



(RESEARCH ARTICLE)



## Fatty acid composition and antioxidant capacity of pomegranate seed oil

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### Abstract

Pomegranate (*Punica granatum*) is a perennial herb with its long history and has been the symbol of health, fertility and spirituality. In addition to being consumed as fresh fruit, pomegranate is consumed as pomegranate juice, syrup, pomegranate syrup and many different product groups. In particular, pomegranate seeds, which are often produced as waste, are evaluated separately in terms of their fatty acid composition and antioxidant capacity. In this study, the fatty acid composition of pomegranate seed oils was determined by gas chromatography/mass spectrophotometry (GC/MS). Total antioxidant activity analysis was performed using DPPH (2, 2-diphenyl-1-picrylhydrazyl) method and antioxidant activity value was determined as trolox equivalent (TE). In the study, it was determined that the antioxidant capacity was 171.44 mg TE / 100g. According to GC / MS analysis, it was determined that the most dominant fatty acid was punicalic Acid (68.12%). Oleic acid (8.51%), linoleic acid (6.71%) and palmitic acid (5.55%) are the highest fatty acids after punicalic acid. It is clear that apart from the widespread consumption of pomegranate fruit, which is very beneficial for health, its seeds can also be used.

**Keywords:** *Punica granatum*; Pomegranate; Punicalic acid; Fatty acid; Seed oil; Antioxidant capacity

### 1. Introduction

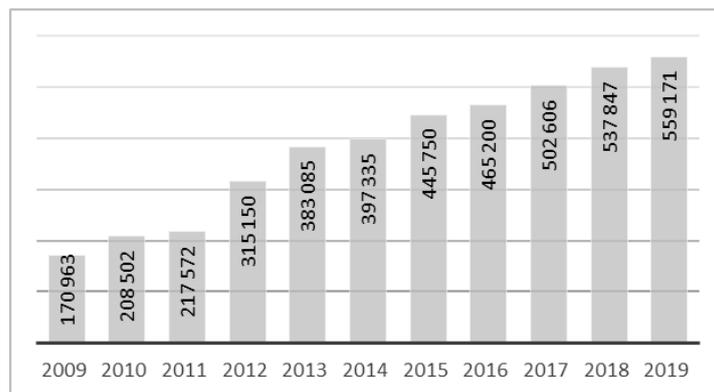
The belief in the healing effects of plants extends from the existence of humanity to the present day. The healing effects of plants are closely related to their active components, and their effectiveness varies from plant species to growing conditions. Among these plants, pomegranate has been used as a medicinal fruit due to its anti-cancer, immunosuppressive activities, antioxidant, antimicrobial, anti-inflammatory, and its use is increasing day by day [1, 2, 3, 4].

The current scientific appellation of the pomegranate, *Punica granatum*, is originated from Pomum (apple) granatus (granular) or seeded apple [5]. It is native to Asia and is a deciduous shrub or small tree. It has been utilized in oriental traditional medicine for a long time [6, 7, and 8]. Pomegranate has been accepted as a manifestation of life, longevity, health, splendor, abundance, awareness, morality and spirituality [9, 10]. Since ancient times, it has been accepted as a "healing food" because it has many beneficial effects on many diseases [1].

Pomegranate is commonly grown in Spain, Azerbaijan, China, Afghanistan, Turkey, USA, Armenia, India, Iran, Uzbekistan, the Middle East, Pakistan, Tunisia, and Israel, dry regions of Southeast Asia, Peninsular Malaysia, the East Indies and tropical Africa [11]. It is reported in different sources that the cultivated pomegranate production area is 3 hundred thousand hectares and the production is 3 million tons. Approximately 76% of this production takes place in India, Iran, China, Turkey and the United States [11, 12, and 13]. Among the substantial pomegranate produces, Turkey

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produced 559.171 tons and 537.847 tons in 2019 and 2018, respectively [14]. And as can be seen in Figure 1 the production level has been rising.



**Figure 1** Pomegranate Production of Turkey over Years (tons) [14].

In addition to being fresh-consumed fruit, pomegranate has also been used for different purposes such as a source of healing [15, 16, and 17]. The Pomegranate plant consists of various parts such as fruit skin, leaves, flowers, seeds, arils, fruit juice, roots and bark [18, 19]. The internal structure of the pomegranate fruit, which has a spherical shape, is irregular and consists of many tightly arranged grains, generally ranging from white to dark red. Each pomegranate seed consists of a seed surrounded by juice. In addition to being a fresh-consumed fruit, pomegranates are fruits valued for their decorative appearance, consumed and marketed as fruit juice, syrup (pomegranate syrup), teas, extracted grains, seed oils and other products. As a healing source, pomegranate juice contains relatively more minerals such as iron and elements such as calcium, selenium, chlorine, cobalt, chromium, cesium, copper, potassium, magnesium, manganese, molybdenum, sodium, rubidium and zinc. Recently, pomegranate consumption has been reported to have health benefits that have shown efficacy against a variety of diseases, including partially atherosclerosis, coronary heart disease, and prostate cancer, and infectious diseases, hypertension. Approximately 100 g edible pomegranate contains 68-72 kcal energy, 0.95-1.0 g protein, 6.1-7 mg vitamin C, 1-3 mg sodium, 3 mg calcium, 16.6-17.17 g carbohydrate, 0.3-0.7 mg iron, 3 mg magnesium, 0.07 mg copper, 259 379 mg Potassium, 0.3 mg niacin and many more nutrients [18, 20, 21, 22, 23, 24].

Pomegranate seeds, leaves and peels have many beneficial phytochemicals such as sterols, lignins and terpenoids. There are alkaloids in bark, root bark and leaves; simple galloyl derivatives in leaves; triglycerides and fatty acids in seed oils and seeds; organic acids, anthocyanins and phenolic acids in fruit juice; simple galloyl derivatives in leaves [25, 26, 27, 28]. There are flavonols in rind [29, 30], fruit [31, 32], bark and leaves [31, 33]. In addition, pomegranate peel, leaf, and fruit peel are rich in ellagitannins and gallotannins [34, 35, 36]. The presence of anthocyanidins and anthocyanins, catechins and procyanidins in peel and juice has been reported [33, 37,38]. The presence of estrogens in juice, seeds and shell extracts [39] and essential oils in seeds has been reported [5, 40]. Dried pomegranate seeds include the steroid estrogen estrone [41] and [42], isoflavone phytoestrogens such as genistein and daidzein, and the phytoestrogen kumestrol [43, 44]. It is known that pomegranate seeds constitute approximately 20% of the whole fruit by weight and are an important source of antioxidants. It has been suggested that among the beneficial effects of pomegranate seeds can prevent DNA damage, reduce the risk of cancer, and also alleviate menopausal symptoms [18, 45, 46, 47].

Punicic acid, the conjugated isomer, is the predominant fatty acid in pomegranate seed oil. This fatty acid, which is a long-chain omega-5 polyunsaturated fatty acid, is a conjugated isomer of linolenic acid like pomegranate seeds, *Momordica charantia*, *Trichosanthes bracteata*, *Trichosanthes kirilowii*, and *Trichosanthes cucumerina* seed oils are among the sources of punicic acid [48, 49]. It is known that punicic acid has antioxidant and antitumor properties. It is known to inhibit the growth of skin cancer, prostate cancer, colon cancer and similar types of cancer [50, 51, 52]. Lansky et al. [53] reported that pomegranate oil as a whole, and punicic acid in particular, causes the invasion (inhibits) of human prostate cancer cells.

Within the scope of this study, it was aimed to determine the fatty acid composition and antioxidant capacity of pomegranate seed, whose rich content was investigated by many studies. The determination of fatty acids belonging to pomegranate seeds rich in bioactive compounds and used in many fields from health to cosmetics will shed light on future studies

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## 2. Material and methods

### 2.1. Material

Pomegranate seeds used in this study were obtained from local companies and kept at room temperature until analysis.

### 2.2. Method

#### 2.2.1. Fatty Acid Analysis

Oil extraction of the pomegranate seeds we preserved was made with n-hexane. Analysis of fatty acids was made according to the method of IUPAC IID19 [54]. Gas chromatography (GC; Perkin Elmer, Shelton, USA) was used to determine the fatty acid composition. Flame ionization detector (FID) and column (30 m × 0.25 mm ID, 0.25- $\mu$ m film thickness) were used for chromatographic separation. The oven temperature was kept for 10 min by increasing to 220 °C by 120 °C (2 min) and 5 °C/min; Injector and detector temperatures were set to 280 °C and 260 °C, respectively. Results were calculated in % with mean deviations.

#### 2.2.2. Determination of DPPH radical scavenging capacity

Free radical scavenging activity was analyzed by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay [55, 56]. 100  $\mu$ l of methanolic sample was homogeneously mixed with fresh 2.9 ml DPPH solution (in 0.1 mM methanol). The mixture was kept at room temperature for 30 minutes in the dark. The mixtures were read using a spectrophotometer at 517nm absorbance. The trial was run in 3 replicates and the values obtained were determined as Trolox equivalent (TE) corresponding to 100g sample.

#### 2.2.3. Statistical analysis

Fatty acid analyzes were carried out in three replicates according to a random trial design. Variance analysis was performed with the SAS-JMP statistical program (SAS Institute Inc., Cary, NC). The means were compared with the least significant difference (LSD) at  $P < 0.05$ .

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## 3. Results and discussion

In this study analysis of total oil amount, fatty acid composition and total antioxidant activity in *Punica granatum* seeds were made. According to the results, the total amount of fat was determined as 1.44g/100g. As a result of GC analysis, the major compound in the fatty acid composition was determined as Punicic Acid (68.12%). This fatty acid was followed by oleic acid (8.51%), linoleic acid (6.71%) and palmitic acid (5.55%), respectively (Table 1).

Total antioxidant capacity was determined as 171.44 mg TE/100g. Punicic acid, which is determined as the major compound, is a long chain polyunsaturated omega-5 fatty acid and it has been emphasized by many researchers that it is the main component of pomegranate fatty acids. In one of these studies, Melo (2012) reported that punicic acid is the dominant fatty acid [3]. Similarly, the amount of punicic acid was found to be 78.3-83.4% by Pande and Akoh [57], 70-76% by K yralan et al. [58], 62.43-73% by Elshaarawy & Nahapetian [59].

Fadavi et al. [60] examined the fatty acid content of the seeds of 25 different pomegranate varieties in Iran. In the study, it was found that punicic acid was the most dominant fatty acid with 31.8-86.6% and it was found to be linoleic acid (0.7-24.4%), oleic acid (0.4-17.4%), stearic acid (0.3-9.9%), palmitic acid (3.7-16.7%) and arachidic acid (0-3.9%) followed. Similarly, all these fatty acids have been identified in our study. Lauric and arachidic acid, which the researchers stated that they could not be detected, were detected in our study, and the presence of lignoceric acid was not detected in our study. It is predicted that these similarities and differences may arise from differences between types and locations. In a different study not mentioning lauric acid and arachidic acid, Hernandez et al. [61] found that in 3 different pomegranate varieties have punicic acid (66.76-79.29%) followed by linoleic (4.98-7.74%), oleic (4.70-5.91%), stearic (% 1.6-2.38) and palmitic (2.99-3.83%) acid. It is noteworthy that only 5 fatty acids were mentioned in the study.

**Table 1** Fatty acid composition of *Punica granatum* Seed (Figures in parentheses are angular transformation values of percentage of response)

	Fatty acid	Percentage (%)
1	Punicic Acid C18:3	68.12 <sup>a</sup> (55.68)
2	Palmitic Acid (C16: 0)	5.55 <sup>cd</sup> (13.62)
3	Palmitoleic Acid (C16: 1)	0.07 <sup>h</sup> (1.53)
4	Stearic Acid (C18: 0)	3.86 <sup>e</sup> (11.34)
5	Oleic Acid (C18: 1n9c)	8.51 <sup>b</sup> (16.96)
6	Linoleic Acid (C18: 2n6c)	6.71 <sup>c</sup> (15.01)
7	Arachidic Acid (C20: 0)	0.83 <sup>f</sup> (5.23)
8	Eicosenoic Acid (C20: 1n9c)	0.95 <sup>f</sup> (5.59)
9	Behenic Acid (C22: 0)	0.35 <sup>g</sup> (3.39)
10	Caproic Acid (C6: 0)	0.05 <sup>h</sup> (1.28)
11	Tricosanoic Acid (C23:0)	0.06 <sup>h</sup> (1.34)
12	Lauric Acid (C12:0)	0.02 <sup>h</sup> (0.88)
13	Myristic Acid (C14: 0)	0.05 <sup>h</sup> (1.29)
14	Heptadecanoic Acid (C17: 0)	0.03 <sup>h</sup> (1.00)
15	Others	4.82 <sup>de</sup> (12.68)

LSD: 1.61\*\*\*, P<0.05\*, P<0.01\*\*, P<0.001\*\*\*, The LSD value was calculated according to the angle transformation value. Different letters (a-h) indicate significant differences by the LSD test (p ≤ 0.05).

Siano et al. [62] compared the physicochemical and biochemical features of the oils of pomegranate (*Punica granatum*), sweet cherry (*Prunus avim*) and pumpkin (*Cucurbita maxima*) seeds. While cherry and pumpkin seed oils contain high amounts of linoleic acid, pomegranate seed oil has been found to contain polyunsaturated fatty acids and monounsaturated fatty acids with eicosapentaenoic acid. They also found catalpic acid, Eptadecanoic acid and trace amounts of nervoic acid in pomegranate seed oil. Differently, while these fatty acids were not detected in our study, fatty acids such as Myristic, Heptadecanoic, Behenic Acid were determined in our study, unlike this study. In addition, researchers have suggested that pomegranate, cherry and pumpkin seed oils can be used as both protective and functional components in food, medicine and cosmetics, and can contribute to the prevention of diseases. In addition, they emphasized that these oils have the potential to create a natural filter against radiation in pharmaceutical, food and cosmetic products because they provide UV light absorption. They found that especially pomegranate and pumpkin seed oils have high absorption in UV-A and UV-B.

Fernandes et al. [63] analyzed the fatty acid and vitamin E compositions of nine pomegranate varieties grown in Spain. It was determined that the total fat content varied between 4.44-13.70% and the most prevailing fatty acid in all cultivars was punicic acid (77.3-83.6%). This was followed by linoleic acid with 3.9-5.4% and oleic acid with 3.1-5.7%, respectively. Differently, in this study, while punicic acid was detected in a lower concentration, linoleic Acid (6.71%) and oleic acid (8.51%) were detected in higher amounts.

Studying the fatty acids of seed oils of 21 different pomegranate varieties, Elfalleh et al. [64] stated that qualitatively, there are no significant differences between varieties in terms of fatty acid composition. Quantitatively, they determined that the dominant fatty acid was linolenic acid (main isomer is punicic acid) (44.51-86.14%). In addition, researchers found oleic acid (3.03-12.88%), linoleic acid (3.57-13.92%), stearic acid (1.68- 15.64%), palmitic acid (3.13-11.82%), lignoceric acid (< 2.53%), gadoleic acid (0.50-4.91%), arachidic acid (< 1.70%) and myristic acid (< 0.85%).

Thitipramote et al. [65] investigated the bioactive compounds, protoanthocyanin contents and their antioxidant activities of pomegranate peel and seed in their study. They obtained the highest bioactive content results from acetone extractions. Antioxidant activities differed, and according to DPPH, better results were obtained from seed extracts (0.034-0.512mg TE/g) from shell extracts (2069-2957 mg TE/g) and the highest results were obtained in extractions

with acetone. Differently, higher antioxidant capacity (171.44 mg TE/100g) was determined in this study, and it is predicted that this may be based on many other reasons from location differences to harvest season.

Guo et al. [66] found that pomegranate fruit had the highest total value in their study on the antioxidant activities of the peel, seed and flesh parts of 28 different fruits. It is noteworthy that pomegranate peels in particular have a very high antioxidant activity.

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#### 4. Conclusion

Pomegranate fruit, which has a long history, has very important chemical components for human health. In this study, oil was obtained from the seeds of the pomegranate plant. As a result of the analysis of the fatty acid composition, it was determined that the dominant fatty acid was punicalic acid (68.12%). Additionally, the presence of oleic acid, linoleic acid, palmitic acid and stearic acid was also detected. This study has also proven that pomegranate seeds, which are mostly produced as a result of fruit processing, have important fatty acids. It is clear that pomegranate seeds can be included more in human diets due to their rich content.

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

The authors declare that there are no conflict of interest.

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