

# Research Progress on the Pharmacological Effects and Quality Analysis Methods of Bupleurum

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**Abstract:** Bupleurum has been used as traditional medicine in China, Japan, South Korea, and other Asian countries for more than 2,000 years. The crude extracts and pure compounds isolated from Bupleurum have a variety of biological activities, such as anti-inflammatory, anti-cancer, antipyretic, antibacterial, anti-viral, liver protection, neuroprotection, and immune regulation. The pharmacological effects of Bupleurum have been classified and summarized in this paper by consulting literatures at home and abroad. At the same time, the quality analysis methods of bupleurum, including high-performance liquid chromatography, thin layer chromatography, ultra-high-performance liquid chromatography mass spectrometry, UPLC-QTOF-MS, and so on, are discussed. This paper provides a reference for further research, development, and application of Bupleurum.

**Keywords:** Bupleurum; Pharmacological action; Quality analysis method; Research progress

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## 1. Introduction

Bupleurum is a herbal remedy that can be found in the Chinese Pharmacopoeia, and it is used in traditional Chinese medicine. It is widely used in China, Japan, South Korea, and other Asian countries to treat influenza, fever, inflammation, malaria, menstrual disorders, and hepatitis. According to ancient Chinese medical documents, Bupleurum can regulate internal and external metabolisms, dispel heat, soothe the liver, and raise the Yang. In recent decades, the research on Bupleurum mainly focuses on its biological activities, including its anti-inflammatory, anti-cancer, antipyretic, antibacterial, anti-viral, liver protection, and immune regulation activities. For example, Saikosaponin D, an active component of Bupleurum, can change the permeability of cell membranes, leading to cell damage and even necrosis, thus playing an anti-tumor role <sup>[1]</sup>. Through PPAR- $\alpha$  signaling pathway, Saikosaponin A improves the degree of hepatic steatosis and reduces insulin resistance <sup>[2]</sup>. In addition, Bupleurum has various pharmacological effects in view of its complex bioactive compounds. With in-depth research on Bupleurum's bioactivity, the research on effective quality analysis methods of Bupleurum is particularly important. There are many Bupleurum-producing areas, and the quality of Bupleurum from different areas varies, making quality control a challenging task. Bupleurum in Southern Hebei, for example, is considered a genuine medicinal material and is of high-quality. It is crucial to maintain stringent quality control. At present, various quality analysis methods have been used in studying Bupleurum, such as high-performance liquid chromatography, thin layer chromatography, and ultra-high-performance liquid chromatography mass spectrometry. Therefore,

this paper focuses on the research progress of Bupleurum's pharmacological effects and quality analysis methods in hope of ensuring the safety and effectiveness of its use and promoting a standardized application of Bupleurum.

## **2. Pharmacological action of Bupleurum**

### **2.1. Anti-inflammatory effect**

Bupleurum has been widely used to treat a variety of chronic inflammatory diseases. The crude polysaccharide isolated from the roots of Bupleurum can reduce myeloperoxidase, tumor necrosis factor (TNF)- $\alpha$ , and nitric oxide, thereby significantly reducing lung injury<sup>[3]</sup>. Saikosaponins in Bupleurum have shown to have anti-inflammatory properties, such as inhibiting inflammatory exudation, strengthening capillary permeability, releasing inflammatory mediators, promoting leukocyte migration and connective tissue proliferation, etc.<sup>[4]</sup>. A study has proven that saponins in Bupleurum exert anti-inflammatory effects, mainly through regulating the metabolism of niacin, nicotinamide, and arachidonic acid<sup>[5]</sup>. In addition, many studies have also shown that some Bupleurum preparations have anti-inflammatory effects in vitro and in vivo models. Chailing (Bupleurum and Poria) decoction and its active components can inhibit the proliferation of mesangial cells and the expansion of mesangial matrix in a rat model of glomerulonephritis<sup>[6]</sup>. In the rat model of experimental chronic pancreatitis, it has been found that Chaihu Shugan Powder inhibits NF- $\kappa$ B and TNF- $\alpha$ , which plays an anti-inflammatory and anti-fibrotic role<sup>[7]</sup>.

### **2.2. Anticancer effect**

The extract of Bupleurum also has anti-cancer effect. Bupleurum acetone extract can inhibit the proliferation of the A549 lung cancer cell line by arresting the cell cycle in G2/M phase, increasing microtubule stability, inhibiting telomerase activity, and activating ERK1/2 and Caspase-3/9<sup>[8]</sup>. In another study, saponins, isolated from Bupleurum, also showed significant anti-proliferative activity in human non-small cell lung cancer A549 cells through Fas-mediated apoptosis<sup>[9]</sup>. Saikosaponin A has been shown to prolong the hypnotic effect of cyclobarbital, suggesting that it plays a role in blocking the drug metabolic pathway in the liver. This inhibition slows down the metabolism of chemotherapeutic drugs in the liver, increasing their efficacy and anticancer effect<sup>[10]</sup>. In addition, a variety of traditional Chinese medicine preparations containing Bupleurum have traditionally been used to treat tumors and cancer. Longdan Xiegan pill, a water-based extract, has a significant growth inhibitory effect on HL60 and HT29 cancer cell lines, indicating that the preparation may have certain chemotherapeutic potential.

### **2.3. Antiviral effect**

It has been reported that the flavonoids of Bupleurum can reduce lung injury and mortality in mice infected with influenza B virus<sup>[11]</sup>. Saikosaponin C showed effective antiviral activity by inhibiting the DNA expression of HBsAg, HBeAg, and HBV. Xiaochaihu (Minor Bupleurum) decoction can inhibit the production of HBV and the expression of HBeAg<sup>[12]</sup>. Similarly, in another study, it has been found that Saikosaponin D, isolated from Bupleurum, showed significant biological activity in inhibiting viral DNA replication<sup>[13]</sup>. Since the outbreak of the novel coronavirus (2019-nCoV), Xiaochaihu decoction has been used as the main prescription. Upon observing the changes of various indicators in five patients after consuming the decoction, it has been found that Xiaochaihu decoction and violet decoction have therapeutic effects on patients with mild to moderate pneumonia infected with the novel coronavirus (2019-nCoV)<sup>[14]</sup>.

### **2.4. Antipyretic effect**

The antipyretic effect of Bupleurum is one of its most important functions. Since ancient times, Bupleurum has been widely used in the treatment of exogenous heat or cold and heat exchange, malaria, as well as

plagues. In particular, Xiaochaihu decoction and Chaige Jieji decoction have been mentioned in the Treatise on Febrile Diseases, a monograph for critical care medicine. By comparing the antipyretic effects of Bupleurum from different habitats, it has been found that Bupleurum from Hebei has better antipyretic effect on the established inflammatory fever model of New Zealand rabbits due to dry yeast <sup>[15]</sup>. In addition, another study found that Xiaochaihu decoction has antipyretic effect on the fever model of Sprague-Dawley (SD) rats. The mechanism of Bupleurum is that it can act on the hypothalamic thermoregulatory center, inhibit the production or release of cAMP, inhibit the upward movement of the set point temperature, and reduce body temperature.

## **2.5. Liver protection**

The meridians of Bupleurum are the liver and gallbladder. It has the effect of soothing the liver and dispersing knots. Bupleurum can restore liver function and promote immune function by significantly reducing the level of transaminase in the body. It has been found that Saikosaponin A has a protective effect on alcoholic fatty liver based on a zebrafish model of alcoholic fatty liver <sup>[16]</sup>. Several researchers have found that Bupleurum can reduce the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and triacylglycerol in rats through CC14 intraperitoneal injection <sup>[17]</sup>. Furthermore, Saikosaponin D has been reported to lower collagen I deposition and ALT levels in rats with liver fibrosis, as well as transforming growth factor (TGF)- $\alpha$ , which further decreases oxidative stress-induced hepatocyte injury <sup>[18]</sup>.

## **2.6. Immunomodulatory effect**

Bupleurum can improve the clearance of immune complexes in patients with nephritis, thereby improving their immune function and the efficacy of steroid drugs as well as reducing or eliminating adverse reactions by regulating the proportion of antigens and antibodies. A number of experimental studies have shown that the immune-enhancing components of Bupleurum chinense are Bupleurum polysaccharide and Bupleurum saponins. Bupleurum polysaccharide, in particular, can improve the spleen coefficient and immune function of mice by increasing the number of natural killer (NK) cells. Saikosaponin D can significantly activate peritoneal macrophages, enhance phagocytic activity, increase the level of lysosomal enzymes, and suppress the response of plaque-forming cells to heterologous erythrocytes by stimulating T and B cells <sup>[19]</sup>. In another study, it has been found that Saikosaponin D can inhibit the proliferation of human T cells that are stimulated by OKT3/CD28 in vitro and play an immunomodulatory role.

## **2.7. Anticonvulsant effect**

It has been reported that Bupleurum can play an anticonvulsant role in pentylenetetrazole-induced seizure in a mice model by regulating energy metabolism and improving ATP content. In addition, Bupleurum can significantly inhibit the cerebral cortical discharge and synaptic transmission of the central nervous system. Saponins can reduce the spontaneous activity of mice, prolong the hypnotic time of pentobarbital, and deepen sleep as it has a sedative effect. By investigating the effects of Bupleurum's volatile oil on the seizure threshold induced by pentylenetetrazole and the maximal electroshock seizure in mice models, it has been found that the volatile oil of Bupleurum has anticonvulsant effect <sup>[20]</sup>.

## **2.8. Other pharmacological effects**

Several studies have evaluated the effect of Bupleurum ethanol extract on the activity of cytochrome 450 isomers by using the six-drug cocktail method. It has been found that Bupleurum has strong inducing activity on CYP2E1, CYP2D6, and CYP3A4, which may lead to potential plant-drug interaction. In previous studies, it has been demonstrated that the hepatoprotective effect of Bupleurum may be attributed

to its autophagy induction. The autophagy activity of Bupleurum plays an important role in alleviating liver disease-related symptoms through its anti-inflammatory, organ protective, and aggregate scavenging functions. In addition, the anticancer effect of Bupleurum can also be attributed to its autophagy induction. Bupleurum has been found to effectively treat depression by regulating the levels of metabolites, hormones, and neurotransmitters through autophagy-mediated lipid metabolism <sup>[21]</sup>.

### **3. Quality analysis methods of Bupleurum**

#### **3.1. High-performance liquid chromatography**

In recent years, researchers have carried out a lot of research work on HPLC, putting forward several useful quality control parameters, which are conducive to the research of modern traditional Chinese medicine. High-performance liquid chromatography has high resolution and high sensitivity. It is widely used in the quality control of traditional Chinese medicine. Yumeng Bi used RP-HPLC to create the fingerprint of Bupleurum aqueous extract, which serves as a new benchmark for the quality control of Bupleurum <sup>[22]</sup>. Up to this point, more than 280 compounds have been isolated and identified from Bupleurum, including flavonoids, lignin, benzyl alcohol derivatives, triterpenoids, and volatile oil. These compounds constitute the basis of Bupleurum's pharmacological action and primary active ingredients <sup>[23]</sup>.

#### **3.2. Thin layer chromatography**

TLC has high specificity and is simple to operate. It is one of the most commonly used identification methods in the national standards of traditional Chinese medicine preparations. In addition, TLC can also identify a single component in complex components, and it is often used to confirm a traditional Chinese medicine or component in TCM compound preparations. Jun Zhang refined the method described in the Chinese Pharmacopoeia by isolating and identifying Saikosaponin A, C, and D with a high degree of separation, which aids in the detection of fake Bupleurum as well as the development and utilization of Bupleurum resources <sup>[24]</sup>. Jingli Li used TLC to study Bupleurum from different producing areas and collection times and found that the saponin content in Bupleurum from different producing areas and collection times is different, which is conducive to further improving the quality control of Bupleurum <sup>[25]</sup>.

#### **3.3. Ultra-high-performance liquid chromatography mass spectrometry**

Ultra-high-performance liquid chromatography mass spectrometry has high separation ability and sensitivity. It combines the advantages of high-performance liquid chromatography and mass spectrometry. The method is stable and can better control the quality of medicinal materials; thus, it is helpful to promote the modernization of traditional Chinese medicine. Hua Cai and others isolated and identified saponins according to mass spectrometry and used them as the basis for the overall quality evaluation of Bupleurum to ensure the safety and controllability of clinical treatment <sup>[26]</sup>. Another study compared and analyzed the differences of chemical components between Northern and Southern Bupleurum based on ultra-high-performance liquid chromatography mass spectrometry, which provided a reference for the research on the ecological planting of Northern Bupleurum <sup>[27]</sup>.

#### **3.4. UPLC-QTOF-MS**

In recent years, significant changes have taken place in the field of content determination of traditional Chinese medicine, from QqQ or IT instruments to high-resolution systems, such as TOF detectors, especially hybrid Q-TOF systems. Q-TOF instrument has been proven to be an excellent solution for drug content determination beyond QqQ and IT MS in view of its fast acquisition speed, full scanning sensitivity, enhanced quality resolution, and accurate quality measurement. In a study, the contents of Saikosaponin A and Saikosaponin D have been determined under different extraction conditions using UPLC-QTOF-MS,

which provided reasonable indicators for the quality control of Saikosaponin A and Saikosaponin D [28]. In addition, Tianshu Liu and other researchers measured the content of Bupleurum based on UPLC-QTOF-MS, which made the identification of chemical components of Bupleurum more comprehensive and accurate as well as laid a foundation for in vivo research and the quality control of Bupleurum [29].

#### 4. Conclusion

In traditional Chinese medicine, Bupleurum has long been used to regulate internal and external metabolisms, disperse evil heat, soothe the liver, and promote Yang Qi. For centuries, it has been widely used to treat various diseases in China, Japan, South Korea, and other Asian countries. Using high-performance liquid chromatography, thin layer chromatography, ultra-high-performance liquid chromatography mass spectrometry, and UPLC-QTOF-MS, essential oils, triterpene saponins, polyacetylene, flavonoids, lignans, fatty acids, sterols, and other compounds can be isolated and identified from Bupleurum. Pharmacological studies have shown that Bupleurum offers a variety of biological effects, such as anti-inflammation, anti-cancer, anti-viral, antipyretic, immune regulation, liver protection, and so on. However, in order to promote the all-round development of Bupleurum and ensure its quality, safety, and controllability, more in-depth research is needed.

Bupleurum is a component of many traditional Chinese medicine compound preparations. Although modern experiments have confirmed that Bupleurum or its isolated monomers have various pharmacological activities, it is of great significance to study the mechanism of action of Bupleurum on the basis of traditional uses. In addition, the pharmacological effects of only a few components, such as saikosaponins, flavonoids, and essential oil, have been reported in literatures, while those of polyacetylene, lignans, and sterols have not been fully studied. The quality analysis methods of Bupleurum are not comprehensive, and these methods need to be further improved to make the separation and sensitivity of each component better.

In short, this review summarizes the pharmacological effects and quality analysis methods of Bupleurum, which is conducive to the improvement of the quality of Bupleurum. In addition, it is also conducive to revealing the chemical components and pharmacodynamic material basis of bupleurum in a more comprehensive manner as well as providing reference for further studies on the pharmacological mechanism and rational use of Bupleurum, which is of great significance to the research progress of Bupleurum.

#### Disclosure statement

The authors declare no conflict of interest.

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