frontiers | Frontiers in Pharmacology

REVIEW
published: 23 June 2022
doi: 10.3389/fphar.2022.930813

Phytochemistry, Pharmacology and Quality Control of Xiasangju: A Traditional Chinese Medicine Formula

Siyuan Wu[1,2†], *Hua Luo*[1,2†], *Zhangfeng Zhong*[1,2], *Yongjian Ai*[3], *Yonghua Zhao*[1,2*], *Qionglin Liang*[3*] and *Yitao Wang*[1,2*]

[1]Macau Centre for Research and Development in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau SAR, China, [2]State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau SAR, China, [3]Department of Chemistry, Center for Synthetic and Systems Biology, MOE Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology, Beijing Key Lab of Microanalytical Methods and Instrumentation, Tsinghua University, Beijing, China

OPEN ACCESS

Edited by:
Michał Tomczyk,
Medical University of Bialystok, Poland

Reviewed by:
Vuyisile Samuel Thibane,
Sefako Makgatho Health Sciences University, South Africa
Ruxandra Stefanescu,
George Emil Palade University of Medicine, Romania

***Correspondence:**
Yonghua Zhao
yonghuazhao@um.edu.mo
Qionglin Liang
Liangql@tsinghua.edu.cn
Yitao Wang
ytwang@um.edu.mo

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Ethnopharmacology, a section of the journal Frontiers in Pharmacology

Received: 28 April 2022
Accepted: 31 May 2022
Published: 23 June 2022

Citation:
Wu S, Luo H, Zhong Z, Ai Y, Zhao Y, Liang Q and Wang Y (2022) Phytochemistry, Pharmacology and Quality Control of Xiasangju: A Traditional Chinese Medicine Formula. Front. Pharmacol. 13:930813. doi: 10.3389/fphar.2022.930813

As a traditional Chinese herbal formula, Xiasangju (XSJ) is widely used in China for antipyresis and influenza treatment. However, XSJ still fails to have a comprehensive summary of the research progress in the last decade. This review summarizes the advanced research on the extraction process, phytochemistry, pharmacological activity, and quality control of XSJ. Current research mainly focuses on quality control and the pharmacological effects of single herbs and active ingredients, but many pharmacological mechanisms of the formula are unclear. The development of active ingredients reflects the active characteristics of triterpenes, phenolic acids and flavonoids, but the hepatotoxicity of *Prunella vulgaris* L. has not been taken into account. XSJ has extensive historical practical experiences, while systematic clinical trials remain lacking. Therefore, it is necessary to study the active ingredients and define the mechanisms of XSJ to develop multiple applications, and further studies on the dose range between its hepatoprotective activity and hepatotoxicity are necessary to improve the safety of the clinical application. In this review, the current problems are discussed to facilitate the reference basis for the subsequent research on the development of XSJ and future application directions.

Keywords: Xiasangju, *Prunella vulgaris* L., *Morus alba* L., *Chrysanthemum indicum* L., phytochemistry, pharmacology, quality control

1 INTRODUCTION

Xiasangju (夏桑菊, XSJ), a traditional Chinese herbal formula, consists of Prunellae spica (*Prunella vulgaris* L., PV), Folium mori/Mulberry Leaf (*Morus alba* L., MA), and Chrysanthemi indici flos (*Chrysanthemum indicum* L., CI), derived from a classic formula "Sangju Yin (桑菊饮)" in the monograph entitled "Analysis of Warm Diseases (Wen Bing Tiao Bian)" by Wu Jutong, a famous febrile disease scientist in the Qing Dynasty (1798 AD) (Xu and Wei, 2013). It has been a common herbal tea formula for a long time in Guangdong, China. Based on more than 200 years of clinical practice and transmission, the modern pharmaceutical process has refined it into XSJ formulations, including XSJ granules, XSJ capsules, XSJ oral liquid, XSJ effervescent tablets, etc. They mainly play the roles of clearing liver fire to improve eyesight, resolving exterior, clearing lung-heat for arresting cough, removing dampness, and diffusing impediment, and relieving sores and toxins, and are

usually used for treating the wind-heat type of cough, whose symptoms include slight fever, cough, dry lips, failing to expel phlegm smoothly, sore throat (Yao et al., 2017a; Chinese Pharmacopoeia Commission, 2020). Recent studies have shown that it also has pharmacological activities such as antioxidant, anti-tumor, antidiabetic activity, hepatoprotective, and renoprotective effects (Ma et al., 2011; Yu et al., 2011). Some health products, such as XSJ health tea is regarded as a refreshing drink and XSJ lozenges contribute to throat moistening.

The prescription of XSJ granules recorded in Pharmacopoeia of the People's Republic of China (Ch.P) 2020 is composed of 500 g of PV, 80 g of CI, and 175 g of MA (Chinese Pharmacopoeia Commission, 2020). The formula is based on "one Sovereign (Jun) two minister (Chen)" of the principle of traditional Chinese medicine (TCM) compatibility. PV is regarded as "Sovereign (Jun) medicinal," which has the functions of clearing fire, brightening eyes, reducing swelling and dispersing nodules, and is suitable for treating redness and swelling of eyes, headache, and vertigo, scrofula, canker sores, enlarged thyroid gland, lymph nodes, breast hyperplasia, and hypertension (Chinese Pharmacopoeia Commission, 2020). CI and MA are the "minister (Chen) medicinals." CI clears heat-toxin, while MA resolves exterior, clears lung-heat, and moistens dryness, and removes liver fire for improving eyesight. The combination of CI and MA has the effects of liver cleansing and detoxification, anti-inflammation and antibacterial, which complements each other and augments the major action of PV. XSJ granule is the 11th category of TCM formula included in the "Drug Standard of the Ministry of Health of the People's Republic of China" (Book XV) (National Health Commission of the People's Republic of China, 2000). The quality standard of XSJ granule has been established by high performance liquid chromatography (HPLC), and the content of rosmarinic acid in PV is determined to be not less than 2.5 mg/bag. However, quality control of the XSJ granule needs to be continuously improved.

The application of XSJ is very popular in China, as an herbal drink in summer, because of its mild, and long-lasting effects. There are no significant side effects like drowsiness and weakness of western medicines for cold and flu. In recent years, with the explosion of the herbal tea beverage market, XSJ granule has been increasingly known by more people relying on their exact efficacy on wind-heat and its cooling taste, gradually exporting to countries outside of China. XSJ granule market has covered throughout Guangdong and South China, and the products are sold to Hong Kong, Macao, the United States, Canada and other countries or regions. In addition, 107 improved formulations of XSJ in the form of granules, tablets, and capsules have received marketing approval from the State Food and Drug Administration of China, and four products exported abroad belong to granule (National Medical Products Administration, 2022).

According to the Global Biodiversity Information Facility database (https://www.gbif.org/) **(Supplementary Figure S1)**, georeferenced records of the three herbs up to 2022 indicate a remarkable regional distribution and abundant resources (Gbif | Global Biodiversity Information Facility, 2022). PV, the common self-heal, selfheal, or heal-all, is an herbaceous plant in the mint family Lamiaceae. There are 10 primary species of PV worldwide, with a total of 7 subspecies, 1 variety, and 2 forms recorded. It was widely distributed in the temperate regions and tropical mountains of Europe and Asia, south-eastern Australia, and North America (Gbif.Org., 2022c). In addition, the primary species of MA recorded 3 varieties and 1 form. MA mainly distributes in the temperate and tropical regions, including South Europe, North America, East and Southeast Asia, south-eastern Australia, and some parts of Africa (Gbif.Org., 2022b). CI was distributed in Eurasia, North America, and other regions, and widely distributed in China, Japan, Korea, and India, with a total of 12 recorded varieties (Gbif.Org., 2022a; Encyclopedia of Life, 2022). The distribution of the three herbs in East Asia relates to their Chinese application patterns. PV has long been used as a self-healing medicine in Europe and the United States (Wagner et al., 2020), mainly used for antifebrile, immunoregulation, anti-inflammation, treating breast disorders, especially for relieving sore throats and fevers, and facilitating wound healing (Bekut et al., 2018). The differences in the folk application habits of XSJ may mainly depend on the geographical distribution of the herbs.

In recent years, the study of each herb of XSJ has been reviewed, but as a formula, the research progress of XSJ over the past decade has yet to be summarized. This review summarizes the advanced research on the extraction process, phytochemistry, quality control, and pharmacological activity of XSJ. Current research mainly focuses on the quality control and pharmacological effects of single herb and its active ingredients, but the pharmacological mechanisms of the formula are unclear. The development of active ingredients reflects the characteristics of triterpenes, phenolic acids, and flavonoids, but the hepatotoxicity of PV has not been taken into account. XSJ has extensive practical experience in TCM, but systematic clinical trials remain lacking. Therefore, it is necessary to study the active ingredients and mechanisms of XSJ to develop multiple applications. Further studies on the dose range between its hepatoprotective activity and hepatotoxicity are necessary to improve the safety of clinical use. In this review, the current problems are discussed to consolidate the reference basis for the subsequent research on the development of XSJ and future application directions.

2 EXTRACTION METHODS AND PURIFICATION PROCEDURES

The extraction process of XSJ is based on the aqueous extraction method, by which the three herbs are decocted in water. Then the alcohol precipitation method is used to obtain the infusion. Lin et al. (2012b) used total flavonoids, total polysaccharides, and paste yield as the index components, and used the orthogonal experimental design to select the optimal extraction process of XSJ as 12 times the amount of water decoction, and then extracted 3 times for 2 h each. At present, the extraction process of XSJ mainly focuses on increasing the content of active ingredients and retaining the aromatic and volatile active ingredients of CI. The original decoction method would lead to the destruction of the active ingredients such as chlorogenic acid and linarin by high

temperatures. Therefore, Guangzhou Xingqun Pharmaceutical has developed a preparation method to extract part of CI separately by taking a small amount of CI to obtain an alcoholic extract, and then combining it with the aqueous extracts of the remaining CI, PV, and MA (**Supplementary Figure S2**) (Sun et al., 2008). This extraction method has been recorded in the Ch.P, and sucrose was used as an adjuvant to prepare yellow-brown colored XSJ granules. In practical production, Xiao et al. (2014) preferred the extraction process of XSJ based on the homogeneous extraction technique, and used the orthogonal test method with the relative mass per herbal extract, dry paste yield and fingerprint peak information as the comprehensive scoring indexes to guide the efficient and rapid extraction of the components in XSJ. The optimal conditions were as follows: 30% ethanol by volume, 45 times the amount, and 70°C extraction temperature.

Some studies have further applied membrane separation technology to the refinement of XSJ extracts to improve the yield of chlorogenic acid and ursolic acid. The process is carried out by inorganic ceramic membranes or organic composite membranes to refine and separate the extracts, and then the permeate is concentrated by nanofiltration or reverse osmosis to obtain the infusion. The results showed that it could effectively prevent the loss of active ingredients during the separation of impurities, and the enrichment rate was higher than that of the original alcohol precipitation process (Sun et al., 2006). Another study had also concluded that the alcohol precipitation method led to too much ethanol, which was detrimental to the safety and taste of the drug, so the extraction solution was obtained by centrifugal filtration followed by low-temperature ultrafiltration (Zhao, 2015). However, given the economic values such as the cost and efficiency of extraction, the main extraction and purification process still retain the alcohol precipitation method.

3 PHYTOCHEMISTRY

3.1 Physiochemical and Structural Features

The TCM formulas have various and complex components. Modern medicine methods of separation, screening and pharmacological activity research have essentially elucidated the material basis of pharmacological effects of formulas and given the scientific connotation to TCM formulas. The isolation and identification of the chemical constituents of XSJ are beneficial in advancing the scientific verification and modern interpretation of their corresponding pharmacological activities. Studies of XSJ primarily include the overall chemical composition and the change of the chemical composition before and after compatibility. Results show that rosmarinic acid from PV, chlorogenic acid from MA and CI, linarin from CI are the three main active ingredients in XSJ, which are considered as markers for quality control (Lin et al., 2013; Chinese Pharmacopoeia Commission, 2020). With the development of modern detection technology, studies have established qualitative and quantitative methods to determine the active ingredients in XSJ, focusing on quality standards and content determination. By LC-MS, NMR, and HPLC-MS, 31 compounds were identified in the ethanol-extracted XSJ spectrum. They were mainly identified as oleanolic acid 1), β-Amirin 2), ursolic acid 3), protocatechuic acid 4), caffeic acid 5), rosmarinic acid 6), salviaflaside 7), chlorogenic acid 8), caffeoylquinic acid 9), dicaffeoylquinic acid 10), kaempferol 11), quercetin 12), apigenin 13), luteolin 14), acacetin 15), diosmetin 16), astragalin 17), kaempferol 3-O-rutinoside 18), quercetin 3-glucoside 19), hyperoside 20), rutin 21), apigenin 7-O-glucoside 22), luteolin 7-O-glucoside 23), tilianin 24), linarin 25), acacetin 7-O-β-D-glucuronopyranosyl-(1→2)[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside 26), diosmetin 7-O-β-D-glucoside 27), β- Sitosterol 28), β-Daucosterol 29), dotriacontanoic acid 30), n-nonadecanol 31). In the study of component analysis, there are two methods of sample preparation, one is for the ethanol extraction of the three herbs according to the prescription ratio (Liu et al., 2012; Zhou, 2012; Zhou et al., 2012; Xin and Tang, 2013), and another is for the methanol extraction of the preparation of XSJ granule (Hua et al., 2013; Lin et al., 2013; Xia et al., 2016a; Xia et al., 2016b; Wu, 2019). The extraction method combined with the different analytical methods and conditions led to differences in the results. Currently, the analysis of active ingredients in XSJ has focused on prescriptions or herbal extracts, while no quantification of active ingredients in biological samples has been reported. This review summarized the chemical components isolated and identified from the formula, as shown in (**Supplementary Table S1**), to provide a reference for the isolation and analysis of XSJ.

3.1.1 Triterpenes

Triterpenes are a class of terpenoids whose original parent nucleus consists of six isoprene units polymerized, and exists in the plant in free form or glycosides and esters forms. PV contains various triterpenes as the major active ingredients for lowering blood pressure (Wang et al., 2019). Therefore, the study of triterpenes in XSJ on lowering blood pressure is beneficial in guiding the pharmacodynamic study. The triterpenes in PV are basically oleanolic acid 1) which is the standard control for determining saponin content (Wu, 2019). However, the isolation study of total saponins in XSJ compounds is lacking. The formula ethanol extract was extracted with petroleum ether, and then separated by silica gel column chromatography to obtain β-amirin 2), ursolic acid 3) (Zhou et al., 2012). The structures of the three isolated and identified triterpenes are shown in (**Supplementary Figure S3A**), where the two main components of triterpenes are oleanolic acid 1) and ursolic acid 3).

3.1.2 Phenolic Acids

Phenolic acids are a large group of active ingredients in XSJ, mainly consisting of phenolic acids and other acids. Seven phenolic acids in XSJ were isolated, and most have significant biological activity (**Supplementary Figure S3B**). Rosmarinic acid 6) is one of the main active ingredients of PV, and salviaflaside 7) is a unique ingredient in PV corm. Rosmarinic acid 6) and salviaflaside 7) have antibacterial, antiviral, antioxidant, anti-inflammatory, immunosuppressive, and antithrombotic activities and so on (Hua et al., 2013). Phenolic acids

treatment can achieve good antioxidant effects by inhibiting or scavenging free radicals. Chlorogenic acid 8) has a strong synergistic antioxidant action and is one of the core polyphenolic monomers of antioxidants, which is a common component of MA and CI (Zhou, 2012; Cai et al., 2014; Xia et al., 2016a). Therefore, rosmarinic acid 6) and chlorogenic acid 8) became the quality markers for XSJ. Moreover, caffeic acid 5) is the synthetic precursor of these phenolic acids polymers (Chen et al., 2018).

3.1.3 Flavonoids

Flavonoids are an important class of plant secondary metabolites, with 2-phenylchromone as the parent nucleus. It is another major component of active ingredients in XSJ, and all three herbs have high total flavonoid content. 17 flavonoids identified are shown in (**Supplementary Figure S3C**) and were chiefly divided into flavones and flavonols. Among them, linarin 25) is the characteristic chemical component of CI, which is the quality marker in the Ch.P (Lin et al., 2013). Also, the Ch.P includes rutin 21) as a quality maker of MA (Sun et al., 2016).

3.1.4 Other Constituents

In addition to triterpenes, phenolic acids, and flavonoids, sterols identified in XSJ include β-sitosterol 28) and β-daucosterol 29) (Zhou et al., 2012) (**Supplementary Figure S3D**). XSJ also contains long-chain fatty acids and alcohols with the molecular formula H_3C-$(CH_2)_{30}$-COOH (30) and H_3C-$(CH_2)_{32}$-CH_2OH 31). The long-chain fatty acids were mainly obtained by NMR analysis (Zhou et al., 2012). XSJ is rich in amino acids, and 12 characteristic amino acids were discovered. Four of them are essential amino acids, namely valine (Val), isoleucine (Ile), leucine (Leu), and phenylalanine (Phe), reflecting the TCM's view of "nourishing yin for lowering fire 养阴扶正以降火," which is in line with the efficacy of XSJ (Ke et al., 2007). The non-essential amino acids include aspartic acid (Asp), serine (Ser), proline (Pro), etc. The active ingredients in the XSJ herbs are complex and diverse, which still requires systematic active ingredient testing. The results of the phytochemical analysis can assist in confirming the exact pharmacological effects of each active ingredient.

4 BIOLOGICAL ACTIVITIES

In TCM theory and experience, XSJ has the effect of clearing heat and detoxifying toxins and is mainly used to treat wind-heat colds, fever, and sore throat. It is suitable for treating red eyes and headache, hypertension, dizziness and tinnitus, sore throat, furuncles, and swellings caused by wind-heat colds (Chinese Pharmacopoeia Commission, 2020). Because of its mild and long-lasting effect, and no side effects such as "drowsiness and weakness" of chemical medicines for cold and flu. With the rapid development of modern pharmacology and biotechnology, the combination of modern pharmacology and phytochemistry has been used to verify the traditional efficacy of XSJ and explain its mechanisms. Modern research has shown that XSJ granule can inhibit the growth and reproduction of various bacteria, and have stronger inhibitory effects on *Staphylococcus aureus* and *hemolytic streptococcus* (Guo, 2010), indicating that the antipyretic and anti-infective effects are closely related to their antibacterial action. There is growing evidence that the active ingredients in XSJ have pleiotropic effects on tumors, lipid, and glucose disorders (diabetes), bacterial infections, immune system disorders, and liver disease (Huang et al., 2007; Ma et al., 2011; Qiu et al., 2011; Yu et al., 2011) (**Supplementary Table S2**).

4.1 Antiviral Effect

Chinese medicines have unique efficacy in the treatment of influenza (Huang et al., 2021). Extracts of XSJ have significant antiviral effects and are commonly used to treat fever, which was granted a quasi-brand name in 1985 and has been commercially available for nearly 40 years (Guangzhou Xingqun Pharmaceutical, 2022). *In vitro* experiments have shown that the lipid-soluble components of XSJ extract can inhibit influenza A H3N2, H5N1, and B viruses co-cultured with Madin-Darby canine kidney (MDCK) cells at a dose 20 times lower than the cytotoxic dose (Zhan and Dong, 2009). XSJ was found to inhibit the proliferation of respiratory syncytial virus (RSV) and to be protective against RSV-infected rats. Its effective concentration was 544.59 μg/ml, and the efficacy was enhanced with an increasing dose of the drug, which also reduced the virus titer in tissues and prevented virus replication *in vivo*, and the anti-RSV effect was similar to that of the same dose of virazole (Huang et al., 2007). Viruses with 1:256~1:2048 dilution of XSJ infusion and 1:128 dilution of XSJ granule have a cytopathogenic effect on Coxsachie (Cox) A16 *in vitro* at the cellular level (Yao et al., 2017b). Meanwhile, XSJ granule produces significant anti-dengue virus (DENV)-1 effects by *in vitro* pre-administration, which has a similar effect to ribavirin injection. MTT results showed that the maximum nontoxic dose of XSJ granule on the C6/36 cells was 7.81 mg/ml. The cytopathic effect of DENV-1 infection was measured after treatment with XSJ granule solution, and the survival rate of cells infected with the virus increased, which significantly attenuated the effect of virus-induced cytopathy. Cellular immunofluorescence assay showed that the relative infection rate of DENV-1 was decreased after pretreatment with different dilutions of XSJ granule. The inhibition of the virus copy number by XSJ granule was obvious. It is suggested that XSJ directly affects DENV-1, probably by direct inhibition of viral infectivity and RNA replication capacity (Yao et al., 2017a; Zhang et al., 2019a).

A study further investigated the effect of XSJ on influenza A H1N1 virus and its mechanism at the molecular level. By observing the effect of XSJ on the replication process of MDCK cells, the half toxic concentration (TC50), half infectious amount (TCID50), and half inhibitory concentration (IC50) were calculated. The action of XSJ on influenza A H1N1 virus inhibited vacancy formation, nuclear export of viral nucleoproteins (NPs) and phosphorylation of nuclear factor kappa-B (NF-κB) pathway-related proteins Ικκα, Ικκβ, NF-κB p50, and NF-κB p65 in the virus strain. XSJ has a significant inhibitory effect on influenza A H1N1 virus, probably through the inhibition of NF-κB pathway-associated protein phosphorylation (Yu et al., 2018).

Aqueous extracts of natural herb PV can interrupt SARS-CoV-2-Spike glycoprotein binding to its receptor angiotensin-converting enzyme 2 (ACE2) and block the viral entry step (Ao et al., 2021). Aqueous extracts of PV also inhibited the HIV-1 (Oh et al., 2011), IHNV (Li et al., 2019), Ebola virus (Zhang et al., 2016), and HSV-1 (Nolkemper et al., 2006) by early interference. The polysaccharide and essential oils from CI inhibited replication of anti-duck hepatitis A virus (Ming et al., 2017; Ming et al., 2019), HSV-1, HAV, and VSV (Youssef et al., 2020). Caffeic acid and chlorogenic acid from aqueous and hydro methanolic extracts of MA inhibited the replication of human CoV and single-stranded RNA viral strains (Thabti et al., 2020). Caffeic acid, the active ingredient of the three herbs of XSJ, can inhibit Hepatitis C virus (HCV) propagation and the proliferation of influenza A virus by interfering mainly with the viral genome replication in the infected cells (Tanida et al., 2015). Caffeic acid has also been shown to have antiviral activity against the herpes simplex virus (DNA virus) and polio virus (RNA virus) (Utsunomiya et al., 2014). The inhibition effect indicated that phenolic acids, like rosmarinic acid (Nolkemper et al., 2006), caffeic acid (Nolkemper et al., 2006; Thabti et al., 2020), ursolic acid (Li et al., 2019), chlorogenic acid (Thabti et al., 2020), were the active ingredients of antivirus.

4.2 Antioxidant Activity

Antioxidant herbs mainly belong to bitter-cold herbs, while oxidizing herbs are mainly warm (Liao et al., 2008). The extract of XSJ contains phenolic acids, flavonoids, polysaccharides, and amino acids, which have the effects of lowering blood pressure, dilating coronary arteries and preventing coronary atherosclerosis, etc. It is a kind of natural organic antioxidant. A study was carried out to compare the scavenging effect of different systems on free radicals in terms of absorbance size by extracting XSJ with ethanol, followed by petroleum ether, ethyl acetate, and n-butanol in turn (Ma et al., 2011). The results showed that all extracted fractions of XSJ had some scavenging effect on hydroxyl radicals, except for the petroleum ether layer. Compounds with catechol functional groups, such as caffeic acid and its derivatives, including rosmarinic acid, and flavonoids, such as rutin, which are active substances for scavenging reactive free radicals, have been reported in extracts of XSJ (Ma et al., 2011). The antioxidant activity of active ingredients was conferred by the structural property of phenolic compounds, which can directly scavenge of reactive oxygen species (ROS) or metal chelation (Habtemariam, 2019).

Two ursane-type triterpenes from ethanol extract of PV induced heme oxygenase-1(HO-1) in HepG2 cells (Jeong et al., 2008) and ursolic acid inhibited nitric oxide (NO) (Ryu et al., 2000; Miceli et al., 2005). Caffeic acid, rosmarinic acid, rutin, quercetin, and luteolin from the total phenols of PV extract increased superoxide dismutase (SOD) activity and decreased malondialdehyde (MDA) content in the serum of tumor-bearing mice (Feng et al., 2010). 1,1-diphenyl-2-picryhydrazyl (DPPH) and 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) free radicals were significantly scavenged by the ethanol extracts of PV, which is mainly determined by ursolic acid, oleanolic acid, rosmarinic acid, salviaflaside, caffeic acid, and hyperoside (Chen et al., 2019). Rosmarinic acid and caffeic acid from PV reduced breakage together with the apoptotic process, eliminated ROS production, and diminished IL-6 release (Vostalova et al., 2010), and induced the expression of efflux transporters through activation of the Nrf2-mediated signaling pathway (Wu et al., 2016). Chlorogenic acid, rutin, astragalin, quercetin, and kaempferol from MA reduced the ROS and NO production (Kwon et al., 2017), and scavenged DPPH radical (Katsube et al., 2009). Total phenolic and flavonoid from CI showed the peroxyl radical-scavenging capacity, and quercetin, luteolin, acacetin, luteolin 7-O-glucoside, linarin, luteolin, chlorogenic acid, apigenin also exhibit such activity (Luyen et al., 2015; Hwang et al., 2016; Kang et al., 2021). As the quality marker of XSJ, rosmarinic acid can scavenge ROS, inhibit lipid peroxidation (Elufioye and Habtemariam, 2019) and activate the AMPK pathway (Feng et al., 2020). And linarin activated Nrf-2 through activating PI3K/Akt signaling pathway in myocardial cell H9C2 and langendorff heart model (Yu et al., 2017). In addition, the aqueous extract of PV may mediate the protective effect of gastric ulcers by inhibiting oxidative stress and promoting regenerative processes in the mucosa. Considerable amounts of hyperoside, rutin, isoquercitrin, small amounts of kaempferol, apigenin, and phenolic acids such as caffeic acid are present in the aqueous extract (Grosan et al., 2020a). The higher the level of total phenolic content and rosmarinic acid content, the stronger the total antioxidant capacity of PV (Sarosi et al., 2011) (**Supplementary Figure S4**).

4.3 Anticancer Activity

The ancient Chinese medical treatise "Zhongzangjing" describes cancer-like symptoms such as "Yong, Yang, Shang, and Zhong," caused by the retention of various pathogens such as heat and dampness. Tumor growth involves the induction of cell cycle processes, avoidance of apoptosis and activation of cell survival pathways. Studies have shown that some of the active ingredients in XSJ can inhibit the proliferation of cancer cells *in vitro* and suppress tumor growth *in vivo* (Yu et al., 2011).

PV extract inhibits the growth of proximal tubular epithelial cells (PTC) *in vivo* and *in vitro via* autophagy, which is associated with the AMPK/mTOR/ULK1 pathway (Song et al., 2021a). PV extract also inhibits the proliferation and migration of thyroid cancer cells both *in vitro* and *in vivo*, which may have been achieved by modulation of the expression of MKI67, PCNA, and CDH1 (Yu et al., 2021). PV total flavonoids have an obvious anti-hepatocarcinoma effect, and the mechanism may be linked to the inhibition of autophagy and promotion of apoptosis in liver cancer cells, which may be related to the activation of the PI3K/Akt/mTOR pathway (Song et al., 2021b). Root extract of PV shows the anticancer by suppressing angiogenesis, inducing apoptosis and cell cycle arrest, whose mechanism may be *via* activating PI3K/AKT signaling pathway (Gao and Xu, 2019). It was also associated with the enhancement of Bax expression and the decrease in the expression of Bcl-2. Ethanol extract of PV activated p53-mediated apoptosis (Kim et al., 2020) and the proapoptotic protein caspase-3 induced cellular apoptotic pathway (Zhu et al., 2018). And the CI Extract induces

apoptosis by suppressing constitutive STAT3 activation in human prostate cancer DU145 cells, especially the methylene chloride fraction acacetin of CI could inhibit the JAK1/2 and STAT3 signaling pathways (Kim et al., 2013). Linarin from CI induced factor-related apoptosis-induced ligand (TRAIL)-regulated apoptosis *via* upregulating Caspases (Xu et al., 2017) and p53 expression, and inhibiting NF-κB/p65 expression (Zhen et al., 2017). Total triterpenes and total phenols from 95% ethanol extract of PV increased the TNF-α level, such as caffeic acid, rosmarinic acid, rutin, quercetin, oleanolic acid, and ursolic acid (Feng et al., 2010; Lee et al., 2019; Zheng et al., 2022). Chlorogenic acid, rutin, quercetin, astragalin, and kaempferol from MA also recovered the effects of endoplasmic reticulum stress-induced resistance to DOX through COX-2 or p38 MAPK-mediated inactivation of the PI3K/Akt pathway (Yang et al., 2020). The activity of luteolin and acacetin-7-O-rutinoside from water extract of CI was confirmed to be similar to that of tyrosinase inhibitor (Choi et al., 2016) (**Supplementary Figure S5**).

In the study results, considering the dose and drug delivery of oral granule, anticancer studies of XSJ are not systematic, so no antitumor effect of XSJ granule has been reported. But these three herbs were shown to inhibit tumor growth in a dose-dependent manner, respectively. Oleanolic acid, ursolic acid, linarin, and other flavonoids may be active compounds showing targeted antitumor activity. Further research on anti-tumor treatment of the whole XSJ formula should be carried out. The emergence of severe side-effects and multidrug resistance (MDR) are big challenges to clinical usage of cancer chemotherapy. Therefore, the synergistic treatment of XSJ reflects its advantages, such as reducing the toxic and side effects of chemotherapy, so it could serve as a putative adjuvant drug with chemotherapy in the future (Yang et al., 2011; Yang et al., 2017).

4.4 Antidiabetic Activity

Diabetes mellitus belongs to the category of "thirst (Xiao Ke)" in Chinese medicine, and the patient may suffer from physical wasting, excessive urination, excessive drinking, excessive eating, and sweet-tasting urine. Aqueous extract of PV attenuated IL-1β-increased NF-κB binding activity and inflammatory cytokine expression (Wu et al., 2012), and disrupted the TGF-β/Smad signaling (Namgung et al., 2017). Rosmarinic acid, caffeic acid, rutin, and quercetin form PV increased serum-insulin, attenuation of α-amylase and α-glucosidase (Raafat et al., 2016). Aqueous extract of MA improved glucose metabolism disorders, ameliorated the antioxidative ability, and decreased insulin resistance *via* IRS-1/PI3K/Glut-4 signaling pathway (Cai et al., 2016; Lyu et al., 2021). Both MA extract (Wu et al., 2017) and CI water extract (Nepali et al., 2018) exert anti-diabetic activity by regulating the AMPK pathway in diet-induced obesity mice models. Chlorogenic acid, caffeic acid, rutin, isoquercitrin astragalin, and dicaffeoylquinic acid from MA improved glucose tolerance and lowered the level of glucose (Ma et al., 2016). Acacetin, apigenin, chlorogenic acid, kaempferol, luteolin, and quercetin from CI improved the fat metabolism (Bai et al., 2018) and inhibited the formation of advanced glycation end products (Tsuji-Naito et al., 2009). Triterpenes acid from PV controlled blood glucose and decreased SOD mRNA expression in pancreatic β cells (Zhou et al., 2013). Total polyphenols, total flavonoid aglycons, quercetin, and kaempferol from MA exhibited a hypocholesterolemic and hypotriacylglyceridemic effects attributed to the stimulatory effect on the β-oxidation of fatty acids. They showed the induction of fatty acid oxidation, inhibition of lipogenesis, and suppression of oxidative stress (Kobayashi et al., 2010). Among them, chlorogenic acid, rutin, quercetin derivatives and β-sitosterol from MA also showed the significant control of lipid and glucose metabolism, attenuation of oxidative stress and insulin resistance improvement (Hunyadi et al., 2012; Sun et al., 2015; Sheng et al., 2018). β-Sitosterol can activate the synthesis and translocation of the transporter GLUT-4 (Ponnulakshmi et al., 2019). In summary, some of the active ingredients of XSJ can improve insulin resistance, increase the sensitivity of surrounding tissues to insulin, and enhance the ability of fat cells to take up and utilize glucose, resulting in lower blood glucose (**Supplementary Figure S6**). It is important to conduct studies with diabetic patients and healthy volunteers to understand how natural products such as MA work in the human body. A double-blind clinical trial reported that 12 weeks of MA extract in patients with type 2 diabetes reduced malondialdehyde (MDA) levels but did not affect other biomarkers of inflammation and oxidative stress (Taghizadeh et al., 2022). Consumption of MA extract for 12 weeks significantly reduced triglyceride, VLDL-cholesterol, and MDA levels, and significantly increased HDL-cholesterol and GSH concentrations in T2DM patients with kidney disease (Taghizadeh et al., 2017). To evaluate the effects of DNJ-rich MA extract on patients with T2D, the results of a clinical trial combining dietary control, exercise and the total alkaloid composition of MA showed that it lowered blood glucose, regulated blood lipids and had fewer side effects compared to acarbose treatment (Liu et al., 2013). However, studies on safety are lacking and studies on the absorption, distribution, metabolism and excretion of compounds in XSJ need to be conducted.

4.5 Antibacterial Activity

Antibacterial herbal medicines are mostly heat-clearing and detoxifying drugs, mostly with anti-microbial and anti-inflammatory effects. XSJ has an antibacterial effect on *Staphylococcus aureus* and *Streptococcus hemolyticus* (Guo, 2010). XSJ extract inhibits *Escherichia coli* (*E. coli*) in human urine samples, and aqueous extract showed higher inhibitory activity than ethanol extract (Komal et al., 2018). The active ingredients chlorogenic acid, caffeic acid, protocatechuic acid, luteolin-7-glucoside, apigenin-7-glucoside, kaempferol-3-glucoside, linarin, apigenin, luteolin, and kaempferol have been observed in CI extract studies to have an inhibition of bacteria activity (Kozyra et al., 2015). CI extract can inhibit gram-positive bacteria, such as *Streptococcus aureus* and *Streptococcus pneumoniae*, as well as *Streptococcus epidermidis*, *Bacillus cereus* and *Bacillus subtilis*. The 50% and 80% methanol extracts and fractions are richer in phenolic compounds that showed antibacterial activity (Kozyra et al., 2015). Silver nanoparticles of CI aqueous extract inhibited *Klebsiella pneumonia*, *E. coli*, and *Pseudomonas aeruginosa*, which contained total flavonoids,

terpenoids, and glycosides (Arokiyaraj et al., 2014; Arokiyaraj et al., 2015). Rosmarinic acid and caffeic acid from PV inhibited Gram-positive bacteria (Psotova et al., 2003), while oleanolic acid and ursolic acid from MA inhibited periodontogenic bacteria (Park et al., 2014). The aqueous and hydroalcoholic extracts of PV showed antibacterial effect against most bacteria, showing measurable activity against Gram-positive bacteria and multi-drug resistant Gram-negative bacteria. It indicates that the extract of PV has antibacterial potential in the adjuvant treatment of multi-drug resistant infections (Grosan et al., 2020b).

Ursolic acid compromised the integrity of the bacterial membrane, inhibited protein synthesis, and elicited the oxidative response in Methicillin-Resistant *Staphylococcus aureus* (MRSA) *via* the AhpC induction (Wang et al., 2016). Caffeic acid could inhibit bacterial enzyme activity, including respiratory enzymes against the representative foodborne bacteria *E. coli* O157:147, *Salmonella Typhimurium*, and *Listeria monocytogenes* (Park and Kang, 2021). Chlorogenic acid induces damage of intracelluar and outer membranes as well as disruption of cell metabolism resulting in *Salmonella Enteritidis* S1 death eventually. It also suppressed the activities of malate dehydrogenase and succinate dehydrogenase, two main metabolic enzymes in the TCA cycle and electron transport chain (Sun et al., 2020). β-Sitosterol showed antibacterial activity against *Staphylococcus aureus* and *E. coli* (Ododo et al., 2016). Since ancient times, these three herbs and XSJ have been used for their antibacterial and anti-inflammatory properties. A randomized clinical trial of chronic infective refractory wounds repair focused on the decoction of PV and showed higher rates of wound repair, bacterial negativity, clinical total efficacy rate and shorter healing times compared to routine wound dressing change. It inhibited the growth of traumatic bacteria and provides a good environment for the growth of granulation. Compared with modern drugs, PV does not produce too many toxic side effects, which is of some relevance especially in the case of antibiotic abuse (Zhao et al., 2021).

4.6 Immunomodulatory Effect

The active ingredient rosmarinic acid in XSJ and aqueous extract of PV inhibited Th1, Th2, and Th17 immune responses *via* inhibiting HMGB1/TLR9 signaling (Guo et al., 2021; Zhu et al., 2022).Triterpenoids, flavonoids, tannins and polysaccharide from PV suppressed Con A-, LPS-, and OVA-induced splenocyte proliferation in the immunized mice, reduced total IgG, IgG1 ,and IgG2b levels significantly, and suppressed the cellular and humoral response (Sun et al., 2005), immuno-stimulating activities through the activation of TLR2, TLR4 ,and CR3 (Li et al., 2015). Ursolic acid, quercetin, β-sitosterol from PV suppressed the activation of NF-κB and interferon regulatory factor 3 (IRF3), and inhibited genes related to antigen presentation pathways (Chen et al., 2020a). Water extract of MA stimulated the production of NO and PGE2 as immune response parameters, and was associated with the increased expression of inducible NO synthase and COX-2 (Kwon et al., 2016). Extract of CI increased the delayed-type hypersensitivity (DTH) reaction, and enhanced antibody generation and IgG and IgM levels in mice sera (Cheng et al., 2005). β-sitosterol in XSJ regulates immunity by increasing viable peripheral blood mononuclear cell (PBMC) numbers and it activates swine dendritic cells (DCs) in culture. It can drive the BMDC response towards a Th1 pattern with IFN-α secretion and the absence of IL-10 (Fraile et al., 2012). Therefore, XSJ has a potential immunomodulatory effect.

4.7 Hepatoprotective Activity

The liver is essential for bile formation, amino acid utilization and ammonia detoxification. In TCM, the liver is an organ susceptible to heat and toxins, so its detoxification capacity is diminished by pathological damage. XSJ is rich in bioactive flavonoids and polyphenols, which restore the balance of the liver's metabolic disorder state. Flavonoids and total phenolic extracts obtained from floral spikes of PV have hepatoprotective potential and free radical scavenging *in vivo* (Ahmad et al., 2020). 80% methanol extract of PV reduced the contents of inflammatory factors and liver function markers, and improved metabolic disorder of liver injury (Deng et al., 2021). Extract from CI reduced the elevated levels of ALT and AST, alleviated abnormal alterations in structure and function and liver (Zhang et al., 2019b), and inhibited bioactivation of hepatotoxicity and downregulated CYP2E1 expression (Jeong et al., 2013). The active ingredient rosmarinic acid scavenged or reduced reactive superoxide or peroxynitrite, decreased indicators of hepatotoxicity, inhibited hepatic stellate cell proliferation, suppressed the activities of TGF-β1, CTGF, and α-SMA, attenuated fibrosis, improved biochemical indicators and histopathological patterns (Osakabe et al., 2002; Rocha et al., 2015; Elufioye and Habtemariam, 2019). β-Sitosterol, rutin, and isoquercitrin from MA extract reduced the production of NO, malondialdehyde, and glutathione levels, and prevented the increase in the hepatic malondialdehyde, playing a pivotal role in the antifibrotic properties (Amer et al., 2013). β-sitosterol downregulated the expression of apoptosis-related genes in the PI3K/Akt pathway and restored the liver enzymes, liver lipid peroxidation markers, total bilirubin, and albumin to their normal levels without inhibitory effect on the CYP2E1 activity (Abdou et al., 2019; Chen et al., 2020b). Apigenin-7-glucoside suppresses the elevation of GPT, GOT, MDA, and 8-OHdG, and inhibits the reduction of GSH in a dose-dependent manner *in vivo* and reduces the damage of hepatocytes *in vitro* (Zheng et al., 2005). The low dosage of oleanolic acid reprogrammed the liver to activate the Nrf2 pathway in mice (Liu, 1995; Jin et al., 2012; Liu et al., 2019). In general, the regulatory and protective effects of XSJ on the liver are caused by its specific active ingredients (**Supplementary Figure S7**).

5 QUALITY CONTROL

Research on quality standards of proprietary Chinese medicines is generally based on thin-layer chromatography (TLC) and HPLC methods. The quality control study of XSJ focuses on applying HPLC methods for the content determination of various components and the establishment of fingerprint profiles. The Drug Standard of the Ministry of Health of the People's Republic

of China, Book XV, shows that the quality control of XSJ includes the identification reaction of chloroform solution saturated with antimony trichloride and the TLC identification of ursolic acid, excluding the content of XSJ fingerprinting and content dete8mination. However, the methods of compound identification or content determination for individual active ingredients can hardly reflect the quality of XSJ comprehensively. To provide a reference for the quality standard of the XSJ granule, most of the studies reported that the fingerprinting of XSJ extracts as a whole had been carried out, focusing on the development and determination of several main active ingredients. Ch.P (2015 and 2020) summarized and extracted the previous studies, mainly based on TLC identification, particle examination, fingerprinting and content determination as indicators for the determination of XSJ granule. The authenticity of XSJ granule was controlled. The quality markers of XSJ granule included in the Ch.P are chlorogenic acid, rosmarinic acid and linarin, and the content determination is calculated by the amount of PV in each bag with rosmarinic acid content. The three quality markers were significantly show in the fingerprint of Xiasangju, and it requires that the similarity between the fingerprint of the test sample and the control fingerprint should not be less than 0.90. The content of rosmarinic acid in PV is determined to be not less than 2.5 mg/bag (Chinese Pharmacopoeia Commission, 2020).

5.1 Quality Standards

With the development of various identification techniques, the researchers identified the authenticity of XSJ granule by HPLC and UPLC. The fingerprint profile of the extract was analyzed as a whole and focused on the sequence and interrelationship of each constituent fingerprint peak. It is important as a quality control method in optimizing the production process. Ke et al. (2008) established fingerprint profiles of 10 batches of XSJ granule with similarity greater than 0.970, and compared the fingerprint profiles of the aqueous decoction, alcoholic precipitation, concentrated solution, and finished products of the herbs, respectively, and found that there were significant differences. Based on establishing the fingerprint profiles of amino acid components (Ke et al., 2007) and alcohol extracts of XSJ, the fingerprint profiles of different brands of XSJ granule were compared, and qualitative and semi-quantitative evaluations were conducted using similarity analysis and cluster analysis (Yao et al., 2012). The control chart comparison method is simple and intuitive, fully reflecting the overall characteristics of XSJ granule and effectively controlling the product quality. Xia et al. (2014) constructed the fingerprint profiles of 12 batches of XSJ granule by UPLC and labelled 16 common peaks with fingerprint similarity above 0.9. HPLC was applied to establish the quality standards of different dosage forms of XSJ granule. The fingerprint profiles of sugar- and sugar-free XSJ granule were analyzed by Principal Components Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA), in which the three main components, salviaflaside, luteolin 7-O-glucoside and linarin, were found to be different (Xia et al., 2016b). And this quality difference may come from the ethanol extraction of CI alone, which facilitates the exudation of its flavonoid fractions. Also, these three compounds are among the most reported substances in the literature as quality control markers. Further, the random forest algorithm was used to process similar samples of XSJ with better results than the PCA and PLS-DA algorithms (Xia et al., 2017). A study on the change of the compound composition of XSJ detected the production of new unknown chemical components and high content of the three herbs after decoction together, and the content of some major chemical components also changed (Peng et al., 2014). The single herb preparation and the negative preparation (missing PV, MA, and CI, respectively) were examined under the same conditions. The HPLC fingerprints showed that six characteristic peaks originated from PV, one from MA, five from CI, one from MA, and CI together, and one from PV, MA, and CI together, and the peak areas of these characteristic peaks changed to different degrees before and after the compatibility (Xia et al., 2016a).

5.2 Content Determination

Quantitative analysis of XSJ focused on the determination of rosmarinic acid (Lin et al., 2012a; Huang, 2013; Lin et al., 2013; Peng et al., 2014; Zhou et al., 2014; Luo et al., 2016; Sun et al., 2016), salviaflaside (Hua et al., 2013; Lin et al., 2013), chlorogenic acid (Lin et al., 2013; Sun et al., 2016), linarin (Zeng and Ding, 2008; Lin et al., 2012a; Lin et al., 2013), luteolin 7-O-glucoside (Sun et al., 2016), rutin (Sun et al., 2016), quercetin (Sun et al., 2016), and total saponins (Wu, 2019). Most samples had the highest contents of chlorogenic acid and rosmarinic acid, followed by salviaflaside, and the other components varied widely among batches of the herbs.

A method was established for the quantitative determination of rosmarinic acid and linarin in XSJ granule by HPLC. 15 batches of samples were extracted by methanol ultrasonication and analyzed within 15 min. The results showed that the amounts of rosmarinic acid and linarin in the measured samples varied considerably, and the content determined to be not less than 1.50 and 0.50 mg/bag (Lin et al., 2012a). HPLC was used to simultaneously determine the contents of rosmarinic acid and salviaflaside in XSJ granule. Using acetonitrile–0.1%-phosphoric acid gradient elution, rosmarinic acid, and salviaflaside peak shape are good, and the separation is ideal, negative without interference, the content of rosmarinic acid is not less than 0.22 mg/g (Hua et al., 2013). Further study determined the contents of chlorogenic acid, salviaflaside, rosmarinic acid, and linarin in XSJ granule simultaneously by Reversed-phase HPLC method, and compared the contents of active ingredients from different manufacturers. The result suggested that the lower limits of the contents of the four active ingredients in the quality standard were 1.50, 0.20, 2.00, and 1.00 mg/bag for chlorogenic acid, salviaflaside, rosmarinic acid, and linarin, respectively (Lin et al., 2013). Based on the study of XSJ granule, quality control of other preparations of XSJ has also been reported in the literature. For example, the quality research of XSJ herbal tea mainly focuses on establishing quality control methods for chlorogenic acid, rosmarinic acid, luteolin 7-O-glucoside, rutin, quercetin and linarin, and contents determination by HPLC (Sun et al., 2016). Since chlorogenic acid is a common component of all three herbs, it is difficult to be

used as a quantitative standard. Rosmarinic acid in PV was used for content determination for quantification.

6 CONCLUSION

After years of practice, XSJ has been practically used to treat influenza and purge heat and toxicity. It is sold in several regions and occupies a certain market share for its good quality. The latest studies of XSJ focus on the development of active ingredients, reflecting the active characteristics of triterpenes, phenolic acids, and flavonoids. XSJ may benefit people with liver disease by preventing or treating liver injury, and may have one or more of antioxidant, antifibrotic, immunomodulatory, or antiviral activities. However, "liver-protective" herbs may also cause liver damage, so it may have to take into account the hepatotoxicity of XSJ. For example, whereas a low dose of oleanolic acid is hepatoprotective, higher doses, and long-term use of oleanolic acid can produce liver injury, characterized by cholestasis. This paradoxical hepatotoxic effect occurs not only for oleanolic acid, but also for other oleanolic acid-type triterpenoids. Dose and length of time of oleanolic acid exposure differentiate the ability of acid to cause hepatoprotection or hepatotoxicity (Liu et al., 2019). And because of the incomplete study of XSJ phytochemical composition, more information can be obtained only from the study of the three herbs.

In herbal formulas, these fundamental problems or phenomena have never been completely resolved. The mismatch of chemical composition and active ingredients has resulted in potent substances that remain unclear, but effective for clinical use. The boundary between pharmacological and toxic effects is unclear, leading to unclear synergistic or antagonistic mechanisms. The *in vivo* delivery process of herbal medicinal substances is not clarified. Therefore, more phytochemical composition, physical structure composed of multiple components, and biological activities of XSJ should be addressed, and further application of advanced technical tools, such as network pharmacology and structural Chinese medicine, to systematically study the complete pharmacological substance basis of the compound. The synergistic treatment of XSJ could be a potential putative adjuvant drug with chemotherapy in the future.

AUTHOR CONTRIBUTIONS

YW, YZ, and QL organized, conceived, and supervised the study. SW wrote the article. HL conceived and revised the manuscript. YA and ZZ supervised the manuscript. All authors have read and approved the final version of the manuscript.

FUNDING

This study received funding from the Macao Science and Technology Development Fund (FDCT 007/2020/ALC), the Shenzhen-Hong Kong-Macau S&T Program (Category C) (SGDX2020110309420200), the Research Fund of University of Macau (CPG2022-00005-ICMS) and Guangzhou Baiyunshan Xingqun Pharmaceutical CO., LTD. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

ACKNOWLEDGMENTS

We thank Dr. Shengpeng Wang (University of Macau) for providing valuable suggestions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.930813/full#supplementary-material

REFERENCES

Abdou, E. M., Fayed, M. A. A., Helal, D., and Ahmed, K. A. (2019). Assessment of the Hepatoprotective Effect of Developed Lipid-Polymer Hybrid Nanoparticles (LPHNPs) Encapsulating Naturally Extracted β-Sitosterol against CCl4 Induced Hepatotoxicity in Rats. *Sci. Rep.* 9, 19779. doi:10.1038/s41598-019-56320-2

Ahmad, G., Masoodi, M. H., Tabassum, N., Mir, S. A., and Iqbal, M. J. (2020). Invivo Hepatoprotective Potential of Extracts Obtained from Floral Spikes of Prunella Vulgaris L. *J. Ayurveda Integr. Med.* 11, 502–507. doi:10.1016/j.jaim.2019.08.003

Amer, O. S. O., Dkhil, M. A., and A-Quraishy, S. (2013). Antischistosomal and Hepatoprotective Activity of Morus Alba Leaves Extract. *Pak. J. Zoology* 45, 387–393.

Ao, Z., Chan, M., Ouyang, M. J., Olukitibi, T. A., Mahmoudi, M., Kobasa, D., et al. (2021). Identification and Evaluation of the Inhibitory Effect of Prunella Vulgaris Extract on SARS-Coronavirus 2 Virus Entry. *PLoS One* 16, e0251649. doi:10.1371/journal.pone.0251649

Arokiyaraj, S., Arasu, M. V., Vincent, S., Prakash, N. U., Choi, S. H., Oh, Y. K., et al. (2014). Rapid Green Synthesis of Silver Nanoparticles from Chrysanthemum Indicum L and its Antibacterial and Cytotoxic Effects: an *In Vitro* Study. *Int. J. Nanomedicine* 9, 379–388. doi:10.2147/IJN.S53546

Arokiyaraj, S., Dinesh Kumar, V., Elakya, V., Kamala, T., Park, S. K., Ragam, M., et al. (2015). Biosynthesized Silver Nanoparticles Using Floral Extract of Chrysanthemum Indicum L.--potential for Malaria Vector Control. *Environ. Sci. Pollut. Res. Int.* 22, 9759–9765. doi:10.1007/s11356-015-4148-9

Bai, Y., Li, K., Shao, J., Luo, Q., and Jin, L. H. (2018). Flos Chrysanthemi Indici Extract Improves a High-Sucrose Diet-Induced Metabolic Disorder in Drosophila. *Exp. Ther. Med.* 16, 2564–2572. doi:10.3892/etm.2018.6470

Bekut, M., Brkić, S., Kladar, N., Dragović, G., Gavarić, N., and Božin, B. (2018). Potential of Selected Lamiaceae Plants in Anti(retro)viral Therapy. *Pharmacol. Res.* 133, 301–314. doi:10.1016/j.phrs.2017.12.016

Cai, S., Sun, W., Fan, Y., Guo, X., Xu, G., Xu, T., et al. (2016). Effect of Mulberry Leaf (Folium Mori) on Insulin Resistance via IRS-1/PI3K/Glut-4 Signalling Pathway in Type 2 Diabetes Mellitus Rats. *Pharm. Biol.* 54, 2685–2691. doi:10.1080/13880209.2016.1178779

Cai, S.-f., Shi, J., Huang, X., and Huang, H. (2014). Xiasangju fufang peiwu de huaxuechengfen bianhua yanjiu [Study on the chemical composition variation of Xiasangju compound compounding]. *J. North Pharm.* 11, 10–11.

Chen, F., Kawashima, A., Luo, Y., Kiriya, M., and Suzuki, K. (2020a). Innate Immune-Modulatory Activity of Prunella Vulgaris in Thyrocytes Functions as a Potential Mechanism for Treating Hashimoto's Thyroiditis. *Front. Endocrinol. (Lausanne)* 11, 579648. doi:10.3389/fendo.2020.579648

Chen, Y., Zhang, X., Guo, Q., Cao, L., Qin, Q., Li, C., et al. (2019). Plant Morphology, Physiological Characteristics, Accumulation of Secondary Metabolites and Antioxidant Activities of Prunella Vulgaris L. Under UV Solar Exclusion. *Biol. Res.* 52, 17. doi:10.1186/s40659-019-0225-8

Chen, Y., Zhang, X., Guo, Q., Liu, L., Li, C., Cao, L., et al. (2018). Effects of UV-B Radiation on the Content of Bioactive Components and the Antioxidant Activity of Prunella Vulgaris L. Spica during Development. *Molecules.* 23. doi:10.3390/molecules23050989

Chen, Z., Wu, A., Jin, H., and Liu, F. (2020b). β-Sitosterol Attenuates Liver Injury in a Rat Model of Chronic Alcohol Intake. *Arch. Pharm. Res.* 43, 1197–1206. doi:10.1007/s12272-020-01271-w

Cheng, W., Li, J., You, T., and Hu, C. (2005). Anti-inflammatory and Immunomodulatory Activities of the Extracts from the Inflorescence of Chrysanthemum Indicum Linné. *J. Ethnopharmacol.* 101, 334–337. doi:10.1016/j.jep.2005.04.035

Chinese Pharmacopoeia Commission (2020). *Pharmacopoeia of the People's Republic of China*. Beijing: China Medical Science Press.

Choi, K. T., Kim, J. H., Cho, H. T., Lim, S. S., Kwak, S. S., and Kim, Y. J. (2016). Dermatologic Evaluation of Cosmetic Formulations Containing Chrysanthemum Indicum Extract. *J. Cosmet. Dermatol* 15, 162–168. doi:10.1111/jocd.12211

Deng, J., Li, L., Lin, L. M., Li, Y. M., Xia, B. H., and Liao, D. F. (2021). Metabolic Mechanism of Prunella Vulgaris in Treatment of Ethanol-Induced Oxidative Stress in Rats Based on Metabonomics. *Zhongguo Zhong Yao Za Zhi* 46, 1813–1821. doi:10.19540/j.cnki.cjcmm.20210122.503

Elufioye, T. O., and Habtemariam, S. (2019). Hepatoprotective Effects of Rosmarinic Acid: Insight into its Mechanisms of Action. *Biomed. Pharmacother.* 112. doi:10.1016/j.biopha.2019.108600

Encyclopedia of Life (2022). *Chrysanthemum indicum* L. Encyclopedia of Life. Available: https://eol.org/(Accessed April 15, 2022).

Feng, L., Jia, X., Zhu, M. M., Chen, Y., and Shi, F. (2010). Antioxidant Activities of Total Phenols of Prunella Vulgaris L. *In Vitro* and in Tumor-Bearing Mice. *Molecules* 15, 9145–9156. doi:10.3390/molecules15129145

Feng, T. Y., Lv, D. L., Zhang, X., Du, Y. Q., Yuan, Y. T., Chen, M. J., et al. (2020). Rosmarinic Acid Improves Boar Sperm Quality, Antioxidant Capacity and Energy Metabolism at 17°C via AMPK Activation. *Reprod. Domest. Anim.* 55, 1714–1724. doi:10.1111/rda.13828

Fraile, L., Crisci, E., Córdoba, L., Navarro, M. A., Osada, J., and Montoya, M. (2012). Immunomodulatory Properties of Beta-Sitosterol in Pig Immune Responses. *Int. Immunopharmacol.* 13, 316–321. doi:10.1016/j.intimp.2012.04.017

Gao, W., and Xu, H. (2019). Root Extract of Prunella Vulgaris Inhibits *In Vitro* and *In Vivo* Carcinogenesis in MCF-5 Human Breast Carcinoma via Suppression of Angiogenesis, Induction of Apoptosis, Cell Cycle Arrest and Modulation of PI3K/AKT Signalling Pathway. *J. BUON* 24, 549–554.

Gbif | Global Biodiversity Information Facility (2022). Species in the GBIF Taxonomy. Global Biodiversity Information Facility. Available: https://www.gbif.org/(Accessed April 25, 2022).

Gbif.Org. (2022a). Chrysanthemum Indicum L. In GBIF Secretariat. GBIF Backbone Taxonomy. doi:10.15468/39omei

Gbif.Org. (2022b). Morus Alba L. In GBIF Secretariat. GBIF Backbone Taxonomy. doi:10.15468/39omei

Gbif.Org (2022c). Prunella Vulgaris L. In GBIF Secretariat. GBIF Backbone Taxonomy. Available: GBIF.org (Accessed April 25, 2022).

Groşan, A., Stefanescu, R., Gurzu, S., Muntean, D. L., Vlase, L., and Vari, C. E. (2020a). Study of the Potential Antiulcerous Action of Hydroalcoholic Extracts from Prunella Vulgaris L. Of Romanian Origin. *Farmacia* 68, 870–881. doi:10.31925/farmacia.2020.5.14

Groşan, A., Vari, C.-E., Ştefănescu, R., Danciu, C., Pavel, I. Z., Dehelean, C., et al. (2020b). Antibacterial and Antitumor Activity of the Species Prunella Vulgaris L. *Rev. Romana De. Med. De. Lab.* 28, 405–417. doi:10.2478/rrlm-2020-0031

Guangzhou Xingqun Pharmaceutical (2022). Xiasangju. Guangzhou Xingqun Pharmaceutical. Available: http://xingqun.com.cn/(Accessed April 15, 2022).

Guo, J. (2010). *Yizhong Xiasangju Guodong [A Kind of Summer Sang Ju Yin Jelly]*. CHINA Patent CN101874574A.

Guo, Q., Qu, H., Zhang, H., and Zhong, X. (2021). Prunella Vulgaris L. Attenuates Experimental Autoimmune Thyroiditis by Inhibiting HMGB1/TLR9 Signaling. *Drug Des. Devel Ther.* 15, 4559–4574. doi:10.2147/DDDT.S325814

Habtemariam, S. (2019). Antioxidant and Anti-inflammatory Mechanisms of Neuroprotection by Ursolic Acid: Addressing Brain Injury, Cerebral Ischemia, Cognition Deficit, Anxiety, and Depression. *Oxidative Med. Cell. Longev* 2019. doi:10.1155/2019/8512048

Hua, R.-f., Yao, J.-x., Li, Z.-j., Fang, T.-z., Jiang, L.-j., and Xu, Z.-d. (2013). HPLC tongshi ceding Xiasangju keli zhong midiexiangsuan yu yimidiexiangsuangan de hanliang [Simultaneous Determination of Rosmarinic Acid and Salviaflaside in Xiasangju Granules of by HPLC]. *Chin. J. Exp. Traditional Med. Formulae* 19, 75–77.

Huang, K., Zhang, P., Zhang, Z., Youn, J. Y., Wang, C., Zhang, H., et al. (2021). Traditional Chinese Medicine (TCM) in the Treatment of COVID-19 and Other Viral Infections: Efficacies and Mechanisms. *Pharmacol. Ther.* 225, 107843. doi:10.1016/j.pharmthera.2021.107843

Huang, X.-j., Hou, W., Zhao, Y.-l., Luo, F., and Yang, Z.-q. (2007). Xiasangju kang huxidaohebao bingdu de shiyan yanjiu [Experimental study on the anti-respiratory syncytial virus of Xiasangju]. *Chin. J. Mod. Drug Appl.* 8, 11–14.

Huang, Y.-l. (2013). HPLC Ceding Xiasangju Keli Zhong Midiexiangsuan Hanliang [Determination of Rosmarinic Acid in Xiasangju Granules by HPLC]. *Chin. J. Ethnomedicine Ethnopharmacy* 22, 15–16.

Hunyadi, A., Martins, A., Hsieh, T. J., Seres, A., and Zupkó, I. (2012). Chlorogenic Acid and Rutin Play a Major Role in the *In Vivo* Anti-diabetic Activity of Morus Alba Leaf Extract on Type II Diabetic Rats. *Plos One* 7, e50619. doi:10.1371/journal.pone.0050619

Hwang, S. H., Paek, J. H., and Lim, S. S. (2016). Simultaneous Ultra Performance Liquid Chromatography Determination and Antioxidant Activity of Linarin, Luteolin, Chlorogenic Acid and Apigenin in Different Parts of Compositae Species. *Molecules* 21. doi:10.3390/molecules21111609

Jeong, G. S., An, R. B., Pae, H. O., Oh, G. S., Chung, H. T., and Kim, Y. C. (2008). Heme Oxygenase-1 Inducing Constituent of Prunella Vulgaris in HepG2 Cells. *Biol. Pharm. Bull.* 31, 531–533. doi:10.1248/bpb.31.531

Jeong, S. C., Kim, S. M., Jeong, Y. T., and Song, C. H. (2013). Hepatoprotective Effect of Water Extract from Chrysanthemum Indicum L. Flower. *Chin. Med.* 8, 7. doi:10.1186/1749-8546-8-7

Jin, P., Tan, X. B., Liu, W. B., and Jia, X. B. (2012). Regulation Mechanism of Triterpenoid Components from Prunella Asiatica on Phase II Detoxifying Enzymes *In Vitro* and *In Vivo*. *Zhongguo Zhong Yao Za Zhi* 37, 3637–3640. doi:10.3109/13880209.2011.611143

Kang, H., Park, C.-H., Kwon, S.-O., and Lee, S.-G. (2021). Antioxidant and Anti-inflammatory Activities of Chrysanthemum Indicum Linne Extracts at Different Ethanol Ratios. *Korean J. Food Sci. Technol.* 53, 416–422. doi:10.9721/KJFST.2021.53.4.416

Katsube, T., Tsurunaga, Y., Sugiyama, M., Furuno, T., and Yamasaki, Y. (2009). Effect of Air-Drying Temperature on Antioxidant Capacity and Stability of Polyphenolic Compounds in Mulberry (Morus Alba L.) Leaves. *Food Chem.* 113, 964–969. doi:10.1016/j.foodchem.2008.08.041

Ke, X.-h., Sun, W.-g., Yao, J.-x., Hua, R.-f., and Chen, J.-f. (2008). Xiasangju keli zhiwentupu de jianli jiqi zai zhiliangkongzhi zhong de yingyong [Fingerprint of Xiasangju Granules and its application in quality control]. *Chin. Tradit. Pat. Med.* 30 (7), 937–941.

Ke, X.-h., Sun, W.-g., Yao, J.-x., and Hua, R.-f. (2007). Xiasangju keli anjisuanlei chengfen zhiwentupu de yanjiu [HPLC fingerprint of amino acids compounds of Xiasangju Granules]. *Chin. Tradit. Pat. Med.* 29 (6), 781–784.

Kim, A., Lee, S. Y., Seo, C. S., and Chung, S. K. (2020). Prunellae Spica Extract Suppresses Teratoma Formation of Pluripotent Stem Cells through P53-Mediated Apoptosis. *Nutrients* 12. doi:10.3390/nu12030721

Kim, C., Kim, M. C., Kim, S. M., Nam, D., Choi, S. H., Kim, S. H., et al. (2013). Chrysanthemum Indicum L. Extract Induces Apoptosis through Suppression of Constitutive STAT3 Activation in Human Prostate Cancer DU145 Cells. *Phytother. Res.* 27, 30–38. doi:10.1002/ptr.4689

Kobayashi, Y., Miyazawa, M., Kamei, A., Abe, K., and Kojima, T. (2010). Ameliorative Effects of Mulberry (Morus Alba L.) Leaves on Hyperlipidemia

in Rats Fed a High-Fat Diet: Induction of Fatty Acid Oxidation, Inhibition of Lipogenesis, and Suppression of Oxidative Stress. *Biosci. Biotechnol. Biochem.* 74, 2385–2395. doi:10.1271/bbb.100392

Komal, S., Kazmi, S. A. J., Khan, J. A., and Gilani, M. M. (2018). Antimicrobial Activity of Prunella Vulgaris Extracts against Multi-Drug Resistant Escherichia Coli from Patients of Urinary Tract Infection. *Pak J. Med. Sci.* 34, 616–620. doi:10.12669/pjms.343.14982

Kozyra, M., Biernasiuk, A., Malm, A., and Chowaniec, M. (2015). Chemical Compositions and Antibacterial Activity of Extracts Obtained from the Inflorescences of Cirsium Canum (L.) All. *Nat. Prod. Res.* 29, 2059–2063. doi:10.1080/14786419.2015.1030341

Kwon, D. H., Cheon, J. M., Choi, E. O., Jeong, J. W., Lee, K. W., Kim, K. Y., et al. (2016). The Immunomodulatory Activity of Mori Folium, the Leaf of Morus Alba L., in RAW 264.7 Macrophages *In Vitro*. *J. Cancer Prev.* 21, 144–151. doi:10.15430/JCP.2016.21.3.144

Kwon, D. H., Jeong, J. W., Choi, E. O., Lee, H. W., Lee, K. W., Kim, K. Y., et al. (2017). Inhibitory Effects on the Production of Inflammatory Mediators and Reactive Oxygen Species by Mori Folium in Lipopolysaccharide-Stimulated Macrophages and Zebrafish. *Acad Bras Cienc* 89, 661–674. doi:10.1590/0001-3765201720160836

Lee, J. H., Moon, J. M., Kim, Y. H., Lee, B., Choi, S. Y., Song, B. J., et al. (2019). Effect of Enzymatic Treatment of Chrysanthemum Indicum Linné Extracts on Lipid Accumulation and Adipogenesis in High-Fat-Diet-Induced Obese Male Mice. *Nutrients* 11. doi:10.3390/nu11020269

Li, B. Y., Hu, Y., Li, J., Shi, K., Shen, Y. F., Zhu, B., et al. (2019). Ursolic Acid from Prunella Vulgaris L. Efficiently Inhibits IHNV Infection *In Vitro* and *In Vivo*. *Virus Res.* 273, 197741. doi:10.1016/j.virusres.2019.197741

Li, C., You, L., Fu, X., Huang, Q., Yu, S., and Liu, R. H. (2015). Structural Characterization and Immunomodulatory Activity of a New Heteropolysaccharide from Prunella Vulgaris. *Food Funct.* 6, 1557–1567. doi:10.1039/c4fo01039f

Liao, H., Banbury, L. K., and Leach, D. N. (2008). Antioxidant Activity of 45 Chinese Herbs and the Relationship with Their TCM Characteristics. *Evid. Based Complement. Altern. Med.* 5, 429–434. doi:10.1093/ecam/nem054

Lin, L.-m., Xia, B.-h., Liu, J.-y., Li, C., He, Y.-c., Yao, J.-x., et al. (2013). RP-HPLC Fa Tongshi Ceding Xiasangju Keli Zhong Lvyuansuan, Yimidiexiangsuangan, Midiexiangsuan He Menghuagan [Simultaneous Determination of Chlorogenic Acid, Salviaflaside, Rosmarinic Acid and Linarin in Xiasangju Granules by RP-HPLC]. *Chin. Tradit. Pat. Med.* 35, 2411–2415.

Lin, L.-m., Xu, Z.-d., Yao, J.-x., Liu, J.-y., Li, C., and Wang, Z.-m. (2012a). Xiasangju Keli Zhiliangbiaozhun Yanjiu [Quality Standards for Xiasangju Granules]. *Chin. Tradit. Pat. Med.* 34, 1500–1505.

Lin, M.-c., Xu, H., Huang, X., Lin, X.-d., and Yu, B.-c. (2012b). Xiasangju tiqugongyi de zhengjiaoyouhua yanjiu [Orthogonal optimization study on the extraction process of Xiasangju]. *J. North Pharm.* 9, 23–24.

Liu, J., Lu, Y. F., Wu, Q., Xu, S. F., Shi, F. G., and Klaassen, C. D. (2019). Oleanolic Acid Reprograms the Liver to Protect against Hepatotoxicants, but Is Hepatotoxic at High Doses. *Liver Int.* 39, 427–439. doi:10.1111/liv.13940

Liu, J. (1995). Pharmacology of Oleanolic Acid and Ursolic Acid. *J. Ethnopharmacol.* 49, 57–68. doi:10.1016/0378-8741(95)90032-2

Liu, Q., Liu, J., Wu, C., and Yang, J. (2013). The Treatments of Total Alkali from Morus Folium Jiangtang Capsule Combined with Diet and Exercise Program for Type 2 Diabetes Mellitus Equivalence Randomized Controlled Study. *J. Pract. Traditional Chin. Intern. Med.* 27, 24–27.

Liu, W.-g., Su, L.-q., and Guo, X.-l. (2012). Zhongyao Xiasangju fufang tiquwu zhong de huangtong lei chenfen yanjiu [Study on flavonoids in the extract of Chinese Medicine compound Xiasangju]. *Int. J. Traditional Chin. Med.* 6, 522–524.

Luo, Y., Fang, T., Yao, J., Xu, Z., Xia, B., Lin, L., et al. (2016). Xiasangju keli zhong midiexiangsuan de hanliangceding [Determination of Rosmarinic Acid in Xiasangju granules]. *Chin. J. Ethnomedicine Ethnopharmacy* 25, 44–46.

Luyen, B. T., Tai, B. H., Thao, N. P., Lee, Y. M., Lee, S. H., Jang, H. D., et al. (2015). The Anti-osteoporosis and Antioxidant Activities of Chemical Constituents from Chrysanthemum Indicum Flowers. *Phytother. Res.* 29, 540–548. doi:10.1002/ptr.5281

Lyu, K., Yue, W., Ran, J., Liu, Y., and Zhu, X. (2021). *In Vivo* therapeutic Exploring for Mori Folium Extract against Type 2 Diabetes Mellitus in Rats. *Biosci. Rep.* 41. doi:10.1042/BSR20210977

Ma, R., Weng, H., and Liang, J. (2016). Screening of Lipase Inhibitors in Folium Mori with Lipase-Linked Magnetic Microspheres by High-Performance Liquid Chromatography and Evaluation in Diabetic Mice. *J. Sep. Sci.* 39, 4474–4483. doi:10.1002/jssc.201600924

Ma, W., Wu, X.-m., and Zhang, K.-g. (2011). Xiasangju tiquwu dui ziyouji de qingchu zuoyong [Free radical scavenging effect of Xiasangju extract]. *Chin. J. Spectrosc. Laboratory* 28, 2313–2316.

Miceli, N., Taviano, M. F., Giuffrida, D., Trovato, A., Tzakou, O., and Galati, E. M. (2005). Anti-inflammatory Activity of Extract and Fractions from Nepeta Sibthorpii Bentham. *J. Ethnopharmacol.* 97, 261–266. doi:10.1016/j.jep.2004.11.024

Ming, K., Chen, Y., Shi, J., Yang, J., Yao, F., Du, H., et al. (2017). Effects of Chrysanthemum Indicum Polysaccharide and its Phosphate on Anti-duck Hepatitis a Virus and Alleviating Hepatic Injury. *Int. J. Biol. Macromol.* 102, 813–821. doi:10.1016/j.ijbiomac.2017.04.093

Ming, K., Yuan, W., Chen, Y., Du, H., He, M., Hu, Y., et al. (2019). PI3KC3-dependent Autophagosomes Formation Pathway Is of Crucial Importance to Anti-DHAV Activity of Chrysanthemum Indicum Polysaccharide. *Carbohydr. Polym.* 208, 22–31. doi:10.1016/j.carbpol.2018.12.035

Namgung, S., Yoon, J. J., Yoon, C. S., Han, B. H., Choi, E. S., Oh, H., et al. (2017). Prunella Vulgaris Attenuates Diabetic Renal Injury by Suppressing Glomerular Fibrosis and Inflammation. *Am. J. Chin. Med.* 45, 475–495. doi:10.1142/S0192415X1750029X

National Health Commission of the People's Republic of China (2000). *Drug Standard of the Ministry of Health of the People's Republic of China*. Beijing: China Medical Science Press.

National Medical Products Administration (2022). Xiasangju. Available: https://www.nmpa.gov.cn/(Accessed April 26, 2022).

Nepali, S., Cha, J. Y., Ki, H. H., Lee, H. Y., Kim, Y. H., Kim, D. K., et al. (2018). Chrysanthemum Indicum Inhibits Adipogenesis and Activates the AMPK Pathway in High-Fat-Diet-Induced Obese Mice. *Am. J. Chin. Med.* 46, 119–136. doi:10.1142/S0192415X18500076

Nolkemper, S., Reichling, J., Stintzing, F. C., Carle, R., and Schnitzler, P. (2006). Antiviral Effect of Aqueous Extracts from Species of the Lamiaceae Family against Herpes Simplex Virus Type 1 and Type 2 *In Vitro*. *Planta Med.* 72, 1378–1382. doi:10.1055/s-2006-951719

Ododo, M. M., Choudhury, M. K., and Dekebo, A. H. (2016). Structure Elucidation of β-sitosterol with Antibacterial Activity from the Root Bark of Malva Parviflora. *Springerplus* 5, 1210. doi:10.1186/s40064-016-2894-x

Oh, C., Price, J., Brindley, M. A., Widrlechner, M. P., Qu, L., Mccoy, J. A., et al. (2011). Inhibition of HIV-1 Infection by Aqueous Extracts of Prunella Vulgaris L. *Virol. J.* 8, 188. doi:10.1186/1743-422X-8-188

Osakabe, N., Yasuda, A., Natsume, M., Sanbongi, C., Kato, Y., Osawa, T., et al. (2002). Rosmarinic Acid, a Major Polyphenolic Component of Perilla Frutescens, Reduces Lipopolysaccharide (LPS)-induced Liver Injury in D-Galactosamine (D-GalN)-Sensitized Mice. *Free Radic. Biol. Med.* 33, 798–806. doi:10.1016/s0891-5849(02)00970-x

Park, M. Y., and Kang, D. H. (2021). Antibacterial Activity of Caffeic Acid Combined with UV-A Light against *Escherichia coli* O157:H7, *Salmonella enterica* Serovar Typhimurium, and Listeria Monocytogenes. *Appl. Environ. Microbiol.* 87, e0063121. doi:10.1128/AEM.00631-21

Park, S.-N., Lim, Y. K., Lim, Y. K., Cho, E., Jo, E., Park, P.-S., et al. (2014). Antimicrobial Activity of Mulberry Leaf against Mutans Streptococci and Periodontopathogens. *Intern. J. Oral Biol.* 39, 201–208. doi:10.11620/ijob.2014.39.4.201

Peng, L.-y., Peng, Y.-l., and Yang, X.-g. (2014). RP-HPLC ceding Xiasangju keli zhong midiexiangsuan de hanliang [Determination of rosmarinic acid in Xiasangju granules by RP-HPLC]. *China Mod. Med.* 21, 8–9+13.

Ponnulakshmi, R., Shyamaladevi, B., Vijayalakshmi, P., and Selvaraj, J. (2019). In Silico and *In Vivo* Analysis to Identify the Antidiabetic Activity of Beta Sitosterol in Adipose Tissue of High Fat Diet and Sucrose Induced Type-2 Diabetic Experimental Rats. *Toxicol. Mech. Methods* 29, 276–290. doi:10.1080/15376516.2018.1545815

Psotová, J., Kolár, M., Sousek, J., Svagera, Z., Vicar, J., and Ulrichová, J. (2003). Biological Activities of Prunella Vulgaris Extract. *Phytother. Res.* 17, 1082–1087. doi:10.1002/ptr.1324

Qiu, Z., Tang, H., Luo, D., and Li, D. (2011). Xiasangju keli dui dashu tinei malaisuanlvbennamin daixie de yingxiang[The Effect of Banlangen Granula on the Metabolism of Chlorphenamine]. *J. NEW Chin. Med.* 43, 106–107.

Raafat, K., Wurglics, M., and Schubert-Zsilavecz, M. (2016). Prunella Vulgaris L. Active Components and Their Hypoglycemic and Antinociceptive Effects in Alloxan-Induced Diabetic Mice. *Biomed. Pharmacother.* 84, 1008–1018. doi:10.1016/j.biopha.2016.09.095

Rocha, J., Eduardo-Figueira, M., Barateiro, A., Fernandes, A., Brites, D., Bronze, R., et al. (2015). Anti-inflammatory Effect of Rosmarinic Acid and an Extract of Rosmarinus Officinalis in Rat Models of Local and Systemic Inflammation. *Basic Clin. Pharmacol. Toxicol.* 116, 398–413. doi:10.1111/bcpt.12335

Ryu, S. Y., Oak, M. H., Yoon, S. K., Cho, D. I., Yoo, G. S., Kim, T. S., et al. (2000). Anti-allergic and Anti-inflammatory Triterpenes from the Herb of Prunella Vulgaris. *Planta Med.* 66, 358–360. doi:10.1055/s-2000-8531

Sárosi, S., Bernáth, J., Burchi, G., Antonetti, M., Bertoli, A., Pistelli, L., et al. (2011). Effect of Different Plant Origins and Climatic Conditions on the Total Phenolic Content and Total Antioxidant Capacity of Self-Heal (Prunella Vulgaris L.). *Acta Hortic.* 925, 49–55. doi:10.17660/ActaHortic.2011.925.5

Sheng, Y., Zheng, S., Zhang, C., Zhao, C., He, X., Xu, W., et al. (2018). Mulberry Leaf Tea Alleviates Diabetic Nephropathy by Inhibiting PKC Signaling and Modulating Intestinal Flora. *J. Funct. Foods* 46, 118–127. doi:10.1016/j.jff.2018.04.040

Song, J., Zhang, Z., Hu, Y., Li, Z., Wan, Y., Liu, J., et al. (2021a). An Aqueous Extract of Prunella Vulgaris L. Inhibits the Growth of Papillary Thyroid Carcinoma by Inducing Autophagy *In Vivo* and *In Vitro*. *Phytotherapy Res.* 35, 2691–2702. doi:10.1002/ptr.7015

Song, Y. G., Kang, L., Tian, S., Cui, L. L., Li, Y., Bai, M., et al. (2021b). Study on the Anti-hepatocarcinoma Effect and Molecular Mechanism of Prunella Vulgaris Total Flavonoids. *J. Ethnopharmacol.* 273, 113891. doi:10.1016/j.jep.2021.113891

Sun, H. X., Qin, F., and Pan, Y. J. (2005). *In Vitro* and *In Vivo* Immunosuppressive Activity of Spica Prunellae Ethanol Extract on the Immune Responses in Mice. *J. Ethnopharmacol.* 101, 31–36. doi:10.1016/j.jep.2005.03.023

Sun, P., Li, G.-j., Zhu, K., and Wang, R. (2016). Xiasangju liangcha zhong duochengfen hanliangceding fangfa de yanjiu [Determination of Active Components in Xisangju Herbal Tea Beverage]. *Food Industry* 37, 278–281.

Sun, W.-g., Fang, T.-z., and He, G.-x. (2006). *Yingyong mofenli jishu dui Xiasangju tiquye jingzhi de xingongyi [A new process of applying membrane separation technology to the refinement of Xiasangju extracts.* CHINA Patent CN1823908A.

Sun, W.-g., Tan, Y.-h., Fang, T.-z., Su, G.-f., Yao, J.-x., Xu, Z.-d., et al. (2008). *Xiasangju zhiji de zhibei fangfa [Preparation method of Xiasangju formulation].* CHINA Patent CN101297864A.

Sun, X., Yamasaki, M., Katsube, T., and Shiwaku, K. (2015). Effects of Quercetin Derivatives from Mulberry Leaves: Improved Gene Expression Related Hepatic Lipid and Glucose Metabolism in Short-Term High-Fat Fed Mice. *Nutr. Res. Pract.* 9, 137–143. doi:10.4162/nrp.2015.9.2.137

Sun, Z., Zhang, X., Wu, H., Wang, H., Bian, H., Zhu, Y., et al. (2020). Antibacterial Activity and Action Mode of Chlorogenic Acid against Salmonella Enteritidis, a Foodborne Pathogen in Chilled Fresh Chicken. *World J. Microbiol. Biotechnol.* 36, 24. doi:10.1007/s11274-020-2799-2

Taghizadeh, M., Soleimani, A., Bahmani, F., Moravveji, A., Asadi, A., Amirani, E., et al. (2017). Metabolic Response to Mulberry Extract Supplementation in Patients with Diabetic Nephropathy: a Randomized Controlled Trial. *Iran. J. Kidney Dis.* 11, 438–446.

Taghizadeh, M., Mohammad Zadeh, A., Asemi, Z., Farrokhnezhad, A. H., Memarzadeh, M. R., Banikazemi, Z., et al. (2022). Morus Alba Leaf Extract Affects Metabolic Profiles, Biomarkers Inflammation and Oxidative Stress in Patients with Type 2 Diabetes Mellitus: A Double-Blind Clinical Trial. *Clin. Nutr. ESPEN* 49, 68–73. doi:10.1016/j.clnesp.2022.03.027

Tanida, I., Shirasago, Y., Suzuki, R., Abe, R., Wakita, T., Hanada, K., et al. (2015). Inhibitory Effects of Caffeic Acid, a Coffee-Related Organic Acid, on the Propagation of Hepatitis C Virus. *Jpn. J. Infect. Dis.* 68, 268–275. doi:10.7883/yoken.JJID.2014.309

Thabti, I., Albert, Q., Philippot, S., Dupire, F., Westerhuis, B., Fontanay, S., et al. (2020). Advances on Antiviral Activity of Morus Spp. Plant Extracts: Human Coronavirus and Virus-Related Respiratory Tract Infections in the Spotlight. *Molecules* 25. doi:10.3390/molecules25081876

Tsuji-Naito, K., Saeki, H., and Hamano, M. (2009). Inhibitory Effects of Chrysanthemum Species Extracts on Formation of Advanced Glycation End Products. *Food Chem.* 116, 854–859. doi:10.1016/j.foodchem.2009.03.042

Utsunomiya, H., Ichinose, M., Ikeda, K., Uozaki, M., Morishita, J., Kuwahara, T., et al. (2014). Inhibition by Caffeic Acid of the Influenza A Virus Multiplication *In Vitro*. *Int. J. Mol. Med.* 34, 1020–1024. doi:10.3892/ijmm.2014.1859

Vostálová, J., Zdarilová, A., and Svobodová, A. (2010). Prunella Vulgaris Extract and Rosmarinic Acid Prevent UVB-Induced DNA Damage and Oxidative Stress in HaCaT Keratinocytes. *Arch. Dermatol Res.* 302, 171–181. doi:10.1007/s00403-009-0999-6

Wagner, C., De Gezelle, J., and Komarnytsky, S. (2020). Celtic Provenance in Traditional Herbal Medicine of Medieval Wales and Classical Antiquity. *Front. Pharmacol.* 11, 105. doi:10.3389/fphar.2020.00105

Wang, C. M., Jhan, Y. L., Tsai, S. J., and Chou, C. H. (2016). The Pleiotropic Antibacterial Mechanisms of Ursolic Acid against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Molecules* 21. doi:10.3390/molecules21070884

Wang, X. N., Yang, Y. T., An, Y. T., and Fang, G. (2019). The Mechanism of Anticancer Action and Potential Clinical Use of Kaempferol in the Treatment of Breast Cancer. *Biomed. Pharmacother.* 117. doi:10.1016/j.biopha.2019.109086

Wu, H., Gao, M., Ha, T., Kelley, J., Young, A., and Breuel, K. (2012). Prunella Vulgaris Aqueous Extract Attenuates IL-1β-induced Apoptosis and NF-Kb Activation in INS-1 Cells. *Exp. Ther. Med.* 3, 919–924. doi:10.3892/etm.2012.524

Wu, J., Zhu, Y., Li, F., Zhang, G., Shi, J., Ou, R., et al. (2016). Spica Prunellae and its Marker Compound Rosmarinic Acid Induced the Expression of Efflux Transporters through Activation of Nrf2-Mediated Signaling Pathway in HepG2 Cells. *J. Ethnopharmacol.* 193, 1–11. doi:10.1016/j.jep.2016.07.021

Wu, J. (2019). Xiasangju youxiao buwei de zongzaogan ceding [Determination of total saponins in the active parts of Xanthium]. *Strait Pharm. J.* 31, 95–97.

Wu, W., Liang, K. L., Chen, B., Su, J., Chen, S. H., and Lyu, G. Y. (2017). Effects of Mori Folium Extract on Diet-Induced Obesity Mechanism in Rats. *Zhongguo Zhong Yao Za Zhi* 42, 1757–1761. doi:10.19540/j.cnki.cjcmm.2017.0069

Xia, B.-h., Pi, S.-l., Zhou, Y.-m., Xie, J.-c., Lin, L.-m., and Li, Y.-m. (2016a). HPLC Yanjiu Xiasangju Keli Peiwu Chengfen Bianhuaguilv [HPLC Study on the Variation Pattern of Dosage Components of Xiasangju Granules]. *J. Chin. Med. Mater.* 39, 1813–1816.

Xia, B. H., Cao, Y., Xie, W. J., Xu, Z. D., Lin, L. M., and Liao, D. F. (2014). Study on UPLC Fingerprint of Xiasangju Granules. *Zhong Yao Cai* 37, 1463–1466.

Xia, B. H., Hu, Y. Z., Xiong, S. H., Tang, J., Yan, Q. Z., and Lin, L. M. (2017). Application of Random Forest Algorithm in Fingerprint of Chinese Medicine: Different Brands of Xiasangju Granules as Example. *Zhongguo Zhong Yao Za Zhi* 42, 1324–1330. doi:10.19540/j.cnki.cjcmm.20170121.020

Xia, B. H., Yan, D., Cao, Y., Zhou, Y. M., Li, Y. M., Xie, J. C., et al. (2016b). Analysis of Different Dosage Forms of Xiasangju Granules on Fingerprints and Models Using High Performance Liquid Chromatography. *Zhongguo Zhong Yao Za Zhi* 41, 416–420. doi:10.4268/cjcmm20160309

Xiao, J., Li, M., Liu, X., Song, L., and Long, X. (2014). Duozhibiao Zonghe Youxuan Xiasangju Junzhi Tiqu Gongyi yanjiu[Study on the Homogenization Extraction of Xiasangju by Comprehensive Evaluation of Multiple Indexes]. *J. Guangdong Pharm. Univ.* 30, 688–692. doi:10.1021/la5030795

Xin, Y.-z., and Tang, W.-z. (2013). Xiasangju fufang zhong duofen lei chenfen de HPLC-MS fenxi [HPLC-MS analysis of polyphenolic components in Xiasangju Compound]. *Shandong J. Traditional Chin. Med.* 32, 828–830.

Xu, M.-g., and Wei, K.-f. (2013). WENBINGTIAOBIAN" Sang Ju Yin Fangzhengtanxi [Analysis of the Evidence of the Formula of Sang Ju Yin]. *Shandong J. Traditional Chin. Med.* 32, 681–682.

Xu, Z. F., Sun, X. K., Lan, Y., Han, C., Zhang, Y. D., and Chen, G. (2017). Linarin Sensitizes Tumor Necrosis Factor-Related Apoptosis (TRAIL)-induced Ligand-Triggered Apoptosis in Human Glioma Cells and in Xenograft Nude Mice. *Biomed. Pharmacother.* 95, 1607–1618. doi:10.1016/j.biopha.2017.08.021

Yang, H. M., Sun, C. Y., Liang, J. L., Xu, L. Q., Zhang, Z. B., Luo, D. D., et al. (2017). Supercritical-Carbon Dioxide Fluid Extract from Chrysanthemum Indicum Enhances Anti-tumor Effect and Reduces Toxicity of Bleomycin in Tumor-Bearing Mice. *Int. J. Mol. Sci.* 18. doi:10.3390/ijms18030465

Yang, L., Wei, D. D., Chen, Z., Wang, J. S., and Kong, L. Y. (2011). Reversal of Multidrug Resistance in Human Breast Cancer Cells by Curcuma Wenyujin

and Chrysanthemum Indicum. *Phytomedicine* 18, 710–718. doi:10.1016/j.phymed.2010.11.017

Yang, M. Y., Wu, C. H., Hung, T. W., and Wang, C. J. (2020). Endoplasmic Reticulum Stress-Induced Resistance to Doxorubicin Is Reversed by Mulberry Leaf Polyphenol Extract in Hepatocellular Carcinoma through Inhibition of COX-2. *Antioxidants* 9. doi:10.3390/antiox9050379

Yao, J.-x., Fang, T.-z., Peng, T.-h., Ji, X.-m., Zhang, X.-c., Zhang, J.-k., et al. (2017a). *Xiasangju zai zhibei fangzhi denggere de yaowu zhong de xinyingyong [New application of Xiasangju in the preparation of drugs against dengue fever]*. CHINA Patent CN107397793A. China National Intellectual Property Administration. Beijing, China.

Yao, J.-x., Fang, T.-z., Peng, T.-h., Pu, Q.-h., Zhang, X.-c., Zhang, J.-k., et al. (2017b). *Xiasangju zai zhibei fangzhi shouzukoubing de yaowu zhong de xinyingyong [New application of Xiasangju in the preparation of drugs against hand, foot and mouth disease]*. CHINA Patent CN107412340A. Beijing, China: China National Intellectual Property Administration.

Yao, J., Ke, X., and Hua, R. (2012). Butong pinpai Xiasangju keli zhiwentupu de duibi yanjiu [Comparative Study on Fingerprint Chromatograms of Different Brands of Xiasangju Granules]. *Traditional Chin. Drug Res. Clin. Pharmacol.* 23, 460–463.

Youssef, F. S., Eid, S. Y., Alshammari, E., Ashour, M. L., Wink, M., and El-Readi, M. Z. (2020). Chrysanthemum Indicum and Chrysanthemum Morifolium: Chemical Composition of Their Essential Oils and Their Potential Use as Natural Preservatives with Antimicrobial and Antioxidant Activities. *Foods* 9. doi:10.3390/foods9101460

Yu, B., Lin, M., Lu, X., Wu, Y., Huang, H., Zhu, Q., et al. (2011). Research on the quality control and health function of Xiasangju [Xiasangju de zhiliangkongzhi he baojiangongneng yanjiu]. *Guide China Med.* 9, 219–221.

Yu, F., Zhang, L., Ma, R., Liu, C., Wang, Q., and Yin, D. (2021). The Antitumour Effect of Prunella Vulgaris Extract on Thyroid Cancer Cells *In Vitro* and *In Vivo*. *Evidence-Based Complementary Altern. Med.* 2021–8869323. doi:10.1155/2021/8869323

Yu, Q., Li, X., and Cao, X. (2017). Linarin Could Protect Myocardial Tissue from the Injury of Ischemia-Reperfusion through Activating Nrf-2. *Biomed. Pharmacother.* 90, 1–7. doi:10.1016/j.biopha.2017.03.025

Yu, S.-m., Li, Y.-h., Jiang, Y., and Wang, L. (2018). Xiasangju dui jiaxing H1N1 liugan bingdu de zuoyong jiqi jizhi [Effect of XiaSangju on influenza A (H1N1) virus and relevant mechanism]. *Chin. J. Biol.* 31, 1099–1103.

Zeng, S.-p., and Ding, Y. (2008). RP-HPLC fa ceding Xiasangju keli zhong menghuagan de hanliang [RP-HPLC determination of buddleoside in Xiaxiangju granules]. *Chin. J. Pharm. Analysis* 28, 2109–2110.

Zhan, H.-q., and Dong, T.-x. (2009). *Yizhong kangliugandu de zhongyao youxiao buwei jiqi zhibei fangfa [An effective part of an anti-influenza virus Chinese medicine and its preparation method]*. CHINA Patent CN100493532C.

Zhang, L., Yao, J.-x., Ji, X.-m., Yu, T.-t., Pu, Q.-h., Tang, R., et al. (2019a). Xiasangju keli tiwai kang Ⅰxing denggere bingdu de zuoyong Inhibitory effects of Xiasangju granules on dengue virus type Ⅰ *In Vitro*]. *Guangdong Med. J.* 40, 1250–1254.

Zhang, X., Ao, Z., Bello, A., Ran, X., Liu, S., Wigle, J., et al. (2016). Characterization of the Inhibitory Effect of an Extract of Prunella Vulgaris on Ebola Virus Glycoprotein (GP)-mediated Virus Entry and Infection. *Antivir. Res.* 127, 20–31. doi:10.1016/j.antiviral.2016.01.001

Zhang, X., Wu, J. Z., Lin, Z. X., Yuan, Q. J., Li, Y. C., Liang, J. L., et al. (2019b). Ameliorative Effect of Supercritical Fluid Extract of Chrysanthemum Indicum Linnén against D-Galactose Induced Brain and Liver Injury in Senescent Mice via Suppression of Oxidative Stress, Inflammation and Apoptosis. *J. Ethnopharmacol.* 234, 44–56. doi:10.1016/j.jep.2018.12.050

Zhao, X. (2015). *Fengmi Xiasangju yinliao de zhibei fangfa [Preparation method of honey Xiasangju drink]*. CHINA Patent CN104287028A.

Zhao, Z., Li, X., Zha, Z., Cui, B., and Li, Y. (2021). A Clinical Observation and Study on Prunella Vulgaris Decoction in Promoting the Repair of Chronic Infective Refractory Wounds. *Adv. Emerg. Med.* 10 (3), 10–14. doi:10.18686/aem.v10i3.199

Zhen, Z. G., Ren, S. H., Ji, H. M., Ma, J. H., Ding, X. M., Feng, F. Q., et al. (2017). Linarin Suppresses Glioma through Inhibition of NF-κB/p65 and Up-Regulating P53 Expression *In Vitro* and *In Vivo*. *Biomed. Pharmacother.* 95, 363–374. doi:10.1016/j.biopha.2017.08.023

Zheng, Q. S., Sun, X. L., Xu, B., Li, G., and Song, M. (2005). Mechanisms of Apigenin-7-Glucoside as a Hepatoprotective Agent. *Biomed. Environ. Sci.* 18, 65–70. doi:10.1016/j.jenvman.2004.08.015

Zheng, X.-Q., Song, L.-X., Han, Z.-Z., Yang, Y.-B., Zhang, Y., Gu, L.-H., et al. (2022). Pentacyclic Triterpenoids from Spikes of Prunella Vulgaris L. With Thyroid Tumour Cell Cytostatic Bioactivities. *Nat. Prod. Res.* 1-9, 1–9. doi:10.1080/14786419.2021.2024532

Zhou, F.-f., Tang, W.-z., Wang, X.-j., and Jia, X.-h. (2012). Xiasangju fufang huaxue chengfen de yanjiu [Study on Chemical Constituents of Xiasangju Formula]. *Food Drug* 14, 107–109.

Zhou, F.-f. (2012). *Xiasangju fufang huaxue chengfen de yanjiu [Study on Chemical Constituents of Xiasangju Formula]. Master Master*. Jinan: University of Jinan.

Zhou, J.-y., Chen, J.-p., and Hu, F. (2014). HPLC fa ceding Xiasangju keli zhong midiexiangsuan de hanliang fenxi [Determination of Rosmarinic acid in Xiasangju Granules by HPLC]. *J. North Pharm.* 11, 9.

Zhou, Q. X., Liu, F., Zhang, J. S., Lu, J. G., Gu, Z. L., and Gu, G. X. (2013). Effects of Triterpenic Acid from Prunella Vulgaris L. On Glycemia and Pancreas in Rat Model of Streptozotozin Diabetes. *Chin. Med. J. Engl.* 126, 1647–1653.

Zhu, J., Zhang, W., Zhang, Y., Wang, Y., Liu, M., and Liu, Y. (2018). Effects of Spica Prunellae on Caspase-3-Associated Proliferation and Apoptosis in Human Lung Cancer Cells *In Vitro*. *J. Cancer Res. Ther.* 14, 760–763. doi:10.4103/jcrt.JCRT_1289_16

Zhu, Q., Muyayalo, K. P., Xu, Q. H., Wang, J., Wang, H., and Liao, A. H. (2022). Prunella Vulgaris Can Improve the Pregnancy Outcomes of Experimental Autoimmune Thyroiditis Rats by Inhibiting Th1/Th17 Immune Responses. *J. Reprod. Immunol.* 149, 103469. doi:10.1016/j.jri.2021.103469

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.