A systematic review of the active saikosaponins and extracts isolated from Radix Bupleuri and their applications

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ABSTRACT

Context: Radix Bupleuri has been used in traditional Chinese medicine for over 2000 years with functions of relieving exterior syndrome, clearing heat, regulating liver-qi, and lifting yang-qi. More natural active compounds, especially saikosaponins, have been isolated from Radix Bupleuri, which possess various valuable pharmacological activities.

Objective: To summarize the current knowledge on pharmacological activities, mechanisms and applications of extracts and saikosaponins isolated from Radix Bupleuri, and obtain new insights for further research and development of Radix Bupleuri.

Methods: PubMed, Web of Science, Science Direct, Research Gate, Academic Journals and Google Scholar were used as information sources through the inclusion of the search terms ‘Radix Bupleuri’, ‘Bupleurum’, ‘saikosaponins’, ‘Radix Bupleuri preparation’, and their combinations, mainly from the year 2008 to 2016 without language restriction. Clinical preparations containing Radix Bupleuri were collected from official website of China Food and Drug Administration (CFDA).

Results and conclusion: 296 papers were searched and 128 papers were reviewed. A broad spectrum of in vitro and in vivo research has proved that Radix Bupleuri extracts, saikosaponin a, saikosaponin d, saikosaponin c, and saikosaponin b2, exhibit evident anti-inflammatory, antitumor, antiviral, anti-allergic, immunoregulation, and neuroregulation activities mainly through NF-κB, MAPK or other pathways. 15 clinical preparations approved by CFDA remarkably broaden the application of Radix Bupleuri. The main side effect of Radix Bupleuri is liver damage when the dosage is excess, which indicates that the maximum tolerated dose is critical for clinical use of Radix Bupleuri extract and purified compounds.

Introduction

With a 2000-year medicinal history, Radix Bupleuri (Chai Hu in Chinese) is believed to be one of the most important herbal medicines in China. The earliest record about Radix Bupleuri in China appeared in Shen Nong Ben Cao Jing, the first Chinese medical book, since then, Radix Bupleuri has been widely used in traditional Chinese medicine (TCM) for its effects of relieving exterior syndrome, clearing heat, regulating the liver-qi, and lifting yang-qi (Sen 1959). It has been used in many traditional Chinese prescriptions, such as Xiao Chai Hu Tang and Chai Hu Shu Gan Yin to treat cold and liver diseases (Chen et al. 2011). The roots are usually the medicinal parts of Radix Bupleuri, and which is often processed into pieces for easy use (Figure 1).

Bupleurum chinense DC. (Apiaceae) and Bupleurum scorzonerifolium Willd. are defined as the original plants of Radix Bupleuri in Chinese Pharmacopeia (National Pharmacopoeia Committee 2010). In fact, many other Bupleurum species are also used as Radix Bupleuri in East Asia, such as Bupleurum falcatum L., which is officially listed in Japanese Pharmacopoeia (Saiko in Japanese (Japanese Pharmacopoeia Editorial Board 2011), and Bupleurum yinchowense Shan and Li, which is recorded in some provincial Pharmacopoeia of China (The Inner Mongolia Autonomous Region Health Department 1988; Food and Drug Administration of Gansu Province 2008). These Bupleurum medicinal plants are widely distributed in the northern hemisphere (Judd 2008), and also commonly used in Eurasia and North Africa for their medicinal properties (Mabberley 2008). As shown in Figure 2, they are perennial herbs with compound umbels, yellowish or rarely purplish bisexual flowers, containing five stamens, crenocarps, and simple, long, slender leaves (Figure 2).

With the development of modern pharmacology, many valuable and important activities of Radix Bupleuri have been discovered, such as anti-inflammatory (Xie et al. 2012), antitumor (Liu & Li 2014), antidepressant (Jin et al. 2013), antiviral (Chiang et al. 2003), hepatoprotection (Wang et al. 2013a), immunoregulation (Ying et al. 2014), and neuromodulation activities (Zhou et al. 2014). All of these potent effects are due to its various secondary metabolites, especially saikosaponins, the content of which is up to 7% of the total dry weight of Radix Bupleuri roots (Ashour & Wink 2011). To date, over 100 glycosylated oleanane-type saponins have been isolated and identified from Radix Bupleuri (Pistelli et al. 1993; Ebata et al. 1996), and some of them have been demonstrated possessing bioactive properties both in vitro and in vivo. Therefore, reviewing and summarizing the pharmacological activities and mechanisms of saikosaponins from Radix Bupleuri is meaningful and important to obtain new insights for further research and development of Radix Bupleuri. In addition, since extracts are the main source of Chinese patent medicines containing Radix Bupleuri, their pharmacological...
properties and mechanisms are also summarized. Moreover, the applications and toxicity studies are discussed to provide a basis for further studies concerning the safety and efficacy of Radix Bupleuri.

In this paper, six main databases, PubMed, Web of Science, Science Direct, Research Gate, Academic Journals, and Google Scholar were used as information sources through the inclusion of the search terms 'Radix Bupleuri', 'Bupleurum', 'saikosaponins', 'Radix Bupleuri preparation', and their combinations, mainly from the year 2008 to 2016 without language restriction. As a result, we searched 296 papers and a total of 128 references were included in the present work.

**Purified saikosaponins from Radix Bupleuri**

In recent years, over 100 different triterpenoid saponins have been isolated from Radix Bupleuri, among them saikosaponin a (SSa), saikosaponin d (SSd), saikosaponin c (SSc) and saikosaponin b2 (SSb2) (Figure 3) are believed to be responsible for the most pharmacological activities of Radix Bupleuri (Liu et al. 2002; Huang et al. 2013). Saikosaponins are oleanane type triterpenoid saponins and divided into seven types according to different aglycones. SSa, SSd and SSc are epoxy-ether saikosaponins (type I), while SSb2, with a different aglycone, is heterocyclic diene saikosaponin (type II) (Lin et al. 2013).

**SSa**

SSa, one of the most important active saikosaponins in Radix Bupleuri (Liang et al. 2014), plays a significant role in anti-inflammatory (Wu et al. 2008, 2010; Han et al. 2011; Lu et al. 2012b; Chen et al. 2013b; Wang et al. 2013b; Zhu et al. 2013; Fu et al. 2015; Kim et al. 2015; Zhao et al. 2015a; Zhou et al. 2015), antitumor (Tsai et al. 2002; Wang et al. 2010a, 2010b), antiviral (Cheng et al. 2006; Chen et al. 2015), neuromodulation (Yu et al. 2012; Xie et al. 2013; Yoon et al. 2012, 2013; Zhou et al. 2014), and...
and immunoregulation (Sun et al. 2009) activities. The various pharmacological activities, mechanisms, models and applications of SSa are given in Table 1.

**Anti-inflammatory activity**

Among all of the pharmacological activities of SSa, the most important one is anti-inflammatory activity. SSa develops its anti-inflammatory activity mainly by inhibiting some inflammation-associated cytokines, proteins and enzymes, and regulating inflammation-related signal pathways, such as nuclear factor-κB (NF-κB) pathway and mitogen-activated protein kinase (MAPK) pathway. In order to better explain the molecular mechanisms of the anti-inflammatory activity of SSa, Figures 4(a,b) are provided to describe its NF-κB pathway and MAPK pathway.

In general, SSa inhibits the expression of pro-inflammatory cytokines, including tumor necrosis factor α (TNF-α), transforming growth factor-β1 (TGF-β1), interleukin 1β (IL-1β), IL-6, and IL-8, and increases the expression of anti-inflammatory cytokine TGF-β1 and IL-10 (Wu et al. 2008, 2010; Han et al. 2011; Lu et al. 2012a; Zhu et al. 2013; Fu et al. 2015; Kim et al. 2015; Zhao et al. 2015a). SSa exerts inhibiting effect on inflammatory associated proteins and enzymes, such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) (Lu et al. 2012b; Zhu et al. 2013; Fu et al. 2015; Kim et al. 2015), extracellular matrix-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) (Chen et al. 2013b; Zhu et al. 2015), and it also suppresses particular proteins, bone morphogenetic protein 4 (BMP-4) (Wang et al. 2013b), platelet-derived growth factor receptor 1 (PDGFR1) (Chen et al. 2013b) and toll-like receptor 4 (TLR4) (Fu et al. 2015) to exert particular effects (Wang et al. 2013b).

NF-κB pathway is an important signal pathway in inflammatory process (Bannon et al. 2015). SSa attenuates inflammation mainly by inhibiting the activation of NF-κB pathway (Wu et al. 2008, 2010; Han et al. 2011; Lu et al. 2012a; Kim et al. 2015; Zhao et al. 2015a; Zhou et al. 2015). As shown in Figure 4(a), these inhibition effects are mainly reflected in two ways. One is inhibitory effects on phosphorylation of kinases, including IκBα, p65 (Zhu et al. 2013; Kim et al. 2015; Zhou et al. 2015), p38 (Han et al. 2011; Chen et al. 2013b; Zhou et al. 2015), JNK (Zhu et al. 2013; Zhou et al. 2015), and Akt (He et al. 2016), and the other is blocking translocation of nuclear factors, including NF-κB (Lu et al. 2012a; Zhu et al. 2013; Kim et al. 2015) and NF-κB/Rel A (Han et al. 2011). The above two inhibition effects are marked by triangle in Figure 4(a).

As shown in Figure 4(b), SSa also has an inhibiting effect on MAPK pathway. It downregulates the phosphorylation of three key kinase, p38 MAPK, c-JNK, and ERK 1/2, which are located in the downstream of MAPK pathway and marked by triangle symbol in Figure 4(b).

For studying the anti-inflammatory activity of SSa, it has been applied to mouse macrophage cells RAW264.7 (Zhou et al. 2015), human umbilical vein endothelial cells (HUVECs) (Fu et al. 2015), mouse embryonic fibroblasts 3T3-L1 (Kim et al. 2015), hepatic stellate cells (HSCs) (Chen et al. 2013b), and human mast cells (HMCs) (Han et al. 2011) in vitro, and has been applied to the livers of Sprague-Dawley rats (Wu et al. 2010) and Wistar rats (Zhao et al. 2015a) in vivo.

**Neuroregulation activity**

SSa plays a significant role on neuroregulation. It exerts antiepileptic mainly by inhibiting N-methyl-D-aspartic acid (NMDA) receptor current, persistent sodium current (Yu et al. 2012) and inactivating K+ current (Xie et al. 2013). It inhibits the activation of p38 MAPK, NF-κB signaling pathways to attenuate neuropathic pain (Zhou et al. 2014), and activates γ-aminobutyric acid (GABA) receptor B to attenuate cocaine-reinforced behavior (Yoon et al. 2012, 2013) and drug addiction (Maccioni et al. 2016). It also counteracts the inflammatory response and neurological function deficits via an anti-inflammatory response and inhibition of the MAPK signaling pathway to ease nerve injury (Mao et al. 2016). SSa has been applied to the hippocamp, CA1 neurons, and spinal cord tissues of Sprague-Dawley rats (Mao et al. 2016; Maccioni et al. 2016; Yu et al. 2012; Xie et al. 2013; Yoon et al. 2012, 2013), and chronic constriction injury rats (Zhou et al. 2014) in vivo, which determined its potential application in epilepsy, chronic constriction injury, nerve injury, and drug addiction.
| Pharmacological activities of SSa | Tissue | Models/cells | In vivo/vitro | Mechanisms | Applications | References |
|----------------------------------|--------|-------------|---------------|------------|-------------|------------|
| Anti-inflammatory activity        | Adipocytes | 3T3-L1 | In vitro | SSa inhibits the expression of inflammatory associated genes and is a potent inhibitor of NF-κB activation. SSa suppresses the production of TNF-α and IL-6 and inhibits the nucleotide-binding oligomerization domain 2 (NOD2)/NF-κB signalling pathway. | Obesity-associated inflammation | (Kim et al. 2015) |
|                                  | Ileum | Male Wistar rats | In vivo | SSa down-regulates BMP-4 expression and inhibits hepatic stellate cell activation. | Sepsis | (Zhao et al. 2015a) |
|                                  | Liver | LX-2 | In vitro | SSa regulates inflammatory mediators and suppresses the MAPK and NF-κB signalling pathways. | Liver fibrosis | (Wang et al. 2013b) |
|                                  | Macrophages | RAW 264.7 | In vitro | SSa inhibits receptor activator of the nuclear factor-κB ligand (RANKL)-induced IκBα phosphorylation, p65 phosphorylation and NF-κB lucerase activity | Lipopolysaccharide (LPS)-induced inflammation | (Zhu et al. 2013b) |
|                                  | Vascular tissue | HUVECs | In vitro | SSa dose-dependently inhibits the production of ROS, TNF-α, IL-8, COX-2 and iNOS in LPS-stimulated HUVECs. | Oxidative damage | (Fu et al. 2015) |
|                                  | Liver | HSC-T6 | In vitro | SSa decreases the expressions of ERK1/2, PDGFR, TGF-β1R, α-smooth muscle actin, and connective tissue growth factor to inhibit proliferation and activation of HSCs. | Liver inflammation and fibrogenesis | (Chen et al. 2013a) |
|                                  | Macrophages | RAW264.7 | In vitro | SSa decreases the expression of hepatic proinflammatory cytokines and NF-κB signal pathway and increases the expression of anti-inflammatory cytokine IL-10. | Inhibition of liver injury | (Wu et al. 2008, 2010) |
|                                  | Inflammatory tissue | HMC-1 | In vitro | SSa decreases the expression of IL-6, IL-1β and TNF-α and suppresses NF-κB signal pathway. | LPS-induced inflammation | (Lu et al. 2012a) |
|                                  | Liver | Sprague-Dawley rats | In vivo | SSa attenuates viral replication, aberrant pro-inflammatory cytokine production and lung histopathology. | Anti-inflammation | (Han et al. 2011) |
|                                  | Human monocytic leukemia cells | THP-1 | In vitro | SSa inhibits oxLDL-induced activation of AKT and NF-kappaB, assembly of NLRP3 inflammasome and production of pro-inflammatory cytokines. | Atherosclerosis | (He et al. 2016) |
| Neuroregulation                  | Hippocampal tissue | Sprague-Dawley rats | In vivo | SSa inhibits NMDA receptor current and persistent sodium current. | Epilepsy | (Yu et al. 2012) |
|                                  | CA1 neurons | Sprague-Dawley rats | In vivo | SSa attenuates cocaine-reinforced behaviour through activation of GABA(B) receptors. | Epilepsy | (Xie et al. 2013) |
|                                  | Spinal cord tissues | Chronic constriction injury rats | In vivo | SSa counteracts the inflammatory response and neurological function deficits via an anti-inflammatory response and inhibition of the MAPK signalling pathway. | Chronic constriction injury | (Zhou et al. 2014) |
|                                  | Hippocampus | Sprague-Dawley rats | In vivo | SSa counteracts the inflammatory response and neurological function deficits via an anti-inflammatory response and inhibition of the MAPK signalling pathway. | Morphine-reinforced behaviour | (Yoon et al. 2012, 2013) |
|                                  | Nervous tissue | Sprague-Dawley rats | In vivo | SSa inhibits this addiction by regulating GABA(B) receptor system. | Nerve injury | (Mao et al. 2016) |
|                                  | Nervous tissue | Sprague-Dawley rats | In vivo | SSa sensitizes cancer cells to cisplatin through ROS-mediated apoptosis. | Drug addiction | (Maccioni et al. 2016) |
| Antitumor activity               | Different cancer cells | A549, SKOV3, HeLa and Siha | In vitro | SSa enhances the enzymatic activities of GS and CNP. | Cancer cell cytotoxicity | (Wang et al. 2010a) |
|                                  | Glioma | C6 glioma cells | In vivo | SSa inhibits cancer cells to cisplatin through ROS-mediated apoptosis. | G6 glioma cells proliferation | (Tsai et al. 2002) |
| Antiviral activity               | Human fetal lung fibroblasts | Human coronavirus 229E | In vitro | SSa intervenes in the early stage of viral replication, such as absorption and penetration. | Coronavirus infection | (Cheng et al. 2006) |
|                                  | Lung tissue | Influenza A virus infected A549 | In vitro | SSa attenuates viral replication, aberrant pro-inflammatory cytokine production and lung histopathology. | Pathological influenza virus infections | (Chen et al. 2015) |
| Immunoregulation                 | Lymphoid tissue | Sprague-Dawley rats | In vivo | SSa inhibits the proliferation and activation of T cells and causes the G0/G1 arrest as well as the induction of apoptosis via mitochondrial pathway. | Inflammatory and autoimmune diseases | (Sun et al. 2009) |
Figure 4. The molecular mechanisms of the anti-inflammatory activity of SSa. (a) shows the NF-κB pathway, (b) shows the MAPK pathway.
SSa exhibits antitumor activity in vitro by sensitizing cancer cells to cisplatin, such as human lung adenocarcinoma cells A549, ovarian cancer cells SKOV3, and cervix cancer cells Hela and Siha, through reactive oxygen species (ROS)-mediated apoptosis (Wang et al. 2010a) and enhancing the enzymatic activities of glutamine synthetase (GS) and \(2,3^\prime\)-cyclic nucleotide 3′-phosphohydrolase (CNP) in rat C6 glioma cells (Tsai et al. 2002). Thus, the combination of SSa with cisplatin could be an effective therapeutic strategy against cancer.

SSa has generally inhibitory effects against human coronavirus 229E (Cheng et al. 2006) and influenza A virus (Chen et al. 2015). It exerts antiviral activity mainly through interference in the early stage of viral replication, such as absorption and penetration (Chen et al. 2015), and attenuating aberrant pro-inflammatory cytokine production (Cheng et al. 2006). These two viruses are cultured in human cells, human fetal lung fibroblasts MRC-5 and A549 cells, respectively.

**Immunoregulation activity**

SSa inhibits the proliferation and activation of T cells and causes the G0/G1 cells arrest as well as the induction of apoptosis via mitochondrial pathway to exhibit its immunoregulation effect in Sprague-Dawley rats (Sun et al. 2009). This may herald a novel approach for further studies of SSa as a candidate for the treatment of autoimmune diseases.

SSd is the epimer of SSa, they have the same basal structure. So, it has some similar pharmacological activities with SSa, such as anti-inflammatory (Lu et al. 2012b), antitumor (Chen et al. 2013a), and immunoregulation activities (Sun et al. 2009; Ying et al. 2014). However, SSd also possesses some specific pharmacological activities, such as anti-allergic (Hao et al. 2012) and
Table 2. The various pharmacological activities, mechanisms, models, and applications of SSd.

| Pharmacological activities of SSd | Tissue | Models/cells | In vivo/in vitro | Mechanisms | Applications | References |
|----------------------------------|--------|--------------|------------------|------------|--------------|------------|
| Antitumor activity               | Liver  | Sprague Dawley rats | In vivo | SSd inhibits the activation of CCAAT/enhancer binding protein (C/EBP) and COX-2. SSd promotes cell apoptosis and induced G1-phase cell cycle arrest. | Human hepatocellular carcinoma | (Lu et al. 2012b) |
|                                  | Thyroid| ARO, 805C, SW1736 | In vivo | SSd suppresses the expression of COX-2 through the p-STAT3/hypoxia inducible factor-1α (HIF-1α) pathway. | Human undifferentiated thyroid carcinoma | (Li & Li 2014) |
|                                  | Liver  | SMMC7721 | In vitro | SSd has effects on induction of apoptosis and cell cycle arrest at G0/G1 phase. | Human hepatocellular carcinoma | (He et al. 2014) |
|                                  | Prostate carcinoma cells | DU145 | In vitro | SSd suppresses TNF-α-induced NF-κB activation and its target genes expression to inhibit cancer cell proliferation, invasion, angiogenesis and survival. | Prostate carcinoma | (Yao et al. 2014) |
|                                  | Different cancer cells | HeLa, HepG2 | In vitro | SSd has a radiosensitizing effect on hepatoma cells under hypoxic conditions by inhibiting HIF-1α expression. | As a combined adjuvant remedy with TNF-α for cancer patients | (Wong et al. 2013a) |
|                                  | Lung carcinoma | A549 | In vitro | SSd induces apoptosis and blocked cell cycle progression by activating Fas/FasL pathway in the G1 phase in A549 cells. | Human non-small cell lung cancer | (Hsu et al. 2004a) |
|                                  | Liver  | HepG2, 2.2.15 | In vivo | SSd induces the apoptosis through the activation of caspase-3 and caspase-7. | Human hepatocellular carcinoma | (Chiang et al. 2003) |
|                                  | Liver  | Hep3B | In vitro | SSd acts as a weak phytoestrogen. | Human hepatocellular carcinoma | Zhou 2003 |
|                                  | Breast carcinomas tissue | MCF-7 | In vitro | SSd has inhibitory effects on NF-κB activation and iNOS, COX-2 and pro-inflammatory cytokines including TNF-α and IL-6. | Acting as a weak phytoestrogen. | (Wang et al. 2010a) |
|                                  | Liver  | SMMC-7721, HepG2 | In vitro | SSd induces autophagy through the formation of autophagosomes by inhibiting SRECA. | Radiotherapy sensitizer in hepatoma radiotherapy | (Wang et al. 2014a, 2014b) |
|                                  | Different cancer cells | HeLa, MCF-7 | In vitro | SSd regulates kidney oxidative stress by inhibiting NF-κB p65 and increasing IκBα activity. | LPS-induced inflammation | (Wong et al. 2013b) |
| Anti-inflammatory activity        | Inflammatory tissue | RAW264.7 | In vitro | SSd suppresses the expression of extracellular matrix-regulated kinase 1/2 (ERK1/2), PDGFR, TGF-β1R, α-smooth muscle actin, TGF-β1 and connective tissue growth factor. | Liver inflammation and fibrogenesis | (Lu et al. 2012a) |
|                                  | Hepatic stellate cells | HSC-T6 | In vitro | SSd down-regulates liver TNF-α, IL-6 and NF-κB p65 expression and increases IκBα activity. | L-selectin-mediated cell adhesion | (Jang et al. 2014) |
|                                  | Human acute monocytic leukaemia cells | THP-1 | In vitro | SSd down-regulates NF-κB and STAT3-mediated inflammatory signal pathways. | Hepatotoxicity and liver injury | (Liu et al. 2014a) |
|                                  | Liver  | C57/BL6 rats | In vivo | SSd reduces the expression of pro-inflammatory cytokines including MIP-2, IL-6 and TNF-α and elevates the expression of anti-inflammatory mediators, such as TGF-β1 and IL-10. | Oxidative damage in the kidney | (Dang et al. 2007) |
|                                  | Liver  | Hepatic fibrosis rats | In vivo | SSd decreases the expression of pro-inflammatory cytokines including MIP-2, IL-6 and TNF-α and elevates the expression of anti-inflammatory mediators, such as TGF-β1 and IL-10. | Inflammation in C6 rat glioma cells | (Kodama et al. 2003) |
|                                  | Kidney | LLC-PK1 | In vitro | SSd possesses a dual effect: an inhibition of PGE2 production without a direct inhibition of cyclooxygenase activity and an elevation of [Ca^{2+}]i. | L-selectin-mediated cell adhesion | (Zhang et al. 2015b) |
|                                  | Nervous tissue | C6 rat glioma cells | In vitro | SSd down-regulates liver TNF-α, IL-6 and NF-κB p65 expression and increases IκBα activity. | Oxidative damage in the kidney | (Dang et al. 2007) |
|                                  | Lung   | VILI rats | In vivo | SSd regulates kidney oxidative stress by inhibiting NF-κB p65 and increasing IκBα activity. | Liver inflammation and fibrogenesis | (Kodama et al. 2003) |
|                                  | Renal tubular epithelial cells | NRK-52E | In vitro | SSd attenuates oxidative injury via upregulation of SIRT1. | DDP-induced kidney injury | (Ma et al. 2015) |
|                                  | Kidney | HK-2 | In vitro | SSd represses ROS-mediated activation of MAPK and NF-κB signal pathways. | High glucose induced kidney injury | (Zhao et al. 2015b) |

(continued)
| Pharmacological activities | Mechanisms | Applications | Models/Cells | References |
|---------------------------|------------|--------------|--------------|------------|
| Immunoregulation          | Lymphoid tissue | Mouse T cells | In vitro | (Wong et al. 2009) |
| Anti-inflammatory activity | Lymphoid tissue | Mouse T cells | In vitro | (Wong et al. 2013) |
| Antitumor activity        | Mouse T cells | In vitro | | (Chiang et al. 2003; Chou et al. 2003) |
| Neuroregulation           | Neuronal cells | PC12 cells | In vitro | (Lin et al. 2016) |

**Pharmacological activities of SSd Tissue Models/cells**

| In vivo / in vitro | Immunoregulation | Application | Mechanism | Model/Cell | Reference |
|-------------------|------------------|-------------|-----------|------------|-----------|
| SSd inhibits the T cell proliferation and activation | In vivo | SSd reduces the differentiation of human DCs and promotes DCs maturation and increases the function of mature DCs. | SSd suppresses the differentiation of human DCs and promotes DCs maturation and increases the function of mature DCs. | DCs | (Hao et al. 2012) |
| SSd suppresses the intracellular calcium mobilization and tyrosine phosphorylation | In vivo | SSd reduces PC12 cells apoptosis by removing ROS and blocking MAPK-dependent oxidative damage. | SSd reduces PC12 cells apoptosis by removing ROS and blocking MAPK-dependent oxidative damage. | PC12 cells | (Lin et al. 2016) |
| SSd suppresses the activity and expression of inhibitor of nuclear factor of kappa B (IκB-α) | In vivo | SSd reduces the expression of B cell lymphoma 2 (Bcl-2) family proteins. | SSd reduces the expression of B cell lymphoma 2 (Bcl-2) family proteins. | B cells | (Li et al. 2014b) |
| SSd increases the expression of p53 and Bax | In vivo | SSd increases the expression of p53 and Bax (Liu & Li 2014; Wang et al. 2014a, 2014b; Yao et al. 2014), activates caspases apoptosis pathway, including the activation of caspases-3 and caspases-7 | SSd increases the expression of p53 and Bax (Liu & Li 2014; Wang et al. 2014a, 2014b; Yao et al. 2014), activates caspases apoptosis pathway, including the activation of caspases-3 and caspases-7 | Cancer cells | (Chiang et al. 2003; Chou et al. 2003; Hsu et al. 2004a) |
| SSd suppresses the activity and expression of iNOS, COX-2, ERK1/2, PDGFR, and signal transducer and activator of transcription 3 (STAT3) | In vivo | SSd suppresses the activity and expression of iNOS, COX-2, ERK1/2, PDGFR, and signal transducer and activator of transcription 3 (STAT3) | SSd suppresses the activity and expression of iNOS, COX-2, ERK1/2, PDGFR, and signal transducer and activator of transcription 3 (STAT3) | Cancer cells | (Chen et al. 2013a; Liu et al. 2014a; Hsu et al. 2004a) |
| SSd suppresses the expression of COX-2, which has been shown to be involved in carcinogenesis | In vivo | SSd suppresses the expression of COX-2, which has been shown to be involved in carcinogenesis | SSd suppresses the expression of COX-2, which has been shown to be involved in carcinogenesis | Cancer cells | (Lu et al. 2012b; He et al. 2014) |
| SSd increases the expression of COX-2, which has been shown to be involved in carcinogenesis | In vivo | SSd increases the expression of COX-2, which has been shown to be involved in carcinogenesis | SSd increases the expression of COX-2, which has been shown to be involved in carcinogenesis | Cancer cells | (Lu et al. 2012b; He et al. 2014) |
| SSd decreases the expression of B cell lymphoma 2 (Bcl-2) family proteins | In vivo | SSd decreases the expression of B cell lymphoma 2 (Bcl-2) family proteins | SSd decreases the expression of B cell lymphoma 2 (Bcl-2) family proteins | Cancer cells | (Lu et al. 2012b) |
| SSd increases the expression of COX-2, which has been shown to be involved in carcinogenesis | In vivo | SSd increases the expression of COX-2, which has been shown to be involved in carcinogenesis | SSd increases the expression of COX-2, which has been shown to be involved in carcinogenesis | Cancer cells | (Lu et al. 2012b) |

**Antitumor activity**

The most important pharmacological activity of SSd is antitumor activity. In order to better explain this important activity, Figure 5 is provided to describe its molecular mechanisms. SSd exhibits the antitumor activity mainly through activation and inhibition, which are marked by rectangle and triangle in Figure 5, respectively. First, SSd increases the expression of p53 and Bax (Liu & Li 2014; Wang et al. 2014a, 2014b; Yao et al. 2014), activates caspases apoptosis pathway, including the activation of caspases-3 and caspases-7 (Chiang et al. 2003; Chou et al. 2003) and the Fas/FasL apoptotic system (Hsu et al. 2004a) in several cancer cell lines in vitro, which are marked by rectangle in Figure 5. Second, SSd decreases the expression of B cell lymphoma 2 (Bcl-2) family proteins (Liu & Li 2014; Wang et al. 2014a, 2014b; Yao et al. 2014), suppresses the expression of COX-2, which has been shown to be involved in carcinogenesis (Lu et al. 2012b; He et al. 2014), and also potentiates TNF-α-mediated cell death via suppression of TNF-α-induced NF-κB activation (Wong et al. 2013a), which are marked by triangle in Figure 5. Besides, SSd also suppresses MCF-7 cells proliferation through the estrogens activity of SSd by the estrogen receptor (Wang et al. 2010a, 2010b), and induces autophagy of apoptosis-resistant cancer cells through the formation of autophagosomes by inhibiting sarcoplasmic/endoplasmic reticulum Ca²⁺ ATPase pump (SERCA) (Wong et al. 2013b).

To date, SSd has been applied in human hepatoma cells HepG2, Hep3B (Chou et al. 2003), SMMC7721 (He et al. 2014), and 2.2.15 cells (Chiang et al. 2003), anaplastic thyroid cancers cells ARO, 8305C, and SW1736 (Liu & Li 2014), prostate carcinoma cells DU145 (Yao et al. 2014), lung cancer cells A549 (Hsu et al. 2004a), cervical carcinoma cells Hela (Wong et al. 2013a, 2013b), and breast carcinoma cells MCF-7 (Wang et al. 2010b) in vitro, and applied in diethylaminoethane (DEA)-treated Sprague Dawley rats in vivo (Lu et al. 2012b), and which indicates its potential in treatment of cancer.

**Anti-inflammatory activity**

SSd also possesses an evident anti-inflammatory activity, and the mechanisms are similar to SSA, as shown in Figure 4(a). On the cytokines level, SSd suppresses pro-inflammatory cytokines including TNF-α, IL-6, macrophage inflammatory protein-2 (MIP-2), and elevates the expression of anti-inflammatory cytokines, such as TGF-β1 and IL-10 (Lu et al. 2012a; Ma et al. 2015; Wang et al. 2015). On the level of proteins and enzymes, it inhibits the activity and expression of iNOS, COX-2, ERK1/2, PDGFR, and smooth muscle actin, NF-κB, and signal transducer and activator of transcription 3 (STAT3) (Chen et al. 2013a; Liu et al. 2014a), and increases the activity and expression of inhibitor of nuclear factor of κB (IκB-α) (Dang et al. 2007), SirT3 (Zhao L et al. 2015), anti-oxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and heat shock protein (HSP) 72 (Zhang et al. 2014). Furthermore, SSd also exhibits its particular anti-inflammatory pattern by inhibiting selectin-mediated cell adhesion (Jang et al. 2014), and possessing a dual effect, an inhibition of prostaglandin E₂ (PGE₂) production without a direct inhibition of cyclooxygenase activity and an elevation of Ca²⁺ (Kodama et al. 2003).
According to the above reports, SSa and SSd are very similar in mechanisms of anti-inflammation, however, there are still several different points, which are listed in Table 3. SSa is able to inhibit phosphorylation of three key kinase in MAPK pathway, which was not reported in researches of SSd. While SSd is able to restrain selectin-mediated cell adhesion, PGE2 production, and elevate the Ca2+ level intracellular, which were not reported in researches of SSa.

For a better understanding of SSd’s anti-inflammatory activity, it has been applied to mouse leukaemic monocyte macrophage macroph RAW264.7 (Lu et al. 2012a), hepatic stellate cells HSC-3, human acute monocyte cells THP-1 (Jang et al. 2014), pig kidney proximal tubular cells LLC-PK1 (Zhang et al. 2014), C6 rat glioma cells (Kodama et al. 2003), renal tubular epithelial cells NRK-52E (Zhao et al. 2015b), and HK-2 (Ma et al. 2015) in vitro, and acetonophen-induced hepatotoxicity C57BL/6 rats (Liu et al. 2014a), hepatic fibrosis model rats (Dang et al. 2007), and ventilator-induced lung injury (VILI) rats (Shin et al. 2015). However, the pharmacological activities of SSd are far weaker than SSa and SSd. To date, reports about pharmacological activities of SSc are very limited. SSa exerts anti-apoptotic effects on HUVECs by suppressing caspase-3 activation and subsequent degradation of FAK and other cell adhesion signals, which is similar to SSa (Lee et al. 2014). Thus, it will be a promising therapeutic candidate for the treatment of vascular endothelial cell injury and cellular dysfunction. Besides, SSc completely prevents the development of nephritis (Chen et al. 2008), but the mechanism of this activity is still unclear. In addition, SSc exhibits antiviral activity by inhibiting hepatitis B virus (HBV) DNA replication (Chiang et al. 2003).

SSc

SSc has the same basal structure with SSa and SSd. They are epoxy-ether saikosaponins belonging to type I saikosaponins (Shin et al. 2015). However, the pharmacological activities of SSc are far weaker than SSa and SSd. To date, reports about pharmacological activities of SSc are very limited. SSc exerts anti-apoptotic effects on HUVECs by suppressing caspase-3 activation and subsequent degradation of FAK and other cell adhesion signals, which is similar to SSa (Lee et al. 2014). Thus, it will be a promising therapeutic candidate for the treatment of vascular endothelial cell injury and cellular dysfunction. Besides, SSc completely prevents the development of nephritis (Chen et al. 2008), but the mechanism of this activity is still unclear. In addition, SSc exhibits antiviral activity by inhibiting hepatitis B virus (HBV) DNA replication (Chiang et al. 2003).

SSb2

SSb2 has a different basic structure compared to SSa, SSd, and SSc. SSb2 is a type II saikosaponin, and it is not considered as a main active compound in Radix Bupleuri. However, SSb2 has fairly inhibitory effects against corona virus and hepatitis C virus (HCV). It mainly interferes with the early stages of viral replication, such as absorption and penetration of the virus (Cheng et al. 2006). SSb2 potently inhibits HCV infection at non-cytotoxic concentrations through efficient inhibition on early HCV entry, including neutralization of virus particles, preventing viral attachment, and inhibiting viral entry/fusion (Lin et al. 2014).

Radix Bupleuri extracts

Many Bupleurum medicinal plants are used as Radix Bupleuri. The pharmacological activities of extracts from seven Bupleurum species, B. chinense (Wen et al. 2011), B. falcatum (Lee et al. 2012a), Bupleurum marginatum Wall. ex DC. (Ashour et al. 2014), B. yinchowense (Li et al. 2013), Bupleurum kaoi L. (Hsu et al. 2004a, 2004b), B. scorzonerifolium (Cheng et al. 2005), and Bupleurum longiradiatum Turkz. (You et al. 2002), are given in Table 4. They have been demonstrated to possess antitumor (Cheng et al. 2003, 2005; Hsu et al. 2004a, 2004b; Chen et al. 2005; Kang et al. 2008; Ashour et al. 2014), antiviral (Wen et al. 2011), anti-inflammatory (Lee et al. 2010; Nakahara et al. 2011), anti-hyperthyroidism (Kim et al. 2012b)

Table 3. The similarities and differences of SSa and SSd in mechanisms of anti-inflammation.

| The possible mechanisms of anti-inflammation                  | SSa | SSd |
|---------------------------------------------------------------|-----|-----|
| Inhibiting pro-inflammatory cytokines and promoting anti-inflammatory cytokines | ✔   | ✔   |
| Inhibiting activity of enzymes associated with inflammation  | ✔   | ✔   |
| Inhibiting activation of NF-κB pathway                        | ✔   | ✔   |
| Inhibiting activation of MAPK pathway                         | ✔   | ✔   |
| Inhibiting selectin-mediated cell adhesion                     | ✔   | ✔   |
| Inhibiting PGE2 production and elevating Ca2+ level intracellular | ✗   | ✔   |

References

For a better understanding of SSd’s anti-inflammatory activity, it has been applied to mouse leukaemic monocyte macrophage macroph RAW264.7 (Lu et al. 2012a), hepatic stellate cells HSC-3, human acute monocyte cells THP-1 (Chen et al. 2013a), pig kidney proximal tubular cells LLC-PK1 (Zhang et al. 2014), C6 rat glioma cells (Kodama et al. 2003), renal tubular epithelial cells NRK-52E (Zhao et al. 2015b), and HK-2 (Ma et al. 2015) in vitro, and acetonophen-induced hepatotoxicity C57BL/6 rats (Liu et al. 2014a), hepatic fibrosis model rats (Dang et al. 2007), and ventilator-induced lung injury (VILI) rats (Wang et al. 2015) in vivo, which determined its potential application for treating hepatitis, pneumonia, nephritis and other inflammation.

Immunoregulation activity

SSd plays its immunoregulation role by regulating the NF-κB, nuclear factor-AT (NF-AT), and activator protein 1 (AP-1) signal pathways to inhibit T cell proliferation and activation (Wong et al. 2009). It has been applied to condylomata acuminata, a disease caused by human papilloma virus (HPV), by reducing the differentiation of human monocyte-derived dendritic cells (DCs) and promoting DCs maturation and increasing the function of mature DCs (Ying et al. 2014).

Anti-allergic activity

β-Conglycinin has been identified as a potential diagnostic marker for severe basophil-dependent allergic reactions to soybean. SSd possesses anti-allergic activity by inhibiting β-conglycinin-induced rat basophilic leukemia-2H3 cell degranulation and suppressing critical incidents in the signal transduction pathway (Hao et al. 2012). Hence it could become an effective herbal therapy for alleviating soybean allergy.

Neuroregulation activity

Neuronal oxidative stress injury has been proven to be associated with many neurodegenerative diseases. SSd exerts neuroregulation activity on neuronal PC12 cells by inhibiting the translocation of the glucocorticoid receptor (GR) to the mitochondria, restoring mitochondrial function, down-regulating the expression of pro-apoptotic-related signalling events and up-regulating anti-apoptotic-related signalling events (Li et al. 2014b). In H2O2-induced oxidative stress PC12 cells, SSd effectively decreases oxidative stress injury by blocking H2O2-induced phosphorylation of ERK, JNK, and p38MAPK to exert neuroregulation activity (Lin et al. 2016). Thus, SSd treatment is an effective method for treating neurodegenerative diseases.
| Species          | Extractive fractions | Extraction method          | Activities                      | Mechanisms                                                                                                                                                                                                 | References                      |
|------------------|----------------------|----------------------------|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| *B. chinensis*   | Aqueous extracts     | Water decoction, 3 h       | Antitumor activity             | Enhancing 5-fluorouracil-induced cytotoxicity in HepG2 hepatoma cells and protecting normal blood lymphocytes.                                                                                                 | (Kang et al. 2008)               |
|                  |                      | Water decoction, 3 h       | Antiviral activity             | Suppressing the effect on regulated activation normal T-cell expressed (RANTES) secretion.                                                                                                               | (Wen et al. 2011)               |
|                  | Methanol TSS extracts| Water decoction, 3 h       | Affect drug distribution       | Inhibiting the activity of β-glucuronidase.                                                                                                                                                                | (Chen et al. 2014)              |
|                  |                      | Methanol, reflux, 4 h      | Neuroregulation                | Suppressing the abnormal activation of hippocampal astrocyte through inhibiting the overexpression of glial fibrillary acidic protein.                                                                       | (Xie et al. 2006)               |
|                  |                      | 95% methanol 5% pyridine, |                               | TSS antagonizes the reserpine-induced akinesia, and ptosis in mice.                                                                                                                                       | (Liu et al. 2014a)              |
|                  |                      | reflux, 4 h                |                               |                                                                                                                                                                                                            |                                 |
| *B. falcatum*    | Ethanol extracts     | 70% ethanol, reflux, 6 h   | Anti-inflammatory activity     | Inhibiting the expression and activation of both metal matrix proteinase (MMP)-2 and MMP-9 after spinal cord injury (SCI) and the mRNA expressions of TNF-α, IL-1β, COX-2, and iNOS.           | (Lee et al. 2010)               |
|                  |                      | 80% ethanol, reflux, 6 h   | Anti-depressant activity       | Reducing depression and anxiety-like behaviors, possibly through central adrenergic mechanism.                                                                                                              | (Lee et al. 2012a)              |
|                  |                      | 80% ethanol, reflux, 6h    | Memory improvement             | Attenuating IMO stress-induced loss of cholinergic immunoreactivity in the hippocampus.                                                                                                                   | (Lee et al. 2009)               |
|                  | Methanol extracts    | Methanol, reflux, 4 h      | Anti-depressant activity       | The mechanism of this activity involves the serotonergic and noradrenergic systems.                                                                                                                       | (Kwon et al. 2010)              |
|                  |                      | Methanol, reflux, 4 h      | Anti-inflammatory activity     | Decreasing the content of alanine transaminase (ALT) in blood serum of the liver injury rats.                                                                                                              | (Nakahara et al. 2011)          |
|                  | Aqueous extracts     | Water decoction, 3 h       | Anti-hyperthyroidism           | Attenuating LT4-induced hyperthyroidisms and normalizing LT4-induced liver oxidative stresses and reducing liver and epididymal fat pad changes.                                                              | (Kim et al. 2012b)              |
| *B. scorzonerifolium* | Acetone extracts   | Acetone, reflux, 4h        | Antitumor activity             | Inducing tubulin polymerization, and activates caspase-3 and caspase-9 in A549 cells, and these effects are related to ERK 1/2 activation and the apoptosis.                                               | (Chen et al. 2005; Cheng et al. 2005) |
| *B. marginatum*  | Methanol extracts    | Methanol, reflux, 4 h      | Anti-infective and antitumor activities | Methanol extracts show a significant anti-trypanosomal activity and moderate activity against *Streptococcus pyogenes* and have the cytotoxicity inducing apoptosis.                                          | (Cheng et al. 2003)             |
| *B. longiradiatum* | Ethyl acetate extracts | Ethyl acetate, reflux, 4 h | Antiangiogenic activity        | It has an inhibitory effect on the tube-like formation of HUVECs.                                                                                                                                         | (Ashour et al. 2014)            |
| *B. yinchowense* | Ethanol TSS extracts | 60% ethanol 0.5% ammonia reflux, 6 h | Neuroregulation               | The neuroprotective mechanism relates with inhibiting the ER stress and the mitochondrial apoptotic pathways.                                                                                           | (You et al. 2002)               |
| *B. kaoi*        | Methanol TSS extracts| Methanol, reflux, 4 h      | Antitumor activity             | The activity of the Fas/Fas ligand apoptotic system participates in the antiproliferative activity of TSS in A549 cells.                                                                                 | (Hu et al. 2016)                |
|                  |                      | Methanol, reflux, 4h       |                               | Extracts from *B. kaoi* show potent antiproliferative effects on human A375.S2 melanoma cells.                                                                                                            |                                 |
and neuroregulation effects (Xie et al. 2006; Lee et al. 2009, 2012b; Li et al. 2013; Liu et al. 2014b).

Five kinds of extraction agents, water, methanol, ethanol, acetone, and ethyl acetate, have been used to extract effective fractions from Radix Bupleuri. Aqueous extracts of Radix Bupleuri are obtained by boiling at 80°C for 3 h, and then evaporating and lyophilizing (Kang et al. 2008; Wen et al. 2011; Kim et al. 2012b; Chen et al. 2014). The method to obtain methanol, ethanol, acetone and ethyl acetate extracts is reflux extraction (You et al. 2002; Cheng et al. 2005; Lee et al. 2010; Liu et al. 2014a). To obtain methanol extracts, Radix Bupleuri is extracted twice by 100% methanol or 95% methanol with 5% pyridine at 70°C for 4 h (Xie et al. 2006; Kwon et al. 2010; Nakahara et al. 2011; Liu et al. 2014a; Ashour et al. 2014). To obtain ethanol extracts, Radix Bupleuri is extracted twice by 60% (Li et al. 2013), 70% (Lee et al. 2010) or 80% ethanol (Lee et al. 2012a) at room temperature for 6 h. To obtain acetone and ethyl acetate extracts, Radix Bupleuri is extracted three times by 100% acetone and 100% ethyl acetate at room temperature for 4 h (You et al. 2002; Cheng et al. 2005).

The pharmacological activities of extracts from B. chinense and B. falcatum have relative in-depth studies. The aqueous extracts of B. chinense possess three activities, antitumor activity on HepG2 hepatoma cells (Kang et al. 2008), antiviral activity on H1N1-infected A549 cells (Wen et al. 2011), and an activity to affect drug distribution (Chen et al. 2014). Methanol total saikosaponins (TSS) extracts of B. chinense have a neuroregulation effect (Xie et al. 2006; Liu et al. 2014a). In chronic kindling rats induced by pentetrazole (PTZ), TSS of B. chinense inhibit gial fibrillary acidic protein (GFAP) over-expression and suppress the abnormal activation of hippocampal astrocyte (Xie et al. 2006). Anti-depressant activity of TSS is investigated by tail suspension test, forced swimming test, and reserpine antagonism test in mice, which demonstrate that it shortens the immobility time of mice in the tail suspension test in a somewhat dose-dependent manner (Liu et al. 2014a).

Both ethanol extracts and methanol extracts of B. falcatum have an anti-inflammatory effect (Lee et al. 2010; Nakahara et al. 2011) with similar mechanisms to SSa. They also possess an anti-depressant activity possibly through central adrenergic mechanism (Kwon et al. 2010; Lee et al. 2012a). Besides, the ethanol extracts of B. falcatum has its specific memory improvement activity by attenuating immobilization (IMO) stress-induced loss of cholinergic immunoreactivity in the hippocampus (Lee et al. 2009). The aqueous extracts of B. falcatum has an anti-hyperthyroidism activity by attenuating leukotriene-4 (LT4)-induced hyperthyroidisms, normalizing LT4-induced liver oxidative stresses and reducing liver and epididymal fat pad changes (Kim et al. 2012b).

The acetone extracts of B. scorzonerifolium exerts stronger antitumor activity on A549 cells mainly through inducing tubulin polymerization (Chen et al. 2005), activating caspase-3 and caspase-9 (Cheng et al. 2005), and inhibiting telomerase activation (Cheng et al. 2003). Methanol extracts of B. marginatum and B. kaoi have an antitumor activity by inducing apoptosis (Ashour et al. 2014) and activating the Fas/Fas ligand apoptotic system respectively (Hsu et al. 2004b), and extracts of B. kaoi have antitumor activity on human A375.52 melanoma cells by inhibiting phosphorylation of JNK, p38 and p53, decreasing level of cytochrome c (Hu et al. 2016). What’s more, the ethanol TSS extracts of B. yinchowense show antidepressant activity by inhibiting the estrogen receptor (ER) stress and the mitochondrial apoptotic pathways (Li et al. 2013), and the ethyl acetate extracts of B. longiradiatum exhibit an antiangiogenic activity by inhibiting the tube-like formation of HUVECs (You et al. 2002).

Applications of Radix Bupleuri in TCM

Radix Bupleuri has been used for more than 2000 years in China since its first record in Shen Nong Ben Cao Jing (Xie et al. 2009). And now, it is officially listed in Chinese Pharmacopeia. In TCM, Radix Bupleuri is mainly used to treat liver diseases, alleviate cold fever, chills, chest pain, regulate menstruation, and improve uterine prolapsed (Zhou 2003). In particular, Radix Bupleuri also plays a significant role in the treatment of malaria (Xue et al. 1996). Importantly, Radix Bupleuri is usually used as a monochor in many traditional Chinese prescriptions.

To date, Radix Bupleuri has been used in about 150 traditional Chinese prescriptions. Among them, Xiao Chai Hu Tang, Chai Hu Gui Zhi Tang, and Xiao Yao San are very famous in TCM. Xiao Chai Hu decoction, including Radix Bupleuri, pinellia (the tuber of Pinellia ternata (Thunb.) Breit., Banxia in Chinese) and skullcap (the root of Scutellaria baicalensis Georgi, Huangqin in Chinese), is used to treat malaria and jaundice. When Radix Bupleuri combines with cassis twig (the twig of Cinnamomum cassia Presl, Guizhi in Chinese), it is called Chai Hu Gui Zhi decoction which is often used for regulating liver-qi, clearing heat, and lifting yang qi. Xiao Yao San, composed of Radix Bupleuri, Poria (Poria cocos (Schw.) Wolf), Radix Paonieae Alba (Paonia lactiflora Pall.), Radix Angelicae Sinensis (Angelica sinensis (Oliv.) Diels), Rhizoma Atractylodis Macrocephalae (Atractylodes macrocephala Koidz.), Herba Menthae (Mentha haplocalyx Briq.), and Rhizoma Zingiberis Recens (Zingiber officinale Rosc.), has been widely used in clinic for treating mental disorders, such as depression and irregular menstruation. In addition, combination with ginseng (Panax ginseng C.A.Mey.) and Radix Astragali (Astragalus membranaceus (Fisch.) Bge.). Radix Bupleuri is also used to treat hemorrhoids, anal and uterine complications, and diarrhea (1998; 1999; World Health Organization 1997). Inspired by the role in regulating metabolism and controlling Yin/Yang as mentioned in the traditional Chinese medicine, Radix Bupleuri is also widely used in Korea and Japan (Van & Wink 2004; Pan 2006).

Applications of Radix Bupleuri in modern Chinese medicine

With the development of TCM modernization, more Radix Bupleuri preparations have been developed, such as Xiao Chai Hu tablets, Chai Hu dripping pills, Chai Hu injection and Chai Hu Shu Gan pills (Li et al. 2014a). The preparations from Radix Bupleuri approved by CFDA from June 2010 to October 2015 are given in Table 5. Among them, Chai Hu injection is the first successful traditional Chinese medicine injection having been used in clinic since 1940s, which is widely used to treat fever caused by influenza or common cold and malaria (Zuo et al. 2013). Moreover, some new dosage forms of Radix Bupleuri have been prepared. A nasal temperature-sensitive in situ gel system is developed, which is more effective for the treatment of fever than the traditional nasal spray (Chen et al. 2010). Another benefit of this novel in situ gel is that it exhibits more noticeable antipyretic effects and remains much more time (Cao et al. 2007). Besides, the...
Table 5. The preparations from Bupleuri Radix approved by CFDA.

| Components                                    | Dosage forms     | China Approved Drug Names (CADN)  | Batch number | Approval date | Drug standard code |
|-----------------------------------------------|------------------|-----------------------------------|--------------|---------------|--------------------|
| Radix Bupleuri extract, poly yamana-shi ester-80, sodium chloride | Injection        | Chai Hu Injection                 | Z61021126    | 07/2013       | 86902434000703     |
| Radix Bupleuri dry extract                   | Tablet           | Chai Hu Cough Tablets             | Z42020845    | 06/2015       | 869018760000227    |
| Radix Bupleuri, scutellaria, pinella, dangshen, ginger, licorice and jujube | Tablet           | Xiao Chai Hu Tablets              | Z20023393    | 10/2015       | 86903050000405     |
| Radix Bupleuri, polyethylene glycol          | Dripping Pill    | Chai Hu Dripping Pills            | Z20020053    | 07/2015       | 86900941000063     |
| Radix Bupleuri, scutellaria, pinella, dangshen, ginger, licorice and jujube | Decoction Pill   | Xiao Chai Hu Decoction Pills      | Z41021830    | 06/2015       | 86903082013140     |
| Radix Bupleuri, scutellaria, pinella, dangshen, ginger, licorice, jujube | Tablet           | Xiao Chai Hu Tablets              | Z34020723    | 05/2015       | 86904366000721     |
| Radix Bupleuri, scutellaria, pinella, dangshen, ginger, licorice, jujube | Capsule          | Xiao Chai Hu Capsules             | Z20090882    | 08/2014       | 86904641002884     |
| Radix Bupleuri, scutellaria, rhubarb, immature bitter orange, pinella, paeniae, jujube, ginger | Tablet           | Da Chai Hu Particles              | Z20080007    | 02/2013       | 86901622002642     |
| Radix Bupleuri, tangerine peel, ligustici, rhizoma cyperi, hovenia dulcis, paeniae, licorice | Pill             | Chai Hu Shu Gan Pills             | Z20073333    | 07/2015       | 86901174000103     |
| Radix Bupleuri extract                        | Oral Liquid      | Chai Hu Oral Liquid               | Z20020107    | 06/2010       | 86903099000244     |
| Radix Bupleuri, sileris, tangerine peel, paeniae, licorice, ginger | Particle         | Zheng Chai Hu Yin Particles       | Z20003013    | 06/2015       | 86901622002086     |
| Radix Bupleuri, sileris, tangerine peel, paeniae, licorice, ginger | Capsule          | Zheng Chai Hu Yin Capsules        | Z20040013    | 07/2015       | 86904930000362     |
| Radix Bupleuri, sileris, tangerine peel, paeniae, licorice, ginger | Mist             | Zheng Chai Hu Yin Mixture         | Z20090749    | 06/2014       | 86901622002666     |
| Radix Bupleuri, scutellaria, pinella, dangshen, ginger, licorice, jujube | Effervescent tablet | Xiao Chai Hu Effervescent Tablets | Z20060458    | 11/2011       | 86900042000085     |
| Radix Bupleuri extract, acetaminophen         | Injection        | Paracetamol and Bupleurum Injection | H52020518  | 09/2010       | 86905510000024     |

Radix Bupleuri suppositoria is very suitable for kids without pain (Wang & Chen 2003).

**Side effects of Radix Bupleuri**

Radix Bupleuri is not defined as a toxic medicine in many official pharmacopeias, such as *Chinese Pharmacopeia* and *Japanese Pharmacopoeia* (National Pharmacopoeia Committee 2010; Japanese Pharmacopoeia Editorial Board 2011). However, in practical use, it exhibits liver, kidney, and blood system toxicity by taking a large dose for a long period, while it shows no side effect without over-dose (Liu et al. 2012). *Chai Hu* injection may cause a hypersensitivity-like response, hypokalemia and renal failure. And one case is reported to die from severe hypersensitivity shock (Wu et al. 2014). So, the safety of Radix Bupleuri preparations is of great concern to us.

Saikosaponins and essential oils are believed to be the main compounds responsible for side effects of Radix Bupleuri (Liu et al. 2012). Essential oils from *B. chinense* cause hepatic injury when the dosage is about 1.5–3.4 times of the clinical daily dosage of Radix Bupleuri oral liquid (Sun & Yang 2011). Saikosaponins from *B. chinense* induce the hepatotoxicity by causing liver cell damage and necrosis administrating continuously to rats for 15 days (Huang et al. 2010). Ssd stimulates mitochondrial apoptosis in hepatocytes to exhibit its hepatotoxicity (Chen et al. 2013a).

Extracts of Radix Bupleuri also show some side effects. Extracts of *B. chinense* induce hepatotoxicity damage through oxidative damage mechanism, and the hepatotoxicity damage caused by the alcohol extracts is more serious than that caused by aqueous extracts (Lv et al. 2009). Furthermore, LD_{50} (50% lethal dose) of the aqueous extracts of Radix Bupleuri after single oral treatment in female and male mice are considered to be over 2000 mg/kg (Kim et al. 2012a). In Kampo (Japanese traditional herbal) medicines, studies of some potential interactions between Radix Bupleuri and other drugs are considered, especially in prescriptions containing Radix Bupleuri, such as *Shosaikoto, Daisaikoto, Saikokeishito, Hochuekkito, Saitobuto* and *Saiketo*. They may lead to anorexia, slight fever, and nausea (Ikegami et al. 2006).

Among other *Bupleurum* species, *B. longiradiatum* is a toxic herb in *Chinese Pharmacopeia* (National Pharmacopoeia Committee 2010), and it cannot be used as Radix Bupleuri. The main toxic compounds in *B. longiradiatum* are acetyl-bupleurotoxin, bupleurotoxin (Zhao et al. 1987) and polyene acetylene compounds, which are able to cause neurotoxicity (Chen et al. 1981).

**Discussion and perspective**

Saikosaponins, especially Ssa and Ssd, are the main active compounds in Radix Bupleuri. They are also prescribed as the marker compounds to evaluate the quality of Radix Bupleuri in *Chinese Pharmacopeia* (National Pharmacopoeia Committee 2010). They possess evident anti-inflammatory, antitumor, neuroregulation, hepatoprotection, immunoregulation, antiviral, and antioxidative activities. And what need to emphasize is that Ssa has a strongest anti-inflammatory effect, and Ssd possesses a strongest antitumor effect compared with other saikosaponins, and both Ssb and Ssc have a better antiviral activity than Ssa and Ssd, which proves
that the activities of different saikosaponins have some extent tendency. Inspired by this feature, we speculate that purified saikosaponin has more concentrated pharmacological activities than extracts.

Recently, more preparations containing Radix Bupleuri have been developed, such as Xiao Chai Hu tablets, Chai Hu dripping pills, Chai Hu injection, and Chai Hu Shu Gan pills (Li et al. 2014a). In these preparations the extracts of Radix Bupleuri, especially saikosaponins (Hu et al. 2011), are the main composition. Although B. chinense and B. scorzonerifolium are the only two original plants of Radix Bupleuri in Chinese Pharmacopeia, many other Bupleurum species are often used as Radix Bupleuri in China. However, the extracts of B. chinensis, B. falcatum, B. marginatum, B. yinchowense, B. kaoi, B. scorzonerifolium, and B. longiradiatum possess different pharmacological activities, such as the antitumor and antiviral activities of B. chinensis extracts, and the anti-inflammatory, anti-hyperthyroidism and neuroregulation activities of B. falcatum extracts. Because the quality, botanic characteristic and property, and pharmacological activities of different Bupleurum species are different, the standardization of Bupleuri Radix extracts is vital for the safe use of Radix Bupleuri.

In addition, there are many other compounds in Radix Bupleuri, such as polysaccharides and essential oils. Polysaccharides in Radix Bupleuri usually exert hepatoprotective and immunoregulation activities. The hepatoprotective effect of Radix Bupleuri polysaccharides is evaluated by measuring aspartate transaminase (AST), alanine transaminase, alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities in the plasma of mice (Zhao et al. 2012), and Radix Bupleuri polysaccharides inhibits complement activation on both the classical and alternative pathways (DI HY et al. 2013). The essential oils of Radix Bupleuri have strong antimicrobial (Ashour et al. 2009) and antifungal activities (Mohammadi et al. 2014). Besides, Radix Bupleuri also contains a little lignans, which exhibit antitumor (Ou et al. 2012) and hepatoprotective activities (Lee et al. 2011, 2012). Since polysaccharides (Tong et al. 2013; Wu et al. 2013) and essential oils (Liu et al. 2009; Yan et al. 2014) have been found to possess excellent pharmacological activities so far, we suppose that the quality evaluation method should be updated to meet the need of clinical therapy.

Radix Bupleuri also exhibits some security problems in the clinic. Since ‘Xiao Chai Hu Decoction event’ occurred in late 1980s in Japan, the clinical safety of Radix Bupleuri has been considered (Wu et al. 2014). The reasons of toxicity are complex and there is a great individual variation in the susceptibility to Radix Bupleuri. The current researches have shown that the toxicity of Radix Bupleuri mainly associated with dosage and drug administration time (Liu et al. 2012). For example, SSd exhibits antitumor activity on carcinoma cell lines with dose-dependence, but when the dosage of SSd increased to a high level it would exert cytotoxicity (Zhang et al. 2015). Usually, Radix Bupleuri is believed to be safe in defined dose prescribed by pharmacopeia.

Disclosure statement
All authors declare that they have no competing interests.

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