The combination of two natural medicines, Chuanxiong and Asarum: A review of the chemical constituents and pharmacological activities

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Abstract
Traditional Chinese medicine has been clinically used in China for many years, with experimental studies and clinical trials having demonstrated that it is safe and valid. Among many traditional natural medicines, Chuanxiong and Asarum have been proven to be effective in the treatment of relieving pain. Actually, as well as analgesic, they have common attributes, such as anti-inflammatory, cardiovascular benefits, and anticancer activities, with volatile oils being their major components. Furthermore, Chuanxiong and Asarum have been combined as drug pairs in the same prescription for thousands of years, with examples being Chuanxiong Chatiao San and Chuanxiongxixintang. More interestingly, their combination has better therapeutic effects on diseases than a single drug. After the combination of Chuanxiong and Asarum forms a blend, a series of changes take place in their chemical components, such as the contents of the main active ingredients, ferulic acid and ligustilide, increased significantly after this progress. At the same time, the pharmacological effects of the combination appearing to be more powerful, such as synergistic analgesic. This review focuses on the chemical constituents and pharmacological activities of Chuanxiong, Asarum, and Chuanxiong Asarum compositions.

Keywords
Asarum, chemical constituents, Chuanxiong, combination, pharmacological activities, synergy

Introduction
Chuanxiong (CX) is the dried root of the plant Szechuan lovage; Latin botanical name: Ligusticum chuanxiong Hort (LCH),1 which is mainly distributed in Dujiangyan, Sichuan Province (China). It was first recorded in the Divine Husbandman’s Classic of Materia Medica (Shen Nong Ben Cao Jing).2 In recent studies, the pharmacological effects of CX are found to be mainly focused on activities including analgesia, anti-inflammatory, cardiovascular benefits, anti-tumor, neuroprotection, and bone protection. Asarum, from China, belongs to the endemic form, Asarum heterotropoides f. mandshuricum (Maximowicz) Kitagawa [A. sieboldii Miquel var. mandshuricum Maximowicz; Asiasarum heterotropoides (F. Schmidt) F. Maekwa var. mandshuricum (Maximowicz) F. Maekawa], whereas f. heterotropoids are restricted to Japan.3 Asarum is demonstrated to exert significant analgesic effects, the same as CX. Furthermore, the combination of CX and Asarum exerts a synergistic analgesic effect, with some classical prescriptions, such as Chuanxiong Chatiao San clinically used to alleviate migraines,4 and Chuanxiongxixintang, used to treat neuropathic headaches.5 The aim of this article is to review the published scientific information on the chemical constituents and pharmacological activities of CX, Asarum, and CX–Asarum compositions for the benefit of further studies.
Pharmacological effects of CX

Analgesic effect. CX is a type of traditional natural medicine and has been used clinically for many years. In the field of traditional Chinese medicine (TCM), it functions by promoting the circulation of blood and alleviates pain and is used for chest and rib pain, physical injuries, irregular menstruation, amenorrhea and dysmenorrhea, abdominal pain, headaches, and rheumatism. Furthermore, a number of clinical trials have demonstrated that CX is related to the treatment of pain; therefore, many researchers have focussed on its mechanism of action from the perspective of modern medicine. Most research has focused on the effects and mechanisms of the main active components of CX on angina pectoris, migraine, dysmenorrhea, and cancer-induced pain. For instance, the relationship between CX extracts and pain relief, for example, TMP, ligustilide (LIG), ferulic acid (FA), senkyunolide, and the volatile oil from Chuanxiong (CXVO).

Initially, Peng et al. found that the CXVO is likely to be the main active ingredient of CX in curing headaches through the use of several classical animal models to explore the mechanism of CX relief pain. Chen et al. have evaluated and compared five essential oils (EOs) as penetration enhancers (PEs) to improve the transdermal drug delivery (TDD) of ibuprofen to treat dysmenorrhea. The results showed that CXVO can be viewed as a potential PE for TDD of ibuprofen to treat dysmenorrhea, and the pain inhibitory intensity of ibuprofen hydrogel was obviously increased with CXVO compared to ibuprofen without EOs. Zhang et al. demonstrated that TMP could relieve angina through the inhibition of acid-sensing ion channels (ASICs) activation. Many TCM prescriptions are routinely used for the effective treatment of migraine, such as xiongfuyin, tianshu fang, chuan xiong chatiaosan, and chuan xiong ge gentang. However, due to the complexity and variability of the compatibility of TCM, the specific mechanism of action is not completely clear. Li et al. placed an emphasis on the mechanism of the treatment of migraine through a novel systems pharmacology-based method which integrates pharmacokinetic filtering, target fishing, and network analysis, and speculated that CX mediates the major targets like PTGS2, ESR1, NOS2, HTR1B, and NOS3 to regulate the vasculature and nervous systems, as well as inflammation and pain-related pathways to benefit migraine patients. Furthermore, many researchers are interested in the effects of CX used in combination to cure migraines.

In recent years, Wu et al. discovered that Chuanxiong Rhizoma and Cyperi Rhizoma (CRCR) showed powerful therapeutic effects on migraine via increasing cerebral blood flow; the main bioactive constituents are FA, senkyunolide A (SA), 3-n-butylphthalide (NBP), Z-ligustilide (Z-LIG), Z-3-butyldienephthalide (BDPH), cyperotundone (CYT), and nookatone (NKT), in which FA, SA, NBP, LIG, and BDPH are effective constituents of CX. Yan et al. discovered that topicaly applied FA emulsion can provide a long-term stable drug concentration in blood and skin and is an effective treatment for pain.

Anti-inflammatory. CX can attenuate the liver and kidney injuries in D-galactose-treated mice by attenuating oxidative stress and inflammatory response and treating diabetes. These research findings suggested that CX extracts LIG, FA, and TMP functioned by inhibiting oxidative stress and inflammation. What is more, CX extracts, especially TMP show anti-inflammatory activity on a variety of inflammatory models. Wei et al. found that TMP was effective in the attenuation of allergic airway inflammatory changes and related chemokines and receptors in an ovalbumin (OVA)-induced asthma model. This action might be associated with inhibition of the STAT3 and p38 MAPK pathways. TMP could effectively reduce the blood–brain barrier (BBB) permeability, which may be closely associated with enhanced splenic anti-inflammatory effects activated by nAChRα7 stimulation and potentially improve cognitive recovery concerning spatial learning and memory permeability. Besides, LIG also has anti-inflammatory activity. Wu et al. discovered that LIG reduces the inflammatory response via the NF-κB pathway and reduces ROS, which may help protect the skin from the sun’s damaging UV rays.

Recently, Zhang et al. selected drug compounds for treating rheumatoid arthritis based on a network pharmacology, with data showing that lefunomide (LEF) combined with LIG worked better than LEF alone, then validated this predicted combination in a prospective clinical trial. Yuan et al. isolated three new compounds from CX, chuanxiongioside A, (2E,4E)-8-(6-O-isotiosyl)-8-oxo-2,7-dimethyloctadienoic acid chuanxiongside C, which exert anti-inflammatory activity.

Cardiovascular benefits. A recent study showed that CX extracts such as TMP, LIG, FA, and senkyunolide, has cardiovascular benefits for the treatment of cardiocerebrovascular diseases. TMP, one of the main active components, exerts significant cardiovascular benefits. Zhang et al. found that TMP significantly attenuated acid-induced ASIC currents, reduced cardiac-ischemia-induced electrical dysfunction and infarction size, and is beneficial to ward ischemic heart disease and angina. Liu et al. studied the spectrum-effect relationship and effective components of LCH and concluded that the effective components of LCH were TMP, FA, cnidilide, and LIG, which have protective effects on myocardial ischemia. In particular, TMP and FA could significantly reduce serum lactic acid in a canine model of acute myocardial ischemia, while LIG significantly reduced the elevation of serum-free fatty acids. Mak et al. added TMP to primary coronary endothelial cells (PCECs), and found that it had potent anti-ER stress capacity through which TMP normalizes soluble epoxide hydrolase (sEH) expression and confers protective effects against Ang-II on the endothelial function of coronary arteries. Further studies demonstrated that reversal of BKCa channel inhibition via suppressing ER stress-mediated loss of β1 subunits contributes to the protective effect of TMP against homocysteine on the coronary dilator function. In addition, LIG, one of the volatile constituents of
### Table 1. The Main Chemical Constituents of Chuanxiong.

| Main chemical constituent | Representative compounds | Ref |
|---------------------------|--------------------------|-----|
| Alkaloid                   | Ligustrazine (1)          | 6,7 |
|                           | Ferulic acid (2)          | 6,8 |
|                           | Vanillic acid (3)         | 9   |
|                           | Caffeic acid (4)          | 10  |
|                           | Protocatechuic acid (5)   | 9   |
|                           | p-hydroxybenzoic acid (6) | 10  |
|                           | L-tryptophan (7)          | 10  |
|                           | Gallic acid (8)           | 10  |
|                           | 3,5-O-dicaffeoylquinic acid (9) | 10 |
|                           | Chlorogenic acid (10)     | 10  |
|                           | (2E,4E)-8-(6-O-inositol)-8-oxo-2,7-dimethyloctadienoic acid (11) | 11  |
|                           | (S)-2-(2-carboxyl-2-hydroxyethylthio) ferulic acid (12) | 12  |
| Phenolic acids            |                          |     |
|                           | Caffeic acid (4)          | 10  |
|                           | Vanillic acid (3)         | 9   |
|                           | Protocatechuic acid (5)   | 9   |
|                           | p-hydroxybenzoic acid (6) | 10  |
|                           | L-tryptophan (7)          | 10  |
|                           | Gallic acid (8)           | 10  |
|                           | 3,5-O-dicaffeoylquinic acid (9) | 10 |
|                           | Chlorogenic acid (10)     | 10  |
|                           | (2E,4E)-8-(6-O-inositol)-8-oxo-2,7-dimethyloctadienoic acid (11) | 11  |
|                           | (S)-2-(2-carboxyl-2-hydroxyethylthio) ferulic acid (12) | 12  |
|                           |                          |     |
| Esters                    | Ligustilide (13)          | 6,10,13 |
|                           | Cnidilide (14)            | 6   |
|                           | Neocnidilide (15)         | 10  |
|                           | Senkyunolide I (16)       | 8,10|
|                           | Senkyunolide H (17)       | 8,10|
|                           | Senkyunolide A (18)       | 8,10,14,15 |
|                           | Senkyunolide B (19)       | 16  |
|                           | 3-butylyphthalide (20)    | 10,15|
|                           | Ligusticumolide A (21)    | 17  |
|                           | Ligusticumolide B (22)    | 17  |
|                           | Ligusticumolide C (23)    | 17  |
|                           | Ligusticumolide D (24)    | 17  |
|                           | Ligusticumolide E (25)    | 17  |
|                           | Ligusticumolide F (26)    | 17  |
|                           | Ligusticumolide G (27)    | 17  |
|                           | Levistolide A (28)        | 18  |
|                           | 3a,8′,6′,3′-diligustilide (29) | 19 |
|                           | 3,3′,8,8′-diligustilide (30) | 19  |
|                           | Ligubenzocycloheptanone A (31) | 20  |
|                           | Chuanxiongin A (32)       | 16  |
|                           | Chuanxiongin B (33)       | 16  |
|                           | Chuanxiongin C (34)       | 16  |
|                           | Chuanxiongin D (35)       | 16  |
|                           | Chuanxiongin E (36)       | 16  |
|                           | Chuanxiongin F (37)       | 16  |
|                           | Coniferil ferulate (38)   | 8,10|
|                           | Thiosenkyunolide C (39)   | 12  |
|                           | (+)-neophathalide A (40)  | 21  |
|                           | (−)-neophathalide A (41)  | 21  |
|                           | (+)-neophathalide B (42)  | 21  |
|                           | (−)-neophathalide B (43)  | 21  |
|                           |                          |     |
| Volatile oils             | β-selminene (44)          | 15  |
|                           | 1,3,5-undecatriene (45)   | 15  |
|                           | Sabinene (46)             | 22  |
|                           | Butylidenepthalide (47)   | 22  |
|                           | (+)-α-pinene (48)         | 22  |
|                           | p-cymene (49)             | 22  |
|                           | γ-terpinene (50)          | 22  |
|                           | Terpinolene (51)          | 22  |
|                           | l-phenyl-l-pentanone (52) | 22  |
|                           | Espatulenol (53)          | 22  |
|                           | Methyl-4-ethylbenzoate (54) | 22  |
|                           | p-phenylenediacetamide (55) | 22  |
|                           | Fenipentol (56)           | 22  |
|                           | 1,2,3,5,6,7-hexahydroinden-4-one (57) | 22  |
|                           |                          |     |
| Other constituents        | (E)-2-methoxy-4-(3-(methylsulfonyl)propenyl)phenol (58) | 12  |
|                           | Chuanxiongoside A (59)    | 11  |
|                           | Chuanxiongoside C (60)    | 11  |
Figure 1. (Continued)
CX, has cardiovascular benefits. Li et al.\textsuperscript{35} experimentally showed that intranasal delivery of Z-LIG enhanced protection against ischemic injury via Nrf2 and HSP70 signaling pathways and that it had prophylactic potential in populations at high risk of stroke.

In another study, Wang et al.\textsuperscript{36} discovered that ester constituents of CX concentration dependently ameliorated myocardial ischemia injury in rats, as manifested by a reduction of the infarction size, a decrease in the serum levels of cardiac enzymes and an improvement of pathological changes through activation of the PI3K/Akt/mTOR signaling pathway. Luo et al.\textsuperscript{37} investigated sodium ferulate (SF) inhibition in a rat model of myocardial hypertrophy induced by coarctation of the abdominal aorta and discovered that SF alleviated such myocardial hypertrophy (induced by coarctation of the abdominal aorta), and these protective effects could be related to the inhibition of protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) signaling pathways. Further studies\textsuperscript{38} demonstrated that a CX ethyl acetate (EA) extract showed strong activity against thrombin (THR) and butanol extract (BA) was more effective in inhibiting factor Xa (FXa) activity, being beneficial to thromboembolic disease.

\textbf{Anti-tumor.} CX possesses potent inhibitory effects on cancer, and its main bioactive constituent TMP inhibits tumor cell proliferation in a dose-dependent way, enhances tumor cell apoptosis, and thus inhibits the growth of cancer. Cao et al.\textsuperscript{39} found that TMP-inhibited cell proliferation of HepG2 cells in a dose-dependent manner, while high concentrations of TMP administration inhibited tumor growth; in addition, TMP-induced autophagy might be a pro-apoptosis process in hepatocellular carcinoma (HCC). Shen et al.\textsuperscript{40} investigated the regulatory effect of TMP on breast cancer cells and its underlying molecular mechanism of action, and revealed that TMP significantly inhibited the viability, migration and invasion rates, and increased the apoptosis of MDA-MB-231 cells in a dose-dependent manner. TMP significantly decreased the gene expression and activity of Akt and increased the activity of caspase-3, this mechanism may be responsible for the inhibition of viability, migration, and invasion, and activation of apoptosis in breast cancer cells. TMP suppressed angiogenesis and tumor growth of lung cancer via blocking BMP/Smad/Id-1 signaling.\textsuperscript{41} Luan et al.\textsuperscript{42} suggested that the TMP-mediated inhibition of human clear cell renal cell carcinoma (ccRCC) cells might occur via inhibition of the NKG2D-related signaling pathway to further suppress epithelial–mesenchymal transition (EMT) progression. TMP significantly downregulated the expression of the C–X–C chemokine receptor type 4 (CXCR4) in WERI-Rb1 cells cultured at high density, whereas it had a minor effect on low-density WERI-Rb1 cells,\textsuperscript{43} thus providing scientific evidence to improve the current situation in that most anticancer drugs damage normal cells. Furthermore, levistolide A (LA) inhibited viability and caused apoptosis of both wild-type and p53
knockout (p53−/−) HCT116 cells through the ROS-mediated ER stress pathway and is used for treating colorectal cancer (CRC).18 Hu et al.19 isolated a series of butylphthalide derivatives (BPDs) 1–8 from CX, with the results showing that BPDs 5 and 6 remarkably inhibited the migration and invasion of cancer cells and that the growth inhibition ability of BPDs against cancer cells had a close relationship with their chemical structures. Recently, many researchers found that plant polysaccharides exhibit significant anti-tumor activity. Hu et al.44 obtained CX polysaccharides through ultrasonic-assisted extraction technology, and found three novel polysaccharide fractions, LCX0, LCX1, and LCX2, of which LCX1 showed relative higher antioxidant activity and inhibitory activity toward the growth of HepG2, SMMC7721, A549, and HCT-116 cells.

Neuroprotection. TMP can exert neuroprotective functions through multiple pathways. Lu et al.45 investigated the possible protective effects and mechanisms of TMP against dopaminergic neuron injury in a rat model of Parkinson’s disease induced by MPTP and suggested that TMP prevents the down-regulation of Nrf2 and GCLc, maintaining redox balance and inhibiting apoptosis, leading to attenuation of dopaminergic neuron damage. TMP promoted NPC migration by increasing the expression and secretion of stromal-cell-derived factor 1 (SDF-1), thus exerting a neuroprotective function as well. Further studies demonstrated that this function was associated with the activation of the PI3K pathways.46 Yan et al.47 found that the PI3K/Akt/Sp1/TopoII signaling pathway was necessary for TMP-induced neuronal differentiation. Furthermore, senkyunolide H (SNH),48 isolated from the rhizome of LCH also has a neuroprotective effect via the ROS-mediated MAPK pathway. Lately, research has shown that49 a promising neuroprotective compound derived from TMP protected against glutamate-induced CGN injury, possibly in part through regulation of the PGC1α/Nrf2 and PI3K/Akt pathways. CX exerts neuroprotection by promoting adult neurogenesis and inhibition of inflammation in the hippocampus of ME cerebral ischemia rats.50 LIG protects PC12 cells from oxygen-glucose deprivation/reoxygenation-induced apoptosis via the LKB1-AMPK/mTOR signaling pathway.51 These new findings provide a novel approach and direction for the study of neuroprotection by CX.

Bone preservation. CX extracts also demonstrate have bone preservation effects, and are commonly used to treat osteoporosis (OP). Wang et al.52 investigated the effects of Du-Huo-Ji-Sheng-Tang (DHJST), a Chinese herbal medicine and its active component CX on osteogenic differentiation and the aging process of human mesenchymal cells (hMSCs), concluding that DHJST and its active component CX are able to promote osteogenic activity and decrease hMSCs senescence as cells age through increasing BMP-2 and RUNX-2 gene expression via the activation of SMAD1/5/8 and ERK signaling. TMP is used to treat glucocorticoid-induced osteoporosis (GIOP). Wang et al.53 discovered that TMP can protect BMSCs from exposure to excess glucocorticoids by promoting autophagy via the AMPK/mTOR pathway and that it might be an effective agent for the prevention and treatment of GIOP. Further studies suggested that TMP down-regulated RANKL and IL-6, promoted osteogenesis and inhibited osteoclastogenesis to ameliorate the change in bone mass in the GIOP state.54 In addition, LIG protected chondrocytes against SNP-induced apoptosis and delayed articular cartilage degeneration via suppression of the JNK and p38 MAPK pathways.55 Recently, Dong et al.56 discovered that the CX ethanol extract (CXE) could be used to treat OP by attenuating cellular reactive oxygen species levels and inhibiting osteoblast apoptosis through PI3K/Akt signaling pathway.

Other pharmacological effects. Studies have demonstrated that TMP can provide protection against memory loss in Alzheimer’s disease (AD) via inhibition of GSK-3β signaling,57 against vascular dementia (VD) through inhibition Bax, cleaved caspase-3 and Bax/Bcl-2 (Table 2).58 Yang et al.24 found that CX has potential inhibitory effects against oxidative stress and inflammation in vitro.59 Rai et al.60 suggested that TMP can be used to treat type-2 diabetes (T2D) via activation of the PI3K/Akt/GLUT-4Y pathway, and suppression of inflammation-induced insulin resistance. Furthermore, a protein-containing polysaccharide (LCP)60 and three novel polysaccharides fractions, LCX0, LCX1, and LCX2, showed strong antioxidant activity from CX.44 In addition, CXVO exerts insecticidal action,15 further studies indicating that Z-LIG exhibited remarkable larvicidal activity against S. litura larvae.51 Moreover, Hu et al.62 discovered that TMP significantly inhibited the expression of cyclin D1, cyclin E1, and cyclin-dependent kinase CDK2, inhibiting the proliferation of hepatic stellate cell (HSC) and treating hepatic fibrosis via the Hh signaling pathway. A recent study showed that CX extract-polyactic acid sustained-release microspheres significantly enhanced both the duration and hepatoprotective efficacy. Ge et al.63 discussed Ligusticum chauxiong extract-polyactic acid sustained-release microspheres (LCE-PLA) in vitro release, cytotoxicity and in vivo hepatoprotective effect, with the results demonstrating that the duration prolonged nearly five-fold and that the in vitro cytotoxicity declined 25% compared with standard LCE.

Asarum (Asarum sieboldii Miq)

The main chemical constituents of Asarum (Asarum sieboldii Miq)

Asarum originates from the dry roots and rhizomes of Aristolochiaceae plants, including Asarum heterotropoides var. Mandshuricum, A. sieboldii var. seoulense or A. sieboldii, with rich chemical composition structure types in the roots and rhizomes, including volatile oils, lignans, flavonoids, alkaloids, amides, and so on. Currently, it is widely believed that the main active components of Asarum plants are volatile oils. These include safrole, methyleugenol, kakuol, and so on. The main chemical components and plant sources of Asarum are shown in Table 3, while the
### Table 2. The pharmacological action and mechanism of Chuanxiong.

| Pharmacological effect | Substance | Mechanism | Ref |
|------------------------|-----------|-----------|-----|
| Analgesic              | CXVO      | —         | 7   |
|                        | TMP       | ↓ASIC     | 23  |
|                        | CX extract| Mediates the major targets such as PTGS2, ESR1, NOS2, HTR1B, and NOS3 | 24  |
|                        | CX extract| Increasing the cerebral blood flow | 25  |
|                        | FA        | —         | 26  |
| Anti-inflammatory       | LIG; FA; TMP| Inhibition of oxidative stress and inflammation | 28  |
|                        | TMP       | ↓STAT3, p38 MAP | 29  |
|                        | TMP       | ↓BBB permeability | 30  |
|                        | LIG       | ↓NF-κB    | 31  |
| Cardiovascular benefits|TMP        | ↓ASIC     | 23  |
|                        | TMP       | Suppressing ER stress-mediated | 34  |
|                        | Z-LIG     | regulation of Nrf2 and HSP70 signaling pathways | 35  |
|                        | LC        | ↑PI3K/Akt/mTOR | 36  |
|                        | SF        | ↓PKC/MAPK | 37  |
|                        | CX EA extract| ↓THR | 38  |
|                        | CX BA extract| ↓FXα | 38  |
| Anti-tumor             | TMP       | Inhibition of cell proliferation of HepG2 cells | 39  |
|                        | TMP       | Inhibition the viability, migration and invasion rates, and increases the apoptosis of MDA-MB-231 cells; decreases the gene expression and activity of Akt; increases the activity of caspase-3 | 40  |
|                        | TMP       | ↓BMP/Smad/Id-1 | 41  |
|                        | TMP       | ↑NKG2D    | 42  |
|                        | TMP       | ↓CXCR4    | 43  |
|                        | LA        | Active ER stress-mediated | 18  |
|                        | Butylphthalide derivatives| inhibition of the migration and invasion of SMMC772 cancer cells | 19  |
|                        | LCX1      | Inhibition of the growth of HepG2 | 44  |
| Neuroprotection        | TMP       | SMMC7721, A549 and HCT-116 cells regulation of Nrf2, GCLc | 45  |
|                        | TMP       | Regulation of the PI3K pathway | 46  |
|                        | TMP       | Regulate PI3K/Akt/Spl/Topoll pathway | 47  |
|                        | SNH       | Regulation of the MAPKs pathways | 48  |
|                        | TMP derivatives | Regulation of the PGClα/Nrf2 and PI3K/Akt pathways | 49  |
|                        | LIG       | Regulation of the LKB1-AMPK-mTOR pathway | 51  |
|                        | LC        | Promotion of adult neurogenesis and inhibition of inflammation | 50  |
| Bone protection        | CX extract| ↑SMAD 1/5/8, ERK pathways; ↑BMP-2, RUNX-2 | 52  |
|                        | TMP       | Regulation of the AMPK/mTOR pathway | 53  |
|                        | TMP       | ↓RANKL, IL-6 | 54  |
|                        | LIG       | ↓JNK, p38MAPK | 55  |
|                        | CXE       | Regulation of the PI3K/Akt signaling pathway | 56  |
| Against memory loss    | TMP       | ↓GSK-3β | 57  |
| Against vascular dementia | TMP    | ↓Bax, cleavage of caspase-3, Bax/Bcl-2 | 58  |
| Antioxidant            | LCP       | —         | 60  |
|                        | LCX; LCX1; LCX2| —         | 44  |
| Insecticidal           | CXVO      | —         | 15  |
|                        | Z-LIG     | —         | 61  |
| Liver protection       | TMP       | ↓Cyclin D1, Cyclin E1, Cyclin-dependent kinase CDK2; regulation of the Hh pathway | 62  |

↓: down-regulated; ↑: up-regulated.

structures of the primary chemical constituents of Asarum are shown in Figure 2.

### The main pharmacological effects of Asarum

Recent research has shown that the main pharmacological function of Asarum includes antipyretic and analgesic effects, anti-inflammatory effects, cardiovascular benefits, and immunosuppressive effects. The pharmacological effects and mechanisms of Asarum are shown later in Table 4.

**Antipyrretic and analgesic effects.** Yuan et al. studied the analgesic effects of Asarum and its mechanisms. These studies demonstrated that the main component responsible
for the analgesic effects of Asarum exists in the EA extract. These analgesic effects may be related to reducing the contents of nitric oxide (NO), prostaglandin E2 (PGE2), and malondialdehyde (MDA), and the activity of nitric oxide synthetase (NOS), and improving the activity of superoxide dismutase (SOD).

Studies have found that Asarum volatile oil has a significant antipyretic effect on normal and experimental fever rabbits and mice. Both the volatile oil and water extract of a single leaf of Asarum have strong analgesic effects. Asarum and its extracts, when used in combination with other TCM preparations, have obvious analgesic effects. They have beneficial effects on different pains such as toothache, neuropathic pain, and headaches, and have improved effects on peripheral pain. However, compared with morphine and pethidine, the effect is slow and lasts a long time, and the exact mechanism of action is still unclear.

Recently, Yang et al. employed an acetic acid-induced writhing, a hot-plate-induced pain test, a formalin-induced patin test and a xylene-induced auricular edema test to evaluate the anti-nociceptive and anti-inflammatory effects of ethyleugenol (a compound derived from Asarum). The results showed that ethyleugenol had significant anti-nociceptive and anti-inflammatory effects and that the mechanism is probably related to the activation of GABA receptors and inhibition of NO levels.

Table 3. The main chemical components of Asarum.

| Main chemical constituents | Representative compound | Plant source | Ref |
|----------------------------|-------------------------|--------------|-----|
| Terpenoids                 | Ocimene (61)            | ①②           | 64  |
|                            | 4-carene (62)            | ①②           | 64  |
|                            | Limonene (63)           | ①            | 65–66 |
|                            | 3-carene (64)            | ①②           | 64,65–70 |
|                            | Camphene (65)           | ①②           | 64,64,69 |
|                            | α-pinene (66)           | ①②③          | 64,67,70 |
|                            | β-pinene (67)           | ①②③          | 64,69,70 |
|                            | Linalool (68)           | ①②           | 66,67 |
|                            | Cineole (69)            | ①②           | 65–67,70,71 |
|                            | Borneol (70)            | ①②           | 65–67,69,70 |
|                            | Patchouli alcohol (71)  | ①            | 69  |
|                            | Terpinolene (72)        | ①②           | 65,66,68 |
|                            | Camphor (73)            | ①②③          | 69,70 |
|                            | Asaricin A (74)         | ①            | 72  |
|                            | Asaricin B (75)         | ①            | 72  |
|                            | Aisasarinol (76)        | ①            | 72  |
|                            | 2-exo-O-β-D-glucosyl-5-hydroxy-borneol (77) | ① | 72 |
|                            | Asiarinol A (78)        | ①            | 72  |
|                            | Carveol (79)            | ①②           | 64  |
|                            | Myrcene (80)            | ①②           | 64  |
|                            | Phellandrene (81)       | ①②           | 64  |
|                            | Bicyclohex-2-ene,2-methyl-5-(1-methylethyl) (82) | ① | 64 |
|                            | Limonene oxide (83)     | ①②           | 64  |
|                            | Thujone (84)            | ①②           | 64  |
|                            | 4-terpenenol (85)       | ①②           | 64  |

Anti-inflammatory effects. Xiong et al. have used xylene-induced Institute of Cancer Research (ICR) mouse ear edema and hot-plate tests to evaluate the anti-inflammatory and anti-nociceptive effects of Asarum at different dose levels. They discovered that both the water extracts and ethanol extracts of Asarum showed considerable anti-inflammatory potency against xylene-induced inflammation. Xu et al. showed that Asarum had an obvious anti-inflammatory effect (by employing the 1.6 g/kg EA extract of Asarum) on ear inflammation of mice caused by xylene and the hyperpermeability of capillarity caused by acetic acid. Moreover, the extract after aristolochia removal also had a reliable anti-inflammatory effect. Phitak et al. found that asarinin has an anti-inflammatory effect by inhibiting some pathways of IL-1β signal transduction: p38 MAPK and C-Jun amino-terminal kinase (JNK). Zhang et al. studied the protective effects of Asarum extract on rats with adjuvant arthritis (AA) and to determine the underlying mechanism, the obtained results demonstrated that Asarum extract significantly increased the phosphorylation of IKKB, IkB, and p65, which results in the activation of the NF-kB signaling pathway and was found to significantly inhibit the phosphorylation of P38 and ERK, thereby blocking the activation of the MAPK signaling pathway.

In recent years, studies have shown that Asarum inhibits the amount of NF-kB in microglia after activation and the
Table 3. (Continued)

| Main chemical constituents | Representative compound | Plant source | Ref |
|----------------------------|--------------------------|--------------|-----|
|                            | Longifolene (86)         | 1 2          | 64  |
|                            | Ledene (87)              | 1 2          | 64  |
|                            | Caryophyllene oxide (88)| 1 2          | 64  |
|                            | Cubenol (89)             | 1 2          | 64  |
|                            | Kaurene (90)             | 1 2          | 64  |
|                            | 2,4-decadienal (91)      | 1 2          | 64  |
|                            | Verbenol (92)            | 1 2          | 64  |
|                            | Piperitone (93)          | 1 2          | 64  |
|                            | (-)-β-elemene (94)       | 1 2          | 64  |
|                            | Thujopsene (95)          | 1 2          | 64  |
|                            | Aristolene (96)          | 1 2          | 64  |
|                            | Spathulenol (97)         | 1 2          | 64  |
|                            | Bisabolol (98)           | 1 2          | 64  |
|                            | Cadinol (99)             | 1 2          | 64  |
|                            | Spathulenol (100)        | 1 2          | 64  |
|                            | 3,5-dimethoxytoluene (102)| 1 2          | 64  |
|                            | Estragole (103)          | 1 2          | 66  |
|                            | Asarone (104)            | 1 2          | 66  |
|                            | Elemicin (105)           | 1 2          | 74  |
|                            | Safrole (106)            | 1 2          | 65  |
|                            | Myristicin (107)         | 1 2          | 67  |
|                            | Kakuol (108)             | 1 2          | 69  |
|                            | Thymol (109)             | 1 2          | 64  |
|                            | Cyclohexene, 1-methyl-4-(1-methylethylidene) (110)| 1 2          | 64  |
|                            | Guaiazulene (111)        | 1 2          | 64  |
|                            | Disobutyl phthalate (112)| 1 2          | 64  |
|                            | 1-(2-hydroxy-5-methylphenyl) ethenone (113)| 1 2          | 64  |
|                            | 4-allylanisole (114)     | 1 2          | 64  |
|                            | Aristololactamosome i (115)| 1          | 77  |
|                            | (-)-Asarinin (116)       | 1 2          | 65  |
|                            | (-)-Sesamin (117)        | 1 2          | 70  |
|                            | Asarinin b (118)         | 1 2          | 72  |
|                            | (1r,2s,5r,6r)-5'-o-methylpluviatill (119)| 1 2          | 72  |
|                            | Xanthoxylol (120)        | 1 2          | 72  |
|                            | Clemaphenol a (121)      | 1 2          | 72  |
|                            | Epipinosinosol (122)     | 1 2          | 72  |
|                            | (-)-Piperitol (123)      | 1 2          | 72  |
|                            | Episesaminone (124)      | 1 2          | 72  |
|                            | Neoasarininoside a (125) | 1 2          | 72  |
|                            | Neoasarininoside b (126) | 1 2          | 72  |
|                            | Neoamarin a (127)        | 1 2          | 72  |
|                            | Neoasarinb (128)         | 1 2          | 72  |
|                            | Neoasarinin c (129)      | 1 2          | 72  |
|                            | Pentadecane (130)        | 1 2          | 64  |
|                            | Verbenone (131)          | 1 2          | 64  |
|                            | Eucarvone (132)          | 1 2          | 65  |
|                            | Nonanal (133)            | 1 2          | 64  |
|                            | 4,6,4′-trihydroxyaurone-4,6-di-o-β-d-glucopyranoside (134)| 1          | 79  |
|                            | Naringenin (135)         | 2 3          | 79  |
|                            | Naringenin-5-o-β-d-glucopyranoside (136)| 2 3          | 79  |
|                            | Naringenin-7-o-β-d-glucopyranoside (137)| 2 3          | 79  |
|                            | Naringenin-5,7-di-o-β-d-glucopyranoside (138)| 2 3          | 79  |
|                            | Naringenin-7,4′-di-o-β-d-glucopyranoside (139)| 2 3          | 79  |
|                            | Chalcononaringenin-2′-o-β-β-d-glucopyranoside (140)| 2 3          | 79  |
|                            | Chalcononaringenin-2′,4′-di-o-β-d-glucopyranoside (141)| 1          | 79  |

1 Asarum heterotropoides var. Mandshuricum 2 A. sieboldii var. seoulense 3 A. sieboldii.
Figure 2. (Continued)
Figure 2. (Continued)
Figure 2. The structures of some of the chemical constituents of Asarum: (a) terpenoids, (b) aromatic compounds, (c) alkaloids, (d) lignans, (e) aliphatic compounds, and (f) glycosyl flavonoids.

Table 4. The pharmacological effects and mechanisms of Asarum.

| Pharmacological effect | Substance               | Mechanism                                                                 | Ref  |
|------------------------|-------------------------|---------------------------------------------------------------------------|------|
| Antipyretic and analgesic effect | Ethyl acetate extract of asarum | ↓NO, PGE2, MDA, NOS, ↑SOD                                                | 80   |
|                        | Asarum volatile oil     | Activation of GABA receptor and inhibition of NO                          | 81   |
|                        | Methyleugenol           |                                                                            | 82   |
| Anti-inflammatory effect | Asarinin                | Inhibition of the IL-1 signal transduction pathway and p38 MAPK and C-Jun amino-terminal kinase (JNK) | 83   |
|                        | Asarum                  | ↑IKKβ, IκB, p65, NF-κB, inhibition of the phosphorylation of P38 and ERK, blocking the activation of the MAPK signaling pathway; | 84   |
|                        | Asarum volatile oil     | Inhibition of the amount of NF-κB in microglia after activation and the inflammatory factors IL-1β and TNF-α | 85   |
|                        | Asarinin and sesamin    | Interacted with the iNOS and MAPK14,                                      | 86   |
|                        | Safrole and sarisan     | Interaction with iNOS, COX-1 and LAT4 H                                   | 86   |
|                        | Methyleugenol           | Interaction with COX-1 and LAT4 H                                         | 86   |
| Immunosuppressive effect | Asarinin                | Inhibition of the expression level of CAM-1 and VCAM-1                   | 87   |
|                        | Asarum                  | Regulation of caspase 3, III, I20, matriptase2, Nf-κB, Rag2, Tmprss6, Prkag3, Nptx2, Antx1, Klk1 I, Ollr77, Cd7, LOC69, C6, LOC68, Cd163, Ampk, Bcl2 and Ccl20, Cd163Ampk, Bcl2 and Ccl20 | 88   |
| Cardiotonic effect     | Asarum extract          | ↑LVP, MAP                                                                  | 89   |
|                        | AEO                     | Enhances the Na+ channel current of myocardial cells                      | 90   |
| Regulate blood pressure | Asarum                  | Bidirectional regulation effect                                           | 91   |
|                        | Asarum                  | Reduces the blood pressure of rabbits                                     | 91   |
| Effect on blood vessels | Sesamin                 | Activation of the MEK/ERK-, PI3K/Akt/eNOS-, p125FAK-, and p38 MAPK-dependent pathways | 93   |
|                        | Sesamin                 | Inhibition of TNF-α-induced NF-κB translocation; activation of TRPV1-calcium signaling, including the phosphorylation of PKA, CaMKII, CaMKKβ, Akt, and AMPK, triggered eNOS activity and NO production; | 94   |
|                        | ESM                     | Inhibition of PMA, TNF-α, IL-1β, and CLP-induced EPCR shedding            | 95   |
| Antibacterial and antiviral effects | Safrole                 | –                                                                         | 96   |
|                        | Asarum                  | Anti-human papillomavirus                                                  | 97   |
|                        | Asarum                  | Damages Gram-positive bacteria                                             | 98   |
|                        | Asarum                  | Direct inactivation effect on NDV                                           | 99   |
|                        | Asarum                  | Interferes with the replication of the swine flu virus                     | 100  |
|                        | Asarum                  | Against the H1N1 influenza virus                                           | 101  |
|                        | L-asarinin, L-sesamin, and kakuol | Antibacterial activities against EC, SAs, PN, PAK, and CA            | 102  |

(Continued)
Table 4. (Continued)

| Pharmacological effect | Substance       | Mechanism                                                                 | Ref  |
|------------------------|-----------------|---------------------------------------------------------------------------|------|
| Relieving cough and    | AEO             | Inhibitory activity toward bacteria                                        | 103  |
| asthma                 | Methyleugenol   | –                                                                         | 104  |
|                        | Asarinin        | Improve immunity and block virus invasion                                  | 105  |
|                        | AEO             | –                                                                         | 106  |
|                        | AP              | Might be related with anti-inflammatory and antioxidant activity          | 107  |
| Anti-aging effect       | Asarum          | ↓iNOS, NO, SOD, GSH-Px, MDA, LOP, scavenging free radicals                 | 108  |
|                        | Methyleugenol   | Inhibited the production of (NO), ↓iNOS                                   | 109  |
|                        | Sesamin, asarinin| –                                                                         | 110  |
|                        | Mg              | Against t-BHP-triggered cytotoxicity via the activation of the AMPK/GSK3β- and ERK-Nrf2 signaling pathways | 111  |
| Improve body metabolism| AP              | –                                                                         | 112  |
| Local anesthesia       | Higenamine      | Strenthenes the heart, dilates blood vessels, relaxes smooth muscle,     | 113  |
| On the nervous system  | Methyleugenol   | Inhibits Na⁺ channels                                                      | 114  |
|                        | Asarum          | Brain CRF and TH expression increases, and brain 5-HT expression         | 115  |
|                        | Methyleugenol   | Inhibits epileptic seizures in SD rats in epilepsy models                 | 116  |
|                        | Asarum          | inhibits the activity of HL-60, BGC-823, KB and Bel-7402)                 | 117  |
|                        | Asarinin        | ↓PKA-CREB-TH system and protects against 6-OHDA-induced cytoxicity, inhibits the continuous activation of the ERK-p38MAPK-JNK1/2-caspase-3 system | 118  |
| in vitro anticancer     |                |                                                                           |      |
| activity               | (−)-asarinin    | Increases the activation of caspase-3, caspase-8, and caspase-9           | 119  |

Inflammatory factors IL-1β and tumor necrosis factor (TNF)-α to prevent and treat neuroinflammation mediated by microglia. Liu et al. conducted network pharmacological analysis of 119 constituents of Asari Radix et Rhizoma, showing that the anti-inflammatory effect of Asari Radix et Rhizoma might be related to eight targets including COX-2, COX-1, iNOS, MAPK14, (LAT)4H, NR3 C1, PPARG and TNF, and so on. Among them, COX-2 is a key target, which interacts with the five characteristic constituents: asarinin, sesamin, safrole, methyleugenol and sarisan. Of these, asarinin and sesamin also interacted with iNOS and MAPK14, and safrole and sarisan also interacted with iNOS, COX-1, and LAT4 H, while methyleugenol interacted with COX-1 and LAT4 H.

Immunosuppressive effects. Zhang et al. administered cyclosporin A and asarinin to rats one day before heart transplant surgery to observe their anti-acute rejection effects and their effects on adhesion molecules. The immunosuppressive effects were similar to ciclosporin (CsA), showing that asarinin may play an important role in suppressing the immune reaction, prolonging the allograft survival times and in protecting the donor organ; the expression level of ICAM-1 and VCAM-1 is increased in suppressing the course of acute rejection, and asarinin can inhibit their expression level. Li et al. showed that Asarum selectively altered the expression of immune-related genes in the lungs by regulating caspase 3, Il1, Il20, matptase2, Nf-xB, Rag2, Tmprss6, Prkag3, Nptx2, Antx1, Klk11, Ollr77, Cd7, LOC69, C6, LOC68, Cd163, Ampk, Bcl2, and Ccl20.

Cardiovascular effects

Cardiotonic effects. The heart-strengthening effect of Asarum extract is similar to that of dopamine, isoproterenol, and noracoonitine. In vitro experiments show that Asarum volatile oil has a significant excitatory effect on the hearts of rabbits, mice, and cardiogenic shock dogs. Its positive muscle strengthening, positive frequency effects, and its mechanism of action are as follows: Asarum extract can increase left ventricular pressure (LVP), and mean arterial pressure (MAP) and cardiac output,89 and its mechanism of action is as follows: Asarum extract can increase left ventricular pressure (LVP), and mean arterial pressure (MAP) and cardiac output in dogs with cardiogenic shock, increase volume, as well as: heart rate, myocardial contractility, and so on. Asarum alcohol extract and Asarum water decoction can obviously excite isolated rabbit and guinea pig hearts, and manifests is to increased coronary blood flow of the isolated heart, accelerate the heart rate, and enhances the contractility of the myocardium. Moreover, Asarum volatile oil can weaken the acute myocardial ischemia caused by rabbit pituitary gland and increase the tolerance of mice to decompression and hypoxia. At the same time, Asarum can markedly enhance the Na⁺ channel current of myocardial cells.90

Regulation of blood pressure. Asarum can reduce blood pressure in patients with elevated blood pressure and increase blood pressure in patients with decreased blood pressure, which represents a bidirectional regulation effect. Huang showed that Asarum volatile oil significantly expands the visceral blood vessels of toads and reduce the blood pressure of anesthetized cats. Astheno infusion can reduce the blood pressure of anesthetized dogs, showing an adrenergic effect. Ma et al. used norepinephrine to simulate rabbit
blood pressure in an experiment on the effect of Asarum extract and found that Asarum water-soluble substances can increase the blood pressure of rabbits acting on norepinephrine and that the volatile oil substances contained in Asarum can reduce the blood pressure of rabbits.

**Effect on blood vessels.** Chung et al.93 suggested that sesamin stimulates angiogenesis in vitro and in vivo through the activation of MEK/ERK-, PI3K/Akt/eNOS-, p125FAK-, and p38 MAPK-dependent pathways, without increasing vascular inflammation, and can be used for the treatment of ischemic diseases and tissue regeneration. Ku et al.95 showed that epi-sesamin (ESM), likely through suppression of TACE expression, induces potent inhibition of PMA, TNF-α, IL-1β, and CLP-induced EPCR shedding. At the same time, ESM should be viewed as a candidate therapeutic agent for the treatment of various severe vascular inflammatory diseases via inhibition of EPCR shedding. ESM therapy reduces PMA-stimulated phosphorylation of p38, extracellular regulatory kinase (ERK) 1/2, and JNK, which should be considered as a candidate therapy for a variety of severe vascular inflammatory diseases by inhibiting EPCR shedding. Furthermore, Pham et al.94 showed that sesamin may be useful for treating or preventing endothelial dysfunction correlated with cardiovascular diseases. What is more, the mechanism of action of sesamin occurs through the inhibition of TNF-α-induced NF-κB translocation, intercellular adhesion molecule-1 expression, and monocyte adhesion. Sesamin also triggers eNOS activity and NO production via activation of TRPV1-calcium signaling, including the phosphorylation of PKA, CaMKII, CaM KKβ, Akt, and AMPK.

**Antibacterial and antiviral effects.** Asarum volatile oil was discovered as a broad-spectrum antifungal drug as early as 1981, and safrole was identified as the main active antifungal ingredient.96 Deng et al.97 screened the anti-HPV effective fraction from Asarum heterotropoides, and found that the water extracts from Asarum heterotropoides were effective against anti-Human papillomavirus, with a minimum effective concentration of 0.4 g/mL. Zhang102 showed that L-asararin, L-sesamin, and kakukol had antibacterial activities against Escherichia coli (EC), Staphylococcus aureus (SA), Pseudomonas aeruginosa (PAK), and Candida albicans (CA). Perumalsamy et al.98 compared the growth-inhibiting and morphostructural effects of seven constituents identified in Asarum heterotropoides root on 14 intestinal bacteria. The results showed that Asarum caused physical damage and morphological changes to Gram-positive bacteria to different degrees. Yu et al.103 showed that the EO had a significant inhibitory activity on five species of tested Fusarium, which occurred in a dose-dependent manner based on the concentration of the EO. In addition, Asarum volatile oil obtained under different process conditions had good inhibitory activity on five kinds of bacteria (Staphylococcus epidermidis, Propionibacterium freudenreichii, Micrococcus luteus, Corynebacterium jeikeium, and Corynebacterium xerosis) producing odor, with the strongest inhibitory activity against M. Luteus bacteria. Among them, the minimum inhibitory concentration (MIC) of methyleugenol in the EO was up to 0.3 mg L⁻¹, which was the main effective component of the anti-bacterial activity.104

Asarum has a direct inactivation effect on NDV, and the therapeutic index is 64.99 Asarum can also interfere with the replication of the swine flu virus, thus acting as an antiviral.100 Wu et al.105 reported a preliminary exploration of the mechanism of Qingfei Paidu decoction against novel coronavirus pneumonia based on network pharmacology and molecular docking technology. It was found that asarinin from Asarum can directly act on human cells, improve immunity and block virus invasion. Yang et al.101 analyzed the Asarum polysaccharides (APs) activity of against H1N1 influenza virus in vitro and its intervention effect on mice with kidney-Yang deficiency syndrome and found that AP had good anti-influenza virus activity in vitro, and could protect mice with kidney-Yang deficiency syndrome by reducing the viral load in lung tissue, decreasing inflammation damage in lung tissue, and by regulating the expression of inflammatory cytokines.

**Relieving cough and asthma.** The EO is the active part of Asarum for relieving coughs and asthma.122 Liu et al.106 established the HPLC fingerprints of different extracts, which were isolated with a gradient series of solvents and analyzed the relationship of cough relieving and asthma preventing with relative peak areas in the finger prints, in order to explore the material basis of Asari Radix et Rhizoma for relieving asthma and cough. The results showed that the constituents corresponded to 12 chromatographic peaks are active basis of Asari Radix et Rhizoma, in which methyleugenol and 11 activity constituents are detected in HPLC fingerprints of Asari Radix et Rhizoma gel, except for the volatile oil. Liu and Li107 evaluated the anti-tussive, anti-inflammatory, and anti-oxidant effects of AP in a guinea pig model for chronic cough and found that the AP from Asarum Heterotropoides have significant anti-tussive activity in the guinea pig model for chronic cough induced by (2-chlorobenzylidene)malononitrile-exposure, which might be related with anti-inflammatory and antioxidant activity.

**Anti-aging effects.** Asarum has a certain anti-aging effect. It can improve the activity of nitric oxide synthase (iNOS), reduce the content of malonaldehyde (MDA), scavenge free radicals, and increase the content of NO, and reduce the damage of oxygen free radicals to cell lipids. At the same time, it can also increase the activity of SOD, enhance the ability of the bodies to scavenge free radicals, and reduce the damage of free radicals to the body.108 Asarum can significantly increase the activity of glutathione peroxidase (GSH-Px) in the heart and liver tissues of old mice and inhibit free radical reactions.123 Choi et al.109 tested the cytoprotective effect of methyleugenol in an in vivo ischemia model, and found that it inhibited the production of NO and decreased the protein expression iNOS, down-regulated the production of pro-inflammatory cytokines in the ischemic brain as well as in immune-stimulated mixed glial cells, indicating that methyleugenol can be useful for the treatment of ischemia/inflammation-related diseases. Liu110 revealed that sesamin and asarinin play significant roles in the antioxidant activities. With asarinin, the total
antioxidant capacity (T-AOC) content of cells increased by 292.6%, SOD increased by 307% and MDA decreased by 413%; with sesamin, the results were 305.2%, 394%, and 41%, respectively, which suggested that sesamin and asarinin can delay cell aging.

More recently, Zhou et al.116 explored the anti-oxidative potential of methyleugenol (Mlg) against tert-butyl hydroperoxide (t-BHP)-triggered oxidative injury and the involvement of anti-oxidative mechanisms. They found that Mlg might exhibit a protective role against t-BHP-triggered cytotoxicity via activation of the AMPK/GSK3β and ERK-Nrf2 signaling pathways. Ren et al.117 optimized the extraction process of APs and evaluated the protective effect of AP on the senescence of vascular smooth muscle cells, showing that AP pretreatment significantly reduced the morphological changes of cellular senescence, improved cell viability, and down-regulated the level of senescence β-galactosidase activities induced by etoposide.

**Improving body metabolism.** As one of the important material bases of dispersing cold, higenamine isolated from Asarum can improve the metabolic function of the body. It has extensive pharmacological effects such as receptor agonism, which can strengthen the heart, dilate blood vessels, relax smooth muscle, enhance lipid metabolism, and increase blood glucose.113

**Local anesthesia.** The anesthetic properties of methyleugenol have been demonstrated by a loss of the righting reflex and a decreased sensitivity to tail pinching in rats and mice, and a loss of the corneal reflex in rabbits.124,125 Wang et al.114 suggested that the antinociceptive and anesthetic effects of methyleugenol result from the inhibitory action of methyleugenol on peripheral Na⁺ channels. Therefore, methyleugenol is a potential candidate as an effective local anesthetic and analgesic.

**Effects on the nervous system.** Kim et al.115 studied the effect of the fragrance inhalation of EO from *Asarum heterotropoides* (EOAH) on depression-like behavior in mice, with their results suggesting that EOAH effectively inhibits depression-like behavioral responses, while brain corticotropin releasing factor (CRF) and tyrosine hydroxylase (TH) expression increases, and brain serotonin (5-HT) expression decreases in mice challenged with stress. Methyleugenol, a monomer in Asarum volatile oil, can inhibit epileptic seizures in epilepsy models of (Sprague–Dawley) SD rats and can prolong the incubation period of epileptiform discharge clusters.116

**In vitro anticancer activity.** Cai et al.117 studied the cytotoxic activity of Asarum on some tumor cell lines using the extract of Asarum. The inhibitory activity of Asarum extract on four tumor cell lines (HL-60, BGC-823, KB and Bel-7402) indicated that Asarum extract had certain anti-tumor effects. Park et al.118 investigated the effects of asarinin on dopamine biosynthesis and 6-hydroxydopamine-induced cytotoxicity in PC12 cells, with the results indicating that asarinin induces dopamine biosynthesis via activation of the PKA-CREB-TH system and protects against 6-OHDA-induced cytotoxicity by inhibiting the continuous activation of the ERK-p38MAPK-JNK1/2-caspase-3 system in PC12 cells.

In their study, Jeong Miran et al.119 showed that (-)-asarmin from the roots of *A. sieboldii* may induce caspase-dependent apoptotic cell death in human cancer cells, and by increasing the activation of caspase-3, caspase-8, and caspase-9 in ovarian cancer cells. Pretreatment with caspase inhibitors attenuated the cell death induced by (-)-asarmin. Aristolobolic acid from Asarum also has a certain anti-tumor effect. The experimental results on aristolobolic acid in animals have shown that it has anti-tumor activity and can enhance the phagocytosis ability of leukocytes,126 but aristolobolic acids have been proved to have some renal toxicity,127 and carcinogenic and mutagenic effects.128,129

**CX Asarum composition**

**Chemical constituents of compositions**

The common components of CX and Asarum are volatile oils, which are the main active ingredients of medicines to promote blood circulation and to relieve pain.130 Volatile oils from this composition are different from CX or Asarum alone, and not only the simple sum of these two drugs. The volatile oil constituents of the compositions are listed in Table 5. Complicated chemical reactions and physical changes appear when CX is compatible with Asarum, the proportion and content of each component will change, and some components will be lost and some new components will be created. Li et al.131 used HPLC to study the change of the chemical components before and after the combination of CX and Asarum, and they found that the contents of the main active ingredients, FA and LIG, increased significantly after this progress, especially the former, which has remarkable analgesic and anti-inflammatory effects, and contributes to enhance the analgesic effect (Figure 3).

**Pharmacological effects of CX–Asarum compositions**

CX is set to become the treatment of choice for headaches.134 Experimental studies and clinical trials have demonstrated that it exerts synergistic analgesic effects when CX combination with Asarum is used to alleviate different types of pain, such as headache, prosopalgia, backache and toothache, especially cold headaches.135 Liu et al.136 have collected Chinese herbal prescriptions for migraine treatment, which were published in the VTTMS, CNKJ, and WanFang databases from January 1989 to March 2018 and that were demonstrated to have clinical effectiveness. They also explored the compatibility rule of TCM in the treatment of migraine, and found that the CX Asarum composition commonly used to treat migraines functioned by activating vital energy and blood circulation. Zhang et al.137 observed the effect of Chuanxiong xintiang in treating neuropathic headaches in 48 cases, with the results showing that the clinical cure rate was 100%. Furthermore, CX Asarum composition is used to treat cold-induced backache
Table 5. Volatile oil constituents of Chuanxiong–Asarum compositions.

| Constituents                                      | Chemical formula | Ref |
|--------------------------------------------------|------------------|-----|
| Isodurenol                                       | C$_{10}$H$_{14}$O | 131 |
| 2-methoxy-4-vinylphenol                          | C$_{6}$H$_{12}$O$_2$ | 131 |
| Isoledene                                        | C$_{7}$H$_{14}$ | 131 |
| β-patchoulene                                    | C$_{13}$H$_{24}$ | 131 |
| β-elemene                                        | C$_{13}$H$_{24}$ | 131 |
| Methyleugenol                                    | C$_{8}$H$_{14}$O$_2$ | 131 |
| Tetradecane                                      | C$_{14}$H$_{30}$ | 131 |
| 1,2,3-trimethoxy-5-methylbenzene                 | C$_{9}$H$_{20}$O$_3$ | 131 |
| (-)-aristolene                                   | C$_{13}$H$_{24}$ | 131 |
| β-copaene                                        | C$_{15}$H$_{24}$ | 131 |
| (+)-calrene                                      | C$_{15}$H$_{24}$ | 131 |
| α-copaene                                        | C$_{15}$H$_{24}$ | 131 |
| α-guaiene                                        | C$_{15}$H$_{24}$ | 131 |
| Myristicin                                       | C$_{11}$H$_{12}$O$_3$ | 131 |
| γ-cadinene                                       | C$_{15}$H$_{24}$ | 131 |
| Cyclopentadecane                                 | C$_{15}$H$_{30}$ | 131 |
| β-eudesmene                                      | C$_{15}$H$_{24}$ | 131 |
| α-longipinene                                    | C$_{15}$H$_{24}$ | 131 |
| Pentadecane                                      | C$_{15}$H$_{32}$ | 131 |
| δ-guaiene                                        | C$_{15}$H$_{24}$ | 131 |
| α-cadinene                                       | C$_{15}$H$_{24}$ | 131 |
| trans-isomyristicin                              | C$_{15}$H$_{12}$O$_3$ | 131 |
| 3,4-methylenedioxypropiophenone                  | C$_{10}$H$_{16}$O$_2$ | 131 |
| Elemicin                                         | C$_{12}$H$_{16}$O$_3$ | 131 |
| Nerolidol                                        | C$_{12}$H$_{16}$O | 131 |
| Asarone                                          | C$_{12}$H$_{16}$O$_3$ | 131 |
| Spathulenol                                      | C$_{13}$H$_{26}$O | 131 |
| Alloaromadendrene                                | C$_{13}$H$_{26}$O | 131 |
| Cedrol                                           | C$_{13}$H$_{26}$O | 131 |
| Valencene                                        | C$_{13}$H$_{26}$O | 131 |
| Patchouli alcohol                                | C$_{13}$H$_{26}$O | 131 |
| 3-butyldiene-1(3H)-isobenzofuranone              | C$_{13}$H$_{12}$O$_2$ | 131 |
| Senkyunolide A                                   | C$_{13}$H$_{16}$O$_2$ | 131 |
| cis-ligustilide                                  | C$_{13}$H$_{16}$O$_2$ | 131 |
| trans-ligustilide                                | C$_{13}$H$_{16}$O$_2$ | 131 |
| n-hexadecanoic acid                              | C$_{16}$H$_{32}$O$_2$ | 131 |
| Hexadecanoic acid, ethyl ester                   | C$_{18}$H$_{36}$O$_2$ | 131 |
| Kaur-16-ene                                      | C$_{20}$H$_{32}$ | 131 |
| 9,12-octadecadienoic acid, methyl ester          | C$_{18}$H$_{24}$O$_2$ | 132 |
| 9,12-octadecadienoic acid (Z, Z)-                | C$_{18}$H$_{24}$O$_2$ | 132 |
| Linoleic acid, ethyl ester                       | C$_{20}$H$_{36}$O$_2$ | 132 |
| α-thujene                                        | C$_{10}$H$_{16}$ | 132 |
| β-pinene                                         | C$_{10}$H$_{16}$ | 132 |
| Camphene                                         | C$_{10}$H$_{16}$ | 132 |
| β-myrcene                                        | C$_{10}$H$_{16}$ | 132 |
| α-phellandrene                                   | C$_{10}$H$_{16}$ | 132 |
| 2-carene                                         | C$_{10}$H$_{16}$ | 132 |
| 3-carene                                         | C$_{10}$H$_{16}$ | 132 |
| 3-isovalyl-5,5-dimethylcyclopentene              | C$_{10}$H$_{16}$ | 132 |
| 1,8-cineole                                      | C$_{10}$H$_{18}$O | 132 |
| (E)-β-ocimene                                    | C$_{10}$H$_{18}$ | 132 |
| β-terpinene                                      | C$_{10}$H$_{18}$ | 132 |
| Terpinolene                                      | C$_{10}$H$_{18}$ | 132 |
| Endo-borneol                                     | C$_{10}$H$_{18}$O | 132 |
| Methyl piperphenol                               | C$_{10}$H$_{12}$O | 132 |
| Carvacrolmethyl ether                            | C$_{11}$H$_{16}$O | 132 |
| 3,5-dimethoxytoluene                             | C$_{6}$H$_{12}$O$_2$ | 132 |

(Continued)
Table 5. (Continued)

| Constituents                        | Chemical formula | Ref |
|-------------------------------------|------------------|-----|
| Safrole                             | C10H10O2         | 132 |
| 3-n-butylphthalide                  | C12H14O2         | 132 |
| 4,5-dihydrobutylphthalide           | C12H16O          | 132 |
| Pentadecanoic acid                  | C15H30O2         | 132 |
| Ethyl hexadecanoate                 | C18H36O2         | 132 |
| Methyl linoleate                    | C19H34O2         | 132 |
| Isopropyl (2E,4Z,8Z,10E)-dodecanetetramide | C16H25NO       | 132 |

Figure 3. The chemical constituents and pharmacological effects of Chuanxiong–Asarum compositions.

and injury backache.\textsuperscript{138} Asarum with the function of promoting the circulation of qi, tongqiao and relieving depression, expelling wind and alleviating pain, is set to become the treatment of choice for tooth disease, soremouth, and headache, blends with CX used to treat tooth disease, especially toothache.\textsuperscript{138} Su et al.\textsuperscript{140} have analyzed effect of 101 prescriptions on allergic rhinitis (AR) and discovered that the most popular TCM appeared to be CX, radix angelicae and Asarum, and CX Asarum composition commonly used in TCM. The main components of this composition are volatile oils, which are the main constituents for curing AR with the function of promoting the circulation of qi and dredging collaterals.

Conclusion

This review focuses on the chemical components and pharmacological actions of CX, Asarum and CX–Asarum compositions. Overall, the chemical components and pharmacological actions of CX and Asarum have been thoroughly investigated. The main active constituents of CX are alkaloids, which demonstrate analgesic, anti-inflammatory, cardiovascular benefits, anti-tumor, neuroprotection, and bone protection, in which TMP exerts significant anti-tumor benefits. Studies revealed that TMP inhibits tumor cell proliferation in a dose-dependent way, appearing as another promising potential cancer drug. The main active ingredients of Asarum are volatile oils, which impact analgesic, anti-inflammatory, and cardiovascular benefits.

Reducing toxicity and increasing therapeutic effects can be achieved by employing the compatibility of medicines. CX compatible with Asarum exerts synergistic analgesic effects, used to alleviate different types of pain, such as headaches, prosopalgia, backache, and toothache, especially cold headache. The main components of CX Asarum composition are volatile oils, which are important constituents with the function of promoting the circulation of the blood and alleviating pain. It is worth to focusing on changing the volatile oil constituents of compositions, however, not little many research has been done in this area. Furthermore, the contents of FA increased significantly after compatibility, which has prominent analgesic effects, and contributes to enhancement of the analgesic effects in treatments.

In conclusion, CX and Asarum had many pharmacological effects and a wide-spectrum of clinical applications. Many experimental studies and clinical trials have demonstrated that CX Asarum composition exerts significant synergistic analgesic effects; therefore, it is of great values study the chemical components and pharmacological
mechanisms of its compositions, in order to provide evidence-based medicine and promote the improvement of clinical treatment. However, CX and Asarum both have anti-inflammatory action, cardiovascular benefits and anticancer activity, and the contents of LIG increased significantly after compatibility, which has prominent cardiovascular benefits. There are also potential synergistic anti-inflammatory effects, cardiovascular benefits, and anticancer effects on combining these two natural medicines. This aspect is worthy of further study and to provide scientific basis for clinical practice and expand clinical applications (Table 6).

Table 6. The list of abbreviations and definitions.

| Abbreviations | Definitions |
|---------------|-------------|
| CX            | Chuanxiong  |
| TMP           | Ligustrazine|
| TCM           | Traditional Chinese medicine |
| LIG           | Ligustilide |
| FA            | Ferulic acid|
| CXVO          | Volatile oil from Chuanxiong |
| EO            | Essential oils |
| PEs           | Penetration enhancers |
| TDD           | Transdermal drug delivery |
| ASICs         | Acid-sensing ion channels |
| CRCR          | Chuanxiong Rhizoma and Cyperi Rhizoma |
| SA            | Senkyunolide A |
| NBP           | 3-β-butylyphthalide |
| Z-LIG         | Z-ligustilide |
| BDPH          | Butylenephthalide |
| CYT           | Cypertundone |
| NKT           | Nookatone |
| OVA           | Ovalumine |
| BBB           | Blood–brain barrier |
| LEF           | Lefunomide |
| LCH           | *Ligusticum Chuanxiong* Hetrt |
| PCECs         | Primary coronary endothelial cells |
| sEH           | Soluble epoxide hydrolase |
| SF            | Sodium ferulate |
| PKC           | Protein kinase C |
| MAPK          | Mitogen-activated protein kinase |
| EA            | Ethyl acetate |
| THR           | Thrombin |
| BA            | Butanol |
| FXa           | Factor Xa |
| HCC           | Hepatocellular carcinoma |
| ccrCC         | Clear cell renal cell carcinoma |
| EMT           | Epithelial–mesenchymal transition |
| CXCR4         | The C–X–C chemokine receptor type 4 |
| LA            | Levistolide A |
| CRC           | Colorectal cancer |
| BPDs          | Butylphthalide derivatives |
| SDF-I         | Stromal-cell-derived factor I |
| SNH           | Senkyunolide H |
| DHJST         | Du-Huo-Ji-Sheng-Tang |
| hMSCs         | Human mesenchymal cells |
| GIOOP         | Glucocorticoid-induced osteoporosis |
| CXE           | Chuanxiong ethanol extract |
| OP            | Osteoporosis |
| AD            | Alzheimer’s disease |
| VD            | Vascular dementia |
| T2D           | Type-2 diabetes |
| LCP           | Polysaccharide from *Ligusticum chuanxiong* hort |
| HSC           | Hepatic stellate cell |
| LCE-PLA       | *Ligusticum chuanxiong* extract-polyacetic acid |
| CX EA         | Chuanxiong ethyl acetate extract |
| CX BA         | Chuanxiong butanol extract |
| LC            | *Ligusticum chuanxiong* |
| NO            | Nitric oxide |
| PGE2          | Prostaglandin E2 |
| NOS           | Nitric oxide synthetase |
| SOD           | Superoxide dismutase |
| JNK           | Jun amino-terminal kinase |
| AA            | Adjuvant arthritis |
| CsA           | Ciclosporin |
| LVP           | Left ventricular pressure |
| MAP           | Mean arterial pressure |
| ESM           | Epi-sesamin |
| ERK           | Extracellular regulatory kinase |
| EC            | Escherichia coli |
| SAs           | Staphylococcus aureus |
| PN            | Pneumonia |
| PAK           | Pseudomonas aeruginosa |
| CA            | *Candida albicans* |
| MIC           | Minimum inhibitory concentration |
| AP            | Asarum polysaccharides |
| iNOS          | Nitric oxide synthase |
| MDA           | Malondialdehyde |
| LOP           | Lipid peroxide |
| GSH-Px        | Glutathione peroxidase |
| T-AOC         | Total antioxidant capacity |
| Mlg           | Methyleugenol |
| PSA           | Asarum polysaccharide |
| t-BHP         | tert-butyl hydroperoxide |
| EOAH          | Essential oil from Asarum heterotropoides |
| CRF           | Corticotropin releasing factor |
| TH            | Tyrosine hydroxylase |
| S-HT          | Serotonin |
| AR            | Allergic rhinitis |
| AEO           | Asarum essential oil |
| SD            | Sprague–Dawley |
| glc           | Glucose |

Author contributions

Q.-C.Y., F.-L.S., and H.-Z.Z. conceived and designed the manuscript; F.-Q.Z., X.B., and Y.-R.Z. gave some advice for improving the manuscript; H.-Z.Z. gave some advice for improving the pictures; Q.-C.Y. and F.-L.S. wrote the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.
Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

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