New Collection of Crude Drugs in *Chinese Pharmacopoeia 2010* II. *Sankezhen* (*Berberis* spp.)

DAN Yang[1], LIU Yan-ze[1], PENG Yong[1], QIAN Zhong-zhi[2], XIAO Pei-gen[1*]

1. Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing 100193, China
2. Chinese Pharmacopoeia Commission, Beijing 100061, China

Abstract: *Sankezhen* (*Berberidis Radix*) is a traditional Chinese materia medica, cold in nature and bitter in taste, for treating syndromes of liver, stomach, and large intestinal meridians, in which berberine and berbamine are the major pharmacological components. *Sankezhen* has been readmitted in *Chinese Pharmacopoeia 2010* following the 1977 version as the roots of *Berberis* spp. e.g. *B. soulieana*, *B. wilsonae*, *B. poiretii*, *B. vernae*, etc. Recent studies showed that *Berberis* spp. were potential phytomedicines with multiple spectrums therapeutic effects and various pharmaceutical parts. Here we reviewed *Sankezhen* in traditional use and phytochemistry, and its major active components berberine and berbamine with potential bioactivities recently discovered, such as antitumor, antidiabetic, antihyperlipidemic, anti-arrhythmic, and neuro-protective activities. It is necessary to mature the quality assessment of *Sankezhen* as a new admission of *Chinese Pharmacopoeia 2010*. Other parts of *Berberis* spp. should be investigated to better develop this herb in medicinal usage.

Key words: alkaloids; *Berberidis Radix*; berberine; berbamine; *Chinese Pharmacopoeia*; ethnopharmacology
DOI: 10.3969/j.issn.1674-6384.2011.04.007

Berberis L. includes about 500 species of spiny or unarmed, evergreen or deciduous shrubs native to Asia, Europe, North Africa, and to North, Central, and South America. Over 200 species spread in the Southwest and Northwest of China, many of which are called *Sankezhen* (three spines) in folk Chinese due to the characteristics of three spines fasciculate on stems. Xiao *et al* (1974) reported that there are only 40－50 medicinal species in this genus which are abundant and widely distributed in China.

Sankezhen (*Berberidis Radix*) has been readmitted in *Chinese Pharmacopoeia 2010* (Pharmacopoeia Committee of P. R. China, 2010) following the 1977 version (Pharmacopoeia Committee of P. R. China, 1977) due to its multiple spectrum therapeutic effects and wide distribution in China. The botanical origins are authorized as dried roots of *Berberis* spp. e.g. *B. soulieana* Schneid., *B. wilsonae* Hemsl., *B. poiretii* Schneid., and *B. vernae* Schneid., etc, which is yet to clarify the botanical origins to date. So, the so-called *Sankezhen* as medicine here and thereafter means the radix of many species of *Berberis* spp. which could be used as the same purpose. Fortunately, the four species in *Chinese Pharmacopoeia 2010* might be potential candidates due to their abundance as the representatives in various regions of China such as Southwest, Northwest, and North China. For example, *B. soulieana* is largely spread in Qinling and Daba mountains, and in Sichuan, Hubei, Gansu, and Shannxi Provinces. *B. wilsonae* is abundant in Southwest China including Sichuan, Yunnan, Guizhou, Gansu, Hubei Provinces, and Tibet. *B. poiretii* grows in North China, such as forests of Daxing'anling and Xiaoxing'anling, and Hebei, Shandong, Shanxi, Shannxi Provinces, and Inner Mongolia, etc. *B. vernae* is popular in Northwest China, especially in Gansu and Qinghai Provinces and Xinjiang Autonomous Region. However, few studies have been intensively carried out on their phytochemistry.

Since *Berberis* spp. spread worldwide and are used for medicinal purpose, here we reviewed *Sankezhen* in traditional use, phytochemistry, and pharmacology recently discovered. Since *Sankezhen* is one of the new

* Corresponding author: Xiao PG
Received: June 7, 2011; Revised: September 1, 2011; Accepted: September 20, 2011
Fund:

admissions of *Chinese Pharmacopeia 2010*, it is in great needs to improve current quality assessment upon this herb. In addition, we briefly introduced other bioactive parts of *Berberis* spp. and the responsible compounds in order to better develop *Berberis* spp. in medicinal use.

Botanical description

Berberis L. is described in *Flora of China* as follows: shrubs, evergreen or deciduous. Branches glabrous or tomentose, spinose or not; spines simple or usually 3－5-fid. Leaves on short shoots, simple, alternate, usually petiolate. Inflorescences solitary or fascicled flowers, racemes, umbels, or panicles. Flowers 3-merous; bracteoles usually 3, caducous, scalelike. Sepals 6, rarely 3 or 9, yellow. Petals 6, yellow, bases nectariferous. Stamens opposite petals; anthers dehiscing by valves; pollen grains sub-spheroidal, exine reticulate. Ovary symmetrically club-shaped; ovules 1－12, rarely to 15, subbasal; styles very short. Fruit a berry, usually red, dark red, or black, globose, ellipsoid, oblong, ovoid, or obovoid. Seeds 1－10, tan to red-brown or black; aril absent. $2n$ = 14 (Ying and Ying,).

Traditional use

Sankezhen has long been used for treating liver, stomach, and large intestinal meridian syndromes in traditional Chinese medicine (TCM) (Committee of *Chinese Herbacology*, 2004). It was first documented in *Tangbencao* that the plants were as small as pomegranate trees in height with yellow barks and bitter taste. *Bencao Shiyi* said that *Sankezhen* were alternative to *Phellodendri Chinensis Cortex* and *Coptidis Rhizoma* in TCM. They are similar in the following aspects, cold in nature and bitter in taste, clearing heat and drying dampness, purging fire for removing toxin, and strengthening the stomach, orally used in treating dysentery, gastroenteritis, hot eyes, toothache, aptha, sore throat, acute hepatitis, chronic cholecystitis, jaundice, and dyspepsia, and externally used to treat swelling and ulcer on the body, hot eyes, burn, eczema, and other skin infection diseases (Xiao *et al*, 1974). However, *Sankezhen* is absent from traditional Chinese prescriptions. Roots and barks of the species in *Berberis* L. are also used as traditional medicines in Korea, India, and Pakistan. *B. koreana* Palibin has been used to treat gastroenteritis, sore throats, fever, and conjunctivitis in Korea (Kim, Choi, and Lee, 2010). *B. asiatica* (Daruharidra) is an important ingredient of the Ayurvedic system in India (Andola *et al*, 2010). The extracts from roots, leaves, and fruits of *B. lyceum* have been used against liver cirrhosis, hepatitis, colic disorder, chest cold, suppressed cough, and burned wound in Pakistan (Gulfraz *et al*, 2008).

It is generally believed that alkaloids, principally berberine are the major compounds in *Sankezhen*. Berberine is a botanic drug in clinic to treat cholera, severe diarrhea, amoebiasis, and intestinal infections, which has been admitted as *Huangliansu* in *Chinese Pharmacopeia*. In recent decades, it has been demonstrated to possess antimicrobial and anticancer bioactivities, and it is also used for cardiovascular disease and central nervous disorder (Tang *et al*, 2009). Although total synthesis of berberine was achieved in 1969, it is largely extracted from the plants of *Berberis* L., which are major natural resources of berberine upon the demand of pharmaceutical industry. In China, Xiao *et al* (1974) reported that the good botanical resources for producing berberine were *B. gagnepainii* var. *lanceifolia*, *B. sanguine*, *B. soulieana*, *B. taronensis*, and *B. reticulate* due to higher yields of berberine (1.08%－3.04% of root dry weight) though the highest yields of berberine (3.95%－4.17 % of root dry weight) were found in *B. virgetorum*, *B. henryana*, and *B. polyantha*. The best botanical resources for the production of berberine rely on population biomass and berberine contents in plants.

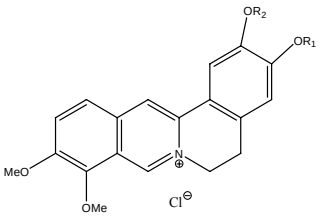
Due to difference in chemical structures, berberine and berbamine are used differently in clinical practices. Berberine is commonly used in gastroenteritis, neonatal conjunctivitis, and tympanitis caused by various microbe with the single dose of 0.1－0.4 g by ig administration and as anti-arrhythmics with the single dose of 0.6－1 g, while berbamine, another major alkaloid in *Sankezhen*, is mainly used in stimulating normal hematopoiesis and anti-arrhythmics in clinical practice.

Chemical components in *Sankezhen* and other parts of *Berberis* spp.

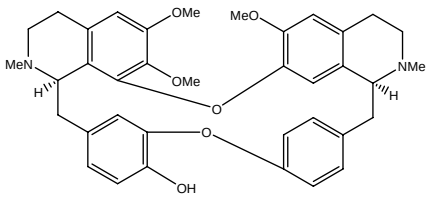
The major compounds of *Sankezhen* are alkaloids,

e.g. berberine, berbamine, jatrorrhizin, palmatine (Fig. 1) and so on, which are also detected in shoots (e.g. trunks, barks, and stems). In addition, other classes of compounds have been isolated and identified in the rest parts of *Berberis* spp. (e.g. polyphenols, triterpens, biphenyls, saponin, pyrrole acids, and tannin) (Gulfraz *et al*, 2008; Rashmia, Rajasekaranb, and Pant, 2008). Since the previous review (Rashmia, Rajasekaranb, and Pant, 2008) summed up the chemical constituents discovered before 2008, we show the alkaloids discovered in the latest four years (Table 1). The major components of roots, twigs, and barks are alkaloids containing aporphines (e.g. magnoflorine), proaporphines (e.g. pronuciferine and pronuciferine *N*-oxide) (Fajardo *et al*, 2009), bisbenzylisoquinolines (e.g. tabienine, isotetrandrine, obamegine, and thaligrisine) (Suau *et al*, 1998), pseudobenzylisoquinolines (e.g. dihydrorugosinone) (Valencia, Weiss, and Shamma, 1984), aporphinebenzylisoquinolines (e.g. 1-*O*-methylpakistanine, kalashine, chitraline, and pakistanine) (Hussain *et al*, 1986), proaporphine-benzylisoquinolines (e.g. pakistannamine) (Hussain *et al*, 1986), benzytetrahydroisoquinolines (e.g. reticuline, glaucine, and thalicmidine) (Karimov *et al*, 1992; Imanshahidi and Hosseinzadeh, 2008), berberines, and protoberberines (e.g. tetrahydroberberine) (Karimov *et al*, 1992) among which berberine, berbamine, jatrorrhizine, and palmatine are abundant in content. Alkaloids are undetectable in fruits and leaves (Koncic *et al*, 2010a). Biphenyls and triterpens were largely isolated from twigs (Kim *et al*, 2009; Kim, Choi, and Lee, 2010). The major chemical constituents of fruits are polyphenols including flavonoids (anthocyanins, flavonols, and flavanols), condensed and hydrolysable tannins, stibenoids (resveratrol), phenolic acids, and phenylpropanoid (Vereskovskii and Shapiro, 1986; Seeram, 2008). Flavonoids also could be isolated from the leaves (Imanshahidi and Hosseinzadeh, 2008).

The yield of crude methanol extract and individual compounds varied among plant organs. Koncic *et al* (2010b) reported that the methanol extract was highest in fruits of *B. vulgaris* and *B. croatica* followed by leaves and lowest in roots or twigs, and berberine was largely found in twigs and roots while negligibly in leaves and fruits. Lv, Wang, and Xiao (1999) found that the amount of seven alkaloids (oxyacanthine,



berberine $R_1+R_2=CH_2$
jatrorrhizine $R_1=H$; $R_2=Me$
palmatine $R_1=R_2=Me$



berbamine

Fig. 1 Four major alkaloids in plants of *Berberis* L.

berbamine, isotetrandrine, columbamine, jatrorrhizine, palmatine, and berberine) from 17 species of *Berberis* L. was the highest in root barks followed by stem barks, root wood, and stem wood. In most roots of the 17 species, berberine was the richest alkaloid while berbamine, jatrorrhizine, and palmatine were irregular in content. Habitat-dependent variations in berberine content have been investigated (Andola *et al*, 2010). The berberine content was significantly higher in *B. asiatica* Roxb. ex. DC. at low altitude than at high altitude, which was significantly influenced by the moisture and potassium percentage of the soil (Andola *et al*, 2010).

Bioactive-guided isolation of bioactive compounds resulted in discovery of many nonalkaloids and alkaloids in this genus (Figs. 2－5). Recent studies showed that biphenyls and triterpenoids were isolated and identified from twigs of *B. kareana*, neither of which has been isolated from Berberidaceae (Kim *et al*, 2009; Kim, Choi, and Lee, 2010). Berbekorin, a novel biphenyl, exhibited cytotoxic activity against the SK-MEL-2 skin melanoma cell line, other biphenyls (e.g. methoxyaucuparin, 3,5-dimethoxybiphenyl-2′-ol, 2′-hydroxy-3,4,5-trimethoxybiphenyl, and ε-cotonefuran) had inhibitory activities against NO production in LPS-activated microglial cell line (BV-2) (Kim *et al*, 2009). Two triterpenoids, betulinic acid 3-β-*trans*-caffeate and betulinic acid 3-β-*cis*-caffeate, showed cytotoxicity against A594, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (Kim, Choi, and Lee, 2010). 1-Methyl

Table 1 Chemical constituents in plants of *Berberis* L. discovered from 2008 to 2011

Compounds	Representative species	Parts	References
Alkaloids			
Proaporphine-type			
pronuciferine *N*-oxide	*B. coletioides*	whole	Fajardo *et al*, 2009
pronuciferine	*B. coletioides*	whole	Fajardo *et al*, 2009
Bisbenzylisoquinoline-type			
tabienine	*B. tabiensis*	stems	Quevedo *et al*, 2008
Benzytetrahydroisoquinoline-type			
thalicmidine	*B. vulgris*	leaves	Imanshahidi and Hosseinzadeh, 2008
Berberine-type			
berlambine	*B. vulgris*	roots	Imanshahidi and Hosseinzadeh, 2008
Biphenyls			
berbekorin	*B. koreana*	trunks	Kim *et al*, 2009
2′-hydroxy-3,4,5-trimethoxybiphenyl	*B. koreana*	trunks	Kim *et al*, 2009
4,5-dihydroxy-3-methoxybiphenyl	*B. koreana*	trunks	Kim *et al*, 2009
eriobofuran	*B. koreana*	trunks	Kim *et al*, 2009
methoxyaucuparin	*B. koreana*	trunks	Kim *et al*, 2009
3,5-dimethoxybiphenyl-2′-ol	*B. koreana*	trunks	Kim *et al*, 2009
3-hydroxy-5-methoxybiphenyl	*B. koreana*	trunks	Kim *et al*, 2009
aucuparin	*B. koreana*	trunks	Kim *et al*, 2009
3,5-dimethoxybiphenyl	*B. koreana*	trunks	Kim *et al*, 2009
3,5-dimethoxybiphenyl-4′-ol	*B. koreana*	trunks	Kim *et al*, 2009
2′-hydroxyaucuparin	*B. koreana*	trunks	Kim *et al*, 2009
cotonefuran	*B. koreana*	trunks	Kim *et al*, 2009
9-hydroxyeriobofuran	*B. koreana*	trunks	Kim *et al*, 2009
cotonefuran	*B. koreana*	trunks	Kim *et al*, 2009
Triterpenoids			
23-*trans*-*p*-coumaroyloxy-2a,3a-digydroxy-olean-12-en-28-oic acid	*B. koreana*	trunks	Kim, Choi, and Lee, 2010
23-*cis*-*p*-coumaroyloxy-2a,3a-digydroxy-olean-12-en-28-oic acid	*B. koreana*	trunks	Kim, Choi, and Lee, 2010
3b-*trans*-caffeoyloxy-2a-hydroxyurs-12-en-28-oic acid	*B. koreana*	trunks	Kim, Choi, and Lee, 2010
3-*O*-*trans*-*p*-coumaroyltormentic acid	*B. koreana*	trunks	Kim, Choi, and Lee, 2010
betulinic acid 3b-*trans*-caffeate	*B. koreana*	trunks	Kim, Choi, and Lee, 2010
betulinic acid 3b-*cis*-caffeate	*B. koreana*	trunks	Kim, Choi, and Lee, 2010
betulinic acid	*B. koreana*	trunks	Kim, Choi, and Lee, 2010
3-epimaslinic acid	*B. koreana*	trunks	Kim, Choi, and Lee, 2010
pomolic acid	*B. koreana*	trunks	Kim, Choi, and Lee, 2010
ursolic acid	*B. vulgris*	fruits	Imanshahidi and Hosseinzadeh, 2008
Flavonoid			
chrysanthemin	*B. vulgris*	fruits	Imanshahidi and Hosseinzadeh, 2008
delphinidin-3-*O*-β-*D*-glucoside	*B. vulgris*	leaves	Imanshahidi and Hosseinzadeh, 2008
pelargonin	*B. vulgris*	fruits	Imanshahidi and Hosseinzadeh, 2008
petunidin-3-*O*-β-*D*-glucoside	*B. vulgris*	fruits	Imanshahidi and Hosseinzadeh, 2008
Flavonol			
hyperoside	*B. vulgris*	fruits leaves	Imanshahidi and Hosseinzadeh, 2008

(To be continued)

(Continued Table 1)

Compounds	Representative species	Parts	References
quercitrin	*B. vulgris*	leaves	Imanshahidi and Hosseinzadeh, 2008
Polyphenols			
delphinidin-3-glucoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
cyanidin-3-glucoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
petunidin-glucoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
peonidin-3-glucoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
malvidin-3-glucoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
delphinidin-3-rutinoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
cyanidin-3-rutinoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
petunidin-3-rutinoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
peonidin-3-rutinoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
malvidin-3-rutinoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
delphinidin-3,5-dihexoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
cyanidin-3,5-dihexoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
petunidin-3,5-dihexoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
peonidin-3,5-dihexoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
malvidin-3,5-dihexoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
delphinidin-3-rutinoside-5-glucoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
petunidin-3-rutinoside-5-glucoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
malvidin-3-rutinoside-5-glucoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
delphinidin-3-sambubioside-5-glucoside	*B. microphylla*	fruits	Ruiz *et al*, 2010
Phenylpropanoid			
caffeic acid	*B. vulgris*	fruit	Imanshahidi and Hosseinzadeh, 2008
chlorogenic acid	*B. vulgris*	fruit	Imanshahidi and Hosseinzadeh, 2008
Coumarin			
aesculetin	*B. vulgris*	fruit	Imanshahidi and Hosseinzadeh, 2008
Carbohydrate			
alpha-glucan	*B. vulgris*	leaf	Imanshahidi and Hosseinzadeh, 2008
pectin	*B. vulgris*	fruit	Imanshahidi and Hosseinzadeh, 2008
polysaccharide	*B. vulgris*	leaf	Imanshahidi and Hosseinzadeh, 2008
β-xylan	*B. vulgris*	leaf	Imanshahidi and Hosseinzadeh, 2008
sucrose	*B. vulgris*	fruit	Imanshahidi and Hosseinzadeh, 2008
vitamin			
ascorbic acid	*B. vulgris*	fruit leaf	Imanshahidi and Hosseinzadeh, 2008
vitamin K	*B. vulgris*	leaf	Imanshahidi and Hosseinzadeh, 2008

malate, a monoterpene, was isolated from the fruits of *B. integerrima* with enhancement of the antibacterial activity of Ampicillin (Alimirzaee *et al*, 2009). 5′-Methoxyhydnocarpin-D and pheophorbide A isolated from the leaves of *B. fremontii* Torrey were identified as flavonolignan and porphyrin, respectively, which were inhibitors of multidrug resistant pump (MDR) in *Staphylococcus aureus* (Stermitz *et al*, 2000b) (Fig. 5). It this study, the correct structure of the first MDR inhibitor was determined as 5′-methoxy- hydnocarpin-D, a flavonolignan, instead of 5′-methoxy-hydnocarpin, an analogue of hydnocarpin reported previously (Stermitz *et al*, 2000a). *O*-Methylisothalicberine, a bisbenzyl-isoquinoline alkaloid isolated from *B. chilensis* Gillies ex Hook, could significantly reduce mean arterial pressure in normotensive anaesthetized rats (Martinez, Torres, and Morales, 1997).

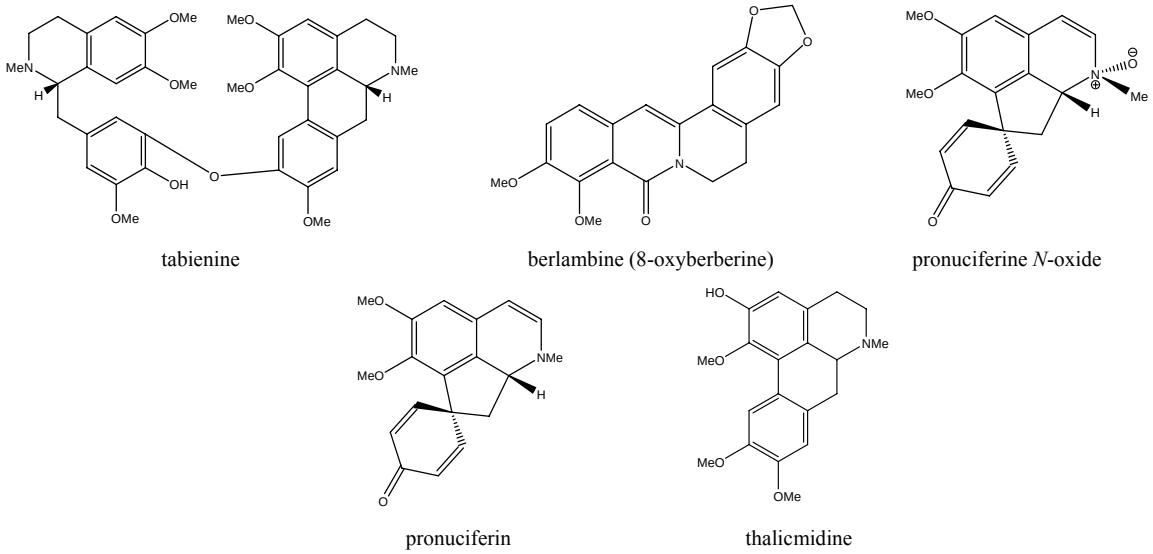


Fig. 2 Alkaloids in the genus *Berberis* L. discovered since 2008

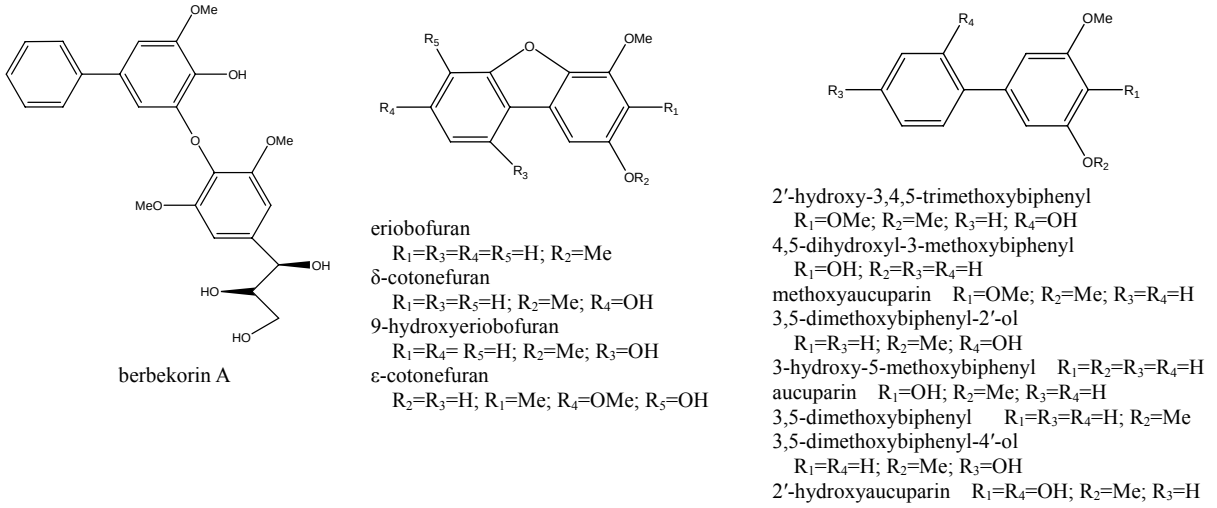


Fig. 3 Biphenyl compounds in plants of *Berberis* L.

Pharmacological activities of berberine and berbamine

Berberine and berbamine, the major components responsible for various bioactivities, have been intensively studied and reviewed (Tang *et al*, 2009; Imanshahidi and Hosseinzadeh, 2008; Kulkarni and Dhir, 2010; Vuddanda, Chakraborty, and Singh, 2010). Here we summarized important potential bioactivities and the corresponding novel pharmacological mechanisms. Since berberine and berbamine are close in chemical structure—monoisoquinoline and bisbenzylisoquinoline respectively, they possess similarity and differences in several pharmacological activities, therapeutic effects, and molecular mechanisms.

Anticancer activity

Berberine and berbamine could induce apoptosis in several tumor cell lines by loss in mitochondrial transmemberane potential and caspase activation, especially caspase-3 (Tang *et al*, 2009; Wang *et al*, 2007; 2009a; 2009b). They, however, possess difference and similarity in the mechanisms of apoptosis, cell-cycle arrest, cell-motility and invasion (Table 2).

Antitumor drugs are characterized by (1) promoting apoptosis including two pathways by activating caspase(s) cascade—Fas/FasL and mitochondria systems, Bcl-2, ICE, Apaf-1, c-myc, P53, ATM, and (2) inhibiting survival kinds of apoptosis e.g. PI3K/Akt, RAS-MAPK, ERK, NF-κB, and NO signaling pathways, and the inhibitor of apoptosis (IAP) family of proteins (XIAP, CIAP1, CIAP2, Survivin) and/or fusion genes encoding oncoproteins. Berberine seems more likely to promote positive to apoptosis,

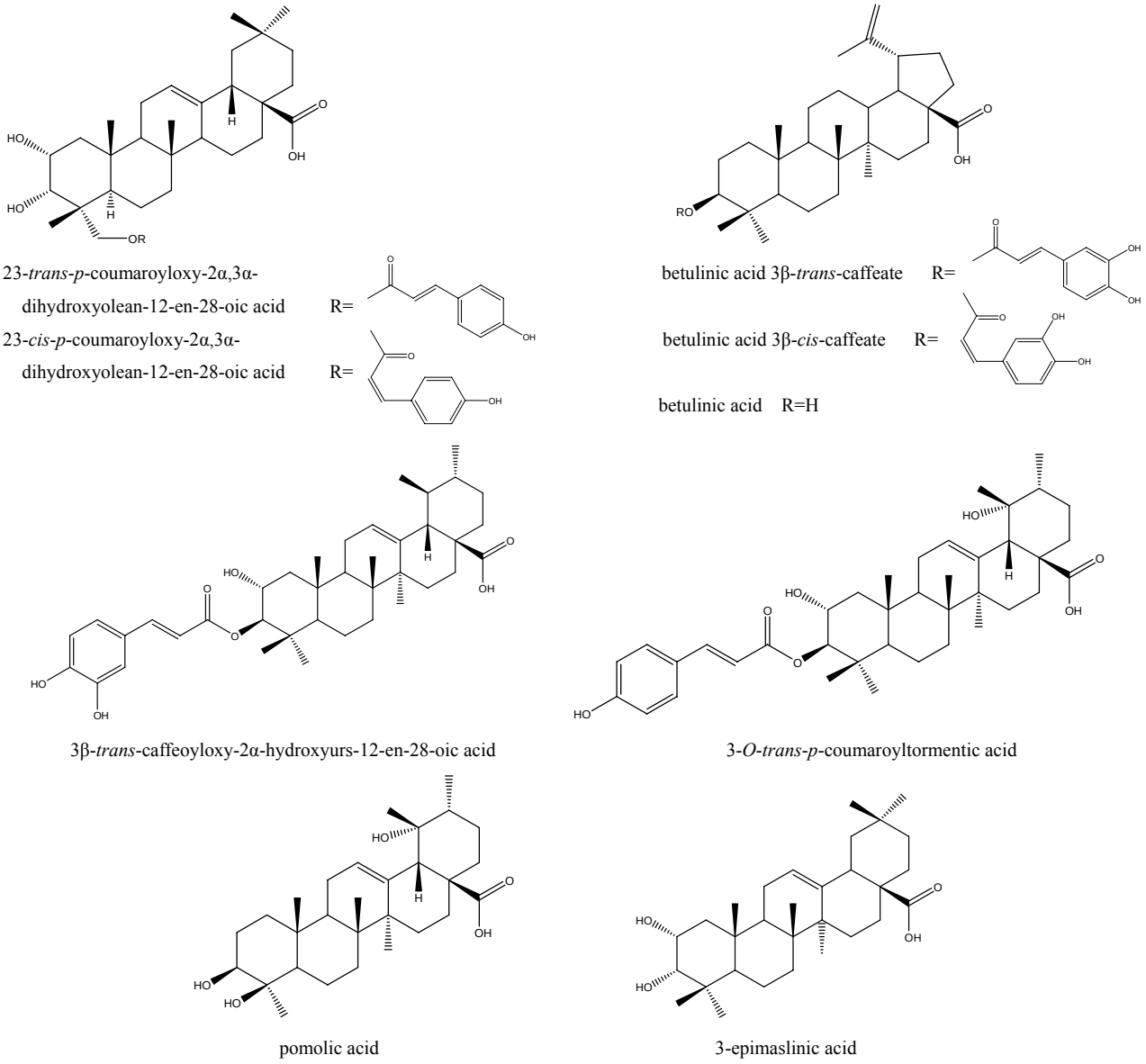
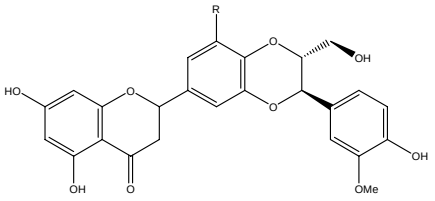


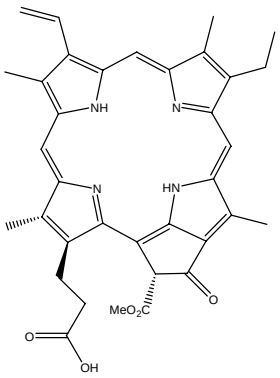
Fig. 4 Triterpenes in plants of *Berberis* L.

while berbamine and its analogues (e.g. 4-chlorobenzoyl berbamine) inclines to inhibit negatively to apoptosis or targeting oncogenes. They both induce Fas/FasL signaling pathways, activate P53, and alter ration of anti-apoptotic and pro-apoptotic members of the Bcl-2 family proteins (Wang *et al*, 2009a) in tumor cells. Berbamine and its analogues could induce apoptosis in tumor cells and multidrug resistant tumor cells by inhibiting PI3K/Akt and NF-κB signaling pathways (Wang *et al*, 2009b; Liang *et al*, 2009; Du *et al*, 2010) and surviving gene's expression (He *et al*, 2006), such as *bcr/abl* fusion gene which induces leukemia-like syndrome *in vivo* (Xu *et al*, 2006; Wei *et al*, 2009b; Ji *et al*, 2002), *mdr-1* and P-GP (Dong *et al*, 2004; Han *et al*, 2003; Wei *et al*, 2009a; 2009b)—the genes and the encoded protein that remove antitumor drugs out of tumor cells. In addition, Sun *et al* (2006) reported that berbamine reduced expression of Hsp90 protein, a molecular chaperone of p210 bcr/abl in chronic myeloid leukemia cells (Ph + K562) (Sun *et al*, 2006). Berbamine is also an inhibitor of telomerase which might play an important role in carcinogenesis and immortalization in human leukemia HL-60 cells (Ji *et al*, 2002).

Berberine and berbamine are able to induce the arrest in various phases of cell cycle. Berberine contributed to G_0/G_1, G_1, G_2, and G_2/M cell cycle arrests, and coordinated suppression of cyclin-dependent kinases (e.g., CDK 2, 4, and 6) and cyclins (e.g., cyclin B, D, and E) (Tang *et al*, 2009), while



5′-methoxyhydnocarpin-D



porphyrin

Fig. 5 Inhibitors of MDR isolated from *B. fremontii*

berbamine and its analogue (e.g. 4-chlorobenzoyl berbamine) induced cell-arrest at G_0/G_1 (Wang *et al*, 2007), G_1 (Liang *et al*, 2009), S (Dai *et al*, 2009), and G_2/M (Liu *et al*, 2010; Du *et al*, 2010), and down-regulated cyclin D_1 (Liang *et al*, 2009), B_1 and a cyclin-dependent kinase Cdc2/p34 (Liu *et al*, 2010), cyclin A, B and a cyclin-dependent kinase CDK1 (Du *et al*, 2010), which indicated *Sankezhen* could inhibit cell cycle at all phases.

Berberine and berbamine at low concentration could significantly inhibit migration ability of several human metastatic cancer cell lines by down-regulation of MMP proteins (e.g. MMP-1, MMP-2, and MMP-9) and some signaling pathways. Several studies showed that MAPK, NF-κB, and RhoA signaling pathways were involved in berberine-treated cells (Ho *et al*, 2009a; 2009b; Tsang *et al*, 2009), while Ca^{2+}-dependent signaling pathway did in berbamine-treated ones (Huang *et al*, 2010). In addition, berbamine could not only decrease MMP proteins but also increase TIMP-1 (Pan *et al*, 2005), and berberine could decrease nuclear transcription factors e.g., c-fos, c-jun, AP-1 (a complex of c-fos and c-jun), and NF-κB (Peng *et al*, 2006; Fukuda *et al*, 1999).

Both berberine and berbamine could inhibit cancer arising from leucocytes, liver, lung, stomach, colon, skin, oral, esophagus, brain, bone, and breast organs (Tang *et al*, 2009; Duan *et al*, 2009; Dai *et al*, 2009; Liang *et al*, 2009; Wang *et al*, 2009a) without toxicity against normal cells (Wang *et al*, 2009b). However, they are different and similar likely in antitumor mechanism largely due to chemical structures that the former is monoisoquinoline and the latter is dimiso-quinoline. It is primarily proposed that the two classes have tendency in opposite aspects of apoptosis, monoisoquinolines have tendency to positive aspect of apoptosis, while dimisoquinolines prefer to negative aspect of apoptosis which could integrate apoptosis. A recent study (Xie *et al*, 2009) seems to support this proposal that berbamine derives are a class of compounds for antileukemia activity by inhibition of the cytoplasm-to-nucleus translocation of NF-κB p65 which plays a critical role in the survival of leukemia stem cells. However, more evidences should be provided.

Immunopromotion and immunosuppression properties

Extracts of *Berberis* spp., such as *B. chengi* Chen, *B. lyceum* Royle, *B. asiatica* Roxb. ex DC., *B. crataegina* DC., and *B. koreana* (Cyran, 1989; Ikram, Ehsanul, and Warsi, 1966; Bhattarai, 1992; Yeşlada and Küpeli, 2002; Kim *et al*, 2010), berberine and berbamine showed immuomodulative properties including up- and down-regulation.

Berberine chloride could ameliorate the spatial memory impairment and increase the expression of IL-1β in improvement of Alzheimer's disease (Zhu and Qian, 2006). Berbamine is an immunopromotor in influenced mice, in which bioactivity of pulmonary macrophage is enhanced (Jin and Sui, 1986).

Berberine suppresses neuroinflammatory response through AMP kinase (AMPK) activation in BV-2 microglia, in which LPS- or interferon (IFN)-*γ*-induced iNOS and COX-2 expression is down-regulated (Lu *et al*, 2010). In an LPS-injured rat model, berberine significantly suppressed the increased TNF-α, IL-1β, and NO in plasma (Zhang *et al*, 2011). Jeong *et al* (2009) also reported that berberine suppressed proinflammatory response through AMPK activation in macrophages. Previous studies showed that berbamine had anti-inflammatory and immunosuppressive properties (Wong *et al*, 1992; Küpeli *et al*, 2002; Ren *et al*, 2008). Ren *et al* (2008) discovered that in EAE mice a novel immunomoduatoric mechanism was induced by

Table 2 IC_{50} values of berbamine and its derivatives against various tumor cell lines at 24, 48, and 72 h·μg^{-1}·mL^{-1}

Cells	Cell lines	IC_{50} / (?)	References
nasopharyngeal	KB	17.8	Marshall *et al*, 1994
cervical	ES-2	13.67	Huang *et al*, 2010
human hepatoma cell	SMMC7721	10.9**	Wang *et al*, 2007
	DEE		Liu *et al*, 2002
	HepG2	34.5*	Wang *et al*, 2009a
	7402	1.167	Liu *et al*, 2002
mouse hepatoma cell	H-22	3.312	Liu *et al*, 2002
human oral squamous cell carcinoma			
chronic myelogenous leukemia	K562 (resistant)	11.1**	Wei *et al*, 2009a
	K562	8**	Sun *et al*, 2006
human leukemia cell	Gleevec-sensitive-K562	8.8	Xu *et al*, 2006
	Gleevec-resistant-K562	11.34	Xu *et al*, 2006
	KG-1	54.4	Xu *et al*, 2006
	HL-60	15.0	Dong, Yang, and Kwan, 1997
	NL-60	< 10*	Ji *et al*, 2002
	K562/Adr (resistant)	21.9	Dong *et al*, 2004
human acute myelogenous leukemia	NB4	3.86**	Zhao *et al*, 2007
	NB4	3.86	He *et al*, 2006
	KM3	8.17	Liang *et al*, 2009
human breast cancer cells	MCF-7	13.0	Dai *et al*, 2009
	MDA-MB-231	13.7	Wang *et al*, 2009b
	MDA-MB-435S	25	Wang *et al*, 2009b
lymphoma cell line	Raji	0.9***	Du *et al*, 2010
	L428	1.16***	Du *et al*, 2010
	Namalwa	1.21***	Du *et al*, 2010
	Jurkat	1.24***	Du *et al*, 2010
human lung cancer	A549	< 20	Duan *et al*, 2009
human myeloma cell	KM3	3.84	Liang *et al*, 2009
human fibrosarcoma cells	HT1080	8.2	Pan *et al*, 2005

*: IC_{50} at 24 h; **: IC_{50} at 48 h; ***: IC_{50} of 4-chlorobenzoyl berbarmine; others: IC_{50} at 72 h

berbamine and probed its specific interactions with molecular targets, *vis*. Berbamine selectively inhibited IFN-γ through the Jak/Stat pathway in $CD4^+$ T cells, in which berbamine selectively promoted STAT4 proteosomal degradation by up-regulating SLIM, a ubiqutin E3 ligase for STAT4.

Berberine and berbamine suppressed the delayed-type hypersensitivity (DTH) reaction response with sheep red blood cells (SRBC) (Ivanovska and Philipov, 1996; Luo *et al*, 1998). Luo *et al* (1998) demonstrated immunosuppressive effect of berbamine by DTH, the mixed lymphocyte reaction, and a skin model of allograft rejection on mice, which indicated that berbamine could be a potential agent in clinical transplantation (Luo *et al*, 1998).

Antidiabetic activity

Berberine has significant effect on carbohydrate metabolites, while no reports for berbamine. Recent preclinical and clinical studies (Zhang *et al*, 2010; Kong *et al*, 2009) showed it acted on glucose metabolism in rats and human of diabetes mellitus type 2 through several mechanisms, such as mimicking insulin, improving insulin action by activating AMPK, reducing insulin resistance through protein kinase

C-dependent up-regulation of insulin receptor expression, inducing glycolysis, and on increstin by promoting GLP-1 secretion, modulating its release, and inhibiting DPP-4 (Steriti, 2010). In addition, berberine has a protective effect for diabetes though β-cell regeneration, anti-oxidant enzyme activity, and decreasing lipid peroxidation (Vuddanda, Chakraborty, and Singh, 2010). Clinical studies showed that berberine was as effective as metformin (3 × 500 mg of each) in the bioactivities of reducing elevated blood glucose. Adverse effects of transient gastrointestinal were fairly common in berberine-administrated patients (Zhang *et al*, 2010).

A recent study (Cui *et al*, 2009) reported that berberine could ameliorate diabetes mellitus type 1 NOD mice by suppressing T cell differentiation. In this study, berberine inhibited Th17 differentiation by activating ERK 1/2 and inhibited Th1 differentiation by inhibiting p38 MAPK and JNK activation, and it controlled the stability of STAT4 through the ubiquitin-proteasome pathway. This finding revealed that the immune regulation property of berberine was another mechanism for type 1 diabetes treatment.

Antihyperlipidemic activity

Lipid metabolic effect of berberine has been observed in human and animals, while no reports for berbamine. The mechanisms included upregulation of low-density lipoprotein receptor (LDLR), promotion of LPL activities and adipose hormones (visfatin and adiponectin), and inhibition of lipid biosynthesis and pro-lipid cell differentiation (Shen *et al*, 2010).

Berberine, only depending on ERK activation but sterol regulatory element binding protein (SREBP), elevates heptatic LDLP expression through a post-transcriptional mechanism that stabilizes the *LDLP* mRNA, and the responsible agent is the 5′ proximal section (AU-rich elements and tetranucleotide UCAU) of the *LDLR* mRNA 3′ untranslated region (3′ UTR), which is a unique mechanism distinct from statins (Kong *et al*, 2004). And *LCLR* mRNA stability is controlled by a group of AU-rich elements binding proteins including hnRNP D, hnRNP I, and KSRP. Berberine interferes with the binding proteins-receptor interaction, which is a likely mechanism for regulating LDLR expression (Li *et al*, 2009). Berberine may be useful supplement to statins due to the following reasons. It is time and dose-dependent to up-regulate heptic *LDLR* mRNA as well as down-regulate proteins convertase subtilisin/kexin type 9 (PCSK9) which is benefit to LDLR degradation. However, statins up-regulate both *LDLR* mRNA and PCSK9 depending on SREBP, which would contract therapeutic effects of statins by reducing PCSK9 expression. A combination of berberine and mevastatin increases LDLR mRNA and protein levels, while suppressing the increase in PCSK9 mRNA levels caused by mevastatin alone (Cameron *et al*, 2008). SP600125, the inhibitor of JNK, could suppress the berberine-induced up-regulation of LDLR mRNA because both ERK and JNK/c-jun are activated by berberine.

Berberine inhibits total cholesterol (TC) and triglyceride (TG) biosynthesis in HepG2 by activating of AMPK, and promotes oxidation of lipid acids as well. The underlying inhibitory mechanism is the same as AICAR–the activator of AMPK, and could be inhibited by PD98059—the inhibitor of MAPK/ERK (Shen *et al*, 2010).

Berberine inhibites 3T3-LI adipocyte different-tiation through PPAR pathway, in which the expressions of PPARα, β/δ, γ, and C/EBPα mRNA and proteins are inhibited. However, berberine up-regulates the expression of PPARα mRNA in hepG2 (Shen *et al*, 2010).

Antihypertensive activity

Previous studies demonstrated blood pressure lowering and vasorelaxatory effects of berberine and berbamine. For berberine, the mechanisms include α1-adrenoceptor antagonistic action in rat and rabbit aorta, ACE-inhibitory activity and direct release of NO/cGMP from rat aortic rings, the activation of tetrapentylamnels, 4-aminopyridine, and Ba^{2+}-sensitive K^+ channels, the inhibition of intracellular Ca^{2+} release from caffeine-sensitive pools, the blocking of *L*-type calcium channels, the potentiation of acetylcholine (Imanshahidi and Hosseinzadeh, 2008), the inhibition of rat vascular smooth muscle cell proliferation and migration *in vitro*, and the promotion of neointima formation after ballon injury *in vivo* or in a rat model (Lee *et al*, 2006). For berbamine, the major mechanism is blocking calcium channels (Guo and Fu, 2005). Han *et al* (2006) reported that *N*-methyl berbamine increased calcium-activated potassium currents in the rat mesenteric resistance vascular smooth muscle cells, which could lead to lower blood pressure.

Anti-arrhythmic activity

Berberine and berbamine are both found with anti-arrhythmic activities. Berbamine is a popular drug of anti-arrhythum through inhibiting ionic channels of sodium, potassium, calcium, etc., negative frequency and negative transduction, improving the diastolic excitation threshold of myocardium, and prolonging effective refractory period of myocardium (Guo and Fu, 2005).

Recent studies showed that berberine which has been used in diabetic patients with ischemic cardio-myopathy might effectively reduce the morbidity and lethality of myocardial infarction related arrythumias. Wang *et al* (2011) revealed that berberine could elicit this effect via I_{K1}/Kir2.1 in rat type 2 diabetic myocardial infarction model.

Anti-osteoporotic activity

Berberine by ig administration inhibited bone resorption of ovariectomized rats. And berberine inhibited osteoclast formation in coculture system of mouse primary osteoclastic cells and bone narrow cells in the presence of 1α, 25-dihydroxyvitamin D3, parathyroid hormone, and interleukin-1α (IL-1α) (Li *et al*, 1999). Hu *et al* (2008) reported that berberine inhibited RANKL-induced osteoclast ad survival through suppressing the NF-κB and Akt pathways. Berbeine attenuated RANKL-induced activation of NF-κB through inhibiting phosphorylation at the activation loop of IκBα kinase β, phosphorylation and degradation of IκBα, and NF-κB p65 nuclear translocation.

Neuroprotective activity

Berberine possesses neuroprotective activities in various animal models of neurodegenertative and neuropsychiatic disorders such as Alzheimer's disease, forebrain ischemia, mental depression, anxiety, pentylenetetrazole, maximal electroshock, kainic acid-induced convulsion (Bhutada *et al*, 2010), and disorders associated with exitotoxicity (Campisi *et al*, 2010) (Table 3).

Table 3 Therapeutic use of berberine in various CNS disorders and the underlying mechanisms

Clinical disease and syndrome	Mechanisms	References
Alzheimer's	an increased level of both IL-1band iNOS resulted in neuronal astabilization	Kulkarni and Dhir, 2010
	acetylcholinesterase inhibiting property	
	cholesterol lowering agent	
Schizophrenia	prolyl oligopeptidase inhibition	
	D2 dopamine receptor antagonist property	
Mental depression	monoamine oxidase property	
	norepinephrine, serotonin and dopamine transporter inhibitor	
	modulatory effect on sigma receptors	
Cerebral ischemia	*N*-methyl-d-aspartate receptors antagonistic activity	
	anti-inflammatory activity	
	reduced hydrogen peroxide-induced free calcium conentration elevation	
Anxiety	modulate sertonergic system in the body	
Glutamate-evoked excitotoxity	ameliorate excessive production of glutamate, protein misfolding and aggregation, mitochondrial fragmentationa nd neurodegenration	Campisi *et al*, 2010
Pentylenetetrazole, maximal electroshock, and kainic acid-induced convulsion	modulate neurotransmitter systems	Bhutada *et al*, 2010

Anti-oxidative activity

Berberine and berbamine have been found to be anti-oxidants (Guo and Fu, 2005, Shirwaikar *et al*, 2006). Thirupurasundari, Padmini, and Devaraj (2009) reported that berberine significantly attenuated the increase on lipid peroxidation and protein bound carbohydrates and enhanced the anti-oxidative status in azoxymethane-induced colon cancer in rats, which

indicated that berberine inhibited neoplastic transformation by the induction of anti-oxidant defence system and ability to induce apoptosis. Berbamine was also an anti-oxidant which scavenged O_2^- in alkaline DMSO and xanthine/xanthine oxidase systems, respectively, in mice livers (Ju and Han, 1990). Zhang, Ba, and Ren (1993) reported that berbamine could increase SOD and GSH-Px activities and reduce lipid peroxidation and O_2^- in rabbits with myocardial infarction. Zhou *et al* (1998) demonstrated that berbamine reduced O_2^- in rats with cerebral ischemia. Extracts of *Berberis* spp. (e.g. *B. aristata*, *B. vaulgaris*, *B. croatica*, and *B. microphylla*) had anti-oxidative activities (Singh and Kakkar, 2009; Koncic *et al*, 2010b; Hanachi, 2009; Ruiz *et al*, 2010), some of which were demonstrated to correlate well with the content of phenols and flavonols (Koncic *et al*, 2010b; Ruiz *et al*, 2010).

Discussion

Determination of botanical origins

Botanical origin is the first important issue in development of quality assessment of *Sankezhen*. As we know, *Sankezhen* has wide botanical origins which spread worldwide. It is essential to determine the representative species, the corresponding producing areas, and the harvest periods which certainly will contribute to variations in chemical composition and contents. As a result, the quality of *Sankezhen* is approaching controllable, the ideal botanical origins are characterized by high yield (e.g. population biomass and desired compound contents) and stabile genetics, which should be further investigated and determined.

Quality and quantity of alkaloids in various parts of *Berberis* spp.

Chemical constituents in the representative species should be intensively isolated and identified, which is the base for quality control and pharmaceutical effects.

Xiao *et al* (1974) qualitatively and quantificationally determined berberine, palmatine, jatrorrhizine, and berbamine from over 40 species by TLC and spectrophotometry. Later studies were focused upon the analysis of contents of those alkaloids in *Berberis* spp. from different habitats and harvest periods, different species and the various parts by HPLC in China, aiming to find out alkaloid distribution in various parts of plants and content variations in different conditions (Lv, Wang, and Xiao, 1999; Yang, Ma, and Qi, 2009). Few studies have been intensively carried out upon chemical compounds of the species of *Berberis* L. in China, and only Wang *et al* (2009c) investigated the chemical constituents in *B. sargentiana*. Moreover, several studies (Kim *et al*, 2009; Kim, Choi, and Lee, 2010) have demonstrated that plants in *Berberis* L. species produced various interesting chemical structures besides the four common alkaloids (see the last paragraph). Thus, we believe that it is very valuable to systematically study chemical components from each species, especially the representatives and the four species admitted in *Chinese Pharmacopeia 2010*. Obviously chemical constituents in detail are quite necessary in the establishment of quality control methods of great specialization. In addition, it is suggested to investigate the certain producing areas and the harvest periods since they greatly affect the contents of chemical compounds, especially for such a herb as *Sankezhen* with rather wide distribution in China.

Development of authentication approaches

Chemical constituents, bioactivities, and botanical origin are the important factors in authentication of a Chinese materia medica. Berberine is the only chemical marker for quality assessment of *Sankezhen* in *Chinese Pharmacopeia 2010*, which is the single-compound analysis approach (Yuan, Zhang, and Xiao, 2011). However, simultaneous analysis on four alkaloids including berberine, berbamine, jatrorrhizine, and palmatine by HPLC-DAD has been reported in several papers (Di *et al*, 2004; Lv, Wang, and Xiao, 1999). HPLC simultaneous analysis on the four chemical markers is increasingly popular in recent years, which is the multi-compound analysis approach (Yuan, Zhang, and Xiao, 2011). HPLC spectrum of the four alkaloids will be more accurate in authentication than one. Sometimes it is difficult to distinguish *Sankezhen* and *Huangbai* due to similarity in spiny. As a result, folk people mis-submit *Sankezhen* as *Huangbai* so multi-compound analysis approach will be a method of more accurate and feasible in identification. Thus HPLC simultaneous determination of four markers might be used for assessing quality of *Sankezhen*.

Obviously other authentication approaches need to be developed to overcome the limitation of each

approach. Bioassay approach is a new quality control method for Chinese materia medica, which shows the advantages in case of uncertainty of chemical compounds and is expected to be well developed in the Guide of *Chinese Pharmacopiea 2010*. This approach addresses the biological properties which closely relate to efficacy and safety. Yan *et al* (2008) assayed antibacterial potency of berberine alkaloids in *Coptis Rhizoma* using microcalorimetry and thermoanalysis, which is a promising method in quality assessment.

DNA-based markers and genomics are the promising methods for authentication of medicinal plants (Hao *et al*, 2009). And DNA barcoding—a method that is the use of short DNA sequences for species identification (Han *et al*, 2010), is expected to be accepted in updating *Chinese Pharmacopeia* due to the advantages of quickness and accuracy. However, it is doubted in the genus of *Berberis* L. because universal plant DNA barcode loci may not work in a complex genus (Roy *et al*, 2010). This is a result obtained from Indian berberis samples, which is not fully convinced in Chinese berberis. Nevertheless, it is quite hard to identify origin plants of *Berberis* L. on market. Therefore, proper methods of DNA-based markers should be established.

Pharmaceutical and therapeutic effects of *Sankezhen*—a prospect natural resource for drug discovery

Sankezhen has wide potential therapeutic effects on diseases from cardiovascular, immune, central nervous, respiratory, and several stubborn diseases such as cancer, diabetes, Alzheimer's disease, etc. However, some of the pharmaceutical effects are uncertain in clinical practice, which indicates that there is a long way from discovery of bioactivities to clinical practice. Fortunately, there is an example for *Sankezhen* studies, that is *Huanglian* (*Coptidis Rhizoma*) and berberine, which have been quite intensively studied and recognized as novel antineoplastic agents (Tang *et al*, 2009), and *Sankezhen* is a substitute of *Huanglian* in TCM to treat gastrointestinal diseases. Nevertheless, we do not suppose that *Sankezhen* is identical to *Huanglian* in all pharmaceutical effects. Since few studies are available in identical property, the two herbs are suggested to be studied in identities and differences including chemical constituents, pharmaceutical bioactivities, and ethnopharmacology (e.g. nature, flavour, and favored channel tropisms).

Other parts of *Berberis* spp. and active ompounds

Twigs, trunks, and barks are bioactive parts of *Berberis* spp. due to accumulation of alkaloids (Table 1), however they are unable to substitute the roots unless clinical efficacy evidences are provided. New classes of chemical compounds (e.g. triterpenes and biphenyls from trunks of *B. koreana*) (Kim *et al*, 2009) and novel compounds from the extract of those parts of the species of *Berberis* L. have been isolated and identified by bioactivity-guided assays, which indicated that this genus was a great reservoir with potential wide spectrum therapeutical effects. Triterpenes from trunks of *B. koreana* are characterized by five rings including oleanane, ursane, and lupine, which are known in antitumor bioactivities against various cancer cell lines (Sun and Li, 2009). Biphenyls are another class of compounds isolated from *Berberis* L. with several substituent groups of $-OCH_3$ and OH, which indicates the bioactivities of depressing center central nervous system and relaxing muscles due to similar chemical structures with magnolol. We suppose that diversity of chemical groups contributes to multispectrum bioactivities of *Berberis* spp. properly in some ways different from alkaloids. However, further studies are needed upon bioassays of those compounds from the plants of *Berberis* L.

Fruits are also the important pharmaceutical part of *Berberis* spp. in some regions or ethnic minorities which are promising pharmaceutical parts and deserve further studies, e.g., in China, the fruits of *B. heteropoda* Schrenk, neutral in nature and invigorating the gastrointestinal tract and clearing away heat and toxic material, are authenticated as the botanical origin in Ha ethnopharmacology to treat gastroenteritis, dysentery, dyspepsia, aphthous stomatitis, and vitamin E deficiency disease (Ha and Xv, 2005). Fruits of *B. henryana* Schneid are used to treat infantile convulsion, release wind and fire from inner body in ethnic minority areas of Sichuan Province in China (Xiao *et al*, 1974). In Croatia, fruits of *B. croatica* and *B. vulgaris* are used for ailments and discomforts of the kidney and urinary tract, gastrointentinal tract, liver disease, bronchial discomfort, spleen ailments, spasms, and as a stimulant for the circulatory system (Koncic *et al*,

2010b). *B. vulgaris* fruit is known for its anti-arrhythmic and sedative effects in Iranian traditional medicine (Fatehi *et al*, 2005). A few papers reported that chemical constitutes of fruits were polyphenols including flavonoids (anthocyanins, flavonols, and flavanols), condensed and hydrolysable tannis, stibenoids (resveratrol), phenolic acids, and phenylpropanoid. However, fruits of *Berberis* spp. in China have never been systematically or intensively studied on chemical constituents and biological activities, which needs to be carried out for the discovery of new botanical resources as crude drugs.

Acknowledgements

Authors thank Mr. Zhai WM of Chinese Pharmacopoeia Commission for assisting references.

References

Alimirzaee P, Gohari AR, Hajiaghaee R, Mirzaee S, Jamalifar H, Monsef-Esfahani HR, Amin Gh, Saeidnia S, Shahverdi AR, 2009. 1-Methyl malate from *Berberis integerrima* fruits enhances the antibacterial activity of ampicillin against *Staphylococcus aureus*. *Phytother Res* 23: 797-800.

Andola HC, Garia KS, Rawal RS, Rawat MMS, Bhatt ID, 2010. Habitat-dependent variations in berberine content of *Berberis asiatica* Roxb. ex. DC in Kumaon, Western Himalaya. *Chem Biodiver* 7: 415-420.

Bhattarai NK, 1992. Folk herbal remedies of Sindupalchok district, Central Nepal. *Fitoterapia* 63: 145-155.

Bhutada P, Mundhada Y, Bansod K, Dixit P, Umathe S, Mundhada D, 2010. Anticonvulsant activity of berberine, an isoquinoline alkaloid in mice. *Epilepsy Behav* 18: 207-210.

Cameron, J, Ranheim T, Kulseth MA, Leren TP, Berge KE, 2008. Berberine decreases PCSK9 expression in HepG2 cells. *Atherosclerosis* 201: 266-273.

Campisi A, Acquaviva R, Mastrojeni S, Raciti G, Vanella A, De Pasquale R, Puglisi S, Iauk L, 2010. Effect of berberine and *Berberis aetnensis* C. Presl. alkaloid extract on glutamate-evoked tissue transglutaminase up-regulation in astroglial cell cultures. *Phytother Res*

Committee of *Chinese Herbacology*, 2004. *Zhonghua Bencao* (*Chinese Herbacology*). Shanghai Scientific & Technical Publishers: Shanghai.

Cui GL, Qin X, Zheng YB, Gong ZW, Ge BX, Zang YQ, 2009. Berberine differentially modulated the activities of ERK, p38 MAPK, and JNK to suppress Th17 and Th1 T cell differentiation in type 1 diabetic mice. *J Biol Chem* 284: 28420-28429.

Cyran HB, 1989. *The Asian Way with Herbs*, vol. 15. PRP Books, NSW, Australia.

Dai XH, Liu R, Zhou Y, Yang M, Fan DM, Xiong DS, Qi J, Yang CZ, Zhu HF, 2009. Effect of calmodulin antagonist *O*-4-ethoxiyl-butyl-berbamine on the proliferation of human breast cancer cell line MCF-7 and its relevant mechanism. *Acta Acad Med Sin* 31: 326-329.

Di DL, Wang Q, Ma ZG, Jiang SX, 2004. Distribution of alkaloids in *Berberis* spp. *J Chin Med Mater* 27: 83-85.

Dong Y, Yang MMP, Kwan CY, 1997. *In vitro* inhibition of proliferation of HL-60 cells by tetrandrine and coriolus versicolor peptide derived from Chinese medicinal herbs. *Pharmacol Lett* 60: 135-140.

Dong QH, Zheng S, Xu RZ, Lv QH, He LM, 2004. Study on effect of berbamine on multidrug resistance Leukemia K562/Adr cells. *Chin J Integr Med* 24: 820-822.

Du HP, Shen JK, Yang M, Wang YG, Yuan XG, Ma QL, Jin J, 2010. 4-Chlorobenzoyl berbamine induces apoptosis and G_2/M cell cycle arrest through the PI3K/Akt and NF-κB signal pathway in lymphoma cells. *Oncol Rep* 23: 709-716.

Duan HY, Luan JL, Liu Q, Yagasaki K, Zhang GY, 2009. Suppression of human lung cancer cell growth and migration by berbamine. *Cytotechnology* 62: 341-348.

Fajardo V, Araya M, Cuadra P, Oyarzun A, Gallardo A, Cueto M, Diaz-Marrero AR, Darias J, Villarroel L, Alvarez C, Mora-Pérez Y, Joseph-Nathan P, 2009. Pronuciferine *N*-oxide, a proaporphine *N*-oxide alkaloid from *Berberis coletioides*. *J Nat Prod* 72: 1355-1356.

Fatehi M, Saleh TM, Fatehi-Hassanabad Z, Farrokhfal K, Jafarzadeh M, Davodi S, 2005. A pharmacological study on *Berberis vulgaris* fruit extract. *J Ethnopharmacol* 102: 46-52.

Fukuda K, Hibiya Y, Mutoh M, Koshiji M, Akao S, Fujiwara H, 1999. Inhibition of activator protein 1 by berberine in human hepatoma cells. *Planta Med* 65: 381-383.

Gulfraz M, Asad MJ, Qaddir G, Mehmood S, Shaukat S, Parveen Z, 2008. Phytochemical constituents of *Berberis lyceum* Royle and *Justicia adhatoda*. *J Chem Soc Pak* 30: 453-457.

Guo ZB, Fu JG, 2005. Progress of cardiovascular pharmacologic study on berbamine. *CJITWM* 25: 765-768.

Ha LD, Xv Q, 2005. Quality assessment of fruits of *Berberis* spp. *Lishizhen Med Mater Med Res* 16: 763.

Han DY, Nie HG, Chen L, Song ZG, Li JM, 2006. Effect of *N*-methyl berbamine on calcium-activated potassium currents in rat mesenteric artery smooth muscle cell. *Chin Pharmacol Bull* 22: 488-491.

Han JP, Liu C, Li MH, Shi LC, Song JY, Yao H, Pang XH, Chen SL. Relationship between DNA barcoding and chemical classification of *Salvia* medicinal herbs. *Chin Herb Med* 2010, 2(1): 16-29.

Han YQ, Yuan JY, Shi YJ, Zhu Y, Wu SL, 2003. Reversal effect of berbamine on multidrug resistance of K562/A02 cells and its mechanism. *J Exper Hemat* 11: 604-608.

Hanachi P, 2009. Using HPLC to determination the composition and anti-oxidant activity of *Berberis vulgaris*. *Eur J Sci Res* 29: 47-54.

Hao DC, Che SL, Xiao PG, Peng Y, 2009. Authentication of medicinal plants by DNA-based markers and genomics. *Chin Herb Med* 2: 250-261.

He ZW, Zhao XY, Xu RZ, Wu D, 2006. Effects of berbamine on growth of leukemia cell line NB4 and its mechanism. *J Zhejiang Univ*: *Med Sci* 35: 209-214.

Ho YT, Yang JS, Li TC, Lin JJ, Lin JG, Lai KC, Ma CY, Wood WG, Chung JG, 2009a. Berberine suppresses *in vitro* migration and invasion of human SCC-4 tongue squamous cancer cells through the inhibitions of FAK, IKK, NF-κB, u-PA and MMP-2 and -9.

Cancer Lett 279: 155-162.

Ho YT, Yang JS, Lu CC, Chiang JH, Li TC, Lin JJ, Lai KC, Liao CL, Lin JG, Chung JG, 2009b. Berberine inhibits human tongue squamous carcinoma cancer tumor growth in a murine xenograft model. *Phytomedicine* 16: 887-890.

Hu JP, Nishishita K, Sakai E, Yoshida H, Kato Y, Tsukuba T, Okamoto K, 2008. Berberine inhibits RANKL-induced osteoclast formation and survival through suppressing the NF-κB and Akt pathways. *Eur J Pharmacol* 580: 70-79.

Huang R, Jiang LL, Sun XM, Zhou Y, Cheng X, Yang M, Xu YF, Xiong DS, Wang CY, Yang CZ, Pan B, Zhu HF, 2010. The metastasis suppressing ability and its mechanism of calmodulin antagonist EBB in human metastasis ovarian clear cell line ES-2. *Chin Pharmacol Bull* 26: 642-645.

Hussain SF, Siddiqui MT, Khan L, Freyer AJ, Guinaudeau H, Shamma M, 1986. Berbamine 2'-beta-*N*-oxide, a new bisbenzyl-isoquinoline from *Berberis brandisiana*. *J Nat Prod* 49(3): 538-539.

Ikram M, Ehsanul M, Warsi SA, 1966. Alkaloids of *Berberis lycium.* Royle-1. *Pakistan J Sci Indust Res* 9: 343-346.

Imanshahidi M, Hosseinzadeh H, 2008. Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. *Phytother Res* 22: 999-1012.

Ivanovska N, Philipov S, 1996. Study on the anti-inflammatory action of *Berberis vulgaris* root extract, alkaloid fractions and pure alkaloids. *Int J Immunopharmacol* 18: 553-561.

Jeong HW, Hsu KC, Lee JW, Ham M, Huh JY, Shin HJ, Kim WS, Kim JB, 2009. Berberine suppresses proinflammatory responses through AMPK activation in macrophages. *Am J Endocrinol Metab* 296: E955-E964.

Ji ZN, Ye WC, Liu GQ, Huang Y, 2002. Inhibition of telomerase activity and *bcl-2* expression in berbamine-induced apoptosis in HL-60 cells. *Planta Med* 68: 596-600.

Jin L, Sui WZ, 1986. Effect of berbamine on immunologic function of influenced mice. *Acta Pharmacol Sin* 7(5): 475-479.

Ju HS, Han ZW, 1990. Anti-oxidant effect of berbamine. *Acta Pharmacol Sin* 11(6): 539-541.

Karimov A, Butayarov AB, Yusupov MM, Mirzamatov RT, Shakirov RSh, 1992. Berberis alkaloids XIII. An investigation of the alkaloids *Berberis heteropoda*. *Chem Nat Comp* 28: 523-524.

Kim BH, Lee J, Shen T, Kim JD, Cho JY, 2010. Anti-inflammatory activity of hot water extract of *Berberis koreana* in lipopolysaccharide-induced macrophage-like cells. *J Med Plants Res* 4: 745-752.

Kim KH, Choi SU, Ha SK, Kim SY, Lee KR, 2009. Biphenyls from *Berberis koreana*. *J Nat Prod* 72: 2061-2064.

Kim KH, Choi SU, Lee KR, 2010. Bioactivity-guided isolation of cytotoxic triterpenoids from the trunk of *Berberis koreana*. *Bioorg Med Chem Lett* 20: 1944-1947.

Koncic MZ, Kremer D, Karlovic K, Kosalec, 2010a. Evaluation of anti-oxidant activities and phenolic content of *Berberis vulgaris* L. and *Berberis croatica* Horvat. *Food Chem Toxicol* 48: 2176-2180.

Koncic MZ, Kremer D, Schuly W, Brantner A, Karlovic K, Kalodera Z, 2010b. Chemical differentiation of *Berberis croatica vs B. vulgaris* using HPLC fingerprinting. *Croat Chem Acta* 83: 451-456.

Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, Wang Y, Wang Z, Si S, Pan H, Wang S, Wu J, Wang Y, Li Z, Liu J, Jiang JD, 2004. Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nat Med* 10(12): 1344-1351.

Kong WJ, Zhang H, Song DQ, Xue R, Zhao W, Wei J, Wang YM, Shan N, Zhou ZX, Yang P, You XF, Li ZR, Si SY, Zhao LX, Pan HN, Jiang JD, 2009. Berberine redueces indulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression. *Meta Clin Exp* 58: 109-119.

Kulkarni SK, Dhir A, 2010. Berberine: A plant alkaloid with therapeutic potential for central nervous system disorders. *Phytoher Res* 24: 317-324.

Kùpeli E, Koşar M, Yeşilada, Başer HC, Başer C, 2002. A comparative study on the anti-inflammatory, antinociceptive and antipyretic effects of isoquinoline alkaloids from the roots of Turkish *Berberis* species. *Life Sci* 72: 645-657.

Lee S, Lim HJ, Park HY, Lee KS, Park JH, Jang Y, 2006. Berberine inhibits rat vascular smooth muscle cell proliferation and migration *in vitro* and improves neointima formation after balloon injury *in vivo*. Berberine improves neointima formation in a rat model. *Atherosclerosis* 186: 29-37.

Li H, Chen W, Zhou Y, Abidi P, Sharpe O, Robinson WH, Kraemer FB, Liu J, 2009. Identification of mRNA binding proteins that regulate the stability of LDL receptor mRNA through AU-rich elements. *J Lipid Res* 50: 820-831.

Li H, Miyahara T, Tezuka Y, Namba T, Suzuki T, Dowaki R, Watanabe M, Nemoto N, Tonami S, Seto H, Kadota S, 1999. The effect of kampo formulae on bone resorption *in vitro* and *in vivo*. II. Detailed study of berberine. *Biol Pharm Bull* 22: 391-396.

Liang Y, Xu RZ, Zhang L, Zhao XY, 2009. Berbamine, a novel nuclear factor κB inhibitor, inhibits growth and induces apoptosis in human myeloma cells. *Acta Pharmacol Sin* 30: 1659-1665.

Liu JW, Qi SL, Zhu HF, Zhang JH, Li Z, Wang T, 2002. The effect of calmodulin antagonist berbamine derivative-EBB on hepatoma *in vitro* and *in vivo*. *Chin Med J Engl* 115: 759-762.

Liu R, Zhang YJ, Chen YH, Qi J, Ren SM, Xushi MY, Yang CZ, Zhu HF, Xiong DS, 2010. A novel clmodulin antagonist *O*-(4-ethoxyl-butyl)-berbamine overcomes multidrug resistance in drug-resistant MCF-7/ADR breast carcinoma cells. *J Pharm Sci* 99: 3266-3275.

Lu DY, Tang CH, Chen YH, Wei IH, 2010. Berberine suppresses neuroinflammatory responses though AMP-activated protein kinase activation in BV-microgia. *J Cell Biochem* 110: 697-705.

Luo CN, Lin X, Li WK, Pu F, Wang LW, Xie SS, Xiao PG, 1998. Effect of berbamine on T-cell mediated immunity and the prevention of rejection on skin transplants in mice. *J Ethnopharmacol* 59(3): 211-215.

Lv GH, Wang LW, Xiao PG, 1999. Determination of alkaloids in berberry (*Berberis* L.) plants and comprehensive utilization of their resources. *Chin Tradit Herb Drugs* 30(6): 428-430.

Marshall SJ, Russell PF, Wright CW, Anderson MM, Phillipson JD, Kirby GC, Warhust DC, Schiff PL, JR, 1994. *In vitro* antiplasmodial, antiamoebic, and cytotoxic activities of a series of bisbenzylisoquinoline alkaloids. *Antimicrob Agents Chemother* 38: 96-103.

Martinez JL, Torres R, Morales M, 1997. Hypotensive effect of *O*-methylisothalicberine, a bisbenzylisoquinoline alkaloid isolated from *Berberis chilensis* on normotensive rats. *Phytotherapy Res*

11: 246-248.

Pan B, Zhou Y, Qi J, Xiong DS, Liu WJ, Qi SL, Cheng YH, Yang CZ, Zhu HF, 2005. Effect of calmodulin antagonist EBB on invision of human fibrosarcoma cell HT1080. *Acta Acad Med Sin* 27: 311-314.

Peng PL, Hsieh YS, Wang CJ, Hsu JL, Chou FP, 2006. Inhibitory effect of berberine on the invasion of human lung cancer cells via decreased productions of urokinase-plasminogen activator and matrix metalloproteinase-2. *Toxicol Appl Pharm* 214: 8-15.

Pharmacopoeia Committee of P. R. China, 1977. *Pharmacopoeia of People's Republic of China*. People's Medical Publishing House: Beijing.

Pharmacopeia Committee of P. R. China, 2010. *Pharmacopoeia of People's Republic of China*. China Medical Science and Technology Press: Beijing.

Quevedo R, Valderrama K, Moreno-Murillo B, Laverde M, Fajardo V, 2008. A new bisbenzyltetrahydroisoquinoline alkaloid from *Berberis tabiensis* (Berberidaceae). *Biochem Syst Ecol* 36: 812-814.

Rashmi, Rajasekaran A, Pant J, 2008. The genus *Berberis* Linn.: A review. *PHCOG Rev* 2: 369-385.

Ren YP, Lu LM, Guo TB, Qiu J, Yang YQ, Liu AL, Zhang JWZ, 2008. Novel immunomodulatory properties of berbamine through selective down-regulation of STAT4 and action of IFN-γ in experimental autoimmune encephalomyelitis. *J Immunol* 181: 1491-1498.

Roy S, Tyagi A, Shukla V, Kumar A, Singh UM, Chaudhary LB, Datt B, Bag SK, Singh PK, Nair NK, Husain T, Tuli R, 2010. Universal plant DNA barcode loci may not work in complex groups: A case study with Indian *Berberis* species. *Plos ONE* 5: e13674.

Ruiz A, Hermosí-Gutiérrez I, Mardones C, Vergara C, Herlitz E, Vega M, Dorau C, P Winterhalter, Von Baer D, 2010. Polyphenols and anti-oxidant activity of Calafate (*Berberis microphylla*) fruits and other native berries from Southern Chile. *J Agric Food Chem* 58: 6081-6089.

Seeram NP, 2008. Berry fruits: Compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. *J Agric Food Chem* 56: 627-629.

Shen YB, Piao XS, Kim SW, Wang L, Liu P, 2010. The effects of berberine on the magnitude of the acute inflammatory response induced by *Escherichia coli* lipopolsaccharide in broiler chickens. *Immunol Health Dis* 89: 13-19.

Shirwaikar A, Shirwaikar A, Rajendran K, Punitha ISR, 2006. *In vitro* anti-oxidant studies on the benzyl tetra isoquinoline alkaloid berberine. *Biol Pharm Bull* 29(9): 1906-1910.

Singh J, Kakkar, 2009. Antihyperglycemic and anti-oxidant effect of *Berberis aristata* root extract and its role in regulating carbohydrate metabolism in diabetic rats. *J Ethnopharmacol* 123: 22-26.

Steriti R, 2010. Berberine for diabeties mellitus type 2. *Nat Med J* 2: 5-6.

Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K, 2000a. Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor. *PNAS* 97: 1433-1437.

Stermitz FR, Tawara-Matsuda J, Lorenz P, Mueller P, Zenewicz L, Lewis K, 2000b. 5′-Methoxyhydnocarpin-D and pheophorbide A: *Berberis* species components that potentiate berberine growth inhibition of resistant *Staphylococcus aureus*. *J Nat Prod* 63: 1146-1149.

Suau R, Rico R, Lopez-Romero JM, Najera F, Cuevas A, 1998. Isoquinoline alkaloids from *Berberis vulgaris* subsp. *australis*. *Phytochemistry* 49: 2545-2549.

Sun CS, Li ML, 2009. Progress of pentacyclic triterpenes in antitumor activities and the machanisms. *Chin J Ethnomed Ethnopharm* 12: 14-16.

Sun JR, Zhang XH, He ZW, Gu Y, Yu YZ, Fang YM, Lu QH, Dong QH, Xu RZ, 2006. The mechanism of apoptosis of chronic myeloid leukemia cells induced by the novel p210 ber/abl inhibitor berbamine. *Natl Med J China* 86: 2246-2251.

Tang J, Feng YB, Tsao SW, Wang N, Curtain R, Wang YW, 2009. Berberine and *Coptidis Ghizoma* as novel antineoplastic agents: A review of traditional use and biomedical investigations. *J Ethnopharm* 126: 5-17.

Thirupurasundari CJ, Padmini R, Devaraj SN, 2009. Effect of berberine on the anti-oxidant status, ultrastructural modifications and protein bound carbohydrates in azoxymethane-induced colon cancer in rats. *Chemico-Biol Inter* 177: 190-195.

Tsang CM, Lau Echo PW, Di K, Cheung PY, Hau PM, Ching YP, Wong YW, Cheung Annie, LM, WanThomas SK, Tong Y, Tsao SW, Feng Y, 2009. Berberine inhibits RhoGTPases and cell migration at low doses but induces G_2 arrest and apoptosis at high doses in human cancer cells. *Intern J Mol Med* 24: 131-138.

Valencia E, Weiss I, Shamma M, 1984. Dihydrorugosinone, a pseudobenzylisoquinoline alkaloid from *Berberis darwinii* and *Berberis actinacantha*. *J Nat Prod* 47: 1050-1051.

Vereskovskii VV, Shapiro DK, 1986. Flavonoids, phenolic acids, and hydroxycoumarins from the fruit of various species of the genus *Berberis*. *Chem Nat Compd* 22: 482-483.

Vuddanda PR, Chakraborty S, Singh S, 2010. Berberine: A potential phytochemical with multispectrum therapeutic activities. *Drug Eval* 19: 1297-1307.

Wang GY, Lv QH, Qi D, Xu RZ, Dong QH, 2009a. Berbamine induces Fas-mediated apoptosis in human hepatocellular carcinoma HepG2 cells and inhibits its tumor growth in nude mice. *J Nat Asian Prod Res* 11: 219-228.

Wang GY, Zhang JW, Lü QH, Xu RZ, Dong QH, 2007. Berbamine induces apoptosis in human hepatoma cell line SMMC7721 by loss in mitochondrial transmembrane potential and caspase activation. *J Zhejiang Univ Sci B* 8: 248-255.

Wang LH, Yu CH, Fu Y, Li Q, Sun YQ, 2011. Berberine elicits anti-arrhythmic effects via IK1/Kir2.1 in the rat type 2 diabetic myocardial infarction model. *Phytother Res* 25(1): 33-37.

Wang S, Liu Q, Zhang Y, Liu K, Yu PF, Liu K, Luan JL, Duan HY, Lu ZQ, Wang FF, Wu EX, Yagasaki K, Zhang GY, 2009b. Suppression of growth, migration and invasion of highly-metastatic human breast cancer cells by berbamine and its molecular mechanisms of action. *Mol Cancer* 8: 81-96.

Wang ZD, Song BA, Yang S, Xue W, Jiang LK, 2009c. Chemical constituents in herb of *Berberis sargentiana* Schneid. *J Guizhou Univ*: *Nat Sci* 26: 11-17.

Wei YL, Liang Y, Xu L, Zhao XY, 2009a. The antiproliferation effect of berbamine on K562 resistant cells by inhibiting NF-κB

pathway. *Anatomical Record* 292: 945-950.

Wei YL, Xu L, Liang Y, Xu XH, Zhao XY, 2009b. Berbamine exhibits potent antitumor effects on imatinib-resistent CML cells *in vitro* and *in vivo*. *Acta Pharmacol Sin* 30: 451-457.

Wong CW, Seow WK, O'Callaghan JW, Thong YH, 1992. Comparative effects of tetrandrine and berbamine on subcutaneous air pouch inflammation induced by interleukin-1, tumor necrosis factor and platelet-activating factor. *Agents Actions* 36: 112-118.

Xiao PG, Song WZ, Liu GS, Chen BZ, Lv JS, 1974. Study on the medicinal plant resources of *Berberis* in China—including their taxonomy, distribution and evaluation medicinal. *Acta Phytotaxon Sin* 12(4): 383-406.

Xie JW, Ma T, Gu Y, Zhang XZ, Qiu X, Zhang L, Xu RZ, Yu YP, 2009. Berbamine derivatives: A novel class of compounds for anti-leukemia activity. *Eur J Med Chem* 44: 3293-3298.

Xu RZ, Dong QH, Yu YZ, Zhao XY, Gan XX, Wu D, Lu QH, Xu XH, Yu XF, 2006. Berbaimine: A novel inhibitor of *bcr/abl* fusion gene with potent anti-leukemia activity. *Leukema Res* 30: 17-23.

Yan D, Jin C, Xiao XH, Dong XP, 2008. Antimicrobial properties of berberines alkaloids in *Coptis chinensis* Franch by micro-calorimetry. *J Biochem Biophys Methods* 70(6): 845-849.

Yang WL, Ma ZG, Qi M, 2009. Determination of alkaloids in various parts of *Berberis* spp. from various producing areas of Gansu Province, China. *Gansu Med J* 128: 349-351.

Yeşlada E, Küpeli E, 2002. *Berberis crataegina* DC. root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats. *J Ethnopharmacol* 79: 237-248.

Ying JS, Ying TS. *Flora of China.* http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=103816.

Yuan HL, Zhang TT, Xiao XH, 2011. Quality control approaches for Chinese herbal medicines. *Chin Herb Med* 3: 17-22.

Zhang H, Wei J, Xue R, Wu JD, Zhao W, Wang ZZ, Wang SK, Zhou ZX, Song DQ, Wang YM, Pan HN, Kong WJ, Jiang JD, 2010. Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression. *Meta Clin Exp* 29: 285-292.

Zhang Q, Piao XL, Piao XS, Lu T, Wang D, Kim SW, 2011. Preventive effect of *Coptis chinensis* and berberine on intestinal injury in rats challenged with lipopolysaccharides. *Food Chem Toxicol* 49(1): 61-69.

Zhang XW, Ba XY, Ren J, 1993. Inhibitive effect of berbamine and verapamil on oxygen free radicals after myocar dial infarction in rabbits. *Chin J Anesthesiol* 13: 335-336.

Zhao XY, He ZW, Wu D, Xu RZ, 2007. Berbamine selectively induces apoptosis of human acute promyelocytic leukemia cells via surviving-mediated pathway. *Chin Med J* 120: 802-806.

Zhou H, Wang L, Hao XM, et al 1998. Effect of berbamine on oxygen free radicals after experimental cerebral ischemia in rats. *Chin J Chin Mater Med* 23: 307.

Zhu FQ, Qian CY, 2006. Berberine chloride can ameliorate the spatial memory impairment and increase the expression of interleukin-I beta and inducible nitric oxide synthesis in the rat model of Alzheimer's disease. *BMC Neurosci* 7: 78-87.