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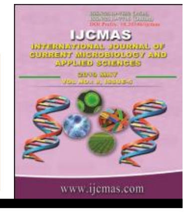


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# Identification of Chemical Content of Fruit and Peel's Extract of Some Varieties' of Pomegranate (*Punica granatum* L.)

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The goal of this research work is analysis of chemical compounds of extract of fruit and peel of some local varieties pomegranate ("Qora qayim", "Qizil anor", "Oq dona/Tuyatish", "Achchik dona"). The experiments were carried out by using methods of standard chemical synthesis. In the ethanol extract (70%) of the fruit peel of varieties "Qora qayim", "Qizil anor", "Oq dona/Tuyatish", "Achchik dona"were identified vitamins A (0.015–0.063 mg/100 ml), $B_1$ (0.034–0.064 mg/100 ml), $B_2$ (0.024–0.052 mg/100 ml), $B_5$ (0.023–0.038 mg/100 ml), $B_6$ (0.025–0.082 mg/100 ml), C (4.805–11.546 mg/100 ml), and E (0.014–0.612 mg/100 ml), as well as macronutrients (K, Ca, Mg, Na) and micronutrients (Mn, Zn, Cu, Se). The results show that the rind of pomegranate fruits (Punica L.) is a polycomplex property by chemical composition and a promising agent in the development of pharmacological drugs.

## Introduction

Pomegranate fruits have been widely used in ancient plants as a source of nutritional value. Pomegranate fruits are attracted by researchers' interest as they are rich for biologically active compounds, microelements and vitamins. The products obtained from the processing of pomegranate fruits are widely used in various juices, meat and fish processing products, pharmacology, and other medicinal products (Cowan, 1999; Dahanukar *et al.*, 2000; Das *et al.*, 2009; Radhika *et al.*, 2011).

Although the pomegranate fruit has been used in the treatment of some internal diseases and skin rashes in folk medicine, the seeds and sheaths remain as residuals (Kovalevskaya *et al.*, 2015). Pomegranate fruit contains 85.4% of water, 10.6% of total sugar, 1.4% of pectin and 0.2–1% of polyphenol compounds (Prakash and Prakash, 2011).

Pomegranate juice is rich with 85.4% of water, 1.4% of pectin, 0.2–1% of polyphenol compounds, and 10.6% of carbohydrates, while the sheath is rich with citric acid and colorful dubile substances (Prakash and Prakash, 2011). Also, the sheath of pomegranate is rich for pectin composition. Pomegranate seeds contain a large amount of fat, about 67% of which are unsaturated fatty acids, of which 40% are linoleic acid. The pomegranate seeds are rich in tocopherol (Gafizov and Semochkina, 1995; Ferrari *et al.*, 2010; Vroegrijk *et al.*, 2011; Afshar *et al.*, 2011; Prakash and Prakash, 2011; Valero *et al.*, 2014; Basiri, 2015; Gafizov, 2015; Kovalevskaya *et al.*, 2015).

It is worthwhile to note that based on the analysis of literature on the pomegranate's chemical composition, the amount of organic and inorganic substances in the juice, seed and crust of pomegranate fruits depends on the region and soil–climatic conditions.

The results obtained in connection with a wide range of physiologically active substances in the juice of pomegranate, peel's and seeds (Aviram *et al.*, 2006; Sreekumar *et al.*, 2014; Akhavan and Barzegar, 2017; Qamar Abbas *et al.*, 2018) show that broader and deeper research is still needed.

Based on the above–mentioned opinions, it is important to study some of the varieties of pomegranate (*Punica granatum* L.) fruits and peel's chemical compounds vegetated in the Mirzachul oasis, which occupies a large area in the Central Asian region, and the fruits and peel's. Therefore, the purpose of this research was to compare the varieties of certain pomegranate varieties' (*Punica granatum* L.) produced in the Mirzachul oasis and the amount of vitamins and macro–microelements contained in the fruit and peel's.

## Materials and Methods

In the researches, some of the local varieties of pomegranate (*Punica* L.) ("Qora qayim", "Qizil anor", "Oq dona/Tuyatish", "Achik dona") cultivated in Syrdarya region of the Mirzachul oasis of Uzbekistan were selected as the object.

Extraction and analysis of ethanol (70%) extract from the fruit and sheath of pomegranate (*Punica* L.) varieties is carried out jointly with the staff of the Laboratory of chemistry of lower molecular compounds at the Institute of Bioorganic chemistry.

High–performance liquid chromatography (HPLC) method of determining the vitamin D group (thiamin – $B_1$, riboflavin – $B_2$, pyrotoxin – $B_6$, pantothenic acid – $B_5$, biotin, cyanocobalamin – $B_{12}$, folic acid) has been reported that it is possible to obtain reliable results (Amidzic *et al.*, 2005; Aslam *et al.*, 2008; Sami *et al.*, 2014).

In the experiments, the method of identification of vitamins within the ethanol (70%) extract of native varieties of pomegranate (*Punica anata* L.) was carried out by using the standard method, published in the literature (Ellekr *et al.*, 2004).

This method (high accuracy: $p<0.05$) was based on the extraction of vitamins in an organic solvent medium and concentration of extracted extracts in the chromatographic method by fractionation of C18 in the method of HPLC and at the next stage, analysis of fractions by fluorescence and spectrophotometric detectors found. The detection of each individual substance in the content of the test sample contained in the test sample was determined by comparing the detection in the chromatographic system to the standard sample size and area of the "peaks" corresponding to the wavelength (Ellekr *et al.,* 2004; Rowayshed *et al.,* 2013). Experiments were carried out by using Agilent (USA) high–performance liquid chromatography equipment equipped with autosampler "Agilent Technologies–1200" (Fig. 1).

The sequence of methodologies for quantitative analysis of vitamins in HPLC is described in detail in the following manual (Ellekr *et al.*, 2004).

In the experiments, vitamins belonging to group B were determined by standard methods (Aslam *et al.*, 2008; Ringling and Rychlik, 2013).

The pomegranate (*Punica* L.) extract was powdered and dissolved in 80 mg of 5 ml of 0.1 normal $H_2SO_4$ solutions and stored at +25°C for 100 minutes. In the next step, using sodium acetate, *pH*=2.5 was filtered and prepared in 5 ml test sample in distilled water. Standard vitamin specimens were prepared using the appropriate methods to compare (Aslam *et al.,* 2008; Ringling and Rychlik, 2013).

Chromatographic process at +25 °C, HPLC–based diode matrix (DAD) detector for firm "Agilent Technologies–1200" (USA) was performed on high–performance liquid chromatography device. Chromatography conditions: column type – Eclipse XDB C18; the length of the column is 150×4,6 mm; sorbent volume – 5 micrometer; flow rate – 1 ml/min. Melting system: A: acetonitrile; B: Acetate buffer (rN–3.0–3.5). Chromatography conditions gradient % A/min: 0–2%/0–4 min; 2–20%/4.1–8 min; 20–0%/8.1–10 min. Detection wavelengths – 204 nm, 245 nm, 254 nm, 290 nm and 325 nm.

The vitamins A and E were found to be 325 nm and 290 nm respectively, according to the standard method (Sami *et al.*, 2014).

## Determination of macronutrients and micronutrients content of pomegranate (*Punica granatum* L.) fruits and peel's

In experiments, the pomegranate (*Punica granatum* L.) fruit and inductively coupled argon plasma were analyzed by Mass–spectrometry method (ISP–MC NEXION–2000, Perkin Elmer USA) (Rowayshed *et al.*, 2013).

The samples were sampled on an analytical scale and placed in autoclave with teflon and the acids breakdown method was used. The shredding works were performed for 35 min at the "Berghof" microwave breaking device (Germany) with MWS–3 + software. The tube was transferred to the measuring tubes as the amount of the resulting solution. Mass spectrometer was analyzed for autosampler analysis. The analysis was based on the multielement standard. The standard concentration of calibration was at concentrations of 10 mg/l (10 ppb) and 100 mg/l (100 ppb). Conditions of analysis: generator (plasma) voltage – 1500 W; flow speed – 0.7 ml/min.; nebulizer – 0.8 l/min.; argon (plasma) – 15 l/min.

## Solvents and chemicals

In the experiments were used methanol, acetonitrile ("Sigma–Aldrich", Germany) for the liquid chromatography technique (mobile phase). Standardized samples were used as standard vitamins (retinol acetate ($C_{22}H_{32}O_2$), α–tocopherol acetate ($C_{31}H_{52}O_2$), pyridoxine hydrochloride, riboflavin, thiamine, ascorbic acid, etc. ("Sigma–Aldrich", Germany).

## Data analysis

The results were statistically processed by a special software package OriginPro v. 8.5 SR1 (EULA, USA). The results of experiments processed mathematically–statistically using standard biometric methods (Lakin, 1990).

## Results and Discussion

The sheath and fruits of the first varieties of pomegranate were separated and mechanically mixed in sterile conditions. The weighted samples weight (5–10 g) were measured ("CAS_CUW220H"; China, the accuracy was 0.01 g) and the tubes (V=300 ml) were added and 50 ml of ethanol solution (70%) was added. Tubular magnetic fridge (Russia) 60 min. It was stored at +110 °C under temperature conditions. In the next step the mixture was extracted 2 times in 25 ml of ethanol solution (70%). The filtrate was dissolved in 100 ml of ethanol (40%) and was incubated for 10 min at 7000 rpm. during centrifugation. The deposition was removed and the resulting solution was used as a porcine extract in the next chemical analysis (Fig. 2).

## Analysis of quantity of vitamins

In the experiments, the following results were obtained in the Mirzachul oasis of the Republic of Uzbekistan, as a result of the HPLC analysis of some of the pomegranate varieties grown in Syrdarya region ("Qora qayim", "Qizil anor", "Oq dona/Tuyatish", "Achik dona") (Fig. 3; Table 1).

Pomegranate (*Punica granatum* L.) contains vitamins of A (0.164 mg/100 g), vitamin $B_1$ (0.123 mg/100 g), vitamin $B_2$ (0.07 mg/100 g), Vitamin C (12.9 mg/100 g), vitamin E (3.99 mg/100 g) that many other researchers have reported (Ellekr *et al.*, 2004).

In addition, using HPFC method, juice of varieties of pomegranate includes vitamin A (44–236.1 mg/100 ml), vitamin $B_1$ (30.8–124.1 mg/100 ml), vitamin $B_5$ (114.9–301.5 mg/100 ml), vitamin $B_6$ (12–90.3 mg/100 ml) was found to be vitamin C (10.4–35.4 mg/100 ml) (Akhavan and Barzegar, 2017). In the next series of experiments, the yield of local varieties of pomegranate varieties and macroelements and microelements in the sheath was analyzed as well (Table 2).

It is necessary to mention that during the conducted researches in the content of pomegranate peel’s (*Punica Granatum* L.) there were determined the followings Ca (338.5 mg/100 g), K (146.6 mg/100 g), Na (66.43 mg/100 g), P (117.9 mg/100 g), Fe (5.93 mg/100 g), Zn (1.01 mg/100 g), Mn (0.8 mg/100 g), Cu (0.6 mg/100 g), Se (1.03 mg/100 g) (Rowayshed *et al.*, 2013).

When analyzing the chemical composition of 23 types of commercial fruit juices by some researchers, K (209.3–251.7 mg/100 ml), P (9.3–15.1 mg/100 ml), Ca (1.1–14.9 mg/100 ml), Mg (2.1–10.4 mg/100 ml) and Na (2–12.8 mg/100 ml) were found (Eksi and Ozhamamci, 2009).

Also, the concentration of the chemical elements contained in the pomegranate plant (Zn, Mn, Cu, Fe, K, etc.) is also related to plant growth conditions, agrochemical processing (Hasani *et al.*, 2012; Hamouda *et al.*, 2016; Korkmaz *et al.*, 2017).

In particular, the concentration of polyphenols, macroelements (P, K, Mg, Ca, Na) and micronutrients (Zn, Cu, Mn, Fe) in the sheath of pomegranate (*Punica granatum* L.) has significantly changed during the ripening of the fruit (Parashar *et al.*, 2014).

Thus, it was determined that the concentration of macroelements K>Ca>P>Mg>Na and microelements concentration Fe>Zn>Cu>Mn has the sequence in the fruiting field (Parashar *et al.*, 2014) (Table 3).

Particularly, the pomegranate (*Punica granatum* L.) contains a large amount of vitamin C, which has a broad spectrum of vegetation–dependent geographical areas, as well as the age of fruit ripening (Kulkarni *et al.*, 2005). In particular, in the research, pomegranate fruits grown in different geographical regions contain 9.91–20.92 mg/100 ml (Tehranifar *et al.*, 2010), 312–1050 mg/100 mg (Dumlu and Gürkan, 2007), 52.8–72 mg/100 ml (Opara *et al.*, 2009).

The difference between the values of quantitative data presented in literature and the results obtained in experiments can be explained by the geographical region, varieties and harvesting periods of plant varieties (*Punica granatum* L.).

**Table.1** The amount of vitamins in the fruit and sheath varieties of pomegranate (mg/100 ml)

| # | Pomegranate varieties | | Vitamins | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | A | $B_1$ | $B_2$ | $B_5$ | $B_6$ | C | E |
| 1 | "Qora qayim" | Peel's | 0.015 | 0.034 | 0.052 | 0.031 | 0.045 | 11.546 | 0.037 |
| | | Fruit | 0.164 | 0.053 | 0.068 | 0.035 | 0.085 | 12.345 | 0.054 |
| 2 | "Qizil Anor" | Peel's | 0.04 | 0.032 | 0.033 | 0.025 | 0.032 | 8.330 | 0.014 |
| | | Fruit | 0.143 | 0.084 | 0.036 | 0.041 | 0.081 | 13.635 | 0.645 |
| 3 | "Oq dona/Tuyatish" | Peel's | 0.056 | 0.052 | 0.024 | 0.032 | 0.059 | 4.805 | 0.036 |
| | | Fruit | 0.132 | 0.069 | 0.048 | 0.036 | 0.074 | 7.092 | 0.547 |
| 4 | "Achik dona" | Peel's | 0.063 | 0.064 | 0.042 | 0.023 | 0.025 | 8.412 | 0.025 |
| | | Fruit | 0.157 | 0.072 | 0.054 | 0.038 | 0.082 | 14.236 | 0.612 |

**Table.2** The amount of macro elements contained in fruit and sheath of pomegranate varieties (mg/100 ml)

| # | Pomegranate varieties | | Macroelements | | | | |
|---|---|---|---|---|---|---|---|
| | | | *K* | *Ca* | *Mg* | *Na* | *P* |
| 1 | "Qora qayim" | Peel's | 0.783 | 0.438 | 0.115 | 0.340 | 0.341 |
| | | Fruit | 226.128 | 8.457 | 12.184 | 3.237 | 32.295 |
| 2 | "Qizil Anor" | Peel's | 0.432 | 0.356 | 0.196 | 0.567 | 0.428 |
| | | Fruit | 198.639 | 11.345 | 11.385 | 5.986 | 29.322 |
| 3 | "Oq dona/Tuyatish" | Peel's | 0.543 | 0.521 | 0.783 | 0.823 | 0.277 |
| | | Fruit | 213.541 | 9.134 | 11.234 | 4.257 | 35.273 |
| 4 | "Achchik dona" | Peel's | 0.674 | 0.670 | 0.234 | 0.451 | 0.450 |
| | | Fruit | 265.465 | 10.283 | 13.254 | 6.672 | 36.349 |

**Table.3** Amount of microelements in the fruit and sheath of pomegranate varieties (mg/100 ml)

| # | Pomegranate varieties | | Microelements | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Fe* | *Mn* | *Zn* | *Cu* | *Se* |
| 1 | "Qora qayim" | Peel's | 0.035 | 0.086 | 0.024 | 0.013 | 0.034 |
| | | Fruit | 0.597 | 0.123 | 0.314 | 0.163 | 0.656 |
| 2 | "Qizil anor" | Peel's | 0.047 | 0.084 | 0.039 | 0.023 | 0.017 |
| | | Fruit | 0.386 | 0.129 | 0.428 | 0.158 | 0.543 |
| 3 | "Oq dona/Tuyatish" | Peel's | 0.086 | 0.075 | 0.042 | 0.074 | 0.058 |
| | | Fruit | 0.219 | 0.126 | 0.510 | 0.164 | 0.498 |
| 4 | "Achchik dona" | Peel's | 0.054 | 0.092 | 0.049 | 0.028 | 0.076 |
| | | Fruit | 0.442 | 0.118 | 0.476 | 0.162 | 0.585 |

**Fig.1** Determination of vitamins in ethanol (50%) extract of native varieties of pomegranate (*Punica* L.) in HPFC method

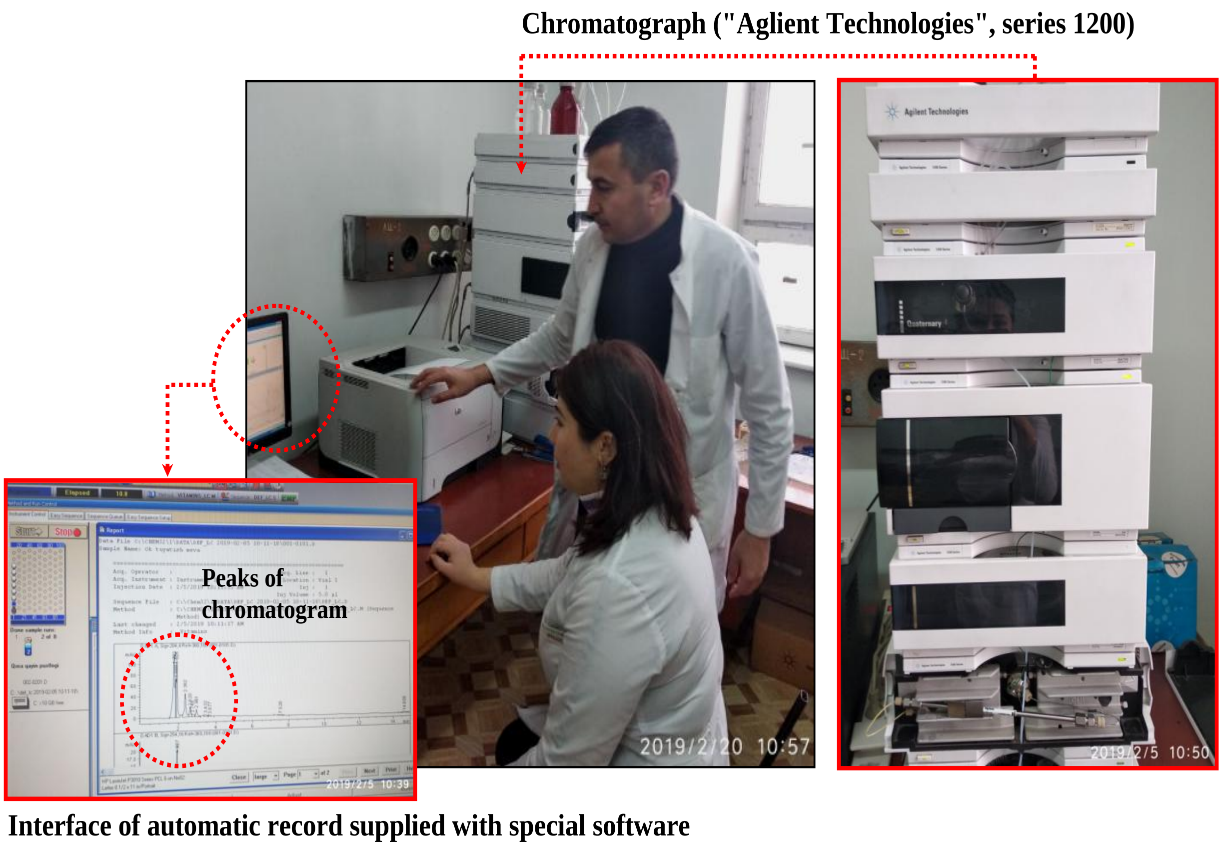



**Fig.2** Process of preparation of ethanol extract of Pomegranate (*Punica granatum* L.) fruit and peel's

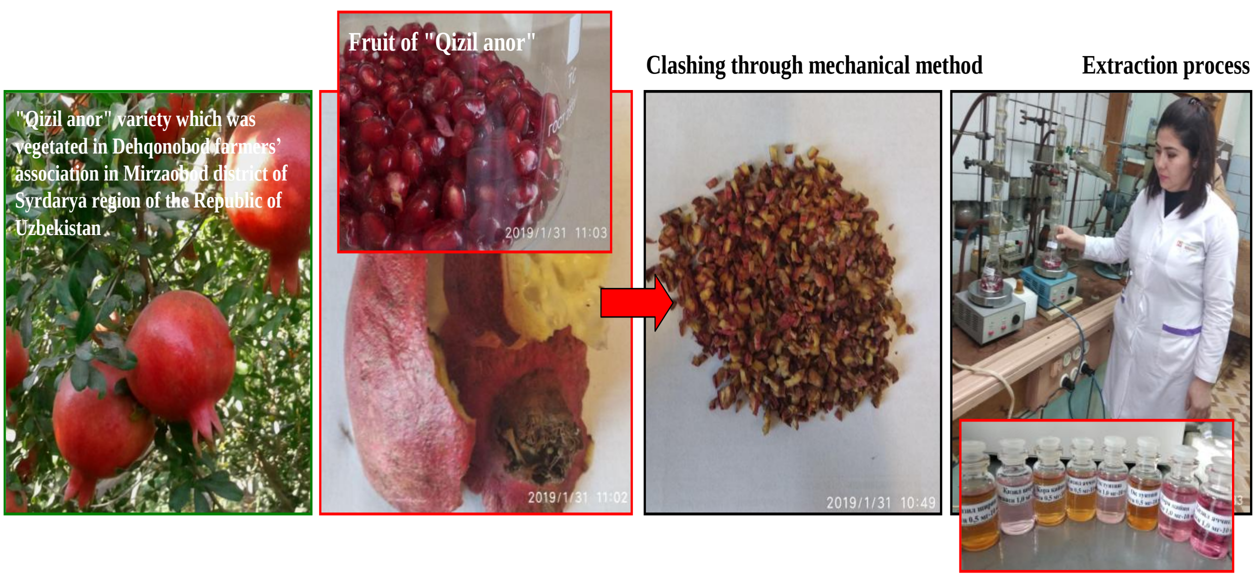
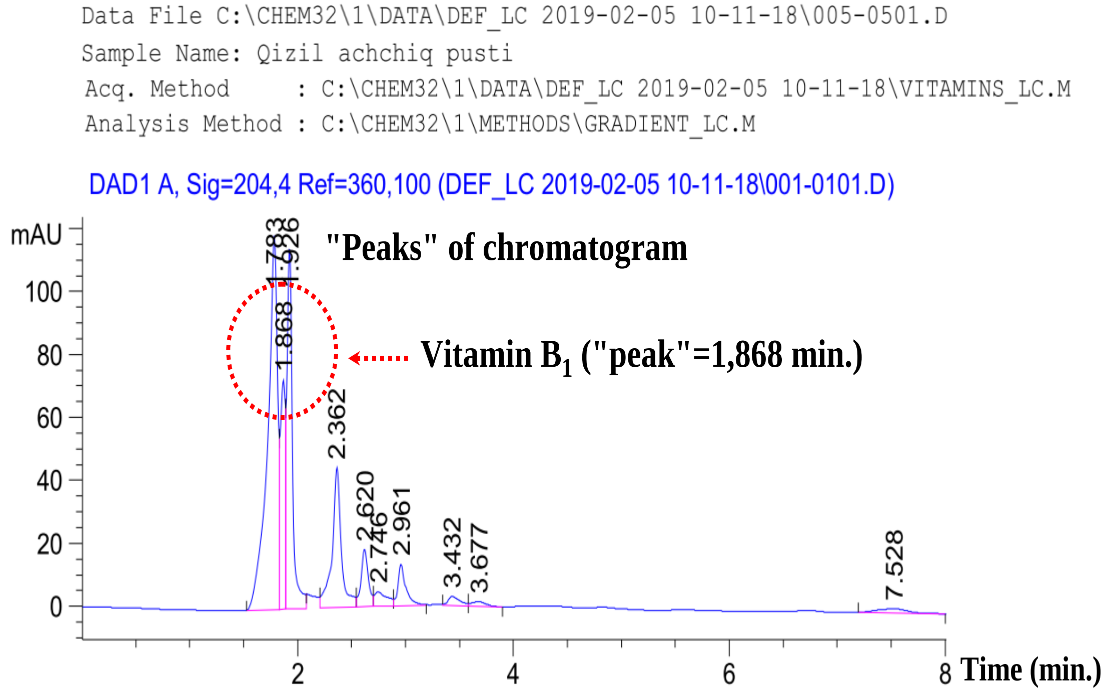


**Fig.3** Chromatogram of pomegranate extract




In conclusion, in this research, the Mirzachul oasis of the Republic of Uzbekistan has been acknowledged as a pomegranate produced in Syrdarya region by the varieties of "Qora qayim", "Qizil anor", "Oq dona/Tuyatish", and "Achchiq Dona" varieties of vitamins (A, B, C, E), as well as macroelements (K, Ca, Mg, Na, R) and microelements (Fe, Mn, Zn, Cu, Se).

The obtained results can be used as a scientific basis for the analysis of the chemical composition and pharmacological activity of pomegranate (*Punica granatum* L.) extract.

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