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RESEARCH INTO PROPERTIES AND STRUCTURE OF BASIC POLYSACCHARIDE IN *PRUNUS DOMESTICA* (CHERRY)

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The paper deals with FTIR and ^{13}C NMR spectroscopic, thermo-gravimetric, radioactive and viscosimetric analyses of the diluted gum water solution. It revealed that there are galactan and arabinan in the basic residue of cherry gum extract in the environs of Shamakhi city (Azerbaijan) with 14% moisture mineral salts of 2-3%. Most of them include e Na^+, Mg^{2+}, Ca^{2+}, K^+, Zn^{2+} Fe^{3+} ions. In the meanwhile Cu^{2+}, Mg^{2+}, Cd^{2+} ions make up 0.001% of total mass. Following FTIR and C NMR analyses, there are mainly –OH, -COOH, -O- functional groups with molecular mass for arabinogalactan fraction of 130 kDa. At the same time, the quantity and activity of radioactive elements such as ^{137}Cs, ^{226}Ra, ^{228}Ra, ^{40}K, ^{235}U, ^{238}U in the content of cherry gum have been established by γ-spectrometer method. It demonstrated that isotopes dominate in ^{238}U and ^{40}K and their activity are respectively 1.31±0.243 and 04±0.16 Bk/gr. The content of these radioactive isotopes in the gum makes it possible to produce arabinogalactan-based sorbents.

***Keywords**: polysaccharides, cherry gum, arabinogalactan, NMR and FTIR spectroscopy, radioactive isotope*

INTRODUCTION

Over the past few years, new approaches to the production of therapeutical preparations based on immobilization of biologically active substances on polymer carriers have been developed [1-3]. Transportation of biologically active substances, including natural polyose and polyaminose extracted from the plants, has widely been used. Note that dextrane, starch, chitosan, cellulose, carboxymethylcellulose have no diaphragm properties as polymer-carriers [4-6]. The arabinogalactan (AG) is increasingly used and offers ever growing promises among natural polyaminoses [7,8]. High-solubility and polyfunctionality of AG enables us to apply it in medicine and various spheres of biotechnology. AG is a biologically active natural polysaccharide, which displays gastro protective, diaphragm and immunomodulatory properties [9-11]. Note that the properties of AG mentioned above make it possible to use it as a carrier of medicine preparations, enzymes and microelements that are necessary for human organism. [12-15]

It should be added that complexes of AG with ions of copper, nickel, cobalt and iron with 60-80% yield have been synthesized. The amount of metal ions is 1-5% and it was established that AG is more perspective as a carrier of biogenic microelements. Complex of AG with iron ferrogal is widely used in medicine as preparations with anti-anemic and immunomodulatory properties [16-17].

AG is also used as polyose with antibacterial and antimutagenous effect and produced as Larex UF, Bio-Max, Clear Tac^{TM} preparations. High dispersibility property enables us to use it as a supplement to food products (Hercules) and vitamin complexes [18-20].The authors analyze some vegetable gum exudates (plum tree, cherry tree) by means of FTIR and ^{13}C NMR spectroscopy, review thermo-gravimetric and rheological properties of aqueous solutions. Also, functional groups and main components of the

analyzed gums have been identified, including galactan, xylan and arabinan. It emphasized that there are differences between the two gums in thermal stability. For the diluted aqueous solutions of the analyzed gums, Huggins constant values and intrinsic viscosity have been identified which are dependent on the structure, molecular weight and interactions between existing functional groups [21].

In the research works mentioned above, the use of AG obtained from different trees are shown. It is well known that species of plants growing in different climatic zones differ one from another by the content of cells and mechanism of processes. In this work, we have studied composition of gum extracted from cherry trees growing in Shamakhi region of Azerbaijan. The number and activity of some metal ions (Na, Mg, K, Ca, Zn, Cu, Mn, Fe, Pb, Cd) and radioactive elements (Cs, Ra, Ra, U, K) have been established in the composition of cherry gum. Characteristics of separated fractions; average molecular mass and amount of –COOH groups studied; their structures examined by FTIR and ^{13}C NMR spectroscopy method.

EXPERIMENTAL PART

Materials

The *Prunus Domestica* (cherry) gum species have been collected as tree exudates in the south-east of Azerbaijan, Shamakhi region (Fig 1). After the collection, gums were dried at room temperature and powdered. AG was extracted from *Prunus Domestica* cherry gum through the use of relevant method [22].

Figure 1. Raw polysaccharide samples from cherry tree, *Prunus Domestica*

It has to be kept in mind that 1 kg cold mass was thermo-stated in the water bath at 20 °C and separated solution extracted through filtering. Residual raw material was extracted twice by distilled water at 60-70 ^{0}C for 3 hours. The obtained solution was mixed, evaporated, and the raw material isolated by centrifuge, polysaccharide was precipitated in ethanol with 96% yield. The precipitate was dissolved in water and dialyzed and lyophilized in distilled water while raw AG was obtained with 5.8% yield. The glucuronic acid is used to determine the reaction with 3.5-dimethylphenolom to take thick H_2SO_4 [23].

AAS analysis: Na, Mg, K, Ca, Zn, Cu, Mn, Fe, Pb and Cd metal ions were determined by flame atomic-adsorption method (AAS Spectra AA 220 FS, VARIAN).

Radioactive analysis: the amount and activity of radio-active elements were reviewed by means of γ-spectrometer HP and detected by Ge-detector with irradiation of samples for 3300 seconds.

Thermogravimetric analysis: Thermal characteristics of the cherry gum powder was determined by thermogravimetric-differential thermal analysis (TG-DTA, Diamond TG/DTA (PerkinElmer), in air at a heating rate of 0.167 °C/s. The analysis was run on 6 mg sample for cherry, placed into a platinum crucible, under dynamic dry atmosphere (100 mL/min), at a heating rate of 10 °C/min within the 30-400 °C temperature range.

^{13}C NMR spectroscopy: The NMR spectrum was recorded in DRX-500 of «Bruker» (Germany) for 3-5% solutions of olygo and polysaccharides with D_2O at 303 K (internal standard is acetone, δ_h 2.225 ppm, δ_s 31.45 ppm). Water solution was thickened in vacuum with rotative evaporator at 313-318 K within 10-20 minutes in the centrifuge with samples dried up.

FTIR spectroscopy: FTIR spectra of the plum and cherry gums were obtained by KBr pellet technique on ATR-FTIR Perkin Elmer instrument, Germany. Scans (32) were performed in the range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1}.

Viscosimetric characterization: Viscometric measurements were carried out using an Ubbelohde viscometer V_2, (α= 0.5; K = 9.78×10^{-4}) at 25 ± 0.01 °C. Several dilutions were made *in situ*.

RESULTS AND DISCUSSION

AAS analysis

According to initial view, AG collected from cherry trees is solid, fragile substance differently shaped, from light-beige to dark-brown colors, insoluble in high-soluble or colloid system to form alcohols and ethers. The gum sugar is refined from mechanical mixtures by filtering, and 1.0 g burns at 900 °C. Note that 0.01405 g ash is processed with 0.1 N HNO_3 and all metal complexes take the form of $Me_x(NO_3)_y$. The amount of metal ions in the solution is measured and the results are given in the table 1.

Table 1. Mass and percentage of some metal ions at cherry gum.

Ion of Me	Na^+	Mg^{2+}	Ca^{2+}	K^+	Zn^{2+}	Cu^{2+}	Mn^{2+}	Fe^{3+}
Concentration, mg/l	10.98	15.27	67.50	6.42	1.168	0.310	0.248	1.766
Amount of metals in cherry gum composition, mg/g	1.098	1.527	6.750	0.642	0.1168	0.0155	0.0124	0.0883
Me,%	0.1098	0.1527	0.675	0.06	0.01	0.0015	0.0012	0.009

As is known, gums are salts (Ca^{2+}, Mg^{2+}, K^+) with high molecular polysaccharides (glucuronic acid, galactouronic acid). Calcium salts occur more often than other salts. Cherry gum consists of 14% water and 2-3.5% mineral substances [24]. Table 1 shows that cherry gum contains 1.2% metal ions. AG is a polysaccharide with linked structure which contains 4.7% glucuronic acid and 0.4% of free -COOH groups while the aqueous solution is noted for acidic character of pH=4;j.66.

A small amount of metal ions does not restrict the use of AG, on the contrary, metal ions in organism take part in various biochemical processes, and their presence in its composition is a positive result. It found that in case where metal ions are absorbed by polyose with 0.1 N HCl, no changes are observed in the activity of biological active compounds.

Thermogravimetric analysis

The thermal decomposition process for cherry gum can be observed: the first phase of decomposition occurs within 30-200 °C temperature range with a maximum degradation rate at 78 °C and mass loss of 12.8%; the second phase (total mass loss is of 64.2%) is noted for multiple decomposition processes. Within the range of 200-272 °C, the maximum rate occurs at 256 °C with mass loss of 32.6%. Within the range of 272-310 °C, the maximum rate occurs at 284 °C with mass loss of 21%. Within the range of 310-400 °C, the maximum rate occurs at 338 °C, with a mass loss of 17.6%.

Radiactive analysis

Thus, the research into cherry gum-based arabinogalactan shows that the amount and activity of some metal ions and radioactive elements have no negative impact on human organism [25].

In order to define the amount and activity of some radioactive elements in the composition of cherry gum purified from mechanical mixtures, 4 g gum sugar is dissolved in 100 ml of deionized water. Then, spectrum of the sample is placed for 3300 seconds in γ-spectrometer (Fig. 2) and the amount and activity of some radioactive elements in the composition of gum sugar are given in Table 2. Table 2 shows that the amount and activity of radioactive elements are on the background level.

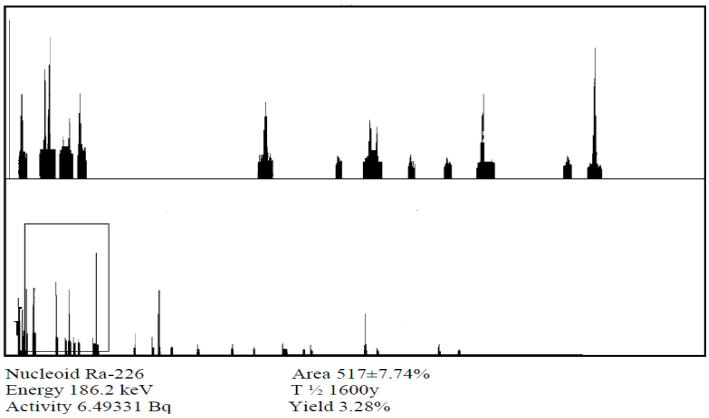

Figure 2. Radio-active elements spectra of cherry gum

Table 2. Amount and radioactive activity of some radionuclides in raw polysaccharide. T=20 ^{0}C. τ=3300 second

Radioactive elements	^{137}Cs	^{226}Ra	^{228}Ra	^{235}U	^{238}U	^{40}K
Activity of 4 g gum in 100ml solution, Bk/kg	<0.39	19.3±0.6	3.8±0.3	2.5±0.4	52.2±9.6	121.7±6.4
Amount of radioactive elements in 1g gum, g	$<3.2\cdot10^{-15}$	$13.1\pm 0.55\cdot10^{-12}$	$9.41\pm0.99\cdot10^{-15}$	$788\pm20\cdot10^{-9}$	$106\pm2\cdot10^{-6}$	$11.7\pm0.6\cdot10^{-6}$
Activity of radioactive elements in 1 g gum,Bk/g	<0.01	0.48±0.02	0.095±0.01	0.063±0.01	1.31±0.24	3.04±0.16

Note that the amount within the range of 10^{-6}-10^{-12} g/g has no impact on physical-chemical properties of macromolecule.

Viscosimetric characterization

The characteristic viscosity of precipitated and nonprecipitated fraction in ethyl alcohol (I) makes up [η] =1.15 and [η] = 0.97 respectively. The fraction deposited in alcohol has a higher viscosity. Equivalent amount of -COOH groups in polysaccharides is 11194 (I) and 14423 (I), and the quantity of them in percent (for 1 gr mass) makes up 0.4 and 0.312%. Relatively high amount of acidic groups in the fraction deposited in alcohol causes AG to be polyelectrolyte and expands its scope of application. According

to characteristic viscosity values [26], the average molecular mass was determined for both fractions: both for I-precipitated fraction M_η=130 kDa and II - nonprecipitated fraction it makes up M_η = 98 kDa.

^{13}C NMR spectroscopy

In previous studies, the methylation analysis was conducted in line with Smith and NMR spectroscopy studies. ^{13}C NMR results show that the main chain in all high-soluble arabinogalactan consists of β-D-(1→3)-Gal*p*-units (Fig.3).

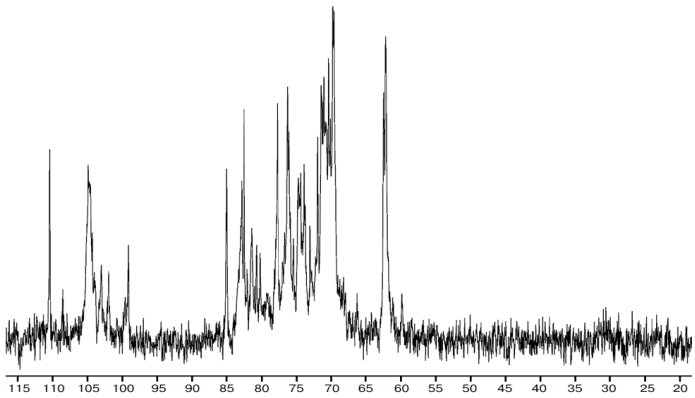

Figure 3. The ^{13}C NMR spectrum of water soluble AG from *Prunus Domestica* (Cherry) gum

The main chain consists of mainly (1→6)-linked galactose, arabinose or glucuronic acid units. The ratios of the signals at δ=82.6 ppm [→3,6)- Gal*p*-(1→, C-3] and δ=62.3 ppm [β-D-Gal*p*-(1→, C-6] were used for the determination of the monomer unit ratio →3,6)-Gal*p*-(1→/Galp-(1→. Owing to the signals (Fig. 3) at δ=102.6–106.1 ppm the monomer unit ratio →3, 6)-Gal*p*-(1→/Gal*p*-(1→+→6)-Gal*p*-(1→ can be determined [27].

FTIR spectroscopy

The FT-IR spectra of AG (Fig. 4) shows a peak at 2962 cm^{-1}, which corresponds to –CH stretching of methyl and propyl groups (symmetric, asymmetric). The peaks at 1654 cm^{-1} and 1538 cm^{-1} are assigned to –COO groups (symmetric stretching). The intense peaks at 1071 cm^{-1} and 1042 cm^{-1} denote stretching of the C-O-C (valence vibration of uronic acids).

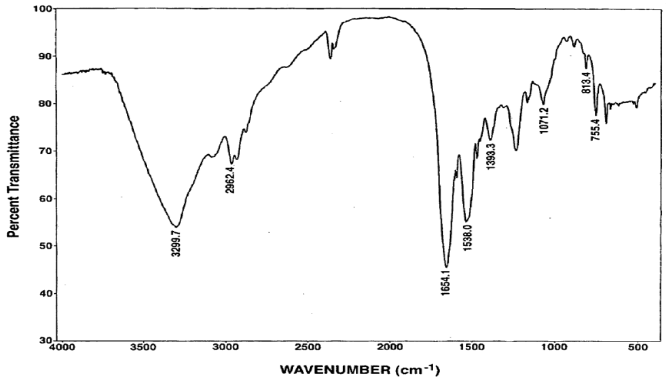

Figure 4. FTIR spectra of cherry gum

The peak at 1420 cm^{-1} in the spectra of AG corresponds to the stretching vibration of >C=O in –COOH functional group. The peak at 1393 cm^{-1} corresponds to the asymmetric stretch of >C=O. The absorption band of the –OH stretch of the -OH groups (stretching vibration) appears in the range of 3300-2700 cm^{-1}. The FT-IR spectra confirm the functional groups content of AG [28].

The major structural conclusions (Fig.5) in this study are drawn from the ^{13}C NMR and FTIR spectroscopy analysis. In addition, the methylation analysis and the carbohydrate monomer composition provided some important structural features: e.g. the presence of unsubstituted (1→3)-bonded Gal*p*-units became apparent by the methylation analysis and the presence of GlcA in the Larch AG sample appeared from the carbohydrate monomer composition. Amounts of these units were probably under the limit of detection for the NMR analysis. Figure 5 shows a suggested average structure for water-soluble Shemakha cherry arabinogalactans.

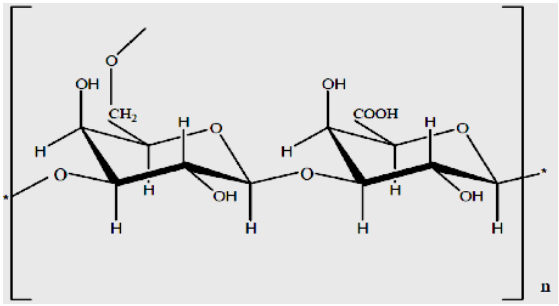

Figure 5. Suggested major structural features of typical arabinogalactan molecule

The structure slightly differs from molar masses and the mass range of these samples was quite broad. It is therefore unlikely that the different types of side chains will manifest themselves in a single molecule. However, a typical cherry arabinogalactan molecule has a shorter main chain than does a typical larch arabinogalactan molecule. It has also more free side units chains and a higher amount of acidic groups than larch arabinogalactan [29]. The low amount of [→6)-Galp-(1→] -units suggest that the probability of side chains with more than two units is very low. There are less acidic side chains and relatively high amount of [→6)-Gal*p*- (1→] units which suggest that the probability of side chains with more than two units is high. It was not possible to suggest the ratio of the three Galp-type side chains.

The sugar unit ratios of the isolated AG samples determined by acid methanolysis and methylation analyses, and ^{13}C NMR spectroscopy were in good agreement with each other. The Gal:Ara ratio for Shamakhi cherry AG determined by ^{13}C NMR proved to be high. This may be explained as being due to the unidentified signals from the anomeric carbons. Some polysaccharide fractions were lost during the isolation procedure.

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PRUNUS DOMESTICA (ALBALI) TƏRKİBİNDƏ ƏSAS POLİSAXARİDLƏRİN QURULUŞ VƏ XASSƏLƏRİNİN TƏDQİQİ

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Araşdırma albalı ağacından əldə edilən selik ekzudatının durulaşdırılmış sulu məhlulunun FTIR və ^{13}C NMR spektroskopiya, termo-gravimetrik, radioaktiv və viskozimetrik analizlərinə həsr olunmuşdur. Müəyyən olunmuşdur ki, Azərbaycanın Şamaxı ərazisindəki albalı kitrəsi ekstraktının əsas tərkibi qalaktan və arabinan qalıqlarından ibarətdir və tərkibində 14% nəm, 2-3% miqdarında isə mineral duzlar saxlayır. Bunlardan üstünlük təşkil edəni Na^+, Mg^{2+}, Ca^{2+}, K^+, Zn^{2+}, Fe^{3+} ionları, Cu^{2+}, Mn^{2+}, Pb^{2+}, Cd^{2+} ionları isə ümumi kütlədə 0.001% tərtibdə olur. NMR və FT-İR analizlərinə əsasən tərkibdə -OH, -COOH, -O- funksional qrupları müşahidə edilmiş və arabinoqalaktan fraksiyasının molekul kütləsinin 130 kDa olduğu təyin edilmişdir. Həmçinin albalı kamedinin tərkibindəki ^{137}Cs, ^{226}Ra, ^{228}Ra, ^{40}K, ^{235}U, ^{238}U kimi radioaktiv elementlərin miqdarı və aktivliyi γ-spektrometri ilə müəyyən edilmişdir. Sübut olunmuşdur ki, tərkibdə ^{238}U və ^{40}K izotopları üstünlük təşkil edir və uyğun olaraq 1.31±0.243 və 04±0.16 Bk/qr təşkil edir. Kamedin belə radioaktiv izotopları özündə toplaması gələcəkdə arabinoqalaktan tərkibdə sobentlərin hazırlanmasına əsas verir.

Açar sözlər: *polisaxarid, albalı kitrəsi, arabinoqalaktan, NMR və İQ spektroskopiya, radioaktiv izotop*

ИССЛЕДОВАНИЕ СОСТАВА И СВОЙСТВ ОСНОВНЫХ ПОЛИСАХАРИДОВ PRUNUS DOMESTICA (ВИШНЯ)

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Исследование посвящено ИК и ^{13}C ЯМР спектроскопическому, термогравиметрическому, радиоактивному и визкозиметрическому анализам разбавленного экзудата водного раствора слизи, полученному из вишневого дерева. Установлено что в окрестностях города Шамахи (Азербайджан) в составе экстракта вишневой камеди в основном остатке имеется галактан и арабинан, влажность составляет 14% и в количестве 2-3% содержатся минеральные соли. Большинство из них составляют Na^{2+} Mg^{2+}, Ca^{2+}, K+, Zn^{2+}, Fe^{3+} ионы. Ионы Cu^{2+}, Mg^{2+}, Cd^{2+} содержатся в основном составе в количестве 0.001%. По анализам

ЯМР и ИК спектроскопии в основном составе наблюдаются -OH, -COOH, -O- функциональные группы и молекулярная масса для фракции арабиногалактана определена в количестве 130 кDa. Также в составе вишневой камеди методом γ-спектроскопии установлены количество и активность радиоактивных элементов ^{137}Cs, ^{226}Ra, ^{228}Ra, ^{40}K, ^{235}U, ^{238}U. Доказано, что в составе изотопы ^{238}U, ^{40}K составляют большинство и активности их равны соответственно 1.31±0.243 и 0.4±0.16 Вк/г. Содержание в составе камеди таких радиоактивных изотопов дает возможность в будущем готовить сорбенты, имеющие в составе арабиногалактаны.

Ключевые слова: *полисахариды, вишневая камедь, арабиногалактан, ЯМР и ИК спектроскопия, радиоактивный изотоп*

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