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Pomegranate bioactive constituents target multiple oncogenic and oncosuppressive signaling for cancer prevention and intervention

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1 **Pomegranate bioactive constituents target multiple oncogenic and**
2 **oncosuppressive signaling for cancer prevention and intervention**

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1 **Abstract**

2 Cancer remains to be the second highest cause of mortality in our society, falling just short of heart
3 disease. Despite major advancement in cancer therapy over the past decade, momentum has been
4 gaining for an alternative approach of using naturally-occurring and dietary agents for cancer
5 prevention and management. Research on pomegranate (*Punica granatum* L.), a fruit of the
6 Punicaceae family, has shown enormous potential for cancer prevention and intervention. In
7 addition to a rich source of polyphenols, including flavonoids and ellagitannins, in its juice,
8 pomegranate also houses hundreds of other phytochemicals in its pericarp, seed, flower, bark,
9 flowers and leaves, These phytochemicals provide powerful antiproliferative, anti-inflammatory,
10 antioxidant, anti-invasive, antimigratory, anti-angiogenic and anti-metastatic effects without
11 significant toxicity. This makes the use of its various extracts a very attractive strategy to our
12 current battle against cancer. This review article presents a systematic, comprehensive and critical
13 review of research on pomegranate-derived products in both cancer prevention and intervention.
14 It discusses the chemical constituents of pomegranate, the results of both preclinical (*in vitro*, *ex*
15 *vivo* and *in vivo*) and clinical studies on the anticancer effect of pomegranate phytochemicals and
16 molecular targets in numerous types of cancers, such as breast, gastrointestinal tract (oral, colon,
17 liver and pancreas), gynecological (uterine and ovarian), hematological (lymphoma, leukemia and
18 myeloma), lung, neurological (glioma), urogenital (bladder and prostate), bioavailability,
19 pharmacokinetics and safety of pomegranate constituents. In order to guide the direction of future
20 research, we have also included current limitations and challenges in the field and our post analysis
21 recommendation.

22 *Key words:* Pomegranate, *Punica granatum*, extracts, phytochemicals, cancer, prevention, therapy,
23 molecular targets

1. Introduction

Pomegranate (*Punica granatum*, L.), a fruit-bearing deciduous large shrub or small tree, is a native of the Himalayas in northern India to Iran [1]. It has been cultivated in various areas of the Middle East, southeast Asia, European Mediterranean region and northern Africa [2]. However, due to its popularity of consumption, the fruit and its juice, it is now cultivated in various regions of the world, including Afghanistan, China, Greece, India, Iran, Italy, Mexico, Morocco, Russia, Spain, Uzbekistan and the United States [3,4]. Globally, Iran is one of the largest pomegranate-producing countries [5]. In the United States, the primary production sites are the drier regions of Arizona and California [1,2].

The pomegranate shrub is a member of the Punicaceae family which is composed of two species, namely *P. granatum* and *P. protopunica*, of which only *P. granatum* is edible. There are more than 1,000 cultivars of *P. granatum* are known to exist [6]. The shrub grows from 5 to 10 m in height, generating an assortment of leaves, flowers, and fruits amongst thorny branches. Pomegranate fruit (classified as a large berry) ranges in color from light red to a green-yellow or purple. The fruit can be 5-20 cm in diameter, about 200-800 g in weight, grenade-shaped and crowned by the pointed calyx. It contains approximately 200-1,400 white, red or purple arils embedded into a spongy, white membrane enveloped by exocarp. The fruit essentially contains three parts: the seeds (~3% of fruit weight) containing around 20% oil, the juice (~30% of fruit weight) and the peels (pericarp) that include the interior network of membranes [4]. The leaves are glossy, opposite or subopposite, narrow oblong, entire, 3–7 cm long and ~2 cm wide. The flowers are mostly bright red (rarely white or yellow), odorless, and about 3 cm in diameter with three to seven petals.

1 The pomegranate fruit finds its use for medicinal purposes in ancient cultures for centuries. It
2 prominently features in various major world religions, such as Buddhism, Christianity, Islam,
3 Judaism, and Zoroastrianism [4]. It represents a symbol of life, longevity, health, femininity,
4 fecundity, knowledge, morality, immortality and spirituality [7]. In the Ancient Greek myth of
5 Persephone's abduction, the pomegranate, known as the "fruit of the dead," signifies life and
6 rebirth. This mythology contributed to the inspiration for utilizing pomegranate in the coat of arms
7 of various medical associations, including British Medical Association, Royal College of
8 Midwives, Royal College of Obstetricians and Gynaecologists and Royal College of Physicians
9 [8]. Zoroastrians believe the fruit represents immortality, whereas in China it represents a time-
10 honored symbol of fertility and prosperity [8].

11 The pomegranate's medicinal value has been recognized in various indigenous and
12 traditional systems of medicine to treat a variety of ailments. The applications of pomegranate are
13 expansive and have notable uses in both Ayurvedic and the Unani systems of medicine. Ayurveda
14 is India's primary form of medicinal system in which pomegranate is regarded as "a pharmacy
15 unto itself" [1]. The bark and roots are believed to possess antihelmintic and vermifuge properties
16 [9], whereas the seeds are known to facilitate urination and prevent urinary disorders [10]. Dried
17 pomegranate peel, bark, leaves and flowers have been used in Indian subcontinent to treat
18 inflammation, diarrhea, nose bleeding, sore throat, hoarseness, periodontitis and ulcers [11,12]. In
19 Unani (a system of medicine practiced in the Middle East and India) and traditional Chinese
20 medicine, pomegranate has been considered a remedy for diabetes [13,14]. In India, Guatemala
21 and Tunisia, water decoction of dried pomegranate peels has been used as astringent and germicide
22 as well as to treat diarrhea, aphthae and ulcers [15-17].

1 Pomegranate fruits are widely consumed in fresh and beverage forms as juice as well as jam,
2 jelly and wine [18]. Additionally, pomegranate leaves are brewed as tea and dried seeds are used
3 as spice [19]. During the last decade, pomegranate fruit (termed as “super fruit”) has been gaining
4 a tremendous reputation as a nutraceutical source as well as functional food for putative health
5 benefits [20] ascribed to the exceptional antioxidant activity of the fruit and juice as compared to
6 other fruits and antioxidant beverages [21-24]. Based on numerous preclinical and clinical studies,
7 pomegranate juice, extracts, and phytoconstituents have been found to be useful for the prevention
8 and/or treatment of cardiovascular disease, hypertension, diabetes, nonalcoholic fatty liver disease,
9 obesity, inflammatory disorders, ulcer, arthritis, microbial infection, dental ailments, acquired
10 immune deficiency syndrome, neurological disorders, erectile dysfunction, male infertility and
11 ultra-violet radiation-induced skin damage [1,4,5,25-33].

12 The abundance of phytochemicals and numerous biological and medicinal properties coupled
13 with its use in traditional medicine [34] have attracted cancer researchers all over the world to
14 explore the anticancer potential of pomegranate. This fact combined with the prevalence of cancer
15 and the numerous severe side effects of traditional first line treatments make the precedent for
16 investigation of dietary prevention and efficacious natural therapies a prudent approach. There has
17 been a plethora of publications that have focused on anticancer role of pomegranate extracts and
18 phytoconstituents [4,35-41]. However, a large number of articles focus on pomegranate’s impact
19 on cancer in an organ-specific system, such as breast cancer [42,43], colon cancer [44,45] and
20 prostate cancer [46,47], rather than comparing its efficacy against cancers of multiple organ
21 systems. Additionally, many studies focused on only a major pomegranate phytochemical group,
22 e.g., polyphenols [37, 48]. Moreover, there has been limited discussion of the molecular targets of

1 the various phytochemicals within pomegranate and how that knowledge can be applied to
2 chemopreventive setting.

3 The purpose of this article is to perform a systematic, comprehensive and critical review of
4 the anticancer and cancer preventive effects of pomegranate phytochemicals with an emphasis on
5 their various molecular targets. The goal is to understand the precise role of pomegranate
6 phytoconstituents in both cancer prevention and intervention. The article analyzes available
7 preclinical *in vitro* and *in vivo* studies as well as clinical reports of the chemopreventive and
8 therapeutic applications of pomegranate in numerous types of cancer. Current limitations,
9 challenges and future directions of research are also discussed.

10

11 **2. Chemical constituents of pomegranate**

12 The chemical composition of pomegranate is very diverse as different parts of the plant,
13 namely, leaves, root, flowers, seed, peel and arils, contain a wide array of phytochemicals, such as
14 phenols, anthocyanins, tannins, carbohydrates, sterols, fatty acids, vitamins and minerals [4,48].
15 Approximately half of the total fruit weight corresponds to the peel (also known as pericarp), **which**
16 **contains several** important compounds, such as phenolics, flavonoids, proanthocyanidin
17 compounds and ellagitannins [4,28]. **The phenolic acids present in pomegranate peel are represented**
18 **by caffeic acid, *p*-coumaric acid, chlorogenic acid, gallic acid and ellagic acid (Fig. 1)** [50,51]. The
19 peel also contains hydrolyzable tannins or ellagitannins, such as corilagin, granatin A and B,
20 tellimagrandin, pedunculagin, punicalagin, and punicalin (Fig. 1). Punicalagin is unique to
21 pomegranate and it is found in the seeds, peel, leaves and juice [28]. **The peel also contains various**
22 **flavonols, such as kaempferol, quercetin and myricetin as well as flavan-3-ols, such as catechin,**
23 **epicatechin and epigallocatechin 3-gallate** [52,53]. The pomegranate fruit's edible parts, arils,

1 contain around 85% water and about 11% total sugars, 1.4% pectin, organic acids, such as citric
2 acid, malic acid and ascorbic acid constitute a small percentage of the composition [48].
3 Pomegranate juice is a rich source for ellagic acid and its derivatives [54]. Apart from these, arils
4 also contain anthocyanins (sugar derivatives of delphinidin, cyanidin and pelargonidin), namely
5 delphinidin-3-glucoside, delphinidin-3,5-diglucoside, cyanidin-3-glucoside, cyanidin-3,5-
6 diglucoside, pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside [55] (Fig. 2).

7 Pomegranate seeds carry a high concentration of total lipids, but lack in polyphenol content
8 [56]. Qualitative analysis carried out by two different studies has confirmed the presence of puni-
9 cid acid (Fig. 3) in seeds [57,58]. Other fatty acids, such as linoleic acid, oleic acid, stearic acid and
10 palmitic acid, were also detected in appreciable amounts ranging from 7 to 3% [58]. Additionally,
11 minor components of oil include triglycerides and glycolipids, such as cerebroside and coumestrol
12 [58]. Pomegranate seeds also contain ellagic acid derivatives, e.g., 3,3'-di-*O*-methyl ellagic acid
13 and 3,3,4'-tri-*O*-methyl ellagic acid, and triterpenoids, e.g., asiatic acid and betulinic acid (Fig. 3).
14 Sterols, namely stigmasterol, sitosterol, cholesterol and sex steroids, such as estrone, testosterone
15 and estriol, were also detected in seeds.

16 Pomegranate leaves contain various ellagitannins, such as punicalin, punicalagin,
17 punicafolin and corilagin [59]. Other phytochemicals, such as apigenin, strictinin, tercatin and
18 number of other flavone glycosides, namely apigenin-4'-*O*- β -D-glucopyranoside, luteonin-4'-*O*-
19 β -D-glucopyranoside, luteonin-3'-*O*- β -D-glucopyranoside and luteonin-3'-*O*- β -D-
20 xylopyranoside, have been reported [60] (Fig 4). Pomegranate root is known to contain different
21 alkaloids, namely pelletierine, N-methylpelletierene and pseudopelletierene) and piperidine
22 [61,62]. Alkaloids, such as sedridine, 2-(2'-hydroxypropyl)- Δ^1 piperidine, 2-(2'-propenyl)- Δ^1
23 piperideine, hygrine and norhygrine, have been reported in the bark [61] (Fig. 5). Additionally,

1 ellagitannins, including punicalin, punicalagin, punicacortein A, punicacortein B, punicacortein C
2 and punicacortein D, have been reported reported in the bark [59]. A number of phytochemicals
3 were also reported in the flowers of pomegranate. Several phytochemicals belong to
4 hydroxybenzoic acids class, such as gallic acid [63]. Triterpenoids, including urosolic acid,
5 oleanolic acid, masilinic acid and asiatic acid, were also reported as pomegranate flower
6 constituents [64] (Fig. 6).

7

8 **3. Pomegranate in cancer prevention and therapy**

9 *3.1. Literature search methodology*

10 For this work, we have followed the Preferred Reporting Items for Systematic Reviews and
11 Meta-Analysis (PRISMA) criteria [65], which is recommended for reporting systematic
12 reviews. Relevant articles were first identified via various databases, such as PubMed,
13 ScienceDirect, Scopus, Web of Science, Springerlink, and Google Scholar, from their inception
14 date through March 2020. There was no restriction on year of publication. Articles were included
15 if the following criteria were satisfied: (i) publications in peer-reviewed scientific journals; (ii)
16 English language articles only; (iii) original research articles; (iv) *in vitro*, *in vivo* and clinical studies
17 that utilized pomegranate-derived constituents. We excluded items if the following criteria were
18 met: (i) conference abstracts, books, book chapters, and unpublished results; (ii) non-English
19 articles; (iii) reviews, systematic reviews, meta-analysis and letters; (iv) publications that did not
20 involve tumor cell lines or animal tumor models. These exclusion criteria were applied to the
21 article pool after review of article title and abstract to remove unqualified articles. Questionable
22 articles were either included or excluded after consensus discussion among all authors. The authors
23 then proceeded to obtain full-length articles using various methods followed by a review of

1 reported studies to summarize important study findings. The overview of literature search and
2 selection process is provided in Fig. 7.

3

4 *3.2. Preclinical studies*

5 3.2.1. Breast cancer

6 *3.2.1.1. In vitro*

7 Pomegranate-derived products have been extensively used for research on breast cancer. Kim
8 et al. [66] conducted a study in which estrogen receptor (ER)-positive (MCF-7) and ER-negative
9 (MB-MDA-231) breast cancer cells were exposed to fermented pomegranate juice polyphenols,
10 fresh pomegranate juice polyphenols and pericarp polyphenols (derived from aqueous extract). It
11 was shown that the fermented juice polyphenols displayed a superior antiproliferative effect to
12 fresh juice polyphenols. Pomegranate seed oil registered a 90% inhibition of cell proliferation and
13 a 75% of cell invasion in MCF-7 cell line as well as a 54% apoptosis in MDA-MB-435 (ER-
14 negative metastatic human breast cancer) cells (Table 1). A follow-up study by Toi et al. [67]
15 showed that the fermented juice polyphenols and seed oil downregulated vascular endothelial
16 growth factor (VEGF), a proangiogenic factor, in MCF-7 cells, where the seed oil upregulated
17 migration-inhibitory factor (MIF) in MDA-MB-231 cells.

18 Louis Jeune et al. [68] reported that pomegranate extract alone or in combination with genistein
19 (an isoflavone present in soybeans) showed significant concentration- and time-dependent
20 cytotoxic and growth-inhibitory effects in MCF-7 cells through induction of apoptosis. An aqueous
21 pomegranate fruit extract inhibited the proliferation of MDA-MB-231 and SUM 149 cells through
22 induction of apoptosis and suppression of constitutive activation of nuclear factor- κ B (NF- κ B).
23 The aqueous extract also inhibited NF- κ B-dependent reported gene expression associated with

1 proliferation, invasion and motility with simultaneous decrease in Ras homolog family member A
2 (RhoA) and RhoC protein expression, indicating the ability of the extract in lowering the metastatic
3 potential of aggressive breast cancer phenotypes [69]. Later, a pomegranate juice or a combination
4 of its components (luteolin, ellagic acid and puniic acid) was found to interfere with multiple
5 processes involved in metastasis of breast cancer cells, such as cell growth, adhesion, migration,
6 chemotaxis and epithelial-to-mesenchymal transition (EMT) [70]. An aqueous extract of whole
7 pomegranate fruit inhibited mammosphere formation in two different cell lines, namely neoplastic
8 mammary epithelial HMLER and breast cancer Hs578T. Incubation of mammospheres with the
9 extract reversed them into adherent cultures, indicating promotion of cancer stem cell
10 differentiation. The extract also reduced cell migration, a hallmark of EMT phenotype, and
11 downregulated genes involved in EMT [71]. In MDA-MB-231 cells, a methanolic extract of
12 pomegranate peel resulted in cell death and markedly inhibited cell migration, accompanied by
13 upregulation of the expression of intracellular cell adhesion molecule 1 (ICAM-1), a protein
14 essential for cell adhesion. The methanolic extract also downregulated the expression of matrix
15 metalloproteinase-9 (MMP-9), fibronectin and VEGF, proteins with crucial roles in cancer cell
16 migration [72]. A follow-up study confirmed proapoptotic, antimigratory and anti-invasive effects
17 and identified additional targets, such as vimentin, ZEB1, β -catenin, and E-cadherin, underscoring
18 the antimetastatic potential of pomegranate peel extract [73].

19 Various ellagitannin-derived compounds, including urolithin B, isolated from pomegranate
20 have been found to exert antiproliferative and antiaromatase activity in ER-positive aromatase-
21 overexpressing MCF-7aro cells. This demonstrates the potential of pomegranate ellagitannin-
22 derived compounds for the prevention of ER-responsive breast cancer [74]. Several conjugated
23 fatty acids, such as α -eleostearic acid, γ -linoleic acid and puniic acid, isolated from pomegranate

1 seed oil inhibited the proliferation of MCF-7 and MDA-MB-231 cells through modulation of ER
2 activity [75]. A methanolic extract of pomegranate pericarp inhibited the binding of [³H]-estradiol
3 (E₂) to ER and suppressed the growth and proliferation of ER-positive MCF-7 cells. The extract
4 was also found to bind with ER and downregulate the transcription of estrogen-responsive reporter
5 gene transfected into breast cancer cells with concomitant downregulation of estrogen-responsive
6 genes, such as ER- α , progesterone receptor (PR) and pS2 [76]. Later, the same group of researchers
7 found that the extract inhibited the MCF-7 cell proliferation induced by 27-hydroxycholesterol
8 (27HC), an endogenous selective estrogen receptor modulator (SERM) that can act through ER-
9 mediated mechanisms. The extract also downregulated the expression of genes, ER- α , PR, pS2,
10 induced by 27HC [77].

11 Joseph et al. [78] isolated galactomannan polysaccharide (PSP001) from the fruit rind of
12 pomegranate which showed *in vitro* cytotoxic effect against Ehrlich ascites carcinoma (EAC), a
13 transplantable mouse carcinoma of possible mammary origin. A standardized extract of
14 pomegranate (Pomella), containing ellagitannins (gallic acid, punicalagin α and punicalagin β),
15 inhibited the proliferation and viability of MMTV-Wnt-1 mouse mammary cancer stem cells,
16 possibly by arresting cell cycle progression in the G₀/G₁ phase and increasing caspase-3 activity
17 in a time- and concentration-dependent manner [79]. Pomegranate juice-derived ellagitanins,
18 such as gallic acid, ellagic acid, hexahydroxydiphenic acid, punicalagins and punicalagins, exhibited
19 antiproliferative activities against BT-549 breast ductal carcinoma cells [80]. Banerjee et al. [81]
20 reported that a pomegranate fruit extract enhanced the action of tamoxifen, a SERM, in both
21 sensitive and tamoxifen-resistant MCF-7 cells through the inhibition of cell viability by inducing
22 cell-death machinery. A methanolic pomegranate fruit peel extract, containing ellagic acid as the
23 most abundant phenolic acid, exhibited antiproliferative effects against MCF-7 cells through

1 antioxidant and proapoptotic mechanisms [82]. Later, the antiproliferative effects of methanolic
2 peel extract in MCF-7 cells was confirmed by another group [83]. Similarly, acetone and
3 methanolic extracts from whole pomegranate peels were reported to possess antiproliferative
4 activities against MCF-7 and MDA-MB-231 cells [84]. A polyphenol-rich extract of pomegranate
5 peel decreased the growth of MCF-7 and doxorubicin-resistant MCF-7 cells (MCF-7/DX) through
6 antioxidant and proapoptotic mechanisms [85]. Polysaccharide (PSP001) isolated from the fruit
7 rind has also been shown to have antitumor activity in MCF-7 breast cancer cells [86]. Punicalagin
8 isolated from pomegranate husk exhibited strong antiproliferative activity against MCF-7 cells and
9 this effect could possibly be due to the ability of the pomegranate constituent to inhibit oxidative
10 DNA adducts [87]. A standardized extract of pomegranate fruit skins (POMX), containing 95%
11 glycone ellagitannins and free ellagic acid, significantly inhibited MCF-7 cell growth in a
12 concentration- and time-dependent manner by inducing cell cycle arrest in G₂/M phase followed
13 by the induction of apoptosis. POMX downregulated genes associated with mitosis, chromosome
14 organization, RNA processing, DNA replication, and DNA repair and upregulated genes involved
15 in regulation of cell proliferation and apoptosis. Furthermore, POMX-mediated downregulation of
16 homologous recombination (HR) genes correlated with increased levels of their predicted
17 miRNAs, such as miRNA-24 (predicted target BRCA1) and miRNA-183 (predicted target
18 RAD50), suggesting that the pomegranate extract may regulate miRNAs involved in DNA repair
19 machinery [88]. A polyphenolic extract prepared from a pomegranate juice concentrate, containing
20 punicalagin A, punicalagin B, delphinidin-3-glucoside, and cynidin-3-glucoside, inhibited the
21 growth of MDA-MB-231 and BT-474 cells, but not non-cancerous MCF-10F and MCF-12F cells.
22 The extract also decreased the expression of specificity protein 1 (sp1), sp3 and sp4 and sp-
23 regulated genes as well as miR-27a and elevated the expression of ZBTB10, a transcriptional

1 repressor, in BT-474 cells. Additionally, the extract induced the expression of SH-2 containing
2 inositol 5'-phosphatase 1 (SHIP-1) which was accompanied by downregulation of miRNA-155
3 and inhibition of phosphoinositide 3-kinase (PI3K)-dependent phosphorylation of Akt [89].

4 In recent years, several investigators studied antibreast cancer effects of pomegranate seeds.
5 Contantini and colleagues [90] characterized conjugated linolenic acids, e.g., punicic acid, as the
6 major components of a hydrophilic fraction (80% aqueous methanol extract) from pomegranate
7 seed oil. In MCF-7 and MDA-MB-231 cells, the hydrophilic seed extract caused a significant
8 decrease in cell viability, increase in the number of cells at G₀/G₁ phase and suppression of VEGF
9 and nine proinflammatory cytokines. An ethanolic or methanolic extract of pomegranate seeds
10 caused inhibition of proliferation of MCF-7 cells though the underlying mechanisms of action
11 were not clearly understood [91,92].

12 There has been at least one study that evaluated the effects of pomegranate flower extracts on
13 proliferation of breast cancer cells. Ethanolic extracts of flowers of seven pomegranate varieties
14 were investigated against MCF-7 cells. The results indicated cytotoxic activities of extracts of two
15 varieties (GR and ZH), possibly due to antioxidant and anti-inflammatory (inhibition of 5-
16 lipoxygenase, 5-LOX) properties [93].

17 Several investigators evaluated the efficacy of novel formulations of pomegranate
18 phytoconstituents against breast cancer *in vitro*. Shirode and colleagues [94] prepared formulations
19 of poly(D,L-lactic-*co*-glycolic acid)-poly(ethylene glycol) (PLGA-PEG) nanoparticles (NPs)
20 loaded with pomegranate extract or individual polyphenols, such as punicalagin and ellagic acid.
21 In MCF-7 and Hs578T cells, the pomegranate nanoformulations had a 2- to 12-fold enhanced
22 effects on cell growth inhibition compared to their free counterparts, and the extract-containing
23 nanoprototype was found to be the most potent. Monodispersed silver-NPs (Ag-NPs) of

1 pomegranate extract developed by green synthesis was found to inhibit the proliferation of MCF-
2 7 cells by reducing DNA synthesis and promoting apoptosis-inducing cell cycle changes [95].
3 Similarly, platinum NPs (Pt-NPs) biosynthesized using pomegranate crusts produced a superior
4 antiproliferative effect when compared to pomegranate peel in MCF-7 cells by inducing apoptosis
5 through cell cycle arrest at G₀/G₁ phase [96]. In order to maximize the chemotherapeutic efficacy
6 of pomegranate extract, it was incorporated in an optimized solid lipid NPs (SL-NPs) formulation.
7 The pomegranate extract-loaded NPs exhibited better cytotoxic profile in MCF-7 cell line
8 compared to that of the free extract [97].

9

10 *3.2.1.2. Ex vivo and in vivo*

11 Utilizing the murine mammary gland organ culture model, Kim and colleagues [66] found
12 that the fermented juice polyphenols displayed a 47% inhibition of cancerous lesions induced by
13 mammary carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) (Table 2). Using the same mouse
14 mammary organ culture model, Mehta and Lansky [98] observed that a purified fraction derived
15 from pomegranate fermented juice polyphenols by high-performance chromatography and
16 pomegranate seed oil elicited a 87% reduction in the number of DMBA-induced mammary lesions
17 compared to control. This indicates the breast cancer preventive potential of pomegranate juice
18 and seed oil.

19 Banerjee et al. [89] conducted the first *in vivo* study to evaluate antitumorigenic effect of
20 pomegranate in breast cancer. Oral administration of a pomegranate juice extract (0.8 mg gallic
21 acid equivalents/kg/day) for 35 days reduced tumor volume and size in athymic nude mice bearing
22 BT474 tumor cells as xenografts (Table 2). The tumor tissues from the extract-treated animals
23 exhibited higher level of apoptosis, decreased expression of Sp1, Sp3, Sp4, VEGF, survivin, NF-

1 κ B p65, pAkt and pPI3K, and increased expression of ZBTB10 and SHIP-1 compared to control
2 tumors. Additionally, the expression of miRNA-27a and miRNA-155 were significantly reduced
3 in tumors in the extract-treated animals. Pomegranate fruit rind-derived galactomannan
4 polysaccharide (PSP001) alone or in combination with doxorubicin produced a significant
5 reduction in the tumor burden and increased the life span of mice containing EAC ascites or EAC
6 solid tumors compared to corresponding control animals [78]. Bishayee and colleagues [99]
7 evaluated for the first time *in vivo* chemopreventive potential of a novel pomegranate emulsion
8 (PE) that consists of most bioactive constituents present in the whole fruit using the classical
9 preclinical mammary carcinoma model utilizing female Sprague-Dawley rats and DMBA. The
10 animals were orally administered (p.o.) with PE (0.2, 1 or 5.0 g/kg), starting 2 weeks before and
11 16 weeks following DMBA exposure. PE caused a striking reduction of DMBA-induced
12 mammary tumor incidence and tumor burden accompanied by reversal of histopathological
13 changes in tumor tissue. PE also dose-dependently suppressed intratumor cell proliferation,
14 induced apoptosis, increased Bax expression, decreased Bcl-2 and manifested a proapoptotic shift
15 in Bax/Bcl-2 ratio. The accompanying gene expression study revealed that PE upregulated the
16 expression of BAD, caspase-3 (CASP3), caspase-7 (CASP7), caspase-9 (CASP9), poly (ADP
17 ribose) polymerase (PARP) and cytochrome c (CYT. C) in mammary tumors. In a follow-up study,
18 it has been found that PE downregulated the expression of ER- α and ER- β and lowered the ratio
19 of ER- α to ER- β . PE also decreased the expression, cytoplasmic accumulation and nuclear
20 translocation of β -catenin and suppressed the expression of cell growth regulatory protein cyclin
21 D1, which represents a downstream target of both ER and Wnt/ β -catenin signaling [100]. Finally,
22 PE decreased the expression of cyclooxygenase-2 (COX-2) and heat shock protein 90 (HSP90),
23 prevented the degradation of inhibitory κ B α (I κ B α), hindered the translocation of NF- κ B from

1 cytosol to nucleus and elevated the expression and nuclear translocation of nuclear factor erythroid
2 2p45 (NF-E2)-related factor (Nrf2) in tumor tissues [101]. In contrast to the aforementioned
3 findings, Lepionka et al. [102] recently reported that oral feeding of pomegranate seed oil increased
4 breast cancer incidence and accelerated tumor growth in female Sprague-Dawley rats subjected to
5 DMBA mammary carcinogenesis, and these results could be due to the influence of seed oil
6 constituents on DMBA metabolism.

7

8 3.2.2. Gastrointestinal tract and associated cancers

9 3.2.2.1. Oral cancer

10 3.2.2.1.1. *In vitro*

11 Seeram et al. [103] demonstrated that pomegranate juice (PJ), total pomegranate tannin (TPT)
12 extract, and punicalagin isolated from pomegranate husk have cytotoxic effects on *in vitro* human
13 oral cancer cells (KB and CAL27). A follow-up study confirmed similar cytotoxic effects of
14 pomegranate juice-derived ellagitannins in KB cells [80]. Similarly, Weisburg et al. [104] found
15 that ellagitannin-enriched pomegranate polyphenol extract exerted cytotoxic effect on various oral
16 cancer cells (CAL27, HSC-2 and SCC 1483) via activation of caspase-3 activity, cleavage of
17 PARP, and reduction of glutathione (GSH) levels.

18

19 3.2.2.2. Colon cancer

20 3.2.2.2.1. *In vitro*

21 Pomegranate juice (PJ), total pomegranate tannin (TPT) extract, and punicalagin isolated from
22 pomegranate husk have cytotoxic effects against a panel of colon cancer cells (HT-29, HCT116,
23 SW480 and SW620). In HT-29 and HCT116 cell lines, the cytotoxic effects were accompanied by
24 apoptosis [103]. Kasimetty et al. [80] reported that pomegranate juice (PJ)-derived ellagitannins

1 induced cell cycle arrest at G₀/G₁ and G₂/M stages, and also inhibited proliferation and clonogenic
2 efficiency of HT-29 tumor cells via activation of caspase-3 activity. Banerjee et al. [105]
3 demonstrated that polyphenolic extract from pomegranate juice exerted cytotoxic, anti-
4 inflammatory and antiangiogenesis effects on HT-29 cells via upregulation of caspase-3, PARP
5 cleavage, downregulation of COX-2, vascular cell adhesion molecule 1 (VCAM-1), VEGF, and
6 NF-κB p65, reduction in phosphorylation of phosphatidylinositol 3-kinase/protein kinase B
7 (PI3K/Akt) and increase in the expression of miRNA-126. Recently, Chen et al. [106] showed that
8 both pomegranate peel-derived ellagitannins alone and ellagitannins in combination with 5-
9 fluorouracil (5-FU) significantly inhibited HT-29 cell proliferation and induced S-phase cell cycle
10 arrest. Concomitant mechanistic studies showed induction of intrinsic apoptosis via dissipation of
11 mitochondrial membrane potential, increased Bax to Bcl-2 ratio, and cleavage of caspase-3 and
12 caspase-9. Galactomannan polysaccharide (PSP001) extracted from pomegranate peel exerted
13 cytotoxic effect on HCT116 cells via apoptosis induction [78]. Subsequent study by the same
14 group [107] observed similar cytotoxic, proapoptotic and additional antimigratory effects on
15 HCT116 cells with PSP001 alone or silver nanoparticles (SNPs) containing PSP001 (SNP@PSP).
16 Larrosa et al. [108] used another *in vitro* colon cancer model (Caco-2) and found that punicalagin
17 extracted from pomegranate peel as well as ellagic acid induced cytotoxicity and S-phase cell cycle
18 arrest, possibly via downregulation of cyclin A and cyclin B1, upregulation of cyclin E, induction
19 of apoptosis, downregulation of Bcl-XL as well as activation of caspase-3 and caspase-9, and
20 release of cytochrome c. The investigation on pomegranate's anticancer properties has expanded
21 from ellagitannin to other fractions of pomegranate extract. Moreira et al. [85] reported that
22 pomegranate peel extract exhibited antiproliferative effect on another colon cancer cell line
23 (LOVO) and its doxorubicin-resistant form (LOVO-DX) via induction of apoptosis, suggesting

1 pomegranate extract's potential to treat chemotherapy-resistant cancers. Similarly, acetone and
2 methanolic extracts from whole pomegranate peels were found to exert cytotoxicity in RKO
3 colorectal cancer cells [84]. Devanesan et al. [109] developed aqueous pomegranate peel extract-
4 derived silver nanoparticles that demonstrated increased cytotoxicity in RKO cells, possible due
5 to programmed cell death via autophagy. Interestingly, Costantini et al. [90] found only a marginal
6 effect of a methanolic extract of pomegranate seed oil against HT29 and HCT 116 colon cancer
7 cells.

8

9 **3.2.2.2.2. *In vivo***

10 Based on encouraging *in vitro* results as presented earlier, several groups have also explored
11 the anticancer properties of various pomegranate extracts in several animal models. Kohno et al.
12 [110] demonstrated that dietary pomegranate seed oil (PSO) reduced the incidence and multiplicity
13 of azoxymethane (AOM)-induced colonic adenocarcinomas in male F344 rats. The associated
14 increase in conjugated linolenic acids (CLA) content of colonic mucosa and liver and the
15 upregulation of peroxisome proliferator-activated receptor gamma (PPAR γ) expression in colon
16 mucosa were proposed as the potential mechanism for antitumor property of PSO. Similarly,
17 Boateng et al. [111] used the same chemical carcinogenesis model and found that treatment with
18 pomegranate juice significantly reduced the frequency of aberrant crypt foci (ACF), which
19 represent early preneoplastic lesions of adenocarcinoma, in rats. The investigators also reported
20 increased glutathione S-transferase (GST) activity in the liver of rats subjected to pomegranate
21 juice treatment compared to control animals. Similarly, Waly et al. [112] used AOM-induced
22 tumor model in Sprague-Dawley rats and found that pomegranate peel extract can reverse the
23 tumor-inducing effects of AOM, via increase in total antioxidant concentration (TAC), elevation

1 in GSH, GST, glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase
2 (SOD) and catalase (CAT). To further expand on the study, the same group [113] reported similar
3 reduction of ACF formation in proximal and distal colon of rats following pomegranate peel
4 extract administration with simultaneous reduction of AOM-induced genotoxicity, elevation in
5 GSH/GSSG ratio, increase in malondialdehyde (MDA) level and suppression of myeloperoxidase
6 (MPO) activity. Banerjee et al. [105] studied the effect of pomegranate juice consumption with the
7 same cancer model and reported similar suppression of total ACF and dysplastic ACF formation
8 in colon. Additionally, they found that the mRNA and protein levels of COX-2, inducible nitric
9 oxide synthase (iNOS), NF- κ B, VCAM-1, insulin growth factor (IGF), insulin growth factor
10 receptor (IGFR), PI3K, Akt, mammalian target of rapamycin (mTOR) as well as the expression of
11 miR-126 were modulated with pomegranate juice treatment. Bastide et al. [114] reported that
12 pomegranate extract containing 12% ellagic acid reduced mucin-depleted foci (MDF, biomarker
13 for colorectal cancer) formation in rats fed dark cooked meat with nitrite (DCNO, cured meat with
14 known carcinogenic property) and received AOM injection. The extract-mediated protection
15 against colon carcinogenesis was associated with full suppression of fecal excretion of nitrosyl
16 iron. Sadik et al. [115] used another *in vivo* colon cancer model, namely 1,2-dimethylhydrazine
17 dihydrochloride (DMH)-induced tumorigenesis in male Wistar albino rats, and found that
18 treatment with a standardized pomegranate fruit extract enhanced survival of tumor-bearing
19 animals, lowered tumor incidence and reduced carcinoembryonic antigen (CEA, a serum tumor
20 marker) in parallel with a normalization of colonic histopathological characteristics. This study
21 also suggested that changes in expression of various Wnt-target genes, including Wnt5a, frizzled
22 receptor-8 (FRZ-8), β -catenin, T cell factor/lymphoid enhancer binding protein (Tcf4/Lef1), c-
23 myc, cyclin D1, adenomatous polyposis coli (APC), and axin1 in colonic tissues potentially

1 contributed to the anticarcinogenic effect of pomegranate constituents. Tortora and colleagues
2 [116] investigated the chemopreventive potential of pomegranate mesocarp, a polyphenol-rich by-
3 product of juice production, by using another colorectal cancer model, Apc-mutated Pirc
4 (F344/NTac-Apcam1137) rats. A mesocarp decoction was found to reduce the number and
5 multiplicity of MDF in experimental animals via induction of apoptosis.

6

7 3.2.2.3. Liver cancer

8 *3.2.2.3.1. In vitro*

9 There are several studies on pomegranate-derived products in *in vitro* liver cancer. While 24-
10 h treatment with pomegranate seed oil did not change the viability of human liver cancer cells
11 (HepG2 and Huh7) [90], pomegranate fruit rind-derived galactomannan polysaccharide (PSP001)
12 [78] and a novel formulation of PSP001 (SNP@PSP) [Padinjarathil et al. 2018] exerted cytotoxic
13 and antimigratory effects in HepG2 cells. Li et al. [117] detected four polyphenols (gallic acid,
14 ellagic acid, punicalagin A&B and punicalin A&B) in various parts of pomegranate, such as peels,
15 flesh, seeds, juice and leaf. Interestingly, the peel and flesh extracts (containing 83% of total
16 phenolic content of pomegranate) exhibited significantly potent cytotoxicity against HepG2 cells,
17 whereas less pronounced effects were observed with other parts. A polyphenol-rich pomegranate
18 peel extract inhibited the proliferation of HepG2 cells with simultaneous induction of cell cycle
19 arrest at the S-phase and apoptosis, increase in the level of reactive oxygen species (ROSs) and
20 cyt. c, elevation in the activity of caspase-3 and caspase-9, upregulation of the expression of Bax
21 and p53 and downregulation of Bcl-2 expression [118]. Saratale and colleagues [119] developed
22 a cost-effective green synthesis of silver nanoparticles (AgNPs) containing pomegranate leaf
23 extract (PGE). This product (PGE-AgNPs) showed remarkable and greater cytotoxicity against

1 HepG2 cells in concentration-dependent manner compared to PGE, possibly through free-radical
2 scavenging activity. Similarly, solid lipid nanoparticles containing pomegranate extract exhibited
3 superior cytotoxic activity in HepG2 cells when compared to the free extract [97].

4

5 *3.2.2.3.2. In vivo*

6 Bishayee and co-investigators [120] examined, for the first time, the mechanism-based
7 chemopreventive potential of a novel pomegranate emulsion (PE) containing numerous
8 phytochemicals, including caffeic acid, corilagin, ellagic acid, ferulic acid, gallic acid,
9 punicalagins, protocatechuic acid, and γ -tocopherol, against diethylnitrosamine (DENa)-initiated
10 and phenobarbital-promoted rat hepatocarcinogenesis that resembles human hepatocellular
11 carcinoma (HCC). Oral administration of PE (1 or 10 g/kg), initiated 4 weeks before DENa
12 treatment and continued for 18 weeks, exhibited a striking chemopreventive effect as evidenced
13 by reduced incidence, number, multiplicity, size and volume of hepatic nodules, precursors of
14 HCC. Both doses of PE also reduced the number and area of γ -glutamyl transpeptidase (GGT)-
15 positive hepatic foci coupled with attenuation of lipid peroxidation (thiobarbituric acid-reactive
16 substances, TBARS) and protein oxidation (protein carbonyls) in DENa-challenged rats. Based
17 on mechanistic studies, PE elevated gene expression of hepatic antioxidant and carcinogen
18 detoxifying enzymes and elevated hepatic Nrf2 at transcriptional and translational levels. In a
19 subsequent study, PE was found to reverse DENa-mediated inflammatory cascades by abrogation
20 of hepatic expression of iNOS, 3-nitrotyrosine (3-NT), heat shock protein 70 (HSP70), HSP90,
21 COX-2 and NF- κ B as well as by hindering nuclear translocation of NF- κ B during experimental
22 hepatocarcinogenesis [121]. Finally, PE has been found to inhibit cell proliferation, as evidenced
23 by reduced expression of proliferating cell nuclear antigen (PCNA), altered cell cycle progression

1 (cyclin D1), and induced apoptosis by upregulating Bax and downregulating Bcl-2 under the same
2 experimental conditions [122]. PE also dose-dependently reduced hepatic β -catenin and elevated
3 glycogen synthase kinase-3 β (GSK-3 β) at protein and mRNA levels, indicating that pomegranate
4 bioactive constituents exert chemoprevention of HCC through antiproliferative and proapoptotic
5 mechanisms by modulating Wnt/ β -catenin signaling pathway. Subsequently, El-Ashmawy et al.
6 [123] confirmed the liver cancer preventive effect of pomegranate constituents and several
7 mechanisms of action as previously reported by Bishayee and colleagues [120-122]. In their study,
8 treatment of DENA-exposed rats with pomegranate hull extract (PHE) for 10 weeks (4 weeks
9 before and 6 week after DENA exposure) increased survival of hepatocarcinogen-treated animals,
10 decreased liver tumor size, reduced the expression of Bcl-2, cyclin D1 and β -catenin and elevated
11 hepatic GSH level. All the aforementioned studies suggest that pomegranate phytochemicals target
12 Nrf2, NF- κ B and Wnt/ β -catenin signaling to confer chemoprevention of HCC.

13
14

15 3.2.2.4. Pancreatic cancer

16 *3.2.2.4.1. In vitro*

17 There is at least one study that investigated the effect on pomegranate on pancreatic cancer
18 cells. Nair et al. [124] reported that a standardized whole fruit extract of pomegranate had
19 antiproliferative effect and altered cell cycle progression arrest via increasing the proportion of
20 PANC-1 pancreatic cancer cells that lack CD44 and CD24 expression, which are associated with
21 increased tumor-initiating activity. Similar results were achieved in another pancreatic cancer
22 (AsPC-1) cell line. Isolated pomegranate phytochemicals, ellagic acid, luteolin and ursolic acid,
23 were found to be only modestly effective in suppressing cell proliferation, suggesting synergistic
24 effects of pomegranate phytochemicals.

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3.2.3. Gynecological cancers

3.2.3.1. Uterine cancers

3.2.3.1.1. In vitro

In one of the earliest studies, a polyphenol-rich whole pomegranate fruit extract showed very little effect on the growth of HeLa human cervical cancer cells [125]. Similarly, a lack of effect was observed with a methanolic extract of pomegranate pericarp against HeLa and HEC-1A human endometrial cancer cells; however, a growth-inhibitory effect was observed in SiHa human cervical cancer cells with very high concentration [76]. Interestingly, pomegranate peel and flesh extracts significantly inhibited the proliferation of HeLa cells whereas the extracts of seeds, leaves as well as juice showed less pronounced effects [117]. Later, Fazio et al. [84] observed antiproliferative effects of acetone and methanolic extracts of pomegranate peels against HeLa and Ishikawa (human endometrial adenocarcinoma) cells. The HeLa cells treated with the methanolic extract exhibited higher caspase-3, caspase-7 and caspase-9 activity, PARP cleavage and apoptosis. Ellagic acid, a polyphenol extracted from pomegranate peel, showed inhibition of invasive ability and promotion of apoptotic characteristics of HeLa cells via increased expression of insulin-like growth factor binding protein 7 (IGFBP7) and simultaneous inhibition of Akt/mTOR signaling [126]. Pomegranate husk-derived punicalagin exhibited antiproliferative activities against three cervical cell lines, namely HeLa, SiHa and CaSki, in a concentration-dependent manner, with CaSki cell line being the most sensitive [87]. The antiproliferative effect of punicalagin in HeLa cells was confirmed by a subsequent study that also showed inhibition of cancer cell migration, downregulation of MMP-2 and MMP-9, upregulation of TIMP-2 and TIMP-

1 3, cell cycle arrest in the G₁ phase, induction of apoptosis by upregulation of Bax, downregulation
2 of Bcl-2 and β -catenin and its downstream proteins, cyclin D1 and c-myc [127].

3

4 3.2.3.2. Ovarian cancer

5 *3.2.3.2.1. In vitro*

6 As one of the most lethal gynecologic malignancies, there is great interest in finding novel
7 treatments for ovarian cancer in the face of emerging chemotherapy resistance. While a methanolic
8 extract of pomegranate pericarp failed to exhibit any cytotoxicity against SKOV3 human ovary
9 cancer cells [76], other studies documented antiproliferative effects of a methanolic peel extract
10 [83] and pomegranate juice-derived ellagitannins [80] in the same cell line without an
11 understanding of the mechanisms of action. Additionally, pomegranate seed extract was found to
12 inhibit the growth of SKOV3 cells with an unknown mechanism [92]. Finally, pomegranate fruit
13 juice and its components, ellagic acid and luteolin, were found to suppress the proliferation and
14 migration of another human ovarian cancer cell line, namely A2780, in a concentration-dependent
15 manner via downregulation of the expression of MMP-2 and MMP-9 [128].

16 *3.2.3.2.2. In vivo*

17 Liu et al. [128] expanded their *in vitro* study to confirm the anticancer effects of pomegranate
18 juice and its phytoconstituents using an *in vivo* ovarian cancer model. In this study, ES-2 human
19 ovarian adenocarcinoma cells were injected to the right hind leg of female nude mice. Two weeks
20 following ES-2 cell injection, the tumor-bearing animals were treated with pomegranate fruit juice
21 (20 mL/kg), ellagic acid (50 mg/kg) or luteolin (50 mg/kg) for approximately 24 days. All three
22 treatments inhibited tumor growth and suppressed the expression of intratumor MMP-2 and MMP-
23 9 without any effect on body or spleen weight of experimental animals compared to control.

1

2 3.2.4. Hematological cancers

3 3.2.4.1. Lymphoma

4 3.2.4.1.1. *In vitro*

5 Various pomegranate constituents demonstrated anticarcinogenic effects in diverse
6 hematologic cancers through a variety of mechanisms. Settheetham and Ishida [129] conducted
7 one of the earliest *in vitro* studies to measure the effect of pomegranate extract on cancer cells.
8 The results indicated that boiled-water extract of pomegranate peel inhibited the growth of human
9 Burkitt's lymphoma cell lines, Raji and P3HR-1, via apoptotic DNA fragmentation. Similarly,
10 pomegranate fruit rind-derived galactomannan polysaccharide (PSP001) was found to exhibit
11 significant cytotoxicity against Dalton's ascites lymphoma, a murine lymphoid cancer cell line,
12 through apoptosis induction [78].

13

14

15 3.2.4.2. Leukemia

16 3.2.4.2.1. *In vitro*

17 Kawaii and Lansky [130] evaluated flavonoid-rich fractions from fresh and fermented
18 pomegranate juice and from an aqueous extract of pomegranate pericarp extract as potential
19 differentiation-inducing agents in HL-60 human acute promyelocytic leukemia (APL) cells. The
20 fractions from fermented juice and pericarp extracts were found to exert strong differentiation-
21 promoting effects with proportional inhibitory effects on HL-60 cell proliferation.
22 Antiproliferative activity was also observed when Jurkat (peripheral blood T-cell leukemia), SUP-
23 B14 (acute lymphoblastic leukemia, ALL), MOLT-3 (ALL), CCRF-CEM (ALL), HL-60 (APL),

1 THP-1 (acute monocytic leukemia, AMCL), K562 (chronic myloid leukemia, CML) and KG1a
2 (acute myelogenous leukemia, AML) cell lines were exposed to 6.25-12.5% of pomegranate juice
3 extracts for up to 48 h. The extract-treated cell lines were arrested in G₀/G₁ phase and showed an
4 increase in apoptotic activity [131]. A follow-up study by the same group illustrated the cytotoxic
5 effect of acetonitrile fraction obtained from pomegranate juice against CCRF-CEM (ALL),
6 MOLT-3 (ALL), HL-60 (APL), and THP-1 (AMCL) cells. Additionally, the cells were arrested in
7 S phase, exhibited caspase-3 activation and decreased adenosine triphosphate (ATP) levels
8 (indicative of metabolically active cells) and underwent apoptosis after exposure to the acetonitrile
9 fraction. Accompanying liquid chromatography mass spectrometry analysis revealed that the
10 acetonitrile fraction contained ellagitannins, ellagic acid, and hydroxycinnamic acid derivatives,
11 but depleted in anthocyanins [132]. A pomegranate pericarp extract (PPE) has been found to
12 contain punicalagin anomers (punicalagin A and B), ellagic acid-hexoside and ellagic acid. Self-
13 assembled NPs made of PPE and gelatin (PPE-gelatin NPs) were found to be less effective than
14 PPE in inducing the early stage of apoptosis in HL-60 cells. However, both PPE-gelatin NPs and
15 PPE had similar effects in inducing the late stage of apoptosis and necrosis [133]. In another study,
16 K562 human CML cell line was exposed to PSP001 polysaccharide (isolated from pomegranate
17 fruit rind), resulting in a concentration- and time-dependent increase in cytotoxic activity [86]. In
18 the same leukemia cells (K562), a pomegranate peel extract promoted growth inhibition via G₂/M
19 phase arrest and apoptosis induction through the upregulation of caspase-9, caspase-3, caspase-7,
20 cyt. c, p21 and p53 and Akt, with simultaneous downregulation of Bcl-2 and Bax [134].

21
22 3.2.4.3. Myeloma

23 *3.2.4.3.1. In vitro*

1 Antiproliferative activity was also encountered in U266 human multiple myeloma (MM) cells
2 treated with 1-500 µg/mL of extracts of pomegranate leaves, stems, and flowers for up to 72 h.
3 The leaf and stem extracts triggered apoptosis with an increase in the loss of mitochondrial
4 membrane potential, whereas the flower extract resulted in slight decrease in MMP [135]. Finally,
5 pomegranate juice resulted in antiproliferative effect, G₀/G₁ phase arrest as well as increase in
6 PPAR γ mRNA expression in three MM cell lines, namely U266, KMS26 and MM1S.
7 Interestingly, pomegranate juice or bortezomib (BTZ), a proteasome inhibitor used as a first line
8 drug to treat MM, exhibited better cytotoxic effect than a combination treatment of U266 cell line.
9 However, pre-treatment with pomegranate juice inhibited the cytotoxic effect of BTZ. On the other
10 hand, treatment of U266 cells with pomegranate juice after BTZ exposure improved the cytotoxic
11 effect [136].

12

13 3.2.5. Lung and respiratory tract cancers

14

15 3.2.5.1. *In vitro*

16 A pomegranate fruit (edible portion) extract was tested against an *in vitro* model of human
17 non-small cell lung cancer (A549 cells). Treatment with the extract at 50-150 µg/mL for 72 h was
18 found to decrease the viability of A549 cells with concurrent arrest of cells at G₀/G₁ phase,
19 induction of p21^{WAF1} and p27^{KIP1} and decrease in the expression of Ki-67, PCNA, cyclin D1,
20 cyclin D2, cyclin E, cyclin-dependent kinase 2 (cdk2), cdk4 and cdk6. Additional findings involve
21 inhibition of phosphorylation of MAPK and Akt, decrease in the expression of PI3K, NF- κ B and
22 IKK α , degradation and phosphorylation of I κ B α and inhibition of NF- κ B-DNA binding activity
23 [137]. Subsequently, other investigators confirmed the antiproliferative activity of methanolic
24 extracts of pomegranate peel and seeds against the same lung cancer cell line [83,92]. The

1 anticancer potential of a polysaccharide (PSP001) isolated from rinds of fully ripened fruits of
2 pomegranate was investigated using KB human nasopharyngeal carcinoma cells with positive
3 results [86]. Moreover, silver nanoparticles (SNPs) containing PSP001 (SNP@PSP) exhibited a
4 time- and concentration-dependent cytotoxic behavior in A549 cells via caspase-mediated
5 apoptosis induction. SNP@PSP also showed antimigratory potential based on wound healing
6 assay [107].

7 The anticancer effect of an extract of pomegranate leaf (containing punicalagin and ellagic
8 acid) have been examined in A549, H1299 (human non-small cell carcinoma) and LL2 (mouse
9 Lewis lung carcinoma) cell lines *in vitro*. In all three cell lines, the extract suppressed proliferation
10 and colony formation. In H1299 cells, the extract arrested cell cycle progression in G₂/M phase
11 and induced apoptosis by decreasing ROS accumulation and mitochondrial transmembrane
12 potential ($\Delta\Psi_m$). Further, the extract blocked H1299 cell migration and invasion in parallel with
13 reduction of MMP-2 and MMP-9 expression [138]. Punicalagin isolated and purified from the pith
14 and carpellary membrane of pomegranate fruit was incubated with A549 and Hep-2 human larynx
15 epithelial cancer cells and demonstrated low cytotoxicity, while still demonstrating antioxidant
16 effects mediated through chelation of metal ions, restitution of lipid oxidation products, and
17 decreased levels of ROS and reactive nitrogen species [139]. Punicalagin extracted from
18 pomegranate husk powder exhibited strong antiproliferative activity against A-549 as well as
19 H1299 cells. The mechanism of antiproliferative effect was proposed to be antioxidant effects,
20 including diminished free radicals and decreased oxidized DNA adducts [87]. The same research
21 group extended their earlier study to document antiproliferative and antimutagenic activities of
22 punicalagin and ellagic acid against A549 and H1299 cells [140].

23

1 3.2.5.2. *In vivo*

2 Based on encouraging *in vitro* results, several investigators evaluated the preventive and
3 therapeutic actions of pomegranate-derived products using various *in vivo* models on lung cancer.
4 In a mouse xenograft model with A549 tumors, pomegranate fruit extract (0.1 and 0.2%, w/v) in
5 drinking water resulted in significant inhibition of tumor growth and increased the survival of
6 animals with tumor volumes equal to or less than 1200 mm³ [137]. However, the investigators did
7 not provide any mechanisms of such antitumor effects. In a second study, benzo(a)pyrene [B(a)P]
8 or N-nitroso-tris-chloroethylurea (NTCU) was used to induce lung tumors in A/J mice. The
9 animals had access to drinking water containing pomegranate fruit extract one week before the
10 carcinogenic challenge. Long-term consumption of pomegranate extract reduced lung tumor
11 multiplicity in both models. Accompanying studies revealed several underlying mechanisms of
12 action, such as inhibition of activation NF- κ B and I κ B α kinase (IKK), degradation and
13 phosphorylation of I κ B α , phosphorylation of mitogen-activated protein kinases (e.g., extracellular
14 signal-regulated kinase 1/2, c-Jun NH₂-terminal kinase 1/2, and p38), phosphatidylinositol 3-
15 kinase (p85 and p110), phosphorylation of Akt, activation of mammalian target of rapamycin
16 (mTOR) signaling, phosphorylation of c-met, and suppression of the markers of cell proliferation
17 (Ki-67 and PCNA) and angiogenesis (iNOS, CD31, and VEGF) [141]. Finally, Husari and
18 coinvestigators [142] used cigarette smoke (known to cause oxidative stress, DNA damage and lung
19 cancer) to induce lung nodules and tumors in AJ mice. The animals drank pomegranate juice,
20 starting one week prior to cigarette smoke (delivered through a smoke generator) and continued
21 for 5 months, which reduced nodule incidence, nodule multiplicity, mean nodular size, tumor
22 incidence and tumor multiplicity. Pomegranate juice supplementation also significantly inhibited
23 intranodular mitotic activity, measured using anti-phosphohistone-H3 (PHH3) antibody, and

1 reduced hypoxia-inducible factor-1 α (HIF-1 α) expression. All these studies underscore the value
2 of pomegranate juice consumption in preventing and/or treating human lung cancer.

3

4 3.2.6. Neurological cancers

5 *3.2.6.1. In vitro*

6 Limited studies have been conducted on the association between pomegranate
7 phytochemicals and antitumorigenic effects in the nervous system. In U87MG human glioma cells,
8 pomegranate-derived punicalagin (1-30 $\mu\text{g}/\text{mL}$) administered for 24 h had demonstrated reduced
9 cell viability in association with increased cyclin E, and decreased cyclin A and cyclin B levels.
10 The treatment with punicalagin also triggered apoptosis as evidenced by PARP cleavage,
11 activation of caspase-9 and decrease in Bcl-2 level. The treatment also increased microtubule-
12 associated protein light chain 3 II (LC3-II) cleavage and caused green fluorescence-LC3 fusion
13 protein (GFP-LC3-II)-stained punctate pattern in U87MG cells, indicating autophagy.
14 Additionally, punicalagin increased the extent of AMPK and p27 phosphorylation which
15 correlated with the induction of autophagic cell death [143]. Ferreira et al. [144] prepared
16 pomegranate seed oil nanoemulsion containing ketoprofen using pullulan as a polymeric stabilizer
17 and evaluated its antitumor activity against C6 rat malignant glioma cells. The nanoemulsion
18 suppressed C6 cell growth; however, the associated mechanisms were not studied. In an extended
19 work, the same research group found that 3% pomegranate seed oil had slightly better cytotoxic
20 profile compared to a nanoemulsion containing the same pomegranate product in C6 cells [145].

21

22 3.2.7. Skin cancer

23 *3.2.7.1. In vitro*

1 Very limited information is available regarding *in vitro* effect of pomegranate in skin cancer.
2 Various ellagitanins, including gallic acid, ellagic acid, hexahydroxydiphenic acid, punicallic
3 and punicalagins, from pomegranate juice displayed moderate antiproliferative activity in SK-
4 MEL human malignant melanoma cells [80]. Pomegranate peel-derived galactonmammann
5 polysaccharide (PSP001) exhibited cytotoxicity against another human malignant melanoma
6 (A375) cells, possibly via induction of apoptosis [78].

7

8 **3.2.7.2. *In vivo***

9 Pomegranate fruit and seeds have been used for research to explore anticancer potential in
10 various *in vivo* skin cancer models. Hora et al. [146] reported for the first time a decrease in skin
11 tumor (papilloma) incidence and multiplicity in female CD-1 mice topically treated with DMBA
12 and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and subjected to 5% pomegranate seed oil
13 treatment. Mechanistically, this is thought to be attributed to the observed decrease in TPA-
14 induced epidermal ornithine decarboxylase (ODC), a marker for skin cancer promotion. Afaq and
15 colleagues [147] investigated the inhibitory effect of pomegranate fruit extract, containing
16 anthocyanins, ellagitanins and hydrolyzable tannins, on DMBA-initiated and TPA-promoted skin
17 tumor formation in CD-1 mice. The results indicated that topical application of the extract
18 displayed reduced tumor incidence, total number of tumors and tumor multiplicity. The extract
19 also afforded significant inhibition of TPA-mediated increase in skin edema and hyperplasia,
20 epidermal ODC activity, expression of ODC and COX-2, phosphorylation of ERK1/2, JNK1/2
21 and p38, activation of NF- κ B and IKK α , and phosphorylation and degradation of I κ B α [147].
22 Later, George and coinvestigators [148] confirmed the antiskin tumor effect and associated
23 mechanisms (e.g., interference with MAPK and NF- κ B signaling) of oral pomegranate fruit extract

1 in the same two-stage (DMBA/TPA) mouse skin tumorigenesis model. Additional findings
2 indicated induction of apoptosis and inhibition of proliferation (based on PCNA expression) and
3 DNA strand breaks in the skin tissue/tumor tissue of experimental animals. A synergic effect was
4 observed when the animals received topical application of diallyl sulfide (DAS), a component of
5 garlic, in addition to oral pomegranate juice.

6

7 3.2.8. Urogenital cancers

8 3.2.8.1. Bladder cancer

9

10 3.2.8.1.1. *In vitro*

11

12 Whole fruits or peels of pomegranate cultivars from different geographic locations were
13 subjected to several extraction methods. These extracts evaluated for polyphenolic content,
14 antioxidant capacity and tested for antiproliferative activity against T24 human bladder cancer
15 cells. The Soxhlet extract of peels from fruits of Israeli origin was found to be the most efficacious
16 with the highest ellagic acid content (responsible for antioxidant activity), whereas the aqueous
17 extract from Israeli whole fruit was the least efficacious with lowest ellagic acid content [149].
18 Pomegranate rind extract containing 13% (w/w) ellagic acid (100 ug) was incubated with EJ
19 human bladder carcinoma cells for 48 h and was found to have antiproliferative and proapoptotic
20 effects. These effects were mediated via increased activity of caspase-3, induction of p53,
21 increased miR-34a levels leading to inhibition of c-myc and CD44 [150]. In another study, T24
22 and J82 urinary bladder urothelial carcinoma cell lines were exposed to pomegranate fruit
23 ethanolic extract at various concentrations (5-200 μ l/mL) for 12-72 h. The results demonstrated
24 that the extract was more effective in suppressing the proliferation of T24 cells than J82 cells.
25 Apoptosis induction in T24 cells was mediated via activation of procaspase-3, procaspase-8,

1 procaspase-9, procaspase-12, increase in Bax/Bcl-2 ratio, increased expression of CCAAT-
2 enhancer-binding protein homologous protein (CHOP) and binding immunoglobulin protein
3 (BiP), therefore activating endoplasmic reticulum stress, mitochondrial damage and death receptor
4 pathways [151]. The same research group later reported that 20 proteins, involved in cell
5 proliferation, apoptosis, cytoskeleton regulation, proteasome activity, and aerobic glycolysis, were
6 differentially expressed in ethanolic extract-treated T24 cells. Additional signaling studies
7 revealed that the extract inhibited T24 cells proliferation through restriction of PTEN/Akt/mTOR
8 pathway via profilin 1 (Pfn1) upregulation and evoked apoptosis through inhibition of X-linked
9 inhibitor of apoptosis protein (XIAP) and Diablo over-expression [152]. In an extended study, it
10 has been found that an ethanolic extract of pomegranate peels exhibited better growth-inhibitory
11 activity against T24 and J82 cells than that of pulp. The ethyl acetate fraction of the peel extract
12 demonstrated the highest anticancer activity attributed to apoptosis [153].

13 3.2.8.1.2. *In vivo*

14 Few investigators confirmed bladder cancer-inhibitory effects of pomegranate products *in*
15 *vivo*. Zhou et al. [150] reported that pomegranate rind extract suppressed the growth of xenografted
16 EJ tumor in mice with concomitant upregulation of the expression of miR-34a. Using another
17 xenograft model, Chang et al. [153] showed that oral administration of ethylacetate fraction of
18 ethanolic extract of pomegranate peel decreased the volume and weight of T24 tumors in mice and
19 evoked intratumor apoptosis.

20

21 3.2.8.2. Prostate cancer

22 3.2.8.2.1. *In vitro*

1 Prostate cancer has one of the highest incidences and mortality rates, and certainly has been
2 one of the biggest forefronts in pomegranate research. Albrecht et al. [154] launched the first *in*
3 *vitro* study on the effects of cold-pressed pomegranate seed oil, supercritical CO₂-extracted seed
4 oil, polyphenol extract of fermented pomegranate juice, and polyphenol extract of pomegranate
5 pericarp on prostate cancer cells (LNCaP, PC-3 and DU-145). These investigators observed
6 antiproliferative, proapoptotic and anti-invasive effects of various pomegranate-derived products
7 via induction of cell cycle arrest at G₂/M checkpoint, apoptosis, increased caspases-3 activity, and
8 upregulation of p21^{WAF1} and mitogen-activated protein kinase-activated protein kinase 2 (MAPK-
9 APK2) expression, along with downregulation of growth arrest and DNA-damage-inducible
10 protein 45α (GADD45α) and c-myc gene expression. Lansky et al. [155] extended the previous
11 study and tested the same pomegranate extracts in various combination and reported synergistic
12 antiproliferative and anti-invasive effects, possibly via downregulation of phospholipase A2
13 (PLA2) expression in DU-145 and PC-3 cells. Malik et al. [156] reported similar antiproliferative
14 and proapoptotic effects with pomegranate fruit extract on PC-3 cells, and further proposed
15 possible mechanisms, including upregulation of Bax, Bak, p21^{WAF1} and p27^{KIP1}, along with
16 downregulation of Bcl-XL, Bcl-2, cyclin D1, cyclin D2, cyclin E, and cdk2, cdk4, cdk6 expression.
17 Seeram et al. [103] also observed antiproliferative effect of pomegranate juice, total pomegranate
18 tannin extract, punicalagin and ellagic acid in two other types of prostate cancer cells (RWPE-1
19 and 22Rv1), possibly via the inhibition of lipid peroxidation. Hong et al. [157] observed
20 antiproliferative and proapoptotic effects of a standardized pomegranate extract (POMx),
21 pomegranate juice, punicalagin and ellagic acid against DU-145, LNCaP and androgen receptor
22 (AR) expressing LNCaP (LNCaP-AR) cells. Subsequent mechanistic study showed
23 downregulation of 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2), aldo-keto reductase

1 family 1 member C3 (AKR1C3), steroid 5 α reductase type 1 (SRD5A1) and AR by all
2 pomegranate-derived products. POMx also significantly inhibited the proliferation of LNCaP cells
3 under both normoxic and hypoxic conditions. Under hypoxic environment, POMx exhibited
4 antiangiogenic effect via downregulation of HIF-1 α and VEGF [158]. Using DU-145 cells, Rettig
5 et al. [159] compared the anticancer effect POMx with that of pomegranate juice and found that
6 POMx was more effective in exhibiting cytotoxicity via induction of apoptosis and inhibition of
7 NF- κ B. In addition to reporting the antiproliferative and proapoptotic effects of POMx on LAPC4
8 cells via upregulation of c-Jun N-JNK phosphorylation, downregulation of insulin-like growth
9 factor-1 (*igf-1*) mRNA expression, and decreased Akt and mTOR activation, Koyama et al. [160]
10 found that co-treatment with POMx and IGF binding protein-3 resulted in an intriguing synergistic
11 stimulation of apoptosis and additive inhibition of cell growth.

12 Wang and colleagues [161,162] conducted several studies to explore the potential of
13 pomegranate juice in the prevention and treatment of prostate cancer metastasis. In the first study,
14 in addition to causing cell death of hormone-refractory prostate cancer cells, pomegranate juice
15 increased cell adhesion and decreased cell migration. Mechanistic results revealed upregulation of
16 genes related to cell adhesion, such as ICAM-1 and E-cadherin and downregulated genes involved
17 in cell migration, such as hyaluronan-mediated motility receptor (HMMR) and collagen type I α 1
18 (COL1A1). Interestingly, anti-invasive microRNAs, such as miR-126, miR-200, miR-205 and
19 miR-335, were upregulated, and at the same time pro-invasive microRNAs, such as miR-21 and
20 miR-373, were downregulated. Additionally, pomegranate juice suppressed pro-inflammatory
21 cytokines, such as IL-6, IL-1 β and IL-12P40, and chemokine, namely regulated on activation,
22 normal T cell expressed and secreted (RANTES) and also inhibited the ability of the chemokine
23 stromal-derived growth factor 1 α (SDF1 α) to chemoattract prostate cancer cells [161]. In an

1 attempt to further identify the active phytochemicals associated with the effects of pomegranate
2 juice reported in the preceding study, the same research group [162] published a secondary study
3 to document that a specific combination of pomegranate juice components (luteolin, ellagic acid,
4 caffeic acid and punicalic acid) inhibited the growth of hormone-dependent and hormone-refractory
5 prostate cancer cells and suppressed their migration and chemotaxis towards SDF1 α , a chemokine
6 with important role in prostate cancer metastasis to the bone. The investigators again reported
7 modulatory effects of pomegranate juice components on another extensive list of target genes,
8 miRNAs and proteins related to cell cycle control, adhesion, migration and metastasis.

9 Several independent investigations documented encouraging effects of pomegranate peels
10 against *in vitro* prostate cancer models. In one study, a methanolic extract of pomegranate peel
11 was found to inhibit the proliferation of PC-3 prostate adenocarcinoma cells [83]. Another study
12 reported that an ethanolic extract of pomegranate peel displayed similar antiproliferative effect on
13 PC-3 cells when compared to anticancer drug taxol as a positive control with elevated nucleosome
14 production in apoptotic cells [163]. Finally, Deng et al. [164] reported that pomegranate peel
15 extract (containing punicalagin and ellagic acid) exerted antiproliferative, proapoptotic, anti-
16 invasive and antimigratory effects on a panel of prostate cancer cells (PC-3, DU-145 and TRAMP-
17 C1) with associated dissipation of mitochondrial membrane potential, accumulation of reactive
18 oxygen species, increased Bax to Bcl-2 ratio, elevated caspase-3 activity, downregulation of
19 MMP-2 and MMP-9 and upregulation of TIMP-2.

20 Following the early success with pomegranate seed-derived products as antiprostata cancer
21 agents, various investigators conducted additional *in vitro* experiments with mixed results. An
22 ethanolic seed extract displayed marginal inhibitory effect on the growth of PC-3 cells [163].
23 Likewise, it has been found that a hydrophilic fraction (80% aqueous methanolic extract) of

1 pomegranate seed oil had minimal effect on the viability of DU-145 cells [90]. On the contrary, a
2 methanolic extract of pomegranate seed exerted antiproliferative activity against PC-3 cells even
3 at low concentrations [92]. Interestingly, a pomegranate whole seed ethanolic extract (containing
4 puniic acid, α -linoleic acid and α -linolenic acid) showed a promising antiproliferative activity
5 against hormone-dependent LNCaP cells with an IC₅₀ value 3-fold lower than that of positive
6 control vinblastine [91].

7 Recently, *in vitro* cytotoxic profile of pomegranate extract-loaded solid lipid nanoparticles
8 was investigated in PC-3 cells with results showing nanoencapsulation of the extract enhanced its
9 anticancer efficacy compared to the free extract [97].

10

11

12

13 3.2.8.2.2. *In vivo*

14 Based on encouraging *in vitro* results, several laboratories extended anticancer investigation
15 on pomegranate using various *in vivo* models of prostate cancer. Parenteral administration of
16 pomegranate pericarp polyphenols and supercritically extracted seed oil significantly inhibited the
17 growth of xenografted PC-3 tumor in athymic BALB/c male homozygous nude mice. However,
18 there were no significant differences in survival of animals between experimental and control
19 groups [154]. Malik et al. [156] showed that pomegranate fruit extract in drinking water suppressed
20 tumor growth, increased survival of tumor-bearing animals and lowered serum prostate-specific
21 antigen (PSA) levels in athymic nude male mice injected with androgen-sensitive CWR22Rv1
22 human prostate carcinoma cells. Seeram et al. [165] also reported significant inhibition of tumor
23 growth with the oral administration of ellagitannins-enriched extract of pomegranate fruit skin in

1 severe combined immunodeficient (SCID) mice injected with LAPC4 cells. Subsequently, the
2 same group [158,159] studied antitumor effects and associated mechanisms of action of POMx on
3 the same xenograft animal model. Rettig et al. [159] reported inhibition of growth of androgen-
4 independent LAPC4 xenografts, intratumor antiproliferative and proapoptotic activities,
5 abrogation of NF- κ B activity in tumor samples together with suppression of serum PSA level.
6 Sartippour et al. [158] found that POMx reduced xenograft size and tumor vessel density via
7 downregulation of HIF-1 α , suggesting antiangiogenesis as an additional anticancer mechanism of
8 ellagitannin-rich pomegranate extract. Wang et al. [166] conducted another investigation on the
9 effects of treatment with a combination of pomegranate juice components (luteolin, ellagic acid
10 and puniceic acid) on male SCID mice bearing PC-3M-luc xenograft or *Pten*^{-/-}; *K-ras*^{G12D} allograft
11 tumors. Intraperitoneal (i.p.) injection of pomegranate juice phytochemicals inhibited the growth,
12 angiogenesis and metastasis of PC-3M-luc tumors via suppression of C-X-C motif chemokine 12
13 (CXCL12/SDF1- α)/C-X-C chemokine receptor type 4 (CXCR4) and Akt signaling axis. In *Pten*-
14 ^{-/-}; *K-ras*^{G12D} tumor model, similar effects were observed with tumor growth and metastasis.

15 Adhami et al. [167] evaluated the effects of pomegranate fruit extract against prostate cancer
16 development utilizing a classical chemoprevention protocol of transgenic adenocarcinoma mouse
17 prostate (TRAMP) model. The animals received 0.1 and 0.2% fruit extract in the drinking water
18 for 6, 14 and 28 weeks. The investigators observed that continuous supplementation of fruit extract
19 conferred significant survival advantage, reduced palpable tumor incidence and hindered
20 metastasis to lungs, liver and lymph nodes over water-fed TRAMP mice. Mechanistically,
21 pomegranate extract resulted in concurrent and considerable inhibition of IGF-I/Akt/mTOR
22 pathway in both prostate and tumor tissues.

23

1 **3.3. Clinical studies**

2 With encouraging preclinical results in various cancers, pomegranate constituents attracted
3 several investigators to conduct clinical studies to gather valuable clinical data, predominantly in
4 the field of breast, colon and prostate cancers.

5 6 *3.3.1. Breast cancer*

7 Interest in pomegranate and breast cancer has been growing over the past decade as evident
8 by the rapid rise of preclinical studies demonstrating pomegranate's efficacy in breast cancer cells
9 and animal models. Kapoor et al. [168] conducted the first clinical study on the effects of
10 pomegranate juice (POM Wonderful 100% juice) on various biomarkers associated with breast
11 cancer risk. Sixty-four healthy postmenopausal women randomly assigned to drink 8 ounces of
12 either 100% commercial pomegranate juice (intervention group) or apple juice (control group) for
13 a period of 3 weeks. Interestingly, the investigators reported that there was no significant decline
14 in serum sex hormones or sex hormone binding globulin (SHBG) in the intervention group
15 compared to control (Table 3). Nevertheless, subgroup analyses revealed significant decline in
16 estrone and testosterone levels in 38 normal weight females (intervention group) compared to
17 control, warranting the need for future investigations to determine the effects of pomegranate in
18 reducing breast cancer risk in normal vs overweight/obese populations.

19 20 *3.3.2. Colon cancer*

21 Nuñez-Sánchez et al. [169] has been the pioneer in advancing the field of pomegranate clinical
22 studies in colorectal cancer. In 2014, they investigated whether ellagic acid derivatives and
23 urolithins (produced by gut microbiota from ellagitannins and ellagic acid) in colon tissues from
24 colorectal cancer (CRC) patients receiving oral pomegranate extract (PE, 900 mg/day for 15 days)

1 with different punicalagin:ellagic acid ratio, such low (PE-1) and high (PE-2). Results showed
2 significant levels of ellagic acid derivatives and urolithins in human colon tissue of CRC patients
3 following consumption of pomegranate products [169]. Nuñez-Sánchez et al. [170] subsequently
4 reported that PE displayed a moderate, but statistically insignificant, modulatory effect on the
5 expression of specific colon tissue microRNAs (miR-646, miR-1249, miR-135b-5p, miR-135b-
6 3p, miR-92b-5p, miR-765, miR-496, miR-181c-3p, and miR-18a-3p) in CRC patient who received
7 900 mg per day of PE-1 or PE-2. In another study, the same PE regimen in CRC patients (as
8 indicated earlier) was significantly associated with a counterbalance effect in the expression of
9 several genes, such as cluster of differentiation 44 (*CD44*), β -catenin (*CTNNB1*), cyclin-dependent
10 kinase inhibitor 1A (*CDKN1A*), epidermal growth factor receptor (*EGFR*) and thymidylate
11 synthase (*TYMS*) [Nuñez-Sánchez et al., 2017]. The investigators also reported that the observed
12 changes were not associated with the presence of urolithins or ellagic acid in colon tissues [171].
13 Recently, González-Sarrías et al. [172] found that the previously described regimen with PE-1 or
14 PE-2 decreased the plasma levels of lipopolysaccharide-binding protein (LBP), a marker of
15 metabolic endotoxemia related to CRC progression, in newly diagnosed CRC patients.

16

17 3.3.3. Prostate cancer

18 Pomegranate-derived products have been subjected to substantial clinical investigation in
19 prostate cancer. The earliest study (an open-label, single-center clinical trial) was conducted by
20 Pantuck et al. [173] who reported that oral consumption of 8 ounces of pomegranate juice (Pom
21 Wonderful, Los Angeles, CA, USA), containing 570 mg total polyphenol gallic acid equivalents,
22 daily up to 54 months significant prolonged the prostate-specific antigen (PSA) doubling time
23 (PSADT) in men with a rising PSA level following primary therapy. Additionally, the researchers

1 incubated LNCaP prostate cancer cells with the serum of prostate cancer patients before and after
2 oral pomegranate juice treatment and reported antiproliferative and proapoptotic effects. There
3 was also a significant reduction in oxidative state and sensitivity to oxidation of serum lipid
4 following pomegranate juice consumption [173]. The same group of investigators later conducted
5 a double-blind, placebo-controlled and multi-institutional investigation with expanded population
6 size. Interesting, in contrary to the earlier observation, this study concluded that there was no
7 significant prolongation of PSADT in prostate cancer patients after treatment with a pomegranate
8 liquid extract (Pom Wonderful). However, they noted a specific subtype of patient, specifically
9 men with manganese superoxide dismutase (MnSOD) AA genotype, had significant prolongation
10 of PSADT after pomegranate extract treatment, suggesting that a certain patient population might
11 have increased benefit from the antiproliferative effects of pomegranate [174]. Paller et al. [175]
12 conducted a randomized, double-blind, multi-center phase II study on the effects of pomegranate
13 fruit extract (POMx, Pom Wonderful) in men with recurrent prostate cancer and observed that
14 there was at least 6 months of prolongation in PSADT with 3 g of POMx per day for 6-18 months,
15 along with 13% of patients demonstrating reclamation of PSA levels. In an attempt to identify a
16 mechanism of action of pomegranate constituent in prostate cancer, Freedland et al. [176] also
17 used POMx to study its effect on tissue 8-hydroxy-2'-deoxyguanosine (8-OHdG), an oxidative
18 stress biomarker, Ki-67 (a marker for proliferation), cancer pS6 kinase (a marker of mTOR
19 activity), and NF- κ B (a measure of inflammation). The dietary intervention with POMx before
20 radical prostatectomy did not significantly lower the level of 8-OHdG compared to placebo group.
21 The changes in other markers as well as serum PSA levels were also similar between two groups.

22 Based on a clinical study conducted in Spain, González-Sarriás et al. [177] reported detection
23 of pomegranate polyphenol metabolites (e.g., ellagic acid metabolites and urolithins) in prostate

1 tissue of prostate cancer patient after pomegranate juice intake (200 mL/day for 3 days), suggesting
2 the metabolites found in the tissue may be the active ingredient responsible for pomegranate's
3 potential therapeutic effects in prostate cancer. However, no significant changes in the expression
4 of three proliferation biomarkers, namely *CDKN1A*, *MKi-67* and *c-Myc*, were noticed in prostate
5 tissue samples following pomegranate juice consumption. Stenner-Liewen et al. [178] conducted
6 a phase IIb, double-blind, randomized, placebo-controlled clinical trial in Switzerland to evaluate
7 the effect of pomegranate juice (Biotta AG, Egnach, Switzerland) intake in patients with prostate
8 cancer. Interestingly, they concluded that there was no difference between the treatment and
9 placebo group with regards to PSA kinetics and pain scores. Finally, Thomas et al. [179] used a
10 whole food supplement blend containing pomegranate whole fruit powder, turmeric powder,
11 broccoli powder and green tea extract to conduct a double-blind, randomized placebo-controlled
12 trial in the United Kingdom. They found that the intervention for 6 months demonstrated
13 significant suppression of rising PSA levels in prostate cancer patients. The therapeutic potential
14 of pomegranate in prostate cancer continues to be controversial, warranting the need for
15 additional clinical trials with long-term study design and appropriate biomarkers of disease
16 progression.

17

18 **4. Bioavailability and pharmacokinetics of pomegranate constituents**

19 Various research studies have been conducted to determine the bioavailability and
20 pharmacokinetics of pomegranate in both preclinical and clinical settings. These studies focused
21 mostly on the metabolites of ellagitannins, punicalagin and ellagic acid.

22 When rats were provided with a diet containing 6% punicalagin (derived from pomegranate
23 husk) for 37 days, their plasma became saturated on the 7th day of the study with a mean

1 concentration of 29 $\mu\text{g/mL}$ of punicalagin. Three isomers of punicalagin were found in the plasma.
2 The researchers also detected five punicalagin metabolites, such as two ellagic acid derivatives,
3 gallagic acid, 3,8-dihydroxy-6H-dibenzo[*b,d*]pyran-6-one glucuronide and 3,8,10-trihydroxy-6H-
4 dibenzo[*b,d*]pyran-6-one, in the kidney and liver [180]. In another study by the same group, rats
5 were fed 6% punicalagin for 37 days to evaluate the bioavailability and metabolism of this
6 pomegranate phytochemical. In plasma, the main detectable entities were punicalagin as well as
7 glucuronides of methyl ether derivatives of ellagic acid. Only 3-6% of ingested punicalagin was
8 detected in the pure or metabolic form in urine and feces. The main urinary metabolites were
9 aglycones or glucuronic conjugates of dihydroxy and trihydroxy-6H-dibenzo[*b,d*]pyran-6-one.
10 The feces contained hydrolyzed metabolites of punicalagin, including aglycones, gallagic acid,
11 ellagic acid, and punicalin, along with microflora ellagic acid derivative, such as 3,8-dihydroxy-
12 6H-dibenzo[*b,d*]pyran- 6-one. Additionally, this study noted two stages of metabolism of
13 punicalagin in rats. In the first stage, hydrolyzed and conjugated punicalagin were the main
14 metabolites. In the second stage, the gut microflora metabolites predominated and were present in
15 the feces as well as their conjugated products in the plasma [181].

16 A later study used pigs that were fed with ellagitannins over a period of 177 days to determine
17 the bioavailability and metabolism of these compounds. The results demonstrated that
18 ellagitannins released ellagic acid in the jejunum and subsequently intestinal flora metabolized
19 ellagic acid sequentially to produce tetrahydroxy- (urolithin D), trihydroxy- (urolithin C),
20 dihydroxy- (urolithin A), and monohydroxy- (urolithin B) dibenzopyran-6-one metabolites which
21 were absorbed from the intestinal walls. The study also explains why urolithins persist for elevated
22 periods of time and equates this fact to enterohepatic circulation. Moreover, the presence of ellagic
23 acid metabolites in bile and urine and its absence in intestinal tissues indicates its absorption in the

1 stomach. In addition, there was no organ accumulation of ellagitannins in lung, liver, heart, kidney,
2 muscle, or adipose tissues in pigs [182].

3 Seeram et al. [183] conducted one of the first clinical studies to investigate the bioavailability
4 of ellagitannins and ellagic acid. One human subject consumed 180 mL pomegranate juice that
5 contained 318 mg punicalagins (major pomegranate ellagitannins) and 25 mg ellagic acid. While
6 no intact ellagitannins were found in the plasma, ellagic acid was detected and reached a maximum
7 concentration of 31.9 ng/mL at 1 h following consumption. Interestingly, ellagic acid was no
8 longer detected in the subject's plasma after 4 h. In a follow-up study, the same research group
9 [184] explored the pharmacokinetics of ellagitannins in 18 healthy volunteers who consumed 180
10 mL of pomegranate juice that contained punicalagins (387 mg/L) and ellagic acid (12 mg/L) one
11 time and blood and urine samples were taken at different time intervals. Ellagic acid was detected
12 in plasma samples of all human subjects with a maximum concentration (C_{\max}) of 0.06 ± 0.01
13 mmol/L, area under the concentration-time curve (AUC) of 0.17 ± 0.02 ($\mu\text{mol} \cdot \text{h}$) $\cdot \text{L}^{-1}$, a time of
14 maximum concentration (t_{\max}) of 0.98 ± 0.06 h and elimination half-life ($T_{1/2E}$) of 0.71 ± 0.08 h. The
15 major ellagic acid metabolites found in plasma and urine were dimethylellagic acid glucuronide
16 and urolithin derivatives. Although ellagic acid was no longer detected in the plasma 5 h after
17 ingestion, urolithin metabolites were still found in the urine 48 h after ingestion. Therefore,
18 urolithins might contribute to long-term therapeutic effects of pomegranate juice consumption
19 beyond the effects exerted by ellagitannins and ellagic acid. The same investigators [185]
20 expanded the previous studies and compared bioavailability of polyphenols from various
21 pomegranate products. Sixteen healthy volunteers sequentially consumed, with one-week washout
22 period between treatments, pomegranate juice, a polyphenol liquid extract preparation and
23 polyphenol powder extract, containing 857, 776 and 755 mg of polyphenols as gallic acid

1 equivalents, respectively. The bioavailability based on plasma ellagic acid levels over a 6 h period
2 did not exhibit statistical differences in AUC for three products. Similar results were observed with
3 the level of urinary urolithon-A glucuronide. However, the t_{\max} was delayed for the powder extract
4 (2.58 ± 0.42 h) compared to juice (0.65 ± 0.23 h) and liquid extract (0.94 ± 0.06 h).

5 Cerdá and colleagues [186,187] conducted several clinical studies to evaluate the
6 bioavailability and metabolism of pomegranate juice ellagitannins. In one study, six healthy
7 participants (four men and two women) drank 1 L of pomegranate juice containing 5.58 g/L of
8 polyphenols, including 4.37 g/L of punicalagin isomers, for 5 days. Intact punicalagin and ellagic
9 acid present in the juice did not show in the plasma or the urine of participants. However, the
10 plasma contained three microbial ellagitannin metabolites, the main metabolite being a derivative
11 of urolithin A. The urine contained six different metabolites and the main metabolite was also the
12 same derivative of urolithin A [186]. In another study, fecal samples donated by six volunteers
13 were incubated with ellagic acid and punicalagin under anaerobic conditions. Urolithin A was
14 produced from both ellagic acid and punicalagin after fermentation in all the fecal cultures after
15 24 h. Urolithin A was produced with varying production rates and concentration in various fecal
16 samples, indicating different composition of fecal microflora in individuals [187].

17 The absorption and metabolism of pomegranate polyphenols were examined on 11 healthy
18 human subjects fed 800 mg of standardized pomegranate extract in a capsule with 330.4 mg of
19 punicalagins and 21.6 mg of ellagic acid. The plasma samples were examined and five major
20 metabolites, namely urolithin A, hydroxy-urolithin A, urolithin A-glucuronide, urolithin B and
21 dimethyl ellagic acid-glucuronide were detected. Like the aforementioned study, the levels of these
22 metabolites varied between subjects and between time intervals. The pharmacokinetic analysis
23 revealed a C_{\max} of 31.9 ng/ml at one hour and a t_{\max} of 33.8 ± 12.7 ng/mL at 1 h [188]. Very recently,

1 García-Villalba and colleagues [189] identified four previously unknown urolithins, namely
2 4,8,9,10-tetrahydroxy urolithin (urolithin M6R), 4,8,10-trihydroxy urolithin (urolithin M7R),
3 4,8,9-trihydroxy urolithin (urolithin CR), and 4,8-dihydroxy urolithin (urolithin AR), in human
4 feces following daily intake of a pomegranate extract (160-640 mg phenolics). The new
5 metabolites were identified only in 19% of the subjects. Moreover, phase II conjugates of the novel
6 urolithins were detected in urine, confirming their absorption, circulation, and urinary excretion.
7 The endogenous production of these "R" urolithins can be considered an additional metabolic
8 feature for volunteer stratification.

9 Based on the results obtained from the aforementioned studies, major pomegranate
10 phytochemicals are absorbed from the gastrointestinal tract and become bioavailable. The main
11 metabolic products of ellagitannins appear to be urolithins which are formed by gut microflora and
12 the difference in gut microflora also explain the contrasting concentrations and the rate of
13 formation in individuals. Nevertheless, further research needs to be conducted regarding the effects
14 of different compositions of microflora on urolithin formation. Moreover, long-term analysis needs
15 to be carried out to determine the effects of prolonged metabolite formation. The metabolites of
16 ellagitannins may be responsible for the long-term therapeutic effects of pomegranate, including
17 its anticancer potential. However, more studies must be conducted to validate this premise.

18

19 **5. Toxicity studies**

20 Pomegranate is a fruit that has been consumed as well as used for its medicinal properties
21 since ancient times. Due to this fact, pomegranate has been generally regarded as safe for
22 utilization in humans. Numerous studies using micriorganisms, animals and human have been
23 conducted regarding the toxicity of various pomegranate products, such as pomegranate juice,

1 whole pomegranate extract, pomegranate seed oil, pomegranate emulsion and pomegranate
2 ellagitannins [190].

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6 *5.1. In vitro*

7 Vidal and colleagues [62] performed one of the earliest studies to evaluate the toxicity of a
8 hydroalcoholic extract of whole pomegranate fruit used in Cuban traditional medicine. Using a
9 chick embryo model, the investigators showed that the extract at a level below 0.1 mg per embryo
10 did not exhibit any toxicity. The genotoxicity of a pomegranate whole fruit hydroalcoholic extract
11 was assessed using various *in vitro* assays to detect DNA damage at different expression levels.
12 The results indicated genotoxic effects of the extract based on point reverse-mutation and mitotic
13 gene-conversion test in microorganisms as well as sister chromatid exchange and chromosomal
14 aberration assays in Chinese hamster ovary cells [191]. Meerts and colleagues [192] conducted a
15 series of *in vitro* experiments to evaluate the toxicity of pomegranate seed oil (PSO) and showed
16 no mutagenicity based on the Ames test as well as chromosomal aberration test in human
17 lymphocytes. Similarly, Heilman et al. [193] indicated that urolithin A, a predominant urolithin
18 isoform found in the plasma and urine following the consumption of ellagitannins and ellagic acid,
19 was not genotoxic.

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21 *5.2. In vivo*

22 In OF-1 mouse model, the median lethal dose (LD₅₀) of a pomegranate fruit extract following
23 intraperitoneal administration to both sexes was found to be 731 mg/kg body weight. In male

1 Wistar rats, a repeated intranasal administration, at a dose of 0.4 or 1.2 mg/kg, produced no toxic
2 effects as evidenced from food intake, weight gain, behavioral observations as well as biochemical
3 and histopathological indices [62]. Patel et al. [194] systematically examined the acute and
4 subchronic toxicity of whole pomegranate fruit extract on mouse and rat models. The extract was
5 standardized to contain 70% polyphenols, including 30% punicalagins, 5% ellagic acid and 0.3%
6 gallic acid. In the acute toxicity study, the oral LD₅₀ of the extract was found to be greater than 5
7 g/kg body weight and the intraperitoneal LD₅₀ of the extract was 187.5 mg/kg (Swiss albino mice)
8 and 217.5 mg/kg (Wistar rats). In the subchronic study, oral administration of the extract up to 600
9 mg/kg body weight/day for 90 days in rats did not lead to any adverse effects based on general
10 observations, ophthalmic examinations, growth patterns, food consumption, organ weights and
11 histopathological assessments. Based on the experimental results, the no observed-adverse-effect
12 level (NOAEL) of the extract was determined as 600 mg/kg body weight/day. Considering the fact
13 that a 6 oz of pomegranate juice contains 266-337 mg of punicalagin, 32-40 times lower than the
14 highest dose tested in this study, it can be concluded that human consumption of pomegranate
15 juice should have no toxicity. Interestingly, another study indicated genotoxic effect of a whole
16 fruit extract based on bone marrow micronucleus assay in Balb/C mice [191]. However, an
17 ethanolic extract of pomegranate fruit or leaf did not exhibit any mutagenic effects in Swiss mice
18 [195].

19 The acute oral toxicity study in female Wistar rats revealed devoid of toxic effects of PSO
20 at 2,000 mg/kg body weight. Dietary administration of PSO at 10,000, 50,000 or 150,000 ppm
21 over a 28-day period elicited no adverse effects or mortality. However, in the 150,000-ppm group,
22 both male and female rats experienced elevated hepatic enzymes (alkaline phosphatase, alanine
23 aminotransferase and aspartate aminotransferase) in plasma and increased liver to body weight

1 ratio. These effects were ascribed to very high fatty acid level in diet rich in PSO without any
2 toxicological relevance. The NOEL in this study was 50,000 ppm PSO (4.3 g/PSO/kg/day) [192].

3 Oral administration of a pomegranate emulsion (10 g/kg body weight), three times a week,
4 for 22 weeks in male Sprague-Dawley rats was devoid of any toxic manifestation as evidenced
5 from growth pattern as well as general observations related to food and water intake and behavioral
6 changes. Additionally, assessment of cardiac performance by transthoracic echocardiography did
7 not reveal any cardiotoxicity in rats [120]. The same extract at 5 g/kg body weight (p.o., three
8 times a week) for 18 weeks did not exhibit any toxicity in female Sprague-Dawley rats [99] as
9 observed previously in male counterparts [120].

10 Various pomegranate phytoconstituents and metabolites have been subjected to safety
11 assessment by different laboratories. A study conducted by Cerdá et al. [180] found no adverse
12 effects of a diet containing 6% punicalagin in rats over the course of 37 days. The dose of
13 punicalagin was increased weekly and the average daily intake of punicalagin was 4.8 g/kg of body
14 weight per day. The authors concluded that this was comparable to a 70 kg human consuming 194
15 L per day of pomegranate juice. No histological abnormalities were noted in the liver or the kidney.
16 Similarly, oral administration of ellagitannins in Sprague-Dawley rats over a time period of 96
17 days did not result in adverse effects or significant differences in eating patterns, growth rates,
18 blood parameter values, and histopathological characteristics of liver and kidney [196]. Heilman
19 et al. [193] described the safety profile of direct oral administration of urolithin A to Wistar rats.
20 A diet containing urolithin up to 5% for 28 or 90 days did not show any alteration in growth pattern
21 as well as blood chemistry and hematology and urinalysis results and also did not indicate any
22 target organ toxicities. The NOAEL was found to be the highest dose tested (i.e., 5% urolithin in
23 diet) or 3,451 and 3,826 mg/kg body weight/day in male and female rats, respectively.

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5.3. Clinical studies

Several clinical studies evaluated the safety aspects of pomegranate-derived products in humans. Pomegranate juice (8 oz) consumption by men with rising PSA level following primary therapy did not produce any serious adverse effects and the treatment was well tolerated [173]. A randomized, multicenter, double-blind, phase II study evaluated the effects of pomegranate extract on men with elevated PSA levels. The researchers did not find any toxic effects of the extract. However, diarrhea was reported as a side effect which occurred in 1.9% and 13.5% of participants taking 1 and 3 g dose, respectively [175]. A follow-up study also reported a lack of serious adverse events in pomegranate extract-treated group as well as in placebo group. Both group experienced nausea and diarrhea [176]. The safety and tolerability of short-term or long-term consumption of pomegranate juice or extract have been confirmed by other investigators [178,179,197-199]. Heber et. al. [200] tested the safety of pomegranate ellagitannin-enriched polyphenol extract (POMx) in overweight individuals in their pilot clinical studies. Participants consumed 0.710 mg, or 1,420 mg of POMx in a capsular form and there were no significant toxic effects reported in any test subjects.

5.4. Assessment of safety profile of pomegranate

The multitude of animal studies suggests that there should be minor to no adverse or toxic effects of pomegranate when used in humans. Minor side effects were found in human clinical trials, such as nausea and diarrhea. In addition, most of the side effects in the animal studies were attributed to components other than the pomegranate compounds. However, given the fact that the

1 aforementioned study by Sanchez-Lamar et al. [191] concluded that whole pomegranate does have
2 mutagenic effects at high concentrations, additional studies need to be conducted on the individual
3 components of pomegranate to determine which components have the greatest mutagenic effects.
4 Further human clinical studies, specifically regarding the toxicity of prolonged use of
5 pomegranate, need to be conducted in order to fully conclude the safety of pomegranate use in
6 humans.

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9 **6. Conclusion and future directions**

10 Known as “a phytochemical reservoir of heuristic medicinal value” [4], pomegranate has
11 acquired a prominent role in traditional medicines for the treatment of various disorders. During
12 the last few decades, pomegranate constituents have been extensively studied for their ability to
13 prevent and treat cancer, establishing its role as a chemopreventative and chemotherapeutic agent
14 with high potential. Nevertheless, the full potential pomegranate in cancer prevention and therapy
15 as well as its molecular targets in cancer are not completely understood. **This work analyzed**
16 **available preclinical *in vitro* and *in vivo* results as well as clinical studies and presented a**
17 **systematic review of available evidences on anticancer effects of pomegranate-derived**
18 **constituents with an emphasis on their various molecular targets.**

19 We have found that pomegranate constituents exhibit cytotoxic and antiproliferative effects
20 against numerous cancer cells. In addition, pomegranate-based products suppressed the
21 development and reduced the growth of chemically-induced or xenografted tumors in rodents. The
22 anticancer effects of pomegranate phytochemicals could be mediated through antioxidant, anti-
23 inflammatory, immune-modulatory, cell death-inducing (apoptosis and autophagy), anti-invasive,
24 antimigratory and antiangiogenic effects as well as modulation of various oncogenic and

1 oncosuppressive signaling pathways (Fig. 8). Based on studies presented here, it is highly likely
2 that numerous pomegranate phytochemicals exhibited synergistic effect in inhibiting the
3 proliferation of tumor cells and/or suppressing the development and growth of tumors. Emerging
4 evidence suggests that bioactive plant phytochemicals exert cancer preventive and anticancer
5 effects when they are used in combination rather than individually. Hence, it plausible that
6 pomegranate phytochemicals may confer the observed chemopreventive and anticancer activities
7 via promotion of multifactorial effects utilizing chemical synergy. Interestingly, pomegranates
8 phytochemicals not only act synergistically with its own phytochemicals, but also with other
9 phytochemicals and even with other established chemotherapeutic drugs to confer superior
10 anticancer action to that of any single compound, providing strong evidence of pomegranate's
11 powerful potential as chemotherapy adjunct. In clinical trial, there was clear evidence that oral
12 ingestion of pomegranate extract result in the accumulation of its active metabolite in various
13 target organs in prostate and colorectal cancer. Furthermore, several cancer-related molecular
14 markers were shown to be modulated by oral intake of pomegranate extract, supporting the
15 preclinical findings. However, due to various limitations in clinical studies, many studies have also
16 reported no significant differences between pomegranate-treated and placebo groups. Yet
17 subsequent subgroup analysis has reported significant effect with pomegranate therapy, thus
18 suggesting that certain population of cancer patient may benefit more from pomegranate's
19 therapeutic activity. This warrant the need for more *in vivo* and clinical data before definite
20 conclusions can be made.

21 Moreover, we have identified several limitations within the current field pomegranate research.
22 First, the poor bioavailability of pomegranate phytochemicals may limit therapeutic outcome.
23 Several clinical studies reported that the poor bioavailability of pomegranate phytochemicals after

1 ingestion of pomegranate supplement can contribute to the disconnection between the observed
2 preclinical and clinical effects. Secondly, most of the *in vitro* studies are carried out using breast,
3 prostate and gastrointestinal tract cancer cells, suggesting the need to diversify and investigate the
4 effects of pomegranate on various other types of cancer. Lastly, few clinical studies are conducted
5 with limited sample size and cancer endpoints; moreover, some preclinical results (anticancer
6 effects and mechanisms) failed to be replicated in clinical trials. Despite the limitations,
7 pomegranate remains to have high potential as a source of as chemotherapeutic and
8 chemopreventive drugs. Based on various safety assessment data, pomegranate products seem to
9 be nontoxic *in vitro*, *in vivo*, and clinically. This suggest that pomegranate research remains
10 extremely valuable as an alternative to traditional chemotherapy to improve efficacy and reduce
11 severity of side effects.

12 Based on the current limitations we have identified, we recommend future investigations to
13 explore the anticancer synergistic effects of pomegranate phytochemicals, compare the effects of
14 pomegranate against cancer across multiple organ systems, identify additional anticancer
15 molecular targets of pomegranate to explain the various anticancer effects of pomegranate, conduct
16 randomized clinical trials with expanded sample size, and analyze cancer endpoints and
17 biomarkers. Improving the bioavailability of pomegranate phytochemicals should also be
18 investgated by using novel formulations and advanced drug delivery systems, such as micelles,
19 liposomes, nanoparticles and nano-emulsions to further potentiate the observed preclinical effects
20 of pomegranate in clinical trials. Additionally, more research should be directed to evaluation of
21 anticancer potential of metabolic products of pomegranate phytochemicals as well as agents
22 derived by colonic microbial metabolism. Based on the overwhelming evidence presented and
23 analyzed in this review, pomegranate constituents evidently remain extremely promising as both

1 chemopreventative and chemotherapeutic agents. This warrants the need for future investigators
2 to further optimize previous studies to establish the full therapeutic potential of this complex fruit
3 to combat a complex disease, such as cancer.

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5 **Conflict of interest**

6 Authors declare no conflicts of interest.

7

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3

4

1 **Legends to figures:**

2

3 **Fig. 1.** Polyphenols present in pomegranate fruit.

4

5 **Fig. 2.** Anthocyanins present in pomegranate fruit.

6

7 **Fig. 3.** Lipids and sterols present in pomegranate seeds.

8

9 **Fig. 4.** Phytochemicals present in pomegranate leaves.

10

11 **Fig. 5.** Alkaloids present in pomegranate root.

12

13 **Fig. 6.** Phytochemicals present in pomegranate flowers.

14

15 **Fig. 7.** PRISMA flow chart describing the process of literature search and study selection related
16 to pomegranate in cancer research. The total number of *in vitro*, *ex vivo*, *in vivo* and clinical studies
17 (160) is greater than the number of studies included in our work (151) because several publications
18 reported results from more than one study type (e.g., *in vitro* and *in vivo* or *in vitro* and *ex vivo*).

19

20 **Fig. 8.** Major anticancer mechanisms and molecular targets of pomegranate-derived constituents
21 based on *in vitro* and *in vivo* studies.

22

Table 1.*In vitro* and effects of pomegranate extracts, fractions and pure compounds on various cancers.

Materials tested	Cell lines used	Anticancer effects	Mechanisms	Conc. & time	References
<i>Breast cancer</i>					
Fermented juice polyphenols, fresh juice polyphenols, pericarp polyphenols, seed oil	MCF-7, MDA-MB-231, MDA-MB-435	Inhibited cell proliferation and invasion	↑Apoptosis; ↓VEGF; ↑MIF	50-350 µg/mL; 4 h	Kim et al. [66]; Toi et al. [67]
Pomegranate extract (with or without genistein)	MCF-7	Caused cytotoxicity and reduced cell survival	↑Apoptosis	1-6 µg/mL; 24, 72 h	Louis Jeune et al. [68]
Aqueous fruit extract	MDA-MB-231, SUM 149	Suppressed proliferation, invasion and motility	↑Apoptosis; ↓NF-κB p65; ↓NF-κB p50; ↓RhoA; ↓RhoC	50-200 µg/mL; 1-5 days	Khan et al. [69]
Pomegranate juice (PJ) or luteolin (L)+ellagic acid (E)+punicalic acid (P)	MCF-7, MDA-MB-231	Inhibited cell growth, stimulated adhesion, suppressed migration and chemotaxis	↑E-cadherin; ↓HMMR; ↓TWIST; ↓IL-8; ↓RANTES; ↓PDGFB	PJ: 1, 5% L+E+P: 1-8 µg/mL; 12-72 h	Rocha et al. [70]
Aqueous fruit extract	HMLER; Hs578T	Inhibited formation and promoted differentiation of mammosphere; inhibited cell migration and increased adhesion	↓TWIST1; ↓HMMR; ↓PI3KCA; ↓AKT1; ↓JNK1; ↓JNK2; ↓ALDH1; ↑CLDN4	5, 10 µg/mL; 48 h	Nallanthighal et al. [71]
Methanolic extract of fruit peel	MDA-MB-231	Inhibited cell migration and invasion	↑Apoptosis; ↑ICAM-1; ↓MMP-9; ↓fibronectin; ↓VEGF; ↓vimentin; ↓ZEB1; ↓β-catenin;	12.5-1,000 µg/mL; 24-72 h	Ahmadiankia et al. [72]; Bagheri et al. [73]

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			↑E-cadherin		
Pomegranate ellagitannin-derived compounds	MCF-7aro	Inhibited cell proliferation	↓Aromatase	1-5 μmol/L; 48 h	Adams et al. [74]
Conjugated fatty acids from seed oil	MCF-7; MDA-MB-231	Exhibited antiproliferative effects	↓ERα; ↓ERβ (MCF-7)	50-100 μM; 5 days	Tran et al. [75]
Methanolic extract of pericarp	MCF-7	Reduced cell viability and proliferation	↓E2 binding to ER; ↓pS2; ↓ERα; ↓PR	10-320 μg/mL; 48 h	Sreeja et al. [76]
Methanolic extract of pericarp	MCF-7	Inhibited 27HC-induced cell proliferation	↓pS2; ↓ERα; ↓PR; ↓NCOR1	100 μg/mL; 48 h	Vini et al. [77]
Polysaccharide from fruit rind (PSP001)	EAC	Exhibited cytotoxic activity		0.001-1,000 μg/mL; 24, 48 h	Joseph et al. [78].
Pomegranate extract	MMTV-Wnt-1 cells	Inhibited cell proliferation and viability	⊥G ₀ /G ₁ ; ↑caspase-3	10-200 μg/mL; 24-96 h	Dai et al. [79]
Pomegranate juice-derived ellagitannins	BT-549	Inhibited cell proliferation		IC ₅₀ =47-198 μM; 48 h	Kasimsetty et al. [80]
Pomegranate extract (with or without tamoxifen)	MCF-7	Inhibited cell viability	↑Apoptosis; ↑Bax; ↓Bcl-2; ⊥G ₀ /G ₁	300 μM; 48 h	Banerjee et al. [81]
Methanolic extract of fruit peel	MCF-7	Reduced cell proliferation	↑Apoptosis; ↑Bax; ↓Bcl-2; antioxidant activity	25-300 μg/mL; 24-72 h	Dikmen et al. [82]
Methanolic extract of fruit peel	MCF-7	Exhibited antiproliferative effect		5-1,000 μg/mL; 72 h	Modaeinama et al. [83]

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Acetone and methanolic extracts of fruit peels	MCF-7; MDA-MB-231	Reduced tumor cell viability	Antioxidant activity	5-960 µg/mL; 72 h	Fazio et al. [84]
Polyphenol-rich extract of fruit peel	MCF-7; MCF-7/DX	Inhibited cell growth	↑Apoptosis; antioxidant activity	1-75 µg/mL; 24 h	Moreira et al. [85]
Polysaccharide from fruit rind (PSP001)	MCF-7	Exerted cytotoxic effect	Antioxidant and immunomodulatory activities	0.001-1,000 µg/mL; 24-72 h	Joseph et al. [86]
Punicalagin from pomegranate husk	MCF-7	Displayed antiproliferative activity	↓Oxidative DNA damage	12.5-200 µg/mL; 72 h	Aqil et al. [87]
Extract of pomegranate fruit skins (POMX)	MCF-7	Inhibited cell growth	↑Apoptosis; ↓G ₂ /M; ↓DNA repair; ↓HR genes; ↑miRNA-24; ↑miRNA-132; ↑miRNA-183	20-100 µg/mL; 24-96 h	Shirode et al. [88]
Polyphenolic extract of pomegranate juice	MDA-MB-231, BT-474	Inhibited proliferation	↑Apoptosis; ↓sp1; ↓sp3; ↓sp4 ↓survivin; ↓VEGF; ↓VEGFR-1; ↓NF-κB; ↓miR-27a; ↓miRNA-155; ↑ZBTB10; ↑SHIP-1; ↓pPI3K; ↓pAkt	2.5-50 µg/mL; 48 h	Banerjee et al. [89]
Hydrophilic fraction of seed oil	MCF-7; MDA-MB-231	Decreased cell viability	↑G ₀ /G ₁ ; ↓VEGF; ↓IL-2; ↓IL-6; ↓IL-12; ↓IL-17; ↓CXCL10; ↓MIP-1α; ↓MIP-1β; ↓MCP-1; ↓TNF-α	0.12-0.6 µL 24 h	Costantini et al. [90]
Ethanol extract of seeds	MCF-7	Exerted antiproliferative activity	Antioxidant activity	5-100 µg/mL; 48 h	Lucci et al. [91]
Methanolic extract of seeds	MCF-7	Registered growth inhibition		5-1,000 µg/mL;	Seidi et al. [92]

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				72 h	
Ethanollic flower extracts	MCF-7	Exhibited cytotoxic activities	Antioxidant activity; ↓5-LOX	IC ₅₀ = 33-35 mg/L; 48 h	Bekir et al. [93]
PLGA-PEG NPs containing pomegranate extract, punicalagin or ellagic acid	MCF-7; Hs578T	Reduced cell growth		1-10 µg/mL; 96 h	Shirode et al. [94]
Ag-NPs of pomegranate extract	MCF-7	Inhibited proliferation	↑Apoptosis	2.5-50 µg/mL; 1-3 days	Sahin et al. [95]
Ag-NPs of pomegranate extract	MCF-7	Inhibited proliferation	↑Apoptosis	2.5-50 µg/mL; 1-3 days	Sahin et al. [96]
SL-NPs of pomegranate extract	MCF-7	Exhibited cytotoxicity		1-500 µg/mL; 24 h	Badawi et al. [97]
<i>Gastrointestinal tract and associated cancers</i>					
Pomegranate juice, tannin extract, ellagic acid and punicalagin	KB, CAL27 (oral)	Displayed antiproliferative activity		12.5-100 µg/mL; 48 h	Seeram et al. [103]
Pomegranate juice-derived ellagitannins	KB (oral)	Inhibited proliferation		IC ₅₀ =153-332 µM; 48 h	Kasimsetty et al. [80]
Pomegranate polyphenolic extract	CAL27, HSC-2, SCC 1483 (oral)	Displayed cytotoxic effects	↑Caspase-3; ↑PARP cleavage; ↓GSH	25-250 g/mL; 24 h	Weisburg et al. [104]

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Pomegranate juice, tannin extract, ellagic acid and punicalagin	HT-29, HCT116, SW480, SW620 (colon)	Displayed antiproliferative activity	↑Apoptosis (HT-39 & HCT116)	2.5-100 µg/mL; 48 h	Seeram et al. [103]
Pomegranate juice-derived ellagitannins	HT-29 (colon)	Inhibited cell proliferation and clonogenic efficiency	↑Caspase-3; ⊥G ₀ /G ₁ ; ⊥G ₂ /M	IC ₅₀ =123-462 µM; 24-72 h	Kasimsetty et al. [80]
Pomegranate extract	HT-29 (colon)	Reduced cell viability	↑Caspase-3; ↑PARP cleavage; ↓COX-2; ↓VCAM-1; ↓NF-κB p65; ↓VEGF; ↓pAkt; ↑miR-126	5- 25 µg/mL; 24-72 h	Banerjee et al. [105]
Peel-derived ellagitannins (PET); PET+5-FU	HT-29 (colon)	Exhibited cytotoxicity	⊥S; dissipation of mitochondrial membrane potential; ↑Bax:Bcl-2; ↑caspase-3; ↑caspase-9	80 µg/mL; 80 µg/mL PET+40 µg/mL 5-FU; 48 h	Chen et al. [106]
Galactomannan polysaccharide (PSP001) from peel	HCT116 (colon)	Induced cancer cell cytotoxicity	↑Apoptosis	0.001-1.000 µg/mL; 72 h	Joseph et al. [78]
PSP001; SNP@PSP	HCT116 (colon)	Displayed cytotoxic and antimigratory effects	↑Apoptosis; ↑caspase-2; ↑caspase-3; ↑caspase-8; ↑caspase-9	0.01-200 µg/mL; 24-72 h	Padinjarathil et al. [107]
Punicalagin extracted from peel	Caco-2 (colon)	Produced cytotoxic effect	↑Apoptosis; ⊥S; ↓cyclin A; cyclin B1; ↑cyclin E; ↓Bcl-XL; ↑caspase-9; ↑caspase-3; ↑cyt. c	1-100 µmol/L; 72 h	Larrosa et al. [108]
Pomegranate peel extract	LOVO; LOVO DX (colon)	Displayed antiproliferative activity	↑Apoptosis	1-75 µg/mL; 48 h	Moreira et al. [85]

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Acetone and methanolic extracts of fruit peels	RKO (colorectum)	Exhibited antiproliferative activity		5-960 µg/mL; 72 h	Fazio et al. [84]
Peel derived silver nanoparticles	RKO (ATCC CRL-2577) (colorectum)	Exerted cytotoxic effect	↑Autophagy	0.3-100 µg/mL; 2-5 days	Devanesan et al. [109]
Methanolic extract of pomegranate seed oil	HT29, HCT 116 (colon)	Displayed marginal effect		0.12-0.60 µL; 24 h	Costantini et al. [90]
Methanolic extract of pomegranate seed oil	HepG2; Huh7 (liver)	Displayed marginal effect		0.12-0.60 µL; 24 h	Costantini et al. [90]
Galactomannan polysaccharide (PSP001) from peel	HepG2 (liver)	Registered cytotoxicity	↑Apoptosis	0.001-1.000 µg/mL; 72 h	Joseph et al. [78]
PSP001; SNP@PSP	HepG2 (liver)	Exhibited cytotoxicity and antimigratory effects	↑Apoptosis; ↑caspase2; ↑caspase-3; ↑caspase-8; ↑caspase-9	0.01-200 µg/mL; 24-72 h	Padinjarathil et al. [107]
Pomegranate juice and extracts of peel, flesh, seeds and leaf	HepG2 (liver)	Inhibited proliferation		20-80 µg/mL; 48 h	Li et al. [117]
Polyphenol-rich ethanolic peel extract	HepG2 (liver)	Reduced cell viability	↑Apoptosis; ↓S; ↑ROS; ↑caspase-3; ↑caspase-9; ↑cyt. c; ↑Bax; ↑p53; ↓Bcl-2	100-300 µg/mL; 24 h	Song et al. [118]
Silver nanoparticles of aqueous leaf extract	HepG2 (liver)	Induced cell mortality	Antioxidant activity	5-200 µg/mL; 24 h	Saratale et al. [119]
SL-NPs of pomegranate extract	HepG2 (liver)	Exhibited cytotoxicity		1-500 µg/mL; 24 h	Badawi et al. [97]
Pomegranate whole fruit extract; ellagic	PANC-1,	Inhibited proliferation	↑G ₀ /G ₁ ; ↓G ₂ ; ↓CD44; ↓CD24	50–250 µg/mL;	Nair et al. [124]

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acid, luteolin and ursolic acid	AsPC-1 (pancreas)			24-96 h	
<i>Gynecological cancers</i>					
Polyphenol-rich fruit extract	HeLa (cervix)	Exerted marginal effect on cell viability		50 µg GAE/mL; 72 h	McDougall et al. [125]
Methanolic extract of pericarp	HeLa, SiHa (cervix); HEC-1A (endometrium)	Registered growth-inhibitory effects (SiHa only)		20-320 µg/mL; 48 h	Sreeja et al. [76]
Pomegranate juice and extracts of peel, flesh, seeds and leaf	HeLa (cervix)	Inhibited proliferation		20-80 µg/mL; 48 h	Li et al. [117]
Acetone and methanolic extracts of fruit peels	HeLa (cervix); Ishikawa (endometrium)	Exhibited antiproliferative activity	↑Apoptosis; ↑caspase-3; ↑caspase-7; ↑caspase-9; ↑PARP cleavage (HeLa only)	5-960 µg/mL; 72 h	Fazio et al. [84]
Ellagic acid from pomegranate peel extract	HeLa (cervix)	Suppressed cell invasion	↑Apoptosis; ↑IGFBP7; ↓Akt; ↓mTOR	2.5 - 10.0 uM; 24 h	Guo et al. [126]
Punicalagin from pomegranate husk	HeLa, CaSki, SiHa (cervix)	Displayed antiproliferative activity	Antioxidant activity	12.5–200 µg/ml; 72 h	Aqil et al. [87]
Punicalagin	HeLa (cervix)	Suppressed proliferation and migration	↑Apoptosis; ↓Bcl-2; ↑Bax; ↓G1; ↓β-catenin; ↓cyclin D1; ↓c-myc; ↓MMP-2; ↓MMP-9; ↑TIMP-2; ↑TIMP-3	12.5- 200 µM; 24-48 h	Tang et al. [127]

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Methanolic extract of pericarp	SKOV3 (ovary)	Exhibited marginal cytotoxicity		20-320 µg/mL; 48 h	Sreeja et al. [76]
Pomegranate peel extract	SKOV3 (ovary)	Exerted cytotoxicity		5 -1,000 µg/ml; 72 h	Modaeinama et al. [83]
Pomegranate juice-derived ellagitannins	SKOV3 (ovary)	Inhibited cell proliferation		IC ₅₀ =44-222 µM; 48 h	Kasimsetty et al. [80]
Methanolic extract of pomegranate seeds	SKOV3 (ovary)	Inhibited cell growth		5-1,000 µg/mL; 72 h	Seidi et al. [92]
Pomegranate fruit juice (PFJ), ellagic acid, and luteolin	A2780 (ovary)	Displayed antiproliferative and antimigratory activities	↓MMP-2; ↓MMP-9	5%, 10% (PFJ), 5-15 mg/mL (ellagic acid & luteolin); 12-48 h	Liu et al. [128]
<i>Hematological cancers</i>					
Aqueous extract of peels	Raji, P3HR-1 (Burkitt's lymphoma)	Reduced cell viability	↑Apoptosis	1.3-6.3 µl/mL; 24, 48 h	Settheetham and Ishida [129]
Galactomannan polysaccharide (PSP001) from peel	Dalton's lymphoma	Exerted cytotoxicity	↑Apoptosis	0.001-1,000 µg/ml; 24, 48 h	Joseph et al. [78]
Fresh and fermented pomegranate juice; pomegranate pericarp extract	HL-60 (APL)	Displayed antiproliferative activity	↑Differentiation	100-400 µg/mL; 3 days	Kawaii & Lansky [130]

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Pomegranate juice extract	Jurkat (T-cell leukemia); SUP-B14 (ALL); MOLT-3 (ALL); CCRF-CEM (ALL); HL-60 (APL); THP-1 (AMCL); K562 (CML); KG1a (AML)	Displayed antiproliferative activity	↑Apoptosis; ⊥G ₀ /G ₁	6.25%, 12.5% (v/v); 24, 48 h	Dahlawi et al. [131]
Acetonitrile fraction from pomegranate juice	CCRF-CEM (ALL); MOLT-3 (ALL); HL-60 (APL); THP-1 (AMCL)	Reduced cell viability	↑Apoptosis; ↓ATP; ⊥S; ↑caspase-3	6.25-25% (v/v); 48 h	Dahlawi et al. [132]
Pomegranate peel extract (PPE) and PPE-gelatin NPs	HL-60 (PML)	Exhibited differential effects on early and late stage apoptosis		0.0156-0.125 μg/mL; 22 h	Li et al. [133]
Galactomannan polysaccharide (PSP001) from peel	K562 (CML)	Exerted cytotoxicity		0.001-1,000 μg/mL; 24-72 h	Joseph et al. [86]
Pomegranate peels extract	K562 (CML)	Displayed antiproliferative activity	↑Apoptosis; ⊥G ₂ /M; ↑caspase-9; ↑caspase-3; ↑caspase-7; ↑cyt. c; ↑p21 ^{WAF1} ; ↑p53; ↓Bcl-2; ↓Bax; ↑Akt	100-800 μg/mL; 72 h	Asmaa et al. [134]
Extracts of pomegranate leaves, stems, flowers	U266 (MM)	Decreased proliferation	↑Apoptosis; ⊥G ₂ /M phase; ⊥S; ↓MMP	1-500 μg/mL; 48, 72 h	Kiraz et al. [135]

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Pomegranate juice with or without BTZ	U266, KMS26, MM1S (MM)	Inhibited proliferation	⊥G ₀ /G ₁ ; ↑PPAR γ	2-12% (v/v); 24 h	Tibullo et al. [136]
<i>Lung and respiratory tract cancers</i>					
Pomegranate fruit extract	A549	Reduced cell viability	⊥G ₀ /G ₁ ; ↑p21 ^{WAF1} ; ↑p27 ^{KIP1} ; ↓Ki-67; ↓PCNA; ↓cyclin D1; ↓cyclin D2; ↓cyclin E; ↓cdk2; ↓cdk4; ↓pMAPK; ↓PI3K; ↓pAkt; ↓NF- κ B; ↓IKK α ; ↓I κ B α ; ↓NF- κ B-DNA binding	50-150 μ g/mL; 2 h	Khan et al. [137]
Methanolic extract of peel	A549	Displayed antiproliferative activity		5 -1,000 μ g/mL; 72 h	Modaeinama et al. [83]
Methanolic extract of seeds	A549	Exerted cytotoxicity		5- 1,000 μ g/mL; 72 h	Seidi et al. [92]
PSP001 polysaccharide	K562	Inhibited cell growth	Antioxidant activity	0.001-1,000 μ g/mL; 72 h	Joseph et al. [86]
SNP@PSP	A549	Exhibited cytotoxic and antimigratory effects	↑Apoptosis; ↑caspase-2; ↑caspase-3; ↑caspase-8; ↑caspase-9	0.01-200 μ g/mL; 24-72 h	Padinjarathil et al. [107]
Leaf extract	A549, H1299, LL2	Inhibited proliferation and colony formation; suppressed migration and invasion	↑Apoptosis; ⊥G ₂ /M; ↓ROS; ↓ $\Delta\Psi$ m; ↓MMP-2; ↓MMP-9	6.25-200 μ g/mL; 24-72 h	Li et al. [138]

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Punicalagin	A549, Hep-2	Exerted cytotoxicity	Antioxidant effects	10-100 μ M;	Kulkarni et al. [139]
Punicalagin	A549, H1299	Displayed antiproliferative activity	Antioxidant effects	12.5-200 μ g/mL; 72 h	Aquil et al. [87]
Punicalagin, ellagic acid	A549, H1299	Displayed antiproliferative activity	Antimutagenic effects	12.5-200 μ g/mL; 48 h	Zahin et al. [140]
<i>Neurological cancers</i>					
Punicalagin	U87MG (human glioma)	Inhibited cell viability	\uparrow Apoptosis; \uparrow cyclin E; \downarrow cyclin A; \downarrow cyclin B; \uparrow PARP cleavage; \uparrow caspase-9; \uparrow autophagy; \uparrow pAMPK; \uparrow pp27	1-30 μ g/mL; 24 h	Wang et al. [143]
Nanoemulsion of seed oil with ketoprofen	C6 (rat glioma)	Suppressed cell growth		50, 100 μ M; 72 h	Ferreira et al. [144]
Pomegranate seed oil or nanoemulsions of seed oil	C6 (rat glioma)	Reduced cell viability		2.61-8.7 % v/v; 24, 48 h	Ferreira et al. [145]
<i>Skin cancer</i>					
Pomegranate juice-derived ellagitannins	SK-ELM	Inhibited cell proliferation		IC ₅₀ =70.5-197 μ M; 48 h	Kasimsetty et al. [80]
Galactonmannan polysaccharide (PSP001) from peel	A375	Exerted cytotoxicity	\uparrow Apoptosis	0.001-1,000 μ g/mL; 24-72 h	Joseph et al. [78]
<i>Urogenital cancers</i>					

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<i>Bladder cancer</i>					
Ethyl acetate and Soxhlet extract of whole fruit or peel	T24	Inhibited cell proliferation	Antioxidant activity	50 µg/mL; 48 h	Masci et al. [149]
Pomegranate rind extract	EJ	Displayed antiproliferative activity	↑Caspase-3; ↑cleaved PARP; ↑p53; ↓c-Jun; ↑miR-34a; ↓c-myc; ↓CD44	25-200 µg/mL; 24-72 h	Zhou et al. [150]
Ethanol extract of pomegranate juice	T24, J82	Exhibited cytotoxic activity	↑Apoptosis; ⊥S phase; ↑cyclin A; ↓cdk1; ↑procaspase-3; ↑procaspase-8; ↑procaspase-9; ↑procaspase-12; ↑Bax:Bcl-2; ↑CHOP; ↑BiP	2.5-200 µg/mL 12-72 h	Lee et al. [151]
Ethanol extract of pomegranate juice	T24, J82	Displayed antiproliferative activity; reduced cell migration	↑PTEN; ↓pAkt; ↓mTOR; ↓XIAP; ↑Pfn1; ↑Diablo	50 µg/mL; 24-72 h	Wu et al. [152]
Ethanol extract of pomegranate peels and pulp	T24, J82	Showed growth-inhibitory activity	↑Apoptosis; ⊥sub-G ₁ ; ↑caspase-3; ↑caspase-8; ↑caspase-9; ↑caspase-12; ↑DR4; ↑DR5; ↑Bax; ↓Bcl-2; ↑Bip; ↑VCP	5-200 µg/mL	Chang et al. [153]
<i>Prostate cancer</i>					
Fermented juice polyphenols, pericarp polyphenols and cold-pressed seed oil	LNCaP, PC-3, DU-145	Displayed antiproliferative and anti-invasive activity	↑Apoptosis; ↑caspase-3; ⊥G ₂ /M; ↑p21 ^{WAF1} ; ↓GADD45α; ↓c-myc; ↑MAPK-APK2	ED ₅₀ = 20-100 µg/mL; 96-168 h	Albrecht et al. [154]

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Fermented juice polyphenols, pericarp polyphenols and seed oil	DU-145, PC-3	Inhibited cell proliferation and invasion	↓PLA2	12.5-100 µg/mL; 0.5-96 h	Lansky et al. [155]
Fruit extract	PC-3	Inhibited cell viability	↑Apoptosis; ↑Bax; ↑Bak; ↓Bcl-XL; ↓Bcl-2; ↑p21 ^{WAF1} ; ↑p27 ^{KIP1} ; ↓cyclin D1; ↓cyclin D2; ↓cyclin E; ↓cdk2; ↓cdk4; ↓cdk6	10-100 µg/mL; 48 h	Malik et al. [156]
Pomegranate juice, total pomegranate tannin extract, punicalagin and ellagic acid	RWPE-1, 22Rv1	Displayed antiproliferative activity	Antioxidant activity	12.5-100 µg/mL; 48 h	Seeram et al. [103]
Fruit extract (POMx), juice, punicalagins and ellagic acid	LNCaP, LNCaP-AR, DU-145	Exhibited antiproliferative activity	↑Apoptosis; ↓HSD3B2; ↓AKR1C3; ↓SRD5A1; ↓AR	3.125-50 µg/mL; 72 h	Hong et al. [157]
POMx	LNCaP	Displayed antiproliferative and antiangiogenic effects	↓HIF-1α; ↓VEGF	1-5 µg/mL; 6 h	Sartippour et al. [158]
POMx	DU-145	Inhibited cell viability	↑Apoptosis; ↓NF-κB	Dilution factor: 50-2,000; 24, 48 h	Rettig et al. [159]
POMx	LAPC4	Inhibited cell growth	↑Apoptosis; ↑pJNK; ↓pAkt; ↓pmTOR; ↓Igf1	10 µg/mL; 72 h	Koyama et al. [160]

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Pomegranate juice	DU145, LNCaP, PC-3	Inhibited cell growth increased cell adhesion and reduced cell migration	↑ICAM-1; ↑E-cadherin; ↓HMMR; ↓COL1A1; ↑miR-335; ↑miR-205; ↑miR-200; ↑miR-126; ↓miR-21; ↓miR-373; ↓IL-6; ↓IL-12p40; ↓IL-1β; ↓RANTES	1%, 5%; 12-72 h	Wang et al. [161]
Combination of juice components (luteolin, ellagic acid, caffeic acid and punicic acid)	DU145, LNCaP, PC-3	Inhibited cell growth, increased cell adhesion and reduced cell migration and chemotaxis	↑E-cadherin; ↓HMMR; ↓TWIST; ↓FSCN1; ↓CDK6; ↓COL1A1; ↓Bcl-2; ↓NEXN; ↓EZH2; ↓CDC25B; ↓ZEB1; ↓ROCK2; ↓CCNB2; ↓CCNB1; ↓DTL; ↓CCNE2; ↑PTEN; ↑CDKN1A; ↑CDKN2A; ↑CLDN1; ↑CDKN2B; ↑miR144; ↑miR-133b; ↑miR-1; ↑miR-122; ↑miR-34c; ↑miR-200c; ↑miR-127; ↑miR-335; ↑miR-124; ↑miR-181a; ↑miR-7; ↑miR-215; ↑miR-15a; ↑Let-7d; ↓miR-20a; ↓miR-21; ↓miR-9; ↓miR-29b; ↓miR-181b	2 or 4 µg/mL for each constituent; 12-72 h	Wang et al. [162]
Methanolic peel extract	PC-3	Displayed antiproliferative activity		5 -1,000 µg/mL; 72 h	Modaeinama et al. [83]

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Ethanollic peel extract	PC3	Displayed antiproliferative effect	↑Apoptosis	10- 600 $\mu\text{g/mL}$; 24 h	Sepehr et al. [163]
Ethanollic peel extract	PC-3, DU-145, TRAMP-C1	Registered antiproliferative, proapoptotic, anti-invasive and antimigratory effects	↓Mitochondrial membrane potential; ↑ROS; ↑Bax; ↓Bcl-2; ↑caspase-3; ↓MMP-2; ↓MMP-9; ↑TIMP2	12.5-200 $\mu\text{g/mL}$; 24-72 h	Deng et al. [164]
Ethanollic seed extract	PC-3	Produced marginal antiproliferative effect		10-600 $\mu\text{g/mL}$; 24 h	Sepehr et al. [163]
Aqueous methahnolic extract of seed oil	DU-145	Displayed minimal effects of cell viability		0.12-0.60 μL ; 24 h	Costantini et al. [90]
Methanollic seed extract	PC-3	Displayed antiproliferative and cytotoxic activity		5- 1,000 $\mu\text{g/ml}$; 72 h	Seidi et al. [92]
Ethanollic seed extract	LNCaP	Inhibited cell proliferation		5-100 $\mu\text{g/ml}$; 48 h	Lucci et al. [91]
SL-NPs of pomegranate extract	PC-3	Exhibited cytotoxic profile		1-500 $\mu\text{g/mL}$; 24 h	Badawi et al. [97]

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Table 2.*Ex vivo and in vivo* effects of pomegranate extracts, fractions and pure compounds on various cancers.

Materials tested	Animal models used	Effects	Mechanisms	Dose & route	Frequency & duration	References
<i>Breast cancer</i>						
Fermented juice polyphenols, chromatographic peak (Peak B) of fermented juice polyphenols, seed oil	DMBA-induced mouse mammary gland lesions (<i>ex vivo</i>)	Reduced the number of lesions		1, 10 µg/mL	10 days	Kim et al. [66]; Mehta and Lansky [67]
Polyphenolic extract of pomegranate juice	Female athymic BALB/c nude mice with xenografted BT474 tumors	Decreased tumor volume and weight	↑Apoptosis; ↓Sp1; ↓Sp3; ↓Sp4; ↓VEGF; ↓survivin; ↓NF-κB p65; ↓pAkt; ↓pPI3K; ↑ZBTB10; ↑SHIP-1; ↓miRNA-27a; ↓miRNA-155	0.8 mg gallic acid equivalents/kg/day; p.o.	Once a day; 35 days	Banerjee et al. [89]
Galactonmannan polysaccharide (PSP001) from pomegranate fruit rind with or without doxorubicin	Female BALB/c mice with EAC tumors (ascites and solid tumors)	Reduced tumor volume and increased survival		100 mg/kg; i.p.	Once a day; 1-14days	Joseph et al., [78]
Pomegranate emulsion	DMBA-induced mammary	Reduced tumor incidence, total	↑Apoptosis; ↓proliferation;	0.2-5.0 g/kg; p.o.	3 times/week; 18 weeks	Bishayee et al. [99]; Mandal &

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	tumorigenesis in female Sprague-Dawley rats	tumor burden and average tumor weight	↑Bax; ↓Bcl-2; ↑Bax:Bcl-2; ↑BAD; ↑CASP3; ↑CASP7; ↑CASP9; ↑PARP; ↑CYT. C; ↓ER-α; ↓ER-β; ↓ERα:ERβ; ↓β-catenin; ↓cyclin D1; ↓COX-2; ↓HSP90; ↓NF-κB; ↓IκBα; ↑Nrf2			Bishayee [100]; Mandal et al. [101]
Pomegranate seed oil	DMBA-induced mammary tumorigenesis in female Sprague-Dawley rats	Increased cancer incidence, tumor multiplicity, total tumor mass and mean tumor mass	Modification of DMBA metabolism	0.15 ml/day; p.o.	21 weeks	Lepionka et al. [102]
<i>Gastrointestinal tract and associated cancers</i>						
Pomegranate seed oil (PGO)	AOM-induced colon carcinogenesis in male F344 rats	Reduced tumor incidence and multiplicity	↑CLA content; ↑PPARγ	0.01% - 1% PGO by weight; diet	32 weeks	Kohno et al. [110]
Pomegranate Juice	AOM-induced colon carcinogenesis in male Fisher 344 rats	Reduced the number of ACF	↑GST	20% fruit juice in drinking water	13 weeks	Boateng et al. [111]
Pomegranate peel extract	AOM induced colon carcinogenesis in	inhibited ACF formation	↑TAC; ↑GSH; ↑GST; ↑GPx; ↑GR; ↑SOD; ↑CAT	1.5 mL/day; p.o.	Daily for 13 weeks	Waly et al. [112]

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	male Sprague-Dawley Rats					
Pomegranate peel extract	AOM induced colon carcinogenesis in male Sprague-Dawley rats	Reduced ACF formation	↓MDA; ↑GSH/GSSG; ↓myeloperoxidase	1.5 mL/day; p.o.	Daily for 16 weeks	Waly et al. [113]
Pomegranate juice	AOM induced colon carcinogenesis in male Sprague-Dawley rats	Suppressed ACF and dysplastic ACF formation	↓COX-2; ↓iNOS; ↓VCAM-1; ↓NF-κB; ↓pNF-κB; ↓IGF; ↓IGFR; ↓p-PI3K; ↓pAkt; ↓pmTOR; ↑miR-126	57.2 mL/day; drinking water	70 days	Banerjee et al. [105]
Pomegranate extract	AOM-initiated and DCNO-promoted colon carcinogenesis in male F344 rats	Decreased the number of MDF		0.6% w/w in DCNO; diet	100 days	Bastide et al. [114]
Pomegranate extract	DMH-induced colon carcinogenesis in male Wistar albino rats	Enhanced survival rate and reduced tumor incidence	↓CEA; ↓Wnt5a; ↓FRZ-8; ↓β-catenin; ↓Lef1; ↓Tcf4; ↓c-myc; ↓cyclin D1; ↑Axin1; ↑APC	3% (wt/wt); diet	30 weeks	Sadik et al. [115]
Pomegranate mesocarp decoction	Male Pirc (F344/NTac-Apc ^{am1137}) rats	Reduced the number and multiplicity of MDF	↑Apoptosis	10,000 ppm (50 mg/kg); diet	6 weeks	Tortora et al. [116]
Pomegranate emulsion	DENA-initiated and PB-promoted	Reduced incidence, number,	↓TBARS; ↓protein carbonyls; ↑Gsta2;	1, 10 g/kg; p.o.	3 times/week for 18 weeks	Bishayee et al. [120];

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	hepatocarcinogenes is in rats	multiplicity, size and volume of hepatic nodules and number and area of GGT-positive foci	↑Gsta5; ↑Gstm1; ↑Gstm7; ↑Gstt1; ↑Nqo1; ↑Ugt1a1; ↑Ugt2b17; ↑Nrf2; ↓iNOS; ↓3-NT; ↓HSP70; ↓HSP90; ↓COX-2; ↓NF-κB			Bishayee et al. [121]
Pomegranate emulsion	DENA-initiated and PB-promoted hepatocarcinogenes is in rats	Inhibited hepatic cell proliferation	↑Apoptosis; ↓cyclin D1; ↑Bax; ↓Bcl-2; ↓Bax:Bcl-2; ↓β-catenin; ↑GSK-3β	1, 10 g/kg; p.o.	3 times/week for 18 weeks	Bhatia et al. [122]
Pomegranate hull extract	DENA-induced hepatocarcinogenes is in rats	Improved survival rate reduced size of hepatic foci	↓Bcl-2; ↓cyclin D1; ↓β-catenin; ↓GSH	6 g/kg; p.o.	3 times/week for 10 weeks	El-Ashmawy et al. [123]
<i>Gynecological cancers</i>						
Pomegranate fruit juice (PFJ), ellagic acid, luteolin	Female nude mice injected with ES-2 cells	Reduced tumor volume and weight	↓MMP-2; ↓MMP-9	20 mL/kg (PFJ), 50 mg/kg (ellagic acid & luteoin)	~24 days	Liu et al. [128]
<i>Lung cancer</i>						
Pomegranate fruit extract	Male athymic(nu/nu) nude mice injected with A549 cells	Induced tumor growth inhibition and improved survival of tumor-bearing animals	N/A	0.1%, 0.2% w/v; dw	~64 days	Khan et al. [137]
Pomegranate fruit extract	Female A/J mice gavaged with B(a)P	Reduced tumor multiplicities	↓NF-κB p65; ↑IκBα; ↓IKKα; ↓pERK1/2; ↓pJNK1/2; ↓pp38;	0.2% w/v; dw	~77-240 days	Khan et al. [141]

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	or topically treated with NTCU		↓PI3K; ↓pAkt; ↓pmTOR; ↓pc-met; ↓Ki-67; ↓PCNA; ↓iNOS; ↓CD31; ↓VEGF			
Pomegranate juice	Male A/J mice exposed to cigarette smoke	Decreased the number and growth of lung nodules and tumors	↓PHH3; ↓HIF-1 α	80 μ mol/kg/day; dw	9 months	Husari et al. [142]
<i>Skin cancer</i>						
Pomegranate seed oil	DMBA-initiated and TPA-promoted skin carcinogenesis in female CD-1 mice	Decreased tumor incidence and multiplicity	↓ODC	100 μ L of 5% oil; topical	Twice/week for 19 weeks	Hora et al. [146]
Pomegranate fruit extract	DMBA-initiated and TPA-promoted skin carcinogenesis in female CD-1 mice	Reduced incidence, total number and multiplicity of tumor	↓ODC; ↓COX-2; ↓pERK1/2; ↓pJNK1/2; ↓pp38; ↓NF- κ B p65; ↓IKK α ; ↓pI κ B α	2 mg/mouse; topical	Twice/week; 29 weeks	Afaq et al. [147]
Pomegranate fruit extract with or without DAS	DMBA-initiated and TPA-promoted skin carcinogenesis in male Balb/c mice	Delayed tumor onset and incidence; Demonstrated regression in tumor volume	↑Apoptosis; ↓pERK1/2; ↓pJNK1; ↓NF- κ B p65; ↓IKK α ; ↓pI κ B α	10%; dw	18 weeks	George et al. [148]
<i>Urogenital cancers</i>						
<i>Bladder cancer</i>						

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Pomegranate rind extract	EJ cells xenografted in male athymic BALB/C nude mice	Suppressed tumor growth (volume)	↑miR-34a	100 mg/kg; p.o.	Once daily; 4 weeks	Zhou et al. [150]
Ethylacetate layer of ethanolic peel extract	T24 cells xenografted in male BALB/cAnN-Foxn1 nude mice	Reduced tumor volume and weight	↑Apoptosis	2-100 mg/kg; p.o.	once daily; 10 weeks	Chang et al. [153]
<i>Prostate cancer</i>						
Supercritical CO ₂ -extracted seed oil, pericarp polyphenols	PC-3 xenograft in athymic BALB/c male homozygous nude mice	Inhibited tumor growth		2 µg/g; s.c.	35 days	Albrecht et al. [154]
Fruit extract	CWR22Rv1 xenograft in athymic nude male mice	Suppressed tumor volume and improved survival of animals with tumor	↓Serum PSA levels	0.1%, 0.2% wt/vol; d.w.	31-39 days	Malik et al. [156]
Ellagitannin-rich pomegranate extract	LAPC4 xenograft in SCID mice	Decreased tumor volume		0.8 mg/mouse; p.o.	5 days/week; 4 weeks	Seeram et al. [165]
POMx	LAPC4 xenograft in SCID mice	Reduced tumor growth	↑Apoptosis; ↓NF-κB; ↓pIκBα; ↓PSA	0.8 mg/mouse; p.o.	5 days/ week; 3 weeks	Rettig et al. [159]
POMx	LAPC4 xenograft in SCID mice	Reduced tumor volume and intratumor blood vessel density	↓HIF-1α	0.8 mg/mouse; p.o.	5 days/ week; 4 weeks	Sartippour et al. [158]

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Combination of luteolin, ellagic acid and punicic acid	PC-3M-luc xenograft and Pten ^{-/-} ; K-ras ^{G12D} allograft tumors in male SCID mice	Inhibited tumor growth and metastasis	↓CXCR4; ↓Gα13; ↓PI3K; ↓pAkt	64 μg/component/day; i.p.	5 days/week; 8 weeks	Wang et al. [166]
Fruit extract	TRAMP mice	Increased survival and inhibited tumorigenesis and metastasis	↓Raptor; ↓Rictor; ↓IGF-1; ↓PI3K; ↓pAkt; ↓pmTOR	0.1, 0.2% wt/vol; d.w.	6-28 weeks	Adhami et al. [167]

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Table 3

Clinical trials of several of pomegranate products in cancer patients.

Phase/Study type; ClinicalTrials.gov identifier	Materials tested	No. of patients	Effects	Dose & route	Frequency & duration	References
<i>Breast cancer</i>						
Randomized clinical trial	Pomegranate juice (POM Wonderful 100% juice)	64	Significant decline in estrone and testosterone in subgroup of females with normal weight	8 fluid oz/day; p.o.	4 oz in the morning and 4 oz in the evening; 3 weeks	Kapoor et al. [168]
<i>Colon cancer</i>						
Phase I-II; NCT01916239	Pomegranate extracts (PE-1: 2 mg/g punicalin, 72 mg/g punicalagin and 294 mg/g ellagic derivatives; PE-2: 5.4 mg/g punicalin, 155 mg/g punicalagin and 28 mg/g ellagic derivatives	26	Increased levels of ellagic acid derivatives and urolithin found in colon tissue of CRC patient	Two 450 mg capsules; p.o.	Daily; 12-18 days	Nuñez-Sánchez et al. [169]
Phase I-II; NCT01916239	Pomegranate extracts (PE-1: 2 mg/g punicalin, 72 mg/g punicalagin and 294 mg/g ellagic acid derivatives;	45	Moderate (but statistically insignificant) modulation in miR-646, miR-1249, miR-135b-5p, miR-135b- 3p, miR-92b-5p, miR-765,	Two 450 mg capsules; p.o.	Daily; 12-14 days	Nuñez-Sánchez et al. [170]

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	PE-2: 5.4 mg/g punicalin, 155 mg/g punicalagin and 28 mg/g ellagic acid derivatives		miR-496, miR-181c-3p, and miR-18a-3p expression, which are linked to common cancer pathways in CRC and Jak-STAT signaling pathway			
Phase I-II; NCT01916239	Pomegranate extracts PE-1: 2 mg/g punicalin, 72 mg/g punicalagin and 294 mg/g ellagic acid derivatives; PE-2: 5.4 mg/g punicalin, 155 mg/g punicalagin and 28 mg/g ellagic acid derivatives	45	Counteracting effect on <i>CD44</i> , <i>CTNNB1</i> , <i>CDKN1A</i> , <i>EGFR</i> and <i>TYMS</i> gene expression.	Two 450 mg capsules; p.o.	Daily; An average of 13.6±7.5 days	Núñez-Sánchez et al. [171]
Phase I-II; NCT01916239	Pomegranate extracts PE-1: 2 mg/g punicalin, 72 mg/g punicalagin and 294 mg/g ellagic acid derivatives; PE-2: 5.4 mg/g punicalin, 155 mg/g punicalagin and 28 mg/g ellagic acid derivatives	57	Decrease in plasma level of LBP	Two 450 mg capsules; p.o.	Daily; An average of 14.4±7.6 days	González-Sarrías et al. [172]
<i>Prostate cancer</i>						

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Phase II	Pomegranate juice, equivalent to 570 mg total polyphenol gallic acid equivalents	46	Prolongation of PSA doubling time	8 oz; p.o.	Daily; 15-54 months	Pantuck et al. [173]
Randomized, double-blind, placebo-controlled study; NCT00336934	Pomegranate liquid extract with 1.6 mmol of total polyphenol	183	Significant prolongation of PSADT for men with <i>MnSOD</i> AA genotype; No significant prolongation of PSADT for other patient population	8 oz; p.o.	Daily; 12 months	Pantuck et al. [174]
Phase II	Pomegranate extract (capsule with 1 g powder)	101	At least 6 months prolongation of PSADT, declining PSA levels in 13% of patients	1 or 3 g of polyphenol extract; p.o.	Daily; 6-18 months	Paller et al. [175]
Phase II; NCT00719030	Pomegranate extract (capsule with 1 g powder)	70	No change in tissue 8-OHdG, Ki67, cancer pS6 kinase, NF-κB and serum PSA levels	2 g of POMx powder; p.o.	One capsule twice daily; Up to 4 weeks	Freedland et al. [176]
	Pomegranate juice with 279 mg of ellagic acid-type phenolics	63	Upon intake of pomegranate juice, main metabolites such as urolithin glucuronides and dimethyl ellagic acid were detected in prostate tissue.	200 mL; p.o.	Daily; 3 days	Gonzalez-Sarrias et al. [177]

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Phase IIb	Pomegranate juice (2294 mg/L polyphenol gallic acid)	102	No differences detected with regard to PSA kinetics and pain scores	500 mL/day; 250 mL/day	500 mL: Every day for 4 weeks; 250 mL: After the first 500 mL every day for 4 weeks, received 250 mL daily for 4 weeks	Stenner-Liewen et al. [178]
Randomized, double-blind, placebo-controlled study	Whole food supplement containing 100 mg of pomegranate whole fruit powder	199	Significant suppression of median percentage rise in PSA	One tablet; p.o.	Three times a day; 6 months	Thomas et al. [179]

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