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Characterization of biochemical compounds in different accessions of pomegranate (*Punica granatum* L.) peels in Iraq

Asaad Mohammed Mahmood[1*], Hiran Luqman Jabar[1]

[1]*Department of Biology, College of Education, University of Garmian, Kalar, Kurdistan Region, Iraq*

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ABSTRACT

The polyphenol complex of pomegranate peel (*Punica granatum* L.), which makes up half of the pomegranate fruit, is important due to its roles in disease prevention. Although several studies examined the phenolic compounds in pomegranate peel, there is no any previous study on this plant part in Iraq using HPLC. This study was carried out to investigate the content of these bioactive compounds in pomegranate peels of 27 accessions collected from five different locations in the North and Middle of Iraq. In total; ten polyphenolic agents, including Gallic acid, Ellagic acid, Hydroxybenzoic acid, Caffeic acid, Luteolin, Quercetin, Catechin, Rutin, Punicalins and punicalagin were detected in the ethanolic extracts of the respective accessions using HPLC technique. Results showed that the range of concentrations (µg/ml) of the phenolic compounds, namely gallic acid (80.6-170.24), ellagic acid (50.32-134.36), punicalagin (5.2-130.32), luteolin (20-122.32), catechin (50.2-121), rutin (41-102.36), hydrobenzoic acid (26.2-101.96), quercetin (27.92-94.36), punicalins (29.92-90.36) and caffeic acid (38.32-81) were significantly varied in the selected accessions. The highest concentrations of all the respective phenolic compounds were characterized in the accession number 27, which was collected from Shahraban. This accession might be a valuable source for pharmaceutical products, specifically anti-cancers.

Keywords: Phenolics, Pomegranate Peel, HPLC, Anti-Cancer Agents, Pharmaceutical Importance.

1. Introduction

Polyphenols are organic chemical compounds and are known as secondary metabolites in plants, they are particularly distributed in fruits and vegetables[1]. Phenolic compounds are commonly featured by having one (simple phenolics) or more (polyphenols) hydroxyl substituents, which are directly attached to one or more aromatic or benzene rings. Phenolic compounds can be structurally classified into four general groups, namely phenolic acids, flavonoids, stilbenoids, and lignans[2]. Phenolics or polyphenolic compounds have been found to have therapeutic effects[3]. Numerous studies confirmed the presence and therapeutic effects of polyphenols, including phenolic acids, flavonoids and tannins in Pomegranate[4]. Pomegranates are shrub plants cultivated in west Asia and specifically around the Mediterranean region, these plants are also cultivated in other parts of the world (America), based on the suitability of the climate for their fruit growth[5]. Due to their medicinal and food industrial uses, Pomegranates have valuably shown attractive interest for researchers[6]. This attraction is a consequence of the occurrence of remarkable nutritional substances in this plant. Such substances include hydrolysable tannins, condensed tannins, flavonols, anthocyanins, and phenolic and organic acids compounds[7]. Despite the fact that Pomegranate peel (PP) represents about half weights of the inedible part of the pomegranate fruit and is considered an industrial waste, it contains well-known polyphenolic compounds at higher levels as found in the juice and seeds[8-10]. It has also been documented that PP is an excellent source of valuable bio compounds, including phenolic acids (hydroxycinnamic and hydroxybenzoic acids), flavonoids (anthocyanins, catechins) and hydrolyzable tannins (ellagic and gallic acids, pedunculagin, punicalin and punicalagin). Such compounds have also been proven with their health benefits[11-13], such as antioxidant, anti-inflammatory, and anti-cancer effects[14-17]. The effectiveness of biological activities of phytochemicals are limited to different environmental elements (such as soil water, soil salinity, soil fertility, temperature, carbon dioxide, illumination, ozone, and others which have a considerable impact on the secondary metabolic process) and biochemical contents[18]. Biochemical properties of pomegranate fruit appeared to be more sensitive to differences in microclimate, and significant differences in the antioxidant and phytochemical properties of pomegranate fruit were differed among fruits grown under different climatic conditions[19]. It is indicated that environmental conditions significantly affect pomegranate the content of total phenolics, including the two

* Corresponding author
E-mail address: asaad@garmian.edu.krd (Instructor).
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hydrolyzable tannins, punicalagin and punicalin, total anthocyanins[20].

Despite the pharmaceutical importance of PP; a few studies have been done on pomegranate accessions in Iraq, which only examined phenolic contents in pomegranate juice. There is no previous study to characterize pomegranate peel contents in Iraq, specifically in the Kurdistan Region. Therefore, our study is the only HPLC characterization of polyphenols in peel extracts of different pomegranate accessions in Iraq. We aimed at exploring the contents of phenolic compounds and specifically anti-cancer agents in pomegranate peels of different accessions collected from diverse locations of Iraq, specifically the Kurdistan region.

2. Material and Methods

2.1 Plant Sampling

Twenty-seven mature and healthy pomegranates of different accessions were collected from four different regions of Iraq. These accessions belong to five central populations grown in the different geographic and mountainous regions of the Iraqi Kurdistan Region (Table 1).

Table 1: Accession numbers, local names and locations of samples.

Accession No.	Local name	Population (location)	Accession No.	Local name	Population (location)
1	Salakhani	Halabja	**15**	Hanar bahari	Hiran
2	Swra xanmy	Halabja	**16**	Mahdawi, armasht	Zaxo (armasht)
3	Shini shirin	Halabja	**17**	Hanar shirin	Zaxo (armasht)
4	Shini trsh	Halabja	**18**	Dekani	Zaxo (armasht)
5	Pirm kayall	Halabja	**19**	Hanar paizi	Zaxo (armasht)
6	Melese	Halabja	**20**	Salimi	Kalar
7	Wonderful	Halabja	**21**	Karbalay (hashri)	Kalar
8	Holland (island)	Halabja	**22**	Shahraban 1	Shahraban
9	Larasha	Halabja	**23**	Shahraban 2	Shahraban
10	Salakhany + melese	Halabja	**24**	Shahraban 3	Shahraban
11	Tapasor	Hiran	**25**	Shahraban 4	Shahraban
12	Trshi twekle tank	Hiran	**26**	Shahraban 5	Shahraban
13	Trshi paizi	Hiran	**27**	Shahraban 6	Shahraban
14	Hanar Shirin	Hiran			

The first population was collected from Halabja (Al-Sulaimanyha Governorate) district, represented by 10 accessions. The second population was involved five accessions from Hiran (Erbil Governorate). The third population was from Armisht (Dohuk Governorate), where four accessions were collected. The fourth population from Kalar (Al-Sulaimanyha Governorate) included two accessions, and the last population consisted from six accessions from Shahraban (Diyalah four accessions). Phenolic compounds were characterized according to their retention times and UV data were compared with commercial standards.

2.2 Extraction of pomegranate peel extract

PP samples the respective accessions were manually removed from fruits and dried under shade. Therefore, samples for accession were mixed and then crushed into fine powders using an electronic grinder. In order to extract phenolic compounds, three grams of the homogenized powder was soaked in the 70% ethanolic solvent. The extraction process was done by Ultrasonic Bath at the ambient temperature for one hour and then followed by fine filtration[21]. 5 mL of the liquid extract was taken and used for extraction yield determination. In order to remove the solvent, a rotary evaporator was used and set at 40°C to the constant mass. The dried extracts were kept in brown glass bottles at 4°C.

2.3 HPLC analysis

Quantification of individual phenolic compounds was performed using reversed phase HPLC analysis. The HPLC chromatographic system was equipped with a UV detector (Chemstation, a Zorbax Eclipse Plus-C18-OSD, 25cm, 4.6 mm column). The column temperature was set at 30ºC using the gradient elution method. Eluent A (methanol) and eluent B (1% formic acid in water (v/v)) was used, as follows: initial 0-6 min, 40 % B; 7-16 min, 50 % B; and flow-rate of 0.7 mL/min. 100 µl were used as the injection volume for samples and standards. The spectra were acquired at 280 nm[21].

2.4 Identification and quantification of compounds

HPLC chromatogram was used to identify and quantify bioactive compounds in PP extracts by retention time, concentration, area and height, respectively. The results of the bioactive compounds of each sample are expressed as mg/ml of extracts[21].

2.5 Statistical analysis

The study was carried out at least in triplicate with constant results. Data were recorded as Mean ± Standard error mean significant differences between means were determined by one-

way analysis of variance (One-Way ANOVA) followed by Duncan's multiple range test ($P < 0.05$) was used to compare ten phenolic compounds abundant in the underlying accessions. All the data were analyzed by IBM SPSS Statistics 29.0.10 software.

3. Result

In order to find the anti-cancer components in pomegranate peels of 27 accession in Iraq. A total of 10 anticancer agents, including four phenolic acids (Gallic acid, Ellagic acid, Hydroxybenzoic acid, Caffeic acid), four flavonoids (Luteolin, Quercetin, catechin, Rutin), and two hydrolysable tannins (Punicalins, punicalagin (PG) were detected in the extracts of different pomegranate peel accessions. The HPLC chromatographic system expressed as µg/ml with a UV detector. The anticancer agents we distinguished in pomegranate peel showed a high amount of gallic acid followed by ellagic acid, punicalagin, luteolin, catechin, rutin, Hydrobenzoic acid, Quercetin, Punicalins and Caffeic acid.

3.1 Gallic acid

In the current study, gallic acid is the most dominant phenolic acid (Figure 1) among underlying pomegranate accessions. The highest gallic acid content (170.24 µg/ml) was detected in accessions 27, followed by accessions 26 and 4 (158.32 and 158.32 µg/ml), respectively. However, the gallic acid content was also statistically significant and the lowest amounts (80.6, 86.32 and 89.96) in accessions 16, 10, and 14, respectively.

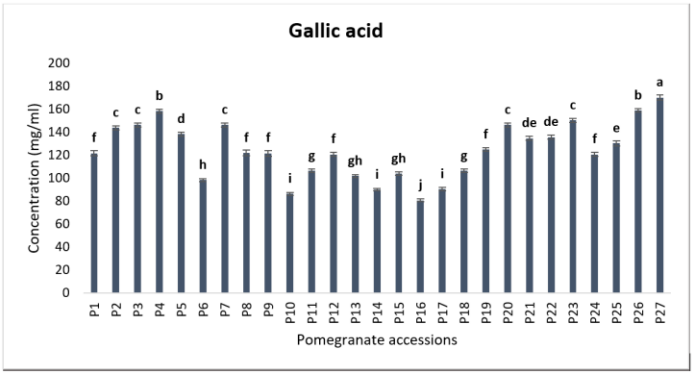

Figure 1: Gallic acid content (µg/ml) in 27 pomegranate accessions collected from different locations in the Iraqi Kurdistan Region. The values are expressed as means ± SE (n= 3), ($P < 0.05$). Means followed by the same letters within a column do not differ significantly according to Duncan's multiple range tests.

3.2 Ellagic acid

The HPLC chromatograms of the ellagic acid in peel extracts is clearly shown in (Figure). In Pomegranate peel extract (PPE), the maximum ellagic acid content was expressed as (µg/ml) and recorded by accession numbers 27 (134.36) and 7 (134.32), which they were statistically significant in comparison with all other accessions. Both accessions 26 (122.24), and 23 (121) observed high amounts of ellagic acid, despite their significant differences with accessions 7 and 27; however, the least recovery of ellagic acid from peel powder of the accessions coded as 18, 16 and14 showed lowest concentrations 50.32, 54.32 and 61.76 µg/ml, respectively.

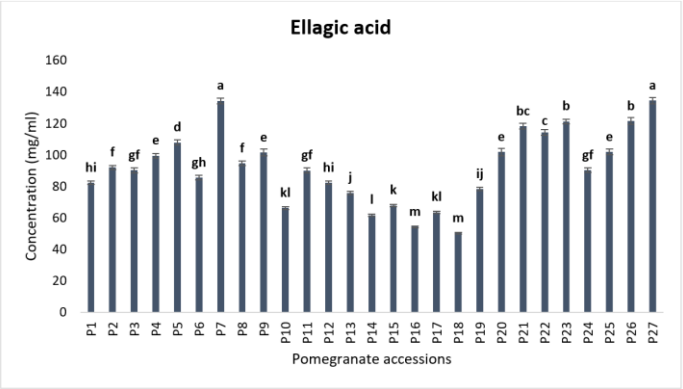

Figure 2: HPLC chromatograms of phenolic compound ellagic acid content (µg/ml) identified in 27 pomegranate peel accessions collected from different region in the Iraqi Kurdistan Region. The values are expressed as means ± SE (n= 3), ($P < 0.05$). Means followed by the same letters within a column do not differ significantly according to Duncan's multiple range tests.

3.3 Punicalagin

The results of punicalagin from 27 different types of pomegranate peel extract are presented in (Figure3). The punicalagin compound was estimated through HPLC and is expressed as µg/ml in peel pomegranate. The results showed the most abundant punicalagin content (130.32) was recorded in accession 27. It is significantly followed by the accession 26 (122.4) that is also statistically significant when it compared with all other respective accessions. The punicalagin content was comparatively very low and statically significant at lowest concentrations (24.32, 25.12 and 26.32) in samples 14, 13 and 16, respectively.

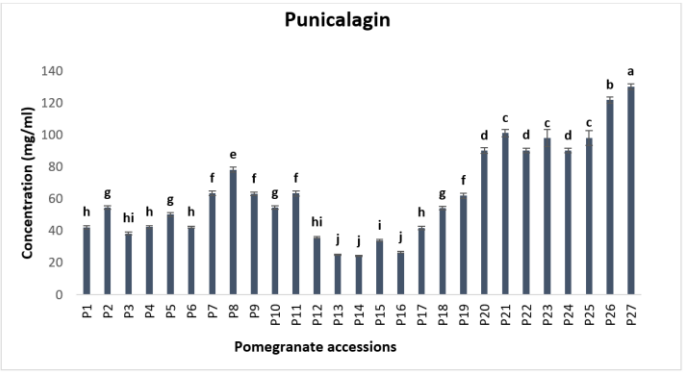

Figure 3: HPLC chromatograms of Punicalagin content (µg/ml) identified in 27 pomegranate peel accessions collected from different regions in the Iraqi Kurdistan Region. The values are expressed as means ± SE (n= 3), ($P < 0.05$). Means followed by the same letters within a column do not differ significantly according to Duncan's multiple range tests.

3.4 Luteolin

The amounts of the phenolic compound luteolin detected in the samples are presented in Figure 4. Results are expressed in µg/ml. The most abundant amount of luteolin (122.32) was demonstrated in samples 27. This result was statistically significant in comparison with all other accessions. The second highest amount (106, 93) of luteolin was detected in accession number 26.

Conversely, the slightest content of luteolin (19.52, 20, 24 and 24.88) was detected in samples 14, 13, 16 and 15, respectively.

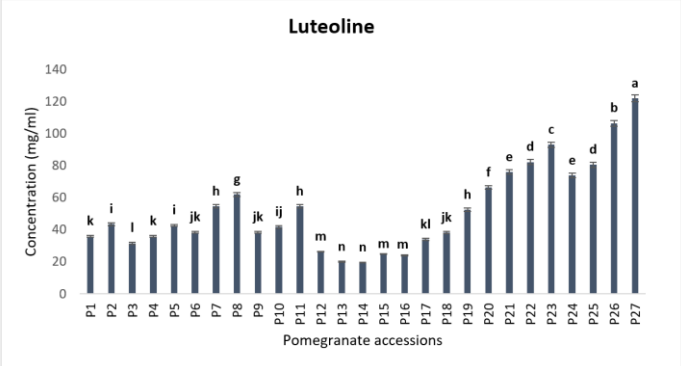

Figure 4: HPLC chromatograms of Luteolin content (µg/ml) identified in 27 pomegranate peel accessions collected from different regions in the Iraqi Kurdistan Region. The values are expressed as means ± SE (n= 3), (P < 0.05). Means followed by the same letters within a column do not differ significantly according to Duncan's multiple range tests.

3.5 Catechin

Identified catechin from 27 different types of PPE are presented in Figure 5. Results show that the accession numbers 27 and 26, observed the highest quantity of catechin (121 and 118.32, µg/ml). Such accessions were significantly different from all the studied accessions. A concentration of 114.32 µg/ml was expressed by the accession 23 which is considered as second significant amount of Catechine in the respective pomegranate accessions. In contrast, the lowest amount of Catechine (58.32, 54.32, 50.32, and 50.2) and) was determined in accessions 15, 18, 1 and 10, respectively, which they are statistically not significant with each other.

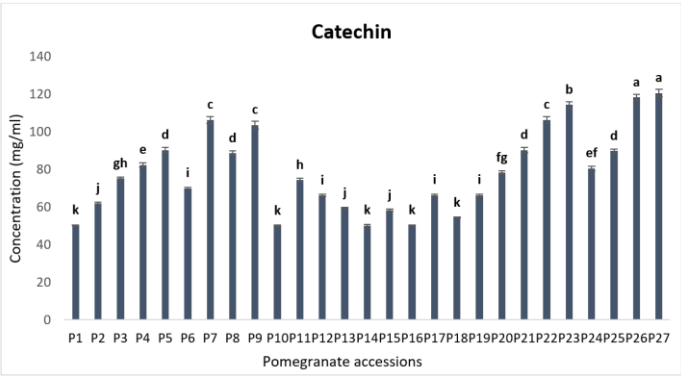

Figure 5: HPLC chromatograms of Catechine content (µg/ml) identified in 27 pomegranate peel accessions collected from different regions in the Iraqi Kurdistan Region. The values are expressed as means ± SE (n= 3), (P < 0.05). Means followed by the same letters within a column do not differ significantly according to Duncan's multiple range tests.

3.6 Rutin

The Rutin compositions of the PPE is given in (Figure 6)t, and all concentrations are expressed in µg/ml. The extreme concentration of the targeted phenolic compound rutin was determined in the accession number 27 (102.36 µg/ml). The results also indicated that the accession number 27 and 20 are statistically significant. On the other hand, the lowest concentrations were detected in accession numbers 13 and 14 with the concentration of (35.92 and 36.48 µg/ml) that statistically show no significant differences with each other.

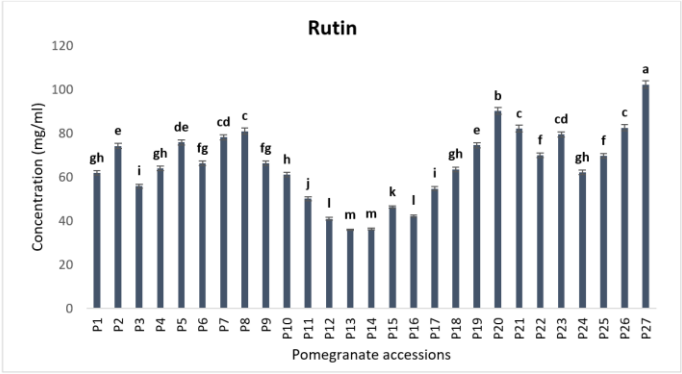

Figure 6: HPLC chromatograms of Rutin content (µg/ml) identified in 27 pomegranate peel accessions collected from different regions in the Iraqi Kurdistan Region. The values are expressed as means ± SE (n= 3), (P < 0.05). Means followed by the same letters within a column do not differ significantly from each other according to Duncan's multiple range tests.

3.7 Hydrobenzoic acid

The extraction results of Hydrobenzoic acid from twenty-seven different types of pomegranate peel powders are presented in Figure 7. The Hydrobenzoic acid compound was estimated through HPLC and is expressed in µg/ml of peel powders. The results indicated that the highest significant amount of Hydrobenzoic acid in both accessions number 27 and 26 peel powders. Among all respective accessions, the highest Hydrobenzoic acid content (101.96) was found in the accession number 27. Each accession 26,10 and 21 observed 90.32, 83.96 and 81.84 Hydrobenzoic acid contents respectively. Though, the lowest amounts (26.2, 26.32, 29.92 and 33.76, respectively) of the Hydrobenzoic acid were detected in accession numbers 16, 14, 13and 12. At the same time, the amount of Hydrobenzoic acid in other accessions observed moderate concentrations.

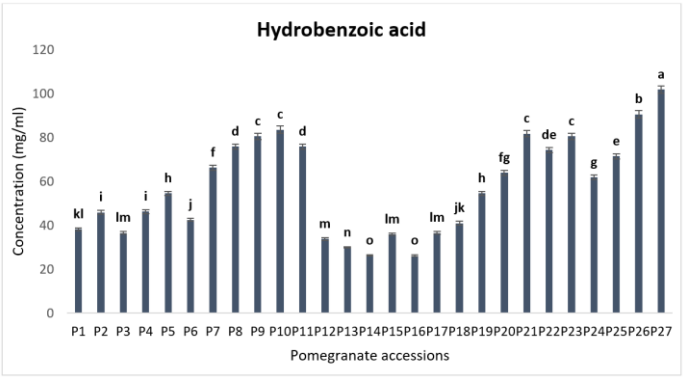

Figure 7: HPLC chromatograms of Hydrobenzoic acid content (µg/ml) identified in 27 pomegranate peel accessions collected from different regions in the Iraqi Kurdistan Region. The values are expressed as means ± SE (n= 3), (P < 0.05). Means followed by the same letters within a column do not differ significantly according to Duncan's multiple range tests.

3.8 Quercetin

The results of the quantification of Quercetin from pomegranate peel powders are shown in Figure 8. The highest amount of

Quercetin is characterized in the accession number 27 at concentration 94.36 µg/ml. This amount is significantly different from all the studied accessions. The results also indicated that peel powders of accession number P8, P21, P23 and P25 are significantly given higher amounts of quercetin in comparison with other studied accessions. accessions numbers 14, 13, 16 and 15 observed the least amount of Quercetin (27.92, 28.44, 32 and 35.96 µg/ml), respectively.

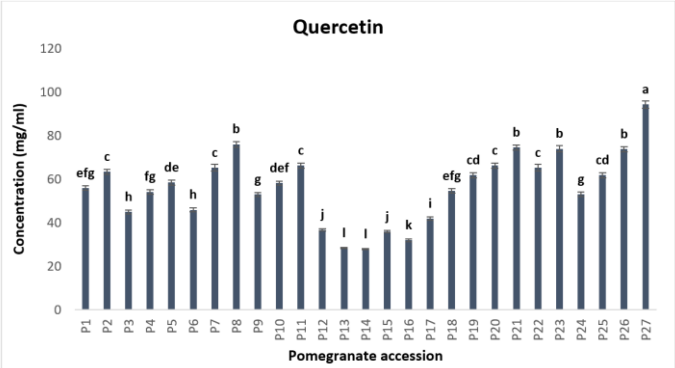

Figure 8: HPLC chromatograms of Quercetin content (µg/ml) identified in 27 pomegranate peel accessions collected from different regions in the Iraqi Kurdistan Region. The values are expressed as means ± SE (n= 3), (P < 0.05). Means followed by the same letters within a column do not differ significantly according to Duncan's multiple range tests.

3.9 Punicalins

Figure 9 shows the quantification of the Punicalins from pomegranate peel powders of 27 accessions collected from different locations in Iraq. The concentration of Punicalins compound is estimated through HPLC and is expressed as µg/ml. Results indicated that the significantly highest amount of punicalins is found in accessions 27 and 21 were (90.36 and 74.36) respectively. There were no significant differences between those two accessions, but they were significantly different from all other underlying accessions. All accessions 20, 22, 23 and 26 significantly observed high Punicalins. The lowest amount of Punicalins (29.92 and 33.96) were found in accessions 16 and 14, respectively, although they were significant when compared with each other.

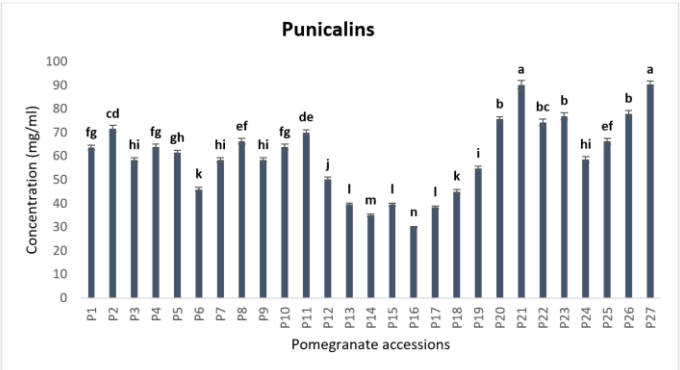

Figure 9: HPLC chromatograms of Punicalins content (µg/ml) identified in 27 pomegranate peel accessions collected from different regions in the Iraqi Kurdistan Region. The values are expressed as means ± SE (n= 3), (P < 0.05). Means followed by the same letters within a column do not differ significantly according to Duncan's multiple range tests.

3.10 Caffeic acid

After extraction and acid hydrolysis, the content of phenolic substances was determined by quantitative HPLC analysis. A typical HPLC chromatogram of PPE is presented in Figure 10. The highest amount of Caffeic acid (81, 78.32, 73.96, and 61.96 µg/ml) was found in the accessions 27, 7, 26 and 21, respectively. All of those accessions were significantly different when compared with each other. The statistically significant and maximum caffeic acid compound (81) was recorded in accession number (27). Whereas, the lowest concentration of caffeic acid was found in the accession number (16,14, (13,10) and (15,8) and concentrations were 36,38.32, 41, and 42.32, respectively.

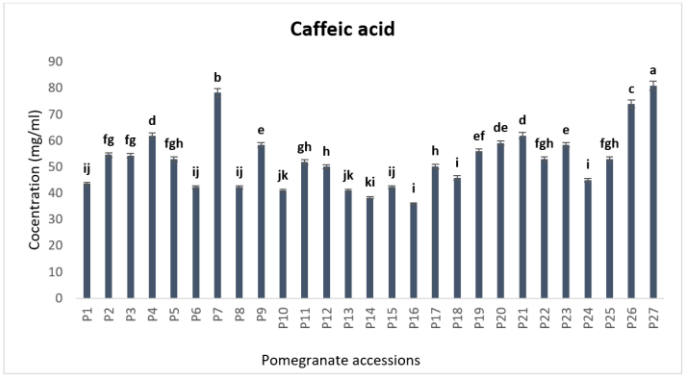

Figure 10: HPLC chromatograms of Caffeic acid content (µg/ml) identified in 27 pomegranate peel accessions collected from different regions in the Iraqi Kurdistan Region. The values are expressed as means ± SE (n= 3), (P < 0.05). Means followed by the same letters within a column do not differ significantly according to Duncan's multiple range tests.

4. Discussion

In the current research, 10 compounds in pomegranate peel extracts were characterized by HPLC and all the results were expressed as µg/ml.

4.1 Gallic acid

The highest contents of gallic acid in pomegranate peel extracts were obtained (170.24, 158.32 µg/ml) from accession numbers 27 and 26, respectively. It was determined that the gallic acid content in 70% ethanolic extract of a pomegranate peel of malas variety was 0.051 mg/g (51 µg/ml)[22]. Furthermore, the concentration of gallic acid from ethanolic extract of pomegranate peels in Saudi Arabia was 0.96 mg/L[23]. In addition, from seven main production areas in China, nine accessions of pomegranate peel showed that the formic acid aqueous extract of pomegranate peel was ranged from (9.62 to 72.77 µg/ml)[24]. While the current study is relatively revealed two to three-fold higher in the gallic acid content. Our results were very high with those previously obtained in Egypt from methanolic extract of pomegranate peel analyzed by HPLC which was 25.00 mg/kg[25].

However, a previous result on gallic acid content in pomegranate peel extracts in Indonesia showed that the 70% ethanolic extract of pomegranate peel by HPLC contained 617 µg/ml gallic acid[26]. It is also shown that the ethanolic extract of pomegranate peel for two pomegranate varieties (i.e., Bhagwa and Ganesh)

using HPLC were 1.60 and 0.70 mg/g (1600 and 700 µg/ml) respectively[27]. Similar results were observed by[28, 29]; they reported that the content of gallic acid from pomegranate peels from Iran and Egypt were 674.9871 µg/ml and 6041.1 µg/ml, respectively. Stojanovic et al. (2017) reported that the gallic acid compounds in Serbia's hydro-methanol 50% pomegranate peel extract was (5.52 mg/g) (5520 µg/ml)[30]. Additionally, gallic acid content range from (0.0–904.2 µg/ml) was identified in six Italian Pomegranate Peel extracts in 50% methanol detected by HPLC-PDA-ESI/MS[31]. Furthermore, methanolic extracts of pomegranate peels were performed using HPLC in Saudi Arabia was 7.89 mg/ml, and 7,890 µg/ml[32]. These variations may be due to several factors including the extraction procedure, differences in fruit accessions, experimental and environmental conditions (19; 20).

4.2 Ellagic acid

The highest characterization of ellagic acid in pomegranate peel extracts were 134.6, 134.3, 122.2 µg/ml obtained from accessions 27, 7 and 26, respectively. The contents of ellagic acid in three different solvents 30%, 50%, 70% ethanolic extraction were 93.01, 91.10, and 88.87 µg/ml, respectively[33]. Furthermore, in Saudi Arabia, ellagic acid content from ethanolic pomegranate peel mixture was 1.50 mg/L (1.50 µg/ml)[23]. Another study obtained (125.61 µg/ml) of ellagic acid in Egyptian pomegranate peel from methanolic extract analyzed by HPLC[25]. Our results observed higher contents in comparison with the above studies on ellagic acid of pomegranate peel. However, ellagic acid contents were ranged from (56.3 – 16,520.3 µg/ml) in six Italian Pomegranate Peel extracts dissolved in 50% methanol and detected by HPLC-PDA-ESI/MS[31]. The most abundant ellagic acid contents were identified in the methanolic extract of pomegranate peels of seven South African accessions and the concentrations were ranged from 46.87 to 209.44 µg/ml[34].

However, our results are lower than 80% ethanolic extract of pomegranate peel in an Iranian (Shirin Pust Ghermez) variety, which observed 628.6004 µg/ml of ellagic acid[28]. Similarly, absolute and 50% ethanolic extracts of pomegranate peels of the El Gabsi variety from Tunisia were given 1.1 and 3.2 mg/g (1,100 and 3,200 µg/ml) of ellagic acid, respectively[35]. Those variations indicate that the different plant varieties, environmental conditions, solvent type and concentrations, and detection methods are directly related to the concentrations of ellagic acid in plants.

4.3 Punicalagin

The highest contents (130.3, and 122.2 µg/ml) of punicalagin in the peel extracts were obtained from accessions 27 and 26, respectively. Our results are lower than the contents of Punicalagin obtained from Iranian pomegranate peel extract 146.55 mg/g (144,550 µg/ml)[22], and two pomegranate varieties (Bhagwa and Ganesh) 1.4 and 0.46 mg/g (1,400 and 460 µg/ml) respectively[27]. Moreover, contents of punicalagin from peel ethanolic extracts (macerated in ethanol and in ethanol-water mixture 50%) of the El Gabsi variety from Tunisia, observed 1.3 and 14.7 mg/g (1,300 and 14,700 µg/ml), respectively[35]. It is also found that the punicalagin content was 96.5 mg/g (96,500 µg/ml)[36] and 98.0 mg/g (98,000 µg/ml)[37]. Another study to examine the influence of genotypes on phenolic compounds observed differences in punicalagin contents of extracts from three different varieties, including Desi, Kandhari and Badana (110.00, 118.60 and 98.70 mg/g) in 50% methanol[38]. The punicalagin has also been identified in six Italian Pomegranate peels extracted in 50% methanol using (HPLC-PDA-ESI/MS), the Punicalagin α content was 500.9–23,092.7 mg/kg and Punicalagin β content was 491.0–24,455.1mg/kg[31]. These results are remarkably higher than the punicalagin content (130.3 to 24.32 µg/ml) in the current study. It is indicated that the underlying pomegranate varieties contain a deficient concentration of Punicalagin compared to the worldwide varieties.

4.4 Luteolin

The highest contents (122.3 and 106.4 µg/ml) of luteolin in pomegranate peel extracts were detected from accessions 27 and 26, respectively. These contents are higher than a previous study in Iran, where authors determined 15. 98 µg/ml of Luteolin[28]. A similar study in Beirut showed the variation in luteolin content in three different extraction methods, namely Solid-liquid extraction (35.8 µg/ml), Ultrasound-assisted extraction (40.3 µg/ml) and Infrared-assisted extraction (49.0 µg/ml) in 50% ethanol analyzed by HPLC[39]. These results are also lower than our results indicating the effect of extraction methods on the content of phenolic compounds, specifically luteolin. Moreover, researchers in Korea identified and quantified luteolin in two commercial pomegranate peels (Italy and Turkey) using HPLC coupled with diode array detection. However, they have not detected this compound in both pomegranate peel extracts[40]. In light of this result, the detection method is another factor that influence luteolin determinations in pomegranates. Another study performed in Italy, authors identified, and quantified luteolin in the peels of different pomegranate accessions that were dried differently, namely PSD (spray-dried Wonderful peel), CFD (Calabrian Wonderful peel freeze-dried), SAFD (South African Wonderful peel freeze-dried) and IC (Indian accession peel freeze-dried) analyzed by Reverse-phase–diode array detector–high-performance liquid chromatography (RP-DAD-HPLC). Results were 0.0025 and 1 mg/g (2.5 and 1000 µg/ml) in IC and CFD, respectively, but luteolin was undetectable in both PSD and SAFD[41]. It can be concluded that the dryness methods will directly affect contents of phenolic compounds, particularly luteolin. The luteolin from the ethanolic extract of pomegranate peel was not detected in an Egyptian variety, indicating the effect of genotypes on the content of luteolin in pomegranate peels[42].

4.5 Catechin

The highest contents of catechin (121, 118.32 and 114.36 µg/ml) in pomegranate peel extracts were obtained from accessions 27, 26 and 23, respectively. These results are higher when compared with those results reported from Iraq, Iran and Saudi Arabia. Those reports show that the catechin contents from ethanolic extracts were 34 µg/ml, 30.751 µg/ml and 21.04 µg/ml, respectively[23, 28, 43]. In another study, Catechin was identified in peels of seven South African pomegranate accessions in methanolic extract of pomegranate peel by HPLC-MS; the highest Catechin contents (28. 85 µg/ml) was detected in *Molla*

de Elche accession[34]. These results are also lower than our results.

However, the peel of pomegranate variety "Wonderful" dissolved in 80% ethanol and analyzed by HPLC was given 864.325 µg/ml of catechin[29]. Catechin concentration [21.8 mg/g, (21,800 µg/ml)] is reported in the 80% ethanolic extract of Pomegranate (cv bhagwa) peels analyzed by HPLC[44]. Catechin contents were 12, 3, 0.03 and 1.4 mg/g (12000, 3000, 30 and 1400 µg/ml) in PSD, CFD, SAFD and IC, respectively[41]. These values are remarkably higher than our results. In light of the above previous results, the content of Catechin is changeable in responses to the geographical distributions of plant sources.

4.6 Rutin

Both accessions 27 and 20 observed the highest contents (102.36 and 90.24 µg/ml) of Rutin in pomegranate peel extracts, respectively. 24.15 µg/ml of Rutin was detected in a previous study of 80% ethanolic pomegranate peel extract (Wonderful variety) in Egypt[29]. Furthermore, the levels of rutin in 60% ethanolic extract of pomegranate peel extract in a Chinese accession was 0.34 mg/100 mg (3.4 µg/ml)[45]. An ethanolic extract of pomegranate peel in an Egyptian accession observed 98.27µg/ml of rutin[42]. A methanolic extract from an Egyptian pomegranate peel was observed (2.65 µg/ml) of Rutin contents[25]. Such results is collectively lower than the highest contents of rutin in the present study. However, 1.5 mg/g (1,500 µg/ml) of rutin was detected in an Indian pomegranate (cv bhagwa) peel dissolved in 80% ethanolic extract[44]. In addition, 0.021, 0.3, 0.56 and 3 mg/g (21, 300, 560 and 3000 µg/ml) of rutin were detected in 95% ethanolic extract of IC, PSD, SAFD and CFD, respectively[41]. These results are higher than the results detected in the current study. It can be concluded that the rutin content in the underlying accessions are relatively high and can be pharmaceutically of value.

4.7 Hydrobenzoic acid

The highest contents of Hydrobenzoic acid in pomegranate peel extracts were 101.96 and 90.32 µg/ml. These results were observed by accessions 27 and 26, respectively. Our results are higher than the hydroxybenzoic acid content 15.68 mg/L (15.68µg/ml) of pomegranate peel[23]. However, in two previous studies, Hydroxybenzoic acid contents were 27.15 mg/100g (271.5 µg/ml) and 88.1278 mg/100g (881.278 µg/ml) when determined from ethanolic extracts of pomegranate peel using HPLC[42, 46]. Furthermore, Hydrobenzoic acid contents in 50% ethanolic extract of pomegranate peels were varied against three different extraction methods, including Solid-liquid extraction (201 µg/ml), Ultrasound-assisted extraction (225 µg/ml) and Infrared-assisted extraction (224 µg/ml)[39]. In comparisons, the latter results are higher than those contents determined in the current study. These differences might be due to the accession types, extraction procedures and machine types.

4.8 Quercetin

The highest contents 94.36 and 75.88 µg/ml of quercetin were detected in both accessions 27 and 8, respectively. Previous studies in Egypt[29, 42], Lebanon[39] and Saudi Arabia[23], authors determined 0.7 µg/gm, 4.39 µg/ml, 46.6 µg/ml and 2.35 µg/ml of quercetin in pomegranate peel extract respectively. Similarly, the concentrations of quercetin was 49.7 µg/ml and 27.7 µg/ml in two commercial (Turkey and Italy) pomegranate peels dissolved in 50% ethanol using HPLC coupled with diode array detection[40]. Those results are lower when compared with findings in the current study. Conversely, quercetin contents were varied in the peel pomegranate, which dried differently. IC (RP-DAD-HPLC), SAFD, CFD and PSD observed 0.02, 0.3, 2 and 3 mg/g (20, 300, 2000 and 3000 µg/ml), respectively[41]. These results indicate that the Quercetin concentrations are significantly affected by the extraction methods. Another study in India reported that the contents of the Quercetin in an ethanolic extract of pomegranate peel in the Ganesh variety was 0.03 mg/g (30 µg/ml) but for the Bhagwa variety was 0.12 mg/g (120 µg/ml)[27]. These results confirm the variability of Quercetin contents against differences in pomegranate varieties and accessions.

4.9 Punicalins

The highest Punicalins contents (90.36 and 74.36 µg/ml) were detected from accessions 27 and 21, respectively. Pomegranate peels from Serbia extracted by 70% ethanol yielded 164. 68 mg/g (164,680 µg/ml)[47]. Similarly, concentrations of Punicalins were ranged from 202.64 to 840.13 µg/ml from nine accessions of pomegranate peels in China extracted with formic acid[24]. Another study in Serbia, the Punicalins contents 708.18 and 747.89 mg/100g (7,081.8 and 7,478.9 µg/ml) in pomegranate peel extracts obtained by microwave-assisted extraction at different power 800 and 470, respectively[48]. HPLC quantification of Punicalins compounds in pomegranate peel extracted in 50% ethanol was 200.02 mg/g (200,200 µg/ml)[30]. Despite using different extraction methods and different varieties, all results are remarkably higher than our findings. It is an indication that the underlying accessions are poor with regards to Punicalins contents.

4.10 Caffeic acid

The highest contents of Caffeic acid in pomegranate peel extracts were 81 and 78 µg/ml, which they detected from accession s 27 and 7, respectively. These contents are higher than a previous study in Saudi Arabia. The authors determined 2.04 µg/ml of Caffeic acid from the ethanolic extract of pomegranate peel[23]. Another study in China, reported that the concentration of Caffeic acid in 60% ethanolic extract of pomegranate peel extract was 0.03 mg/100 mg (3 µg/ml)[45]. In addition, another study in Egypt observed 11.916 µg/ml of Caffeic acid in an ethanolic extract of PP[49]. These results are somewhat different from a study in Beirut that showed variations in Caffeic acid contents (199 µg/ml, 234 µg/ml and 326 µg/ml) in three 50% ethanolic extraction methods, including Solid-liquid, Ultrasound-assisted and Infrared-assisted extractions, respectively[39]. Other authors showed that contents of Caffeic acid in 80% ethanolic extract from pomegranate peels of Wonderful variety in Egypt and Shirin Pust Ghermez variety in Iran were 1220.37 µg/ml and 299.17µg/ml, respectively[28, 29].

Collectively, significant variations of phenolic compounds are observed in the underlying accessions. These variations might be due to the ontogenetic variability, which influences the biosynthesis and bioaccumulation of metabolic processes of

phenolics in opposite directions. For example, when plants are exposed to various destructive factors during ontogenies, including droughts or excessive rainfall and nutrient deficiencies, the necessity of continuous production and transportation of appropriate protective m6etabolites to various organs will become of higher value[50]. Moreover, plant ages of the studied leaf materials might be another factor for the diverse contents of the studied phenolic compounds. It is well documented that plant phenols are qualitatively and quantitatively varied at different genetic levels, and physiological and developmental stages. Such patterns are closely affect by environmental factors, including biotic, and abiotic stresses[51]. Our study reveals that wild and cultivated pomegranates are rich natural sources of phenolics, and are rich in active compounds with significant health-promoting benefits. This plant can be considered as agro-industrial plants in Iraq and the Kurdistan Region.

Conclusions

In conclusion, the highest content of the ten phenolic compounds, namely Gallic acid, Ellagic acid, Hydroxybenzoic acid, Caffeic acid, Luteolin, Quercetin, catechin, Rutin, Punicalins and punicalagin were characterized in the accession number 27. Among all phenolic compounds, Gallic acid allocated the highest content, averaging from 80.6- 170.24 to µg/ml. This accession might be an essential source of antioxidants, anti-inflammation and ant-cancer agents. It will be credible to investigate a deeper and more genetic and epigenetic detailed insight into the phenolic compositions in pomegranate peel of the accession 27, which may subsequently facilitate the health-promoting utilization of phenolics, particularly Gallic acid.

Conflict of interests

Authors have no conflict of interests

Authors contribution

Asaad M. Mahmood: Supervision, conceived and designed the analysis, edited the paper writing

Hiran Luqman Jabar: Collected samples, performed characterizations, performed the analysis, wrote paper drafts.

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