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Review

A review of *Penthorum chinense* Pursh for hepatoprotection: Traditional use, phytochemistry, pharmacology, toxicology and clinical trials

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ABSTRACT

Ethnopharmacological relevance: In China, *Penthorum chinense* Pursh (*P. chinense*) has been used for hundreds of years traditionally for alleviating symptoms by excessive intake of alcohol as well as in the treatment of traumatic injury, edema and liver diseases. Recently, *P. chinense* and its extract have been developed into tea, drinks or medicines for treatment of liver diseases, including hepatic virus infections, alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD) and liver fibrosis.

Aim of the study: The main purpose of this review is to provide a critical appraisal of the existing knowledge on the phytochemical data, quality control aspect, pharmacological, as well as toxicological and clinical studies performed on *P. chinense*, including the identification of scientific gaps.

Materials and methods: A detailed literature search was conducted using various online search engines, such as Pubmed, Scopus, Google Scholar, Mendeley, Web of Science as well as China National Knowledge Infrastructure (CNKI) database.

Results: In the pharmacological studies, there clearly are links between local/traditional uses and the biomedical investigations. Most pharmacological studies indicated potential liver protective effects in experimental models of chemicals-induced liver injury, acute and chronic alcoholic liver injury, NAFLD, liver fibrosis and viral infection, potentially through antioxidant effects, balancing key liver enzyme levels, inhibition of hepatic virus DNA replication, inhibition of hepatic stellate cells activation and inflammation either *in vitro* or *in vivo*. In some models, the effects of *P. chinense* is comparable with the one of silymarin. Clinical studies have suggested that *P. chinense* is safe and effective in treating several liver diseases, although most of them are not double-blinded and placebo-controlled studies. Toxicology studies show that *P. chinense* has no obvious toxicity or side effects in animals or human. Flavonoids, lignans, coumarins, polyphenols and organic acids have been identified. However, only a few studies have investigated the active compounds (mainly flavonoids and lignans) and molecular mechanisms of *P. chinense*.

Conclusion: P. chinense seems to be safe and shows relevant liver protecting effects. Therefore, it might be a promising candidate for developing as new hepatoprotective agents. However, a lack of understanding of the active compounds and mechanisms of action needs further attention.

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Abbreviations

$ABTS^+$	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ACC	acetyl-CoA carboxylase
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate transaminase
ATGL	triglyceride lipase
CAT	catalase
CCl_4	carbon tetrachloride
CHO	cholesterol
CYP2E1	cytochrome P450 2E1
DAD	diode array detection
DBIL	direct bilirubin
DPPH	2,2-diphenyl-1-picrylhydrazyl
FAS	fatty acid synthase
FRAP	ferric ion reducing antioxidant power
FTICR	Fourier transform ion cyclotron resonance
HA	hyaluronic acid
HDL-C	high density lipoprotein cholesterol
HSC	hepatic stellate cell
GC	gas chromatography
GSH	glutathione
HHDP	hexahydroxydiphenoyl
HO-1	heme oxygenase-1
HPLC	high performance liquid chromatography
IL-1β/6	interleukin-1β/6
KEAP-1	Kelch-like ECH-associated protein 1
LDL	low density lipoprotein
LPS	lipopolysaccharides
LN	laminin
NAD(P)H	nicotinamide adenine dinucleotide phosphate
MDA	malonaldehyde
MS	mass spectrometry
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
NEFA	nonesterified fatty acid
NO	nitric oxide
NRF2	nuclear factor erythroid 2-related factor 2
P. chinense	*Penthorum chinense* Pursh
PGHG	pinocembrin-7-O-[3″-O-galloyl-4″, 6″-hexahydroxydiphenoyl]-β-glucose
p-HSL	phosphorylation of hormone-sensitive lipase
PPARα	peroxisome proliferator-activated receptor α
QC	quality control
ROS	reactive oxygen species
SCD1	stearoyl-CoA desaturase 1
SIRT3	silent mating type information regulation 2 homolog 3
SOD	superoxide dismutase
SREBP1c	sterol regulatory element binding protein-1c
t-BHP	tert-butyl hydroperoxide
TBIL	total bilirubin
TC	total cholesterol
TG	triacylglycerol
TNF-α	tumor necrosis factor-α
WAT	white adipose tissue
α-SMA	α-smooth muscle actin

1. Introduction

Penthorum chinense Pursh (“Gan-Huang-Cao” or “Che-Gen-Cai” in Chinese; *Penthoraceae*) is widely used in China's local and traditional medical systems and is also available as a vegetable or functional drink. According to the basic theory of traditional Chinese medicine (TCM), *P. chinense* has the functions of alleviating heat, diuresis, detoxification as well as promoting blood circulation. Its traditional use has been typically linked to the therapeutic applications towards several liver diseases including jaundice, cholecystitis, non-alcoholic and alcoholic fatty liver, and infectious hepatitis (He et al., 2019; Jeong et al., 2019; Lu et al., 2012), which can be traced back to Ming dynasty (1400s). Till today, the locals frequently use this herb to prevent or treat liver diseases.

In the past decades, the hepatoprotective effect of *P. chinense* has aroused much attentions. It is worth noting that the extract of *P. chinense* is individually involved in 12 Chinese patent pharmaceutical preparations, which have been approved by the China Food and Drug Administration (CFDA) and are being produced by more than 28 Chinese pharmaceutical companies. *P. chinense* is now commercially available as many pharmaceutical formulations in the market such as tablet, granule, capsule and pill under the trademark of “Gansu” (Sun et al., 2001). *P. chinense* preparations are particularly used for liver protection and treatment of acute viral hepatitis and chronic liver diseases. Liver diseases, such as viral hepatitis, alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), liver cirrhosis and hepatocellular carcinoma (HCC), are one of the main health issues and causes of death worldwide (Younossi et al., 2016). As reported in 2014, about 300 million people were affected by liver diseases in China (Wang et al., 2014a,b). Notably, due to the change of lifestyle, the number of Chinese patients with chronic liver diseases (*e.g.*, ALD, NAFLD and HCC) has dramatically increased in recent years (Wang et al., 2014a,b). Unfortunately, only few agents that are effective, economic and safe enough are available to protect against liver diseases. According to the traditional applications, *P. chinense* is considered as a potential candidate and can be further developed and used for treatment of liver diseases.

Recent renewed interests in *P. chinense* have greatly increased the number of pharmacological research to potentiate the use of *P. chinense* as a liver-protecting agent. However, due to the variations in experimental design, disease models and dosage used across studies, there is an intensive need to re-assess all the available results. Furthermore, although many studies have focused on phytochemistry and pharmacology of *P. chinense*, till now, only a few have investigated the potential activities of the main constituents such as polyphenols and other compounds of *P. chinense* as well as the underlying molecular mechanisms. Therefore, a link bridging phytochemical and pharmacological studies on *P. chinense* is needed. The collection and identification of scientific gaps in the literatures remains essential.

This review thus gives a comprehensive summary and evaluation of current research progress on *P. chinense* from aspects of phytochemistry, pharmacology, toxicology and clinical trials, which highlights the existing evidences for hepatoprotection of *P. chinense* and points out scientific gaps to facilitate future research.

2. Methodology

A literature search was performed using online scientific databases including Pubmed, Scopus, Google Scholar, Mendeley, Web of Science as well as the China National Knowledge Infrastructure (CNKI). No time limit was indicated, and examples of search terms were “*Penthorum chinense*”, “Gan-Huang-Cao (In Chinese)”, “Che-Gen-Cai (In Chinese)”, “biological activities”, “liver protection”, “phytochemicals”, “toxicity”, “clinical observations”, *etc*. This review mainly focuses on data collection from primary sources of traditional uses, pharmacological activities, phytochemical constituents, toxicology and clinical investigations and provides beneficial information for future research perspectives. The research papers were subjected to the guidelines proposed by

Mullane and Williams (2015), in order to ensure the credibility, relevance and sustainability of the study process in the biomedical field.

Additional data was also sourced from relevant theses and dissertations from China via CNKI. Ethnobotanical books documenting *P. chinense* were consulted to find information on the applications of the plant in traditional medicine. Botanical name and the family of *P. chinense* was confirmed using the "International Plant Names Index (IPNI)" (https://www.ipni.org/n/274678-1).

3. Traditional and current use

P. chinense is mainly distributed in eastern Asia, such as eastern Russia, Mongolia, China, Japan, Korea, and Laos. It is also called as oriental penthorum. *P. chinense* (Fig. 1) is firstly recorded in an ancient flora, Jiu-Huang-Ben-Cao (1406 AD), in China. Later on, another Chinese local flora, Tian-Bao-Ben-Cao (1900s), described the details of medical applications of *P. chinense*. Based on the theory of TCM, *P. chinense* is "warm" in property and attributes to the "liver" and "kidney" meridians, with the functions of "alleviating heat", "diuresis", "detoxification", and "promoting blood circulation". It is traditionally documented that *P. chinense* can be used for treatment of traumatic injury, edema, dysuria, carbuncle as well as liver diseases like jaundice and cholecystitis. In Korea, people usually use *P. chinense* to treat contusions and skin bruises due to the effect of improving blood flow. *P. chinense* is traditionally applied in decoction or as a functional drink. Till today, the locals in China often apply this plant to alleviate symptoms due to excessive intake of alcohol as well as for treatment of traumatic injury, edema and liver diseases such as jaundice, cholecystitis, fatty liver, and infectious hepatitis.

In China, the Gulin county (Luzhou, Sichuan province) in southwest China, where the Miao minority has inhabited, is considered as the geo-authentic habitat of *P. chinense*. In these local areas, *P. chinense* as a liver-protecting herb is specifically used to deal with liver diseases. Due to its good efficacy, *P. chinense* is called as an "immortal herb" by the locals. *P. chinense* is also locally available as a kind of vegetable.

Currently, several preparations derived from the hot-water extract of *P. chinense* are available in the forms of tablets, granules, capsules and pills in market under the trade name of "Gansu" in China. The indications of "Gansu" include several hepatic diseases such as chronic

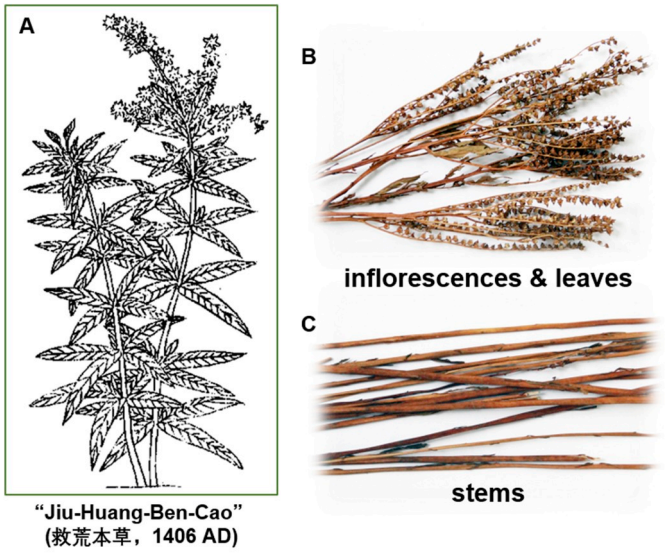


Fig. 1. The morphological characteristics of material of *P. chinense*. (A) Original plant picture adapted from Jiu-Huang-Ben-Cao, 1406 AD, (B) Inflorescences and leaves of P. chinense, (C) Stems of *P. chinense*.

active hepatitis, hepatitis B and acute viral hepatitis. Generally, the whole grass of *P. chinense* can be used for medical purposes. To be specific, the stems are commonly applied for preparations, while the leaves are especially made into tea or drinks (Fig. 1). Therefore, the modern medical applications of *P. chinense* have mainly focused on liver diseases.

A summary of traditional and modern use of *P. chinense* is given in Table 1. In the past decades, repeated evidences from *in vitro*, *in vivo* and clinical observations have demonstrated that *P. chinense* exhibited remarkable hepatoprotection effects with little adverse effects. To promote the understanding of traditional and modern use of *P. chinense*, current research on phytochemistry, pharmacology, and clinical evaluation as well as scientific gaps will be further discussed in the following sections.

4. Phytochemistry and quality control

4.1. Phytochemistry

The phytochemical study on *P. chinense* started since 1990s. In 1998, Chen et al. isolated and characterized 6 compounds from *P. chinense* extract, including several flavonoids and organic acids such as quercetin, quercitrin, quercetin-3-*O*-glucoside and gallic acid (Chen et al., 1998). Subsequently, a wide range of compounds were isolated from this herb. Great progress has also been made in recent years. It is shown that a total of 88 compounds have been isolated and purified from *P. chinense*. The major types of constituents are identified as flavonoids, organic acids, coumarins, lignans, (poly)phenols, sterols, and others. Since most phenylpropanoids (flavonoids, coumarins and lignans) belong to polyphenols, the majority of constituents from *P. chinense* are actually polyphenol-type. It is reported that 26 volatile compounds, mostly as ketones and esters, are also identified in volatile oil of *P. chinense* by gas chromatography-mass spectrometry (GC-MS) (Feng et al., 2003). However, these volatile ingredients will not be included in this review. Table 2 lists all constituents derived from *P. chinense* by several research groups.

4.1.1. Flavonoids and glycosides

Till now, 28 flavonoids and their glycosides have been found in *P. chinense*. Their structures are shown in Fig. 2. Compound **1–14** are identified as derivatives of flavone and flavonol, while **15–22** are flavanone and flavanonol analogues. Most of them are derived from quercetin, kaempferol and pinocembrin, with 15 glycosides characterized. In particular, pinocembrin-7-*O*-[4″,6″-hexahydroxydiphenoyl]-*β*-D-glucose (**20**) and pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-*β*-D-glucose (**21**) are resulted from substitution of a hexahydroxydiphenoyl (HHDP)-glucose group and a galloyl-HDDP-glucose group at C-7 position of the structure of pinocembrin, respectively. Notably, the presence of a HDDP or a galloyl-HDDP group in flavonoids may be one of the key characteristics of *P. chinense* constituents.

In addition, a xanthone, mangiferin (**23**), along with catechin (**24**) are found in *P. chinense*. Chalcones (**25** and **26**) and dihydrochalcones (**27** and **28**) are also identified. It is shown that flavonoids and their glycosides are one of main types of constituents in *P. chinense*. Pharmacological evaluations have demonstrated that they were at least partially responsible for the hepatoprotective effect, which will be specifically discussed in section 5.

4.1.2. Organic acids

A total of 9 organic acids (Fig. 3) were identified in *P. chinense*. A saturated fatty acid, palmitinic acid (**29**) along with 5 hydroxy-benzoic acids (**30–34**) was purified by several research groups. Besides, chebulic acid (**35**), brervifolin-caboxylic acid (**36**) and ferulic acid glucopyranoside (**37**) were also found.

Table 1
Traditional and modern indications of *P. chinense*.

P. chinense	Traditional use	Modern applications
Documentations	Jiu-Huang-Ben-Cao (救荒本草, 1406 AD); Tian-Bao-Ben-Cao (天寶本草, 1900s); Chinese Materia Medica (中華本草, 1999); Chinese Materia Medica Grand Dictionary (中藥大辭典, 2006)	Sichuan Chinese Metaria Medica Standards (2010); Drug Standard of Ministry of Public Health of the People's Republic of China (1998)
Formulations	Decoction, functional drink & vegetableDecoction	Tablets, Granules, Capsules, Pills, etc.
Functions & Indications	Functions: removal of heat, diuresis, detoxification, and promoting blood circulation. Indications: traumatic injury, edema, jaundice, cholecystitis, fatty liver, infectious hepatitis, dysuria and carbuncle	Functions: hepatoprotection. Indications: liver diseases of chronic active hepatitis, hepatitis B and acute viral hepatitis

4.1.3. Coumarins and lignans

Both coumarin and lignan belong to phenylpropanoids that are characterized with an aromatic ring and a C3 propene tail. Scopoletin (**38**) is the only coumarin-type compound isolated from *P. chinense*. A total of 21 compounds are lignan-type. Zhang et al. firstly isolated three neolignans, namely penthorin A (**42**), (7′*E*)-2′,4,8-trihydroxy-3-methoxy-2,4′-epoxy-8,5′-neolign-7′-en-7-one (**43**) and 9,9′-*O*-diferuloyl-(−)-secoisolariciresionl (**53**), from the ethanol extract of *P. chinense*. Since then, several novel lignans, including **39–42**, **44–47**, and **54–59**, were continually found in *P. chinense*. Notably, these lignans show diverse skeleton structures. Compounds **48–52** are characterized as bisepoxylignans, while most other lignans are neolignans. The structures of coumarins and lignans isolated from *P. chinense* are shown in Fig. 4. Although there is currently limited evidence for the activities of these lignans, they might be important chemical markers of *P. chinense*.

4.1.4. (Poly)Phenols

Polyphenols are a kind of structural class with the presence of multiple phenol structural units. Many phenylpropanoids including flavonoids, coumarins and lignans are also polyphenols. Here, we discuss the polyphenols which are non-phenylpropanoids.

In *P. chinense*, 18 polyphenols (Fig. 5) were isolated and purified. Most of them are formed by esterification of garlic acid or its derivatives to a glucose. Two ellagitannins namely thonningianin A (**63**) and B (**64**) were repeatedly found in *P. chinense*. Another four new ellagitannins penthorumnin A-D (**69–72**), which carry a (R)-HHDP group at C-4/C-6 of the glucose moiety, were identified by Era et al. (2018). Since polyphenols are secondary metabolites in plant and are involved in defense against ultraviolet radiation or aggression by pathogens, they often possess significant activities of antioxidation, anti-inflammation, anti-oxidative stress and anticancer (Bernatova, 2018; Cai et al., 2017; Eskandari et al., 2019; Nabavi et al., 2018; Nowdijeh et al., 2019). Therefore, these polyphenols might be important chemical markers of *P. chinense*.

4.1.5. Others

Other compounds (**78–88**) isolated from *P. chinense* include sterols, triterpenoids, monoglyceride, and so on. It should be noted that *P. chinense* also contains polysaccharides (Deng et al., 2009; Lin et al., 2018).

4.2. Quality control

According to the Drug Standard of Ministry of Public Health of the Peoples Republic of China, quercetin is chosen as a quality control (QC) marker for Gansu preparations. Specifically, quantification of quercetin by high performance liquid chromatography (HPLC) is required. Although quercetin is one of main bioactive constituents of *P. chinense*, it is not specific, and thus monitoring only one marker for QC of Gansu preparations is difficult to ensure product quality.

QC process is not only essential for batch-to-batch stability of products, but also a prerequisite for further pharmacological or toxicological assessment. Several studies have been recently carried out for QC of *P. chinense*. A qualitative analysis combined with a fingerprint method and a quantitative analysis of multiple components may be an ideal strategy. Sun et al. performed fingerprint analysis of 9 batches of *P. chinense* extracts using HPLC with UV detection at 254 nm and 26 peaks were identified (Sun et al., 2018). Furthermore, simultaneously detection of 17 compounds, mostly flavonoids and polyphenols, were carried out using HPLC with mass spectrometry (MS) (Sun et al., 2018). It is showed that thonningianins A was most abundant in *P. chinense* with levels ranging from 14.8 to 44.9 mg/g, and the total contents of all detected compounds was 41.8–96.8 mg/g. In another study (Guo et al., 2015), a high-resolution MS, fourier transform ion cyclotron resonance MS (FTICR-MS), was employed for identification of constituents from *P. chinense*. A total of 27 compounds, including phenolic acids and derivatives of quercetin, pinocembrin and kaempferol were identified (Guo et al., 2015). Subsequently, fingerprint analysis and quantification of main constituents by HPLC with diode array detection (DAD) method was established (Guo et al., 2015). A similar report can be found using chromatographic fingerprinting combined with multi-components quantitative analysis of 17 batches of *P. chinense* (Deng et al., 2015).

Despite the significant progress that has been made for QC of *P. chinense*, it is still challenging to select ideal chemical markers due to the currently lack of solid evidences for active constituents responsible for pharmacological effects of *P. chinense*. Since polyphenols are known to exhibit considerable bioactivities, most studies have chosen phenolic compounds such as flavonoids (e.g., quercetin, pinocembrin and kaempferol and their derivatives) and organic acids (e.g., gallic acid and brevifolin carboxylic acid) as QC markers.

5. Hepatoprotective activity of extract and compounds derived from *P. chinense* Pursh

Since *P. chinense* is traditionally used for treatment of liver diseases, pharmacological research has been extensively performed to investigate the hepatoprotective effects and mechanisms of extract and compounds derived from this plant. Oxidative stress in liver is commonly considered as one of key factors for liver injury and development of liver diseases (Cichoż-Lach and Michalak, 2014; Parola and Robino, 2001; Zeng et al., 2017). Hence, investigations on the hepatoprotective effects and mechanisms of *P. chinense* extract and/or compounds were performed by anti-oxidation capacity assay based on chemical reagents ($ABTS^+$, DPPH and lipid peroxidation) and oxidants (*t*-BHP) induced liver cell damage models *in vitro*. Additionally, the hepatoprotective effects and mechanisms of *P. chinense* and/or compounds were also tested on chemical reagents (carbon tetrachloride) or diets (excessive

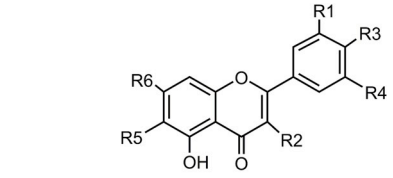
Table 2
Chemical constituents isolated from *P. chinense*.

NO	Name	Refs.
Flavonoids and glycosides (28)		
1	kaempferol	(Fu et al., 2013; Wang et al., 2016; Zhang et al., 2017a,b)
2	kaempferol-3-*O*-α-L-arabinfuranoside	(Sun et al., 2018; Wang et al., 2016)
3	kaempferol-3-*O*-α-L-rhamnopyranoside/afzelin	(Fu et al., 2013; Wang et al., 2016; Zhang et al., 2017a,b)
4	apigenin	(Sun et al., 2018; Wang, 2012; Zhang et al., 2007a,b,c)
5	quercetin	(Feng et al., 2001; Fu et al., 2013; Wang et al., 2016; Zhang et al., 2017a,b,c; Zhang and Yang, 2002)
6	quercetin-3-*O*-glucoside	Wang (2012)
7	quercitrin/quercetin-3-*O*-β-D-rhamnoside	(Huang et al., 2018; Sun et al., 2018; Wang et al., 2006; Zhang et al., 2017a,b)
8	quercetin-3-*O*-α-L-arabinfuranoside	Sun et al. (2018)
9	quercetin-3-*O*-β-D-xyloside	Wang et al. (2014a,b)
10	quercetin-3-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranoside	Fu et al. (2013)
11	luteolin	(Sun, 2016; Wang, 2012)
12	rutin	Sun et al. (2018)
13	scutellarin-7-*O*-rhamnoside	Sun et al. (2018)
14	avicularin	Zhang et al. (2017a,b)
15	naringenin-7-*O*-glucoside	Sun (2016)
16	pinostrobin	He et al. (2015a,b)
17	pinocembrin	(Fu et al., 2013; He et al., 2015a,b; Wang et al., 2016)
18	pinocembrin-7-*O*-glucoside	(Fu et al., 2013; He et al., 2015a,b; Sun et al., 2018; Wang et al., 2016; Wang et al., 2006)
19	5-methoxy-pinocembrin-7-*O*-β-D-glucoside	(He et al., 2015a,b; Wang et al., 2016)
20	pinocembrin-7-*O*-[4″,6″-hexahydroxydiphenoyl]-β-D-glucose	(Huang et al., 2014, 2015a; Sun et al., 2018; Wang et al., 2006; Zhang et al., 2017a,b)
21	pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-D-glucose (PGHG)	(Huang et al., 2014, 2015a; Lu et al., 2012; Sun et al., 2018; Zhang et al., 2017a,b)
22	alpinetin-7-*O*-β-D-glucopyranoside	Huang et al. (2018)
23	mangiferin	Wang (2012)
24	catechin	(Fu et al., 2013; Sun et al., 2018; Wang, 2012)
25	pinostrobin chalcone	He et al. (2015a,b)
26	(E)-3-phenyl-1-(2,4,6-trihydroxyphenyl) prop-2-en-1-one	He et al. (2015a,b)
27	2′,4′,6′-trihydroydihydrochalcone-4′-β-D-glucoside	(Huang et al., 2018; Sun et al., 2018)
28	6'-hydroxy-2'-methoxy-dihydrochalcone-4'-*O*-β-D-glucopyranoside	Zhang et al. (2017a,b)
Organic acids (9)		
29	palmitinic acid	Wang (2012)
30	3,5-dihydroxy-benzoic acid	Wang et al. (2016)
31	gallic acid	(Fu et al., 2013; Sun et al., 2018; Wang et al., 2006; Zhang and Yang, 2002)
32	protocatechuic acid	(Fu et al., 2013; Sun et al., 2018)
33	2,4,6-trihydroxybenzoic acid	Feng et al. (2001)
34	vanillic acid	Wang (2012)
35	chebulic acid	Wang (2012)
36	brervifolin-caboxylic acid	Wang (2012)
37	ferulic acid glucopyranoside	Huang et al. (2015b)
Coumarins and lignans (22)		
38	scopoletin	Wang (2012)
39	penthorin B	He et al. (2015a,b)
40	(7′Z,8R)-2′,4,8-trihydroxy-3-methoxy-2,4′-epoxy-8,5′-neolign-7′-ene-7-one	Liu et al. (2018)
41	(7′Z,8R)-8-hydroxy-3-methoxy-2,4′-epoxy-8,3′-neolign-7′-ene-7-one-4-*O*-β-glucopyranoside	Liu et al. (2018)
42	penthorin A/(7′*E*)-2′,4,8-trihydroxy-3-methoxy-2,4′-epoxy-8,5′-neolign-7′-en-7-one	(He et al., 2015a,b; Zhang et al., 2007a,b,c)
43	(7′*E*)-2′,4,8-trihydroxy-3-methoxy-2,4′-epoxy-8,5′-neolign-7′-en-7-one	Zhang et al. (2007a,b,c)
44	(7′E,8R)-8-hydroxy-3-methoxy-2,4′-epoxy-8,3′-neolign-7′-ene-7-one-4-*O*-β-glucopyranoside	Liu et al. (2018)
45	(7′E)-2′,3,4-trihydroxy-2,4′-epoxy-8,5′-neolign-7′-en-7-one	Deng (2012)
46	(7′E)-3,4,8-trihydroxy-2,4′-epoxy-8,3′-neolign-7′-en-7-one	Deng (2012)
47	(7′E)-3,4-dihydroxy-2,4′-epoxy-8,3′-neolign-7′-en-7-one	Deng (2012)
48	(+)-syringaresinol	He et al. (2015a,b)
49	(+)-medioresinol	He et al. (2015a,b)
50	(+)-pinoresinol	He et al. (2015a,b)
51	(+)-episyringaresinol	He et al. (2015a,b)
52	(+)-epipinoresinol	He et al. (2015a,b)
53	9,9′-*O*-diferuloyl-(−)-secoisolariciresionl	Zhang et al. (2007a,b,c)
54	penchinone A	He et al. (2015a,b)
55	penchinone B	He et al. (2015a,b)
56	(4′E)-2,3′-dihydroxy-3-methoxy-6′-methanone-benzophenone-4-*O*-β-D-glucopyranoside	Huang et al. (2018)
57	(4′E)-2,4-dihydroxy-3-methoxy-6′-methanone-benzo-phenone-3′-*O*-β-D-glucopyranoside	Huang et al. (2018)
58	penchinone C	He et al. (2015a,b)
59	penchinone D	He et al. (2015a,b)
(Poly)Phenols (18)		
60	2,6-dihydroxyacetophenone-4-*O*-β-D-glucoside	(Deng, 2012; Huang et al., 2015b; Sun et al., 2018; Zhang and Yang, 2002)
61	2,6-dihydroxyacetophenone-4-*O*-[4',6'-(S)-hexahydroxydiphenoyl]-β-D-glucose	Huang et al. (2014)
62	brevifolin	Deng (2012)
63	thonningianin A	(Huang et al., 2014, 2015a; Lu et al., 2012; Sun et al., 2018)

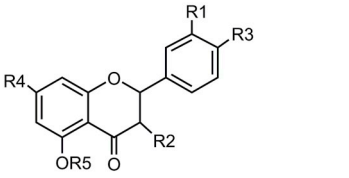
(*continued on next page*)

Table 2 (*continued*)

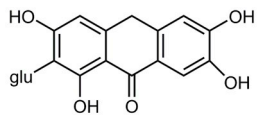
NO	Name	Refs.
64	thonningianin B	(Huang et al., 2014; Sun et al., 2018)
65	(E)-phenylpropene-3-methoxyphenyl-[6″-*O*-galloy]-4-*O*-β-D-glucopy-ranoside	Huang et al. (2015b)
66	2,6-dihydroxyacetophenone-5-(2′-methylene-2(5H)-furanone)-4-*O*-β-D-glucopyranoside	Huang et al. (2015b)
67	2′,6′-dihydroxydihydrochalcone-4′-*O*-[3″-*O*-galloyl]-β-D-glucopyranoside	Huang et al. (2015b)
68	1-*O*-sinapoyl-β-D-glucopyranoside	Huang et al. (2015b)
69	penthorumnin A	Era et al. (2018)
70	penthorumnin B	Era et al. (2018)
71	penthorumnin C	(Era et al., 2018; Huang et al., 2014)
72	penthorumnin D	Era et al. (2018)
73	phyllanemblinin F	Era et al. (2018)
74	chebulic acid	Era et al. (2018)
75	bergenin	Fu et al. (2013)
76	11-*O*-galloylbergenin	Fu et al. (2013)
77	4-galloylbergenin	Fu et al. (2013)
Others (11)		
78	β-sitosterol	(Lu et al., 2012; Zhang et al., 2007a,b,c)
79	β-daucosterol	(Sun, 2016; Wang, 2012)
80	lupeol	(Wang, 2012; Zhang et al., 2007a,b,c)
81	ursolic acid	(Wang, 2012; Zhang et al., 2007a,b,c)
82	betulic acid	Wang (2012)
83	2β,3β,23-trihydroxy-urs-12-ene-28-oic acid	Zhang et al. (2007a,b,c)
84	helicin	Zhang et al. (2017a,b)
85	1-*O*-(β-*D*-glucopyranosyl)-(2*S*,2′*R*,3*R*,4*E*,8*E*)-2-(2′-hydroxyhexadecanoylamino)-4,8-octdecadiene-1,3-diol	Zhang et al. (2007a,b,c)
86	glyceryl monopalmitate	Zhang et al. (2007a,b,c)
87	glyceryl monolaurate	Zhang et al. (2007a,b,c)
88	n-butyl-β-D-fructopyranoside	Deng (2012)



1 R1=H R2=OH R3=OH R4=H R5=H R6=OH
2 R1=H R2=*O*-ara R3=OH R4=H R5=H R6=OH
3 R1=H R2=*O*-rha R3=OH R4=H R5=H R6=OH
4 R1=H R2=OH R3=OH R4=H R5=H R6=OH
5 R1=OH R2=OH R3=OH R4=H R5=H R6=OH
6 R1=OH R2=*O*-glu R3=OH R4=H R5=H R6=OH
7 R1=OH R2=*O*-rha R3=OH R4=H R5=H R6=OH
8 R1=OH R2=*O*-ara R3=OH R4=H R5=H R6=OH
9 R1=OH R2=*O*-xyl R3=OH R4=H R5=H R6=OH
10 R1=OH R2=*O*-xyl-gal R3=OH R4=H R5=H R6=OH
11 R1=OH R2=H R3=OH R4=H R5=H R6=OH
12 R1=H R2=*O*-rha-glu R3=OH R4=OH R5=H R6=OH
13 R1=H R2=H R3=OH R4=H R5=OH R6=*O*-rha
14 R1=H R2=*O*-ara R3=OH R4=H R5=H R6=OH



15 R1=H R2=H R3=OH R4=*O*-glu R5=H
16 R1=H R2=H R3=H R4=*O*-CH_3 R5=H
17 R1=H R2=H R3=H R4=OH R5=H
18 R1=H R2=H R3=H R4=*O*-glu R5=H
19 R1=H R2=H R3=H R4=*O*-glu R5=CH_3
20 R1=H R2=H R3=H R4=*O*-s1 R5=H
21 R1=H R2=H R3=H R4=*O*-s2 R5=H
22 R1=H R2=H R3=H R4=*O*-glu R5=CH_3



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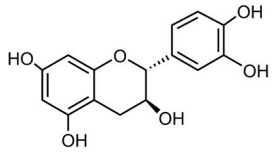

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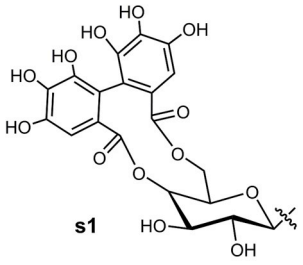
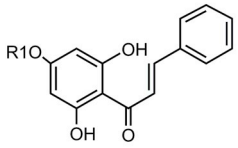
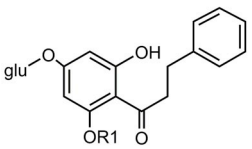


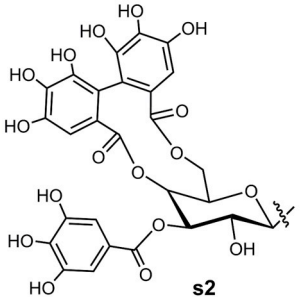
s1



25 R1=CH_3
26 R1=OH



27 R1=H
28 R1=CH_3



s2

Fig. 2. Structures of flavonoids and glycosides from *P. chinense*.

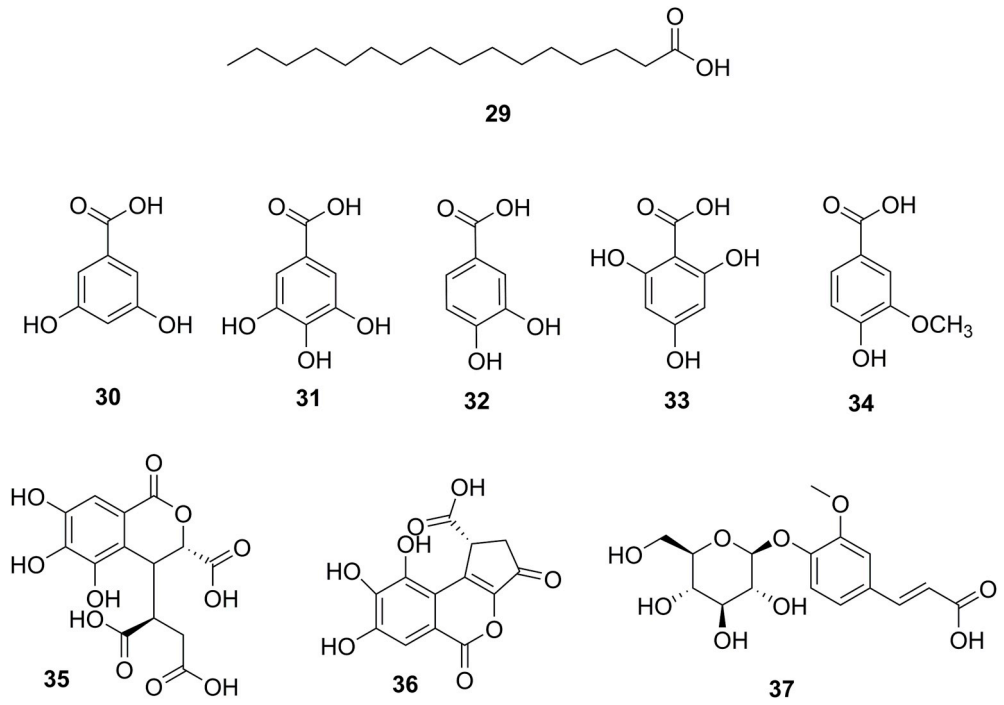


Fig. 3. Structures of organic acids from *P. chinense*.

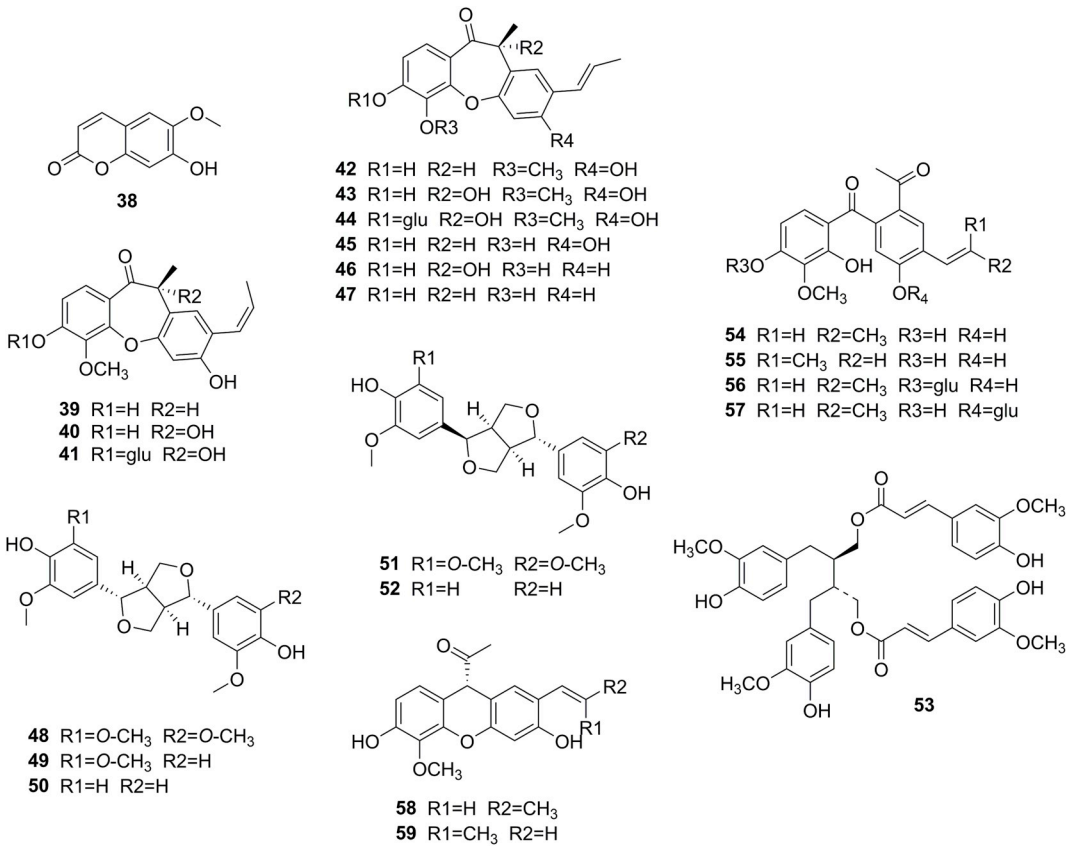


Fig. 4. Structures of coumarins and lignans from *P. chinense*.

alcohol and high fat diet) induced liver injury in animal models (Fig. 6). In this part, the hepatoprotective effects of *P. chinense* extract and compounds *in vitro* and *in vivo* will be comprehensively reviewed (Table 3). Reports which failed to demonstrate how extract was prepared or did not describe experimental details have been excluded. It should be noted that although a few studies are included in Table 3, they still lack a detail about quality control of herbal preparation and/or no positive control is used in the pharmacological experiments. The information provided by these studies may be possibly not reproducible.

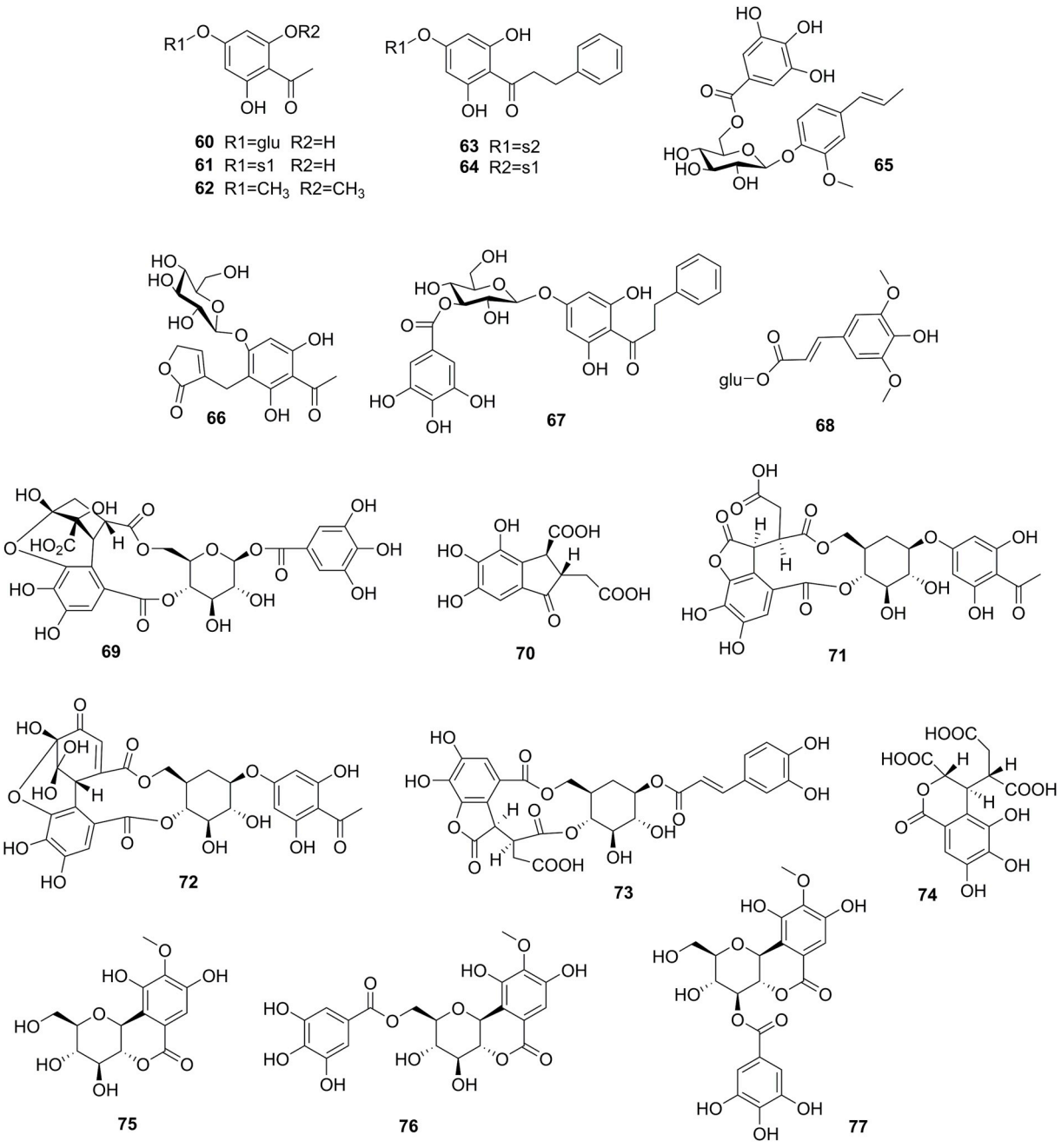


Fig. 5. Structures of polyphenols from *P. chinense*.

5.1. Antioxidant and anti-inflammatory activity in vitro

Generally, the antioxidant activity potential of natural products is assessed by various chemical methods, including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), ferric reducing antioxidant powder (FRAP) and oxygen radical absorbance capacity (ORAC). However, it should be noted that these *in vitro* results are of no biological or pharmacological relevance.

The anti-oxidation and DNA damage protection effects of 75% ethanol extract of *P. chinense* (Total phenols: 324.86 ± 32.68 mg/g; Total flavonoids: 487.56 ± 55.12 mg/g) reported by Xia and colleagues were tested by $ABTS^{+}$, hydroxyl radical scavenging, DPPH tests and lipid peroxidation methods. The results indicated that extract of *P. chinense* exhibited potent DPPH, ABTS and hydroxyl radicals scavenging activities, inhibition of lipid peroxidation, and possessed protective activity against DNA damage (Xia et al., 2012). Lu et al. reported that two polyphenols (thonningianin A and PGHG) isolated from ethyl acetate fraction of *P. chinense* extract had antioxidant activity in DPPH and FRAP radical scavenging test, in which vitamin C was taken as a control for parallel comparison (Lu et al., 2012). Apart from investigation on the antioxidant capacity of polyphenols in *P. chinense*, the activity of polysaccharides was also investigated (Lin et al., 2018). A kind of acidic polysaccharides (PCPP-1a; MW, 47.3 KDa; composed of Man, Rib, Rha, GlcUA, GalUA, Glc, Gal, Xyl, Ara, and Fuc) and total polysaccharides showed strong $ABTS^{+}$ radical scavenging, DPPH radical scavenging, and Fe^{2+} chelating activities.

The anti-inflammatory effect of some fractions of *P. chinense* was also evaluated *in vitro*. For instance, the inhibition effect of PCPP-1a and total polysaccharides (10–100 μg/mL) in nitric oxide (NO), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) release were tested in lipopolysaccharides (LPS) activated RAW264.7 macrophages (Lin

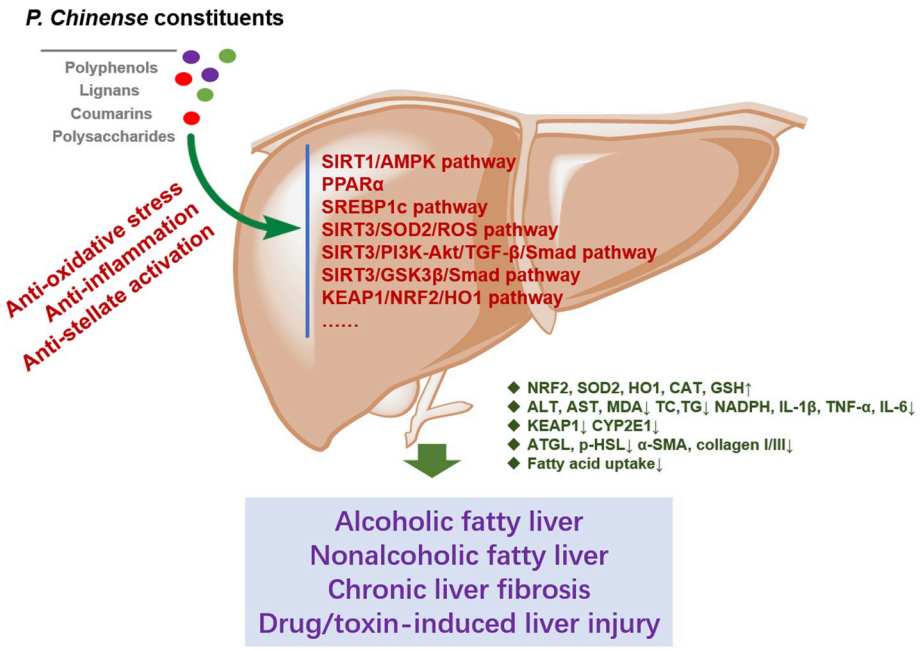


Fig. 6. Hepatoprotection effect of *P. chinense in vitro* and *in vivo*. *P. chinense* and its constituents show potent effects of anti-oxidative stress, anti-inflammation and anti-stellate activation in several cell models as well as animal models of alcoholic and nonalcoholic fatty liver disease, drug/toxin-induced liver injury, liver fibrosis, hepatocarcinoma, *etc*. The underlying molecular mechanisms include the regulation of several signaling pathways such as SIRT1/AMPK, PPARα, SREBP1c, SIRT3/SOD2/ROS, SIRT3/PI3K-Akt/TGF-β/Smad, SIRT3/GSK3β/Smad, and KEAP1/NRF2/HO-1 pathways.

et al., 2018). Both PCPP and PCPP-1a showed a significant inhibitory effect on TNF-α and IL-1β release at concentration of 50 μg/mL, however, the significant inhibition of NO release was only observed when the concentration reached at 100 μg/mL in LPS stimulated RAW264.7 macrophages. However, these *in vitro* assays are usually of limited pharmacological relevance *in vivo*.

5.2. Chemicals-induced acute liver injury

Tert-butyl hydroperoxide (*t*-BHP) and carbon tetrachloride (CCl_4) are common chemical inducers of liver injury, which are frequently used for establishment of models of liver damage *in vitro* or *in vivo* (Choi et al., 2015; Yang et al., 2010). *t*-BHP as an organic peroxide is metabolized by cytochrome P450 enzymes (CYPs) into free radical intermediates, which promotes lipid peroxidation and reactive oxygen species (ROS), and forms covalent bonds with cellular molecules initiating cell damage (Lee et al., 2005). CCl_4 directly impairs hepatocytes via changing permeability of cell membranes, and can also be transformed into active intermediates by CYPs causing lipid peroxidation. Notably, a single dose of CCl_4 can induce acute liver injury, while long-term administration causes liver fibrosis and cirrhosis (Aterman, 1962). In this section, we assess the protective effect of *P. chinense* against *t*-BHP or CCl_4 induced acute liver injury. The models based on CCl_4-mediated chronic liver diseases will be discussed later.

Hu et al. showed that, in *t*-BHP-induced LO2 cells damage model, boiling water extract of *P. chinense* (at 25–400 μg/mL; containing gallic acid, isoquercitrin, quercitrin, quercetin and kaempferol: 5.5, 14.1, 10.4, 0.8 and 0.1 mg/g, respectively) protected LO2 cells from being damaged by oxidant (*t*-BHP, 200 μM, 6 h) through decreasing the intracellular ROS and ROS-induced cell apoptosis, with the minimum effective concentration at 200 μg/mL (Hu et al., 2015a,b). Using the same model, researchers found that a fraction (≥50 μg/mL) and quercetin (≥50 μM) from extract of *P. chinense* showed protective effects using a bioassay-guided strategy, and the mechanisms for protective effect may be related to activation of the intracellular anti-oxidation response system (upregulation nuclear factor erythroid 2-related factor 2 (NRF2), superoxide dismutase-2 (SOD-2) and heme oxygenase-1 (HO-1), and downregulation of Kelch-like ECH-associated protein 1 (KEAP-1), which facilitated cells to counteract with *t*-BHP-induced apoptosis (Wang et al., 2016). These results suggest that *P. chinense* protects liver cells from *t*-BHP-induced oxidative stress *in vitro*. However, the evidences from *in vivo* studies are lacking.

In CCl_4 (10 mM, 24 h) induced sub-acute hepatotoxicity in LO2 cells, 70% ethanol extract of *P. chinense* (≥12.5 μg/mL) showed stronger protective effect regarding the ability to alleviate levels of alanine aminotransferase (ALT), aspartate transaminase (AST) and malonaldehyde (MDA), compared with that of 95% ethanol and water extracts (both ≥ 50 μg/mL), where vitamin C was taken as a positive control (at 12.5–200 μg/mL) (Zhang et al., 2013). Although all the subfractions (extracted by different organic solutions, respectively, including petroleum ether, ethyl acetate, *n*-BuOH) obtained from 70% ethanol fraction (at 12.5–200 μg/mL, all with the minimum effective concentration of 12.5 μg/mL) were effective in protecting liver cells from being damaged by CCl_4, further study found that ethyl acetate extracts showed the highest hepatoprotective activity in decreasing the release of ALT, AST and MDA (Zhang et al., 2013). This study shows that different extracts of *P. chinense* and the subfractions are effective in protecting against CCl_4-induced liver cell damage, which facilitates the finding of the active ingredients. But whether the *in vitro* results have relevance for *in vivo* study needs further investigation.

In CCl_4 (intraperitoneally injected with 10% CCl_4 in olive oil solution (*v/v*, 2 mL/kg B.W.)) induced acute mice liver injury model, Wang et al. demonstrated that water extract of *P. chinense* (gavaged with 5.15 and 10.3 g crude herbal material equivalent (CHME)/kg B.W. once daily for 1 week prior to the challenge with CCl_4) significantly enhanced the activities of SOD and catalase (CAT), decreased the levels of MDA (only effective at 10.3 g CHME/kg B.W.), restored the glutathione (GSH, only effective at 10.3 g/kg B.W.) in the liver, which impaired the hepatocyte apoptosis induced by oxidative stress. In this experiment, silymarin (gavaged with 100 mg/kg B.W.) was taken as a positive control, which showed comparable effect with *P. chinense* at 10.3 g CHME/kg (Wang et al., 2017). It should be noted that the dosage of *P. chinense* for mice in this experiment is calculated as equivalent of crude herbal material but not the amount of extract from *P. chinense*. Protein analysis indicated that pretreatment with water extract of *P. chinense* up-regulated the expression of CYP2E1 and NRF2, and increased the nuclear translocation and activation of NRF2 in mice liver to enhance the capacity of counteracting with CCl_4-induced oxidative stress (Wang et al., 2017), which suggested that the water extract of *P. chinense* has a protective effect in CCl_4-induced liver damage in mice.

Overall, *P. chinense* protects liver cells against *t*-BHP or CCl_4 induced liver injury potentially through anti-oxidative stress effect via

Table 3
Hepatoprotective effect of extracts and compounds derived from *Penthorum chinense* Pursh.

Extracts (quality control) or compounds	Model	Concentration/Dosage	Key findings	Ref.
***P. chinense* Extracts**				
75% Ethanol extract of *P. chinense* (Total phenols: 324.86 ± 32.68 mg/g; Total flavonoids: 487.56 ± 55.12 mg/g)	$ABTS^{+}$, hydroxyl radical scavenging, DPPH tests; Lipid peroxidation (positive control: ascorbic acid, trolox)	0.5–50 μg/mL	Anti-oxidation; lipid peroxidation↓	Xia et al. (2012)
Boiling water extract of *P. chinense* (gallic acid, isoquercitrin, quercitrin, quercetin and kaempferol: 5.50, 14.1, 10.4, 0.8 and 0.1 mg/g)	*t*-BHP-induced liver damage in LO2 cells (positive control: tiopronin)	100, 200 and 400 μg/mL	Attenuating *t*-BHP-induced ROS and apoptosis: Bax and cleaved products of caspase-9, caspase-7 and PARP↓, Bcl-2↑	Hu et al. (2015a,b)
95% ethanol extract and quercetin	50% ethanol intramuscular injection/rats/alcoholic fatty liver (positive control: tiopronin)	95% ethanol extract: 2 and 4 g CHME/kg; quercetin: 25 and 50 mg/kg B.W.	ALT, AST, TC, TG↓; the protective effect of extract was better than that of the same amount of quercetin	Li (2015)
95% ethanol extract and quercetin (1.25% in 95% ethanol extract)	Feed with 1.5% $FeSO_4$ provender/continuously gavage with 50% ethanol for 6 weeks/rats/alcoholic fatty liver (positive control: tiopronin)	95% ethanol extract: 2 and 4 g CHME/kg B.W.; quercetin: 50 and 25 mg/kg B.W.	ALT, AST, TG, TC (except for quercetin at 25 mg/kg B.W.) ↓;	Yuan et al. (2012)
95% ethanol extract and quercetin (1.25% in 95% ethanol extract)	Feed with 1.5% $FeSO_4$ provender/continuously gavage with 50% ethanol for 6 weeks/rats/alcoholic fatty liver (positive control: tiopronin)	95% ethanol extract: 2 and 4 g CHME/kg B.W.; quercetin: 50 and 25 mg/kg B.W.	ALT, AST, TC, TG↓; the protective effect of extract was better than that of the same amount of quercetin	Yuan et al. (2011)
Water extract	Feed with 1.5% $FeSO_4$ provender/continuously gavage with 45% ethanol/rats/alcoholic fatty liver (positive control: tiopronin)	4.2, 8.4 and 16.7 g CHME/kg B.W.	AST, TG, NEAF↓	Xiao et al. (2014b)
35, 55, 75 and 95% ethanol extract	High fat diet fed (contained with 1.5% $FeSO_4$), 52% ethanol and 15% glucose gavage/rats/alcoholic fatty liver (positive control: tiopronin)	3 g CHME/kg B.W.	TNF-α, IL-6, TC, LDL, TG↓, HDL↑; Suppress oxidative stress: MDA↓, SOD↑;	Tang et al. (2016)
35, 55, 75 and 95% ethanol extract	High fat diet (contained with 1.5% $FeSO_4$) and continuously gavage with 52% ethanol for 8 weeks/rats/alcoholic fatty liver (positive control: tiopronin)	0.489, 0.498, 0.504 and 0.288 g extract/kg/d	TG, LDL, TC, ALT, AST↓, HDL↑; Inflammatory factors in serum: NADPH, IL-1β, TNF-α and IL-6 ↓; Oxidative stress in liver: MDA↓, SOD↑; Expression of inflammatory factors in liver: CYP2E1, IL-1β, TNF-α and IL-6↓; 95% ethanol extract showed the best effects in TC, LDL, ALT, AST, MDA, IL-1β, TNF-α↓ and HDL, SOD↑	Zhang (2015)
35, 55, 75 and 95% ethanol extract	High fat diet fed (contained with 1.5% $FeSO_4$), 50% ethanol gavage/rats/alcoholic fatty liver (positive control: tiopronin)	3 g CHME/kg B.W.	AST, TC, TG, LDL-C↓;	Li et al. (2016)
95% ethanol extract	High-fat diet and ethanol (0.1 mL/10 g rat i.g) (positive control: tiopronin)	20, 40 and 80 mg extract/kg B.W.	ALT, AST, TC, TG↓	Hu et al. (2015a,b)
Water extract	Continuously gavage with lipid emulsion for 9 weeks/rats/nonalcoholic fatty liver (positive control: fenofibrate)	4.2, 8.4, 16.7 g CHME/kg B.W.	Regulation of lipid metabolism: CHO, TG, NEFA, T-CHO↓, HDL-C↑; liver enzyme: ALT↓; antioxidation: activity of GSH-Px	Xiao et al. (2014a)
Total flavones	High fat diet/mice/non-alcoholic fatty liver (positive control: fenofibrate)	0.33 and 0.66 g extract/kg B.W.	AST, ALT, TBil, TG↓, HDL-C↑ (0.33 and 0.66 g/kg B.W.); CHO, LDL-C↓ (0.33 g/kg B.W.)	Tan et al. (2017)
Gansu Granules	Subcutaneous injection of 40% CCl_4 for 8 weeks/rats/liver fibrosis (no positive control)	1.0, 2.0 and 4.0 g CHME/kg B.W.	Liver protection: ALT, AST, ALP↓; Anti-liver fibrosis: MDA, HA, α-SMA, collagen I and III ↓	Qu et al. (2011)
Extract for preparation of Gansu Granules	Ligation of the common bile duct/rats/bile duct obstructive liver fibrosis (positive control: ursodeoxycholic acid)	1.67 g CHME/kg B.W.	ALT, AST, TBIL, ALP, DBil, globulin↓, ALB, globulin/albumin ↑	Xie et al. (2018)
Total flavones (content 65%, 45 g/kg crude herbal material)	Continuously gavage with ethanol for 24 weeks/rats/alcoholic fatty liver (no positive control)	20, 40 and 80 mg extract/kg (equivalent to 0.44, 0.89 and 1.8 g CHME/kg)	ALT, AST, PC-III, HA, LN, Hyp↓; Suppression of liver fibrosis: MDA↓, SOD, GSH-Px, GSH↑, TNF-α, IL-6↓	Shi and Zhuo (2015)
Water extract	Bile duct ligation induced obstructive jaundice and α-naphthyl isothiocyanate (ANIT)-induced cholestasis for rats (no positive control)	4.5, 9.0 and 18.0 g CHME/kg B.W.	TBil, ALP, GGT, ALT, AST↓; obvious protective and jaundice-relieving effects	Zhang and Hunag (2008)
Water extract	HepG 2.2.15 cells (no positive control)	264 μg/mL	Inhibitory rate for HBV was more that 50%	Zhao et al. (2002)
Boiling water extract of *P. chinense* (pinocembrin-7-*O*-β-D-glucoside: 3.49 mg/g)	Acute alcohol-induced liver injury in mice (no positive control)	5.2 and 10.3 g CHME/kg B.W. once daily for 7 consecutive days	Suppressing ROS: MDA↓, GSH, SOD and CAT↑, CYP2E1↓; Inhibiting alcohol-induced lipolysis of white adipose tissue: ATGL and p-HSL↓, fatty acid uptake↓	Cao et al. (2015a,b)

(*continued on next page*)

Table 3 (*continued*)

Extracts (quality control) or compounds	Model	Concentration/Dosage	Key findings	Ref.
Boiling water extract of *P. chinense* (pinocembrin-7-*O*-β-D-glucoside: 3.49 mg/g)	Chronic alcohol-induced liver injury in mice (positive control: silymarin)	5.2 and 10.3 g CHME/kg B.W. once daily for 7 consecutive days	Lipid accumulation, inflammatory cytokines↓; Suppressing ROS: MDA↓, GSH, SOD and GPx↑, CYP2E1↓; Enhancing oxidant defense system: NRF2 and HO-1↑	Cao et al. (2015a,b)
Boiling water extract of *P. chinense* (pinocembrin-7-*O*-β-D-glucoside: 3.49 mg/g)	Carbon tetrachloride-induced acute liver injury (positive control: silymarin)	5.2 and 10.3 g CHME/kg B.W. once daily for 7 consecutive days	The efficacy of *P. chinense* extract at 10.3 g/kg B.W. was comparable to that of silymarin at 100 mg/kg B.W.; Inhibiting hepatocyte apoptosis: SOD/CAT↑, CYP2E1↓, MDA↓, NRF2↑	Wang et al. (2017)
70% ethanol extract of *P. chinense*	Carbon tetrachloride-induced HL-7702 cells (positive control: vitamin C)	12.5–200 μg/mL	ALT, AST, MDA↓	Zhang et al. (2013)
Aqueous extract of *P. chinense*	Human immortalized LX-2 cells and rat immortalized HSC-T6 cells (no positive control)	25, 50 and 100 μg/mL	α-SMA, collagen I↓	Zhou et al. (2018)
Compounds isolated from *P. chinense*				
Polysaccharide, PCPP-1a (47.3 KDa, acidic polysaccharides composed of Man, Rib, Rha, GlcUA, GalUA, Glc, Gal, Xyl, Ara, and Fuc)	$ABTS^+$, FRAP, and DPPH tests (positive control: trolox); LPS-treated RAW264.7 macrophages (no positive control)	0.5–6 mg/mL; 10–100 μg/mL	Anti-oxidation; Inflammatory factors: NO, TNF-α and IL-1β release↓	Lin et al. (2018)
Pinocembrin-7-*O*-β-D-glucoside Pinocembrin 5-methoxy-pinocembrin-7-*O*-β-D-glucoside	Non-alcoholic injured HepG2 cell model induced by free fatty acid (no positive control)	0.1, 1 and 10 μM 1, 10 and 100 μM 1, 10 and 100 μM	Protective effect: Anti-oxidative stress: MDA↓, SOD/GSH-Px↑; Regulation of lipid metabolism: SIRT1/AMPK/SREBP1c pathway↑	Guo et al. (2018)
Pinocembrin	Human immortalized HSCs LX-2 cells and rat immortalized HSC-T6 cells (no positive control)	10, 20 and 40 μM	Suppression of the activation of LX-2 and HSC-T6 cells: Expression and activity of SIRT3↑, SOD2↑, PI3K-AKT↓, TGF-β and Smad↓, GSK3β↑	Zhou et al. (2018)
(7′Z)-2′,4-dihydroxy-3-methoxy-2,4′-epoxy-8,5′-neolign-7′-ene-7-one (+)-episyringaresinol (+)-epipinoresinol	Acetaminophen-induced LO2 damage (no positive control)	5 μM	Protective effect	He et al. (2015a,b)
2,6-dihydroxyacetophenone-4-*O*-[4',6'-(S)-hexahydroxydiphenoyl]-β-D-glucose Thonningianin A	HSC-T6 cells (no positive control)	–	Anti-proliferation (IC_{50} values: 12.7 and 19.2 μM)	Huang et al. (2014)
Pinocembrin-7-*O*-[3″-*O*-galloyl-4″,6″-hexahydroxydiphenoyl]-β-glucose (PGHG) Thonningianins A	DPPH, FRAP tests (positive control: vitamin C); SMMC-7721 cells (positive control: 5-FU)	15.6–250 μg/mL (DPPH), 62.5–1000 μg/mL (FRAP), 12.5–100 μg/mL (SMMC-7721)	Anti-oxidation and anti-hepatocarcinoma	Lu et al. (2012)
Gallic acid, thonningianin A, PGHG, catechin, thonningianin B, pinocembrin-7-*O*-HHDP-β-glucose, protocatechuic acid, quercetin	SMMC-7721 (no positive control) Alcohol-induced liver injury in mice (no positive control)	12.5–200 μg/mL 18 mg/kg	Anti-hepatocarcinoma Protective effect	Sun et al. (2018)
Quercetin	*t*-BHP induced LO2 damage (no positive control)	40, 80 and 160 μM	Inhibiting *t*-BHP-induced apoptosis: BCL-2 and BCL-xL↑, NRF2↑, SOD-2 and HO-1↑, KEAP-1↓	Wang et al. (2016)

Note: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); ALT, alanine aminotransferase; AST, aspartate transminase; CHME, crude herbal material equivalent; CHO, cholesterol; CAT, catalase; CYP2E1, cytochrome P450 2E1; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant powder; GSH, glutathione; GSH-Px/GPx, glutathione peroxidase; HA, hyaluronic acid; HDL, high density lipoprotein; HO-1, heme oxygenase-1; HSC, hepatic stellate cell; IL-6, interleukin-6; KEAP-1, Kelch-like ECH-associated protein 1; LDL, low density lipoprotein; LN, laminin; MDA, malonaldehyde; NADPH, nicotinamide adenine dinucleotide phosphate; NEFA, nonesterified fatty acid; NRF2, nuclear factor erythroid 2-related factor 2; ORAC, oxygen radical absorbance capacity; PC III, procollagen III; ROS, reactive oxygen species; SIRT3, silent mating type information regulation 2 homolog 3; SOD, superoxide dismutase; SREBP1c, sterol regulatory element binding protein-1c; t-BHP, tert-butyl hydroperoxide; TBil, total bilirubin; TC, serum total cholesterol; T-CHO, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-α; α-SMA, α-smooth muscle actin.

regulation of CYP2E1 and NRF2/KEAP1 pathways. Most studies have used water extract of *P. chinense*. However, an *in vitro* study suggests that 70% ethanol extract of *P. chinense* is more effective than the water extract. The *in vivo* relevance should be further investigated. Moreover, in-depth research into the exploration of mechanisms of action is advocated in future.

5.3. Alcoholic liver disease

Heavy as well as prolonged alcohol consumption is considered as one of key factors for all preventable deaths and leading causes for liver diseases, such as fatty liver, liver steatosis and cirrhosis (Control and Prevention, 2004). Oxidative stress, disrupted NO signaling, and mitochondrial dysfunction are proposed to be key molecular pathways that accelerate or worsen steatosis and initiate progression to steatohepatitis and fibrosis (Mantena et al., 2008). According to the traditional application of *P. chinense* for treatment of liver disease, the alleviation effects of extract of *P. chinense* for ALD *in vivo* was also investigated by researchers.

The protective effect of water extract of *P. chinense* has been conducted on animal models of alcohol-induced acute liver injury (ALI) and alcoholic fatty liver disease (AFLD) induced by chronic intake of alcohol. Cao et al. established ALI in mice by ethanol gavage (4.7 g/kg B.W.; every 12 h for a total of three doses). They found that boiling water extract of *P. chinense* (5.2 and 10.3 g CHME/kg B.W. once daily for 7 days prior to ethanol gavage), containing 3.49 mg/g pinocembrin-7-*O*-β-D-glucoside, protected mice liver from being damaged through suppressing the activity of CYP2E1 to decrease ROS and MDA level in liver, and down-regulating the expressions of adipose triglyceride lipase (ATGL), phosphorylation of hormone-sensitive lipase (p-HSL) and fatty acid uptake capacity in liver to impair lipolysis of white adipose tissue (WAT). However, in this experiment, no positive control was parallelly used (Cao et al., 2015a,b). In an AFLD rat model induced by daily intragastric gavage of 45% ethanol for 9 weeks, water extract of *P. chinense* (at 4.2, 8.4 and 16.7 g CHME/kg B.W.) was effective in decreasing the serum AST as well as liver triglyceride (TG) and nonesterified fatty acid (NEFA) in rats, however, the other liver index, such as ALT, total bilirubin (TBil), cholesterol (CHO), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and MDA, were not significantly improved by treatment with *P. chinense* (at neither low, middle nor high dosage) (Xiao et al., 2014b). These results showed slight protective effect of *P. chinense* for AFLD. On the contrary, in another AFLD mice model (fed with modified Lieber-DeCarli alcohol liquid diet with composition of 11% carbohydrate, 18% protein, 35% fat and 36% ethanol for 4 weeks), Cao et al. reported that the increase of serum ALT (effective at both low and high dosages) and AST (only effective at high dosage) levels and inflammatory cytokines (i.e., TNF-α (only effective at high dosage), IL-6 (both effective at low and high dosage) induced by chronically intake of alcohol for C57BL/6 mice was significantly alleviated by administration of water extract of *P. chinense* (5.15 and 10.3 g CHME/kg B.W.) (Cao et al., 2015a,b). Protein assay found that *P. chinense* treatment decreased the expression of CYP2E1 while increased expression of NRF2 and anti-oxidant protein HO-1 in liver of AFLD mice (Cao et al., 2015a,b). Silymarin (86 mg/kg B.W.) was used as positive control, which showed comparable effect with *P. chinense* at 10.3 g CHME/kg. In this experiment, animals were freely access to the daily prepared food containing with ethanol, which is quite different from that using a gavage manner. The manner of modeling is quite ideal and is in line with the real situation, although the daily dosage of ethanol for each animal cannot be normalized during the experiment procedure. Taken together, the water extract of *P. chinense* (at a dosage around 5–10 g CHME/kg) shows a protective effect against both acute and chronic alcohol-induced liver injury. CYP2E1 and NRF2 pathways may contribute to this effect. The precise mechanisms as well as the active principles still need future exploration.

Several studies also investigated the effect of ethanol extract of *P. chinense* on alcoholic liver injury. Tang et al. found that different extracts (extracted by 35, 55, 75 and 95% ethanol) of *P. chinense* (3 g CHME/kg B.W.) were all effective in decreasing the TNF-α, IL-6, total cholesterol (TC), LDL, TG and MDA levels, and upregulating HDL level and SOD activity in rat liver after daily gavage of 52% ethanol (v/v) and 15% sugar (v/v) solution combined with high fat diet containing 1.5% $FeSO_4$ for 8 weeks (Tang et al., 2016; Zhang, 2015). These extracts also significantly decreased the release of inflammatory factors in serum (NADPH, IL-1β, TNF-α and IL-6) and the expression of proteins (CYP2E1, IL-1β, TNF-α and IL-6) in rat liver (Zhang, 2015). However, some details about the experiments were not fully described in the article, for example how long for administration of the extract of *P. chinense*, and only one dosage was used. Notably, it is demonstrated that the 95% ethanol extract of *P. chinense* was the most potent (except regulation of TG, CYP2E1 and IL-6) in regulating the inflammatory factors and liver antioxidant response in rats compared with that of the other three extracts (35, 55 and 75% ethanol, respectively) (Tang et al., 2016; Zhang, 2015). In a similar study, the liver protective effect of extracts of *P. chinense* (3 g CHME/kg B.W. gavage for 4 weeks after modeling) prepared by different solvents (35, 55, 75 and 95% ethanol) were further compared in rats with daily gavage ethanol and high fat diet. All these extracts were effective in decreasing the AST, TC, TG and LDL-C in rat model (Li et al., 2016). However, the authors found that extracts of *P. chinense* extracted by 75 and 95% ethanol showed more obvious hepatoprotective effects in rats compared with that of extracts of *P. chinense* extracted by 35 and 55% ethanol (Li et al., 2016). This experiment was repeatedly performed by authors, however, some details about the experiment were lost, such as the concentration of ethanol gavage to animals, procedures for modeling as well as administration process for animals, which may weaken the reproducibility of results and conclusions. Furthermore, Li et al. found that both 95% ethanol extract of *P. chinense* (2 and 4 g CHME/kg B.W.) and quercetin (25 and 50 mg/kg B.W.) were effective in decreasing the serum ALT, AST, TC and TG levels in liver of rats by intramuscular injection with 50% ethanol for 4 weeks and fed with diet containing 1.5% $FeSO_4$ (Li, 2015). The hepatoprotective effects of 95% ethanol extract of *P. chinense* and quercetin were simultaneously compared in this experiment, and it was found that the protective effect of 95% ethanol extract of *P. chinense* was better than that of equivalent amount of quercetin in this extract (Li, 2015). Similar results were also obtained by Yuan et al. (Yuan et al., 2011, 2012). The above experiments were repeatedly performed to investigate anti-ALD effect of both *P. chinense* (2 and 4 g CHME/kg B.W.) and quercetin (25 and 50 mg/kg B.W.) on the same animal model, in which tiopronin (50 mg/kg B.W.) was taken as positive control. However, no further research was performed to investigate the underlying mechanisms for regulation of ALD by *P. chinense* and quercetin.

Additionally, in mice with chronic alcohol (5.0 mL/kg, 50% alcohol administered for 7 days) induced liver hepatitis, Sun et al. reported that gallic acid, ellagitannins, PGHG, thonningianin A and B (all administered with 18 mg/kg B.W.) isolated from *P. chinense* were effective in suppression of hepatocytes necrosis with inflammatory cell infiltration induced by ethanol (Sun et al., 2018). These polyphenolic compounds may be important constituents responsible for hepatoprotection of *P. chinense*. In this experiment, no specific liver indexes were detected except for the liver pathological observation.

Collectively, although the animal models are established using varied methods, the results implicate that both water (at a dosage around 5–10 g CHME/kg) and ethanol (at a dosage around 2–4 g CHME/kg) extracts of *P. chinense* are effective for hepatoprotection in alcoholic liver injury. Some suggest that 95% ethanol extract may be

more effective compared to the aqueous extract. Quercetin, gallic acid, ellagitannins, PGHG, thonningianin A and thonningianin B are potential active ingredients.

To present, only a few studies evaluated the underlying mechanisms of *P. chinense*. In future, more efforts should be made to explore the potential bioactive compounds in extract, and to determine the underlying molecular mechanisms instead of repeatedly investigate on the effectiveness of different extracts on animals.

5.4. Non-alcoholic fatty liver

Asymptomatic hepatic steatosis, nonalcoholic steatohepatitis (NASH) and cirrhosis are included in the spectrum of NAFLD (Cai et al., 2019; Liu et al., 2019). During this progress, increasing oxidative stress, upregulated inflammatory mediators, and dysregulated apoptosis trigger liver inflammation (producing NASH) and fibrosis (Lewis and Mohanty, 2010). The prevalence of NAFLD is between 12% and 15% among general adult Chinese population, which is still increasing in China (Fan and Farrell, 2009). Hence, research and development of *P. chinense* for treatment or prevention of NAFLD is attracting more and more researchers' attention in China in recent years.

Results from Xiao et al.'s study demonstrated that *P. chinense* water extract (4.2, 8.4 and 16.7 g CHME/kg B.W., administration from the third week of modeling to the endpoint of experiment) was effective in anti-lipid metabolism disorder (decreasing the serum CHO, TG, NEFA, TC, and increasing HDL-C levels), lowering liver enzyme (ALT) as well as enhancing liver antioxidant capacity (increasing the liver content of GSH-Px) to protect rats' liver from being damaged by excessive lipid storage in rats with continuously and daily gavage administration of lipid emulsion (10 mL/kg, consist with cholesterol, pig oil, egg yolk powder and sodium cholate, 1:5:3:0.5:0.5, w/w) for 9 weeks (Xiao et al., 2014a). However, the chemical compositions about extract of *P. chinense* gavage to animals was not described by authors.

Tan et al. reported that both the total flavonoids () from *P. chinense* (0.33 and 0.66 g extract/kg B.W., ethyl acetate fraction of water extract of *P. chinense* purified on polyamide column, from the third week of modeling to the endpoint of experiment) and Gansu Granules (a preparation of *P. chinense* water extract, 8.4 g CHME/kg B.W., from the third week of modeling to the endpoint of experiment) significantly decreased the serum AST, ALT, TBIL and TG, and increasing HDL-C levels in mice with continuously gavage administration of lipid emulsion (10 mL/kg, consist with cholesterol, pig oil, egg yolk powder and sodium cholate, 1:5:3:0.5:0.5, w/w) for 8 weeks (Tan et al., 2017). Low dosage of the total flavonoids from *P. chinense* (0.33 g extract/kg B.W.) showed significant effects in decreasing the serum CHO and LDL-C levels in mice, however, the high dosage (0.66 g extract/kg B.W.) and Gansu Granules showed no obvious improving effects for these two index (Tan et al., 2017). Moreover, the anti-NAFLD effect of flavones from water extract of *P. chinense* was simply evaluated, and the underlying mechanisms were scarcely studied. The equivalent dosage of total flavonoids to that of *P. chinense* crude material was not mentioned in this study.

Notably, three main flavonoids from *P. chinense*, pinocembrin-7-*O*-β-D-glucoside (0.1–10 μM), pinocembrin (1–100 μM) and 5-methoxy-pinocembrin-7-*O*-β-D-glucoside (1–100 μM), showed significant regulatory effects in free fatty acid (FFA, oleic acid/palmic acid, 2:1, 0.8 mM) induced hepatic steatosis in HepG2 cells (Guo et al., 2018). These three flavonoids showed obvious antioxidant activity for HepG2 cells (up-regulation of SOD and GSH-Px, and suppression of MDA), and molecular mechanism study demonstrated that these three compounds activated the SIRT1/AMPK pathway, which further regulated the expression of peroxisome proliferator-activated receptor α (PPARα), sterol regulatory element binding protein-1c (SREBP1c) and the downstream targets fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and stearoyl-CoA desaturase 1 (SCD1) (Guo et al., 2018). This experiment was just performed on cell model *in vitro* and further research work should focus on validation the anti-hepatic steatosis on animal models. Additionally, in many studies, the content of these flavones in the extract of *P. chinense* as well as their effects on NAFLD should be emphasized.

Although the previous research works have preliminarily demonstrated the effectiveness of *P. chinense* in treatment of NAFLD, further investigations using more rigor experimental design are needed. The quality control of extracts used should be performed to ensure reproducibility of studies. Furthermore, some studies suggest that flavonoids are important active ingredients for *P. chinense* to treat NAFLD. SIRT1/AMPK, PPARα and SREBP1c may be potential targets. Future research is advocated for further elucidation.

5.5. Liver fibrosis

The effects of *P. chinense* extract or Gansu Granules in improving liver fibrosis *in vitro* and *vivo* were also extensively investigated by researchers. Qu et al. reported that Gansu Granules (gavage administration with 1, 2 and 4 g CHME/kg B.W. at the beginning of modeling) showed obvious hepatoprotective effects for rats with subcutaneous injection of 40% (v/v) CCl_4 solution for 8 weeks (Qu et al., 2011). Gansu Granules significantly decreased the serum ALT, AST, alkaline phosphatase (ALP), hyaluronic acid (HA) and MDA levels in rats of model group (Qu et al., 2011). Gansu Granules also significantly alleviated liver fibrosis and inhibited the formation of α-SMA, collagen I and III in liver of rats stimulated by CCl_4 (Qu et al., 2011). However, only a few liver fibrosis markers were examined in this experiment, and the underlying mechanisms were not fully investigated. Another report also demonstrated that *P. chinense* stream extract (for preparation of Gansu Granules, 16.7 g CHME/kg B.W.) significantly decreased the rats' serum ALT, AST, TBiL, ALP, DBil and globulin, and increased the albumin levels and ratio of albumin/globulin in bile duct obstructive liver fibrosis model by ligation of the common bile duct for rats (Xie et al., 2018). Shi et al. reported that the total flavonoids from *P. chinense* (0.44, 0.89 and 1.8 g CHME/kg B.W., hot water extract followed by precipitation with 75% ethanol solution) showed obvious liver protective effects (decreasing serum ALT, AST, PC-III, HA, laminin (LN) and liver Hyp and MDA) through enhancing the liver capacity of antioxidant (increasing activity of SOD and content of GSH-Px, GSH in liver) and anti-inflammatory (decreasing the release of TNF-α, IL-6 in serum) in alcoholic liver fibrosis rats model by continuous intragastric administration of ethanol for 24 weeks (Shi and Zhuo, 2015). The results indicate that *P. chinense* has an anti-fibrotic effect in different liver fibrosis models. However, the dosage used in different studies varied from 1 to 16.7 g CHME/kg.

Several studies have investigated the underlying mechanisms of anti-fibrotic effect of *P. chinense* and its active ingredients using *in vitro* cell models. Thonningianin B and 2,6-dihydroxyacetophenone-4-*O*-[4',6'-(*S*)-hexahydroxydiphenoyl]-β-D-glucose showed significant anti-proliferation effect in human stellate HSC-T6 cells among the 6 tested polyphenols from *P. chinense* (Huang et al., 2014). Zhou et al. reported that *P. chinense* extract (25–100 μg/mL) and pinocembrin (10–40 μM) significantly suppressed the activation of LX-2 and HSC-T6 cells (down-regulating the expression of α-SMA and collagen I), which provided evidence that extract of *P. chinense* and pinocembrin were effective in regulation of liver fibrosis *in vitro* (Zhou et al., 2018). In this process, pinocembrin activated silent mating type information regulation 2 homolog 3 (SIRT3)/SOD2/ROS, SIRT3/PI3K-Akt/TGF-β/Sma- and Mad-related proteins (Smad) and SIRT3/GSK3β/Smad signaling pathways in LX-2 and HSC-T6 cells (Zhou et al., 2018). Although these

Table 4
Clinical trials of *Penthorum chinense* related products.

Drug	Subjects	Study design	Intervention	Primary endpoint	Outcome	Quality of evidence[a]	Refs.
Gansu tablet *(Gulin Gansu Pharmaceutical;0.3 g/tablet)*	60 patients of both sex aged 21–59 with chronic hepatitis B	Randomized, 2-parralell group study	Group 1 (n = 30): Peginterferon α-2a Solution, 180 μg/time, once weekly, for 48 weeks; Group 2 (n = 30): The above therapy plus Gansu tablet (1.5 g/time, three times a day), for 48 weeks.	Serum ALT/AST/HA/LN, HBV-DNA, SF-36 score	Gansu tablets combined with Peginterferon α-2a Solution show better efficacy (improvement of liver function and increase of negative rates of HBV-DNA) than Peginterferon α-2a alone. No Gansu-related adverse effect.	Ib	Chen et al. (2018)
Gansu granule *(Sichuan Langzhong Pharmaceutical; 3 g/bag)*	116 patients of both sex aged 29–65 with chronic hepatitis B	Randomized, 3-parralell group study	Group 1 (n = 56): ATP 40 mg, CoA 100 IU, inosine 0.4 g, once daily, for 12 weeks; Group 2 (n = 60): The above therapy plus Gansu granule (1 bag/time, three times a day), for 12 weeks; Group 3: healthy volunteers (n = 30).	Disease symptoms, serum indicators of liver fibrosis (HA, LN, IV-C, PC-III)	Significant improvement of disease by combined treatment of Gansu granule compared to ATP/CoA/Inosine therapy. No adverse effect.	Ib	Wu and Zou (2003)
Gansu granule *(Sichuan Langzhong Pharmaceutical; 3 g/bag)*	160 patients of both sex aged 29–65 with chronic hepatitis B	Randomized, 2-parralell group study	Groups 1 (n = 78): Diammonium glycyrrhizinate 30 mL, Tiopronin 0.3 g, once daily, for 12 weeks; Group 2 (n = 82): The above therapy plus Gansu granule (1 bag/time, three times a day), for 12 weeks.	Disease symptoms, serum ALT/AST/TBil/γ-GT, serum HA/LN/IV-C, PGA index	Significant improvement of disease by combined treatment of Gansu granule compared to conventional therapy. No adverse effect.	Ib	He et al. (2007)
Gansu granule *(Sichuan Langzhong Pharmaceutical; 3 g/bag)*	193 patients of both sex aged 19–64 with chronic hepatitis B	Randomized, 2-parralell group study	Groups 1 (n = 81): ATP/CoA/inosine, plus Yiganning Pill, (115.5 mg/time, three times a day), for 12 weeks; Group 2 (n = 112): ATP/CoA/inosine, plus Gansu granule (3 g/time, three times a day), for 12 weeks.	Disease symptoms, serum HBV-DNA/HA/PC-III	Gansu improves liver fibrosis. No adverse effect.	Ib	Sun et al. (2002)
Gansu granule *(Sichuan Gulin Gansu Pharmaceutical; 9 g/bag)*	90 patients of both sex aged 31–62 with alcoholic fatty liver	Single-blind, randomized, 2-parralell group study	Groups 1 (n = 45): Diammonium glycyrrhizinate, 150 mg, three times a day, for 12 weeks; Group 2 (n = 45): Gansu granule, 1 bag/time, three times a day, for 12 weeks.	Disease symptoms, serum ALT/AST/GGT/TG	Gansu shows better efficacy than diammonium glycyrrhizinate in improving alcoholic fatty liver. No adverse effect.	Ib	Qin (2013)
Gansu granule *(Sichuan Gulin Gansu Pharmaceutical; 3 g/bag)*	169 patients of both sex aged 18–56 with non-alcoholic fatty liver	Randomized, 2-parralell group study	Groups 1 (n = 84): 150 mg Ursodeoxycholic acid plus 4 Fufangyiganling Pills, three times a day, for 12 weeks; Group 2 (n = 85): Gansu granule, 1 bag/time, three times a day, for 12 weeks. Both groups receive vitamin C and B2.	Disease symptoms, serum ALT/AST/TBil/γ-GT/TC/GS, body weight	Gansu improves lipid metabolism and decreases blood sugar and weight, showing better efficacy compared to group 1. No adverse effect.	Ib	Shu (2017)

(*continued on next page*)

Table 4 (*continued*)

Drug	Subjects	Study design	Intervention	Primary endpoint	Outcome	Quality of evidence[a]	Refs.
Gansu granule *(Sichuan langzhong Pharmaceutical; 3 g/bag)*	630 patients of both sex aged 8–74 with (chronic and acute) viral hepatitis	Randomized, 2-parralell group study	Groups 1 (n = 244): Yiganling pill, 115.5 mg/time, three times a day, for 12 weeks; Group 2 (n = 386): Gansu granule, 1 bag/time, three times a day, for 12 weeks. Both groups receive vitamin C/ATP/CoA.	Disease symptoms, serum HA/PC-III/ALT/TBil	Gansu improves disease symptoms and decreases indices of liver fibrosis, showing better efficacy compared to group 1. No adverse effect.	Ib	Sun et al. (2001)
Gansu granule *(Hospital preparations; 20 g/bag)*	150 patients aged 20–70 with compensated cirrhosis of hepatitis B	Randomized, 2-parralell group study	Groups 1 (n = 75): 1.44 g Hugan pill plus 1.28 g Yiganling pill, three times a day, for 8 weeks; Group 2 (n = 75): Gansu granule, 1 bag/time, twice a day, for 8 weeks. Both groups receive vitamin C/ATP/CoA.	Disease symptoms, PVID, serum HBV-M/HBV-DNA	Gansu group (group 2) shows better efficacy than group 1 regarding alleviating disease symptoms and improving virus infection. No adverse effect.	Ib	Qin (2013)
Gansu tablet *(Sichuan Gulin Gansu Pharmaceutical; 0.3 g/tablet)*	37 patients of both sex with acute icteric hepatitis	Randomized, 2-parralell group study	Groups 1 (n = 11): Fufang linzhi syrup, 5 g/time, twice daily, for 4 weeks; Group 2 (n = 26): Gansu tablet, 1.5 g/time, three times a day, for 4 weeks.	Disease symptoms, serum bilirubin and SGPT	Significant better efficacy in Gansu group.	Ib	Chen (1987)
Gansu granule *(Sichuan langzhong Pharmaceutical; 3 g/bag)*	60 patients of both sex with fatty liver	Randomized, 2-parralell group study	Groups 1 (n = 28): Zhibituo capsule (2 capsules/time, twice daily) plus vitamin B6 (30 mg/time, three times a day) for 12 weeks; Group 2 (n = 32): The above therapy plus Gansu capsule (6 g/time, three times a day), for 12 weeks.	Disease symptoms, serum ALT/AST/TG/TC	Significant better efficacy in Gansu group. No adverse effect.	Ib	Chen and Li (2003)
Penthorum chinense decoction pieces *(Sichuan Neautus Pharmaceutical)*	100 patients of both sex aged around 52 with cirrhosis and liver ascites	Randomized, 2-parralell group study	Groups 1 (n = 50): Furosemide plus Diammonium glycyrrhizinate, for 4 weeks; Group 2 (n = 50): The above therapy plus *P. chinense* (1.6 g/time, twice daily), for 4 weeks.	Disease symptoms, serum ALT/AST/TBil and A/G	Significant better efficacy in Gansu group.	Ib	Shu (2017)

Note: ALT, alanine aminotransferase; AST, aspartate transminase; A/G, albumin-globulin ratio; GGT, galactosyl glucosyltransferase; γ-GT, γ-glutamyltranspeptidase; HA, hyaluronic acid; HBV-DNA, hepatitis B virus DNA; HBV-M, HBV serum marker; IV-C, collagen IV; LN, laminin; PC III, procollagen III; PVID, portal vein diameter; TG, triglyceride; TC, serum total cholesterol; TBil, total bilirubin; SF-36, the MOS item short from health survey; SGPT, serum glutamic pyruvic transaminase.

[a] According to WHO, FDA and EMEA: Ia - meta-analyses of randomized and controlled studies; Ib - evidence from at least one randomized study with control; IIa - evidence from at least one well-performed study with control group; IIb - evidence from at least one well-performed quasi-experimental study; III - evidence from well-performed non-experimental descriptive studies as well as comparative studies, correlation studies and case-studies; and IV - evidence from expert committee reports or appraisals and/or clinical experiences by prominent authorities.

results demonstrated the anti-liver fibrosis effect of extract of *P. chinense* or compounds *in vitro*, the effectiveness and molecular mechanisms of improving liver fibrosis is still needed to be validated on animal models.

Taken together, *in vivo* functional assays have demonstrated the effectiveness of *P. chinense* in improving liver fibrosis. Some results also shed light on the potential molecular mechanisms, which have implications for future research.

5.6. Others

He et al. showed that (−)-(7′Z, 8S)-2′,4-dihydroxy-3-methoxy-2,4′-epoxy-8,5′-neolign-7′-ene-7-one, (+)-episyringaresinol and (+)-epipinoresinol (with inhibition rates of 15.0%, 10.5%, and 26.2%, respectively) isolated from an ethyl acetate soluble portion of water decoction of *P. chinense* significantly protected LO2 cells from being damaged by acetaminophen at a concentration of 5 μM, in which bicyclol was taken as the positive control with inhibition rate of 33.2% under the tested condition (He et al., 2015a,b), however, all the 7 isolates from this fraction were inactive in protecting LO2 cells from being damaged by H_2O_2 at concentration at concentration from 5 to 50 μM (He et al., 2015a,b).

Additionally, the water extract of *P. chinense* showed obvious protective and jaundice-relieving effects in bile duct ligation induced obstructive jaundice and α-naphthyl isothiocyanate (ANIT)-induced cholestasis for rats (decreasing the serum TBIL, ALP, GGT, ALT and AST levels) (Zhang and Hunag, 2008).

Lu et al. reported that thonningianin A and PGHG showed significant cytotoxicity (12.5–100 μg/mL) in SMMC-7721 cells, and thonningianin A exhibited better anti-proliferation activity than 5-fluorouracil (positive control) (at concentration of 50–100 μg/mL) (Lu et al., 2012). In another study, anti-proliferation activity of 17 compounds from *P. chinense* extract in SMMC-7721 cells (12.5–200 μg/mL), and gallic acid (IC_{50}, 36.52 μg/mL), thonningianin A (IC_{50}, 38.66 μg/mL) and PGHG (IC_{50}, 43.25 μg/mL) showed better anti-proliferation activity in SMMC-7721 cells compared with that of other 14 compounds and 5-fluorouracil (positive control, IC_{50}, 45.28 μg/mL) (Sun et al., 2018). However, all these studies are mainly based on *in vitro* experiments. Whether these compounds have anticancer effect *in vivo* is still unknown. There are also concerns about the difference between chemopreventive and anti-cancer agents. Notably, a clear cut for liver-protection and anti-cancer effects of *P. chinense* and its constituents should be established.

Moreover, Zhao et al. tested the inhibitory effect of *P. chinense* extract on HBV replication in HepG 2.2.15 cells (the secretion of HBsAg and HBeAg), and it was found that the inhibitory rate of the water extract of *P. chinense* was 54.79% at the concentration of 264 mg/mL (Zhao et al., 2002). However, all these results just showed the anti-hepatic virus replication effect of *P. chinense* extract on cell lines, the exact effect should be further investigated on animals.

6. Clinical study

Clinical trials of *P. chinense* using randomized controlled methodology have been carried out since 1980s. Most of publications (of varying methodological rigor) on clinical efficacy of *P. chinense* and its preparations are documented in locally-published journals in China. These studies have focused on the effects of *P. chinense* on several liver diseases such as chronic hepatitis B and other viral hepatitis, acute icteric hepatitis, ALD as well as NAFLD. The main documented clinical studies are summarized in Table 4.

Repeated evidences have demonstrated that *P. chinense* has a therapeutic effect on chronic hepatitis B and other viral hepatitis. In 2018, Zheng et al. performed Meta-analysis of the anti-hepatitis B effect of a *P. chinense* preparation, Gansu granule, with 7 randomized controlled trials included (Zheng et al., 2018). A total of 893 cases are analyzed. The results showed that Gansu granules significantly decreased serum markers of liver function (e.g., ALT, AST, GGT and TBil) and fibrosis (e.g., HA, LN, IV-C and PC-III) to a larger extend compared to control group. A similar Meta-analysis also supports these findings (Yu et al., 2012). In Sun et al.'s study, Gansu granules were effective in treating not only hepatitis B but also hepatitis A, C and E (Sun et al., 2001). Notably, it has been demonstrated that Gansu preparations could facilitate eradication of virus, alleviation of fibrosis and protection of liver function in the treatment of viral hepatitis. Chen et al. showed that *P. chinense* extract (1.5 g/time, three times a day, for 30 days) significantly reduced serum total TBil and glutamic pyruvic transaminase (SGPT) in patients with acute icteric hepatitis (Chen, 1987). Besides, *P. chinense* is well tolerated in patients (Chen, 1987).

Furthermore, several studies indicate that *P. chinense* preparations, either alone or in combination with other anti-oxidants, could promote alleviation the symptom of ALD and NAFLD (Chen and Li, 2003; Hu and Yuan, 2013; Zhang et al., 2007a). In particular, *P. chinense* can improve lipid metabolism and decrease blood sugar and body weight.

Current knowledge on clinical trials of *P. chinense* demonstrate that its current clinical use is generally in line with the traditional applications. Most studies using *P. chinense* extract have showed an efficacy superior to other conventional drug treatments such as diammonium glycyrrhizinate. However, it should be noted that most of these trials lack a detailed description of methodology (e.g., methods for randomization, blind, and criteria for selecting and excluding patients), rendering them generally of low quality. Moreover, these studies did not describe details on quality control of *P. chinense*. Inconsistency in quality of different batches of *P. chinense* makes it hard for comparison and assessment of results. In addition, these trials have used only one dosage, which might lead to bias in results. Based on the above analysis, it is recommended that more high-quality trials on *P. chinense* and its preparations using rigorous study design are performed in future.

7. Toxicology

Traditionally, *P. chinense* is recorded as a non-toxic edible herb. According to clinical observations, there are no *P. chinense*-related side effects or toxicity in patients observed after a single treatment or in combination with other therapies such as furosemide, diammonium glycyrrhizinate, vitamin B6, ATP/CoA/inosine, and peginterferon α-2a, even using a dosage as high as 6 g (three times a day, for 12 weeks) (Chen and Li, 2003; Chen et al., 2018; Qin, 2013; Shu, 2017).

Guo et al. systematically evaluated the toxicity of *P. chinense* in mouse and rat (Guo et al., 2016). It is shown that the maximum tolerated dose (MTD) of oral *P. chinense* is more than 15 g/kg either in mouse or rat (Guo et al., 2016). After a 30-day oral administration of *P. chinense* in rats, there was no significant change in body weight increase, food intake, food utilization, organ index, biochemistry index of blood compared to control group ($p > 0.05$) (Guo et al., 2016). There were no parent pathological alterations of major organs. Moreover, *P. chinense* exhibited no genetic, maternal, embryonic toxicities or teratogenicity (Guo et al., 2016). It is suggested that *P. chinense* carries with a quite safe profile in animals and human.

8. Conclusion and perspectives

To the best of our knowledge, there are only few drugs that are available for handling liver disease. Since the number of patients with liver disease is rapidly increased, new drugs and novel therapeutic targets are highly advocated. Based on the current research achievements from aspects of phytochemistry, pharmacological studies as well as clinical trials, it is believed that *P. chinense* is potent with liver-protection effects and is deserved for further research and development for treatment of liver diseases, including virials hepatitis, ALD as well as NAFLD. Silymarin, a standardized extract of the milk thistle seeds, is one of herbal medicines that have been applied for treating liver

diseases (Feher and Lengyel, 2012). It contains a mixture of flavonolignans (polyphenols). In several animal models, the protective effect of *P. chinense* extract (10.3 g CHME/kg) was found comparable with that of silymarin (86 mg/kg or 100 mg/kg). *P. chinense* extract are mainly composed of many flavonoids, lignans, coumarins, phenolic acids and polysaccharides. Continuous efforts should be made for identification of representative bioactive constituents in *P. chinense* for liver protection, which may be followed by systematic pharmacokinetics-pharmacodynamics and action mechanism study on suitable animal models and humans.

The papers reviewed in this work have been assessed with some problems, regarding the experimental design and methods. Most of clinical investigations did not describe clearly about experimental design such as methods for randomization, blind, and criteria for selecting and excluding patients. Some pharmacological studies did not have a proper design due to a lack of positive control. Only one single dose of *P. chinense* was used in some animal experiments (Li et al., 2016; Xie et al., 2018), which makes the results less reliable. Some papers reviewed did not include an ethical statement on approval of animal experiments, regarding both study design and proper procedures. Notably, a lack of QC process on *P. chinense* extract is one of biggest gap in pharmacological studies (Hu et al., 2015a,b), which may lead to low reproducibility across studies. Furthermore, regarding the dosage used, most studies have calculated it as equivalent of crude herbal material per body weight, while in some reports it is expressed as the amount of extract per body weight without giving details on extraction yields (Hu et al., 2015a,b). It is difficult to do a direct comparison across these studies. In addition, none of *in vitro* studies described the passage number or doubling time of cell lines used. Cell-based *in vitro* or *in silica* studies are performed to assess antioxidant or anti-inflammatory effect of *P. chinense* without further investigation of action mechanisms or *in vivo* relevance. Given the current research progress as well as scientific gaps identified, future works should mainly focus on the following aspects.

Taken together all the previous pharmacological studies on *P. chinense*, it is found that the underlying mechanisms for *P. chinense* on liver protection still need further investigation in future, for example, what kind of constituents are responsible for its action and which molecular targets and pathways are responsible for its actions. Advanced technologies, such as high throughput screening, target fishing as well as metabolomics technologies, should be applied for identifying bioactive constituents in *P. chinense*. Although some reports have shown evidences that polyphenols have liver protective effect both *in vitro* and *in vivo*, however, the active ingredients of *P. chinense* are still not fully understood. To date, a total of 88 compounds have been isolated from *P. chinense*, and most of them belong to polyphenols (flavonoids, coumarins, lignans, organic acid, and others) as well as polysaccharides. Some of the studies focused on *in vitro* activity assessment of constituents such as pinocembrin, pinocembrin-7-*O*-β-D-glucoside, thonningianin A, thonningianin B, quercetin as well as polysaccharides. Thus, future in-depth studies are still needed to establish a link between phytochemical and pharmacological study on *P. chinense*.

Furthermore, although the pharmacological effect of *P. chinense* has been proved in different animal models, the molecular targets are scarcely explored in most studies. It is suggested that *P. chinense* has effects of anti-inflammation, anti-oxidative stress, anti-apoptosis and regulating redox balance. Upon treatment of *P. chinense* and its constituents, the CYP2E1, KEAP1/NRF2, PPAR, SIRT1/3, PPARα, and SREBP1c pathways are potentially affected in hepatocytes or stellate cells. Nevertheless, more efforts should be made to clarify the molecular mechanisms of *P. chinense* for liver protection in different models.

Water extract of *P. chinense* is traditionally used for treatment of liver disease, but some organic solutions (*e.g.*, ethanol) are also applied for extraction of *P. chinense* in recent studies, which may lead to significant different composition of constituents for pharmacological study. Notably, several previous studies reported that 95% ethanol extract of *P. chinense* showed better protective effects than that of water extract. Future works may further compare different extracts of *P. chinense* for treatment of specific liver disease but not just limited to its traditional application manner. However, it should be noted that although some of results from *in vitro* studies using *P. chinense* extracts seem to be promising, it is highly possible that the conclusions may be erroneous because *in vitro* studies do not take oral bioavailability and biotransformation of constituents during absorption into consideration.

Clinical trials are essential for validation the safety and efficacy of preparations from *P. chinense* in treating liver diseases. Although numerous works on preparations from *P. chinense* have been performed by clinical researchers in recent years, most of them should be performed in a more strict and standard way, in which randomized double-blind and placebo-controlled experiments should be performed, in order to ensure the credibility of safety and efficacy of preparations from *P. chinense*.

Additionally, although some documentations declare the preventive effect of *P. chinense* for liver diseases, there are currently no scientific data to clearly support it. Therefore, future research works are still required to investigate on whether *P. chinense* can prevent liver abnormality.

Collectively, based on current studies on *P. chinense*, we believe it is a promising candidate for further developing as a new liver protecting herbal medicine. Nevertheless, to achieve the goal, in-depth research based on advanced cross-discipline technologies are needed to elucidate active ingredients and/or fractions as well as underlying molecular mechanisms.

Author contribution

Xu Wu proposed concept of the review. Huimin Huang, Zhangang Xiao, Jing Shen and Yueshui Zhao collected and analyzed documentations. Jianhua Yin and Parham Jabbarzadeh Kaboli prepared tables. Anqi Wang and Xu Wu drew the figures. Xu Wu, Jing Li, Anqi Wang, Mingxing Li, Jiliang Cao and Huimin Huang drafted the manuscript. Chi Hin Cho and Yitao Wang critically revised the manuscript. All authors read and approved the final version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest in this study.

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