· Reviews ·

Traditional Use, Pharmacology, Toxicology, and Quality Control of Species in Genus *Bupleurum* L.

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Abstract: Many species of genus *Bupleurum* L. have been pharmaceutically used mainly in Asia and Europe for thousand years. Their roots are the most popular ingredients in Chinese materia medica prescriptions for the treatment of inflammatory diseases and auto-immune diseases. A plenty of chemical constituents have been isolated and identified from the species in *Bupleurum* L., such as saikosaponins, polysaccharides, volatile oils, flavonoids, polyacetylenes, lignins, and coumarins, most of which possess a variety of biological activities, especially for the hepatoprotective effect, antitumor activity, immunoregulation, and febrifuge efficacy. Therefore, the species in genus *Bupleurum* L. could be potential herbs of immunomodulator, antineoplastic, anti-oxidant, etc. Meanwhile, as potential toxicities have been discovered in some constituents, it is urgent to establish a comprehensive quality evaluation system to ensure the safety and efficiency of herbs. This paper reviews on the phytochemical and pharmacological studies, especially for the toxicology and quality control of the species in *Bupleurum* L., which is a reference for the species in this genus for safe usage and further development.

Key words: biological activity; *Bupleurum* L.; phytochemistry; quality control; toxicology
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Introduction

The plants of genus *Bupleurum* L., including approximately 200 species in the world, are mainly located in Northern Hemisphere, Eurasia, and North Africa (Su *et al*, 1998). Among them, 26 species and six varieties could be the botanical resources of *Bupleuri Radix* in China (Pan, 2006), though only *B. chinense* DC. and *B. scorzonerifolium* Willd. were authenticated species in *Pharmacopoeia of People's Republic of China* (Pharmacopoeia Committee of P. R. China, 2010).

Bupleuri Radix, a traditional Chinese herb used as febrifuge, is a common ingredient in the prescriptions of Chinese materia medica (CMM) for the treatment of fever, flu, distending pain in the chest, and menstrual disorder (Ikegami *et al*, 2006). For example, Xiaochaihu Decoction, Dachaihu Decoction, Xiaoyao Pill, Chaihushugan Powder, Chaihuguizhi Decocton, and Buzhongyiqi Decoction are all famous prescriptions with certain therapeutic effects (Pan, 2006). Recent studies have indicated that *Bupleuri Radix* has the bioactivities of antivirus, anti-ulcer, antitumor, immunoregulation, proliferation, antidepressive, and hepatoprotective effects (Cheng *et al*, 2006; Matsumoto *et al*, 2002; Su *et al*, 2002; Wang *et al*, 2009; Cho *et al*, 2010; Zhao *et al*, 2010; Guo *et al*, 2012; Liu *et al*, 2006). In addition, the extract of *Bupleuri Radix* has a significant bioactivity of anti-influenza A H1N1 *in vitro* (Su *et al*, 2011). However, there are also some potent toxicities in it, as a result, a great attention has been paid

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for the quality control in the good practice in traditional Chinese medicine (GP-TCM) research (Uzuner *et al*, 2012). In this paper, the species of *Bupleurum* L. in phytochemical, pharmacological, and toxicological studies, and the quality control studies are reviewed.

Traditional uses

The species in *Bupleurum* L., first recorded in *Shen Nong's Herbal Classic* 2000 years ago, are widely used in the ancient folk medicine. Traditional medicinal formulae containing *Bupleuri Radix* displayed the excellent therapeutic effects on some common diseases, so they were largely propagated for pharmaceutical demand. According to a survey on the frequency and pattern of CMM prescriptions for chronic hepatitis in Taiwan, Longdan Xiegan Decoction, a famous prescription containing *Bupleuri Radix*, was demonstrated to be the most popular prescription among the 91 080 subjects treated by CMM for chronic hepatitis (Chen *et al*, 2008).

Many prescriptions including *Bupleuri Radix* are very effective, and some of them have been widely used with some new applications so far. Indeed, there are about 60 formulae including *Bupleuri Radix* in *Pharmacopoeia of People's Republic of China 2010*. Accordingly, it is a good source of CMM for the treatment of fever, flu, inflammatory reaction, infectious diseases, amenorrhea, deafness, dizziness, vomiting, dry throat, diabetes mellitus, etc.

Pharmacology of main components

Principal saikosaponins and their bioactivities

Saikosaponins are the major secondary metabolites in the species of *Bupleurum* L., amounting to approximately 1% to 2% of the total dry weight of roots, and in some species they reached 7% (Lin *et al*, 2004). More than 120 glycosylated oleanane-type and ursane-type saponins have already been isolated from the species in *Bupleurum* L. (Ashour and Wink, 2011), of which saikosaponin-A, -C, and -D are the most active saikosaponins. They are demonstrated to have exact pharmacological activities.

Due to the high content and potent pharmacological activities, saikosaponin-A was considered as a marker for the quantity control of *Bupleuri Radix* (Zhu *et al*, 2006; Liau *et al*, 2007). It is demonstrated that saikosaponin-A possessed numerous pharmacological activities, such as anticancer, anti-inflammation, antiallergy, anti-oxidation, anticonvulsion, accelerated apoptosis, etc.

Saikosaponin-A potently suppressed concanavalin A (Con A)-stimulated IL-2, IFN-γ, and TNF-α production in T cells of mice. It significantly inhibited the proliferation and activation of T cells activated by Con A in a concentration-dependent manner. It also caused G_0/G_1 arrest of the activated T cells through down-regulating the protein levels of CDK6 and cyclin D3 and up-regulating the protein level of $p27^{kip}$ (Sun *et al*, 2009). Saikosaponin-A also inhibited the passive allergic skin reactions in rats and suppressed the asthmatic bronchoconstriction in sensitized guinea pigs (Park *et al*, 2002). These results have confirmed that saikosaponin-A had the immunosuppressive activity.

Saikosaponin-A had a significant antitumor effect. It could sensitize the cancer cells to cisplatin-induced apoptosis through ROS accumulation, which may be attributed to the combination of saikosaponins and cisplatin. It was found to be an effective therapeutic strategy (Wang *et al*, 2010a). Chen *et al* (2003) have reported the apoptotic mechanism was induced by saikosaponin-A in human breast MDA-MB-231 and MCF-7 cancer cells. According to Chen *et al* (2003), saikosaponin-A could cause an obvious increase in the sub-G1 population of cell cycles.

Saikosaponin-A was also a potential anti-oxidant in biological body. It was investigated that the supplementation with saikosaponin-A significantly decreased the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as liver cholesterol and triglyceride levels in plasma of rats with CCl_4-induced liver injury (Wu *et al*, 2008). As a result, the supplementation with saikosaponin-A could impair hepatic lipids and lipid peroxidation; it also could enhance the anti-oxidant defense to protect the liver injury induced by CCl_4, but more clinical trials are needed in order to develop saikosaponin-A into an anti-oxidant.

Saikosaponin-D is similar to saikosaponin-A in chemical structure, so is in pharmacological activities. It was reported that saikosaponin-D potently suppressed the expression of T cells in mice and inhibited the propylene glycol monomethyl acetate and ionomycin-

stimulated IL-2 production in T cells of mice (Leung *et al*, 2005). The saikosaponin-D could inhibit the activation of T cell similar to saikosaponin-A, however, they were different in the inhibitory mechanisms.

Hsu, Kuo, and Liu (2004) reported that the pro-apoptotic effect of saikosaponin-D had been discovered in two liver cancer cell lines, Hep G2 and 3B. It has been proved that saikosaponin-D could prevent the progression of the cell cycle at G_1 phase, and inhibit the cell survival signaling. Therefore saikosaponin-D decreased the cell proliferation and induced apoptosis. It could also inhibit the cell growth of human lung cancer cell line A549 by inducing apoptosis and blocking cell cycle progression in G_1 phase (Hsu *et al*, 2004). These results may verify the antitumor activity of saikosaponin-D.

An *in vitro* test showed that saikosaponin-D acted as a weak phytoestrogen. It has a proliferation-stimulating effect on MCF-7 cells, and the effect is associated with a marked increase in the number of MCF-7 cells in S phase. Besides, it could activate the estrogen responsive elements luciferase activity, and the estrogenic effect was mediated through the estrogen receptors (Wang *et al*, 2010b).

Saikosaponin-D was also a potent macrophage activator. It significantly activated the peritoneal macrophages in terms of the enhancement of the phagocytosis, increased the level of acid phosphatase, induced the activity of suppressing the cell growth, and promoted the expression of immune-associated antigen on the cell surface. The activities of saikosaponin-D were found to be much stronger than those of typical saponins ginsenoside Rg_1 and glycyrrhizin (Kumazawa *et al*, 1989). Saikosaponin-D was also provided with a potent cytotoxicity, and it seemed that the activity might be beneficial to cure some diseases (Chiang *et al*, 2003). Furthermore, saikosaponin-D possessed a significant hepatoprotective effect, and this might be associated with its anti-lipid peroxidation activity.

Saikosaponin-C contains a glycon different to other saikosaponins, which is a trisaccharide of glucose-glucose-rhamnose and may lead to some different pharmacological activities. Researchers have compared the inhibitory effects of saikosaponin-A, -C, and -D on the nephrotoxic serum nephritis of mice, and the results indicated that saikosaponin-C almost completely controlled the development of nephritis, while others had lower activity (Chen *et al*, 2008). The superior efficacy endues saikosaponin-C with great developing value. Saikosaponin-C also has been shown to possess cytotoxicity, which might play a role in inhibiting HBV-DNA replication (Chiang *et al*, 2003). Moreover, saikosaponin-C possessed an ability of stimulating cell proliferation, and it has been proved that saiko-saponin-C could induce the growth and migration of endothelial cells, as well as the formation of capillary tube (Shyu *et al*, 2004).

There are also some other saikosaponins that have been isolated and found to have inhibitory effect on Na^+, K^+-ATPase, especially for saikosaponin-B1, B2, and B4 (Zhou *et al*, 1996). In addition, there are also some saponins which are not exclusive to the species of *Bupleurum* L., such as buddleja saponin IV and malonyl buddleja saponin IV, two saponins isolated from the species of *Bupleurum* L., were proved to possess hepatoprotective effects too (Guinea *et al*, 1994). Total saponin is suggested to be an important source of drugs, because individual saponin may have diverse bioactivities. As a result, saponins from the species of *Bupleurum* L. may be developed to treat some diseases effectively.

Principal polysaccharose and their bioactivities

Numerous polysaccharides isolated from plants have been proven to possess a wide range of biological functions with weak side effects, and some of them have already been applied in clinic. There have been many kinds of polysaccharides with the diverse bioactivities obtained from the species of *Bupleurum* L.

Crude polysaccharides extracted from *Bupleuri Radix* could enhance phagocytic activities and inhibit the productions of proinflammatory mediators induced by lipopolysaccharide (Cheng *et al*, 2010). Wang *et al* (2009) has certified the immunoregulation effects of crude polysaccharides of *Bupleuri Radix*, it enhanced the phagocytic functions of macrophages, and inhibited the lipopolysaccharide-induced productions of NO and proinflammatory cytokines. In addition, crude poly-saccharides was benefit to the treatment of systemic lupus erythematosus which was induced by CJ-S131 in BALB/c mice (Wang *et al*, 2009); these pharma-cological activities might be associated with the curative effect of *Bupleurum* polysaccharides (BP) on

autoimmune disease.

D3-S1, a kind of polysaccharide isolated from hot water extract of the roots of *B. smithii*, with an average molecular weight of 2000, has been proven to have an anti-complement activity and a strong inhibitory effect on laking (Xu *et al*, 2007). D3-S1 with more potent bioactivities than heparin, provided an initial approach by using natural products to develop the drugs that directly targeted to the complement components (Xu *et al*, 2007).

BCPS-1, a pure polysaccharide fraction isolated from *B. chinense*, with an average molecular weight of about 29 000, has a significant anti-oxidative activity (Sun *et al*, 2010). According to studies, the DPPH radical scavenging activity of BCPS-1 reached 76.5% at 8 mg/mL (Sun *et al*, 2010), suggesting that BCPS-1 could be potentially used as a natural anti-oxidant.

BP III is mainly composed of arabinose and galactose with an average molecular weight of 8100 (Xiao *et al*, 2005); it had a radio protective effect on rats (Luo *et al*, 1995). It was reported that BP III increased the survival rate of mice under 8.5 Gy-irradiated by 45%, and it also had a good defending effect on some indexes such as spleen weight, DNA contents of bone marrow, hematopoietic cell colony, and so on (Luo *et al*, 1995).

There are abundant pectic polysaccharides isolated from *Bupleuri Radix*, some of which have remarkable biological activities. Bupleuran 2IIb, with a molecular weight of 23 000 containing 75.5% uronic acid, has a potent in complement-activating activity and an immune complex clearance-enhancing activity, and the activities are attributed to its neutral carbohydrate chains (Yamada *et al*, 1989; Matsumoto *et al*, 1993). Bupleuran 2IIc, with a molecular weight of 63 000, containing 93.6% galacturonic acid, was an anti-ulcer pectin, which could stimulate lymphocytes and deplete adherent cells or T cells. In addition, it showed a potent mitogenic activity and B-cell proliferation activity (Yamada *et al*, 1989; Yamada, 1994; Sakurai *et al*, 1999).

Researchers (Yang *et al*, 2009; Zhang *et al*, 1989) have reported that the crude polysaccharide from *Bupleuri Radix* had a radio protective effect on rats under 8.2 Gy-irradiated, especially for the protective effect on hematopoietic function. Moreover, it could improve immunity in rat and recover the immuno-suppression. Recently, the structure-activity relationships of polysaccharides have also been studied. For instance, the anti-oxidation of SPB_0, a crude polysaccharide extracted by ultrasound-assisted method, is stronger than that of the crude polysaccharide extracted by hot water. SPB_0 may attribute to ultrasonic degradation which diminished the linear scale of the polysaccharide molecular and the polysaccharides of smaller molecular have a stronger free radical scavenging activity (Li *et al*, 2009a). Another study (Li *et al*, 2009b) has demonstrated the aggregations of an acidic polysaccharide from the species of *Bupleurum* L. in different conditions, indicating that we could control the conformation of polysaccharides by adjusting their extraction conditions, in order to regulate their bioactivities.

Principal bioactivities of volatile oil

As a group of the dominant constituents from the species of *Bupleurum* L., the volatile oil is found to be a source of febrifuges and antiphlogistics. Moreover, it has also been developed into cosmetic uses (Pelletier *et al*, 2012). So far, more than 200 constituents have been identified in more than 20 species (Pan, 2006). In view of their efficient pharmacological activities, volatile oils have been the main ingredient in the injection and dripping pills with the species of *Bupleuri* L., which are commonly used in modern medicine. To make a better use of volatile oils, the extraction method for volatile oils has been optimized (Li *et al*, 2004), and the fingerprint profile of volatile oil has been established (Sun *et al*, 2011; Ni *et al*, 2009).

Anti-inflammatory activity of the volatile oils extracted from the species of *Bupleurum* L. have been determined in many experiments. α-Pinene and β-pinene are considered to be the major components which could inhibit the inflammation caused by cotton wool (Lorente *et al*, 1989). α-Pinene and β-caryophyllene are recognized as the key anti-inflammatory components, which are effective to the carrageenin-induced hind paw edema in adrenal ectomized rats; and Δ-3-carene was proved to have a similar function (Martin *et al*, 1993; Ocete *et al*, 1989; Gil *et al*, 1989); assessed for the inhibition of both prostaglandin E(2) production and lipoxygenase, researchers have found that the volatile oils also showed a significant anti-

inflammatory activity. According to the assessing study, the IC_{50} value was 63.64 μg/mL for lipoxygenase, and the inhibitory rate of prostaglandin E(2) at the dose of 25 μg/mL was 26.04% (Ashour *et al*, 2009). The results above revealed that there were many compounds in the volatile oils which could act as available anti-inflammatory agent.

The volatile oils exhibited a moderate antimicrobial activity, which showed a significant *in vitro* anti-microbial activity against the two kinds of Gram positive pathogens, *Streptococcus pyogenes* and *S. agalactiae*, with the minimum inhibitory concentration (MIC) values ranging from 0.125 to 4.000 mg/mL; it also had an effect on four bacterial species, and the inhibitory zones and MIC for the bacterial strains were in the range of 7.0—18.0 mm and 250—500 μg/mL, respectively (Ashour *et al*, 2009; Shi *et al*, 2010). Its antimicrobial activity was limited, and could be an effective way for broadening its antibiogram if combinating with other drugs. The fungicidal activity of the volatile oils was also studied. The volatile oil at the concentration of 5.0 mL/L could inhibit the sporulation of *Plasmopara halstedii*, a parasite fungus species in sunflower seedlings. It seemed that the volatile oils could activate the defense response of sunflower seedlings against the pathogen invasion. Besides, the volatile oils showed a variability of inhibition among 14 fungal species (Fernández-Ocaña *et al*, 2004; Maxia *et al*, 2011).

The volatile oils also showed an anti-oxidative activity, as evidenced by the low IC_{50} value of 566.2 μg/mL for DPPH radical and the inhibition of lipid peroxidation (induced by $FeSO_4$, H_2O_2, and CCl_4) with IC_{50} values of 275.2, 296.9, and 118.7 μg/mL, respectively. Previous studies (Shi *et al*, 2010; Ashour *et al*, 2009) showed that the IC_{50} values for DPPH and deoxyribose were 3.66 mg/mL and 17.4 μg/mL, respectively. The sedation effect of the volatile oils was verified, as it exhibited an antispasmodic activity in rat uterine contractions which was caused by oxytocin (Ocete *et al*, 1989; Lorente *et al*, 1989).

The essential oils possessed a strong cytotoxicity against many cancer cells. The MTT assay showed that the IC_{50} value of 46.01 μg/mL indicated the highest activity in the CCRF-CEM cell line after 24 h treatment (Ashour *et al*, 2009).

Furthermore, the fatty acid compositions of some species have the relatively high contents of unsaturated fatty acids, such as oleic, linoleic, and α-linolenic acids, thus the oils from the species of *Bupleurum* L. could be considered as a good source of unsaturated fatty acids in food and oil industry (Saracoglu *et al*, 2012).

Principal bioactivities of flavonoids

The statistic data showed that more than 20 flavonoids have been isolated from some species of *Bupleurum* L., most of which were glycoside, including three categories by their aglycones (quercetin series, kaempferol series, and isorhamnetin series) (Zhang *et al*, 2007; Pan *et al*, 2002). Pharmacological studies (Feng *et al*, 2002) demonstrated that the flavonoids in the species of *Bupleurum* L. had the inhibitory effects on influenza virus. The total flavonoids from the aerial part of *B. chinense* (TFB) could significantly relieve the lung pathological processes of the infected mice. In addition, eugenin and saikochromone potently inhibited the CD28-costimulated activation of human peripheral blood T cells (Chang *et al*, 2003). Moreover, flavonoids in oral S/B remedy (containing *Scutellaria baicalensis* and *B. scorzonerifolfium*) were recognized to be therapeutically useful in the treatment of central nervous system neurodegenerative diseases (Lin *et al*, 2011).

Other components

The species of *Bupleurum* L. have the plenty of phenols with a free radical scavenging activity and an anti-oxidative capacity. Song *et al* (2010) reported that there was a high positive correlation between anti-oxidative capacities and total phenolic components. Many other components have also been isolated from the species of *Bupleurum* L., such as lignins and coumarins, some of which were reported to possess the varieties of bioactivities. The presence of these components has greatly enriched the chemical composition diversity of the species in *Bupleurum* L. On the other hand, there will be more requirements to develop the natural source, especially for its quality control.

Toxicological studies

There are many beneficial contents in the species of *Bupleurum* L., but their toxicities are inevitable. For example, Xiaochaihu Decoction, a herbal formulae containing *Bupleuri Radix*, has been demonstrated to have subacute toxicity (Shin *et al*, 2012). The single

oral dose toxicity of aqueous extracts from *Bupleuri Radix* has also been demonstrated in healthy mice (Kim *et al*, 2012). Saikosaponins and the volatile oil, the main effective components, are recognized to have both acute toxicity and chronic toxicity; some poisonous contents are also isolated from only a few species.

Toxicity of saikosapinons

Saikosaponins are demonstrated to be the major chemical components responsible for hepatoprotective effect, according to the clinical applications and modern studies. Their intensive hepatotoxicities were mainly related to the multi-channel oxidative injury. As reported, saikosaponins could induce the chronic toxicity in rats, and the mainly target organ was liver (Huang, Zhao, and Sun, 2011). Rats could be decreased in weight after saikosaponins treatment in which abnormal liver function and hepatic damage occurred. These lesions gradually aggravated with dose increasing (Liu *et al*, 2012; Huang, Liu, and Sun, 2011). It was also reported that the administration of saikosaponins for a long time or at a high dose may cause serious liver injury or even death in rats, and there are certain time- and dose-toxicity relationships in cumulative toxicity of saiko saponins to rats. Further- more, total saponins extract in the species of *Bupleurum* L. exhibited a potent acute toxicity on rats with a LD_{50} value of 2255.6 mg/(kg·d). Saikosaponins could lead to some acute toxicity symptoms, such as negligent action, abdominal lying sleeping, rapid heart rate, shortness of breath, and so on (Huang and Sun, 2010). It has been widely recognized that the hemolytic activity of saikosaponin-D is caused by its complex with cholesterol on erythrocyte membrane, leading to membrane disruption and cell lysis, which could be decreased by liposomes (Ding *et al*, 2005).

Toxicity of volatile oils

A recent study (Sun and Yang, 2011a) showed that the volatile oils from *B. chinense* could induce the chronic hepatotoxicity and hepatotoxical injury in rats along with some abnormal changes in the activity of serum ALT, AST, and some other hepatic indexes. The dosage-time-toxicity relationship has been clarified. The volatile oils from *B. chinense* also possessed an acute toxicity to rats and mice, inducing restlessness, accelerated heart rate, abnormal walking, breathlessness, and consecutive convulsion, and the LD_{50} values of the volatile oils were 2.081 and 3.118 mL/kg, respectively (Sun *et al*, 2011). The mechanism of the volatile oil for hepatotoxic injury was revealed that the volatile oils could suppress the respiratory control rate, phosphorus/ oxygen rate, respiratory oxygen uptake, contents of ATP, and activities of ATPases, while increase the activity of iNOS and the contents of TNF-α, IL-6, IL-10, and NO both in serum and liver could increase. As a result, they caused the hepatotoxicity in rats by inhibiting function of hepatic mitochondrion and influence the energy metabolism of the liver (Sun and Yang, 2011b; Yang and Sun, 2011).

Toxicity of polyacetylenes

Polyacetylenes are the poisonous components of some species in *Bupleurum* L., such as *B. longiradiatum* Turcz., *B. angustissimum* (Franch.) Kitagawa, *B. smithii* var. *parvifolium*, and *B. bicaule* Helm. Recently, many polyacetylenes have been isolated, and an HPLC-DAD-MS method has been developed for the qualitative and quantitative analysis of polyacetylenes in the species of *Bupleurum* L. It was revealed that polyacetylenes were only accumulated in the species above, so they were considered to be poisonous species (Huang *et al*, 2009; 2011). The bioassay tests showed that (2*Z*,8*E*,10*E*)-14*S*-hydroxy-heptadecatriene-4,6-diyn-1-yl acetate and mTPA esters of bupleurotoxin were cytotoxic compounds with IC_{50} values of 9.4 and 4.9 μmol/L, respectively, and they also possessed a cytotoxic activity against cancer cell lines (Huang *et al*, 2009). Acetylbupleurotoxin and upleurotoxin are found to completely inhibit the tube-like formation of human umbilical venous endothelial cells, but they do not exhibit the antitumor activity on BDF1 mice bearing Lewis lung carcinoma cells (You *et al*, 2002).

Recently, an aryltetraline lactone lignan has found to exhibit the cytotoxic activity with IC_{50} values of 12.14 and 16.90 mmol/L after 24 h treatment for HepG2 and HeLa cells, respectively (Ashour *et al*, 2012).

Quality evaluation studies

Because of the complex constituents in *Bupleuri Radix*, it is necessary to study the quality control and quantitative analysis of the active components in it. Recently, its quality evaluating technologies and strategies have been widely studied, and we have made a summary on them as follows.

Due to its various pharmacological properties, saikosaponins have been regarded as the important quality indexes, and based on their content property, the quality profiles have been established by different methods. HPLC was a commonly used analysis technique, by which Li *et al* (2005) analyzed 17 samples of different species of *Bupleuri Radix* from various habitats. With an optimized condition, saikosaponin-A and -D are converted into saikosaponin-B1 and -B2 completely and quantitatively before being analyzed by HPLC. This method could be used as a routine analytical one for the quantitative analysis of saikosaponin-A and -D in *Bupleuri Radix*, which are the detecting indexes in *Pharmacopoeia of People's Republic of China 2010*. High performance capillary zone electrophoresis (HPCE) is also a powerful technique to study the compounds in the complex extracts, and a fast HPCE method has been developed for the determination and separation of saikosaponin-A, -C, and -D in the extracts of *B. chinense* from different areas (Lin *et al*, 2005). A rapid resolution liquid chromatography coupled with evaporative light scattering detection method (RRLC-ELSD), demonstrated to be highly effective for the quality evaluation of the species in *Bupleurum* L., has been developed for the simultaneous determination of six major saikosaponins (Huang *et al*, 2009). LC-MS allows a comprehensive analysis of the saikosaponins in the species of *Bupleurum* L. and will be helpful for the quality control of the crude materials and their related formulations (Huang *et al*, 2008). Combined with accelerated solvent extraction, a rapid-resolution LC-MS method for the efficient extraction, rapid separation, online identification, and accurate determination of the saikosaponins has been developed because it was rapid, efficient, accurate, and suitable for the quality control of *Bupleuri Radix* (Yang *et al*, 2010). Saikosaponins were also regarded as the target markers of some traditional medicinal formulations containing *Bupleuri Radix*, the analysis of saikosaponin-A and -C could be obtained readily by the use of LC/ESI/ion trap tandem mass spectrometry (Liau *et al*, 2007). Immunoassay has been used in the semiquantitative analysis of saikosaponins. The Eastern blotting technique for the analysis of saikosaponins has been reported to be a simple method for the quality control of *Bupleuri Radix* and some formulas with it (Zhu *et al*, 2007).

Flavonoids have been regarded as the referential indexes to evaluate the quality of *Bupleuri Radix* due to numerous biological activities, characteristic category and contents. With HPLC-UV for the simultaneous determination of flavonoids, Zhang *et al* (2010) have established a quantitative method to investigate the contents and the distribution of flavonoids in the different species of *Bupleurum* L., which was useful for the reliable identification of 12 compounds. The establishments of HPLC and RP-HPLC fingerprints have provided a scientific basis for the development of the comprehensive utilization and the quality control of *Bupleuri Radix* (Li *et al*, 2009; Yue *et al*, 2010).

The volatile oil is another group of characteristic component in the species of *Bupleurum* L. The GC-MS analysis showed that *n*-hexadecanoic acid could be detected in all samples, indicating that it might be the feature chemical component of the volatile oils from the species of *Bupleurum* L. The volatile oil showed the various inhibitory bioactivities against the fungi tested (Xie *et al*, 2008; Maxia *et al*, 2011).

Multi-component analysis methods together with the fingerprints are considered to be useful tools for the quality control of herbs. An efficient method has been established to evaluate the efficacy of *Bupleuri Radix* by integrating the principal component analysis with the canonical correlation analysis based on UV spectral fingerprint, and HPLC-ELSD and HPTLC analyses could be considered the complementary measure of the quality control (Ni *et al*, 2009; Tian *et al*, 2009).

Conclusion

The present studies have mainly concentrated on the phytochemical and pharmacological aspects of the species in *Bupleurum* L. Accordingly, the species in *Bupleurum* L. are rich in triterpenoid-saponins (the so-called saikosaponins), flavonoids, volatile oils, and polysaccharides. In addition, there are some bioactive constituents of lower contents isolated from the species of *Bupleurum* L., such as lignins and coumarins, and they contribute to phytochemical diversity of the species in *Bupleurum* L.

The diverse pharmacological activities should attribute to the abundant chemical constituents in the species of *Bupleurum* L.

According to the studies, it is clear that the further development for the species of *Bupleurum* L. has a broad prospect. *Bupleuri Radix* is a famous febrifuge in traditional use, also known for the good curative effect on pain and inflammation associated with flu and common cold. Recently, it has been proven to be effective to depression, influenza A H1N1, and it also has the hepatoprotective and antimicrobial activities. Moreover, it is a potential immunomodulator, anti-neoplastic, anti-oxidant, and so on.

At the same time, some active constituents (e.g. saikosaponins and the volatile oils) are demonstrated to have some side effects; many components in *Bupleuri Radix* have both acute and chronic toxicities. Therefore, to control the use dosage and time in a proper extent is vital for ensuring the safety medication of *Bupleuri Radix*.

Quality control is thought to be vital for ensuring the safety and efficacy medication of *Bupleuri Radix*. And a comprehensive quality evaluation system is necessary, which should contain the safety assessment and efficiency evaluation. Specifically, the first step is to regularize the germplasm, thus to eliminate the toxic and the inferior species; then is to detect the contents and toxicity of the toxic components, such as pesticide residues and toxic secondary metabolites (e.g. poly-acetylenes), which could be determined by chemical analysis and bioassay approach; finally, multi-component analysis methods are regarded to be reliable for the assessment of the effective components, and combined with the advanced analytical techniques, such as HPLC-MS, GC-MS, and FT-IR, which will play a potent role in the efficiency evaluation of *Bupleuri Radix*.

All in all, the species in *Bupleurum* L. are potential natural medicine resources with a variety of bioactive constituents and abundant pharmacological activities. It is necessary to develop a comprehensive quality evaluation system to ensure the safety and efficiency. It is strongly believed that the application of the species of *Bupleurum* L. has a broad prospect.

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