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Pomegranate: Constituents, Bioactivities and Pharmacokinetics

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ABSTRACT

Pomegranate (*Punica granatum* L.), a species of Punicaceae, has recently become of great interest to the scientists who engage themselves in pharmaceutical, nutriological and pharmacological research, and new drug development, due to its distinctive multiple officinal parts and multiple bioactivities such as hypolipidemic, antioxidant, antiviral, anti-neoplastic, antibacterial, anti-diabetic, anti-diarrheal, and helminthic effects. In the present review, we reported the research on pomegranate as to chemical constituents, bioactivities and pharmacokinetics in combination with the recent publications. The constituents of this plant were thoroughly reviewed, and the biosynthetic pathways of major compounds were discussed based on our research results and those reported in the literatures. The bioactivities categorized by vascular protection, digestive protection, anti-pathogenic microbes, anticancer, anti-diabetes, immuno-modulation, and others were summarized based on our experimental and the reported data. This article also included the review of pharmacokinetic experiments conducted in our laboratory and toxic investigation reported up to date which may be in favor of the research and development of new drugs.

Keywords: constituents, bioactivity, pharmacokinetics, *Punica granatum*

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INTRODUCTION

Pomegranate (*Punica granatum* L.) has been used in various regions and folk or traditional medical systems as a food supplement or a medicine because of its enormous compounds with lots of activities and without toxicity. In addition to edible use, almost all parts of this plant are used pharmaceutically worldwide, for example, the pericarp is used by Chinese for the treatment of diarrhea, metrorrhagia, metrostaxis and bellyache; the flower is used as a food supplement to treat diabetes mellitus in Unani medicine; the bark and root are believed to have anthelmintic and vermifugic properties in Ayurvedic medicine; the fruit is employed by South Africa people for the treatment of diarrhea (*Zhonghua Bencao*, Vol. 15, 1998; Ahmad and Beg 2001; Mathabe *et al*. 2006; Lansky *et al.* 2007; Al-Zoreky 2009). Many researchers have focused their interests and investigation on this wonderful shrub for the purpose of finding miraculous compounds for human health. In order to facilitate the further investigation and exploit of the said plant, we summarized herein the research achievements of our laboratory and the advances in chemistry, bioactivity and biosynthesis of partial constituents.

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Invited Review

CONSTITUENTS

Many efforts have been made during the last several decades to investigate the constituents of pomegranate, leading to the isolation and structure characterization of many compounds which are categorized into polyphenols including tannins and flavonoids, alkaloids, organic acids, etc.

Tannins

Hydrolysable tannins of diverse structures including ellagitannins and gallotannins constitute the most prevalent compounds presenting in various parts of pomegranate (**Table 1**). Condensed tannins, however, are rarely found in this plant.

Ellagitannins are mainly found in the pericarp, bark, seeds and flowers (Tanaka *et al.* 1986; Wang *et al*. 2004, 2006a). For example, punicalin and punicalagin are the major constituents of pericarp and also exist in the bark; however, they are practically undetected in the leaves. This kind of compounds is the derivatives of ellagic acid which is abundant in every part of pomegranate and its content can be as high as 0.1% in the flowers and up to 0.2% in the pericarp and leaves. The biosynthesis of ellagitannins in pomegranate follows the common pathway of this kind of compounds. Ellagic acid, which is biosynthesized by hexahydroxy-diphenyldicarboxylic acid through lactonization between the carboxyl and hydroxyl groups, can be considered as the central compound (**Fig. 1**). The hydroxyl groups of ellagic acid are substituted to form various derivatives of ellagic acid, such as 3-*O*-methylellagic acid, 3,3′-di-*O*-methylellagic acid, 4,4′-di-*O*-methylellagic acid, 3,3′,4′-tri-*O*-methylellagic acid, and 3′-*O*-methyl-3,4-methylenedioxyellagic acid. Ellagic acid and its derivatives generate glycosides through glycosidation with saccharides. Several hexahydroxy-diphenyldicarboxylic acids can be polymerized into polymers through esterification between the carboxyl groups of one monomer and the hydroxyl groups of another one (Wang *et al*. 2006b).

Gallotannins, which are mostly found in the leaves and seldom reported in other parts of pomegranate, usually consist of a couple of galloyl groups and, therefore, can be con-

Table 1 Major tannins of pomegranate.

Compound name	Plant part*	Bioactivity	Reference
Brevifolin	L	Hypolipidemic; antioxidant; hepatoprotective	Nawwar *et al.* 1994; Wang *et al.* 2005
Brevifolin carboxylic acid	L		Nawwar *et al.* 1994a
Ethyl brevifolincarboxylate	F, L	Antiviral	Hussein *et al.* 1997
Brevifolin carboxylic acid monopotassium sulphate	L		Hussein *et al.* 1997
3,4,8,9,10-penta-hydroxydibenzo[*b*,*d*]pyran-6-one	L		Nawwar *et al.* 1994a
Casuarinin	P	Antiviral; antioxidant	Satomi *et al*. 1993
Corilagin	L, P	Anti-hypertensive; anti-neoplastic	Satomi *et al*. 1993; Nawwar *et al.* 1994b
Ellagic acid	F, J, L, P	Anti-neoplastic; skin-whitening	Amakura *et al*. 2000a; Wang *et al.* 2004
Gallagyldilacton	P	Anti-inflammatory; anti-neoplastic	Satomi *et al.* 1993
Gallic acid	F, J, L, P	Anti-inflammatory; anti-mutagenic; antioxidant; antiviral	Amakura *et al*. 2000a; Huang *et al.* 2005a
Methyl gallate	P		Kasimu *et al.* 2009
1,2,3-tri-*O*-galloyl-β-^{4}C1-glucose	L		Nawwar *et al.* 1994b
1,2,4-tri-*O*-galloyl-β-glucose	L		Hussein *et al.* 1997
1,3,4-tri-*O*-galloyl-β-glucose	L		Hussein *et al.* 1997
1,2,6-tri-*O*-galloyl-β-^{4}C1-glucose	L		Nawwar *et al.* 1994b
1,4,6-tri-*O*-galloyl-β-^{4}C1-glucose	L		Nawwar *et al.* 1994b
1,2,4,6-tetra-*O*-galloyl -β-D-glucose	L		Tanaka *et al.* 1985
1,2,3,4,6-peta-*O*-galloyl -β-D-glucose	L		Tanaka *et al.* 1985
3,6-(R)-hexahydroxydiphenoyl-(α/β)-^{1}C4-glucose	L		Nawwar *et al.* 1994b
1,4-di-*O*-galloyl-3,6-(R)-hexahydroxydiphenoyl-β-glucose	L		Hussein *et al.* 1997
1,2-di-*O*-galloyl-4,6-*O*-(S)-hexahydroxydiphenoyl β-D-glucopyranoside	F	Anti-neoplastic	Xie *et al.* 2008
2-*O*-galloylpunicalin	B,W		Tanaka *et al*. 1986
2,3-(S)-HHDP-D-glucose	B		Tanaka *et al*. 1986; Liu *et al*. 2007
6-*O*-galloyl-2,3-(S)-HHDP-D-glucose	B		Tanaka *et al*. 1986
Granatin A	P		Tanaka *et al*. 1990
Granatin B	P	Anti-inflammatory	Tanaka *et al*. 1990; Liu *et al*. 2007
3-*O*-methylellagic acid	W		El-Toumy *et al*. 2003
3,3′-di-*O*-methylellagic acid	S	Antioxidant	Wang *et al.* 2004
4,4′-di-*O*-methylellagic acid	W		El-Toumy *et al.* 2003
3,3′,4′-tri-*O*-methylellagic acid	S	Antioxidant	Wang *et al.* 2004
3′-*O*-methyl-3,4-methylenedioxyellagic acid	W		El-Toumy *et al.* 2003
Pedunculagin	P	Anti-neoplastic; antioxidant	Satomi *et al.* 1993
Pomegranatate	F		Wang *et al.* 2006a
Punicacortein A	B	Anti-HIV	Tanaka *et al*. 1986
Punicacortein B	B	Anti-HIV	Tanaka *et al.* 1986
Punicacortein C	B	Anti-HIV	Tanaka *et al.* 1986
Punicacortein D	B	Anti-HIV	Tanaka *et al.* 1986
Punicafolin	L	Anti-neoplastic	Nawwar *et al.* 1994b
Punicalagin	B, L, P, R	Antioxidant; anti-hypertensive	Gil *et al.* 2000; Anand *et al.* 2004; Liu *et al*. 2007
Punicalin	B, L, P, R	Antioxidant; anti-HIV	Tanaka *et al.* 1986; Gil *et al.* 2000; Liu *et al.* 2007
Punigluconin	B	Antioxidant	Tanaka *et al*. 1986
Tellimagrandin	P	Anti-neoplastic	Satomi *et al.* 1993
Diellagic acid rhamnosyl(1→4)glucopyranoside	W		Sayed *et al.* 2002
5-*O*-galloylpunicacortein D	W		Sayed *et al.* 2002

*B: bark; F: flowers; J: juice; L: leaves; P: pericarp; S: seeds; W: wood.

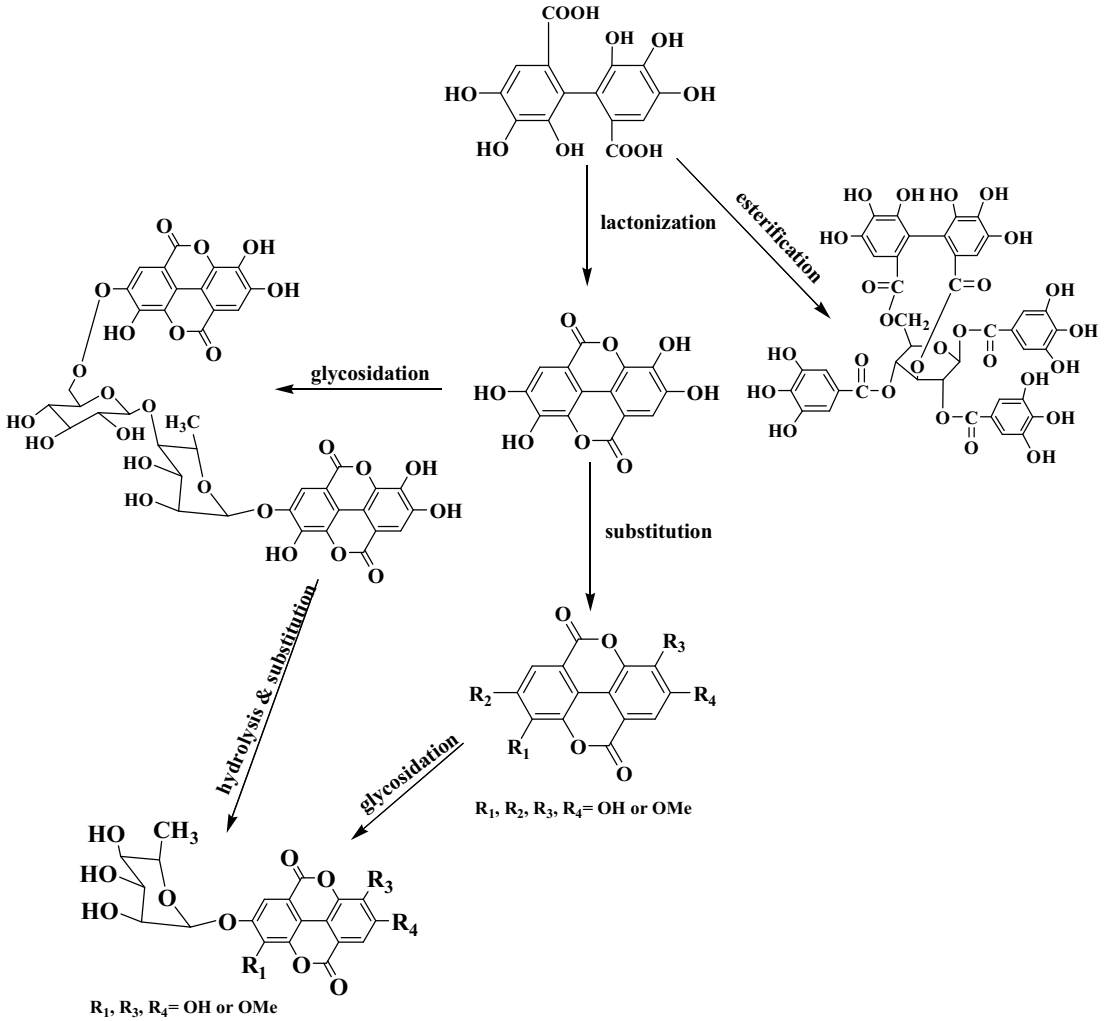

Fig. 1 Biosynthetic pathway of ellagitannins in pomegranate.

sidered as the derivatives of gallic acid (Li HX *et al*. 2002). From the perspective of biosynthesis, these compounds are, like ellagitannins, synthesized through esterification, lactonization and glycosidation between the moieties from single or multiple molecule(s) (Wang *et al*. 2006b).

Flavonoids

Flavonoids isolated from pomegranate (**Table 2**) include flavones, flavonols, anthocyanidins and flavan-3-ols. The brilliant colors of pericarp and juice are attributed to anthocyanidins and flavan-3-ols, of which the content decrease or increase with the time of ripening. Anthocyanidins reported in pomegranate usually present in the form of glycoside with aglycons of delphinidin, cyanidin and pelargonidin, while flavan-3-ols found in this plant only present in the unglycosylated form including catechin, epicatechin, epigallocatechin and their derivatives. Flavones and flavonols constitute the major flavonoids of pericarp and leaves, which frequently exist as glycosides with aglycons of luteolin, kaempferol, quercetin, apigenin and naringin.

Alkaloids

Alkaloids were mainly found in the bark of both stem and root as well as the juice of pomegranate (**Table 3**). There are mainly two kinds of alkaloids including piperidines and pyrrolidines reported in this plant. Usually, piperidines have a skeleton of six-member ring and pyrrolidines have a skeleton of five-member ring. Both the species and content of piperidines are relatively more than those of pyrrolidines. Piperidines were reported in both the bark of stem and root, for example, isopelletierine, pseudopelletierine, and *N*-methylisopelletierine are the major alkaloids of the stem bark, while 2-(2′-hydroxypropyl)-Δ1-piperidine, 2-(2′-propenyl)-Δ1-piperidine, and norpseudopelletierine are abundant in the root bark (Neuhofer *et al.* 1993).

Piperidines are biosynthesized through a combined pathway with lysine as the precursor and acetoacetyl coenzyme A as the major source of their side chains (Keogh *et al.* 1970). Lysine first transforms into tetrahydropyridine through a series of reactions including oxidation, cyclization and decarboxylation. Then, it condenses with acetoacetyl-coenzyme A into isopelletierine. Isopelletierine is the branch point of this pathway. Methylation of isopelletierine yields *N*-methylisopelletierine which further transforms into various piperidines in this plant while condensation of isopelletierine with another molecule of tetrahydropyridine yields anaferine in other plant species such as *Withania somnifera*. The detailed possible biosynthetic pathway is shown in **Fig. 2**.

Pyrrolidines reported hitherto only include hygrine and norhygrine which mainly exist in the bark of root with very low content. In addition, indoleamines with indole ring, such as serotonin, tryptamine and melatonin are also detected in the juice of pomegranate (Badria 2002).

Organic acids

The seeds are rich in unsaturated fatty acids including punicic acid, linoleic acid, oleic acid, palmitic acid, stearic acid, and linolenic acid, of which the total content account for 15.26% of the weight of seeds (Li *et al*. 1994; Wang *et al.* 1999; Schubert *et al.* 1999). The juice mainly contains

Table 2 Major flavonoids of pomegranate.

Compound name	Plant part*	Bioactivity	Reference
Apigenin	L	Antioxidant; anti-inflammatory; anti-neoplastic	Nawwar *et al.* 1994a
Apigenin 4′-*O*-glucopyranoside	L		Nawwar *et al.* 1994a
Catechin	J, P	Anti-neoplastic; antioxidant	de Pascual-Teresa *et al.* 2000
Catechol	J	Antioxidant; anti-neoplastic	Liu HX *et al.* 2002
Cyanidin	P	Antioxidant	Noda *et al.* 2002
Cyanidin 3-*O*-glucoside	J	Antioxidant	Hernández *et al.* 1999
Cyanidin 3,5-di-*O*-glucoside	J	Antioxidant	Hernández *et al.* 1999
Delphinidin 3-*O*-glucoside	J		Hernández *et al.* 1999
Delphinidin 3,5-di-*O*-glucoside	J		Hernández *et al.* 1999
Epicatechin	J, P	Anti-neoplastic	de Pascual-Teresa *et al.* 2000
Epigallocatechin 3-gallate	J, P	Anti-neoplastic	de Pascual-Teresa *et al.* 2000
Flavan-3-ol	J, P	Anti-neoplastic	de Pascual-Teresa *et al.* 2000
Isoquercetin	J	Hepatoprotective	Liu HX *et al.* 2002; Rena *et al.* 2009
Kaempferol	P	Antioxidant; anti-inflammatory	van Elswijk *et al.* 2004
Kaempferol-3-*O*-glucoside	P	Antioxidant	van Elswijk *et al.* 2004
Kaempferol-3-*O*-rhamnoglycoside	P	Anti-hypertensive	van Elswijk *et al.* 2004
Luteolin	P	Antioxidant; anti-inflammatory	van Elswijk *et al.* 2004
Luteolin 7-*O*-glucoside	P	Antioxidant	van Elswijk *et al.* 2004
Luteolin 4′-*O*-glucopyranoside	L	Antioxidant	Nawwar *et al.* 1994a
Luteolin 3′-*O*-glucopyranoside	L		Nawwar *et al.* 1994a
Luteolin 3′-*O*-xylopyranoside	L		Nawwar *et al.* 1994a
Naringenin-4′-methylether-7-*O*-α-L-arabinofuranosyl(1→6)-β-D-glucopyranoside	B		Srivastava *et al.* 2001
Naringin	P	Antiviral; antibacterial	Lansky *et al.* 2007
Pelargonidin	P	Anti-oxidant; antibacterial	Noda *et al.* 2002
Pelargonidin 3-*O*-glucoside	J	Antioxidant	Hernández *et al.* 1999
Pelargonidin 3,5-di-*O*-glucoside	J		Hernández *et al.* 1999
Procyanidin	J	Antioxidant; anti-neoplastic; anti-inflammatory	Liu *et al.* 2002
Prodelphinidin	P	Antioxidant	Plumb *et al.* 2003
Punicaflavone	F		Ali M *et al.* 2006
Quercetin	J, P	Anti-neoplastic; antioxidant; antiviral	Artik 1998
Quercetin-3,4-dimethylether-7-*O*-α-L-arabinofuranosyl(1→6)-β-D-glucopyranoside	B	Antioxidant	Chauhan *et al.* 2001
Rutin	P, J	Antioxidant; anti-hypertensive; antiviral	Artik 1998

*B: bark; F: flowers; J: juice; L: leaves; P: pericarp.

Table 3 Major alkaloids of pomegranate.

Compound name	Plant part*	Bioactivity	Reference
Tryptamine	J		Badria 2002
Serotonin	J	Antioxidant	Badria 2002
Melatonin	J	Antioxidant; anti-inflammatory; hepatoprotective	Badria 2002
Peelletierine	B, P, R		Neuhofer *et al.* 1993; Vidal *et al.* 2003
N-methylpelletierene	B, R		Neuhofer *et al.* 1993
Pseudopelletierene	B, R		Neuhofer *et al.* 1993
Norpseudopelletierene	R		Neuhofer *et al.* 1993
Sedridine	R		Neuhofer *et al.* 1993
2-(2′-hydroxypropyl)Δ1-piperideine	R		Neuhofer *et al.* 1993
2-(2′-propenyl)Δ1-piperideine	R		Neuhofer *et al.* 1993
N-(2′,5′-dihydroxyphenyl)pyridium chloride	L		Andreas *et al.* 2007
Hygrine	R	Anti-neoplastic	Neuhofer *et al.* 1993
Norhygrine	R		Neuhofer *et al.* 1993
N-acetyl-sedridine	B, R		Neuhofer 1990

*B: bark; F: flowers; J: juice; L: leaves; P: pericarp; R: root.

straight chain fatty acids, of which citric acid and malic acid are the major compounds with the content of up to 4.85 and 1.75 g/L, respectively (Neuhofer 1990). In addition, tartaric acid, oxalic acid and succinic acid were also found in the juice (Poyrazoglu *et al.* 2002). Phenolic acids consisting of caffeic acid, fumalic acid, chlorogenic acid and *p*-coumaric acid usually present in the juice and/or pericarp (Artik 1998; Amakura *et al.* 2000b).

Triterpenes and steroids

Triterpenes without glycosidation are frequently found in the flowers and the seeds of pomegranate. These compounds including ursolic acid, oleanolic acid, maslinic acid, punicanolic acid, friedelin, betulinic acid and Asiatic acid usually appear in the form of pentacyclic triterpenoids with a C-28 carboxyl and a double bond between C-12 and C-13 (Batta and Rangaswami 1973; Ahmed *et al.* 1995; Krishna *et al.* 2002; Huang *et al.* 2005b). Steroids are only found in the seeds, which consist of sterols, such as cholesterol, stigmasterol, camesterol, β-sitosterol and daucosterol, and sex steroids, such as 17-α-estradiol, estrone, testosterone and estriol (Heftmann *et al.* 1966; Dean *et al.* 1971; Abd El Wahab *et al.* 1998; Wang *et al.* 2004; Lansky *et al.* 2005; Xie *et al.* 2008).

Others

Other compounds including saccharides, coumarins and lignans were also reported in the pomegranate. For example, coumestrol, coniferyl 9-*O*-[β-dapiofuranosyl(1→6)]-*O*-β-D-glucopyranoside, sinapyl 9-*O*-[β-dapiofuranosyl(1→6)]-*O*-β-D-glucopyranoside, phenethyl rutinoside and icariside D1 were isolated from the seeds (Wang *et al*. 2004), and

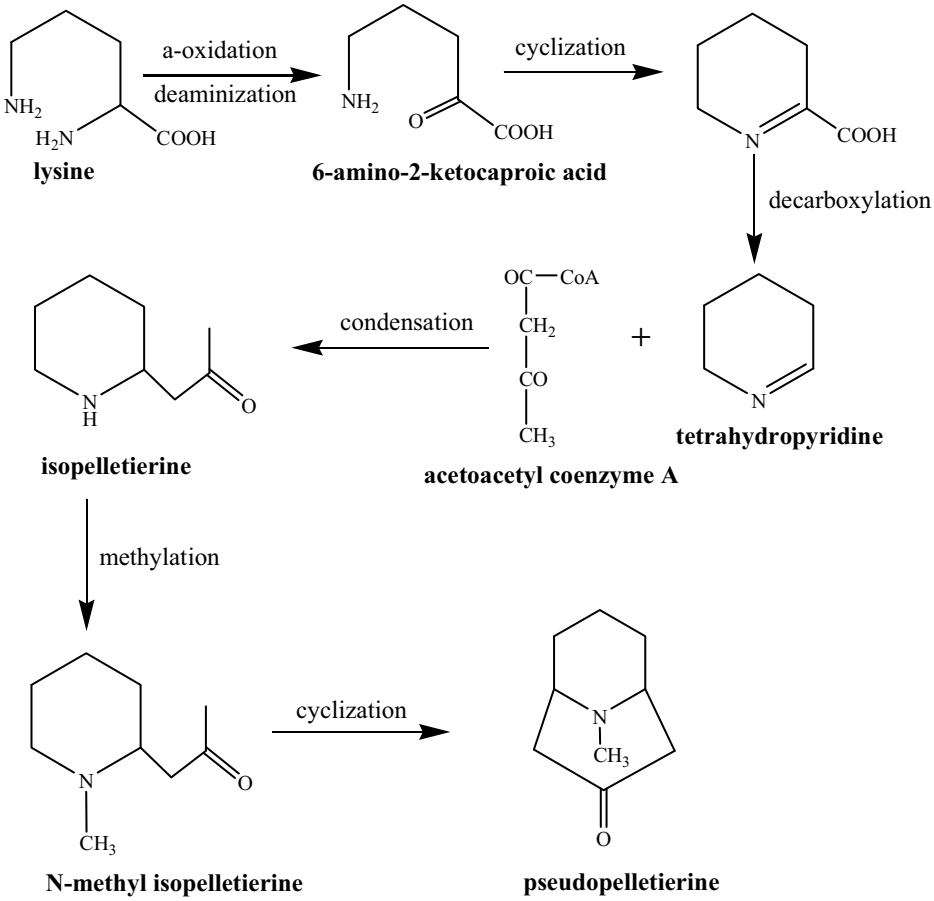

Fig. 2 Biosynthetic pathway of piperidines in pomegranate.

glucose, fructose and sucrose were found in the juice (Cui *et al*. 2004).

BIOACTIVITIES

Vascular protection

Dietary patterns are widely recognized as contributors to cardiovascular and cerebrovascular disease. Several epidemiological studies suggest that the regular consumption of foods and beverages rich in polyphenols such as tannins and flavonoids is associated with a reduced risk of several pathological conditions ranging from hypertension to coronary heart disease, atherosclerosis, stroke and dementia (Hamendra and Anand 2007; Dilip and Arjan 2009). Pomegranate is such a plant containing large amount of these polyphenols and, therefore, has been extensively investigated for vascular protection.

1. Anti-oxidation

Many of the papers published upon pomegranate in the last decades have focused on the antioxidant action *in vitro*, *ex vivo* and *in vivo* because it is the most important bioactivity of pomegranate and constitutes the basis of other activities including lipid regulatory, anti-inflammatory, anti-neoplastic and anti-diabetic effects (Lansky *et al*. 2007). The antioxidant substances mainly exist in the leaves and the fruit including seed, juice and pericarp. It was reported that the juice and aqueous extract of leaves can effectively scavenge free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS), superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH$) and nitric oxide (NO), and the effect is significantly superior than that of the extracts from other fruits (Halvorsen *et al.* 2002; Xu *et al.* 2005; Gurpreet *et al.* 2006; Guo *et al.* 2007). The juice can inhibit the production of oxLDL *in vitro* (Fuhrman and Aviram 2001) and effectively decrease the oxLDL level in rats *in vivo* (Xu *et al*. 2005).

The antioxidant constituents of pomegranate mainly comprise the compounds with phenolic hydroxyl groups and double bonds including tannins, flavonoids and unsaturated fatty acids. Significant and positive linear correlations were found between total antioxidant capacities and phenolic contents, indicating that phenolics are the dominant antioxidant constituents of pomegranate (Surveswaran *et al.* 2007).

Tannins, which are a kind of excellent antioxidant found in almost all parts of pomegranate, usually contain several hexahydroxydiphenoyl groups or galloyl groups. Both of these two groups possess the abilities to provide protons as well as to form stable free radicals which enable them to be the major active groups in the molecule of tannins (Wang *et al*. 2005). Furthermore, it was reported that the hexahydroxydiphenoyl group is more potent as to antioxidant effect than the galloyl group (Wei *et al.* 2000). The linkage among tannin monomers and the existing status of the phenolic hydroxyl groups are also the important factors affecting the antioxidant activities of tannins. The antioxidant effect is more potent as the tannin monomers bound together through hydrolysable bonds, i.e., the esteric bond and the glycosidic bond, while the antioxidant effect decreases dramatically as the tannin monomers bound into condensed tannins through carbon-carbon bonds (Wei *et al.* 2000). The free phenolic hydroxyl group due to its vulnerability to oxidant can favorably affect the antioxidant effect of tannins, which is evidenced by that 3,3′4′-tri-*O*-methylellagic acid is less potent *in vitro* than 3,3′-di-*O*-methylellagic acid which has one more phenolic hydroxyl group than the former (Wang *et al*. 2004). Two tannins, namely, ellagic acid and punicalagin are considered to play an important role in the antioxidant activity of tannins in pomegranate (Sestili *et al.* 2007). Ellagic acid, which can react with free radicals due to its ability to chelate with metal ions, is a potent antioxi-

dant against lipid peroxidation in mitochondrion and microsome (Oswa *et al.* 1987). Punicalagin, *viz*, 2,3-(S)-hexahydroxydiphenoyl-4,6-(S,S)-gallagyl-D-glucose is the major antioxidant compound in the pericarp and juice. It makes the greatest contribution to the antioxidant action of the pericarp and the juice due to its inhibition on lipid peroxidation. This kind of inhibiton is attributed to its ability to provide electrons so as to eliminate the free radicals resulted from lipid peroxidation (Anand *et al.* 2004; Lansky *et al.* 2007). This compound, however, can hardly be absorbed into the circulation and only its three microbiological metabolites, namely, 3,8-dihydroxy-6*H*-dibenzo[b,d]pyran-6-one glucuronide, trihydroxy-6*H*-dibenzo[b,d]pyran-6-one and hydroxy-6*H*-dibenzo[b,d]pyran-6-one glucuronide were detected in the blood and urine, indicating the antioxidant bioactivity may be ascribed to the metabolism of intestinal flora (Kulkami *et al.* 2004).

Flavonoids also make a great contribution to the antioxidant activity of pomegranate due to their effect in free radicals elimination (Wang *et al.* 2006b; Suo *et al.* 2009). It seems that flavonoids from pomegranate possess a significant antiperoxidative activity. It was evidenced by that the concentrations of malondialdehyde, hydroperoxides and conjugated dienes in the liver, heart and kidney were significantly reduced and the activities of the enzymes such as catalase, SOD, glutathione peroxidase, glutathione reductase and the concentration of glutathione in the tissue were significantly enhanced after the rats were orally administered with total flavonoids from pomegranate (Sudheesh and Vijayalakshmi 2005). The major flavonoids in pomegranate, particularly catechin, quercetin, kaempferol and equol, play a significant role in the photoprotective effects on UVB-induced skin damage which is demonstrated by the increased expression level of procollagen type I and decreased expression level of matrix metallopr-proteinase-1 (MMP-1) (Park *et al*. 2010). Phenolic hydroxyls, in particular, those at carbon-5 and -7 (such as those in the molecules of luteolin, delphinidin and cyaniding), or at the *ortho*-positions of B ring of flavonoids (such as those in the molecules of luteolin and pelargonidin) are the key groups responsible for the antioxidant effect (Noda *et al.* 2002).

Other compounds containing phenolic hydroxyl groups or unsaturated double bonds such as lignans including coniferyl 9-*O*-[α-D-apiofuranosyl(1→6)]-*O*-α-D-glucopyranoside and sinapyl 9-*O*-[α-D-apiofuranosyl(1→6)]-*O*-α-D-glucopyranoside (Wang *et al*. 2004) and unsaturated fatty acids (Elgareo *et al.* 1995) are also the active antioxidant constituents of pomegranate. Therefore, the antioxidant activity of pomegranate is a multi-factorial effect and chemical synergic action of multiple compounds, which was also supported by the comparative test results (Seeram *et al.* 2005).

2. Lipid regulation

The extracts of leaves, pericarp, juice and seeds were reported to have a beneficial effect on blood lipid regulation which was demonstrated to be ascribed to their antioxidant activity (Li *et al.* 1999; Aviram *et al.* 2000; Kaplan *et al.* 2001; Meng *et al.* 2005; Li WM *et al.* 2006, 2007; Lei *et al.* 2007a). The crude extract of pericarp and the EtOAc extract thereof can effectively lower the levels of serum TC, TG, LDL-C and FFA, and increase the level of serum HDL-C in mice (Li YF *et al.* 2005). We have reported that tannins are the active fraction of the leaves for lipid regulation, which can decrease TC, VLDL, TC/HDL and triglycerides, and increase HDL and thus play a positive role in the treatment of lipid metabolic disorder and obesity (Lei *et al.* 2007a). Its mechanism may correlate with the inhibition on HMG-CoA reductase, pancreatic lipase and ACAT, as well as suppression of energy intake (Cheng *et al.* 2005; Meng *et al.* 2005). Further investigation performed by our laboratory showed that ellagic acid and other two constituents, SY 2 and SY 3, are the major active tannins responsible for lipid-lowering activity, and ellagic acid is the most effective one (Lei *et al.* 2007b).

3. Anti-hypertension

The anti-hypertensive action of pomegranate is very potent and it acts in a multimechanical way; however, the major responsible constituents seem to be polyphenols. These anti-hypertensive polyphenols were mainly reported in the juice which can significantly lower the level of serum angiotensin-converting enzyme by 36% and decrease the systolic pressure by 5% in a dose-dependant manner. It has been found that the protective effect of juice against vascular disease may correlate with its inhibition on oxidant stress and serum ACE activity (Xu *et al.* 2005; Wang *et al.* 2008). Results from a small randomized human trial showed that consumption of juice (50 mL of pomegranate juice, containing 1.5 mM of total polyphenols) by patients with severe carotid artery stenosis induced decrease of carotid intima-media thickness (IMT) and reduction of systolic blood pressure (Aviram *et al.* 2004). Many polyphenols such as punicalagin can increase the production of endothelial nitric oxide (NO) which is a relaxing factor produced in the endothelium via activation of endothelial NO synthase (eNOS) and acts as a vasodilator (Li and Frostermann 2000; Ignarro *et al*. 2006; de Nigris *et al.* 2007). In addition, the extract of pericarp can protect the endothelium cell of human umbilical vein from injury induced by oxidative stress, indicating its antihypertensive effect (Li YF *et al.* 2006).

Digestive protection

1. Gastroprotection

Pericarp and leaves of pomegranate were reported to possess gastroprotective activity which usually resulted mechanically from their astringent property, and inhibition of bacteria. The antioxidant effect and the effective compounds were pointed to polyphenols.

The polyphenols in the pericarp can significantly protect rats from ethanol-induced gastric mucosal damage and decrease the incidence of gastric lesions by 53-80% as well as improve the healing of gastric ulcer with a curative ratio of 97.4%. This effect is believed to be related to the astringent property of tannins which are able to bind with protein so as to accelerate the healing of ulcer or trauma (Murthy *et al.* 2004; Ajaikumar *et al.* 2005). However, this protective effect may also correlate with the antibacterial activity of the pericarp because it was reported that the aqueous extract of pericarp significantly inhibited the growth of *Helicobacter pylori* (HP) resistant to metronidazole, a major pathogenic bacterium for gastritis or gastric ulcer, with an IC_{50} of 29.9 mg/mL *in vitro* (Hu *et al*. 2006). Another investigation performed by Ajaikumar *et al*. (2005) showed that after administrated with 70% methanolic extract of pericarp (250 and 500 mg/kg) the aspirin- and ethanol-induced gastric ulceration in rats were inhibited by 22.37, 74.21% and 21.95, 63.41%, respectively, and antioxidant levels such as superoxide dismutase (SOD), catalase, glutathione (GSH) and glutathione peroxidase (GPx) levels were markedly increased and were found more or less equal to the normal values *in vivo*. Obviously, this investigation revealed the gastroprotective activity of the extract through antioxidant mechanism (Ajaikumar *et al.* 2005).

The extract of the leaves abundant with tannins was demonstrated to be a good gastric protective agent. It can increase the activity of pepsin, improve the secretion of bile, enhance the intestine peristalsis, inhibit the secretion of gastric acid, and decrease the incidence of gastric ulcer (Li *et al.* 1998, 2003).

2. Hepatoprotection

Hepatoprotective effect of pomegranate was recently observed by a few of animal experiments *in vivo*, but the detailed mechanism and the effective compounds have not been determined. The extract of flowers exhibited hepatoprotective effect against ferric nitrilotriacetate (Fe-NTA)

induced hepatotoxicity in mice which was considered to be probably resulted from the potent antioxidant activity of polyphenols in this extract (Kaur *et al.* 2006; Çelik *et al.* 2009).

3. Anti-diarrhea and anti-helminthes

Both organic extract and aqueous extract of the leaves, roots, bark, stem, and rhizome exhibited anti-diarrhea activity for which the antibacterial effect against *Escherichia coli*, *Shigella sonnei*, *S. flexneri* and *Salmonella typhi* of the hydrolysable tannins may be responsible (Mathabe *et al.* 2006). The methanol extract of the seeds also significantly inhibited castor-oil induced diarrhea and PGE_2 induced enteropooling in rats; however, the active principle(s) and exact mechanism(s) have not been revealed (Das *et al.* 1999). Base on above experiment results and the theory of traditional Chinese medicine, we presume that the antidiarrheal effect of pomegranate derives from its astringent property which is resulted, predominantly, from tannins.

The pericarp has been used in China to dispel intestinal parasite since ancient time. Its mechanism was believed to be induction of continual intestinal tract contraction (*Zhonghua Bencao*, Vol. 15).

Anti-pathogenic microbes

1. Anti-bacteria

Besides the *Helicobacter pylori*, *Escherichia coli*, *Salmonella typhi* and microorganisms of *Shigella* mentioned above, the extracts of pomegranate also exhibit significant inhibiting effect against the common pathogenic bacteria especially Gram positive pathogens. It was reported that both methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) strains of *Staphyloccocus aureus* were susceptible to the extracts of pericarp or fruit and the subsequent enterotoxin production was inhibited by these extracts, indicating the therapeutic use of pomegranate for bacterial infection (Machado *et al.* 2003; Braga *et al.* 2005). Additionally, other bacteria such as *Streptoccus hemolyticus*, *Vibrio cholerae*, *Bacillus paratyphosus*, *Proteus bacillus*, *Proteus vulgaris*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Candida albicans*, etc. were also reported to be susceptible to the extracts of pomegranate (Navarro *et al.* 1996; *Zhonghua Bencao* Vol 15, 1998; Prashanth *et al.* 2001; Al-Zoreky 2009). The antibacterial active constituents of pomegranate were demonstrated to be tannins such as ellagitannins and flavonoids (Machado *et al.* 2003; Wang *et al.* 2008; Al-Zoreky 2009).

2. Antivirus

Polyphenols especially tannins of pomegranate play a key role in the antiviral effect because of their special property of protein precipitation which adversely affect the enzymes involved in the life cycle of virus. Zhang *et al*. reported that the aqueous extract of pericarp containing high content of tannins significantly inactivated HSV-2 and HBV via inhibiting the DNA polymerase in a dose dependent manner *in vitro* (Zhang *et al.* 1995, 1997). Like the pericarp, the juice also containing enormous tannins showed an appreciable effect in inhibition or elimination of HIV-1 (Neurath *et al.* 2004). The polyphenol extract inhibited the replication of human influenza A/Hong Kong (H3N2) *in vitro*, suppressed replication of influenza A virus in MDCK cells, and inhibited agglutination of chicken red blood cells (cRBC) caused by influenza virus. Four major polyphenols in pomegranate, namely ellagic acid, caffeic acid, luteolin, and punicalagin were evaluated for anti-influenza effect and the results demonstrated that punicalagin is the most effective one. Punicalagin blocked replication of the virus RNA, inhibited agglutination of chicken RBC induced by virus and had a virucidal effect (Haidari *et al.* 2009).

Anticancer

Carcinogenesis is a very complex cascade and can be affected by multi-factors. Pomegranate can interfere with the occurrence and development of tumors in many procedures including inflammation development, angiogenesis, apoptosis, proliferation and invasion.

1. Anti-inflammation

Although physiological or acute inflammation is a beneficial host response to tissue damage, it may lead to such immune-associated diseases as rheumatoid arthritis, inflamematory bowel disease (IBD) and cancer as timely resolution is delayed (Balkwill *et al*. 2005). Chronic inflammation can lead to early changes associated with the development of cancer through attraction of soluble pro-inflammaotry mediators e.g. TNF-α, interleukins (e.g. IL-6 and IL-8), transcription activation factors (e.g. NF-kB), and bioactive lipids such as eicosanoids (e.g. prostaglandin E2 and lipoxygenase derived products) (Lansky *et al*. 2007). The anti-inflammatory compounds in pomegranate were mainly investigated in the seeds and the results exhibited polyphenols and fatty acids were the major anti-inflammatory constituents. The extract from cold pressed seed oil of pomegranate mainly consisting of polyphenols and fatty acid showed 31-44% inhibition of sheep cyclooxygenase and 69-81% inhibition of soybean lipoxygenase, while the extract from fermented juice showed 21-30% inhibition of soybean lipoxygenase (Schubert *et al*. 1999). The polyphenols in cold pressed seed oil were also reported by another research group to suppress inflammatory cell signaling in colon cancer cells (Adams *et al*. 2006). The major ingredient of fatty acids, punicic acid, is a well-known anti-inflammatory compound which inhibits the development of inflammation through suppressing the biosynthesis of prostaglandin (Nugteren *et al*. 1987).

2. Anti-angiogenesis

It is important for the tumor growth and metastasis that new blood vessels can regenerate and develop timely to supply oxygen and nutrients to the tumor cells. The juice and seed oil of pomegranate could adversely affect the angiogenesis in chicken chorioallantoic membrane (CAM) *in vivo* by downregulating the pro-angiogenic vascular endothelial growth factor (VEGF) in MCF-7 estrogen dependent breast cancer cells, and upregulating macrophage migration inhibitory factor (MIF) in MDA-MB-231 cells (Toi *et al*. 2003).

3. Apoptosis induction

Apoptosis, also called the programmed cell death, is a tightly regulated cascade of cell death. Effective induction of apoptosis is considered as a promising approach for cancer treatment. The fruit including pericarp, juice and seeds was reported to induce apoptosis both *in vitro* and *in vivo*. Both the lipid and aqueous fractions of the pericarp extract were demonstrated to possess selective apoptotic potential in different hormone-independent cancer cell lines (Lansky *et al*. 2007). Aqueous pericarp extract resulted in apoptotic DNA fragmentation and suppression of growth in two human Burkitt's lymphoma cell lines, Raji and P3HR-1 (Settheetham and Ishida 1995). The juice, polyphenols in the pericarp and the seed oil potently induce apoptosis in human prostate cancer cells *in vivo* (Albrecht *et al*. 2004).

4. Proliferation and invasion inhibition

The extracts of pomegranate pericarp have been demonstrated to interfere with the proliferation of cells in different human cancer cell lines (Settheetham and Ishida 1995; Mavlyanov *et al*. 1997; Albrecht *et al*. 2004; Kawaii and Lansky 2004). The extracts selectively inhibited the cancer cells and minimally affected the normal cells, suggesting

their potential therapeutic use in the tumor treatment.

Suppressing tumor cell invasion is also a property of pomegranate extract in tumor inhibition. Albrecht *et al*. reported that the juice, polyphenols in the pericarp and the seed oil suppressed the proliferation of xenograft and the invasion of human prostate cancer cells by 60% *in vivo* (Albrecht *et al*. 2004). And Kim *et al*. found that the seed oil inhibited invasion of estrogen sensitive MCF-7 human breast cancer cells *in vitro* across an artificial Matrigel^{TM} membrane at doses less than 10 μg/mL (Lansky *et al*. 2007).

Anti-diabetes

Flowers of pomegranate have been used as an anti-diabetic medicine in Unani medicine and as a supplement in the diet therapy in many countries. The flowers can significantly lower the blood glucose level of type 2 diabetes animals with different possible mechanisms including enhancement of mRNA expression, improvement of insulin receptor sensitivity, increment of peripheral glucose utilization, etc.

The methanol extract of pomegranate flower (500 mg/kg, daily) inhibited glucose loading-induced increase of plasma glucose levels in Zucker diabetic fatty rats (ZDF), enhanced cardiac PPAR-γ mRNA expression and restored the down-regulated cardiac glucose transporter (GLUT)-4 (the insulin-dependent isoform of GLUTs) mRNA, suggesting that the anti-diabetic activity of methanol extract of pomegranate flowers may result from improved sensitivity of the insulin receptor. Phytochemical investigation demonstrated that gallic acid in the methanol extract of flowers is mostly responsible for this activity (Tom *et al*. 2005). Oral administration of aqueous extract of pomegranate flowers at doses of 250 and 500 mg/kg for 21 days resulted in a significant reduction in fasting blood glucose, TC, TG, LDL-C, VLDL-C and tissue LPO levels coupled with elevation of HDL-C, GSH content and antioxidant enzymes in comparison with diabetic control group. These results suggested that the aqueous extract of flowers can be used, as a dietary supplement, in the treatment and prevention of chronic diseases characterized by atherogenous lipoprotein profile, aggravated antioxidant status and impaired glucose metabolism (Bagri *et al*. 2009). Oral administration of the aqueous-ethanolic (50%, v:v) extract of pomegranate flowers led to significant blood glucose lowering effect in normal, glucose-fed hyperglycaemic and alloxan-induced diabetic rats. This effect can be due to increased peripheral glucose utilization. Retardation of intestinal glucose absorption may also be partly responsible for inhibition of hyperglycaemia in glucose-fed rats (Jafri *et al*. 2000). Li *et al*. reported that the extract of pomegranate flowers improved postprandial hyperglycemia in type 2 diabetic and obese animal model, and this effect at least partially resulted from inhibiting intestinal α-glucosidase activity (Li YH *et al*. 2005).

Immunomodulation

The crude extract of pericarp and seeds possess immunomodulatory activity and its mechanism was preliminary revealed; however, the individual compound responsible for this activity has not been identified. The aqueous suspension of pericarp has appreciable immunostimulatory activity in rabbits. It stimulated both the humoral and cell-mediated immune responses which were evidenced by the enhanced inhibition of leucocyte migration and increased antibody titer to typhoid-H antigen (Ross *et al*. 2001). The seed oil enables the production of immunoglobulins in the spleen cells of mice and probably improves the function of B cells *in vivo* (Yamasaki *et al*. 2006).

Others

Other bioactivities of pomegranate include: the aqueous extract and the seed oil can promote proliferation of hypodermis and epidermis, respectively (Aslam *et al*. 2006); the aqueous extract of pericarp can suppress the sperm fertility and prevent the rabbit from pregnancy (Sun *et al*. 1994); the petroleum ether extract of the seeds exhibited potent estrogenic activity which can be antagonized by progesterone (Li *et al*. 2002).

PHARMACOKINETICS AND TOXICITY

The excellent pharmacokinetic profile as well as the minimal toxicity and side effect are the key elements for new drugs being approved to enter clinical trial. It was reported that among the drug candidates failed to be used clinically, 39% of them was due to poor pharmacokinetic profile and 21% of them was due to safety issue caused by toxicity and side effect (Yang *et al.* 2004). We have studied the pharmacokinetics of two important compounds of pomegranate leaf tannins in order to ascertain their absorption, distribution and metabolism. The concentration-time profile was fitted with an open two-compartment system with lag time and its max concentration of ellagic acid in plasma was 213 ng/ml only 0.55 h after oral administration of the extract at 0.8 g/kg. This pharmacokinetic profile indicated that ellagic acid has poor absorption and rapid elimination after oral administration of pomegranate leaf extract, and part of it was absorbed from the stomach (Lei *et al.* 2003). Further experiment *in vitro* showed that ellagic acid in pomegranate leaf tannins could be transported into the HepG2 cells, which was in correlation with total cholesterol alteration in the cells (Lan *et al.* 2008). Another major tannin brevifolin also exhibited the similar kinetic characteristics to ellagic acid, including rapid absorption, distribution and elimination. These results suggested that the compounds with similar structure usually have similar pharmacokinetic properties, and thus we can characterize the pharmacokinetic profile of total tannins based on the information obtained on theses two compounds.

Only the bark and root were reported to be toxic and this toxicity was alleged to be related to their alkaloid content (Tripathi and Singh 2000). The LD_{50} of the whole fruit extract, determined in OF-1 mice of both sexes after intraperitoneal administration was 731 mg/kg (Vidal *et al*. 2003). In an investigation to study the acute and subchronic toxicity of a standardized pomegranate fruit extract containing 30% punicalagins, acute oral LD_{50} in Wistar rats and Swiss albino mice was greater than 5000 mg/kg body weight, and the subchronic no-observed-adverse-effect level (NOAEL) was determined as 600 mg/kg body weight/day (Patel *et al*. 2008). Based on above reports, the pomegranate is safe if used at the normal dosage.

CONCLUSION

The chemical constituents of almost all parts of pomegranate have been investigated, as well as a lot of bioactive and pharmacokinetic studies *in vivo* and/or *in vitro* have been carried out on individual compounds or extract of some parts. These results provided us a solid basis for the development and utilization of pomegranate as both pharmaceuticals and dietary supplement. However, the compound-bioactivity relationship and structure-bioactivity relationship were studied relatively less in-depth. The investigation in this area can lead us thoroughly understand this plant and provide a foundation for safe and efficient use.

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