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CHROMATOGRAPHIC ANALYSIS OF POMEGRANATE PEEL EXTRACTS OBTAINED WITH VARIOUS SOLVENTS

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Abstract

This study involved the extraction of pomegranate peel from four local varieties – Qoradon qizil, Achchiqdona, Qozoqi, and Qayum – using various organic solvents. The composition of the resulting extracts was analyzed using high-performance liquid chromatography (HPLC), and the antioxidant activity of the compounds extracted from the pomegranate peels was compared. Water, ethyl acetate, and ethanol were used as solvents, and the analysis was conducted at a wavelength of 254.4 nm.

Keywords: *Karadon kyzyl, Achchikdona, Kazoki, Kayum, antioxidant, gallic acid, ellagic acid*

Introduction

As a solution to the shortage of food and other consumer goods observed in the world today, rapid development of the agricultural sector is shown as a solution to current problems. Along with the horticulture industry, the processing of products obtained from them and the efficient use of secondary products retain their importance. Among the research carried out by scientists of the world, there are many developments that are scientifically based and put into production, which make it possible to organize measures to find a solution to such problems.

Within the framework of measures for the development of the Republic of Uzbekistan until 2030, tasks have been clearly defined regarding the development of effective methods of growing horticulture, viticulture and many other agricultural products. Numerous experimental works are being carried out on the productivity of pomegranate varieties grown in our country and their adaptation to soil and climatic conditions.

By studying and analyzing pomegranate varieties that can be grown in the regions, it is possible to make a significant contribution to the future results of these plans. At this point, taking into account the plans of the Fergana Valley, Kashkadarya and Surkhandarya regions, as well as the existing pomegranate orchards in other regions of the country, it is aimed to increase the indicator of pomegranate cultivation and productivity in the republic's agriculture in the next decade, and increase the production volume to 600,000 tons per year.

Literature Review

Currently, the value of biologically active compounds, which are necessary for the human body, have healing properties, is of particular importance. Examples of biologically active substances are proteins, carbohydrates, lipids, enzymes, vitamins, hormones, macro-micro elements and many other primary and secondary metabolites. Among these main biologically active compounds, the role of secondary biologically active substances that stimulate metabolism in the body and have a high effect is also important, one of such substances is gallic acid and ellagic acid formed as its residues, and other tannins, as well as nitrogen-containing and acid-based vital compounds, each of them plays an irreplaceable and very important role in the life activity of the human body (Braga, L.C., Shupp, J.W., Cummings, C., Jett, M., Takahashi, J.A., Carmo, L.S., Nascimento, A.M.A., 2005; Menezes, S.M.S., Cordeiro, L.N., & Viana, G.S.B., 2006).

Pomegranate is distinguished from other fruits by its medicinal properties. The fruit contains on average 25–32% sugar, 0.2––2.5% organic acids, 10% tannin, up to 3.4% proteins, 2–6% pectin, as well as iron, cobalt, iodine, C, PP, A vitamins. The amount of vitamin C in fruits is 250–1300 mg. This causes an increase in interest in its consumption (Hosseini, B., Saedisomeolia, A., Wood, L.G., Yaseri, M., & Tavasoli, S., 2016).

These natural antioxidants play a role in preventing free radical oxidation, aging process and the development of many diseases in the human body. Ellagic acid is a polyphenolic compound with strong antioxidant properties. It combines with various biomacromolecules, for example, proteins and collagen, and has the property of improving the immunity in the body. Ellagic acid has been found to have high effectiveness against cancer, this substance slows down the development of tumors due to its high effect (Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., & Cheng, S., 2006).

Pomegranate peel is rich in ellagic acid, in addition, pomegranate fruit peel forms complex compounds, which further increases the effect of the substance. Another positive effect of ellagic acid is due to its skin-tightening properties. For medical purposes, ellagic acids isolated from pomegranate peel are effectively used in various biochemical methods. Ellagic acid has many effects, including important effects such as narrowing the pores of the organ system in the body, reducing sebum secretion, and improving the formation of tissues that improve skin lubrication (Bell, C., & Hawthorne, S., 2008).

Figure 1. *Pomegranate peel and its dried and crushed sample*

Extraction of ellagic acid from this raw material is recognized as a technology with high efficiency due to the fact that the production technology is somewhat simple and high productivity (Usta, C., Ozdemir, S., Schiariti, M., & Puddu, P.E., 2013).

The purpose of the work is to carry out quantitative analyzes of gallic and ellagic ac-

ids, which have antioxidant properties, in the local pomegranate skin.

Methods

In the extraction stages, 3 samples of dried and ground biomass of pomegranate peel of 4 different varieties, 50 g each, corresponding to 3 types of solvents, are measured. each of the measured samples was placed in separate containers (500 ml flat-bottomed flasks) into which equal amounts of all solvents (water, ethyl acetate, and ethyl alcohol) were poured (250 ml per sample) and extracted in a magnetic stirrer for 30 minutes, after which the samples were left at room temperature for 48 hours. At the end of the specified time during the process, the liquid part of each sample is poured separately, and the remaining part is poured with the same amount of solvent as the initial volume, and it is kept in the unit of time specified in the initial stage. In this way, the extraction is carried out three times, and all the extractants (the liquid part of the sample of the extraction process obtained from the three steps of the pomegranate peel using solutions) are collected in one container depending on the pomegranate variety. Extract concentration process, rotor evaporation was carried out using RE100-Pro equipment. In this case, the process methodology, the rotation speed of the rotor is 60 rpm, the temperature is 38–40 °C and the pressure is 0.9 Pa. The duration of the process is on average 60–90 minutes when evaporating the extract obtained through organic solvents (ethyl acetate and ethyl alcohol) to a residual amount of 10% of the solution content. When evaporating aqueous extracts, the temperature of the water bath rises to 70–75 °C, the duration of the process is 90–120 minutes, compared to 25–30% residual content. Each concentrated sample is collected in separate containers, and at the next stage, their qualitative and quantitative analysis is carried out.

In the second stage of the research, modern chromatographic methods were used to analyze the amounts of gallic and ellagic acids in the extracts extracted from the peel of different varieties of pomegranate. Gallic and ellagic acids from polyphenols contained in the sample were determined using liquid chromatography to organize the work process.

5–10 g of the sample is taken on an analytical balance and placed in a 300 ml flat flask. Add 50 ml of 70% ethanol solution. The mixture was heated at 70–80 °C with vigorous stirring for 1 hour, equipped with a magnetic stirrer, reflux condenser, and then stirred at room temperature for 2 hours. The mixture is cooled and filtered. 25 ml of 70% ethanol is added to the remaining part and it is re-extracted for the 2nd time. The filtrates were combined and filled to the mark with 70% ethanol in a 100 mL volumetric flask. The resulting solution is spun in a centrifuge at a speed of 6000–8000 rpm for 20–30 minutes. The resulting solution is taken from the upper part for analysis.

First, working standard solutions and then prepared working solutions were introduced into the chromatograph. High-performance liquid chromatography is used in the analysis. The experimental conditions of the chromatographic environment adapted for the analysis include the following: Chromatograph Agilent-1200 (equipped with an autodoser), Column Exlipse XDB C 18 (obraschenno-faznyy), 5 μm, 4.6 × 250mm, Diode matrix detector (DAD), 254 nm, 272 nm identified. Flow rate 0.8 ml/min, eluent phosphate buffer: acetonitrile: 0–5 min 95:5, 6–12 min 70:30, 12–13 min 50:50, 13––15 min 95:5, thermostat temperature 30 °C, the amount of sample included in the process is 10 μl (vcol). Studies are conducted on 3 solvent-extracted solutions of each sample, so that a total of 12 extracts were tested in each step (4 × 3).

Results and discussion

During the research, 250 ml of the 4 types of pomegranate varieties selected for the extraction process in different solvents (water, ethyl alcohol, ethyl acetate) make a total volume of 750 ml of solvent in three stages. One-stage extraction takes 48 hours, with all three stages totaling 144 hours. Pomegranate skin taken in solution is shaken up to 3 times a day using a shaker (Shaker GFL-3017) to increase the solubility of substances in extraction.

Table 1. *Amount of reagent used for the extraction of pomegranate peel and extraction period*

№	Varieties of pomegranate 50 gr	Water ml	Ethanol ml 144 soat	Ethyl acetate ml
1.	Karadon kyzyl	250	250	250
		250	250	250
		250	250	250
2.	Achchikdona	250	250	250
		250	250	250
		250	250	250
3.	Kazoki	250	250	250
		250	250	250
		250	250	250
4.	Kayum	250	250	250
		250	250	250
		250	250	250

Note: the solvent volumes given in the table are selected for a 50 g sample, increasing the sample volume may result in a decrease or increase in the amount of solvents

Based on the results of the analysis, different ratios of solvents and extraction periods were carried out based on several experiments, and the optimal amounts as well as time units were selected as the results given in Table 1. As the samples age, the mixture becomes a brown mass (see Figure 2). After the fractionation and filtration process, the liquid contents have a dark red, bright appearance (see Figure).

Figure 2. *Extracted samples*

Figure 3. *The separated extract fluids*

Since the residual moisture content of the samples during drying is 3–6%, it is well absorbed by the crushed bark during the extraction stages. The liquid part of the substances dissolved in three different solvents is poured into separate containers according to the type of pomegranate and the type of solvent. The amount of extract obtained is different, the reason for this can be said that the rate of absorption and retention of the solvents in the plant source is different, therefore the amounts show a difference, but not much from each other (see Table 2).

Table 2. *Amounts of extract from pomegranate varieties*

№	Varieties of pomegranate	Water ml	Ethanol ml	Ethyl acetate ml
1.	Karadon kyzyl	375	550	345
2.	Achchikdona	345	530	340
3.	Kazoki	180	540	360
4.	Kayum	370	530	340

A total of 750 ml of solvents were included in the three-stage extraction process, and the amount of extracts obtained from them is different for several reasons, for example, the amount of extract obtained from the peel of black red pomegranate is 375 ml, the amount of ethyl alcohol extract is 550 ml, and the amount of ethyl acetate is 345 ml. The amount of solvents used in the extraction of pomegranate peels of other varieties almost repeats these volumes. With the exception of the skin of the Kazoki pomegranate variety in the aqueous solution, the reason is that the amount of this solution decreases in a sharply different volume compared to the others. From this, it can be concluded that, due to the preservation of solvents in the source and their evaporation over time, among them, the alcohol extract dominates in terms of quantity. In addition, it is observed that some of the samples retain a large amount of aqueous solution in their cells.

Figure 4. *Chromatograms of the analysis of ellagic and gallic acids in the peel of a pomegranate variety (studied in a-ethyl acetate, b-ethyl alcohol and c-aqueous solutions)*

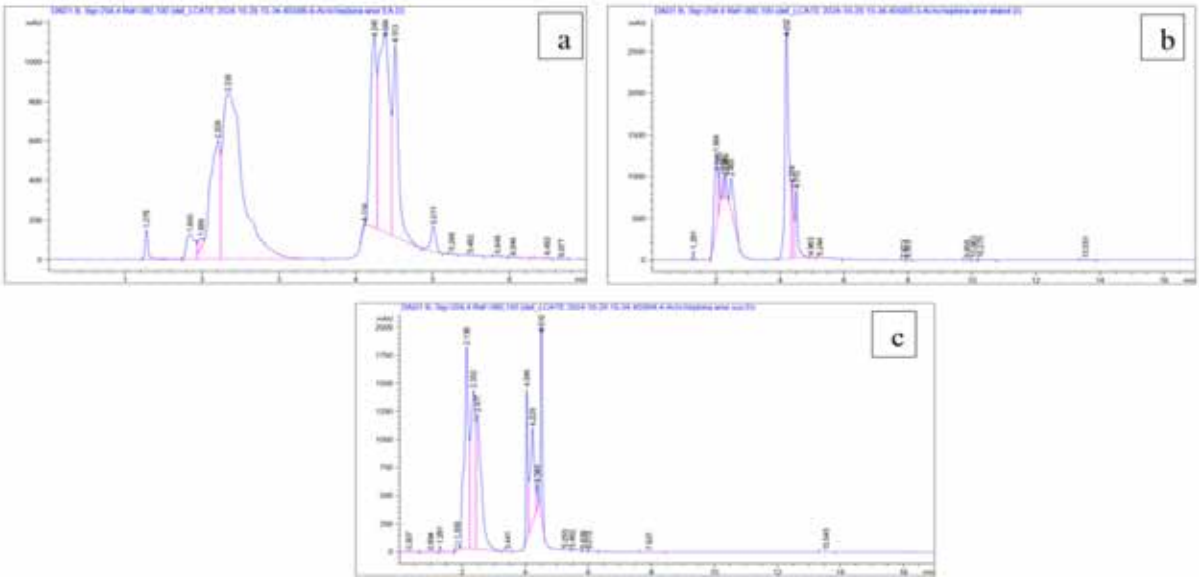
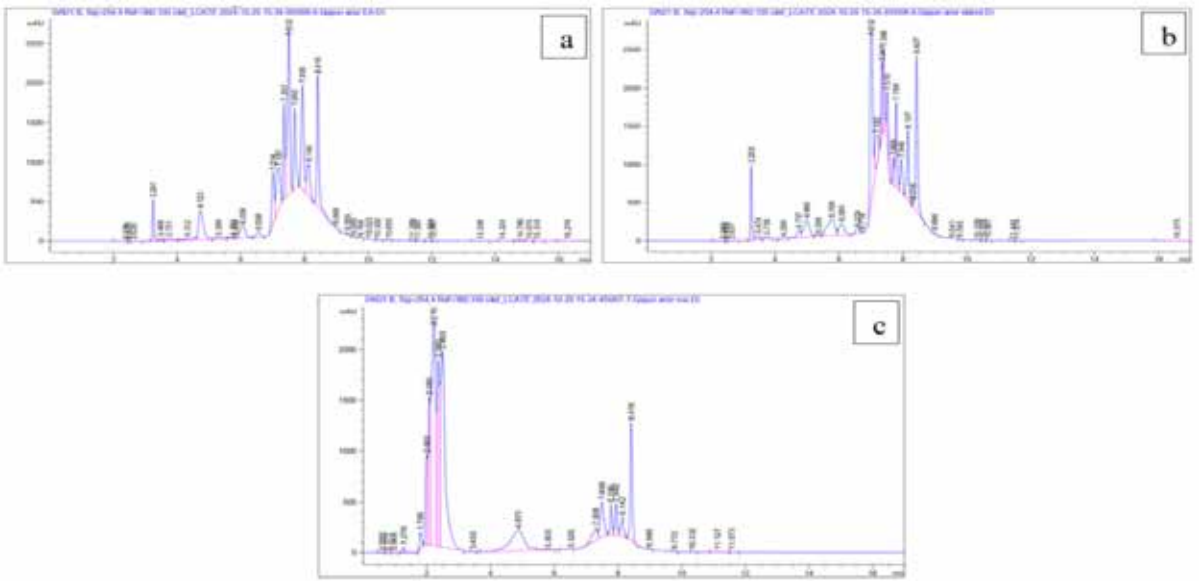


Figure 5. *Chromatograms of the analysis of ellagic and gallic acids in the peel of Kayum pomegranate variety (studied in a-ethyl acetate, β-ethyl alcohol and c-aqueous)*



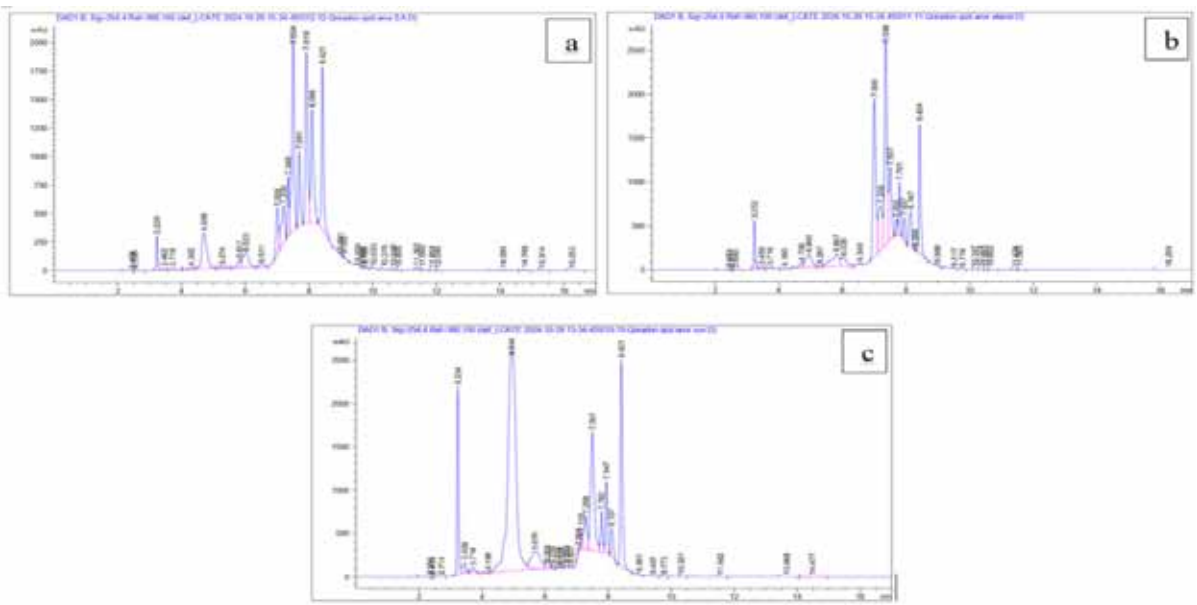
High performance liquid chromatography (HPLC) analysis results. This method is a high-precision, reliable analysis method for the analysis of the quantity and quality of biologically active substances. According to the analysis of polyphenol substances in pomegranate peel, the number of intense peaks visible in the obtained chromatogram, as well as their intensity indicators, led to accurate information about the quality and quantity of the substances.

Intense peaks in these chromatograms indicate the presence of both polyphenols. According to it, it was found out that the substance coming out of the column in the first 2–4 minutes is ellagic acid in accordance with the standard.

Also, according to the standard chromatogram, the retention period of gallic acid in the column corresponds to an average interval of 4–6 minutes, the chromatogram of the analyzed solution showed intensive peaks related to gallic acid during these minutes. It can be seen that the periodicity of substances leaving the column has not changed in all three solution samples isolated from bitter pomegranate peel.

The compositional chromatograms of Kayum pomegranate peel have a slightly different appearance compared to other pomegranate varieties, including the fact that the content of ellagic and gallic acids in the extracts obtained through all three types of solvents is relatively low, besides, it can be seen that the content of other polyphenol substances in the content of three different solvents is present in large quantities.

Figure 6. *Chromatograms of the analysis of ellagic and gallic acids in the peel of Karadon kyzyl pomegranate variety (studied in α-ethyl acetate, β-ethyl alcohol and c-aqueous solutions)*

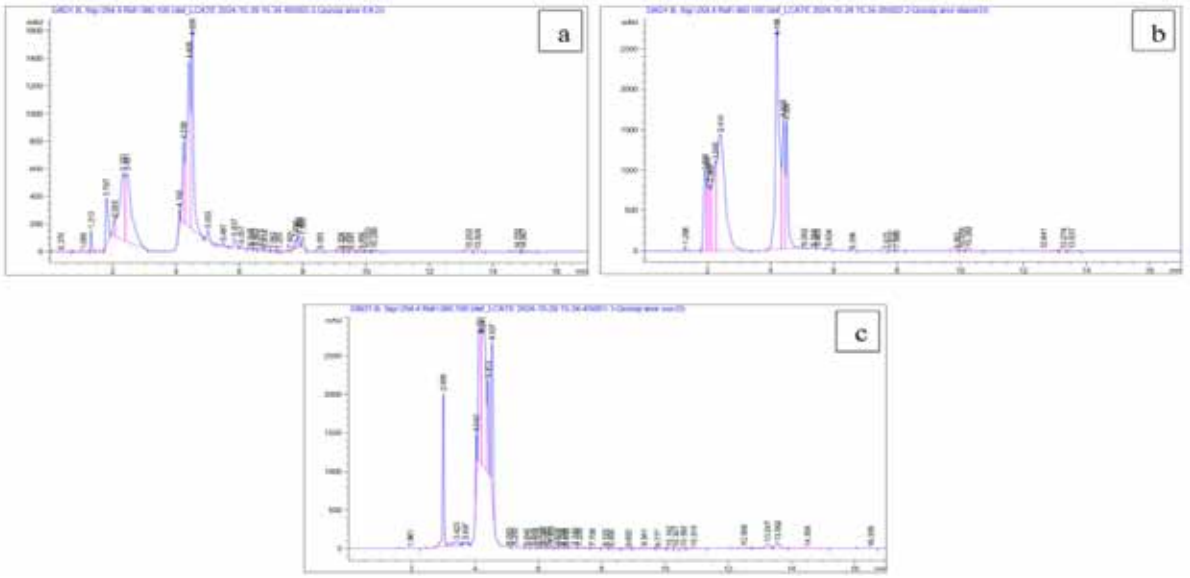


If we pay attention to the chromatograms of the extracts isolated from the Karadon kyzyl pomegranate variety (given in Figure 6), the intense peaks corresponding to the amounts of ellagic acid show that the extracts dissolved in all solvents contain the amount of ellagic acid, but the amount of gallic acid is significantly less in the ethyl alcohol solution. In all three of these chromatograms, it was found that there are polyphenol-based substances with a relatively long retention period in the column.

According to the quantitative analysis of gallic and ellagic acids, which contain polyphenols in Kazoki pomegranate peel, the peaks in the obtained chromatogram prove that the solutions mainly consist of two types of polyphenolic bases, i.e., ellagic and gallic acids. As a result of the results of the chromatograms, it can be said that the amount of polyphenol-based substances in the peel of all the selected pomegranate varieties is high enough. Among such substances, ellagic and gallic acids, which are studied in this

work and have important therapeutic functions for the human body, can be mentioned as an example.

Figure 7. *Chromatograms of the analysis of ellagic and gallic acids in the skin of the Kazoki pomegranate variety (studied in a-ethyl acetate, b-ethyl alcohol and c-aqueous solutions)*



By calculating the amount of substances appearing in the chromatograms, it was possible to know their exact size. According to it, the exact amount of two types of polyphenols dissolved in each solvent is quite different from each other. Table 3 below shows the results of chromatographic quantitative analysis of the amount of gallic and ellagic acid in the peel of pomegranate varieties.

Table 3. *Results of chromatographic analysis of the contents of gallic and ellagic acids in the samples*

Polyphenol name	**Achchikdona variety (ethyl acetate)**	**Achchikdona variety (ethanol)**	**Achchikdona variety (water)**	**Kayyum variety (ethyl acetate)**	**Kayum pomegranate (ethanol)**	**Kayyum pomegranate (water)**
			Concentration mg/g			
Gallic acid	0.058986	0.171384	0.07421	0.312362	0.00424	0.009564
Ellagic acid	0.225619	0.11314	0.16023	0.050285	0.060209	0.035067
Polyphenol name	Karadonkyzyl variety (ethyl acetate)	Karadonkyzyl variety (ethanol)	Karadonkyzyl variety (water)	Kazoki variety (ethyl acetate)	Kazoki variety (ethanol)	Kazoki variety (water)
Gallic acid	0.110716	0.056253	0.075428	0.038633	0.003345	0.047914
Ellagic acid	0.112478	0.097261	0.066825	0.052269	0.022138	0.060870

The results of chromatographic studies show that the content of substances in different solvents is different. For example, in the extracts obtained from the peel of the Achchikdona pomegranate variety, using ethyl acetate solution, it can be seen that the content of ellagic acid (0.225619 mg/gr) is higher than that of gallic acid (0.058986 mg/gr). On the contrary, in the ethyl alcohol extract of the same variety, it can be seen that the content of gallic acid (0.171384 mg/gr) is significantly higher than that of ellagic

acid (0.11314 mg/gr). In the aqueous extract of the Achchikdona pomegranate peel, the content of ellagic acid (0.16023 mg/gr) is also recorded in a larger amount than that of gallic acid (0.07421 mg/gr). The solubility of polyphenols from the peel of this pomegranate variety was recorded at the highest rate in ethyl acetate. The lowest solvent content among the samples was observed in the aqueous solution.

It can be seen that the extracts obtained from the peel of the Kayum pomegranate variety using ethyl acetate solution contain a high amount of gallic acid (0.312362 mg/gr) compared to ellagic acid (0.050285 mg/gr). It was found that the ethyl alcohol extract of this variety contains a low amount of gallic acid (0.00424 mg/gr) compared to ellagic acid (0.060209 mg/gr). The aqueous extract of the Kayum pomegranate peel also contains a low amount of ellagic acid (0.009564 mg/gr) compared to gallic acid (0.035067 mg/gr). The solubility of both substances in solvents from the peel of the Kayum pomegranate variety, according to the results, is high in ethyl acetate solution and highest in ethyl acetate. In this case, the aqueous solution also contains a low amount of gallic and ellagic acid.

Extracts obtained from the peel of the Karadon kyzyl pomegranate variety using ethyl acetate solution contain a low amount of gallic acid (0.110716 mg/gr), compared to ellagic acid (0.112478 mg/gr). It can be seen that the content of gallic acid (0.056253 mg/gr) in the ethyl alcohol extract of the same variety has a low amount of ellagic acid (0.097261 mg/gr). The content of ellagic acid (0.038633 mg/gr) in the aqueous extract of the Karadon kyzyl pomegranate peel is also noted, which is low in comparison to gallic acid (0.066825 mg/gr). The ethyl acetate solution also showed the best results in the Karadon kyzyl peel, and it can be said that the content of gallic and ellagic acid in this solvent is almost equal.

Extracts from the peel of the Kazoki variety, obtained using ethyl acetate solution, contain a lower amount of gallic acid (0.038633 mg/gr), compared to ellagic acid (0.522694 mg/gr). It can be seen that the content of gallic acid (0.003345 mg/gr) in the ethyl alcohol extract of the same variety is lower than that of ellagic acid (0.221384 mg/gr). The content of ellagic acid (0.047914 mg/gr) in the aqueous extract of the Kazoki pomegranate peel was also shown to be lower than that of gallic acid (0.608707 mg/gr). Compared to extracts from the peel of other pomegranate varieties, the amounts of the Kazoki pomegranate variety in all solvents were recorded at similar levels.

Conclusion

The fruit peels of four types of pomegranate varieties selected for the study were first dried and ground. The samples were extracted in three different solvents, and the amount of extract in them was measured separately. This revealed the ability of the crushed fruit peels to sorb three different solutions.

The composition of the extracts dissolved in three types of solvents from each sample was analyzed using high-performance liquid chromatography (HPLC). When the amounts of ellagic and gallic acid in the extracted extracts were studied, it was determined that each solvent contained these polyphenol-based substances, and their retention time on the chromatographic column was in accordance with the standard, which was ellagic and gallic acid. The resulting chromatograms were analyzed and the amount of substances was determined.

Based on the results of the chromatographic analysis, the amounts of polyphenol-based ellagic and gallic acids in each solvent were compared to each other and an optimal solvent analysis was performed, according to which it was determined that ethyl acetate had the highest efficiency among all solvents.

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