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# Traditional use, germplasm identification, phytochemistry, pharmacology of *Bupleuri Radix*: a review

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## Abstract

*Bupleuri Radix* (BR) is a traditional Chinese medicinal herb with a history of 2000 years of medicinal use. It is primarily used for heat-clearing, liver-soothing, Yang-ascending and sinking-reversing effects. It has huge clinical value in traditional Chinese medicine (TCM) and ethnomedicine. There are more than 200 species in the genus *Bupleurum* of the Apiaceae family, but their classification and taxonomy have not been unified and clarified. Numerous active compounds have been isolated and identified from BR, such as saikosaponins, volatile oils, polysaccharides, polyacetylenes, lignans, flavonoids and such like, which possess anti-inflammatory, antioxidant, anti-tumor, anti-cancer, hepatoprotective, immunomodulatory and anti-depressant effects. This review provides an overview of the traditional use, germplasm identification, phytochemistry and pharmacological activities of BR. The current research progress of BR is summarized and the innovation points are highlighted, aiming to provide baseline information and expert references for quality control and rational usage of BR in the post-pandemic era.

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## Introduction

BR includes a series of plant species of the genus *Bupleurum*, Apiaceae family, which are widely distributed in the Northern Hemisphere. Currently, there are about 241 confirmed species in *Bupleurum*, and 29 additional species level taxa which still have no clear-cut status (www.gbif.org). As a kind of TCM, the medicinal use of BR can be traced back to *Shen Nong's Classic of the Materia Medica*, a herbal medicine text dating back to 2,000 years ago, which describes the nature, efficacy and clinical applications of BR[1]. Although multiple species of BR can be used as herbal medicine, only the dried roots of *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd are officially listed as authentic medicinal materials in the *Chinese Pharmacopoeia 2020 edition*[2]. There are different opinions regarding the specific medicinal parts of BR. The *Shen Nong's Classic of the Materia Medica* only describes its nature and efficacy without specifying the exact plant part used. The first written record specifying the root as the medicinal part of BR can be found in the *Wu Pu Bencao*, a book from the 3rd century AD[3]. Until the end of the Qing Dynasty, it was discovered that the above-ground parts, and even the whole plant of BR, possessed medicinal properties in the Yangtze River region, which led to the gradual use of non-root parts and even the entire plant in medicine[4]. So far, besides the officially listed *B. chinense* and *B. scorzonerifolium*, local medicinal standards in regions such as Gansu, Yunnan, Shandong and Sichuan province (China) still include other *Bupleurum* species and non-root parts of BR.

The main bioactive component of BR is saikosaponins (SSs), with saikosaponin a (SSa) and saikosaponin d (SSd) being the chemical standards used in the *Chinese Pharmacopoeia 2020 edition* to evaluate the quality. In addition to SSs, BR contains volatile oils, polysaccharides, polyacetylenes, lignans, flavonoids, fatty acids, sterols, coumarins, alkaloids and many other active components, which exert various effects such as anti-inflammatory, antioxidant, anti-tumor, anticancer, antidepressant, antimicrobial, antifungal, hepatoprotective and immunomodulatory activities by modulating multiple signaling pathways. Furthermore, BR is often used in combination with other drugs to treat symptoms such as the common cold with fever, malaria, liver stagnation with Qi stagnation, chest and rib pain, prolapse of the rectum, uterine prolapse and irregular menstruation[5]. Recent studies have shown that Qing Fei Jie Du Decoction, which contains BR as the principal herb, had significant therapeutic effects in mild COVID-19 infections[6].

Therefore, this article provides a systematic review of BR in terms of traditional use, germplasm identification, phytochemistry and pharmacological activities. By extensively reviewing domestic and international literature, we summarize and synthesize the research progress in both basic research and application fields. The current challenges in the development of BR are also highlighted, so as to provide a valuable reference for further exploration and development of its medicinal value.

## Traditional use

BR has a long history of medicinal use in China. It is slightly cold in nature, bitter and pungent in taste, and belongs to the liver and gallbladder meridians. In Korea, many medicines,

including BR, are made into oral liquid, which is called *Tang*[7]. In the *Japanese Pharmacopoeia*, there are prescriptions for the various parts of *Bupleurum falcatum* L.[8]. In TCM, it has the functions of dispersing and heat-clearing, liver-soothing and Yang-ascending. However, caution should be taken when using BR against excessive liver Yang, internal liver wind, Yin deficiency with excessive fire and rebellious Qi. This is owing to the unique 'ascending and dispersing' properties of BR, which require the physician to have a good understanding of the indications and dosage. Ancient medical practitioners emphasized the taste, properties and therapeutic effects of BR, particularly its use in treating conditions like fever and liver-related diseases. Many classical BR containing prescriptions and formulations have been passed down through the ages and continue to be highly regarded in modern medical practice. Presently, the traditional applications of BR have been expanded and deepened. The ongoing research has uncovered previously unknown active compounds in BR, broadening its efficacy to encompass various areas such as anti-inflammatory, antioxidant and liver-protective effects. Modern scientific studies have further elucidated the pharmacological actions and active components of BR, diversifying its applications in clinical medicine (Fig. 1).

BR is often combined with other herbs to form various TCM formulas and prescriptions for the treatment of various diseases (Supplemental Tables S1 & S2). For example, BR is often used in combination with *Puerariae lobatae Radix*, as seen in Chai Ge Jie Ji Decoction against colds and fever. BR is also frequently paired with *Scutellariae Radix* in Xiao Chai Hu Decoction, which is used for treating Shao Yang syndrome with alternating chills and fever, bitter taste in the mouth, dry throat, a sense of fullness in the chest and ribcage. The ability of BR to disperse heat and treat alternating chills and fever is related to the anti-inflammatory and antiviral activities of BR compounds, such as SSs and volatile oils. Additionally, compounds such as SSs and flavonoids possess hepatoprotective and antidepressant properties, making them valuable for treating liver stagnation and Qi obstruction. When combined with *Cyperi rhizoma* and *Paeoniae Radix Alba*, BR is effective in treating liver Qi stagnation with chest and ribcage pain, which is the basis of TCM formula Chai Hu Shu Gan San. BR is also combined with *Angelicae Sinensis Radix*, *Paeoniae Radix Alba* and *Menthae Haplocalycis Herba* in TCM formula Xiao Yao San (XYS), which show the synergistic actions against liver Qi stagnation, irregular menstruation and menstrual pain. In treating raising Yang and lifting sinking Qi, such as Qi deficiency and sinking, BR is often used in combination with Qi-tonifying herbs like *Ginseng Radix Et Rhizoma* and *Astragali Radix*, which are the components of Bu Zhong Yi Qi Decoction. This formula is used for addressing symptoms like fatigue, poor appetite, loose stools, chronic diarrhea, prolapse of the stomach and uterine prolapse. The neuroregulatory, antidepressant, anxiolytic and anti-inflammatory properties of BR partially explain these therapeutic concepts.

TCM processing, a unique pharmaceutical technique, follows TCM theories and is employed to modify the properties of medicinal herbs according to their intrinsic nature, as well as the requirements of formulation, compatibility and clinical applications. In most cases, BR is administered in dosage forms such as granules, oral liquid, injection, decoction pieces and compound preparations with other herbal medicines. After processing, medicinal herbs, including BR, can exhibit enhanced therapeutic effects, reduced toxicity, altered pharmacokinetic and pharmacological properties, targeted meridian tropism, modified tastes and odors[9]. Various processing methods, such as vinegar-processing, wine-processing, honey-processing and turtle blood-processing, change physical and chemical characteristics of BR, resulting in differential therapeutic effects. It should be noted that different processing

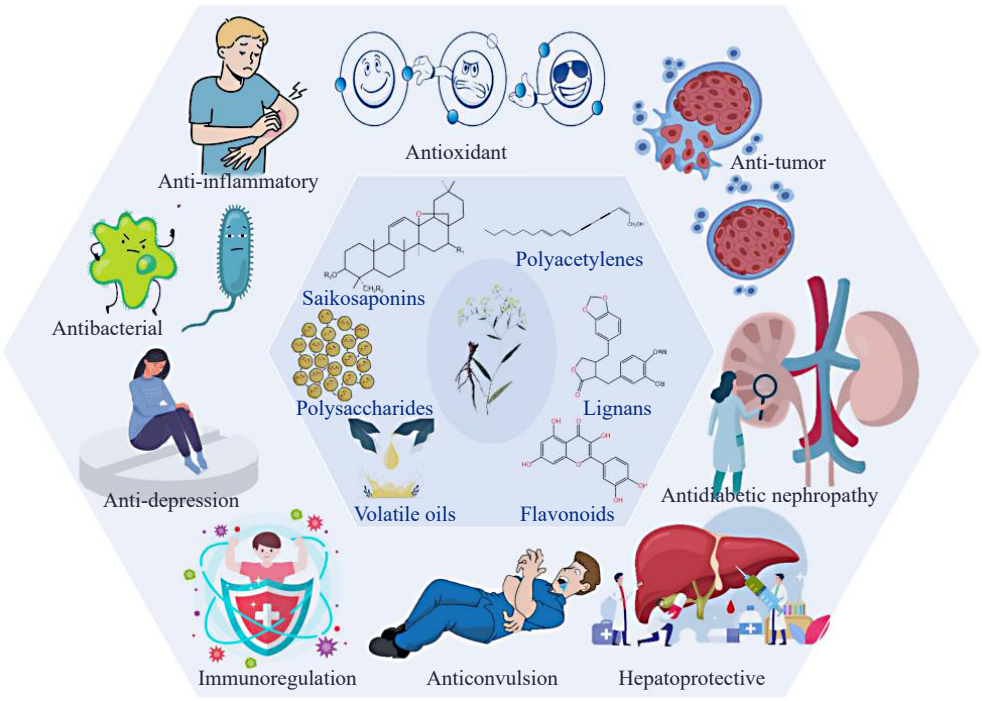


**Fig. 1** Pharmacological activities of *Bupleuri Radix*.

methods applied to BR can lead to variations in the content of active ingredients, toxicity levels, pharmacological actions and therapeutic effects[10–15]. In addition to the processing standards specified in the *Chinese Pharmacopoeia*, different regions in China have developed their own processing standards based on specific species and local medicinal practices. Overall, the processing methods, dosage forms and compatibility with other herbs significantly influence the therapeutic outcomes associated with BR.

In addition, health supplements, cosmetics, and personal care products involving BR extracts (BRE) have gradually entered the market (www.yaozh.com). The related products mainly consist of extracts from *B. chinense*, *B. scorzonerifolium* and a few other *Bupleurum* species. These products are primarily used as astringents, moisturizers, emollients and skin conditioning agents.

## Germplasm identification

### History

Due to multiple source species, their similar morphology, as well as diverse historical records regarding the medicinal species, names and usage methods of BR, it is challenging for modern scholars to differentiate them. In the Eastern Han Dynasty, the *Shen Nong's Classic of the Materia Medica* referred to it as *Ci Hu* and *Di Xun*. The late Han Dynasty's *Miscellaneous Records of Famous Physicians* referred to it as *Shan Cai*, *Ru Cao* and *Yun Hao*[1,16]. In the Ming Dynasty, Li Shizhen's *Compendium of Materia Medica* firstly recorded the name *Chai Hu*, which has been used until today[17]. During the Han and Tang Dynasties, the origins of BR were not distinguished, including *B. chinense* and its related species, *B. scorzonerifolium*, and even *non-Bupleurum* plants. The books from this period provided limited descriptions of the morphology of BR, making it impossible to determine the exact species as known today. In the Song Dynasty, the documentation of *Bupleurum* species became clearer, with more detailed morphological descriptions. For instance, the *Materia Medica Arranged According to Pattern* of that time recorded the morphological characteristics and distribution of BR, enabling the identification of three species: *B. chinense*, *B. scorzonerifolium* and *B. scorzonerifolium* f. pauciflorum[18]. During the Ming and Qing Dynasties, there was a serious problem of adulteration and counterfeiting of BR, with significant mixing and adulteration of processed slices, which even led to fatalities. Initially, people did not distinguish between BR and *Stellaria dichotoma var. lanceolata*, a taxon of Caryophyllaceae family. For a long time, they were considered the same medicinal herb within *Bupleurum*. In the Ming Dynasty, Ni Zhumo and Miao Xiyong differentiated *S. dichotoma var. lanceolata* from *Bupleurum* based solely on their efficacy, but the former was still wrongly used as a *Bupleurum* herb[19,20]. Until the Qing Dynasty, with the research of Zhao Xuemin, *S. dichotoma var. lanceolata* was separated from *Bupleurum*[21].

### Origin and authenticity

*Bupleurum* is widely distributed in the temperate regions of the Northern Hemisphere. The southwestern China and Mediterranean region are two major centers of origin of *Bupleurum*[22,23]. The software Reconstruct Ancestral State in Phylogenies (RASP) for phylogenetic ancestral trait reconstruction, was used to study the origin of *Bupleurum* in China. By analyzing the ITS (Internally Transcribed Spacer) and rps16 sequences, southern China was inferred to be the center of origin of China *Bupleurum*, and the distribution types and dispersion pathways of *Bupleurum* species were also elucidated[24].

Through the historical investigation of the regional authenticity of BR, Zhao et al. discovered that in ancient times, the main production areas of BR expanded from Shanxi and Henan Province to the surrounding regions. During the Song Dynasty, Yinzhou (now Yulin, Shaanxi Province) was recognized as the authentic production area of BR. In modern times, Hebei, Shanxi and Shaanxi have become the main production areas of *B. chinense*[25]. The growth environment and cultivation conditions in different regions can influence the genetic material of BR, resulting in distinct regional characteristics in the DNA of BR. The RAPD (Random Amplified Polymorphic DNA) technique has been widely applied in the identification of plant varieties, pedigree analysis and studies of evolutionary relationships. It can accurately determine the regional authenticity of *B. chinense*. Also, it can accurately identify genuine BR and similar species[26–28].

### Identification method

*Bupleurum* is distributed in all regions of China except for Hainan Province, and there are multiple *Bupleurum* species growing together in various production areas. The main medicinal species currently used are *B. chinense*, *B. scorzonerifolium*, *B. smithii* Wolff var. *parvifolium* Shan et Y. Li and *B. marginatum* var. *Stenophylium* (Wolff) Shan et Y. Li. Apart from *B. smithii* Wolff var. *parvifolium* Shan et Y. Li, the other three *Bupleurum* species, as well as *B. falcatum* L., are the main cultivated species in China[29]. However, the sharp decline of wild *Bupleurum* resources has resulted in a scarcity of commercial supply. Therefore, it is necessary to accurately identify *Bupleurum* germplasm to provide a medicinal basis for the transition from wild to cultivated and large-scale production. Currently, the systematic identification studies of *Bupleurum* mainly include traditional morphological methods, fingerprint spectrum, DNA barcoding, molecular markers, chloroplast genome comparisons and combined multi-technology approaches.

#### *Traditional methods*

The morphological observations, literature references and field guides are traditionally utilized to achieve convenient and efficient identification[30]. Early on, Shan examined the morphological characteristics of several *Bupleurum* species in China, summarized their features and distribution, and compiled a key for the identification of China *Bupleurum*[31]. Zhang proposed a convenient method for identifying common counterfeits of *Bupleurum*, and suggested that their origin and characteristics can be used for identification[32]. Traditional methods serve as the main means of field identification, allowing for quick differentiation of plants anytime and anywhere. However, they are influenced by various factors, and are not suitable for newly discovered species; their accuracy in identification is relatively low.

#### *Fingerprint spectrum*

The fingerprint spectrum is a comprehensive and quantitative identification method based on the systematic study of chemical components in TCM. It provides a visual means of identification and can be used to determine the authenticity, quality and stability of BR. Xiao et al. conducted research on BR

from different regions using GC-MS (Gas Chromatography-Mass Spectrometry) fingerprint spectrum and found that the separation of its volatile oils in the GC-MS fingerprint spectrum was good enough for evaluating the quality of BR[33]. Ye et al. established a fingerprint spectrum of *B. chinense* using UPLC-Q-TOF-MS (Ultra Performance Liquid Chromatography-Quadrupole-Time of Flight-Mass Spectrometry) and demonstrated its application value in the rapid identification of *B. chinense* from different regions[34]. Additionally, the combination of HPLC (High Performance Liquid Chromatography) and chemometrics can be used to analyze the component characteristics and differences of *B. chinense* from different regions[35,36]. Jin et al. analyzed the infrared spectra of genuine and non-genuine roots of BR using ATR-FTIR (Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy) and found significant differences in the infrared absorption peak intensities. Also, the DWTS (Discrete Wavelet Transform Spectroscopy) can clearly display differences between genuine and non-genuine roots, and has a high recognition rate of the former[37]. In the future, the combination of fingerprint spectrum and analytical chemistry will play a greater role in the quality control of TCM. It is expected to become an important identification technology in the quality standard system.

### *Molecular biology identification*

Molecular biology identification is a complement to traditional identification methods, and is increasingly being used in plant classification, phylogenetics and other areas. It provides a scientific basis for the identification of *Bupleurum* species, the study of phylogenetic relationships, the assessment of seed and medicinal plant quality, which offer new insights into the precise identification of TCM materials.

DNA barcoding utilizes standardized, sufficiently variable, easily amplifiable and relatively short DNA segments, and is a rapidly developing bio-identification system based on the specificity within species and diversity between taxa, which enables rapid and automated identification of organisms. It is nearly impossible to distinguish every species solely based on a single gene or DNA fragment. However, by combining multiple DNA barcode sequences within the genome, hierarchical barcoding can be achieved, gradually narrowing down the scope and ultimately achieving the goal of species identification[38]. Chen et al. analyzed the guiding principles and applications of DNA barcoding technology in the identification of Chinese medicinal materials, and proposed the use of ITS sequences, including ITS1 and ITS2, as a universal DNA barcode for medicinal plants[39,40]. Compared to other identification techniques, the ITS sequences have a higher plant identification rate and demonstrate good repeatability and universality. They can be used as a basis for the identification of BR and the identification of seeds from various *Bupleurum* species. Combining traditional morphological methods and ITS sequences can be used to effectively distinguish counterfeit BR products found in the market[41–44].

In addition, non-sequencing DNA molecular markers can be used for the identification of BR. Zhan et al. utilized ISSR (Inter Simple Sequence Repeat) and SSR (Simple Sequence Repeat) molecular markers to construct the first genetic map of BR, which included 13 linkage groups and 80 loci. This map laid the foundation for genetic mapping of traits, positional cloning and molecular marker-assisted selection breeding of BR[45]. Wu et al. used SSR markers to identify cultivated germplasm of BR, and generated preliminary SSR fingerprint data that could distinguish different BR varieties[46]. These molecular marker-based identification techniques not only overcome the limitations of plant morphology and environmental conditions, but also possess advantages such as high accuracy, sensitivity and rapidity in plant identification.

### *Chloroplast genome*

Compared to the nuclear genome, the chloroplast genome exhibits less sequence variation, making the identification results more stable and reliable. It has become a research hotspot as a DNA barcode sequence and can be used as a super barcode for studying plant systematics, phylogenetics and species identification. Furthermore, it can be used for subspecies identification, varieties identification and even individual identifications[47,48]. Zhang et al. utilized conventional DNA barcoding techniques to identify cultivated *Bupleurum* species while also verifying their complete chloroplast genomes and developing a new chloroplast marker[49]. Huang et al. reported the chloroplast genome sequences of Mediterranean *Bupleurum* species[50]. The phylogenetic analysis revealed that Mediterranean and East Asian *Bupleurum* species form two separate major branches. Multiple studies based on the chloroplast genome and DNA barcoding indicated that all *Bupleurum* species form a monophyletic clade with high bootstrap support, providing phylogenetic information for further identification[23,51–54]. Today, with the advancement of chloroplast genome sequencing technologies, obtaining plant genome data has become efficient and fast. Also, the sequencing costs have significantly decreased. This progress will greatly promote the development of chloroplast genomics in *Bupleurum* genus and other plant taxa, providing new perspectives for plant identification.

## Phytochemistry

### Saikosaponins

SSs are highly abundant in the BR roots. They exhibit various pharmacological activities, including anti-inflammatory, anticancer, antiviral, immunomodulatory and neuroregulatory effects[55]. Currently, about 180 SSs have been isolated and identified from BR, all of which belong to the class of pentacyclic triterpenoids (Supplemental Table S3). They can be classified into two chemotypes: oleanane-type and ursane-type[56]. The $^{1}$HNMR metabolic fingerprinting efficiently differentiated two main scaffolds of SSs, which can also be used to detect SSs and hydrocarbon aldehydes[57]. SSs generally contain glucose, fructose, xylose and arabinose as sugar moieties. After deglycosylation in the gut, their permeability greatly changed[58]. The ER (Efflux Ratio) values indicate that SSd was actively absorbed, while SSa and $SSb_2$ underwent passive diffusion. In addition to the seven reported types of sapogenins (epoxy ether, heterocyclic diene, 12-ene, homocyclic diene, 12-ene-28-carboxylic acid, heterocyclic diene-30-carboxylic acid and 18-ene), Sui et al. reported seven additional SS aglycones[59,60] (Fig. 2). SSa, SSc and SSd are the major active components and belong to the epoxy ether (type I) category, while $SSb_2$ is a heterocyclic diene (type II) that mainly exhibits antiviral activity. However, the exact reasons for the differences in bioactivities among SSs remain elusive, and it is possibly due to the ether bonds in the chemical structures of SSs[61]. By comparing the activity

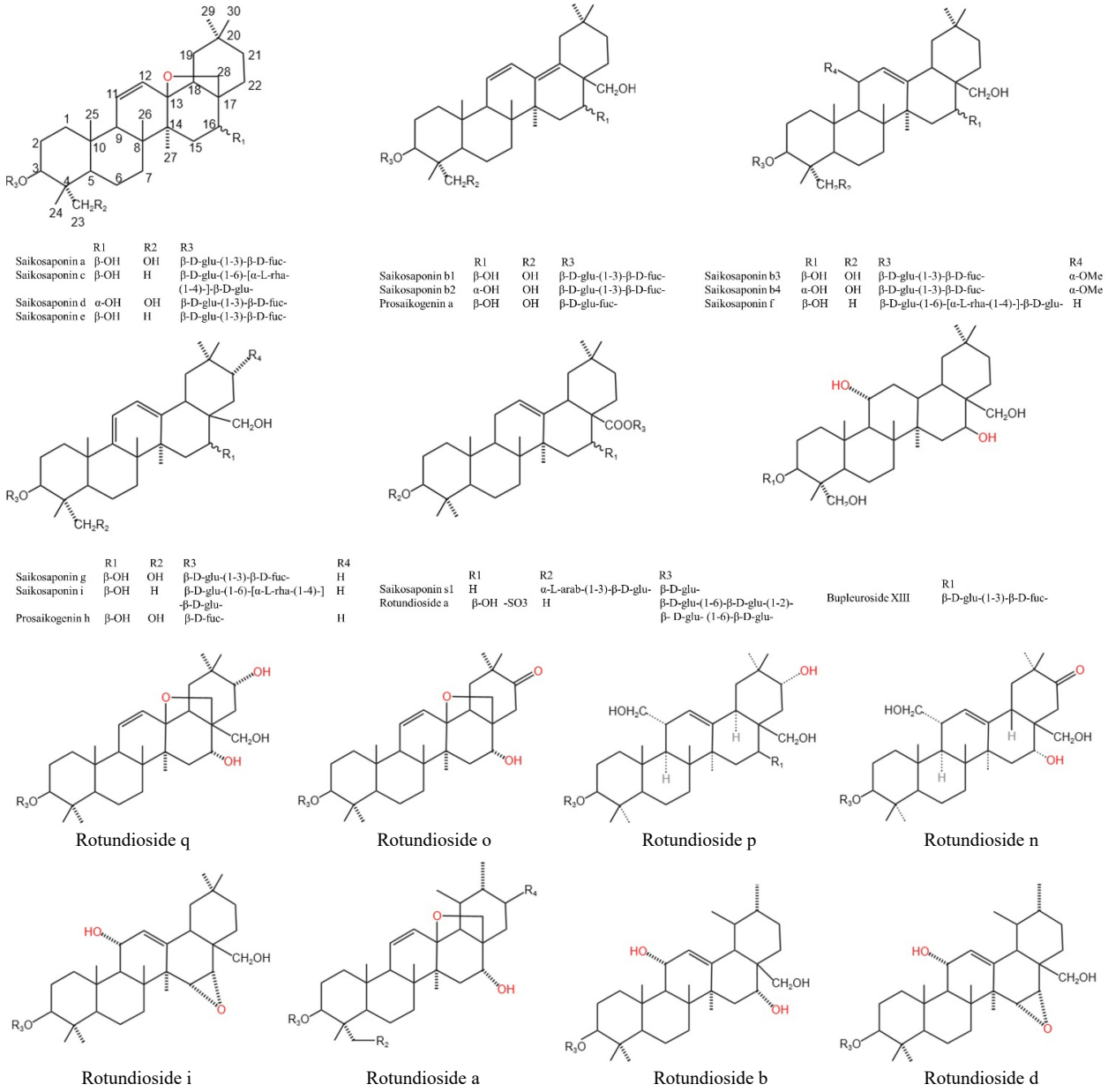


**Fig. 2** The structures of saikosaponins.

differences between SSa and SSd and observing the structures of SS aglycones, it is inferred that the $R_1$ group may also be a factor contributing to the differences in SSs activity. Both SSa and SSd are stereoisomers, differing only in the position of the $R_1$ group, yet they exhibit specific variations in bioactivity.

## Volatile oils

Volatile oils of BR exhibit significant effects such as antipyretic, antibacterial, anti-inflammatory and anticonvulsant properties. Certain volatile oil components, such as (2E,4E)-2,4-decadienal, also demonstrate repellent effects against storage insects[62]. Currently, hundreds of volatile oils components have been identified from BR, e.g., terpenes, alkenes, aldehydes, esters and alcohols, and the fatty compounds are the most (Supplemental Table S4). Apart from variations in the concentrations of major compounds, nearly all of them contain a series of fatty derivatives[63]. Volatile oils are present in various parts of BR, with high concentrations of $\beta$-ocimene observed in all parts. Comparatively, the root of BR contains a greater variety of volatile oils components, while the stems, flowers, leaves and fruits have higher levels of volatile oils contents[64]. Moreover, the composition and content of volatile oils are dynamic, and the production of volatile oils occurs in the early growth and development stages of BR. The flowering period is characterized by the most significant changes in composition, with each phenological stage containing common terpene compounds such as $\beta$-myrcene, trans-$\beta$-ocimene, limonene, (E,E)-$\alpha$-farnesene, $\alpha$-copaene, $\beta$-elemene and other similar terpenes[65]. They have different isomeric forms, and the most effective quantitative and qualitative analysis technique is GC-MS[66,67]. An increasing number of volatile oils are being discovered and identified, which are useful in chemotaxonomy and plant systematics[68].

### Polysaccharides

Polysaccharides are large molecules composed of a complex series of monosaccharide units. They are one of the fundamental substances that maintain normal functioning of life activities, and most polysaccharides derived from plants are relatively non-toxic. The BR polysaccharides exhibit a wide range of types, with diverse structural variations and bioactivities. They primarily exert anti-inflammatory, anti-aging, anti-ulcer, anti-tumor, immunoregulation and mitosis-promoting effects *in vivo*[69–73] (Supplemental Table S5). These polysaccharides are mainly composed of galacturonic acid, galactose, glucose, arabinose, xylose, ribose, rhamnose, mannose, and others. Multiple studies have found that the physiological effects and bioactivities of polysaccharides are related to their molecular weight, monosaccharide composition and glycosidic bond composition, as well as linkage patterns, total carbohydrate content, uronic acid content, protein content and advanced conformation characteristics[74–76]. The bioactivity of BR polysaccharides is highly dependent on their chemical structure. Most polysaccharides contain arabino-furanose units linked by 1,5 and 1,3,5 connections. Some BR polysaccharides possess unique triple-helical structures with specific molecular recognition capabilities and high functionality[72,77].

### Polyacetylenes

Polyacetylenes are unstable compounds that contain one or more unique carbon-carbon triple bond functional groups, which are widely distributed in higher plants such as Apiaceae, Araliaceae and Asteraceae families, as well as in bryophytes, lichens and fungi[78]. Some polyacetylenes have been isolated from *Bupleurum* (Supplemental Table S6). They are mainly concentrated in the roots and possess various bioactivities, including neuroprotective, antidepressant, anti-tumor and antiplatelet aggregation effects[79–82]. However, they are also the main toxic components, e.g., bupleurotoxin, acetylbupleurotoxin and oenanthotoxin[83,84]. The molecular mechanisms of polyacetylene toxicity involve inhibiting GABA ($\gamma$-Aminobutyric acid) receptors and inducing brain damage in mice, as revealed through global and targeted metabolomics analysis[85]. Therefore, when using polyacetylene containing drugs from BR, it is important to pay attention to dosage and proper usage to avoid medical accidents.

### Lignans

Lignans are widely distributed in various parts of plants, including the wood, resin and other tissues. They are formed by the polymerization of two molecules of phenylpropanoid derivatives. Lignans exhibit multiple bioactivities such as antioxidant, anticancer, and immune-modulating effects. Currently, over 60 different lignans have been isolated from *Bupleurum* (Supplemental Table S7 & Fig. 3), most of which are glycosides. Based on the additional bridging patterns between the two $\beta$-bonded phenylpropanoid units, lignans can be classified into four major subtypes and their derivatives: dibenzylbutyrolactone, aryltetralin, aryltetrahydrofuran and tetrahydrofuran lignans[56]. The activity of lignans is closely related to their unique phenylpropane structure. The tertiary hydroxyl group in the main structure tends to decrease their antioxidant activity, while lignans with an anaerobic secondary benzyl position exhibit higher antioxidant activity[86,87].

### Flavonoids

Flavonoids play a significant role in plant growth and development by effectively controlling key steps in cell growth and differentiation, thus regulating the development of the whole plant and individual organs[88]. Currently, over 100 flavonoid compounds have been isolated from *Bupleurum* (Supplemental Table S8), which possess various bioactivities such as antioxidant, antimicrobial and hepatoprotective effects. They are derived from the phenylpropanoid metabolic pathway and have a basic structure consisting of a C15 benzene ring with a C6-C3-C6 framework. Most of them are derivatives of flavanols, including rutin, isorhamnetin and quercetin, as well as other glycosides[89,90] (Fig. 4). The distribution of flavonoids in *Bupleurum* species differs from that of SSs; they are of low amounts in the roots but are highly abundant in the leaves. The main leaf flavonoid is rutin, accounting for more than 85% of the total flavonoid content, which is followed by quercetin, while isorhamnetin has the lowest proportion among the leaf flavonoids[91]. Flavonoids can also serve as indicators for the identification of *Bupleurum*[92].

### Other components

There are other active components in *Bupleurum*, such as fatty acids, sterols, coumarins, alkaloids, and others, most of which also possess antibacterial, anticancer, antioxidant activities. However, there is limited research on them, possibly due to their lower content in BR, difficulties in isolation, or less pronounced activity. Traditionally BR roots are used as medicine, and there was little attention paid to the aboveground parts. In the future, research on the activities of BR can be expanded to different plant parts, exploring the activity and functionality of different components in these parts, thereby enriching the diversity of therapeutic effects. Furthermore, efforts can be made to gradually improve the quality evaluation system of BR.

## Pharmacological activities

### Anti-inflammation

Various active components in BR have anti-inflammatory effects. For ALI (Acute lung injury) inflammation caused by LPS (Lipopolysaccharide), SSs significantly reduced pathological damage such as lung edema, lowered the transcript levels of IL-6 (Interleukin-6), IL-1$\beta$ (Interleukin-1$\beta$), TNF-$\alpha$ (Tumor Necrosis Factor $\alpha$) in serum and lung tissue, and decreased the expressions of NF-$\kappa$B (Nuclear factor kappa-B), TLR4 (Toll-Like Receptor 4) related proteins. SSs induced the inactivation of IKK (Inhibitor kappa-B Kinase), I$\kappa$B$\alpha$ (Inhibitor kappa-B $\alpha$), NF-$\kappa$B signaling in LPS-induced RAW 264.7 macrophages, inhibited the release of pro-inflammatory mediators such as iNOS (inducible Nitric Oxide Synthase) and COX-2 (Cyclooxygenase-2), thereby suppressing inflammatory responses[93–96]. The anti-inflammatory effect of SSa was associated with the activation of LXR$\alpha$ (Liver X Receptor $\alpha$), ABCA1 (ATP-Binding Cassette Transporter A1) signaling pathway, which depleted cholesterol to disrupt lipid rafts, reduced TLR4 translocation to lipid rafts and inhibited oligomerization, thereby alleviating LPS-mediated oxidation and inflammation[97]. SSa also significantly reduced brain damage, improved neurofunctional recovery, and reduced water content in brain tissue. After treatment with SSa,

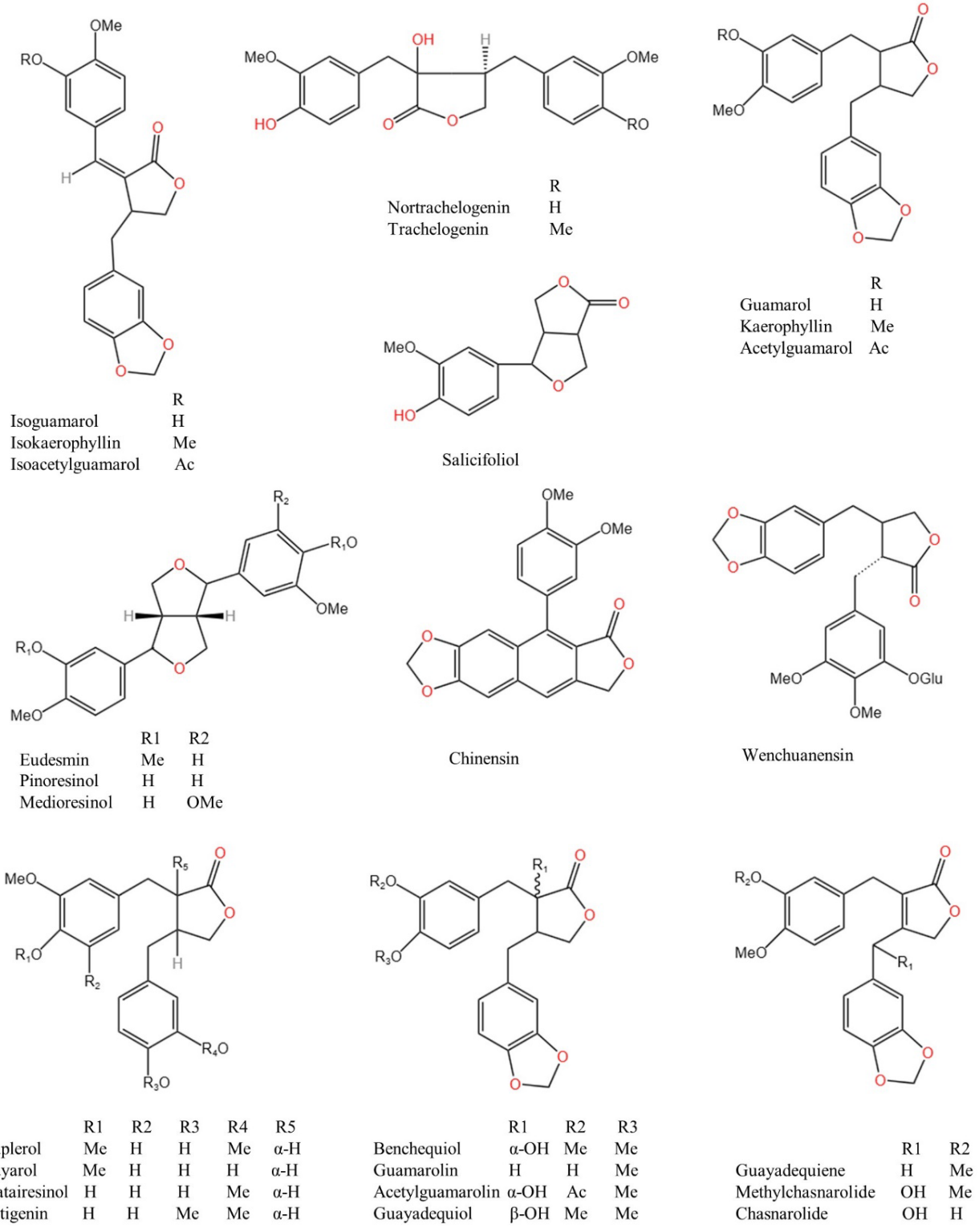


**Fig. 3** The structures of lignans.

NF-$\kappa$B was elevated, serum HMGB1 (High Mobility Group Box-1 Protein) levels were significantly reduced, and the levels of inflammatory cytokines were downregulated, thus alleviating inflammatory damage[98]. SSa had a certain anti-inflammatory effect in Nav1.7 cells, and also had an inhibitory effect on heat pain and formalin pain in mice[99].

In AD (Atopic Dermatitis), SSa and SSc inhibited the extracellular signal-regulated kinases (ERKs) 1/2, c-Jun N-terminal kinases 1/2 and p38 mitogen-activated protein kinase (MAPK) pathways[100]. These SSs downregulated the expression of EGR1 (Early Growth Response Factor 1), thereby inhibiting the expression of TNF-$\alpha$-induced TSLP (Thymic Stromal

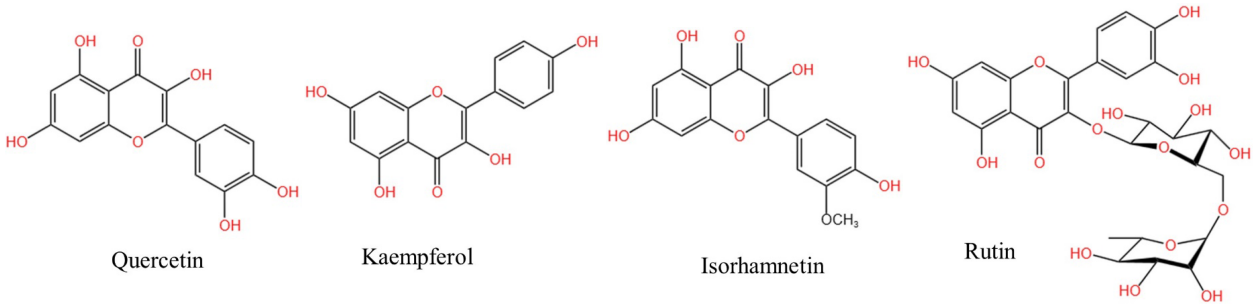


**Fig. 4** The structures of flavonoids.

Lymphopoietin). In mice, local application of SSa or SSc can reduce AD-like skin damage induced by 2,4-dinitrochlorobenzene. SSd improved the intestinal inflammation by reducing the release of pro-inflammatory cytokines TNF-$\alpha$, IL-1$\beta$, and IL-6, and increasing the transcripts of anti-inflammatory cytokine IL-10[101].

SSs analogs isolated from BR also interfered with NF-$\kappa$B activity and inhibited the NF-$\kappa$B signaling pathway[102]. The extraction method of active components in BR can be improved, and supercritical extracts showed stronger anti-inflammatory properties compared to steam-distilled ones[103]. In summary, SSs are one of the main anti-inflammatory components, which induce the expression of anti-inflammatory factors and inhibit the release of pro-inflammatory mediators through various pathways, ultimately achieving the goal of inflammation reduction and pain relief. However, they had varying degrees of cytotoxicity, so caution should be taken in their use[104].

## Antioxidant

Various components isolated from BR, such as SSs, flavonoids, polysaccharides and their extracts, have exhibited antioxidant effects. SSs significantly reduce the levels of ROS (Reactive Oxygen Species) and MDA (Malondialdehyde), a lipid peroxidation product, while enhancing the activities of antioxidant enzymes such as SOD (Superoxide Dismutase), CAT (Catalase) and GSH-Px (Glutathione Peroxidase). The antioxidant effects may be associated with the activation of nuclear transcription factor Nrf2 (NF-E2-related factor 2) signaling pathway[105]. SSa can be used to treat smoke-induced pneumonia in mice[106], with significant antioxidant effects in lung tissues and reduced levels of myeloperoxidase and MDA.

Total flavonoids extracted from BR possess the ability to scavenge ABTS radicals. However, the scavenging abilities of flavonoids extracted from different parts of the plant vary noticeably. The leaf flavonoids had stronger ABTS radical-scavenging ability than that of root flavonoids, and the overall antioxidant capacity was significantly higher in leaves than in other parts[107]. Similarly, BRP (BR Polysaccharides) demonstrated significant capabilities in scavenging hydroxyl radicals and superoxide radicals[108]. A green and efficient method for extracting BRP using a combination of recyclable NADES (Natural Deep Eutectic Solvents) and UAEE (Ultrasound-Assisted Enzyme Extraction) has been developed, and the obtained BRP had specific antioxidant capacities against DPPH, ABTS and hydroxyl radicals. Repeated use of NADES hasd minimal impact on the extraction efficiency of BRP, with the highest extraction rate achieved on the third reuse[109]. Furthermore, the vinegar-processing enhanced the antioxidant activity of BR, which was achieved by altering the structure of polysaccharides, reducing the damage to SM/Cer (Sphingomyelin/Ceramide) and triggering a noticeable antioxidant stress response[110].

BR extract (BRE) also possesses certain antioxidant activity. In SH-SY5Y cells, the serum deprivation reduced cell viability, increased ROS generation, decreased SOD activity, downregulated the anti-apoptotic Bcl-2 (B-cell lymphoma-2) and upregulated the pro-apoptotic Bax (Bcl-2 associated X protein)[111]. BRE dose-dependently reversed the oxidative stress induced by serum deprivation, and exerted antioxidant and proliferation promoting effects. In liver damage of fish, BRE pretreatment upregulated the gene expression of antioxidant GPx and MnSOD, and reduced the gene expression of pro-apoptotic caspase-3, caspase-9 and p53 (protein 53). The antioxidant mechanisms involve enhancing the activities of antioxidant enzymes in damaged liver cells, inhibiting cell apoptosis and immune-inflammatory responses, which are possibly related to the regulation of Nrf2/ARE (Antioxidant Response Element) and TLRs-Myd88-NF$\kappa$B signaling pathways[112,113]. Moreover, oral administration of a certain concentration of BRE inhibited LT4 (Levothyroxine)-induced hypothyroidism and related reductions in body and epididymal fat, as well as liver damage, while enhancing the liver's antioxidant defense system[114]. BRE dose-dependently inhibited the increase of lipid peroxidation induced by LT4, increased GSH content, SOD and catalase activities. It effectively improved LT4-induced hyperthyroidism and organ damage. Currently, BRE shows great potential in antioxidant activity. However, the specific antioxidant components and their related mechanisms still await further clarification. In summary, the antioxidant effects of BR can be attributed to the activity of individual active components as well as the synergistic effects of multiple active components, which activate antioxidant enzymes and regulate the cell cycle.

## Anti-tumor

Various active components and extracts in BR exhibit anti-tumor effects. BR alone or in combination with other physical and chemical approaches can be used for the prevention and treatment of various tumors.

SSd could be effective for the prevention and treatment of OS (Osteosarcoma), as it acted as a functional tumor suppressor by activating the p53 signaling pathway in OS, upregulating the mRNA and protein levels of p53 and its downstream targets, including p21, p27, Bcl-2-like protein 4 and cleaved caspase-3. SSd downregulated the mRNA and protein levels of cyclin D1, thus inhibiting OS proliferation[115]. Combining SSd and SP600125, a specific inhibitor of JNK that competes with adenosine triphosphate, synergistically demonstrated

anti-tumor effects on OS cells by inducing apoptosis through the mitochondrial apoptotic pathway and death receptor pathway, which was superior as compared to SSd alone[116].

SSs dose-dependently and time-dependently inhibit the growth and proliferation of tumor cells. SSd can significantly induce the mRNA expression of HepaRG cells and improve the relative activity of CYP1A2 (Cytochrome P450 family). Also, SSd can induce the expression of CYP1A2 protein and induce both mRNA and protein expression of CYP2D6 (Cytochrome P450 family)[117]. In clinical applications, when drugs metabolized by CYP1A2 and CYP2D6 are used in combination with preparations containing SSd or BR, the blood concentration and action of these drugs should be carefully observed to avoid or take advantage of potential drug interactions. In HepG2 cells, SSd activated pro-apoptotic caspase-3 and caspase-7, induced cell apoptosis, reduced anti-apoptotic protein levels and led to the cleavage of PARP (Poly ADP-Ribose Polymerase). It primarily controlled liver cancer proliferation by suppressing the expression of COX-2 through the p-STAT3/C/EBP$\beta$ signaling pathway, rather than inhibiting the HBV (Hepatitis B virus) proliferation. This suppressive effect is similar to that of PTK inhibitor AG490, suggesting a potential interchangeability between the two[118,119]. Under hypoxic conditions, SSd can reverse the tumor promoting effects of hypoxia, suppress the expression of SUMO1 (Small Ubiquitin-like Modifier 1) and GLI (Glioma-associated oncogene homolog) proteins and selectively activate SENP5 (a SUMO-specific protease), thereby inhibiting the malignant phenotype of liver cancer cells[120]. However, the exact impact of SSd on tumors exposed to radiation remains largely unknown. Both SSd monotherapy and radiotherapy can inhibit the growth of liver cancer cells and increase apoptosis rate[121]. Additionally, SSd enhanced the effects of radiation on SMMC-7721 cells, which may be related to its negative influence on the G0/G1 and G2/M checkpoints of cell cycle. Therefore, SSd holds promise as a radiation sensitizer. Yet, the precise mechanism of action of SSd in human liver cells is still uncertain, and its inhibitory effect on liver cancer-inducing factors such as HBV requires further verification.

SSs exhibit therapeutic effects on breast cancer as well. SSd induced apoptosis in human breast cancer MDA-MB-231 cells by activating the p38 MAPK signaling pathway, while also inhibiting the formation of autophagic lysosomes to prevent autophagic degradation[122]. To enhance the efficiency of SSd in breast cancer treatment, a macrophage biomimetic drug delivery system was developed. T7 peptide-conjugated macrophage membranes were coated onto the surface of SCMNPs (Poly (lactic-co-glycolic acid) nanoparticles). SCMNPs effectively inhibited the growth and metastasis of breast cancer through vascular endothelial growth factor receptor, AKT (protein kinase B) and ERK pathways associated with angiogenesis. SCMNPs demonstrated targeted specificity towards cancer cells, with features such as immune evasion, selective accumulation and enhanced cellular uptake. This biomimetic system provides a complementary treatment paradigm for precise and effective breast cancer therapy[123]. Furthermore, there is a certain correlation between the balance of T helper cells (Th1) and Th2 cells and the anti-tumor immune response in breast cancer[124]. The SSa treatment increased the expressions of IL-12, IL-12 receptors and phosphorylated STAT4 (Signal transducer and activator of transcription 4, reduced the levels of IL-4 and IL-10, and promoted Th1 differentiation. This shift in the Th1/Th2 balance towards Th1 may subsequently inhibit breast cancer growth. These potential mechanisms further activated the IL-12/STAT4 pathway, and induced differentiation towards Th1 cells.

The anticancer effects of BRP and SSs could be comparable. BCP (Acidic water-soluble polysaccharides) inhibited the growth of H22 tumors in mice and protected thymus and spleen tissue from damage, which may be related to S-phase arrest[72]. In mice, BCAP-1 (Alkaline-extracted polysaccharides) significantly inhibited the growth of Sarcoma 180 tumors[125] by increasing the secretion of TNF-$\alpha$ into serum, upregulating the transcription of TNF-$\alpha$ and iNOS, inducing the phosphorylation of macrophage p65, and reducing the expression of I$\kappa$B. Therefore, BRP could be a novel immunostimulant that activates the immune system and can be used for protective treatment in cancer patients.

The phytometabolites of BR could also regulate MDR (Multidrug Resistance) in cells, which remains a challenging issue in tumor treatment. MDR cells overexpress P-gp (P-glycoprotein) encoded by the MDR1 gene, which pumps anticancer drugs out of cancer cells, reducing the effective drug concentration within the cells and posing a major obstacle to successful tumor chemotherapy. Reversing MDR can restore the sensitivity of MDR cells to drugs. SSa and SSd had similar mechanisms of action in MDR of human breast cancer cells, which effectively reversed MDR by reducing the P-gp expression and activity of P-gp-mediated MDR[126,127]. Additionally, SSs from VBBR (Vinegar-Baked BR) enhanced the effects of liver-targeted anticancer drugs by inhibiting MDR-associated transporters P-gp, MRP1/MRP1, MRP2/MRP2, and increasing their distribution in the liver, thereby enhancing the activity of anticancer drugs therein[128,129]. The active components in BR have MDR-reversing effects and can improve the efficacy of chemotherapy drugs while preventing the occurrence of MDR. However, it is important to avoid using anticancer drugs that have antagonistic effects with these components.

## Hepatoprotective activity

The hepatoprotective effect of BR is mainly achieved by inhibiting oxidative stress, regulating enzyme levels (such as GST, AST, ALP), gene expression capacity and promoting liver cell regeneration. In the liver, both SSa and SSd enhance the antioxidant defense, reverse damaged SOD activity, eliminate ROS, inhibit lipid peroxidation and ultimately protect liver cells. SSd also prevented APAP (acetaminophen) -induced hepatitis and liver injury by inhibiting NF-$\kappa$B and STAT3 signaling pathways and inducing the expression of anti-inflammatory cytokine IL-10[130]. SSs alleviated liver injury induced by D-GalN (D-Galactosamine) and LPS in mice[131]. Pretreatment with BRE can alleviate APAP induced ALI in mice, which involves the regulation of various enzymes in the liver, reducing the consumption of liver GSH, and lowering serum AST and ALT levels[132]. The protective mechanism is achieved by inhibiting the reduction produced by rat CYP2E1 and CYP3A protein expression, thereby suppressing CYP450-mediated APAP metabolism, reducing excessive formation of NAPQI and alleviating induced liver toxicity. This mechanism likely occurs at the stage of protein translation rather than gene transcription and requires further research for confirmation.

Inhibition and reduction of oxidative stress can prevent liver fibrosis and cirrhosis. In rats, extracts of BR root had a protective effect against DMA (Dimethylnitrosamine)-induced liver

fibrosis, which was achieved by enhancing the production of liver GSH, promoting liver cell regeneration, and regulating IFN-g (Interferon-g) and IL-10 in serum[133]. BRE can also significantly reduce ALT (Serum alanine aminotransferase), AST (Aspartate aminotransferase), MDA and SOD levels induced by DEN (Diethylnitrosamine), thereby alleviating liver cirrhosis in rats[134].

The hepatoprotective effect of VBBR was explored using NMR (Nuclear magnetic resonance) metabolomics. It is associated with energy metabolism, lipid metabolism, ketone body metabolism, glutathione metabolism, amino acid metabolism, and nucleotide synthesis[135]. VBBR in combination with rhubarb extract can inhibit the efflux transporters P-gp and MRP2 (MDR-associated protein 2), increase the influx transporter OATP2 (Organic anion-transporting polypeptide 2), thereby increasing the concentration of rhubarb acid in the rat liver, and achieving a synergistic effect on liver diseases[136]. The hepatoprotective effect of VBBR may be due to the vinegar processing might increase the concentration of certain active components in BR, and the specific mechanism needs further verification.

Some BR containing TCM formulas, such as XYS and SNS (Si Ni San), have good hepatoprotective effects. XYS reduced the activities of AST and ALT in the serum in a rat CUMS (Chronic unpredictable mild stress) model, increased the levels of SOD and GSH-Px in the liver, and decreased the contents of MDA, IL-6, and IL-1$\beta$. It helped maintain ammonia balance and promote energy metabolism to achieve antidepressant and hepatoprotective effects[137]. SNS can alleviate $CCl_4$ (Carbon tetrachloride) induced ALI as well as chronic liver injury induced by alcohol and sucrose, reduce serum AST and ALT levels, and improve liver tissue morphology[138]. It also decreased the expression of AFP (Alpha-fetoprotein) in WB-F344 cells and promoted the expressions of ALB (Albumin) and CK19 (Cytokeratin 19). Treatment with SMS (SNS-medicated serum) induced the accumulation and nuclear translocation of $\beta$-catenin, which bound with the receptor LEF1 in the nucleus, regulated factors such as c-Myc and Cyclin D1 and activated the Wnt/$\beta$-catenin signaling pathway to induce the differentiation of liver stem cells and promote liver regeneration in rats. The network pharmacology predicted that SNS may exert hepatoprotective effects by regulating potential targets IL-6, VEGFA, EGFR, PPARG, CASP3 and related signaling pathways HIF-1, TNF, and PI3K-Akt, thereby exhibiting anti-inflammatory, anti-oxidative stress effects, inhibiting apoptosis, and protecting the liver[139]. However, it is still unclear if there are other signaling pathways involved in liver cell differentiation, and the exploration of targets of XYS and SNS will be the focus of future research.

## Immunomodulatory activity

The active components of BR have positive effects in the immune system, possibly by enhancing immune function and reducing immune evasion, thereby exerting immunomodulatory effects. It has been found that the pathogenesis of HT (Hashimoto's thyroiditis), one of the common autoimmune diseases, is related to macrophage polarization[140]. Treatment with SSd can alleviate the effects of HT on multiple targets such as IL-6, IL-10, and can act on signaling pathways related to macrophage polarization, e.g., MAPK and JAK-STAT. It reduced the infiltration of thyroid lymphocytes and serum levels of TPOAb antibodies in HT mice. It also regulated the polarization of M1/M2 macrophages in the spleen, systemically and locally inhibiting the IFN-$\gamma$ expression of th1-type cell and IL-17 expression of th17-type cell in the thyroid, thereby reducing the severity of HT and playing a preventive and therapeutic role. In rats with experimental autoimmune thyroiditis, the expressions of NLRP3, ASC, Cleaved Caspase-1 and IL-1$\beta$ proteins increased, indicating the activation of NLRP3 inflammasome. What is delightful is that SSa reversed the expression of these proteins and prevented the thyroid inflammation[141]. SSs can also exert anti-inflammatory effects by promoting the secretion and release of endogenous glucocorticoids[142]. They mainly activate the functions of macrophages and lymphocytes, enhance non-specific and specific immune responses in the body, thereby playing an immunomodulatory role. These not only promote the growth of immune organs spleen and thymus, protect the immune organs, but also improve cellular immune function and enhance the body's anti-tumor ability by regulating the levels of T lymphocyte subsets in peripheral blood.

BRP can be used as immunostimulants, as it restores and improves humoral and cellular immune functions. BRP significantly increased the number of macrophages, enhanced the function of natural killer cell, increased the concentration of virus-specific antibody and lymphocyte transformation rate, and delayed hypersensitivity reaction[143]. BRP also augmented the phagocytic function of macrophages and inhibited the production of NO and pro-inflammatory cytokines IL-1, IL-6 and TNF induced by LPS[144].

In stimulated cells with excessive inflammation, the extracts of BR root may drive macrophages and lymphocytes towards Th2 anti-inflammatory polarization, which reduced ROS generation, increased the secretion of peripheral blood mononuclear cell (PBMC) chemokines such as IL-1$\beta$ and IL-12p70, and upregulated the differentiation of THP-1 monocytes into macrophage-like cells[145]. For the prevention and treatment of COVID-19, the active components of BR also exhibited certain immune effects, which mainly act through anti-inflammatory pathways, inhibit the release of inflammatory factors, maintain cell homeostasis and reduce DNA damage[146,147]. The potential active components and molecular mechanisms of BR provide theoretical support and pharmacological basis for its further development and utilization against COVID-19.

## Antidepressant activity

The antidepressant effect of BR may be mediated through the upregulation of CREB (cAMP-response element binding protein) and BDNF (brain-derived neurotrophic factor) expression via the PI3K/Akt/GSK-3$\beta$ signaling pathway. Both BR itself and its herbal formulations had significant antidepressant effects. BR helped protect and restore the cell metabolism through neuro-regulation[148]. The BR TSS (Total saikosaponins) increased the expression of synaptic proteins and induced AMPA receptor and mTOR (Mammalian Target of Rapamycin) signaling pathways. TSS not only enhanced the expression of synaptic proteins, hippocampal CA3 synapsin-1 and phosphorylated GluR1 Ser 845, but also upregulated downstream regulatory factors such as ERK, AKT and mTOR. Furthermore, TSS can stabilize $Ca^{2+}$ homeostasis, regulate Bcl-2 family, inhibit endoplasmic reticulum stress and mitochondrial apoptosis pathways, partially reverse pathological changes induced by corticosterone and protect neurons[149]. In the rat CUMS model of

depression, SSa exhibited antidepressant effects, which increased body weight, improved sucrose preference, elevated neurotransmitter levels and reduced inflammatory responses[150–152]. SSa upregulated the expression levels of PRKACA and CREB proteins in the cAMP/PKA/CREB signaling pathway, lowered Bax and Caspase-3 levels, inhibited hippocampal neuronal apoptosis and increased dopamine levels.

PBR (Low-polarity components) of BR also show therapeutic effects in CUMS rats[153]. PBR improved depressive behaviors, such as reduced growth rate, anhedonia, and decreased locomotor activity. It significantly decreased the levels of ALT and AST while increasing levels of cytokeratin-18 fragments. Additionally, PBR dose-dependently reversed amino acid metabolism, energy metabolism, sphingolipid metabolism, and fatty acid $\beta$-oxidation. The antidepressant effect of PBR may be related to the cecum content and gut microbiota, as it increased the diversity of beneficial gut microbiota, reduced the abundance of harmful bacteria and regulated the metabolic homeostasis of endogenous biomarkers[154–157]. However, the exact mechanisms underlying the effects of BR components on metabolites and gut microbiota remain unclear, and further experimental research is needed to confirm their potential mechanisms.

BR formulations are widely used in clinical practice for the treatment of severe depression due to their multi-component, multi-target and multi-pathway characteristics. BR monotherapy or combined therapy with antidepressant drugs may regulate oxidative stress, neural plasticity, immune response and neuroprotection. They can alleviate the severity of depression by regulating important metabolic pathways, such as amino acid metabolism, energy metabolism and lipid metabolism[158–162]. The combination of BR formulations with antidepressant drugs enhanced the efficacy of antidepressants and reduced the adverse reactions. XYS is a representative TCM formula for the treatment of depression. It can regulate various aspects of depression through multiple targets, involving metabolism, neuroendocrine function and neuroimmune response. XYS regulated multiple targets associated with necrosis, alleviated depression by modulating necrosis-mediated inflammatory signaling pathways, suggesting that necrosis targets may be scrutinized for treating depression[163]. In addition, autophagy plays a crucial role in the development of depression, which affects the expression of GLUT4 in the hypothalamus. XYS intervention effectively reversed depressive behaviors in CUMS mice, upregulated hypothalamic neuronal autophagy and GLUT4 expression, improved hypothalamic glucose metabolism and related indicators[164]. The current researches of XYS reveal its biological mechanisms of antidepressant action from multiple perspectives, providing insights for deeper investigations.

## Antimicrobial activity

BRE has varying levels of inhibition against different bacteria and fungi[165]. The responsible components of BR might be volatile oils, and a large amount of monoterpenes, such as $\alpha$-pinene, $\beta$-pinene and limonene, had good killing effects against *Cryptococcus neoformans* and *Trichophyton rubrum*. Furthermore, they significantly altered the ultrastructure of *Candida albicans* and *Trichophyton rubrum*, inhibited the hyphae of *C. albicans*, and disrupted mature biofilms[166]. The volatile oils from the aerial parts of *B. montanum* and *B. plantagineum* exhibited the highest antimicrobial activity against *Streptomyces griseus*, *Staphylococcus aureus*, *Enterococcus faecalis* and *C. albicans*[167]. The polyacetylene (8S-heptadeca-2-Z-9-Z-diene-4,6-diyne-1,8-diol) from *B. salicifolium* was toxic to *Artemia salina* and inhibited Gram-positive bacteria[168]. Additionally, when fungal spores were inoculated into sunflower seedlings treated with volatile oils of *B. gibraltarium*, a significant inhibition of spore production was observed. The pre-treatment with volatile oils could activate the defense response of sunflower seedlings against pathogenic invasion and inhibit the antibacterial activity of *C. albicans*[169]. The volatile oils of BR may directly act on microorganisms, disrupting their cell membrane structure and metabolic functions and activating the body's defense response to exert antimicrobial effects.

In addition, SSa had a protective effect against *Salmonella-induced* pullorum disease, which was associated with the upregulation of LXR$\alpha$-ABCG1/ABCA1 pathway; SSa reduced cholesterol in lipid rafts of HD11 cells and inhibited the invasion of *Salmonella pullorum* into HD11 cells[170].

Due to the increasing antibiotic resistance, preventing and controlling infections have become more challenging. Currently, research has focused on synthesizing novel antimicrobial agents using plant materials, which have been widely applied in biomedicine, cosmetics, bioremediation and healthcare industries. The antibacterial Bc-AgNPs (Silver nanoparticles) synthesized from BRE demonstrated a certain antibacterial activity, which strongly inhibited Gram-negative bacteria as compared to Gram-positive ones[171]. Similarly, silver oxide and zinc oxide nanoparticles synthesized based on BR had significant antibacterial activity against *Escherichia coli* and methicillin-resistant *S. aureus*. Furthermore, the nanoparticles and biofunctionalized bacterial cellulose membranes showed remarkable bactericidal activity against various drug-resistant pathogens[172]. In summary, novel bio-based antimicrobial agents have strong functionality and bioactivity, providing possibilities for the application of nanomaterial-based antimicrobial agents in the post-antibiotic era.

## Other bioactivities

SSa can significantly reduce the severity and duration of epileptic seizures and increase the latent period of epilepsy[173]. By inhibiting the mTOR signaling pathway, it downregulated the expression of p-mTOR, p-70S6K, IL-1$\beta$ and TNF-$\alpha$ in the hippocampus, reduced the protein expression of p-mTOR and p-70S6K, thereby inhibiting hippocampal neuronal apoptosis and epileptic seizures. BRE, including SSs, volatile oils and water-soluble components, also exhibited anticonvulsant effects[174]. SSs and volatile oils had a significant antagonistic effect on MES (Maximal Electroshock)-induced seizures, while the water-soluble components effectively counteracted pentylenetetrazole induced seizures. Moreover, linoleic acid of BR may have antiepileptic effects, and the two alcohols of BR may also exert antagonistic effects on chemically-induced seizures.

BRP can alleviate inflammation and fibrosis in the kidney of diabetic mice, which may be attributed to its inhibition of HMGB1-TLR4 signaling pathway and reduction of NF-$\kappa$B activity[175]. BRP significantly reduced blood creatinine levels, urinary albumin excretion, and kidney swelling, which effectively inhibited the progression of renal injury in mice. It decreased the expression of TNF-$\alpha$ and IL-6, as well as Col IV (Type IV collagen), FN (Fibronectin) and $\alpha$-SMA ($\alpha$-Smooth Muscle Actin), thereby alleviating diabetic nephropathy. Furthermore, BRP

can improve the gut barrier and regulate the gut microbiota, thereby reducing kidney and colonic inflammation and ameliorating streptozotocin-induced diabetic kidney disease[176]. The Meta-analysis revealed that combined treatment with DCHD (Da Chaihu Decoction) provided advantages over conventional therapy alone in T2DM (Type 2 diabetes mellitus), as it further regulates glucose and lipid metabolism, reduces insulin resistance, improves pancreatic function and lowers BMI[177]. DCHD alone also has certain blood glucose-regulating effects, but due to limitations in the quality and quantity of included studies, the efficacy and safety of DCHD remain uncertain.

## Conclusions and prospects

The depth and breadth of research on BR has greatly expanded, resulting in a significant increase in published articles. The applications have extended beyond medicinal use to include the fields of health supplements and cosmetics. Moreover, the processing methods of BR have evolved to combine traditional and modern techniques. However, the safety and practicality of these products need to be carefully assessed to ensure the absence of side effects. Additionally, emphasis should be placed on the quality of BR itself, exploration of its active ingredients, pharmacological mechanisms and clinical applications.

### Establishment of a quality standard system

Ensuring the quality and efficacy of BR is crucial for reliable research and application. Currently, BR faces several challenges such as scarcity of wild resources, inadequate market supply and genetic contamination[178]. The presence of multiple regional varieties, including non-medicinal parts or other species falsely claiming to be BR, severely affects its quality and accurate usage. Therefore, it is necessary to investigate, identify and classify BR germplasms to establish scientific identification systems, reliable quality evaluation indicators and quality control methods. These methods could include chemical fingerprinting, determination of active ingredient content and analysis of environmental interactions. Such measures will ensure the stability and safety of BR products and provide guidance for its accurate medicinal use.

### In-depth exploration of active ingredients

Using advanced techniques such as UPLC, MS and NMR, numerous active ingredients have been identified in BR, including SSs, volatile oils, polysaccharides, polyacetylenes, flavonoids and others. However, the current researches focus on SSs and volatile oils, with limited exploration of the aerial parts and other phytometabolites. Future studies should optimize extraction and separation techniques to obtain higher purity and stability of active ingredients. The structure-activity relationship of BR constituents should be investigated. The details of pharmacological activities and mechanisms of action, such as modulation of cell signaling pathways, regulation of inflammatory mediators and influence on the immune system, should be elaborated, and the potential of BR in treating neurological disorders, cardiovascular diseases, liver conditions and other health concerns should be further explored. Additionally, further investigations using genomics, proteomics, metabolomics and other advanced/high throughput technologies should be applied in BR studies to reveal the intricate interactions between BR active constituents and their targets. Future studies can focus on the variation and content differences of BR chemicals of different varieties, geographical origins and growth environments, and correlate such differences with pharmacological activities.

### Strengthening clinical application research

The clinical research of BR remains relatively limited. Future efforts should include conducting more randomized controlled clinical trials and observational studies to further investigate and validate the role and efficacy of BR in clinical applications. Evaluating BR effects, safety and side effects in specific disease treatments will provide a more robust scientific basis for its rational application. Moreover, studying the interactions between BR and other drugs, including their pharmacokinetic and pharmacodynamic influences, can be very enlightening in exploring potential synergistic effects and determining appropriate combination strategies to enhance treatment efficacy and minimize adverse reactions.

In conclusion, this review has summarized the traditional uses, germplasm identification, phytochemistry and pharmacological activities of BR. Future researches should focus on establishing quality control systems, identifying novel chemical components, elucidating the mechanisms of pharmacological activities, verifying and evaluating clinical applications, and studying drug interactions. These endeavors will provide more reliable evidence for the scientific research and clinical application of BR, promoting its further development and utilization in diverse fields.

## Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Yu M, Wei J; data collection: Zhao J, Zeng C; analysis and interpretation of results: Chen H, Xin Chao; draft manuscript preparation: Zeng C, Wang B. All authors reviewed the results and approved the final version of the manuscript.

## Data availability

All data generated or analyzed during this study are included in this published article.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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