



Review

Pomegranate peel and fruit extracts: A review of potential anti-inflammatory and anti-infective effects

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ABSTRACT

Ethnopharmacological relevance: *Punica granatum* L. (Punicaceae) has been used for centuries in many cultures for the prevention and treatment of a wide number of health disorders such as inflammation, diabetes, diarrhea, dysentery, dental plaque and to combat intestinal infections and malarial parasites. **Aim of the review:** This review aims at providing an up-to-date overview of the chemical constituents, traditional uses, phytochemistry, pharmacology and toxicology of *Punica granatum* L. Moreover, the focus of this review is the possible exploitation of this species to treat different diseases and to suggest future investigations.

Materials and methods: An extensive and systematic review of the extant literature was carried out, and the data under various sections were identified by using a computerized bibliographic search via PubMed, Web of Science and Google Scholar. All abstracts and full-text articles were examined. The most relevant articles were selected for screening and inclusion in this review.

Key findings: A variety of pomegranate ethnomedical uses have been recorded. Additionally, over the last decade, there has been a dramatic increase of interest in pomegranate as a medicinal and nutritional product due to its newly identified potential health effects, which include treatment and prevention of cancer and cardiovascular diseases. **From the toxicological perspective, pomegranate fruit juice, extracts and preparations have been proven to be safe.**

Conclusions: The ethnopharmacological relevance of pomegranate is fully justified by the most recent findings indicating the fruit is a medicinal and nutritional agent useful for treating a wide range of human disorders and maladies. Further investigations are needed to fully understand the mode of action of the active constituents and to fully exploit pomegranate's preventive and therapeutic potential.

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Abbreviations: PoP, Pomegranate peel; PoPx, Pomegranate peel extract; PoMx, Pomegranate extracts; DPPH, Diphenyl-1-picrylhydrazyl; ROS, Reactive oxygen species; LDL, Low density lipoproteins; Ox LDL, Oxidized low density lipoproteins; SPRE, Standardized pomegranate rind extract; IC, Inhibitory concentration; iNOS, Inducible nitric oxide synthase; COX-2, Cyclohexogenase-2; PGE₂, Prostaglandin-2; NF-kB, Nuclear factor kappa B; MIC, Minimum inhibitory concentration; OMARIA, Orissa malaria research indigenous attempt; NOAEL, No observed adverse effects level; LD, Lethal doses; LPS, Lipopolysaccharide.; FRAP, Ferric reducing antioxidant power; PPAR-γ, Peroxisome proliferator-activated receptor gamma; PON 1, Paraoxonase 1; MMP-9, Metalloprotease-9; TNF, Tumor Necrosis Factor

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1. Introduction

Pomegranate (*Punica granatum L. Punicaceae*; the common name is derived from Latin words *ponus* and *granatus*), a seeded or granular apple, is a delicious fruit consumed worldwide. The fruit is native to Afghanistan, Iran, China and the Indian sub-continent. The ancient sources of pomegranate linked Iran to Pakistan, China and eastern India, where pomegranates had been under cultivation for thousands of years. From the west of Persia (modern day Iran), pomegranate cultivation stretched through the Mediterranean region to the Turkish European borders and American southwest, California and Mexico (Celik et al., 2009; Lansky and Newman, 2007).

Pomegranate peels are characterized by an interior network of membranes comprising almost 26–30% of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid). These compounds are concentrated in pomegranate peel (PoP) and juice, which account for 92% of the antioxidant activity associated with the fruit (Afaq et al., 2005; Negi et al., 2003; Zahin et al., 2010). Fig. 1.

Gallic acid, ellagic acid and punicalagin, in addition to their free radical-scavenging properties, also possess antibacterial activities against intestinal flora, particularly enteric pathogens, i.e., *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, as well as *Vibrio cholerae* (Aviram et al., 2008; Lu et al., 2007; Pai et al., 2011; Taguri et al., 2004).

The therapeutic potential of PoP has been widely recognized by different cultures. In Egyptian culture, several common ailments such as inflammation, diarrhea, intestinal worms, cough and infertility have been treated by exploiting pomegranate peel extract (PoPx). The exceptional antioxidant potential and strong medicinal properties of PoP led the international scientific community to initiate intensive research in the last decade to further investigate its role in human health (Lansky and Newman, 2007).

Several studies have demonstrated the antimicrobial, antihelminthic, and antioxidant potential of the active ingredients of pomegranate extracts (PoMx), suggesting their preventive and curative role in gastro-mucosal injuries, cancer chemoprevention, ethanol- and acetone-induced ulceration and diabetic oxidative damage (Al-zoreky and Nakahara, 2003; Arun and Singh, 2012; Negi et al., 2003). The mechanism of antimicrobial activity of

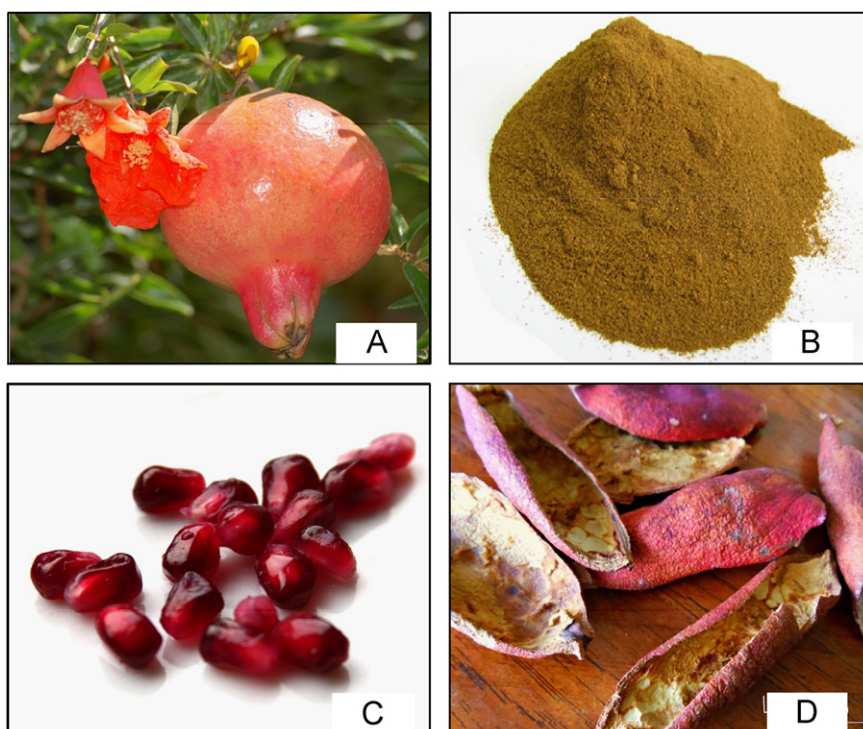


Fig. 1. Pomegranate fruit (A) and its anatomical components, pomegranate peel powder (B) pomegranate seeds (C) and sundried pomegranate peel (D).

pomegranate peel phenolics involves precipitation of membrane proteins resulting in microbial cell lysis. The ethnopharmacological profile of PoPx makes it a valuable traditional asset due to its antimicrobial and anti-mutagenic properties. Moreover, the phytochemical concentration of PoP is high enough to be effective without further enrichment with the extracts of any other fraction of the fruit (Sestili et al., 2007).

2. Traditional medicinal uses

A variety of cultures and traditions in both the developing and developed worlds recommend pomegranate peel to treat common health problems. Traditionally, aqueous PoP extract is obtained by boiling for 10–40 min. The extract has been used to treat diarrhea, dysentery, and dental plaque, in addition to being used as a douche and enema agent (Lansky et al., 2004). Similarly, diarrhea, intestinal worms, bleeding noses and ulcers have been treated in Indian Subcontinent with dried PoP, plant bark and flower infusions. PoPx is gargled as a liquid to relieve sore throat and hoarseness. Topical application of the rind powder can aid in healing bleeding gums and plaque in patients with periodontitis (Amrutesh, 2011). Oral ingestion of 5–10 g of peel powder is recommended two to three times a day for the treatment of hyperacidity.

3. Bioactivities of pomegranate peel ellagic acid and punicalagin

The antioxidant activity of PoP is associated with its phenolic compounds in the form of anthocyanins, gallotannins, ellagitannins, gallagyl esters, hydroxybenzoic acids, hydroxycinnamic acids and dihydroflavonol, however, ellagitannins characterized by ellagic acid, gallic acid and punicalagin are the predominant phenolics of the fruit (Cerdeira et al., 2003; Larrosa et al., 2006). Ellagic acid occurs in free and bound forms (EA-glycosides and ellagitannins). The efficacy of ellagic acid as a tool to treat some low to mild and chronic disorders with very low rates of progression has been widely documented in the literature. Moreover, it has been shown to be a potential candidate as a chemopreventive agent for cancer treatment (Kelloff et al., 1994). Beside its other well-known ethnopharmacological properties, ellagic acid has been demonstrated to reduce white fat deposits and triglycerides levels accumulated in the body during regular intake of high-fat diets (Lei et al., 2007).

Several studies have confirmed the cytoprotective effects of ellagic acid from PoPx on oxidatively injured living cells, oxidative DNA damage and depletion of the non-protein sulfhydryl pool. Higher ellagic acid concentrations are directly associated with the antioxidant activity of PoPx. The ellagic acid contents of the fruit peel and fruit juice have been reported to be 10–50 mg/100 g and 1–2.38 mg/100 ml, respectively (Akbarpour et al., 2009; Lu and Yuan, 2008; Seeram et al., 2004).

Pomegranates have the highest concentration of punicalagin among the most commonly consumed fruits. Studies have shown that punicalagin has antioxidant, antifungal and antibacterial properties. The alpha and beta forms of pomegranate punicalagin are polyphenolic hydrolysable tannins and isomers of 2,3-(S)-hexahydroxydiphenyl-4,6-(S,S)-gallagyl-D-glucose. Punicalagin, being water soluble, hydrolyzes into smaller polyphenolic compounds in the small intestine under normal physiological conditions. Punicalagin comprises 11–20 g/kg of the peel powder ellagitannins. The punicalagin available in pomegranate juice is a major antioxidant polyphenol and also has antiproliferative activity, inhibiting proliferation from 30% to 100%, against all cell lines (Fischer et al., 2011; Seeram et al., 2005) Fig. 2.

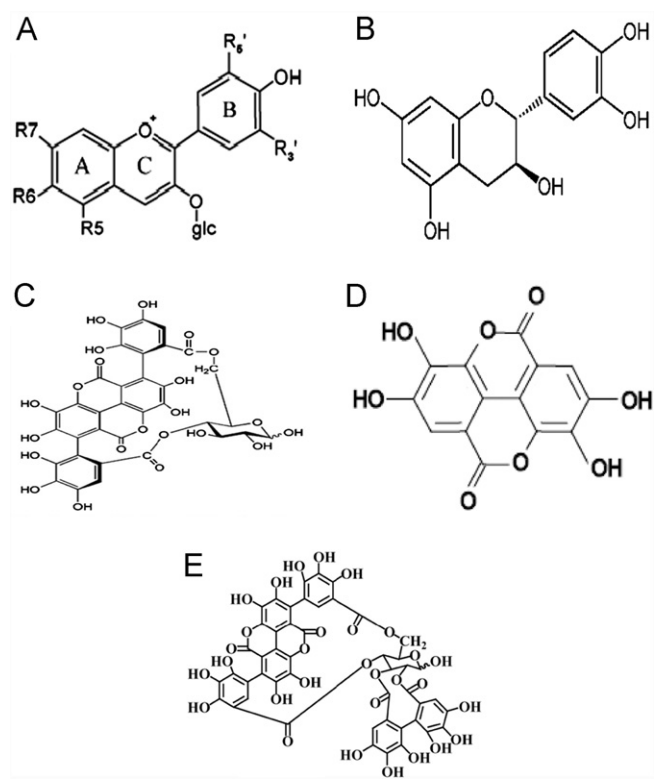


Fig. 2. General structure of the important phenolic compounds of pomegranate peel (A) Anthocyanin (B) Catechins (C) Punicalin (D) Ellagic acid (E) Punicalagin.

4. Peel phenolics extraction modeling

Industrial scale extraction of phenolic compounds from PoP is carried out by using solvents such as methanol, ethanol, acetone, chloroform and ethyl acetate. Polar solvents have greater antioxidant extraction capability compared to non-polar solvents. The use of different solvents other than water for peel phenolic extraction are reported to yield different phenolic content ratios and associated antioxidant activity (Negi and Jayaprakasha, 2003; Negi et al., 2003; Zahin et al., 2010). Phenolics extracted from dried PoP with ethyl acetate, acetone, methanol and water demonstrate higher antioxidant activity, but aqueous extracts exhibit higher anti-mutagenic activity than methanolic extracts. Due to the adequate polarity of methanol, methanolic extracts of pomegranate peel possess higher antioxidant activity than other solvents (Ajaikumar et al., 2005; Iqbal et al., 2008; Negi et al., 2003; Singh et al., 2002; Zahin et al., 2010).

The type of solvent, solid–liquid ratio, extraction temperature and size of the peel particles have been shown to significantly affect antioxidant extraction. Small-sized peel particles increase surface area of the powder and decrease solvent transfer time across the particles subsequently yielding greater antioxidant extraction efficiency. Similarly, the total phenolic content and antioxidant activity of PoPx increase as an inverse function of peel particle size. Partitioning water in methanol extracts of PoP between 2% acetic acid and ethyl acetate increases ellagic acid yield (7.06–13.63%) and diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (38.21–14.91 µg/mL) (Panichayupakaranant et al., 2010a). Under the most economical method, PoPx was generated by grinding the dried peels of the fruit up to a 0.2-mm mesh size and subsequently extracted at a water/peel ratio of 50/1 (w/w) at 25 °C for 2 min. In one study, such a procedure yielded 11.5% total phenol bearing a 22.9% antioxidant content, corresponding to DPPH scavenging activity of 6.2 g/g (Qu et al., 2010).

The extraction of polyphenols with water requires a higher extraction temperature, a condition that increases the total yield but decreases whole antioxidant activity. Up to 262.7 mg/g of hydrolyzable tannic acid equivalents from PoP could be extracted with pressurized (102.1 atm) de-ionized water at 40 °C. Up to 116.6 mg/g of punicalagin, the secondary component of PoP could also be extracted on a dry matter basis under the same conditions (Cam and Hisil, 2010). Pomegranate phenolics extraction modeling has become very sophisticated in the recent years. The fractionation of the active components of extracts and the identification of the most appropriate extraction techniques to raise antioxidant and anti-mutagenic activity of extracts are steps toward recognizing the actual ethnopharmacological basis of this useful fruit.

In conclusion, utilizing methanolic extractions as fine peel powders (up to 0.2 mm) at < 40 °C increases the anti-oxidative properties of extracts. Amberlite resin vacuum-aspirated column chromatography and high-speed chromatography are the most appropriate techniques for maximizing the yield of total phenols as well as active biomolecules such as punicalagin and ellagic acid.

5. Antioxidant potential

Reactive oxygen species (ROS) produced during normal cellular metabolic processes or derived from the exposure to ionizing radiation or xenobiotics are well-recognized causal factors in a wide number of chronic diseases, including CVD and cancer. The toxic effects of ROS depend on their capacity to damage relevant and sensitive biological substrates, such as DNA, RNA, proteins and membrane lipids. ROS include superoxide radicals, lipoperoxides, hydrogen peroxide, and hydroxyl free radicals. The use of indiscriminate chemotherapy to ameliorate oxidative damage in humans has not been regarded as a preferable therapeutic tool to treat malignancies. Phytonutrients and nutraceuticals, when used as anti-oxidative and anti-proliferative therapeutic agents, open up new avenues for preventing and treating certain malignancies. Therefore, as fruits and vegetables are natural reservoirs of antioxidants, their pharmacological potential should be explored. Many studies in the literature have confirmed PoP as a remarkable source of antioxidants, i.e., ellagitannins and flavonoids. PoP has been reported to carry 90% pure 3.5% ellagic acid (Lu and Yuan, 2008) and 4.23% (42.36 mg rutin equivalent/g) total flavonoids (Viuda-Martos et al., 2012).

PoPx, as evaluated by Ferric reducing antioxidant power (FRAP) assay, was found to be the richest source of antioxidants among peel extracts of the most commonly consumed fruits. Similarly, PoP demonstrated 2.8-fold higher antioxidant activity compared to pomegranate seed and leaf extracts (Guo et al., 2003; Okonogi et al., 2007; Negi et al., 2003; Tehranifar et al., 2011). The free radical scavenging activity of PoP phenolics involves electron donation to free radicals that converts them to relatively more stable compounds. Studies have confirmed that the antioxidant activity of plants extracts depends on the concentration of phenolic compounds, and the antioxidant power of PoPx has been found to linearly increase with the concentration of peel phenolics up to the level of 400 µg/g (Naveena et al., 2008a; Negi and Jayaprakasha, 2003). Several studies have substantiated the chemoprotective role of the POM antioxidant fraction against chemically induced injuries. These studies suggest an inhibitory and protective role against oxidative damage in carcinogenesis and its progression. In vivo studies have validated the attenuation of oxidative stress by pomegranate ellagitannins in the treatment of placental dysfunction. Prenatal ingestion of the metabolic fractions of ellagitannins in juice form reduced oxidative stress and apoptosis in placental tissues, confirming the in vitro results of

sub-culturing placental tissues of pomegranate administered to expectant mothers suffering from hypoxia (Chen et al., 2012; Celik et al., 2009).

6. Nutraceutical properties of pomegranate and peel extracts

6.1. Cardiovascular Protective role

Atherosclerosis is one of the leading causes of death, particularly in developed countries where a higher percentage of atherosclerotic deaths are observed. Low density lipoproteins (LDL) accumulate in the interior layers of blood vessels and then undergo oxidation, a process that generates harmful species. Inhibition of LDL oxidation is considered to be a good strategy to prevent the accumulation of foam cells and, ultimately, cholesterol deposits in the arteries. Due to its excellent antioxidant activity, PoPx has the potential to inhibit LDL oxidation and thus retard the progression of atherosclerosis with a significant reduction of arterial foam cell levels. The pomegranate polyphenols punicalagin, gallic acid, and to lesser extent ellagic acid, increase hepatocyte paraoxonase 1 expression and secretion in a dose-dependent manner, thereby reducing the risk of atherosclerosis development (Khateeb et al., 2010; Rosenblat and Aviram, 2009).

The cardiovascular disease preventive effects of PoP ellagitannins (10–100 µM) have been observed in vitro; however, relatively lesser cardioprotective effects of pomegranate ellagitannins were noticed in vivo. These results suggest the cardioprotective effects are associated with lower bioavailability of the antioxidant fractions in the fruit (Larrosa et al., 2010). The cardioprotective effects of PoMx (100 mg/kg) in a rat model have been more recently demonstrated (Hassanpour Fard et al., 2011) via a reduction in creatine kinase-MB, lactate dehydrogenase and glutathione. There have been many reports on the positive effects of POM polyphenols and their possession of oxidation sensitive genes, nitrous oxide synthase expression inhibition potential, (de Nigris et al., 2005) and downregulation of redox sensitive ELK-1 and p-JUN genes and endothelial nitrous oxide expression under induced endothelial wall shear stress (Larrosa et al., 2006, 2010). Aside from the biochemical properties associated with its extracts, pomegranate peel powder has also been evaluated as a dietary fiber source for the treatment of hypercholesterolemia and atherosclerosis. Dietary supplementation with peel powder at a concentration of 5, 10 and 15 g/100 g for a period of four weeks significantly reduced serum total cholesterol, triglycerides, LDL and lipid peroxidation levels in hypercholesterolemic rats (Hossin, 2009).

6.2. Anti-inflammatory and anti-allergic properties

The weight of compelling scientific evidence regarding the therapeutic benefits of pomegranate and its fractions has built a scientific consensus that pomegranate rind methanolic extract has the ability to inhibit inflammation and allergies (Panichayupakaranant et al., 2010b). The anti-inflammatory components of PoP, i.e., punicalagin, punicalin, strictinin A and granatin B significantly reduce production of nitric oxide and PGE2 by inhibiting the expression of pro-inflammatory proteins (Lee et al., 2008; Romier et al., 2008). Evidently, inflammatory cells including neutrophils, macrophages and monocytes may inflict damage to nearby tissues, an event thought to be of pathogenic significance in a large number of diseases such as emphysema, acute respiratory distress syndrome, atherosclerosis, reperfusion injury, malignancy and rheumatoid arthritis (Babior, 2000).

A recent study on isolated human neutrophils indicated that aqueous PoPx directly inhibited neutrophil myeloperoxidase activity and the enzymatic production of hypochloric acid from

hydrogen peroxide at a 50 ng/ml concentration (Bachoual et al., 2011). One group of investigators (Ouachrif et al., 2012) reported anti-inflammatory properties of the PoPx following intraperitoneal (25, 50 and 100 mg/kg) and intra-cerebroventricular (10, 25 and 50 µg/3 µl/rat) administration. The experiments indicated pain index reduction of 52–82% and a significant reduction in egg albumin induced hind paw inflammation at the same intraperitoneal dosage levels. Similarly, another study elucidated a strong inhibitory effect against inflammation stimulators during carrageenan-induced paw edema in mice following oral administration of granatin B (2.5 and 10 mg/kg). Significant inhibitory effects were observed after 6 h of peel-active component administration when compared to indomethacin (Lee et al., 2010). Successful in vitro and in vivo assays indicated that PoPx and hydrolysable tannins, in the form of standardized active components, are a very effective treatment measure against inflammatory disorders.

6.3. Anticancer properties

Cancer is the leading cause of death in both developed and developing countries. This disease presents higher mortality rates among the low- and middle-income populations. The significant determinants for greater mortality in poorer countries are primarily related to the absence of appropriate health systems. One study reported 12.7 million new cancer cases with 7.6 million cancer deaths occurring in 20 world regions in 2008. Early detection and implementation of appropriate preventive measures to reduce the cancer burden have been recommended (Ferlay et al., 2010; Kanavos, 2006; Sloan and Gelband, 2007).

ROS represent a causal and/or concausal factor in the development of cancer. Extensive ROS damage to DNA ultimately leads to somatic mutations and organ malignancies. At the cellular and tissue level, copper and iron binding sites of macromolecules serve as central sites for the production of free radicals. Such site-specific free radical generation is inhibited by chelation of metal ions by antioxidants of biological origin, e.g., flavonoids (Chevion, 1988; Sies, 1997). Moreover, ellagic acid and punicalagin arrest cancer cell growth by inducing apoptosis—a multistep cell death program. Antioxidants, either from synthetic or plant sources, are thought to be a preventive approach against cancerogenesis. Natural antioxidants, despite the presence of synthetic ones in the market, have gained a wide acceptance due to their high safe edible limits.

6.4. Prostate and colon cancer

The anticarcinogenic effects of the fruit ellagitannins have been associated with its hydrolyzed products, specifically ellagic acid and punicalagin that induced apoptosis in colon cancer cells (HT-29, HCT116) and prostate cancer cells at a concentration of 100 µg/ml. These effects were mediated through some of the fundamental pathways, such as introducing cytochrome c in cell cytosol and by up-regulation of Bax and down-modulation Bcl-2 (Larrosa et al., 2006; Malik et al., 2005; Seeram et al., 2004,2005). Treatment of Los Angeles prostate cancer cells (LAPC4) with standardized PoMx (10 µg/ml) containing 37% punicalagin is based on the inhibition of cell proliferation and the induction of apoptosis. PoMx treatment in combination with IGFBP-3 reduced the activity of cell growth promoters (Akt and mTOR) performing a pro-apoptotic function in inhibiting cancer cells growth (Koyama et al., 2010). In vitro incubation of human prostate cancer cells and umbilical vein endothelial cells with 37% standardized ellagitannin-rich PoMx inhibits proliferation of both prostate cancer and umbilical vein endothelial cells under hypoxic and normoxic conditions. The IC_{50} of ellagitannins-enriched PoMx for endothelial

cells proliferation was 6.7 ± 0.5 µg/ml and 2.2 ± 0.2 µg/ml under reduced and normal oxygen supply, respectively. Similar inhibitory effects were observed following oral administration to mice of ellagitannin-rich PoMx for a period of four weeks after subcutaneous injection with prostate cancer cells. Indeed, a large reduction in cancer cell proliferation, prostate cancer xenograft size and tumor vessel density were observed (Sartippour et al., 2008). Koyama et al. (2010) demonstrated a complete inhibition of PoMx-induced apoptosis with a 100 ng/ml co-treatment of prostate cancer cells with IGF-1. However, there remains much that needs to be learned about the positive and negative synergistic effects of PoMx combined with cell growth factors.

6.5. Melanogenesis/skin cancer

PoPx (IC_{50} = 182.2 µg/ml) inhibits melanocyte proliferation and melanin synthesis by inhibiting tyrosinase activity. The magnitude of inhibition is comparable to arbutin. The protective role of orally administered PoPx, as compared to a water fed group, containing 90% ellagic acid, at a concentration of 1000 mg/kg, has been shown in brown guinea pigs to inhibit skin pigmentation induced by exposure to UV radiation (Yoshimura et al., 2005).

Several studies have confirmed the ability of PoPx and POM ellagitannins (500–10,000 mg/L) to inhibit free radical generation in UVA- and UVB-irradiated human skin, thus protecting it from DNA fragmentation, skin burns and depigmentation. This human skin DNA base damage is attributable to monochromatic light that activates photosensitizers leading to the generation of genotoxic single oxygen species (Kasai et al., 2006; Manasathien et al., 2011; Pacheco-Palencia et al., 2008). Another study explained the role of PoMx in mediating UVB-induced skin damage. Epidermal pretreatment with PoMx (5–10 µg/0.1 ml/well) prior to UVB-induced (60 mJ/cm²) skin damage inhibits the matrix metalloproteinases compounds involved in the degradation of skin connective tissues and collagen components and the markers of oxidative stress and genotoxicity (Afaq et al., 2009).

6.6. Breast cancer

PoPx has been shown to induce apoptosis in human breast cancer cells (MCF-7). In past studies, the application of PoPx and genistein presented significantly higher MCF-7 inhibitory and cytotoxic effects in treating breast cancer cells. Moreover, PoPx exhibited the potential to inhibit cell proliferation and angiogenesis marker expression, phosphorylation of p38 and C-Jun mitogen-activated protein kinases and activation of pro-survival signaling pathways. PoPx has further been shown to inhibit nuclear factor κ B (NF- κ B)-dependent reporter gene expression associated with proliferation, invasion, and motility in aggressive breast cancer phenotypes (Jeune et al., 2005; Khan et al., 2009). Quite a few reports have presented a plausible explanation for endocrinal breast cancer therapy in post-menopausal women where a non-significant response to estrogen receptor-positive MCF-7 breast tumors was observed. PoMx exhibiting anticancer properties were successfully tested at a concentration of 300 µg/ml in combination with 1 µM tamoxifen to sensitize and enhance the activity of the latter, thereby inhibiting resistant MCF-7 cell proliferation (Aiyer et al., 2012; Banerjee et al., 2011).

Breast cancer cell proliferation and the generation of estrogen receptor-positive tumors are estrogen-stimulated activities that could be checked by anti-aromatase compounds. Urolithin B, a pomegranate ellagitannin metabolite, was identified by a microsomal aromatase assay as the active ingredient with maximum anti-aromatase activity, in addition to inhibition of testosterone-induced MCF7 cell proliferation (Adams et al., 2010). Although in vitro

cultured cell and animal anti-estrogenic studies on pomegranate extracts constituents have been successfully conducted, human studies are required to clarify the effect of PoMx and PoPx nutraceuticals on serum hormone levels and associated activity in a more systematic way.

6.7. Antimicrobial potential of peel extracts

Polyphenols, flavonoids, condensed and hydrolysable tannins extracted from fruits, vegetables, herbs and spices have been explored as potential agents for treating or preventing a wide range of infections (Cowan, 1999; Naz et al., 2007; Taguri et al., 2004).

The antimicrobial mechanisms of phenolic compounds involve the reaction of phenolics with microbial cell membrane proteins and/or protein sulfhydryl groups that yield bacterial death due to membrane protein precipitation and inhibition of enzymes such as glycosyltransferases (Haslam, 1996; Naz et al., 2007; Vasconcelos et al., 2003).

Food-borne diseases and urinary tract infections are conventionally treated on the Indian Subcontinent using PoPx (El-Sherbini et al., 2010; Gopalakrishnan and Benny, 2009), while PoP ellagitannins, punicalagin, ellagic acid and gallic acid, as natural antimicrobial agents, have been widely exploited against *Staphylococcus aureus* and hemorrhagic *Escherichia coli* for their ability to precipitate membrane proteins and inhibit enzymes such as glycosyltransferases, leading to cell lysis (Braga et al., 2005; Haslam, 1996; Machado et al., 2003; Naz et al., 2007; Vasconcelos et al., 2003; Voravuthikunchai et al., 2005).

In vivo and in situ application of an 80% methanolic extract of PoP presented an inhibitory effect against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Yersinia enterocolitica* (Al-Zoreky, 2009). However, higher doses of PoPx (24.7 mg/ml) have been reported to be the minimum bactericidal concentration for *Listeria monocytogenes*.

6.8. Anti-influenza and anti-malarial responses

Pomegranate hydrolysable tannins, including punicalin, punicalagin, gallic acid and ellagic acid, exhibit antiviral properties that modulate respiratory infections and influenza (Gil et al., 2000; Nonaka et al., 1990). The antiviral properties have been attributed to inhibition of RNA replication of the influenza virus by pomegranate-purified polyphenol extract. Punicalagin compounds with inhibitory concentrations of up to 40 µg/ml have been shown to be the most active in blocking viral RNA replication (Haidari et al., 2009). Similarly, peel phenolics inactivate viruses via direct structural damage and indirect intercellular inhibition of viral replication. Glycoprotein-enveloped viruses have been reported to be more susceptible to structural damage by polyphenols compared to non-enveloped viruses (Kotwal, 2008).

Another recent study elucidated the antiviral potential of pomegranate polyphenols. The study reported that a short-time frame (5 min) exposure of avian and human influenza viruses (H1N1, H3N2, and H5N1) to 800 µg/ml pomegranate polyphenols resulted in a 3-log reduction of viral titer at room temperature (Sundararajan et al., 2010). However, polyphenol antiviral activity was lower against H5N1 influenza virus isolated from birds. The virucidal effects of pomegranate phenolics are associated with the interaction of pomegranate phenolics with enveloped glycoprotein i.e., hemagglutinin, yielding loss of red blood cell agglutination. In addition, pomegranate phenolics interact with viral proteins during the intercellular steps to inhibit influenza viral replication (Haidari et al., 2009).

OMARIA, an Ayurvedic formulation derived from dried PoP powder, is a basic nutraceutical used for the treatment of malaria in the *Plasmodium falciparum*- and *Plasmodium vivax*-endemic rural

area of the eastern province of Orissa, India. Interestingly, chloroquine-resistant strains of *Plasmodium falciparum* have been found to be sensitive to the toxic action of PoP methanolic extracts.

Tannin-enriched POM methanolic extract tested against hemozoin (6 µg/ml) stimulated THP-1 cells antagonistically and reduced MMP-9 secretion by 78% and 95% at concentrations of 50 and 100 µg/ml, respectively. Approximately 65% and 79% inhibition of MMP-9 secretion in THP-1-stimulated cells was observed with a 10-µM concentration of purified ellagic acid and punicalagin, respectively (Dell'Agli et al., 2010).

6.9. Wound healing potential

Epithelialization, antioxidant immunity and characteristic biochemical properties are the common features of the wound healing process that prevail in injured tissues. Topical administration of PoPx can be recommended for dead tissue, incisional and excisional wound models. Improved epithelialization, breaking strength and contraction of incised wounds, along with increased hydroxyproline content, dry weight and breaking strength of granulated tissues, can be observed in the healing process of PoPx-treated wounds. In studies, oral administration of a 100 mg/kg aqueous extract of PoPx to Wistar rats and topical application of PoPx formulated with hydrophilic gel resulted in significant improvement in all wound models (Adiga et al., 2010; Murthy et al., 2004).

Methanolic PoP extracts act as potent inhibitors of gastric mucosal injuries. Oral administration of 70% methanolic PoMx at 250 and 500 mg/kg inhibited the progression of ulcers by 22.37% and 74.21%, respectively, with aspirin-induced gastric ulcers and 21.95% and 63.41%, respectively in rats with ethanol-induced gastric ulcers (Ajaikumar et al., 2005).

7. Toxicological limits

Considering the worldwide diffusion of PoPx in dietary supplements, it is important to determine if any toxicological effects can occur from chronic and sub-chronic consumption. PoPx is a rich source of phytochemicals that can produce toxic effects at higher consumption rates or from long-term administration (Vidal et al., 2003).

The paucity of literature hampers our clear understanding of the safe limits of pomegranate phytochemicals at NOEL. Oral and intraperitoneal administration PoPx is recommended, but LD varies in both cases. In one study the extract oral LD₅₀ in rats and mice was greater than 5 g/kg body weight, while in the same study the intraperitoneal LD₅₀ in rats and mice was reported to be 217 and 187 mg/kg body weight, respectively. In addition to acutely toxic doses, the NOEL for PoMx following sub-chronic administration (90 day) to Wistar rats was identified as 600 mg/kg/day (Patel et al., 2008). Similarly, no observed changes were reported for blood parameters and serum enzymes of humans orally administered 1420 mg/day of ellagitannin-enriched phenolic compounds for a 28-day period (Heber et al., 2007).

The first study dealing with the possible genotoxicity of pomegranate (Amorin, 1995) indicated no mutagenic effect in rats treated with an aqueous extract similar to those used in folk medicine. More recently, the genotoxicity of a hydroalcoholic pomegranate whole fruit extract was tested both in vitro and in vivo. The results indicated that to be toxic, doses greater than 70 mg/kg body weight were necessary (Sanchez-Lamar et al., 2008). The genotoxic effects reported by these researchers raised certain concerns over the safety of high intakes of PoMx in humans. The authors suggested further investigations be undertaken to determine the

extent to which the whole fruit extract or its components can be consumed without risk to human health.

8. Conclusions

Pomegranate fruit and its peel exhibit a high antioxidant potential. They have gained a wide acceptance for their pharmacological activities against serious maladies such as prostrate, colon and liver cancers, stomach ulcers, cardiovascular diseases and digestive disorders. The cytoprotective and inhibitory effects of this fruit and its peel demonstrate the potential to prevent some human carcinomas. As ethnopharmacological utilization of the fruit and peel extract is prevalent in a variety of cultures to cure common disorders without any consideration to its phytochemical profile and toxicological limit, safety verification and clinical trials are needed prior to its pharmacological exploitation by modern medicine. A more integrated approach is needed to use pomegranate fruit and its peel for the treatment of diarrheal disorders, especially in the developing countries with poorer hygienic practices and unsanitary conditions. Similarly, in vivo trials are needed to assess the potential of the fruit to treat fatal in vivo fungal proliferation. The prophylactic potential of the fruit and its peel against viral epidemics and pandemics, specifically influenza, may open up new avenues for research in the nutritional and medical science domains.

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