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Pomegranate Peel as a Potential Weapon against Oxidative Stress: A Mini Review

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Abstract

Radicals, such as Reactive Oxygen Species (ROS), are extremely reactive oxidants formed as natural by-products of normal processes within the human body. Their number upsurges when antioxidant levels are not enough and subsequently they react with DNA, lipids and proteins to cause damage. Phenolic compounds are most bountiful natural antioxidants in nature usually found in both edible and non-edible parts of plants, and have numerous biological effects including antioxidant, anti-microbial, anti-inflammatory, anti-viral, analgesic and antipyretic activities. Their isolation from plant sources has drawn increasing attention as a low-value source of antioxidants. Although the developments in analytical techniques have played a momentous role in detection of a wide range of phenolics yet success still relies on isolation method. Natural ingredients in food products are unceasingly gaining popularity everywhere and the use of plant extracts in cosmetic formulations are on rise. Clinical trials for dosage efficacy and drug designing like encapsulation of phenolics for effective delivery can be engaged in treating several calamitous diseases like atherosclerosis, coronary heart diseases, cancer, diabetes and neuronal diseases.

Keywords: Reactive oxygen species; Pomegranate; Antioxidant; Polyphenols; Oxidative stress

Causes and Consequences of Oxidative Stress

Reactive Oxygen Species (ROS) are ceaselessly generated by partial or fragmentary reduction of molecular oxygen which is an irresistible after-effect of aerobic metabolism. Apart from production by endogenous system, these are also generated by several foreign agents [1,2]. The most influential primary ROS are depicted in Figure 1. Fluctuating environmental conditions such as extremes of temperature, soil water availability, presence of salts, heavy metal exposure, UV radiation, intensity of light, aerobic exercise, excess ions, and air pollution may lead to loss of redox homeostasis due to elevated production and build-up of pro-oxidants and thus leading to troublesome oxidative damage in cells [3-8]. Additionally, some secondary oxidative products are also produced in cells (Figure 2).

Among all ROS, the most active species is hydroxyl radical ($OH^{\cdot}$), which is ordinarily produced from hydro-peroxides by Fenton reaction, employing some reducing agents and metallic ions having the tendency to wobble between different valencies (Cr, Mn, Fe, Ce). Its malicious effect takes place on a number of biological molecules including carbohydrates, unsaturated lipids, proteins, nucleic acids etc [9]. Balaban et al., [10] reported that 0.2-2% of oxygen expended in the cell is radiated as ROS. At higher concentrations, ROS are extremely harmful and can sooner or later lead to cellular damage and death. The boundless levels of ROS cause oxidative damage to nucleic acids, proteins and cofactors required for various enzyme activities [11-13]. On the other hand, at relatively low or moderate concentrations, they become the climactic component of a signalling pathway disturbing up-regulation of anti-oxidative pathway to safeguard tolerance against oxidative damage [14-17]. ROS participate in several physiological processes, for instance, cell growth, necrosis, gene expression, energy production, apoptosis, protease activities, phagocytosis, intercellular signalling and/or synthesis of biologically important compounds [18,19]. They play a pivotal role in the maintenance and amendment of cellular processes, conducting hormone concentration, chemical equilibrium and enzyme activation [20].

Attenuation of ROS

Oxidative stress is wide-spread in all organisms living under aerobic conditions. It is widely known to participate in the pathogenesis of numerous diseases (e.g. AIDS, cancer, Alzheimer's disease etc.) and gets extravagant during aging and exercise [21]. Consequently, many organisms

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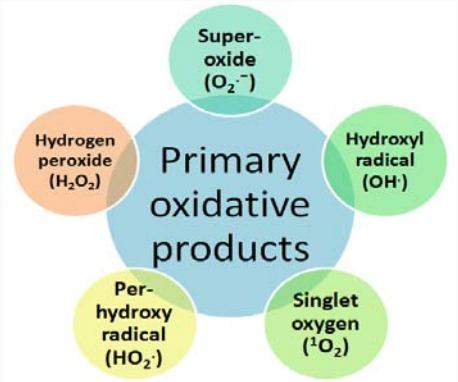

Figure 1: Examples of primary oxidative products.

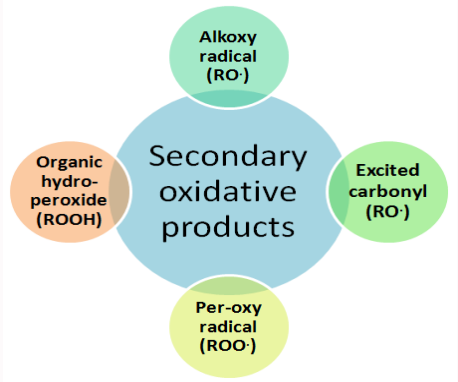

Figure 2: Examples of secondary oxidative products.

have derived diverse protection or repair mechanisms to counteract it. Cellular ROS are regulated by the networking of complex antioxidant machineries in living systems. Nature has bequeathed living systems with plentiful antioxidant molecules like redox enzymes (such as superoxide dismutase, catalase, peroxidase, peroxiredoxin etc.), chemical reducers (such as tocopherols, ascorbic acid etc.), and some repair systems (e.g. lipases, nucleic acid excision systems) to wipe out the oxidatively damaged components of complex molecules. Discrete redox enzymes use an array of reducing molecules such as NADH and NADPH to eradicate the ROS [22]. It is imperative for the cells to control ROS turn-over or tightly administer its endogenous titer so that it can evade oxidative injury and handle its signalling role efficiently. Living organisms possess thoroughly regulated systems to maintain scanty ROS levels i.e., their production and elimination are well balanced culminating in specified steady-state ROS level. Nonetheless, under specific circumstances this balance can be messed up. The reasons may include exalted level of endogenous and exogenous compounds causing autoxidation coupled with ROS production; deficiency of reserves of low molecular mass antioxidants; inactivation of antioxidant enzymes; reduction in production of antioxidant enzymes and low molecular mass antioxidants; and some combinations of two or more of the above mentioned factors.

Action of Antioxidants

Antioxidants support the organisms to tackle with oxidative stress. These substances have the potential to prevent the effects of very deleterious reactive free radicals such as ROS. Misra *et al.*, [23] proclaimed that antioxidants achieve it by interacting with these free radicals and extricate away free radical intermediates to terminate the chain reactions and also oxidize themselves to escape further oxidation reactions.

The human antioxidant system is divided into two major groups: enzymatic and non-enzymatic antioxidants. Former group is further sub-divided into primary and secondary enzymatic defenses. Primary defense is composed of three crucial enzymes (i) Glutathione Peroxidase (GPx) which donates two electrons to reduce peroxides by forming selenoles and also eliminates peroxides as potential substrate for the Fenton reaction (ii) Catalase (CAT) that converts hydrogen peroxide into water and molecular oxygen and has one of the biggest turnover rates known to man, allowing just one molecule of catalase to convert 6 billion molecules of hydrogen peroxide and finally (iii) Superoxide Dismutase (SOD) that converts superoxide anions into hydrogen peroxide as a substrate for catalase [24]. These enzymes, therefore, either prevent the formation of free radicals or neutralize them. Secondary enzymatic defence includes Glutathione Reductase (GR) and Glucose-6-Phosphate Dehydrogenase (G6PDH). GR reduces glutathione (antioxidant) from it's oxidized to reduced form, thus recycling it to continue neutralizing more free radicals. G6PDH regenerates NADPH (Nicotinamide Adenine Dinucleotide Phosphate - coenzyme used in anabolic reactions) thereby creating a reducing environment [25,26]. These two enzymes do not neutralize free radicals directly but have supporting roles to other endogenous antioxidants. However, under oxidative stress conditions, enzymatic antioxidants are not sufficient and exogenous non-enzymatic antioxidants (dietary antioxidants) are required to maintain optimum cellular functions.

Numerous non-enzymatic endogenous antioxidants like vitamins (A,C and E), enzyme cofactors (Q10), nitrogen compounds (e.g. uric acid), and peptides (e.g. glutathione) are present in the cells. Glutathione is a tripeptide and protects the cells against free radicals either by donating a hydrogen atom or an electron. It is also very crucial in the regeneration of other antioxidants like ascorbate [27]. Despite its remarkable efficiency, the endogenous antioxidant system does not satisfy and humans depend on various types of antioxidants present in the diet to maintain free radical concentrations at low levels [28].

Synthetic Antioxidants

Main examples of synthetic radical terminators include Butylated Hydroxyl Anisole (BHA), Butylated Hydroxyl Toluene (BHT), tertiary butyl hydroquinone and gallates (dodecyl gallate, propyl gallate and octylgallate) [29,30]. Most of the synthetic antioxidants are composed of radical terminators which discontinue free radical chains to avoid lipid oxidation. European Food Safety Authority (EFSA) established revised Acceptable Daily Intakes (ADIs) of 0.25 mg/kg bw/day for BHT and 1.0 mg/kg bw/day for BHA elucidated that the exposure of adults and children was unlikely to exceed these intakes [31,32]. Recent reports reveal that these compounds may be involved in a number of health risks including cancer and carcinogenesis. Pokorný [33] noted that Tertiary Butyl Hydroquinone (TBHQ), the most potent synthetic antioxidant, is not allowed for food application in Japan, Canada and Europe. Some of them get deposited in adipose tissue because of low solubility in water [30]. High doses of TBHQ exerted negative health effects such as DNA damage and stomach tumors in laboratory animals [34]. BHA has been reported to act as a tumor initiator and promoter in some animal tissues [35].

Natural Antioxidants: A Better and Safer Approach

Due to these safety concerns, there is a rising trend among food scientists to supplant these synthetic antioxidants with natural ones, which in general are surmised to be safer [33]. The ongoing change in life-style promotes the search for more natural antioxidants with higher therapeutic potential which can replace synthetic drugs with unwanted side-effects [36]. Considerable naturally occurring sources of antioxidants are fruits [37], vegetables and whole grains [38]. Antioxidant activity is a pivotal parameter used to characterize various plant materials. This activity is analogous with compounds capable of safe-guarding a biological system contradictory to the potential adverse effects of oxidative processes. Antioxidants have received elevated attention in the last years from nutritionists and medical researchers for their budding activities in the prevention of several deteriorating diseases such as cancer and cardiovascular disorder, as well as for treatment of lung cancer, esophagus, cardiovascular disorders and breast cancer [39-42].

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is the most ordinarily used substrate for rapid evaluation of antioxidant activity due to its stability in the radical form and ease of assay [43]. This assay is popular for providing decisive information regarding the antioxidant ability of tested compounds [44]. Substances which are capable of performing this reaction can be considered as antioxidants and therefore, are radical scavengers [45]. ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay is known to be a hasty method for estimation of antioxidant activity and could be used as a tool to screen samples and cultivars in order to obtain high content of natural antioxidants in foods [46].

Polyphenolics as Biological Antioxidants

All natural antioxidants differ with respect to their composition, physical and chemical characteristics, mechanisms and sites of actions and molecular weights [47]. They can vary from simple molecules (phenolic acids, flavonoids, phenylpropanoids) to highly polymerised compounds (lignins, tannins, melanins) [48]. Polyphenolic secondary metabolites have >8000 phenolic structures and are most copious and broadly dispersed group of substances in plant kingdom [49]. Phenolics may act as phytoalexins, anti-feedants, and contributors to plant pigmentation, antioxidants and protective agents against ultraviolet light [50]. They are also important for ensuring the construction of lignin and regulating plant growth and disease resistance. Molecular studies have revealed that phenolics can exert modulatory actions in cells by interacting with a wide spectrum of molecular targets central to the cell signalling machinery. These include activation of Mitogen-Activated Protein Kinase (MAPK), Protein Kinase C (PKC), phase II antioxidant detoxifying enzymes, serine/threonine protein kinase Akt/PKB, down-regulation of pro-inflammatory enzymes (COX-2 and iNOS) through the activation of Peroxisome Proliferator-Activated Receptor gamma (PPARγ), tyrosine kinases, NF-κB, c-JUN, regulation of calcium homeostasis, inhibition of phosphoinositide 3-kinase (PI 3-kinase), as well as modulation of several cell survival/cell-cycle genes [51]. Plant phenolics are usually involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contribute to colours. Polyphenols also significantly affect many food properties including bitterness, astringency, colour, flavor, odor and oxidative stability.

Phenolic compounds provide amusing health benefits such as antioxidant, anti-bacterial, immune-modulatory, wound healing, anti-viral, anti-inflammatory, anti-allergic, anti-thrombotic, hepatoprotective, anti-carcinogenic and vasodilatory actions [52,53]. Phenolic acids, flavonoids and hydrolysable tannins behave as protective agents of DNA in response to free radicals. These compounds inactivate carcinogens, bind to the enzymes that stimulate pro-carcinogen and also activate the enzymes responsible for detoxification of xenobiotics [54].

Soobrattee *et al.*, [55] reported that many of these biological functions have been associated with their free radical scavenging and antioxidant activity. Phenolic compounds achieve ideal structural chemistry for free radical scavenging action because they have phenolic hydroxyl groups that are amendable to donate a hydrogen atom or an electron to a free radical; drawn-out conjugated aromatic system for delocalization of an unpaired electron [56]. Thus, in addition to their protective effect by selectively inhibiting or stimulating key protein in the cell signalling cascades, they possess tremendous antioxidant capacity. Extracts from rosemary, grape seed, pomegranate by-products, oregano and various other spices have been evaluated for their antioxidant capacity in meat and poultry products [57]. Mattiello *et al.*, [58] showed the efficacy of polyphenolics of pomegranate to reduce the cardiovascular coincidences in high-risk patients. Extraction of polyphenols from plant sources has drawn increasing attention as a low-value source of antioxidants [59]. This process is influenced by several factors such as type and concentration of solvent, extraction time, extraction temperature, sample-to-solvent ratio, rate of agitation, pH etc. [46-60]. Research performed by Ilaiyaraja *et al.*, [61] proclaimed that phenolic compounds are usually more soluble in polar solvents. It has also been reported that aqueous mixtures of solvents give better extraction yield as compared with pure solvents [62].

Pomegranate as a Source of Polyphenolics

Fruits, vegetables and their waste products are compelling sources of bioactive compounds such as polyphenolics, flavonoids, alkaloids, antibiotics, natural colourants and plant growth components [63]. Encompassed by other fruits, pomegranate (*Punica granatum* L.) is zenithal in bioactive phytochemicals embodying total polyphenols and flavonoids [64]. It is a fruit-bearing deciduous tree in Lythraceae (formerly in Punicaceae) family and popularly known as Chinese apple, Apple with many seeds or Apple of Carthage. Currently, it is cultivated in Africa, South and Central Asia, South Caucasus, North and South America and in the Mediterranean region. It is largely used in Indian sub-continent as a conventional and folk medicine [65]. The fruit is a spherical berry with a thick reddish husk which comprises ~50% of the total fruit weight and is often discarded as a waste [65,66]. The innermost component of the husk is a white, thin-walled mesocarp that forms enclosures which consists of edible arils with seeds inside it. The arils are purple or deep red in colour because of high content of polyphenols, majority of them being the anthocyanins [67]. Very high level of phenolic content has been found in juice and peels of pomegranate fruits grown in hot desert climate as compared to those grown in Mediterranean climate [68]. Many studies have shown an ample variety of phytochemicals in distinctive extracts of Pomegranate Peels (POP) [69-71].

In the last decade, pomegranate processing industry has undeniably expanded due to the acceptance and great demand of pomegranate juice which imparts bountiful health benefits [72,73].

Table 1: Total phenolic contents in various parts of pomegranate.

Sr. No.	Part of pomegranate	TPC (mg/g)	Reference
1.	Peel	128.4	[95]
2.	Peel	139.4	[96]
3.	Peel	249.4	[69]
4.	Pulp	17.9	[95]
5.	Seed	24.4	[69]

Pomegranate has been endorsed as a medicinal food of great attention for curative purposes, relieving ailments such as colic, colitis-diarrhea, leucorrhea, dysentery, paralysis, and headache [74,75]. Its juice shows the crowning antioxidant capacity among other frequently consumed polyphenol-rich beverages and fruit juices, emcompassing red wine, green tea and orange, grapefruit, grape or cranberry juice [76,77]. Production of every ton of concentrated pomegranate juice produces 5.0-5.5 tons of waste pomegranate peels which is a major environmental distress for the pomegranate processing industry [72]. The peel is customarily used for the treatment of ulcer and inflammation, and it has established antioxidant and anti-bacterial activities [78-80]. Pomegranate peel contains disparate groups of polyphenols with sturdy antioxidant activities [81]. Other parts including pulp and seeds of the fruit also possess comparatively lower amount of Total Phenolic Compounds (TPC) as depicted in Table 1.

The peel is a great source of bioactive compounds such as anthocyanins, gallagyl esters, catechin, epicatechin, hydroxycinnamic acids, ellagitannins, gallotannins, rutin,hydroxybenzoic acids, dihydroflavonol and many others. Such bioactive compounds are answerable for many biological activities such as anti-mutagenic, apoptotic, antimicrobial, anti-inflamatory etc [82-88]. As a result of high levels of phenolic acids, flavonoids and other polyphenolic compounds, pomegranate peel can be used as a proficient scavenger of several reactive oxygen species [89]. Remarkably high polyphenolic contents in POP make it a significant source of natural antioxidants to be used as food additive. For instance, sunflower oil fortified with POP extracts presented delayed oxidation processes [90]. While massive data exists in favor of use of polyphenols from green tea, rosemary, berry fruits, thyme, sage, and other herbs as natural antioxidants [91,92], interest in the antioxidant properties of polyphenols from pomegranate has recently developed.

Malviya *et al.*, [81] investigated the antioxidant potential of POP of Ganesh variety using water, methanol, ethanol and their mixtures, and proclaimed that highest DPPH and ABTS scavenging activity was detected when methanol or a mixture with water (70% ethanol: 30% water) were used as the extracting solvents. Effects of ethanol concentration, solid to solvent ratio, extraction time and temperature on ultrasound assisted extraction of pomegranate peel were interpreted by Živković *et al.*, [93] using response surface methodology. A multi-response optimization study based on central composite design granted the forecasting of optimal conditions for higher rate of extraction of total polyphenolics, gallic acid, ellagic acid, punicalin and punicalagin from POP. Extraction time of 25 min, solid to solvent ratio of 1:44, ethanol concentration of 59% and extraction temperature of 80°C were affirmed optimal in the case of the POP phenolics [93]. Heber *et al.*, [94] reported the safety of pomegranate ellagitannin-enriched polyphenol capsules in normal and obese individuals in doses up to 1420 mg per day for four weeks [95,96].

Conclusion

In this mini review, we have collected data on the oxidative stress caused by various reactive oxygen species and its alleviation by natural compounds. Presence of polyphenolics might be responsible for substantial antioxidant potential of pomegranate peel. The summarized information could be useful to pomegranate industry in identifying and developing cultivars having commercial value. Pomegranate peel is an agro-industry waste and its use as food additive or nutraceutical *via* industrial process may be economical and reduce substantial amount of bio-waste.

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Author's Contribution

Sonam Sihag collected the literature, investigated the references, and drafted the original manuscript. Ajay Pal conceptualized the theme, supervised and edited the manuscript.

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