Annals of Phytomedicine 12(1): 93-104, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.1.105

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Review Article : Open Access

Pomegranate (*Punica granatum* L.) seed: A review on nutritional profile, functional food properties, health benefits, and safety aspects

S. Yamini, Vinod Kumar Paswan◆, Abdelrazeq M. Shehata*, Mahipal Choubey, Durga Shankar Bunkar and Vinay Venkatesh Varada*****

Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India
** Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo -11651, Egypt*
*** Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India*
**** Animal Nutrition Division, ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India*

Article Info

Article history
Received 20 May 2023
Revised 22 June 2023
Accepted 23 June 2023
Published Online 30 June-2023

Keywords
Phytochemicals
By-products
Dried pomegranate seed residues
Fatty acids composition
Pharmacological properties

Abstract

Pomegranate (*Punica granatum* L) is a superfruit with long medicinal history across the world as a healthy and nourishing ancient fruit. The principal waste generated by the processing of pomegranate fruit is a considerable number of seeds, and the disposal of this debris becomes an environmental problem. Pomegranate seeds residues (PSR) possess a high concentration of phytochemicals (a-tocopherols, polyphenols, flavonoids and anthocyanins) and an excellent source of crude fibers. About 7-27% of the total seed weight is composed of pomegranate seed oil (PSO), which contains a high amount of conjugated fatty acids, mainly punicic acid (PA) and linolenic acid, which have various pharmacological applications. The main properties are antioxidant, anticancer, nephroprotective, reducing insulin resistance, antiatherogenic, antidiabetic, memory effects, estrogenic, and dental effects. Therefore, this review presents a detailed nutritional profile, primary and secondary metabolites in dried pomegranate seed residue and oil and their mechanisms of action in pharmacological and clinical applications.

1. Introduction

Numerous epidemiological, observational, preclinical, and human intervention studies have shown that eating a diet rich in plant-based foods lowers the risk of developing chronic diseases (Moore *et al.*, 2020). In this case, pomegranate has become popular because of notion that it has health benefits that go beyond its nutritional value (Abiola *et al.*, 2018). The pomegranate fruit (*Punica granatum* L.) is a fruit-bearing deciduous shrub native to Iran (Persia) with cultivation spreading widely throughout the Mediterranean region, Afghanistan, Spain, Egypt, Russia, China, Japan, and California (U.S.). Pomegranate processing by industries produces an enormous volume of residue, and the disposal of this material raises environmental concerns. A byproduct of pomegranate processing, pomegranate seed makes up around 20% (w/w) of the entire fruit. Pomegranate seeds may have the potential to be a rich source of nutrients and antioxidants, according to recent studies. Dried pomegranate seed residue has a high concentration of bioactive substances such as hydrolysable tannins (ellagitannin, punicalagin, punicalin, and pedunculagin), flavonoids, anthocyanins, and other phenolic compounds (Li *et al.*, 2016).

Corresponding author: Dr. Vinod Kumar Paswan
Assistant Professor, Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005
E-mail: vkpaswan.vet@gmail.com; vkpaswan.dsft@bhu.ac.in
Tel.: +91-9454894274

Dried pomegranate seed residue has a high concentration of insoluble fibers (35 to 66% hemicellulose and cellulose) that could be used as dietary fibers to improve the laxative effect by reducing constipation and antihyperglycemic activity in diabetes (Maphosa and Jideani, 2016). It also has several essential minerals, such as potassium, calcium, phosphorus, magnesium, nitrogen, iron, and sodium.

When extracted using both traditional and modern methods, the oil makes up between 7 and 27% of the pomegranate seeds. Pomegranate seed oil (PSO) has many health benefits due to its high concentration of polyunsaturated fatty acids (PUFAs), especially the conjugated fatty acids punicic acid, 13-trienoic acid (Shabbir *et al.*, 2017), tocopherols and phytosterols.

This article comprehensively depicts the nutritional value of pomegranate seeds and how they could be used as an ingredient in foods that are both healthy and palatable. It also emphasizes the safety features of pomegranate seed consumption, paving the way for future research to develop a variety of functional meals with pomegranate seeds or their extracts within acceptable limits.

2. Nutritional profile and chemical composition

Researchers found that dried pomegranate seed has numerous health benefits and nutraceutical properties (Wong *et al.*, 2021; Banerjee *et al.*, 2017) based on the results of *in vivo* and *in vitro* studies as well as proximate analysis. The age of the fruit, the kind of soil, the location, the climate and methods of drying, all affect the nutritional composition of dried pomegranate seed residue. The chemical composition of dried pomegranate seed residue includes moisture 6-

13%, crude fiber 27-43%, crude protein 4-15%, fat 11-26%, carbohydrate 12-25%, crude ash 1-2% (Abiola *et al.*, 2018). The mean values of minerals in 100 g of dried pomegranate seed residue reported by Haleem (2016) were potassium (K) - 761.28 mg, calcium (Ca) - 498.74 mg, sodium (Na) - 385.83 mg, magnesium (Mg) - 243.39 mg, manganese (Mn) - 61.08 mg, selenium (Se) - 39.45 mg, zinc (Zn) - 12.71 mg, copper (Cu) - 4.18 mg, and iron (Fe) - 3.62 mg. Potassium and calcium are involved in managing the activity of muscles and nerves and maintaining normal cardiac function. Potassium regulates the body's fluid balance and selenium (Se) is an essential trace element that functions as an antioxidant. Along with the remarkable mineral profile, dried pomegranate seed residue is also infused with an appreciable number of water-soluble and fat-soluble vitamins (Haleem, 2016). Ascorbic acid, thiamine and riboflavin were found to be major water-soluble vitamins and vitamin-E as the major fat-soluble vitamin (Haleem, 2016).

2.1 Proteins

Storage proteins account for 17% of the dry seed weight of pomegranate (Elfalleh *et al.*, 2011). Analyses of the storage protein content in the dried pomegranate seed residue have shown that globulins and albumins account for about 43% and 32% of the total storage proteins. The non-storage protein component in pomegranate seeds amounts to 19% of the overall protein content. A study that used 2DE (Two-dimensional gel electrophoresis) and LC-MS/MS analysis demonstrated that dried pomegranate seed residue had a profile of seven water-soluble, non-storage proteins. Yang *et al.* (2012) found that most protein spots were made up of polypeptides with molecular masses between 10,000 and 25,000 Da. Small heat shock proteins, class 1 small heat shock protein, glyceraldehyde-3-phosphate dehydrogenase, fructose bisphosphate, enolase, alcohol dehydrogenase, and proteasome subunit alpha type proteins were identified as non-storage proteins in dried pomegranate seed residue (Yang *et al.*, 2012). In addition to acting as a source of essential amino acids for animal and human nutrition, seed storage proteins also serve as a storage site for various bioactive peptides with preventive effects against oxidative-stress, hypertension, immunomodulation, inflammation, and others (Salem *et al.*, 2023; Cruz-Chamorro *et al.*, 2023). Through *in vivo* digestion or food processing, these peptides, which are still suppressed inside the basic structure of seed storage proteins, can be released. Upon consumption, these bioactive peptides may have the potential to influence the primary body systems; namely, the digestive, cardiovascular, immune, and nervous systems based on the amino acid sequence of the peptides (Ashaolu *et al.*, 2023).

2.2 Amino acid profile of dried pomegranate seed residue

A pomegranate seed contains 14.45 g/100 g dry weight of total amino acid; glutamine, leucine, aspartate, glycine, and arginine are the major amino acids (Elfalleh *et al.*, 2011). There are very few studies carried out by researchers on the amino acid profile of dried pomegranate seed powder, which was assessed in comparison to the reference protein pattern of FAO/WHO (1973), as shown in Table 1.

As illustrated in Table 1, the dried seed powder of *Punica granatum* L. possesses essential amino acids, methionine and cysteine (sulphur containing amino acids), phenylalanine, and tyrosine (aromatic amino acids), in higher amounts compared to the reference protein pattern of FAO/WHO (1973) recommended for adults. Lysine, leucine, valine, and histidine (essential amino acids) were also found to be present (Haleem, 2016; Rowayshed *et al.*, 2013). Tryptophan could not be found because of its degradation upon acid hydrolysis. Therefore, the utilization of available dried pomegranate seed residue for incorporation in human and animal feed deficient in essential amino acids has greater economic importance.

Table 1: Amino acid profile of dried pomegranate seed powder compared with reference protein pattern of FAO/WHO (1973)

Amino acids	Dried pomegranate seed powder (g/100 g protein)	FAO/WHO value (g / 100 g protein)
Essential amino acids		
Methionine+Cysteine	4.6	3.5
Lysine	1.89	5.5
Leucine	6.5	7.0
Phenyalanine+Tyrosine	6.8	6.0
Valine	3.9	3.8
Histidine	2.7	
Isoleucine	4.8	
Total essential amino acids	31.19	
Non-essential amino acids		
Glutamic	25.6	
Glycine	7.10	
Arginine	10.76	
Alanine	4.46	
Aspartic	8.85	
Proline	5.19	
Serine	4.46	
Total non-essential amino acids	66.42	

Source: Haleem, 2016; Rowayshed *et al.*, 2013.

2.3 Lipid profile and phospholipids of dried pomegranate seed residue

The phospholipids and lipid types of dried pomegranate seed residues were investigated by Haleem (2016). The results showed that there were 8 different types of polar and non-polar lipids in total lipids, with triacylglycerols making up 83.35% of total lipids. Phospholipids, monoacylglycerols, 1,2-diacylglycerols, sterols, 1,3-diacylglycerols, and hydrocarbon plus sterol esters were also present (Haleem, 2016). Phosphatidyl choline and phosphatidyl ethanol amine were the two phospholipids that predominated in PSO, as indicated in Table 2.

Table 2: Lipid components of dried pomegranate seed oil

Lipid components	% of total components
Phospholipids	3.6
Monoacylglycerols	0.92
1,2- diacylglycerols	1.08
1,3-diacylglycerols	4.31
Sterols	4.12
Free fatty acids	0.74
Triacylglycerols	83.35
Hydrocarbons plus sterol esters	1.84
Phosphatidyl serine	3.01
Lysophosphatidyl choline	10.84
Phosphatidyl inositol	14.20
Sphingomyelin	5.84
Phosphatidyl choline	19.09
Phosphatidyl ethanol amine	31.45
Phosphatidyl glycerol	3.67
Phosphatidic acid	1.90

Source: Haleem, 2016.

2.4 Sterols and tocopherol composition

PSO is high in phytosterols such as β-sitosterol which is abundant, followed by campesterol, stigmasterol, and tocopherols such as á and β-tocopherol. Many research studies have revealed that α-sitosterol has sedatory effects, analgesic, immunomodulatory, antimicrobial, anticancer, anti-inflammatory, and protective effects against non-alcoholic fatty liver disease (NAFLD), the lipid-lowering effect, hepatoprotective, respiratory disease protection, wound healing effect, digestive problems, antioxidant and antidiabetic properties. According to Hajib *et al.* (2021), 33.44 mg/100 g of total seed oil content extracted from cold-pressed 'Sefri' variety of pomegranate fruit contained tocopherol, of which γ-tocopherol (190.47 mg/100 g oil) was the major, followed by α-tocopherol (74.62 mg/100 g oil) and δ-tocopherol (53 mg/100 g oil). Kola *et al.* (2021), determined total tocopherol, total sterol and total triacylglycerols in cold- pressed seed oil extracted from pomegranate of Turkish origin, and the results show that the total tocopherol content was 258.40 mg/100 g, major tocopherol were α-tocotrienol (1898 µg/g), γ-tocopherol (531.38 µg/g) and α-tocopherol (153.67 µg/g), respectively. Total sterol composition was 930 mg/100 g of which β-sitosterol was abundant at 83%, followed by campestral (8.2%), stigmasterol (4.2%), sitostanol (3.31%), and others at lower concentrations. Total triglyceride composition was reported to be 11-12%, of which, stearic-punicic-punicic acid (SPuPu) was 4.34% higher, oleic-punicic-linoleic acid (OPuL) was 1%, and palmitic-punicic-linoleic acid (PPuL) was 0.71%.

2.5 Fatty acid composition of dried pomegranate seed oil

Approximately 80% of the seed oil content consists of conjugated octadecatrienoic fatty acids. The seed oil contains a high amount of polyunsaturated (n-3) fatty acids (PUFAs). The majority of the conjugated linolenic acids (CLnAs) in pomegranate seed oil are punicic acid (Abiola *et al.*, 2018). Punicic acid (C18:3-9 cis,11 trans, and 13cis), also known as trichosanic acid, is a molecule of conjugated α-linoleic acid (CLnA) with structural similarities to conjugated linoleic acid (CLA) and α-linolenic acid (LnA), a major polyunsaturated (n-3) fatty acids (PUFAs) constituting about 76% of pomegranate seed oil, followed by linoleic acid (C18:2), and arachidonic acid. Figure 1A illustrates the total fatty acid composition of pomegranate seed oil (Ferrara *et al.*, 2014). Saturated fatty acids have the following compositions: myristic (C14:0), palmitic (C16:0), stearic (C18:0) and arachidic acid (C20:0) (Figure 1B) (Jing *et al.*, 2012; Fernandes *et al.*, 2015). The monounsaturated fatty acids (MUFAs) reported to be present in pomegranate seed oil include oleic acid (C18:1), Stearoleic acid (C18:1), and erucic acid (C22:1) (Figure 1C) (Rowayshed *et al.*, 2013). Among polyunsaturated fatty acids (PUFAs), punicic acid (C18:3) was found to be abundant followed by linoleic acid (C18:2(n-6)), linolenic, arachidonic, eicosapentanoic and docosatetraenoic acids (Rowayshed *et al.*, 2013; Wu and Tian, 2017) (Figure 1D).

According to Ferrara *et al.* (2014), oil obtained from dried pomegranate seeds of 13 sweet and sour genotypes of pomegranate from Italy and Israel constituted total lipids varying from 10.5-26.7% in sweet genotypes and 5-17% in sour genotypes, 16 fatty acids, punicic acid being the major fatty acid at 74.5%, followed by linoleic, palmitic, stearic and oleic acids. Hajib *et al.* (2021) studied the fatty acid composition of seed oil obtained from cold pressed "Sefri" variety of pomegranate, and the results obtained included saturated fatty acid of about 4.74 g/100 g of which palmitic acid was the abundant saturated fatty acid (SFA) with 2.64 g/100 g, stearic acid with 1.73 g/100 g and arachidic acid with 0.37 g/100 g of total saturated fatty acids. The total unsaturated fatty acid (UFA) accounted for 95.13 g/100 g, of which polyunsaturated fatty acid (PUFA) with 91.07 g/100 g was predominant and the remaining 4.06 g/100 g was MUFA. Among PUFA, punicic acid was higher and accounted for 75 g/100 g, catalpic acid at 6.7 g/100 g, α-eleostearic acid with 3.73 g/100 g and β-eleostearic acid at 1.43 g/100 g of total PUFA. Kola *et al.* (2021), determined the fatty acid composition of cold pressed seed oil extracted from pomegranate of Turkish origin and observed that total saturated fatty acid (SFA) was 9.17%, total unsaturated fatty acid (UFA) was 88.9% in the seed oil, of which polyunsaturated fatty acid (PUFA) accounted for 80.7%, which was major, followed by monounsaturated fatty acid (MUFA) at 8.17%. The major UFA is punicic acid (76%), followed by lignoceric acid (4.6%), linoleic acid (4.2%), oleic acid (4.1%), and nervonic acid (3.4%).

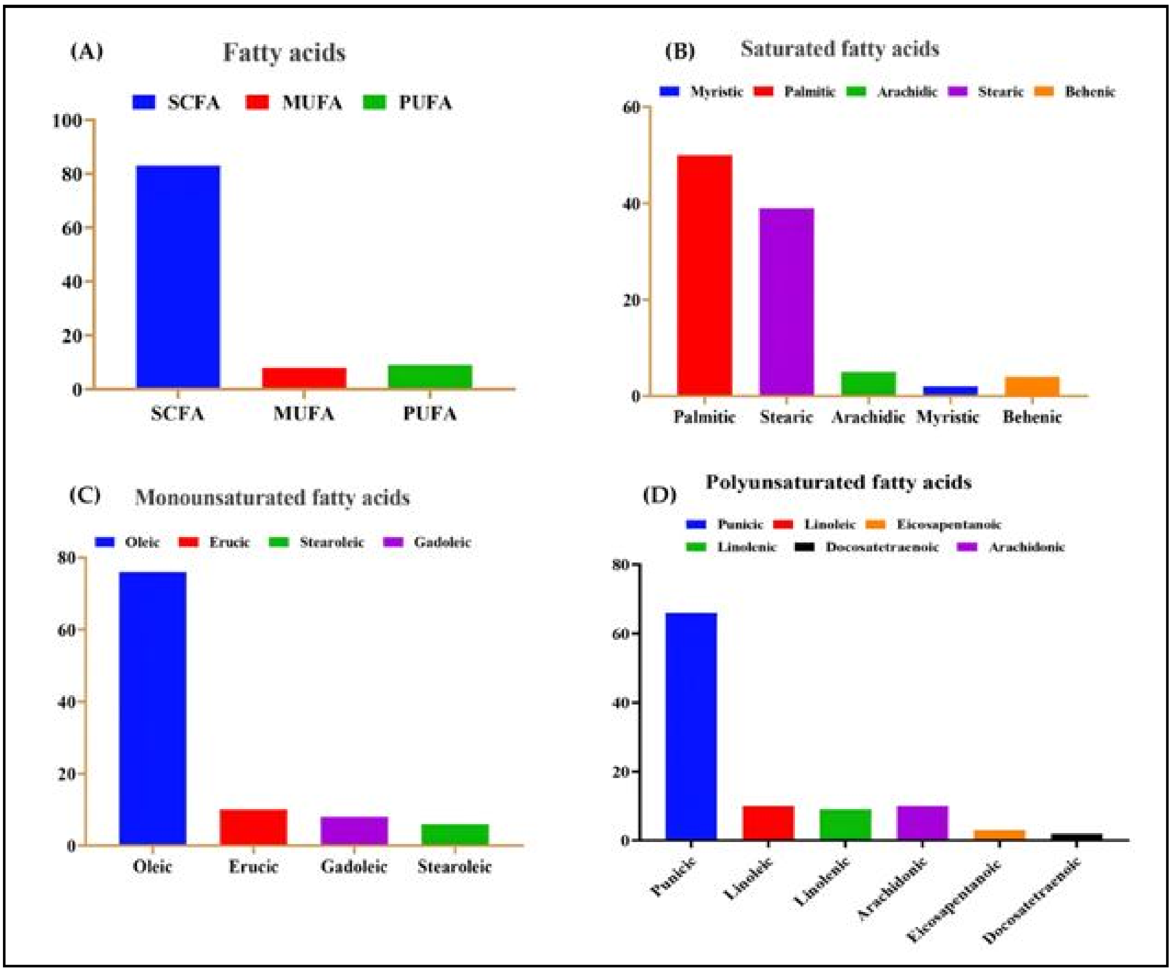

Figure 1: Schematic representation of total fatty acids in dried pomegranate seed oil from cultivars of different regions. (A) Seed oils by Ferrara *et al.* (2014), (B) Jing *et al.* (2012); Fernandes *et al.* (2015), (C) Rowayshed *et al.* (2013) and (D) Rowayshed *et al.* (2013); Wu and Tian, (2017).

3. Secondary metabolites

The pomegranate seeds are rich in antioxidants; namely, phenolics, flavonoids, alkaloids, saponins, and tannins. Because of differences in cultivars and growing circumstances, phytochemical levels and antioxidant activity in pomegranate seeds have been observed to vary. Shahidi and Ambigaipalan (2015) determined the total phenolic content (mg GAE/g of sample) in all three forms (free, esterified and insoluble bound fractions) with 1.38, 1.39 and 0.62, in dried pomegranate seed residues. Phenolic acids, flavonoids, tannins and anthocyanins of free, esterified and insoluble bound fractions from dried pomegranate seed residues were also identified using HPLC-DAD-ESI-MS/MS, and the results obtained are as shown in the Table 3.

3.1 Phenolic acid derivatives

Jing *et al.* (2012) evaluated the total phenolic acids in the seeds of four commercial Chinese pomegranate cultivars, the results showed the presence of 3, 4-dihydroxybenzoic, 4-hydroxybenzoic, syringic, vallic and ferulic acid. Shahidi and Ambigaipalan (2015) identified 13 phenolic acids in pomegranate seed extracts using UV spectra and MS data. The following acids were identified in pomegranate seeds: protocatechuic acid, vanillic acid, gallic acid, brevifolin carboxylic acid, p-hydroxybenzoic acid hexoside, cis-and trans-caffeic acid hexoside, a derivative of caffeic acid hexoside, vanillic acid hexoside, ferulic acid hexoside, catechin, quercetin hexoside, cis- and trans-dihydro-kaempferol-hexoside, ellagic acid, ellagic acid pentoside, ellagic acid deoxyhexose and ellagic acid hexoside, valoneic acid bilactone, digalloyl hexoside, and galloyl-hexahydroxydiphenoyl-hexoside (Shahidi andAmbigaipalan, 2015). Catechin, on the other hand, was exclusively found in the free phenolic fraction of PS, with its distinctive molecular ion (M-H) m/z 289. Based on the retention time, UV spectra, and MS data of the original standard, elagic acid (m/z 301) and MS2 fragments m/z 185, 229, 257, and 283 were found in all fractions of PS. Fischer *et al.* (2011) determined the phenolic acid contents in the pomegranate seed extracts using HPLC-DAD-ESI-MS/MS, and the results obtained showed that gallic acid (~1037 µg/100 g DW) was the abundant phenolic acid followed by cyanidin-pentoside-hexoside, valoneic acid bilactone, brevifolin carboxylic acid, vanillic acid 4-glucoside and dihydrokaempferol-hexoside. PS yielded 1164 g/100 g DW total phenolic acid (Shahidi andAmbigaipalan, 2015). Using HPLC-ESI-MS, He *et al.* (2011) identified the phenolic acid derivatives present in the seed extracts of Chinese pomegranate seed extracts. Coumaric acid, caffeic acid glycoside dimer, and fabric acid derivative were the primary phenolic acids identified (He *et al.*, 2011).

3.2 Flavonoids

Flavonoids are cyclized diphenylpropanes that are often conjugated with sugars and exist as O-or C-form glycosides, though they can also exist as free aglycones. Flavonoids also possess potential pharmacological activity, such as free radical scavenging, estrogenic action, anti-inflammatory actions and anti-cancer activity (Meena *et al.*, 2020)

Flavonoids, which include flavones, flavonol, isoflavones, flavanones, flavanonol, flavanol, and anthocyanidin, contain the typical C6-C3-C6 carbon skeleton with varying amounts of hydroxylation and methoxylation. Fischer *et al.* (2011) confirmed that pomegranate seed of German origin included eight flavonoids, including (+)-catechin, dihydroxygallocatechin, naringenin hexoside, quercitrin-3-O-rhamnoside, quercetin hexoside, kaempferol-3-O-glucoside, and cis- and trans-dihydrokaempferol-hexoside. According to He *et al.* (2011), four flavan-3-ol compounds were discovered with the same λ_{max} of 280 nm in Chinese pomegranate seed extracts using HPLC-ESI-MS. These compounds include procyanidin trimer type C, procyanidin dimer type B, procyanidin dimer, and (E) catechin. Jing *et al.* (2012) evaluated total flavonoid contents of pomegranate seed flour extracts of four Chinese cultivars using a slightly modified colorimetric method, and the results obtained showed that the total flavonoid content in 80% aqueous methanol ranged from 0.37 - 0.58 mg CAE/g in pomegranate seeds. Peng (2019) determined the total flavonoid contents of seeds and sprouts of three different pomegranate cultivars and found that the flavonoid content of seeds ranged from 0.51 mg/g to 0.68 mg/g, while that in sprouts ranged from 1.97 mg/g to 2.48/g. Pomegranate seed extracts had total phenolic and flavonoid levels of 77.93 g gallic acid equivalent/mg and 16.66 g (CAE)/mg, respectively (Manasathien *et al.*, 2012). Pomegranate seed oil of Tounsi variety had a total flavonoid content of 59.46 mg (CEq)/g of sample, respectively (Amri *et al.*, 2017). Bashandy *et al.* (2019) evaluated the effects of PS flavonoids on doxorubicin (DOX)-induced hepatotoxicity in albino Wistar rats. During four weeks, rats were given DOX (2.5 mg/kg intraperitoneal injection) and/or 2Gy irradiation. Every day, 100 mg/kg of pomegranate seed powder was administered orally. The researchers analysed blood levels of hepatic markers such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) to assess liver function. Many liver biomarkers increased in the DOX and/or gamma groups relative to the control group, then decreased after PS treatment. PS treatment against DOX toxicity significantly (p<0.05) reduced high MDA levels and stabilized tissue glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activity, according to the researchers.

Table 3: The content of phenolic acids, flavonoids, tannins and anthocyanins of free, esterified and insoluble-bound fractions from dried pomegranate seed residues

Phenolics	Free	Esterified	Insoluble-bound	References
	(mg/ 100 g dry weight)			
Phenolic acids				
Protocatecheuic acid	12.9	13	1.2-79	Shahidi and Ambigaipalan, 2015; Krimer *et al.*, 2013
Gallic acid	12.9-117.8	58.1-68.9	70.7-959	Shahidi and Ambigaipalan, 2015; Krimer *et al.*, 2013
p-Hydroxybenzoic hexoside	1.1-20.5	2.2	0. 6	Shahidi and Ambigaipalan, 2015; Krimer *et al.*, 2013
Vanillic	0.6	3.3	1.2	Krimer *et al.*, 2013
Vanillic acid hexoside	19.9	-	-	Shahidi and Ambigaipalan, 2015
cis-Caffeic acid hexoside	3.2	-	-	Shahidi and Ambigaipalan, 2015
trans-Caffeic acid hexoside	-	2.7	-	Shahidi and Ambigaipalan, 2015
Ferulic acid hexoside	3.8	-	-	Shahidi and Ambigaipalan, 2015
Flavonoids				
Catechin (m/z 289)	18.6	-	-	Shahidi and Ambigaipalan, 2015
Naringenin hexoside	25.87	-	-	Shahidi and Ambigaipalan, 2015
Quercetin hexoside	0.10	-	-	Shahidi and Ambigaipalan, 2015
Hydrolysable tannins				
Ellagic acid	172	17.5	29	Shahidi and Ambigaipalan, 2015
Valoneic acid bilactone I	0.34	-	-	Shahidi and Ambigaipalan, 2015
Valoneic acid bilactone II	-	2.69	-	Shahidi and Ambigaipalan, 2015
Galloyl-HHDP-hexoside	0.41	-	-	Shahidi and Ambigaipalan, 2015
Anthocyanins				
Cyanidin-3,5-*O*-diglucoside	4.6	-	-	Gulsunoglu *et al.*, 2019
Cyanidin-3-*O*-glucoside	4.5	-	-	Gulsunoglu *et al.*, 2019

3.3 Anthocyanins

Phytochemicals such as anthocyanins, which are water-soluble natural pigments belonging to the family of flavonoids and responsible for the red, purple, blue, or black color appearance of fruit depending on their pH, are found in large quantities in pomegranate fruit. Fruits and flowers mostly contain anthocyanins in their cell vacuoles. The presence of six anthocyanins in dried pomegranate seed residues was investigated (Ben-Simhon *et al.*, 2015). Fischer *et al.* (2011) determined twelve anthocyanins in pomegranate seed using UV spectra (520 nm) and MS/MS data in positive mode. Major anthocyanins were cyanidin-3-O-pentoside, pelargonidin-3-O-glucoside, cyanidin-3-O-glucoside and delphidin-3-O-glucoside. Gölükcü (2015) found that freeze-dried samples had the greatest anthocyanin concentration (679.76 mg/kg), followed by vacuum, convective, and sun-dried samples in declining order. According to the results of this investigation, freeze drying is the best technique for drying pomegranate arils. According to Yilmaz *et al.* (2017), drying method, drying temperature, and product thickness all had a substantial impact on anthocyanin concentration. Hota *et al.* (2017) discovered that anthocyanin concentration (OD) varied substantially between samples, with the highest (0.907) in steam-blanched, cabinet-dried samples and the lowest in sun-dried control samples. According to research by Bhat *et al.* (2014) on the effects of drying methods and packaging on the stability of dried wild pomegranate arils, the mechanical cabinet dryer produced the highest anthocyanin content of 33.08 mg/100 g, followed by the solar cabinet dryer's 28.22 mg/100 g and sun drying's 25.56 mg/100 g. Bchir *et al.* (2012) determined total anthocyanin content in air dried pomegranate seeds at 40ºC, 50ºC and 60ºC and found that with increased temperature of drying, the anthocyanin value decreased due to polyphenol oxidase reaction, and the finished product contains 40, 24, and 20 mg/100 g FM of anthocyanin, respectively. Kaute *et al.* (2018) observed anthocyanin content in dried seeds of different lines of pomegranate packed in polythene bags and aluminium laminated pouches and found the content varying from 57.67 to 79.74 mg/100g DM.

3.4 Hydrolysable tannins

Hydrolysable tannins are a type of secondary metabolites that has been shown to have antibacterial, fungicidal, and antiviral activities. They are often numerous esters of gallic acid and glucose. Pomegranates are high in hydrolysable tannins in addition to anthocyanins.

Hydrolysable tannins from the peels, seeds, leaves, and flowers of the pomegranate cultivar 'Gabsi' were examined by Elfalleh *et al.* (2011), total hydrolysable tannins varied from peel (139.63 ± 4.25), through the seeds (29.57 ± 4.54), leaves (128.02 ± 4.49), and flowers (148.24 ± 10.29) in terms of mg TAE/g DW, depending on the type of organ. The number of natural antioxidants found in various pomegranate sections is highlighted by these findings, which may help to explain why traditional healers are drawn to pomegranate trees and why they are thought of as medicinal plants. According to Fischer *et al.* (2011) ellagic acid, which followed the pattern of free > insoluble bound > esterified, was the main hydrolysable tannin found in dried pomegranate seed residue (220 µg/100 g DW).

4. Biological activities of various constituents of dried pomegranate seed residue

4.1 Antimicrobial property

The antibacterial activity of pomegranate seed extracts from Egyptian and Taif varieties was studied by Gaber *et al.* (2015). The results showed that at a concentration of 60 mg/ml, the seed extracts were most effective against bacteria. Most of the extract was shown to inhibit the common bacteria such as *Escherichia coli, Staphylococcus aureus, and Pseudomonas* sp. Kupnik *et al.* (2021) investigated the antibacterial activity of ethanolic and aqueous extracts of fresh and lyophilized seeds from various pomegranate fruit samples using the disc diffusion method (DDM). The results revealed that all of the seed extracts reduced the development of *Bacillus cereus* at both starting doses, but only the ethanol lyophilized seed extract inhibited the growth of *Staphylococcus aureus* and *Staphylococcus pyrogenes*. The effects of several pomegranate ingredients on the prevention and mitigation of diabetes, oxidation, inflammation, and cancer have been studied *in vitro*, in animal trials, and in human trials (Table 4) (Costantini *et al.*, 2014; Mirmiran *et al.*, 2010; Boroushaki *et al.*, 2013; Hui *et al.*, 2013; Amri *et al.*, 2017).

4.2 Antioxidant property

The principal reactive oxygen species (ROS) known to cause oxidative damage in the human body are superoxide anion (O_2^{-}), hydrogen peroxide (H_2O_2), peroxyl radical (ROO^{-}), hydroxyl radical (HO^{-}), and singlet oxygen ($^1O_2^{-}$). Although, enzymatic antioxidants in live cells are capable of turning reactive oxygen/nitrogen species into safe species, dietary antioxidant-rich foods are beneficial to health. Benchagra *et al.* (2021) studied the impact of methanolic extract of both pomegranate peel (PPPE) and pomegranate aril (PAPE) of 'Sefri' cultivar on lipid peroxidation in human for evaluating oxidative injury, quantified by measuring TBARS. Both the methanolic extracts reduced lipid peroxidation when compared to the control. PPPE (100 and 200 g/ml) reduced MDA levels by 39.81% and 52.58% (p <0.01), respectively, as compared to the control. PAPE (100 and 200 g/ml) reduced TBARS by 17.32% (p<0.05) and 23.4% (p<0.01), respectively, compared to PPPE. The antioxidant effects of pullulan nano-based pomegranate seed oil film (PSONCF) in the atopic dermatitis in mice was studied by Cervi *et al.* (2021), the dorsal skin cells, melanocytes and keratinocytes produced reactive species which also produced exacerbated reactive lipid species by reacting with lipid molecules which accelerates the development of atopic dermatitis was treated by PSONCF by restoring the biochemical parameters. PSONCF system was found to be retained in the stratum corneum and had the potential to penetrate and permeate the inner layers of skin tissue.

Anti-inflammatory property

The hydrophilic fraction (80% aqueous methanol extract) of pomegranate seed oil (PSO) was tested by Costantini *et al.* (2014) for its anti-inflammatory effects in human colon (HT29 and HCT116), liver (HepG2 and Huh7), breast (MCF-7 and MDA-MB-231) and prostate (DU145) cancer cell lines. Results showed that punicic acid and related chemicals significantly reduce cell viability and increase the number of cells in the G0/G1 phase of the cell cycle as compared to untreated cells (Costantini *et al.*, 2014). Hydrophilic PSO extracts were also found to have a dose-dependent anti-inflammatory activity, with the levels of VEGF and nine pro-inflammatory cytokines (IL-2,

IL-6, IL-12, IL-17, IP-10, MIP-1, MCP-1, and TNF-α) decreasing with increasing concentrations. The anti-inflammatory effects of pullulan nano-based pomegranate seed oil film (PSONCF) in the atopic dermatitis in mice was studied by Cervi *et al.* (2021), the results showed that both free pomegranate seed oil (PSO) and PSONCF inhibited the cyclooxygenase enzymes (COX) 1 and 2. In an animal model of skin injury brought on by UVB radiation, Marchiori *et al.* (2019) found that the pomegranate seed oil (PSO), which was included as a polymeric shell in the nanocapsules, sustained the anti-inflammatory action of a topical formulation containing silibinin. Ibrahim *et al.* (2016) investigated the molecular mechanisms involved in hepatoprotective effects against oxidative stress in a rat model of carbon tetrachloride (CCl_4)-induced liver injury, and found that pomegranate reduced Carbon tetrachloride (CCl_4)-induced sterol regulatory element-binding protein-1c (SREBF-1c) mRNA expression and down-regulated the anti-inflammatory factors alpha 2-macroglobulin (α-2M) and IL-10. Superoxide dismutase (SOD), Glutathione S-transferase (GST) and catalase (CAT) expression was also found to be reduced. According to Colombo *et al.* (2013), pomegranate peel, flower, seed, and juice extracts have significant anti-inflammatory activity in the gut. The anti-inflammatory activity of pomegranate may be attributed to its immunological regulatory impact on T and B lymphocytes and macrophages (Colombo *et al.*, 2013). Houston *et al.* (2017) found that topically administered pomegranate tannins and pomegranate rind extract had a substantial anti-inflammatory action on cyclooxygenase-2 expression in *ex vivo* skin. However, the biological activities of various components of dried pomegranate seed or oil are briefly interpreted in the Figure 2.

4.4 Anticancer effects

The primary cause of a number of cutaneous illnesses, including skin cancer, is excessive exposure to solar UVB and, to a lesser degree, UVA radiations. García-Villalba *et al.* (2019) discovered four previously unknown urolithins, namely 4,8,9,10-tetrahydroxy urolithin (urolithin M6R), 4,8,10-trihydroxy urolithin (urolithin M6R), and 4,8,10-trihydroxy urolithin (urolithin M7R), 4,8,9-trihydroxy urolithin (urolithin CR), and 4,8-dihydroxy urolithin (urolithin AR) were found in human faeces after consuming a pomegranate extract (160-640 mg phenolics) daily. Only 19% of the individuals had the novel metabolites identified. Furthermore, phase II conjugates of the new urolithins were found in urine, indicating that they were absorbed, circulated, and excreted by the kidneys. Endogenous synthesis of these "R" urolithins might be thought of as a further metabolic trait for volunteer stratification.

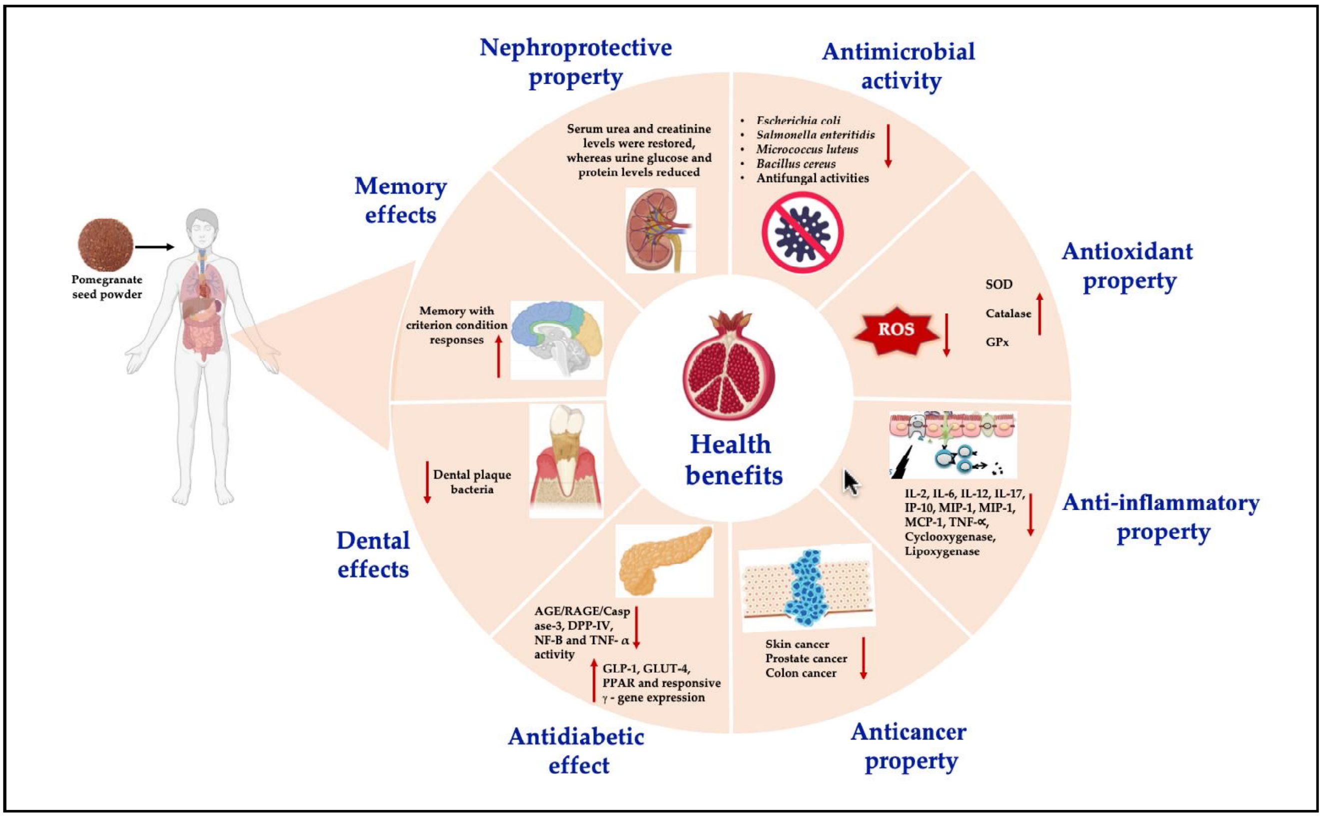

Figure 2: Biological activities of various components of dried pomegranate seed/oil.

Sreeja *et al.* (2012) investigated anti-proliferative activity of methanolic extract of pomegranate (PME) at 20-320 ìg/ml of on human breast (MCF-7, MDA MB-231) endometrial (HEC-1A), cervical (SiHa, HeLa), ovarian (SKOV3) carcinoma and normal breast fibroblast (MCF-10A) cells and found that the PME inhibited the binding of [3 H]-estradiol (E2) to the ER and lowered the growth and proliferation of ER-positive MCF-7 cells and downregulated the transcription of estrogen-responsive reporter gene transfected into breast cancer cells. Joseph *et al.* (2013) extracted galactomannan polysaccharide (PSP001) from pomegranate fruit peel, which demonstrated in vitro cytotoxicity against Ehrlich ascites cancer (EAC), a transplantable mouse tumour of potential mammary origin. A standardised extract of pomegranate

(Pomella) containing ellagitannins (gallic acid, punicalagin, and punicalagin) inhibited the proliferation and viability of MMTV-Wnt-1 mouse mammary cancer stem cells, possibly by arresting cell cycle progression in the G0/G1 phase and increasing caspase-3 activity in a time- and concentration-dependent manner. In a study conducted by Sahin *et al.* (2018), the acetone and methanolic extracts of whole pomegranate peels were shown to have antiproliferative properties against MCF-7 and MDA-MB-231 cells. Similarly, platinum nanoparticles (Pt-NPs) biosynthesized from pomegranate crusts outperformed pomegranate peel in MCF-7 cells by inducing apoptosis through cell death at G0/G1 phase (Fazio *et al.*, 2018). Table 4 summarizes the other clinical and pharmacological actions of pomegranate seed oil/seed residue.

4.5 Improved gut health

The utilization of pomegranate has the potential to enhance the composition and balance of microorganism in the gut. The modes of action of pomegranate primarily revolve around the activation of nuclear factor-erythroid factor 2-related factor 2 (Nrf2), nuclear factor kappa B (NF-kB), and mitogen-activated protein kinase (MAPK) signaling pathways. Recently, computational molecular docking investigations have indicated that pomegranate extract and its phytochemicals possess the potential to hinder the interaction between the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) and the angiotensin-converting enzyme 2 (ACE2) receptor (Sharma *et al.*, 2021). In a recent study conducted by Song *et al.* (2022), the researchers investigated the impact of consuming pomegranate fruit pulp (PFP) on the gut microbiota in mice, and the study demonstrated that the consumption of pomegranate fruit pulp (PFP) resulted in a decrease in the abundance of firmicutes and proteobacteria, while promoting an increase in the abundance of bacteroidetes. Similarly, Al-Al-Gubory *et al.* (2016), incorporated pomegranate peel at a concentration of 10% in the animal diet and observed a modulatory effect on the activity of sodium oxide dismutase 1 (SOD1) and glutathione, while reducing the level of malondialdehyde (MDA) in the small intestine of ruminants compared to the control diet. According to Hollebeeck *et al.* (2012a), pomegranate peel and seed oil extracts showed preventive action against chronic inflammation and intestinal damage associated with necrotizing enterocolitis in an *in vitro* model of human intestine. The polyphenols in pomegranate, particularly ellagitannins, exhibited protective effects against ulcerative colitis by reducing ERK1/2 activity through downregulation of the mTOR downstream pathway (Hollebeeck *et al.*, 2012b).

5. Some potential applications of pomegranate seed powder and oil

The utilization of natural flocculants in the coagulation-flocculation process for water and wastewater treatment has gained significant interest owing to its straightforward approach and inherent safety. The utilization of response surface methodology (RSM) by Shabanizadeh *et al.* (2023) allowed for the optimization of the performance of Pomegranate seeds in treating paper recycling wastewater (PRWW). The optimization process considered pH, dosage, and settling time as variables. In order to examine the characteristics of the flocs and the flocculation-coagulation mechanism, various analyses such as ZP, FTIR, EDX, XRD, BET, and SEM were conducted. The optimal conditions, which resulted in the highest removal rates, were determined to be a Pomegranate seed dosage of 2391.91 mg/l, a pH value of 10.37, and a settling time of 25 minutes. Under these conditions, impressive removal rates of 87% for chemical oxygen demand (COD) and 98% for turbidity were achieved, with a high coefficient of determination ($R^2 = 0.988$). The ZP analysis revealed that the addition of pomegranate seeds increased the ZP of the PRWW from -11.7 mV to an isoelectric point (ZP ~ 0 mV), leading to the coagulation of particles through a charge neutralization mechanism (Shabanizadeh *et al.*, 2023). Ebrahim *et al.* (2023) utilized pectin extracts of pomegranate peel as natural thickening agent in textiles. The IR spectrum was used to characterize the modified pectin achieved through the addition of hexamine. The findings demonstrated that the utilization of pectin and modified pectin in printing resulted in enhanced color intensity compared to the standard sample printed with sodium alginate and Dell p. There was increase in the color strength (K/S) values for various fabric types, with sequential percentage increases of 3% for cotton, 13% for wool, 109% for polyester, and 25% for polyacrylic. Similarly, improvements of 49%, 64%, 12%, and 22% were observed for polyester. The fastness properties of the printed fabrics, including resistance to washing, dry and wet rubbing, acidic and alkaline perspiration, and light, demonstrated satisfactory to excellent levels. Additionally, enhancements were observed in fabric roughness, tensile strength, elongation at the break, and crease recovery. Moreover, fabrics printed with both pectin and modified pectin exhibited high resistance against gram-negative and gram-positive bacteria, as well as fungi (Ebrahim *et al.*, 2023). Costa *et al.* (2022) conducted an assessment to determine the viability of utilizing complex coacervation of PSO (pomegranate seed oil) with whey protein and gum arabic in order to create a functional ingredient suitable for incorporation in food formulations. Although, the spray-dried PSO microparticles displayed reduced retention of punicic acid, which could be attributed to its conversion into more heat-stable trans isomers, the study concluded that the utilization of PSO was more advantageous. Sogut *et al.* (2019) conducted a study aiming to enhance the stability of carrageenan and whey protein isolate blended films by incorporating PSO (pomegranate seed oil). The experimental results indicated that the film samples containing PSO exhibited significantly reduced water vapor permeability and solubility values. Moreover, the addition of PSO improved the elongation capacity of the films, indicating a plasticizing effect. The release rates of PSO in response to stimuli such as ethanol and acetic acid were also investigated, revealing a faster release of PSO in ethanol due to its higher capacity for solubilizing essential oils. Furthermore, whey protein isolate films demonstrated a higher diffusion rate of PSO, resulting in increased antimicrobial activity in these films (Sogut *et al.*, 2019).

6. Safety aspects

Even though seed oils have a lot of potential, more research is needed to find out if they are safe and if they have any side effects before, they can be used in consumer food and drinks. Despite the promise of seed oils, more study is needed to examine their safety and potential harmful effects before they may be employed at the consumer level in the food and beverage sector. Many investigations on the various components obtained from dried pomegranate dried seeds/oil have been conducted, however, no harmful effects in the tested dosage have been reported. When suitable sanitation protocols are not followed during the extraction, the formation of mycotoxins may raise toxicity problems in seed oils. Punicalagin, a polyphenol antioxidant, was shown to be non-toxic when against herpes simplex virus type-1 in *ex vivo* study model (Luo *et al.*, 2020).

Table 4: Other clinical and pharmacological effects of pomegranate seed oil/seed residue

In vivo studies	Clinicalstatus	Dose	Time	Effect	References
Wistar male rats	Nephroprotective	0.32-0.64 mg/kg PSO	24 h	Serum urea and creatinine levels were restored, whereas urine glucose and protein levels were reduced. Hydroperoxide production, biohydrogenation of CLnAs, chelation of transition metals, and the reduction of peroxy radicals and Cu2+-induced lipid peroxidation are all possible outcomes of this process.	Boroushaki *et al.*, 2013
Female Wistar	Memory	100-800 mg/2 ml/kg rats orally supplementation of pomegranate seed extract (PSE)	14 days	Improved memory with criterion condition responses in permanent cerebral ischemia in rats by treating ameliorated passive and active memory impairments with bilateral common carotid arteries occlusion.	Sarkaki *et al.*, 2013
Human trial	Breast cancer	20-40 µM punicic acid	-	Inhibited cell proliferation and induced apoptosis in estrogen sensitive and insensitive breast cancer cell lines, dependenton lipid peroxidation and the PKC pathway.	Hui *et al.*, 2013
		100-1000 µg/ml of Pomegranate seed oil	-	Inhibit the proliferation of T24 and J82 bladder carcinoma cell lines.	Lee *et al.*, 2013
Human cell lines	Bladder cancer	10-50 µg/mlof pomegranate ethanolic extract	72 h	Treatment groups had increased femoral length, weight, volume, density, and fourth lumbar hardness. OVX rats' femoral bones showed a substantial rise in calcium levels, ash content, and ash weight.	Yogesh, 2020
Ovariectomized (OVX) female albino rats	Osteoporosis	100-500 mg/kg of Ethanolic extract of pomegranate seed	90 days	Increased bone formation markers and alkaline phosphatase activity by 32 and 69%.Improved growth of tibia and bone mineral density (BMD).	Shaban *et al.*, 2017
Ovariectomized western albino eats		500 µl/kg body weight /day of pomegranate seed oil	12 weeks	Altered lipid profile in blood and prevent fat buildup in the body, as seen by a weight loss. According to these researchers, PSO extracts had a correcting impact on LDL-C and TG levels, lowering total cholesterol as compared to the HFD group.	Amri *et al.*, 2017
Rats	Obesity	2 ml/kg of pomegranate seed oil (PSO) daily	12 weeks		

According to Costa *et al.* (2019), the oxidative stability of PSO is poor, which is attributed to the oxidizability of CLnA found in it. The pomegranate seed oil (PSO) components delayed intestinal absorption and fast metabolism provide the path for increased bioavailability. As a result, several PSO production methods have been studied.

In a study conducted by Meerts *et al.* (2009), The safety and toxicological effects of pomegranate seed oil (PSO) were assessed through laboratory tests conducted outside a living organism (*in vitro*), such as Ames and chromosomal aberration tests, as well as through experiments conducted within a living organism (*in vivo*), including tests for acute toxicity and 28-day toxicity in Wistar rats. PSO showed no mutagenicity in the absence or presence of metabolic activity at precipitating concentrations of 5000 g/plate (Ames test) or 333 g/ml (chromosome aberration test). At 2000 mg PSO/kg body weight, the acute oral toxicity investigation yielded no noteworthy results. PSO was dosed at concentrations of 0, 10,000, 50,000, and 150,000 ppm in a 28-day food toxicity study, resulting in a mean consumption of 0-0, 825-847, 4269-4330, and 13,710-14,214 mg PSO/kg body weight per day in men and females, respectively. Increased hepatic enzyme activities determined in plasma (aspartate, alanine aminotransferase, and alkaline phosphatase) and increased liver-to-body weight ratios were observed at 150,000 ppm dietary exposure to PSO (Meerts *et al.*, 2009).

7. Conclusion

Pomegranate consumption has grown as a result of its supposed health advantages. Pomegranate and its derivatives, particularly dried pomegranate seed residue, contain various chemical constituents with possible physiological activity. This review article outlines some of the pharmacological and toxicological mechanisms and properties of dried pomegranate seed residue and oil, and it has been demonstrated that there is a substantial body of evidence that supplementation with dried pomegranate seed residue/oil may protect against a wide range of diseases. Cancer, diabetes, inflammation, neurotoxicity, oral and skin issues are among these ailments. High antioxidant content of pomegranate seed oil may be responsible for its positive effects. As a result, it is advised that dried pomegranate seed residue be used to treat and protect against specific illnesses and disorders, although the underlying mechanisms of this protection should be investigated further.

Acknowledgment

The corresponding author acknowledges the IoE Scheme research grant from Banaras Hindu University, Varanasi, India.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abiola, T. (2018). Proximate composition, phytochemical analysis and *in vivo* antioxidant activity of pomegranate seeds (*Punica granatum*) in female albino mice. Reviews, pp:439.

Al-Gubory, K. H.; Blachier, F.; Faure, P. and Garrel, C. (2016). Pomegranate peel extract decreases small intestine lipid peroxidation by enhancing activities of major antioxidant enzymes. Journal of the Science of Food and Agriculture, **96**(10):3462-3468.

Amri, Z.; Lazreg-Aref, H.; Mekni, M.; El-Gharbi, S.; Dabbaghi, O.; Mechri, B. and Hammami, M. (2017). Oil characterization and lipids class composition of pomegranate seeds. Bio. Med. Research International, pp:17.

Ashaolu, T. J. and Suttikhana, I. (2023). Plant based bioactive peptides: A review of their relevant production strategies, in vivo bioactivities, action mechanisms and bioaccessibility. International Journal of Food Science and Technology, **58**(5):2228-2235.

Banerjee, J.; Singh, R.; Vijayaraghavan, R.; MacFarlane, D.; Patti, A. F. and Arora, A. (2017). Bioactives from fruit processing residues: Green approaches to valuable chemicals. Food Chemistry, **225**:10-22.

Bashandy, M. A.; Ibrahim, D. F.; Hasan, H. F. and El-Sharkawy, M. A. (2019). Effect of Punica granatum seeds on doxorubicin and gamma radiation-induced hepatotoxicity in Wistar rats. Int. J. Biol. Res, **4**:44-50.

Bchir, B.; Besbes, S.; Karoui, R.; Attia, H.; Paquot, M. and Blecker, C. (2012). Effect of air-drying conditions on physicochemical properties of osmotically pre-treated pomegranate seeds. Food and Bioprocess Technology, **5**:1840-1852.

Benchagra, L.; Berrougui, H.; Islam, M. O.; Ramchoun, M.; Boulbaroud, S.; Hajjaji, A. and Khalil, A. (2021). Antioxidant effect of moroccan pomegranate (*Punica granatum* L. *sefri variety*) extracts rich in punicalagin against the oxidative stress process. Foods, **10**(9):2219.

Ben-Simhon, Z.; Judeinstein, S.; Trainin, T.; Harel-Beja, R.; Bar-Ya'akov, I.; Borochov-Neori, H. and Holland, D. (2015). A" White" anthocyanin-less pomegranate (*Punica granatum* L.) caused by an insertion in the coding region of the leucoanthocyanidin dioxygenase (LDOX; ANS) gene. PloS one, **10**(11):e0142777.

Bhat, M. M.; Thakur, N. S. and Jindal, N. (2014). Studies on the effect of drying methods and packaging on quality and shelf life of dried wild pomegranate arils. Asian Journal of Dairying and Foods Research, **33**(1):18-24.

Boroushaki, M. T.; Arshadi, D.; Jalili-Rasti, H.; Asadpour, E. and Hosseini, A. (2013). Protective effect of pomegranate seed oil against acute toxicity of diazinon in rat kidney. Iranian Journal of Pharmaceutical Research: IJPR, **12**(4):821.

Cervi, V. F.; Saccol, C. P.; Sari, M. H. M.; Martins, C. C.; da Rosa, L. S.; Ilha, B. D. and Cruz, L. (2021). Pullulan film incorporated with nanocapsules improves pomegranate seed oil anti-inflammatory and antioxidant effects in the treatment of atopic dermatitis in mice. International Journal of Pharmaceutics, **609**:121144.

Colombo, E.; Sangiovanni, E. and Dell'Agli, M. (2013). A review on the anti-inflammatory activity of pomegranate in the gastrointestinal tract. Evidence-Based Complementary and Alternative Medicine, pp:13.

Costa, A. M. M.; Gaspar, B. R. A.; Calado, V.; Tonon, R. V. and Torres, A. G. (2022). Microencapsulation of pomegranate (*Punica granatum* L.) seed oil by complex coacervation: Stability and application in an instant caffè latte beverage. Food Chemistry, **381**:132199.

Costantini, S.; Rusolo, F.; De Vito, V.; Moccia, S.; Picariello, G.; Capone, F. and Volpe, M. G. (2014). Potential anti-inflammatory effects of the hydrophilic fraction of pomegranate (*Punica granatum* L.) seed oil on breast cancer cell lines. Molecules, **19**(6):8644-8660.

Cruz-Chamorro, I.; Santos-Sánchez, G.; Álvarez-López, A. I.; Pedroche, J.; Lardone, P. J.; Arnoldi, A.; and Carrillo-Vico, A. (2023). Pleiotropic biological effects of Lupinus spp. protein hydrolysates. Trends in Food Science and Technology. PLOS, pp:410

Ebrahim, S. A.; Othman, H. A.; Mosaad, M. M. and Hassabo, A. G. (2023). Eco-friendly natural thickener (Pectin) extracted from fruit peels for valuable utilization in textile printing as a thickening agent. Textiles, **3**(1):26-49.

Elfalleh, W.; Tlili, N.; Nasri, N.; Yahia, Y.; Hannachi, H.; Chaira, N. and Ferchichi, A. (2011). Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (*Punica granatum*) fruits. Journal of food science, **76**(5):C707-C713.

Fazio, A.; Iacopetta, D.; La Torre, C.; Ceramella, J.; Muià, N.; Catalano, A. and Sinicropi, M. S. (2018). Finding solutions for agricultural wastes: Antioxidant and antitumor properties of pomegranate Akko peel extracts and α-glucan recovery. Food and Gunction, **9**(12):6618-6631.

Fernandes, L.; Pereira, J. A. C.; Lopéz-Cortés, I.; Salazar, D. M. and Ramalhosa, E. C. (2015). Physicochemical changes and antioxidant activity of juice, skin, pellicle and seed of pomegranate (cv. Mollar de Elche) at different stages of ripening. Food Technology and Biotechnology, **53**(4):397-406.

Ferrara, G.; Giancaspro, A.; Mazzeo, A.; Giove, S. L.; Matarrese, A. M. S.; Pacucci, C. and Gadaleta, A. (2014). Characterization of pomegranate (*Punica granatum* L.) genotypes collected in Puglia region, Southeastern Italy. Scientia Horticulturae, **178**:70-78.

Fischer, U. A.; Carle, R. and Kammerer, D. R. (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD–ESI/MSn. Food Chemistry, **127**(2):807-821.

Gaber, A.; Hassan, M. M.; Dessoky, E. D. S. and Attia, A. O. (2015). *In vitro* antimicrobial comparison of Taif and Egyptian pomegranate peels and seeds extracts. Journal of Applied Biology and Biotechnology, **3**(2):012-017.

García-Villalba, R.; Selma, M. V.; Espín, J. C. and Tomaìs-Barberaìn, F. A. (2019). Identification of novel urolithin metabolites in human feces and urine after the intake of a pomegranate extract. Journal of Agricultural and Food Chemistry, 67(40):11099-11107.

Gölükcü, M. (2015). The effects of drying methods, packaging atmosphere and storage time on dried pomegranate aril quality. Journal of Agricultural Sciences, 21(2):207-219.

Gulsunoglu, Z.; Karbancioglu-Guler, F.; Raes, K. and Kilic-Akyilmaz, M. (2019). Soluble and insoluble-bound phenolics and antioxidant activity of various industrial plant wastes. International Journal of Food Properties, 22(1):1501-1510.

Hajib, A.; Nounah, I.; Harhar, H.; Gharby, S.; Kartah, B.; Matthäus, B. and Charrouf, Z. (2021). Oil content, lipid profiling and oxidative stability of "Sefri" Moroccan pomegranate (*Punica granatum* L.) seed oil. OCL, 28: 5.

Haleem, A. E. (2016). Evaluation and application of some phytochemicals in dried pomegranate seed residues and guava seeds. Alexandria Journal of Food Science and Technology, 13(1):19-30.

Hollebeeck, S.; Larondelle, Y.; Schneider, Y. J. and During, A. (2012b). The use of pomegranate (Punica granatum l.) phenolic compounds as potential natural prevention against IBDs. Inflamm. Bowel Dis. Adv. Pathog. Manag. Intech-Publ. Belg, pp:275-300.

Hollebeeck, S.; Winand, J.; Hérent, M. F.; During, A.; Leclercq, J.; Larondelle, Y. and Schneider, Y. J. (2012a). Anti-inflammatory effects of pomegranate (Punica granatum L.) husk ellagitannins in Caco-2 cells, an in vitro model of human intestine. Food and Function, 3(8):875-885.

Hota, M.; Dahiya, D. S. and Kumar, S. (2017). Effect of Various Drying Methods on Drying Time and Quality of Pomegranate (*Punica granatum* L.) Arils. Int. J. Curr. Microbiol. App. Sci., 6(4):1711-1717.

Houston, D. M.; Bugert, J.; Denyer, S. P. and Heard, C. M. (2017). Anti-inflammatory activity of Punica granatum L. (Pomegranate) rind extracts applied topically to *ex vivo* skin. European Journal of Pharmaceutics and Biopharmaceutics, 112:30-37.

Hui, C., Qi, X.; Qianyong, Z.; Xiaoli, P.; Jundong, Z. and Mantian, M. (2013). Flavonoids, flavonoid subclasses and breast cancer risk: A meta-analysis of epidemiologic studies. PloS one, 8(1):e54318.

Jing, P. U.; Ye, T.; Shi, H.; Sheng, Y.; Slavin, M.; Gao, B. and Yu, L. L. (2012). Antioxidant properties and phytochemical composition of China-grown pomegranate seeds. Food Chemistry, 132(3):1457-1464.

Joseph, M. M.; Aravind, S. R.; George, S. K.; Varghese, S. and Sreelekha, T. T. (2013). A galactomannan polysaccharide from *Punica granatum* imparts *in vitro* and *in vivo* anticancer activity. Carbohydrate Polymers, 98(2):1466-1475.

Kaute, A.R.; Panchal J.B.; Gaikwad, R.S. and Dhemre J.K. (2018). Evaluation of promising lines of pomegranate for preparation of anardana. J Pharmacogn Phytochem, 7(6):2319-2325.

Kola, O.; Erva, P.; Reis, A. M.; Devrim, O. B.; Emrah, M. and Burcak, T. (2021). Fatty acids, sterols and triglycerides composition of cold pressed oil from pomegranate seeds. La Rivista Italiana Delle Sostanze Grasse, 98:197-204.

Krimer, M. V.; Vaštag, Ž.; Popoviæ, L.; Popoviæ, S. and Perièin-Starèeviè, I. (2014). Characterisation of black cumin, pomegranate and flaxseed meals as sources of phenolic acids. International Journal of Food Science and Technology, 49(1):210-216.

Kupnik, K.; Primožiè, M.; Vasiæ, K.; Knez, Ž. and Leitgeb, M. (2021). A Comprehensive study of the antibacterial activity of bioactive juice and extracts from pomegranate (*Punica granatum* L.) peels and Seeds. Plants, 10(8):1554.

Lee, S. T.; Lu, M. H.; Chien, L. H.; Wu, T. F.; Huang, L. C. and Liao, G. I. (2013). Suppression of urinary bladder urothelial carcinoma cell by the ethanol extract of pomegranate fruit through cell cycle arrest and apoptosis. BMC Complementary and Alternative Medicine, 13:1-11.

Li, R.; Chen, X. G.; Jia, K.; Liu, Z. P. and Peng, H. Y. (2016). A systematic determination of polyphenols constituents and cytotoxic ability in fruit parts of pomegranates derived from five Chinese cultivars. Springer Plus, 5(1):1-9.

Luo, Z.; Kuang, X. P.; Zhou, Q. Q.; Yan, C. Y.; Li, W.; Gong, H. B. and He, R. R. (2020). Inhibitory effects of baicalein against herpes simplex virus type 1. Acta Pharmaceutica Sinica B., 10(12):2323-2338.

Manasathien, J.; Indrapichate, K.; and Intarapichet, K. O. (2012). Antioxidant activity and bioefficacy of pomegranate *Punica granatum* Linn. peel and seed extracts. Global Journal of Pharmacology, 6(2):131-141.

Maphosa, Y. and Jideani, V. A. (2016). Dietary fiber extraction for human nutrition: A review. Food Reviews International, 32(1):98-115.

Marchiori, M. C. L.; Rigon, C.; Camponogara, C.; Oliveira, S. M. and Cruz, L. (2017). Hydrogel containing silibinin-loaded pomegranate oil based nanocapsules exhibits anti-inflammatory effects on skin damage UVB radiation-induced in mice. Journal of Photochemistry and Photobiology B: Biology, 170:25-32.

Meena, N. L.; Verma, P.; Pande, R.; Kumar, M.; Watts, A. and Gupta, O. P. (2020). Bioavailability and Nutritional Analysis of Flavonoids. Plant Phenolics in Sustainable Agriculture, 1:135-156.

Meerts, I. A. T. M.; Verspeek-Rip, C. M.; Buskens, C. A. F.; Keizer, H. G.; Bassaganya-Riera, J.; Jouni, Z. E. and Van de Waart, E. J. (2009). Toxicological evaluation of pomegranate seed oil. Food and Chemical Toxicology, 47(6):1085-1092.

Moore, M. P.; Cunningham, R. P.; Dashek, R. J.; Mucinski, J. M. and Rector, R. S. (2020). A fad too far? Dietary strategies for the prevention and treatment of NAFLD. Obesity, 28(10):1843-1852.

Peng, Y. (2019). Comparative analysis of the biological components of pomegranate seed from different cultivars. International Journal of Food Properties, 22(1):784-794.

Rowayshed, G.; Salama, A.; Abul-Fadl, M.; Akila-Hamza, S. and Emad, A. M. (2013). Nutritional and chemical evaluation for pomegranate (Punica granatum L.) fruit peel and seeds powders by products. Middle East Journal of Applied Sciences, 3(4):169-179.

Sahin, B.; Aygün, A.; Gündüz, H.; ªahin, K.; Demir, E.; Akocak, S. and ªen, F. (2018). Cytotoxic effects of platinum nanoparticles obtained from pomegranate extract by the green synthesis method on the MCF-7 cell line. Colloids and Surfaces B: Biointerfaces, 163:119-124.

Salem, M. A.; El-Shiekh, R. A.; Aborehab, N. M.; Al Karmalawy, A. A.; Ezzat, S. M.; Alseekh, S. and Fernie, A. R. (2023). Metabolomics driven analysis of *Nigella sativa* seeds identifies the impact of roasting on the chemical composition and immunomodulatory activity. Food Chemistry, 398: 133906.

Sarkaki, A.; Rezaiei, M. and Rafieirad, M. (2013). Improving active and passive avoidance memories deficits due to permanent cerebral ischemia by pomegranate seed extract in female rats. The Malaysian Journal of Medical Sciences: MJMS, 20(2):25.

Shaban, N. Z.; Talaat, I. M.; Elrashidy, F. H.; Hegazy, A. Y. and Sultan, A. S. (2017). Therapeutic role of Punica granatum (pomegranate) seed oil extract on bone turnover and resorption induced in ovariectomized rats. The journal of nutrition, health and aging, **21**:1299-1306.

Shabanizadeh, H. and Taghavijeloudar, M. (2023). A sustainable approach for industrial wastewater treatment using pomegranate seeds in flocculation-coagulation process: Optimization of COD and turbidity removal by response surface methodology (RSM). Journal of Water Process Engineering, **53**:103651.

Shabbir, M. A.; Khan, M. R.; Saeed, M.; Pasha, I.; Khalil, A. A. and Siraj, N. (2017). Punicic acid: A striking health substance to combat metabolic syndromes in humans. Lipids in Health and Disease, **16**(1):1-9.

Shahidi, F. and Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. Journal of Functional Foods, **18**:820-897.

Sharma, P. and Shanavas, A. (2021). Natural derivatives with dual binding potential against SARS-CoV-2 main protease and human ACE2 possess low oral bioavailability: A brief computational analysis. Journal of Biomolecular Structure and Dynamics, **39**(15):5819-5830.

Sogut, E.; Balqis, A. I.; Hanani, Z. N. and Seydim, A. C. (2019). The properties of ê-carrageenan and whey protein isolate blended films containing pomegranate seed oil. Polymer Testing, **77**:105886.

Song, H.; Shen, X.; Chu, Q. and Zheng, X. (2022). Pomegranate fruit pulp polyphenols reduce diet induced obesity with modulation of gut microbiota in mice. Journal of the Science of Food and Agriculture, **102**(5):1968-1977.

Sreeja, S.; Kumar, T. R. S.; Lakshmi, B. S. and Sreeja, S. (2012). Pomegranate extract demonstrate a selective estrogen receptor modulator profile in human tumor cell lines and *in vivo* models of estrogen deprivation. The Journal of Nutritional Biochemistry, **23**(7):725-732.

Wong, T. L.; Strandberg, K. R.; Croley, C. R.; Fraser, S. E.; Venkata, K. C. N.; Fimognari, C. and Bishayee, A. (2021). Pomegranate bioactive constituents target multiple oncogenic and oncosuppressive signaling for cancer prevention and intervention. In Seminars in Cancer Biology (Vol. 73, pp. 265-293). Academic Press.

Wu, S. and Tian, L. (2017). Diverse phytochemicals and bioactivities in the ancient fruit and modern functional food pomegranate (Punica granatum). Molecules, **22**(10):1606.

Yang, H.; Li, M.; Qi, X.; Lv, C.; Deng, J. and Zhao, G. (2012). Identification of seven water-soluble non-storage proteins from pomegranate (*Punica granatum* Linn.) seeds. Food Science and Technology International, **18**(4):329-338. doi:10.1177/1082013211428008

Yýlmaz, F. M.; Yüksekkaya, S.; Vardin, H. and Karaaslan, M. (2017). The effects of drying conditions on moisture transfer and quality of pomegranate fruit leather (*pestil)*. Journal of the Saudi Society of Agricultural Sciences, **16**(1):33-40.

Yogesh, H. S. (2020). Evaluation of antiosteoporosis activity of ethanolic extract of *Punica granatum* Linn. seeds in ovariectomized-induced osteoporosis rats. International Journal of Green Pharmacy (IJGP), **14**(1).

Citation	S. Yamini, Vinod Kumar Paswan, Abdelrazeq M. Shehata, Mahipal Choubey, Durga Shankar Bunkar and Vinay Venkatesh Varada(2023). Pomegranate (*Punica granatum* L.) seed: A review on nutritional profile, functional food properties, health benefits, and safety aspects. Ann. Phytomed., 12(1):93-104. http://dx.doi.org/10.54085/ap.2023.12.1.105.