Topical Review Article

A Review Study on *Punica granatum* L

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Abstract

Punica granatum L (pomegranate) is a deciduous shrub, native to Iran. Nowadays, besides its use as a fruit, its medicinal properties have attracted the interest of researchers of many countries. Pomegranate fruit has medicinal properties such as anti-inflammatory and antibacterial activities. The pomegranate seed oil has inhibitory effect on skin and breast cancers. The pomegranate seed oil has phytoestrogenic compounds and the fruit is rich in phenolic compounds with strong antioxidant activity. Ellagic acid is one of the main components of pomegranate with phenolic structure and antioxidant activity. This review article presents the recently published findings on different aspects of this plant focusing on its medicinal properties

Keywords

pomegranate, antioxidants, free radicals, ellagic acid, breast cancer

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Introduction

Punica granatum L (pomegranate) is a deciduous shrub, native to Iran. Pomegranate has extensively been used as a source of traditional medicine.[1,2] Pomegranate fruit has medicinal properties such as anti-inflammatory and antibacterial activities. The pomegranate seed oil has inhibitory effect on skin and breast cancers. The pomegranate seed oil has phytoestrogenic compounds and the fruit is rich in phenolic compounds with strong antioxidant activity. The fruit and bark of pomegranate are used against intestinal parasites, dysentery, and diarrhea.[1] The juice and seeds are considered a tonic for throat and heart. It is used to stop nose and gum bleeds and treating hemorrhoids.[3]

Today, *Punica granatum* L. as a fruit not only attracts a lot of public interest but research is also focused on its medicinal properties and food industry.[3] So a wide range of research studies have already been launched in this field. However, there are not enough studies performed on the medicinal properties of pomegranate. This review article presents the recently published findings on different aspects of this plant, with a focus on its medicinal properties.

Geographical Origin and Distribution of Pomegranate

Pomegranate (*Punica granatum* L.) is one of the first domesticated fruits that has been cultivated from past times. It is indigenous to Iran and neighboring countries that gradually developed in central Asia regions to Himalaya, Eyalet of Anatolia, Middle East, and Mediterranean area. It also thrive in Arizona and California, and has been cultivated in the Mediterranean region, South Asia, and the Middle East countries; Kandahar in Afghanistan is famous for its high-quality pomegranate. Today, pomegranate is cultivated in most regions of the world, including Iran, Spain, Italy, Afghanistan, America, India, China, Russia, Uzbekistan, Morocco, and Greece.[4] Iran is one of the biggest producers of pomegranate in the world. In Iran, Markazi, Yazd, Fars, Khorasan, and Kerman provinces have the highest production rates.[1]

Morphology and Echophysiology of Pomegranate

Pomegranate is a shrub that reaches to 1.5 to 5 m in height, with more or less irregular and thorny branches and glossy leaves that appears as a deciduous shrub in temperate regions and as evergreen in frigid regions. *Punica granatum* L belongs to the Punicaceae family and is the smallest plant family that includes 1 genus and 2 species, including the following: *Punica granatum* (edible pomegranate) is indigenous to Iran and Mediterranean regions, and *Punica protopunica* (inedible) is endogenous to Socotra islands in Pacific Ocean. The other characteristics are:

Leaves: Leaves are seen as reciprocal in newly grown branches and as integrated in spores.

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Flowers: 1-5 flowers, one of them terminal and the rest marginal, short or without peduncle, their color is red and rarely yellow or white, odorless, and two-sex.

Fruit: Balausta in light red color to greenish yellow and rarely in some species dark purple. It is 5 to 20 cm in diameter and its weight varies from less than 200 g to more than 800 g.

Seed: Seeds are produced in high amounts, are triangular, albumin free, and embedded in aril.[5]

Pomegranate is one of the well-known fruits having long-term history in Iran and Middle East. This plant is mostly easy to grow in desert margins with hot dry summer and scorching heat of the sun and rather cold winter having saline water and soil. This wide compatibility range is considered as favorable ecophysiological condition for *Punica granatum* L, and so it is called Kavir ruby. Pomegranate grows in a wide range of climatic condition and can adapt itself to different types of soils. However, this plant is sensitive to soils that have low drainage and its growth in this condition is low and product quality is decreased. The best soil condition to cultivate pomegranate is deep sandy clay soils and the most growth, performance and quality of product is in regions with hot and long summers. This product can be grown up to an altitude of about 1600 m above sea level. Some species of this product are well grown in low heights and some other in higher heights. One of the most important limitations of cultivating pomegranate is its sensitivity to coldness. Pomegranate is damaged in temperatures lower than 12°C and so sweet pomegranates are more sensitive than sour ones. In this plant, temperature below 7°C is needed and fruit needs long and hot summers to ripen.[4,5]

The Metabolites of *Punica granatum* L

The metabolites in various parts of the *Punica granatum* L fruit and tree include various kinds of sugars, organic acids, polyphenols, flavonoids, anthocyanins, fatty acids, alkaloids, vitamins, and so on. The main sugars included in *Punica granatum* L extract are composed of glucose, fructose, sucrose, and maltose, while the vitamins are C, B_1, B_2, and beta-carotene. Additionally, malic acid, fumaric acid, oxalic acid, succinic acid, citric acid, and tartaric acid are among the major organic acids in *Punica granatum* L. Alkaloids found in pomegranate peel include ellagic acid, gallic acid, chlorogenic acid, cinnamic acid, hydroxy protocatechuic acid, hydroxy benzoic acid, caffeic acid, ferulic acid, coumaric acid, *p*-coumaric acid, and *o*-coumaric acid, pelletierine, isopelletierine, methylpelletierine, pseudopelletierine, punicalagin, punicalin, phloridzin, quercetin, and catchin.[6,7] Furthermore, the flavonoids of *Punica granatum* L are luteolin, kaempferol, and narigenin found in glycoside forms.[7,8] The color of pomegranate is induced by its compounds, specially anthocyanin. Anthocyanin is a glycoside releasing a glucose molecule and a glycone ring (anthocyanidin).[9] Six anthocyanins are responsible for red color of edible parts of *Punica granatum* L derived from pelargonidin (orange and red colors), cyanidins (red and deep red colors), and delphinidins (blue and purple colors); these are 3,5-diglucoside delphinidin, 3-glycoside delphinidin, 3,5-diglucoside cyanidin, 3-glycoside cyanidin, 3,5-diglucoside pelargonidin, and 3-glycoside pelargonidin.[9,10]

As long as *Punica granatum* L is matured, the color of the fruit is gradually changed; however, this transformation in color is very slow and increases after the middle stage of maturation. In the earlier stages of development, the amount of diglucoside anthocyanins seem to be higher than the monoglucoside forms; however, the trend changes inversely at the end of the development and ripening stages.[9]

Also the proportion and type of the anthocyanins varies depend on different cultivars.[11] Antioxidant activity of pomegranate fruit can be ascribed to the presence of some components like ascorbic acid and phenolic compounds, including punicalagin, punicalin, gallic acid, ellagic acid, and anthocyanins. These compounds are affected by fruit development as the highest antioxidant activity was observed in newly formed fruits (20 days).[6,7] As the fruit grows, the antioxidant activity reduces due to the decline in the level of ascorbic and phenolic acids.[12] Besides, antioxidant activity affected by phenolic compounds and ascorbic acid varies among different varieties of pomegranate plant (Sarkhosh A, Zamani Z, Fatahi R, unpublished data). The oil derived from pomegranate seeds has recently received more attention to be used in industrial process and also in providing necessary fatty acids.[13] Saturated and unsaturated fatty acids account for the main proportions of fatty acids composites. The ratios of fatty acids vary between 63 and 272 g/ kg dry weight of seed among different varieties.[13] In the most studied cultivars of pomegranate the predominant fatty acids are linolenic (74% to 88%) and linoleic acid (5% to 16%).[14] Furthermore, some fatty acids, including oleic acid, palmitic acid, stearic acid, palmitoleic acid, arachidonic acid, lauric acid, and caprylic acid are identified in different varieties of *Punica granatum* L. Studies have shown that unsaturated fatty acids are major components of lipids in sweet cultivars of pomegranate fruit.[13] Pomegranate seeds oil include steroidal estrogens (γ-tocopherol, 17-α-estradiol, stigmasterol, β-estriol sitosterol, and testosterone) nonsteroidal compounds (compestrol, coumestrol).[15]

Application of Pomegranate in Traditional Medicine

Flowers, leaves, bark of young shoots and roots, fruit peel, and pomegranate sauce have been traditionally used.[16] All components of *Punica granatum* L fruit with abundant tannins show relatively strong astringent effects. Several infusions or decoctions of the plant flowers have been used in traditional medicine to treat simple diarrhea, vaginal discharge, and also this extract accompanied with pomegranate peel have usually been gurgled to relieve pancreas inflammation of the pacreas.[17] Refreshing juice of *Punica granatum* L fruit is recommended to heal gallbladder diseases. The fruit contains strong tannin considered as bitter nutrition. Its decoction appears to be helpful for treating diseases such as ordinary diarrhea, dysentery,

and stomach disorders.[17] Tannin content of pomegranate seed, however, is not remarkable and it is usually used to treat women vaginal discharge and wound healing.[18] Fresh or dried root barks or ethanol extracts of pomegranate are used to remove intestinal parasites due to the alkaloid substances. It is also used in traditional medicine because of the antibacterial and anti-inflammatory properties.[17,18]

Pharmacological Properties of Pomegranate

Fermented juice of pomegranate fruit has been found to have antioxidant activity. Extracted juice of pomegranate flowers can reduce blood sugar and lipids.[19] Flavonoids and tannins of pomegranate juice can prevent the growth of cancer cells.[20] Flavonoids observed in the watery extract and fruit peel have shown estrogenic activity.[21] In addition, luteolin and naringenin have indicated an activity similar to the hormone usually secreted prior to pregnancy in women. Polyphenols of fermented extract of pomegranate fruit potentially appear to have antioxidant activity and pericarp tannins may increases antioxidant potential of fruit extract.[22] The stronger activities in polyphenols of fermented extract than nonfermented extract is likely due to the breakdown of flavonoids sugar complexes during fermentation that the final products will contain high concentrations of free polyphenols (with high biological activity).[20] It has been proven that flavonoids contained in peels are found in glycoside forms.[20] They have no estrogenic activity in glycoside forms while they behave inversely when they are released and hydrolyzed.[23] The estrogenic effect of pericarp polyphenols and pomegranate fermented juice is possibly referred to their binding to the estrogen receptors like estrogenic flavonoids, including kaempferol, quercetin, naringenin, luteolin, caumestrol, and weak 17-α-estradiol estrogen so that it prohibits estrogenic activity of 17-β-estradiol. Watery parts of pomegranate can inhibit estrogen-dependent and estrogen-independent breast cancer cells. However, this inhibitory effect has been reported to be doubled for estrogen-dependent case.[23,24] Ellagic acid and gallic acid are among the constituents observed in pomegranate peels, and the former is a dimeric derivative of gallic acid and is found mostly in higher plants, such as fruits and nuts.[25] Ellagic acid shows antimutant, antiviral, antioxidant, and skin-bleaching activity and has already been added to food as an antioxidant in Japan.[26] Antioxidant capacity of extracts derived from pomegranate peel in producing phospholipid complex has been measured. The base of method is established on the recovery of molybdenum(VI) to molybdenum(V) using antioxidant compounds and creation of green molybdenum(V) compounds with maximum absorption at 695 nm.[27] Antioxidant capacity of extracts from pomegranate peels is due to the presence of the phenols such as ellagic tannins, ellagic acid, and gallic acid.[20,28] Antimutagenic and anticarcinogenic properties of the extracts were examined against the azide sodium by the Ames test. The experiment showed that juice extract of pomegranate peel can inhibit mutation and cancer using azide sodium in 2 species of salmonella.[29] The results of the experiment showed that juice has the lowest antioxidant activity and the highest antimutagenic activity while methanol extract acts in the opposite way.

These abilities can be ascribed to the presence of phenols and also their capacities in regulating the free radicals.[29-32] The seeds and thin whitish membrane in pomegranate constitutes 13% of the fruit. These 2 parts play a key role in diagnosis of the disease, health of edible part of the fruit, especially in the prevention of the fruit discoloration and browning. Such tissues should be an enriched resource of active biological substances. More recent studies have shown that the aforementioned parts of the pomegranate fruit are enriched with a strong antioxidant called punicalagin that controls superoxide and free radicals of DPPH (1,1-diphenyl-2-picrylhydrazyl).[22,33] The best extraction and separation way is done by methanol. Punicalagin is capable to regulate the activity of superoxide and DPPH radicals.[34] Moreover, it can inhibit lipid peroxidation due to the presence of hydroxyl groups in their structures and peroxidation of the chain termination (by removing peroxide radicals).[24,34] The antioxidant activity of different parts of fruit (peel, flesh, and seed) has been examined. This approach is based on the reduction of a colored form of trivalent iron compound called ferric-tripyridyltriazin in the presence of divalent iron antioxidants. This method is known as FRAP (ferric reducing antioxidant power assay). In accordance with this method, FRAP value of peel, flesh, and seed were 82.11, 3.1, and 0.72, respectively.[34]

In an additional research, the blood plasma derived from person who had taken pomegranate juice containing ellagic acid (25 mg) and ellagitannins (318 mg, especially punicalagin) was analyzed.[35] The main purpose of the earlier study was to assess the amount and time duration of ellagic acid bioavailability in plasma following consumption. According to the results, the highest ratio of ellagic acid in the blood plasma was measured at 0.5, 1, 2, 3, 4, 5, and 6 hours after taking pomegranate juice. The highest and lowest ratios were observed at 1 hour and 4, 5, and 6 hours after consumption, respectively.

The presence of the free ellagic acid in the blood plasma is induced by its breakdown under biologic pH of stomach. Thus, it can be used as a biologic marker in bioavailability studies confirming the consumption of ellagic acid from food resources.[25]

Polyphenols of oil prohibit the activity of eicosanoid and cyclooxygenase enzymes. 18C trans fatty acids known as conjugated linoleic acid, structurally related to punicic acid, possess cancer arresting properties.[13]

Protective chemical effects of pomegranate seed oil were investigated for possible skin cancer development. Skin cancer is among the most prevalent types of cancer in the United States. About 1 000 000 cases of skin cancer and consequently 9000 deaths were reported in 2002.[36] Increased incidence of this type of cancer is mainly due to the permanent exposure of skin to the environmental cancer-causing agents, especially chemicals and ultraviolet radiation.[36] The previous experiments in order to prevention from skin cancer have proven the functionality and application of natural products extracted from onion and garlic oil. Skin cancer induced by chemicals and

ultraviolet ray include 3 steps: (1) initiation, (2) increment, and (3) promotion. Initiation step can be stimulated by application of a skin carcinogen, that is, 7,12-dimethyl benzanthracene (DMBA) in vivo (this reaction is necessarily irreversible); however, it is noteworthy that applying such a substance for one time cannot produce considerable tumors and it is only possible through permanent application of a tumor-promoting agents like 12-*O*-tetradecanoylphyl phorbol 13-acetate (TPA). Such tumor-promoting can provoke an enzyme known as ornithinedecarboxylase (ODC) with a limited activity scope in polyamine synthesis and also an important molecular agent to chemical prevention from skin cancer.[36] Pomegranate seed oil consists of 80% conjugated fatty acids, particularly punic acid and octadecatri enoic acid.[13,37] Punic acid acts as an inhibitor of prostaglandin biosynthesis as well as a cytotoxin for cancer w2a cells; such activity is possibly due to the inhibitory effect against fat peroxidation. Punic acid of pomegranate seed oil inhibits prostaglandin biosynthesis (promote ornithine decarboxylase enzyme activity at lower concentration).[23] Also this oil can prevent DMBA- and TPA-induced skin cancer.[37,38]

Inhibitory activities of prostaglandin as well as antioxidant activity of polyphenols extracted from both pomegranate seed oil and its fermented extract have widely been reported for prevention from human breast cancer.[36,39] Inhibitory impact of the watery and oily parts of the fruit has been reported on breast cancer cells in vivo. Such parts prohibit the activity of enzymes responsible for active estrogen biosynthesis (17-β-estradiol). Since the watery and oily parts of the fruit are chemically different, they probably act via different mechanisms in the prevention of cancer.[34,38,39] Pomegranate seed oil is considered as biosynthesis inhibitor E2 (17-β-estradiol) catalyzed by 17-β-hydroxysteroid enzyme. It also prevents invasion of cancer cells and also can strengthen and encourage apoptosis.[13] Extracted polyphenols of pomegranate seed oil can potentially prevent cyclooxygenase activity; however, these activities were not observed in the polyphenols of fermented extract.[20,40] Inactivation of the mentioned enzyme prevents the proliferation of breast cancer cells, its severity, and also the transformation of mammals' alveoli tissue (milk-producing units) to cancer mode.[40]

Clinical Applications of Pomegranate

Pomegranates are biologically unique and potent source of many of the body's physiological factors having significant effects on human health.[41] Pomegranate fruit has been recommended as a pharmaceutical and food ingredient in treatment of acquired immune deficiency syndrome (HIV/AIDS) due to the enrichment of divers bioflavonoids, inhibition of free radicals, as well as lipooxygenases inhibition (the enzymes that transform arachidonic acid to leukotrienes).[41]

The pomegranate peels have traditionally used to treat ordinary diarrhea and dysentery. The focus of future studies is concentrated on producing natural antidiarrhea remedy from pomegranate peel for over-the-counter or prescription-based medications.[42] Pomegranate is identified as an antiparasitic fruit for both humans and livestock.[42] Flavonoids contained in the fruit not only have powerful antioxidant activity but they also have inhibitory effects on enzymes since the juice and oil behave as potential nutritional supplement in enhancing longevity as well as preventing from heart disease and cancer.[37]

The extracted oil of pomegranate can effectively prohibit the production of prostaglandin or leukotriene through inhibition of cycloxygenase and lipoxygenase eicosanoid enzymes so that it may increase the application of oil or its derivatives as internal or external anti-inflammatory substances.[37]

The recent focus on the use of phytoestrogenic compounds in medicine for the prevention and treatment of menopause, osteoporosis, and cardiovascular diseases induced by reduced estrogen and cancer may possibly increase the prospect of using pomegranate seed oil and juice for postmenopausal women to be replaced by internal and external phytoestrogen as an alternative to hormone replacement therapy (HRT).

Discussion

Pomegranate possesses a wide range of compounds, including polyphenols, alkaloids, and vitamins with potent free radical scavenging properties.[43] Free radicals can cause oxidative stress, which may induce damage to biomolecules, leading to many chronic diseases, such as cancer,[44,45] diabetes,[46,47] atherosclerosis,[48,49] Alzheimer's disease,[50,51] nephrotoxicity,[52,53] hepatotoxicity,[54,55] pain,[56,57] and other degenerative diseases. Antioxidant activity of pomegranate fruit has been attributed to the presence of some components like ascorbic acid and phenolic compounds, including punicalagin, punicalin, gallic acid, ellagic acid, and anthocyanins.[2] Pomegranate tannins, especially ellagitannins are also the most abundant polyphenols that have shown potent free radical scavenging properties. Punicalagins and ellagitannins are converted to urolithins by bacteria in the intestine, and these to have antioxidant activity.[37] The red color of pomegranate juice has been attributed to anthocyanins, such as cyanidin, pelargonidin glycosides, and delphinidin, which have potent antioxidant activity.[37]

Preclinical and clinical researches have revealed that plants antioxidants are effective in prevention and treatment of free radical–induced complications such as low-density lipoprotein oxidation,[58,59] heart disease,[60,61] diabetes,[62,63] cancer,[64,65] cognition problem,[66,67] and infectious diseases.[68-70] Therefore, the medicinal properties of pomegranate, at least in part, can be attributed to its components with antioxidant activities. If it is true, other medicinal plants with antioxidant activity[71-89] might have the same properties.

These compounds with antioxidant activity are affected by fruit development. The highest antioxidant activity has been observed in newly formed fruits (20 days).[6,7] As the fruit grows, the antioxidant activity reduces because of the decline in the level of ascorbic and phenolic acids.[12] Besides, the antioxidant activity varies among different varieties of pomegranate plant (Sarkhosh A, Zamani Z, Fatahi R, unpublished data). Therefore, different varieties of pomegranate may not have the same medicinal property and they are better consumed fresh, while in the early stages of development.

Other components of pomegranate should be analyzed and evaluated to realize their role in the treatment of diseases.

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Author Contributions

All the authors contribute equally toward writing the first draft of the manuscript. MRK revised and edited the final version.

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