qualitative and quantitative analysis.

**Key words:** Ordinary horse chestnut (*Aesculus hippocastanum* L.) % Medicinal plant material % High performance liquid chromatography (HPLC) % Organic acids % Phenolic compounds % Sugars % Escin

### INTRODUCTION

Chronic venous insufficiency is a debated issue in the practice of medicine and is a serious problem for modern society. The disease is widespread, has a trend to growth and is registered in 15-40 % of the adult population. CVI is accompanied by a decrease in venous capacity, venous congestion and impaired lymphatic drainage. Heavy proceeding of the chronic form and frequent resulting disability gives all grounds to call CVI "a disease of civilization". Relevance of the disease study and the search for ways to improve the effectiveness of pharmacotherapy for a total structure of disease, necessitate the development and introduction of new medical practice of herbal medicine. At present, in the pharmaceutical market of Russia there is a large number of drugs with anti-inflammatory and venous activity. However, much of these semi-synthetic drugs are of synthetic and semi-synthetic origin [1-7].

Ongoing studies have shown the prospects of development of herbal drugs on the basis of the common horse chestnut [8].

Pharmacological action of the horse chestnut is associated with the presence of triterpene saponins, flavonoids, pectin, carotenoids, sugars and organic acids in it. Preparations based on horse chestnut increase the tone of the veins and accelerate blood flow in the veins, which prevents the formation and rise of the phenomena of thrombosis [9].

Now for the qualitative and quantitative analysis of medicinal plants a modern method of HPLC is used; its benefits are the ability to study almost any objects without any restrictions on their physical and chemical properties and possible automation of control process [10].

The aim of this work is to study biologically active substances contained in the dry extract from the leaves of ordinary horse chestnut with high-performance liquid chromatography.

Methods. The qualitative composition of the biologically active substances from the leaves of dry extract of ordinary horse chestnut was studied using HP liquid chromatograph of "GILSTON" company with subsequent computer processing of the results using Multichrom for "Windows".

Determining the phenolic compounds as the stationary phase the researchers used the steel column with the size 4.6 x 250 mm. Kromasil C18, particle size - 5 microns. The mobile phase was methanol-water-concentrated phosphoric acid in a ratio of 400:600:5. Analysis was performed at room temperature. Eluent flow rate was 0.8 ml / min, duration of the analysis - 70 min. Detection was carried out using a UV detector at 254 nm wavelength.

For investigation 0.15 g (accurate weighing) of dry extract was placed into a volumetric flask with a capacity of 25 ml, 20 ml of 50% ethyl alcohol was added, stirred until dissolved and added with the same solvent up to the mark.

In parallel, in 70% ethyl alcohol researchers prepare 0.05% solutions of comparison: rutin, quercetin, luteolin, luteolin-7-glucoside, hyperoside, hesperidin, apigenin, epicatechin, taxifolin, kaempferol, esculetin, tannin, epigallocatechin gallate, gallic acid, caffeic acid, chlorogenic acid, cichoric acid, cinnamic acid, neohlorogen acid and ferulic acid. 0.05 g (accurately weighed) of the standard sample is placed into a volumetric flask with 100 ml capacity, 20 ml of 70% ethyl alcohol is added and stirred until dissolved and the volume is added with the same solvent to the mark.

Test solution and reference solutions 50 µl each were introduced into the chromatographer and chromatographed using the above procedure.

To study organic acids as the stationary phase researchers used metal column with the size 7.8 x 300 mm REZEX ROA Organic Acids, particle size - 8 nm. The mobile phase was 0.005 M solution of sulfuric acid. Eluent flow rate was 0.5 ml/min and analysis duration - 96 minutes. Detection was carried out using a UV detector at 210 nm wavelength.

0.10 g of dry extract (accurately weighed) was placed in a flask with the volume of 50 ml, 30 ml of water was added and stirred; the mixture was filtered through the ashless fold paper - blue ribbon filter into a volumetric flask with 50 ml volume and the volume was added with the solvent to the mark (test solution).

For comparison, a series of reference solutions of organic acids is prepared. Accurately weighed ascorbic, citric, oxalic, malic, succinic, tartaric and lactic acids 0.025 g each are placed in a 50 ml volumetric flask, dissolved in 25 ml of a 0.005 M solution of sulfuric acid and added with the same solvent to the mark.

Test solution and reference solutions 50 µl each are introduced into the chromatograph and chromatographed using the above procedure.

When studying the sugars as the stationary phase researchers used the steel column with the size 6.5 x 300 mm ALTECH OA-1000 Organic Acids. The mobile phase was 0.01 M sulfuric acid. Eluent flow rate was 0.8 ml/min. The column temperature was 20°C and the length of analysis - 100 minutes. Detection was carried out using a UV detector at a wavelength of 190 nm.

For investigation 0.10 g of accurately weighed dry extract was placed in a 50 ml flask, 30 ml of water was added and stirred; the mixture was filtered through ashless fold paper-blue ribbon filter into a 50 ml volumetric flask and volume was added with solvent to the mark. For comparison, the reference solutions of sugars were prepared.

Accurately weighed rhamnose, xylose, maltose, sorbitol, glucose, lactose, arabinose, sucrose, fructose and galactose 0.60 g each was placed into a 50 ml volumetric flask, dissolved in 25 ml of 0.01 M sulfuric acid solution, added with the same solvent up to the mark and mixed.

Test solution and reference solutions 50 µl each are introduced into the chromatograph and chromatographed using the above procedure.

To identify escin, as the stationary phase researchers used the steel column Kromasil C18 with the size 4.6x250mm, particle size - 5 microns. The mobile phase was acetonitrile - 0.1 M phosphoric acid in a ratio of 20:80. Analysis was carried out at a temperature of 45°C. The feed rate of the eluent was 1 ml/min and the analysis duration - 60 minutes. Detection was carried out using a UV detector at 220 nm wavelength.

Approximately 0.10 g of accurately weighed dry extract of chestnut leaves was placed into a 100 ml conical flask, then 25 ml of 0.1 M potassium hydroxide solution was added, attached to a backflow condenser and heated for 30 minutes. Uniform boiling was maintained. The mixture was cooled and quantitatively transferred to a 50 ml volumetric flask with distilled water. The volume was added with the same solvent to the mark. Stirring (solution A). 1 ml of solution A was placed into a 25 ml volumetric flask and added with distilled water to the mark (test solution).

In parallel, the solution of standard sample of escin is prepared in the same way. With this view approximately 0.01 g of accurately weighed escin SS was placed in a 100 ml cone flask, 12.5 ml of 0.1 M KOH solution was added and followed the above described procedure. After cooling, the mixture was transferred into a 25 ml volumetric flask and added with water and to the mark (SS).

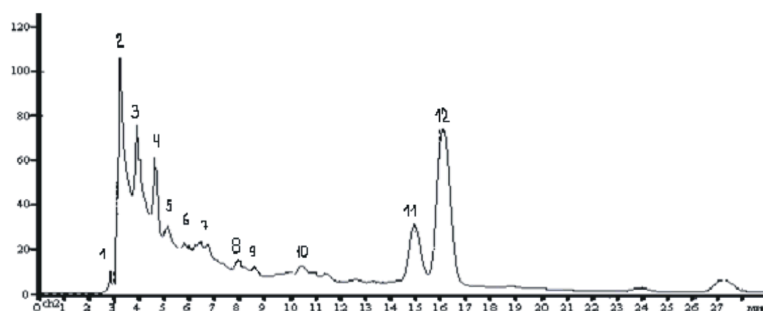


Fig. 1: HPLC-chromatogram for identification of phenolic compounds of the dry extract from the leaves of ordinary horse chestnut: 1 – tannin; 2 - gallic acid; 3 – epigallocatechin-Gallate; 4 - cichoric acid; 5 - chlorogenic acid; 6 – catechin; 7 – esculetin; 8 - caffeic acid; 9 - ferulic acid; 10 – epicatechin; 11 – luteolin-7-glucoside; 12 - hyperoside.

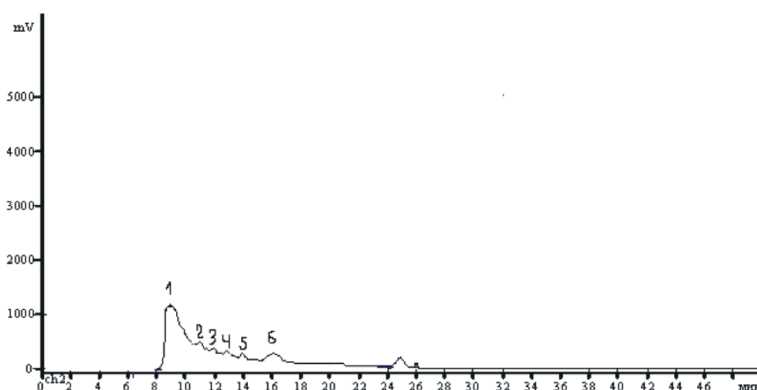


Fig. 2: HPLC-chromatogram for identification of organic acids in the dry extract from the leaves of ordinary horse chestnut: 1 - oxalic acid; 2 - Citric acid; 3 - tartaric acid; 4 - malic acid; 5 - ascorbic acid; and 6 - lactic acid.

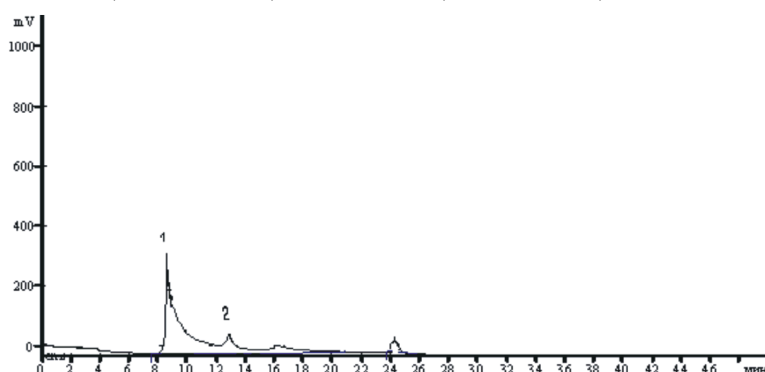


Fig. 3: HPLC-chromatogram for identification of sugars in the aqueous solution of the dry extract from the leaves of ordinary horse chestnut: 1 - glucose; 2 - fructose.

Test solution and reference solution 50 µl each were introduced into the chromatograph and chromatographed using the above methodology.

**Key Part:** In a dry extract from the leaves of the ordinary horse chestnut, we have identified such biologically active substances as flavonoids. HPLC chromatogram is shown in Figure 1.

As a result of HPLC analysis we have identified the qualitative composition of phenolic compounds which number was 12. Among the phenolic compounds the dominant are: gallic acid (14.6%), hyperoside (17.42%), EGC-Gallate (12.98%), luteolin-7-glukozid (8.07%) and cichoric acid (7.06%).

Figure 2 shows a chromatogram of organic acids.

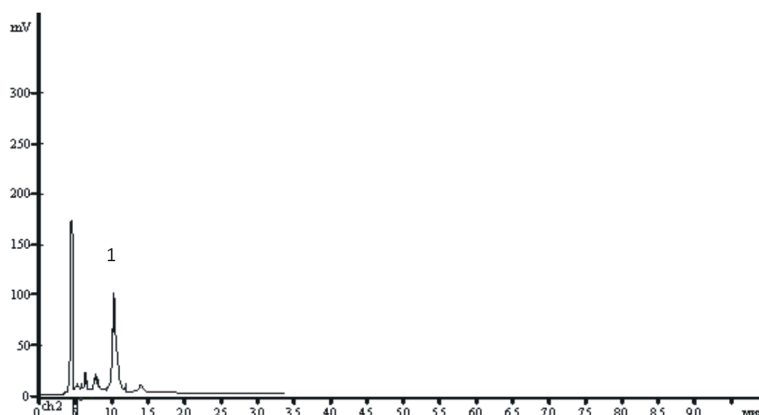


Fig. 4: HPLC-chromatogram of escin - 1.

During the studies we have found the presence of 6 organic acids. In a dry extract from the leaves of the chestnut the following acids have been identified: citric (7.25%), tartaric (6.45%), malic (5.78%), ascorbic (4.27%), oxalic acid (62.95%) and lactic acid (13.29%).

The research results for sugars are shown in Figure 3. It was revealed that the dry extract is rich in glucose (87.68%) and fructose (12.32%).

HPLC confirmed the presence of escin in a dry extract from the leaves of the ordinary horse chestnut; the chromatogram is shown in Figure 4.

### CONCLUSION

The study of dry extract from the leaves of ordinary horse chestnut confirms the presence of phenolic compounds and escin which increase the tone of the veins and accelerate blood flow in the veins [11].

It is essential that other biologically active substances contained in ordinary horse chestnut (organic acids, sugars) potentiate the anti-inflammatory effect of escin. Thus, in the presence of natural flavonoid complex of chestnut the escin activity increases 5 times.

### Findings:

- C HPLC method was used to study the composition of phenolic compounds, organic acids and sugars; and escin was identified in a dry extract from the leaves of ordinary horse chestnut.
- C The presence of 12 phenolic compounds, 6 organic acids and 2 sugars was proved and their ratio was determined.

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