Gene xxx (xxxx) 147426

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Pinellia genus: A systematic review of active ingredients, pharmacological effects and action mechanism, toxicological evaluation, and multi-omics application

Cheng Chen [a, b], Yunting Sun [c, *], Zhijing Wang [a, b], Zhihua Huang [a, b], Yuqing Zou [a, b], Feifei Yang [a, b], Jing Hu [a, b], Huijuan Cheng [a, b], Chenjia Shen [d, *], Shuling Wang [a, b, *]

[a] School of Pharmacy, Hangzhou Normal University, Hangzhou, Zhejiang 311121, China
[b] Key Laboratory of Elemene Class Anti-Cancer Chinese Medicines, Engineering Laboratory of Development and Application of Traditional Chinese Medicines, Collaborative Innovation Center of Traditional Chinese Medicines of Zhejiang Province, Hangzhou Normal University, Hangzhou, Zhejiang 311121, China
[c] Hangzhou TCM Hospital Affiliated to Zhejiang Chinese Medical University, Hangzhou, Zhejiang 311121, China
[d] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China

ARTICLE INFO

Edited by: Houqing Zeng

Keywords:
Active ingredient
Cytotoxicity
Omics
Pharmacological characteristic
Pinellia ternata

ABSTRACT

The dried tuber of *Pinellia ternata* (Thunb.) Breit, Pinelliae Rhizoma (PR, also named 'Banxia' in Chinese), is widely used in traditional medicine. This review aims to provide detail summary of active ingredients, pharmacological effects, toxic ingredients, detoxification strategies, and omic researches, etc. Pharmacological ingredients from PR are mainly classified into six categories: alkaloids, amino acids, polysaccharides, phenylpropanoids, essential oils, and glucocerebrosides. Diversity of chemical composition determines the broad-spectrum efficacy and gives a foundation for the comprehensive utilization of *P. ternata* germplasm resources. The pharmacological compounds are involved in inhibition of cancer cells by targeting various pathways, including activation of immune system, inhibition of proliferation and cycle, induction of apoptosis, and inhibition of angiogenesis. The pharmacological components of PR act on nervous system by targeting neurotransmitters, activating immune system, decreasing apoptosis, and increasing redox system. Lectins, one major class of the toxic ingredients extracted from raw PR, possess significant toxic effects on human cells. Inflammatory factors, cytochrome P450 proteins (CYP) family enzymes, mammalian target of rapamycin (mTOR) signaling factors, transforming growth factor-β (TGF-β) signaling factors, and nervous system, are considered to be the target sites of lectins. Recently, omic analysis is widely applied in *Pinellia* genus studies. Plastome genome-based molecular markers are deeply used for identifying and resolving phylogeny of *Pinellia* genus plants. Various omic works revealed and functional identified a series of environmental stress responsive factors and active component biosynthesis-related genes. Our review summarizes the recent progress in active and toxic ingredient evaluation, pharmacological effects, detoxification strategies, and functional gene identification and accelerates efficient utilization of this traditional herb.

1. Introduction

Pinellia ternata (Thunb.) Breit, a valuable medical plant belonging to the Araceae family, is widely cultivated in Eastern Asia (Mao and He, 2020). In summer, mature Pinelliae Rhizome (PR) is produced from *P. ternata* tuber and commonly used as traditional Chinese medicine (TCM) for thousands of years (Tian et al., 2008). The genus *Pinellia* comprises many classic species, such as *P. tripartita*, *P. pedatisecta*, *P. integrifolia*, *P. ternata*, *P. cordata*, *P. peltata*, *P. polyphylla*, *P. yaoluopingensis* and *P. fujianensis* (Ji et al., 2014). The diversity of species and varieties determines the chemical composition complexity of genus *Pinellia* plants.

During the past years, the chemical and active constituents of PR have been deeply studied by various analytical methods (Seo and Shin,

Abbreviations: PR, Pinelliae Rhizoma; TCM, taditional Chinese medicine; TNF-α, tumor necrosis factor-alpha; GC, gas chromatography; MS, mass spectrometry; TADC, tumor-associated dendritic cells; MAPK, mitogen-activated protein kinase; XTSJ, Xiaotan Sanjie; YKSCH, Yokukansankachimpihange; GABA, γ-Aminobutyric acid; 5-HT, 5-hydroxytryptamine; DA, dopamine; FOXO3, forkhead box 3a; HUR, Hu antigen R; SOD, superoxide dismutase; MDA, malonaldehyde

* Corresponding authors at: School of Pharmacy, Hangzhou Normal University, Hangzhou, Zhejiang 311121, China (S. Wang).
E-mail addresses: syt182@163.com (Y. Sun), shencj@hznu.edu.cn (C. Shen), wsling222@163.com (S. Wang).

https://doi.org/10.1016/j.gene.2023.147426
Received 20 February 2023; Received in revised form 5 April 2023; Accepted 7 April 2023
0378-1119/

2022). In PR, different types of chemical compounds, including alkaloids, amino acids, phenylpropanoids, volatile oils, and lectins, are considered to be pharmacological and/or toxicological constituents (Ji et al., 2014; Seo and Shin, 2022). PR extracts possess multiple pharmacological and clinical effects, such as anti-depressant, expectorant, antitumor, anti-inflammation, antiemetic, and wound-healing (Kong et al., 2018; Mao and He, 2020). On the other hand, toxicological studies have also pointed out PR's serious side effects, such as reproductive toxicity, mucosal irritation and hepatotoxicity (Sun et al., 2019). Recently, two reviews on ethnopharmacological, phytochemical, pharmacological and toxicological evaluation, and quality control of *Pinellia ternata* have been published (Bai et al., 2022; Peng et al., 2022). With the development of technology, omics analysis has been widely applied in *Pinellia* studies, including genetic diversity, environmental responses, continuous cropping, and functional gene identification (Shu et al., 2019; Ma et al., 2020). The present review is a comprehensive analysis of the active ingredients, pharmacological effects and action mechanism, detoxification, and application of omics in *Pinellia* genus studies.

2. Isolation of active ingredients from *Pinellia* plants

2.1. Alkaloids

Alkaloids are the major active components in both of the cultured (0.48 %) and planted (0.14 %) *Pinellia* plants (Zhao and Su, 1990). Total alkaloids from PR exert anti-epileptic effects on the pilocarpine-induced epilepsy model by modulating the GABAergic system (Deng et al., 2020). A chemical investigation of *P. pedatisecta* stem tuber isolated three species specific alkaloids, including 9-((5-methoxypyridin-2-yl) methyl)-9*H*-purin-6-amine, 4-(2-(2,5-dioxopyrrolidin-1-yl)ethyl) phenyl acetate, and *N*-(9-((5-methoxypyridin-2-yl)methyl)-9*H*-purin-6-yl)acetamide, which display cytotoxicity against human cervical cancer HeLa cells (Lin et al., 2007).

Ephedrine is an adrenergic drug with potential harmful effects on human cells. In the 1970 s, Oshio's group firstly isolated and purified L-ephedrine hydrochloride by nuclear magnetic resonance technology from *P. ternata* extracts (Oshio et al., 1978). By liquid chromatography time-of-flight mass spectrometer (LC-TOF-MS) analysis, no ephedrine was detected in various *Pinellia* species in Japanese market (Yahagi et al., 2021). Conversely, Fang's group has successfully extracted and separated ephedrine from *Pinellia* plants by molecular imprinted polymer coated ionic liquid-based silica method (Fang et al., 2020). These studies showed that the existence of berberine in *P. ternata* is unstable.

2.2. Amino acids

Amino acids are another major type of active components in the stem tubers of various *Pinellia* species, such as *P. ternata* and *P. pedatisecta* (Liang et al., 2013). Using microchip electrophoresis with electrochemical detection method, two important amino acid type ingredients, methionine and glycine, were detected (Shih et al., 2018).

2.3. Polysaccharides

Polysaccharides and glycosides are the majority of PR carbohydrates (Mao and He, 2020). A combination of gradient ion-exchange chromatography and gel-permeation chromatography was used to analyze the polysaccharides composition of a water-soluble extraction from *Rhizoma Arisaematis*. The main component of total neutral polysaccharides-II is glucose, and the main components of total acidic polysaccharides-II are mannose, glucose, arabinose, rhamnose, and galactose (Chen et al., 2012). Chen's group extracted crude polysaccharides from the dried tubers of *P. pedatiseeta*, composition of which are mannose, fucose, glucose, rhamnose, arabinose, and galactose (Li et al., 2021). By Fourier-transformed infrared and nuclear magnetic resonance (NMR) combined techniques, a pyranose containing α-configuration and β-configuration is detected in in PR polysaccharides extraction (Hu et al., 2019).

In addition to simple ethanol–water extraction, PR polysaccharides can also be extracted by ultrasonic method. The ultrasound-assisted extraction method is applied to characterize the bioactivity of polysaccharides from PR Praeparatum Cum Alumine (Liu et al., 2017).

2.4. Phenylpropanoids

Various chromatographic techniques are used to separate and purify the phenylpropanoids in genera *Pinellia* plants. For example, (*E*)-p-coumaryl alcohol, 3, 4-dihydroxycinnamyl alcohol, sachaliside I and coniferin are isolated and their inhibitory effects on tumor necrosis factor-alpha (TNF-α) production is determined *in vitro* by microplate colorimetric method (Han et al., 2007).

2.5. Essential oils and glucocerebrosides

Chemical composition of the volatile oils from raw PR are investigated by capillary gas chromatography (GC) and GC–MS analysis. PR processes different essential oils that are enriched in perillaldehyde, rosmarinic acid, magnolol and gingerol (Sumino et al., 2012). A total of 114 chemicals, including four main oil components, β-cubebene (8.8 %), atractylon (7.8 %), methyl eugenol (6.2 %), and δ-cadinene (5.3 %), are isolated from the essential oils from PR. Furthermore, several major odor-active compounds, such as safrole, β-vatirenene, paeonol, α-humulene, and β-phenylnaphthalene, are detected by the GC-olfactometry (Iwasa et al., 2014).

Sphingolipids play important roles in regulation of cell proliferation, cell differentiation, and apoptosis. Using 2-D NMR and electrospray ionization mass spectrometry / collision induced dissociation mass spectrometry (ESI-MS/CID-MS) technologies, glucocerebrosides from PR are fully characterized (Rozema et al., 2012)(Fig. 1).

3. Pharmacological effects of *Pinellia* plants

3.1. Antitumor

3.1.1. Antitumor components in Pinellia

PR can be used to treat various cancers, such as cervical cancer, ovarian cancer, breast cancer, leukemia, liver cancer, cholangiocarcinoma, and gastric cancer (Ji et al., 2014). Many PR active ingredients, including proteins, polysaccharides, agglutinins, stigma sterols, lipids, and other secondary metabolites, significantly inhibit the proliferation of cancer cells.

Total protein extracts of *P. pedatisecta* have a significant inhibitory effect on ovarian cancer cell line SKOV3 by inducing cell apoptosis in a dose- and time-dependent manner (Zhou et al., 2016). Agglutinin, a mannose specific plant lectin, plays an important role in antitumor. A prokaryotic expressed recombinant *P. pedatisecta* agglutinin domain B protein enhances the phagocytosis of drug resistant cancer cell line K562/ADR (Chen et al., 2013). Another recombinant *P. ternata* agglutinin exhibits anti-proliferative activity on hepatoma cells in a dose-dependent manner (Xu et al., 2012b). Furthermore, trypsin inhibitor isolated from PR significantly inhibits the proliferation of human gastric adenocarcinoma cell line BGC-823, in a concentration- and dose-dependent manner (Zu et al., 2014).

Addition to proteins, various types of secondary metabolites are reported to be involved in various antitumor processes. A water-soluble polysaccharide (RAP-W1) has inhibitory effect on human breast cancer cell line MCF-7 (Chen et al., 2012). Li's group isolated and purified a novel PR polysaccharide, which has significant inhibitory effect on the proliferation of different cancer cell lines, such as SNU-245, CL-6, Sk-ChA-1 and MZ-ChA-1 (Li et al., 2016). Lipid-soluble extracts from the

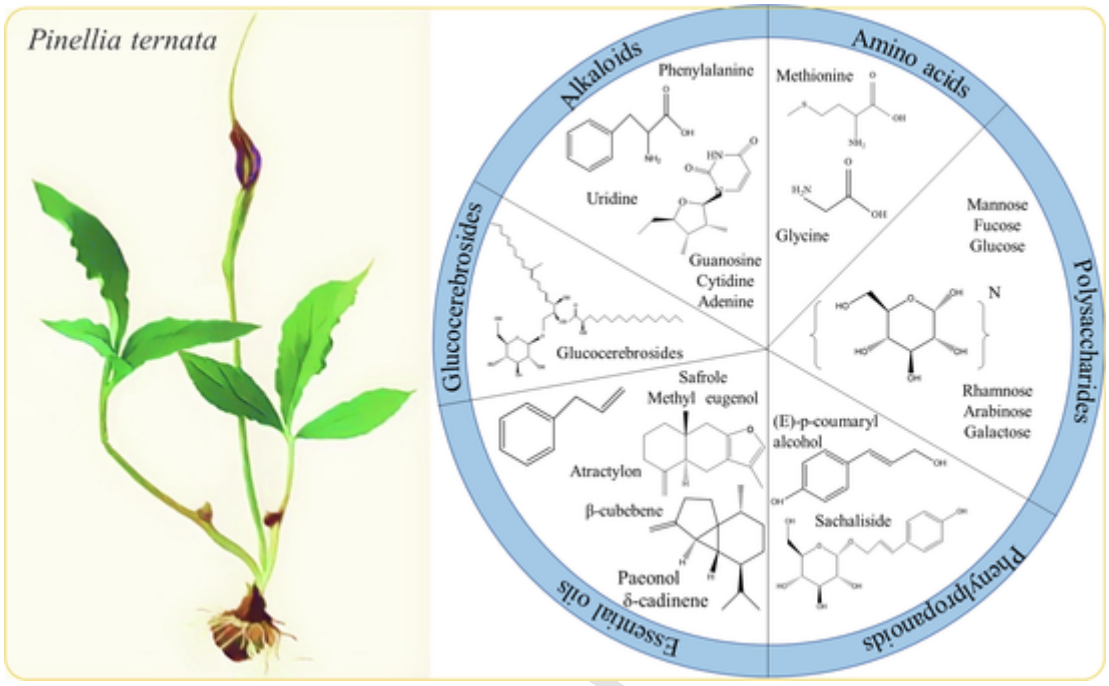

Fig. 1. Active ingredients from *Pinellia* plants. Different types of chemical compounds, including alkaloids, organic acids, amino acids, phenylpropanoids, volatile oils, and lectins, are considered as pharmacological constituents.

tuber of *P. pedatisecta* can induce the apoptosis of human cervical cancer cells, such as CaSki and HeLa cells, and exert anti-tumor effects in a dose- and time-dependent manner (Li et al., 2010). Several other secondary metabolites, 8-octadecenoic acid, stigmasterol, ferulic acid, and naringenin, in Citri Reticulatae Pericarpium-PR herb pair were considered to be the material basis for gastric cancer treatments (Song et al., 2021). Two flavonoids from PR, naringenin and luteolin, can induce the apoptosis in human gastric cancer cell lines SGC7901 and AGS (Zhang et al., 2021).

3.1.2. *Mechanism of active components of Pinellia in anti-tumor*

The imbalance of intracellular signal pathways, leading to abnormal cell proliferation and apoptosis, is one of the major causes of cell carcinogenesis (Sever and Brugge, 2015). Several previous studies have proved that *Pinellia*, along or combination with other TCMs, play significant roles in anti-tumor by regulating multiple cell signal transduction pathways.

3.1.2.1. Activation of immune system. Immune systems, including T/B lymphocyte cells, macrophages, and cytokines such as interleukin 1 (IL-1) and TNF-α, play an important role in anti-tumor. In mouse model, PR activates the immune responses and improves tumor microenvironment by affecting T lymphocyte cells and tumor-associated dendritic cells (TADC) (Huang et al., 2018). Further study showed that PR involved in the treatments of TADC by modulating the anti-tumor $CD4^+$ and $CD8^+$ responses and activating the JAK2-STAT pathway (Wang et al., 2019b; Wang et al., 2021). RAP-W1, a water-soluble polysaccharide, is reported to be involved in the activation of T cells by increasing Th1/Th2 cytokine ratio and causing the inhibition of tumor growth (Chen et al., 2012). PR extracts promote cytotoxic T lymphocyte responses by restoring the function of TADC, thereby exerting anti-cancer effects. Lupeol and stigmasterol are the main components of the Citri Reticulatae Pericarpium-PR herb pair (Song et al., 2021). Treatment of lupeol and stigmasterol mixture significantly suppresses the cell viability, migration, and morphogenesis of human umbilical vein endothelial cells by decreasing the expression level of TNF-α and vascular endothelial growth factor receptor 2 signaling pathways (Kangsamaksin et al., 2017).

Autophagy is a genetically controlled cellular process that regulates immune responses by controlling the production of cytokines (Jiang et al., 2019). PR is a one of the major constituents of the Chinese medicine formula 'Weikang Keli', which induces autophagy in SGC-7901 gastric cancer cells (Huo et al., 2013). Interestingly, differential accumulation of autophagy-associated proteins were detected in stigmasterol-treated gastric cancer cells. Further study showed that stigmasterol simultaneously induces protective autophagy by inhibiting Akt/mTOR pathway, suggesting a potential role of PR in autophagy-associated cell immune responses (Zhao et al., 2021a). Further research found that PR can induce autophagy in tumor cells by inhibiting the nuclear Nrf2 and mitogen-activated protein kinase (MAPK) pathways.

3.1.2.2. Inhibition the proliferation and cycle of cancer cells. Different studies have investigated that the effective components of raw PR can inhibit the proliferation of human cancer cells, compress surrounding tissues and accelerate cell death (Yen et al., 2014). For examples, PR extracts have significant inhibitory effects on the cell proliferation and cell cycle of HepG2 and Bel7402 cell lines. Li's group found that the polysaccharides from PR can significantly inhibit the proliferation of SNU-245, CL-6, Sk-ChA-1 and MZ-ChA-1 cell lines by regulating the intrinsic mitochondrial apoptotic pathway (Li et al., 2016).

The proliferation cycle of tumor cells can be divided into four phases: G1, S, G2 and M. The active ingredients of PR are reported to play a role in blocking cell proliferation cycle. Ethanol extract of PR significantly inhibits the proliferation of SGC7901 cells by decreasing the expression levels of *V-ATPase* and *NHE1* genes (Zhang et al., 2011). A novel lipid-soluble extract from the tuber of *P. pedatisecta* arrests SiHa cells at the G0/G1 phase and CaSki cells at G2/M, respectively (Zhang et al., 2017b). The effective components of 'Pinelliae Decoction' can inhibit the tumor-forming ability of BGC-823 cells by inducing G2 phase arrest and inhibiting cell proliferation (Liu et al., 2016).

3.1.2.3. Induction of apoptosis. Apoptosis, also known as programmed death, consists of two main apoptotic pathways involving a series of

Caspases (Khan et al., 2022). PR herbal-acupuncture solution induces the apoptosis of human cervical cancer SNU-17 cells by Bax-related caspase-3 activation pathway (Yoon et al., 2006). Cisplatin is a first-line drug for cervical cancer. Co-treatment of the extracts from the tuber of *P. pedatisecta* and cisplatin significantly induces the apoptosis in the SiHa and CaSki cell lines (Zhang et al., 2017b). The rhizome of *P. pedatisecta* exhibits an inhibitory effect on tumor growth by up-regulating of p53-dependent apoptosis-associated proteins, including B cell lymphoma 2 (Bcl-2) associated X protein and cleaved caspase-3 and caspase-9 (Zhang et al., 2017a). Transcriptomic analysis identified a number of apoptosis-related differentially expressed genes, such as the caspase family members, *P. pedatisecta* tuber extract treated ovarian cancer cell line SKOV3, suggesting that *P. pedatisecta* tuber extract could induce the apoptosis of SKOV3 cells (Zhou et al., 2016). 'Ruan-jian Sanjie' decoction, composed of *P. ternata*, *Prunella vulgaris*, *Cremastra appendiculata* and *Sargassum pallidum*, shows potential cytotoxicity against breast cancer cells by activating of caspase-3/7 and caspase-9, and the apoptotic cascade (Zhao et al., 2017). *P. pedatisecta* agglutinin, a specific mannose-binding plant lectin, has strong cytotoxicity to cancer cells by colocalizing with methylosome and inducing cell death (Lu et al., 2012). In PR extract, naringenin and luteolin are reported to have anti-inflammatory and pro-apoptotic effects on gastric cancer cells by regulating downstream target genes, such as *PTGS2*, *NOS2*, *CASP3*, and *BCL2* (Zhang et al., 2021).

3.1.2.4. Inhibition of angiogenesis. It was reported that 'Xiaotan Sanjie' (XTSJ), a TCM decoction prescription containing PR, can inhibit the adhesion, migration and invasion of SGC-7901 gastric cancer cells. Protein and mRNA expression analysis showed that XTSJ decoction inhibits the angiogenesis in gastric cancer through IL-8-linked regulation of the VEGF pathway (Shi and Wei, 2015). Another study showed that XTSJ decoction also suppresses the tumor angiogenesis of gastric cancer cell line MKN-45 by manipulating Notch-1 signaling pathway (Yan et al., 2014). *In vivo*, lupeol and stigmasterol, two main active ingredients from PR, disrupts tumor angiogenesis and reduces the growth of cholangiocarcinoma tumor (Kangsamaksin et al., 2017) (Fig. 2).

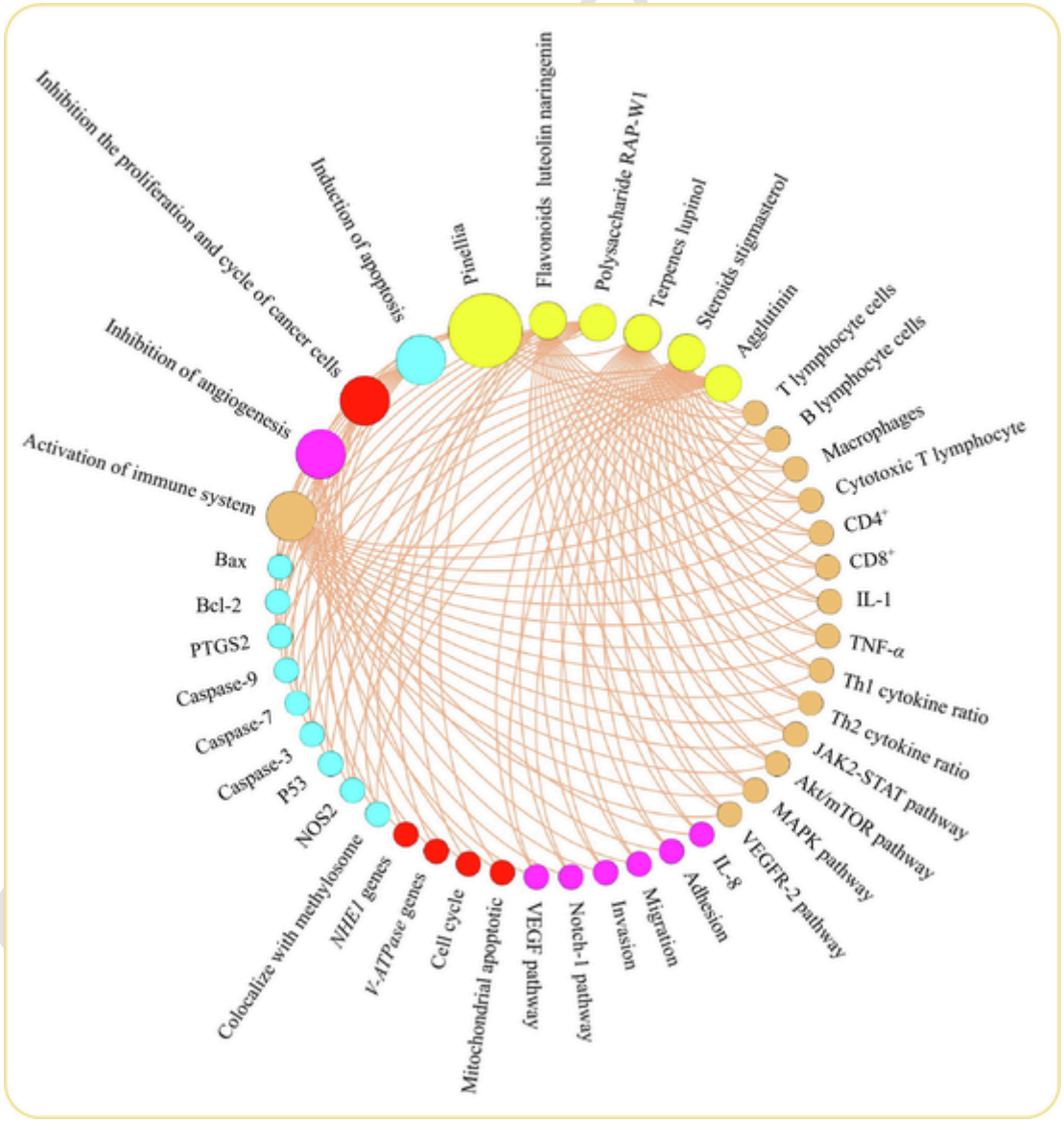

Fig. 2. Mechanism of Pinellia active components in anti-tumor. Yellow background indicated different types of active components; blue background indicated angiogenesis pathway-related factors; red background indicated cell proliferation and cycle pathway-related factors; purple background indicated angiogenesis pathway-related factors; and orange background indicated immune system-related factors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Effects on the central nervous system

3.2.1. Active ingredients affecting the nervous system

The effects of active ingredients from PR on the nervous system, including anti-aging, anti-epileptic, anti-depressant, anti-anxiety, sedative and hypnotic, are reported by many groups. The PR active ingredients, such as adenosine, succinic acid, baicalein, β-sitosterol, and stigmasterol, exert neuroprotective effects (Xu et al., 2018). Adenosine and succinic acid, the main active ingredients of the ethanol PR extract, play roles in anti-aging by reducing the deposition of lipofuscin and inhibiting the expression of senescence β-galacosidase encoding gene (Tang et al., 2020). Total alkaloids from PR reduce the incidence and frequency of spontaneous recurrent seizures (Deng et al., 2020). PR adenosine promotes the quality of sleep by increasing rapid eye movement sleep and reducing wakefulness (Lin et al., 2019). Network pharmacology and molecular docking identify-three antidepressant active components from PR, baicalein, β-sitosterol, and stigmasterol (Xiao et al., 2022).

Besides, many chemical compositions extracted from PR-related decoctions are also reported to have effects on nervous system. For example, polysaccharides from the 'Banxia-Houpu' decoction attenuate the abnormalities in the serotonergic and dopaminergic system of animal depression models (Yi et al., 2009). Several secondary metabolites, including behenyl alcohol, luteolin, β-sitosterol, stigmasterol, baicalein and cavidine, extracted from 'Shuangxia' decoction, achieve sedative and hypnotic effects (Guo et al., 2020). Three metabolites from 'BanXia-YiYiRen' decoction, baicalein, β-sitosterol, and stigmasterol, play a role in the treatment of insomnia, which is characterized by difficulty in falling asleep and/or maintaining sleep (Wang et al., 2022).

3.2.2. Mechanisms of Pinellia affected nervous system

3.2.2.1. Regulation of neurotransmitters. *Pinellia* total alkaloids are considered to be the main component with neuromodulatory effect. γ-Aminobutyric acid (GABA) is the most inhibitory neurotransmitter (Gonzalez et al., 2015). The expression levels of several GABA-related genes, such as *GAD65*, *GAT-1*, *GABA-T*, and GABAAR α4, α5, γ2 and δ subunits encoding genes, are significantly regulated by the treatment of PR alkaloids (Deng et al., 2020). Dietary zinc deficiency over-activates the glutamatergic nervous system and induces excitotoxic cell death (Takeda, 2010). PR together with Citrus Unshiu Peel suppress the aggressiveness of mice under zinc deficiency by decreasing excess glutamatergic neuron activity in hippocampus (Tamano et al., 2016). Further study suggests that 'Yokukansankachimpihange' (YKSCH) reduces the aggressive behavior of mice by reducing the concentrations of glutamate, GABA, 5-hydroxytryptamine (5-HT), and dopamine (DA) (Tamano et al., 2010). YKSCH ameliorates Glu-induced aggressive behaviors, which is mediated by 5-HT1A receptor stimulation (Tabuchi et al., 2017). A major chemical constituent of Yokukansan, hesperidin, was reported to be an active principle attributed to the antianxiety effects by regulating the serotonergic neurotransmission pathway (Ito et al., 2013). 'Chailong Jieyu Pill', a TCM containing PR, diminishes monoamine neurotransmitters and regulates the expression levels of mineralocorticoid receptor and glucocorticoid receptor encoding genes in the hippocampus (Feng et al., 2018).

Ethanol fraction of PR praeparatum is reported to possess good sedative, hypnotic and anticonvulsant activities, which may be related to the GABAergic system (Wu et al., 2011). 'Shuangxia' decoction, which is composed of PR and *Prunella vulgaris*, alleviates *p*-chlorophenylalanine-induced insomnia by increasing the content of 5-HT and decreasing the content of DA and norepinephrin (Sun et al., 2020). Polysaccharides from the 'Banxia-Houpu' decoction exert antidepressant-like effects by increasing the 5-HT and DA levels (Yi et al., 2009).

3.2.2.2. Effects on immune system and apoptosis. *N*-butyl alcohol extracts from PR alleviate neurological deficit in a dose-dependent manner by decreasing TNF-α and interleukin-1β (IL-1β) levels. Further study showed that ischemia-induced neuron apoptosis can be inhibited by the pretreatment of *N*-butyl alcohol extracts of PR (Ye et al., 2016). 'Shuangxia' decoction plays a role in the treatment of insomnia by reducing the levels of inflammatory factors, such as IL-1, TNF-α, in a dose-dependent manner (Sun et al., 2020). Ethanol extracts of PR can inhibit PC12 cell apoptosis by enhancing telomerase activity and relieving cell cycle arrest at the G2/M phase. Quantitative real-time PCR and western blot further identify several cell cycle factors, including up-regulated SIRT1, forkhead box 3a (FOXO3), Bcl-2, active regulator of SIRT1, RPS19BP1, and Hu antigen R (HUR), and down-regulated Bax, p53 and Deleted in Breast Cancer 1 (Tang et al., 2020).

3.2.2.3. Regulate oxidative stress. Treatment of *N*-butyl alcohol extracts from PR protect against the classic middle cerebral artery occlusion-induced brain injury by increasing superoxide dismutase (SOD) activity and decreasing malonaldehyde (MDA) content (Ye et al., 2016). Ethanol extracts of PR reduce reactive oxygen species (ROS) accumulation, lipofuscin deposition, and the expression of senescence β-galactosidase encoding gene (Tang et al., 2020). The extracts of *P. pedatisecta* Schott rhizome, increases plasma lipid peroxidation, reduces whole blood nitric oxide, and inhibits Na(+)/K(+)-ATPase activities in a concentration-dependent manner, which may be responsible for neurotoxicity (Huang et al., 2011) (Fig. 3).

4. Toxic ingredients and detoxification strategies

4.1. Potential toxic ingredients

Although enriched in active compounds, raw PR has significant toxic effects on liver, reproduction organs, and mucosal system (Zhao et al., 2013; Yu et al., 2015a; Xie et al., 2016). However, the toxic ingredients in PR have not been well identified yet.

Many research groups have pointed out that lectins are the major toxic ingredients in raw toxic *Pinellia* tubers. For examples, the contents of lectin in raw tuber materials of *P. pedatisecta* and *P. ternata* are 7.3 % and 4.9 %, respectively (Yu et al., 2019). Homodimer of 12.09 kDa subunits of lectin is separated and purified from PR by a combination of hydrophobic chromatography and DEAE-ion exchange chromatography (Zuo et al., 2012). Bioactive recombinant *P. ternata* agglutinin is separated and purified by Ni-NTA agarose affinity chromatography (Lin et al., 2003). *P. ternata* agglutinin, a mannose-binding lectin that induces apoptosis of Bel-7404 cells, is extracted by mannose-Sephrose 4B affinity chromatography and SDS polyacrylamide gel electrophoresis (Zhou et al., 2014). Furthermore, needle-like calcium oxalate crystals also were considered to be toxic components causing mucosal irritation (Peng et al., 2022).

4.2. Toxic effect

P. pedatisecta lectin stimulates macrophages to release a number of ROS and inflammatory factors, activating the MAPK and nuclear factor-κB (NF-κB) signal pathway-related inflammatory reactions (Yu et al., 2015c; Wang et al., 2019a). PR also can significantly vary the intermediary metabolism in pregnant rats (Xie et al., 2016). For example, raw PR significantly inhibits the CYP3A activity in rats, which is the most abundant liver P450 enzymes involving in drug metabolism (Ingelman-Sundberg, 2004; Wu et al., 2015). Raw PR induces cardiotoxicity by inhibiting of mTOR signaling and activation of the TGF-β pathway (Su et al., 2016). To explore the fetal toxicity of raw PR in mice, an isobaric tags for relative and absolute quantification (iTRAQ) based proteomic analysis identifies 37 nervous system development-

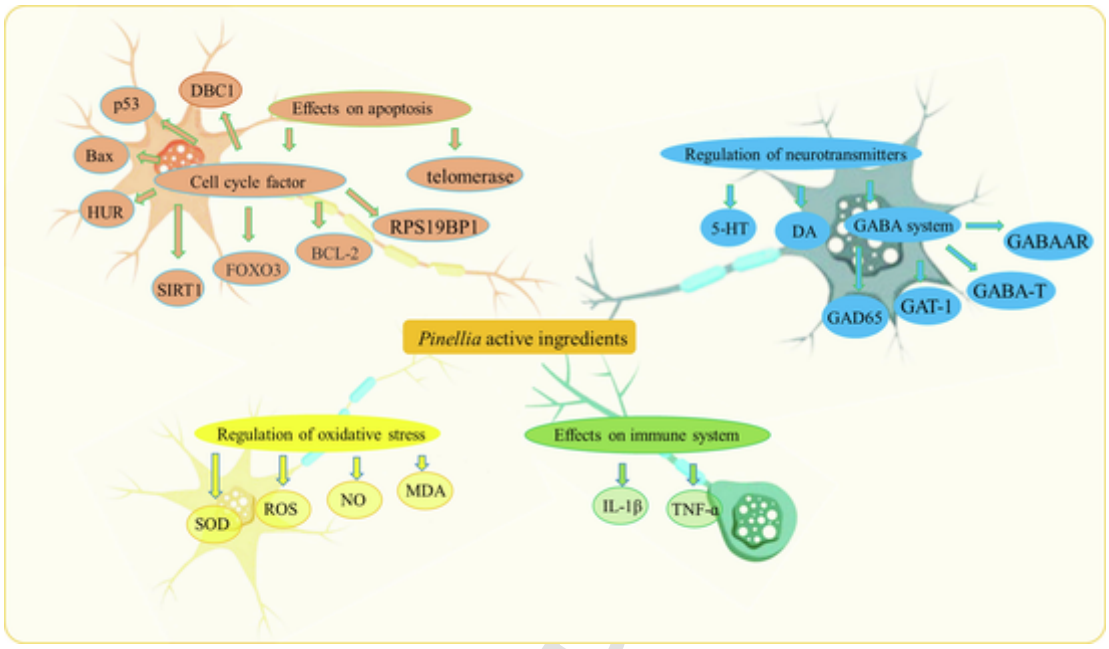

Fig. 3. Mechanisms of *Pinellia* active components affecting nervous system. Light red background indicated cell cycle-related factors; blue background indicated neurotransmitters; yellow background indicated oxidative stress-related factors; and green background indicated immune system-related factors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

related differentially accumulated proteins, suggesting that raw PR may cause a fetal abnormality of nervous system (Xu et al., 2018).

4.3. Detoxification strategies

Because PR is toxic, it is necessary to reduce its toxicity and complete the detoxification before using it. Unprocessed dried tuber of *P. ternata* contains raphides, which can cause severe acrid irritation of the oral and laryngopharynx mucosa. For many years, several classic methods have been used to attenuate the toxicity of raw PR.

Alum-processing, a traditional method to attenuate the toxicity of PR, decreases the activities of several water-soluble principles along with decreasing toxicity (Sun et al., 2019). Al^{3+} in the alum interacts with oxalic acid in raphides to form a stable complex, promoting the dissolution of raphides, which significantly changes the structure and sequence of lectin (Yu et al., 2015a). Animal experiments show that ginger juice and alumen PR is less toxic than raw PR (Wu et al., 1993). Chemical analysis indicated that gingerol from ginger juice plays an effective role in PR detoxification and inhibition of PR-induced inflammation (Yu et al., 2015b). Further study showed that lipophilic raphides can be specifically denatured by ginger extract (Fueki et al., 2020). A recent study investigated that lime and licorice processing on PR significantly reduces the level of toxic lectin proteins (Li et al., 2020). In addition to chemical methods, genetic methods can also play a certain role in reducing toxicity. However, the genetic transformation system of *P. ternata* has not yet been established.

5. Application of omics in *Pinellia* genus studies

5.1. Analysis of genetic diversity

Complex geographic regions cause distinctive biological traits, leading the identification of Chinese herbal medicine a difficult work (Chen et al., 2021b). To date, several physical and chemical methods have been developed to analysis of the genetic diversity of *P. ternata* genus. For example, an ultrafast and colorimetric detection system is successfully applied to PR identification (Chen et al., 2021a). A comprehensive profiling method comprising a fast ultrasonic extraction, integrated with GC–MS and liquid chromatography atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS) analysis, is used to appraise the *Pinellia* species (Lee et al., 2016). However, the traditional methods are time-consuming and inefficiency.

Chloroplast-based molecular markers are widely used for identifying and resolving phylogeny of plants (Negi et al., 2021). The chloroplast is an important self-replicating organelle containing its own circular double-stranded genome. The chloroplast genome processes adequate levels of polymorphism, making it a suitable molecule for studies on phylogeny and evolution (Zhai et al., 2019). Recently, several complete chloroplast genomes of *Pinellia* genus plants, such as *P. ternata*, *P. pedatiseeta*, *P. cordata*, *P. peltata*, have been published (Han et al., 2016; Cai et al., 2020; Henriquez et al., 2020; Luo et al., 2021). A number of protein coding genes, rRNA genes, tRNA genes are identified in the chloroplast genome, providing sufficient genetic information to design molecular markers (Renner and Zhang, 2004). Based on the protein coding genes, phylogenetic analysis showed that *P. peltata* is original than other *Pinellia* genus plants and *P. ternata* shows a close relationship to *P. pedatiseeta* (Cai et al., 2020). Furthermore, eight mutational hotspot regions, including psbI-trnG-UCC, psbM-rpoB, ndhJ-trnT-UGU, trnP-UGG-trnW-CCA, ndhF-trnN-GUU, ndhG-ndhE, ycf1-rps15 and trnR-ycf1, were determined based on the chloroplast genomes of *Pinellia* (Cui et al., 2022). With the increase of plastome sequences, it is possible to accurately evaluate the genetic diversity of *Pinellia* genus.

5.2. Analysis of environmental stress responses

Plants face the challenge of environmental stress during their growth and development (Feng et al., 2023). *Pinellia* genus plants, sensitive to high temperature over 35°C, are always cultivated in the shade and moist environments (Xue et al., 2021). Several omic studies are applied to reveal the internal mechanism involving in the thermotolerance of *P. ternata*. Transcriptomic analysis of *P. ternata* reported that a number of short-term heat responsive genes, such as *HSF*s, *NAC*s, *WRKY*s, and *bZIP*s, are regulated by spermidine and melatonin treatments (Ma et al., 2020). A comparative proteomic analysis identified four important heat stress responsive proteins, including cytosolic class I small heat shock protein I/II, mitochondrial small heat shock protein,

and glycine-rich RNA-binding protein, suggesting new insights into the responses of *P. ternata* to heat stress (Zhu et al., 2013). Ultra-high performance liquid chromatography mass spectrometry (UHPLC-MS) based metabolomic analysis identified that several organic acids, such as mevalonic acid, 12,13-dihydroxy-9*Z*-octadecenoic acid, urocanic acid, and γ-aminobutyric acid, are enriched in the *P. ternata* in shaded environment (Xue et al., 2021). Interestingly, environmental stress has led to the accumulation of a large number of active components in *P. ternata*. On the other hand, some active ingredients can help plants resist environmental stresses.

In addition to abiotic stresses, omic study is performed to reveal the insights into host responses to microbial invasion. For example, transcriptomic analysis determines the defense responses of *P. ternata* T2 plus line, indicating a transcript reprogramming under *Pectobacterium carotovorum* infestation (Shu et al., 2021).

5.3. Analysis of the effects of Pinellia genus plants on soil microbial structure

Continuous cropping is challenge for medicinal plants, especially for *Pinellia* plants. Increased demand urges farmers to specialize in *Pinellia* crops annually, leading to crop pests and soft-rot diseases (Zhao et al., 2021b). Continuous cropping changes soil physical properties, especially in microbial biomass and composition (He et al. 2019). High-throughput ITS2 sequencing is a powerful tool to reveal the community shifts between the continuous and rotational cropping soils. In 2019, Zhao's group showed that the abundances of the genera *Podospora* and *Alternaria* are significantly enriched by rotational cropping, which may play a major role in increasing the rhizosphere soil fertility (He et al., 2019a). Liu' group discovered that *Rhizobium*, *Pseudomonas*, *Flavobacterium*, *Sphingomonas*, *Rhizobacter*, and *Arthrobacter* are enriched in *P. ternata* crop rotation soils (He et al., 2019b). Recently, another study showed that microbial population in the rhizosphere of *P. ternata* intercropped soil is significantly larger than that of *P. ternata* sole-cropped soil. Specifically, *Acidobacteria* and *Anaerolineaceae* are dominant microbes in the intercropped soils (Noushahi et al., 2021). These high-throughput sequencing provided several potential microorganisms that can be used for remediation of deterioration in continuous *P. ternata* cropping soil.

5.4. Analysis of miRNAs and their targets

Plant miRNAs generally control gene expression by guiding targeted mRNA cleavage. Degradome sequencing is an effective approach to identify miRNAs and their target genes (Shomron and Levy, 2009). Ten years ago, an *in situ* synthesized custom miRNA microarray was performed, identifying a total of 99 miRNAs belonging to 22 miRNA families in *P. pedatiseeta* and a total of 54 miRNAs belonging to 23 miRNA families in *P. ternata* (Wang et al., 2012; Xu et al., 2012a). Microarray and degradome sequencing detects a total of 101 miRNAs and 18 downstream target transcripts in *Pinellia* plants (Dong et al., 2013). The underground tubers of *Pinellia* plants are commonly used as an antitussive, expectorant, and anticancer drug (Lin et al., 2007). MiRNA319 shows the highest expression level in tubers, suggesting its regulatory role in tuber formation (Xu et al., 2012a).

5.5. Gene clone and functional identification

It is very difficult to identify and clone functional genes in non-model medicinal plants (Yu et al., 2017; Yu et al., 2022). Suppression subtractive hybridization library of *P. ternata* is constructed, yielding 303 expressed sequences tags. Tag sequence analysis identified four heat stress responsive transcripts, including *sHSP*, *HSP90*, *HSP70*, and *SAD* (Lu et al., 2013). High-throughput sequencing is easy and cheap to obtain genomic information of *Pinellia* plants. In 2016, Li's group identified a total of 53,544 unigenes and Chen's group identified 89,068 unigenes of *P. ternata* with the Illumina HiSeq™ 2000 sequencing platform (Huang et al., 2016; Zhang et al., 2016). In 2020, Cao's group identified 76,755 unigenes of *P. ternata* (Ma et al., 2020). In 2021, another group identified 75,551 unigenes of *P. ternata* T2 plus line (Shu et al., 2021). In 2022, Liang's group analyzed the transcriptome profiling and revealed the mechanisms of the allelopathic effects of phenolic acids on *P. ternata* (He et al., 2022). In 2022, another group revealed the essential role of exogenous brassinolide on the alkaloid biosynthesis pathway in *P. ternata* by transcriptomic analysis (Guo et al., 2022). Full-length transcriptome also is performed to identify the completed gene sequences of *P. ternata*. By third generation single-molecule real-time sequencing, a large number of unigenes (136,163 non-redundant transcripts) are identified in *P. ternata*. (Xue et al., 2019). A large amount of gene sequence information makes it possible to identify functional genes.

Ephedrine, a classic type of alkaloids, is the main active ingredient in mature PR. A complete understanding of the biosynthesis of phenylpropylamino alkaloids still need to be elucidated in *P. ternata*. (Ge and Hao, 2009). Based on transcriptomes, possible biosynthesis pathway of ephedrine in *P. ternata* is proposed, including *PAL*, *CNL*, *CHD*, *KAT*, *BALDH*, *CHY*, *AO4*, *PDC*, and *AHAS* etc. (Zhang et al., 2016). Furthermore, several candidate genes, such as *PIF3*, *HY5*, *CAB151*, *RUB*, *SOD*, *CAT*, *POD*, *GR*, *ARF1/19*, *PIN1/6*, *XET*, *RGP*, *SUS*, and *CHS*, involved in sprout tumble formation and tuber growth are identified by a full-length transcriptome (Xue et al., 2019). These genes can be applied to the genetic improvement and molecular breeding of *P. ternata*.

6. Conclusion and prospect

P. ternata has a long cultivation history over 2000 years. As a TCM originated from *P. ternata* tubers, PR possesses multiple pharmacological and clinical effects. This review provided a comprehensive summary of active ingredients, pharmacological effects, toxic ingredients and detoxification strategies, and omic researches on marker screening and functional gene identification, etc.

Recently, the habitat of wide *P. ternata* has been seriously destroyed. Researches on active ingredients are conducive to the comprehensive utilization of *P. ternate* germplasm resources. Active ingredients from PR are mainly classified into six categories: alkaloids, amino acids, polysaccharides, phenylpropanoids, essential oils, and glucocerebrosides. The diversity of active ingredients determines the broad-spectrum efficacy of *P. ternata*.

PR has been proved to possess significant anti-tumor effects. The primary and secondary metabolites, such as polysaccharides, agglutinins, stigma sterols, lipids, were reported to play important roles in anti-tumor. The active components of PR are involved in the inhibition of cancer cells by regulating multiple pathways, including activation of immune system, inhibition the proliferation and cycle, induction of apoptosis, and inhibition of angiogenesis. Effects of PR extracts on the central nervous system also were reported recently. Several active ingredients, such as adenosine, succinic acid, baicalein, β-sitosterol, and stigmasterol, exert neuroprotective effects. The active components of PR are involved in the treatments of nervous system by regulating neurotransmitters, activating immune system, decreasing apoptosis, and increasing redox system. In the future, investigation of specific target genes and drug-binding sites is a key step to explore the effect and mechanism of PR active components.

PR has significant toxic effects on human cells, and lectins were considered to be the major toxic ingredients of raw PR. Inflammatory factors, CYP family enzymes, mTOR signaling factors, TGF-β signaling factors, and nervous system are potential targets of raw PR toxic ingredients. Several classic methods, such as alum-processing, ginger juice processing, lime and licorice processing, were used to attenuate the toxicity of raw PR.

Recently, omic analysis was widely applied in *Pinellia* genus studies. Chloroplast genome-based markers were deeply used for identifying and resolving phylogeny of *Pinellia* genus plants. With the progress of technology, more plastome sequences will be completed gained, making accurately evaluation of the genetic diversity of *Pinellia* genus possible. A large number of omic works revealed a series of environmental stress responsive factors, and functional genes involved in active component biosynthesis. Omic studies provided several valuable candidates to accelerate the breeding of *Pinellia* genus plants. With the progress of single cell sequencing technology, gene expression and metabolite accumulation at higher spatial and temporal resolution can be expected.

Funding.

This work was supported by the National Natural Science Foundation of China (China, 82074052), Zhejiang Province Chinese medicine science and technology plan (2022ZZ024 and 2022ZB228), Zhejiang Province Education Department projects (China, 202147432), Shandong Provincial Natural Science Foundation Innovation and Development Joint Project (ZR2021LZY039).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Bai, J., Qi, J., Yang, L., Wang, Z., Wang, R., Shi, Y., 2022. A comprehensive review on ethnopharmacological, phytochemical, pharmacological and toxicological evaluation, and quality control of *Pinellia ternata* (Thunb.) Breit. J. Ethnopharmacol. 298, 115650.

Cai, Z., Wang, H., Wang, G., 2020. Complete chloroplast genome sequence of *Pinellia ternata* (Thunb.) Breit, a medicinal plants to China. Mitochondrial DNA B Resour. 5, 2107–2108.

Chen, R., Ding, S., Wei, Y., Yu, J., Xu, R., Luo, X., Fan, G., Yin, H., Bian, J., 2021a. Ultrafast identification of Pinelliae Rhizoma using colorimetric direct-VPCR. 3. Biotech. 11, 493.

Chen, Z.Y., Lai, C.J., Wei, X.Y., Gong, L., Qiu, Z.D., 2021b. Research progress on identification methods of growth years of traditional Chinese medicinal materials. Zhongguo Zhong Yao Za Zhi 46, 1357–1367.

Chen, G., Xu, J., Miao, X., Huan, Y., Liu, X., Ju, Y., Han, X., 2012. Characterization and antitumor activities of the water-soluble polysaccharide from Rhizoma *Arisaematis*. Carbohydr. Polym. 90, 67–72.

Chen, K., Yang, X., Wu, L., Yu, M., Li, X., Li, N., Wang, S., Li, G., 2013. *Pinellia pedatisecta* agglutinin targets drug resistant K562/ADR leukemia cells through binding with sarcolemmal membrane associated protein and enhancing macrophage phagocytosis. PLoS One 8, e74363.

Cui, N., Chen, W., Li, X., Wang, P., 2022. Comparative chloroplast genomes and phylogenetic analyses of *Pinellia*. Mol. Biol. Rep. 49, 7873–7885.

Deng, C.X., Wu, Z.B., Chen, Y., Yu, Z.M., 2020. Pinellia total alkaloids modulate the GABAergic system in hippocampal formation on pilocarpine-induced epileptic rats. Chin. J. Integr. Med. 26, 138–145.

Dong, M., Yang, D., Lang, Q., Zhou, W., Xu, S., Xu, T., 2013. Microarray and degradome sequencing reveal microRNA differential expression profiles and their targets in *Pinellia pedatisecta*. PloS one 8, e75978–e.

Fang, L., Xie, J., Lin, L., Tian, M., Row, K.H., 2020. Multi-phase extraction of ephedrine from *Pinellia ternata* and herbal medicine using molecular imprinted polymer coated ionic liquid-based silica. Phytochem. Anal. 31, 242–251.

Feng, S., Kailin, H., Zhang, H., Chen, C., Huang, J., Wu, Q., Zhang, Z., Gao, Y., Wu, X., Wang, H., Shen, C., 2023. Investigation of the role of TmMYB16/123 and their targets (TmMTP1/11) in the tolerance of *Taxus media* to cadmium. Tree Physiol. tpad019.

Feng, G.K., Ma, X.J., Chen, Y.Y., Bian, G.R., Yang, C., Gu, B.D., 2018. Effects of chailong jieyu pill on behavior, monoamine neurotransmitters, and corticosteroid receptors in a rat model of anxiety disorder. Evid. Based Complement Alternat. Med. 2018, 5489215.

Fueki, T., Tanaka, K., Obara, K., Kawahara, R., Namiki, T., Makino, T., 2020. The acrid raphides in tuberous root of *Pinellia ternata* have lipophilic character and are specifically denatured by ginger extract. J. Nat. Med. 74, 722–731.

Ge, X., Hao, W., 2009. Phytochemical properties and quality evaluation methods of *Pinelliae ternata*. China Pharm. 18, 3–5.

Gonzalez, M.I., Grabenstatter, H.L., Cea-Del Rio, C.A., Cruz Del Angel, Y., Carlsen, J., Laoprasert, R.P., White, A.M., Huntsman, M.M., Brooks-Kayal, A., 2015. Seizure-related regulation of GABAA receptors in spontaneously epileptic rats. Neurobiol. Dis. 77, 246–256.

Guo, C., Chen, Y., Wu, D., Du, Y., Wang, M., Liu, C., Chu, J., Yao, X., 2022. Transcriptome analysis reveals an essential role of exogenous brassinolide on the alkaloid biosynthesis pathway in *Pinellia Ternata*. Int. J. Mol. Sci. 23, 10898.

Guo, J., Lou, M.P., Hu, L.L., Zhang, X., 2020. Uncovering the pharmacological mechanism of the effects of the Banxia-Xiakucao Chinese Herb Pair on sleep disorder by a systems pharmacology approach. Sci. Rep. 10, 20454.

GUOHan, L., Chen, C., Wang, B., Wang, Z.Z., 2016. The complete chloroplast genome sequence of medicinal plant *Pinellia ternata*. Mitochondrial DNA A DNA Mapp Seq Anal 27, 2921-2.

Han, M.H., Yang, X.W., Zhong, G.Y., Zhang, M., 2007. Bioactive constituents inhibiting TNF-alpha production in fresh rhizome of *Pinellia ternata*. Zhongguo Zhong Yao Za Zhi 32, 1755–1759.

He, Z., Chen, H., Liang, L., Dong, J., Liang, Z., Zhao, L., 2019a. Alteration of crop rotation in continuous *Pinellia ternate* cropping soils profiled via fungal ITS amplicon sequencing. Lett Appl Microbiol 68, 522–529.

He, Z., Mao, R., Dong, J.E., Liang, Z., Zhang, H., Liu, L., 2019b. Remediation of deterioration in microbial structure in continuous *Pinellia ternata* cropping soil by crop rotation. Can. J. Microbiol. 65, 282–295.

He, Z., Wang, Y., Yan, Y., Qin, S., He, H., Mao, R., Liang, Z., 2022. Dynamic analysis of physiological indices and transcriptome profiling revealing the mechanisms of the allelopathic effects of phenolic acids on *Pinellia ternata*. Front. Plant Sci. 13, 1039507.

Henriquez, C.L., Abdullah, Ahmed, I., Carlsen, M.M., Zuluaga, A., Croat, T.B. and McKain, M.R., 2020. Evolutionary dynamics of chloroplast genomes in subfamily *Aroideae* (Araceae). Genomics 112, 2349-2360.

Hu, M., Liu, Y., Wang, L., Wang, J., Li, L., Wu, C., 2019. Purification, characterization of two polysaccharides from *Pinelliae Rhizoma* praeparatum cum alumine and their anti-inflammatory effects on mucus secretion of airway epithelium. Int. J. Mol. Sci. 20, 3553.

Huang, H., Zhang, M., Yao, S., Zhang, M., Peng, J., with the Pinellia pedatisecta advisory, g., 2018. Immune modulation of a lipid-soluble extract of Pinellia pedatisecta Schott in the tumor microenvironment of an HPV(+) tumor-burdened mouse model. J. Ethnopharmacol. 225, 103-115.

Huang, X., Jing, Y., Liu, D.J., Yang, B.Y., Li, M., 2016. Whole-transcriptome sequencing of Pinellia ternata using the Illumina platform. Genet. Mol. Res. Gmr 15, gmr8062.

Huang, C.F., Yang, R.S., Liu, S.H., Hsieh, P.C., Lin-Shiau, S.Y., 2011. Evidence for improved neuropharmacological efficacy and decreased neurotoxicity in mice with traditional processing of *Rhizoma Arisaematis*. Am. J. Chin. Med. 39, 981–998.

Huo, J., Qin, F., Cai, X., Ju, J., Hu, C., Wang, Z., Lu, W., Wang, X., Cao, P., 2013. Chinese medicine formula "Weikang Keli" induces autophagic cell death on human gastric cancer cell line SGC-7901. Phytomedicine 20, 159–165.

Ingelman-Sundberg, M., 2004. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. Trends Pharmacol. Sci. 25, 193–200.

Ito, A., Shin, N., Tsuchida, T., Okubo, T., Norimoto, H., 2013. Antianxiety-like effects of Chimpi (dried citrus peels) in the elevated open-platform test. Molecules 18, 10014–10023.

Iwasa, M., Iwasaki, T., Ono, T., Miyazawa, M., 2014. Chemical composition and major odor-active compounds of essential oil from PINELLIA TUBER (dried rhizome of *Pinellia ternata*) as crude drug. J. Oleo Sci. 63, 127–135.

Ji, X., Huang, B., Wang, G., Zhang, C., 2014. The ethnobotanical, phytochemical and pharmacological profile of the genus *Pinellia*. Fitoterapia 93, 1–17.

Jiang, G.M., Tan, Y., Wang, H., Peng, L., Chen, H.T., Meng, X.J., Li, L.L., Liu, Y., Li, W.F., Shan, H., 2019. The relationship between autophagy and the immune system and its applications for tumor immunotherapy. Mol. Cancer 18, 17.

Kangsamaksin, T., Chaithongyot, S., Wootthichairangsan, C., Hanchaina, R., Tangshewinsirikul, C., Svasti, J., 2017. Lupeol and stigmasterol suppress tumor angiogenesis and inhibit cholangiocarcinoma growth in mice via downregulation of tumor necrosis factor-alpha. PLoS One 12, e0189628.

Khan, H., Bangar, A., Grewal, A.K., Bansal, P., Singh, T.G., 2022. Caspase-mediated regulation of the distinct signaling pathways and mechanisms in neuronal survival. Int. Immunopharmacol. 110, 108951.

Kong, X., Yang, M., Abbas, H.M.K., Wu, J., Li, M., Dong, W., 2018. Antimicrobial genes from *Allium sativum* and *Pinellia ternata* revealed by a *Bacillus subtilis* expression system. Sci. Rep. 8, 14514.

Lee, J.Y., Park, N.H., Lee, W., Kim, E.H., Jin, Y.H., Seo, E.K., Hong, J., 2016. Comprehensive chemical profiling of Pinellia species tuber and processed Pinellia tuber by gas chromatography-mass spectrometry and liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry. J. Chromatogr. A 1471, 164–177.

Li, G.L., Jiang, W., Xia, Q., Chen, S.H., Ge, X.R., Gui, S.Q., Xu, C.J., 2010. HPV E6 down-regulation and apoptosis induction of human cervical cancer cells by a novel lipid-soluble extract (PE) from *Pinellia pedatisecta* Schott *in vitro*. J. Ethnopharmacol. 132, 56–64.

Li, Y., Li, D., Chen, J., Wang, S., 2016. A polysaccharide from *Pinellia ternata* inhibits cell proliferation and metastasis in human cholangiocarcinoma cells by targeting of Cdc42 and 67kDa Laminin Receptor (LR). Int. J. Biol. Macromol. 93, 520–525.

Li, S.H., Yu, H.L., Wu, H., Zhang, Y.B., Wang, W., 2020. Effect of *Pinelliae Rhizoma* processed with lime and licorice on toxic lectin protein. Zhongguo Zhong Yao Za Zhi 45, 2546–2551.

Li, X., Zhang, X., Yang, W., Guo, L., Huang, L., Li, X., Gao, W., 2021. Preparation and characterization of native and autoclaving-cooling treated *Pinellia ternate* starch and its impact on gut microbiota. Int. J. Biol. Macromol. 182, 1351–1361.

Liang, Z., Zhang, J., Wong, L., Yi, T., Chen, H., Zhao, Z., 2013. Characterization of secondary metabolites from the raphides of calcium oxalate contained in three araceae family plants using laser microdissection and ultra-high performance liquid

chromatography-quadrupole/time of flight-mass spectrometry. Eur. J. Mass Spectrom. (Chichester) 19, 195–210.
Lin, S., Nie, B., Yao, G., Yang, H., Ye, R., Yuan, Z., 2019. *Pinellia ternata* (Thunb.) Makino Preparation promotes sleep by increasing REM sleep. Nat. Prod. Res. 33, 3326–3329.
Lin, J., Yao, J., Zhou, X., Sun, X., Tang, K., 2003. Expression and purification of a novel mannose-binding lectin from *Pinellia ternata*. Mol. Biotechnol. 25, 215–222.
Lin, J., Zhou, X., Gao, S., Liu, X., Wu, W., Sun, X., Tang, K., 2007. cDNA cloning and expression analysis of a mannose-binding lectin from *Pinellia pedatisecta*. J. Biosci. 32, 241–249.
Liu, X.P., Ming, H.X., Li, P.Q., 2016. Intervention effect of pinelliae decoction for purging stomach-fire on malignant transformation of bone marrow mesenchymal stem cells in the gastric cancer microenvironment. Am. J. Transl. Res. 8, 2937–2946.
Liu, Y.J., Mo, X.L., Tang, X.Z., Li, J.H., Hu, M.B., Yan, D., Peng, W., Wu, C.J., 2017. Extraction optimization, characterization, and bioactivities of polysaccharides from *Pinelliae Rhizoma* praeparatum cum alumine employing ultrasound-assisted extraction. Molecules 22, 965.
Lu, Q., Li, N., Luo, J., Yu, M., Huang, Y., Wu, X., Wu, H., Liu, X.Y., Li, G., 2012. *Pinellia pedatisecta* agglutinin interacts with the methylosome and induces cancer cell death. Oncogenesis 1, e29.
Lu, H., Xue, T., Zhang, A., Sheng, W., Zhu, Y., Chang, L., Song, Y., Xue, J., 2013. Construction of an SSH library of *Pinellia ternata* under heat stress, and expression analysis of four transcripts. Plant Molecular Biology Reporter 31, 185–194.
Luo, R., Du, S., Luo, X., 2021. The complete plastome sequence of *Pinellia peltata* Pei (Araceae). Mitochondrial DNA B Resour 6, 2112–2113.
Ma, G., Zhang, M., Xu, J., Zhou, W., Cao, L., 2020. Transcriptomic analysis of short-term heat stress response in Pinellia ternata provided novel insights into the improved thermotolerance by spermidine and melatonin. Ecotoxicol. Environ. Saf. 202, 110877.
Mao, R., He, Z., 2020. *Pinellia ternata* (Thunb.) Breit: A review of its germplasm resources, genetic diversity and active components. J Ethnopharmacol 263, 113252.
Negi, R.K., Nautiyal, P., Bhatia, R., Verma, R., 2021. RbcL, a potential candidate DNA barcode loci for aconites: conservation of himalayan aconites. Mol Biol Rep 48, 6769–6777.
Noushahi, H.A., Zhu, Z., Khan, A.H., Ahmed, U., Haroon, M., Asad, M., Hussain, M., Beibei, H., Zafar, M., Alami, M.M., Shu, S., 2021. Rhizosphere microbial diversity in rhizosphere of Pinellia ternata intercropped with maize. 3 Biotech 11, 469.
Oshio, H., Tsukui, M., Matsuoka, T., 1978. Isolation of l-ephedrine from "Pinelliae Tuber". Chemical & Pharmaceutical Bulletin 26, 2096–2097.
Peng, W., Li, N., Jiang, E., Zhang, C., Huang, Y., Tan, L., Chen, R., Wu, C., Huang, Q., 2022. A review of traditional and current processing methods used to decrease the toxicity of the rhizome of *Pinellia ternata* in traditional Chinese medicine. J Ethnopharmacol 299, 115696.
Renner, S.S., Zhang, L.B., 2004. Biogeography of the Pistia clade (Araceae): based on chloroplast and mitochondrial DNA sequences and Bayesian divergence time inference. Syst Biol 53, 422–432.
Rozema, E., Popescu, R., Sonderegger, H., Huck, C.W., Winkler, J., Krupitza, G., Urban, E., Kopp, B., 2012. Characterization of glucocerebrosides and the active metabolite 4,8-sphingadienine from *Arisaema amurense* and *Pinellia ternata* by NMR and CD spectroscopy and ESI-MS/CID-MS. J Agric Food Chem 60, 7204–7210.
Seo, C.S., Shin, H.K., 2022. Simultaneous analysis for quality control of traditional herbal medicine, gungha-tang, using liquid chromatography-tandem mass spectrometry. Molecules 27, 1223.
Sever, R., Brugge, J.S., 2015. Signal transduction in cancer. Cold Spring Harb Perspect Med 5.
Shi, J., Wei, P.K., 2015. Xiaotan Sanjie decoction inhibits interleukin-8-induced metastatic potency in gastric cancer. World J Gastroenterol 21, 1479–1487.
Shih, T.T., Lee, H.L., Chen, S.C., Kang, C.Y., Shen, R.S., Su, Y.A., 2018. Rapid analysis of traditional Chinese medicine *Pinellia ternata* by microchip electrophoresis with electrochemical detection. J Sep Sci 41, 740–746.
Shomron, N., Levy, C., 2009. MicroRNA-biogenesis and Pre-mRNA splicing crosstalk. J Biomed Biotechnol 2009, 594678.
Shu, F., Han, J., Ndayambaje, J.P., Jia, Q., Sarsaiya, S., Jain, A., Huang, M., Liu, M., Chen, J., 2021. Transcriptomic analysis of *Pinellia ternata* (Thunb.) Breit T2 plus line provides insights in host responses resist *Pectobacterium carotovorum* infection. Bioengineered 12, 1173–1188.
Shu, B.O., Ying, J., Wang, T., Xia, M., Zhao, W., You, L., 2019. Microbiota and chemical compounds in fermented *Pinelliae Rhizoma* (Banxiaqu) from different areas in the Sichuan Province, China. Pol J Microbiol 68, 83–92.
Song, S., Huang, W., Lu, X., Liu, J., Zhou, J., Li, Y., Shu, P., 2021. A network pharmacology study based on the mechanism of Citri Reticulatae Pericarpium-Pinelliae Rhizoma in the treatment of gastric cancer. Evid Based Complement Alternat Med 2021, 6667560.
Su, T., Tan, Y., Tsui, M.S., Yi, H., Fu, X.Q., Li, T., Chan, C.L., Guo, H., Li, Y.X., Zhu, P.L., Tse, A.K., Cao, H., Lu, A.P., Yu, Z.L., 2016. Metabolomics reveals the mechanisms for the cardiotoxicity of *Pinelliae Rhizoma* and the toxicity-reducing effect of processing. Sci Rep 6, 34692.
Sumino, M., Saito, Y., Ikegami, F., Hirasaki, Y., Namiki, T., 2012. Efficient preparation of Hangekobokuto (Banxia-Houpo-Tang) decoction by adding Perilla herb before decoction is finished. Nat Prod Commun 7, 1619–1622.
Sun, L.M., Zhang, B., Wang, Y.C., He, H.K., Chen, X.G., Wang, S.J., 2019. Metabolomic analysis of raw *Pinelliae Rhizoma* and its alum-processed products via UPLC-MS and their cytotoxicity. Biomed Chromatogr 33, e4411.
Sun, Y., Zhang, N., Qu, Y., Cao, Y., Li, J., Yang, Y., Yang, T., Sun, Y., 2020. Shuangxia decoction alleviates p-chlorophenylalanine induced insomnia through the modification of serotonergic and immune system. Metab Brain Dis 35, 315–325.
Tabuchi, M., Mizuno, K., Mizoguchi, K., Hattori, T., Kase, Y., 2017. Yokukansan and Yokukansankachimpihange ameliorate aggressive behaviors in rats with cholinergic degeneration in the nucleus basalis of meynert. Front Pharmacol 8, 235.
Takeda, A., 2010. Insight into glutamate excitotoxicity from synaptic zinc homeostasis. Int J Alzheimers Dis 2011, 491597.
Tamano, H., Kan, F., Oku, N., Takeda, A., 2010. Ameliorative effect of Yokukansan on social isolation-induced aggressive behavior of zinc-deficient young mice. Brain Res Bull 83, 351–355.
Tamano, H., Yusuke, E., Ide, K., Takeda, A., 2016. Influences of yokukansankachimpihange on aggressive behavior of zinc-deficient mice and actions of the ingredients on excessive neural exocytosis in the hippocampus of zinc-deficient rats. Exp Anim 65, 353–361.
Tang, D., Yan, R., Sun, Y., Kai, G., Chen, K., Li, J., 2020. Material basis, effect, and mechanism of ethanol extract of *Pinellia ternata* tubers on oxidative stress-induced cell senescence. Phytomedicine 77, 153275.
Tian, Y.Q., Ding, P., Yan, X.H., Hu, W.J., 2008. Discussion on quality control of preparations with cortex moutan in volume I pharmacopoeia of People's Republic of China (2005 edition). Zhongguo Zhong Yao Za Zhi 33, 339–341.
Wang, B., Dong, M., Chen, W., Liu, X., Feng, R., Xu, T., 2012. Microarray identification of conserved microRNAs in *Pinellia pedatisecta*. Gene 498, 36–40.
Wang, Y., Huang, H., Yao, S., Li, G., Xu, C., Ye, Y., Gui, S., 2019b. A lipid-soluble extract of *Pinellia pedatisecta* Schott enhances antitumor T cell responses by restoring tumor-associated dendritic cell activation and maturation. J Ethnopharmacol 241, 111980.
Wang, Y., Lu, C., Huang, H., Yao, S., Xu, C., Ye, Y., Gui, S., Li, G., 2021. A lipid-soluble extract of *Pinellia pedatisecta* Schott orchestrates intratumoral dendritic cell-driven immune activation through SOCS1 signaling in cervical cancer. J Ethnopharmacol 267, 112837.
Wang, W., Mao, S., Yu, H., Wu, H., Shan, X., Zhang, X., Cui, G., Liu, X., 2019a. *Pinellia pedatisecta* lectin exerts a proinflammatory activity correlated with ROS-MAPKs/NF-kappaB pathways and the NLRP3 inflammasome in RAW264.7 cells accompanied by cell pyroptosis. Int Immunopharmacol 66, 1–12.
Wang, L., Wang, P., Chen, Y., Li, C., Wang, X., Zhang, Y., Li, S., Yang, M., 2022. Utilizing network pharmacology and experimental validation to explore the potential molecular mechanisms of BanXia-YiYiRen in treating insomnia. Bioengineered 13, 3148–3170.
Wu, H., Cai, B.C., Shi, X.L., Ye, D.J., 1993. The effect of stimulation and toxicity of rhizoma Pinelliae processed by ginger juice on animals. Zhongguo Zhong Yao Za Zhi 18 (408–10), 446–447.
Wu, J., Cheng, Z., He, S., Shi, J., Liu, S., Zhang, G., Zhu, L., Liu, L., Liu, Z., Lin, N., Lu, L., 2015. Pinelliae rhizoma, a toxic chinese herb, can significantly inhibit CYP3A activity in rats. Molecules 20, 792–806.
Wu, X.Y., Zhao, J.L., Zhang, M., Li, F., Zhao, T., Yang, L.Q., 2011. Sedative, hypnotic and anticonvulsant activities of the ethanol fraction from Rhizoma Pinelliae Praeparatum. J Ethnopharmacol 135, 325–329.
Xiao, Y.G., Wu, H.B., Chen, J.S., Li, X., Qiu, Z.K., 2022. Exploring the potential antidepressant mechanisms of *Pinellia* by using the network pharmacology and molecular docking. Metab Brain Dis 37, 1071–1094.
Xie, H.H., Xu, J.Y., Xie, T., Meng, X., Lin, L.L., He, L.L., Wu, H., Shan, J.J., Wang, S.C., 2016. Effects of *Pinellia ternata* (Thunb.) Berit. on the metabolomic profiles of placenta and amniotic fluid in pregnant rats. J Ethnopharmacol 183, 38–45.
Xu, J.Y., Dai, C., Shan, J.J., Xie, T., Xie, H.H., Wang, M.M., Yang, G., 2018. Determination of the effect of *Pinellia ternata* (Thunb.) Breit. on nervous system development by proteomics. J Ethnopharmacol 213, 221–229.
Xu, T., Wang, B., Liu, X., Feng, R., Dong, M., Chen, J., 2012a. Microarray-based identification of conserved microRNAs from *Pinellia ternata*. Gene 493, 267–272.
Xu, T., Wang, B., Wang, L., Zhang, Y., Lv, Z., 2012b. *Pinellia ternata* agglutinin produced in Bombyx mori cells exhibits bioactivity. Acta Biochim Pol 59, 231–236.
Xue, T., Zhang, H., Zhang, Y., Wei, S., Chao, Q., Zhu, Y., Teng, J., Zhang, A., Sheng, W., Duan, Y., Xue, J., 2019. Full-length transcriptome analysis of shade-induced promotion of tuber production in *Pinellia ternata*. BMC Plant Biol 19, 565.
Xue, T., Xiong, Y., Shi, J., Chao, Q., Zhu, Y., Duan, Y., Sheng, W., Teng, J., Xue, J., 2021. UHPLC-MS-based metabolomic approach for the quality evaluation of Pinellia ternata tubers grown in shaded environments. J Nat Med 75, 1050–1057.
Yahagi, T., Atsumi, T., Mano, S., Kikuchi, Y., Hara, Y., Furukawa, M., Yang, Z., Matsuzaki, K., 2021. Quality evaluation of Pinellia tuber by LC-TOF/MS targeted to ephedrine. J Nat Med 75, 692–698.
Yan, B., Liu, L., Zhao, Y., Xiu, L.J., Sun, D.Z., Liu, X., Lu, Y., Shi, J., Zhang, Y.C., Li, Y.J., Wang, X.W., Zhou, Y.Q., Feng, S.H., Lv, C., Wei, P.K., Qin, Z.F., 2014. Xiaotan Sanjie decoction attenuates tumor angiogenesis by manipulating Notch-1-regulated proliferation of gastric cancer stem-like cells. World J Gastroenterol 20, 13105–13118.
Ye, Y., Li, J., Cao, X., Chen, Y., Ye, C., Chen, K., 2016. Protective effect of n-butyl alcohol extracts from Rhizoma *Pinelliae Pedatisectae* against cerebral ischemia-reperfusion injury in rats. J Ethnopharmacol 188, 259–265.
Yen, M.H., Lee, J.J., Yeh, C.F., Wang, K.C., Chiang, Y.W., Chiang, L.C., Chang, J.S., 2014. Yakammaoto inhibited human coxsackievirus B4 (CVB4)-induced airway and renal tubular injuries by preventing viral attachment, internalization, and replication. J Ethnopharmacol 151, 1056–1063.
Yi, L.T., Zhang, L., Ding, A.W., Xu, Q., Zhu, Q., Kong, L.D., 2009. Orthogonal array design for antidepressant compatibility of polysaccharides from Banxia-Houpu decoction, a traditional Chinese herb prescription in the mouse models of depression. Arch Pharm Res 32, 1417–1423.
Yoon, J.S., Seo, J.C., Han, S.W., 2006. Pinelliae Rhizoma herbal-acupuncture solution induced apoptosis in human cervical cancer cells, SNU-17. Am J Chin Med 34, 401–408.
Yu, C., Guo, H., Zhang, Y., Song, Y., Pi, E., Yu, C., Zhang, L., Dong, M., Zheng, B., Wang, H., Shen, C., 2017. Identification of potential genes that contributed to the variation

in the taxoid contents between two *Taxus* species (*Taxus media* and *Taxus mairei*). Tree Physiology 37, 1659–1671.
Yu, C., Huang, J., Wu, Q., Zhang, C., Li, X.L., Xu, X., Feng, S., Zhan, X., Chen, Z., Wang, H., Shen, C., 2022. Role of female-predominant MYB39-bHLH13 complex in sexually dimorphic accumulation of taxol in *Taxus media*. Hortic Res 9, uhac062.
Yu, H.L., Mao, S.H., Zhao, T.F., Wu, H., Pan, Y.Z., Shu, C.Y., 2015b. Antagonistic effect of gingerols against TNF-alpha release, ROS overproduction and RIP3 expression increase induced by lectin from Pinellia ternata. Zhongguo Zhong Yao Za Zhi 40, 3630–3635.
Yu, H., Pan, Y., Wu, H., Ge, X., Zhang, Q., Zhu, F., Cai, B., 2015a. The alum-processing mechanism attenuating toxicity of Araceae *Pinellia ternata* and *Pinellia pedatisecta*. Arch Pharm Res 38, 1810–1821.
Yu, H.L., Zhao, T.F., Wu, H., Pan, Y.Z., Zhang, Q., Wang, K.L., Zhang, C.C., Jin, Y.P., 2015c. *Pinellia ternata* lectin exerts a pro-inflammatory effect on macrophages by inducing the release of pro-inflammatory cytokines, the activation of the nuclear factor-kappaB signaling pathway and the overproduction of reactive oxygen species. Int J Mol Med 36, 1127–1135.
Yu, H.L., Wang, W., Wu, H., Shen, M., Zhang, Y.B., Li, S.H., 2019. Effect of processing on toxic components lectin from four kinds of Araceae toxic medicines. Zhongguo Zhong Yao Za Zhi 44, 5398–5404.
Zhai, W., Duan, X., Zhang, R., Guo, C., Li, L., Xu, G., Shan, H., Kong, H., Ren, Y., 2019. Chloroplast genomic data provide new and robust insights into the phylogeny and evolution of the Ranunculaceae. Mol Phylogenet Evol 135, 12–21.
Zhang, G.H., Jiang, N.H., Song, W.L., Ma, C.H., Yang, S.C., Chen, J.W., 2016. *De novo* sequencing and transcriptome analysis of *Pinellia ternata* identify the candidate genes involved in the biosynthesis of benzoic acid and ephedrine. Front Plant Sci 7, 1209.
Zhang, C.A., Wu, F., Mao, Z.J., Wei, Z., Li, Y.J., Wei, P.K., 2011. Effects of ethanol extract of Rhizome Pinelliae Preparata on intracellular pH value of human gastric adenocarcinoma cells. Zhong Xi Yi Jie He Xue Bao 9, 894–900.
Zhang, L., Xiao, Y., Yang, R., Wang, S., Ma, S., Liu, J., Xiao, W., Wang, Y., 2021. Systems pharmacology to reveal multi-scale mechanisms of traditional Chinese medicine for gastric cancer. Sci Rep 11, 22149.
Zhang, M., Yu, Y., Zhang, H., Huang, H., Cai, Q., Kang, Y., Li, G., Xu, C., 2017a. Synergistic cytotoxic effects of a combined treatment of a *Pinellia pedatisecta* lipid-soluble extract and cisplatin on human cervical carcinoma in vivo. Oncol Lett 13, 4748–4754.
Zhang, M., Zhang, H., Yu, Y., Huang, H., Li, G., Xu, C., 2017b. Synergistic effects of a novel lipid-soluble extract from *Pinellia pedatisecta* Schott and cisplatin on human cervical carcinoma cell lines through the regulation of DNA damage response signaling pathway. Oncol Lett 13, 2121–2128.
Zhao, L. and Su, X., 1990. [Comparative analysis of alkaloid components in cultured and planted *Pinellia ternata* (Thunb.) Breit]. Zhongguo Zhong Yao Za Zhi 15, 146-7, 189-90.
Zhao, Y., Qin, X., Tian, X., Yang, T., Huang, J., 2021b. Effects of continuous cropping of *Pinellia ternata* (Thunb.) Breit. on soil physicochemical properties, enzyme activities, microbial communities and functional genes. Chemical and Biological Technologies. Agriculture 8, 43.
Zhao, T.F., Zhang, Q., Zhang, W., Wu, H., Yu, H.L., Wang, H.Z., 2013. Study on inflammatory effect of toxic raphides from *Pinellia ternate* and its correlation with macrophages. Zhongguo Zhong Yao Za Zhi 38, 1041–1045.
Zhao, H., Zhang, X., Wang, M., Lin, Y., Zhou, S., 2021a. Stigmasterol simultaneously induces apoptosis and protective autophagy by inhibiting Akt/mTOR pathway in gastric cancer cells. Front Oncol 11, 629008.
Zhao, X., Zhao, J., Hu, R., Yao, Q., Zhang, G., Shen, H., Yague, E., Hu, Y., 2017. Ruanjian Sanjie decoction exhibits antitumor activity by inducing cell apoptosis in breast cancer. Oncol Lett 13, 3071–3079.
Zhou, W., Gao, Y., Xu, S., Yang, Z., Xu, T., 2014. Purification of a mannose-binding lectin *Pinellia ternata* agglutinin and its induction of apoptosis in Bel-7404 cells. Protein Expr Purif 93, 11–17.
Zhou, L., Xu, T., Zhang, Y., Zhu, M., Zhu, W., Wang, Z., Gu, H., Wang, H., Li, P., Ying, J., Yang, L., Ren, P., Li, J., Xu, Z., Ni, L., Bao, Q., Chen, J., 2016. Transcriptional network in ovarian cancer cell line SKOV3 treated with *Pinellia pedatisecta* Schott extract. Oncol Rep 36, 462–470.
Zhu, Y., Zhu, G., Guo, Q., Zhu, Z., Wang, C., Liu, Z., 2013. A comparative proteomic analysis of *Pinellia ternata* leaves exposed to heat stress. Int J Mol Sci 14, 20614–20634.
Zu, G., Wang, H., Wang, J., Dou, Y., Zhao, W., Sun, Y., 2014. Rhizoma Pinelliae trypsin inhibitor separation, purification and inhibitory activity on the proliferation of BGC-823 gastric adenocarcinoma cells. Exp. Ther. Med. 8, 248–254.
Zuo, Z., Fan, H., Wang, X., Zhou, W., Li, L., 2012. Purification and characterization of a novel plant lectin from *Pinellia ternata* with antineoplastic activity. Springerplus 1, 13.