Eurasian Medical Research Periodical		Evaluation Of Medicinal Properties Of Chestnut Fruit Used In The Treatment Of Varicose Diseases By Chemical Composition	
Madaminova Gulassal Abdurauf qizi		Master's Student, Department of Chemistry. Andijan State University, Andijan, Uzbekistan	
Xojiqulov Azizbek Sobirovich		PhD in Chemical Sciences, Senior Teacher of the Department of Chemistry, Andijan State University, Andijan, Uzbekistan	
Asqarov Ibrohim Rahmonovich		Doctor of chemical sciences, professor, chairman of the Academy of Medicine of Uzbekistan	
ABSTRACT	The article provides information on the importance of vitamins used in the treatment of varicose veins, and the determination of the amount of water-soluble vitamins in an aqueous extract of chestnut fruits using the "High-Performance Liquid Chromatography method.		
Keywords:		anti-varicose drugs, treatment with vitamin C, vitamin B ₁ , vitamin B ₉ , vitamin B ₂ , vitamin B ₁₂ , vitamin B ₆ , chestnut seeds, ointments, compresses, nutritional supplements and creams, traditional and scientific medicine.	

Introduction

Recently, the pharmaceutical market of our country has significantly expanded the range of drugs used for the treatment and prevention of varicose veins. Medicinal products used in the treatment and prevention of venous diseases divided into several groups: herbal are preparations of origin, which are considered to be compounds expressed by individual biologically active substances, of origin, obtained as a result of chemical reactions and resulting from the synthesis of combined biologically active fractions synthetic drugs and chemical compounds [1].

A certain amount of vitamins is necessary for the proper functioning of the human body. The correct complex of vitamins with varicose veins helps to alleviate the symptoms of the disease and is also useful for the prevention of vascular pathology. All trace elements do not affect the circulatory system. Therefore, we should focus on the most important vitamins for venous veins and other veins of the legs. In order for the body to fully assimilate micronutrients, the human body must have enough vitamin C [2]. Used by doctors synthetic drugs can treat varicose veins, but these synthetic drugs do not always produce the expected effect and often have various negative consequences due to side effects. By treating the disease with plants and eliminating the causes of the disease through phytotherapy, plants have a minimum number of contraindications, side effects, and medicinal herbs in addition to the treatment of the disease. vitamins and other nutrients to the tissue

substances wealth with that and has several advantages such as affordability [3].

Taking into account these circumstances, it can be considered that the treatment of varicose veins with folk medicine methods has a good effect. That is why ointments, compresses, mixtures, food supplements, as well as tinctures, are used for external use in the treatment of varicose veins by folk medicine methods [4].

Horse chestnut has been widely used in folk medicine and scientific medicine since ancient times. Currently, horse chestnut is actively studied in order to create drugs with angioprotective activity. However, only a few organs of horse chestnut are used in practice, which makes it possible to study and introduce new types of medicinal plant materials based on horse chestnut into practical pharmacology. Prospects for studying the creation of medicines based on horse chestnut raw materials are related to the richness of its chemical composition [5].

In folk medicine and scientific medicine, information about the effectiveness of horse chestnuts in the treatment of vascular diseases and circulatory disorders is used in practice. A tincture made from chestnut seed powder has been used to treat varicose ulcers, haemorrhoids, gout, and chronic colitis [6].

Chestnut appeared in scientific and practical medicine at the end of the 19th century through the work of the French doctor A. Artaud de Veveyga. In 1896, A. Artaud de Vevey published his articles and observations on the successful treatment of haemorrhoids and varicose veins using the chestnut tincture. In addition, horse chestnut was considered an effective tool in the treatment of prostatitis and adenoma. In the middle of the 20th century, venotonics based on horse chestnut began to be produced in Germany [7].

Experimental part

Used reagents and equipment. Vitamin B₁₂ was obtained from "Rhydburg Pharmaceuticals" (Germany), and vitamins B₁, B₂, B₆, B₉ and C from "DSM Nutritional Products GmbH" (Germany). HPLC-grade water, acetonitrile, chemically pure-grade acetic acid and sodium hydroxide reagents were used. Quantification of water-soluble vitamins in the plant was carried out on an LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu, Japan.

Preparation of standard solutions. Solutions of vitamins C (CAS 50–81–7), B₁ (CAS 70–16–6), B₆ (CAS 65–23–6) and B₁₂ (CAS 68–19–9) (100 mg/l) of each vitamin It is prepared by dissolving 5 mg in 50 ml of HPLC grade water. Standard solutions of vitamins B2 (CAS 83-88-5) and B₉ (CAS 59-30-3) were prepared by dissolving 5 mg of these vitamins in 50 ml of 0.025% sodium hydroxide solution. Then all the B vitamins were mixed and a total solution was prepared. (the stock solution was stored in sealed brown vials at -18 °C to prevent decomposition. Working standards of these vitamins up to 5, 10, 15, and 20 mg/l were prepared by diluting the common solution.

Preparation of plant extract. For the extraction of water-soluble vitamins, 2 g of the test sample was weighed with an accuracy of 0.01 g on a scale manufactured by OHAUS company (USA) NV222, placed in a 100 ml conical flask, and 50 ml of 0.1 N HCl solution was added. The mixture was stirred at 60 °C for 15 minutes in an MS-H280 Pro heater magnetic stirrer manufactured by DLAB (China). The mixture was then cooled, filtered and made up to 100 ml with water in a volumetric flask. 1.5 ml of the extract was filtered through a 0.45 µm syringe filter and placed in a vial and used for analysis.

Chromatographic conditions. Standard solutions and sample extracts LC-40 Nexera Lite high-performance liquid chromatograph consisting of LC-40D pump, SIL-40 autosampler, SPD-M40 photo-diode array detector (PDA) and LabSolutions ver. 6.92 software was analyzed. Shim pack GIST C18 (150 × 4.6 mm; 5 µm, Shimadzu, Japan) reverse-phase column and a gradient mobile phase consisting of acetonitrile (A) and a 0.5% solution of acetic acid in water (B) (Table 1) was used. The injection volume was set at 10 μ L, the flow rate at 0.9 mL/min, and the column thermostat temperature at 35 °C. The analytical signal (peak area) of each vitamin was recorded at three wavelengths of 280, 265 and 244 nm (Figures 1-3).

Time	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
0.76	0	100
2.26	17	83
5.26	17	83
5.32	0	100
11	Termination	



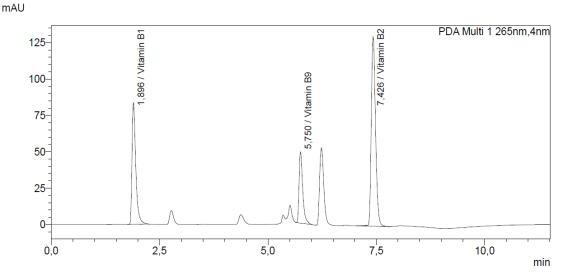


Figure 1. Chromatogram at 265 nm of standard solutions of vitamins B₁, B₂ and B₉.

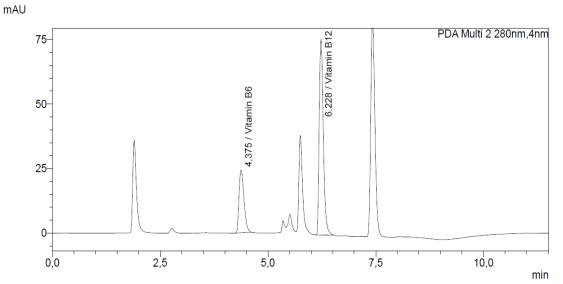


Figure 2. Chromatogram at 280 nm of standard solutions of vitamins B₆ and B₁₂.

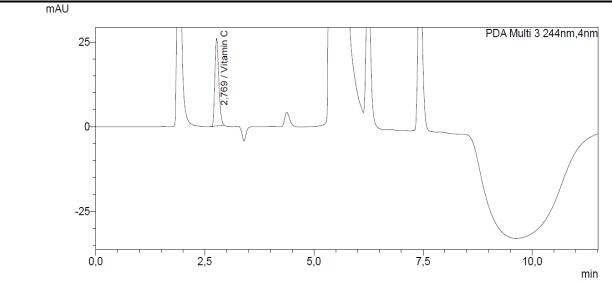
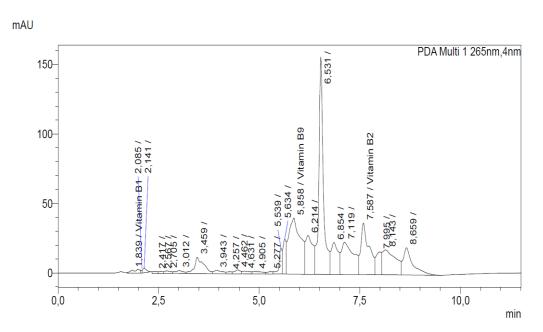


Figure 3. Chromatogram of vitamin C standard solution at 244 nm.

Results and discussion

The amount of water-soluble vitamins in the aqueous extracts extracted from the skin of chestnut seeds was determined by the HPLC (High-Performance Liquid Chromatography) method. A chromatogram of the extract of the tested sample in 0.1 N HCl was obtained (Fig. 4) and the results were processed and presented in Table 2.



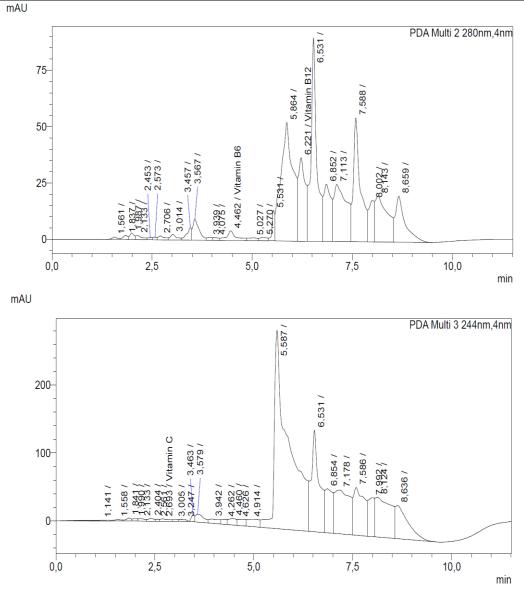


Figure 4. Chromatogram of sample extract in 0.1 N HCl.

Table 2. Amounts and recention times of vitamins in chestnut seeu nusk extracts.	Table 2. Amounts	s and retention times of vitamins in chestnut seed husl	k extracts.
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	0.1 N HCl			
Vitamin	Holding time,	Concentration,	Amount in 100 g sample, mg	
	sec	mg/l		
Vitamin B ₁	1,839	3,078	7,695	
Vitamin B9	5,858	43,285	108.21	
Vitamin B ₂	7,587	10,238	25,595	
Vitamin B ₆	4,462	7,287	18,218	
Vitamin B ₁₂	6,221	16,571	41,428	
Vitamin C	2,693	7,747	19,368	

The amount of water-soluble vitamins in aqueous extracts extracted from chestnut seeds was determined by the HPLC method. A chromatogram of the extract of the tested sample in 0.1 N HCl was obtained (Fig. 5) and the results were processed and presented in Table 3.

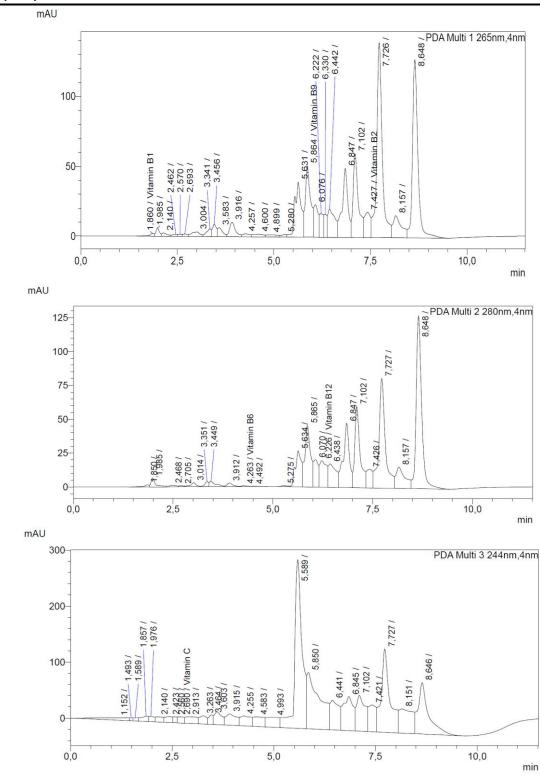


Figure 5. Chromatogram of sample extract in 0.1 N HCl

Table 3. Vitamin content and retention times in chestnut seed extracts.			

	0.1 N HCI		
Vitamin	Holding time, sec	Concentration, mg/l	Amount in 100 g sample, mg
Vitamin B ₁	1,860	3,805	9.5125
Vitamin B9	5,864	25,318	63,295

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Vitamin B ₂	7,427	3,562	8,905
Vitamin B ₆	4,263	1,415	3.5375
Vitamin B ₁₂	6,226	8,972	22.43
Vitamin C	2,690	13,019	32,548

Conclusion

- 1. Methods for the preparation of aqueous extracts of chestnut seeds and seed husks have been improved.
- 2. Water-soluble vitamins contained in chestnut seeds and seed husks were dissolved and the amount of vitamins in the aqueous extracts was determined by the "High-Performance Liquid Chromatography" method.
- 3. Chromatograms of extracts of chestnut seeds and seed husk samples in 0.1 N HCl were obtained, and the results were processed by the method of mathematical statistics to determine the amount of vitamins in 100 grams of the sample.
- 4. Taking into account that chestnut seeds and seed skins are rich in vitamins, it has been scientifically explained that it can be used in the treatment of varicose veins.

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