Antioxidant Activity, Antibacterial Activity and Phenolic Composition of Pomegranate Fruit: A Review

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Abstract

A fruit known for its high levels of phytochemicals, notably polyphenolic compounds that have been linked to several health advantages, is the pomegranate (Punica granatum). The objective of this study was to assess the antioxidant, antibacterial, and phenolic content of pomegranate extracts from various fruit portions. Pomegranate seeds, peel, and juice extracts were obtained by the extraction procedure. Several in vitro techniques, such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test and the ferric reducing antioxidant power (FRAP) assay, were used to measure antioxidant activity. The findings showed that all pomegranate extracts had substantial antioxidant capacity, with the peel extract showing the greatest activity, followed by the seed and juice extracts. A panel of pathogenic bacteria, comprising both Gram-positive and Gram-negative species, were used to assess the antibacterial activity of the pomegranate extracts. The antibacterial activity of the extracts varied, with the peel extract having the most inhibitory effects on the types of bacteria that were put to the test. Pomegranate extracts may be useful as natural antimicrobial agents due to their antibacterial characteristics. High-performance liquid chromatography (HPLC) was also used to analyze the phenolic makeup of the pomegranate extracts. Several phenolic chemicals, including ellagic acid, punicalagins, anthocyanins, and derivatives of quercetin, which are recognized for their antioxidant and antibacterial characteristics, were found, according to the research. The phenolic profile differed amongst the fruit’s various components, with the peel extract exhibiting the highest level of these beneficial substances.

Keywords

Antioxidant, Antibacterial, Phenolic, Growth, Pomegranate

Introduction

An antioxidant is something that, when present at low quantities compared to those of an oxidizable substrate, considerably slows down or stops that substrate from oxidizing. According to epidemiological research, eating fruits and vegetables with a high phenolic content lowers the risk of developing cardiovascular disease, stroke, and cancer [1, 2]. Boiling the dried pomegranate peel in water has several health advantages, including easing menopausal symptoms in women and soothing sore throats. It also reduces stress and cholesterol levels [3]. Due to the presence of flavonoids such as catechin, epicatechin, epigallocatechin-3-gallate, flavan-3-ol, kaempferol, kaempferol-3-β-glucoside, kaempferol-3-O-rhamnoglucoside, luteolin, luteolin 7-O-glucoside, pelargonidin, prodelphinidin, quercetin, and rutin [4]. As well as alkaloids like N-methylpelleretine, pseudopelleretine, and pelleretine. Researchers have shown that the antioxidant activity of pomegranates is three times higher than that of red wines and green teas [5]. Raw and processed pomegranate fruits, such as juice, jam, jelly, vinegar, wine,
and supplements containing extract, are often consumed [6]. The total antioxidant capacity, total phenol content, ellagitannins, and anthocyanin concentration of pomegranate fruits are some characteristics that may be used to monitor their quality as they ripen, juices and fresh fruits now have more nutritional value because to these ingredients. Additionally, they are useful for assessing the quality of fruit juice as well as how fruit is handled throughout processing and storage [7]. When added to juice, pomegranate peel extract increases the process' intensity by causing the chemical that causes haze to create its deposit more quickly [8]. Pomegranate juice, even fermented pomegranate juice, has recently been shown to have strong antioxidant activity [6, 9]. The pomegranate peel is said to have several advantages, including biological qualities that are antibacterial, antiviral, antioxidant, anti-inflammatory, and anti-neoplastic [3]. Pomegranate fruit intake has been linked to health advantages, including lowered levels of oxidative stress, atherogenic alterations to LDL, and platelet aggregation. Regular use of foods high in flavonoids may lower elderly men's risk of dying from coronary heart disease [10].

Antioxidants are known to include a large proportion of phenolic compounds and have a great capacity to neutralize free radicals. Individual flavonoid compounds' antioxidant properties include the capacity to neutralize free radicals and to activate phase II enzymes like quinone reductase [11, 6]. Condensed tannins, commonly referred to as pro-anthocyanidins, are a subgroup of the flavonoid family that also contains prodelphinidins. They are called delphinidins because after acid hydrolysis, they yield delphinidin. Pro-anthocyanidins exhibit a wide range of biological actions, according to epidemiological studies as well as in vitro research. They can function as complex proteins, metallic ions, antioxidants, radical scavengers, and more [12]. Based on wet weight, the by-product, known as pomegranate marc, contains roughly 78% peel and 22% seeds. Currently, most of the research focuses on pomegranate fruit antioxidants, oil, and juice extraction and product qualities [13]. Varietal, geographic, seasonal, and maturity variations, as well as processing settings, are known to have a significant impact on the makeup of both fruits and juices. According to some reports, solvents may not have an impact on the antioxidant properties, but they may change how pomegranate extracts are made [14-16].

The goal of this work was to identify the antioxidant-rich pomegranate peel and seed extract fractions and to assess their antioxidant activity, phenolic composition, and potential therapeutic applications (Figure 1).

**Antioxidant Activity and Phenolic Composition**

Azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS), Ferric reducing antioxidant power (FRAP), and 1, 1-diphenyl-2-picrylhydrazine (DPPH) are used to measure the antioxidant capacity. Pomegranate juices (PJ) were also assessed for total polyphenols (TP), Total flavonoid (TF), Total anthocyanins content (TAC), and qualitative and quantitative assessments of specific phenolic components by High-performance liquid chromatography (HPLC). Gallic, chlorogenic, caffeic, ferulic, ellagic, catechin, epicatechin, phloridzin, quercepin, and rutin were among the phenolic compounds found in the examined cultivars [16]. Three times more antioxidant activity was found in commercial PJ (18–20 TEAC) than in red wine and green tea (6–8 TEAC). Commercial juices made from entire pomegranates had more activity than experimental juices made just from the arils (12 – 14 TEAC). Commercial juices included the pomegranate tannin punicalagin (1500 – 1900 mg/L), but only traces of this substance were found in the experimental juice made from arils in the lab, according to HPLC-DAD and HPLC-MS studies of the juices [6]. High performance liquid chromatography was used to separate the galloallocatechin and other prodelphinidins from pomegranate peel. By using LC-DAD-MS and MS-MS, the compounds galloallocatechin, galloallocatechin-(4-8)-catechin, galloallocatechin-(4-8)-galloallocatechin, and catechin-(4-8)-galloallocatechin were all recognized, purified, and quantified. These chemicals' antioxidant capacities were evaluated. In the aqueous phase, prodelphinidin dimers were substantially more active antioxidants than galloallocatechin monomer. However, only one of the dimers (galloallocatechin-(4-8)-catechin) substantially outperformed the monomer in the lipid phase to prevent phosphatidylcholine vesicle lipid peroxidation [12].

Ganesh variety pomegranate peels were extracted using several solvents, including water, methanol, and ethanol, either alone or in conjunction with water. 50% ethanol and 50% water produced the highest yield (16.3 1.9%). The methanol and 70% ethanol: 30% water extracts were found to have the greatest DPPH and ABTS inhibitory activity (79.5 - 6.5 and 94.6 - 6.10, respectively). The aqueous extract has the greatest phenol concentration (438.3 - 14.15). Four bacterial strains were used to investigate the antibacterial activity of peel extracts. With a greater phenolic content and antioxidant activity, 70% ethanol: 30% water and 100% water extract have the potential to be used in nutraceuticals [4]. Out of the peel, pulp, and seed, pomegranate peel has the highest antioxidant activity. They used a combination of ethanol, methanol, and acetone to extract antioxidants from pomegranate peel. Based on the findings, pomegranate peel extract had a much better antioxidant capacity than pomegranate pulp extract. Additionally, peel extract contained more total phenolics, flavonoids, and proanthocyanidins than pulp extract did. Peel extract's potent antioxidant properties may be due to the high concentration of phenolics it contains. We concluded that pomegranate peel extract, which is higher in natural antioxidants than pulp extract, looked to have more promising as a health supplement [17]. Twenty pomegranate cultivars' physico-chemical traits...
and antioxidant activity were investigated. Fruit weight, skin percentage, aril percentage, and juice percentage all fell within the ranges of 196.89 - 315.28 g; 32.28 - 59.82%, 37.59 - 65%, and 26.95 - 46.55%, respectively. The total soluble solids content ranged from 11.37 (Brix) to 15.07 (Brix), the pH ranged from 3.16 to 4.09, the titratable acidity content ranged from 0.33 g-100 g to 2.44 g - 100 g, and the total sugars content ranged from 13.23 g - 100 g to 21.72 g - 100 g. Ascorbic acid concentrations varied from 9.91 mg per kg to 20.92 mg per kg. The total anthocyanin content ranged from 5.56 mg per kg to 30.11 mg per kg. The range of the total phenolics ranged from 295.79 mg per kg to 985.37 mg per kg. Between 15.59 and 40.72% of antioxidant activity was observed [18].

Ethyl acetate, methanol, and water were used to extract antioxidant-rich fractions from pomegranate (P. granatum) skins and seeds. Utilizing the DPPH and carotene–linoleate model systems, the methanol extract of peels demonstrated 83 and 81% antioxidant activity, respectively, at 50 ppm. Similarly, the pomegranate peel methanol extract had the greatest antioxidative activity out of all the extracts, with 22.6 and 23.2% antioxidant activity at 100 ppm. The methanol extract demonstrated 56, 58, and 93.7% inhibition using the thiobarbituric acid technique, hydroxyl radical scavenging activity, and LDL oxidation, respectively, at a concentration of 100 ppm and is utilized for food preservation as well as their usage as health supplements and neutraceuticals [19]. The findings demonstrated that neither the yield nor the quantity or activity of antioxidants in either the peel or the seeds were significantly impacted by the drying process. The quantity and content of the antioxidants derived from the peel were greater than those from the seeds. Reduced particle size, increased water/ sample ratio, and temperature all boosted antioxidant yield and content; nevertheless, antioxidant activity was limited at high extraction temperatures. Given the high antioxidant yield (11.5%) and content (22.9%), as well as DPPH scavenging activity of 6.2 g/g, the recommended extraction conditions were peel particle size of 0.2 mm, water/peel ratio of 50/1 (w/w), temperature of 25 °C, and extraction time of 2 min [13]. Pomegranate juice, seed, and peel all have significant phenolic component content and antioxidant action. Peels, juice, and seeds of pomegranates were collected from three regions: Natanz, Shahreza, and Doorak. Pomegranate peel, seed, and juice extract antioxidant activity, total phenolic, flavonoid, and flavanol concentrations were examined. In comparison to the seeds and liquids, the pomegranate peel extract demonstrated high levels of antioxidant activity. The antioxidant activity of the Doorak peel was 58% higher than that of the other two peels. Pomegranate peel was shown to have significantly more total phenolics, flavonoids, and flavanols than the fruit’s seeds and liquids. The high antioxidant capacity of pomegranate, particularly the peel, has made it possible to employ them as natural food preservatives, according to the results [20].

Using direct infusion quadrupole–time of flight (Q-TOF), eight ethanol extracts of pomegranate peels (PPEs) were evaluated for their antioxidant, antibacterial, and antiproliferative properties. Punicalagin, granatin, and their derivatives were found to be the most common chemicals in pomegranate peels, according to mass spectrometry research. The total phenolic content of the examined extracts was high (5766.44 to 10599.43 mg GAE/100 g), and the antioxidant activity was high (DPPH and FRAP assays: 7551.31 - 7875.42 and 100.25 - 176.60 mol TE/100 g, respectively). Minimum concentrations of bactericidal agents are between 0.8 and 6.4 mg/ml. The examination of PPEs’ antiproliferative activity also demonstrated their considerable potency, with IC50 values ranging from 0.132 mg/ml to 0.396 mg/ml [21]. This study looks at the antioxidant properties, organic acid content, and sugar level of pomegranate drinks available in Turkish markets. They assessed the total phenolic content, 2,2-diphenyl-1-picrylhydrazyl’s potential to scavenge free radicals, and the ability of seven commercial PJs to reduce ferric. Capillary zone electrophoresis was used to assess the organic acid and sugar content in juices. The findings demonstrated the very high total phenolic contents and antioxidant capacity of commercial PJ. The two main sugars discovered were fructose and glucose. Citric and malic were the two main acids. The F/G ratio, organic acid profiles, TPs, and antioxidant capacity readings all indicated that one of the juices may have been adulterated [22]. Supercritical fluid extraction was compared to the extraction of phenolic components from pomegranate peel using two different techniques and five different solvents (acetone, methanol, ethanol, water, and ethyl acetate). Tannic acid was used as a reference for the Folin–ciocaltu reagent, which was used to determine the total phenolic compounds. Most phenolic compounds from pomegranate peel extracts (PPE) were created by acetone with sonication, according to the overall findings. The antioxidant activity of pomegranate peel extract was higher than that of the synthetic antioxidants butylated hydroxyanisole (BRA) and butylated hydroxytoluene (BHT) at a concentration of 0.05%. It is possible to think of the pomegranate peel extract as a rich source of natural antioxidants due to its comparatively strong antioxidant activity [23]. Utilizing in vitro studies, the antioxidant capacity of pomegranate fruit peels was assessed. Peels extracted with 80% methanol produced greater yields (45.4%) and total phenolics (27.4%) than peels extracted with water (WE) or ether (EE). ME had a stronger decreasing capability than WE or EE (P 0.05). ME outperformed –catechin in terms of its ability to scavenge DPPH radicals (%). The peels of pomegranates have phenolic content, DPPH scavenging ability, and reducing power [24].

**Antibacterial activity due to phenolic composition**

P. granatum extracts were successively examined for their antibacterial activity in vitro using petroleum ether, chloroform, methanol, and water. The methanolic extract was discovered to be the most efficient against all tested microbes. All the studied extracts had some antibacterial activity, but the methanolic extract was particularly effective against Proteus vulgaris and Bacillus subtilis [25]. The antibacterial efficacy of P. granatum peel EtOH extract (PGPE) against 16 strains of salmonella both in vitro and in vivo. The range of PGPE’s minimum inhibitory doses was 62.5 - 1000 x 03 BCg ml-1. A PGPE infection followed by an initial Salmonella typhimurium infection was given to mice. The amount of live S. typhimurium collected from faeces and mortality were both shown to be significantly affected by the extract. Although clinical symptoms and his-
tological damage were infrequently seen in treated mice, the untreated controls exhibited evidence of lethargic behavior and liver and spleen histological damage. When considered as a whole, the findings of this research show that PGPE has the potential to be a successful therapy for salmonellosis [26]. In Iranian traditional medicine, “Golnar” - a term for the flowers of the plant P. granatum L. (Punicaceae) var. pleniflora has been used to cure and prevent foodborne illnesses. According to this study’s analysis of total phenolic and flavonoid content, the methanol fraction had the greatest levels of phenolic and flavonoid content, while the chloroform fraction had the lowest levels (3.8 mg GAE/g and 1.1 mg RE/g, respectively). The antibacterial activities of the fractions were positively correlated with the total phenolic and flavonoid content, with the methanol fraction having the highest antibacterial effect against Staphylococcus aureus (MIC 0.19 mg/ml) and chloroform extract having the lowest antibacterial activity against Escherichia coli (MIC 25 mg/ml) [27]. In Indian traditional medicine, the pericarp of P. granatum Linn. is frequently used as a crude medicament to cure diarrhea as well as other conditions such as astringency, antihelminthic, asphodiassic, laxative, diuretic, stomachic, cardiotonic, and refrigerant. The findings showed that all species, except for Pseudomonas aeruginosa, were resistant to the antibacterial action of the extracts made from P. granatum pericarp. Maximum antibacterial activity was shown by the methanol extract against Shigella dysenteriae serotype 1, Salmonella typhi, and S. typhimurium. The results indicate that a suitable bioactive component (or compounds) may be created from P. granatum pericarp as an alternative medicine for the treatment of bacterial strains that cause GTF infections [28]. The extraction of pelargonidin-3-galactose, cyanidin-3-glucose, gallic acid, quercetin, and myricetin from the pomegranate fruit’s methanolic extract followed antibacterial activity-directed isolation. All these chemicals show notable antimicrobial efficacy against Corynebacterium, Staphylococcus, Streptococci, B. subtilis, Shigella, Salmonella, Vibrio cholera, and E. coli species. Gallic acid demonstrated the highest antibacterial activity against all the tested sensitive strains, while the activity of the other pure compounds was almost the same because of the compounds’ structural similarity. This was discovered by comparing the activity of all the isolated pure compounds. All pure compounds were shown to be bactericidal due to their phenolic structures [29].

**Medicinal effect and health benefits of pomegranate**

Animal models used in *in vivo* investigations on the whole fruit or juice, peel, and flowers show antiulcer effects. Although other individual ellagitannins may have contributed to the mixture’s biological activity, ellagic acid was the predominant factor in this result. Pomegranate extracts from peels, blossoms, seeds, and juice, among other preparations, exhibit a notable anti-inflammatory effect in the stomach [30]. The fruit is known as “nature’s power fruit” and has potent anti-inflammatory, antibacterial, and antioxidant effects. Recent research has also revealed possible anticancer activity. Potentially effective against bacterial infections is *P. granatum*. Utilizing nutritional agar medium (HIMEDIA), antibacterial activity was determined using the agar-well diffusion technique. Along with sugar, the pomegranate’s antibacterial properties were investigated [5]. The individuals had comprehensive oral prophylaxis and were instructed to forego oral hygiene practices for 24 h. Each person had their dental plaque sampled both before and after washing with 30 ml of unsweetened pomegranate juice. Plaque samples were cultivated on Rogosa SL Agar and Mitis Salivarius Agar medium. The signed rank test by Wilcoxon was employed for statistical analysis. The effectiveness of pomegranate rinse against dental plaque bacteria was demonstrated by the results. The amount of streptococci (23%) and lactobacilli (46% of colony forming units) significantly decreased. The ruby red seeds could be an option for treating the gums in tooth plaque [31]. The interest in this red fruit has dramatically expanded as a result of studies looking into the health advantages of pomegranate juice. Rich in polyphenols, which have intriguing antioxidant effects, is pomegranate juice. Pomegranate juice may have antioxidant, anti-inflammatory, anti-hypertensive, and anti-atherogenic properties, according to several *in vitro* and animal investigations. As a result, encouraging outcomes against cancer, diabetes, and other disorders have been documented [32]. The impact of various dosages of a pomegranate extract with HPLC characterization on the nociceptive behavior brought on by formalin in mice. It was also evaluated how well the extract protected against stomach damage brought on by ethanol and non-steroidal anti-inflammatory medicines. In both rounds of the formalin test, pomegranate lowered nociception, indicating that both central and peripheral activity can suppress nociception. Pomegranate also provided protection against ethanol-induced gastric lesions and prevented indomethacin-induced stomach damage. The current findings support the use of pomegranate (poly)phenolics for pain relief and for their anti-inflammatory activities [33].

Various disease risk factors, such as high blood pressure, high cholesterol, oxidative stress, hyperglycemia, and inflammatory activity, can be prevented or treated using pomegranates. It has been established that some pomegranate constituents, such polyphenols, may have antioxidant, anti-inflammatory, and anticarcinogenic properties. Pomegranate juice has greater antioxidant potential than red wine and green tea, which are produced by ellagitannins and hydrolysable tannins. Pomegranate juice helps lessen lipid peroxidation, free radicals, and macrophage oxidative stress. Pomegranate fruit extract also inhibits cell division and triggers apoptosis, which may contribute to its anticarcinogenic benefits. Additionally, ellagitannins prevent the promoter suppression of several inflammatory indicators and their production [23]. Pomegranate juice has been found to significantly reduce the risk of atherosclerosis, act as an antioxidant, lower blood pressure, and reduce inflammation. Intima media thickness in cardiac patients on medication and atherosclerotic lesion areas in immune-deficient animals were both dramatically decreased by pomegranate juice. Additionally, it reduced systolic blood pressure, serum angiotensin converting enzyme activity, and lipid peroxidation in type 2 diabetic individuals as well as hypertensive patients. Considering this, more clinical research into the possible cardioprotective effects of pomegranate juice is warranted, and current research shows it may be wise to incorporate this fruit juice into a diet that promotes heart health [34]. The management and avoidance of inflammatory illnesses like arthritis. The floral part’s pet-ether, dichloromethane,
and methanol fractions were selected for pharmacological testing, as well as their analgesic and anti-inflammatory effects in an animal model. Carragennan-induced rat paw edema was used to test the anti-inflammatory activity. The acetic acid-induced writhing test was used to assess the analgesic efficacy in mice. Pet-ether, dichloromethane, and methanol fractions at 200 mg/kg dosage levels demonstrated 75.77% in the acetic acid-induced writhing test in mice [35].

Conclusion

The peel extract of pomegranates showed the most powerful antibacterial and antioxidant benefits, according to the study’s result. Pomegranate extracts may be made from several portions of the fruit. Pomegranate extracts are interesting for future use in functional foods, medications, and nutraceuticals due to the variety of phenolic components that contribute to their bioactivity. The precise mechanisms behind these beneficial benefits and their potential therapeutic uses require more study.

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Conflict of Interest

None.

References


