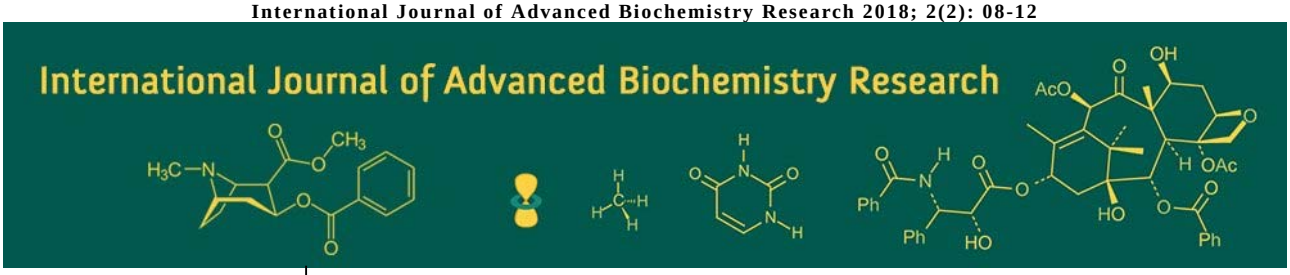

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Chemical investigation of Pomegranates and Its Health Benefits

Amit Parashar and Shailendra Badal

Abstract
In this paper, the chemical investigation and medicinal properties of *Punica granatum* L. (Punicaceae) have been noticed. In the past years, studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been discussed. Here we focus on treatment and prevention of different disease such as cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance etc. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis and obesity.

Keywords: *Punica granatum* L., pomegranate, chemical composition, medicinal treatments

Introduction
The pomegranate, Punica granatum L., belongs to the family Punicaceae which includes only one genus and two species, the other one, little-known, being P. protopunica Balf. Peculiar to the island of Socotra. It is a sacred fruit conferring powers of fertility, abundance, and good luck. It also features significantly in the ceremonies, art, and mythology of the Egyptians and Greeks and was the personal emblem of the Holy Roman Emperor, Maximilian. Pomegranate is the symbol and heraldic device of the ancient city of Granada in Spain – from which the city gets its name. (Parashar *et al*., 2009) [45],

The genus name, *Punica*, was the Roman name for Carthage, where the best pomegranates were known to grow. Pomegranate is known by the French as grenade, the Spanish as granada, and literally translates to seeded ("granatus") apple ("pomum") (Abdurazakova *et al*., 1968) [1].

The pomegranate tree typically grows 12 to16 feet, has many spiny branches, and can be extremely long lived, as evidenced by trees at Versailles, France, known to be over 20 years old.

The leaves are glossy and lance-shaped, and the bark of the tree turns gray as the tree ages. The flowers are large, red, white, or variegated and have a tubular calyx that eventually becomes the fruit. The ripe pomegranate fruit can be up to five inches wide with a deep red, leathery skin, is grenade-shaped, and crowned by the pointed calyx. The fruit contains many seeds (arils) separated by white, membranous pericarp, and each is surrounded by small amounts of tart, red juice.

The pomegranate is native from the Himalayas in northern India to Iran but has been cultivated and naturalized since ancient times over the entire Mediterranean region. It is also found in India and more arid regions of Southeast Asia, the East Indies, and tropical Africa. The tree is also cultivated for its fruit in the drier regions of California and Arizona (Albrecht *et al*., 2004, Parashar *et al*., 2015) [2].

In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. In Ayurvedic medicine the pomegranate is considered "a pharmacy drug itself" and is used as an antiparasitic agent, (Aviram and Dornfeld, 2001) a "blood tonic", (Batra *et al*., 1968., Parashar *et al*., 2015) [4] and to heal aphthae, diarrhea, and ulcers (Batta and Rangaswami, 1973) [5].

Pomegranate also serves as a remedy for diabetes in the Unani system of medicine practiced in the Middle East and India (Baytop, 1963) [6].

The current detonation of interest in pomegranate as a medicinal and nutritional product is evidenced by a search from 2000 to present, revealing over 130 new scientific papers pertaining to its health effects. Between 1950 and 1999 only 20 such publications were available. (Borir, 1980) [7].

The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and protection from ultraviolet (UV) radiation. Other potential applications include infant brain ischemia, Alzheimer's disease, male infertility, arthritis, and obesity. The following abbreviations for various pomegranate extracts will be used throughout the article:

1. Pomegranate juice – PJ
2. Pomegranate by-Extract – PBE
3. Fermented pomegranate juice – FPJ
4. Cold-pressed seed oil – CPSO
5. Pomegranate peel extract – PPE
6. Pomegranate pulp juice – PPJ
7. Pomegranate fruit extract – PFE
8. Pomegranate buds extract – PBE
9. Hydroalcoholic crux of pomegranate – HACP
10. Gel-based pomegranate extract – GPBE

Alkaloid

It was indicated that alkaloid was present at the rate of 0.45 to 0.70% in the body rinds, and over 5% in the roots; but none was found in the fruit rinds (Brieskorn and Keskin, 1954; Caceres *et al*., 1987 Parashar *et al*., 2012) [8, 9]. It was also indicated that pseudopelletierine, pelletierine, isopelletierine, methyl pelletierine 1-pelletierine, dl-pelletierine and methyl isopelletierine were found in composition of the root, body and branch rinds of *P. granatum*. (Chidambara *et al*., 2002; Dean *et al*., 1971) [10, 11].

It was detected that saturated alkaloids present in the root and body rinds are not present in the leaves, whereas 2-(2-propenyl)-piperidine of unsaturated alkaloids was present in the leaf extract (Du *et al*., 1975, Parashar *et al*., 2018) [12, 34].

Tannin and similar compounds

It was stated that punicacortein A, B, C, D in the structure of hydrolysable C-glycoside, which is a new ellagitannin, as well as punigluconin which contains one gluconic acid; and also casuariline and casuarine were present in the fresh body roots of *P. granatum* (Drillien and Viel, 1963; Fayez *et al*., 1963) [13, 14]. Punicafolin as well well four ellagitannins and two gallotannins were isolated from the leaves (Parashar *et al*., 2013) [38], These were indicated to be granatin A and B, strictinin, corilagin, 1,2,4,6-tetra-O-galloyl D-glucose with 1, 2, 3, 4, 6-penta-O-galloyl D-glucose (Feldman and Markh, 1970) [15]. *Pericarpium Granati* on the other hand contains granatin A and B with punicalin and punicalagin (Gabbasova and Abdurazokova, 1968; Gil *et al*., 2000) [16, 17].

Anthocyanosides

Anthocyanosides are present in the fruit and flower sections of the plant. In comparison of antho cyanoside content of partly purified fruit rind extract and pomegranate seeds; it is stated that pelargonidin-2-glucoside and pelargonidin-2, 4-diglucoside found in high amounts in the rinds are present in less amounts in the seeds. Cyanidin-2-glucoside and cyanidin-2, 4-diglucoside were detected in both seeds and fruit rinds.

On the other hand, it was not possible to detect in the fruit rinds delfinidin-2, 4-diglucoside and delfinidin-2-glucoside, the major anthocyan in pomegranate juice (Guo *et al*., 2008; Hartwell, 1971) [18]. Flowers contain pelargonidin-2, 4-diglucoside (Hartwell, 1971). It is additionally stated that the amount of anthocyan varies by altitude of the location where the plant grows; and diminishes and disintegrates by keeping it waiting (Guo *et al*., 2008, Parashar *et al*., 2014) [18, 37].

Flavonoids

Flavonoids which display vitamin P activity are present in *P. granatum*. It is indicated that the fruits contain compounds in structure of flavonoid, quercetol in particular (Heftman *et al*., 1966).

Triterpenic acids

Presence of ursolic acid, one of the compounds in triterpenic structure, was determined in different sections of the pomegranate plant. Amount of ursolic acid is at the rate of 0.55% in the leaves and flowers as it reaches to 0.9% in the fruit rinds (Isamuhamedov and Akramov, 1982) [19].

Polyholosides

Free SUGERS (fructose, glucose, and raffinose in low amounts), pectic substances, hemicellulose A and B, and water-soluble polyholosides are found in *P. granatum*. It was determined that the fruit rinds contained polyholoside at the rate of 4.62% (Jurkovic *et al*., 1976; Keogh and Donovan, 1970, Parashar *et al*., 2012) [20, 21].

In result of pectin-related studies conducted on the fruit rinds, it was revealed that mannose, galactose, rhamnose, arabinose, glucose and galacturonic acid were present in its composition. They were found to be present in the form of calcium pectate in the lamella (Khodzhaeva and Yuldasheva, 1985) [22].

Other compounds

It is found that Sitosterol, maslinic acid, asiatic acid and alkanes are present in the composition of pomegranate flower. It was expressed that D-hamitol, ellagic acid and gallic acid were present in its alcoholic extract (Hartwell, 1971). It is stated that in the pomegranate juice almost all the amino acids are present; while valine and methionine are in a very high concentration (Koleva *et al*., 1981; Konowalchuk and Speirs, 1976) [23, 24]. It was found that pomegranate juice also contained invert sugar, thiamin, vitamin C, riboflavin and protein (Heftman *et al*., 1966, Lad and Frawley, 1986, Malik *et al*., 2005, Parashar *et al*., 2017) [25, 27, 35]. Moreover, organic acids such as citric acid, malic acid and oxalic acid are present in the pomegranate juice, with 18.21% carotenoid and carotene being present in the edible part of the fruit (Nakov *et al*., 1982; Naqvi *et al*., 1991; Okuda *et al*., 1980) [28, 29, 30]. Composition of phenolic acids in cultivated and wild pomegranate fruits was determined, and it was reported to contain vanillic acid, neochlorogenic acid, chlorogenic acid, sinapic acid, kumic acid, ferulic acid and caffeic acid (Pantuck *et al*., 2006) [31].

Pomegranate seeds contain 3.5 g/kg of estrone, with its surface parts containing 9.2 g/kg and flowers containing 3.2 g/kg of that (Rosenblat *et al*., 2006; Saxena and Vikram, 2004) [32]. When fatty acid composition of the seeds were examined; punicic acid, 4-methyl lauric acid, 1,3-dimethyl stearic acid, sterols (stigmasterol, sitosterol), phospholipids (phosphatidyletanolamine, phosphatidylcholine, phosphatidylinositol) along with mono, di- and triglycerides and free fatty acids were detected (Santagati *et al*., 1984; Sergeeva, 1973). Prepa-rations made up of different sections of *P. granatum* have been applied to cancer therapy (Sharaf, 1966). The fruit extract shows antiviral activity (Schubert *et*

al., 1999), and also antimicrobial effect due to its anthocyans (Tanaka *et al*., 1986, Parashar *et al*., 2013) [38].

Biochemical constituents

Over the past decade, important progress has been made in establishing the medicinal mechanisms of pomegranate and the individual constituents responsible for them. Extracts of all parts of the fruit appear to have therapeutic properties (Borir, 1980) [7] and some studies reported that the bark, roots, and leaves of the tree have medicinal benefit as well. Three current researches seems to indicate the most therapeutically beneficial pomegranate constituents are ellagic acid ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones.

Antioxidant Mechanisms

An *in vitro* assay using four separate testing methods demonstrated pomegranate juice and seed extracts have 3 to 4 times the antioxidant capacity of either red wine or green tea. (Tanaka *et al*., 1986b). Pomegranate extracts have been shown to scavenge free radicals and decrease macrophage oxidative stress and lipid per oxidation in animals (Tanaka *et al*., 1985) and increase plasma antioxidant capacity in elderly humans (Torres and Fresno, 1970).

Studies in rats and mice confirm the antioxidant properties of a pomegranate by-product (PBP) extract made from whole fruit minus the juice (Parashar *et al*., 2012) showing a 27-percent reduction in oxidative stress in Mouse peritoneal macrophages (MPM), a 56% decrease in cellular lipid peroxide content (Parashar *et al*., 2016) [36], and a 49% increase in reduced glutathione levels (Tanaka *et al*., 1985). *In vitro* assay of a fermented pomegranate juice (FPJ) extract and a cold pressed seed oil (CPSO) extract found the antioxidant capacity of both are superior to red wine and similar to green tea extract (Zelepukha *et al*., 1975 Parashar *et al*., 2012). A separate study in rats with CCl_4 induced liver damage demonstrated pretreatment with a pomegranate peel extract (PPE) enhanced or maintained the free-radical scavenging activity of the hepatic enzymes catalase, super oxide dismutase, and peroxidase, and resulted in 56% reduction of lipid peroxidation values compared to controls (Tsuyuki *et al*., 1981, Parashar *et al*., 2009) [46]. Research in humans has shown a juice made from pomegranate pulp (PPJ) has superior antioxidant capacity to apple juice. Using the FRAP assay (ferric reducing/antioxidant power), Guo *et al*. (2008) [18] found 250 ml PPJ daily for four weeks given to healthy elderly subjects increased plasma antioxidant capacity from 0.95 to 2.37 mmol (Tanaka *et al*., 1986a Parashar *et al*., 2011) [39] while subjects consuming apple juice experienced no significant increase in antioxidant capacity. (Parashar *et al*., 2010) [40].

In addition, subjects consuming the PPJ exhibited sig-nificantly decreased plasma carbonyl content (a bio-marker for oxidant/antioxidant barrier impairment in various inflammatory diseases) compared to subjects taking apple juice. Plasma vitamin E, ascorbic acid, and reduced glutathione values did not differ significantly between groups, leading researchers to conclude pomegranate phenolics may be responsible for the observed results (Torres and Fresno, 1970, Parashar *et al*., 2010) [41].

Clinical applications

Prostate cancer

Among males in the India, Pakistan, China, United States and other Western countries, prostate cancer is the second-leading cause of cancer-related death. *In vitro* studies show several PFEs inhibit prostate cancer cell growth, induce apoptosis of several prostate cancer cell lines (including highly aggressive PC-3 prostate carcinoma cells), suppress invasive potential of PC-3 cells, and decrease proliferation of PJ-162 prostate cancer cells (Khodzhaeva *et al*., 1985; Ulja, 1972; Veres, 1977) [22].

The extracts resulted in a 99% suppression of PJ-162 prostate cancer cell invasion across a Matrigel matrix. CPSO extract or FPJ extract alone resulted in 76% suppression of invasion, and combining any two extracts induced 80% suppression. Studies in mice have also demonstrated PFE inhibits prostate tumor growth and decreases prostate specific antigen (PSA) levels (Veres 1977, Wills *et al*., 1986, Parashar *et al*., 2012).

These promising results led some of the same researchers to conduct a two-stage phase II clinical trial in men with recurrent prostate cancer and rising PSA levels. All eligible patients had previous surgery or radiation therapy for prostate cancer, Gleason scores (a grading system for predicting the behavior of prostate cancer) ≤7, rising PSA value of 0.2 to 5.0 ng/ml, no prior hormonal therapy, and no evidence of metastases. Baseline PSA doubling times were established for 50 participants who were then started on eight ounces PJ (570 mg total polyphenol gallic acid equivalents) daily until meeting disease progression end points. End points measured were: effect on PSA levels, serum lipid peroxidation and nitric oxide levels, *In vitro* induction of proliferation and apoptosis of LNCaP cells in patient serum containing pomegranate constituents, and overall safety of extract administration (40 based on preliminary results achieved in phase I), 24 additional patients were enrolled and 46 patients were evaluated over 13 months in both stages of the trial. Of these, 35% (n=16) demon-strated decreased PSA levels, the primary trial endpoint– average decrease=27%; median decrease =18%; range 5 to 85%. Four of 46 patients (8.7%) met objective response criteria and exhibited >50% reduction in PSA values, meeting criteria for a phase III trial.

In addition, an average 40% reduction in serum oxidative state was observed in patients accompanied by a significant reduction in serum lipid peroxidation com-pared to baseline. Nitric oxide serum metabolites mea-sured at nine months after study initiation revealed an average 23% increase, which significantly correlated with baseline PSA levels. An *in vitro* arm of the trial using patient serum investigated whether PJ consumption had any effect on growth rates or apoptosis of prostate cancer cells in culture.

Serum collected at nine months after study initiation and incubated decreased cell growth by an average of 18% in 84% of patients compared to baseline. An average of 20.5% in-crease in apoptosis in 70% of patients was also noted. This study indicated that PJ or PJ constituents may have promise as a therapy for prostate cancer, particularly recurrent type with rising PSA levels; phase III studies are currently underway (Yurtaev, 1959, Parashar, *et al*. 2015).

Hypertension

A small clinical trial demonstrated PJ inhibits serum angiotensin converting enzyme (ACE) and reduces systolic blood pressure in hypertensive patients. Ten hypertensive subjects (ages 62 to 77; seven men and three women) were given 50 ml/ day PJ containing 1.5 mmol total polyphenols

for two weeks. Two of seven patients were also diabetic and two were hyperlipidemic. Seven of 10 subjects (70%) experienced a 36% average decrease in serum ACE activity and a small, but signi-ficant, five percent decrease in systolic blood pressure (Yurdasheva *et al*., 1978, Parashar *et al*., 2013) [38].

Alzheimer's disease

The neuroprotective properties of pomegranate polyphenols were evaluated in an animal model of Alzheimer's disease. Transgenic mice with Alzheimer's like pathology treated with PJ had 50% less accumulation of soluble amyloid-beta and less hippocampal amyloid deposition than mice consuming sugar water, suggesting PJ may be neuroprotective. Animals also exhibited improved learning of water maze tasks and swam faster than control animals (Zelepukha *et al*., 1975).

Conclusion

An explosion of interest in the numerous therapeutic properties of *P. granatum* over the last decade has led to numerous *in vitro*, animal, and clinical trials. Pomegranate is a potent antioxidant, superior to red wine and equal to or better than green tea. In addition, ant carcinogenic and anti-inflammatory properties suggest its possible use as a therapy or adjunct for prevention and treatment of several types of cancer and cardiovascular disease.

The possibility that pomegranate extracts may also have an effect on several other disease processes, such as Alzheimer's disease, osteoarthritis, neonatal brain injury, male infertility, and obesity, underscores the need for more clinical research.

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