

## REVIEW

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# A review on the extraction of polyphenols from pomegranate peel for punicalagin purification: techniques, applications, and future prospects

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Pomegranate peel is rich in polyphenols with punicalagin as the dominant compound but is always regarded as agricultural waste. This review focuses on the extraction of polyphenols as well as the purification, food industry applications and health effects of punicalagin. Considering polyphenol extraction, solvent extraction is the most commonly used method because it offers the highest total phenolic content; however, it is time consuming and energy intensive. Therefore, advanced methods are used to enhance its extraction efficiency, such as enzyme-assisted solvent extraction, resulting in the highest extraction yield. As for the purification of punicalagin from polyphenols, liquid chromatography is the most widely used method, and the highest purity is achieved with semi-high-pressure liquid chromatography (HPLC). Medium-pressure liquid chromatography (MPLC) and high-speed countercurrent chromatography (HSCCC) have similar effects, but relatively fewer studies have adopted these two methods. Besides, punicalagin has outstanding antioxidant properties and can thus be added to functional foods to extend their shelf life. Moreover, it shows great antibacterial effects on drug-resistant pathogens. Its anti-inflammatory potential is governed by its ability to treat infection and hyperimmune-related disorders. This work provides a comprehensive review of methods for extracting and purifying valuable compounds from pomegranate peel, particularly punicalagin, and highlights its potential applications in functional foods and health therapies.

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## Sustainability spotlight

Pomegranate peel, a major byproduct of the food industry, is generated at an estimated 1.5 million tons annually according to the Food Agricultural Organization (FAO). This review explores sustainable methods to repurpose this waste by extracting polyphenols and purifying punicalagin from pomegranate peel. Potential applications of punicalagin include its use as a natural food preservative, antioxidant in nutraceuticals, and agent in energy storage. This work aligns with the UN's sustainable development goals 2 (zero hunger), 3 (good health and well-being), 9 (industry and innovation), and 12 (responsible consumption and production). The re-utilization of food waste not only contributes to reducing environmental waste but promotes the development of preservatives and nutraceuticals, supporting a circular economy in the food and pharmaceutical industries.

## 1. Introduction

Pomegranate (*Punica granatum* L.), a deciduous shrub or small tree belonging to the Punicaceae family, is commonly grown and consumed in the Middle East, Europe, and Southeast Asia.<sup>1</sup> The pomegranate fruit possesses significant commercial value and finds extensive utilization in the fruit processing and beverage industries, particularly for the manufacturing of juice and soft drinks. However, a significant amount of nonedible waste is generated from this fruit during its processing, which is usually disposed of as agro-waste.<sup>2,3</sup> According to the Food Agricultural Organization (FAO), industrial pomegranate-processing centers generate an estimated 1.5 million tons of

waste annually.<sup>4</sup> The fruit consists of three parts, *i.e.*, leathery exocarp (peel), fleshy mesocarp, and seeds, including arils.<sup>5</sup> After juicing, a combination of peel, fleshy mesocarp, seeds, and aril residues remain. The combination can be manually fractionated into the following: peel (hard and tough) and seed residuals (a mixture of fleshy mesocarp, seeds, and aril residues). The peel accounts for about 40–50% of the total weight of the fruit.<sup>6</sup> According to Bar-Ya'akov *et al.*,<sup>7</sup> pomegranate peel is a source of several important bioactive compounds, such as phenolic compounds including ellagitannins, flavonoids, and anthocyanins, which are known to have potent antioxidant activities, thus suggesting its good potential for health-related applications. Thus, pomegranate waste can be valorized for use in the nutraceutical and pharmaceutical industries.<sup>8</sup>

Polyphenols belong to an organic chemical class with multiple phenolic structural units. Different parts of the

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pomegranate fruit, including seeds and peel, consist of up to 48 phenolic compounds, including flavonoids, anthocyanins, gallo-tannins, hydroxycinnamic acid, hydroxybenzoic acid, and hydrolyzed tannins such as ellagitannins and gallagyl esters.<sup>9</sup> Various biological activities of phenolic compounds in pomegranate peel extracts have been reported. These compounds regulate gene expression, reduce inflammation, and function as antioxidants, helping support immune functions in humans.<sup>10,11</sup> Pomegranate peel extract has antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, and antigenotoxic activities.<sup>12–14</sup> The important health properties of pomegranate products are mainly due to the strong biological activity of water-soluble hydrolyzable ellagitannins, whereby punicalagin accounts for up to 70% of the polyphenols found in commercially available pomegranate juice.<sup>15</sup> A major polyphenol of pomegranate is punicalagin, which exists in the  $\alpha$  and  $\beta$  forms (Fig. 1). This is a unique high-molecular-weight water-soluble compound, serving as the predominant ellagitannin in the pomegranate fruit.<sup>16–18</sup> Punicalagin shows multiple bioactivities, including antibacterial, anti-inflammatory, antioxidant, and anti-atherosclerosis activities.<sup>19–22</sup> These properties suggest that punicalagin has significant potential for use in functional foods and nutritional supplements.<sup>23</sup>

In the food industry, there is an increasing interest in mild, non-thermal approaches that effectively reduce microbial

activity, while preserving the physical, chemical, nutritional, and sensory characteristics of raw materials.<sup>24</sup> In most current studies, researchers are focused on the extraction of polyphenols from pomegranate peel, while targeted extraction methods for specific compounds such as punicalagin during the crude extraction stage remain relatively limited. Punicalagin makes up the majority of polyphenols in pomegranate peel, and therefore extraction methods targeting polyphenols as a group can also serve as an indirect reflection of punicalagin content. In this respect, initially this review introduces the methods for the crude extraction of polyphenols from pomegranate peel, followed by approaches for the purification of punicalagin. Although the traditional methods of maceration and solvent extraction are commonly used, currently active research in the area of extraction includes new and environmentally friendly methods such as supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), and enzyme-assisted extraction (EAE). The techniques employed to purify punicalagin include liquid chromatography (LC), high-speed countercurrent chromatography (HSCCC), and medium-pressure liquid chromatography (MPLC). Overall, this review aims to explore the various extraction methods for obtaining polyphenols from pomegranate peel, with punicalagin being the predominant compound, together with the purification

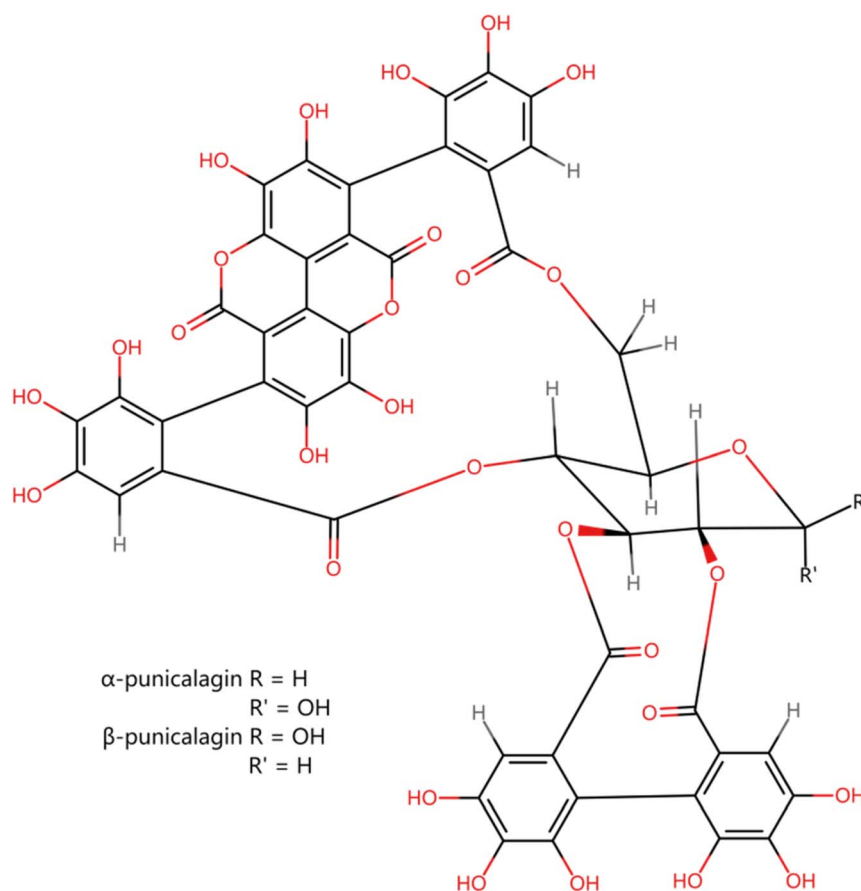


Fig. 1 Structure of punicalagin (PubChem CID: 44584733).



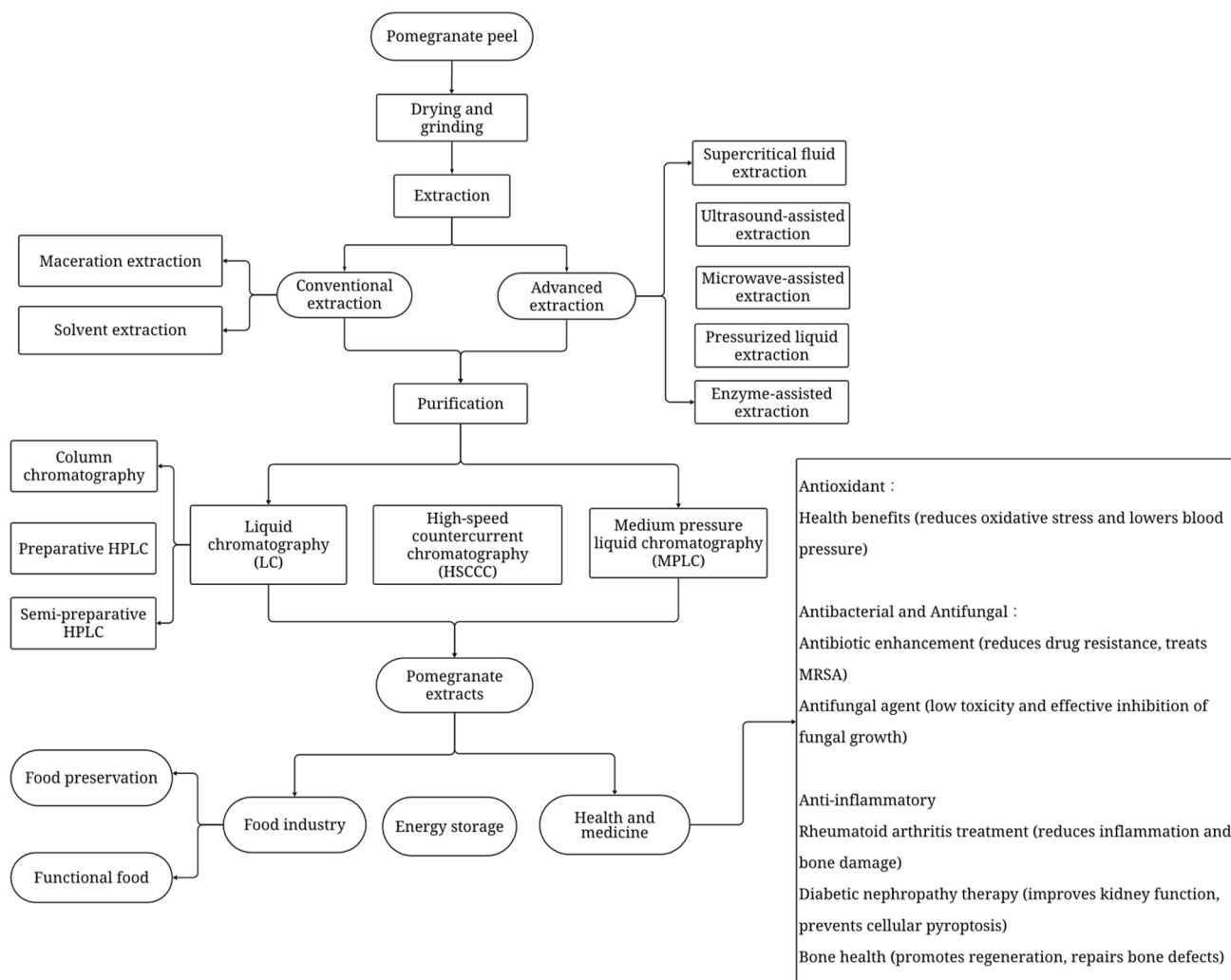


Fig. 2 Overview of techniques used in pomegranate peel processing for extraction of polyphenols and purification of punicalagin.

techniques targeting punicalagin and its applications in the food industry and pharmaceutical use. The overall framework and structure of this review are summarized in Fig. 2.

## 2. Extraction techniques

### 2.1 Conventional extraction methods

**2.1.1 Maceration.** Maceration is one of the most common methods used for the extraction of bioactive compounds from plant material. The plant material is soaked in an organic solvent at room temperature, after which the solvent penetrates the plant cells and dissolves the active substances therein.<sup>25</sup> Maceration is favored because of its simplicity and low cost, requiring only a basic lab setup, which makes it widely accessible for various experiments.

El-Beltagi *et al.*<sup>26</sup> obtained pomegranate peel powder using ethanol, methanol, and water. Briefly, 20 g powdered pomegranate peel was macerated in 100 mL of the above-mentioned solvents separately, shaking at 200 rpm at 37 °C for 24 h three times. The results indicated that the best extraction of phenolic

compounds was in distilled water, with the highest content of total phenols of  $513.8 \pm 4.0$  mg gallic acid equivalent (GAE)/100 g dry weight, flavonoids of  $45.3 \pm 0.5$  mg quercetin equivalent (QE) per g dry weight, and extraction yield of 0.55 g/10 g peel. During the maceration process described by Ranjha *et al.*,<sup>27</sup> mixed solvents were used, involving different concentrations of methanol, ethanol, and acetone. The sample-to-solvent ratio was maintained at 1 : 15 at a temperature of 40 °C in a shaking water bath for a period of 20 h. Their findings indicated that 50% methanol gave the highest extraction yield at 30.87%, while 75% methanolic extract exhibited the highest total phenolic content of 51.04 mg GAE per g and the total flavonoids reached as high as 27.61 mg QE per g. Thus, the type of solvent used is very important during the process of maceration. For example, water is a good solvent in extracting phenolic compounds from pomegranate peel, as reported by El-Beltagi *et al.*,<sup>26</sup> while 50% methanol gives superior extraction yields in comparison with 75% methanol according to Ranjha *et al.*<sup>27</sup> Alternatively, Ranjha *et al.*<sup>27</sup> compared the conventional maceration technique with sonication, where the yields



obtained using 50% methanol in sonication were the highest at about 31.45%. More importantly, both the phenolic and flavonoid contents increased by about 40% in the sonication method compared to that from maceration, indicating a significant improvement over traditional maceration methods.

In summary, the advantage of maceration is its inherent simplicity and low cost. Dynamic processes such as shaking or magnetic stirring may improve the interaction of the solvent with the plant material. Thus, the integration of dynamic processes, the choice of solvent, and advanced technologies such as ultrasonication can enhance its efficiency to a large degree. Therefore, to obtain the highest yield and quality of extracted bioactive chemicals using maceration, further procedures should be employed.

**2.1.2 Solvent extraction.** Solvent extraction, also known as chemical extraction or leaching, is a process whereby the constituent of a solid is extracted using a liquid solvent.<sup>28</sup> It is one of the widespread methods employed industrially and in a laboratory that embraces different variants, which include methods such as liquid–liquid extraction, liquid–solid extraction, and supercritical fluid extraction.<sup>29</sup> Solvent extraction is quite efficient for the purification and concentration of a certain component from complex mixtures. The extraction efficiency of compounds from pomegranate peels varies significantly depending on the solvent used. However, the implementation of solvent extraction is challenged by the environmental impact of solvents and the release of volatile organic compounds in the process.<sup>28</sup>

Negi and Jayaprakasha<sup>30</sup> extracted pomegranate peels using ethyl acetate, acetone, methanol, and water. Among these solvents, methanol extraction showed the most promising result, returning an extract yield of 9.4% compared with the minimal yield of 1.04% using ethyl acetate. The phenolic content in water, ethyl acetate, acetone and methanol extracts was found to be 140, 170, 400, and 460 mg per g catechin equivalent, respectively.

Wang *et al.*<sup>31</sup> reported that methanol was the most effective solvent for extracting phenolics from pomegranate peels, giving the highest total extract yield of 8.26% compared to that of water of 5.90%, ethanol of 1.55%, acetone of 0.37%, and ethyl acetate of 0.18% at a low temperature of 40 °C. However, although ethyl acetate resulted in the lowest extract yield, it produced the highest content of total phenolics in the extracts, reaching 20.24%, which suggests that ethyl acetate can have a better recovery and preservation of phenolics despite its lower yield. Meanwhile, considering toxicity and cost, water has a comparable value with methanol or ethyl acetate as a better extraction solvent. Under similar conditions but at higher temperatures of >95 °C, water could also achieve a high extractive yield of 11.15%. There is a delicate balance between the characteristics of the solvent and the operating conditions, both of which significantly influence the extraction outcome. Generally, methanol and water appeared to be good solvents for recovering phenolic contents from pomegranate peels, and this also refers to their mixture. Kennas and Amellal-Chibane<sup>32</sup> also depicted that 50% aqueous methanol resulted in the highest extraction yield of  $37.33\% \pm 5.3\%$  and total phenolics of

$625.525 \pm 6.83$  mg GAE per g compared to ethanol, methanol, acetone, and water (Table 1).

These studies highlight the relevance of the proper selection of solvent and extraction conditions according to the target compounds. Specifically, under the optimal conditions of any extraction technology, the extraction efficiency of different solvents would be different due to the interaction between the extraction technology and chemical properties. In general, selecting a suitable solvent and optimizing the extraction conditions are two main factors that can maximize the yields of valuable compounds separated from pomegranate peel.

## 2.2 Advanced extraction methods

**2.2.1 Supercritical fluid extraction (SFE).** Supercritical fluid extraction (SFE) is a method used to extract active ingredients from plant materials by utilizing the unique properties of a supercritical fluid. A supercritical fluid is a substance that after being heated above its critical temperature and compressed beyond its critical pressure, comes to a state where distinct liquid and gas phases no longer exist. In this supercritical state, the fluid behaves as a single phase with properties of both a gas and a liquid, which can diffuse into solid matrices like a gas and dissolve substances like a liquid.<sup>31</sup>

Panja<sup>31</sup> also explained that carbon dioxide is the most attractive fluid for SFE due to its non-toxic, non-flammable and chemically inert properties, which is also available in high quality and large quantities. CO<sub>2</sub> has a low critical temperature of 31 °C, indicating that little heating will be needed to achieve its critical point, making it economically feasible both for laboratory and pilot plant operations. Notably, the critical pressure of CO<sub>2</sub> is very high, *i.e.*, 73 atm. Despite this high pressure, the benefits of using CO<sub>2</sub> as a solvent, particularly in terms of safety and environmental impact, often outweigh the challenges associated with high-pressure conditions.

The research by Bustamante *et al.*<sup>33</sup> showed that small changes in pressure and temperature, together with the addition of cosolvents such as ethanol, can effectively change the solvency power of supercritical fluids, thereby influencing the yield and quality of extracts. These researchers demonstrated that operating within the range of 300–400 bar and 40–50 °C with 20% ethanol optimized the extraction of punicalagin from pomegranate residues. Modifying these parameters affects the density and solvent capacity of supercritical CO<sub>2</sub>, impacting the dissolution rates of specific bioactive compounds.

Ara and Raofie<sup>34</sup> analyzed the volatile chemicals and essential oils extracted from pomegranate peels and found that pressure had a significant impact on the yield of the extraction process. It was noticed that supercritical CO<sub>2</sub> extraction required less time compared to the traditional hydro-distillation method. As a result, it is more efficient given that it requires less energy to operate, making SFE more sustainable. The response surface approach shows that there is room for refinement in the extraction parameters during the optimization of the SFE settings. This means that each extraction parameter will need to be systematically modified to achieve the highest yields and purity. This approach illustrates the great





Table 1 Methods for the extraction of polyphenols from pomegranate peel

Extraction method	Sample preparation	Optimal conditions	Extraction yield	Ref.
Maceration extraction	Purchase location: supermarket, Assiut, Egypt Treatment: peels dried at 50 °C in an oven	Sample to solvent ratio: 1 : 5 (w/v) Solvent: distilled water	Extraction yield: 0.55 g/10 g peel Total phenolic content: 513.8 ± 4.0 mg gallic acid equivalent (GAE)/100 g dry weight	26
	Ripeness: fully ripe, no injury or infection	Shaking speed: 200 rpm at 37 °C for 24 h	Total flavonoid content: 45.3 ± 0.5 mg quercetin equivalent (QE) per g dry weight	
Maceration extraction	Form of sample: peel powder, stored at room temperature in dark plastic bags			
	Purchase location: local fruit market, Islamabad, Pakistan	Solvent: 50% methanol	50% methanol: extraction yield: 30.87%	27
	Treatment: peels dried at 50 °C for 48 h to less than 10% moisture	Solvent: 75% methanol	75% methanol: total phenolic content: 51.04 mg GAE per g	
	Ripeness: fully matured and ripened	Sample to solvent ratio: 1 : 15	75% methanol: total flavonoid content: 27.61 mg QE per g	
Solvent extraction	Form of sample: peel powder, stored in airtight polyethylene zip bags at 4–6 °C	Temperature: 40 °C		
	Purchase location: local markets, India	Shaking water bath: 20 h Solvent: methanol		30
	Treatment: Peels dried under natural conditions for 5 days	Sample to solvent ratio: 1 : 4 (w/v)	Highest extraction yield: 9.4% Phenolic content in methanol extract: 460 mg g <sup>-1</sup> in catechin equivalents	
	Form of sample: peel powder (60-mesh), extracted with multiple solvents	Temperature: 30 °C		
Solvent extraction	Purchase location: stiebs Pomegranate Products (Madera, CA, USA)	Stirring speed: 200 rpm on a magnetic stirrer Duration: 1 h		
	Treatment: peels dried at 40 °C to 8% moisture	Solvent and temperature: methanol, 40 °C	Methanol, 40 °C, 240 min: highest extract yield of total phenolics 8.26% Water, 95 °C, 2 min: highest extract yield 11.15%	31
Solvent extraction	Form of sample: ground peel, stored at –18 °C, separated by mesh size	Solvent and temperature: ethyl acetate, 40 °C	Ethyl acetate, 40 °C, 240 min: highest content of the total phenolics in extract 20.24%	
	Purchase location: Bouira, Algeria	Optimized solvent: 50% aqueous methanol	Highest extraction yield: 37.33% ± 5.3%	32
	Treatment: peels air-dried for 2 weeks (moisture content: 8.64%)	Sample to solvent ratio: 1 : 20 (w/v)	Total phenolics: 625.525 ± 6.83 mg GAE per g	
	Ripeness: collected between October and November	Temperature: room	Flavonoids: 52.68 ± 1.97 µg QE per mg of dry extract	
Supercritical CO <sub>2</sub> extraction	Form of sample: peel powder, stored in dark conditions at 4–6 °C	Shaking speed: 500 rpm		
	Purchase location: local producer, Vicuña, Valle de Elqui, Chile	Time: overnight Solvent: 20% ethanol	300 bar, 50 °C: extract yield: 8.5%	33
	Treatment: peels freeze-dried for 72 h	Pressure: 300 bar, 400 bar	400 bar, 40 °C: punicalagin: 9.5%	
	Ripeness: not specified	Temperature: 40 °C, 50 °C	400 bar, 40 °C: total phenolic content: 8.94 mg GAE per g	
	Form of sample: ground peels (30–40 mesh), stored at –20 °C			
	Purchase location: Meybod, Yazd, Iran	Methanol volume: 150 µL		34



Table 1 (Contd.)

Extraction method	Sample preparation	Optimal conditions	Extraction yield	Ref.
Supercritical fluid extraction	Treatment: peels air-dried for 2 weeks	Pressure: 350 atm	Oleic acid, palmitic acid and (–)-borneol was major compound in extracts The optimum extraction yield was 1.18% (w/w) for SFE Temperature: 55 °C	
Form of sample: ground peels (0.5 mm), stored at 4 °C		Ripeness: obtained in October 2012 Extraction time: 30 min		
Supercritical fluid extraction	Purchase location: Alfred Galke GmbH, Bad Grund, Germany Treatment: lyophilized peels	Fluid: SC CO <sub>2</sub> Co-solvent: ethanol	20 MPa: polyphenolic components: 11 561.84 µg g <sup>−1</sup> Predominant compound: ellagic acid content of 7492.53 µg g <sup>−1</sup> 10 MPa: the highest SFE yield (12.34%)	35
	Form of sample: ground lyophilized peels, used for extractions with various solvents	Pressure: 20 MPa, 10 MPa Temperature: 40 °C Power: 140 W		
Ultrasound extraction	Purchase location: local markets Treatment: peels dried at 40 °C with air circulation Form of sample: ground peels, stored at −20 °C, fractioned by sieves	Solvent: 70% ethanol–water mixture Temperature: 60 °C Extraction time: 30 min Power: 140 W Solvent: 50% ethanol–water mixture Temperature: 45 °C Extraction time: 30 min Ethanol concentration: 53%	Solvent: 70% ethanol–water mixture, temperature: 60 °C, extraction time: 30 min Extraction yield: 45.38% Ferric reducing antioxidant power: 63.37 mmol Fe <sup>2+</sup> /100 g Solvent: 50% ethanol–water mixture, temperature: 45 °C, extraction time: 30 min Total phenolics: 8923.24 mg GA/100 g dry weight	36
Ultrasonic extraction	Purchase location: Sichuan Huili Guoguo Fruit Food Co., Ltd., Chengdu, China Treatment: lyophilized peels, passed through a 100-mesh sieve Ripeness: full-ripe stage Form of sample: ground lyophilized peel powder, stored at 4 °C Purchase location: Sahakari Bhandar, a local fruit market, Mumbai, India Treatment: peels dried at 45 °C in a vacuum oven for 36 h Form of sample: peel powder, passed through a 500 µm mesh sieve and stored in a water and airtight high-density polyethylene (HDPE) pouch at −18 °C	Sample to solvent ratio: 1 : 25 (w/v) Ultrasonic power: 757 W Extraction time: 25 min Solvent type: 50% ethanol (v/v) Sample and solvent ratio: 2.17 g/100 mL, sonication power: 116 W Duty cycle: 80%	Yield of punicalagin: 505.89 ± 1.73 mg per g dry weight Yield: 0.48 g g <sup>−1</sup> Total phenolics: 177.54 mg GAE per g Total flavonoids: 35.71 mg QE per g	37
Pulsed ultrasound extraction		Extraction time: 6 min	Antioxidant capacity: 160.54 mg GAE per g	38



Table 1 (Contd.)

Extraction method	Sample preparation	Optimal conditions	Extraction yield	Ref.
Ultrasound with dynamic maceration extraction	Purchase location: pomegranates (Rabab-e-Neiriz cultivar) were obtained from local shop in Tabriz, Iran Treatment: peels dried at 25 °C for 5 days	Solvent: ethanol–water 60 : 40 (v/v)	Anthocyanin content: 21.65 mg cyn-3-glc/100 g with browning index: 54.92 Temperature: 61.8 °C, stirring speed: 1000 rpm, yield of extract (%) 38.14%	39
	Form of sample: peel powder, passed through a 325-mesh screen and stored in a plastic zip-lock bag at –18 °C	Sample and solvent ratio: 1 : 50 g mL <sup>–1</sup> Sonication time: 70 min	Temperature: 56.2 °C, stirring speed, 515 rpm: DPPH scavenging activity 90.35% Temperature: 62.2 °C, stirring speed, 1000 rpm: TPC 283.18 mg GAE/100 g dry weight pomegranate peel extract	
		Power: 400 W Temperature: 61.8 °C, 56.2 °C, 62.2 °C Dynamic maceration conditions: 24 h at 25 °C, stirring speed: 1000 rpm, 515 rpm Solvent: water		
Vacuum microwave-assisted aqueous extraction	Purchase location: pomegranate peels were collected after juicing process conducted at “Rodones S.A.” (Greece) using the “wonderful” pomegranate fruit variety Treatment: peels frozen after juice processing, milled into 2 kg samples Form of sample: peel powder, stored at –20 °C until extraction	Temperature: 61.48 °C Time: 10 min	Extraction temperature: 61.48 °C, extraction time: 10 min, microwave power: 3797.24 W, water to raw material ratio: 39.92 L kg <sup>–1</sup>  High total polyphenol content (5.542 mg GAE per g fresh pomegranate peel/min)	40
		Microwave power: 3797.24 W Water to raw material ratio: 39.92 L kg <sup>–1</sup> Solvent type: 50% aqueous ethanol		
		Sample to solvent ratio: 1 : 60 (w/v)	Yield: 199.4 mg GAE per g dry peel	
Microwave-assisted extraction	Purchase location: local producer, Rodi Hellas, Greece Treatment: peels dried at 40 °C for 48 h	Power: 600 W Process time: 4 min Irradiation power: 470 W	Antioxidant activity: 94.91% radical scavenging activity Punicalagin content: 143.64 mg per g dry weight	41
	Form of sample: ground peels, stored at –30 °C, with mean particle size of about 0.1 mm	Irradiation power: 800 W		
	Purchase location: a natural locality in village do, Bosnia and Herzegovina Treatment: peels air-dried at room temperature for 4–6 days Ripeness: collected in November Form of sample: ground peels, with particle size between 0.75–2 mm	Solvent: 50% ethanol	Extraction yield (EY): 41.31%  Total phenolics (TP): 20.61 g GAE/100 g Total flavonoids (TF): 3.29 g CE/100 g gallic acid: 53.54 mg/100 g Punicalin: 747.89 mg/100 g Punicalagin: 218.32 mg/100 g Irradiation power: 800 W; ellagic acid: 96.69 mg/100 g	
Microwave-assisted extraction			Irradiation power: 470 W	42

Table 1 (Contd.)

Extraction method	Sample preparation	Optimal conditions	Extraction yield	Ref.
Ultrasound and pressurized liquid extraction	Purchase location: pomegranate fruits (wonderful variety) cultivated in California (USA), purchased at an establishment in Limeira-SP (Brazil)	Solvent type: water, ethanol 70%	Water, 70 °C, 480 W and 3 cycles: highest yield of 61.72 ± 7.70 mg g <sup>-1</sup>	43
	Treatment: peels dried at 70 °C for 48 h	Temperature: 70 °C, 60 °C	Water, 70 °C, 400 W: α-punicalagin: 11.48 ± 0.21 mg per g dry weight	
	Ripeness: not specified	Power: 480 W, 400 W	Water, 70 °C, 400 W: β-punicalagin: 24.55 ± 0.61 mg per g dry weight	
	Form of sample: ground peels, with particle sizes of 0.177–1.410 mm, stored at –20 °C	Pressure: 10 MPa	Ethanol 70%, 60 °C, 400 W: ellagic acid: 1.28 ± 0.09 mg per g dry weight	
Pressurized liquid extraction	Purchase location: commercial farm, Vallenar, Atacama Region, Chile	Process temperature: 200 °C	Water, 70 °C, 400 W: sum of phenolic compounds: 43.27 ± 3.03 mg per g dry weight	44
	Treatment: peels dried at 60 °C for 16 h in an air-drying tunnel	Solvent: 77% ethanol	Total phenolic content (TPC) of 164.3 ± 10.7 mg GAE per g dry weight	
	Ripeness: ripening stage, collected in April 2017	At a pressure of 1500 psi for 20 min	Punicalagin content of 17 ± 3.6 mg per g dry weight	
	Form of sample: ground peel powder, passed through a 20 mesh sieve, stored at room temperature	Solvent: ethanol	Pressure: 40 bar: α-punicalagin: 48 ± 2 mg/100 g	
Pressurized liquid extraction	Purchase location: Ite district, Jorge Basadre province, Tacna, Peru	Temperature: 60 °C	β-Punicalagin: 146 ± 11 mg/100 g	45
	Treatment: peels dried at 40 °C for 72 h	Pressure: 40 bar, 80 bar	Ellagic acid: 25.6 ± 0.3 mg/100 g	
	Ripeness: not specified	Solvent: water	Pressure: 80 bar: global extraction yield: 45% on dry basis	
	Form of sample: ground peels, with average particle size of 0.50 mm, stored at –20 °C	Temperature: 40 °C	100 bar, 5 bar N <sub>2</sub> -Pi, 400 W: α-punicalagin: 14.87 ± 0.36 mg g <sup>-1</sup>	
Pressurized liquids assisted by ultrasound combined with an expansion gas	Purchase location: pomegranates grown in California (USA) of the variety wonderful were purchased from a market located in Limeira-SP	Pressure: 100 bar, 200 bar	100 bar, 5 bar N <sub>2</sub> -Pi, 400 W: β-punicalagin: 37.13 ± 1.44 mg g <sup>-1</sup>	46
	Treatment: peels dried at 40 °C for 48 h	N <sub>2</sub> -Pi: 5 bar	200 bar, 5 bar N <sub>2</sub> -Pi, 400 W: ellagic acid: 0.96 ± 0.05 mg g <sup>-1</sup>	
	Form of sample: ground peels, with particle sizes of 0.5–1.0 mm and >1.0 mm, stored at –20 °C	US-Pwr: 400 W	100 bar, 5 bar N <sub>2</sub> -Pi, 400 W: SPC (sum of phenolic compounds): 53.11 ± 1.79 mg g <sup>-1</sup>	
		Solvent: water	Total extraction yield: 41% ± 1.9% (w/w) per gram of dried pomegranate peel	
Enzymatic extraction combined with high pressure	Purchase location: Serpa, Portugal	High pressure: 300 MPa, 15 min	Antioxidant activity: 334 ± 2.8 mg trolox equivalents per g dry weight	47
	Treatment: peels dried at 40 °C until reaching 6% moisture	Enzymatic: 4% (v/v) pectinase (novozyme) and 4% (v/v) cellulase (novozyme)	Total phenolic compounds: 207 ± 2.8 mg GAE per g dry weight	
	Ripeness: ripe			
	Form of sample: ground peels, stored at –20 °C			





Table 1 (Contd.)

Extraction method	Sample preparation	Optimal conditions	Extraction yield	Ref.
Enzyme-assisted extraction combined with microwave extraction	Treatment: dried PP powder treated with viscozyme for 1 h, followed by microwave-assisted extraction Ripeness: not specified Form of sample: ethanol extract	Solvent: 30% ethanol  Enzymatic treatment: 0.6% (w/v) viscozyme for 1 h Response surface methodology (RSM) optimal condition: power 443.5 W, time: 131.0 min, and sample to solvent ratio: 23.6 : 1	Predicted value  TPC: 305.5 mg GAE per g Antioxidant activity using ferric reducing antioxidant power (FRAP): 1788 $\mu\text{mol TE per mg}$  Antioxidant activity using cupric reducing antioxidant activity (CUPRAC): 2641 $\mu\text{mol TE per mg}$ Actual experimental value: TPC: 326.7 $\pm$ 21.3 mg GAE per g Antioxidant activity using FRAP: 1751 $\pm$ 64 $\mu\text{mol TE per mg}$ Antioxidant activity using CUPRAC: 2589 $\pm$ 87 $\mu\text{mol TE per mg}$	48
Enzyme-assisted extraction combined with supercritical fluid extraction or solvent extraction	Purchase location: local agro-processing units, Faisalabad, Pakistan  Treatment: peels dried under ambient conditions Form of sample: peel powder, passed through an 80-mesh sieve	Enzyme pre-treatment: enzyme: cocktail enzyme (a mixture of cellulase, pectinase, and protease; 50 : 25 : 25) at 3.8% concentration Temperature: 49 °C  pH: 6.7  Time: 85 min Supercritical fluid extraction: CO <sub>2</sub> flow rate: 2 g min <sup>-1</sup> Ethanol injection rate: 0.2 g min <sup>-1</sup> Temperature: 55 °C Pressure: 300 bar Solvent extraction as comparison: solvent: 80% ethanol	Enzyme- assisted solvent extraction: extract yield: 32.19% $\pm$ 1.26% Total phenolics: 301.53 $\pm$ 7.86 mg GAE per g          Enzyme-assisted supercritical fluid extraction: extract yield: 32.19% $\pm$ 1.26% Total phenolics: 301.53 $\pm$ 7.86 mg GAE per g	49
Enzyme-assisted extraction combined with ultrasound extraction	Purchase location: local market, Tezpur, Assam, India Treatment: peels dried at 45 °C for 48 h Ripeness: maturity Form of sample: peel powder, stored at 4 °C	Solvent: water  Sample to solvent ratio: 1 : 10 Ultrasound treatment: 41.45 min Enzyme: viscozyme Enzyme concentration: 1.32 mL/100 mL Incubation temperature: 44.85 °C Incubation time: 1.82 h	TPC (mg GAE per g dry solid): 19.77  TFC (mg QE per g dry solid): 17.97 Radical scavenging activity: 74.213%	50

applicability and efficacy of SFE in extracting various phytochemicals such as polyphenols and essential oils. Kupnik *et al.*<sup>35</sup> emphasized pressure as the main parameter increasing the solubility of bioactive compounds in a relevant mixture of supercritical CO<sub>2</sub> and ethanol. Under the optimal conditions of 20 MPa, the highest levels of ellagic acid were obtained from lyophilized pomegranate peel.

SFE technology is characterized by the use of nontoxic and non-flammable CO<sub>2</sub>, combined with co-solvents, mostly ethanol. Using the optimum pressure and temperature conditions, the extraction efficiency and bioactive compound yields can be greatly enhanced. Thus, SFE technology has wide applicability and effectiveness in the extraction of various phytochemicals and essential oils.

**2.2.2 Ultrasound-assisted extraction (UAE).** Ultrasound-assisted extraction (UAE) has developed into a key technique for the extraction of bioactive compounds from plant materials. It involves the use of ultrasound waves in the frequency range of 20 kHz to 2 MHz for disruption of the plant matrix and improving the penetration of solvents.<sup>51</sup> Basically, this method relies on sonication, including the creation and collapse of cavitation bubbles near the plant matrix give rise to high-pressure, high-temperature microjets. These microjets crash onto the surface of the plant with great force, breaking up its matrix and ensuring that the solvent comes into direct contact with the active ingredients of the cell, thereby enhancing their leaching into the solvent more effectively.<sup>52</sup>

Tabaraki *et al.*<sup>36</sup> investigated the UAE conditions for extracting phenolic-rich by-products from pomegranate peel using response surface methodology with central composite design. They achieved an extraction yield of 45.38% and ferric reducing antioxidant power of 63.37 mmol Fe<sup>2+</sup>/100 g under the optimum extraction conditions of power of 140 W, 70% ethanol–water mixture, temperature of 60 °C, and extraction time of 30 min. Employing a 50% ethanol–water mixture at 45 °C for 30 min, they obtained the highest total phenolic content of 8923.24 mg GAE/100 g dry weight. The extraction yield increased by 6.38% when the concentration of ethanol was increased from 30% to 70%. Thus, an appropriate ethanol content was found to significantly improve the extraction efficiency.

Similarly, Liu *et al.*<sup>37</sup> investigated the UAE conditions for punicalagin, which is the most abundant polyphenol in pomegranate peel, using response surface methodology coupled with a Box-Behnken experimental design. The optimized conditions were an ethanol concentration of 53%, a sample-to-liquid ratio of 1:25 w/v, an ultrasonic power of 757 W, and an extraction duration of 25 min. These extraction parameters resulted in the extraction of a high punicalagin content of 505.89 ± 1.73 mg per g dry weight. Combined with statistical optimization techniques such as response surface methodology (RSM), UAE becomes a highly adaptable method for the optimization of the extraction parameters to achieve elevated yields and purity. This method is effective both for the broad-spectrum extraction of phenolics and the isolation of particular polyphenols, such as punicalagin.

More and Arya<sup>38</sup> introduced pulsed UAE, which enhanced the traditional UAE by reducing the energy consumption, while maintaining a high extraction efficacy. Using a solvent of 50% ethanol (v/v) to extract bio-actives from pomegranate peel, but with a lower sonication power of 116 W and an 80% duty cycle for a brief 6 min duration, a high extraction yield of 48% was achieved, with a total phenolic content of 177.54 mg GAE per g.

Another novel approach in this regard is the research on ultrasonication in conjunction with other modes of extraction. As depicted by Andishmand *et al.*,<sup>39</sup> ultrasonication combined with dynamic maceration was employed to recover phenolic compounds from pomegranate peel, with the optimal conditions identified using response surface methodology. A sonication time of 70 min at 400 W, coupled with dynamic maceration for 24 h at 25 °C and a stirring speed of 1000 rpm yielded the highest phenolic extract of 38.14% and a total phenolic content of 283.18 mg GAE per 100 g dry weight at approximately 60 °C. This synergistic approach demonstrated the potential of integrating ultrasonic energy with mechanical agitation to enhance the extraction efficiency. The combined method outperformed its individual techniques when used independently, offering a more effective strategy for the recovery of bioactive compounds in industrial applications.

The cumulative results show the huge potential of ultrasound technologies in terms of increasing the yield, shortening the processing time, and greater sustainability in industry. This technology is considered to be innovative for the extraction of natural compounds for the production of functional foods and health products because of the adaptability of UAE and its derivatives, including pulsed UAE, and the way it combines dynamic maceration with other approaches.

**2.2.3 Microwave-assisted extraction (MAE).** Microwaves are electromagnetic waves that can penetrate materials and induce molecular motion. They can quickly and uniformly heat solvents, therefore increasing the pace of extraction, while reducing the thermal damage to delicate components. The simplicity, rapidity, and cleanliness of this approach make it highly desirable for the recovery of bioactive chemicals. Furthermore, employing high microwave-absorbing solvents such as dimethyl sulfoxide (DMSO), ethanol, and methanol can increase the extraction efficiency.<sup>51</sup>

Basically, there are two mechanisms for microwave extraction, *i.e.*, dielectric heating and ionic conduction. Dielectric heating is based on the principle that the rotation of dipole moments in molecules rapidly aligns and realigns with the changing electromagnetic field to generate heat *via* molecular friction. Alternatively, when ions in solution align with an electromagnetic field, their movement through the solvent generates heat. These mechanisms enhance effective heat generation inside the solvent, hence improving the extraction process. This indicates that microwave-assisted extraction promotes effective heat generation inside the extracting media, hence improving the extraction process.<sup>53–55</sup>

Kaderides *et al.*<sup>41</sup> reported that the optimized microwave-assisted extraction conditions for phenolics from pomegranate peels were 50% aqueous ethanol, a ratio of 60/1 mL g<sup>−1</sup> for solvent/solid and 600 W for the microwave power. This setup



significantly shortened the process time to a mere 4 min with an extraction yield of 199.4 mg GAE per g dry peel and punicalagin content of 143.63 mg per g dry matter. Therefore, the short processing time and excellent efficiency of microwave-assisted extraction make it more attractive compared to approaches such as ultrasound-assisted extraction, requiring more time to produce similar results.

Skenderidis *et al.*<sup>40</sup> reported the vacuum microwave-assisted extraction (MAE) of pomegranate peels, presenting a valuable tool for the pomegranate juice industry to execute effective and economically viable green extraction technology on an industrial scale. This was aimed at increasing the phenolic content and antioxidant activity using merely water, avoiding organic solvents. It was found that the optimum conditions for extraction temperature, duration, microwave power, and water-to-raw material ratio are 61.48 °C, 10 min, 3797.24 W, and 39.92 L kg<sup>-1</sup>, respectively, amounting to a high total polyphenol content of 5.542 mg GAE per g fresh pomegranate peel per min. By reducing the process time, energy, and solvent costs, while maximizing the quality (indicated by total phenolic content and antioxidant capacity) and avoiding harmful solvents, this method effectively converts a fruit by-product into a functional ingredient with high antioxidant activity.

Additionally, Vradić *et al.*<sup>42</sup> compared the impact of subcritical water and microwave-assisted extraction on the phenolic compounds in pomegranate peel. This study proved that microwave-assisted extraction using a lower microwave power and 50% ethanol was more efficient than subcritical water extraction. High-quality polyphenol-rich extracts with no presence of 5-hydroxymethylfurfural (HMF), which is associated with high temperature processes, were produced. In this work, microwave-assisted extraction was described as a low-cost green methodology that supports the circular economy due to the optimization of natural resource utilization and reducing food waste.

Overall, microwave-assisted extraction has been demonstrated to be a successful method for extracting bioactive chemicals from a considerable number of natural products, including pomegranate peel. In this process, the solvents are rapidly heated, which drastically reduces the extraction time and maintains the integrity of thermally sensitive compounds. Some other benefits include the fact that it is a relatively green methodology, uses less solvent, and high cleanliness, all aligning with the principles of a circular economy.

**2.2.4 Pressurized liquid extraction (PLE).** Pressurized liquid extraction (PLE) is based on applying elevated pressure and temperature conditions to ensure the extraction of compounds from solid and semi-solid samples. In this approach, liquid extraction can be carried out above the boiling point of the solvents used at atmospheric pressure, increasing the solubility and matrix desorption, and hence extraction efficiency.<sup>43,46</sup>

PLE and its advanced versions, such as ultrasound-assisted PLE (UAPLE), represent very promising approaches for the recovery of bioactive compounds from fruit by-products, where the economic evaluation of UAPLE indicates its viability for industrial-scale application.<sup>56</sup> According to Toledo-Merma

*et al.*,<sup>45</sup> high extraction yields of  $\alpha$ -punicalagin ( $48 \pm 2$  mg/100 g),  $\beta$ -punicalagin ( $146 \pm 11$  mg/100 g), and ellagic acid ( $25.6 \pm 0.3$  mg/100 g) could be achieved using PLE by modifying the temperature to about 60 °C and pressure to 40 bar. At higher pressures, such as 80 bar, it could recover even greater quantities. This simply proves that PLE can be tailored to suit the diverse requirements of compounds, making this process more economically viable.

Alternatively, Sumere *et al.*<sup>43</sup> reported an improvement in extraction efficiency from pomegranate peel with the introduction of ultrasound into PLE. Using 70% aqueous ethanol, temperature of 70 °C, ultrasound power of 480 W and pressure of 10 MPa, the highest extraction yield of  $61.72 \pm 7.70$  mg g<sup>-1</sup> was achieved. Santos *et al.*<sup>46</sup> further improved UAPLE by adding an expansion gas and maintained the heat-sensitive chemicals. With a lower temperature of 40 °C, Santos *et al.*<sup>46</sup> obtained an  $\alpha$ -punicalagin content of  $14.87 \pm 0.36$  mg g<sup>-1</sup> and  $\beta$ -punicalagin content of  $37.13 \pm 1.44$  mg g<sup>-1</sup> from pomegranate peel with a much lower temperature than 200 °C employed by García *et al.*<sup>44</sup> and greater extraction yield than that obtained by Toledo-Merma *et al.*<sup>45</sup>

Overall, UAPLE with expansion gas seems to offer good extraction yields and effectiveness with the least amount of damage to the environment. Further research on the improvement of these methods should still be done, mainly by adding ultrasound and searching for the best concentrations of solvents and temperatures to make them even more useful.

**2.2.5 Enzyme-assisted extraction (EAE).** Enzyme-assisted extraction (EAE) is a promising approach to replace the traditional solvent-based methods for the extraction of bioactive compounds from plant materials such as pomegranate peel. Enzymes play a key role in EAE to catalyze the processes for easier extractability, modification, or generation of new complex natural compounds.<sup>57</sup>

EAE is an advanced approach based on the ability of enzymes to catalyze reactions with high specificity and regioselectivity in aqueous media and mild conditions, which would not damage the integrity of the bioactive compounds, as reported by Gardossi *et al.*<sup>58</sup> Physical parameters such as temperature and pressure are crucial for maximizing the extraction efficiency. For instance, Alexandre *et al.*<sup>47</sup> employed a combination of pectinase and cellulase under high-pressure conditions at 300 MPa for 15 min, achieving the highest total extraction yield of  $41\% \pm 1.9\%$  (w/w) per gram of dried pomegranate peel and a total phenolic content of  $207 \pm 2.8$  mg GAE per g dry weight.

Similarly, physical extraction methods can also increase the efficiency when combined with EAE. The combination of cellulolytic enzymes with microwave-assisted extraction was reported to highly increase the phenolic yield and antioxidant activities of pomegranate peel extracts.<sup>48</sup> According to Mushtaq *et al.*,<sup>49</sup> the combination of enzymes and supercritical fluid extraction resulted in a high level of total phenolics of  $301.53 \pm 7.86$  mg GAE per g and the combination of enzymes and solvent extraction resulted in a high yield of  $65.89\% \pm 2.64\%$ , which is also the highest yield among the methods in Table 1. Again, Nag and Sit<sup>50</sup> combined ultrasound with the enzymatic method to recover the total phenolic content (TPC) of 19.77 mg GAE per g dry solid and total flavonoid content of (TFC) 17.97 mg



quercetin equivalent per g dry solid from pomegranate peels. This study demonstrated that ultrasound-assisted enzymatic extraction is a promising method for obtaining polyphenols from pomegranate peels as well as other agricultural and food waste, offering an effective alternative to chemical solvent extraction. The selection of enzymes is crucial, given that the aforementioned studies identified that pectinase, cellulase, and Viscozyme are effective enzymes for extracting bioactive compounds from pomegranate peel.<sup>47–49</sup>

Therefore, EAE is an easy and time-effective way for extracting bioactive compounds from pomegranate peel. The ability of EAE to act in synergy with other extraction technologies, together with its environmental and economic advantages, make it one of the best options among the advanced methods. Enzyme cost is one of the major hurdles in the commercial application of enzyme technology.<sup>59</sup> In this respect, the combination of enzymes with other modern technologies such as ultrasound can balance the cost to a great extent, while using ultrasound and enzymes can be even more cost-effective given that the extraction yield of polyphenols is higher, with the energy consumed by ultrasonic equipment being much lower.<sup>50</sup> Thus, future research should focus on optimizing enzyme combinations and exploring new physical methods that will further enhance the yield and reduce the cost of EAE for its wide application in the food and pharmaceutical industries.

### 3. Purification

Purification is an essential step following the extraction procedure for the commercial use of bioactive chemicals.<sup>60</sup> Chromatography effectively separates, detects, identifies, and measures the components in complex mixtures<sup>61</sup> with its performance influenced by factors such as adsorption and molecular weight. Liquid chromatography (LC) is a common method that has been applied to substances such as pomegranate peel extract because of its ability to separate and identify biomolecules and other active components with the use of a liquid mobile phase and a stationary phase.<sup>62</sup> Within LC, various techniques have been greatly utilized, such as high-performance liquid chromatography (HPLC) and supercritical fluid chromatography.<sup>63</sup> Recent studies emphasized the use of liquid chromatography (LC), high-speed countercurrent chromatography (HSCCC), and medium-pressure liquid chromatography (MPLC) to obtain high-purity products from pomegranate peel extracts.

#### 3.1 Liquid chromatography (LC)

Liquid chromatography (LC) includes preparative high-performance liquid chromatography and semi-preparative HPLC. LC is the most feasible compound isolation method. Nowadays, a set of equipment is combined into a single purification system for increasing the yield of purified compounds. Macroporous resins are high loading, permeable and multi-functional, and hence they are combined with LC as a pretreatment.<sup>64</sup> For example, HPD-300 resin possesses an excellent capacity to enrich total polyphenols, where the

maximum loading concentration improved the extraction ratio of crude pomegranate from 18.02% to 68.45%.<sup>65</sup> Subsequently, the most effective macroporous resin for punicalagin was assessed. Liu *et al.*<sup>37</sup> examined six standard macroporous resins (201-7, D101, AB-8, HPD-100, HPD-300, and HPD-826), and the results of the adsorption studies showed that D101 is the optimum. The purity of punicalagin reached 71.85% by UAE combined with D101.

The integration of other purification procedures, such as preparative HPLC (Table 2), may result in improvements in both the purity and yield of the target compounds. Preparative high-performance liquid chromatography (prep-HPLC) serves as an important approach for the large-scale separation of natural products. Big columns, high flow rates, and small particle sizes are combined to obtain high-resolution and efficient separation. The term “preparative” normally implies that the process is done on a large scale, and its improved capabilities, together with the reduction in cost make it affordable by most research organizations, allowing the exact purification of complicated mixtures.<sup>71,72</sup> Prep-HPLC can be used with macroporous resins to purify polyphenols from plant extracts. Liu *et al.*<sup>37</sup> achieved punicalagin with a purity of 71.85% by purification using D101 macroporous resin. The purity of the extract increased to 92.15%, while the yield reached  $58.90 \pm 1.10$  mg when a pre-HPLC on C18 column was used for purification of the initial extract. According to Fischer *et al.*,<sup>66</sup> the purification of punicalagin involved lyophilizing and extracting pomegranate peels with aqueous methanol, followed by preparative HPLC using a C18 column with gradient elution, and detection at 280 nm, achieving a purity of 93%. Lu *et al.*<sup>67</sup> achieved even greater purity with a one-step purification using prep-HPLC. The optimum choice for purifying punicalagin was the combination of methanol and trifluoroacetic acid (TFA) in water as the mobile phase, from which 81.7 mg of punicalagin with a purity of 98.05% was obtained from 300 mg of crude pomegranate husk extract. Considering this high purity, the parameters of the mobile phase, stationary phase and the flow rate can be used as a reference for the purification of punicalagin. Similarly, Oudane *et al.*<sup>68</sup> achieved an extraordinarily high purity with a comparable mobile phase. They extracted 20 g of ground *Punica granatum* with methanol, and then processed the crude extract with a series of steps, and finally obtained 23 mg of >99% pure punicalagin using semi-preparative HPLC with a reversed-phase C18 column and a linear gradient of methanol and TFA. The combination of column chromatography with prep-HPLC has been proven to be very efficient for the isolation of high-purity molecules such as punicalagin.

#### 3.2 High-speed countercurrent chromatography (HSCCC)

High-speed countercurrent chromatography (HSCCC) has been developed since the mid-1980s with its objective to increase the efficiency and stability of countercurrent chromatography and shorten the separation time by using liquid–liquid partitioning for efficient preparative separation.<sup>73,74</sup> Its low solvent usage, good reproducibility, and large loading capacity for solutes make this technique popular for the separation of phenolics in natural compound extraction.<sup>37,75</sup> HSCCC has been extensively





Table 2 Purification methods of punicalagin from pomegranate peel

Method	Material conditions	Operation process	Purity	Ref.
D101 resin-based column chromatography and prep-HPLC	D101 resin column with 10–80% methanol, C18 column with 5–30% methanol	Using a D101 resin column, sequentially eluted with water and 10–80% methanol (gradient) at a flow rate of 30 mL min <sup>−1</sup> , then purified using a prep-HPLC system with a C18 column and a gradient of 5–30% methanol (gradient) over 60 min at a flow rate of 20 mL min <sup>−1</sup>	D101 resin-based column chromatography: 71.85% prep-HPLC: 92.15%	37
Preparative HPLC	Phenomenex Aqua C18 column, 2% acetic acid in water, 0.5% acetic acid in water and methanol (10/90, v/v)	The purification of punicalagin and pedunculagin I involved lyophilizing and extracting pomegranate peels with aqueous methanol, followed by preparative HPLC using a C18 column with a gradient elution, and detection at 280 nm	93% of punicalagin	66
Preparative HPLC	Reversed phase C18 column (19 × 300 mm, 7 µm, symmetry Prep™, methanol (eluent A) and 0.1% TFA in water (eluent B))	Extracting ground pomegranate husk with 50% acetone, concentrating the extract, and using preparative liquid chromatography with a C18 column	81.7 mg punicalagin at 98.05% purity was obtained from 300 mg crude extract containing 30% punicalagin	67
Semi-preparative HPLC purification	Reversed-phase C18 column (250 mm 10 mm, 5 mm, Grace-Vydac) The mobile phase consisted of a linear gradient of eluent A (0.1% TFA aqueous) and eluent B (0.09% TFA in 70% aqueous acetonitrile)	The optimum purification condition for punicalagin purification was 14% methanol in 0.1% trifluoroacetic acid solution as the mobile phase. The flow rate was 12 mL min <sup>−1</sup> and isolated punicalagin was detected at 378 nm The purification process involved extracting 20 g of ground <i>Punica granatum</i> with methanol, evaporating, dissolving in water, centrifuging, filtering, lyophilizing, and finally purifying 100 mg of the crude extract using semi-preparative HPLC with a linear gradient of methanol and TFA, resulting in 23 mg of pure punicalagin	>99% based on HPLC analysis at 254 nm	68
High-speed countercurrent chromatography (HSC)	Multilayer coiled column Solvent system: mixing butyl alcohol–TFA–water (100 : 1 : 100, v/v)	Filling the column with the lower phase, pumping the upper phase at 2.0 mL min <sup>−1</sup> while running at 800 rpm, injecting the sample after hydrodynamic equilibrium, and monitoring the effluent with a UV detector at 254 nm to collect peak fractions	A 350 mg amount of the crude extract was separated, yielding 105 mg of punicalagin at a high-purity of over 92%	69
Medium pressure liquid chromatography (MPLC)	C18 column. The mobile phase was composed of 3% aqueous acetic acid (solvent A) and methanol (solvent B)	Ellagitannins were purified by injecting pomegranate polyphenols into a C18 column using an MPLC system, with isocratic elution (5% methanol), followed by column washing and reconditioning, collecting fractions, drying, suspending in water, freezing, freeze-drying, and characterizing by HPLC-ESI-MS	Isolation of punicalagin with a purity exceeding 97.9%	70





used to separate the ingredients from natural products and pharmaceuticals, especially polyphenols in various plant materials.<sup>76</sup> For example, Sun *et al.*<sup>77</sup> achieved over 95% purity for many acids and flavonoids from *Sorbus pohuashanensis* fruits. Besides, HSCCC was used to separate phenolic acids from jackfruit peels,<sup>78</sup> apple pomace<sup>79</sup> and grape skins.<sup>80</sup>

It is worth noting that thus far, only a few reports describing the applicability of the HSCCC technique in the purification of punicalagin from pomegranates has been found. Lu *et al.*<sup>69</sup> extracted punicalagin from pomegranate husk with HSCCC using a butyl alcohol-TFA-water (100 : 1:100, v/v) solvent solution. They purified 105 mg of punicalagin (92% purity) and 80 mg of gallic acid (75% purity) from a 350 mg crude sample.

Other improvements have been made to the system including better detection devices, column design, proper solvent selection, and modeling of the process, all imparting an improved efficiency and cost-effectiveness to HSCCC. Innovations such as improved online monitoring and non-aqueous solvent solutions for the highly hydrophobic polyphenols are expected to promote this to an even higher standard. Future improvements in model-based process design and user-friendly chromatography modeling software are expected to enhance the industrial adoption of HSCCC technologies, making them simpler and more convenient for large-scale use.<sup>76</sup>

### 3.3 Medium-pressure liquid chromatography (MPLC)

Medium-pressure liquid chromatography (MPLC) is a preparative technique developed in the 1970s that enables the separation of organic compounds efficiently. It operates under a certain pressure, allowing the use of smaller particle size and a wider range of stationary phases, overcoming the limiting sample loading of low-pressure liquid chromatography (LPLC) and giving separations that are faster and more accurate.<sup>81</sup>

Aguilar-Zárate *et al.*<sup>70</sup> isolated punicalagin from pomegranate husk using MPLC, obtaining 97.9% purity and displaying significant antioxidant capacity with IC<sub>50</sub> values of 109.53 and 151.50 µg mL<sup>-1</sup> for 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals, respectively. Similarly, Wang *et al.*<sup>82</sup> reported similar results with a 5% methanol and 0.1% TFA in water mobile phase to extract 339 mg of punicalin at 95.9% purity in 40 min, as well as 59.7 mg of gallic acid with 78% purity. To achieve the extraction of compounds possessing substantial antioxidant properties, MPLC can be applied in the food, pharmaceutical, and healthcare industries to a great extent. Although there are few reports on purifying punicalagin using MPLC, existing research still shows its efficacy. Future research should be aimed at optimization of the MPLC conditions and the application of the process on an industrial scale to get closer towards the possible purification of punicalagin and other major compounds of chemical interest from natural sources.

## 4. Applications

Punicalagin is a polyphenol compound found in *Punica granatum*, *Lafoensia pacari*, and the genus *Terminalia*.<sup>83</sup> Punicalagin was isolated from *Lafoensia pacari* leaves for the first time by

Carneiro *et al.*<sup>84</sup> It was obtained at a concentration of 197 mg g<sup>-1</sup> of dried leaf, which is higher than the average amount found in pomegranate husk, reported to be 82.4 mg g<sup>-1</sup>.<sup>85</sup> In *Terminalia ferdinandiana*, punicalagin was found to be 74 mg/100 g dry weight in fruits and 49 mg/100 g dry weight in leaves.<sup>86</sup> It appears that *Punica granatum* and *Lafoensia pacari* have higher levels of punicalagin, emerging as the main sources of punicalagin. Additionally, pomegranate peel is a common material for obtaining punicalagin due to its low cost, mass production, and widespread availability.

Punicalagin has attracted great interest due to its antibacterial capabilities and potential for application in multiple areas. An overall summary of its applications is presented in Fig. 3. Gosset-Erard *et al.*<sup>87</sup> found that punicalagin is a key antibacterial agent in pomegranate peel extracts, with minimum inhibitory concentration (MIC) values ranging from 0.3 to 1.2 µg mL<sup>-1</sup>, effectively targeting 10 out of 13 Gram-positive bacteria, 2 out of 3 Gram-negative bacteria, and one yeast strain. Hayrapetyan *et al.*<sup>88</sup> discovered that a 7.5% v/v liquid pomegranate extract (24.7 mg dry pomegranate extract per mL) demonstrated significant antimicrobial activity. In meat pâté refrigerated at 4 °C, pomegranate extract at its minimal bactericidal concentration (MBC) reduced the growth of *L. monocytogenes* by 4.1 log CFU g<sup>-1</sup> over 46 days, compared to that of the control of log 9.2 CFU g<sup>-1</sup> by day 18. Demir<sup>89</sup> also found that adding 1% punicalagin to meatballs had considerable antibacterial activity, with an MIC of 1.87 mg mL<sup>-1</sup> against *L. monocytogenes* and *Salmonella typhimurium*. In addition, punicalagin considerably reduced the free fatty acid (FFA), peroxide value (POV), and thiobarbituric acid reactive substance (TBARS) levels during storage. Cooper *et al.*<sup>90</sup> explored the mechanistic effects of punicalagin on bacterial cells. Using high-throughput mass spectrometry and quantitative isobaric labeling, the researchers discovered that punicalagin impairs iron homeostasis in *Staphylococcus aureus*, causing major changes in the bacterial proteome. Punicalagin therapy inhibited the accumulation of proteins and enzymes required for iron uptake, while inducing an SOS response to damaged deoxyribonucleic acid (DNA), which indicates the usage of punicalagin in limiting bacterial colonization by interfering with critical metabolic pathways. Moreover, punicalagin has antibacterial properties by enhancing the efficacy of antibiotics. For instance, punicalagin inhibited *Escherichia coli* ATP synthase, which was helpful to avoid antimicrobial resistance.<sup>91</sup> Compared with traditional prescriptions, patients took lower dosage of punicalagin for recovering from bacterial infections. Furthermore, punicalagin enhanced the sensitivity towards methicillin-resistant *Staphylococcus aureus* (MRSA) by muting related genes.<sup>92</sup> Punicalagin together with cefotaxime eliminated the symptoms of MRSA-induced pneumonia in mice.<sup>93</sup> These therapeutic effects prove the feasibility of applying punicalagin in treating drug-resistant bacterial infections. Also, punicalagin can be a strong antifungal drug with low side effects and is appropriate for application in pharmaceutical practice. Punicalagin exhibited significant antifungal activity with MIC values in the range of 0.5 to 4.0 µg mL<sup>-1</sup> against *C. neoformans* complex. Additionally, it showed low cytotoxicity in



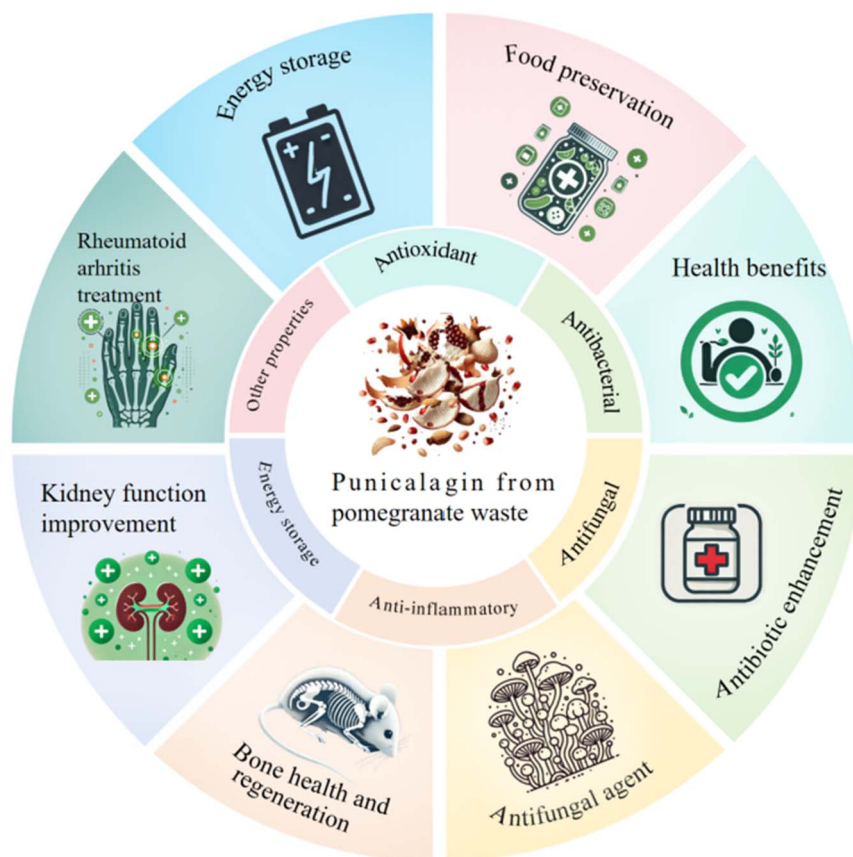


Fig. 3 Overview of the applications of punicalagin in foods, health products, and energy storage.<sup>86–100</sup>

human cell lines and no haemolytic potential in animal cells, further confirming its good potential as a safe antifungal agent.<sup>94</sup> The results of inhibition of different bacteria demonstrate the potential of punicalagin as a highly effective natural bio-preservative, which possesses the ability to improve the shelf life and safety of meat products. In summary, punicalagin exhibits strong antibacterial and antifungal effects.

Punicalagin has attracted significant attention as a plant-derived anti-inflammatory agent due to its strong anti-inflammatory effects and potential applications in medical treatments and disease management.<sup>95</sup> Studies have shown that punicalagin effectively reduces inflammatory markers, including NO, TNF- $\alpha$ , and IL-6, in a concentration-dependent manner, thereby inhibiting inflammation and protecting cartilage cells.<sup>96</sup> In rheumatoid arthritis treatment, punicalagin alleviated the disease progression in mouse models, mitigating the arthritis severity and minimizing bone destruction.<sup>101</sup> Punicalagin exhibited significant renoprotective and anti-inflammatory effects in diabetic nephropathy. In diabetic mice induced by a high-fat diet (HFD) and streptozotocin (STZ), 8 weeks of punicalagin administration led to a significant reduction in blood urea nitrogen (BUN), serum creatinine (CREA), and urinary albumin/creatinine ratio (UACR). It also alleviated glomerular hypertrophy and interstitial hyperplasia, improving kidney function. Furthermore, punicalagin inhibited inflammatory proteins such as IL-1 $\beta$  and caspase-1, effectively

suppressing inflammation and preventing pyroptosis.<sup>97,102</sup> Additionally, punicalagin supports bone health and shows promise in treating periodontal diseases. It promotes bone regeneration and repairs defects caused by inflammation, making it a safe and promising candidate for bone repair therapy. Specifically, punicalagin stimulated osteogenic activity in MC3T3-E1 cells under periodontal inflammatory conditions and inhibited osteoclast formation in bone marrow-derived macrophages (BMMs) through the RANKL/OPG pathway.<sup>103</sup> In conclusion, punicalagin exhibits strong anti-inflammatory and therapeutic effects, offering potential applications in treating rheumatoid arthritis, diabetic nephropathy, and bone-related diseases through its ability to reduce inflammation, protect tissues, and promote regeneration.

Meanwhile, punicalagin also possesses a considerable antioxidant effect. According to Gil *et al.*,<sup>98</sup> HPLC-DAD and HPLC-MS analyses revealed that commercial juices made from pomegranate arils containing 1500–1900 mg L<sup>-1</sup> of punicalagin possessed three times higher antioxidant activity than red wine and green tea. Also, da Silva Veloso *et al.*<sup>99</sup> explored the use of pomegranate epicarp extracts as a natural functionalizing ingredient in Brazilian pastry products. Fourteen phenolic compounds were identified through HPLC-DAD-ESI/MS. Besides, the antioxidant properties of punicalagin also contribute to its potential health benefits. According to Wang *et al.*,<sup>100</sup> punicalagin demonstrated effects on angiogenesis and



oxidative stress in pregnancy-induced hypertensive rats. It was shown that punicalagin significantly reduced the diastolic, systolic, and mean arterial blood pressure in the affected rats.

Alternatively, Talekar *et al.*<sup>104</sup> demonstrated the use of punicalagin-rich phenolics in energy storage applications. The yield of punicalagin-rich phenolics amounted to 71.2% of the total phenolic extract. They were further processed to develop hard carbon electrodes, which performed well in sodium batteries and showed a good electrochemical performance, while displaying the sustainability and efficiency of using the wastes of pomegranate peels in high-performance applications. Overall, punicalagin is extracted from the peels of pomegranate and used for a wide range of applications, including natural antibacterial, anti-fungal, anti-inflammatory, antioxidant agents, and as a compound in energy storage systems.

## 5. Conclusion and future perspectives

This study emphasizes the potential of pomegranate peel, a by-product of pomegranate processing, as a great source of bioactive compounds with commercial value. Repurposing these materials can convert waste into viable resources for functional foods and health goods. Punicalagin, the predominant and crucial polyphenol in pomegranate peel, was the focus of this review. This review discussed the extraction of polyphenols (punicalagin being the most abundant component), purification, and prospective applications of punicalagin, considering its many bioactive properties.

Regarding extraction procedures, this review addressed both conventional and advanced methods. Among the methods, solvent extraction using 50% aqueous methanol attained an astonishingly high total phenolic content of  $625.525 \pm 6.83$  mg GAE per g. However, conventional extraction methods are usually time and energy consuming, which reduces their cost-effectiveness. Alternatively, a combination of traditional and advanced methods, enzyme-assisted solvent extraction, exhibited higher effectiveness and attained the highest extraction yield of  $65.89\% \pm 2.64\%$  compared to others.

Chromatographic technologies are the most common techniques for purification and often with promising results. Prep-HPLC can achieve a quite high purity of 92.15–98.05%, and semi-HPLC can even reach an extremely high purity of 99%. Other methods, including medium-pressure liquid chromatography (MPLC) and high-speed countercurrent chromatography (HSCCC), also result in purities between 92% and 97.9%. Overall, prep-HPLC and semi-HPLC are recommended due to their higher purification efficiency.

Punicalagin has promising potential in both the food industry and health potential given that various studies support its antioxidant, antibacterial, antifungal, and anti-inflammatory properties. Punicalagin possesses the potential to be a natural preservative, improving the shelf life, functional qualities, and nutritional value of food products. Moreover, its antibacterial and anti-inflammatory properties indicate its possible therapeutic uses, including the mitigation of inflammation-related

disorders, reduction in antibiotic use, and treatment of drug-resistant bacterial infections.

However, although punicalagin shows great potential in the food and medical field, its massive production is still immature. Thus, future research should focus on developing cost-effective extraction and purification techniques to enable its mass production and commercialization, thereby optimizing the use of pomegranate peels and delivering their health benefits to consumers. Meanwhile, given that most therapeutic trials have been conducted on mice or *in vitro* cell models, further studies are needed to confirm its safety and effective dosage. More research is also required to ensure the safe and efficient use of punicalagin in enhancing medical therapies and promoting human health.

## Data availability

No primary research results, software or code have been included and no new data were generated or analyzed as part of this review.

## Conflicts of interest

There are no conflicts to declare.

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