Isolation of Tannins Pistacia Vera

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Abstract

The chemical composition of the leaves of Pistacia vera has been studied for the first time. Plant material was successively extracted with chloroform and 70% acetone. The acetone extract was distilled off on a rotary evaporator to an aqueous residue. The aqueous portion was then fractionated with ethyl acetate. Individual compounds were separated by column chromatography and their chemical structure was established by physicochemical (UV, IR, NMR, mass spectroscopy) research methods.

Keywords: Anacardiaceae, Pistacia vera, extraction, column chromatography (CC), thin layer chromatography (TLC), acid hydrolysis, stepwise hydrolysis, flavonoids, phenolic compounds, hydrolysable tannins, gallotannins, high performance liquid chromatography (HPLC), mass spectrometry.

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1. Introduction

It is not for nothing that the volume of drugs created on the basis of natural compounds isolated from plants in the world pharmaceutical markets is currently 40-50 percent. Many phytopreparations, unlike synthetic agents, are characterized by the fact that they can be used for a long time without side effects, demonstrating the property of a broad-spectrum effect on the human body. In particular, drugs containing carbohydrates, macro-and microelements, vitamins, hydrolyzing tannins, flavonoids, phenol carbonic acids and compounds of other classes contained in plants of the Anacardiaceae family are widely used in medical practice, which have the property of effective action created on their basis. It is important to examine the chemical composition of the leaves of the plant Pistacia vera, which is part of this family, to carry out R & D work on the extraction of flavonoid and hydrolyzing tannins, research on their chemical structure and biological activity. Pistacia vera L. the plant is a tree in the family Anacardiaceae that grows to a height of 5-7 meters, sometimes up to 10 meters. The leaves are complex with an odd feather, often 3, sometimes 5-7 round-ovoid or ellipsoid, thick, flat-edged, light green petals, arranged in series on the branches using a band. Pistachios bloom in March-May, the fruit ripens in August-September. It grows in mountainous areas of Central Asia in rocky areas, forest slopes, Mountain foothills and hills, forming large pistachio gardens. It is grown on industrial plantations in the mountainous regions of the Caucasus and Central Asia. The Leaf is collected in the summer months,

and the shade is dried on the ground. When the fruit is ripe, the spoilage on the Leaf is picked and dried in the open air-in the sun. The wort contains 30-50% additives, the leaf contains 13-14% additives, vitamin C and organic acids, and the seed contains up to 60% fats. In folk medicine, its seeds have been used as an analgesic in the liver and stomach colic, in anemia, against vomiting, antitussive and anti-tuberculosis, improving heart function and stimulating sperm production. Pistacia vera, alcoholic extracts and biological additives isolated from the vera plant have been used in medicine to treat many diseases. It has been found to have effective effects against stroke, stomach ulcers, sedatives, neuroprotectors, amenorrhea, bruises, Chest Diseases, circulatory system, dysentery, rheumatism, liver cirrhosis inflammation and wound diseases, among others. Fats are used as many dietary foods that cleanse human facial skin and as an important source of energy. Also of great importance is the presence of magnesium, phosphorus, calcium, sodium, tocopherol, carotenes, lutein, selenium and phytosterols, linolenic acid, which are essential for human health [1-3].

Polyphenols isolated from the ground surface of the plant are P. aeruginosa, P. mirabilis, L. monocytogenes, E. hirae, E. faecium, B. subtilis, S. epidermidis, S. aureus, C. albicans, C. Parapsiloz, A. niger, E. coli has been found to have high activity against microorganisms [4, 5]. Extracts rich in polyphenol substances isolated from the plant have been shown to have a very strong antioxidant property as well as to be used as anti-diabetic agents in the body due to the reduction of blood sugar levels due to the ingestion of α-glycosidase and α-amylase enzymes [6-8]. The fact that Pistacia vera polyphenols have high anti-cancer activity for having cytotoxic effects has also been cited in the scientific literature [9]. Substances isolated from Pistacia species have also been found to have antiatherogenic, hypoglycemic, antioxidant, antiprotozoal, analgesic and anti-inflammatory activities [10-11]. Aqueous extracts of leaves and nuts have been found to have neuroprotective, hepatoprotection and anti-vomiting properties [12]. According to data in the scientific literature on the chemical composition of the plant monoterpenes [13], tetracyclic triterpenoids [14], flavonoids [15], and other phenolic substances, including gallic acid and its dressing [16-19], essential oils [20], have been isolated.

2. Methods

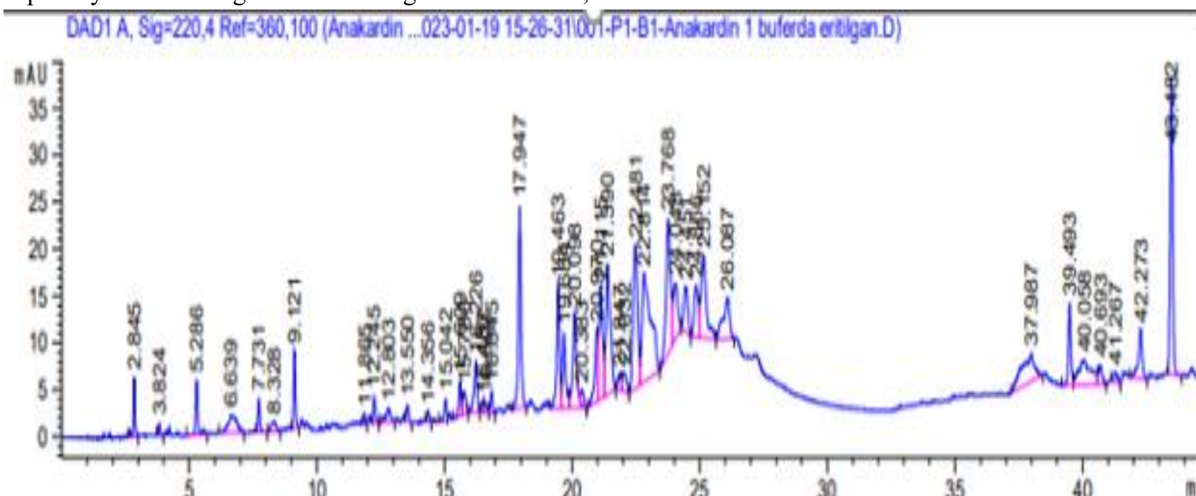
In order to study the chemical composition of plant leaves, in October 2025, 13 kg of plant raw materials were harvested from the Kizilsuv mountain ranges of the Bostonlik district and dried in a cool place without sunlight. It was extracted with chloroform in a ratio of 1:4 (50-55 oC, 2 hours, 3 times) for the purpose of removing 5 kg from dried plant raw materials, grinding, and cleaning from compounds of a coloring and lipophilic nature. Then the raw materials were dried at room temperature for 24 hours (that is, until the smell of chloroform disappears) and extracted with 70% aqueous acetone in a ratio of 1:5 (50-60 oC, 2 hours, 3 times). Combining water acetone extracts, the acetone was driven in a vacuum and the water part was obtained. Then the separated 9.4 liters of aqueous part was processed several times with ethyl acetate, obtaining a fraction of 30 liters of ethyl acetate. The ethyl acetate fraction was dehydrated by 2.5 kg of anhydrous Na_2SO_4 and 4 liters of ethyl acetate concentrate were obtained by increasing its concentration using a rotor evaporator under vacuum. The concentrate was precipitated and filtered by mixing with chloroform. By drying the extracted sediment at room temperature, in a vacuum-drying cabinet, the sum of 600 g (with 12.3% excess) of polyphenol compounds was isolated.

The highly effective liquid chromatography (HELC) method was used to study the chemical composition of the resulting polyphenols. According to the results obtained, it will be known that the composition of polyphenols contains compounds belonging to more than 20 phenol compounds (Pic. 1). IR-SPIRIT IK-Fure spectrometer produced by the Japanese firm Shimadzu in order to determine what kind of functional group of substances contains the aggregate of polyphenols isolated from the plant, IQ-spectrum results were obtained and gave absorption signals characteristic of gallic acid and its properties in 77% similarity when we analyzed the apparatus in comparison with the results obtained before.

3. Results and Discussion

We can see that the sum of the extracted polyphenols contains a compound that contains a very large amount of gallic acid residues. In order to determine the molecular masses of compounds in the aggregate of polyphenols isolated from the above ethyl acetate fraction, mass-spectrum results were obtained in the chromato-mass spectrum Q-TOF LC-MS Agilent Technologies series 6520V apparatus for determining the molecular masses and composition of substances under the conditions described below: in this, the ionization source - ESI, drying gas current - 5 l/min, drying gas temperature – 300 °C, voltage: cone skimmer-20V, fragmentary voltage mass: V mode Ms 100-2500 m/z, target mode Ms/Ms 50-2,500 m/z, Collision

energy (collision energy) – 35.50 yeV. Ionization method: negative. Mass spectrum of samples using chromatograph developed by the firm Agilent Technologies series 1200, column Zorbach SBC18.3 µm, 0.5×150 mm. Mobile phase: a - 0.1% Ant acid solution, B-acetonitrile + 0.1% ant acid.



Pic. 1. Results from the HELC method of summing the polyphenols extracted from the plant.

Chromatography conditions: solvents: a - acetonitrile, B-0.1% trip thoracic acid buffer (pH=3). Buffer concentration gradient with acetonitrile: 0-15 min - acetonitrile 15% (v/v), 15-27 min-acetonitrile 30 % (v/v), 27-42 min-acetonitrile 95 % (v/v), 42-45 min - acetonitrile 15% (v/v), Flow Rate – 0.6 ml/min. Absorption (wavelength) 220, 254, 280 nm. The temperature of the thermostat (column) is 30 °C.

When the results obtained in the mass spectrum were analyzed, it was found that the sum of polyphenols contained gallotanins belonging to the classes of high molecular phenolic compounds and compounds with masses belonging to the compounds that made the flavonoids characteristic of gallic acid (Table 1).

Table 1.

Mass spectra of compounds containing polyphenols

R_t, min	Compounds	M_m	$[M\text{-}H]^-$	MS/MS fragments, m/z
1.89	Galloil glucose	332	331	169 (36.01), 137 (100), 125 (7.17)
2.076	Gallic acid	170	169	125 (100)
9.696	Myricetin geksozid	480	479	316 (100),179 (6.10), 271 (12)
10.294	Quercetin galloyl glucoside	616	615	301 (78) 463 (34)
10.479	Quercetin glucoside	464	463	301 (100); 300 (75); 271 (5); 179 (5)
10.777	Octagalloyl glucose	1392	1391	1243, 1091, 939, 787, 635, 483, 331, 169
11.171	Pentagalloyl glucose	940	939	769, 787, 635, 483, 331, 169
11.991	Hexagalloyl glucose	1092	1091	939, 787, 769, 635, 617, 483, 465, 447, 431, 331, 295, 169
12.696	Heptagalloyl glucose	1244	1243	1091, 939, 787, 635, 483, 331, 169
13.329	Octagalloyl glucose	1392	1391	1243, 1091, 939, 787, 635, 483, 331, 169
13.926	Nonagalloyl glucose	1548	1547	1395, 1243, 1091, 939, 787, 635, 483, 331, 169

15.325	Decagalloyl glucose	1700	1699	1547, 1395, 1243, 1091, 939, 787, 635, 483, 331, 169
16.43	Nonadecagalloyl glucose	1852	1851	1699, 1547, 1395, 1243, 1091, 939, 787, 635, 483, 331, 169

The polyphenols were dissolved in 10 ml of methanol by pulling 100 mg from the substance to extract the individual compounds from the aggregate. Sephadex was divided into two fractions by washing with 50% and 80% aqueous ethanol in a colon (2.2x34 sm) containing LH-20 adsorbent. Purple compounds of 34 mg were isolated from the first fraction and 49 mg from the second. When examining the composition of the resulting substances in a highly effective liquid chromatography method, we found that fraction 1 contains five substances, while fraction 2 contains six substances. The first fraction obtained was re-colonized chromatography using the Sephadex LH-20 adsorbent for the purpose of extracting and cleaning individual compounds from the composition. As an eluent, ethanol-water was separated from 40% to 96% of alcohol fractions that contained 5 substances in themselves when washed in an increasing order of concentration. Water-methanol in Toyopearl HW 40 F adsorbent in order to obtain the separation of substances extracted from the second fraction into separate compounds (10, 20, 30, 40, 50, 60, 70 %) methanol was washed out in order of increasing concentration. In the process of washing, substances falling from the column were repeated in a thin-layer chromatography (TLC) method, as a result of the interleaving of similar fractions, individual substances were divided into stored fractions, and 6 substances were separated using methods of lowering and dipping substances into crystals. Chemical structures of compounds were determined using physicochemical methods of individual isolated substances.

Substance 1 white crystal, liquefaction temperature 150-151°C. Rf 0.51 (system 1: n-butanol-acetic acid-water 4:1:5), 0.25 (2-system: chloroform-methanol-water 75: 22: 3). UV-spectrum (MeOH, λmax, nm, lg ε): 205 (4.0), 213 (4.2), 216 (4.0), 227 (4.1), 279 (3.5). IQ-spectrum (KBr, sm-1): 3496, 3281 (OH), 3063, 2669 (C-H), 1667 (C=A), 1611, 1541, 1426 (C=C). Mass spectrum molecular mass 170, m/z [M-H]- 169, MS/MS 125 (100). 1H PMR-spectrum (CD3)2co, 600 MHz. m.u.): 7.15 (2H, s, H-2,6). 13C NMR-spectrum (CD3)2co, 150 MHz, m.u.): 109.9 (C-2, 6), 122.4 (C-1), 138.6 (C-4), 145.8 (C-3, 5), 170.8 (C-7). The results obtained were compared with the data presented in the literature, identifying this substance with gallic acid [21].

Substance 2 is a light-yellow amorphous powder. UV-spectrum (MeOH, λmax, nm, lg ε): 217.278 nm. Mass spectrum molecular mass 788, m/z [M-H]- 787, MS/MS 617, 169, 125, 123. 1H PMR-spectrum (600 MHz acetone-d6): d H galloyl: 7.20 (2H, s), 7.15 (2H, s), 7.07 (2H, s), 7.04 (2H, s), glucose ring 6.03 (1H, d, J=7.8 Hz, H -1), 5.68(1H, t, J=9.6 Hz, H-3), 5.48 (1H, T, C=9.6 Hz, H-2), 5.29 (1H, D, C=5.1 Hz, 4-oh), 4.48(1H, D, C=10.5 Hz, H-6). 13C NMR spectrum (125 MGz CD3COCD3): d C 166.4(-CO-), 166.1(-CO-), 165.7(-CO-), 165.3 (-CO-), glucose ring: 95.4 (C-1), 75.8 (C-2), 73.8 (C-3), 69.4 (C-4), 72.4 (C-5), 63.0 (C-6).

Hydrolysis products with the participation of 5% HCl yielded glucose Rf 0.35 (System 2: n-butanol-pyridine-water 6:4:3), Rf 0.21 (System 3: methylethylketone : acetic acid : methanol 55:5:2), 1-opener (aniline phthalate reagent), and gallic acid Rf 0.51 (system 1). When the hydrolysis products were quantitatively analyzed (glucose levels were tested according to the ferrocyanide method and gallic acid levels according to the colorimetric method), it was found that glucose and gallic acid were formed in a ratio of 1:4. Comparing the results obtained with the data presented in the literature, this substance was identified with 1,3,4,6-tetra-O-galloyl-β-D-glucose [22].

Substance 3 is a yellow crystalline substance. UV-spectrum (MeOH): λmax (log ε) 264 (3.85), 350 (3.58) nm; IQ-spectrum (KBr) νmax cm−1: 3310, 1662, 1602, 1040; ESI-MS m / z: 463.0882 [M-H]-. MS/MS 487, 465, 303, 301, 257, 251. 1H PMR-spectrum (600 MHz, DMSO) δ: 7.70 (1H, d, J = 2.4 Hz, H-2'), 7.56 (1H, dd, J = 2.4, 8.8 Hz, H-6'), 6.84 (1H, d, J = 8.6 Hz, H-5'), 6.40 (1H, d, J = 2.0 Hz, H-8), 6.20 (1H, D, J = 2.0 Hz, H-6), 5.45 (1H, D, J = 7.4 Hz, H-1''), 3.22–3.59 (6h, M, H-2''-6''). 13C-NMR-spectrum (150 MHz, DMSO) δ: 156.81 (C-2), 133.80 (C-3), 177.91 (C-4), 161.72 (C-5), 99.15 (C-6), 164.68 (C-7), 93.98 (C-8), 156.64 (C-9), 104.43 (C-10), 122.07 (C-1'), 115.68 (C-2'), 121.65 (C-6'), 116.68 (C-5'), 148.94 (C-4'), 101.36 (C-1''), 74.58 (C-2''), 76.99 (C-3''), 70.42 (C-4''), 78.04 (C-5'') and 61.46 (C-6''). Comparing the results obtained with the results presented in the literature, we found that this substance is quercetin-3-O-β-D-glucopyranoside [22].

Substance 4 light yellow amorphous powder, Rf 0.68

(System 1), melting point 278-280 0C (with fragmentation). UV-spectrum (MeOH, λmax, nm): 231, 231, and 278. mass spectrum ESI-MS negative analysis, m/z 939.1146 [M-H]-, MS / MS - decay products 769, 787, 635, 483, 331, 169. 1H PMR-spectrum (CD3OD), glucose ring: δ 6.23 (d, 8.3 Hz, H-1), 5.89 (t, 9.6 Hz, H-3), 5.61 (t, 9.6 Hz, H-4), 5.58 (dd, 9.6, 8.3 Hz, H2), 4.40 (m, H-5), 4.51 (d, 12.2 Hz, H-6A, 4.37 (DD, 12.2, 4.2 Hz, h-6). Galloyl parts: δ 7.10, 7.04, 6.97, 6.94, 6.89 (s, 2H).; 13C-NMR-spectrum (CD3OD), glucose ring: 93.9 (C-1), 74.3 (C-5), 74.1 (C-3), 72.1 (C-2), 69.8 (C-4), 63.1 (C-6). Galloyle pieces: 167.9, 167.3, 167.0, 166.9, 166.2 (carbonyl group signaling), 146.6, 146.5, 146.4, 146.4, 146.3 (C-3, C-5), 140.9, 140.5, 140.4, 140.2, 140.1 (C-4), 121.0, 120.3, 120.2, 120.1, 119.6 (C-1), 110.6, 110.44, 110.38, 110.36, 110.3 (C-2, C-6).

Hydrolysis products carried out in the presence of 5% HCl were found to produce glucose and gallic acid in a ratio of 1:5. Comparing the results obtained with the data presented in the literature, this substance was identified with 1,2,3,4,6-five-O-galloil-β-D-glucose [21].

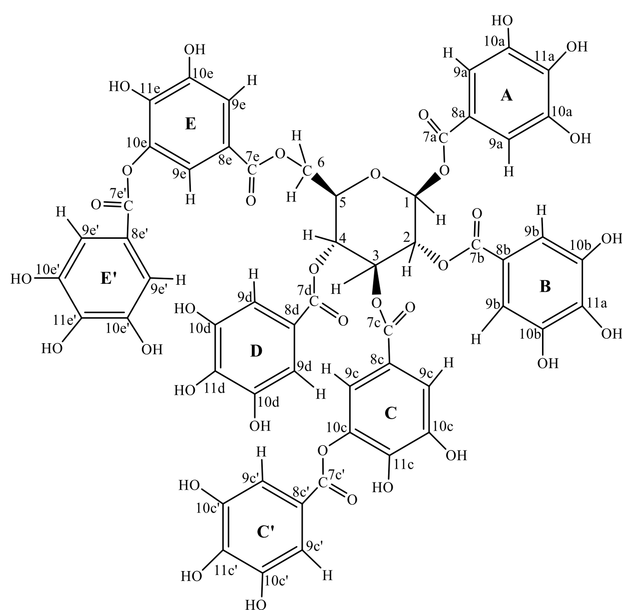
Substance 5 light yellow amorphous powder, Rf 0.54 (System 1), melting point 298-301 0C (with fragmentation). UV-spectrum (MeOH, λmax, nm): 230, and 279. Mas spectrum ESI-MS negative analysis, m/z 1092 [M-H]-, MS / MS - decay products 939, 787, 769, 635, 617, 483, 465, 447, 431, 331, 295, 169. 1H PMR-spectrum (CD3OD), glucose ring: δ 6.14 (d, 8.3 Hz, H-1), 5.81 (m, 9.6 Hz, H-3), 5.53 (m, 9.6 Hz, H-4), 5.58 (dd, 9.6, 8.3 Hz, H2), 4.30 (m, H-5), 4.25 m (d, 12.2 Hz, H-6A), 4.25 (DD, 12.2, 4.28 Hz, h-6). Galloyl group δ 9a: 6.94 (m, 2H), 9b 6.82 (m, 2H), 9c 6.90 (m, 2H), 9d 6.86 (m, 2H),), 9E 6.96 (d, 2H),), 9c' 7.02 (d, 2H). 13C-NMR-spectrum (CD3OD), glucose ring: 93.12 (C-1), 71.48 (C-5), 73.46 (C-3), 69.13 (C-4), 73.46 (C-5), 63.83 (C-6). Galloyle group: 166.82 (C-7a), 118.74 (C-8a), 111.02 (C-9a), 146.04 (C-10A), 140.82 (C-11a), 167.32 (C-7b), 119.74 (C-8b), 110.02 (C-9b), 146.12 (C-10b), 140.02 (c-11b), 166.31 (c-7c), 119.01 (c-8c), 110.09 (c-9c), 146.52 (c-10c), 140.56 (c-11c), 165.28 (c-7c'), 120.20 (c-8c'), 110.76 (c-9c'), 146.56 (c-10c'), 140.62 (c-11c'), 166.32 (c-7d), 119.70 (c-8d), 110.12 (c-9d), 141.12 (c-10d), 145.67 (c-11D), 167.46 (c-7e), 120.74 (c-8e), 109.82 (c-9E), 145.92 (c-10E), 139.07(c-11e).

Hydrolysis products carried out in the presence of 5% HCl were found to produce glucose and gallic acid in a ratio of 1:6. Comparing the results obtained with the data presented in the literature, this substance was identified with 3-O-bisgalloyl-1,2,4,6-four-O-galloyl-β-D-glucose [23].

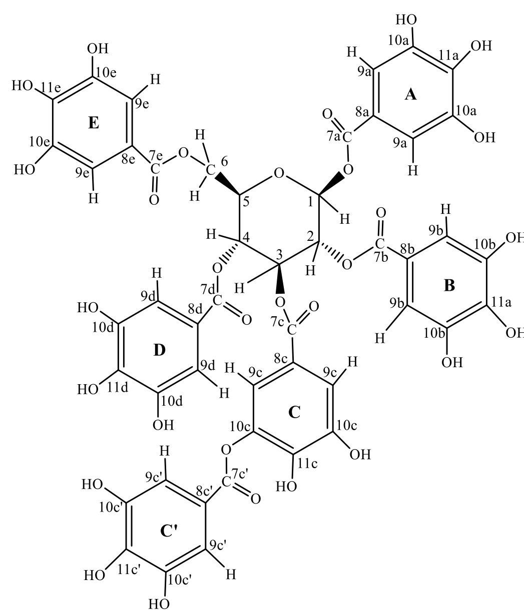
Substance 6 light yellow amorphous powder, Rf 0.42 (System 1), melting point. 298-301 0C (with fragmentation). UB-spectrum (MeOH, λmax, nm): 230, and 279. Mas spectrum ESI-MS negative analysis, M/z 1092 [M-H]-, MS / MS - decay products 939, 787, 769, 635, 617, 483, 465, 447, 431, 331, 295, 169. 1H PMR-spectrum (CD3OD), glucose ring: δ 6.28 (d, 8.3 Hz, H-1), 5.58 (d, 8.3 Hz, H-2), 5.98 (m, 9.6 Hz, H-3), 5.62 (m, 9.6 Hz, H-4), 5.58 (dd, 9.6, 8.3 Hz, H2), 4.54 (m, H-5), 4.45 M (D, 12.2 Hz, h-6), 4.25 (DD, 12.2, 4.28 Hz, h-6). Galloyle group δ 9a: 6.96 (d, 2H), 9b 6.92 (d, 2H), 9c 6.93 (9, 2H), 9d 6.94 (9, 2H), 9E 7.31 (d, 2H),), 9E' 7.25 (d, 2H). 13C-YaMR-spectrum (CD3OD), glucose ring: 93.88 (C-1), 72.48 (C-5), 74.46 (C-3), 70.13 (C-4), 74.46 (C-5), 63.83 (C-6). Galloyle group: 166.32 (C-7A), 119.74 (C-8a), 110.76 (C-9a), 146.60 (C-10A), 140.82 (C-11a), 167.12 (C-7b), 120.74 (C-8b), 110.6 (C-9b), 146.57 (C-10b), 140.4 (c-11b), 167.31 (c-7c), 120.42 (c-8c), 110.45 (c-9c), 146.42 (c-10c), 140.3 (c-11c), 167.12 (c-7d), 120.3 (c-8d), 110.42 (c-9d), 146.42 (c-10d), 140.34 (C-11D), 167.24 (c-7e), 121.14 (c-8e), 117.62 (c-9E), 147.55 (C-10e), 140.39 (C-11e). 166.72 (C-7e'), 120.56 (C-8e'), 110.96 (C-9E'), 146.66 (C-10e'), 140.56 (C-11e').

Hydrolysis products carried out with the participation of 5% HCl were found to have glucose and gallic acid in the composition: in a ratio of 6. Comparing the results obtained with the data presented in the literature, this substance was identified with 6-O-bisgalloyl-1,2,3,4-four-O-galloyl-β-D-glucose [24-25].

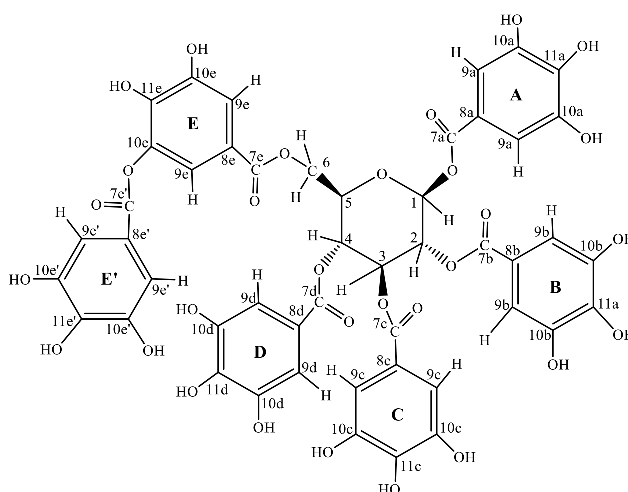
Currently, the results of the Mass-, NMR-, HMBC-spectrum and biological and pharmacological activities of these isolated compounds are being studied.



3,6-bis-galloil-1,2,4- four-galloil -D-glucose



3-bis-galloil-1,2,4,6- four-galloil -*β-D*-glucose



6-bisgalloil-1,2,3,4-four-galloil-D-glucose

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