

## A critical review of *Andrographis paniculata*

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### Abstract

*Andrographis paniculata*, a traditional medicinal plant, is widely used to treat various disorders. According to traditional Chinese medicine (TCM) theory, it is intensely bitter in taste and cold-natured. Andrographolide and its derivatives are the major bioactive compounds that show anti-inflammatory, anti-tumor, anti-diabetes, cardiovascular protection, neuroprotective, and hepatoprotective effects. In this review, we will focus on the application of TCM prescriptions and the modern bioactivities of *Andrographis Herba*. Due to the low content of andrographolide and derived lactones in the original species and the complexity of chemical structure, there is an urgent need to develop biotechnological methods for obtaining andrographolide and its derivatives sustainably. Nevertheless, the andrographolide biosynthetic pathway still needs to be fully elucidated in *A. paniculata*. Therefore, we further review recent progress in revealing the andrographolide biosynthetic pathway, combined with heterologous synthesis strategies of other plant diterpenoids to help create a cell factory with higher production of andrographolide in the future.

**Citation:** Chen X, Ren J, Yang J, Zhu Z, Chen R, et al. 2023. A critical review of *Andrographis paniculata*. *Medicinal Plant Biology* 2:15 <https://doi.org/10.48130/MPB-2023-0015>

### Introduction

*Andrographis paniculata* (Burm. F.) Nees is one of the most widely recognized medicinal plants, which has been utilized for thousands of years as a traditional Chinese medicine. It is an annual herbaceous plant belonging to the genus *Andrographis*, the botanical family Acanthaceae in the order of Lamiales<sup>[1]</sup>. Macroscopically, the erect herb is 50–70 cm tall. The upper part of the stem is quadrilateral, and the lower part is nearly round. The stem texture is fragile and easy to break. Leaves are simple, opposite, shortly petiolate, or almost sessile. The leaf blade is flattened or ovate-lanceolate, 2–7 cm long and 1–3 cm wide, with an acuminate apex, reticulate venation, and cuneate-decurrent base. The margin of the leaf blade is entire or undulated<sup>[1]</sup>. In the theory of traditional Chinese medicine (TCM), the medicinal property of *A. paniculata* belongs to 'cold', which exhibits the effect of clearing heat, detoxifying, cooling blood, and reducing swelling<sup>[1]</sup>. *A. paniculata* has a long history of application in TCM theory, as well as the traditional Indian medicine system-Ayurveda. *A. paniculata* was first introduced into the Chinese Pharmacopoeia (ChP) in 1977 and was approved by the Food and Drug Administration (FDA) to include into the United States Pharmacopoeia (USP). According to the ChP, the dried leaves and above-ground stems are made into a Chinese medicine *Andrographis Herba* (Chuanxinlian, CXL), which acts as a natural immune booster with anti-infection, anti-inflammatory, antiviral, and analgesic effects<sup>[2,3]</sup>.

The main active constituents of CXL include lactones, diterpenoids, flavonoids, diterpenoids lactones, diterpenoid glycosides, especially labdane diterpenoids lactones such as andrographolide (C<sub>20</sub>H<sub>30</sub>O<sub>5</sub>), neoandrographolide (C<sub>26</sub>H<sub>40</sub>O<sub>8</sub>), 14-deoxyandrographolide (C<sub>20</sub>H<sub>30</sub>O<sub>4</sub>) and dehydroandrographolide (C<sub>20</sub>H<sub>28</sub>O<sub>4</sub>)<sup>[1,3]</sup>. Many studies have shown that the

diterpene compound andrographolide and its derivatives are the main active components in CXL and play an essential role in treating COVID-19 infection through TCM<sup>[4]</sup>. Notably, the content of active ingredients is the core of the quality of CXL, which was proven previously to be affected by the area of growing places, the time of harvesting and processing (bud stage with the highest content of andrographolide), and the storage time. Considering that the content of andrographolide in leaves is significantly higher than that in stems, the 2015 edition of ChP requires that the proportion of leaves must be at least 30% to ensure the quality of the medicinal herb and the clinical efficacy<sup>[1]</sup>. Besides, the total amount of active andrographolide and dehydrated andrographolide in CXL must be more than 1.5%, according to the ChP. However, there is no unified international standard for CXL, and the current imperative is to establish a consistent quality standard and grade evaluation.

South India and Sri Lanka are widely acknowledged as the origin and center of genetic diversity for the plant *A. paniculata*, which possesses a broad range of germplasm resources. Nowadays, it is prevalent and widely spread from Southeast Asian countries (India, Sri Lanka, China, Cambodia, Malaysia, and Thailand) to North America and the West Indies<sup>[5]</sup>. Interestingly, it has been speculated that *A. paniculata* in Thailand and Malaysia may have come from India<sup>[6]</sup>. There are about 20 species of *Andrographis* plants, two of which are distributed in China, including the cultivated species (*A. paniculata*) and the wild types (*A. laxiflora* var. *glomerulifera*)<sup>[7]</sup>. *A. paniculata* was introduced into China in the 1950s and primarily cultivated in Guangxi Zhuang Autonomous Region and Guangdong Province, which produce more than 90% of China's total production (Fig. 1)<sup>[8]</sup>. Others are scattered in Fujian, Jiangxi,

Sichuan, and Anhui provinces. Furthermore, the germplasm of Chinese *A. paniculata* is mainly from Guangdong and Hainan. After over 80 years of being introduced and cultivated, the origin of *A. paniculata* remains relatively singular, with a highly similar genetic background and limited potential for evolution within the population<sup>[6]</sup>.

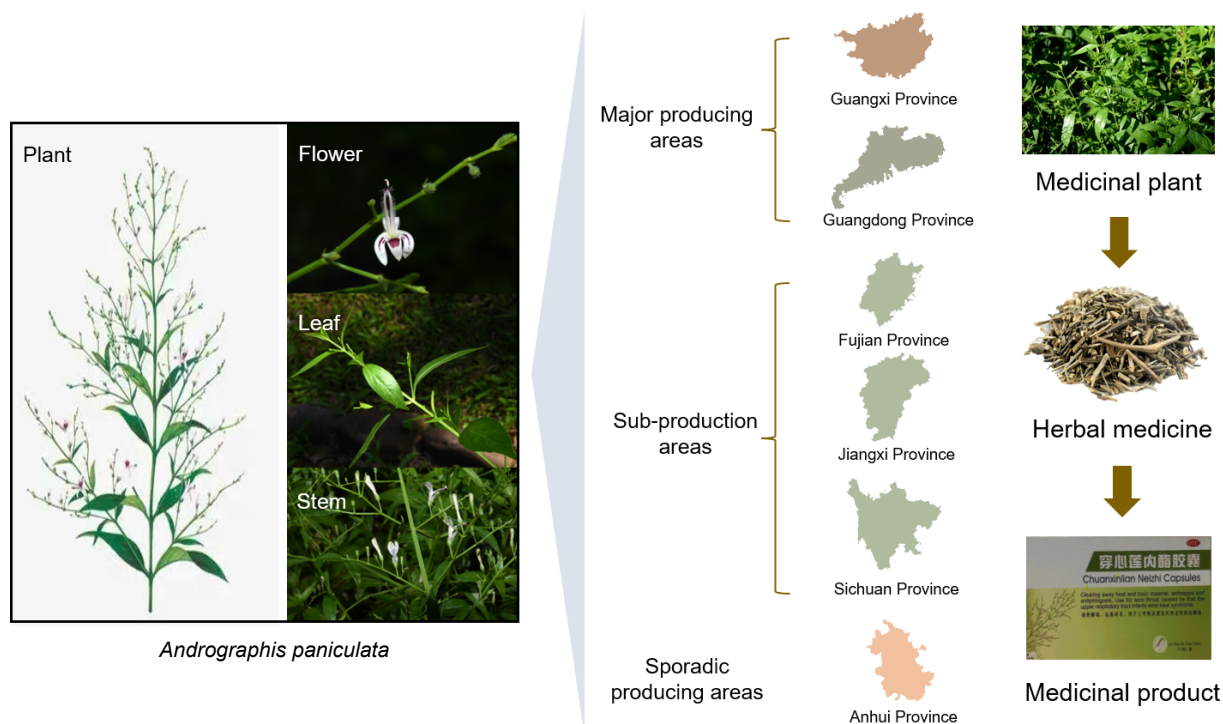
Due to the significant increase in the demand for CXL (~10,000 tons per year in China), the diversity of wild plant resources has been severely threatened in recent years. On the other hand, the long-term artificial cultivation, the different geographical regions, the natural environment, and production management have led to gene variation and isolation, interspecies hybridization, and even apparent differentiation phenomenon, resulting in unstable herb quality and heterogeneous andrographolide content of *A. paniculata*<sup>[5]</sup>. Therefore, collecting germplasm resources and identifying high-quality plant varieties have become the primary task of *A. paniculata* breeding. Germplasm materials of *A. paniculata* mutant have been obtained by specific mutagenesis techniques<sup>[6]</sup>. With the development of high-throughput sequencing technology, scientists have begun to study the genetic and metabolic processes of *A. paniculata* at the omics level. Notably, whole-genome databases have been assembled<sup>[2,9]</sup>, which contained a large amount of gene annotation information and identified three terpenoid synthases, two cytochrome P450 monooxygenases, and a UDP-dependent glycosyltransferase related to andrographolide biosynthesis<sup>[2]</sup>. It is conceivable that combined with the seven basic leucine zipper (bZIP) transcription factors<sup>[10]</sup> and seven WRKY transcription factors<sup>[11]</sup> found in the transcriptomes that may be involved in regulating the andrographolide biosynthesis, more precise molecular breeding of *A. paniculata* and efficient heterologous synthesis of active andrographolide will be possible.

In this review, we will focus on the pharmacological activity of CXL and reveal that andrographolide and its derivatives are the main active substances of CXL, from the theory of TCM to modern pharmacological research. Subsequently, we will review the biosynthesis pathway of andrographolide and the heterologous synthesis strategies of other plant diterpenoids. It is expected that a higher yield of andrographolide can be achieved by cell factories in the future, eliminating the need to harvest *A. paniculata* plants.

## Theoretical effect and application of *Andrographis Herba* in TCM

*Andrographis Herba* (Chuanxinlian, CXL) is a herb with a nontraditional significance imported from abroad, also named Yijianxi or Sifanglian in some places<sup>[7]</sup>. The medical book *Lingnan Medicinal Collection Record* has recorded that CXL can cure snake bites and manage internal injury cough<sup>[12]</sup>. According to the Four Natures and Five Flavors theory of Traditional Chinese Medicine, the 'bitter' and 'cold' Chinese herbal medicine CXL belongs to the heart meridian of hand-shaoyin, the lung meridian of hand-taiyin, the large intestine meridian of hand-yangming and the bladder meridian of foot-taiyang<sup>[1]</sup>. Specifically, CXL can clear heat and detoxify, cool blood and detumescence, dry dampness, and eliminate dysentery<sup>[12]</sup>. CXL is widely used in the clinical practice of TCM, and its efficacy is like *Coptidis Rhizoma* (Huanglian, HL). It is usually taken orally with 6-9 g added into decoction or powder<sup>[1]</sup>.

When used to clear heat and detoxify, CXL collaborates with *Lonicerae Japonicae Flos* (Jinyinhua) and *Scutellariae Radix* (Huangqin) to treat the symptoms of lung heat cough, or sore throat caused by upper respiratory tract infection, tonsillitis, bronchitis, pneumonia, and other diseases. Furthermore, as CXL



**Fig. 1** Schematic diagram of the main origin in China and the industrialization process of *Andrographis paniculata*.

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plays the role of cooling blood and detumescence, it is usually used in combination with *Houttuynia herba* (Yuxingcao), *Coicis Semen* (Yiyiren), and *Phragmites Rhizome* (Lugen) to treat lung carbuncle characterized by cough, chest pain, vomiting purulent blood and sputum. And in the function of drying dampness and eliminating dysentery, CXL always cooperates with *Coptidis Rhizoma* (Huanglian), *Talcum* (Huashi), *Gardeniae Fructus* (Zhizi), *Artemisia Scopariae Herba* (Yinchen, YC), *Phellodendri Chinensis Cortex* (Huangbai) or *Sophorae Flavescentis Radix* (Kushen) to cure dampness-heat diarrhea, jaundice, and gonorrhoea. CXL can also detoxify snake bite poisoning by external use with glycerin after grinding. In addition to these primary functions, CXL can assist other medicinal herbs in their therapeutic effects. For example, when decocted with *Gypsum Fibrosum* (Shigao), the two drugs work together to clear up excessive heat syndrome in Qi and treat the symptoms of high fever, sweating, irritability, thirst, and tachycardia. Conclusively, due to its bitter and cooling properties, CXL can guide and assist in the treatment of many damp-heat syndromes, playing a crucial role in TCM prescriptions.

With the increasing recognition of the medicinal value of CXL, it has been used in many TCM formulas. Here, we summarized formulas containing CXL (Table 1). These formulas can be divided into seven types: heat-clearing drugs, damp-resolving drugs, exterior-relieving drugs, sore and ulcer drugs, phlegm-resolving drugs, astringent drugs, and reinforcing drugs. Meanwhile, dosage forms include tablets, capsules, pills, injections, granules, medicinal extracts, suppositories, powders, and pastilles. Among them, the most significant formula type is the heat-clearing drugs (44.4%) (Fig. 2a), while tablets dosage is the most (57.8%) (Fig. 2b). Especially one of the most famous formula tablets in China, *Fufang Dantong Pian* (tablet), composed of *Isodi Lophanthoidis* (Xihuangcao), YC, CXL, *Rhei Radix et Rhizome* (Dahuang) and 7-hydroxy-4-methylcoumarin (Dantong), has the effect of clearing heat, improving choleric and relieving spastic pain. Moreover, *Fufang Dantong Pian* could also solve problems or diseases such as acute and chronic cholecystitis, cholangitis, gallbladder, biliary calculi complicated with infection, cholecystectomy syndrome, biliary tract dysfunction, etc.<sup>[13,14]</sup>. Another typical formula containing CXL is called *Ganmaoqing Pian* (tablet)<sup>[15]</sup>, which treats the wind-heat common cold with symptoms of fever, headache, nasal congestion, sneezing, sore throat, and body aches. Besides, there are also granules and capsules of *Jinji Keli* (granule)<sup>[16]</sup> and *Jinji Jiaonang* (capsule)<sup>[17]</sup> containing CXL, which are used to treat damp-heat adnexitis by invigorating spleen and dehumidification and activating blood circulation. *Shanghai Sheyao Pian*<sup>[18]</sup>, a tablet with CXL as the main component, is commonly used in clinics to treat various poisonous snake bites, providing essential support for patients' health and well-being.

## Pharmacological activities and mechanism of andrographolide

Andrographolide and its derivatives are the principal bioactive compounds of the medicinal plant *A. paniculata*<sup>[4]</sup>, which have anti-inflammatory, anti-tumor, anti-diabetes, cardiovascular protection, neuroprotective effect, hepatoprotective effect, and others (Fig. 3).

## Anti-inflammatory effects and mechanism of andrographolide

As mediators of intercellular interactions, cytokines are crucially involved in initiating and perpetuating inflammatory disorders. The inhibition of inflammatory mediators represents an effective therapeutic strategy for both acute and chronic inflammatory conditions. Andrographolide exerts its anti-inflammatory effects through many pathways and targets, including regulating the synthesis and release of inflammatory mediators.

According to the research, andrographolide has been shown to alleviate airway inflammation by neutrophil infiltration in lung tissue in a mouse model of ovalbumin (OVA)-induced asthma by blocking cytokines regulated by helper T cell 17 (Th17) and inhibiting the expression of Janus tyrosine-protein kinase one and signal transducer and activator of transcription 3 (JAK1/STAT3) signaling pathway, thereby reducing exaggerated allergic inflammation response of the upper respiratory tract<sup>[49]</sup>. In the complete Freund's adjuvant (CFA)-induced acute paw edema inflammation model, andrographolide can significantly reduce the degree of inflammatory swelling in mice. It has exerted anti-inflammatory effects by inhibiting a series of inflammation-related molecules, such as COX-2, NF- $\kappa$ B, p-p38, CD40, tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , and IL-6, suggesting that andrographolide possesses strong anti-inflammatory properties<sup>[50]</sup>. Inflammatory disorders are often associated with activating NOD-like receptor protein 3 (NLRP3) inflammasomes by various stimuli, including monosodium urate (MSU). Upon activation, NLRP3 inflammasomes release a large amount of interleukin (IL)-1 $\beta$ , contributing to the pathogenesis of numerous inflammatory diseases. In a mouse model of MSU-induced arthritis, treatment with andrographolide has been found to reduce monocyte infiltration and IL-1 $\beta$  release in the knee joint, indicating its potential therapeutic effect in ameliorating inflammation associated with NLRP3 inflammasome activation<sup>[51]</sup>. Moreover, the miR-27-3p/matrix metalloproteinase 13 (MMP-13) signaling axis may represent another potential therapeutic target for treating and preventing osteoarthritis progression. Andrographolide has been demonstrated to mitigate cartilage damage and stimulate chondrocyte anabolism by regulating MMP-13 through the miR-27-3p signaling pathway<sup>[52]</sup>. Additionally, andrographolide has been found to ameliorate acute colitis in mice *via* the activation of the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway. The disease activity index (DAI) score has been significantly reduced, and colon shortening has been markedly improved in the andrographolide-treated group<sup>[53]</sup>.

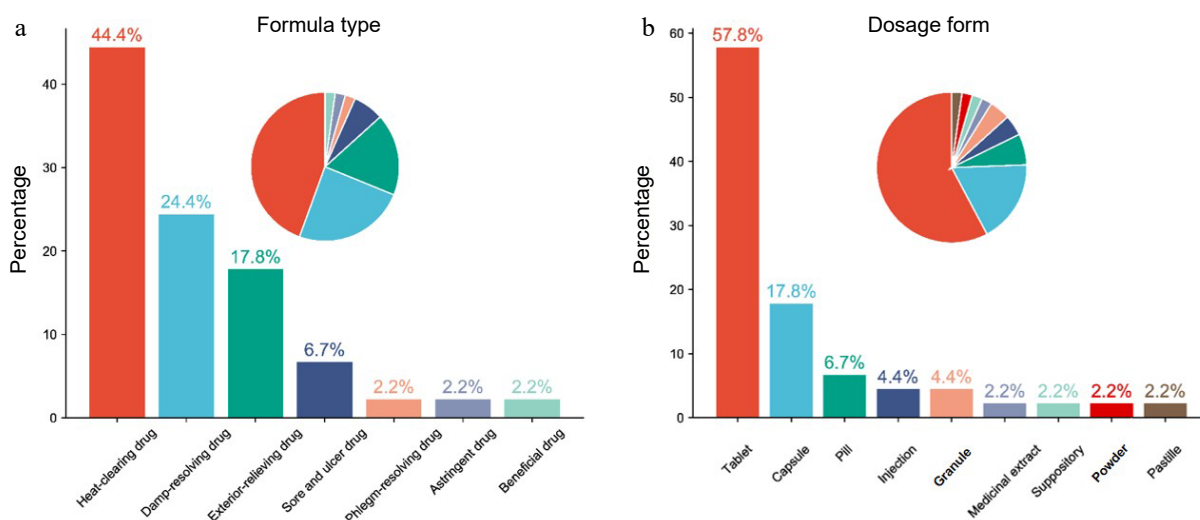
It can be concluded that the primary mechanism through which andrographolide exerts anti-inflammatory effects includes inhibiting inflammatory cell activity and suppressing inflammatory mediators' expression. In terms of clinical trials of drugs, the evaluation of the efficacy and safety of andrographolide sulfonate in the treatment of acute tonsillitis and acute bronchitis has entered Phase VI (NCT numbers: NCT03134443, NCT03132623). Meanwhile, the clinical study of andrographolide in treating acute exacerbations of chronic bronchitis has also entered Phase VI (NCT number: NCT03132610).

**Table 1.** TCM formulas containing *Andrographis Herba* (Chuanxinlian, CXL).

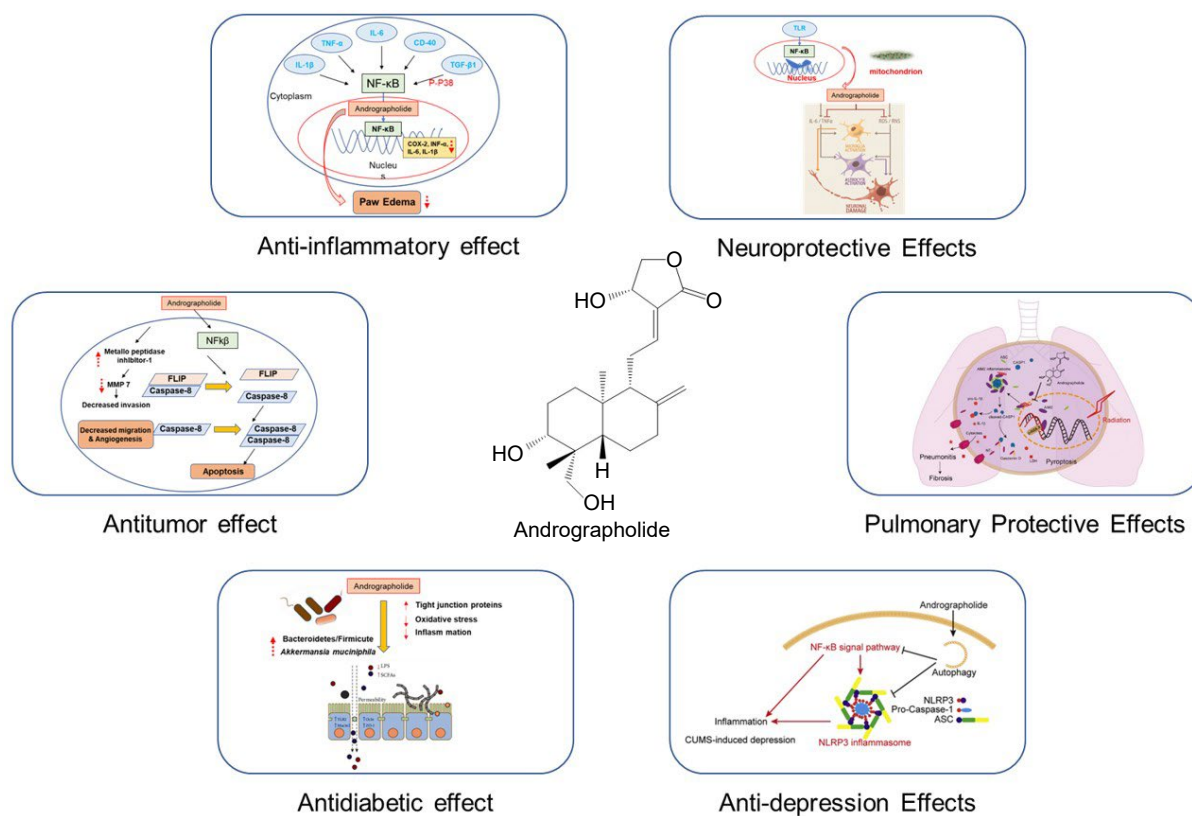
Formula name	Components	Formula type	Dosage form
Liaofeiing Pian <sup>[19]</sup>	Stemona Radix (Baibu, BB), <b>CXL</b> , Imperatae Rhizoma (Baimaogen), Bletillae Rhizoma (Baiji)	Reinforcing	Tablet
Qingre Anchuang Wan <sup>[20]</sup> Qingre Anchuang Pian <sup>[21]</sup>	Japonicae Flos (Jinyinhua, JYH), Rhei Radix et Rhizome (Dahuang, DH), <b>CXL</b> , Taraxaci Herba (Pugongying), Margarita (Zhenzhu, ZZ), Sophorae Tonkinensis Radix et Rhizoma (Shandougen), Glycyrrhizae Radix et Rhizoma (Gancao, GC), Gardeniae Fructus (Zhizi)	Blood-activating and stasis eliminating	Pill Tablet
Waiyong Zijin Ding <sup>[15]</sup>	Cremastrae Pseudobulbus Pleiones Pseudobulbus (Shancigu), Cinnabaris (Zhusha), Galla Chinensis (Wubeizi), Realgar (Xionghuang, XH), Knoxiae Radix (Hongdaji), <b>CXL</b> , Euphorbiae Semen (Qianjinzi), Notoginseng Radix et Rhizoma (Sanqi), Borneolum Syntheticum (Bingpian, BP), Caryophylli Flos (Dingxiang)		Pastille
Kangfu Xiaoyan Shuan <sup>[22]</sup>	Sophorae Flavescentis Radix (Kushen), Patriniae Herba (Baijiangcao), Viola Herba (Zihuadiding), <b>CXL</b> , Taraxaci Herba (Pugongying), Suis Fellis (Zhudan), Arnebiae Radix (Zicao), ALOE (Luhui)	Dampness-resolving	Suppository
Fufang Chuanxinlian Pian <sup>[23]</sup> Fuke Qianjin Pian <sup>[24]</sup>	<b>CXL</b> , Isatidis Folium (Daqingye, DQY) Radix Flemingiae (Qianjinba, QJB), Zanthoxyli Radix (Danmianzhen), Rosae Laevigatae Radix (Jinyinggen, JYG), <b>CXL</b> , Mahoniae Caulis (Gonglaomu, GLM), Codonopsis Radix (Dangshen), Spatholobi Caulis (Jixueteng, JXT), Angelicae Sinensis Radix (Danggui)		Tablet Tablet
Fufang Dantong Pian <sup>[13]</sup> Fufang Dantong Jiaonang <sup>[14]</sup> Fufang Huangqin Pian <sup>[25]</sup>	Isodi Lophanthoidis (Xihuangcao, XHC), Artemisia Scopariae Herba (Yinchen), <b>CXL</b> , DH and 7-hydroxy-4-methylcoumarin (Dantong), Scutellariae Radix (Huangqin, HQ), Polygoni Cuspidati Rhizoma Et Radix (Huzhang), <b>CXL</b> , GLM		Tablet Capsule Tablet
Fufang Kumu Xiaoyan Pian <sup>[26]</sup> Lixieling Pian <sup>[27]</sup>	<b>CXL</b> , Picrasmae Ramulus et Folium (Kumu, KM) Bistortae Rhizoma (Quanshen), <b>CXL</b> , Sophorae Flavescentis Radix (Kushen, KS)		Tablet Tablet
Zhilining Pian <sup>[28]</sup> Jinji Keli <sup>[16]</sup> Jinji Jiaonang <sup>[17]</sup>	<b>CXL</b> , KS, Aucklandiae Radix (Muxiang) JYG, JXT, QJB, GLM, Zanthoxyli Radix (Liangmianzhen), <b>CXL</b>		Tablet Granules Capsule
Xiaoyan Zhike Pian <sup>[29]</sup>	Elaeagnus Pungens Folium (Hutuiziye), Platycodonis Radix (Jiegeng), Pseudostellariae Radix (Taizishen), BB, Papaveris Pericarpium (Yingsuke), Ephedrae Herba (Mahuang, MH), Fructus Vitis Negundo (Huangjingzi), Adenophorae Radix (Nanshashen), <b>CXL</b>	Expectorant	Tablet
Qinggan Chuanxinlian Pian <sup>[30]</sup> Cuilian Jiedu Pian <sup>[31]</sup>	<b>CXL</b> , Gnetum parvifolium (Maimateng) Selaginellae Uncinatae Herba (Cuiyuncao), <b>CXL</b> , Radix Helicteris Angustifoliae (Shanzhima, SZM), Radix Et Caulis Ilicis Asprellae (Gangmei, GM), Vitis Negundo Herba (Wuzhigan), Menthae Haplocalycis Herba (Bohe, BH)	Exterior disorder-relieving	Tablet Tablet
Ganmaoqing Pian <sup>[32]</sup> Ganmaoqing Jiaonang <sup>[32]</sup> Ganmaokang Jiaonang <sup>[33]</sup>	Isatidis Radix (Banlangen, BLG), DQY, Spanishneedles Herb (Jinzhanyinpan), GM, SZM, <b>CXL</b> <b>CXL</b> , Chrysanthemi Indici Flos (Yejuhua, YJH), Solidaginis Herba (Yizhihuanghua), Herb of Marginate Rockbell (Lanhuashen), Stauntoniae Caulis et Folium (Yemugua)		Tablet Capsule Capsule
Kangle Biyan Pian <sup>[34]</sup>	Xanthii Fructus (Cang'erzi), Magnoliae Flos (Xinyi), Angelicae Dahuricae Radix (Baizhi), MH, <b>CXL</b> , HQ, Saposhnikoviae Radix (Fangfeng), Pogostemonis Herba (Guanghuoxiang), Moutan Cortex (Mudanpi), BH	Heat-clearing	Tablet
Zhiganjia Pian <sup>[35]</sup> Houkang San <sup>[36]</sup>	SZM, <b>CXL</b> , Tadehagi Triquetri Herba (Hulucha), Euodiae Leptae Folium et Ramulus (Sanchaku), BLG, Notopterygii Rhizoma et Radix (Qianghuo), BH BP, ZZ, Ginseng Radix et Rhizoma (Renshen), Borax (Pengsha), Natrii Sulfas Exsiccat (Xuanmingfen), L-Menthol (Bohenao), Trichosanthis Radix (Tianhuafen), <b>CXL</b> , Indigo Naturalis (Qingdai), GC		Tablet powder
Lianan Xiaoyan Pian <sup>[37]</sup> Lianzhi Xiaoyan Pian <sup>[38]</sup> Lianzhi Xiaoyan Jiaonang <sup>[39]</sup> Fufang Honggencao Pian <sup>[40]</sup>	<b>CXL</b> , KM <b>CXL</b> , SZM Alviae Prionitidis Herba (Honggencao), Houத்துyniae Herba (Yuxingcao), JYH, YJH, <b>CXL</b>		Tablet Tablet Capsule Tablet
Houshuning Pian <sup>[41]</sup> Qianxi Pian <sup>[42]</sup> Qinghuo Zhimai Pian <sup>[43]</sup> Qinghuo Zhimai Jiaonang <sup>[44]</sup> Xiaoyan Lidan Pian <sup>[45]</sup> Xinxue Pian <sup>[46]</sup> Xinxue Keli <sup>[47]</sup>	Herba Hedyotidis (Baihua Sheshecao), <b>CXL</b> , SZM <b>CXL</b> , Senecionis Scandentis Hebra (Qianliguang) <b>CXL</b> , ZZ, Ophiopogonis Radix (Maidong) <b>CXL</b> , XHC, KM Magnetitum (Cishi), Gypsum Fibrosum (Shigao), Talcum (Huashi), Gypsum Rubrum (Hanshuishi), Saltpeter (Xiaoshi), Natrii Sulfas (Mangxiao), ZZ, Lophatheri Herba (Zhuye), Radix Serratulae Chinensis (Guangshengma), <b>CXL</b> , ZZ, Aquilariae Lignum Resinatum (Chenxiang), BP		Tablet Tablet Tablet Capsule Tablet Tablet Granules
Yanhou Xiaoyan Wan <sup>[48]</sup>	Bufonis Venenum (Chansu), <b>CXL</b> , Ramulus et Folium Schefflerae (Qiyelian), ZZ, BP, XH, Plant Soot (Baicaoshuang)		Pill
Shang Hai She Yao Pian <sup>[18]</sup>	<b>CXL</b> , Ecliptae Herba (Mohanlian)		Tablet



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**Fig. 2** Classification of formulas containing *Andrographis herba*. (a) Classification according to formula type. (b) Classification according to dosage form.



**Fig. 3** Pharmacological activities of andrographolide.

**Antitumor effects and mechanism of andrographolide**

Andrographolide has been shown to possess antitumor activity by activating, regulating, and modulating the expression of various genes. In anti-prostate cancer, andrographolide has been found to inhibit cell proliferation and migration, induce G1/G0 cell cycle arrest, and suppress tumor growth and metastasis in mice<sup>[54]</sup>. Similarly, andrographolide has been demonstrated to impede breast tumor growth and metastasis in mice and inhibit the *in vitro* proliferation, migration, and

invasion of MCF-7 breast cancer cells<sup>[55]</sup>. Furthermore, andrographolide can block breast cancer invasion by upregulating tissue inhibitors of metalloproteinase 1 (TIMP1) and downregulating the expression of MMP-7<sup>[56]</sup>. In the case of anti-cervical cancer, andrographolide can dose-dependently inhibit cell proliferation, promote apoptosis, induce cell cycle arrest at the G1/S phase, and significantly reduce the expression of inducible nitric oxide synthase (iNOS), which is associated with poor survival and increased tumor aggressiveness in cervical

cancer<sup>[57]</sup>. Besides, andrographolide has been shown to increase both early and late apoptosis of colorectal cancer cells by inducing G0/G1 phase cell cycle arrest, upregulating pro-apoptotic protein Bax and downregulating anti-apoptotic gene *Bcl-2*<sup>[58]</sup>. Additionally, andrographolide can work synergistically with other anti-tumor drugs to induce colon cancer cell apoptosis through nuclear condensation, phosphatidylserine externalization, and Caspase-3 activation<sup>[59]</sup>. The role of andrographolide in palliative treatment for advanced esophageal cancer has entered phase III clinical trials (NCT number: NCT04196075).

### Treatment of cardiovascular diseases and diabetes with andrographolide

By using a cerebral ischemia-reperfusion injury (CIRI) animal model, it has been demonstrated that the protective effects of andrographolide on CIRI may be attributed to reducing neuroinflammation and cellular apoptosis<sup>[60]</sup>. Additionally, andrographolide has been found to relieve endothelial dysfunction in mice with coronary heart disease by modulating peroxisome proliferator-activated receptor (PPAR) and NF- $\kappa$ B signaling pathways<sup>[61]</sup>. As is well-known, inhibiting cellular apoptosis, regulating serum lipid levels, improving hemodynamics, and suppressing oxidative and inflammatory cytokine expression all play crucial roles in mitigating the pathogenesis of cardiovascular disease. Research has presented that andrographolide possesses specific abilities in regulating glucose metabolism, as demonstrated by reducing serum high-density lipoprotein cholesterol (HDL-C) levels and increasing low-density lipoprotein cholesterol (LDL-C)/HDL-C in mice<sup>[62]</sup>. Type 2 diabetes (T2DM) is closely related to intestinal barrier dysfunction. Andrographolide has been shown to improve glycemic control by enhancing intestinal barrier function and increasing microbial diversity of *Akkermansia muciniphila in vitro*<sup>[63]</sup>. Furthermore, nano-emulsion-based andrographolide delivery can enhance andrographolide anti-diabetic activity<sup>[64]</sup>. Notably, the evaluation of the pharmaco-metabolomics of andrographolide and metformin under fasting conditions in healthy people has entered phase 1 clinical trial evaluation (NCT number: NCT04161404).

### Neuroprotective effects and mechanism of andrographolide

In the pathogenesis of Alzheimer's disease, andrographolide plays a role in regulating multiple vital steps. It has been shown to lower acetylcholinesterase (AChE),  $\beta$ -amyloid 1-42 ( $A\beta$ 1-42), and p-tau levels in response to Alzheimer's disease induced by streptozotocin (STZ) in mice. This neuroprotective effect is achieved through anti-inflammatory, antioxidant, and modulation of neurotransmitter pathways<sup>[65]</sup>. Andrographolide can also significantly reduce the expression levels of total  $A\beta$  burden, IL-6, 4-hydroxynonenal, and N-tyrosine adducts in the rat brain<sup>[66]</sup>. In addition, it decreases the expression of Toll-like receptor 2 (TLR2), leukocyte differentiation antigen 14 (CD14), chemokine ligand 3 (CCL3), and Toll-like receptor 1 (TLR1) in APP/PS1 mice, thereby alleviating symptoms of Alzheimer by reducing neuroinflammation<sup>[67]</sup>. Furthermore, andrographolide exhibits an antidepressant effect by improving the mood of mice exposed to various unpredictable stressors. This antidepressant property is attributed to promoting the hippocampal brain-derived neurotrophic factor (BDNF) signaling pathway<sup>[68]</sup>.

### Hepatoprotective and pulmonary protective effects of andrographolide

Andrographolide has a beneficial effect on the model of ethanol-induced alcoholic liver disease (ALD). After drug treatment, it can improve serum aminotransferase levels, liver function, lipid accumulation, and liver reactive oxygen species levels and alleviate liver pathological damage and oxidative stress in ALD mice. The mechanism is related to the downregulation of NF- $\kappa$ B and TNF- $\alpha$  expression<sup>[69]</sup>. Multiple studies have reported the protective effects of andrographolide on lung tissue. In a rat model of pulmonary fibrosis induced by bleomycin (BLM), andrographolide can ameliorate lung tissue fibrosis by inhibiting lung fibroblast proliferation, differentiation, and extracellular matrix deposition through the regulation of Smad-dependent and Smad-independent pathways mediated by transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1)<sup>[70]</sup>. Benefiting from the anti-inflammatory activity, andrographolide can significantly alleviate inflammatory lesions in lung tissue, such as pulmonary edema and alveolar wall thickening, by reducing inflammatory cell infiltration and the secretion of proinflammatory cytokines (IL-1 $\beta$ , IL-6)<sup>[71]</sup>.

### Other effects

Andrographolide has been suggested for use with melatonin for treating COVID-19 based on its antipyretic, anti-inflammatory, antioxidant, antiviral, and endoplasmic reticulum stress regulation properties. Docking calculations have been performed between andrographolide and the binding sites of SARS-CoV-2<sup>[72]</sup>. A phase III clinical trial is ongoing (NCT05019326) to evaluate the effects of *A. paniculate* and *Boesenbergia Rotunda* vs control in asymptomatic COVID-19 patients. The 'Xi Yan Ping' injection is listed in the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 10) for the clinical treatment of COVID-19 in China<sup>[73]</sup>. The active ingredient of the injection is andrographolide total sulfonate, which has been proven with the functions of clearing heat, detoxification, and anti-inflammatory of patients infected with SARS-CoV-2.

Andrographolide has also shown promise in the treatment of osteoporosis by promoting bone formation and enhancing osteoblast differentiation, in which the mechanism is regulating the osteoprotegerin (OPG)/receptor activator of the NF- $\kappa$ B ligand (RANKL) signaling pathways<sup>[74]</sup>. In addition, andrographolide exhibits potent antimalarial activity against *Plasmodium falciparum* 3D7, potentially by inhibiting glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) expression and NF- $\kappa$ B activity<sup>[75]</sup>. Meanwhile, it can reverse chloroquine resistance by enhancing the inhibitory effect of chloroquine on plasmodial hemoglobin formation<sup>[76]</sup>.

In conclusion, andrographolide has been shown to have various pharmacological effects, including anti-inflammatory, anti-tumor, treatment of cardiovascular diseases and diabetes, protection of the nervous system, and hepatoprotection. However, most studies on the effects of andrographolide on the nervous and cardiovascular systems have been limited to laboratory stages using cell and animal models. Therefore, most of these pharmacological effects need more clinical research data to support them, which requires further investigation to fully understand andrographolide's absorption, distribution, and metabolic processes *in vivo* and the pathways through which it targets organs and exerts therapeutic effects.

## The original biosynthetic pathway of andrographolide

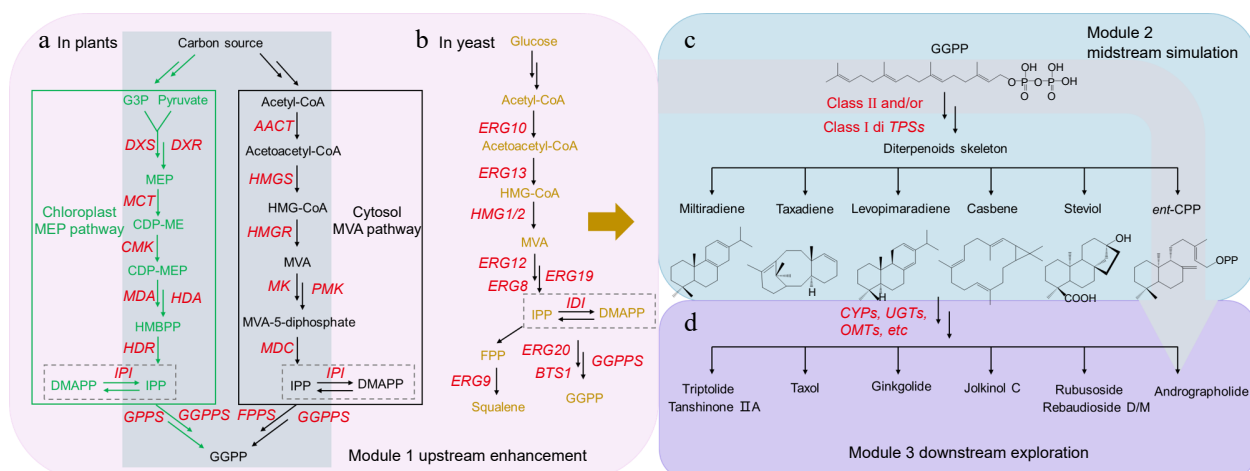
Andrographolide is regarded as the principal constituent responsible for the therapeutic properties of CXL, which is a diterpene lactone derived from C5 unit isopentenyl diphosphate (IPP) and dimethyl allyl pyrophosphate (DMAPP). Notably, diterpenoids (C<sub>20</sub>) are a vast class of structurally diverse metabolites of medicinal plants and are derived from the precursor (*E,E,E*) - geranylgeranyl diphosphate (GGPP)<sup>[2]</sup>. Diterpenoids are required for plant growth or development and carry critical ecological and agronomic functions. Furthermore, diterpenoids have a wide range of commercial applications in the cosmetic, food additive, and pharmaceutical industries<sup>[77]</sup>.

Like diterpenoids, the biosynthesis of andrographolide can be divided into three modules (Fig. 4a, c & d, annotated in gray color): the formation of precursor GGPP, derived from cytosolic mevalonate (MVA) pathway and plastidial 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway; the construction of carbon skeletons through cyclization and isomerization catalyzed by class II diTPSs (copalyl diphosphate synthases - like, CPSs) and/or class I diTPSs (kaurene synthases - like, KSLs); and the post - modification of the molecular skeletons, including oxidation, glycosylation, and acylation. In the first module (Fig. 4a), the MVA pathway in plants starts with acetyl-CoA and is finally converted into IPP<sup>[78]</sup>, which is orderly catalyzed by six enzymes including acetyl-CoA acetyltransferase (AACT), 3-hydroxy-3-methylglutaryl-CoA (HMGs), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), mevalonate kinase (MVK), phosphomevalonate kinase (PMK), and mevalonate 5-diphosphate decarboxylase (MVD). Meanwhile, The MEP pathway consists of seven enzymatic steps orderly catalyzed by 1-deoxy-D-xylulose-5-phosphate synthase (DXS), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), MEP cytidyltransferase (MCT), 4-(cytidine 5-diphosphate)-2-C-methylerythritol kinase (CMK), 2-C-methyl-D-erythritol 2,4-cyclo diphosphate synthase (MDS), hydroxymethyl butenyl 4-diphosphate synthase (HDS), and 4-hydroxy-3-methyl but-2-enyl diphosphate reductase (HDR). Subsequently, HDRs catalyze the last reaction in the MEP pathway; to be specific, HMBPP (1-hydroxy-2-methyl-2-butenyl-4-

diphosphate) is converted to a mixture of IPP and DMAPP 5:1–6:1<sup>[79]</sup>. Isopentenyl diphosphate isomerase (IPI) is mainly responsible for the reversible conversion between IPP and DMAPP.

For the second module (Fig. 4c), diterpenoid synthetases (diTPSs) play a role in the formation of diterpene backbones in plant *A. paniculata*, which can be divided into two categories (class I and class II) following conserved modular structure ( $\alpha$ ,  $\beta$  and  $\gamma$ ) within the proteins. Functionally, class II diTPSs (CPSs) initiate the cyclization of GGPP by protonation of a double bond into several bicyclic prenyl diphosphates with specific stereo configuration ((+)-CPP, *ent*-CPP), which are subsequently converted by class I diTPSs (KSLs) into labdane-related diterpenes (LRDs). Three class II diTPSs (*ApCPS1*, *ApCPS2*, and *ApCPS3*) probably participate in distinct diterpenoid metabolic pathways of *A. paniculata*<sup>[2]</sup>. *ApCPS2* potentially converts GGPP to *ent*-CPP, as shown by biochemical characterization of the recombinant protein and accumulation of tissue-specific *ent*-LRDs<sup>[80]</sup>. While *ApCPS1* has been demonstrated to produce both *ent*-CPP and (+)-CPP, *ApCPS3* can only make (+)-CPP of normal stereochemistry as shown by GC-MS chromatograms of extracts from cultures expressing these proteins in *E. coli* also engineered to produce GGPP<sup>[2]</sup>. Moreover, two KSLs (*ApKSL1* and *ApKSL2*) have been just recognized to consume *ent*-CPP and (+)-CPP but are not related to producing the *ent*-copalol or *ent*-labdatriene, the precursors of andrographolide and derived diterpenoids<sup>[2]</sup>.

In addition, specific functional modifications of LRDs primarily include the addition of hydroxyl group and glycosylation, which can be mediated by the activities of cytochrome P450 monooxygenases (CYPs) and glycosyltransferases (GTs) individually (Fig. 4d). Furthermore, multi-omics data integration revealed some genes encoding CYPs, which may participate in the biosynthesis of andrographolide and related diterpenoids without being proved by functional studies<sup>[2]</sup>. And, *ApUGTs* are known as glycosyltransferases which are responsible for the formation of neoandrographolide, a glycosylated andrographinin. Recently, *ApUGT12* (UGT86C11) has been established to be closely involved in synthesizing neoandrographolide in *A. paniculata*<sup>[81]</sup>. Given the discovery of fewer key enzymes related



**Fig. 4** Biosynthetic pathway of andrographolide (the original biosynthetic pathway of andrographolide is annotated in gray color) and heterologous synthesis strategies of diterpenoids and derivatives in microbes. (a) The formation of diterpenoid's precursor (*E,E,E*) - geranylgeranyl diphosphate (GGPP) in original plants. (b) The formation of GGPP in yeast. (c) The midstream module to generate diterpenoids skeletons. (d) The downstream module to create various diterpenoids with different pharmacological activities.

to the biosynthetic pathway of andrographolide, there is still a need for further exploration and verification of the black box.

## Heterologous production of diterpenoids and derivatives in microbes

The medicinal characteristics of the plant *A. paniculata* are attributed to diterpene lactones and their derivatives, like andrographolide and neoandrographolide. Due to the limited availability of andrographolide from natural sources<sup>[82]</sup> and problems regarding the commercial cultivation of *A. paniculata*<sup>[83]</sup>, it was proposed that biotechnological interventions may be exploited for the sustained production of andrographolide. Over the last 20 years, progress has been made in designing and constructing synthetic biology systems in yeast and bacteria, which serve as cell factories to produce second metabolites belonging to medical plants. Metabolic engineering in microorganisms can provide a cost-effective alternative to gain medicinal diterpenoids such as forskolin<sup>[84]</sup>. However, heterologous production of andrographolide in microbes implies at least partial or total replication of the andrographolide biosynthetic pathway, which is not fully elucidated in *A. paniculata*. Fortunately, the production of diterpenoids and derivatives from other medicinal plants in microbial cell factories has been achieved, including the precursor of taxol (taxadiene)<sup>[85]</sup>, tanshinone analogs (miltiradiene)<sup>[86]</sup>, sclareol<sup>[87]</sup>, etc. Thus, the strategies and methods of constructing cell factories to produce these diterpenes can be learned when we need to utilize microbes for the green production of andrographolide.

Corresponding to the original diterpenoid biosynthetic pathway in plants, diterpenoid cell factory construction is divided into three modules: upstream pathway enhancement (Fig. 4b), midstream pathway simulation, and downstream pathway exploration. In terms of upstream pathway enhancement, systematic multivariate analysis has been used to determine a balance between the IPP production and the skeleton formation, which could maximize the productive capacity of taxadiene<sup>[85]</sup>. This optimization strategy resulted in an *E. coli* strain containing an additional copy of the upstream pathway (to create isoprenoid precursors, IPP, and DMAPP) under the control of Trc promoter producing 2000-fold more taxadiene titers than those only expressing the native MEP pathway. Furthermore, to enlarge the endogenous precursor pools of GGPPS substrates (IPP and DMAPP), the copy number of rate-limiting steps (DXS, IDI, ISPD, ISPF) in the MEP pathway was amplified to five by additional expression, generating a 600-fold increase of levopimaradiene<sup>[88]</sup>. Notably, central metabolism and transcription factors have been manipulated in *Saccharomyces cerevisiae* to further rewire the global cellular metabolism for enhancing the supply of acetyl-CoA and NADPH, which resulted in a 160-fold increase in the production of sclareol<sup>[87]</sup>.

For the midstream modules that form the diterpenoid skeleton, the construction of cell factories has focused on simulating diterpene synthases in plants. Extensive screening of diterpene synthases and their combinations from plants that can produce diterpenoid skeletons may be an effective strategy to improve heterologous productivity in specific hosts<sup>[89]</sup>. The most efficient combination of *Coleus forskohlii* TPS1 and *Salvia miltiorrhiza* KSL1 has finally been selected from the enzyme library for the high-level production of miltiradiene<sup>[89]</sup>. Besides,

51 functional combinations of class I and II diTPSs have been constructed in yeast by imitating the modularity of diterpenoid biosynthesis in plants, generating diverse diterpene skeletons<sup>[90]</sup>. Notably, the combination of diterpene synthases TwTPS9 and TwTPS27 from *Tripterygium wilfordii* has been introduced into yeast to produce the diterpene dehydroabiatic acid, an intermediate of triptolide. Furthermore, the chloroplast transit peptide-truncated TwTPS9 and TwTPS27 raised the production of miltiradiene by about 7.1 fold<sup>[91]</sup>. Similarly, the fused expression of SmCPS and SmKSL led to a 2.9-fold increase in miltiradiene production by shortening the distance and avoiding the block between two active sites<sup>[92]</sup>, which is inspired by the fact that the class II and class I diterpene synthases in plants commonly form complexes in plastids.

The conversion process from a diterpenoid skeleton to various compounds with specific pharmacological activities primarily occurred in the downstream modification module. Nevertheless, this module is unknown in most diterpenoid-producing plants. Therefore, microbial cell factories are used not only for metabolite production but also for identifying critical enzymes. Six CYPs have been examined in high-titer casbene-producing yeast strain JWY509 to verify the ability to produce jolkinol C. The optimal combination of JcCYP71D495 (C9OX2) and JcCYP726A20 (C5OX2) from *Jatropha curcas* has been selected to yield 400 mg/L jolkinol C<sup>[93]</sup>. In addition, two tandemly duplicated CYP82Ds from *T. wilfordii* have been found to catalyze the aromatization of miltiradiene in *S. cerevisiae* and successfully produced triptolide precursor 14-hydroxy-dehydroabiatic acid<sup>[94]</sup>. Compared with diversification mediated by P450 oxidation, glycosylated diterpenoids in plants are relatively less. GjUGT94E13 and GjUGT74F8 from *Gardenia jasminoides* have been functionally characterized to catalyze the glycosylation of crocetin by using an *E. coli* cell factory<sup>[95]</sup>. Moreover, the classic case extensively studied is steviol glycosides (SGs). Based on the understanding that the crystal structure of the SrUGT76G1 (*Stevia rebaudiana*) can be used to accommodate various steviol-derived ligands, the substrate-binding pockets of EUGT11, SrUGT76G1, and UGTSL2 have been modified to convert stevioside/rebaudioside A to sweeter rebaudioside D/M<sup>[96,97]</sup>.

## Conclusions and perspective

*A. paniculata* is a well-known traditional medicinal herb that is utilized in India, China, Thailand, and Malaysia to treat infections and inflammation. It is widely distributed in Southeast and South Asia. According to the theory of TCM, it is intensely bitter in taste and cold-natured. Modern pharmacological research has discovered that the plant possesses various medicinal properties such as anti-tumor, anti-inflammatory, anti-viral, cardio-protecting, neuro-protectant, and hepato-protectant. Although andrographolide has been developed into many dosage forms for versatile applications in clinical treatment, we still need to pay more attention to the safety of andrographolide preparations, especially injection (which accounts for about 4.4% of the market). Since Chinese Food and Drug Administration (CFDA) has been warning of the severe allergic reactions after injecting 'Xi Yan Ping' in adults and forbidden using 'Yan Hu Ning' injections in children. Thus, future directions for developing pharmaceutical preparations need more extensive and comprehensive pharmacological



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experimental evidence and a clear mechanism and target organ of andrographolide.

Notably, andrographolide and related diterpene lactones have been identified as the primary therapeutic active component. Due to the high demand for andrographolide and its semi-synthetic derivatives, *A. paniculata* is commercially cultivated worldwide. Limited by the low content of andrographolide (3.8–31.2 mg/g DCW) and low plant growth rate, the price of purified andrographolide and its derivatives is about \$100,000/kg<sup>[98]</sup>. Chemical synthesis of andrographolide under laboratory conditions is inefficient due to its complex structure and specific stereo-conformation. Therefore, it is imperative to adopt other biotechnological tools to produce andrographolide efficiently. However, the biosynthetic pathway of andrographolide has not been fully elucidated. Urgent research is focused on understanding the enzymatic process involved in the formation of andrographolide and the complete pathways. High-throughput approaches, including multi-omics and comparative omics analysis combined with computational tools and artificial intelligence (AI), are excellent ways to narrow down the gene candidates from large gene families. Meanwhile, heterologous expression systems (stable expression in microbes and transient expression in tobacco leaves<sup>[99]</sup>) can provide diverse platforms for functionalizing key enzymes and act as green factories to produce andrographolide efficiently. In conclusion, bioreactor technologies can rapidly scale up andrographolide production by constructing cell factories with a complete understanding of the other diterpenoids' biosynthetic pathways.

### Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Zhang L, Chen R, Chen X; data collection and analysis: Chen X, Ren J, Yang J, Zhu Z; draft manuscript preparation: Chen X, Ren J, Yang J, Zhu Z, Chen R. All authors reviewed the results and approved the final version of the manuscript.

### Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

### Acknowledgments

We appreciate Huadong Li (Shanghai Kangqiao Chinese Medicine Tablet Co., Ltd) for photos of *Andrographis paniculata*. We also thank Luyao Yu (Naval Medical University, Shanghai, China) for discussing andrographolide bioactivities. This work was supported by the National Key Research and Development Program of China (No. 2022YFC3501703), the National Natural Science Foundation of China (No. 32000231, 32170274, 31970316, 82225047), and the Postgraduate Innovation Fund of Interdiscipline and New Medicine from School of Medicine of Shanghai University (China).

### Conflict of interest

The authors declare that they have no conflict of interest. Lei Zhang is the Editorial Board member of *Medicinal Plant Biology* who was blinded from reviewing or making decisions on the

manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and the research groups.

### Dates

Received 11 May 2023; Accepted 11 September 2023; Published online 31 October 2023

### References

1. Commission SP. 2020. *Pharmacopoeia of the People's Republic of China*. Beijing: China Medical Science and Technology Press. 280 pp.
2. Sun W, Leng L, Yin Q, Xu M, Huang M, et al. 2019. The genome of the medicinal plant *Andrographis paniculata* provides insight into the biosynthesis of the bioactive diterpenoid neoandrographolide. *The Plant Journal* 97:841–57
3. Koteswara Rao Y, Vimalamma G, Venkata Rao C, Tzeng YM. 2004. Flavonoids and andrographolides from *Andrographis paniculata*. *Phytochemistry* 65:2317–21
4. Adiguna SP, Panggabean JA, Atikana A, Untari F, Izzati F, et al. 2021. Antiviral activities of andrographolide and its derivatives: Mechanism of action and delivery system. *Pharmaceuticals* 14(11):1102
5. Raina AP, Gupta V, Sivaraj N, Dutta M. 2013. *Andrographis paniculata* (Burm. f.) Wall. ex Nees (kalmegh), a traditional hepatoprotective drug from India. *Genetic Resources and Crop Evolution* 60:1181–89
6. Chen R, Wang X, Song Y, Zhu Y, Wang P, et al. 2014. Genetic diversity analysis of *Andrographis paniculata* in China based on SRAP and SNP. *China Journal of Chinese Materia Medica* 39(23):4559–65(in Chinese)
7. Editorial Committee Of Flora Of China CAOS. 2002. *Flora of China*. Beijing: Science Press. pp. 204–5
8. Chen D, Zhong C, Lin Y. 2020. Research progress on germplasm resources, breeding, and cultivation of medicinal plant *Andrographis paniculate* (Burm. f.) Nees. *Jiangsu Agricultural Sciences* 48:34–40(in Chinese)
9. Liang Y, Chen S, Wei K, Yang Z, Duan S, et al. 2020. Chromosome level genome assembly of *Andrographis paniculata*. *Frontiers in Genetics* 11:701
10. Guan R, Xu S, Lu Z, Su L, Zhang L, et al. 2022. Genomic characterization of bZIP transcription factors related to andrographolide biosynthesis in *Andrographis paniculata*. *International Journal of Biological Macromolecules* 223:1619–31
11. Zhang R, Chen Z, Zhang L, Yao W, Xu Z, et al. 2021. Genomic characterization of WRKY transcription factors related to andrographolide biosynthesis in *Andrographis paniculata*. *Frontiers in Genetics* 18(11):601–89
12. Xiao B. 2009. *Lingnan Medicinal Collection Record*. Guangzhou: Guangdong Science and Technology Press (Photocopy based on 1932 Xiao Linglan Room print, in Chinese).
13. Pan HT, Wang JJ, Huang JL, Shuai YL, Li J, et al. 2020. Ranibizumab plus fufang xueshuantong capsule versus ranibizumab alone for exudative age-related macular degeneration. *Journal of International Medical Research* 48(9):1–10
14. Wang X, Zhao C. 2002. Study on quantitative method of dehydroandrographolide in compound Dantong Tablet. *Journal of Guangdong Pharmaceutical University* 18(4):285–87(in Chinese)
15. Huang S, Yang Q, Zhao L, Huang H, Deng H. 2018. A retrospective analysis of external application of Zijinding combined with SihuangShuimi for subacute thyroiditis. *Journal of New Chinese Medicine* 50:65–67(in Chinese)
16. Xu H. 2008. Preparation and clinical application of Jinji granules. *Heilongjiang Medicine Journal* 21(5):58–59(in Chinese)

17. Qiu H, Li M, Liao Z. 2011. Simultaneous determination of andrographolide and dehydrographolide in Jinji Capsules by HPLC. *Chinese Journal of Hospital Pharmacy* 31:1142–43(in Chinese)
18. Shanghai TCM Pharmaceutical Factory No. 2. 1975. Shanghai snake medicine. *Chinese Journal of Pharmaceuticals* 7:36–37(in Chinese)
19. Feng J, Li S, Xu J. 2021. Therapeutic effect of Liapfeining Tablet combined with 2HRZE/4HR in the treatment of active pulmonary tuberculosis and its effect on levels of PCT, IL-10 and TNF- $\alpha$ . *Drug Evaluation Research* 44:550–55(in Chinese)
20. Tang D, Yan X. 2017. Determination of chlorogenic acid, gardeni-side, andrographolide and dehydroandrographolide in Qingre-ichuang Pills by HPLC. *Traditional Chinese Drug Research and Clinical Pharmacology* 28:223–27(in Chinese)
21. Zhang C. 2018. Effect of Qingre Anchuang Tablet combined with Adaparine gel on acne. *Drugs and Clinic* 33:2705–8(in Chinese)
22. Ji N, Zhao L, Feng X, Luo L, Liang T, et al. 2015. Clinical observation of Kangfu Xiaoyan Supposant combined with levofloxacin and microwave physiotherapy in the treatment of chronic pelvic inflammatory disease. *China Practical Medicine* 10:15–17(in Chinese)
23. Zhang H, Pan X, Xu X, Deng Q, Wang D. 2015. Study on thin layer identification of Formula Chuanxinlian Tablets. *Chinese Traditional Patent Medicine* 37:460–62(in Chinese)
24. Pan J, Zuo KN, Wang XM, Zhang MP, Wang ZH, et al. 2021. Systematic review and meta-analysis of randomized controlled trials of Fuke Qianjin tablet. *Evidence-Based Complementary And Alternative Medicine* 2021:8861631
25. Shen X, Huang Y. 2011. Determination of dehydroandrographolide in Fufang Huangqin Tablets by HPLC. *Strait Pharmaceutical Journal* 23:93–94(in Chinese)
26. Lu Q. 2016. Determination of five components in Formula Kudu Xiaoyan Tablets by RP-HPLC. *Chinese Traditional Patent Medicine* 38:2590–93(in Chinese)
27. Yang J, Wu S. 1996. Determination of andrographolide in Lixieling Tablets by HPLC. *Chinese Traditional Patent Medicine* 18(6):11–12(in Chinese)
28. Liu Y, Guo L, Liu C. 1999. TLC Qualitative analysis of Xiaoyan Zhililing Tablets. *Chinese Traditional Patent Medicine* 21(5):231(in Chinese)
29. Zhao X, Shen X. 2006. Determination of dehydroandrographolide in Xiaoyan Zhike tablets by HPLC. *Chinese Journal of Experimental Traditional Medical Formulae* 12(7):14–15(in Chinese)
30. Zhang J. 2001. Study on the quality standard of Qinggan Chuanxinlian Tablets. *Chinese Traditional and Herbal Drugs* 32(9):Journal of TCM Univ. of Hunan(in Chinese)(in Chinese)
31. Guo J, Xiang F, Lin B. 2006. Determination of dehydroandrographolide in Cuilian Jiedu Tablets by RP-HPLC. *Herald of Medicine* 25(11):1211–12(in Chinese)
32. Feng X, Fang S, Gao Y, Liu J, Chen W. 2018. Current research situation of nephrotoxicity of Chinese herbal medicine. *China Journal of Chinese Materia Medica* 43:417–24(in Chinese)
33. Zou G, You X, Zhang Y, Wang G, Jiang H. 2008. New method of multi-components quantitation by one marker new method for quality evaluation of Guanmaikang capsula. *China Journal of Chinese Materia Medica* 33(15):1828–31(in Chinese)
34. Yang X, Zhao K, Zhai H, Wang H. 2022. Determination of andrographolide and dehydroandrographolide in Kangle Biyan Tablets by dual wavelength HPLC. *Drug Standards of China* 23:51–54(in Chinese)
35. Zhou X, He Y, Wu S. 2008. Determination of acetaminophen and morph guanidine hydrochloride in Zhiganjia Tablets. *China Pharmaceuticals* 17:24(in Chinese)
36. Du J, Mo J. 2012. Determination of andrographolide and dehydroandrographolide in Houkang Powder by HPLC. *Journal of Chinese Medicinal Materials* 35(4):656–57(in Chinese)
37. Zhang K, Lu Z, Wang Q, Liu F, Wang M, et al. 2022. Pharmacokinetic Study of Four Major Bioactive Components of Liandan Xiaoyan Formula in Ulcerative Colitis and Control Rats Using UPLC-MS/MS. *Frontiers in Pharmacology* 13:936846
38. Tian G, Chen M. 2009. Determination of dehydrographolide and andrographolide in Lianzhi Xiaoyan tablets by RP-HPLC. *Jiangxi Journal of Traditional Chinese Medicine* 40(9):72–73(in Chinese)
39. Lin T, Cheng L. 2010. Study on the quality standard of Lianzhi Xiaoyan Capsule. *Chinese Traditional Patent Medicine* 32(12):2096–100(in Chinese)
40. Zeng R, Yan M, Liu J. 2006. Determination of andrographolide and dehydrated andrographolide in Honggencao Capsules by HPLC. *Journal of TCM University of Hunan* 26(4):25–26+28(in Chinese)
41. Wu Y, Huang X. 2006. Determination of andrographolide and dehydroandrographolide in Houshuning Capsules. *Lishizhen Medicine and Materia Medica Research* 17(12):2458–59(in Chinese)
42. Li L, Tang Z, Liang Y, Wu K, Yu J, et al. 2021. Determination of Qianxi Tablet and its effect on ulcerative colitis induced by DSS. *Lishizhen Medicine and Materia Medica Research* 32(5):1075–78(in Chinese)
43. Zhang J, Qiao Y, Gao X, Zhang Q, Ma Q, et al. 2013. Rapid recognition and identification of chemical constituents in Qinghuo Zhimai tablets by SPE-HPLC-ESI-MS(n). *China Journal of Chinese Materia Medica* 38:186–92(in Chinese)
44. Su J, Liu Y. 2016. Establishment of HPLC chromatogram of Qinghuo Zhimai Capsule and determination of three components. *Chinese Traditional Patent Medicine* 38(4):810–15(in Chinese)
45. Wang M, Liu F, Yao Y, Zhang Q, Lu Z, et al. 2021. Network pharmacology-based mechanism prediction and pharmacological validation of Xiaoyan Lidan formula on attenuating alpha-naphthylisothiocyanate induced cholestatic hepatic injury in rats. *Journal of Ethnopharmacology* 270:113816
46. Liang Y, Nong X, Wei M. 2007. Study on quality standard of Xinxue Tablets. *Lishizhen Medicine and Materia Medica Research* 18(8):1953–54(in Chinese)
47. Jia J, Lin Q. 2006. HPLC analysis of andrographolide and 14-dexo-11,12-didehydroandrographolide in Xinxue granule. *Journal of Guangdong Pharmaceutical University* 22(3):272–74(in Chinese)
48. Ding H. 2007. Determination of dehydroandrographolide in Yanhou Xiaoyan Pills by HPLC. *Research and Practice on Chinese Medicines* 21(3):38–39(in Chinese)
49. Yu Q, Shi Y, Shu C, Ding X, Zhu S, et al. 2021. Andrographolide Inhibition of Th17-Regulated Cytokines and JAK1/STAT3 Signaling in OVA-Stimulated Asthma in Mice. *Evidence-Based Complementary And Alternative Medicine* 2021:6862073
50. Gupta S, Mishra KP, Kumar B, Singh SB, Ganju L. 2020. Andrographolide attenuates complete freund's adjuvant induced arthritis via suppression of inflammatory mediators and pro-inflammatory cytokines. *Journal of Ethnopharmacology* 261:113022
51. Lo C, Lii C, Hong J, Chuang W, Yang Y, et al. 2021. Andrographolide inhibits IL-1 $\beta$  release in bone marrow-derived macrophages and monocyte infiltration in mouse knee joints induced by monosodium urate. *Toxicology And Applied Pharmacology* 410:115341
52. Chen S, Luo Z, Chen X. 2020. Andrographolide mitigates cartilage damage via miR-27-3p-modulated matrix metalloproteinase13 repression. *The Journal of Gene Medicine* 22(8):e3187
53. Kim N, Lertnimitphun P, Jiang Y, Tan H, Zhou H, et al. 2019. Andrographolide inhibits inflammatory responses in LPS-stimulated macrophages and murine acute colitis through activating AMPK. *Biochemical Pharmacology* 170:113646
54. Mitra Ghosh T, Kansom T, Mazumder S, Davis J, Alnaim AS, et al. 2022. The andrographolide analogue 3A.1 synergizes with taxane derivatives in aggressive metastatic prostate cancers by upregulation of heat shock proteins and downregulation of MAT2A-mediated cell migration and invasion. *The Journal of Pharmacology And Experimental Therapeutics* 380:180–201

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55. Li J, Huang L, He Z, Chen M, Ding Y, et al. 2021. Andrographolide Suppresses the Growth and Metastasis of Luminal-Like Breast Cancer by Inhibiting the NF- $\kappa$ B/miR-21-5p/PDCD4 Signaling Pathway. *Frontiers In Cell and Developmental Biology* 9:643525
56. Beesetti SL, Jayadev M, Subhashini GV, Mansour L, Alwasel S, et al. 2019. Andrographolide as a therapeutic agent against breast and ovarian cancers. *Open Life Sciences* 14:462–69
57. Pasha A, Kumbhakar DV, Doneti R, Kumar K, Dharmapuri G, et al. 2021. Inhibition of inducible nitric oxide synthase (iNOS) by andrographolide and *in vitro* evaluation of its antiproliferative and proapoptotic effects on cervical cancer. *Oxidative Medicine and Cellular Longevity* 2021:6692628
58. Khan I, Mahfooz S, Saeed M, Ahmad I, Ansari IA. 2021. Andrographolide inhibits proliferation of colon cancer SW-480 cells via downregulating notch signaling pathway. *Anti-Cancer Agents in Medicinal Chemistry* 21:487–97
59. Khan I, Mahfooz S, Ansari IA. 2020. Antiproliferative and apoptotic properties of andrographolide against human colon cancer DLD1 cell line. *Endocrine Metabolic & Immune Disorders-Drug Targets* 20:930–42
60. Li Y, Xiang LL, Miao JX, Miao MS, Wang C. 2021. Protective effects of andrographolide against cerebral ischemia-reperfusion injury in mice. *International Journal of Molecular Medicine* 48(4):186
61. Shu J, Huang R, Tian Y, Liu Y, Zhu R, et al. 2020. Andrographolide protects against endothelial dysfunction and inflammatory response in rats with coronary heart disease by regulating PPAR and NF- $\kappa$ B signaling pathways. *Annals of Palliative Medicine* 9:1965–75
62. Alipanah-Moghadam R, Mehri A, Manafi F, Malekzadeh V, Nemati A, et al. 2021. Andrographolide, a novel inducer of apelin gene expression. *Journal of Ethnopharmacology* 280:114487
63. Su H, Mo J, Ni J, Ke H, Bao T, et al. 2020. Andrographolide exerts antihyperglycemic effect through strengthening intestinal barrier function and increasing microbial composition of *Akkermansia muciniphila*. *Oxidative Medicine and Cellular Longevity* 2020:6538930
64. Syukri Y, Taher M, Martien R, Lukitaningsih E, Nugroho AE, et al. 2021. Self-nanoemulsifying delivery of andrographolide: Ameliorating islet beta cells and inhibiting adipocyte differentiation. *Advanced Pharmaceutical Bulletin* 11:171–80
65. Patel R, Kaur K, Singh S. 2021. Protective effect of andrographolide against STZ induced Alzheimer's disease in experimental rats: possible neuromodulation and  $A\beta_{(1-42)}$  analysis. *Inflammopharmacology* 29:1157–68
66. Lindsay CB, Zolezzi JM, Rivera DS, Cisternas P, Bozinovic F, et al. 2020. Andrographolide reduces neuroinflammation and oxidative stress in aged *Octodon degus*. *Molecular Neurobiology* 57:1131–45
67. Zhang J, Zheng Y, Zhao Y, Zhang Y, Liu Y, et al. 2021. Andrographolide ameliorates neuroinflammation in APP/PS1 transgenic mice. *International Immunopharmacology* 96:107808
68. Zhang JJ, Gao TT, Wang Y, Wang JL, Guan W, et al. 2019. Andrographolide Exerts Significant Antidepressant-Like Effects Involving the Hippocampal BDNF System in Mice. *International Journal Of Neuropsychopharmacology* 22:585–600
69. Song Y, Wu X, Yang D, Fang F, Meng L, et al. 2020. Protective Effect of Andrographolide on Alleviating Chronic Alcoholic Liver Disease in Mice by Inhibiting Nuclear Factor Kappa B and Tumor Necrosis Factor Alpha Activation. *Journal of Medicinal Food* 23:409–15
70. Li J, Feng M, Sun R, Li Z, Hu L, et al. 2020. Andrographolide ameliorates bleomycin-induced pulmonary fibrosis by suppressing cell proliferation and myofibroblast differentiation of fibroblasts via the TGF- $\beta$ 1-mediated Smad-dependent and -independent pathways. *Toxicology Letters* 321:103–13
71. Gao J, Peng S, Shan X, Deng G, Shen L, et al. 2019. Inhibition of AIM2 inflammasome-mediated pyroptosis by Andrographolide contributes to amelioration of radiation-induced lung inflammation and fibrosis. *Cell Death & Disease* 10:957
72. Banerjee A, Czinn SJ, Reiter RJ, Blanchard TG. 2020. Crosstalk between endoplasmic reticulum stress and anti-viral activities: A novel therapeutic target for COVID-19. *Life Sciences* 255:117842
73. General Office Of The National Health Commission PRC, Comprehensive Department Of The National Administration Of Traditional Chinese Medicine PRC. 2023. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 10). *Infectious Disease Information* 36(1):18–25
74. Tantikanlayaporn D, Wichit P, Suksen K, Suksamrarn A, Piychaturawat P. 2020. Andrographolide modulates OPG/RANKL axis to promote osteoblastic differentiation in MC3T3-E1 cells and protects bone loss during estrogen deficiency in rats. *Biomedicine & Pharmacotherapy* 131:110763
75. Hassan WRM, Basir R, Ali AH, Embi N, Sidek HM. 2019. Anti-malarial and cytokine-modulating effects of andrographolide in a murine model of malarial infection. *Tropical Biomedicine* 36:776–91
76. Ibraheem ZO, Majid RA, Sidek HM, Noor SM, Yam MF, et al. 2019. *In vitro* antiplasmodium and chloroquine resistance reversal effects of andrographolide. *Evidence-Based Complementary And Alternative Medicine* 2019:7967980
77. Shao J, Sun Y, Liu H, Wang Y. 2021. Pathway elucidation and engineering of plant-derived diterpenoids. *Current Opinion in Biotechnology* 69:10–16
78. Englund E, Andersen-Ranberg J, Miao R, Hamberger B, Lindberg P. 2015. Metabolic engineering of *Synechocystis* sp. PCC 6803 for production of the plant diterpenoid manoyl oxide. *ACS Synthetic Biology* 4:1270–78
79. Murthy HN, Dalawai D. 2021. Biotechnological production of diterpenoid lactones from cell and organ cultures of *Andrographis paniculata*. *Applied Microbiology and Biotechnology* 105:7683–94
80. Misra RC, Garg A, Roy S, Chanotiya CS, Vasudev PG, et al. 2015. Involvement of an *ent*-copalyl diphosphate synthase in tissue-specific accumulation of specialized diterpenes in *Andrographis paniculata*. *Plant Science* 240:50–64
81. Srivastava P, Garg A, Misra RC, Chanotiya CS, Ghosh S. 2021. UGT86C11 is a novel plant UDP-glycosyltransferase involved in labdane diterpene biosynthesis. *Journal of Biological Chemistry* 297:101045
82. Nyeem A, Mannan A, Nuruzzaman M, Kamrujjaman K, Das S. 2017. Indigenous king of bitter (*Andrographis paniculata*): A review. *Journal of Medicinal Plants Studies* 5(2):318–24
83. Purkayastha J, Sugla T, Paul A, Solleti S, Sahoo L. 2008. Rapid *in vitro* multiplication and plant regeneration from nodal explants of *Andrographis paniculata*: a valuable medicinal plant. *In Vitro Cellular & Developmental Biology - Plant* 44:442–47
84. Forman V, Bjerg-Jensen N, Dyekjær JD, Møller BL, Pateraki I. 2018. Engineering of CYP76AH15 can improve activity and specificity towards forskolin biosynthesis in yeast. *Microbial Cell Factories* 17:181
85. Ajikumar PK, Xiao WH, Tyo KEJ, Wang Y, Simeon F, et al. 2010. Isoprenoid pathway optimization for Taxol precursor overproduction in *Escherichia coli*. *Science* 330:70–4
86. Mao Y, Ma Y, Chen T, Ma X, Xu Y, et al. 2020. Functional integration of two CYP450 genes involved in biosynthesis of tanshinones for improved diterpenoid production by synthetic biology. *ACS Synthetic Biology* 9:1763–70
87. Cao X, Yu W, Chen Y, Yang S, Zhao ZK, et al. 2023. Engineering yeast for high-level production of diterpenoid sclareol. *Metabolic Engineering* 75:19–28
88. Leonard E, Ajikumar PK, Thayer K, Xiao WH, Mo JD, et al. 2010. Combining metabolic and protein engineering of a terpenoid biosynthetic pathway for overproduction and selectivity control. *Proceedings of the National Academy of Sciences of the United States of America* 107:13654–59
89. Hu T, Zhou J, Tong Y, Su P, Li X, et al. 2020. Engineering chimeric diterpene synthases and isoprenoid biosynthetic pathways

- enables high-level production of miltiradiene in yeast. *Metabolic Engineering* 60:87–96
90. Andersen-Ranberg J, Kongstad KT, Nielsen MT, Jensen NB, Pateraki I, et al. 2016. Expanding the landscape of diterpene structural diversity through stereochemically controlled combinatorial biosynthesis. *Angewandte Chemie (International Edition)* 55:2142–46
  91. Forman V, Callari R, Folly C, Heider H, Hamberger B. Forman V, Callari R, Folly C, Heider H, Hamberger B. Production of putative diterpene carboxylic acid intermediates of triptolide in yeast. *Molecules* 22(6):981
  92. Zhou YJ, Gao W, Rong Q, Jin G, Chu H, et al. 2012. Modular pathway engineering of diterpenoid synthases and the mevalonic acid pathway for miltiradiene production. *Journal of the American Chemical Society* 134:3234–41
  93. Wong J, de Rond T, D'Espaux L, van der Horst C, Dev I, et al. 2018. High-titer production of lathyrene diterpenoids from sugar by engineered *Saccharomyces cerevisiae*. *Metabolic Engineering* 45:142–48
  94. Zhang Y, Gao J, Ma L, Tu L, Hu T, et al. 2023. Tandemly duplicated CYP82Ds catalyze 14-hydroxylation in triptolide biosynthesis and precursor production in *Saccharomyces cerevisiae*. *Nature Communications* 14:875
  95. Pu X, He C, Yang Y, Wang W, Hu K, et al. 2020. *In vivo* production of five crocins in the engineered *Escherichia coli*. *ACS Synthetic Biology* 9:1160–68
  96. Yang T, Zhang J, Ke D, Yang W, Tang M, et al. 2019. Hydrophobic recognition allows the glycosyltransferase UGT76G1 to catalyze its substrate in two orientations. *Nature Communications* 10:3214
  97. Liu Z, Li J, Sun Y, Zhang P, Wang Y. 2020. Structural Insights into the Catalytic Mechanism of a Plant Diterpene Glycosyltransferase SrUGT76G1. *Plant Communications* 1:100004
  98. Pandey G, Rao C. 2018. Andrographolide: its pharmacology, natural bioavailability and current approaches to increase its content in *Andrographis paniculata*. *International Journal of Complementary & Alternative Medicine* 11(5):355–60
  99. Nett RS, Lau W, Sattely ES. 2020. Discovery and engineering of colchicine alkaloid biosynthesis. *Nature* 584:148–53



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