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Review

Pomegranate (*Punica granatum* L.): A medicinal plant with myriad biological properties - A short review

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The pomegranate, *Punica granatum* L., which can be found throughout the Mediterranean region, in Southeast Asia, California and Arizona in USA was in ancient times referred as possessing powers of fertility, abundance and good luck (fruit). The biological properties of extracts (antimicrobial, antioxidant, anticancer, anti-inflammatory, among other properties) obtained from several parts of pomegranate is reported in the present work. Due to such properties, the extracts have been used in therapeutics, such as in the prevention of infection, inflammation, cancer, among other applications. However, other aspects are also referred in the present work such as the good practices of culture and fruit preservation, search of new compounds, selection of cultivars through biotechnological techniques for obtaining juice or fruits ready to eat. Such compilation of information was based on the search in the ISI Web of Knowledge (Thomson Reuters) from 2009 up to the beginning of October 2010.

Key words: *Punica granatum* L., polyphenols, biological activities.

INTRODUCTION

Phytotherapy is considered as a complementary approach for preventing and treating simple disease, although well grounded in medical tradition, it often lacks proper scientific validation (Cravatto et al., 2010). These authors focused the search on clinical investigations concerning some plants, including *Punica granatum* L. (pomegranate). In addition to the its ancient historical uses (it was lauded in the Old Testament of the Bible, Koran, the Jewish Torah, and the Babylonian Talmud as a sacred fruit conferring powers of fertility, abundance, and good luck) pomegranate is used in diverse systems of medicine as ailments. According to the review article made by Julie (2008) about the therapeutic applications of pomegranate, the author refers that in Ayurvedic medicine, the pomegranate is used as antiparasitic agent, a blood tonic, and to heal aphtae, diarrhoea and ulcers. In the Unani system, practiced in the Middle East and India, and according to the same review, pomegranate was described also a remedy for diabetes (Julie, 2008).

The edible part of the fruits contains acids, sugars, vitamins, polysaccharides, polyphenols and minerals, however, several factors may contribute to the chemical changes, including cultivars, environmental conditions, ripening, storage and postharvest treatments, which may

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affect fruit quality and health beneficial compounds (Schwartz et al., 2009a).

A recent review reported the chemical constituents of diverse parts of *P. granatum* as well as their potential for prevention and treatment of inflammation and cancer. The authors refer that in pericarp, leaf and flower can be detected phenols (flavonoids and tannins) being some of them unique. Complex polysaccharides have also been detected and characterized in the peels. In seeds, triacylglycerols constituted the oil, with a high content of punicic acid. In this oil, the authors also reported the presence of sterols, steroids and cerebroside in very small amounts. In addition to the seed oil, lignin and their derivatives have also been reported possessing remarkable antioxidant activities (Lansky and Newman, 2007). In the same review article, the authors highlighted the major components of pomegranate seeds, juice, pericarp, bark and leaf as well as their pharmacological activity in mammalian cells relevant to the prevention and/or treatment of malignant cell growth, from 2000 to 2006. The mechanisms claimed by the authors referred in that review article included increased apoptosis, decreased inflammation, decreased metastasis and invasion, as well as a decrease in drug resistance (Lansky and Newman, 2007). Antixodiant activities associated with different pomegranate components were also summarized by the authors.

One year later, other review article (Julie, 2008) revealed that pomegranate juice may be fruitful as a therapy for prostate cancer, particularly recurrent type of cells; in atherosclerosis by inhibiting the lipid peroxidation in plasma and in lipoproteins as well as the collagen-induced platelet aggregation in human platelets *ex vivo*; in hyperlipidemia owing to the decrease absorption and increased faecal excretion of cholesterol as well as possible effects on HMG-CoA reductase and sterol O-acyltransferase, two enzymes key to cholesterol metabolism. Pomegranate juice was also reported effective in hypertension by decreasing Angiotensin-Converting Enzyme (ACE) activity; reducing myocardial ischemia and improving myocardial perfusion; in diabetes through a significant effect on atherogenesis through reduced oxidative stress; in periodontal disease and denture stomatitis. Other benefits include the combat to some bacterial infections, erectile dysfunction, male infertility, Alzheimer's disease, obesity. The authors also refer those works concerning the pharmacokinetic (metabolism and availability) of ellagitannins present in pomegranate juice as well as the potential drug interactions and safety of pomegranate extracts.

As a crop, pomegranate is extensively cultivated in Iran, India, Afghanistan and Mediterranean countries, and to some extent in the USA, China, Japan and Russia (Narzary et al., 2010a). It is one of the most valuable fruits and is grown on a commercial scale also in Iran (Ramezanian et al., 2009). Pomegranate fruit is consumed directly as fresh seeds, but can also be used for making juice, jelly, grenadine or as flavouring and colouring agents. In addition, this species has been proved to possess therapeutic properties, therefore with an economical and ecological importance (Al-Said et al., 2009; Akbarpour et al., 2010).

The last review about the therapeutic effects of pomegranate found in the ISI Web of Knowledge (Thomson Reuters) was that of Julie (2008). Therefore, the present work intends to make a review about this subject using the same database from 2009 up to 3rd October, 2010. Search keys used for the research were: *P. granatum* (topic) and 2009 to 2010 (year published).

Over 200 works were found including abstracts, proceedings, articles and patents. In some few cases references other than 2009 to 2010 can be cited only as introductory to the more recent works.

The predominant theme was indubitably the biological properties of pomegranate, nevertheless other approaches were also found: pests and diseases in pomegranate crop (Bardas et al., 2009; Mondal and Mani, 2009; Qasen, 2009; Spadaro et al., 2010; Wohlfarter et al., 2010a; Wohlfarter et al., 2010b); pomegranate on the combat of microorganisms affecting plants (Guo et al., 2009; Hassan et al., 2009; Tayel et al., 2009a; Osorio et al., 2010), seeds during storage (Gandhi et al., 2010) or against Lymphocystis Disease Virus (LDV) in the fish olive flounder *Paralichthys olivaceus* (Harikrishnan et al., 2010). Other themes could also been found such as methods, analysis of several compounds from pomegranate and influence of some factors such as cultivar, fruit development on the chemical composition (Al-Said et al., 2009; Kýralan et al., 2009; Liu et al., 2009; Martin et al., 2009; Sassano et al., 2009; Schwartz et al., 2009b; Zhang et al., 2009; Akbarpour et al., 2010; Caligiani et al., 2010; Panichayupakaranant et al., 2010; Qu et al., 2010). New compounds found for the first time in *P. granatum* were also reported (Bagri et al., 2009a; Bonzanini et al., 2009; Tantray et al., 2009; Bagri et al., 2010; Kho et al., 2010) as well as the physical properties of pomegranate (Celik and Ercisli, 2009).

In technological terms, the development of new machines for the automatic sorting of pomegranate arils were also developed and reported by some authors (Blasco et al., 2009) as well as new drinks combining lemon and pomegranate juices (González-Molina et al., 2009) or the powder of pomegranate as a natural textile dyestuff (Adeel et al., 2009).

The appearance and quality of fresh fruits is a primary criterion in making purchasing decisions. Environment, agriculture conditions and postharvest treatments are fundamental for the acceptance of fruit by the consumers and several works reporting this theme could be found. Pomegranate is drought tolerant, winter hardy and can thrive well under desert conditions, frequently affected by high salinity, being proline considered as a possible drought stress indicator in pomegranate fruits (Halilova

and Yildiz, 2009).

Studies regarding the selection of the most salinity-resistant cultivar from Iran (Okhovatian-Ardakani et al., 2010) or the effect of salt stress on the responses of evapotranspiration, crop coefficient and growth of 2 varieties of pomegranate (*P. granatum* L. vars. 'Wonderful' and 'SP-2) irrigated with saline water were reported (Bhantana and Lazarovitch, 2010). Fertilization, including the use of biofertilizers, being one of the alternatives the use of nitrogen-fixing bacteria and arbuscular mycorrhizal fungi (Khanizadeh et al., 1995; Ghazi, 2006) have showed to contribute to the enhance of the growth and production of fruit plants significantly (Khorsandi and Yasdi, 2009; Khorsandi et al., 2009; Ramezanian et al., 2009).

Other studies performed by Schwartz et al. (2009a) also demonstrated the importance of environmental conditions on the colour, taste and antioxidant capacity of 11 pomegranate accessions' fruits grown in Mediterranean and desert climates in Israel. The application of sunscreen is not novelty since Melgarejo et al. (2004) have already shown that the use of kaolin sunscreen treatments had reduced sunburn damage. Nevertheless, recent studies continue to develop new products for application as sunscreen of pomegranate fruits (Weerakkody et al., 2010).

The effect of different scion-rootstock combinations on the vigour, tree size, yield and fruit quality of some Iranian commercial cultivars of pomegranate (Vazifeshenas et al., 2009), as well as the titratable acidity and the red colour parameters of this fruit were studied by some authors (Dafni-Yalin et al., 2010) for enabling breeders to select and breed genotypes having higher colour and desirable taste, and therefore helping industries to produce better pomegranate juices based on consume demand.

Being pomegranate for fresh consumption of arils or juice, the appearance and colour of the skin of the fruit or juice and freshness are very crucial. Therefore, preservation and shelf-life extension have been the major goal of many works over time. Spermidine and calcium chloride (Ramezanian et al., 2010); polyamide plastic (Sadeghi and Akbarpour, 2009); individual film wrapping of pomegranate in combination with fludioxonil (D'Aquino et al., 2010) were all valuable treatments for improving storability of pomegranate fruits.

Biotechnology has been the tool for characterize pomegranate cultivars. There are many molecular marker systems available to characterize genetic resources and cultivars (Staub et al., 1996).

Randomly Amplified Polymorphic DNA (RAPD) markers and fruit traits were used by some authors (Zamani et al., 2010) for determining the molecular and pomological diversity among the popular pomegranate cultivar from the Eastern Mediterranean region of Turkey, respectively.

On the other hand, Melgarejo et al. (2009) evaluated a genetic method to identify pomegranate cultivars. The procedure was based on the Restriction Fragment Length Polymorphisms (RFLP) and Polymerase Chain Reaction (PCR) techniques in 10 pomegranates accessions from the varietal groups 'Mollar de Elche', 'Mollar de Albatera', 'Mollar de Orihuela', 'Valencianas' and 'Bordes'.

The studies performed by Narzary et al. (2009a) revealed that directed amplification of minisatellite DNA (DAMD) and RAPD profiles of wild pomegranates were able to characterize rapidly genotypes as well as DAMD revealed more polymorphism in comparison to RAPD.

Inter Simple Sequence Repeat Profiles (ISSR) was used by Narzari et al. (2009b) in order to investigate the genetic diversity of natural pomegranate populations in India.

Microsatellite-AFLP (M-AFLP) techniques were used by Curro et al. (2010) and Pirseyedi et al. (2010) in *P. granatum*. According to these authors, such markers reported for the first time should allow studies of the population structure and genetic diversity of pomegranate to be performed in the future.

Other aspect in biotechnology on pomegranate is the regeneration of this plant by *in vitro* techniques. Kanwar et al. (2010) concluded that cotyledons excised from *in vitro* germinated seedlings were the most responsive explants for callus induction and plant regeneration.

BIOLOGICAL PROPERTIES AND THERAPEUTIC APPLICATIONS

A large number of articles concerning antimicrobial, antioxidant, anti-inflammatory, anticancer and immune-suppressive activities of pomegranate in the period of 2009 to 2010 (October) were found. Protective effects on hepatic function or on the glucose and lipid metabolism were also reported among other biological properties summarised below.

Antimicrobial activity

In the review article made by Julie (2008), the capacity of preventing infections of extracts of pomegranate was already well documented. Nevertheless, studies concerning this issue have continued. Food-borne illnesses are still a major concern for consumers, the food industry and food safety authorities. Therefore, works searching natural antimicrobials against these pathogens is a great goal of the scientists. Diverse extracts of pomegranate fruit peels, mainly 80% methanolic extract of peel revealed to be a potent inhibitor of *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Yersinia enterocolitica* using both *in vitro* (agar diffusion) and *in situ* (food) methods. The authors attributed such activity to the presence of phenols since the activity of the samples was related to its higher

content in peels (Al-Zoreky, 2009). The combination of pomegranate juice and pomegranate polyphenols was also effective against food-borne viral infectivity. In the absence of culturable human noroviruses, feline calicivirus (FCV/F9), murine norovirus (MNV/1), and MS2 (ssRNA) bacteriophage were used as foodborne viral surrogates. That combination was capable of causing reduction of foodborne viral surrogates FCV-F9, MNV-1 and MS2 (especially on low titers). The authors concluded that these samples appear to be promising natural remedies for preventing or reducing human norovirus infections (Su et al., 2010).

In addition, pomegranate purified polyphenol extract inhibited influenza virus having also a synergistic effect with oseltamivir. Influenza virus causes epidemics and pandemics in human population. Such virus has several zoonite hosts, therefore cannot be eradicated from human populations. Influenza continues to be a major cause of mortality and morbidity, although the vaccines and antiviral therapies (Haidari et al., 2009). These authors concluded that punicalagin present in the pomegranate extract had virucidal capability and inhibited influenza virus RNA proliferation independent on the virucidal effect. In addition, the authors also reported the synergistic effect of purified polyphenol extract with oseltamivir. Such combination and according to the authors has advantages because can contribute to a potentially greater potency, better clinical efficacy and dose-sparing, less toxicity and side effects, and greater cost-effectiveness.

Paracoccidioidomycosis is the most prevalent systemic mycosis in Latin America (Coutinho et al., 2002). In the absence of drug therapy, the disease is usually fatal. The treatment of this mycosis is long (1-2 years) or even more. Johann et al. (2010) studying the activity of extracts of some plants used in Brazilian traditional medicine against the pathogenic fungus that causes this mycosis, *Paracoccidioides brasiliensis*, reported that the hexane fraction from stems of *P. granatum* exhibited better antifungal activity against the three clinical isolated than other parts of the plant or other fractions of the same plant. Nevertheless other extracts of plants revealed to be significantly more effective against that fungus than *P. granatum*.

Candida species are harmless saprophyte yeasts, a normal component of the human biota in the gastrointestinal tract and oral and vaginal mucosa. They can cause superficial infections such as thrush and vaginitis. Nevertheless, whether the immune defences of the host become compromised, they can cause severe systemic infections (Endo et al., 2010). These authors reported that punicalagin isolated from pomegranate peels possessed strong activity against *Candida albicans* and *Candida parapsilosis* as well as the combination of punicalagin and fluconazole showed a synergistic interaction. By transmission electron microscopy, treated cells with punicalagin showed a thickened cell wall, changes in the space between cell wall and the plasma membrane, vacuoles, and a reduction in cytoplasmic content. Denture stomatitis is commonly associated with *C. albicans* although other *Candida* species have been isolated from lesions. Ethnobotanical studies performed in Brazil had demonstrated the utilization of pomegranate in oral health (Santos et al., 2009).

Plasmodium falciparum and *Plasmodium vivax* are endemic in the eastern province of Orissa (India), in which malaria constitutes a major health problem for the population. Sun-dried rind of the immature fruit of *P. granatum* is presently used as a herbal formulation with the name of OMARIA in that province of India, for the therapy and prophylaxis of malaria. Dell'Agli et al. (2009) studied the *in vitro* antiplasmodial activityof methanolic extracts of a tannin-enriched fraction and of compounds/metabolites of the antimalarial plant, to estimate the curative efficacy of such extracts and to explore the mechanisms of action of the antiplasmodial compounds. They conclude that methanolic extracts of pomegranate inhibited parasite growth *in vitro* for D10 and W2 strains. They also reported that punicalagins, punicalins, ellagic acid and its glucosydes were present in such extracts, nevertheless the *in vivo* studies showed that the extracts did not present any *in vivo* efficacy in the murine model. The authors suggest that these negative results of pomegranate extracts *in vivo* might be attributed to the low bioavailability as well as the kinetic of conversion of ellagic acid to inactive metabolites urolithins.

The consumption of pomegranate products leads to a significant accumulation of ellagitannins in the large intestines, where they interact with complex gut microflora (Bialonska et al., 2009a). According to the studies performed by these authors the commercial extract of pomegranate byproduct provided by POM Wonderful (Los Angeles, CA) and punicalagins inhibited the growth of pathogenic clostridia and *Staphylococcus aureus*. Nevertheless the probiotic lactobacilli and bifidobacteria were not affected by ellagitannins. The growth inhibition toward pathogenic bacteria and according to the same authors could be attributed to the lower media pH due to the presence of punicalagins. However, this study was only performed *in vitro* and the authors also refer the importance of phenols metabolism that occurs in gut. As reported in the last paragraph, punicalagins and ellagic acid are metabolized to urolithins by colonic bacteria and the activity may be changed, therefore they suggest the need for performing additional studies using human fecal microbiota.

Tayel and El-Tras (2009) demonstrated that methanol, ethanol and water extracts of pomegranate peels were effective against *C. albicans* growth. In addition, they also proved that pomegranate peel extract aerosol was an efficient method for complete sanitizing of semi-closed places against *C. albicans* growth, and thereby could contribute for preventing *C. albicans* contamination and

growth in suspected places.

Two separated works from different authors demonstrated the importance of physico-chemical properties of pomegranate on the antimicrobial activity. In one of them, the authors related such antimicrobial activity to the pomegranate antioxidant activity since the extent of the inhibitory effects of the pomegranate extracts are almost always attributed to their phenolic and anthocyanin content of fruits (Duman et al., 2009). These authors found that the bioactivity of aril extracts on the microorganisms tested (*Bacillus megaterium*, *Pseudomonas aeruginosa*, *S. aureus*, *Corynebacterium xerosis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Kluvyeromyces marxianus*, *Rhodotorula rubra* and *C. albicans*) had high total flavonols, phenolics, anthocyanins and organic acids. Opara et al. (2009) reported that the best activity against *S. aureus* and *P. aeruginosa* were found in fruit peel fractions, particularly from Oman, which was coincident with the highest levels of vitamin C detected in these samples. The remaining samples were imported. Factors that enhanced the amounts of this vitamin in fruit peels also contributed to the best antimicrobial activity, and according to the authors, sun drying fruit peels induced retention of vitamin C and therefore the antimicrobial activity detected in the assays.

Recently, there has been an increasing tendency towards the prevention of microbial attack on textiles (Han and Yang, 2005). Çaliş et al. (2009) studied the antimicrobial effect of four commercially available dye powders obtained from *Rubia tinctorum*, *Allium cepa*, *Mentha* sp. and *P. granatum* on *S. aureus* ATCC 25923, *Shigella sonnei* RSKK 877, *E. coli* ATCC 35218, *B. megaterium* RSKK 5117, *B. subtilis* RSKK 244, *B. cereus* RSKK 863, *P. aeruginosa* ATCC 29212, *Streptococcus epidermidis*, *Salmonella* 21.3 and *P. aeruginosa* 27853. The natural dyes with antimicrobial activities found in solution were then assayed in textile substrate (wool fabric). Maximum inhibition rate obtained against *Bacillus subtilis* of wool samples dyed with *P. granatum* was 80%. These results may suggest that these natural dyes possess antimicrobial activity either in solution or in substrate.

Antioxidant activity

The antioxidant activity was almost determined *in vitro* conditions and several methods could be used for its determination. Some works focus on the comparative studies of antioxidant activities of diverse fruits and vegetables (Stangeland et al., 2009). Generally, pomegranate possessed the best antioxidant activity, independent on the antioxidant test assayed and generally with significant linear correlation between phenolics concentration and antioxidant capacity (Elfalleh et al., 2009). These authors demonstrated correlation coefficients of total phenols and total flavonoids to the antioxidant activity measured through the ABTS and DPPH methods either in peel or juice.

Pomegranate could be expected be suitable for food processing in which thermal devices are used, because of their heat resistance. Devatkal et al. (2010) using pomegranate rind powders in cooked goat meat patties in a hot air oven until the internal temperature reached 80°C (approximately 15 min at 170°C) and then packaged in low density polyethylene bags and stored at 4°C, for 12 days obtained good results. With such results, these authors consider that this by-product from fruit processing may be an economic source of natural antioxidants replacing easily the synthetic antioxidants. Without cooking, pomegranate rind power revealed also to be also a good antioxidant on the preservation of raw ground goat meat during refrigerated storage (4°C), aerobically packaged in low density polyethylene bags for 6 days, even in the presence of salt which generally promotes lipid oxidation (Devatkal and Naveena, 2010).

The effect of cultivars from Iran, Georgia, Turkey and Israeli on antioxidant activity also target of study of some authors (Borochov-Neori et al., 2009; Mousavinejad et al., 2009; Pande and Akoh, 2009). All authors reported considerable variation in some of the chemical composition profile (lipids, phenols, organic acids, vitamins, sugars) and antioxidant properties of pomegranate samples, independent on the antioxidant method performed. Borochov-Neori et al. (2009) also described the importance of harvesting season on the phenolic content as well as on the antioxidant activity. Arils of fruit ripening later in the season contained more soluble phenolics and exhibited a higher antioxidant activity measured by the ferric reducing ability (FRAP) assay. Other authors also reported the diversity of antioxidant potency of various cultivars of pomegranate form Iran but at the same time they also demonstrated the importance of the solvent used for extracting bioactive compounds on the degree of antioxidant activity (Sadeghi et al., 2009). Several works have demonstrated that peel, seeds, juice of pomegranate have antioxidant activity, nevertheless, after ingestion those compounds responsible for such activity, mainly tannin components, such as punicalagins and ellagic acid, are metabolized by gut bacteria into urolithins, which readily enter systemic circulation. Bialonska et al. (2009b) studied the antioxidant activities of seven urolithins derivatives in a cell-based assay. They have chosen this method because it reflects bioavailability of the test compound to the cells, and the antioxidant activity is evaluated in the cellular environment and in terms of inhibition of intracellular generation of reactive oxygen species. They found that urolithins exhibited a significant antioxidant activity correlated with the number of hydroxyl groups as well as lipophilicity of the molecules.

Glucose and lipid metabolism

According to the review article made by Li et al. (2008) *P.*

granatum flowers was already prescribed in Unani and Ayurvedic medicines for the treatment of diabetes. The effect protective of pomegranate flowers' extracts was investigated by some authors on serum lipid profile, pancreatic lipid peroxidation and activities of both enzymatic and non-enzymatic antioxidant status in streptozotocin-induced diabetic rats (Bagri et al., 2009b). These authors reported that in these rats, there was an increase in blood glucose level, total cholesterol, triglycerides, low-density lipoproteins cholesterol, very low density lipoproteins, lipid peroxidation level with decrease in high density lipoprotein cholesterol, reduced glutathione content and antioxidant enzymes namely, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, superoxide dismutase and catalase. The administration of aqueous pomegranate flowers' extracts reversed these parameters, suggesting the authors that pomegranate could be used as dietary supplement in the treatment and prevention of chronic diseases characterised by atherogenic lipoprotein profile, aggravated antioxidant status and impaired glucose metabolism (Bagri et al., 2009b).

Based on the fact of pomegranate leaf extracts, containing abundant ellagitannins had a potent white fat depot-lowering action in obese mice induced by a high-fat diet, and also decreased the cellular levels of triglycerides by a single orally administered lipid emulsion as well as a noticeable activity of modulating lipid metabolite of high-lipid rat and inhibiting HMG2CoA redutase *in vitro*, Lan et al. (2009) investigated if pomegranate leaf tannins could modulate the lipid metabolism and act on liver cells. For this purpose, the authors investigated the transport behaviour of ellagic acid through HepG2 cell (a kind of liver cellular line) in culture with pomegranate leaf tannins, in order to evaluate if the possible target of ellagic acid is in cells. At the same time, the total cholesterol in the cells was tested to find out whether the transportation of ellagic acid has an influence on total cholesterol. Their results demonstrated for the first time that ellagic acid in pomegranate leaf tannins could be transported into the cells, which was in correlation with total cholesterol alteration in the cells (Lan et al., 2009).

Anti-cancer

Singh and Singh (2009) effectuated an ethnobotanical study of medicinal plants in Chandauli District, one of the less studied regions of India, using semi-structured interviews, field observations, preference and direct matrix ranking with traditional medicine practitioners. They were able to document 40 medicinal plants belonging to 27 families. *P. granatum* was found to be an ingredient of a powder along with whole plant of *Vernonia cinerea* Less. (AS38) and leaves of *Crataeva nurvala* that after heated with castor and coconut oils was used in the external treatment of breast cancer.

In addition to that ethnobotanical study in India, pomegranate has been target of studies in laboratories and cancer centres that have showed its properties against diverse types of cancers. Two recent review articles reported the laboratory and clinical evidence of cancer chemoprevention or treatment of pomegranate (Adhami et al., 2009: Amin et al., 2009). Amin et al. (2009) reported that pomegranate fruit, pomegranate juice, pomegranate seed and seed oil act in prostate, breast, skin, colon, lung, oral and leukaemia cancers, through antioxidant, antiproliferation (growth inhibition, cell cycle disruption and apoptosis), antiangiogenisis and anti-inflammatory mechanisms of action. Ellagic acid, one of the constituents of pomegranate juice and seed oils are reported as acting against cancer of skin, pancreas, breast, prostate, colon, intestine, oesophagus, bladder, oral, leukaemia, liver and neuroblastoma, which mechanisms of action are similar to those described for pomegranate. Ellagic acid acts synergistically with cisplatin, vinorelbine, quercetin, resveratrol, cyclosporine A, 6-gingerol and selenomethionine. The same authors gave information about ongoing clinical trials with natural compounds, including pomegranate, in several parts of the world. Either for prostate therapy or prevention, the several sites reported (M.D. Anderson Cancer Center; University of California, Los Angeles; Radiant Research; Roll International Corporation; Jonsson Comprehensive Cancer Center; University of California, Irvine; and University of Oslo) are in the great majority in the status of recruiting and/or phases I-III trials (Amin et al., 2009). The other review article Adhami et al. (2009) focused on the effects of pomegranate fruit on diverse types of cancer (breast, prostate, lung, colon, and skin) as well as the mechanisms involved. They also reported that extracts of pomegranate or the juice are generally more active than individual or purified compounds. They suggest the existence of a synergistically effect of all compounds present in extracts, explaining therefore the inhibition of multiple targets observed in many studies.

After ingestion, polyphenols present in pomegranate are metabolised. Ellagitannins are transformed by human colon flora into urolithins. In a mouse model, these metabolites preferentially accumulate in prostate, colon, and intestinal tissues. Compounds inhibiting the cytochrome P450 enzyme, CYP1B1, activity seem to exert beneficial effects at three stages of prostate cancer development. In a recombinant CYP1B1-mediated ethoxyresorufin-O-deethylase (EROD) assay, pomegranate ellagitannins/microbial metabolites were examined for their CYP1B1 inhibitory activity. Urolithins, punicalins and punicalagins were also tested for their 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced CYP1B1 inhibitory activity in the 22Rv1 prostate cancer cell line. Urolithins were also studied for their cellular uptake and inhibition of TCDD-induced CYP1B1 expression (Kasimetty et al., 2009). After these studies,

the authors concluded that are effective inhibitors of CYP1B1 enzyme activity and expression.

Concerning breast cancer, Sturgeon and Ronnenberg (2010) described the *in vitro* cell culture studies, animal studies and available data about the property of pomegranate to prevent breast cancer as well as the possible mechanisms involved. Epidemiological studies have demonstrated that elevated serum levels of the estrogens estrone and estradiol and lower levels of sex-hormone binding globulin, after menopause substantially increased the risk of breast cancer. After menopause, most circulating estrogen is derived from the conversion of adrenal androgens to estrone by aromatase in adipose tissue. Some of estrone is further converted to estradiol, which is the most biologically active estrogen in breast tissue. The authors reported that fermented pomegranate juice had higher aromatase inhibitory activity than unfermented polyphenols extracted from pomegranate peels, maybe due to breakage of flavonoid-sugar complexes during fermentation. Cyclooxigenase inhibition by the constituents of the pomegranate fruit, seed oils or pure compounds (ellagic acids and anthocyanins) induce the decrease of prostaglandin E2 (PGE2) that has been shown to downregulate aromatase expression. Ellagic acid seems to exhibit apoptosis, inhibits activation of inflammatory pathways, and inhibits angiogenesis. In addition to aromatase that converts adrenal androgens to estrone, there is 17β-hydroxysteroid dehydrogenase that converts estrone to estradiol. several fractions of pomegranate juice was demonstrated to inhibit this enzyme or have antiestrogenic activity. These assays being performed in animal models need to be confirmed in humans (Sturgeon and Ronnenberg, 2010).

Some authors found that punicic acid inhibited the proliferation of estrogen insensitive breast cancer cell line (MDA-MB-231) and an estrogen sensitive cell line developed from the MDA-MB-231 cells (MDA-ERα7), as well as induced apoptosis in both type of cells. Such inhibition of proliferation was due to lipid peroxidation of cells and activation of protein kinase C (PKC) (Grossmann et al., 2010). Tran et al. (2010) revealed that punicic acid and α-eleostearic acid present in seed oil of pomegranate inhibited the estrogen receptors ERα and ERβ depending on the dose. For example, lower doses of punicic acid acted as agonist for both receptors and antagonist at higher concentrations. α-Eleostearic acid acted as antagonist only against ERα and ERβ at higher concentrations of those for punicic acid. Both acids were effective in producing effective inhibition of cancer cell proliferation: MCF-7 (ER-positive human breast cancer cells) and MDA-MB-231 (ER-negative human breast cancer cells). The same work also demonstrated that those two acids promote ER mRNA expression in a manner analogous to that produced by estradiol for both ERα and ERβ only in MCF-cells. Both acids are Selective Estrogen Receptor modulator (SERM) (Tran et al., 2010). In a short communication, Khan (2009) compiled the studies about the role of pomegranate in colon cancer and according to their compilation, pomegranate fruit was described as being able to reduce the number of aberrant cyptic foci (ACF) of the colon in male F-344 rats before and after treatment with colon-specific chemical carcinogen, azoxymethane; and to increase the hepatic glutathione S transferase (GST) activity in rats. Pomegranate seed oil was also capable to reduce the incidence of colonic cancer in rats. Punicalagin, ellagic acid and total pomegranate tannin inhibited the proliferation of colon cancer cell (SW480, HT29, HCT116, SW620). Mechanistically, punicalagin and ellagic acid induce apoptosis of colon cancer cells, inducing caspase 9 and procaspase 3, member of caspase family of proteases. The author also referred that pomegranate juice, punicalagin and total pomegranate tannin suppressed tumour necrosis factor-alpha (TNFα) and COX-2 in animal and *in vitro* models. Pomegranate juice also inhibited phosphorilation of nuclear factor-kB (NF-kB). DNA binding of this transcription factor was suppressed only by punicalagin (Khan, 2009).

A set of cellular signals critical for the development and homeostasis of multicellular animals are those elicited by Wnt proteins. In the canonical Wnt pathway, the signal produced by the binding of Wnt ligands to cell surface receptors is transmitted through a cytoplasmic protein called disheveled (Dvl) to inhibit the activity of a complex of cellular proteins that phosphorylate another protein, β-catenin, and target it for destruction. Therefore, Dvl-mediated inhibition of the β-catenin destruction complex results in increased levels of cellular β-catenin and translocation of β-catenin into the nucleus. In this place, β-catenin activates transcription factors of the LEF/TCF families and initiates transcription of a spectrum of target genes that affect tissue proliferation, differentiation, and tumorigenesis. In colon cancer, a large percentage of the tumour arises from activating mutations in the Wnt pathway (Sharma et al., 2010). Sharma et al. (2010) studied the effects of urolitinins, ellagic acid and ellagitannin-rich fruit extracts on Wnt signalling in a human 293T cell line using a luciferase reporter of canonical Wnt pathway-mediated transcriptional activation. After this study, they concluded that urolithins produced in the colon from ellagitannins present in pomegranate are inhibitors of the canonical Wnt signalling pathway at physiologically relevant concentrations. Thus and according to the authors, the colon is not a excretory organ, but also an active place for the production of physiologically relevant metabolites through microbiota transformation of dietary components.

Anti-inflammatory activity

The NO production induced by lipopolyssacharide (LPS) in RAW 264.7 macrophage cells was inhibited by pomegranate aqueous extracts. The extracts used and in

the concentrations tested did not present any cytotoxicity (Kumar-Roiné et al., 2009). Lee et al. (2010) also demonstrated the capacity of pomegranate extracts for inhibiting NO production by RAW 264.7 macrophage cells. But in addition, they also find that pomegranate (100 mg/Kg) significantly decreased carrageenan-induced mice paw oedema for several hours (5 h at the maximum). At the same time, the authors using a column chromatography combined with *in vitro* bioassay-guided fractionation found that punicalagin, punicalin, strictinin A, and granatin B were able to inhibit NO production as well as iNOS expression in RAW 264.7 cells. Granatin B was that showed the strongest iNOS and COX-2 inhibitory effects, and exhibited these effects in the inhibition of paw swelling and PGE2 level in carrageenan-induced mice. According to these results, the authors proposed that granatin B could be used as a standard marker for the anti-inflammatory effect of pomegranate (Lee et al., 2010).

Mast cells derived mediators induce oedema, destroy connective tissue, and are involved in lymphocyte chemotaxis and infiltration and in pathological fibrosis in rheumatoid arthritis joints. Activation of the myeloid precursor cell line KU8I2 results in the degranulation accompanied by the production of chemical mediators such as histamine, proteases, metabolites of arachidonic acid and several inflammatory and chemotactic cytokines including IL-6 and IL-8. Such cells are suitable models for studying the activation of degranulation of human mast cells (Rasheed et al., 2009). These authors using KU8I2 cells stimulated with phorbol-12-myristate-13-acetate (PMA) plus calcium ionophore A23I87 (PMACl) studied the inhibitory effect of polyphenol-rich pomegranate fruit extract on pro-inflammatory cytokine gene expression and production by quantitative real time-PCR, and cytokine-specific ELISA assays. The effect of polyphenol-rich pomegranate fruit extract on the activation of mitogen-activated protein kinases (MAPKs) and the NF-kB in PMACl stimulated KU8I2 cells. They found that polyphenol-rich pomegranate fruit extract decreased PMACl stimulated inflammatory gene expression and production of IL-6 and IL-8 in KU8I2 cells. The inhibitory effect of the extract on the pro-inflammatory cytokines was MAPK subgroups c-jun N-terminal kinase (JNK- and extracellular-regulated kinase (ERK) dependent. The extract also inhibited NF-kB by inhibiting IkB-degradation in human basophil cells. The results obtained by the authors suggest that polyphenol-rich pomegranate fruit extract exerts its inhibitory effect on IL-6 and IL-8 expression via modulation of the activation and DNA binding activity of NF-kB (Rasheed et al., 2009).

Orissa Malaria Research Indigenous Attempt (OMARIA) in Orissa, India, used herbal formulation containing the sun-dried rind of the immature fruit of pomegranate for the therapy and prophylaxis of malaria. A complication of the infection by *P. falciparum* is an inflammatory cytokine-driven disease associated to an up-regulation and activity of metalloproteinase-9 (MMP-9) and to the increase of TNF production. Dell'Agli et al. (2010) used a fraction enriched in tannins obtained from the methanolic extract of the fruit rind as well as ellagic acid, punicalagin and urolithin metabolites for evaluating its capacity to inhibit the secretion of MMP-9 induced by *P. falciparum* haemozin or TNF in human THP-1 monocytic leukaemia cells. Extract, pure compounds and urolithins inhibited the secretion of MMP-9 induced by haemozin or TNF at transcriptional level due to the lower levels of MMP-9 mRNA. The fraction enriched in tannins and pure compounds also inhibited MMP-9 promoter activity and NF-kB driven transcription. In addition to the anti-malarial activity of fruit rind of pomegranate, it can also act in the inhibition of the pro-inflammatory mechanisms involved in the onset of cerebral malaria (Dell'Agli et al., 2010).

Other biological activities

In addition to the biological activities of pomegranate reported so far in the present work, others were also found during our database research. They include for example, the reversible inhibition of human plasma thrombin at physiological pH values by the pomegranate extracts (endocarp and mesocarp) which were mainly constituted by catechin along with gallic acid, epicatechin and ellagic acid (Cuccioloni et al., 2009). The hyperbolic nature of the inhibition curves also revealed that the polyphenol-thrombin complex behaves as a non-cooperative reversible system. The authors also suggested a moderate-affinity binding site on the enzyme for the pomegranate polyphenols (Cuccioloni et al., 2009).

The effect of pomegranate juice and the polyphenol-rich extracts from pomegranate fruit on platelet aggregation, calcium mobilisation production of thromboxan A2 induced by collagen and arachidonic acid revealed that both types of samples were able to reduce all platelet responses studied; polyphenol-rich extracts from pomegranate fruits showed a strong action in reducing platelet activation, being active at concentrations similar to those after ingested (Mattiello et al., 2009). The results obtained by these authors demonstrated that the cardiovascular health benefits of pomegranate may in part be related to the ability of polyphenols to inhibit platelet function. Other study using isoproterenol that induces cardiac necrosis in rats demonstrated for the first time that a pre-supplementation with pomegranate juice for 30 consecutive days before administration of isoproterenol on days 29 and 30th showed lesser increase in heart weight, infarct size, plasma marker enzymes, lipid peroxidation, Ca^{2+} ATPase and a significant protective effect in endogenous enzymatic and non-enzymatic antioxidants when compared to those animals only treated with

isoproterenol (Jadeja et al., 2010).

Afaq et al. (2009), using pre-treatment of human reconstituted skin (EpiDerm™ FT-200) with pomegranate-derived products found an inhibition of UVB-induced CPD and 8-OHdG as well as protein oxidation and PCNA protein expression. They also reported an inhibition of UVB-induced MMPs (collagenase, gelatinase, stromelysin, marilysin, elastase and tropoelastin) in pretreated of epiderm with pomegranate-derived products. They also described a decrease of in UVB-induced protein expression of c-Fos and phosphorylation of c-Jun. Thus, the authors considered that all pomegranate-derived products (juice, extract and oil) may be useful against damages in skin induced by the exposition to UVB (Afaq et al., 2009).

Some authors demonstrated that pomegranate juice prevented the negative effects of iodoacetate (used for inducing osteoarthritis) in mice. At the highest concentration tested (20 ml/Kg of juice), chondrocyte organization was preserved by approximately 50%, and the number of cells was locally increased with less damage to proteoglycan in the epiphyseal plate over a period of 2 weeks. No inflammation cells or cell proliferation was in the synovial fluid were observed in all groups of treated-mice (Hadipour-Jahromy and Mozaffari-Kermani, 2010).

Ulcerative colite induced in mice by dextran sulphate sodium ameliorated after treatment with flower pomegranate extract and its ellagic acid rich fraction. The colonic inflammation was significantly attenuated as well as histamine, myeloperoxidase and oxidative stress (Singh et al., 2009).

Pomegranate and more 5 plants are used in Algerian Sahara traditional pharmacopoeia for inhibiting the calcium crystallization and therefore to prevent urolithiasis, being calcium oxalate one of the main constituents of urinary stones. Sekkoum et al. (2010) studied the effect of the extracts of plants, including from pomegranate, on the kinetics of oxalate crystallization made *in vitro*. The aqueous extract of pomegranate under the concentration of 1 mg/ml, exerted an inhibitory effect on crystalline aggregation but does not influenced the crystalline growth. Abbasi et al. (2009) reported 30 plants species used in remote zones of Pakistan for the treatment of jaundice and hepatitis, after compiling 95 responses to questionnaires. In the same work, the authors reported the chemical constituents of such plants compiled from relevant sources. Fruits and seeds of pomegranate were reported in this work as used for the treatment of jaundice and hepatitis. Dried fruits and seeds must be grounded and taken together with sugar thrice a day for three weeks after dissolution of three teaspoons in one cup of water. Other way to administered this remedy reported by the authors consisted in two teaspoons of dried rind powder mixed with sugar and taken orally along with water at morning for a week.

The hepatopreventive capacity of pomegranate beverage (flowers) was evaluated by Celik et al. (2009) in rats exposed to trichloroacetic acid (TCA). This ability was evaluated by measuring level of serum enzymes, antioxidant defence systems and lipid peroxidation content in several organs of rats. The rats submitted to TCA and pomegranate samples showed lower levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), LDH, creatine kinase (CK), malonaldehyde content in liver, brain, kidney and hearth tissues. An increase of the activity of GST in liver, brain and spleen was observed. Pomegranate as immunosuppressive agent was also reported by Amirghofran (2010) in traditional Iranian medicine.

CONCLUSION

The pomegranate has been an inexhaustible source of research going from chemistry in the search for new compounds, techniques of production and conservation, biotechnology in search of more profitable varieties, to the biological effects as anti-microbial antioxidant, anti-inflammatory, anticancer, anti-diabetic, among other applications. However, many biological studies that are described are still performed *in vitro* or in animals whose metabolic pathways are not exactly the same that those occurring in humans. However, clinical trials are in progress exploring the beneficial effects of pomegranate extracts. Several fields of application of pomegranate along with other species have been patented in these last two years, showing therefore the importance of such plant: skin care (Gross and Gross, 2009); for preventing skin dryness and skin aging (Cho et al., 2010); for preventing and/or inhibiting the effect of psychoemotional stress on the hair (Briese et al., 2010); for improving the appearance of the skin (Jacobs, 2010); for improving and retaining the moisture level in the skin (Vilinsky, 2010); for waist and tummy firming (Khan and Zaidi, 2009); for treating alopecia through external application (Jo et al., 2009); to prevent dental diseases e.g. plaque (Babu et al., 2009); for treating sickle cell disease (Desai and Desai, 2009; Desai, 2010); among other patents not referred in the present work.

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