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Review Article

Antimicrobial Activity of Pomegranate Peel and Its Applications on Food Preservation

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Pomegranate (*Punica granatum* L.) fruit is being cultivated since the civilization is known, and its production and consumption have been increased since the last century due to the scientific confirmation of its health benefits. Pomegranate fruits, fruit juice, its seeds, and peels are known to have higher contents of bioactive compounds, viz., phenolic acids, flavonoids, and hydrolysable tannins. The peels of pomegranate fruits are the major by-products produced during food processing of pomegranate enriched in antioxidants and broad-spectrum antimicrobial agents and can prevent food deterioration even. This health potential of pomegranate is known to vary significantly upon the varieties, growing conditions, cultivation practices, stages of the development, and the extraction methods. Herein, the biochemical composition of the pomegranate peel extract (PPE), its efficacy in food preservation, and antimicrobial activities are discussed to provide a comprehensive guide for farmers, food processing, and storage sectors and academia.

1. Introduction

Pomegranate (*Punica granatum* L.) plants are among the first cultivated plants by humanity; however, its consumption had been limited most commonly as a result of the hassle of extracting the juicy arils [1]. Due to the increasing number of scientific studies about its health benefits, production and consumption of pomegranate fruits have been increasing since the beginning of the 21st century. Pomegranate fruits are consumed as both fresh and processed mainly in the forms of juice, oil, wine, and jams. Both the fruits and its peel are known to have high levels of numerous phytochemicals, including phenolic acids, flavonoids, and tannins. This diverse characteristic of phytochemicals is thought to be responsible for its high antioxidant potential and health benefits [2]. During processing, a considerable amount of by-products are developed from peels and is known to have high contents of hydrolysable tannins (HTs) [3]. Recently, by-products of pomegranates, especially pomegranate peel extract (PPE), have been increasing attention due to its scientifically confirmed therapeutic properties such as antioxidant, antimicrobial, anticancer, antiulcer, and anti-inflammatory activities [4, 5]. Numerous scientific studies have suggested that PPE exhibits excellent antimicrobial activity against several foodborne pathogens and improves the postharvest storability of food products [6, 7]. This paper will describe and discuss the recent advancements about the biochemical composition, antimicrobial potential, and food preservation characteristics of PPEs.

2. Biochemical Composition of Pomegranate Peel

Pomegranate peels have high levels of numerous phytochemicals [2]. It has been reported that PPE is high in bioactive compounds, mainly phenolic acids, flavonoids, and hydrolysable tannins [8, 9]. The primary phenolic acids identified from PPEs are ellagic acid, gallic acid, caffeic acid, chlorogenic acid, syringic acid, ferulic acid, vanillic acid, p-coumaric acid, and cinnamic acid [10–14]. Phenolic acids concentration significantly varies among varieties and is highly depending upon the geographical location, climatic conditions, and cultivation practices [9, 13]. One of the main parameters defining the concentration of the phenolic acids was noted as the peel colour where the varieties with dark red colour reported to have higher phenolic acids concentration than the light-coloured varieties [15]. Additional to phenolic acids, PPEs are an excellent source of flavonoids. Flavonoid content and composition are also known to vary significantly among varieties and growing conditions; however, it was also noted that the fruit developmental stage influence the flavonoid content and composition [6, 16, 17]. Besides, PPEs are reported to have rich sources of tannins. The reported tannins are ellagitannins, punicalagin, granatins, punicalin, pedunculagin, castalagin, corilagin, gallagyldilactone, and tellimagrandin [18, 19]. Almost 49 compounds, the majority of which were flavonoids, phenolic acids, and tannins [9, 19–26] isolated from pomegranate peels, are summarized in Figure 1.

The antioxidant activity of the PPEs is attributed to the phenolic acids, flavonoids, and tannins. Among these, ellagitannins are noted to be most responsible for the antioxidant activity of the pomegranate peels [9]. It was also stated that both concentrations of phytochemicals and antioxidant activity are highly dependent upon the solvents used for the peel extraction. Previous studies also suggested that the methanolic extracts of the pomegranate peels exhibit higher antioxidant activity as compared with other extractions methods [27]. The concentrations of phenolic acids, flavonoids, and tannins in PPEs mainly depend on the extraction method. For example, Orak et al. [28] reported the highest concentrations of tannins identified from methanol extracts, as compared with water and ethanol extracts. Acetone extracts of pomegranate peels were also noted to have higher antioxidant activity than the water and ethanol extracts [19].

3. The Extract Process of Pomegranate Peel

One of the most widely used methods for pomegranate peel extraction is the methanol extraction method reported by Dahham et al. [29]. In this method, first of all, the fine peel powders are obtained by an electric blender and oven-dried at 40°C for 24 h. Then, the powders are sieved through a 24-mesh, and 10 g of powder sample is extracted with 250 ml of 80% methanol at room temperature (~25°C) for 24 h. The final extract is then filtered and used. If the aqueous extract is needed for use, the powdered sample (10 g) is extracted with 100 ml of distilled water and used. The drying temperature and duration was reported as 50°C for 48 h in some other studies [11].

Water, ethanol, and acetone are the other solvents reported to be used instead of methanol for the extraction of the pomegranate peel [19, 28]. Most of the published literature about the different extraction methods/solvents recommended that the methanol extraction method is better than others in terms of the abundance of phenolic compounds and increase antioxidant activity [28, 30–32].

The extraction method is reported to have a strong influence on the biochemical composition of PPEs. The conventional techniques generally need high amounts of solvents and results with low extraction yields. Moreover, the use of high temperature is reported to result in degradation in the extracts [33]. Recently, high-pressure extraction is observed and noted as a practical methodology, which results with no detrimental effects on bioactive compounds. Additionally, to be a green technology, the high-pressure extraction is faster and has a high yield [34].

Another important extraction method is the enzymatic extraction. It was reported to assist in the extraction of bioactive compounds. The mechanism behind the enzymatic extraction involves some cell wall degrading enzymes (i.e., pectinases). These enzymes break down the cell wall and improve the extraction of the bioactive compounds [35, 36].

4. Antimicrobial Activity of Pomegranate Peel

Pomegranate has a broad spectrum of antimicrobial effects, which has an apparent inhibitory effect against Gram-negative, Gram-positive bacteria (Table 1), fungi, and mould (Table 2). However, different extracts from different parts of pomegranate have various antimicrobial activities. The study of many scholars showed that the antimicrobial activity of PPE was more potent than other parts, and the antimicrobial activity of PPE was related to the total flavonoids and tannins content. PPE is well known for its antimicrobial activity against bacterial and fungal pathogens [29, 37, 38]. However, the number of studies which investigated the effects of PPE against plant pathogenic bacteria and fungi is limited [11, 32, 39, 40]. PPE was noted to have antibacterial effects on different foodborne pathogens including *Escherichia coli*, *Fusarium sambucinum*, *Penicillium italicum,* and *Bacillus subtilis* [11, 31, 38, 41]. In one of these comprehensive studies, the antimicrobial activity of extracts derived from different parts of pomegranates (peel, seeds, juice, and whole fruits) was tested on seven bacteria (*Bacillus coagulans*, *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*). The maximum inhibitory of all bacteria was noted from the peel extracts of pomegranates [29]. Pomegranate peel was also tested as an incorporation agent into bio-based films and was found to improve the antibacterial activity of materials. In one of these studies, Ali et al. [42] reported that PPE inhibited the growth of *S. aureus* (Gram-positive) and *Salmonella* (Gram-negative). As discussed in Section 3, the extraction method can significantly influence the antimicrobial activity of pomegranate peels. In a recent study, antimicrobial effects of high pressure (300 and 600 MPa) of the pomegranate peels were tested by [13]. In this study, the maximum antioxidant activity and phenolic content were observed at the extraction of 300 MPa; thus, the higher antimicrobial activity against pathogenic bacteria was noted from 300 MPa. The ethyl acetate extract of pomegranate peel was previously reported to include high concentrations of tannins which are very active against *Staphylococcus aureus*

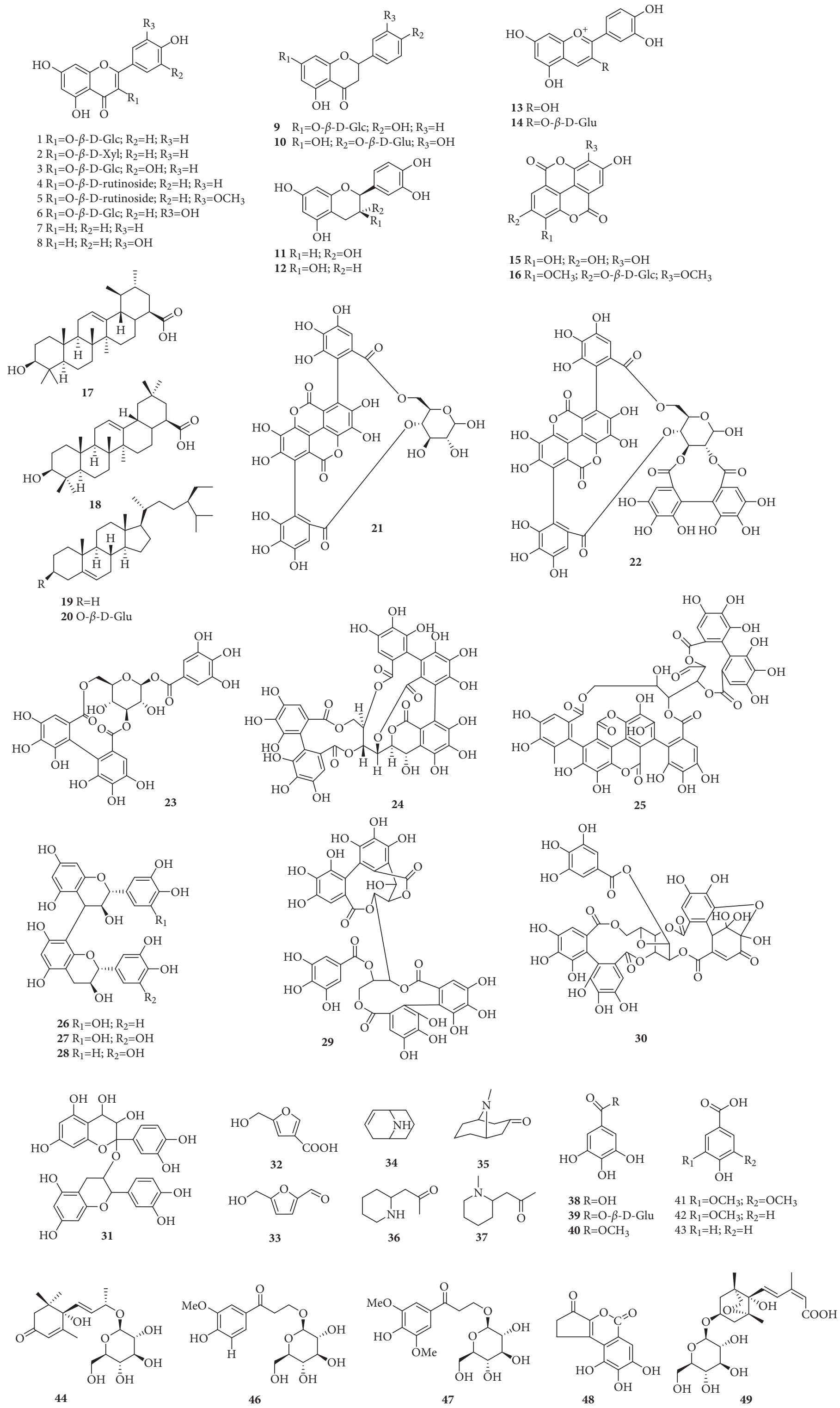

Figure 1: Chemical structures of reported compounds from pomegranate peels.

strains [43]. Water extract of pomegranate peel was also reported to control Gram-positive significantly and Gram-negative bacteria including *E. coli*, *B. subtilis*, *Enterobacter aerogenes*, *Serratia marcescens*, *Brucella spp.*, *Saccharomyces cerevisiae,* and *Rhodotorula glutinis* [44].

In a different study, the effects of PPEs for controlling the growth and development of *Fusarium sambucinum* were tested in in vitro as curative and preventive. The researchers noted that the PPE (20 mg/ml) exhibited complete inhibition of spore germination and 75.5%

Table 1: Antibacterial effects of pomegranate peel against different pathogens.

Bacteria	Peel preparation and dose	Mechanism of action	Reference
Eight different food contaminants/pathogenic bacteria	Under high pressure at 300 MPa	Due to high antioxidant activity and high total phenolic content	[13]
B. coagulans, *B. cereus*, *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*	10 g sample (sample = 10 g peel extracted with 250 ml 80% methanol) extracted with 100 mL water	Estimated to be a result of high phytocompounds including phenols, tannins, and flavonoids.	[29]
S. aureus and *Salmonella*	2, 4, 6, 8, 10, 12, and 14% PPE with 20% polyethylene glycol	Inhibition of films against targeted microorganisms	[42]
S. aureus	The ethyl acetate extract of pomegranate peel	Due to the high concentrations of tannins	[43]
Gram-positive and Gram-negative bacteria including *E. coli*, *B. subtilis*, *E. aerogenes*, *S. marcescens*, *Brucella* spp., *S. cerevisiae*, and *R. glutinis*.	Water extracts of pomegranate peels (50/100 w/v)	Due to high tannins content	[44]

Table 2: Antifungal effects of pomegranate peel against different pathogens.

Fungi	Peel preparation and dose	Mechanism of action	Reference
F. sambucinum	The methanol extract of pomegranate peels ($20\ mg{\cdot}ml^{-1}$)	Due to the high contents of phenolic compounds	[11]
P. digitatum and *Saccharomyces cerevisiae*.	0.061–0.304 g dry methanolic extract mL^{-1}	Inhibit cellular receptors of pathogens	[32]
Brown rot (caused by *Monilinia laxa* and *M. fructigena*)	Aqueous PPEs (9.93 and $12.84\ mg{\cdot}ml^{-1}$)	Due to the high total phenolic and flavonoid contents	[45]
B. cinerea, P. digitatum, and *P. expansum*	20 h of incubation with PPE (1.2 and $12\ g{\cdot}L^{-1}$)	Due to the high concentrations of ellagitannins	[46]
F. oxysporum f. sp. *lycopersici*	Water extract (80%), methanol extract (40%), and ethanol extract (20%) of pomegranate peel	Due to the high levels of total phenol and punicalagin contents.	[47]
P. digitatum	Incorporation of 0.361 g water PPE into locust bean gum and chitosan coatings	Due to the high contents of phenolic compounds	[48]

inhibition on mycelia growth of fungi [11]. In a recent study, El Khetabi et al. [45] studied the in vitro and in vivo effects of aqueous PPE on the brown rot (caused by *Monilinia laxa* and *M. fructigena*). They reported an inhibition varying from 76.65% to 90% on the control of mycelia growth. Intense fungicidal activity of PPE was then reported against *Botrytis cinerea*, *Penicillium digitatum,* and *Penicillium expansum* by Nicosia et al. [46]. 20 h of incubation with PPE resulted with almost complete inhibition of all fungal spores at lemons and grapefruits. A recent study investigated the antifungal activity of 21 different pomegranate genotypes [47]. Researchers noted that all of the tested genotypes have varying antifungal activities, against *Fusarium oxysporum* f. sp. *lycopersici*, where some of them completely inhibit the fungus, and some others had deficient inhibitory activity. The two different extracts of pomegranate peel (water and methanol) were incorporated with chitosan and locust bean gum for controlling *P. digitatum* at orange fruits. Results suggested that the addition of 0.361 g water PPE into both locust bean gum and chitosan coatings significantly reduce the *P. digitatum* by 28% and 49%, respectively [48]. Phenolic profile and antimicrobial activity of "Gabsi" PPEs were studied by Kharchoufi et al. [32]. Similar to the previous studies of the researchers, the water and methanol solvents were tested separately. Researchers noted different results than their other studies and recommended that the methanol extraction is more effective against the *P. digitatum* and *Saccharomyces cerevisiae*.

There are still significant obstacles for the industrial use of PPEs to control postharvest pathogens. This limitation is due to the unclear mode of action of PPE, high costs, lack of curative effect, significant variation among varieties, climatic conditions, cultivation practices, and extraction methods and limited range of activity against different fungal pathogens [9, 49].

5. Biochemical Changes and Food Preservation of Pomegranate Peel

PPE is a valuable by-product for the food preservation industry. As discussed previously, PPE is a rich source of bioactive compounds, including tannins consisting of ellagic acid and gallic acid [42, 50, 51]. Most of the bioactive compounds, which are abundant in the PPE, were previously tested as natural additives for improving the preservation quality of food [52]. PPE was yet tested alone or in combination with edible films and coatings for food preservation. Renewable, bio-based, environment friendly active packaging systems, which are usually composed of biopolymers such as proteins, lipids, and polysaccharides [53], have been extensively used in food packaging since the beginning of the 21st century [54]. Legume seeds as a good source of plant proteins (25–28%) have a good potential as a bio-based film [55].

Moreover, incorporation of natural compounds (with high contents of antioxidant activity, phenolic compounds, and essential oils) into bio-based films is known to improve the activity of edible coatings and enhance the storage duration of food products [56]. Among these, fruit peels constitute an essential part, which is a rich source of bioactive compounds. PPEs have also been widely used in the formulation of bio-based edible coatings/films [57]. The PPE was also noted to reduce the water vapour permeability of chitosan-based film material and its antimicrobial activity [58]. Another recent study showed that the incorporation of different concentrations of PPE with mung bean protein films provides a biofunctional edible film for packaging of food products [59]. The incorporation of PPE was reported to give flexibility to the films, increase the thickness and water vapour permeability, and decreases the moisture content. It was determined that PPE retains its semicrystalline structure in bio-based edible films and improves the efficacy of the material [42]. In a similar study, incorporation of PPE in chitosan was tested for food packaging [60]. The addition of PPE to active polyvinyl alcohol (PVA) composite film also resulted in high antioxidant activity and antibacterial ability [61].

The incorporation of PPE (1% w/v) into chitosan (1% w/v) and alginate (2% w/v) coatings was then reported to improve the postharvest storability of "Allahabad safeda" guava fruits. Results suggested that the edible coatings reduced the respiration rate, protected ascorbic acid content, total phenolics, total flavonoids content, and antioxidant activity, and maintained the overall fruit quality [62]. The phenolics in foods capture free radicals, produced during oxidative stress, and prevent the deterioration of the foods [63]. Several studies with other edible coatings confirm that the edible coatings reduce the respiration rates and help to maintain the postharvest quality of fresh fruits [64, 65]. Reduction in respiration rate results with a decrease in the enzyme activity and thereby resulting in a reduction of ascorbic acid oxidation [66] and improves the postharvest storability of foods. Higher respiration rate results in the breakdown of total phenols, and this accelerates the ageing process. Thus, the reduction of the respiration rate by the edible coatings enriched with PPE results with a high amount of phenolic compounds [31] and improved storability of the foods.

Rather than the direct influence on the pathogens, PPEs also induce plant resistant to pathogens. In one of these studies, PPE was reported to cause a transcriptomic response at orange fruits. It was noted that PPE upregulates 273 significant genes and downregulates eight genes. Changes in the gene expressions were noted to enrich antibiotic biosynthesis and induce defence pathways [67].

6. Prospective

Pomegranate peel is the waste produced in the process of pomegranate food processing, accounting for 20–30% of the total weight of pomegranate. The high antioxidant activity, inhibition of lipid peroxidation, and broad-spectrum antimicrobial efficiency of pomegranate peel play an intrinsic quality foundation for its development as a food preservative. To make pomegranate peel more widely used in food preservation, the following aspects need to be further studied and explored:

(1) Study on active antimicrobial components of pomegranate peel. Pomegranate peel composition is complicated. Although the chemical composition of pomegranate peel was well studied, the number of specific studies about the mode of action is relatively few. Thus, the direct causes of antimicrobial activities of pomegranate peel are still unclear, and there are other antimicrobial substances in pomegranate peel, which still need further research.

(2) Study on the antibacterial mechanism of pomegranate peel. Pomegranate peel has an excellent bacteriostatic effect, but the mechanism of its bacteriostatic effect has not been studied intensely. Furthermore, research on the mechanism of bacteriostatic impact will be helpful to promote the application of pomegranate peel in the field of food preservation.

(3) Study on the compatibility of pomegranate peel and other chemical food preservatives. Food preservatives from different sources have a synergistic preservative effect. Studying the optimization of the formula of pomegranate peel and other chemical preservatives can enhance the preservative effect and reduce the amount of chemical preservative.

Data Availability

The data used to support the findings of this study are included within this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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