# *Punica granatum*

## Scientific Name

*Punica granatum* **L.**

## Synonyms

*Granatum punicum* St.-Lag., *Punica florida* Salisb., *Punica grandiflora* hort. ex Steud., *Punica multiflora* hort. ex Siebold & Voss, *Punica nana* L., *Punica spinosa* Lam.

## Family

Lythraceae, also placed in Punicaceae.

## Common/English Name

Pomegranate

## Vernacular Names

*Afghanistan*: Poste-Anar;
*Albanian*: Shegë;
*Arabic*: Darabhte-Naiy, Gulnar, Julnar, Rana, Roman, Rumman Shajratur Rumman;
*Armenian*: Noor, Nur;
*Azerbaijan*: Hap, Nar;
*Brazil*: Roma, Romeira, Romazeira;
*Burmese*: Talebin, Thale, Salebin;

*Catalan*: Magraner;
*Chinese*: An Shih Hu, Shi Liu, Shi Liu Pi;
*Croatian*: Nar, Šipak;
*Czech*: Granátové Jablko, Granátovník, Granátovník Obecný, Granátovník Punský, Marhaník;
*Danish*: Granatæble;
*Dutch*: Granaatappel, Granaatboom;
*Eastonian*: Granaatõun, Harilik Granaadipuu;
*Esperanto*: Granato, Granatujo;
*Finnish*: Granaattiomena;
*French*: Balaustier, Grenade, Grenadier, Grenadier Commun, Grenadier D'europe, Pommier De Carthage;
*Georgian*: Broceuli;
*German*: Echte Granate, Granatbaum, Granatapfel, Granatapfelbaum, Granatapfelstrauch, Grenadine;
*Greek*: Rodi, Ródi, Rodia, Rodiá;
*Guatemala*: Granad;
*Hebrew*: Rimmon, Rimon;
*Hungarian*: Gránátalma, Közönséges Gránátalma, Pomagránát, Termesztett Gránátalma;
*Icelandic*: Granatepli, Kjarnepli;
*India*: Dalim (Assamese), Dalim, Dalimgachh (Bengali), Dadim, Danoi, Daroona, Darooni (Dogri), Dadam, Dadamna Bee (Gujarati), Amar, Anaar, Anar, Anar-Ka-Per, Anar-Ke-Per, Anardana, Auar, Dalimo, Dalimu, Dalmiya, Daram, Daran, Darim, Darimu, Daroo, Daru, Dhalim, Dharimb, Dharu, Doran, Gulnar-Ka-Per, Nirgal, Ringal, (Hindu), Daadima, Daalimbe, Daalimbe Mara, Dalimba, Dadima, Dadimbe, Dalimabay, Dalimba, Dalimbare, Dalimbe, Dalimbe-Gida, Dalimbuhannu, Dhalimbe, Huli

Daalimbe Mara, Hulidalimbe, Hushidalimbe, Husidalimbe (Kannada), Dalimb (Konkani), Dadimam, Dadiman, Madala, Matalam, Matalam-Cheti, Matalanarakam, Pumatalam, Raktabijam, Talimadalam, Talimatalam, Urumampalam, Urumampazham, Uruyampalam (Malayalam), Kaphoi, Kamphoi (Manipuri), Anārdānā, Anardana, Daalimb, Dalimb, Dalimba, ḍāḷīmb, ḍāḷīmbāce Dāṇe (Marathi), Theibuhfai (Mizoram), Dalimba, Nagarata, Theibuhfai (Oriya), Anar (Punjabi), Bijapura, Dadima, Dadima-Phalam, Dadimah, Dadimam, Dadimaphalam, Dadimasara, Dadimavrikshaha, Dadimba, Dalika, Dantabijaka, Darimba, Ija, Karaka, Kuchaphala, Kuttima, Lohitapushpaka, Lohitapuspaka, Madhubija, Milapatra, Milapatraka, Mukhavallabha, Nagarata, Parvarut, Phalamla, Phalashadava, Pindapushpa, Pindira, Raktabija, Raktapushpa, Shukadana, Shukavallabha, Sunila, Suphala, Svadvamla, Valkaphala, Vrittaphala (Sanskrit), Arocakana-cani, Arulmaram, Arumaram, Atalai, Catimataki, Catipancu, Cerukkam, Cikappumatalai, Civappuccantanikacceti, Civappuccantanikam, Civappumatalai, Cukacanam, Cukatanam, Cukavallam, Cukkilestam, Ekamuli, Inippu Matalai, Inippumatalai, Inippumatulai, Irattapittapicakam, Irattavicam, Irattavitacceti, Irucakam, Irucukam, Kalkapalam, Kalumal Madalai, Karkapalacceti, Karkapalam, Kavaiyal, Kovarttanam, Kucapalam, Kucapalamaram, Kukarumulimpam, Kurucattam, Kuttinam, Maathulai, Madalai, Madalai-Ch-Chedi, Madalai-P-Pazham, Madalam, Madalangkai, Madalum Vayr, Madhalai, Madhulam, Madhulami, Madulai, Madulam, Madulungam, Magilam Palam, Malaki, Mandulai, Manimatari, Mani-picam, Manipicamaram, Manivicam, Maniviciramam, Maniviciramaram, Maralam, Maralam, Maralamaram, Marayam, Matalaimacaki, Matalaimacakimaram, Matalam, Matalampu, Matalunkam, Mathalai, Mathulai, Matulai, Matulainkam, Matulam, Matulankam, Matulankam, Matulankamaram, Matulunkam, Matulunkamaram, Matuvicam, Nallamatulai, Narumatulam, Narumatulam, Nattumatalai, Palacatavam, Palacatavamaram, Palacavatam, Palapuracceti, Palapurakam, Palapurakamaram, Picapuram, Picapuram, Pintirakacceti, Pintirakam, Pintiram, Piraputam, Pu-Madalai, Pulimadalai, Pulippumatulai, Pumadalai, Rumman, Tacanapicam, Tadimadalai, Tadimam, Talimpamayati, Tantapicakam, Tantapicam, Tantapijakam, Tatimakkani, Tatimam, Tatimatulai, Tittippumatulaimaram, Tittippuppalai, Tucakamatulai, Tucakatitam, Tucakatitamaram, Tuccam, Tuccam, Tusagam, Urucakam, Urumamapalam, Uruntanirpputpi, Utirapantam, Varaimatalacci, Varaiyutakam, Vinnarakam, Vinnarakamaram, Vintapurakam, Viraiyotakam, Virotam, Viruttapalam (Tamil), Daadimamu, Daadimba, Daalimba Chettu, Daalimma, Daanimma, Dadima, Dadima-Chetu, Dadimamu, Dadimba, Dadima Pandu, Dadimma, Dalimba, Dalimba-Chettu, Dalimma, Dalunimma, Danima, Danimma, Danimmapandu, Danimma-Chettu, Dhanimmapandu, Karakamu, Pullada-animma, Pulladanimma, Puvvudaanimma, Puvvudanimma, Thiyyadaanimma, Tiyyad-animma (Telugu), Aab-E-Amar, Amardana, Anaar, Anar, Anar Dana, Anar Dona, Anar Shirin, Anardana, Anaspal, Goolnar, Gul Amar, Gul Anar, Gul-I-Anar, Gulnar, Gulnar (Gulnar Farsi), Hab-I-Qilqil, Naspal, Poast Anar, Rub Amar Shirin, Rub Anar, Rub Anar Shirin, Rub Anar Tursh, Rub-I-Anar Shirin, Sharbat Anarshirin, Tub-I-Anar Shirin (Urdu);

***Indonesia***: Delima, Gangsalan;

***Iran***: Ìgolnar-E-Farsiî;

***Italian***: Granato, Melagrano, Melogranato, Melograno, Melograno Bonsai, Melograno Da Fiore, Pomo Granato, Pomo Punico;

***Japanese***: Sekiryu, Zakuro;

***Kazakh***: Anar, Anar Ağaşı, Anar Ağašy, Ahap;

***Khmer***: Totum;

***Korean***: Seok Ryu, Seongnyu, Sokryunann, Songnyu;

***Kurdish***: Henar;

***Laotian***: Kok Mak Phi La,Ph'iilaa;

***Luganda***: Nkomawawanga;

***Malaysia***: Buah Delima;

***Maltese***: Rummiena;

***Nepal***: Anaar, Daariim, Darmi;

***Norwegian***: Granateple;

***Pakistan***: Anar;

***Papiamento***: Granatapel;

***Persian***: Anaar, Darakhte-Gulnar, Darakhte-Nar, Dulim, Dulima, Gulnar, Gulnar (Flowers), Nar;

*Philippines*: Delima, Granada (Tagalog);
*Polish*: Granat, Granatowiec Właściwy;
*Portuguese*: Romã, Romãnzeira, Romãnzeira-De-Jardim, Romeira;
*Romanian*: Rodie;
*Russian*: Granat, Granatnik;
*Samoan*: Limoni;
*Sardinian*: Melagranada;
*Serbian*: Hap;
*Slovak*: Granátové Jablko, Granátové Semená, Granátovník Púnsky;
*Slovenia*: Granatno Jabolko;
*Spanish*: Granada, Granado, Grenada, Grenadier, Mangraner, Mangrano;
*Swedish*: Granatäpple;
*Swahili*: Komamanga, Kudhumani;
*Syrian*: Ruman;
*Thai*: Ma Ko, Thap Thim (Central), Phila (Nong Khai), Bakoh (Northern);
*Tongan*: Pomikanite;
*Turkish*: Rumman, Nar, Nar Cğacı;
*Ukrainian*: Granat;
*Vietnamese*: Cây Lựu, Lựu, Thap Lựu;
*Yiddish*: Milgraym.

## Origin/Distribution

The pomegranate tree is native from the Middle east to the Himalayas in northern India. It has been cultivated and naturalised since ancient times throughout the Mediterranean region of Asia, Caucasus, northern Africa and Europe. The fruit has manifold uses as it is today and was featured in Egyptian mythology and art, in the Old Testament of the Bible and in the Babylonian Talmud. From its native range, it was introduced to central and southern India and southeast Asia. It was reported growing in Indonesia in 1416. It was introduced into Latin America and California by the Spanish in 1796, it is now grown in California and Arizona. It has been widely cultivated throughout India and drier parts of southeast Asia and tropical Africa. The most important growing regions are Egypt, China, Afghanistan, Turkey, Syria, Pakistan, Bangladesh, Iran, Iraq, India, Myanmar and Saudi Arabia. There are some commercial orchards in Israel on the coastal plain and in the Jordan Valley.

## Agroecology

Pomegranate is primarily mild-temperate to subtropical and naturally adapted to regions with cool winters and hot summers, but can also be grown in warm tropical areas, such as in southern India, southeast Asia and various islands in the Caribbean. Areas with mean annual temperature of 20–24°C is ideal. It suffers severe, irrecoverable injuries at temperatures below –11.0°C. The plant thrives in a semi-arid condition with mean annual rainfall of 500 to 1,000 mm and is extremely drought-tolerant. It does not flower and fruit well in very humid and wet climates. It is cultivated up to altitudes of 2,000 m as occur throughout the western range (Baluchistan, N. & S. Waziristan, NWFP, Kurram, Dir, Chitral) in Pakistan. The species is adaptable to a wide range of soil types including soils on which other fruit species will not grow. It thrives on calcareous soil, alkaline soil, gravelly soil and on deep, acidic loams. For commercial cultivation well-drained, heavy, light and medium soils are preferred although it can withstand seasonal waterlogging. Irrigation is required to sustain high yields in drier areas.

## Edible Plant Parts and Uses

The fruit is relished fresh, out of hand by quartering the fruit and lifting out the rind to exposed the juice-laden arils around the seeds, both of which are eaten. The fruit is also consumed as juice which is the basis for lemonades or a beverage similar to wine. In the Middle east, Caucasus and India, pomegranate juice is a very popular beverage. For beverage purposes, the juice is usually sweetened. Pomegranate juice is widely made into grenadine syrup for use in mixed drinks, cocktails and often processed into wine. In Saudi Arabia, the juice sacs may be frozen intact or the extracted juice may be concentrated and frozen, for future use. The juice can be processed into jellies by the addition of pectin and sugar.

Pomegranate is also used in food and as a spice condiment. Fresh pomegranate arils are used in preparation of curd rice *Dadhojanam* (Telugu) in Andhra Pradesh in India. In northern India, the

reduced juice is used for desserts and for marinating and tenderising meat due to its proteolytic enzymes. Dried pomegranate arils are used in various cuisines such as trail mix, granola bars, or as toppings for ice-cream, yogurt and salads. Dried whole arils are commonly sold in ethnic Indian subcontinent markets. They impart a subtle, sweet-sour and tart flavour popular in Punjab and Gujarat. Dried pomegranate seeds, '*anardana*', has culinary importance as spice for vegetable and legume dishes in Northern India and in Pakistani cuisine. These dried seeds are used as an acidic condiment for chutney and curry preparation. The dried seeds can also be ground and used, which results in a deeper flavoring in dishes and prevents the seeds from getting stuck in teeth. Seeds of the wild pomegranate variety known as *daru* from the Himalayas are renown as quality sources for this spice. In Turkey, pomegranate seeds are also used in salads and sometimes as garnish for desserts such as *güllaç*. In Greece and Cyprus, pomegranate is used to make *kolliva*, a mixture of pomegranate seeds and sugar.

Pomegranate juice can also be processed into a concentrate, syrup and sauces for juice in food dishes and desserts. In Iran, a traditional recipe *fesenjan* is made from a thick pomegranate sauce and ground walnuts used for duck and poultry or in a popular pomegranate soup *ash-e nar*. In Azerbaijan, pomegranate sauce *narsharab*, made from pomegranate juice, is served with fish or *tika kabab* (grilled, roasted or stewed meat). In Turkey, pomegranate sauce called *nar ekşisi* is used as a salad dressing, to marinate meat, or simply to drink straight. Pomegranate syrup used in *muhammara*, a roasted red pepper, walnut, and garlic spread popular in Syria and Turkey. Pomegranate is also popular in Greek cuisine such as *kollivozoum*i, a creamy broth made from boiled wheat, pomegranates and raisins, legume salad with wheat and pomegranate, traditional Middle Eastern lamb kebabs with pomegranate glaze, pomegranate eggplant relish, and avocado-pomegranate dip. Pomegranate is also processed into a liqueur and fruit confectionery used as ice-cream toppings or mixed with yogurt and jam on toast.



**Plate 1**   Foliage of pomegranate shrub

## Botany

A deciduous, much-branched, small tree or shrub 1.5–6 m high with a smooth, dark grey bark (Plate 1). Branches are terete, opposite and branchlets usually ending in spines. Leaves are opposite, glabrous, coriaceous, glossy green, entire, simple, oblong-lanceolate (Plates 1, 2 and 3) to obovate or elliptic, $19–35(−50) \times 8–12$ $(−15)$ mm, subpetiolate, apex sub-actue to obtuse. Flowers are large, showy, scarlet red or white, bisexual, to 4 cm across, solitary or clustered at the shoot apex (Plates 2 and 3). Calyx campanulate, reddish or purplish with six triangular, persistent lobes, Petals 6, broadly obovate, wrinkled, alternating with the sepal lobes, stamens numerous, multiseriate, persistent, inserted on flower tube, Ovary subglobose, inferior with three cells in two-series, style one thick, reddish, stigma simple slightly bilobed. Fruit globose to subglobose, 6–8 cm in diameter, pale red to scarlet to purple or brownish,; the rind thick and coriaceous (Plates 4, 5, 6 and 7). Internally, the fruit is partitioned by thin leathery yellow septa into compartments filled with transparent sacs (arils) filled with tart, flavourful, fleshy, juicy, red, pink or whitish pulp (Plates 7 and 8). In each sac, there is one white, pink or red, angular, soft or hard seed 10–13 mm long.

**Plate 2**  Pomegranate flowers and leaves



**Plate 4**  Maturing pomegranate fruits



**Plate 3**  Close-up flowers, leaves and young fruits



**Plate 5**  Graded harvested pomegranate on sale in a supermarket

## Nutritive/Medicinal Properties

### *Fruit Nutrients*

Food value of raw, pomegranate fruit (refuse 44% skin and membrane) per 100 g edible portion based on the California Wonderful variety is as follows

(USDA 2012): water 77.93 g, energy 83 kcal (346 kJ), protein 1.67 g, total lipid (fat) 1.17 g, ash 0.53 g, carbohydrate 18.70 g; fibre (total dietary) 4 g, total sugars 13.67 g, minerals – calcium 10 mg, iron 0.30 mg, magnesium 12 mg, phosphorus

**Plate 6** Bruised, ungraded pomegranate fruit on sale in local market



**Plate 7** Whole ripe fruit and halved to show the edible aril sacs



**Plate 8** Close up view to show the aril sacs

36 mg, potassium 236 mg, sodium 3 mg, zinc 0.35 mg, copper 0.158 mg, manganese 0.119 mg, selenium 0.5 µg; vitamins – vitamin C (total ascorbic acid) 10.2 mg, thiamin 0.067 mg, riboflavin 0.053 mg, niacin 0.293 mg, pantothenic acid 0.377 mg, vitamin B-6 0.075 mg, folate (total)

38 µg, total choline 7.6 mg, vitamin E (α-tocopherol) 0.60 mg, vitamin K (phylloquinone) 16.4 µg, lipids – fatty acids (total saturated) 0.120 g, 12:0 (lauric acid) 0.006 g, 14:0 (myristic acid) 0.006 g, 16:0 (palmitic acid) 0.070 g, 18:0 (stearic acid) 0.038 g; fatty acids (total monounsaturated) 0.093 g, 16:1 undifferentiated (palmitoleic acid) 0.012 g, 18:1 undifferentiated (oleic acid) 0.077 g, 20:1 (gadoleic acid) 0.004 g; fatty acids (total polyunsaturated) 0.079 g, 18:2 undifferentiated (linoleic acid) 0.079 g, total *trans* fatty acids 0.009 g, campesterol 1 mg and β-sitosterol 4 mg.

Nutrient value of bottled pomegranate juice per 110 g edible portion (USDA 2012) is water 85.95 g, energy 54 kcal(228 kJ), protein 0.15 g, total lipid 0.29 g, carbohydrate 13.13 g, total dietary fibre 0.1 g, total sugars 12.65 g, glucose 6.28 g, fructose 6.37 g, ash 0.49 g, calcium 11 mg, iron 0.10 mg, magnesium 7 mg, phosphorus 11 mg, potassium 214 mg, sodium 9 mg, zinc 0.09 mg, copper 0.021 mg, manganese 0.095 mg, selenium 0.3 µg; vitamins – vitamin C (total ascorbic acid) 0.1 mg, thiamin 0.015 mg, riboflavin 0.015 mg, niacin 0.233 mg, pantothenic acid 0.285 mg, vitamin B-6 0.040 mg, folate (total) 24 µg, total choline 4.8 mg, vitamin E (α-tocopherol) 0.38 mg, vitamin K (phylloquinone) 10.4 µg. fatty acids (total saturated) 0.077 g, 12:0 (lauric acid) 0.004 g, 14:0 (myristic acid) 0.004 g, 16:0 (palmitic acid) 0.0744 g, 18:0 (stearic acid) 0.024 g; fatty acids (total monounsaturated) 0.050 g, 16:1 undifferentiated (palmitoleic acid) 0.008 g, 18:1 undifferentiated (oleic acid) 0.049 g, 20:1 (gadoleic acid) 0.003 g; fatty acids (total polyunsaturated) 0.050 g, 18:2 undifferentiated (linoleic acid) 0.050 g. The edible portion of pomegranate fruit constituted 52% of the total fruit weight, comprising 78% juice and 22% seeds (El-Nemr et al. 1990). The fresh juice contained 85.4% moisture, 10.67% total sugars, 1.4% pectin, 0.1 g/100 mL total acidity (as citric acid), 0.7 mg/100 mL ascorbic acid, 19.6 mg/100 mL free amino nitrogen and 0.05 g/100 mL ash. The seeds were a rich source of total lipids (27.2%), protein (13.2%), crude fibre (35.3%) and ash (2.0%), and also contained 6.0% pectin and 4.7% total sugars. The iron, copper, sodium, magnesium and zinc contents of the juice were lower than

those of seeds, except potassium which was 49.2 ppm in the juice. The seed lipids had a refractive index of 1.518, melting point 13.0°C, iodine value 74.2, acid number 1.1, unsaponifiable matter 0.7%, saponification value 188.9, ester value 187.8 and glycerol content 10.3%. The lipids contained 11 fatty acids, with caprylic (36.3%), the predominant acid, followed by stearic acid (22.5%); linoleic acid (10.3%) and oleic acid (5.1%). The saturated fatty acids of the seed lipids constituted 83.6% of the total fatty acids content.

Vitamin C content in 20 different Turkish cultivars of pomegranate had a range of 312–1.050 mg/100 g, oil content a range of 2.41–3.73%, sterol content a range of 5.78–8.43%, anthocyanin content a range of 2,100–4,400 mg/L, potassium a range of 250–1,200 ppm, calcium a range of 35–326 ppm, magnesium a range of 176–427 ppm, iron a range of 21–46 ppm, sodium a range of 35–76 ppm, and phosphorus a range of 12–43 ppm (Dumlu and Gürkan 2007). Elfalleh et al. (2009) found total sugars of pomegranate juice comprised about 7 g/100 mL fructose and about 8 g/100 mL glucose, soluble proteins about 7 g/L, 9.46 mg/100 mL of phosphorus, and 271.94 mg/100 mL of potassium. The peel contained 9.43 and 210.86 mg/100 g of phosphorus and potassium respectively. The sodium contents were nearly 7 mg/100 mL in both peel and juice.

Nutrient composition of the juice for most components was comparable to the whole fruit. Protein and fat values were higher in the whole fruit compared to the juice due to the seeds, which are 10% of the aril (juice sac) weight (Thomas and Gebhardt 2008). Pomegranate aril juice was reported to provide about 16% of an adult's daily vitamin C requirement per 100 mL serving, and to be a good source of vitamin B5 (pantothenic acid), potassium and antioxidant polyphenols. The tocopherol (α-tocopherol, γ-tocopherol, and δ-tocopherol) contents were, respectively, 165.77, 107.38, and 27.29 mg/100 g from dry pomegranate seed (Elfalleh et al. 2011a, b).

A total of 18 compounds were found in pomegranate aroma profiles, including monoterpenes, aldehydes, alcohols, monoterpenoids and linear hydrocarbons (Calín-Sánchez et al. 2011). The most abundant compounds were *trans*–2-hexenal, 3-carene, α-terpinene and α-terpineol. The total concentration of volatiles ranged from 1.7 to 10.9 g/kg. Overall consumer preference of pomegranate juices was associated with the presence of monoterpenes such as α-pinene, β-pinene, β-myrcene, limonene and γ-terpinene. The presence of aldehydes such as hexanol, hexanal and *cis*-3-hexenol was correlated with poor overall consumer liking. High overall consumer liking was associated with intense and acceptable fresh pomegranate odour and flavour (high scores of satisfaction degree), medium intensity of red colour and low sourness.

## Other Fruit Phytochemicals

Pomegranate is a fruit rich in polyphenols that include flavonoids, tannins and hydrolyzable tannins (Gil et al. 2000; Seeram et al. 2005a, b). Pomegranate contain a complex mixture of gallotannins, ellagitannins, ellagic acid and anthocyanins (Madrigal-Carballo et al. 2009). Pomegranate juice was found to be rich in tannins, anthocyanins, ellagic acid derivatives, and hydrolyzable tannins (Gil et al. 2000). The predominant organic acid in pomegranate was citric acid followed by malic acid (Pande and Akoh 2009). The peel fraction had the highest total hydrolyzable tannins content (4,792.3–6,894.8 mg/100 g of FW).

A total of 35 dimers of flavanol-anthocyanin adducts were detected, consisting of mono- and disubstituted hexoside derivatives of the adducts between the flavan-3-ols (epi)gallocatechin, (epi)catechin and (epi)afzelechin and the anthocyanidins delphinidin, cyanidin and pelargonidin in pomegranate juice (Sentandreu et al. 2010). Anthocyanidins found in pomegranate fruit included: delphinidin, cyanidin, and pelargonidin (Noda et al. 2002). Pomegranate fruit was reported to contain ellagic acid, gallagic acid, punicalins and punicalagins (Reddy et al. 2007); ellagic acid, caffeic acid, luteolin and punicic acid (Lansky et al. 2005a, b); pelargonidin-3-galactose, cyanidin-3-glucose, gallic acid, quercetin, and myricetin (Naz et al. 2007); gallic

acid, methyl gallate, ellagic acid, (+) catechin, isoquerecitrin, D-mannitol, ursolic acid, oleanolic acid, β-sitosterol and daucosterol (Rena et al. 2009). Pomegranate fruit was found to have a low level of indolamines (8–12 μg/g serotonin, 4–9 μg/g tryptamine, and 13–29 ng/100 g melatonin) (Badria 2002). Gözlekçi et al. (2011) found that in all the Turkish pomegranate cultivars the highest levels of total phenolic content were obtained from the peel extracts. The total phenolic content ranged from 1,775.4 to 3,547.8 mg gallic acid equivalent (GAE)/L among the cultivars. However, the total phenolic content of pomegranate juice and seed extract ranged from 784.4 to 1,551.5 mg GAE/L and 117.0–177.4 mg GAE/L, respectively. Four phenolic compounds were identified and quantified in pomegranate peel and pulp: 2 hydroxybenzoic acids (gallic and ellagic acids) and 2 hydroxycinnamic acids (caffeic and *p*-coumaric acids) (Elfalleh et al. 2011a).

Ellagitannins isolated from pomegranate pericarp included: inhibitors punicalin, punicalagin, granatin B, gallagyldilactone, casuarinin, pedunculagin, tellimagrandin I, gallic acid, granatin A, corilagin and ellagic acid (Satomi et al. 1993). Pomegranate fruit peel had been reported to be a rich source of hydrolyzable tannins called ellagitannins (ETs); pomegranate ETs were found to show potent antioxidant, antiatherosclerotic and anticancer activities (Seeram et al. 2005b). The major fruit peel ETs were punicalagin (80–85% w/w) and ellagic acid (EA; 1.3% w/w) and unquantified amounts of punicalin and ellagitannin-glycosides (hexoside, rhamnoside and pentoside). Pomegrante fruit peel is currently an underutilized food by-product with potential to develop phytoceuticals with potential health benefits or to develop products for use in the cosmetic and food biopreservative industries (Seeram et al. 2005b). Prodelphinidins and gallocatechins including gallocatechin, gallocatechin-(4–8)-catechin, gallocatechin-(4–8)-gallocatechin and catechin-(4–8)-gallocatechin were identified from pomegranate peels (Plumb et al. 2002). Luteolin, luteolin 7-*O*-glucoside, kaempferol, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rhamnoglycoside and quercetin were found in the fruit peel (van Elswijk et al. 2004). Of the ellagi-

tannins isolated from pomegranate pericarp, seven namely punicalin, punicalagin, granatin B, gallagyldilactone, casuarinin, pedunculagin and tellimagrandin I were found to be active carbonic anhydrase inhibitors and four namely gallic acid, granatin A, corilagin and ellagic acid to be weakly active inhibitors. The type of inhibition by three and seven punicalagin and gallagyldilactone was found to be noncompetitive. A new antifungal peptide designated as pomegranin with a molecular mass of 11 kDa, was isolated from fresh pomegranate peels (Guo et al. 2009). Epicatechin, epigallocatechin 3-gallate, flavan-3-ol, catechin were found in the fruit peel and juice (de Pascual-Teresa et al. 2000). Pomegranate fruit and juice were found to contain the following lignans: isolariciresinol, medioresinol, matairesinol, pinoresinol, secoisolariciresinol and syringaresinol (Bonzanini et al. 2009). Total lignin contents in the seeds was determined as 36.1 μg/g, in wood knots 17.8 μg/g, in fruit pulp 11.2 μg/g and in the endocarp.3.3 μg/g. Syringaresinol was most abundant in the seed (23.5 μg/g), pinoresinol in knots (8.9 μg/g), pulp (7.4 μg/g) endocarp (3.3 μg/g) and juice (2.1 μg/g). Lignans were also found in two concentrated juices and three pomegranate beverages at levels of 0.4–4.4 μg/g. In addition to the peel, mesocarp, and twigs, lignans were detected in two juices obtained from entire pomegranate fruits, four commercial juices, and three encapsulated pomegranate extracts (Fischer et al. 2012). Isolariciresinol was the predominant lignan with contents of 5.0, 10.5, and 45.8 mg/kg dry matter in processed pomegranate mesocarp, peel, and twigs, respectively.

Six anthocyanin pigments identified as delphinidin 3-glucoside, delphinidin 3,5-diglucoside, cyanidin 3-glucoside, cyanidin 3,5-diglucoside, pelargonidin 3-glucoside and pelargonidin 3,5-diglucoside were found to be responsible for the red colour of pomegranate juice (cv 'Mollar') (Gil et al. 1995). The fruit skin contained only the cyanidin and pelargonidin derivatives. Generally, there was an increase in juice pigmentation with fruit ripening. The concentration of pigments in juice obtained from mature pomegranates ranged between 50 and 100 μg of anthocyanin per gram

fresh weight of arils. Six anthocyanin pigments delphinidin 3-glucoside and 3,5-diglucoside, cyanidin 3-glucoside and 3,5-diglucoside and pelargonidin 3-glucoside and 3,5-diglucoside were found to be responsible for the red color of pomegranate juice (Hernández et al. 1999). Generally, juice pigmentation increased as the fruit ripened. In the early fruit-ripening stages, delphinidin 3,5-diglucoside was the major pigment, followed by cyanidin 3,5-diglucoside, while in the later stages, the monoglucoside derivatives cyanidin 3-glucoside and delphinidin 3-glucoside increased considerably. The pelargonidin derivatives were always present in small amounts. RP-HPLC analysis of pomegranate arils' anthocyanins revealed mono- and diglucosylated delphinidins and cyanidins as the major anthocyanins and pelargonidins as minor components (Borochov-Neori et al. 2011). Anthocyanin accumulation changed inversely to the season's temperatures. Cyanidins were generally more abundant but delphinidin accumulation was enhanced in cooler season. Monoglucosylated anthocyanins prevailed at cooler temperatures and subsided during seasonal warming with a concomitant rise in diglucoside proportion.

The major anthocyanins detected in the 15 Iranian pomegranate varieties were as follows: delphinidin 3-glucoside (2.19–16.29 mg/L), delphinidin 3,5-diglucoside (2.36–63.07 mg/L), pelargonidin 3-glucoside (0.26–1.36 mg/L), pelargonidin 3,5-diglucoside (0.01–8.11 mg/L), cyanidin 3-glucoside (5.78–30.38 mg/L), and cyanidin 3,5-diglucoside (4.39–166.32 mg/L) (Alighourchi et al. 2008). The major anthocyanins in the juice of 6 Iranian pomegranate cultivars were delphinidin 3,5-diglucoside (372–5,301 mg/L), cyanidin 3,5-diglucoside (242–2,361 mg/L), delphinidin 3-glucoside (49–1,042 mg/L) and pelargonidin 3,5-diglucoside (7–90 mg/L) (Mousavinejad et al. 2009). The cultivar, Saveh Black Leather had the highest level of ellagic acid (160 mg/L). Pomegranate juices obtained from six Iranian pomegranate cultivars were found to have 15.77–19.56 total soluble solids content (Brix), pH values of 3.06–3.74, titrable acidity concentration from 0.51 to 1.35 g/100 g, total sugars content from 16. to 22.76 g/100 g (Faroogh), total antho-

cyanins 7.93–27.73 mg/100 g, ascorbic acid 8.68–15.07 mg/100, total phenolics content 526.40–797.49 mg tannic acid/100 g, The total tannins level 18.77–38.21 mg tannic acid/100 g, condensed tannins from 12.14 mg to 12.57 catechin/100 g, antioxidant activity from 46.51 to 52.71% (Zarei et al. 2010). Phenolics, flavonoids, anthocyanins, and tannins of pomegranate juices, obtained from nine Tunisian ecotypes were quantified by El Kar et al. (2011). Phenolics ranged from 1,570 to 3,299 mg gallic acid equivalents/L and flavonoids from 135 to 156 mg quercetin equivalent/L of juice. Highest anthocyanin content was 156 mg cyanidin–3-glucoside equivalent/L and highest tannin content was 2,550 mg catechin equivalent/L of juice. Tartaric and quinic acids were confirmed in pomegranate juice at concentrations of 1–5 and ~1 mg/L, respectively (Ehling and Cole 2011).

Twenty-one volatile compounds were found in fresh pomegranate juices from nine Spanish cultivars, including aldehydes, monoterpenes, and alcohols (Melgarejo et al. 2011). The most abundant compounds were hexanal, limonene, *trans*-2-hexenal, and *cis*-3-hexenol. The presence of monoterpenes (α-terpineol) was correlated with overall consumer preference of pomegranate juice while high aldehydes (*trans*-2-hexenal) concentrations were correlated with poor overall consumer liking. 5-Hydroxymethyl furfural was determined to be at a significant level in traditional sour concentrate of pomegranate juice (Orak 2009). Pomegranate was known to contain estrogens (estradiol, estrone, and estriol) (Mori-Okamoto et al. 2004). Polysaccharide (PSP001) was isolated from pomegranate rind (Joseph et al. 2012).

### Phytochemicals in Seeds

Pomegranate seed oil was found to have 8% saturated fatty acids, 10% monounsaturated, 10% diunsaturated and approximately 70% conjugated acid, most probably punicic acid (El-Shaarawy and Nahpetian 1983). Pomegranate seed was found to have high contents of α-tocopherol (161.2–170.1 mg/100 g) and γ-tocopherol (80.2–92.8 mg/100 g). The seeds of *Punica granatum*

also contained ursolic acid and β-sitosterol along with a long straightchain hydrocarbon – nonacosene (Ahmed et al. 1995). Presence of estrogens and glycosides were also detected. Estrone, an estrogen, was identified in pomegranate seeds (Heftmann et al. 1966). Cold pressed pomegranate seed oil was found to contain punicic acid (65.3%), palmitic acid (4.8%), stearic acid (2.3%), oleic acid (6.3%), linoleic acid (6.6%) and three unidentified peaks from which two (14.2%) were probably isomers of punicic acid (Schubert et al. 1999). Pomegranate seed had an average lipid content of 19.2% with punicic acid as the predominant fatty acid (Pande and Akoh 2009). Pomegranate seed oil was found to be richin1-*O*-*trans*,*cis*,*trans*-9,11,13-octadecatrienoyl glycerol and also to have small amounts of 1-*O*-isopentyl-3-*O*-octadec-2-enoyl glycerol and the known *cis*-9-octadecenoic, octadecanoic and eicosanoic acids (Fatope et al. 2002). Pomegranate seed oil (PGO) was reported to be rich in 70% *cis*(c)9,*trans*(t)11,c13-18:3 as conjugated linolenic acids (CLA) (Kohno et al. 2004). A triglyceride, di-*O*-punicyl-*O*-octadeca-8*Z*,11*Z*,13*E*-trienylglycerol, was isolated and characterized from the seeds of *Punica granatum* from India and Iran (Yusuph and Mann 1997). Four compound were isolated from pomegranate seeds namely coniferyl 9-*O*-[β-d-apiofuranosyl(1→6)]-*O*-β-d-glucopyranoside (1) and sinapyl 9-*O*-[β-d-apiofuranosyl(1→6)]-*O*-β-d-glucopyranoside (2), 3,3′-di-*O*-methylellagic acid (3), 3,3′,4′-tri-*O*-methylellagic acid (4) (Wang et al. 2004). Pomegranate seed oil from 21 pomegranate cultivars was found to have mainly unsaturated fatty acids (about 88%) (El Kar et al. 2011). The predominant fatty acid was linolenic acid (44.51–86.14%), followed by linoleic acid (3.57–13.92%), oleic acid (3.03–12.88%), palmitic acid (3.13–11.82%), stearic acid (1.68–15.64%), gadoleic acid (0.50–4.91%), lignoceric acid (<2.53%), arachidic acid (<1.70%) and myristic acid (<0.85%). Pomegranate seed linolenic acid isomers, punicic acid and α-eleostearic acid were found in pomegranate seeds (Tran et al. 2010).

A high yield (3.1–4.2%) of unsaponifiable matter containing tocopherol, aliphatic alcohol (including policosanol), squalene, phytosterols

and triterpene was obtained from pomegranate seed oil (Caligiani et al. 2010). The levels of squalene (up to 800 mg/kg), policosanol (118–185 mg/kg), β-sitosterol (up to 8,069 mg/kg) and cycloartenol (5,916–7,766 mg/kg) were found while β- and δ-tocopherol were the most abundant vitamin E forms. The seed oil of *P. granatum* may be an interesting alimentary source of substances of nutraceutical value involved in the modulation of cholesterol metabolism. Linolenic acid isomers like punicic acid and α-eleostearic acid were reported from pomegranate seeds (Tran et al. 2010). Qualitatively, the pomegranate fatty acid composition of 21 pomegranate cultivars (15 Tunisian and 6 Chinese) seed oil was identical comprising mainly unsaturated about 88% (Elfalleh et al. 2011b). The predominant fatty acid was linolenic acid (44.51-86.14%), followed by linoleic acid (3.57-13.92%), oleic acid (3.03-12.88%), palmitic acid (3.13-11.82%), stearic acid (1.68-15.64%), gadoleic acid (0.50-4.91%), lignoceric acid ( < 2.53%), arachidic acid ( < 1.70%) and myristic acid ( < 0.85%). (Wang et al. 2004) isolated the following bioactive compounds from pomegranate seeds: coniferyl 9-*O*-[β-d-apiofuranosyl(1→6)]-*O*-β-d-glucopyranoside; sinapyl9-*O*-[β-d-apiofuranosyl(1→6)] -*O*-β-d-glucopyranoside; 3,3′-di-*O*-methylellagic acid; 3,3′,4′-tri-*O*-methylellagic acid; phenethyl rutinoside; icariside D1 and daucosterol. A new class III chitinase (pomegranate seed chitinase) with a molecular weight of approximately 30 kDa was isolated and purified from pomegranate seeds (Yang et al. 2011). This chitinase was found to naturally bind calcium ions with high capacity and low affinity, suggesting it to be a calcium storage protein. This enzyme was found to be widely distributed in the stroma of amyloplasts of the embryonic cells, suggesting that amyloplasts in seeds could serve as an alternative plastid for calcium storage.

### *Phytochemicals in Flowers*

Two new β-sitosterol esters elucidated as stigmast-5-en-3β-ol-3β-dodecanoate (β-sitosterol laurate) and stigmast-5-en-3β-ol-3β-tetradecanoate (β-sitosterol

myriste) along with the known compounds n-tricosane, n-heptacosanyl n-hexanoate olean-5,12-dien-3β-ol-28-oic acid and olean-12-en-3β-ol-28-oic acid were isolated from pomegranate flowers (Bagri et al. 2009b). A new polyphenol compound named pomegranatate, together with, ellagic acid, 3,3′,4′-tri-*O*-methylellagic acid, ethyl brevifolincarboxylate, urolic and maslinic acids, and daucosterol were isolated from the ethanolic extract of the flowers of *Punica granatum* (Wang et al. 2006). Maslinic acid exhibited antioxidant activity as evaluated by measurement of LDL susceptibility to oxidation. A taraxastane-type trit-erpene, punicanolic acid; two galloyl glucoses, 1,2,6-tri-*O*-galloyl β-D-glucopyranoside, 1,2-di-*O*-galloyl-4,6-*O*-(S)-hexahydroxydiphenoyl β-D-glucopyranoside; flavones, luteolin; triterp-nenes oleanolic acid, maslinic acid; and β-sitosterol were isolated from pomegranate flowers (Xie et al. 2008).

## *Phytochemicals in Leaves*

An alkaloid 2-(2-propenyl)-Δ1-piperideine was isolated from pomegranate leaves (Roberts et al. 1967).

Pomegranate leaves were found to contain tannins granatin A, granatin B, corilagin, strictinin, 1,2,4,6- tetra-*O*-galloyl—β-D-glucose and 1,2,3,4,6 –penta-*O*-galloyl-β-D-glucose and an ellagitannin, punicafolin elucidated as 1, 2, 4-tri-*O*-galloyl-3, 6-(R)-hexahydroxydiphenoyl-β-D-glucose (Tanaka et al. 1985, 1990). Pomegranate leaves were found to be rich in polyphenols: brevifolin carboxylic acid, brevifolin, corilagin, 3,6-(*R*)-hexahydroxydiphenoyl-(α/β)-$^1C_4$-glucopyranose, 1,2,6-tri-*O*-galloyl-β-$^4C_1$-glucopyranose, 1,4,6-tri-*O*-galloyl-β-$^4C_1$-glucopyranose, ellagic acid, 3,4,8,9,10-pentahydroxydibenzo[*b,d*]pyran-6-one, granatin-B and punicafolin (Nawwar et al. 1994b); N-(2′,5′-dihydroxyphenyl) pyridinium chloride, as well as the known flavone glycosides, apigenin 4′-*O*-β-glucopyranoside, luteolin 4′-*O*-*P*-glucopyranoside, luteolin 3′-*O*-β-glucopyranoside and luteolin 3′-*O*-β-xylopyranoside (Nawwar et al. 1994a); ellagitannin, punicafolin, tannins, granatins A and B, corilagin,

strictinin, 1,2,4,6-tetra-*O*-galloyl-β-D-glucose and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (Tanaka et al. 1985); gallotannins, 1,2,4-tri-*O*-galloyl-β-glucopyranose and 1,3,4-tri-*O*-galloyl-β-glucopyranose together with the hitherto unknown ellagitannins, 1,4-di-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenyl-β-glucopyranose and brevifolin carboxylic acid 10-monopotassium sulphate (Hussein et al. 1997). A hydroquinone pyridinium alkaloid in the form a mixture of a conjugated and a cross-conjugated heterocyclic mesomeric betaine was isolated from the leaves of *Punica granatum* (Schmidt et al. 2005). Balwani et al. (2011) isolated a novel compound, 2-methyl-pyran-4-one-3-*O*-β-d-glucopyranoside from pomegranate leaves.

## *Phytochemicals in Stem Bark/Root*

These alkaloid isopelletierine, methylisopelletierine, pelletierine, pseudopelletierine were isolated from pomegranate bark (Chilton and Partridge 1950; Wibaut et al. 1954) and roots (Chilton and Partridge 1950); isopelletierine, methylisopelletierine and ψ pelleterine from bark (Wibaut and Hollstein 1957); and n-acetyl-sedridine from bark and root (Neuhöfer 1990). The bark is rich in punicotannic acid (about 22%) and also contains gallic acid, mannite and four alkaloids isopelletierine, methylisopelletierine, pelletierine, pseudopelletierine (Grieve 1971). The following alkaloids were isolated from pomegranate bark and roots: pelletierine, methylisopelletierine, pseudopelletierine and from roots norpseudopelletierine, sedridine, 2-(2′-hydroxypropyl) Δ1-piperidine; 2-(2′-propenyl)Δ1-piperidine, hygrine and norhygrine (Neuhöfer et al. 1993). Tannins and related compound were isolated from pomegranate bark and included punicalin and punicalagin elucidated as to 4, 6-(S, S)-gallagyl-D-glucose (1) and 2,3-(S)-hexahydroxy-diphenoyl-4,6-(S, S)-gallagyl-D-glucose (2), respectively and a hydrolyzable tannin, 2-*O*-galloyl-4,6-(S,S)-gallagyl-D-glucose (Tanaka et al. 1986a); ellagitannins, punicacorteins A, B, C and D, punigluconin, casuariin and casuarinin (Tanaka et al. 1986b). Punicacorteins

A, B, C and D were established as novel C-glycosideic ellagitannins, the former two possessing a unique tetraphenyl (gallagyl) ester group, and the latter two containing a galloyl group in place of the gallagyl group, while punigluconin was elucidated as 2,3-di-*O*-galloyl-4,6-(S)-hexahydroxydiphenoyl gluconic acid. A flavonoid diglycoside, quercetin-3,4′-dimethyl ether-7-*O*-α-L-arabinofuranosyl (1→6)-β-D-glucopyranoside, quercetin, pelargonidine-3,5-diglucoside and ellagic acid were isolated from pomegranate bark (Chauhan and Chauhan 2001). The heartwood of *Punica grantum* was found to contain ellagitannins: diellagic acid rhamnosyl (1→4) glucopyranoside and 5-*O*-galloylpunicacortein D, tannin metabolites, punicacortein D, punicalin, punicalagin and 2-*O*-galloylpunicalin (El-Toumy and Rauwald 2002); ellagic acid rhamnosides: 3-*O*-methylellagic acid 4-*O*-α-L-rhamnopyranoside and 3,4′-*O*-dimethylellagic acid 4-*O*-α-L-rhamnopyranoside together with brevifolincarboxylic acid, 3-*O*-methylellagic acid and 4,4′-*O*-dimethylellagic acid (El-Toumy and Rauwald 2003); 3′-*O*-methyl-3,4-methylenedioxyellagic acid, as well as eight known ellagitannins and gallotannins (El-Toumy et al. 2001). A new dimeric gallic acid glycoside named humarain was isolated from stem bark of *Punica granatum* (Tantray et al. 2009).

*Punica granatum* is a unique medicinal plant with a long and extensive ethnomedicinal uses since ancient times. Various parts of the plant viz. seed, aril, fruit juice, peel, leaf, flower, bark, and roots have been reported to contain bioactive phytochemicals with interesting medicinal values and pharmacological activities. The phytochemistry and pharmacological properties of pomegranate plant parts suggest a wide range of clinical applications for the treatment and prevention of ailments such as cancer as well as other diseases where chronic inflammation is believed to play an essential etiologic role (Lansky and Newman 2007). The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. In the past two decade, numerous in-vitro, in-vivo and preclinical studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage (Jurenka 2008). Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity.

### Antioxidant Activity

Aqueous and ethyl acetate extracts of pomegranate arils, juice and peels exhibited good antioxidant activity (Ricci et al. 2006). Pomegranate juice, peel, and seed oil antioxidants were confirmed by ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) methods (Elfalleh et al. 2011). The highest values were recorded in peels with 25.63 mmol trolox equivalent/100 g and 22.08 mmol TE/100 g for FRAP and ORAC assay, respectively. The tocopherol (α-tocopherol, γ-tocopherol, and δ-tocopherol) contents were, respectively, 165.77, 107.38, and 27.29 mg/100 g from dry pomegranate seed. Four phenolic compounds were identified and quantified in pomegranate peel and pulp: 2 hydroxybenzoic acids (gallic and ellagic acids) and 2 hydroxycinnamic acids (caffeic and *p*-coumaric acids). Results showed that the antioxidant potency of pomegranate extracts was correlated with their phenolic compound content. In particular, the highest correlation was reported in peels. High correlations were also found between peel hydroxybenzoic acids and FRAP ORAC antioxidant capacities. Identified tocopherols appeared to contribute in major part to the antioxidant activity of pomegranate seed oil.

Gil et al. (2000) found that the antioxidant activity of commercial pomegranate juices (18–20 TEAC) was three times higher than those of red wine and green tea (6–8 TEAC). Commercial juices extracted from whole pomegranates showed higher antioxidant activity than in experimental juices obtained from the arils only (12–14 TEAC). Further, they found that commercial juices contained the pomegranate abundant tannin

punicalagin (1,500–1,900 mg/L) while only traces were detected in the experimental juice obtained from arils showing that pomegranate industrial processing extracts some of the hydrolyzable tannins present in the fruit rind. Also, anthocyanins, ellagic acid derivatives, and hydrolyzable tannins were found in the pomegranate juices. The results of studies by Tzulker et al. (2007) showed that the antioxidant activity in pomegranate aril juice correlated significantly to the total polyphenol and anthocyanin contents. However, the homogenates prepared from the whole fruit exhibited an approximately 20-fold higher antioxidant activity than the level found in the aril juice. Unlike the arils, the antioxidant level in the homogenates correlated significantly to the content of the four hydrolyzable tannins in which punicalagin was predominant, while no correlation was found to the level of anthocyanins.

Pomegranate juice was found to be a potent inhibitor of superoxide anion-mediated disappearance of nitric oxide (Ignarro et al. 2006). It was much more potent than Concord grape juice, blueberry juice, red wine, ascorbic acid, and DL-$\alpha$-tocopherol. As little as three µl of a six-fold dilution of pomegranate juice, in a reaction volume of 5,000 µl, produced a marked antioxidant effect, whereas 300 µl of undiluted blueberry juice or nearly 1,000 µl of undiluted Concord grape juice were required to produce similar effects. pomegranate juice and other antioxidant-containing products were found to augment the anti-proliferative action of nitric oxide (DETA/NO) on vascular smooth muscle cell (rat aorta) proliferation. and other antioxidant-containing products were found to augment the anti-proliferative action of NO on vascular smooth muscle cell (rat aorta) proliferation. Pomegranate juice was much more effective than the other products tested and elicited no effects when tested alone in the absence of added NO. Pomegranate juice elicited no effects on eNOS protein expression or catalytic activity and did not enhance promoter activity in the eNOS gene. The observations indicated that pomegranate juice possessed potent antioxidant activity that resulted in marked protection of nitric oxide against oxidative destruction

Pande and Akoh (2009) in their study found the highest antioxidant capacity to be in pomegranate leaves followed by peel, pulp, and seed. The tannin rich mixtures from pomegranate by-product exhibited $IC_{50}$ values against reactive oxygen species (ROS) generation at 0.8–19 µg/mL. The antioxidant capacity (ORAC) of pomegranate juice was 2,860 µmol TE/100 g pomegranate juice which was comparable to blueberry and grape juice (Thomas and Gebhardt 2008). Oral administration of flavonoid rich fractions from pomegranate fruits to rats at a dose of 10 mg/kg/day exhibited potential antiperoxidative activity (Sudeesh and Vijayalakshmi 2005). Malondialdehyde, hydroperoxides and conjugated dienes levels in the liver were significantly decreased antioxidative enzymes catalase, superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase were significantly elevated. Glutathione content in the tissues were also increased. Pomegranate fermented juice and cold pressed seed oil exhibited potent antioxidant activity almost equivalent to butylated hydroxyanisole (BHA) and green tea (*Thea sinensis*), but significantly higher than that of red wine (*Vitis vitifera*) (Schubert et al. 1999). Flavonoids extracted from cold pressed pomegranate seed oil exhibited 31–44% inhibition of sheep cyclooxygenase and 69–81% inhibition of soybean lipoxygenase. Flavonoids extracted from pomegranate fermented juice displayed 21–30% inhibition of soybean lipoxygenase but showed no significant inhibition of sheep cyclooxygenase. Total polyphenols in cold pressed pomegranate seed oil showed a concentration by weight of approximately 0.015%. Fatty acid composition in cold pressed pomegranate seed oil showed punicic acid (65.3%) along with palmitic acid (4.8%), stearic acid (2.3%), oleic acid (6.3%), linoleic acid (6.6%) and three unidentified peaks from which two (14.2%) are probably isomers of punicic acid.

Acetone extract (70%) of pomegranate fruit displayed scavenging activity against hydroxyl (·OH) and superoxide (O2·-) radicals (Noda et al. 2002). Its three major anthocyanindins, delphinidin, cyanidin, and pelargonidin, scavenged O2·- in a dose-dependent fashion with $ID_{50}$ values of

2.4, 22, and 456 μM, respectively but did not effectively scavenge nitric oxide. The anthocyanidins inhibited a Fenton reagent ·OH generating system. Further, the anthocyanidins inhibited hydrogen peroxide-induced lipid peroxidation in the rat brain homogenates with $ID_{50}$ values 0.7, 3.5, and 85 μM, respectively for delphinidin, cyanidin, and pelargonidin (Noda et al. 2002). In another study, pomegranate elagitannins – ellagic acid, gallagic acid, punicalins and punicalagins from pomegranate fruit showed $IC_{50}$ values of 1.1, 3.2, 2.3 and 1.4 μM, respectively, against reactive oxygen species (ROS) generation and no toxicity up to 31.25 μg/mL against HL-60 cells (Reddy et al. 2007). The good antioxidant action of punicalagin a high molecular weight polyphenol isolated from pomegranate fruit pith and carpellary membrane was expressed not only through its scavenging reactions but also by its ability to form metal chelates (Kulkarni et al. 2007). Binding of punicalagin with bovine serum albumin and metal ions such as iron and copper revealed different binding affinities, whereas its binding with DNA was very weak and non-specific. In-vitro cytotoxic studies against three cell lines, namely, Vero (normal African green monkey kidney cell line), Hep-2 (human larynx epithelial cancer cell line), and A-549 (human small cell lung carcinoma cell line) showed that punicalagin, was toxic only at higher concentration.

Studies found that pomegranate peel had the highest antioxidant activity among the peel, pulp and seed fractions of 28 kinds of fruits commonly consumed in China as determined by FRAP (ferric reducing antioxidant power) assay (Guo et al. 2003). In a subsequent study (Li et al. 2006) pomegranate peel extract was shown to have markedly higher antioxidant capacity than the pulp extract in scavenging or preventive capacity against superoxide anion, hydroxyl and peroxyl radicals as well as inhibiting CuSO4-induced LDL oxidation. The contents of total phenolics, flavonoids and proathocyanidins were also higher in peel extract than in pulp extract. The large amount of phenolics contained in peel extract may cause its strong antioxidant ability. The authors concluded that pomegranate peel extract

appeared to have more potential as a health supplement rich in natural antioxidants than the pulp extract. Separate studies showed pomegranate peel extracts to have both antioxidant and antimutagenic properties and may be exploited as biopreservatives in food applications and neutraceuticals (Negi et al. 2003). All the pomegranate peel extracts (ethyl acetate, acetone, methanol and water) exhibited marked antioxidant capacity, but the water extract was the lowest. The order of antioxidant capacity varied because of differential responses at four concentrations (25, 50, 75 and 100 μg/mL) in each solvent (Negi et al. 2003). Studies in male rats showed that pomegranate fruit peel extract decreased lipid peroxidation in hepatic, cardiac, and renal tissues and serum glucose concentration (Parmar and Kar 2008). Pomegranate peels were found to contain potent antioxidant contents, as evidenced by free radical DPPH scavenging value of 3.58 μg/mL and ABTS scavenging value of 7.364 mM Trolox equivalent antioxidant capacity/100 g dry weight (Elfalleh et al. 2009). Aqueous and alcoholic extracts of pomegranate rind showed good antioxidant effect with $IC_{50}$ ranging from 34.78 to 135.27/mL for aqueous and 40.03–105.93 μg/mL for alcoholic extracts (Rajan et al. 2011). Phenolic compounds, tannins and flavonoids were the major phytochemicals present in both the extracts. The aqueous and alcoholic extract yielded 122.33 and 176 mg/g gallic acid equivalent phenolic content, 135.33snf 81.33 mg/g quercetin equivalent flavonoid and 81.66 and 114.23 mg/g tannic acid equivalent tannins respectively.

Plumb et al. (2002) found that the prodelphinidin dimers from pomegranate peels were potent antioxidants in the aqueous phase, being much more effective than the gallocatechin monomer in scavenging of the radical cation of 2,2-azinobis (3-ethyl-benzothiazoline-6-sulphonate, ABTS) relative to the water-soluble vitamin E analogue Trolox C (expressed as Trolox C equivalent antioxidant capacity, TEAC). In the lipid phase, only one of the dimers (gallocatechin-(4–8)-catechin) was significantly more effective than the monomer in the inhibition of lipid peroxidation of phosphatidylcholine vesicles. The water, methanol, acetone and ethyl acetate (EtOAc) extracts of

pomegranate peel phenolics showed enhanced inhibitory effect on lard peroxidation as the phenolic concentrations increased (Zhang et al. 2007). Acetone extract exhibited the highest antiliperoxidant activity followed by water, methanol and EtOAc extracts. Acetone extract at 0.1% (w/w) and water extract at 0.2% (w/w) exhibited an antiliperoxidant effect close to that of tea polyphenols (0.02%, w/w) and higher than that of BHT (butylated hydroxytoluene) (0.02%, w/w). At 0.2% (w/w), acetone extract exerted a higher inhibitory activity on lard oxidation than that of tea polyphenols and BHT. Studies by Guo et al. (2007) showed that showed that red pomegranate peel extract had the best effect on the scavenging ability of superoxide anion with lowest $IC_{50}$ value (4.01 μg/mL) among all pomegranate extracts (peel, juice, and seed of three varieties). The peel extract of white pomegranate had the best scavenging ability on hydrogen peroxide with the lowest $IC_{50}$ value (0.032 μg/mL) of the nine extracts. The seed extract of white pomegranate could scavenge hydroxide radical most effectively of the nine extracts (the $IC_{50}$ value 1.69 μg/mL). The seed extract of white pomegranate (the $IC_{50}$ value was 3.67 μg/mL) was the most powerful on the DNA damage-preventing effect of the extracts. The results of studies by Xu et al. (2008) indicated that pomegranate peel extracts exerted protective effects on oxidative stress in mice loaded with restraint stress which may be attributed to its free radical scavenging activity and lipid peroxidation inhibitory effect. The extract decreased alanine aminotransferase and malondialdehyde levels and increased antioxidant capacity in the liver and glutathione levels in plasma as compared with restraint stress control mice. The methanol fraction of pomegranate peel showed highest antioxidant activity by all the four in vitro assays viz. DPPH free radical scavenging, phosphomolybdenum, FRAP (Fe(3+) reducing power) and CUPRAC (cupric ions (Cu(2+)) reducing ability) comparable to ascorbic acid and butylated hydroxy toluene (BHT) followed by activity in ethanol, acetone, and ethyl acetate fractions (Zahin et al. 2010a).

In cell free-systems, preparations from various parts of pomegranate displayed displayed good antioxidant capacity as assayed by 1,1-diphenyl-2-picrylhydrazyl (DPPH), chemiluminescence luminol/xanthine/xanthine oxidase and lipoxygenase assays, with relative potency sequence of rind extract > pomegranate juice > aril juice (Sestili et al. 2007). However, only the rind extract was capable of preventing the deleterious effects – cytotoxicity, DNA damage and depletion of non-protein sulphydrils (NPSH) pool, caused by treatment of cells with hydroxide peroxide, tert-butylhydroperoxide or oxidized lipoproteins (Ox-LDL) via a mechanism which was postulated to involve both direct scavenging of radical species and iron chelation. The results suggested that the aril juice the major and tasty part of pomegranate fruit, did not contain ellagic acid and punicalagin (i.e. the polyphenols highly represented in the rind which appeared to be responsible for the antioxidant capacity) in amounts sufficient to exert cytoprotection in oxidatively injured, living cells. Based on these results, the authors advocated that development and evaluation of rinds-only based derivatives of pomegranate for antiatherogenic preventive purposes in humans should be encouraged.

The antioxidant activity (percentage of inhibition of on peroxidation in linoleic acid system) of CPJ (traditional sour concentrate of pomegranate juice) was determined to be higher (85.91%) than that of PJ (pomegranate juice) (79.06%) (Orak 2009). During the concentration process, the reducing sugars, glucose and fructose level of CPJ showed an increase to 46.46, 23.89, and 22.53%, respectively. In CPJ the amounts of sodium, iron, zinc, copper and lead were found lower than those of PJ. In contrast, potassium and magnesium mineral contents increased during concentration. The total phenolics were also found to be 3,246 and 9,870 μg/mL in PJ and CPJ, respectively. The total anthocyanin content of PJ was found to be 492.9 mg/L but it was not determined in CPJ. 5-Hydroxymethyl furfural was determined to be at a significant level in CPJ as a result of the heat process.

Sezer et al. (2007) found that pomegranate and red wines decreased low-density lipoprotein (LDL) diene levels following a 30-min incubation period compared with controls. However, pure pomegranate wine demonstrated a greater antioxidant effect on diene level (110 μmol/mg of LDL protein)

than pure red wine (124 μmol/mg of LDL protein). The phenol levels of pomegranate and red wines (4,850 mg/L gallic acid equivalents and 815 mg/L gallic acid equivalents, respectively) were in accordance with their total antioxidant activity (39.5 and 33.7%, respectively).

Four compound from pomegranate seeds namely coniferyl 9-*O*-[β-d-apiofuranosyl (1→6)]-*O*-β-d-glucopyranoside (1) and sinapyl 9-*O*-[β-d-apiofuranosyl(1 → 6)]-*O*-β-d-glucopyranoside (2), 3,3′-di-*O*-methylellagic acid (3), 3,3′,4′-tri-*O*-methylellagic acid (4) displayed antioxidant activity, which was evaluated by measurement of low-density lipoprotein (LDL) susceptibility to oxidation and by in-vitro determination of malondialdehyde (MDA) levels in the rat's brain (Wang et al. 2004).

Ethanolic extract of pomegranate flowers was found to contain a large amount of polyphenols and to exhibit potent reducing ability, both indicative of potent antioxidant ability (Kaur et al. 2006). The extract showed 81.6% antioxidant activity in DPPH model system. The flower extract was found to significantly scavenge superoxide ($O^{2-}$) (by up to 53.3%), hydrogen peroxide ($H_2O_2$) (by up to 30%), hydroxyl radicals ($^-OH$) (by up to 37%) and nitric oxide (NO) (by up to 74.5%). The extract also inhibited ($^-OH$) induced oxidation of lipids and proteins in vitro. These results indicated pomegranate flower extract to exert a significant antioxidant activity in-vitro.

Daily consumption of pomegranate juices was found to be potentially better than apple juice in improving antioxidant function in the elderly (Guo et al. 2008). As the plasma ascorbic acid, vitamin E, and reduced glutathione contents did not differ significantly between the apple and pomegranate groups in the study, the phenolics may be the functional components contained in pomegranate juice that accounted for the observations.

## *Anticancer Activity*

Recent in-vitro studies and preclinical animal studies have shown that pomegranate extracts selectively inhibit the growth of breast, prostate, colon and lung cancer cells (Adhami et al. 2009). An initial phase II clinical trial of pomegranate juice in patients with prostate cancer reported significant prolongation of prostate specific antigen doubling time. Some of these researches are further elaborated herein.

Various parts of the pomegranate fruit e.g. seed oil, juice, fermented juice and peel extract, had been shown to exert suppressive effects on human breast cancer cells in-vitro and in this context, three estrogenic compounds, i.e. luteolin, quercetin and kaempferol, were detected in the fruit peel extract (van Elswijk et al. 2004). Studies showed pomegranate fruit possessed chemopreventive and adjuvant therapeutic potential for human breast cancer (Kim et al. 2002). Polyphenols from fermented pomegranate juice, pericarp, and oil inhibited aromatase activity by 60–80% indicating its ability to effect a blockade of endogenous active estrogen biosynthesis. Fermented juice and pericarp polyphenols, and whole seed oil, inhibited 17-β-hydroxysteroid dehydrogenase Type 1 from 34 to 79%, at concentrations ranging from 100 to 1,000 μg/mL in the sequence seed oil >>fermented juice polyphenols>pericarp polyphenols. Lyophilized fresh pomegranate juice elicited a 55% inhibition of the estrogenic activity of 17-β-estradiol; whereas the lyophilized juice by itself displayed only minimal estrogenic action. Inhibition of cell lines by fermented juice and pericarp polyphenols was according to estrogen-dependent (MCF-7) >>estrogen-independent (MB-MDA-231)>normal human breast epithelial cells (MCF-10A). In both MCF-7 and MB-MDA-231 cells, fermented pomegranate juice polyphenols consistently displayed about twice the anti-proliferative effect as fresh pomegranate juice polyphenols. Pomegranate seed oil elicited 90% inhibition of proliferation of MCF-7 at 100 μg/mL medium, 75% inhibition of invasion of MCF-7 across a Matrigel membrane at 10 μg/mL, and 54% apoptosis in MDA-MB-435 estrogen receptor negative metastatic human breast cancer cells at 50 μg/mL. In a murine mammary gland organ culture, fermented juice polyphenols effected 47% inhibition of cancerous lesion formation induced by the carcinogen 7,12-dimethylbenz[a] anthracene (DMBA). Pomegranate seed oil and fermented pomegranate juice polyphenols were found to have anti-angiogenic activity (Toi et al.

2003). In-vitro studies showed that these pomegranate fractions strongly suppressed vascular endothelial growth factor in normal human breast epithelial cells (MCF-10A) and in estrogen sensitive (MCF-7) human breast cancer cells, but upregulated migration inhibitory factor in estrogen resistant (MDA-MB-231) human breast cancer cells. An anti-proliferative effect on angiogenic cells was shown in human umbilical vein endothelial cell (HUVEC) and in myometrial and amniotic fluid fibroblasts, and inhibition of HUVEC tubule formation was also demonstrated in an in-vitro model employing glass carrier beads. Additionally, they showed a significant reduction in new blood vessel formation using the chicken chorioallantoic membrane (CAM) model in-vivo. In another study, pretreatment of mouse mammary organ culture with pomegranate fermented juice polyphenols (W), a high-performance liquid chromatographic (HPLC) peak separated from W (peak B), or pomegranate seed oil prior to exposure to the to the carcinogen 7,12-dimethylbenz[a] anthracene (DMBA) resulted in a 42% reduction in the number of lesions for W compared with control, peak B and pomegranate seed oil each effected an 87% reduction (Mehta and Lansky 2004). Both pomegranate extracts and genistein inhibit the growth of MCF-7 breast cancer cells through induction of apoptosis, with combination treatment being more efficacious than single treatments (Jeune et al. 2005). More recent studies demonstrated that pomegranate fruit extract dose-dependently inhibited NF-kB-dependent reporter gene expression associated with proliferation, invasion, and motility in aggressive breast cancer phenotypes while suppressing RhoC and RhoA protein expression (Khan et al. 2009). The bioactive components of the fruit extract comprised mainly ellagitannins and phenolic acids in the aqueous fruit extract and conjugated octadecatrienoic acids in the lipid fruit extract derived from seeds. The results suggested a role of pomegranate fruit extract in lowering the metastatic potential of aggressive breast cancer species. Pomegranate extract inhibited the proliferation and viability of MMTV-Wnt-1 mouse mammary cancer stem cells in-vitro in a time- and concentration-dependent manner (Dai et al.

2010). Its constituents ellagic ursolic acid and luteolin also caused a time- and concentration-dependent reduction of cell proliferation and viability, suggesting that they contribute to the inhibitory effect of the extract, while caffeic acid had no effect. The methanolic pomegranate fruit peel extract was found to reduce cell proliferation and induce apoptosis on MCF-7 breast cancer cells (Dikmen et al. 2011). In addition, expression of the pro-apoptotic gene Bax was increased, and that of the anti-apoptotic gene Bcl-2 was decreased after pomegranate extract treatment. The extract exhibited high antioxidant activity and yielded total phenolic content of 331.28 mg of gallic acid equivalents/g of extract with ellagic acid as the most abundant constituent.

Among the ten pomegranate ellagitannin-derived compounds (namely ellagic acid, gallagic acid, urolithins A and B and their acetylated, methylated, and sulfated analogues), urolithin B (UB) was shown to most effectively inhibit aromatase activity in a live breast cancer cell assay (Adams et al. 2010). UB significantly inhibited testosterone-induced MCF-7aro cell proliferation. The remaining test compounds also exhibited antiproliferative activity, but to a lesser degree than UB. The results suggested pomegranate ET-derived compounds to have potential for the prevention of estrogen-responsive breast cancers. Pomegranate seed linolenic acid isomers, punicic acid and α-eleostearic acid were found to be selective estrogen receptor modulators (SERMs) in-vitro (Tran et al. 2010). Punicic acid inhibited ($IC_{50}$) estrogen receptor (ER) α at 7.2 μM, estrogen receptor β at 8.8 μM. α-eleostearic acid (AEA) inhibited ERα/ERβ at 6.5/7.8 μM. Punicic acid agonized ERα/ERβ ($EC_{50}$) at 1.8/2 μM, antagonizing at 101/80 μM. α-eleostearic acid antagonized ERα/ERβ at 150/140 μM. Both isomers induced ERα and ERβ mRNA expression in MCF-7 breast cancer cells, but not in MDA-MB-231 breast cancer cells. Punicic acid, an omega-5 fatty acid in pomegranate seed oil, was found capable of inhibiting breast cancer proliferation (Grossmann et al. 2010). Proliferation was inhibited 92 and 96% for MDA-MB-231 and MDA-ERα7 cells,

respectively. Further punicic acid induced apoptosis in the MDA-MB-231 and MDA-ERα7 cells by 86 and 91%, respectively compared to untreated control cells and disrupted cellular mitochondrial membrane potential. The results suggested the breast cancer inhibitor properties of punicic acid were dependent on lipid peroxidation and the protein kinase C signalling pathway.

Treatment of human lung carcinoma A549 cells with pomegranate fruit extract resulted in a decrease in the viability of A549 cells and dose-dependent arrest of cells in G0-G1 phase of the cell cycle (Khan et al. 2007a, b). Treatment of cells with pomegranate fruit extract inhibited (i) phosphorylation of MAPK proteins, (ii) PI3K, (iii) phosphorylation of Akt at Thr308, (iv) NF-kappaB and IKKα, (v) degradation and phosphorylation of IkappaBα, and (vi) Ki-67 and PCNA. Oral administration of pomegranate fruit extract (0.1 and 0.2%, wt/vol) to athymic nude mice implanted with A549 cells resulted in a significant inhibition in tumour growth. Treatment of mice with pomegranate juice prior to exposure to carcinogens benzo(a)pyrene (B(a)P) and N-nitroso-tris-chloroethylurea (NTCU), resulted in statistically significant lower lung tumour multiplicities than mice treated with carcinogens only (Khan et al. 2007a). Treatment of cells with pomegranate fruit extract caused inhibition of (a) activation of nuclear factor-kappaB and IkappaBα kinase, (b) degradation and phosphorylation of IkappaBα, (c) phosphorylation of mitogen-activated protein kinases (extracellular signal-regulated kinase 1/2, c-Jun NH(2)-terminal kinase 1/2, and p38), (d) phosphatidylinositol 3-kinase (p85 and p110), (e) phosphorylation of Akt at Thr(308), (f) activation of mammalian target of rapamycin signaling, (g) phosphorylation of c-met, and (h) markers of cell proliferation (Ki-67 and proliferating cell nuclear antigen) and angiogenesis (inducible nitric oxide synthase, CD31, and vascular endothelial growth factor) in lungs of B(a)P- and NTCU-treated mice. Overall, the results suggested that pomegranate fruit extract could be a useful chemopreventive/chemotherapeutic agent against human lung cancer.

Flavonoid-rich polyphenol fractions from the pomegranate fruit had been reported to exert anti-proliferative, anti-invasive, anti-eicosanoid, and pro-apoptotic actions in breast and prostate cancer cells and anti-angiogenic activities in-vitro and in-vivo (Kawaii and Lansky 2004). They found that various fruit extracts had proportional inhibitory effects on human HL-60 promyelocytic leukemia cell proliferation. Fermented pomegranate juice and aqueous extract of pomegranate pericarps were found to be strong promoters of differentiation in all settings, while fresh juice extract showed only a relatively mild differentiation-promoting effect. Li et al. (2011) found that pomegranate ellagitannins bound with gelatin to form self-assembled nanoparticles. Ellagitannins encapsulated in nanoparticles were less effective in inducing the early stage of apoptosis on human promyelocytic leukemia cells HL-60. But they had similar effects in inducing late stage of apoptosis and necrosis. Differentiation refers to the ability of cancer cells to revert to their normal counterparts, and its induction represents an important noncytotoxic therapy for leukemia, and also breast, prostate, and other solid malignancies (Kawaii and Lansky 2004).

Pomegranate emulsion treatment (1 or 10 g/kg) to rats, started 4 weeks prior to the dietary carcinogen diethylnitrosamine (DENA) challenge and continued for 18 weeks thereafter, showed striking chemopreventive activity demonstrated by reduced incidence, number, multiplicity, size and volume of hepatic nodules, precursors of hepatocellular carcinoma (Bishayee et al. 2011). Both doses of the emulsion significantly attenuated the number and area of γ-glutamyl transpeptidase-positive hepatic foci compared with the DENA control. The emulsion also attenuated DENA-induced hepatic lipid peroxidation and protein oxidation and elevated protein and messenger RNA expression of the hepatic nuclear factor E2-related factor 2 (Nrf2).

The methanolic extract of *Punica granatum* flowers was exhibited inhibitory effect on tumour necrosis factor-α (TNF-α, 1 ng/mL)-induced cytotoxicity in L929 (murine fibroblast) cells (Xie et al. 2008). A new taraxastane-type triterpene, punicanolic acid (1), was isolated from

the active fraction (ethyl acetate-soluble fraction) together with four triterpenes (2–5), two galloyl glucoses (6, 7), two flavones (8, 9), and β-sitosterol. Among the constituents, 1, oleanolic acid (2), maslinic acid (4), 1,2,6-tri-*O*-galloyl β-D-glucopyranoside (6), 1,2-di-*O*-galloyl-4,6-*O*-(S)-hexahydroxydiphenoyl β-D-glucopyranoside (7), and luteolin (8) significantly inhibited TNF-α-induced cytotoxicity in L929 cells at 30 μM.

Four pure chemicals, ellagic acid (E), caffeic acid (C), luteolin (L) and punicic acid (P), all important components of the aqueous compartments or oily compartment of pomegranate fruit exhibited anticancerous activities by inhibiting human PC-3 prostate cancer cell invasion of Matrigel artificial membranes (Lansky et al. 2005a). All compounds significantly inhibited invasion when employed individually. When C, P, and L were equally combined at the same gross dosage (4 μg/mL) as when the compounds were tested individually, a supra-additive inhibition of invasion was observed. Pomegranate cold-pressed seed oil, fermented juice polyphenols (W), and pericarp polyphenols (P) each acutely inhibited in-vitro proliferation of human prostate cancer, LNCaP, PC-3, and DU 145 human cancer cell lines (Albrecht et al. 2004). These effects were mediated by changes in both cell cycle distribution and induction of apoptosis. For example, the androgen-independent cell line DU 145 showed a significant increase from 11 to 22% in G(2)/M cells by treatment with pomegranate oil (35 μg/mL) with a modest induction of apoptosis. In other cell lines/treatments, the apoptotic response predominated, for example, in PC-3 cells treated with pomegranate pericarp polyphenols, at least partially through a caspase 3-mediated pathway. All agents potently suppressed PC-3 invasion through Matrigel, and furthermore pomegranate pericarp polyphenols and seed oil demonstrated potent inhibition of PC-3 xenograft growth in athymic mice. Overall, the study demonstrated significant antitumour activity of pomegranate-derived materials against human prostate cancer. In another study, combinations of the anatomically discrete pomegranate fractions: fermented pomegranate juice polyphenols (W), pomegranate

pericarp (peel) polyphenols (P) or pomegranate seed oil (Oil) exhibited synergistic prostate cancer suppression (Lansky et al. 2005b). Supra-additive, complementary and synergistic effects were proven in all models. Proliferation effects were additionally evaluated with CompuSyn software median effect analysis and showed a concentration index CI < 1, confirming synergy.

Pomegranate fruit extract (PFE) exhibited antiproliferative and proapoptotic activities against human prostate cancer cells (Malik et al. 2005; Malik and Mukhtar 2006). PFE (10–100 μg/mL; 48 h) treatment of highly aggressive human prostate cancer PC3 cells resulted in a dose-dependent inhibition of cell growth/cell viability and induction of apoptosis. Immunoblot analysis revealed that PFE treatment of PC3 cells resulted in (i) induction of Bax and Bak (proapoptotic); (ii) down-regulation of Bcl-X(L) and Bcl-2 (anti-apoptotic); (iii) induction of WAF1/p21 and KIP1/p27; (iv) a decrease in cyclins D1, D2, and E; and (v) a decrease in cyclin-dependent kinase (cdk) 2, cdk4, and cdk6 expression. Findings established the involvement of the cyclin kinase inhibitor-cyclin-cdk network during the antiproliferative effects of PFE. Oral administration of PFE (0.1 and 0.2%, wt/vol) to athymic nude mice implanted with androgen-sensitive CWR22Rnu1 cells resulted in a significant inhibition in tumour growth concomitant with a significant decrease in serum prostate-specific antigen levels. The results suggested that pomegranate juice may have cancer-chemopreventive as well as cancer-chemotherapeutic effects against prostate cancer in humans. In a phase II, Simon two-stage clinical trial for men with a rising prostate-specific antigen (PSA), daily consumption of pomegranate juice was found to have a positive effect following surgery or radiation for prostate cancer (Pantuck et al. 2006). There were no serious adverse events reported and the treatment was well tolerated. Mean PSA doubling time significantly increased with treatment from a mean of 15 months at baseline to 54 months posttreatment. In-vitro assays comparing pretreatment and posttreatment patient serum on the growth of human prostate cancer LNCaP showed a 12% decrease in cell proliferation and a 17%

increase in apoptosis, a 23% increase in serum nitric oxide, and significant reductions in oxidative state and sensitivity to oxidation of serum lipids after versus before pomegranate juice consumption. In further studies, a standardized ellagitannins (ETs)-enriched pomegranate extract (PE), significantly inhibited LAPC-4 xenograft growth in severe combined immunodeficient (SCID) mice as compared to vehicle control Seeram et al. 2007). Ellagic acid and several synthesized urolithins were shown to inhibit the growth of human prostate cancer CaP cells in-vitro. The chemopreventive potential of pomegranate ETs and localization of their bioactive metabolites in mouse prostate tissue suggested that pomegranate may play a role in CaP treatment and chemoprevention.

The results of studies demonstrated that an ellagitannin-rich pomegranate extract could inhibit tumour-associated angiogenesis as one of several potential mechanisms for slowing the growth of prostate cancer in chemopreventive applications (Sartippour et al. 2008). A pomegranate extract standardized to ellagitannin content (POMx) inhibited the proliferation of LNCaP and HUVEC cells significantly under both normoxic and hypoxic conditions. HIF-1$\alpha$ (hypoxia-inducible factor-1$\alpha$) and VEGF (vascular endothelial growth factor) protein levels were also reduced by POMx under hypoxic conditions. POMx decreased prostate cancer xenograft size, tumour vessel density, vascular endothelial growth factor (VEGF) peptide levels and HIF-1$\alpha$ expression after 4 weeks of treatment in severe combined immunodeficient (SCID) mice. Studies showed that pomegranate extract inhibited androgen-independent prostate cancer growth through a nuclear factor-kappaB-dependent mechanism (Rettig et al. 2008). Pomegranate extract (PE) inhibited NF-kappaB and cell viability of prostate cancer cell lines in a dose-dependent fashion in vitro. Maximal PE-induced apoptosis was dependent on PE-mediated NF-kappaB blockade. In the LAPC4 xenograft model, PE delayed the emergence of LAPC4 androgen-independent xenografts in castrated mice through an inhibition of proliferation and induction of apoptosis. The scientist also

showed that Pomegranate polyphenols inhibited gene expression and androgen receptor (AR) most consistently in the human prostate cancer LNCaP-AR cell line (Hong et al. 2008). Therefore, inhibition by pomegranate polyphenols of gene expression involved in androgen-synthesizing enzymes and the AR may be of particular importance in androgen-independent prostate cancer cells and the subset of human prostate cancers where AR is up-regulated. Koyama et al. (2010) demonstrated that pomegranate extract derived from rind and arils (minus seeds) inhibited cell proliferation and induced apoptosis in human LAPC4 prostate cancer cells by modulation of the IGF-IGFBP (insulin growth factor – insulin growth factor binding proteins) axis. Pomegranate extract treatment also decreased IGF-1 mRNA expression in a dose-dependent manner indicating that its actions also involved tumour-specific suppression of IGF-1.

Pomegranate peel extracts increased the levels of oxygen radical absorbance capacity (ORAC) in plasma and the density of lecithin and the levels of Zn in prostatitic rats (Kuang et al. 2009). It decreased the levels of malondialdehyde of prostate and the activity of acid phosphatase and the number of white blood cell and adjusted the levels of NO in plasma compared with the prostatitis model group. The results indicated that pomegranate peel extracts could markedly improve the protective function of oxidation resistance. Pomegranate ellagitannins/microbial metabolites were found to have CYP1B1 (a target in prostate cancer chemoprevention) inhibitory activity in prostate cancer cells (Kasimsetty et al. 2009). Urolithin A, a microbial metabolite, was the most potent uncompetitive inhibitor of CYP1B1-mediated ethoxyresorufin-*O*-deethylase (EROD) activity, exhibiting two-fold selectivity over CYP1A1, while urolithin B was a noncompetitive inhibitor with three-fold selectivity. The punicalins and punicalagins exhibited potent CYP1A1 inhibition with 5–10-fold selectivity over CYP1B1. Cellular uptake experiments demonstrated a five-fold increase in urolithin uptake by 22Rv1 cells. Western blots of the CYP1B1 protein indicated that the urolithins interfered with the expression of CYP1B1 protein. Thus,

urolithins were found to display a dual mode mechanism by decreasing CYP1B1 activity and expression. Wang et al. (2011) showed that in addition to causing cell death of hormone-refractory prostate cancer cells, pomegranate juice also increased cell adhesion and decreased cell migration of the unkilled cells. Pomegranate juice was found to upregulate genes involved in cell adhesion such as E-cadherin, intercellular adhesion molecule 1 (ICAM-1) and down-regulated genes involved in cell migration such as hyaluranan-mediated motility receptor (HMMR) and type I collagen. In addition, pomegranate juice significantly decreased the level of secreted pro-inflammatory cytokines/chemokines such as IL-6, IL-12p40, IL-1β and RANTES, thereby having the potential to decrease inflammation and its impact. Pomegrante juice also inhibited the ability of the chemokine SDF1α to chemoattract these cancer cells. Faria et al. (2007) found that pomegranate juice consumption decreased total hepatic cytochrome P450 (CYP) content as well as the expression of CYP1A2 and CYP3A in male mice. Prevention of procarcinogen activation through CYP activity/expression inhibition may be involved in pomegranate juice's effect on tumour initiation, promotion, and progression

Pomegranate juice showed greatest antiproliferative activity against all cell lines namely human oral (KB, CAL27), colon (HT-29, HCT116, SW480, SW620) and prostate (RWPE-1, 22Rv1) tumour cells by inhibiting proliferation from 30 to 100% (Seeram et al. 2005a). At 100 μg/mL, pomegranate juice, ellagic acid, punicalagin and a standardized total pomegranate tannin (TPT) extract induced apoptosis in HT-29 colon cells. However, in the HCT116 colon cells, ellagic acid, punicalagin and TPT but not pomegranate juice induced apoptosis. The trend in antioxidant activity was pomegranate juice > TPT > punicalagin > ellagic acid. Their data indicated the superior bioactivity of pomegranate juice compared to its purified individual polyphenolic active ingredients illustrating the multifactorial effects and chemical synergy of the action of multiple compounds. In further studies, they (Adams et al. 2006) found that pomegranate juice

significantly suppressed TNF-α-induced COX-2 protein expression by 79%, total pomegranate tannin extract (TPT) 55%, and punicalagin 48% in HT-29 colcon cells. In addition, pomegranate juice reduced phosphorylation of the p65 subunit and binding to the NFkappaB response element 6.4-fold, TPT suppressed NFkappaB binding ten-fold, punicalagin 3.6-fold, whereas ellagic acid was ineffective. Pomegranate juice also abolished TNFα-induced AKT activation, needed for NFkappaB activity. Pomegranate fruit rich in ellagitannins may have beneficial effects against colon cancer. In the stomach and gut, ellagitannins were reported to be hydrolyzed to release ellagic acid (EA) and were converted by gut microbiota to urolithin A (3,8-dihydroxy-6H-dibenzopyran-6-one) type metabolites (Sharma et al. 2010). They reported that pomegranate ellagitannin extract, ellagic acid, and their colonic metabolite, urolithin A inhibited Wnt signaling, which plays a pivotal role in human colon carcinogenesis, suggesting that ET-rich foods may have potential against colon carcinogenesis and that urolithins were relevant bioactive constituents in the colon. Studies by González-Sarrías et al. (2009) showed that elagic acid and its colonic metabolites, urolithin-A (3,8-dihydroxy-6H-dibenzo[b,d] pyran-6-one) and urolithin-B (3-hydroxy-6H-dibenzo[b,d]pyran-6-one), modulated phase I and phase II detoxifying enzymes in colon cancer Caco-2 cells. Ellagic acid and urolithins may exert some blocking chemopreventive effects in the colon but this effect may be critically affected by interfering factors, such as the food matrix nature. Saruwatari et al. (2008) found that pomegranate juice potently inhibited the sulfoconjugation of 1-naphthol in Caco-2 human colon carcinoma cells. The inhibition was both dose- and culture time-dependent, with a 50% inhibitory concentration ($IC_{50}$) value of 2.7% (vol/vol). Punicalagin, the most abundant antioxidant polyphenol in pomegranate juice, was also found to strongly inhibit sulfoconjugation in Caco-2 cells with an $IC_{50}$ of 45 μM. additionally pomegranate juice and punicalagin both inhibited phenol sulfotransferase activity in Caco-2 cells. The data also suggested that constituents of pomegranate

juice, most probably punicalagin, impaired the enteric functions of sulfoconjugation and that this may have effects upon the bioavailability of drugs and other compounds and may be related to the anticarcinogenic properties of pomegranate juice. Pomegranate seed oil (PGO) rich in 70% *cis*(c)9,*trans*(t)11,c13-18:3 as conjugated linolenic acids (CLA) could suppress by azoxymethane -induced colon carcinogenesis, and the inhibition was associated in part with the increased content of CLA in the colon and liver and/or increased expression of peroxisome proliferator-activated receptor (PPAR) γ protein in the colon mucosa (Kohno et al. 2004). Pomegranate extract was found to induce cell cycle arrest and alter cellular phenotype of human pancreatic cancer cells PANC-1 and AsPC-1 (Nair et al. 2011)

Studies by Weisburg et al. (2010) showed that pomegranate extract exerted greater antiproliferative effects towards cancer (such as HSC-2 carcinoma), than to normal, cells, isolated from the human oral cavity. The antiproliferative mechanism of pomegranate extract was, in part, by induction of oxidative stress. The mode of cell death was by apoptosis, as activation of caspase-3, and cleavage of PARP. Reduction of caspase-3 activation and of PARP cleavage in cells co-treated with pomegranate extract and either cobalt or pyruvate, respectively, as compared to pomegranate extract alone, indicated that apoptosis was through the prooxidant nature of pomegranate extract.

Pomegranate seed oil (5%) significantly decreased mice skin tumour incidence, multiplicity, and 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced ornithine decarboxylase activity, an important event in skin cancer promotion (Hora et al. 2003). The results suggested the potential of pomegranate seed oil as a safe and effective chemopreventive agent against skin cancer. Afaq et al. (2005a, b) demonstrated that topical application of pomegranate fruit extract (PFE) prior to 12-*O*-tetradecanoylphorbol-13-acetate (TPA) application on mouse skin afforded significant time-dependent inhibition, against TPA-mediated increase in skin edema and hyperplasia, epidermal ornithine decarboxylase (ODC) activity and protein expression of ODC and cyclooxygenase-2.

Also, application of PFE resulted in inhibition of TPA-induced phosphorylation of ERK1/2, p38 and JNK1/2, as well as activation of NF-kappaB and IKKα and phosphorylation and degradation of IkappaBα. Pretreatment of PFE on TPA-induced skin tumour promotion in 7,12-dimethylbenz(a)anthracene-initiated CD-1 mouse substantially reduced tumour incidence and lower tumour body burden when assessed as total number of tumours per group, percent of mice with tumours and number of tumours per animal as compared to animals that did not receive PFE. Skin application of PFE prior to TPA application also resulted in a significant delay in latency period from 9 to 14 weeks and afforded protection when tumour data were considered in terms of tumour incidence and tumour multiplicity. Studies by George et al. (2011) suggested that pomegranate fruit extract (PFE) and diallyl sulfide (DAS) in combination afforded better suppressive activity of mouse skin tumours than either of these agents alone. PFE and DAS alone delayed onset and tumour incidence by ~ 55 and ~ 45%, respectively, while their combination at low doses synergistically decreased tumour incidence more potentially (~84%,). Further, regression in tumour volume was seen with continuous combinatorial treatment. The inhibition was associated with decreased expression of phosphorylated ERK1/2, JNK1 and activated NF-κB/p65, IKKα, IκBα phosphorylation and degradation in skin tissue/ tumour.

Polysaccharide (PSP001) isolated from pomegranate rind was found to have antioxidant, antitumour and immunomodulatory properties (Joseph et al. 2012). PSP001 exhibited a dose-dependent enhancement in antioxidant activity using concentrations from 10 to 1,000 μg/mL when evaluated using various assays such as, ferric reducing antioxidant power assay, linoleic acid emulsion thiocyanate assay, and superoxide, hydroxyl and nitric oxide radical scavenging assays except for the DPPH assay for which the highest activity was obtained at 200 μg/mL. PSP001 exhibited anticancer activity with $IC_{50}$ values of 97.21 and 52.8 μg/-mL following 72 h incubation for MCF-7 (breast cancer), and K562(leukemia) cells, respectively.

## Antimutagenic Activity

All the pomegranate peel extracts (ethyl acetate (EtOAc), acetone, methanol and water) decreased sodium azide mutagènicity in *Salmonella typhimurium* strains (TA100 and TA1535), either weakly or strongly (Negi et al. 2003). At 2,500 μg/plate all the extracts showed strong antimutagenicity. The antimutagenicity of the water extract was followed by acetone, EtOAc and methanol extracts. The methanol pomegranate peel fraction with promising antioxidant activity showed antimutagenic activity against sodium azide and methyl methane sulphonate with percent inhibition of mutagenicity ranging from 66.76 to 91.86% in a concentration-dependent manner using the Ames Salmonella/microsome assay (Zahin et al. 2010a). Similar trend of inhibition of mutagenicity (81.2–88.58%) against indirect mutagens (2-aminofluorene and benzo(a)pyrene) was also recorded. Phytochemical analysis by HPLC, LC-MS of total phenolic content revealed high content of ellagitannins which might be responsible for promising antioxidant and antimutagenic activities of *P. granatum* peel extract.

Methanol extract of *Punica granatum* flowers (15 mg/plate) showed the highest antimutagenic activity in *Salmonella typhimurium* TA 98 and TA 100, respectively (Wongwattanasathien et al. 2010). The protective effects of these flower extracts might be due to the presence of antimutagenic components that were supposed to be flavonoids.

## Antiviral Activity

Studies demonstrated that tannin from the pericarp of *Punica granatum* was an effective agent against genital herpes simplex virus (HSV-2) (Zhang et al. 1995). The tannin not only inhibited HSV-2 replication, but also showed stronger effects of killing virus and blocking its absorption to cells. *Punica granatum* extract showed anti- human herpes simplex virus type 1 (HSV-1) activity, which was possibly contributed by the polyphenolic compounds in the herbal extract (Li

et al. 2004). Studies by Neurath et al. (2005) indicated that HIV-1 entry inhibitors from pomegranate juice adsorbed onto corn starch and the resulting complex blocked virus binding to CD4 and CXCR4/CCR5 and inhibited infection by primary virus clades A to G and group O. Their results suggested the possibility of producing an anti-HIV-1 microbicide from inexpensive, widely available sources. Pomegranate juice containing polyphenols, β-sitosterol, sugars and ellagic acid) was reported to inactivate HIV and further shown to inactivate influenza, herpes viruses and poxviruses (Kotwal 2008). A formulation consisting of fulvic acid, a complex mixture of compounds was previously reported to render vaccinia virus, HIV and SARS virus non-infectious. Recently, both fulvic acid and pomegranate juice were shown to inactivate genetically diverse strains of influenza including H5N1, further confirming the broad spectrum nature of these agents. Sundararajan et al. (2010) found that the acidity of pomegranate juice and concentrated liquid extract contributed to rapid anti-influenza activity, but this was not a factor with pomegranate polyphenols powder (93%) extract. Studies using pomegranate powder extract showed that 5 min treatment at room temperature with 800 μg/mL pomegranate polyphenols resulted in at least a 3log reduction in the titers of influenza viruses PR8 (H1N1), X31 (H3N2), and a reassortant H5N1 virus derived from a human isolate. However, the antiviral activity was less against a coronavirus and reassortant H5N1 influenza viruses derived from avian isolates. Electron microscopic analysis indicated that viral inactivation by pomegranate polyphenols was primarily a consequence of virion structural damage.

Pomegranate polyphenol extract was shown to have anti-influenza virus properties (Haidari et al. 2009). Of four major polyphenols in pomegranate polyphenol extract (PPE) (ellagic acid, caffeic acid, luteolin, and punicalagin) punicalagin was the effective, anti-influenza component. Punicalagin blocked replication of the virus RNA, inhibited agglutination of chicken RBC's by the virus and had virucidal effects. Further, the combination of PPE and oseltamivir synergistically increased the anti-influenza

effect of oseltamivir. The data showed PPE inhibited the replication of human influenza A/ Hong Kong (H3N2) virus in-vitro. Exposure of foodborne virus surrogates feline calicivirus (FCV-F9), murine norovirus (MNV-1), and MS2 (ssRNA) bacteriophage to pomegranate juice and pomegranate polyphenols resulted in titer reductions after one hour at room temperature, suggesting promise for use in hurdle technologies and/or for therapeutic or preventive use (Su et al. 2010).

## Antimicrobial Activity

Ethanolic extracts of *Garcinia mangostana*, *Punica granatum* and *Quercus infectoria* were found to have good antimicrobial activity of nine Thai medicinal plants with MICs for methicillin-resistant *Staphylococcus aureus* (MRSA) isolates of 0.05–0.4, 0.2–0.4 and 0.2–0.4 mg/mL, respectively, and for *S. aureus* ATCC 25923 of 0.1, 0.2 and 0.1 mg/mL, respectively (Voravuthikunchai and d Kitpipit 2005). MBCs for MRSA isolates were 0.1–0.4, 1.6–3.2 and 0.4–1.6 mg/mL, and for *S. aureus* ATCC 25923 were 0.4, 3.2 and 1.6 mg/mL, respectively. *Punica granatum* was found to have anti-quorum-sensing activity and may be useful in combating pathogenic bacteria and reduce the development of antibiotic resistance (Koh and Tham 2011; Zahin et al. 2010b). In another study the ethanolic extract of *P. granatum* exhibited bacteriostatic and bactericidal activities against two enterohemorrhagic *Escherichia coli* strains (Voravuthikunchai and Limsuwan 2006). The ethanolic extract of *P. granatum* had MICs of 0.49–1.95 mg/mL and MBCs of 1.95–3.91 mg/mL. The extract also demonstrated ability to modulate hydrophobicity characteristics of the bacteria. Pomegranate aril extracts exhibited antimicrobial effect on seven bacteria: (*Bacillus megaterium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Corynebacterium xerosis*, *Escherichia coli*, *Enterococcus faecalis*, *Micrococcus luteus*), and three fungi (*Kluyveromyces marxianus*, *Rhodotorula rubra*, *Candida albicans*) (Duman et al. 2009). The MIC values for active pomegranate extracts ranged between 30 and >90 μg/mL.

Powdered pomegranate peel, fennel, cumin and acacia bark all showed antifungal activity with pomegranate showing the highest inhibition of *Candida albicans* (Pai et al. 2010). Ethanol extract of *P. granatum* exhibited strong antibacterial activity against *Escherichia coli* (Sharma et al. 2009).

Studies showed that *Punica granatum* (pomegranate) methanolic extract (PGME) dramatically enhanced the activity of all antibiotics tested (Braga et al. 2005a). Synergic activity was detected between PGME and the five antibiotics tested, chloramphenicol, gentamicin, ampicillin, tetracycline, and oxacillin, ranging from 38 to 73%. Using PGME (0.1×MIC) in combination with ampicillin (0.5×MIC), cell viability was reduced by 99.9 and 72.5% in methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) populations, respectively. PGME increased the post-antibiotic effect (PAE) of ampicillin from 3 to 7 h. Pomegranate extract inhibited *Staphylococcus aureus* growth and subsequent enterotoxin production (Braga et al. 2005b). Of several Thai medicinal plants, the ethanol extract of *P. granatum* fruit rind displayed the most outstanding in-vitro antibacterial activity with MIC of 0.39 and 12.5 mg/mL and MBC of 1.56 and 12.5 mg/mL against *Staphylococcus aureus* and *Escherichia coli* respectively (Chansakaow et al. 2005). The extract was found to contain both hydrolysable and condensed tannins. The methanol pomegranate pericarp extract exhibited maximum antibacterial activity against *Salmonella typhimurium*, *Salmonella typhi* and *Shigella dysenteriae* Serotype 1 (Pradeep et al. 2008). Studies showed that the antibacterial activity of pomegranate rind can be enhanced by the addition of metal salts and vitamin C (McCarrell et al. 2008). Pomegranate rind extracts (PRE) exhibited activity against the Gram positive organisms at 24 h were inactive against Gram negative bacteria. Addition of $Cu^{2+}$ salts to PRE solutions extended the activities resulting in no detectable growth being observed for the PRE/$Cu^{2+}$ combination against *Escherichia coli*, *Pseudomonas*

*aeruginosa* and *Proteus mirabilis*. Minimal antimicrobial activity was observed following incubation with $Fe^{2+}$, $Mn^{2+}$ or $Zn^{2+}$ salts alone or in combination with PRE against any of the organisms in the test panel. The addition of vitamin C markedly enhanced the activities of both PRE/$Fe^{2+}$ and PRE/$Cu^{2+}$ combinations against *Staphylococcus aureus*.

Pelargonidin-3-galactose, cyanidin-3-glucose, gallic acid, quercetin, and myricetin isolated from the methanolic extract of pomegranate fruit exhibited appreciable activity against species of *Corynebacteria*, *Staphylococcus*, *Streptococcus*, *Shigella*, *Salmonella*, *Bacillus subtilis*, *Vibrio cholera*, and *Escherichia coli* (Naz et al. 2007). However, all these compounds were more inhibitory against Gram-positive species. Gallic acid exerted the highest inhibitory activity against all the tested bacteria. Various tannin-rich fractions from pomegranate byproduct and the ellagitannins, ellagic acid (1), gallagic acid (2), punicalins (3), and punicalagins (4) displayed antimicrobial activity when assayed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Cryptococcus neoformans*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Aspergillus fumigatus* and *Mycobacterium intracellulare* (Reddy et al. 2007). Compounds 2 and 4 showed activity against *P. aeruginosa*, *C. neoformans*, and MRSA. A new antifungal peptide designated as pomegranin, isolated from fresh pomegranate peels, was found to inhibit mycelial growth of the fungi *Botrytis cinerea* and *Fusarium oxysporum* with an $IC_{50}$ of 2 and 6.1 μM, respectively (Guo et al. 2009).

Lyophilized pomegranate juice (LPJ) exhibited antilisterial activity in-vitro and in ground beef (Lucas and Were 2009). Against five *Listeria monocytogenes* strains, LPJ had a MIC of 1.50–1.75% (wt/vol). The LPJ (0, 30, 60, and 120 min of heating) significantly inhibited growth of all five *L. monocytogenes* strains in refrigerated ground cooked beef by 1.80–4.61 log CFU/g at day 21. Heating did not negatively impact LPJ antilisterial activity.

Ethanol peel extract of pomegranate exhibited in-vitro and in-vivo antimicrobial activity against *Salmonella typhimurium* (Choi et al. 2011). The minimal inhibitory concentrations of their extract were in the range of 62.5–1,000 μg/mL. in a *S. typhimurium* infection mouse model. The extract was found to have significant effects on mortality and the numbers of viable *S. typhimurium* recovered from faeces. Although clinical signs and histological damage were rarely observed in the treated mice, the untreated controls showed signs of lethargy and histological damage in the liver and spleen. The results of this study indicated that the peel extract had the potential to provide an effective treatment for salmonellosis.

Studies on patients with denture stomatitis showed that gel extract of *P. granatum* may be used as a topical antifungal agent for the treatment of candidosis associated with denture stomatitis (Vasconcelos et al. 2003). In subsequent studies, *Punica granatum* phytotherapeutic gel and miconazole (Daktarin oral gel) exhibited antimicrobial effect against three standard streptococci strains (*Streptococcus mutans*, *Streptococcus sanguis* and *Streptococcus mitis*), *S. mutans* clinically isolated *and Candida albicans* either alone or in association (Vasconcelos et al. 2006). The minimum inhibitory concentrations of adherence of *Punica granatum* gel against the test organisms were: 1:16 for *S. mutans* (ATCC), *S. mutans* (CI) and *S. sanguis*; 1:128 for *S. mitis* and 1:64 for *C. albicans*. The minimum inhibitory concentrations of adherence of miconazole against the same organisms were: 1:512, 1:64, 1:4, 1:128 and 1:16, respectively. In experiments with three and four associated microorganisms, the *Punica granatum* gel had greater efficiency in inhibiting microbial adherence than the miconazole. The results of this study suggest that this phytotherapeutic agent might be used in the control of adherence of different microorganisms in the oral cavity. Studies showed that the hydroalcoholic extract from *Punica granatum* fruits was very effective against dental plaque microorganisms, decreasing the colony forming units per milliliter (CFU/mL) by 84% (Menezes et al. 2006). While similar values were observed with chlorhexidine, used as standard and positive control (79% inhibition). However, another study found that the gel containing 10% *Punica granatum* extract was not efficient in preventing supragingival dental plaque formation and

gingivitis (Salgado et al. 2006). Methanolic extract of pomegranate peel exhibited antibacterial activity against oral pathogens: *Staphylococcus aureus* and *S. epidermidis* (Abdollahzadeh et al. 2011). Only at concentration of 8 mg/mL and 12 mg/mL the extract was effective against *Lactobacillus acidophilus*, *Streptococcus mutans* and *Streptococcus salivarius*. The extract did not inhibit *Actinomyces viscosus* and *Candida albicans*.

Pomegranate rind extract (PRE) singularly showed limited efficacy against methicillin-sensitive and -resistant *Staphylococcus aureus* (MSSA, MRSA) respectively but in combination with Cu(II) ions (cupric sulphate), it exhibited moderate antimicrobial effects against clinical isolates of MSSA, MRSA and Panton-Valentine Leukocidin positive community acquired MSSA (PVL positive CA-MSSA) isolates. (Gould et al. 2009).

## Anti-gingivitis/Amtiplaque Activity

Sastravaha et al. (2005) showed that adjunctive local delivery of extracts from *Centella asiatica* in combination with *P. granatum* significantly improved clinical signs of chronic periodontitis such probing pocket depth, attachment level, gingival index at 3 and 6 months and of bleeding index at 6 months in the test group as compared to control. No significant differences in plaque index were found between the two treatment modalities. The test group also showed statistically greater reduction of interleukin IL-1β at both 3 and 6 months and lower IL-6 concentration. A study of young adults showed that 4 weeks of thrice daily mouth rinsing with the extract improved salivary measures relevant to oral health including gingivitis (DiSilvestro et al. 2009). Salivary changes observed included a reduction in total protein (associated with plaque forming bacteria readings), activities of aspartate aminotransferase (an indicator of cell injury) and α-glucosidase activity (a sucrose degrading enzyme). The changes also included increased activities of the antioxidant enzyme ceruloplasmin and radical scavenging capacity.

Pomegranate mouth-rinse was found to have an antiplaque effect (Bhadbhade et al. 2011).

Pomegranate extract was efficacious against *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* strains in-vitro. Pomegranate mouth-rinse could be explored as a long-term antiplaque rinse with prophylactic benefits.

## Probiotication Activity

Probiotication improved the antioxidant activity of sweet pomegranate aril juice from 74.4 to 91.82%, and sour pomegranate juice from 82.64 to 97.8% (Fazeli et al. 2011). Based on the ferric reducing antioxidant power (FRAP) value, the reducing power of the probioticated pomegranate juices was also much stronger than the nonprobioticated juices. The FRAP values for sweet and sour probioticated pomegranate juices were 97.34 and 120.7 mmol/L, respectively, which were notably higher than 85.87 and 93.4 mmol/L for sweet and sour nonprobioticated juices. Total counts of *Lactobacillus casei* GG increased by about three log in sweet and two log in sour juices after 48 h incubation. Both fermented and nonfermented juices exhibited a potent and wide-spectrum antibacterial effect, with the highest activity against *Pseudomonas aeruginosa* with the sweet juice showing wider zones of growth inhibition. The results showed that probiotication of sweet and sour pomegranate juices could add to their beneficial antioxidant activities. Pomegranate byproducts and punicalagins inhibited the growth of pathogenic clostridia and *Staphylococcus aureus* (Bialonska et al. 2009b). The growth of probiotic lactobacilli and bifidobacteria were generally not affected by ellagitannins. The effect of pomegranate ellagitannins on bifidobacteria was species- and tannin-dependent. The growth of *Bifidobacterium animalis* ssp. *lactis* was slightly inhibited by punicalagins, punicalins, and ellagic acid. Pomegranate ellagitannin-enriched polyphenol extract (POMx) supplementation significantly enhanced the growth of *Bifidobacterium breve* and *Bifidobacterium infantis.*

Bialonska et al. (2009a) found that products of the intestinal microbial transformation of pomegranate ellagitannins may account for systemic

antioxidant effects. While moving through the intestines, pomegranate ellagitannins namely ellagic acid and punicalagins are metabolized by gut bacteria into urolithins that readily enter systemic circulation. Their study found that the antioxidant activity of urolithins was correlated with the number of hydroxy groups as well as the lipophilicity of the molecule. The most potent antioxidants were urolithins C and D with $IC_{50}$ values of 0.16 and 0.33 μM, respectively, when compared to $IC_{50}$ values of 1.1 and 1.4 μM of the parent ellagic acid and punicalagins, respectively. The dihydroxylated urolithin A showed weaker antioxidant activity, with an $IC_{50}$ value 13.6 μM, however, the potency was within the range of urolithin A plasma concentrations.

### Antiatherogenic Activity

Numerous laboratory research, animal and human pilot studies had reported on the effectiveness of pomegranate fruit, pomegranate juice and pomegranate fruit polypehnols in reducing heart disease risk factors LDL oxidation, blood pressure, serum angiotensin converting enzyme (ACE) activity, cholesterol esterification, macrophage oxidative status, and macrophage foam cell formation, all of which are steps in atherosclerosis and cardiovascular disease (Aviram et al. 2000, 2002, 2004, 2008; Aviram and Dornfeld 2001; Kaplan et al. 2001; Esmaillzadeh et al. 2004; Fuhrman et al. 2005; de Nigris et al. 2005; Rosenblat et al. 2006a, b; Fuhrman and Aviram 2007; Bagri et al. 2009). In healthy humans, pomegranate juice consumption decreased LDL susceptibility to aggregation and retention and increased the activity of serum paraoxonase by 20% (Aviram et al. 2000). Paraoxanase an HDL-associated esterase, could protect against lipid peroxidation. In apolipoprotein E-deficient Eᵒ mice, oxidation of LDL by peritoneal macrophages was reduced by up to 90% after pomegranate juice consumption and this effect was associated with reduced cellular lipid peroxidation and superoxide release. The uptake of oxidized LDL and native LDL by mouse peritoneal macrophages obtained after pomegranate juice

administration was reduced by 20%. Further, pomegranate juice supplementation of Eᵒ mice reduced the size of their atherosclerotic lesions by 44% and also the number of foam cells compared with control Eᵒ mice supplemented with water. The potent antiatherogenic effects in healthy humans and in atherosclerotic mice may be attributable to its antioxidative properties. Anti-atherosclerotic properties was attributed to pomegranate potent anti-oxidative characteristics. After consumption of pomegranate juice, a 36% reduction in serum angiotensin converting enzyme (ACE) activity and a 5% reduction in systolic blood pressure were noted in hypertensive patients (Aviram and Dornfeld 2001). Similar dose-dependent inhibitory effect (31%) of pomegranate juice on serum ACE activity was observed also in-vitro. Additional studies showed that pomegranate juice supplementation to atherosclerotic mice reduced macrophage lipid peroxidation, cellular cholesterol accumulation and development of atherosclerosis (Kaplan et al. 2001). Pomegranate juice supplementation reduced each of the proatherogenic variables. It significantly induced serum paraoxonase activity and reduced mouse peritoneal macrophage (MPM) lipid peroxide content compared with placebo-treated mice and control mice. Pomegranate juice administration to apolipoprotein E-deficient Eᵒ mice significantly reduced the oxidized (Ox)-LDL MPM uptake by 31% and MPM cholesterol esterification and increased macrophage cholesterol efflux by 39% compared with age-matched, placebo-treated mice. Pomegranate juice consumption reduced macrophage Ox-LDL uptake and cholesterol esterification to levels lower than those in 4-month-old, unsupplemented controls. Pomegranate juice supplementation to Eᵒ mice with advanced atherosclerosis reduced the lesion size by 17% compared with placebo-treated mice. In a separate study, supplementation of young (2-month-old) Eᵒ mice for 2 months with a tannin fraction isolated from pomegranate juice reduced their atherosclerotic lesion size, paralleled by reduced plasma lipid peroxidation and decreased Ox-LDL MPM uptake. Studies indicated that the proatherogenic effects induced by perturbed shear stress in cultured human coronary artery endothelial cells could be reversed by

chronic administration of pomegranate juice (de Nigris et al. 2005, 2007). Pomegranate juice concentrate and pomegranate fruit extract rich in punicalagin reduced the activation of redox-sensitive genes ELK-1, p-JUN, p-CREB, and increased eNOS expression (which was decreased by perturbed shear stress) in cultured endothelial cells and in atherosclerosis-prone areas of hypercholesterolemic mice. Furthermore, oral administration of pomegranate juice to hypercholesterolemic mice at various stages of disease reduced significantly the progression of atherosclerosis and isoprostane levels and increased nitrates. de Nigris et al. (2006) found that pomegranate juice reverted the potent down-regulation of the expression of endothelial nitric-oxide synthase (NOSIII) induced by oxidized low-density lipoprotein (oxLDL) in human coronary endothelial cells. Their data suggested that pomegranate juice could exert beneficial effects on the evolution of clinical vascular complications, coronary heart disease, and atherogenesis in humans by enhancing the nitric-oxide synthase bioactivity.

Aviram et al. (2002) reported that pomegranate polyphenols protected low-density lipoprotein (LDL) against cell-mediated oxidation via two pathways, including either direct interaction of the polyphenols with the lipoprotein and/or an indirect effect through accumulation of polyphenols in arterial macrophages (Aviram et al. 2002). Pomegranate polyphenols were shown to reduce the capacity of macrophages to oxidatively modify LDL, due to their interaction with LDL to inhibit its oxidation by scavenging reactive oxygen species and reactive nitrogen species and also due to accumulation of polyphenols in arterial macrophages; hence, the inhibition of macrophage lipid peroxidation and the formation of lipid peroxide-rich macrophages. Additionally, pomegranate polyphenols increased serum paraoxonase activity, resulting in the hydrolysis of lipid peroxides in oxidized lipoproteins and in atherosclerotic lesions. These antioxidative and antiatherogenic effects of pomegranate polyphenols were demonstrated in-vitro, as well as in-vivo in humans and in atherosclerotic apolipoprotein E deficient mice. Dietary supplementation of polyphenol-rich pomegranate juice to atherosclerotic mice significantly inhibited the development of atherosclerotic lesions and this may be attributed to the protection of LDL against oxidation.

Subsequent studies indicated that that pomegranate juice consumption by patients with carotid artery stenosis CAS decreased carotid carotid intima-media thickness (IMT) and systolic blood pressure and these effects could be related to the potent antioxidant characteristics of pomegranate juice polyphenols (Aviram et al. 2004). For all studied parameters, the maximal effects were observed after 1 year of pomegranate juice consumption. Further consumption of pomegranate juice, for up to 3 years, had no additional beneficial effects on IMT and serum paraoxonase 1 (PON 1) activity, whereas serum lipid peroxidation was further reduced by up to 16% after 3 years of pomegranate juice consumption. The antiatherogenic properties of pomegranate juice (PJ) were attributed to its antioxidant potency and to its capacity to decrease macrophage oxidative stress, the hallmark of early atherogeneis (Rosenberg et al. 2006). Pomegranate juice polyphenols and sugar-containing polyphenolic anthocyanins were shown to confer PJ its antioxidant capacity. Their study showed that PJ sugar consumption by diabetic mice for 10 days resulted in a small but significant decrement in their peritoneal macrophage total peroxide levels and an increment in cellular glutathione content, compared to mouse peritoneal macrophages harvested from control diabetic mice administrated with water. These antioxidant/antiatherogenic effects could be due to the presence of unique complex sugars and/or phenolic sugars in PJ. They further showed the anti-oxidative characteristics of PJ unique phenolics punicalagin and gallic acid could be related, at least in part, to their stimulatory effect on macrophage paraoxonase 2 (PON2) expression, a phenomenon which was shown to be associated with activation of the transcription factors PAPR γ and AP-1 (Shiner et al. 2007). Similar results were obtained by pomegranate byproduct (which includes the whole pomegranate fruit left after juice preparation) (17 or 51.5 μg of gallic acid equiv/kg/day) administration to apolipoprotein e-deficient mice that resulted in attenuation

of atherosclerosis development as a result of decreased macrophage oxidative stress and reduced cellular uptake of oxidized low-density lipoprotein (Rosenblat et al. 2006). In-vitro studies showed that preincubation of J774.A1 macrophages with pomegranate juice resulted in a significant reduction in Ox-LDL degradation by 40% (Fuhrman et al. 2005). Macrophage cholesterol biosynthesis was inhibited by 50% after cell incubation with pomegranate juice. This inhibition, however, was not mediated at the 3-hydroxy-3 methylglutaryl coenzyme A reductase level along the biosynthetic pathway. It was concluded that pomegranate juice-mediated suppression of Ox-LDL degradation and of cholesterol biosynthesis in macrophages could lead to reduced cellular cholesterol accumulation and foam cell formation.

Studies in Iran reported that consumption of concentrated pomegranate juice may modify heart disease risk factors in hyperlipidemic (cholesterol $\geq$ 5.2 mmol/L or triacylglycerol $\geq$ 2.3 mmol/L) patients (Esmaillzadeh et al. 2004, 2006). After consumption of concentrated pomegranate juice, significant reductions were seen in total cholesterol, low-density lipoprotein (LDL)-cholesterol, LDL-cholesterol/high-density lipoprotein (HDL)-cholesterol, and total cholesterol/HDL-cholesterol. But, there were no significant changes in serum triacylglycerol and HDL-cholesterol concentrations. Anthropometric indices, physical activity, kind and doses of oral hypoglycemic agents, and the intakes of nutrients and flavonoid-rich foods showed no change during the concentrated pomegranate juice consumption period. Rosenblat et al. (2006) reported that pomegranate juice consumption by diabetic patients did not affect serum glucose, cholesterol and triglyceride levels, but it resulted in a significant reduction in serum lipid peroxides and TBARS (thiobarbituric acid reactive substance) levels by 56 and 28%, whereas serum SH (sulfhydryl) groups and paraoxonase 1 (PON1) activity significantly increased by 12 and 24%, respectively. In the patients versus controls monocytes-derived macrophages (HMDM), they observed increased level of cellular peroxides (by 36%), and decreased glutathione content (by 64%).

Pomegranate juice consumption significantly reduced cellular peroxides (by 71%), and increased glutathione levels (by 141%) in the patients' HMDM. The patients' versus control HMDM took up oxidized LDL (Ox-LDL) at enhanced rate (by 37%) and pomegranate juice consumption significantly decreased the extent of Ox-LDL cellular uptake (by 39%). They thus concluded that pomegranate juice consumption by diabetic patients did not worsen the diabetic parameters, but rather resulted in anti-oxidative effects on serum and macrophages, which could contribute to attenuation of atherosclerosis development in these patients.

Pomegranate juice was found to have potent antiatherogenic activity (Fuhrman and Aviram 2007). In-vitro studies demonstrated a pomegranate juice dose-dependent antioxidant capability against lipid peroxidation in plasma (by 33%), in LDL (by 43%), and in HDL (by 22%). Pomegranate juice consumption by hypertensive patients reduced their systolic blood pressure (by 6%), along with inhibition (by 40%) of angiotensin converting enzyme (ACE). Pomegranate juice supplementation to atherosclerotic apolipoprotein E-deficient (E°) mice reduced their atherosclerotic lesion size by 44% and the number of foam cells in their lesion. Consumption of pomegranate juice by ten patients with carotid artery stenosis (CAS) for 1 year reduced the patients' carotid intima-media thickness (IMT) by 32%. These effects were associated with ex-vivo reduced lipid peroxidation in plasma and in isolated lipoproteins in humans and mice. Furthermore, pomegranate juice consumption by humans increased the activity of their serum paraoxonase (PON1), an HDL-associated esterase that protects against lipid peroxidation. Macrophage atherogenicity was studied in mouse peritoneal macrophages (MPM) harvested from E° mice. Following pomegranate juice consumption, uptake of oxidized LDL and cell-mediated oxidation of LDL by macrophages was reduced by 88 and by 20%, respectively, in association with reduced cellular lipid peroxidation, reduced superoxide anion release due to decreased NADPH-oxidase activation, and elevated glutathione content. In-vitro studies demonstrated that

pomegranate juice reduced macrophage Ox-LDL degradation by 40%, and macrophage cholesterol biosynthesis by 50%. Overall, the results of the above studies demonstrated that pomegranate juice consumption had very potent antiatherogenic properties, which could be associated mainly with pomegranate juice hydrolysable tannin antioxidative properties.

In a recent study (Aviram et al. 2008) pomegranate juice (PJ), fruit peels (POMxl, POMxp), arils (POMa), seeds (POMo), and flowers (POMf), extracts all were found to possess antioxidative properties in-vitro. After consumption of pomegranate juice, fruit peel, aril and flower extracts the atherosclerotic lesion area in atherosclerotic apolipoprotein e-deficient (E 0) mice was significantly decreased by 44, 38, 39, 6, or 70%, respectively, as compared to placebo-treated group, while pomegranate seed oil had no effect. Pomegrante flower consumption reduced serum lipids, and glucose levels by 18–25%. Consumption of the extracts except for the seed oil resulted in a significant decrement, by 53, 42, 35, 27, or 13%, respectively, in MPM (mouse peritoneal macrophage) total peroxides content, and increased cellular paraoxonase 2 (PON2) activity, as compared to placebo-treated mice. The uptake rates of oxidized-LDL by E (0)-MPM were significantly reduced by approximately 15% after consumption of juice and the two fruit peel extracts. Similar results were obtained on using J774A.1 macrophage cell line. Finally, pomegranate phenolics (punicalagin, punicalin, gallic acid, and ellagic acid), as well as pomegranate unique complexed sugars, could mimic the antiatherogenic effects of the pomegranate extracts. Rock et al. (2008) reported that after 4 weeks of pomegranate juice consumption by male patients, basal serum oxidative stress was significantly decreased by 35%, whereas serum concentrations of thiol groups significantly increased by 25%. Moreover, HDL-associated paraoxonase 1(PON1), arylesterase, paraoxonase, and lactonase activities increased significantly after pomegranate juice consumption by 34–45%, as compared to the baseline levels. PON1 protein binding to HDL was significantly increased by 30% following pomegranate juice consumption, and the enzyme became more stable. In male patients that consumed pomegranate polyphenol extract and in female patients that consumed pomegranate juice, a similar pattern was observed, although to a lesser extent. These beneficial effects of pomegranate consumption on serum PON1 stability and activity could lead to retardation of atherosclerosis development in diabetic patients.

Results of a randomized, double-blind, parallel trial involving men (45–74 years old) and women (55–74 years old) with moderate coronary heart disease risk suggested that in subjects at moderate coronary heart disease risk, pomegranate juice consumption had no significant effect on overall carotid intima-media thickness progression rate but may have slowed carotid intima-media thickness progression in subjects with increased oxidative stress and disturbances in the triglycerides-rich lipoprotein/high-density lipoprotein axis (Davidson et al. 2009).

## Antihyperlipidemic/Antiobesity Activity

Lei et al. (2007) reported that the pomegranate leaf extract could inhibit the development of obesity and hyperlipidemia in high-fat diet induced obese mice. Mice treated with the extract presented a significant decrease in body weight, energy intake and various adipose pad weight percents and serum, serum total cholesterol (TC), triglyceride (TG), glucose levels and TC/high-density lipoprotein cholesterol (HDL-C) ratio after 5 weeks treatment. Further, the extract significantly attenuated the raising of the serum TG level and inhibited the intestinal fat absorption in mice given a fat emulsion orally. The effects were postulated to be partly mediated by inhibiting the pancreatic lipase activity and suppressing energy intake. Yamasaki et al. (2006) found that mice fed dietary pomegranate seed oil (PSO) high in levels of punicic acid showed significant increases in serum triacylglycerol and phospholipid levels but not in total cholesterol. Punicic acid could be detected in serum, liver, and adipose tissues in mice fed the 0.12 or 1.2% PSO diet. Oral administration of streptozotocin-induced diabetic Wistar rats with of 250 and 500 mg/kg

of aqueous pomegranate flower extract for 21 days resulted in a significant fall in fasting blood glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol, very low density lipoprotein, lipid peroxidation level (Bagri et al. 2009). Pomegrante extract elevated levels of high density lipoprotein cholesterol (HDL-C), reduced glutathione (GSH) and the antioxidative enzymes, glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT). McFarlin et al. (2009) found that weight gain in high fat diet mice was associated with an increase in biomarkers of cholesterol profile, glucose sensitivity, adipose tissue accumulation and systemic low-grade inflammation. despite a similar level of energy intake, high-fate diet mice had a greater concentration of leptin and a lower concentration of adiponectin compared to high fat + pomegranate seed oil diet mice. Pomegranate seed oil, a rich source of 9-*cis*, 11-*trans* conjugate linolenic acid, only altered body weight accumulation, final body weight, leptin, adiponectin and insulin. Pomegranate seed oil intake was associated with an improvement in insulin sensitivity, suggesting that risk of developing type two diabetes may have been reduced; however, CVD risk did not change. Lan et al. (2009) demonstrated that ellagic acid in pomegranate leaf tannins could be transported into HepG2 cells and this correlated with total cholesterol alteration in the cells.

Vroegrijk et al. (2011) found that pomegranate seed oil, a rich source of punicic acid, ameliorated high-fat diet induced obesity and insulin resistance in mice, independent of changes in food intake or energy expenditure. compared to high fat diet mice, its intake resulted in a lower body weight and improved peripheral insulin sensitivity but did not affect liver insulin sensitivity. In a randomized, double-blind, placebo-controlled clinical trial of 20 obese adult volunteer, pomegranate juice administration for 1 month did not modify insulin secretion and sensitivity in the obese patients, however, the natural evolution to increased weight and adiposity was halted (González-Ortiz et al. 2011).

Rosenblat and Aviram (2011) found that the inhibitory effect of pomegranate juice on triglyceride biosynthesis could be attributed to a direct effect of pomegranate juice on the activity of diacylglycerol acyltransferase 1 (DGAT1) the rate-limiting enzyme in triglyceride biosynthesis. Pomegranate juice and its constituent punicalagin significantly and dose-dependently decreased the triglyceride content and triglyceride biosynthesis rate in J774A.1 macrophages or in C57BL/6 mouse peritoneal macrophages. Both pomegranate juice and punicalagin increased (1.7-fold) mouse peritoneal macrophages paraoxonase 2 (PON2) mRNA expression, and PON2 was previously shown to inhibit DGAT1 activity. However, the addition of PJ or punicalagin (50 μM) to microsomes from PON2-deficient mouse peritoneal macrophages still resulted in a significant reduction (50–58%) in DGAT1 activity.

## Antihypertensive Activity

In a randomised block design study of student volunteers, supplementation of pomegranate juice caused a fall in diastolic blood pressure and this could be related to ROS scavenging activity rather than to angiotensin-converting enzyme inhibitors (Wright and Pipkin 2008)

Oral administration of pomegranate juice extract (100 and 300 mg/kg) to angiotensin-II treated rats for 4 weeks significantly reduced the mean arterial blood pressure and vascular reactivity changes to various catecholamines (Waghulde et al. 2010). Pomegranate juice administration significantly decreased the serum levels of ACE (angiotensin converting enzyme) and the levels of thiobarbituric acid reactive substances (TBARS); while enzyme activity of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSH) in kidney tissue showed a significant elevation in pomegranate juice treated angiotensin-II induced hypertensive rats. The results suggested that pomegranate juice extract could prevent the development of high blood pressure induced by angiotensin-II probably by combating the oxidative stress and antagonizing the physiological actions of angiotensin-II. Chronic administration of pomegranate fruit juice (PJ) extract (100 and 300 mg/kg; p.o.

for 4 weeks) reduced the mean arterial blood pressure and vascular reactivity changes to various catecholamines and also reversed the biochemical changes induced by diabetes and angiotensin II (Ang II) (Mohan et al. 2010b). Acute subcutaneous administration of Angiotensin II causes a rise in blood pressure in streptozotocin-induced diabetic Wistar rats. PJ treatment also caused a significant decrease in levels of thiobarbituric acid reactive substances (TBARS) in the kidney and pancreas while activities of enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GSH) showed significant elevation. PJ treatment prevented the tubular degenerative changes induced by diabetes. The results suggested that the PJ extract could prevent the development of high blood pressure induced by Ang II in diabetic rats probably by combating the oxidative stress induced by diabetes and Ang II and by inhibiting ACE activity.

## Antidiabetic Activity

Pomegranate in particular its flowers, seeds, and juice have been employed for the treatment of various diseases in traditional Unani and Ayurvedic systems of medicine in India but only the flower has been prescribed for the treatment of diabetic disorders (Li et al. 2008; Katz et al. 2007). The mechanisms for it hypoglycaemic effects are largely unknown, though recent research suggested pomegranate flowers and juice may prevent diabetic sequelae via peroxisome proliferator-activated receptor (PPAR) $\alpha/\gamma$ binding and nitric oxide production (Katz et al. 2007; Huang et al. 2005a, b; Li et al. 2008; Xu et al. 2009). Pomegranate compounds associated with such effects include oleanolic, ursolic, and gallic acids (Katz et al. 2007). Another study suggested that *Punica granatum* flower (PGF) extract inhibited increased cardiac fatty acid uptake and oxidation in the diabetic condition (Huang et al. 2005b). PGF extract and its component oleanolic acid enhanced peroxisome proliferator-activated receptor (PPAR)-$\alpha$ luciferase reporter gene activity in human embryonic kidney 293 cells. This effect was completely suppressed by a selective PPAR-$\alpha$ antagonist MK-886, consistent with the presence of PPAR-$\alpha$ activator activity in the extract and this component. The findings suggested that PGF extract improved abnormal cardiac lipid metabolism in Zucker diabetic fatty rats by activating PPAR-$\alpha$ and thereby lowering circulating lipid and inhibiting its cardiac uptake. Excess triglyceride (TG) accumulation and increased fatty acid (FA) oxidation in the diabetic heart contribute to cardiac dysfunction. In subsequent in-vitro studies, the scientists (Huang et al. 2005a) demonstrated that 6-week oral administration of methanol extract from PGF (500 mg/kg, daily) inhibited glucose loading-induced increase of plasma glucose levels in Zucker diabetic fatty rats (ZDF), a genetic animal model for type two diabetes, whereas it did not inhibit the increase in Zucker lean rats (ZL). The treatment did not lower the plasma glucose levels in fasted ZDF and ZL rats. Further, RT-PCR results demonstrated that the PGF extract treatment in ZDF rats enhanced cardiac PPAR-$\gamma$ mRNA expression and restored the down-regulated cardiac glucose transporter (GLUT)-4 (the insulin-dependent isoform of GLUTs) mRNA. These results suggest that the anti-diabetic activity of PGF extract may result from improved sensitivity of the insulin receptor. From the in-vitro studies, it was demonstrated that the PGF extract enhanced PPAR-$\gamma$ mRNA and protein expression and increased PPAR-$\gamma$-dependent mRNA expression and activity of lipoprotein lipase in human THP-1-differentiated macrophage cells. Phytochemical investigation demonstrated that gallic acid in PGF extract was mostly responsible for this activity. Further in-vitro studies showed that *Punica granatum* flower extract and its components oleanolic acid, ursolic acid, and gallic acid inhibited lipopolysaccharide-induced NF-kappaB activation in macrophages. The findings indicated that *Punica granatum* flower extract reduced cardiac fibrosis in Zucker diabetic fatty rats, at least in part, by modulating cardiac ET-1 and NF-kappaB signalling. Recent studies suggested that pomegranate flower (PGF) medicine ameliorated diabetes and obesity-associated fatty liver, at least in part, by activating hepatic expression of genes responsible for fatty acid oxidation (Xu et al. 2009). PGF-treated ZDF

rats showed reduced ratio of liver weight to tibia length, hepatic triglyceride contents and lipid droplets. These effects were accompanied by enhanced hepatic gene expression of peroxisome proliferator-activated receptor (PPAR)-α, carnitine palmitoyltransferase-1 and acyl-CoA oxidase (ACO), and reduced stearoyl-CoA desaturase-1. In contrast, PGF showed minimal effects on expression of genes responsible for synthesis, hydrolysis or uptake of fatty acid and triglycerides.

PGF treatment also increased PPAR-α and ACO mRNA levels in HepG2 cells. Li et al. (2008) reviewed the dual PPAR-α/-γ activator properties of pomegranate flower and its potential treatment of diabetes and its associated complications. PPARs are nuclear transcription factors and are the major regulators of lipid and glucose metabolism. PPAR-α is involved in the regulation of fatty acid (FA) uptake and oxidation, inflammation and vascular function, while PPAR-γ participates in FA uptake and storage, glucose homeostasis and inflammation. Synthetic PPAR-α or PPAR-γ agonists have been widely used in the treatment of dyslipidaemia, hyperglycaemia and their complications. However, they are associated with an incidence of adverse events. Given the favourable metabolic effects of both PPAR-α and PPAR-γ activators, as well as their potential to modulate vascular disease, combined PPAR-α/-γ activation has recently emerged as a promising concept, leading to the development of mixed PPAR-α/-γ activators.

Hontecillas et al. (2009) demonstrated that punicic acid (PUA), a conjugated linolenic acid isomer found in pomegranate, caused a dose-dependent increase PPAR α and γ reporter activity in 3 T3-L1 pre-adipocyte cells and bound although weakly to the ligand-binding domain of human PPAR γ. Dietary PUA decreased fasting plasma glucose concentrations, improved the glucose-normalizing ability, suppressed NF-kappaB activation, TNF-α expression and upregulated PPAR α- and γ-responsive genes in skeletal muscle and adipose tissue. PUA improved glucose homeostasis and suppress obesity-related inflammation

Studies in India showed that pomegranate seed extract (150, 300 and 600 mg/kg) administered orally to streptozotocin (STZ)-induced diabetic rats caused a significant reduction of blood glucose levels by 47 and 52%, respectively, at the end of 12 h (Das et al. 2001). Kim et al. (2011) found that administration of pomegranate extract to streptozotocin (STZ)-induced diabetic mice for 4 weeks improved postprandial glucose regulation. Further elevated Na(+)-dependent glucose uptake by brush border membrane vesicles isolated from STZ mice was normalized by pomegranate treatment. The results suggested that pomegranate extract could play a role in controlling the dietary glucose absorption at the intestinal tract by decreasing sodium-coupled glucose transporter SGLT1 expression, and may contribute to blood glucose homeostasis in the diabetic condition.

Oral administration of pomegranate flower (PGF) extract markedly lowered plasma glucose levels in non-fasted Zucker diabetic fatty rats (a genetic model of obesity and type two diabetes), whereas it had little effect in the fasted animals, suggesting it affected postprandial hyperglycemia in type two diabetes (Li et al. 2005). The extract was found to markedly inhibit the increase of plasma glucose levels after sucrose loading, but not after glucose loading in mice, and it had no effect on glucose levels in normal mice. In-vitro, PGF extract demonstrated a potent inhibitory effect on α-glucosidase activity ($IC_{50}$: 1.8 μg/mL). These findings strongly suggested that PGF extract improved postprandial hyperglycemia in type two diabetes and obesity, at least in part, by inhibiting intestinal α-glucosidase activity. Postprandial hyperglycemia plays an important role in the development of type two diabetes and has been proposed as an independent risk factor for cardiovascular diseases. In a recent paper, Bagri et al. (2009a) reported that oral administration of pomegranate aqueous extract at doses of 250 and 500 mg/kg for 21 days to STZ-induced diabetic rats resulted in a significant reduction in fasting blood glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), very low density lipoprotein (VLDL), and tissue lipid peroxidation

levels coupled with elevation of high density lipoprotein cholesterol (HDL-C), glutathione (GSH) content and antioxidant enzymes in comparison with diabetic control group. The results suggested that PG could be used, as a dietary supplement, in the treatment of chronic diseases characterized by atherogenous lipoprotein profile, aggravated antioxidant status and impaired glucose metabolism and also in their prevention.

In-vitro studies showed that pomegranate juice polyphenols increased recombinant paraoxonase-1 binding to high-density lipoprotein (HDL) beyond their antioxidative effect (Fuhrman et al. 2010). Further recombinant paraoxonase-1 was found to be associated more efficiently with HDLs isolated from diabetic patients after pomegranate juice consumption versus HDLs isolated before pomegranate juice consumption.

## Antiinflammatory and Antiarthritic Activity

Studies by Ahmed et al. (2005) showed that pomegranate fruit extract or compounds derived from it may inhibit cartilage degradation in osteoarthritis and may also be a useful nutritive supplement for maintaining joint integrity and function. The extract inhibited interleukin (IL)-1β induced expression of matrix metalloproteinases by suppressing the activation of mitogen-activated protein kinases and nuclear factor-kappaB in human chondrocytes in-vitro. Pomegranate methanol extract was found to dose-dependently inhibit tumour necrosis factor α (TNF-α) production in lipopolysaccharide (LPS) stimulated cells (Jung et al. 2006). The data suggested that the extract may suppress LPS-stimulated TNF production through inhibition of Nfkappab in BV2 microglia cells.

Shukla et al. (2008b) reported that consumption of hydrolyzable tannins-rich pomegranate extract potently delayed the onset and reduced the incidence and severity of collagen-induced arthritis in mice. Pomegranate extract -fed mice had reduced joint infiltration by the inflammatory cells, and the destruction of bone and cartilage were alleviated. Levels of interleukin IL-6 were significantly decreased in the joints of pomegranate-fed mice with collagen-induced arthritis. In mouse macrophages, pomegranate extract abolished multiple signal transduction pathways and downstream mediators implicated in the pathogenesis of rheumatoid arthritis. In another study, rabbit plasma samples collected after oral ingestion of polyphenol rich pomegranate fruit extract were found to inhibit the IL-1β-induced PGE2 and NO production in chondrocytes (Shukla et al. 2008a). These same plasma samples also inhibited both COX-1 and COX-2 enzyme activity ex-vivo but the effect was more pronounced on the enzyme activity of COX-2 enzyme. The studies suggested that pomegranate fruit extract-derived bioavailable compounds may exert an anti-inflammatory effect by inhibiting the inflammatory cytokine-induced production of PGE2 and NO in-vivo. Pomegranate extract rich in polyphenols was found to inhibit the interleukin-1β-induced activation of MKK-3, p38α-MAPK and transcription factor RUNX-2 in human osteoarthritis chondrocytes (Rasheed et al. 2010). This pharmacological actions of pomegranate extract suggest that the extract or its derived compounds may be developed as MKK and p38-MAPK inhibitors for the treatment of osteoarthritis and other degenerative/inflammatory diseases. In a pilot12 week open-labelled study, pomegranate consumption reduced the composite Disease Activity Index (DAS28) and tender joint count in rheumatoid arthritis patients, and this effect could be related to the antioxidative property of pomegranates (Balbir-Gurman et al. 2011). The results suggested dietary supplementation with pomegranates may be a useful complementary strategy to attenuate clinical symptoms in rheumatoid arthritis patients.

Supplementation of obese Zucker rats with pomegranate fruit extract (PFE) or pomegranate juice (PJ) significantly decreased the expression of vascular inflammation markers, thrombospondin (TSP), and cytokine TGFβ1, whereas seed oil supplementation had a significant effect only

on TSP-1 expression (de Nigris et al. 2007a). Plasma nitrate and nitrite (NO(x)) levels were significantly increased by PFE and PJ. In addition, the effect of PFE in increasing endothelial NO synthase (eNOS) expression was comparable to that of PJ. The data suggested possible clinical applications of PFE in metabolic syndrome (clinical conditions such as obesity, hypertension, dislipidemia, and diabetes).

In-vivo studies revealed that aqueous pomegranate peel extract inhibited neutrophil myeloperoxidase activity and attenuated lipopolysaccharide-induced lung inflammation in mice (Bachoual et al. 2011). Inhibition of myeloperoxidase activity by pomegranate extract could explain its antiinflammatory action.

Balwani et al. (2011) demonstrated that 2-methyl-pyran-4-one-3-*O*-β-d-glucopyranoside (MPG) isolated from pomegranate leaves, inhibited TNFα-induced cell adhesion molecules expression by blocking nuclear transcription factor-κB (NF-κB) translocation and activation. The results suggested that MPG could be useful as a novel lead molecule for developing future antiinflammatory agents. Oral pomegranate extract decreased reactive oxygen species concentration and acute inflammation in the tympanic membrane in rats after myringotomy (Kahya et al. 2011). The density of inflammatory cells was significantly less in rats treated with the extract and the lamina propria thickness and vessel density were also significantly reduced.

### Hepatoprotective Activity

Pretreatment of Wistar rats with a methanolic extract of pomegranate peel followed by carbon tetrachloride treatment retained catalase, peroxidase, and superoxide dismutase to values comparable with control values, whereas lipid peroxidation was reduced by 54% as compared to control (Chidambara Murthy et al. 2002). Histopathological studies of the liver supported the hepatoprotective effects exhibited by the extract by restoring the normal hepatic architecture. Kaur et al. (2006) demonstrated

that pre-treatment of mice with pomegranate flower extract at a dose regimen of 50–150 mg/kg body weight for a week significantly and dose dependently protected against ferric nitrilotriacetate (Fe-NTA)-induced oxidative stress as well as hepatic injury. The extract conferred up to 60% protection against hepatic lipid peroxidation and preserved glutathione (GSH) levels and activities of antioxidant enzymes viz., catalase (CAT), glutathione peroxidase (GPX) glutathione reductase (GR) and glutathione-S-transferase (GST) by up to 36, 28.5, 28.7, 40.2 and 42.5% respectively. A protection against Fe-NTA induced liver injury was apparent as inhibition in the modulation of liver markers viz., aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin and albumin in serum. The histopathological changes produced by Fe-NTA, such as ballooning degeneration, fatty changes, necrosis were also alleviated by the extract. The flower extract was found to significantly scavenge superoxide radicals by up to 53.3%, hydrogen peroxide by up to 30%, hydroxyl radicals by up to 37% and nitric oxide by up to 74.5%. The extract also inhibited (.)OH induced oxidation of lipids and proteins in vitro. The potent antioxidant property of the flower extract was postulated to be responsible for its hepatoprotective effects. In another study, pomegranate flower infusion was found to exhibit hepatoprotective and antioxidant effect against trichloroacetic acid (TCA)-exposed rats (Celik et al. 2009). The infusion significantly decreased levels of aspartate aminotransferase and alanine aminotransferase; increased significantly glutathione S-transferase activity in the liver, brain and spleen and maintained superoxide dismutase level in the liver.

Intake of pomegranate juice by mice for weeks was found to confer hepatic protection against protein and DNA oxidation (Faria et al. 2007b). There was also a significant decrease in GSH (reduced glutathione) and GSSG (oxidized glutathione), without change in the GSH/GSSG ratio. All studied enzymatic activities (superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST) and glutathione

reductase (GR) and catalase) were found to be decreased by pomegranate juice treatment. Also, glutathione S-transferase and glutathione synthetase transcription were also decreased in this group. Chronic pomegranate peel extract administration to rats alleviated the bile duct ligation (BDL)-induced oxidative injury of the liver and improved the hepatic structure and function (Toklu et al. 2007). Plasma antioxidant capacity and hepatic glutathione levels were significantly depressed by BDL but were increased back to control levels in the pomegranate extract-treated BDL group. Increases in tissue malondialdehyde levels and myeloperoxidase activity due to BDL were reduced back to control levels by pomegranate extract treatment. Similarly, increased hepatic collagen content in the BDL rats was reduced to the level of the control group with extract treatment.

### Cardioprotective Activity

Sumner et al. (2005) showed that daily consumption of pomegranate juice may improve stress-induced myocardial ischemia in patients who have coronary heart disease in a randomized, placebo-controlled, double-blind study. After 3 months, the extent of stress-induced ischemia decreased in the pomegranate group (SDS −0.8±2.7) but increased in the control group (SDS 1.2±3.1). This benefit was observed without changes in cardiac medications, blood sugar, hemoglobin A1c, weight, or blood pressure in either group. Mohan et al. (2010a) demonstrated that pre-treatment of male Wistar rats with pomegranate juice (100 and 300 mg/kg, p.o.) and its butanolic extract(100 mg/kg., p.o.) for a period of 21 days significantly inhibited the effects of isoproterenol-induced myocardial infarction such as heart rate, pressure rate index, ECG patterns, levels of lactate dehydrogenase, creatine kinase, superoxide dismutase and catalase in the serum and vascular reactivity changes. Treatment with PJ and B-PJ (100 mg/kg., p.o.) alone did not alter any of the parameters as compared to vehicle-treated Wistar rats. Hassanpour et al. (2011) found that pomegranate fruit extract displayed

cardioprotective doxorubicin (Dox)-induced cardiotoxicity in rats. Rats administered the extract showed decreased QT and increase in heart rate compared to the Dox group Significant decrease in creatine kinase-MB isoenzyme, lactate dehydrogenase and no such significant decrease in aspartate aminotransferase were observed as compared to the Dox group. There was significant increase in the level of reduced glutathione, whereas inhibition of lipid peroxidation and increase in superoxide dismutase concentration was not significant in the extract treated group compared to the Dox group. Histopathological study of the extract -treated group showed slight protection against myocardial toxicity induced by Dox.

### Gastroprotective/Antiulcerative Activities

*P. granatum* fruit peel extract elicited 100% precipitation of ovine haemoglobin in-vitro and when orally administered to ethanol-induced gastric-damaged rats produced a significant decrease in gastric lesions (Gharzouli et al. 1999). The acid content of the stomach was significantly increased by pomegranate (368%) suggesting that monomeric and polymeric polyphenols could strengthen the gastric mucosal barrier. Administration of 70% methanolic pomegranate rind extract inhibited aspirin- and ethanol-induced gastric ulceration (Ajaikumar et al. 2005). Treated animals showed increased antioxidant levels of superoxide dismutase (SOD), catalase, glutathione (GSH) and glutathione peroxidase (GPx) and decreased level of tissue lipid peroxidation. No erosion of gastric mucosa, sub-mucosal edema and neutrophil infiltration was observed in treated animals. Pomegranate tannins (500, 150, 50 mg/kg) significantly inhibited ulcerative formation induced by both water immersion stress and pylorus ligation, and decreased the gastric mucosa damages induced by intragastric absolute ethanol, in dose-dependent manner in rats (Lai et al. 2009). Its antiulcer effect was found to be due to increasing secretion of adherent mucus and free mucus from the stomach wall, which may inhibit

generation of oxygen-derived free radicals, and decrease the consumption glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD), decrease absolute alcohol-induced elevation of malondialdehyde and maintain content of NO at normal level. *Punica granatum* peel extract (PPE) supplementation of irradiated rats reduced oxidative damage in the ileal tissues and protected against ionizing radiation-induced enteritis and leukocyte apoptosis in rats, probably by a mechanism associated with the decreased production of reactive oxygen metabolites and enhancement of antioxidant mechanisms (Toklu et al. 2009). PPE treatment reversed all these biochemical indices induced by irradiations such as the decrease in glutathione and total antioxidant capacity associated with increases in malondialdehyde levels, myeloperoxidase activity, collagen content of the tissue with a concomitant increase 8-hydroxy-2′-deoxyguanosine (an index of oxidative DNA damage) and increases in pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and lactate dehydrogenase. histopathological alterations and the increase in leukocyte apoptosis and cell death induced by irradiation was also reversed by PPE.

Oral administration of aqueous methanolic extract of pomegranate (490 and 980 mg/kg bw) significantly reduced the ulcer lesion index produced by alcohol, indomethacin, and aspirin, at both doses in rats (Alam et al. 2010). In pylorus-ligated rats the extract significantly reduced the ulcer lesions, gastric volume, and total acidity and prevented the ulceration by increasing the pH and mucus secretion.

Oral administration of pomegranate extract and its ellagic acid rich fraction (100 and 200 mg/kg) significantly attenuated dextran sulfate sodium -induced colonic inflammation in mice along with attenuation of histamine, myeloperoxidase and oxidative stress (Singh et al. 2009). The antiulcerative effect was comparable to sulphasalazine (100 mg/kg, p.o.) and sodium cromoglycate (40 mg/kg i.p). The authors stated that the antiulcerative effects may be attributed to mast cell stabilizing, antiinflammatory and antioxidant actions. Pomegrante peel extracts exhibited remarkable in-vitro anti-*Helicobacter pylori* activity against the clinical isolates of *H. pylori* (mean of inhibition zone diameter ranging from 16 to 40 mm/50 μg disc). *Helicobacter pylori* infection causes lifelong chronic gastritis, which can lead to peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer.

## Nephroprotective Activity

Pretreatment of rats with hydroalcoholic extract of pomegranate flowers (125 and 250 mg/kg p.o. twice daily for 3 days) significantly attenuated hypertonic glycerol-induced myoglobinuric renal dysfunction in a dose-dependent manner (Singh et al. 2011). The mechanism of renoprotective effects of *Punica granatum* was found to involve activation of PPAR-γ and nitric oxide-dependent signalling pathway.

## Immunomodulatory Activity

Pomegranate fruit rind powder at the dose of 100 mg/kg orally as aqueous suspension was found to stimulate the cell-mediated and humoral components of the immune system in rabbits (Gracious Ross et al. 2001). The pomegranate powder elicited an increase in antibody titer to typhoid-H antigen. It also enhanced the inhibition of leucocyte migration in Leucocyte Migration Inhibition test and induration of skin in delayed hypersensitivity test with Purified Protein Derivative (PPD) confirming its stimulatory effect on cell-mediated immune response. Punicalagin isolated from pomegranate fruit was found to be a potent immune suppressant, based on its inhibitory action on the activation of the nuclear factor of activated T cells (NFAT). Punicalagin downregulated the mRNA and soluble protein expression of interleukin-2 from anti-CD3/anti-CD28-stimulated murine splenic CD4+ T cells and suppressed mixed leukocytes reaction (MLR) without exhibiting cytotoxicity to the cells. In vivo, the punicalagin treatment inhibited phorbol 12-myristate 13-acetate (PMA)-induced chronic ear edema in mice and decreased CD3+ T cell infiltration of the inflamed tissue. The results suggested that punicalagin could be a potential candidate for the therapeutics of various

immune pathologies. Yamasaki et al. (2006) found that dietary pomegranate seed oil (PSO) high in levels of punicic acid (9c, 11 t, 13c-octadeca-trienoic acid), may enhance B-cell function in mice. Splenocytes isolated from mice fed 0.12 or 1.2% PSO produced larger amounts of immuno-globulins G and M but not immunoglobulin A irre-spective of stimulation with or without phorbol 12-myristate 13-acetate and the calcium ionophore A23187. Dietary PSO did not affect the percent-ages of B cells or CD4-positive or CD8-positive T cells in splenocytes. A polysaccharide (PSP001) isolated from pomegranate rind was found to have immunomodulatory activity (Joseph et al. 2012). PSP001 showed in-vitro growth stimulatory effect on isolated normal lymphocytes, and a prolifera-tive index of 1.21 at a concentration of 1,000 μg/mL was obtained, indicating immunomodulatory activity.

### Wound Healing Activity

Wistar rats with excision wounds treated with 5% water-soluble gel formulated from the methano-lic extract of dried pomegranate rind, showed good complete wound healing after 10 days (Chidambara Murthy et al. 2004). In comparison in rats treated with 2.5% gel, healing was observed on day 12, and in the positive control animals receiving the blank gel took 16–18 days for com-plete healing. Collagen content in terms of hydroxyproline level increased by two-fold in the group treated with 5.0% gel. The gel extract was found to contain gallic acid and catechin as major components. Aslam et al. (2006) found that pomegranate seed oil, but not aqueous extracts of fermented juice, peel or seed cake, stimulated human keratinocyte proliferation in monolayer culture. Contrariwise, pomegranate peel aqueous extract (and to a lesser extent, both the fermented juice and seed cake extracts) stimulated type I procollagen synthesis and inhibited matrix metal-loproteinase-1 (MMP-1; interstitial collagenase) production by dermal fibroblasts, but had no growth-supporting effect on keratinocytes. The results suggested that pomegranate peel aqueous extract could promote regeneration of dermis and pomegranate seed oil could promote regeneration

of epidermis. Pomegranate peel methanol extract-based ointment significantly enhanced wound contraction and the period of epithelial-ization as assessed by the mechanical (contrac-tion rate, tensile strength), the biochemical (increasing of collagen, DNA and proteins syn-thesis) and the histopathological characteristics in guinea pigs (Hayouni et al. 2011). The extract displayed antioxidant activity as potent as natural and synthetic compounds (Trolox, Butylated hydroxyanisole, Quercetin). In addition, the extract exhibited significant antibacterial and antifungal activity against *Pseudomonas aerugi-nosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella anatum*, *Salmonella typhimurium*, *Streptococcus pneumo-niae*, and fungi *Candida albicans*, *Candida glabrata*, *Trichopyton rubrum* and *Aspergillus niger*. The results suggested that the pomegranate formulated ointment may be used as skin repair agent without hazard to human health. The etha-nol extract of *P. granatum* flowers showed significant wound healing activity when topically administered in rats (Pirbalouti et al. 2010). The extract significantly increased the rate of wound contraction and collagen turnover.

### Photoprotective Activity

In-vitro studies using normal human epidermal keratinocytes, showed that pre-treatment with pomegranate fruit extract rich in anthocyannins and hydrolyzable tannins protected against the adverse effects of UV-B radiation by inhibiting UV-B-induced modulations of nuclear factor kappa B (NF-kappaB) and mitogen-activated protein kinases (MAPK) pathways (Afaq et al. 2005a, b). Similarly, they reported pomegranate fruit extract to be an effective agent for amelio-rating UVA-mediated skin damages by modulat-ing cellular pathways in-vitro (Syed et al. 2006). UVA-mediated cellular damage occurs primarily through the release of reactive oxygen species and is responsible for immunosuppression, photodermatoses, photoaging and photocarcino-genesis. Pretreatment of normal human epider-mal keratinocytes with the extract (60–100 μg/mL) for 24 h before exposure to UVA resulted in

a dose-dependent inhibition of UVA-mediated phosphorylation of signal transducers and activators of transcription 3 (STAT3), protein kinase B/AKT and mitogen activated protein kinases (MAPKs) viz. extracellular signal-regulated kinase (ERK1/2). The extract pretreatment also inhibited UVA exposure-mediated increases in Ki-67 antigen and PCNA (proliferating cell nuclear antigen) and increased the cell-cycle arrest induced by UVA in the G1 phase and the expression of Bax and Bad (proapoptotic proteins), while suppressing Bcl-X(L) antiapoptotic protein expression. Studies by Zaid et al. (2007) showed that pretreatment of human immortalized HaCaT keratinocytes with polyphenol-rich pomegranate fruit extract inhibited UVB-mediated decrease in cell viability, decrease in intracellular glutathione content and increase in lipid peroxidation. immunoblot analysis showed that pretreatment of HaCaT cells with pomegranate fruit extract inhibited UVB-induced (1) upregulation of MMP-1, -2, -7 and -9, (2) decrease in TIMP-1, (3) phosphorylation of MAPKs and (iv) phosphorylation of c-jun, whereas no effect was observed on UVB-induced c-fos protein levels. The results suggested that pomegranate fruit protected HaCaT cells against UVB-induced oxidative stress and markers of photoaging and could be a useful supplement in skin care products.

Pomegranate fruit extract (5–60 mg/L) was effective at protecting human skin fibroblasts from cell death following UV irradiation (Pacheco-Palencia et al. 2008). This photoprotective effect was postulated to be related to a reduced activation of the pro-inflammatory transcription factor NF-kappaB, suppression of proapoptotic caspase-3, and an increased G0/G1 phase, associated with DNA repair. Higher polyphenolic concentrations (500–10,000 mg/L) were required to achieve a significant reduction in UV-induced reactive oxygen species levels and increased intracellular antioxidant capacity. Pretreatment of reconstituted human skin "Epiderm" with pomegranate-derived products POMx juice, POMx extract and pomegranate oil inhibited UVB-induced cyclobutane pyrimidine dimers (CPD), 8-dihydro-2′-deoxyguanosine (8-OHdG), protein oxidation and proliferating cell nuclear antigen (PCNA) protein

expression (Afaq et al. 2009). Further, pretreatment of Epiderm with pomegranate-derived products resulted in inhibition of UVB-induced collagenase (MMP-1), gelatinase (MMP-2, MMP-9), stromelysin (MMP-3), marilysin (MMP-7), elastase (MMP-12), and tropoelastin. MMP-2 and MMP-9 activities were also inhibited. Overall, the results suggested that all three pomegranate-derived products may be useful against UVB-induced damage to human skin. Park et al. (2010) using cultured human skin fibroblasts, demonstrated that pomegranate fruit rind extract rich in polyphenols catechin, quercetin, kaempferol, and equol significantly protected against UVB-induced skin damage. The synthesis of collagen was increased and the expression of MMP-1 was decreased.

Oral feeding of pomegranate fruit extract to mice provided substantial protection from the adverse effects of UVB radiation via modulation in early biomarkers of photocarcinogenesis (Afaq et al. 2010). The extract inhibited UVB-induced: skin edema; hyperplasia; infiltration of leukocytes; lipid peroxidation; hydrogen peroxide generation; ornithine decarboxylase (ODC) activity; and ODC, cyclooxygenase-2 and proliferating cell nuclear antigen protein expression. The extract enhanced repair of UVB-mediated formation of cyclobutane pyrimidine dimers (CPDs) and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). The extract inhibited UVB-mediated nuclear translocation of NF-κB; activation of IKKα; and phosphorylation and degradation of IκBα. Additionally, the extract further enhanced UVB-mediated increase in tumour suppressor p53 and cyclin kinase inhibitor p21. In further studies, Khan et al. (2012) reported that oral feeding of pomegranate fruit extract to SKH-1 hairless mice inhibited UVB-induced epidermal hyperplasia, infiltration of leukocytes, protein oxidation and lipid peroxidation. Immunoblot analysis demonstrated that oral feeding of pomegranate fruit extract to mice inhibited UVB-induced (1) nuclear translocation and phosphorylation of nuclear factor kappa B/p65, (2) phosphorylation and degradation of IκBα, (3) activation of IKKα/IKKβ and (4) phosphorylation of mitogen-activated protein kinase proteins and c-Jun. Pomegranate fruit extract consumption also inhibited UVB-induced protein expression

of (1) COX-2 and iNOS, (2) PCNA and cyclin D1 and (3) matrix metalloproteinases-2,-3 and -9 in mouse skin. Overall, the data showed that pomegranate fruit extract consumption afforded protection to mouse skin against the adverse effects of UVB radiation by modulating UVB-induced signalling pathways.

In a double-blind, placebo-controlled trial involving female subjects age 20–40s, Kasai et al. (2006) found that oral administration of an ellagic acid-rich pomegranate extract had an inhibitory effect on a slight pigmentation (stains and freckles, brightness of face) in the human skin caused by UV irradiation.

### Skin Whitening Activity

Methanolic pomegranate extract showed 53.4% in-vitro mushroom tyrosinase inhibitory activity (Adhikari et al. 2008). A pomegranate rind extract was found to have skin whitening activity (Yoshimura et al. 2005). The extract exhibited inhibitory activity against mushroom tyrosinase in-vitro comparable to that of the skin whitening agent, arbutin. When taken orally the extract inhibited UV-induced skin pigmentation on the back of brownish guinea pig. The results suggested the skin-whitening effect of the extract was probably due to inhibition of the proliferation of melanocytes and melanin synthesis by tyrosinase in melanocytes. A pomegranate polysaccharide fraction inhibited the formation of advanced glycation end-products (AGEs) by 28% and also inhibited the formation of fructosamine in the BSA/Glucose system (Rout and Banerjee 2007). The fraction inhibit 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-Azinobis[3-ethylbenzothiazoline-6-sulfonate] ABTS(+) radical activities by 69 and 88%, respectively with 4 μg/mL concentration It also inhibited mushroom tyrosinase by 43% at 10 μg/mL concentration suggesting its efficacy as a potential skin whitener.

### Anti-hyperoxaluria Activity

Pomegrante juice was shown to have a protective effect against ethylene glycol-induced nephrolithiasis in rats (Tugcu et al. 2008). Ethylene glycol caused hyperoxaluria characterised by severe crystalization in renal tubules and granulovacuolar epithelial cell degeneration, marked elevation in malondialdehyde and nitric oxide levels and decrease of reduced glutathione (GSH) in rats There was no crystal formation in the rats treated with ethylene glycol and pomegranate juice. Administration of pomegranate juice at medium and high dosage to rats was found to have inhibitory effects on renal tubular cell injury and oxidative stress caused by oxalate crystal deposition by reducing ROS, iNOS, p38-MAPK, and NF-kB expression (Ilbey et al. 2009).

### Antidiarrhoeal Activity

Rats treated with methanol pomegranate seed extract exhibited significant inhibitory activity against castor-oil induced diarrhoea and PGE2 induced enteropooling (Das et al. 1999). The extract also displayed a significant reduction in gastrointestinal motility in charcoal meal test in rats.

### Antiplatelet Aggregation Activity

Pomegranate juice and polyphenol-rich pomegranate fruit extract reduced platelet aggregation, calcium mobilization, thromboxane A(2) production, and hydrogen peroxide formation, induced by collagen and arachidonic acid (Mattiello et al. 2009). The polyphenol – rich fruit extract was more potent in reducing platelet activation. Studies showed that pomegranate fruit components (mainly ellagic acid) modulated human thrombin amidolytic activity (Cuccioloni et al. 2009).

### Antiosteoporotic Activity

Pomegranate fruit ethanol extract was found to significantly increase the growth of osteoblastic MC3T3-E1 cells and caused a significant elevation of alkaline phosphatase (ALP) activity and collagen content in the cells (Kim and Choi 2009). Treatment the extract decreased the TNF-α-induced production of interleukin IL-6 and nitric oxide in osteoblasts.

## Uterine Contractile Activity

Promprom et al. (2010) found pomegranate seed extract to be a potent stimulator of phasic activity in rat uterus. Pomegranate seed extract and β-sitosterol, the main constituent of the extract (16%), increased spontaneous contractions of the rat uterus in a concentration-dependent manner. Their data suggested that the uterotonic effect was due to nonestrogenic effects of β-sitosterol acting to inhibit K channels and sarcoplasmic reticulum calcium ATPase and thereby increasing contraction via calcium entry on L-type calcium channels and myosin light chain kinase.

## Antidementia and Central Nervous System Activities

Two β-secretase (BACE1) inhibitors (anti-dementia agents) were isolated from pomegranate rind and identified as ellagic acid and punicalagin with $IC_{50}$ values of $3.9 \times 10^{-6}$ and $4.1 \times 10^{-7}$ M (Kwak et al. 2005). Ellagic acid and punicalagin were less inhibitory to α-secretase (TACE) and other serine proteases such as chymotrypsin, trypsin, and elastase, thus indicating that they were relatively specific inhibitors of BACE1. β-Secretase is an aspartic-acid protease involved in the pathogenesis of Alzheimer's disease

Studies showed that ethanolic extract of *P. granatum* seeds significantly exhibited the anxiolytic activity animal models of elevated plus maze test, barbiturate-induced sleeping time, tail suspension test, hot-plate and tail-flick tests (Kumar et al. 2008). The extract (250 and 500 mg/kg) significantly increased the sleeping latency and reduced the sleeping time. Tail suspension test showed that the extract (250 and 500 mg/kg) was able to induce a significant decrease in the immobility time, similar to imipramine, a recognized antidepressant drug. Tail-flick and hot-plate tests exhibited antinociceptive property of pomegranate extract, similar to morphine, a recognized antinociceptive agent. Phytochemical screening and measurement of reducing power revealed the central nervous system (CNS) activity of ethanol extract of pomegranate seeds may be due to its antioxidative profile.

Supplementation of pomegranate flowers led to improvements in learning and memory performances of streptozotocin-induced diabetic rats (Cambay et al. 2011). Supplementation of pomegranate flowers restored the elevated levels of lipid peroxidation and decreased level of glutathione towards their control values. Daily pomegranate flower supplementation to diabetic rats reduced the increase in glial-fibrilar acidic protein (GFAP) contents induced by diabetes in the hippocampus. The observations suggested that pomegranate flower supplementation decreased oxidative stress and amelioratedimpairment in learning and memory performances in diabetic rats and may be clinically useful in treating neuronal deficit in diabetic patients.

## Neuroprotective Activity

Loren et al. (2005) found that maternal dietary supplementation with pomegranate juice was neuroprotective in an animal model of neonatal hypoxic-ischemic brain injury. Dietary supplementation with pomegranate juice resulted in markedly decreased brain tissue loss (>60%) in all three brain regions assessed, with the highest pomegranate juice dose having greatest significance Pomegranate juice also diminished caspase-3 activation by 84% in the hippocampus and 64% in the cortex. In further studies, the scientists showed that pomegranate polyphenols and resveratrol reduced caspase-3 activation following neonatal hypoxic-ischemic injury (West et al. 2007). In separate study, transgenic mice (APP(sw)/Tg2576) treated with pomegranate juice had significantly less (approximately 50%) accumulation of soluble Aβ42 and amyloid deposition in the hippocampus as compared to control mice (Hartman et al. 2006). Mice administered pomegranate juice learned water maze tasks more quickly and swam faster than controls. The results suggest that pomegranate juice may be useful in Alzheimer's disease and warrant further studies. Choi et al. (2011b) found that the ethanol pomegranate extract mitigated $H_2O_2$-induced oxidative stress in PC12 cells. Additionally, the extract inhibited neuronal cell

death caused by Aβ-induced oxidative stress and Aβ-induced learning and memory deficiency in mice.

## Embryo and Sperm Protective Activity

Studies showed that pomegranate fruit extract exhibited embryo protective effect against adriamycin-induced oxidative stress in chick embryos (Kishore et al. 2009). Pre-administration of pomegranate fruit extract significantly ameliorated to normal, embryo gross morphological deformities and significant changes in the levels of biochemical parameters in amniotic fluid observed in the adriamycin-treated group.

Pomegranate juice consumption by healthy male rats provided an increase in epididymal sperm concentration, sperm motility, spermatogenic cell density and diameter of seminiferous tubules and germinal cell layer thickness and antioxidant activity, and it decreased abnormal sperm rate when compared to the control group (Türk et al. 2008). A significant decrease in malondialdehyde level and marked increases in glutathione, glutathione peroxidase and catalase activities, and vitamin C level were observed in rats treated with different doses of pomegranate juice. Studies showed that ethanolic extract of pomegranate could be useful for the treatment of the deleterious effect of lead acetate administration on sperm production in rats (Leiva et al. 2011). The extract exhibited antioxidant activity similar to that of ascorbic acid and prevented lead acetate -induced spermatogenic disruption in rats. Its antioxidant activity could explain its capacity to reverse the damage produced by lead acetate on spermatogenesis.

## Amelioration of Erectile Dysfunction Activity

In a randomized, placebo-controlled, double-blind, crossover study involving male patients with mild to moderate erectile dysfunction, of the 42 subjects who demonstrated improvement in Global Assessment Questionnaires (GAQ) scores after beverage consumption, 25 reported improvement in erectile function after drinking pomegranate juice (Forest et al. 2007). Subjects were more likely to have improved scores when pomegranate juice was consumed. Although overall statistical significance was not achieved, this pilot study suggested the possibility that larger cohorts and longer treatment periods may achieve statistical significance. Studies in rabbits with atherosclerosis-induced erectile dysfunction showed that pomegranate extract significantly improved intracavernosal blood flow, erectile activity, smooth muscle relaxation and fibrosis of the atherosclerotic group in comparison with the atherosclerotic group receiving placebo, but did not normalize them to the age-matched control levels (Zhang et al. 2011). Pomegranate extract appeared more effective in diminishing oxidative products, preventing superoxide dismutase and aldose reductase gene upregulation, and protecting mitochondrial, endothelial and caveolae structural integrity of the atherosclerotic group. The study showed that dietary antioxidants could improve arteriogenic erectile dysfunction.

## Estrogenic Activity

Pomegranate known to contain estrogens (estradiol, estrone, and estriol) exhibited estrogenic activities in mice (Mori-Okamoto et al. 2004). Administration of pomegranate extract (juice and seed extract) for 2 weeks to ovariectomized mice prevented the loss of uterus weight and shortened the immobility time compared with 5% glucose-dosed mice (control). Further, ovariectomy-induced decrease of bone mineral density was normalized by administration of the pomegranate extract. The bone volume and the trabecular number were significantly increased and the trabecular separation was decreased in the pomegranate-dosed group compared with the control group. Some histological bone formation/resorption parameters were significantly increased by ovariectomy but were normalized by administration of the pomegranate extract. These changes suggested that the pomegranate extract inhibited

ovariectomy-stimulated bone turnover. The authors concluded that pomegranate may be clinically effective on a depressive state and bone loss in menopausal syndrome in women.

## Cytochrome P450-3A (Drug-Drug Interaction) Activity

Studies in human volunteers, found that in human liver microsomes, the mean 50% inhibitory concentrations ($IC_{50}$) for pomegranate juice (PJ) and grapefruit juice (GFJ) versus CYP3A (triazolam α-hydroxylation) were 0.61 and 0.55%, (v/v) respectively without preincubation of inhibitor with microsomes (Farkas et al. 2007). After preincubation, the $IC_{50}$ for PJ increased to 0.97% whereas the $IC_{50}$ for GFJ decreased to 0.41% suggesting mechanism-based inhibition by GFJ but not PJ. Administration of PJ also did not affect C(max), total area under the curve (AUC), or clearance of oral midazolam. However, GFJ increased midazolam C(max) and AUC by a factor of 1.3 and 1.5, respectively, and reduced oral clearance to 72% of control values. The results suggested PJ did not alter clearance of intravenous or oral midazolam, whereas GFJ impaired clearance and elevated plasma levels of oral midazolam. Jarvis et al. (2010) reported a potential interaction between pomegranate juice and warfarin as laboratory studies hade shown that pomegranate juice inhibited cytochrome P450 enzymes involved in warfarin metabolism. In a an open-label, randomized, single-center, two-period crossover study in healthy Japanese volunteers, a single subtherapeutic doses of midazolam following 2 weeks consumption of pomegranate juice did not significantly alter the pharmacokinetic profile of midazolam compared with that of the control (Misaka et al. 2011).

## Effect on Chronic Obstructive Pulmonary Disease

Results of a 5-week randomized, double-blind, placebo-controlled study involving 30 patients suggested that polyphenol-rich pomegranate juice (PJ) supplementation added no benefit to the current standard therapy in patients with stable chronic obstructive pulmonary disease (Cerdá et al. 2006). The high TEAC (Trolox Equivalent Antioxidant Capacity) of PJ could not be extrapolated in-vivo probably due to the metabolism of its polyphenols by colonic microflora. The understanding of the different bioavailability of dietary polyphenols was thus critical before claiming any antioxidant-related health benefit.

## Ergogenic Activity

Elbow flexion strength was significantly higher during the 2- to 168-h period post-exercise with pomegranate juice compared with that of placebo (Trombold et al. 2011). Elbow flexor muscle soreness was also significantly reduced with pomegranate juice compared with that of placebo and at 48 and 72 h post-exercise. Isometric strength and muscle soreness in the knee extensors were not significantly different with pomegranate juice compared with those using placebo. The results indicated a mild, acute ergogenic effect of pomegranate juice in the elbow flexor muscles of resistance trained individuals after eccentric exercise.

## Carbonic Anhydrase Inhibition Activity

Seven highly active ellagitannin inhibitors against carbonic anhydrase, punicalin (2), punicalagin (3), granatin B (5), gallagyldilactone (7), casuarinin (8), pedunculagin (9) and tellimagrandin I (10), and four weakly active ellagitannin inhibitors, gallic acid (1), granatin A (4), corilagin (6) and ellagic acid (11), were isolated from pomegranate pericarps (Satomi et al. 1993). The type of inhibition by compounds (3) and (7) using *p*-nitrophenyl acetate as a substrate, was noncompetitive. Carbonic anhydrase inhibitors are used as antiglaucoma drugs, and many potent carbonic anhydrase inhibitors have also been shown to inhibit the growth of several tumour cell lines in-vitro and in-vivo providing interesting leads for developing novel antitumour therapies (Supuran et al. 2004)

## Analgesic Activity

Using the hot plate method in mice, pomegranate flower extracts showed significant analgesic activity at a dose of 50 mg/kg body weight (Chakraborthy 2008). Maximum analgesic activity was observed at 60 min after drug administration, which was equivalent to the standard drug used morphine sulphate.

## Antiplasmodial/Anti-protrozoal Activities

Two milliliters of aqueous extract of pomegranate roots exhibited higher activity on cultures from *Entamoeba histolytica* than from *Entamoeba invadens* strains, producing growth inhibitions of about 100 and 40% respectively (Segura et al. 1990). Alkaloid concentrations of 1 mg/mL had no amoebicide activity, however tannins at concentrations of 10 µg/mL for *E. histolytica*, and 100 µg/mL for *E. invadens* were sufficient to produce an growth inhibition about 100%. Tannic acid was also tested on the cultures of *E. histolytica* producing a high inhibitory activity on growth, this effect was produced at 0.01 mg/mL similar to that observed with the tannin mixture.

The methanolic extract of pomegranate was reported to in-vitro inhibit the growth of the malarial parasite, *Plasmodium berghei* (Dell'Agli et al. 2009). In another study, gallagic acid and punicalagin from pomegranate by-product exhibited antiplasmodial activity against *Plasmodium falciparum* D6 and W2 clones with $IC_{50}$ values of 10.9, 10.6, 7.5 and 8.8 µM, respectively (Reddy et al. 2007). Pomegranate extract exhibited strong antimalarial activity against *Plasmodium falciparum* (Valdés et al. 2010).

*P. grantum* plant extract also exhibited in-vitro activity against the vaginal parasite, *Trichomonas vaginalis* (El-Sherbini et al. 2009).

Hydroalcoholic pomegranate extract inhibited the growth of intracellular amastigotes of *Leishmania amazonensis* with $IC_{50}$ value of 69.6 µg/mL (García et al. 2010). Additionally, a low toxicity on macrophage from BALB/c mice was observed.

## Anthelminthic Activity

Wibaut and Hollstein (1957) found that the anthelminthic activity of pomegranate bark extract was mainly due to isopelletierine, methylisopelletierine while ψ pelleterine was less active

## Molluscicidal Activity

The molluscicidal activity of *P. granatum* bark and *Canna indica* root against the snail, *Lymnaea acuminata* was found to be both time and dose dependent (Tripathi and Singh 2000). The toxicity of *P. granatum* bark was more pronounced than that of *C. indica*. The 24 h $LC_{50}$ of the *C. indica* was 6.54 mg/L whereas that of the bark of *P. granatum* was 4.39 mg/L. The ethanol extract of *P. granatum* (24 h $LC_{50}$: 22.42 mg/L) was more effective than the ethanol extract of *C. indica* (24 h $LC_{50}$: 55.65 mg/L) in killing the test animals. *P. granatum* and *C. indica* may be used as potent molluscicides since the concentrations used to kill the snails were not toxic to the fish *Colisa fasciatus*, sharing the same habitat with the snail. In a subsequent study, Tripathi et al. (2004) reported that sub-lethal 24 h exposure to active fraction of pomegranate bark separately or in combination with Canna roots significantly inhibited the activity of acetylcholinesterase, acid/alkaline phosphatase, Na(+)K(+)ATPase and lactic dehydrogenase in the nervous tissue of *Lymnaea acuminata.*

## Pharmacokinetics/Bioavailability of Pomegranate Phytochemicals

Lei et al. (2003) found that ellagic acid, the principal bioactive component of pomegranate leaf extract, had poor absorption and rapid elimination after oral administration pomegranate leaf extract, and part of it was absorbed from stomach. Studies in rats showed that only 3–6% of the ingested punicalagin was detected as such or as metabolites in urine and faeces (Cerdá et al. 2003b). Only traces of punicalagin metabolites

being detected in liver or kidney. The transformation of ellagic acid derivatives to 6H-dibenzo[b,d] pyran-6-one derivatives in the rat was confirmed. Studies of Cerdá et al. (2004) found that the potential systemic biological effects of pomegranate juice ingestion should be attributed to the colonic microflora metabolites rather than to the polyphenols present in the juice. Neither the potent antioxidant punicalagin nor ellagic acid present in pomegranate juice were detected in both plasma and urine on ingestion of pomegranate juice. Three microbial ellagitannin-derived metabolites were detected: 3,8-dihydroxy-6H-dibenzo[b,d]pyran-6-one glucuronide, an unidentified aglycone (tentatively, trihydroxy-6H-dibenzo[b,d]pyran-6-one) and hydroxy-6-H-dibenzo[b,d]pyran-6-one glucuronide in the plasma and urine. The metabolites did not show significant antioxidant activity compared to punicalagin from pomegranate juice. In separate studies, ellagic acid was detected in human plasma at a maximum concentration (31.9 ng/mL) after 1 h post-ingestion of pomegranate juice but was rapidly eliminated by 4 h (Seeram et al. 2004). Six hours post-ingestion of pomegranate juice, ellagic acid (EA) was detected in plasma of all healthy human volunteers with a maximum concentration of 0.06 μmol/L, area under concentration time curve of 0.17 (μmol × h) × L(-1), time of maximum concentration of 0.98 h, and elimination half-life of 0.71 h (Seeram et al. 2006). Ellagic acid metabolites, including dimethylellagic acid glucuronide (DMEAG) and hydroxy-6H-benzopyran-6-one derivatives (urolithins), were also detected in plasma and urine in conjugated and free forms. DMEAG was found in the urine obtained from 15 of 18 subjects on day 0, but was not detected on d −1 (day before) or +1 (day after), demonstrating its potential as a biomarker of intake. Urolithin A-glucuronide was found in urine samples from 11 subjects on d 0 and in the urine from 16 subjects on d +1. Urolithin B-glucuronide was found in the urine of three subjects on d 0 and in the urine of five subjects on d+1. The scientists asserted that urolithins, formed by intestinal bacteria, may contribute to the biological effects of pomegranate juice as they may persist in plasma and tissues

and account for some of the health benefits noted after chronic juice consumption. Studies by Seeram et al. (2008) found that pomegranate juice, pomegranate polyphenol liquid extract and pomegranate polyphenol powder extract provide similar levels of plasma and urinary ellagitannin metabolites such as urolithin A, in human subjects. There was a delay in time of maximum concentration of pomegranate powder extract compared to pomegranate juice and pomegranate polyphenol liquid. Mertens-Talcott et al. (2006) found ellagic acid from pomegranate extract to be bioavailable at 1 h after consumption by healthy volunteers. Its metabolites urolithin A, urolithin B, hydroxyl-urolithin A, urolithin A-glucuronide, and dimethyl ellagic acid-glucuronide were found in the plasma. The antioxidant capacity measured with the oxygen radical absorbance capacity (ORAC) assay was increased with a maximum effect of 32% after 0.5 h, whereas the generation of reactive oxygen species (ROS) was not affected.

### Toxicological/Safety Studies

Vidal et al. (2003) found that in chick embryo model doses of hydroalcoholic pomegranate fruit extract of less than 0.1 mg per embryo were not toxic. The $LD_{50}$ of the extract, determined in OF-1 mice of both sexes after intraperitoneal administration, was 731 mg/kg. Confidence limits were 565–945 mg/kg. At the doses of 0.4 and 1.2 mg/kg of extract, the repeated intranasal administration to Wistar rats produced no toxic effects in terms of food intake, weight gain, behavioural or biochemical parameters, or results of histopathological studies. Cerdá et al. (2003a) found that repeated oral administration of high doses of the pomegranate ellagitannin punicalagin to rats for 37 days was not toxic. Punicalagin and related metabolites were identified in plasma, liver, and kidney. Five punicalagin-related metabolites were detected in liver and kidney, that is, two ellagic acid derivatives, gallagic acid, 3,8-dihydroxy-6H-dibenzo[b,d] pyran-6-one glucuronide, and 3,8,10-trihydroxy-6H-dibenzo[b,d]pyran-6-one. Feedstuff intake,

food utility index, and growth rate were lower in punicalagin treated rats during the first 15 days without significant adverse effects, which could be due to the lower nutritional value of the punicalagin-enriched diet together with a decrease in its palatability (lower food intake). No significant differences were found in punicalagin treated rats in any blood parameter analyzed (including the antioxidant enzymes glutathione peroxidase and superoxide dismutase) with the exception of urea and triglycerides, which remained at low values throughout the study. Clinical studies by Heber et al. (2007) demonstrated the safety of a pomegranate ellagitannin-enriched polyphenol dietary supplement in overweight individuals with increased waist size and provided evidence of antioxidant activity in humans reflected by a significant reduction in thiobarbituric acid reactive substances (TBARS) linked with cardiovascular disease risk. Patel et al. (2008) found that the no observed-adverse-effect level (NOAEL) for a standardized pomegranate fruit extract was determined as 600 mg/kg body weight/day, the highest dose tested in rats. Compared to the control group, administration of the extract did not result in any toxicologically significant treatment-related changes in clinical observations, ophthalmic examinations, body weights, body weight gains, feed consumption, clinical pathology evaluations and organ weights.

Toxicological evaluation of pomegranate seed oil (PSO) showed that the no observable adverse effect level (NOAEL) was 50,000 ppm PSO (=4.3 g PSO/kg body weight/day) (Meerts et al. 2009). No mutagenicity of PSO was observed in the absence and presence of metabolic activation up to precipitating concentrations of 5,000 μg/plate (Ames test) or 333 μg/mL (chromosome aberration test). The acute oral toxicity study revealed no significant findings at 2,000 mg PSO/kg body weight.

Results from reversion and gene-conversion test in microorganisms, sister chromatid exchanges, micronuclei and sperm-shape abnormality assays in mice, clearly showed that the hydroalcoholic extract of pomegranate whole fruit was genotoxic when tested both in-vitro and in-vivo (Sánchez-Lamar et al. 2008).

## Traditional Medicinal Uses

The bark of the roots, the flowers, the rind of pomegranate fruit and the seeds, are official in many pharmacopoeias. Various parts of the pomegranate plant have been extensively used for thousands of years in traditional medicine in the Middle East, Ancient Greece and Asia (Burkill 1966; Grieve 1971; Stuart 2012); and in India such as in the Ayurveda and Unani systems of medicine (Nadkarni and Nadkarni. 1982; Sharma et al. 2002; Kapoor 2000; Pradeep et al. 2008). The fruit rind and stem bark have been used as a traditional remedy for diarrhoea, dysentery and intestinal parasites. Pomegranate pericarp has been commonly employed as a crude drug in Indian traditional medicine for the treatment of diarrhoea as well as for use as an astringent, antihelminthic, asphrodisacs, laxative, diuretic, stomachic, cardiotonic and refrigerant. The seeds and juice are considered as bitter and astringent and employed as a tonic for hear and throat ailments. The astringent qualities of the flower sap, fruit rind and tree bark are considered useful remedies for nose bleeds and gum bleeds, toning skin, (after mixing with mustard oil) firming-up sagging breasts and treating haemorrhoids. A syrup prepared from the fruit is useful in all bilious complaints. The juice of the fresh fruit is much esteemed in dyspepsia and as a cooling, thirst-quenching beverage in fever and sickness. The fruit juice is also found beneficial in leprosy. Pomegranate fruit juice has been used as eyedrops to treat cataracts.

Dried, pulverized flower buds are employed as a remedy for bronchitis. Pomegranate has been reported as a remedy for diabetes in the Unani system of medicine practiced in the Middle East and India. The ancient Greeks and Egyptians used the fruit rind, flowers and root bark as astringents and the last as vermicide for treating tapeworms. In Malaysia, the root bark is used as vermifuge and powdered root bark is administered to children for stomach pains. The root is also used for diarrhoea and tits sap used for treating sore-eyes. Leaves are used in jamu preparations with a raft of other herbal ingredients for many medicinal complaints. Pounded leaves are

used in a complex bolus for stomach ache and the fruit juice is recommended for coughs. In Singapore, the root bark has been used in as a component in a compound infusion or decoction taken by women for 40 days after childbirth. Other traditional uses of the fruit rind and root include as a treatment for snakebite (Jain and Puri 1984), diabetes (Singh 1986), burns (Siang 1983), leprosy and assorted gynecological problems (Singh et al. 1980). In Sri Lanka, the fresh fruit has been used as a refrigerant to lower fever (Arseculeratne et al. 1985).

In the Philippines, a decoction of the tender leaves is used as a gargle for affections of the buccal cavity. The rind of the fruit is used internally in decoction as anthelmintic and taenifuge. In Mexico, a decoction of the flowers is gargled to relieve oral and throat inflammation. In Korea, traditional uses of the fruit and rind include as an anthelminthic and for phlegm, cholethiasis, tineapedis and laryngitis.

## Other Uses

*Punica granatum* is a drought tolerant tree suitable for arid and semi-arid zone afforestation. Pomegranate has deep rooting system and is used for erosion control, planted along rivers to stabilize banks. An ideal suitable ornamental plant for gardens and amenity parks. Pomegranate grows along well as intercrop with grapes in Mediterranean countries. The tree is sometimes used for fencing and planted as boundary plants. Pomegranate leaf litter decomposes slowly and is suitable for mulching. The leaves are foraged by domesticated stock. Ink can be made by steeping the leaves in vinegar. Both the fruit rind and the flowers yield dyes for textiles. The light-coloured wood is hard and durable, mostly used in making farm implements, walking-sticks and in woodcrafts as it is only available in small dimension. Tree branches are used as firewood. The bark is used in tanning and dyeing providing the yellow hue for Moroccan leather. Root bark yields a black ink rich in tannins. In Japan, an insecticide is derived from the bark.

Studies revealed that pomegranate peel can prepared as an adsorbent in treating industrial effluents containing phenols and safely disposed of by stabilizing into cement (Bhatnagar and Minocha 2009). Studies showed that that pomegranate peel waste can be used as adsorbent beneficially for nickel removal from aqueous solution (Bhatnagar and Minocha 2010). Pomegranate husk when converted into activated carbon exhibited its ability to remove hexavalent chromium from wastewater (Nemr 2009).

Studies showed that the nutritive value and the antioxidant capacity of pomegranate peel could be enhanced by ensiling into a favorable health-promoting constituent of feedlot beef cattle diet (Shabtay et al. 2008). Dietary supplementation with fresh peels promoted significant increases in feed intake and α-tocopherol concentration in the plasma, with positive tendency toward increased weight gain of bull calves.

The pomegranate fruit is steeped in religious and cultural significance in Judaism, Christianity, Islam, Hinduism religions, Persian, Armenian, Azerbaijani and Chinese cultures (Wikipedia 2012).

## Comments

Pomegrante germinates readily from seeds and are established from seedlings, rooted hardwood cuttings, from air layering and suckers.

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