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DETERMINATION OF ARABINOSE AND GALACTOSE CONTENT IN DRY EXTRACT OF WALNUT BARK (JUGLANS REGIA)

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Abstract
The biologically active substances contained in the dry extract of walnut shells were studied. This extract was obtained from various raw materials, and the results of comparative analysis of arabinose and galactose in its content were presented.

Keywords: Juglans regia, diabetes, antioxidant, insulin, inflammation, biologically active substance, arabinose, galactose.

Introduction

Have been using various parts of plants to treat diseases . 38-40% of the medicinal products used in medicine in our republic are preparations obtained from plants. The synthesis of some important biologically active substances (glycosides, alkaloids, terpenes, saponins, steroids, phenolic compounds and other biologically active substances) used in the treatment of some serious diseases is a complex process. In the current rapidly developing era, there are many negative factors that lead to nervous disorders, which leads to an increase in various diseases in humans. One of such diseases is diabetes mellitus [1].

The walnut tree has been known to mankind for 7,000 years. The ancient Romans called the walnut Juglans regia. Currently, at least 30 species of walnut are cultivated in the world. However, only 3 varieties are used in medical medicine. used: J. regia (Persian, English walnut), J. nigra (black walnut) and J. Cinerea (gray walnut).

The isolation and identification of active ingredients in *J. regia* , *as well as* the study of the pharmacological properties of these active compounds , are currently of great interest. Recently, naphthoquinones from the nut have been observed to inhibit enzymes required for SARS-CoV-2 viral protein synthesis . In addition, their anticancer properties have been studied [2].

J. regia , it was found that it can be used in several diseases. In particular, **This article** presents the latest scientific results on the antimicrobial, antioxidant, and antidiabetic properties of various chemical compounds identified and isolated from various solvents and parts of *J. regia [2].*

Diabetes mellitus is a group of metabolic diseases that affect a large proportion of the world's population. It is characterized by chronic hyperglycemia, mainly caused by defects in insulin secretion or insulin action. It is estimated that by 2030, the number of people with diabetes in the world will reach 366 million. Although the incidence of diabetes is increasing day by day , no treatment other than insulin and oral hypoglycemic drugs has yet been developed [3].

The prevalence of diabetes is increasing rapidly worldwide. In particular, the number of patients with diabetes is highest in India (31.7%), China (20.8%), and the United States (17.7%). By 2030,

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it is estimated that India, China, and the United States will have the highest prevalence of diabetes [4].

Moroccan scientists Ilhame Bourais et al. studied the antidiabetic, anti-inflammatory, and antioxidant activities of Juglans regia. The results of the study, conducted using methanol as a solvent to obtain the extract of J. regia , showed its antidiabetic activity [5].

Antariksh Kumar and others, scientists from the Department of Pharmaceutical Sciences, Sardar Bhagwan Singh University, India, conducted scientific research on the bark, leaves, and rind of the Juglans regia plant and proved that different parts of Juglans regia have various beneficial properties. In particular, walnuts have been confirmed in their experiments to have several pharmacological activities, such as antioxidant, anthelmintic, insecticide, hepatoprotective, anti-inflammatory, anti-diabetic, anti-cancer, anti-depressant, wound healing, and immunomodulatory [6].

Research conducted by Daniela Soto-Madrid and others from the Department of Food Science and Technology, University of Santiago de Chile (USACH) has shown that walnut green shell extract aimed to evaluate the effect of the extracts on the antioxidant and antimicrobial properties, and emphasized the effect of the extracts on the kinetic parameters of *Escherichia coli growth.* In addition, walnut green shell extracts showed antimicrobial properties against pathogenic bacteria. This study demonstrated the antimicrobial and antioxidant activities of walnut green shell [7].

Scientists from the Scientific Center for Infectious Diseases of Tabriz University of Medical Sciences studied four parts of the walnut: leaf, shell, kernel, and green shell. Studies on the green shell of Persian walnut showed that catechin, epicatechin, myricetin, and quercetin are the main compounds. Substances belonging to the flavonoid group of polyphenol compounds are present in the walnut shell. The presence of naphthoquinones proved its antimicrobial effect [8].

New Zealand scientists Petri Widsten et al. have shown that switching from monosaccharides to disaccharides such as arabinose and galactose improves insulin control [9].

Based on the above data, positive results have been obtained in the use of walnuts in various diseases, including diabetes mellitus. This property is explained by the complex effect of a number of biologically active substances in its composition, as well as arabinose.

The purpose of the study

Purpose of the study Contains dry extract from walnut shells Determination of galactose and arabinose amounts.

Material and methods

The analysis was carried out on the following modern equipment: Degasser G1379A degasser, G1311A Quat Pump, G1313A ALS autosampler, Colcom G1316A column oven, RID G1362A refractometric detector and Agilent ChemStation Rev. Agilent 1260 liquid chromatograph equipped with a data processing system. Micropipettes with a volume of 100 and 1000 µl, "VWR", Finland. Analytical balance AnD GR-202 (accuracy 0.00001 g), "AnD", Japan. Water deionizer Millipore Simplicity, "Millipore", France. Ultrasonic bath S 30 H Elmasonic, "Elma", Germany. Filter nylon 0.45 microns 13 mm. Arabinose standard, imp. Galactose standard, imp. Acetonitrile for HPLC "Sigma-aldrich", USA.

The analytical conditions determined during the method were: -isocratic elution mode, mobile phase composition acetonitrile/water in a volume ratio of 82/18. Volumetric elution rate 1.0

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ml/min; injection volume 10 µl; column thermostat temperature 35 ° C; retention times of standards: -arabinose -4.7 ± 0.2 min, galactose -5.9 ± 0.2 min.

Results

Quantitative analysis was performed on an Agilent 1260 high-performance liquid chromatograph. In the development of the method, a standard solution was prepared. To ensure long-term storage, the standard solution was mixed with acetonitrile in a ratio of 1/1. Working solutions of the standards were prepared by diluting the standard solution with a 1/1 mixture of water and acetonitrile. 1 g of the test sample (accurately weighed) was dissolved in 50 ml of deionized water. The sample was mixed until completely dissolved at room temperature. The solution was then filtered through a 0.45 micron pore membrane filter. The filtered solution was mixed with acetonitrile in a ratio of 1/1 and analyzed.

Figures 1 and 2 below show the chromatograms of arabinose and galactose standard samples.

It is known that biologically active substances in plants may be preserved in different amounts depending on the condition and type of raw material. Therefore, a comparative analysis of arabinose and galactose in dried walnut shells and freshly harvested walnut green shells was also conducted in the research. The samples used were dry extract of freshly harvested walnut shells and dry extract of its dried shells. Results 3 and presented in Figures 4.

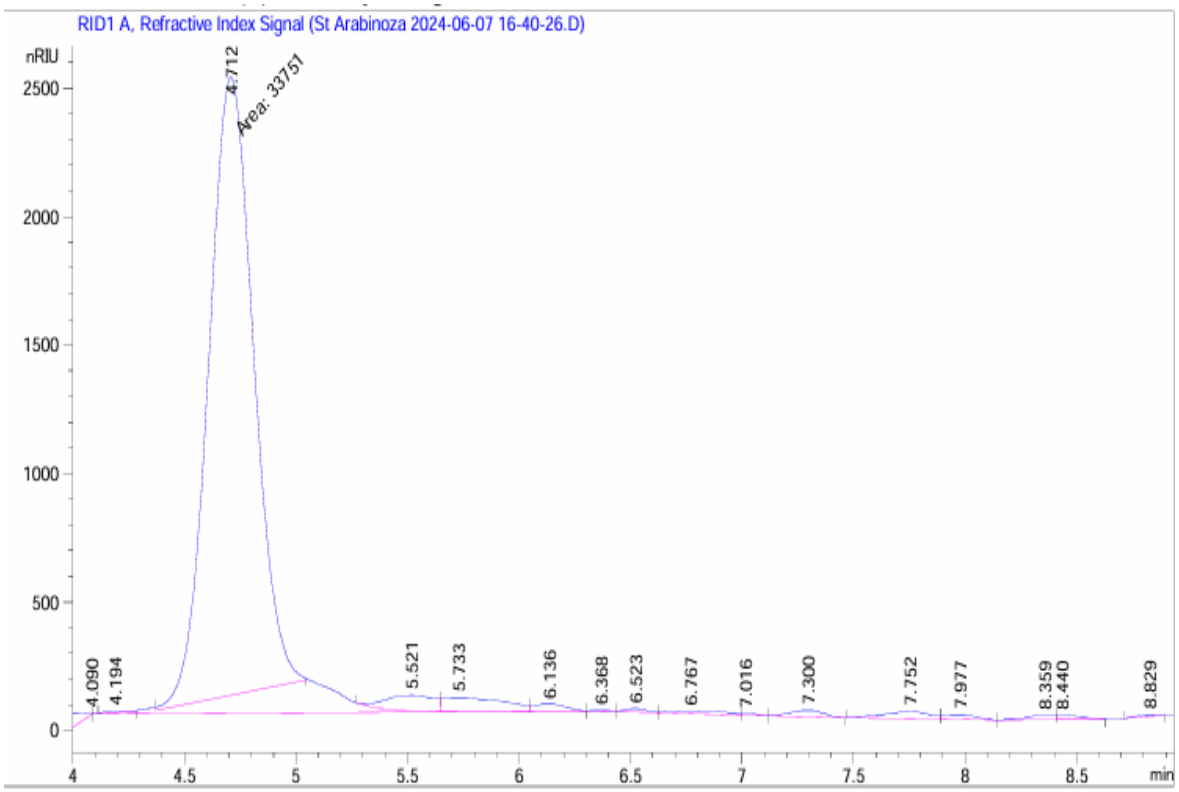

Figure 1. Chromatogram of a standard sample of arabinose

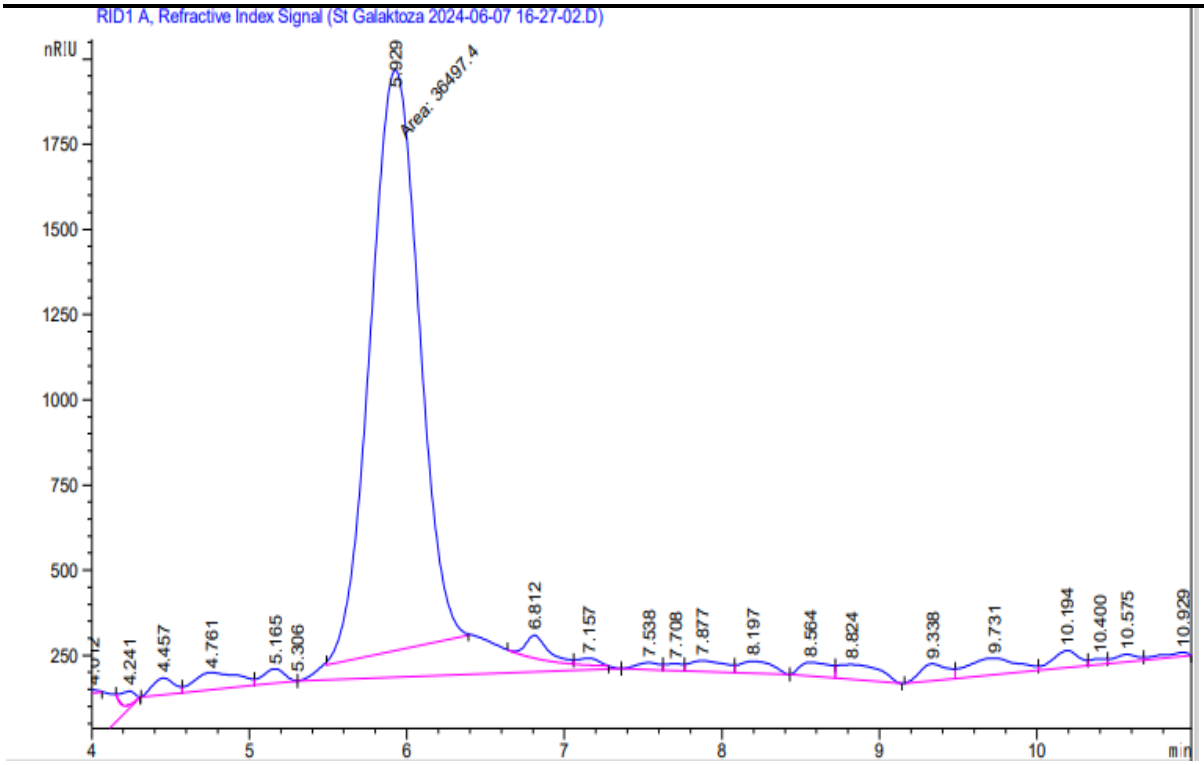

Figure 2. Chromatogram of a standard sample of galactose

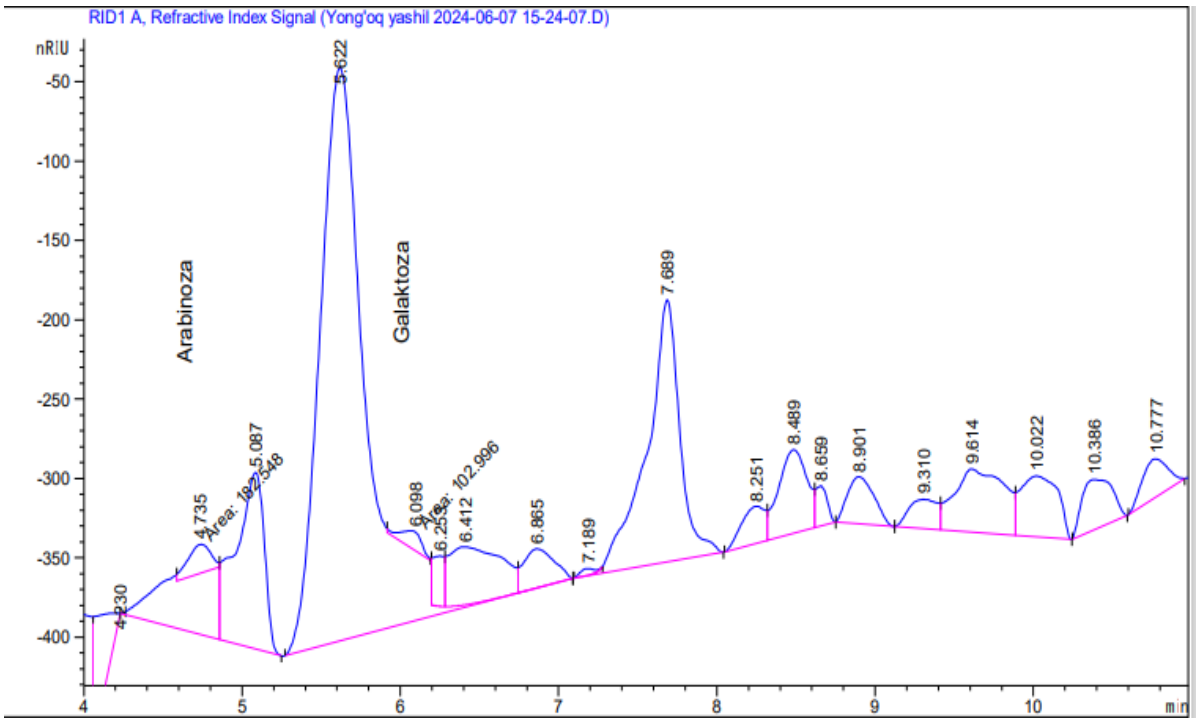

Figure 3. Chromatogram of dry extract from green walnut shells.

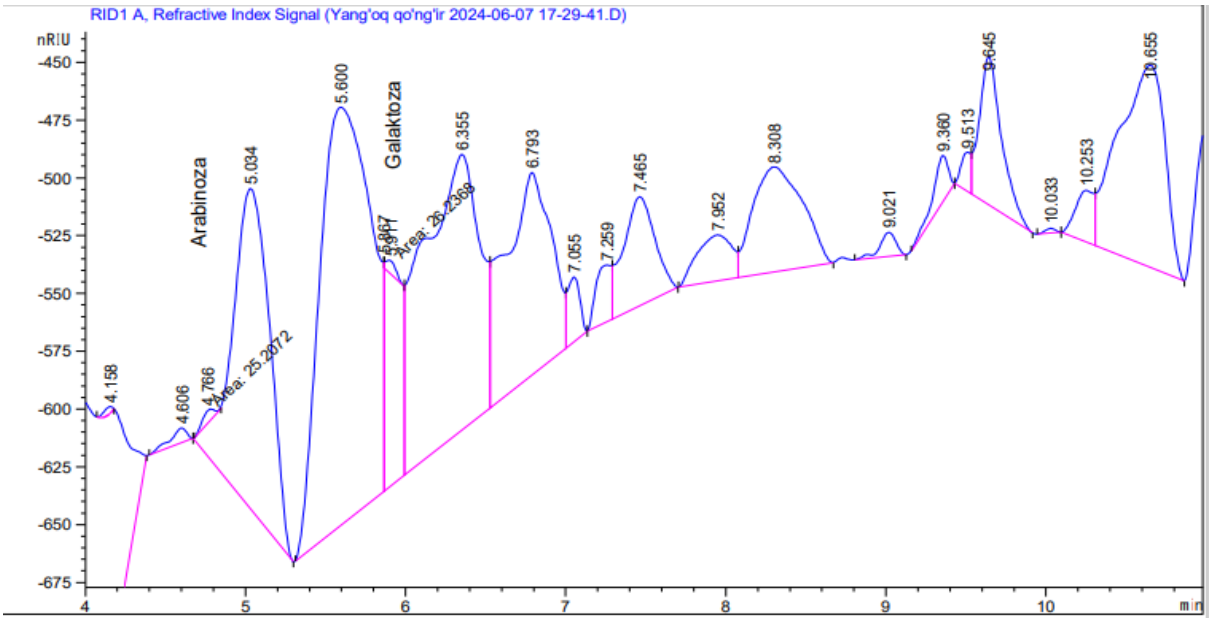

Figure 4. Chromatogram of a dry extract from dried walnut shells

The results of this experiment are presented in Table 1.
The amounts of galactose and arabinose were determined using the following formula:

$$X = \frac{S_{ob} \cdot 0{,}1 \cdot 500 \cdot 1000}{S_{st} \cdot 10 \cdot 100 \cdot 50}$$

Here:
S_{ob}-Peak area in the chromatogram
0.1-Standard hydrocarbon concentration
500- Solvent content after modification
1000 – Convert from mcg to mg
S_{st} ·– Peak area in the standard chromatogram
10 – Amount sent from sample to test
100- Amount received for modification
50 – Amount taken from the initial sample.

Table 1 **Results of the analysis of arabinose and galactose in dry extract from walnut shells using the YuSSX method**

Carbohydrates	Concentration mg /g	
	Walnuts (freshly harvested)	Walnut (dried shell)
Arabinose	0.031	0.0044
Galactose	0.016	0.0041

Conclusion

Quantitative analysis to the results according to Greek fire green 0.31% arabinose, 0.16 % galactose in the bark and dried brown in the shell and 0.044% arabinose, 0.041% galactose existence was determined.

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