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THE STUDY OF CHEMICAL STRUCTURE OF THE ARMENIAN FLORA'S APRICOT GUM

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Abstract— Recently in industrial purposes there is a wide use of gelatin, modified cellulose, dextrins and their synthetic analogues, which are anyway imperfect and have side effects. In this point of view the plantar polysaccharides become a plot of increasing interest of researchers. These substances make up the main moiety of a man nutritional range and because of that are widely applicable in the food and confectionary production. It comprises multiple common groups of organic compounds, which are together with proteins and fats are of vital significance for activity of all the living organisms.

Nowadays, there is a necessity to develop specific methods of standardization of the apricot gum, Gummi Armeniacae (GA). Usage of GA of native origin is as an alternative for Gummi Arabicae, known for its significance as an effective emulsifier, stabilizer and the dietary fiber in medicine and food industry. Because of that we set forth an aim to investigate the GA chemical structure and, so, to develop its new standardization method for GA Specification creation.

By means of the column chromatography the gum polar fraction's (placed onto Al2O3 of IV activity) purification rational method was developed, what could be also used for the gums other polar (arabinic and basorinic) fractions purification and identification purposes.

By means of the HPLC method the GA was standardized. The following monosaccharides: arabinose, galactose, glucose, xylose and ramnose neutral sugars and the glucuronic sugar were identified in the GA hydrolyzate.

In the native GA the low-molecular substances (catechol, hydroquinone and pyrogallol) were detected, what testify about the cambium layer participation in the tree's gum formation process. (***Abstract***)

Keywords— ***Keywords: Apricot tree, Gummi Armeniacae, polysaccharides, GCMS, HPLC.*** (key words)

I. Introduction

Recently in industrial purposes there is a wide use of gelatin, modified cellulose, dextrins and their synthetic analogues, which are anyway imperfect and have side effects. In this point of view the plantar polysaccharides become a plot of increasing interest of researchers. These substances make up the main moiety of a man nutritional range and because of that are widely applicable in the food and confectionary production. It comprises multiple common groups of organic compounds, which are together with proteins and fats are of vital significance for activity of all the living organisms [1,2].

It is noteworthy, that previously polysaccharides were applied just as auxiliary substances in different medical preparations manufacturing, meanwhile nowadays they are observed as biologically active ones. The biologically active plantar polysaccharides are being used in the medical practice for prevention and treatment of diseases of different etiology. Besides, polysaccharides are the potential modifiers of biochemical reactions: they possess antiulcer, emollient, enveloping, anti-inflammatory, antimicrobial and mucolytic activity [3,4].

The plantar derivative, the gum's exudates are of great interest among polysaccharides. Actually, there are some phytochemical investigations related to different gums of plantar origin, particularly, to the Acacia gum- Acacia senegal L., Acacia seyal L., to the Guar gum - Cyamopsis tetragonoloba, to the Prosopis gum- Prosopis spp. and others [5,6].

From the high expediency point of view the most actual are the plantar polysaccharides of Armenia's flora considering an increased interest to the local sources all over the world, so it is becoming an issue of great importance. Different gums are known even from the ancient times, information of which is accessible the Arabic and Armenian literature sources of Abu Ali Ibn Sina and Amirdovlat Amasiaci (XV cc). That testifies about the apricot, plum and almond gums used as remedy for treatment of different diseases, including urolithiasis [7]. But the apricot tree gum having this prehistory did not get its worthy designation in the research aspect after 1960-s, just being a raw material for "Guazolum" plasma substitute

production in the former Soviet Union times, since it was used during the First Word War [8].

From the woody gum-producing Armenian flora (the apricot, peach, plum, almond trees; and plants from other families: Elaegnus and Tragacant) the apricot trees are specifically focused on, as they are one of the main fruit tree cultures in Armenia and occupy great areas in the Ararat valley, Kotayk, Aragatsotn and in the foothills of Vaik.

In this regard the apricot tree gum (Gummi Armeniacae) became of crucial significance, being an ecologically pure product and a potent one completely to substitute a rather expensive gummiarabic or its synthetic analogues use in the food production [9].

Nowadays, there is a necessity to develop specific methods of standardization of the apricot gum, Gummi Armeniacae (GA). Usage of GA of native origin is as an alternative for Gummi Arabicae, known for its significance as an effective emulsifier, stabilizer and the dietary fiber in medicine and food industry [10,11]. Because of that we set forth an aim to investigate the GA chemical structure and, so, to develop its new standardization method for GA Specification creation.

Material and methods

The present study was conducted on the gums collected from the apricot trees (Armeniaca vulgaris Lam.) growing in the Armavir region of Armenia during the trees juice movement period in early spring. An experimental part of the study was performed at the Pharmacognosy Department of YSMU after M. Heratsi, as well as in “FDA Lab” analytical laboratory of the pharmaceutical company ”Tonus-Less” LTD.

The apricot gum’s polar fraction purification using the column chromatography method 10 g. of GA was solved in 50 ml of distilled water. The solution was left for a day in order to swell, and then was heated till its complete dissolving. The solution got in a preparative manner was fully placed onto Al2O3 of IV activity in 1:3 ratios (the amorphous powder was formed as a result). The powder got was transferred into the column filled with 3.0 g polyamide (WOELM). The column lavage was done by distilled water. The aqueous extracts by means of vacuum rotary device were vaporized till dry reminder. As a result an amorphous powder of light orange tint (polysaccharide fraction) was formed. In order to confirm the chemical content of the polysaccharides, the latter were subjected to hydrolysis by means of 2M sulfuric acid (1:50) during 24 hours.

Determination of the neutral and acidic monosaccharides in the gum’s hydrolyzate

Analysis was done in the “Tonus-Less” LTD – “FDA Lab” analytical laboratory by means of the High-performance liquid chromatography (HPLC) method in the following chromatographic systems: the first chromatographic system was a column- VA 300/7.8 NUCLEOGEL, SUGAR 810 Ca, (Machinery-Nagel Germany), mobile phase – 0,1 % of H2SO4 aqueous solution (pH=2), mobile phase flow velocity – 1 ml/min, the column thermostat temperature - 60 CO, detection – RI; the second chromatographic system was a column- EC 250/4 NUCLEOGEL, Carbohydrate, (Machinery-Nagel Germany), mobile phase – acetonitrile : water (85:15), mobile phase flow velocity – 1 ml/min, the column thermostat temperature - 30 CO, detection – RI.

The following monosaccharides: glucose, xylose, galactose, ramnose, arabinose and the glucuronic acids purified as per Sigma- Aldrich served as standards having purity grade not less than 95% according to the gas-liquid chromatography data. In order to determine the saccharides amounts in the gums examined samples the standard saccharides calibrating graphs were used. For this purpose the aliquots of the sugar solutions (0.2 sm2) and the prepared solutions of different concentrations by dissolving in water were then used. Based on the data got the calibrating graphs expressing peak areas and the substance densities correlations were mounted.

Detection of non-polar (low-molecular) compounds fraction by the gas chromatography-mass spectrometry method (GCMS)

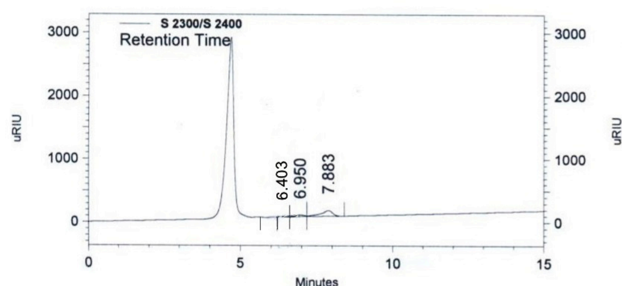
For this purpose the GA aqueous solution in 1:20 ratio was prepared. The aliquot volume made up 2 mcl. Gas chromatograph with the mass selective detector manufactured by BRUKER (USA), the chromatographic column OPTIMA-FFAP-0.25mm, 60m x 0.25mm (ID.MACHINERY-NAGEL, Germany) were used. The flow of helium carrier gas was 1.0 ml/min, volume of injected sample -2 mcl, the evaporator temperature was220oC, a temperature gradient was 50o C (2 min),heated to 250o C (2.5o C/min), retention – 5 min, split-division – 5.

The qualitative analyze was based on the comparison of retention times (Rt) of the GA composites, the samples components and pure substances with the mass spectra data of the library catalog NIST. The components contents were calculated as per the gas chromatographic peak areas. The components identification was carried out by their retention times and the peak increase. The components quantification was determined by the method of internal normalization.

II. Results and discussion

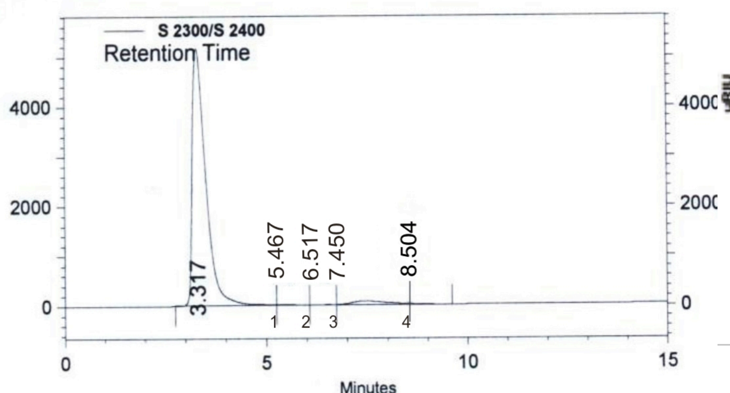
Results of chromatography of sugars standard samples’ (arabinose, galactose, ramnose, xylose and glucose) different concentration solutions in the first chromatographic system showed that in the standard solution of arabinose (4,5 mg/ml) the retention time was Rt=8.183, in the standard solution of galactose (3,5 mg/ml) - Rt=7.067, in the standard solution of glucose (40 mg/ml) – Rt=6.367, in the standard solution of xylose (3,6 mg/ml) - Rt=6.983 and in the standard solution of ramnose (2,7 mg/ml) - Rt=7. 317. A comparative analysis of the gum’s hydrolyzate and the sugars standard samples chromatograms showed that in the first chromatographic system the hydrolyzate retention times recorded on chromatogram (Rt1=6.403, Rt2=7.067, Rt3=7.883) correspond to those of glucose, galactose and arabinose samples (Fig. 1).

Fig. 1. Chromatogram of the apricot tree gum's hydrolyzate (HELC) in the first chromatographic system (column- VA 300/7.8 NUCLEOGEL, SUGAR 810 Ca, mobile phase – 0,1 % of H_2SO_4 aqueous solution (pH=2), mobile phase flow velocity – 1 ml/min, the column thermostat temperature - 60 CO, detection – RI).
1-glucose (Rt1=6.403), 2- galactose (Rt2=7.067),
3- arabinose (Rt3=7.883).



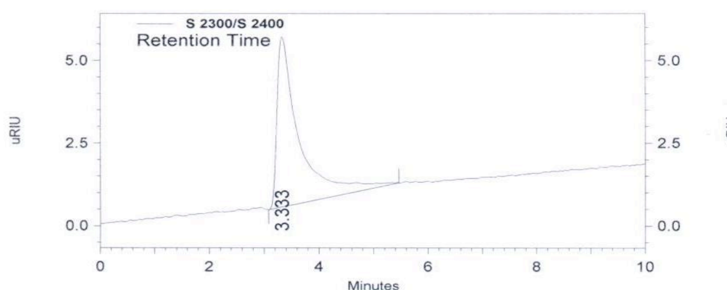
Results of chromatography of sugars standard samples' (arabinose, galactose, ramnose, xylose and glucose) different concentration solutions in the second chromatographic system showed that in the standard solution of arabinose (4,5 mg/ml) the retention time was Rt=7.26, in the standard solution of galactose (3,5 mg/ml) - Rt=10.883, in the standard solution of glucose (40 mg/ml) – Rt=8.500, in the standard solution of xylose (3,6 mg/ml) - Rt=6.367 and in the standard solution of ramnose (2,7 mg/ml) – Rt=5.433. A comparative analysis of the gum's hydrolyzate and the sugars standard samples chromatograms showed that the recorded retention times on the hydrolyzate chromatogram in the second chromatographic system (Rt1=5.467, Rt2=6.517, Rt3=7.450 and Rt4=8.504) correspond to those of ramnose, xylose, arabinose and glucose samples (Fig.2).

Fig.2. Chromatogram of the apricot tree gum's hydrolyzate (HELC) in the second chromatographic system (column- EC 250/4 NUCLEOGEL, Carbohydrate, mobile phase – acetonitrile : water (85:15), mobile phase flow velocity – 1 ml/min, the column thermostat temperature - 30 CO, detection – RI.
1-ramnose (Rt1=6.403), 2- xylose (Rt2=6.517), 3- arabinose (Rt3=7.450), 4-glucose (Rt4=8.504).



Thus, the chromatography results of the apricot gum's polysaccharides complex hydrolyzate show, that the polysaccharides monomer sugars were arabinose, galactose, xylose, as well as ramnose, structures of which were confirmed also by 1H and 13C magnetic resonance imaging (MRI)spectrographic method [4]. In the apricot gum hydrolyzate the glucuronic acid's acidic sugar was identified also, the retention time of what in the hydrolyzate corresponded to that of the standard sample (Rt=3.333) (Fig. 3, 4).

Fig.3.Chromatogram of the glucuronic acid standard sample.



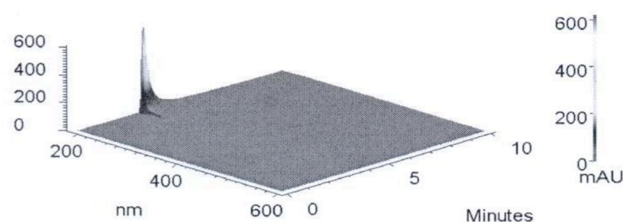
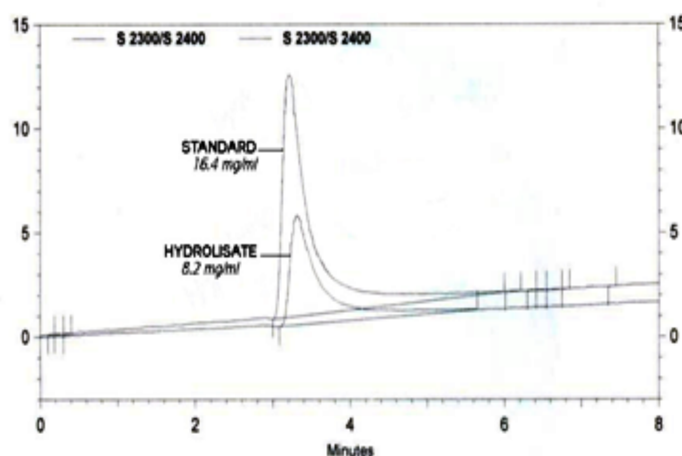
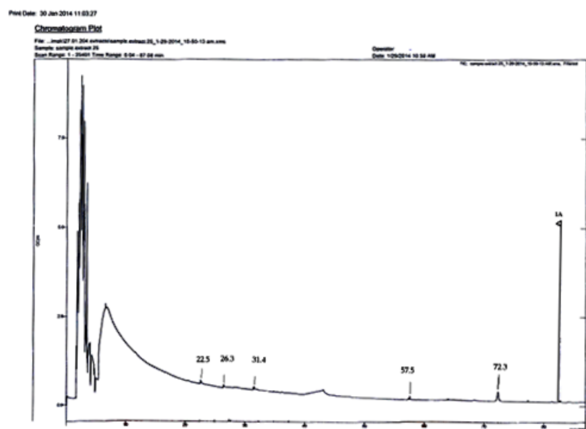


Fig.4. Chromatogram of the apricot gum's hydrolyzate and of the glucuronic acid standard sample (Rt= 3.333).



The low-molecular compounds qualitative analysis based on the gum's composites retention times (Rt), the samples constituents and the pure substance mass-spectra and the NIST-mass-spectrometric data bank comparison showed that in parallel to the main polysaccharides fraction in GA there were also the substances of phenol origin. The latter, as it is known, actively participate in tree heartwood's and pith's metabolism, and so in the cellular membranes recovery and the gum formation processes. According to the chromatogram (Fig.5) in the Rt1=22.5, Rt2=26.3, Rt3=31.4 retention times values the simplest phenols' - catechol, hydroquinone, and pyrogallol peaks were recorded; and their amounts made up 7.58%, 4.27% and 5.69%, correspondingly. The revealed non-polar (low-molecular) compounds presence testifies about the heartwood cambium layer immediate participation in the gum formation process.

Fig. 5. The low-molecular compounds in non-polar fraction of GA on the gas chromatogram recorded by means of chromato-mass-spectrographic method
1-catechol (1,2 dihydroxibenzol) Rt1=2.25; 2- hydroquinone (1,4 dihydroxibenzol) Rt2=26,3; 3- pyrogallol (1,2,3 trihydroxibenzol) Rt3=31.4.



The literature classical sources testify about the fact that the simplest phenols, such as pyrogallol, hydroquinone and catechol provide a strong antiseptic and antimicrobial activity. Scientifically justified these data are expressed in the GA antimicrobial effect [12].

III. Conclusions:

- By means of the column chromatography the gum polar fraction's (placed onto Al2O3 of IV activity) purification rational method was developed, what could be also used for the gums other polar (arabinic and basorinic) fractions purification and identification purposes.
- By means of the HPLC method the GA was standardized. The following monosaccharides: arabinose, galactose, glucose, xylose and ramnose neutral sugars and the glucuronic sugar were identified in the GA hydrolyzate.
- In the native GA the low-molecular substances (catechol, hydroquinone and pyrogallol) were detected, what testify about the cambium layer participation in the tree's gum formation process.

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