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COMPREHENSIVE ANALYSIS OF POLYPHENOLIC AND MINERAL COMPOUNDS IN POMEGRANATE (PUNICA GRANATUM L.) PEEL EXTRACT

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ABOUT ARTICLE

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Abstract: Pomegranate peel, a by-product of juice production, contains bioactive compounds with significant nutritional and therapeutic properties.

This study aimed to isolate and characterize polyphenolic compounds from Punica granatum L. peel and to evaluate their nutritional, functional, and mineral composition [1-2].

Extraction was performed using distilled water at 60 °C, followed by precipitation with gelatin. High-performance liquid chromatography (HPLC) and atomic absorption spectroscopy (AAS) were used for compound identification [3-5].

The tannin content in pomegranate peel was 77.8%, with rutin, isorhamnetin, gallic acid, and hyperoside being the main flavonoids identified. Mineral analysis revealed the

presence of Ca, Na, Mg, Fe, Cu, and Al in the tannin powder [6-9].

Pomegranate peel extract demonstrated high polyphenolic content with potential applications as functional food ingredients and nutraceuticals[10-12].

Introduction. In traditional medicine, pomegranate has been used to treat atherosclerosis, anemia, dysentery, hypertension, colitis, hemoptysis, gastric ulcers, liver and kidney diseases, to heal wounds caused by burns and cuts, restore hormonal balance, and relieve nervousness, irritability, and headaches. The most reliable therapeutic practices with pomegranate have historically been developed among the populations in regions where this fruit is most widespread.

It should be noted that pomegranate peel contains many biologically active compounds, such as polyphenols, dietary fibers, vitamins, minerals, etc. Furthermore, their presence is associated with the prevention and treatment of several chronic metabolic diseases, including cardiovascular diseases, diabetes, and obesity. The astringent properties of pomegranate peel, attributed to tannins, were traditionally recommended for men experiencing “seminal loss” and for treating dysentery. In general, it was used to reduce the frequency and intensity of various secretions. Moreover, preparations from the peel were used for helminth infections, rectal prolapse, and disorders of motor function (such as poor coordination and paralysis).

Thus, the bioactive components contained in pomegranate peel can be employed as functional ingredients, ensuring better utilization of by-products and providing additional value for the food industry. In this regard, we studied the phenolic compounds in the extract and the precipitated tannin of pomegranate peel.

Materials and Methods. For the experiment, we selected the bitter pomegranate variety Shakhrisabz. This is a thick-skinned, sour-tasting fruit, harvested in October–November. This variety was registered in the State Register of Uzbekistan in 1859. The peel of this variety contains 10–15% tannins. The antioxidant activity of pomegranate peel is provided by polyphenolic hydroxyl groups, which can reduce the level of free radicals. Moreover, the hydroxyl groups of catechins in complex and condensed tannins give them the ability to chelate iron and transition metals.

The object of the study was pomegranate peel obtained after juice extraction. Phenolic compounds represent the main secondary metabolites of the shikimic acid, pentose phosphate, and phenylpropanoid pathways, and they include a large number of water-soluble substances. According to the literature, the total amount of phenolic compounds in pomegranate peel ranges from 18 to 510 mg/g of dry matter, depending on the cultivar, extraction solvents, and extraction methods. Their structures consist of at least one aromatic ring with one or more hydroxyl substituents.

To determine the chemical composition of pomegranate peel and to isolate polyphenolic compounds, the following investigations were carried out. The experiments performed were divided into three stages:

1. Determination of the chemical composition of pomegranate peel.
2. Isolation of polyphenolic compounds.
3. Determination of the mineral content of the polyphenolic compounds.

Extraction Procedure. For this purpose, pomegranate peel was extracted with distilled water at 60 °C for 3 hours, repeated three times. The extract was centrifuged, filtered, and precipitated with a gelatin solution. Aqueous solutions formed precipitates with alkaloids and gelatin solutions. The modified Leventhal–Kursanov method represents a combination of tannins with a gelatin precipitate.

To isolate the compounds, the extractant was treated with 2.5% gelatin in a 10% alkaline solution. The gelatin used in the experiment was obtained from bones, skin, and connective tissues of animals, and its composition was verified using a BUCHI spectrophotometer. The quality characteristics of the gelatin are presented in Figure 1 and Table 1.

The light absorption characteristics of gelatin were determined using a BUCHI spectrophotometer.

Table 1

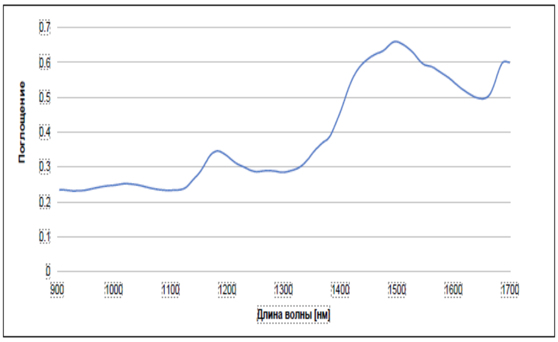

Figure 1. Quality characteristics of gelatin

Chemical composition of gelatin, %	
Moisture	7,6
Protein	59,87
Minerals	14,33
Polysaccharides	10,74

It can be observed that the protein content in gelatin is about 60%. Tannins and polyphenolic compounds bind to the protein and are subsequently separated.

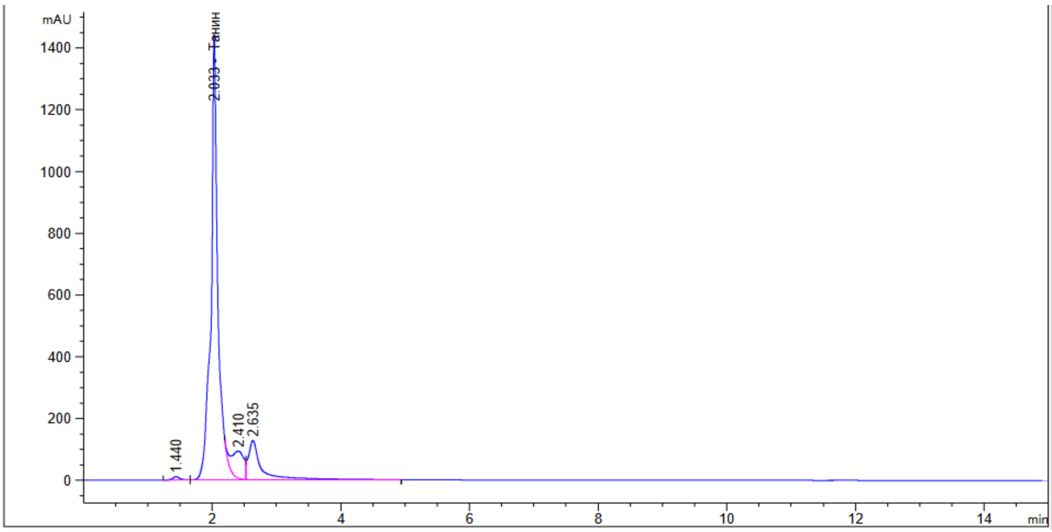

Figure 2. Tannin content in pomegranate peel.

The tannin content in the powder obtained in this analysis was 77.786%. In the following experimental procedure, the chemical composition of tannin was studied and its amount in the precipitated and liquid fractions was compared. The precipitate was referred to as "powder," while the liquid fraction was referred to as the remaining water-soluble extract.

After extraction, the obtained powders (flavonoids) and the remaining water-soluble fraction were analyzed according to GOST 55312-2019. After treatment with a gelatin solution, the resulting precipitate represented a tannin–gelatin complex, which was separated and dried. This yielded a yellow tannin powder bound with gelatin.

The aqueous fraction was also collected separately and its composition was compared with that of the precipitate. The aim was to determine the amount of substances that remained unprecipitated in the liquid fraction.

The tannin content in the precipitated powder was determined using high-performance liquid chromatography (HPLC).

Precipitated powder.

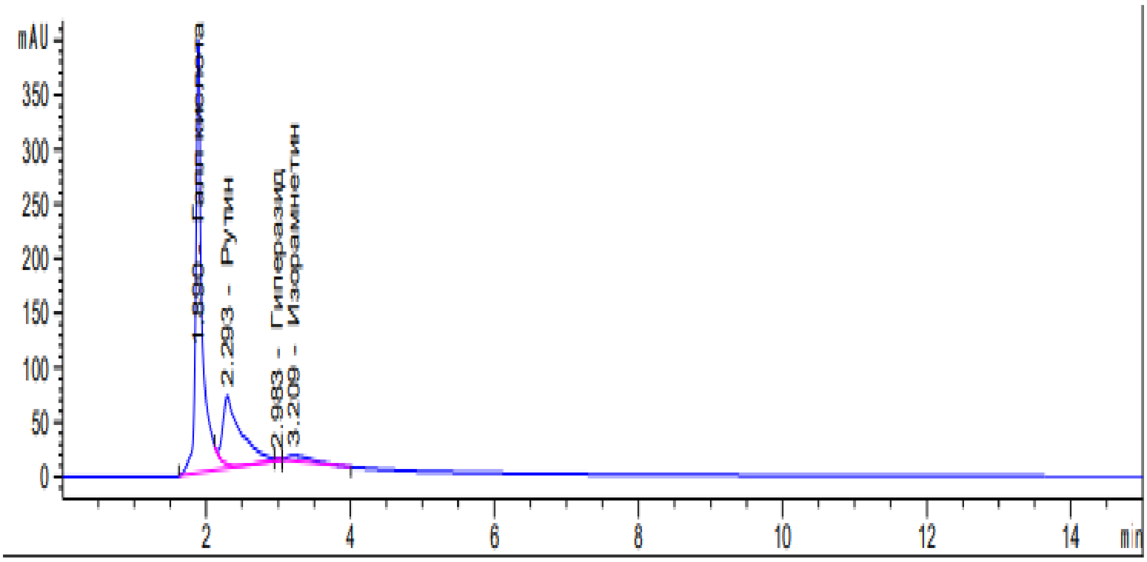

Figure 3. Tannin content in the powder

Since gelatin is used for the extraction of tannins, the protein content in this powder was determined by the Kjeldahl method according to GOST 13496-2012.

Method: In GOST 13496-2012, dedicated to the analysis of protein in tannin, two catalyst formulations are provided:

Catalyst 1: 10.0 g copper sulfate, 100.0 g potassium sulfate, and 2.0 g selenium were weighed, placed into a mortar, and the mixture was thoroughly ground to obtain a homogeneous fine powder.

Catalyst 2: 10.0 g copper sulfate and 300.0 g potassium sulfate were weighed, placed into a mortar, and thoroughly ground to obtain a homogeneous fine powder.

Results and Discussion. In addition to copper sulfate and selenium, mercury salts (Hg_2SO_4) can serve as catalysts for sample decomposition in the Kjeldahl method. To accelerate the decomposition process, oxidizing agents may also be added instead of catalysts, such as potassium permanganate, potassium dichromate, or hydrogen peroxide (as was applied in our case). In general, the method is intended for determining nitrogen in organic compounds.

The total protein content in the powder was found to be 41.125%.

HPLC Analysis of Flavonoids

The conditions for flavonoid analysis by HPLC were as follows: mobile phase – acetonitrile and trifluoroacetic acid solution at pH 2.6 (35:65); flow rate – 1.2 mL/min; column temperature – 27 °C; detection – UV at λ = 360 nm.

Additional HPLC parameters included: column temperature – 20 °C (or ambient), spectrophotometric detector wavelength – 210 nm, diode-array detector range – 200–600 nm, flow rate of eluent – 0.8–1.0 mL/min, and injection volume – 5–10 µL.

The flavonoid content of the aqueous extract after tannin precipitation was determined and is presented in Figure 3.

1. (the remaining aqueous fraction)

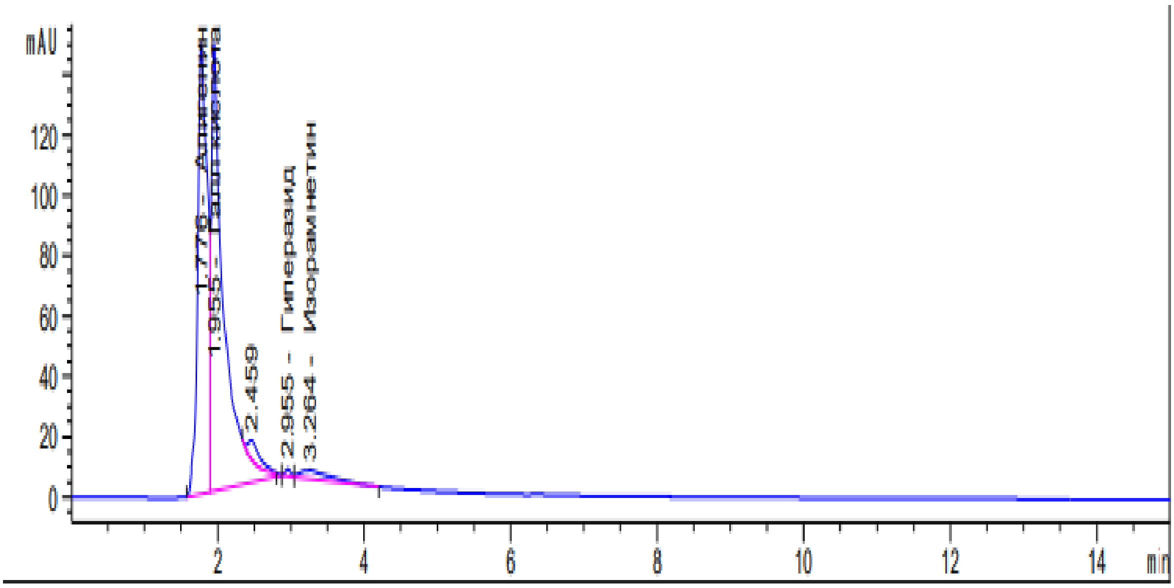

Figure 4. Flavonoid content in the remaining aqueous fraction.

The amount of flavonoids in the precipitated powder and in the aqueous fraction is presented in Table 2

Test results of Flavonoid content

Table 2

№	Sample type	Flavonoid content *mg/100g*						
		Robinin	Rutin	Apigenin	Isorhamnetin	Gallic acid	Hyperosiden	Quercetin
1	The remaining aqueous fraction	-	-	160,252	2,812	42,281	3,125	-
2	Precipitated powder	-	206,197	-	10,025	629,381	4,375	-

As shown in Table 2, apigenin did not form a complex with the gelatin remaining in the aqueous fraction. In small amounts, rutin, robinin, quercetin, isorhamnetin (2.812 mg/100 g), gallic acid (42.281 mg/100 g), and hyperoside (3.125 mg/100 g) were not detected.

In the precipitated powder, rutin (206.197 mg/100 g), isorhamnetin (10.025 mg/100 g), gallic acid (629.381 mg/100 g), and hyperoside (4.375 mg/100 g) were present, while quercetin and robinin were absent.

Furthermore, after the extraction of phenolic compounds from pomegranate peel, the residual peel was examined for the presence of tannins and phenolic compounds. This was performed according to the method described above, and the results are presented in Figure 4.

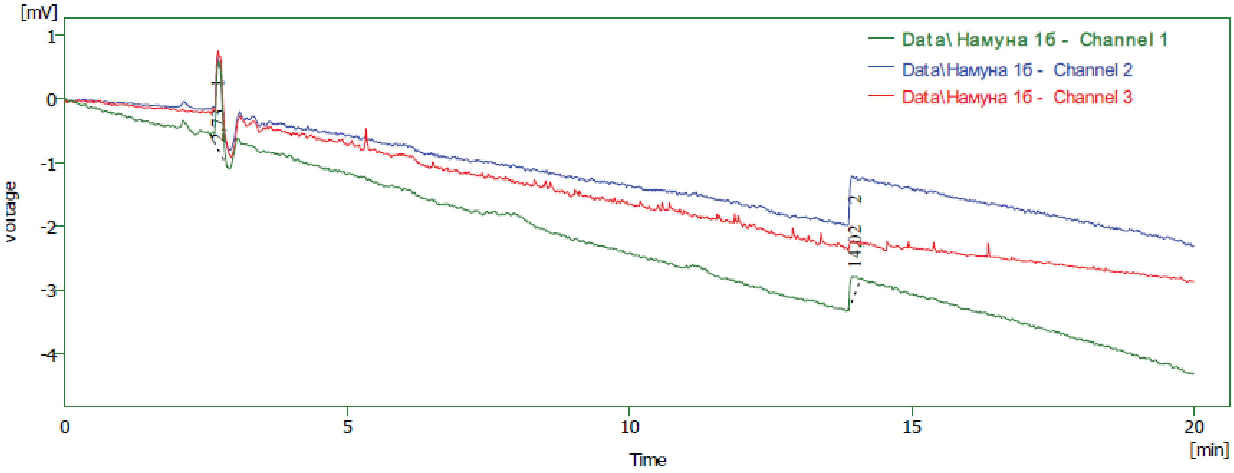

Figure 4. Phenolic compounds in the residual peel

This graph shows that the amount of polyphenolic compounds remaining in the pomegranate peel was insignificant and consisted mainly of water-soluble tannins.

Subsequently, we investigated the mineral substances present in the aqueous solution after tannin precipitation and in the powder fraction. The amounts of macro- and microelements in the obtained tannin powder and in the remaining aqueous fraction were determined according to GOST 31640-2012. Minerals are essential components of nutrition with highly diverse physiological functions. They play an important role in plastic processes and in the formation and development of body tissues, particularly in the skeletal system.

The determination of microelements was carried out using atomic absorption analysis. This method is a quantitative elemental analysis based on atomic absorption spectra in the range of 190–850 nm. The main components of the AAS instrument include: a light source emitting a narrow spectral line of the analyzed element, an atomizer for converting the analyte into atomic vapor, a monochromator for isolating the characteristic spectral line, and an electronic system for detecting, amplifying, and processing the analytical signal, usually presented as a graph.

The mineral composition was determined and is presented in Table 3

Mineral composition

Table 3

Mineral	Powder	Aqueous solution	Absorption index (nm)
Silver (Ag)	-	-	328,068
Aluminum (Al)	99,88	-	396,152
Mercury (Hg)	0,1943	0,0440	194,159

Arsenic (As)	-	1,114	193,698
Barium (Ba)	9,098	-	233,527
Calcium (Ca)	1379	-	315,887
Cadmium (Cd)	0,0185	0,0163	228,802
Cobalt (Co)	0,2507	0,3462	228,615
Chromium (Cr)	3,654	1,285	205,552
Copper (Cu)	7,670	0,2287	327,396
Iron (Fe)	52,56	1,881	259,940
Magnesium (Mg)	222	-	279,553
Manganese (Mn)	0,5052	-	257,610
Sodium (Na)	8673	-	588,995
Nickel (Ni)	1,299	0,7917	221,648
Lead (Pb)	-	-	220,353
Selenium (Se)	-	-	196,028
Strontium (Sr)	-	-	421,552
Zinc (Zn)	-	-	206,200

As shown in Table 3, the elements aluminum, barium, calcium, chromium, copper, iron, magnesium, and sodium in the tannin powder are present as salts of polyphenolic compounds, whereas in the aqueous fraction heavy metals such as arsenic, chromium, and nickel remain.

Conclusion. Thus, polyphenolic compounds of pomegranate are applied in medicine for the treatment of atherosclerosis, anemia, dysentery, hypertension, colitis, hemoptysis, gastric ulcers, liver and kidney diseases, for wound healing in cases of burns and cuts, for restoring hormonal balance, as well as for relieving nervousness, irritability, and headaches. In addition, due to their antioxidant, stabilizing, and coloring properties, tannin powders have found wide application in the light, food, and pharmaceutical industries [13-17].

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