



## The Phenolic Content and Antioxidant Capacity of Pumpkin, Rosehip and Pomegranate Seeds

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### ABSTRACT

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Food waste is a significant problem worldwide. These food wastes, often discarded without any preliminary processing, can be rich in bioactive substances. In this study, the aim was to identify the phenolic compounds in pumpkin, rosehip and pomegranate seeds, which are frequently consumed in winter. For this purpose, ultrasonically assisted methanolic extracts were prepared from seeds separated from other waste parts (such as shells, stems, and leaves). The results indicated that pomegranate seeds had the highest total phenolic content, with  $45.6 \pm 3.1$  mg GAE/g sample ( $P < 0.05$ ). Similarly, pomegranate seeds also showed the highest total antioxidant capacity in both CUPRAC ( $114.7 \pm 2.6$  mg torolox/g sample) and DPPH ( $71.2 \pm 3.8$  mg torolox/g sample) analyses ( $P < 0.05$ ). In phenolic profiling analysis using HPLC-PDA, syringic acid was the most abundant compound in pumpkin seeds, (-)-catechin in rosehip seeds, and punicalagin derivatives in pomegranate seeds.

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### Introduction

Food waste, including leftovers and pre-cooked food, is a biodegradable material generated from a various source, such as homes, hotels, and food processing industries. Utilizing waste and byproducts from food processing and underutilized agricultural products, has recently gained increased attention. A significant amount of food resources or industrial food residues could be preserved if these materials, or even portions of them, are converted into new products. Such applications help maximize the available resources and contribute to creating a variety of new foods, feed, fertilizer, medicine and cosmetics. Maximizing the nutritional and industrial potential of waste, underutilized agricultural products, and byproducts is crucial. Consequently, this can lead to a substantial reduction in waste disposal.

Fruit waste, a major source of municipal waste, contains natural bioactive compounds (Deng et al., 2012). Although synthetic bioactive compounds are approved for food use in many countries, consumer acceptance is declining. As a result, there is a growing interest in using natural bioactive compounds instead of synthetic ones

(Nieto et al., 2023). Recent studies have shown that various fruit and vegetable by-products are an ideal source of bioactive compounds, which can be reintroduced into the food chain as natural additives or within food matrices to create functional foods and nutraceuticals (Vilas-Boas et al., 2021). For instance, grape seed and peel extracts positively impact human health, offering benefits such as anti-inflammatory effects, hyperglycemia prevention through improved insulin sensitivity, and radioprotective properties (Vodnar et al., 2017). The peel, seeds, leaves, and pomace from blueberries are also rich sources of anthocyanins, flavonoids, and phenolic acids (Tylewicz et al., 2018). In this regard, winter fruits like pomegranate, rosehip, and pumpkin have substantial potential applications.

In Türkiye, 1001 tons of organic rose hips were produced on 47998 decares of land (Tarım ve Orman Bakanlığı, 2022a), while the total amount of pumpkin production was recorded as 744300 tons in 2022 (FAO, 2024), and pomegranate production is carried out on approximately 300 thousand decares of land and the

amount of pomegranate production was determined as 600021 tons (Tarım ve Orman Bakanlığı, 2022b). In a study examining the amount of agricultural waste in Türkiye, it was recorded that among the horticultural crops grown in our country, pomegranate fruit has a production volume of 537847 tons and 8745 tons of it is waste, similarly, 21802 tons of waste is generated from 87207 tons of pumpkin production (Ünlü et al., 2023)

Rosehip (*Rosa canina L.*), a member of the *Rosaceae* family, is widely distributed geographically (Chrubasik et al., 2007). Rosehip seeds contain phytochemicals such as carotenoids (2.92 µg/g), phenolic compounds (2554 µg/g), and ascorbic acid (1798 µg/g). Moreover, rosehip-seed oil is rich in polyunsaturated fatty acids (Ilyasoğlu, 2014). Rosehip is valued in various production processes due to its numerous nutritional and health benefits, including its use in treating infections, inflammatory illnesses, flu, chronic pain and in skin care and antiulcer therapies (Guimarães et al., 2010). The pumpkin (*Cucurbita pepo L.*) is commonly used in traditional medicine and as a food source. Its plant extracts, derived from various parts, exhibit a wide range of biological activities, including antibacterial, antidiabetic, anticancer, hypocholesterolemic, antioxidant, immunomodulatory, antimutagenic, anthelmintic, and anti-bladder stone properties (Krimmer-Malešević, 2020). Pumpkin seeds contain essential nutrients, including ash (4.1–5.27%), fiber (2.3%), micro- and macroelements, fats (38–49%) and protein (25.9–35.5%), and omega-3 and omega-6 fatty acids (Karaş et al., 2024). Although often discarded, pumpkin seeds are high in fatty acids and amino acids, which can enhance food flavor when used as an ingredient or byproduct (Lemus-Mondaca et al., 2019). Pomegranates (*Punica granatum L.*), with over 500 cultivars available globally (Passafiume et al., 2019) are known in traditional medicine for their antibacterial, antifungal, antiviral, antiinflammatory, anthelmintic, and antioxidant compounds (Viuda-Martos et al., 2010). Pomegranates are most consumed raw or in fruit juices, jellies, and jams (Alexandre et al., 2019). After juice extraction, the rind and seeds constitute approximately 54% of the fruit (Ko et al., 2021). Despite this, most pomegranate by-products are still discarded (Alexandre et al., 2019; Fourati et al., 2020), causing environmental issues. Pomegranate seeds are an excellent source of unsaturated fatty acids, including arachidic, palmitic, palmitoleic, oleic, linolenic, stearic, and linoleic acids (Eikani et al., 2012). They also contain significant amounts of polyphenols, including hydroxybenzoic acids, tannins, anthocyanins, flavonoids, and hydroxycinnamic acids (Falcinelli et al., 2017; Smaoui et al., 2019). Pomegranate seeds offer numerous health benefits, including anticancer, antiinflammatory, antioxidant, and antimicrobial effects in vivo (Viuda-Martos et al., 2010a).

In the literature most of the studies focused on the fatty acid content and proximate analysis of these selected seed samples. Therefore, this study aimed to investigate the potential phenolic content and antioxidant activity of selected seeds. Pumpkin, rosehip, and pomegranate samples were purchased and separated into flesh, peel, and seeds. The seed samples were subjected to targeted analysis to determine their bioactive content.

## Material and Method

### Sample Preparation

Rosehip, pumpkin and pomegranate were randomly purchased as triplicates from a local market in Istanbul. The seeds were separated from the selected varieties and, all seed groups were pooled as replicates. Liquid nitrogen in a precooled IKA A11 Grinder (Staufen, Germany) was used for grinding the samples. Ground samples were then freeze dried in a freeze dryer (ALPHA 1-2 LDplus, Osterode am Harz, Germany) at  $-60^{\circ}\text{C}$  with 0.001 mbar until a constant weight was reached and stored at  $-20^{\circ}\text{C}$  until further analysis.

### Chemicals

In the present study, all chemicals and reagents used were either analytical or HPLC grade. All chemicals and external standards (gallic acid, *p*-hydroxy benzoic acid, syringic acid, ellagic acid, ferulic acid, cyanidin-3-O-diglucoside, peonidin-3-O-glucoside, (-)-catechin, (-)-epicatechin, (-)-epigallocatechin gallate, and kaempferol) were purchased from Sigma-Aldrich (Taufkirchen Germany) for polyphenol determination.

### Extraction Procedure

The extraction protocol was carried out with a weighed 0.1 g freeze-dried powder and mixed with 4 mL of 75% MeOH containing 0.1% formic acid, following the method described in a previous study (Bakır et al., 2023). The mixture was ultrasonicated for 15 min at  $4^{\circ}\text{C}$  (USC900TH, VWR ultrasonic cleaner, Radnor, PA, USA) and then centrifuged for 10 min at  $4^{\circ}\text{C}$  and  $9500 \times g$  (Hettich Universal 32R, Tuttlingen, Germany). The extracts were filtered through a 0.45 µm membranes filter, and the supernatants were kept at  $-20^{\circ}\text{C}$  until analysis.

### Total Phenolic Content

Total phenolic content (TPC) was calculated using the Folin-Ciocalteu reagent as previously described (Singleton et al., 1999). Briefly, 100 µL of sample solution, 900 µL of distilled water, and 1.5 mL of Folin-Ciocalteu reagent (0.2 N) were mixed and allowed to react for five minutes. Then, 1.2 mL of a 7.5%  $\text{Na}_2\text{CO}_3$  solution was added. Following 90 minutes of incubation at room temperature, the absorbance was measured at 765 nm. The results were reported as mg gallic acid equivalent (GAE)/g of sample, with a linear range of 0.001-0.6 mg/mL ( $R^2 = 0.997$ ).

### Total Flavonoid Content

Total flavonoid content (TFC) was measured following the method of Dewanto et al. (2002). In summary, 1.25 mL of distilled water was added to 250 µL of the sample in an analysis tube. Next, 75 µL of 5%  $\text{NaNO}_2$  solution was added, and the mixture was left for 6 minutes. Then, 150 mL of a 10%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  solution was added. After 5 minutes, 0.5 mL of 1 M NaOH was added, and the total volume was adjusted to 2.5 mL with distilled water. Absorbance was measured at 510 nm against a blank. Results were reported as mg rutin equivalent/100 g of sample (linear range: 0.001-0.8 mg/mL,  $R^2 = 0.991$ ) and mg catechin equivalent/g of sample (linear range: 0.001-0.6 mg/mL,  $R^2 = 0.997$ ).

### Determination of Total Antioxidant Capacity

The total antioxidant capacity (TAC) of seed samples was evaluated using two common methods for fruits and vegetables. The standard curve was prepared using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and results were expressed as  $\mu\text{mol trolox/g}$  sample. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis was performed according to the method of Kumaran and Karunakaran (2006). In brief, 100  $\mu\text{L}$  of sample was combined with 2 mL of DPPH solution (0.1 mM) and kept for 30 minutes at room temperature in the dark. Absorbance was measured at 517 nm (linear range: 0.001-0.2 mg/mL,  $R^2=0.995$ ). The cupric reducing antioxidant capacity technique (CUPRAC) technique was also applied (Apak et al., 2004). In summary, 25 mL of Neocuproine solution (0.039 g in 96% EtOH), 250 mL of  $\text{NH}_4\text{Ac}$  buffer solution (19.27 g in distilled water), and 250 mL of  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  solution (0.4262 g in distilled water) were used. 1 ml of each prepared solution and 1 mL of distilled water were added to an analysis tube containing 100  $\mu\text{L}$  of the sample. The mixture was incubated for 30 minutes, and absorbance was measured at 450 nm ( $R^2=0.997$ ; linear range: 0.001-0.8 mg/mL).

### HPLC Analysis of Seeds Phenolic Profile

An HPLC connected to a photodiode array (HPLC-PDA) detector was used to assess the phenolic profiles of the prepared sample extracts. HPLC analysis followed the method described by Capanoglu et al. (2008). The extracts placed in 1 mL vials and analyzed on a Waters W600 HPLC system equipped with a PDA (Waters 996) detector. The stationary phase a Luna C18 reverse phase column (Phenomenex, Utrecht, The Netherlands) maintained at 40°C. The mobile phase consisted of solvent A (distilled water+0.1% (v/v) trifluoroacetic acid) and solvent B (acetonitrile+0.1% (v/v) trifluoroacetic acid). A linear gradient flow was applied as follows: 95% A and 5% B at 0 min; 65% A and 35% B at 45 min; 25% A and 75% B at 47 min; returning to the initial state at 54 min. The flow rate was a 1 mL/min. Chromatograms were recorded at 280, 312, 360, and 520 nm. Identification was based on retention times and unique UV spectra, and quantification was performed using external standard curves. Standard calibration curves, with linearity ( $R^2 \geq 0.992$ ) in the range of 1–200  $\mu\text{g/mL}$ . The LOD and LOQ were 0.2–0.4 ppm and 0.6–1.2 ppm respectively. Standards were used to express results as mg standard/100 g sample.

### Statistical Analysis

Data were analyzed using the SPSS Statistics Program (21st version, IBM, New York, NY, USA) via one-way analysis of variance (ANOVA). Tukey's Range Test was applied to determine significant differences between TFC, TPC, TAC, and HPLC-PDA ( $P<0.05$ ). All analyses were conducted in triplicate, and results were presented as mean value $\pm$ standard deviation.

## Results and Discussion

### TPC and TFC of Seeds

Variations in TPC and TFC of seed samples are demonstrated in Figure 1. Statistical analysis showed significant differences among samples for both assays

( $P<0.05$ ). Pomegranate seeds had the highest TPC at  $45.6\pm 3.1$  mg GAE/g sample. In contrast, rosehip and pumpkin seeds have much lower amounts ( $4.5\pm 0.32$  mg GAE/g sample and  $1.23\pm 0.06$  mg GAE/g sample, respectively). Elmastas et al. (2017) pointed out rutin and catechin as the two most prevalent phenolic compounds in Rosa species, while Park et al. (2010) identified equol, kaempferol, quercetin, and catechin as major flavonoids in pomegranate, with catechin specifically found in seeds. On the other hand, different levels of rutin and catechin were found in pumpkin seeds (Ahmed et al., 2024). Given this situation, TFC values of samples were calculated using both catechin and rutin equivalents, considering the diversity in dominant flavonoids among seed samples.

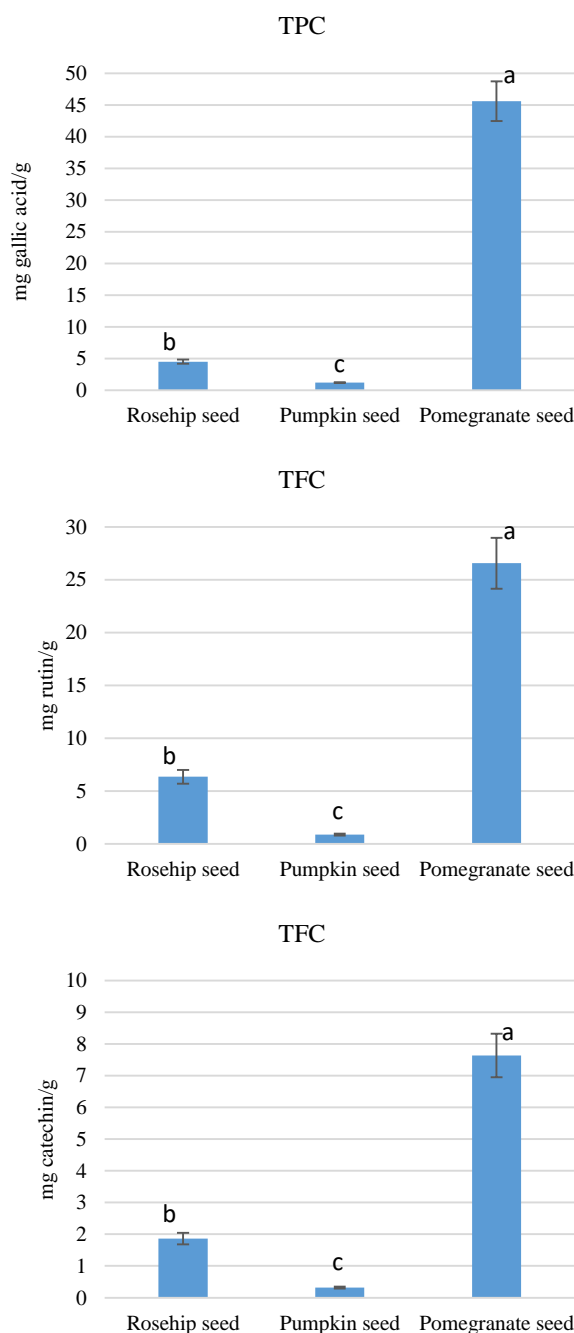


Figure 1. TPC and TFC analysis of samples

Table 1. TAC values of seed samples.

Samples	CUPRAC	DPPH
Rosehip seed	5.98±0.53 <sup>b</sup>	21.54±0.31 <sup>b</sup>
Pumpkin seed	2.99±0.27 <sup>b</sup>	2.60±0.15 <sup>b</sup>
Pomegranate seed	458.36±10.37 <sup>a</sup>	284.62±15.26 <sup>a</sup>

\*Results were expressed as  $\mu\text{mol trolox/g}$  sample. Different letters refer the statistical differences between samples in same column ( $P < 0.05$ ).

The highest TFC was observed in pomegranate seeds ( $7.6 \pm 0.7$  mg catechin/g sample and  $26.6 \pm 2.4$  mg rutin/g sample), followed by rosehip seeds ( $1.9 \pm 0.2$  mg catechin/g sample and  $6.4 \pm 0.7$  mg rutin/g sample) ( $P < 0.05$ ). Despite differences in catechin ( $0.32 \pm 0.03$  mg /g sample) and rutin ( $0.89 \pm 0.1$  mg/g sample) content, TFC values in both equivalents were not statistically significant in pumpkin seeds. ( $P > 0.05$ ). The low metabolite content of samples could cause these results.

For rosehip, TPC ranged from  $31.08 \pm 0.19$  to  $52.94 \pm 0.47$  mg GAE/g in Gümüşhane, Türkiye (Demir et al., 2014), while varieties from Erzurum, Türkiye, showed values between  $10.74 \pm 3.09$  to  $14.35 \pm 2.62$  mg GAE/g (Macit et al., 2023). In contrast, a study on rosehip seeds from Gumushane, Türkiye found  $2.55$  mg GAE/g (Ilyasoğlu, 2014), and in Pakruojis District, Lithuania, rosehip seeds had a range from  $130.04$  to  $207.31$  mg GAE/100 g for five different species (Medveckienė et al., 2020). Similarly, regarding pumpkin seeds, Nigerian seeds dried at  $40^\circ\text{C}$  had a TPC of  $32.90$  mg GAE/g (Akomolafe et al., 2016). Another study on South-Western Nigeria pumpkin seeds showed raw samples at  $4.28$  mg GAE/g increased to  $13.35$  mg GAE/g after roasting at  $100^\circ\text{C}$  for 30 min (Akomolafe, 2021). Sargodha, Pakistan pumpkin seeds, had a TPC of  $224.61 \pm 1.60$  mg GAE/100 g powder (Hussain et al., 2021). The drying method could explain the relatively lower TPC content of pumpkin seed comparing to literature. Moreover that, TPC for pomegranate seeds in California ( $3.39$  mg GAE/g sample) was almost three times that found in pomegranate juice ( $1.03$  mg GAE/g sample) (Ambigaipalan et al., 2017). Pomegranate seeds from Natanz, Shahreza, and Doorak in Iran had TPC values between  $72.4 \pm 10.02$  mg and  $73 \pm 13.35$  mg GAE/g sample (Derakhshan et al., 2018). These findings showed that there is a wide range variation at TPC and TFC content of seed samples. This variation could be attributed to differences in plant origin and growing conditions.

TFC values for rosehip genotypes collected from different parts of mountain Rtanj (Serbia) were reported as  $196.26$  mg rutin/g sample (Tumbas et al., 2012), while *Rosa moschata* from the Kullu Valley in Northwestern India, showed TFC values of  $498 \pm 0.50$  mg rutin/g in water and  $557.33 \pm 0.57$  mg rutin/g in methanol (Shashni & Sharma, 2022). In *Rosa canina* and *Rosa pimpinellifolia*, TPC was recorded at  $22.42 \pm 1.26$  mg catechin/g and  $26.43 \pm 5.76$  mg catechin/g, respectively (Macit et al., 2023). These differences could stem from varying plant material, growing condition and solvents used in extraction. The TFC of Sargodha, Pakistani pumpkin seeds (sun- and hot air-dried) was higher than that of the peel and flesh, at  $139.37 \pm 1.07$  mg catechin/100 g powder (Hussain et al., 2021). Roasting of China's Beitun pumpkin seeds increased TPC and TFC, with the highest values at  $200^\circ\text{C}$  being roughly 1.56 and 2.81 times higher than that of unroasted seeds (Peng et al., 2021). This increase might

result from cell structure breakdown and potential Maillard reaction products that react with Folin-Ciocalteu reagent, explaining higher literature values (Peng et al., 2021). Similarly, in a study, the TFC content of pomegranate seed samples from the Alentejo region of Portugal (Campos et al., 2022) varied between 7-21 mg catechin/g sample. TFC of some pomegranate seed samples from Natanz, Shahreza, and Doorak provinces in Iran ranged from  $7.55 \pm 2.12$  mg rutin/g sample to  $38 \pm 6.38$  mg rutin/g sample (Derakhshan et al., 2018.). In Moroccan pomegranates, TFC in the peel and seeds varied from  $52.13$  to  $62.64$  mg rutin/g and from  $1.76$  to  $2.11$  mg rutin/g, respectively (Sabraoui et al., 2020). Notably the TFC content of peel extracts from Beni Mellal, Berkane, and Settat was found to be 36, 24, and 25 times higher, respectively, than the TFC content in seeds. On the other hand, red grape seeds had the highest TFC value ( $330.60$  mg catechin/g), which is approximately 2.5 times higher than that of white grape seeds ( $133.08$  mg catechin/g) and 300 times higher than of pulp (averaging  $1.08$  mg catechin/g), according to a study on grape seeds and pulp (Wongnarat & Srihanam, 2017). The TPC and TFC contents reported in this study differ from those in other studies in the literature. Variations may be due to differences in plant material, sample preparation, and extraction conditions used in analysis. Additionally, some studies found higher TPC or TFC content in peels, while others found higher in seeds.

#### TAC of Seeds

TAC levels of samples were measured using two different assays and are presented in Table 1. According to the results, the antioxidant activity of pumpkin and rosehip seeds was quite similar ( $P > 0.05$ ), whereas both differed significantly from that of pomegranate seeds ( $P < 0.05$ ). Pomegranate seeds contained a higher amount of antioxidative compounds. Additionally, this study provides an opportunity to compare the CUPRAC and DPPH assays. Generally, the CUPRAC assay yields elevated results compared to the DPPH assay. Here, pomegranate seeds demonstrated a higher trolox equivalent with CUPRAC assay ( $P < 0.05$ ), while rosehip seed exhibited a lower equivalent ( $P < 0.05$ ). Despite the different working mechanisms of the TAC assays, TAC levels of pumpkin seeds did not differ significantly between the two assays ( $P > 0.05$ ).

It is crucial to evaluate the antioxidant activity of plant tissues in relation to their biological activity, as compounds possessing antioxidant potential can protect the human body from the harmful effects of free radicals, slow down aging, and help prevent formation of tumors (Cai et al., 2004). As a result, a large number of studies on the antioxidant activity of various plants have been published in the scientific literature. Due to the complex mechanisms underlying antioxidant activity, which depend on a variety of factors, including temperature, solvent, phenolics'

chemical structure, and the medium's pH, it is not feasible to determine the antioxidant activity of a product using a single method (Viuda-Martos et al., 2010). The purple DPPH radical is reduced to 1,1-diphenyl-2-picrylhydrazine in the DPPH assay, which works with hydrophobic systems. The reducing power of antioxidants can be measured by CUPRAC assay, which has the advantages of neutral pH of 7.0, which is close to biological systems, greater reagent stability than radical reagents, and compatibility with both hydrophilic and lipophilic solvents (Apak et al., 2004).

In one study, rosehip seed methanolic extract demonstrated 108  $\mu\text{mol}$  trolox/g sample antioxidant activity with CUPRAC assay (Ozyurt et al., 2016), while Ilyasoglu (2014) reported 10.4  $\mu\text{mol}$  trolox/g rosehip seed at fresh weight basis using ABTS analysis. For pumpkin seeds antioxidant activity ranged from 0.443 to 1.220  $\mu\text{mol}$  trolox/g on a fresh weight basis using DPPH assay (Nawirska-Olszańska et al., 2013). In pomegranate seeds, 55.5  $\mu\text{mol}$  trolox/g antioxidant activity was recorded for samples from Sanlurfa, Türkiye in a study where samples were subjected to open air drying at room temperature for 5 days (Alsataf et al., 2021), whereas 0.36  $\mu\text{mol}$  of trolox/g sample was found in samples from California, USA by using the DPPH radical scavenging assay in a study where samples firstly subjected to defatting with hexane and then drying under room temperature (Ambigaipalan et al., 2017). Antioxidant activity in fruits and vegetables is influenced by several variables, including cultivar, plant parts, climate, and fruit ripeness stage, as well as the pre-treatments of samples. Thus, the observed variation between seeds in this study and in the literature is reasonable.

### Phenolic Profile of Seed Samples

Individual phenolic compounds are presented in Table 2, highlighting the diversity of phenolic compounds in seeds. Rosehip seeds contain high amounts of flavanols,

including catechin, epicatechin, and epigallocatechin gallate. Additionally, a flavanol, kaempferol, was also detected (5.9 $\pm$ 0.02 mg/100 g sample), along with gallic acid (8.7 $\pm$ 0.08 mg/100 g sample). In a study, catechin and 21 other phenolic compounds were identified using LC-MS/MS in rosehip seed powder (Gavarić et al., 2023), demonstrating consistency with the present findings.

Pumpkin seeds contain primarily hydroxybenzoic acid derivatives, with syringic acid as the dominant compound (22.4 $\pm$ 2.59 mg/100 g sample), followed by p-hydroxybenzoic acid and gallic acid. The primary chemical composition and oil properties of seeds from a Tunisian pumpkin (*Cucurbita maxima*) variety called Béjaoui were examined (Rezig et al., 2012). Protocatechuic, syringic, caffeic, p-coumaric, vanillic, and ferulic acids were the six phenolic acids found; syringic acid was the most prevalent phenolic acid at 7.96 mg/100 g. The seed oil of the Béjaoui variety (a Tunisian variety) did not contain gallic or p-hydroxybenzoic acids, with concentrations recorded at 0.20 and 0.26 mg/100 g, respectively (Rezig et al., 2012). Although p-hydroxybenzoic acid is the most prevalent phenolic acid found in pumpkins, other phenolics like caffeic, p-coumaric, ferulic, sinapic, protocatechuic, vanillic, syringic acid, and p-hydroxybenzaldehyde have also been detected (Krimer-Malešević, 2020). HPLC analysis of raw pumpkin seed extract also identified nicotine, rutin, quinine, caffeic acid, and chlorogenic acid (Akomolafe, 2021). Ellagic acid is the most abundant phenolic compound in pomegranate seeds (165 $\pm$ 1.45 mg/100 g sample), along with substantial amounts of punicalagin derivatives. Pomegranate is one of the richest fruits in phenolic compounds, containing punicalagin, anthocyanins, punicalin and gallic and ellagic acids. Seed extracts from Moroccan pomegranates showed ellagic acid concentrations ranging from 1.1-1.6 mg /g sample (Sabraoui et al., 2020).

Table 2. HPLC-PDA detected compounds in seeds

Compound	Pumpkin seed	Rosehip seed	Pomegranate seed
Hydroxybenzoic acids			
Gallic acid	8.8 $\pm$ 0.21	8.7 $\pm$ 0.08	7.3 $\pm$ 0.04
p-hydroxy benzoic acid	11.0 $\pm$ 1.01	N.D.	N.D.
Syringic acid	22.4 $\pm$ 2.59	N.D.	N.D.
Ellagic acid	N.D.	N.D.	165 $\pm$ 1.45
Hydroxycinnamic acids			
Ferulic acid	1.0 $\pm$ 0.01	N.D.	N.D.
Hydrolyzable tannins			
Punicalagin derivative 1	N.D.	N.D.	45.9 $\pm$ 1.29
Punicalagin derivative 2	N.D.	N.D.	191.3 $\pm$ 1.15
Anthocyanins			
Cyanidin-3-O-diglucoside	N.D.	N.D.	10.9 $\pm$ 0.55
Peonidin-3-O-glucoside	N.D.	N.D.	11 $\pm$ 0.27
Flavanols			
(-)-Catechin	N.D.	5.2 $\pm$ 0.24	N.D.
(-)-Epicatechin	N.D.	5 $\pm$ 0.33	N.D.
(-)-Epigallocatechin gallate	N.D.	3.7 $\pm$ 0.21	N.D.
Flavonols			
Kaempferol	N.D.	5.9 $\pm$ 0.02	N.D.

\*Results were expressed as mg standard/100 g sample. Punicalagin derivatives were calculated as ellagic acid equivalent. N.D.: Not detected.

The antioxidant property of the pomegranate extracts is illustrated to the presence of phenolic acid derivatives, flavonoids, punicalin, and hydrolyzable tannins, including punicalagins, anthocyanins, and ellagic acid derivatives (Kalaycıoğlu & Erim, 2017), while the most common phenolics, protocatechuic acid, caffeic acid, quercetin, p-coumaric acid, gallic acid, and ferulic acid are responsible the antioxidant activity in pumpkins (Babbar et al., 2015), and rosehip fruits are generally rich in bioactive compounds with antioxidant activity, particularly in flavonoids, carotenoids, tannins, phenolic acids, mineral compounds, and fatty and organic acids (Ayati et al., 2019). The total amount of quantified compounds was correlated with the TAC assays and results indicated that both applied TAC assay exhibited high correlation with them ( $R^2 > 0.99$ ). General overview indicated consistency with the literature data. However, the number of identified compounds varies with the geographical origin, growing condition of plant, and harvesting season. Moreover, pre-treatment application, storage condition, differences at extraction protocols could change the qualified phenolic compounds from seeds.

Discarding of food wastes is a problem that can be solved easily by utilizing these food wastes as alternative sources. Since pumpkin fruit has excellent phytochemistry and can have positive health effects, the food and pharmaceutical industries can use all these pumpkin parts as therapeutic agents by isolating and characterizing the bioactives in the form of powders or extracts instead of throwing them away during processing (Hussain et al., 2022). Using the trolox equivalent antioxidant capacity assay, it was also discovered by Ilyasoglu (2014), that the seed oil and rosehip seeds exhibited high antioxidant activity. Pomegranates contain a lot of aril, which makes up between 50 and 70 % of the fruit overall and is composed of 78% juice and 22% seeds (Mohagheghi et al., 2011). Pomegranate seeds, which are produced in large quantities each year as a by-product of the juice and concentrate industries, could be used more profitably in the food industry rather than as animal feed or in manufactured cosmetics (Kalamara et al., 2015).

## Conclusion

In scope of the present study, pomegranate, rosehip and pumpkin seeds were investigated for their bioactive components. The TPC, TFC and TAC of the samples were compared. Variations in individual phenolic compounds suggest these seeds have the potential to be valuable sources of naturally occurring bioactive compounds. These bioactive compounds can be utilized as functional ingredients that can replace artificial food additives such as nutraceuticals and preservatives in the food, pharmaceutical and other industries. Utilizing seeds obtained from food waste will be advantageous in minimizing discarded food parts and enhancing sustainability for a better world. The different phenolic substances of the seed included in this study may allow them to serve different purposes. Further studies should be carried out in this direction, specially design of new studies to assess proposed materials as food additives is recommended.

## Declarations

### Conflicts of Interest

There is no conflict of interest disclosed by the author.

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