Review

DOI: 10.5582/bst.2016.01110

Advance in studies on traditional Chinese medicines to treat infection with the hepatitis B virus and hepatitis C virus

Jufeng Xia[1], Yoshinori Inagaki[1], Peipei Song[2], Tatsuo Sawakami[1], Norihiro Kokudo[1], Kiyoshi Hasegawa[1], Yoshihiro Sakamoto[1], Wei Tang[1,*]

[1] *Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan;*
[2] *Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa-shi, Chiba, Japan.*

Summary **Traditional Chinese medicine (TCM), as a type of complementary and alternative medicine (CAM), is a sophisticated and time-honored form of healthcare in China. Many TCMs are widely used to treat hepatitis B and hepatitis C in countries like China, Japan, and South Korea. Since conventional clinical preparations like interferon-α cause obvious dose-dependent adverse reactions and drug resistance, TCMs and related bioactive compounds have garnered increasing attention from physicians and medical researchers. Thus far, a number of TCMs and compounds have been used to inhibit the hepatitis B virus (HBV) or hepatitis C virus (HCV) *in vitro*, *in vivo*, and even in clinical trials. The current review summarizes TCMs and related compounds that have been used to inhibit HBV or HCV. Most of these medicines are derived from herbs. HepG2.2.15 cells have been used to study HBV *in vitro* and Huh7.5 cells have been similarly used to study HCV. Ducks have been used to study the anti-HBV effect of new medication *in vivo*, but there are few animal models for anti-HCV research at the present time. Thus far, a number of preclinical studies have been conducted but few clinical trials have been conducted. In addition, a few chemically modified compounds have displayed greater efficacy than natural products. However, advances in TCM research are hampered by mechanisms of action of many bioactive compounds that have yet to be identified. In short, TCMs and related active compounds are a CAM that could be used to treat HBV and HCV infections.**

Keywords: Traditional Chinese medicine, active compounds, HBV, HCV, clinical trials

1. Introduction

Hepatitis is a type of inflammation occurring in the liver. Acute hepatitis can be self-limiting and progress to chronic hepatitis or it can lead to acute liver failure in rare instances (*1*). Chronic hepatitis may progress to fibrosis, cirrhosis, or liver cancer. Hepatitis viruses are the most common cause of hepatitis around the world. There are 5 major hepatitis viruses, types A, B, C, D, and E. These five types are of great significance because of their morbidity and mortality and their potential for causing outbreaks and spreading extensively (*2*). Types B and C cause chronic liver disease in hundreds of millions of people worldwide, especially in Africa and Central and East Asia, and these 2 types are the most common cause of cirrhosis and liver cancer (*3*). Although a few antivirals have been used to inhibit the hepatitis B virus, they also lead to obvious dose-dependent adverse reactions and drug resistance (*4*). Interferon-α (IFN-α) was the world's first medication to treat chronic hepatitis B virus (HBV) infection (*5*). However, IFN-α does not yield satisfactory therapeutic outcomes and it leads to several adverse reactions such as flu-like syndrome, fatigue, drowsiness, and low blood counts (*6*). Lamivudine, also called 3TC, was a major

Released online in J-STAGE as advance publication June 25 2016.

**Address correspondence to:*
Dr. Wei Tang, Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, the University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: tang-sur@h.u-tokyo.ac.jp

Table 1. Extracts in and formulations of TCMs to treat HBV

Common name	Composition	Research stages	Tested in	Anti-HBV activity	Combination
Aqueous extract of *B. nivea*	*Boehmeria nivea* (Linn.) Gaudich	Cell experiment	HepG2.2.15 cells	Inhibits HBsAg, HBeAg, and HBV DNA	-
Ethanol extract of Hu-Zhang	*Polygonum cuspidatum*	Cell experiment	HepG2.2.15 cells	Inhibits HBsAg and HBeAg	-
Aqueous extract of *S. media*	*Stellaria media* (L.) Vill.	Cell experiment	HepG2.2.15 cells	Inhibits HBsAg and HBeAg	-
Ethyl acetate extract	*Ligularia atroviolacea*	Cell experiment	HepG2.2.15 cells	Inhibits HBsAg	-
Aqueous extract of *R. Astragali*	*Radix Astragali*	Cell experiment and animal experiment	HepG2.2.15 cells and duck	Inhibits HBeAg, HBV DNA, and DHBV DNA	-
Ethanol extract of *O. javanica*	*Oenanthe javanica*	Cell experiment and animal experiment	HepG2.2.15 cells and duck	Inhibits HBsAg, HBeAg, and DHBV DNA	-
Xiao-Chai-Hu-Tang	*Scutellariae radix et al*.	Cell experiment	HepA2 cells	Inhibits HBV DNA	-
Qizhu granules	*Astragalus et al*.	Clinical trial	Patients	Inhibits HBV DNA and HBeAg	Lamivudine
Fu-Zheng-Jie-Du-Tang	*Cuscuta chinensis* Lam. *et al*.	Clinical trial	Patients	Inhibits HBsAg and increases IFN-γ	-
Cinobufacini	*Bufo Bufo gargarizans* Cantor	Cell experiment and clinical trial	HepG2.2.15 cells and patients	Inhibit HBsAg, HBeAg, HBcrAg, and HBV DNA	IFN-2α 2b

medication to treat HBV infection, but its therapeutic outcomes are also accompanied by a number of adverse reactions and it leads to drug resistance with long-term administration (*7*). Recently, the most common therapy for chronic hepatitis C virus (HCV) has been IFN-α plus ribavirin. Both markedly inhibit the virus. However, both also cause various adverse reactions as well. Adverse reactions to IFN-α have previously been mentioned. Ribavirin is a major ribonucleic analog that is used to suppress HCV, but it often leads to a series of adverse reactions such as anemia, pain, fever, and trouble breathing (*8,9*). Besides these adverse events, most antivirals are costly. Therefore, novel drugs are urgently needed to treat HBV and HCV infection. Accordingly, alternative and complementary medicines (ACM) are being increasingly used.

Traditional Chinese medicine (TCM), as a type of ACM, is a sophisticated and time-honored form of healthcare in China (*10*). Many TCMs are widely used to treat hepatitis B in China and a number of other countries (*11*). In China, TCM is used as CAM treatment and accounts for 30-50% of all medications used, with a low cost and low level of toxicity (*12*). In the US, the 2002 National Health Interview Survey (NHIS) revealed that 19% of adults used some form of herbal medicine within the previous year (*13*). TCMs are complicated mixtures of active compounds. Although the active compounds in TCMs have not been fully isolated and identified, a few have been found to be potential antivirals (*14*). Bioactive compounds extracted from natural products have characteristics similar to chemically synthesized medications and they can be easily assimilated and metabolized by the human body (*15*). Moreover, TCMs can be obtained from various organisms without the need for laborious or industrial chemosynthesis. Thus, TCMs could be a good candidate for antiviral development. Over the past few years, TCMs have garnered increasing attention from investigators searching for effective antiviral compounds.

2. Extracts in and formulations of TCMs to treat HBV

The effects of major extracts in and formulations of TCM to treat HBV infection, including *Boehmeria nivea* (Linn.) Gaudich, *Polygonum cuspidatum*, Stellaria media (L.) Vill., *Ligularia atroviolacea*, *Radix Astragali*, *Oenanthe javanica*, Xiao-Chai-Hu-Tang, Qizhu granules, Fu-Zheng-Jie-Du-Tang, and cinobufacini, are summarized here. Research has mainly focused on cell experiments, animal experiments, and clinical trials. Anti-HBV activities of these formulations in preclinical experiments and clinical trials are listed in Table 1.

Boehmeria nivea (Linn.) Gaudich is a type of perennial ratoon herbal plant, the root of which is used in TCM and possesses a number of pharmacological characteristics. A recent study found that the expression of hepatitis B virus surface antigen (HBsAg) and hepatitis B virus envelope antigen (HBeAg) decreased

in HepG2.2.15 cells treated with the *Boehmeria nivea* extract. Moreover, the concentration of HBV DNA detected in HepG2.2.15 cells in culture medium decreased significantly after treatment (*16*).

Polygonum cuspidatum, also known as Hu-Zhang in China, is a type of TCM herb. *P. cuspidatum* which has a wide range of pharmacological effects, has been used to treat hot flashes, heart disease, cancer, and liver disease. An ethanol extract and resveratrol isolated from *P. cuspidatum* were found to be able to inhibit HBV at 10 μg/mL *in vitro* (*17*).

Stellaria media (L.) Vill. is a TCM that has been used for hundreds years in China to mainly treat skin diseases, arthritis, and bronchitis. A study in HepG2.2.15 cells reported that 30 μg/mL of *S. media* effectively decreased the expression of HBsAg 27.92% and the expression of HBeAg 25.35% after administration for 6 days (*18*).

Ligularia, also known as leopard plant, is a group of herbaceous perennial plants that is mainly distributed in the inland areas. *Ligularia atroviolacea* has been traditionally used as an herbal medicine to treat hepatitis B, asthma, hemoptysis, and pulmonary tuberculosis. Shi *et al*. reported that an ethyl acetate extract of *L. atroviolacea* inhibited the secretion of HBsAg in HepG2.2.15 cells (*19*).

Radix Astragali is the dried root of *Astragalus membranaceus* (Fisch.) Bunge and *Astragalus mongholicus* Bunge (Fabaceae). Extracts of *R. Astragali* have been found to markedly decrease the concentration of HBeAg and HBV DNA in 116 clinical blood samples after 2 months of treatment and to inhibit levels of duck hepatitis B virus (DHBV) DNA in models of duck viral hepatitis B (*20*).

The TCM *Oenanthe javanica* has been used to treat inflammation for many years in China. Han's research group used the HepG2.2.15 cell line and a model of DHBV infection to evaluate the anti-HBV effects of an ethanol extract of *O. javanica*. Results indicated that *O. javanica* suppressed HBsAg and HBeAg *in vitro* and that it also inhibited DHBV replication in duck models (*21*).

Xiao-Chai-Hu-Tang (XCHT) has been widely used to treat various liver diseases in Asian countries. It consists of 7 different constituents, with *Scutellariae radix* accounting for most of its therapeutic effect. A study by Tseng *et al*. indicated that XCHT might inhibit HBV viral gene expression and DNA replication by regulating hepatic transcriptional machinery *in vitro* (*22*).

Qizhu granules (QZG) are a TCM consisting of several herbs such as *Astragalus*. In a clinical trial, 103 patients with chronic hepatitis B were divided into 2 groups. All patients received lamivudine. The treatment group also received QZG. After 1 year of treatment, the treatment group tested positive for HBV DNA at the same rate as the control group, but the level of secreted HBeAg in the treatment group differed significantly from that in the control group (*23*). Thus, the authors contended that a combination of lamivudine and QZG would have therapeutic efficacy.

Several systematic reviews of TCM have reported that Fu-Zheng-Jie-Du-Tang (FZJDT) is able to inhibit HBV. FZJDT consists of a number of herbs, such as *Cuscuta chinensis* Lam., *Eucommia ulmoides* Oliver, and *Poria cocos* (Schw.) Wolf. He *et al*. designed a clinical trial in which three hundreds of patients infected with HBV were divided into a treatment group and control group. After 52 weeks of treatment, the treatment group had a more marked decrease in the mean concentration of serum HBsAg than the control group did. The treatment group had a significant increase in IFN-γ, suggesting that FZJDT was able to modulate host immune function (*24*).

Cinobufacini, also known as Hua-Chan-Su, is a water-soluble extract made from toad skin (*Bufo bufo gargarizans* Cantor). Cinobufacini has long been used in China as an anti-tumor agent, an analgesic, an anti-inflammatory, and an anti-microbial. In a cell experiment, cinobufacini was reported to inhibit HBV replication by suppressing serum levels of HBsAg, HBeAg, hepatitis B core-related antigen (HBcrAg), and HBV DNA (*25*). Results of a clinical trial by Yu *et al*. suggested that cinobufacini combined with IFN-2α 2b significantly inhibited HBV replication (*26*).

3. Bioactive compounds in TCMs to treat HBV

Although TCMs have facilitated drug development and clinical treatment, the full potential of those medicines has yet to tapped because of their complex composition and varying quality (*27*). Over the past few years, rapid and substantial advances have been made in development of TCMs with the increasing emergence of novel theories, methods, techniques, and instruments (*28*). Numerous bioactive compounds have been discovered in TCMs and their functions have been studied. A few bioactive compounds isolated from TCM have been reported to possess anti-HBV activity (*29,30*). The current review identified recent studies on bioactive compounds in TCMs with anti-HBV activity. These compounds have been found to be effective and warrant intensive study in the treatment of hepatitis B (Table 2). The results of the studies in question have been classified into 4 findings: *i*) inhibiting the secretion of HBsAg, HBeAg, and/or HBcrAg; *ii*) inhibiting HBV DNA; *iii*) inhibiting both *i*) and *ii*); and *iv*) inhibiting HBV proliferation *via* other pathways.

i) Inhibiting the secretion of HBsAg, HBeAg, and/or HBcrAg. A study by Dai *et al*., isolated 13 bioactive compounds from the TCM *Viola diffusa* Ging, and results suggested that 2β-hydroxy-3,4-seco-friedelolactone-27-oicacid,2β, 28β-dihydroxy-3,4-seco-friedelolactone-27-oicacid, and 2β,30β-dihydroxy-3,4-seco-friedelolactone-27-lactone suppressed HBsAg and HBeAg in HepG2.2.15 cells (*31*). The rhizome of *Cyperus rotundus*

Table 2. Bioactive compounds in TCMs to treat HBV

Compounds	Herbs	Tested in	Anti-HBV activity	IC_{50}	Mechanism
2β,30β-dihydroxy-3,4-seco-friedelolactone-27-lactone	*Viola diffusa Ging*	HepG2.2.15	Inhibits HBsAg and HBeAg	33.7 μM/26.2 μM	Unclear
Sesquiterpenoid	*Cyperus rotundus*	HepG2.2.15	Inhibits HBsAg and HBeAg	46.6 μM/162.5 μM	Unclear
Swermacrolactone	*Swertia*	HepG2.2.15	Inhibits HBsAg and HBeAg	0.02 μM/0.02 μM	Unclear
Piperine	*Piper longum Linn.*	HepG2.2.15	Inhibits HBsAg and HBeAg	0.13 mM/0.16 mM	Unclear
Gentiocrucine	*Swertia macrosperma*	HepG2.2.15	Inhibits HBsAg and HBeAg	3.14 mM/3.35 mM	Unclear
1,2,4,6-tetra-O-galloyl-β-D-glucose	*Phyllanthus emblica L.*	HepG2.2.15	Inhibits HBsAg and HBeAg	6.25 μg/mL/3.13 μg/mL	Unclear
Bufalin and cinobufagin	*Bufo bufo gargarizans Cantor*	HepG2.2.15	Inhibits HBsAg and HBeAg	-	Unclear
Aserythrocentaurin	*Swertia delavayi*	HepG2.2.15	Inhibits HBV DNA	0.05 mM	Unclear
Dehydroandrographolide	*Andrographis paniculata*	HepG2.2.15	Inhibits HBV DNA	22.6 μM	Unclear
Menisdaurin	*Saniculiphyllum guangxiense*	HepG2.2.15	Inhibits HBV DNA	0.32 mM	Unclear
Chrysophanol 8-O-β-D-glucoside	*Rheum palmatum L.*	HepG2.2.15	Inhibits HBsAg, HBeAg, and HBV DNA	36.98 ± 2.28 μg/mL	Suppress DNA polymerase
Wogonin	*Scutellaria radix*	HepG2.2.15 and duck model	Inhibits HBsAg, HBeAg, HBV DNA, and DHBV DNA	4 μg/mL	Suppress DNA polymerase
Vanitaracin A	Fungus	HepG2.2.15	Inhibits entry of HBV	0.61 ± 0.23 μM	Block NTCP
Saponin	*Hydrocotyle sibthorpioides*	HepG2.2.15 and duck model	Inhibits HBsAg, HBeAg, HBV DNA, and DHBV DNA	56.9 μM	Suppress core,s1,s2, and X gene
Cepharanthine hydrochloride	*Stephania cepharantha Hayata.*	HepG2.2.15	Inhibits HBV proliferation	31.89 ± 5.77 μM	Inhibit Hsc70

is a well-known TCM with a number of formulations that is used to treat hepatitis. Thirty-seven sesquiterpenoids were isolated from the fraction of *C. rotundus* using liquid chromatography-mass spectrometry (LC-MS). Six of those sesquiterpenoids may contribute to the anti-HBV activity of *C. rotundus* (*32*). Plants of the genus Swertia (Gentianaceae), annual or perennial herbs, are thought to have hepatoprotective activity but whether they contained anti-HBV compounds was unclear. A study by Wang *et al*. found 3 new secoiridoids, swermacrolactones A-C, that were able to inhibit the expression of HBsAg and HBeAg in HepG2.2.15 cells with a 50% inhibitory concentration (IC_{50}) of 0.02 and 0.02 mM, respectively (*33*). *Piper longum* Linn., a slender aromatic climber that is widely distributed in the world's tropical and subtropical areas, is used as a TCM. Recently, 11 active compounds were extracted from P. longum Linn. and 4 of those compounds were found to significantly inhibit the secretion of HBsAg and HBeAg in HepG2.2.15 cells (34). *Swertia macrosperma* is a congeneric species of *Swertia mileensis* (Gentianaceae) that has been listed in the Chinese Pharmacopoeia (1977-2010 editions) because of its therapeutic effect on hepatitis B. In a recent study, 5 bioactive compounds, gentiocrucines A-E, were isolated from *S. macrosperma* and *S. angustifolia*, and those compounds were found to inhibit both the secretion of HBsAg and HBeAg in HepG2.2.15 cells, with an IC_{50} of 3.14 and 3.35 mM (*35*). A polyphenolic compound, 1,2,4,6-tetra-O-galloyl-β-D-glucose, was isolated from *Phyllanthus emblica* L. (Euphorbiaceae), and treatment with that TCM decreased levels of both HBsAg and HBeAg in the supernatant of cultured HepG2.2.15 cells (*36*). Unlike the TCMs derived from herbs as have been mentioned thus far, bufalin and cinobufagin are active compounds that are derived from an animal source, the skin of *Bufo bufo* gargarizans Cantor (Bufonidae) (37). A chemiluminescent enzyme immunoassay revealed that bufalin and cinobufagin effectively decreased the concentration of HBsAg, HBeAg, and HBcrAg in HepG2.2.15 cells in culture medium (*27*).

ii) Inhibiting HBV DNA. Fifteen active compounds were isolated from *Swertia delavayi* using silica gel, Sephadex LH-20, and Rp-18 column chromatography (*38*). Six of those compounds, aserythrocentaurin,

Table 3. Extracts in and formulations of TCMs to treat HCV

Common name	Composition	Research stages	Tested in	Anti-HCV activity	Combination
Extract of *A. annua*	*Artemisia annua*	Animal experiment	BALB/c mice	Increases antibody levels	HCV/NS3 DNA vaccine
EtOAc extract of *G. Chinese*	*Galla chinensis*	Cell experiment	_	Inhibits HCV proliferation	_
Actinobacteria extract	*Termite*	Cell experiment	MDBK cells	Inhibits BVDV proliferation	_
Xiao-Chai-Hu-Tang	*Radix Bupleuri et al.*	Cell experiment and clinical trial	Phase II trial	Decreases HCV titer	_

erythrocentaurindimethylacetal, swertiakoside A, 2'-O-acetylswertiamarin, 1,5,8-trihydroxy-3-methoxyxanthone, and isovitexinm, markedly inhibited HBV DNA replication, with an IC_{50} of 0.05-1.46 mM. *Andrographis paniculata*, a well-known TCM described as Chuan-Xin-Lian in all editions of the Chinese Pharmacopoeia, is widely used to suppress inflammation (*39*). Two compounds, dehydroandrographolide and andrographolide, isolated from *A. paniculata* were reported to inhibit HBV DNA replication with an IC_{50} of 22.6 and 54.1 μM. *Saniculiphyllum guangxiense*, also known as Bian-Dou-Ye-Cao, was little known since it was rarely collected or observed (*40*). Recently, 8 active compounds were isolated from *S. guangxiense*, and one, menisdaurin, significantly inhibited HBV DNA replication with an IC_{50} of 0.32 mM.

iii) Inhibiting the secretion of HBsAg, HBeAg, and HBV DNA. The herb *Rheum palmatum* L. is a TCM that has been reported to be able to inhibit herpes simplex virus (HSV) and coxsackie virus (*41*). Chrysophanol 8-O-β-D-glucoside, an active compound isolated from *R. palmatum* L., significantly inhibited HBV DNA replication and expression of viral antigens, with an IC_{50} of 36.98 ± 2.28 μg/mL. An endogenous HBV DNA polymerase activity assay suggested that chrysophanol 8-O-β-D-glucoside might inhibit HBV DNA by suppressing DNA polymerase activity. Similarly, an active constituent, wogonin, from *Scutellaria radix* also was found to inhibit HBV DNA by inactivating DNA polymerase (*42*). In HepG2.2.15 cells, wogonin effectively inhibited HBV antigen expression and DNA replication with an IC_{50} of 4 μg/mL after 9 days of treatment. Wogonin decreased DHBV DNA, with an IC_{50} of 0.57 μg/mL, in ducks infected with DHBV.

iv) Inhibiting HBV proliferation *via* other pathways. A new tricyclic polyketide, vanitaracin A, was part of a secondary metabolite pool extracted from a fungus, and vanitaracin A specifically inhibited HBV (*43*). Vanitaracin A did not directly block the process of HBV replication. Instead, it interacted with sodium taurocholate cotransporting polypeptide (NTCP), an HBV entry receptor, and it disrupted its bile acid transport activity (*44*). Vanitaracin A was similarly found to inhibit proliferation of the hepatitis D virus (HDV). *Hydrocotyle sibthorpioides* (Apiaceae) is a TCM used to treat inflammation and hepatitis B in China. An active compound, saponin, was isolated from *H. sibthorpioides*, and a study suggested that saponin would be able to decrease antigens in a culture medium containing HepG2.2.15 cells and that it would be able to inhibit DHBV DNA replication in ducks infected with HBV by suppressing the activity of core, s1, s2, and X gene promoters in the HBV genome (*45*). Lamivudine-resistant strains of HBV have emerged with the widespread clinical use of lamivudine. Recently, two research teams respectively reported that cepharanthine hydrochloride and oxymatrine inhibited HBV proliferation by inhibiting heat stress cognate 70 (Hsc70), which is a host protein used for HBV replication (*46,47*).

4. Extracts in and formulations of TCMs to treat HCV

The ways in which major extracts in and formulations of TCMs, including *Artemisia annua*, *Zingiberaceae*, *Galla Chinese*, and Xiao-Chai-Hu-Tang, inhibit HCV infection are summarized here. Research has mainly focused on cell experiments and clinical trials. Anti-HCV activities of these extracts in and formulations in preclinical experiments and clinical trials are listed in Table 3.

Artemisia annua, the dried aerial section of *A. annua* L. of the *Compositae* family, is a TCM that is used clinically to treat a fever or malaria and to enhancing immunity. Results of a study by Bao *et al*. suggested that an extract of *A. annua*, when used as an adjuvant of HCV/Nonstructural protein 3 (NS3) DNA vaccine, increased antibody levels *in vivo* and promoted IFN-γ secretion by increasing a Th1-type cellular immune response (*48*). *Galla chinensis*, also called Wu-Bei-Zi, is a TCM that is commonly used to treat dysentery, bleeding, coughing, sweating, and rectal prolapse. An ethyl acetate (EtOAc) extract of *G. Chinese* was found to inhibit HCV proliferation by inhibiting NS3 protease, which is a 70-kDa cleavage product of the HCV polyprotein that acts as a serine protease (*49*). In addition to drugs derived from herbs, drugs derived from animals also can inhibit HCV activity. Extracts from termite-associated bacteria were reported to inhibit bovine viral diarrhea virus (BVDV), which is used as a surrogate model for *in vitro* antiviral studies of HCV (*50*).

Table 4. Bioactive compounds in TCMs to treat HCV

Compounds	Herbs	Tested in	Anti-HBV activity	IC_{50}	Mechanism
Grosheimol and cynaropicrin	*Cynara cardunculus* L.	Huh7/Scr cells, Huh7.5.1 Cl.2 cells	Inhibits HCV entry	1.0 μM and 1.3 μM	Unclear
Delphinidin	*Tea*	Huh-7, HEK 293T	Inhibits HCV entry	3.7 ± 0.8 μM	Impairing HCV attachment to the cell surface
Saikosaponin b2	*Bupleurum kaoi root*	HuH7.5, S29 cells	Inhibits HCV entry	16.13 ± 2.41 μM	Neutralizing particles and preventing attachment
Oleanolic acid derivative	*Glycyrrhiza species*	293T cells	Inhibits HCV entry	1.4 μM	Inhibits HCV attachment to host cell CD81 receptor
Ursolic acid	*Ligustrum lucidum*	HepG2 cells	Inhibits HCV proliferation	3.1 μg/mL	Suppressing NS5B polymerase
Aqueous extract	*Fructus Ligustri Lucidi*	HeLa cells	Inhibits HCV proliferation	10 μg/mL	Suppressing NS5B polymerase
Vitisin B	Grapevine root	Huh7.5 cells and rats	Inhibits HCV proliferation	3 nM	Suppressing NS3 protease
3-Deacetyl-3-cinnamoyl-azadirachtin	*Azadirachta indica*	-	Inhibits HCV proliferation	-	Suppressing NS3 protease
Honokiol	*Magnolia*	Huh7.5.1 cells	Inhibits HCV proliferation	1.2 μM	Suppressing NS3, NS5A, NS5B
Pheophorbide A and pyropheophorbide	*Morinda citrifolia*	Huh7.5 cells	Inhibits HCV proliferation	0.2 μg/mL and	Suppressing RNA replication
Silybin B	*Silybum marianum*	Huh7.5.1 cells	Inhibits HCV proliferation	0.3 μg/mL	Inhibiting HCV core proteins and drug-metabolizing enzymes

Xiao-Chai-Hu-Tang (XCHT) is a formula consisting of 7 herbs that has been used to treat liver diseases in East Asia. In a phase II trial, XCHT was found to decrease levels of HCV (*51*). However, the investigators cited several limitations of their trial, such as sampling bias, inter-observer variability, slow accrual, and a high drop-out rate. Trial results also indicated that XCHT caused interstitial pneumonitis.

5. Bioactive compounds in TCMs to treat HCV

A number of bioactive compounds contained in TCMs have been found to act as HCV inhibitors. The current review identified recent studies on bioactive compounds in TCMs with anti-HCV activity. These compounds have been found to be effective and warrant intensive study in the treatment of hepatitis C (Table 4). The results of the studies in question have been classified into 3 findings: *i*) inhibiting HCV entry; *ii*) inhibiting NS protein; and *iii*) other activities.

i) Inhibiting HCV entry. Entry is the first step for HCV to infect host cells. HCV entry into host hepatocytes is a complicated and multistep process that involves the HCV envelope glycoproteins E1 and E2 and several host factors like CD81 (*52*). Thus far, several active compounds from TCM have been found to be able to interrupt the interaction between the HCV envelope proteins and receptors of host cells. *Cynara cardunculus* L. was used by Egyptians to treat diverse symptoms of hepatitis, such as jaundice and ascites. Two compounds isolated from *C. cardunculus* L., grosheimol and cynaropicrin, were studied for their anti-HCV activity (*53*). Results indicated that these compounds inhibited HCV by suppressing HCV entry into host cells and that they blocked cell-free infection and cell-cell transmission. Calland *et al*. found that the flavonoids delphinidin and epigallocatechin-3-gallate (EGCG) (EGCG is found in green tea) inhibited HCV entry by changing the viral particle structure that facilitated HCV attachment to the host cell surface (*54*). Similarly, a study by Lin *et al*. identified an active compound called saikosaponin b2, which was isolated from the TCM

Bupleurum kaoi root, as an inhibitor of HCV entry (*55*). Saikosaponin b2 played a role in neutralizing viral particles, inhibiting viral attachment, and suppressing viral fusion. In addition to these natural products, many derivatives of natural active compounds are found in TCMs. A study by Yu *et al*. found that oleanolic acid slightly inhibited HCV entry with an IC_{50} of 10 μM. Hydroxylation at the C-16 position markedly increased the anti-HCV effect of oleanolic acid with an IC_{50} of 1.4 μM. Chemical modified oleanolic acid inhibited HCV entry by interrupting the interaction between HCV envelope protein E2 and the receptor CD81 in host cells (*56*).

ii) Inhibiting NS protein. HCV contains a series of nonstructural (NS) proteins such as NS2, NS3, NS4A, NS4B, NS5A, and NS5B (*57*). These proteins act as proteases, replicating viral RNA, or cofactors with other NS proteins. A study by Wozniak *et al*. found that the natural compound ursolic acid, isolated from *Ligustrum lucidum*, inhibited HCV proliferation by suppressing NS5B viral RNA polymerase (*58*). Similarly, another study found that an aqueous extract from Fructus *Ligustri Lucidi blocked* HCV entry by inhibiting NS5B viral RNA polymerase (*59*). Except for NS5B polymerase, NS3 viral proteases have seldom been studied. Lee *et al*. reported that vitisin B, a resveratrol tetramer isolated from grapevine root, was able to inhibit HCV replication by binding to and suppressing NS3 *in vitro* and *in vivo*. A combination of vitisin B and sofosbuvir (an inhibitor of NS5B polymerase) had synergistic anti-HCV activity (*60*). Ashfaq *et al*. found that 3-Deacetyl-3-cinnamoyl-azadirachtin, isolated from *Azadirachta indica*, inhibited HCV by binding with NS3 protease and neutralizing it (*61*). A study by Lan *et al*. revealed that honoliol, a compound derived from *Magnolia*, was able to block HCV infection by inhibiting NS3, NS5A, and NS5B (*62*). According to that study, a combination of honoliol and interferon-α displayed synergistic anti-HCV activity.

iii) Other activities. In addition to inhibiting HCV entry, compounds also inhibit viral infection after entry. Ratnoglik *et al*. found that pheophorbide A and pyropheophorbide, two active compounds isolated from *Morinda citrifolia*, inhibited HCV infection by interrupting viral RNA replication and viral protein production (*63*). Silybin B, a silymarin isolated from *Silybum marianum*, inhibited HCV infection in 2 ways (*64*). Silybin B was reported to inhibit HCV by decreasing HCV core proteins and it was also reported to increase antiviral efficacy by inhibiting major drug-metabolizing enzymes (CYP2C9, CYP3A4/5, and UDP-glucuronsyltransferases).

6. Adverse reactions

Although TCMs and related compounds have been reported to be able to improve the efficacy of monotherapy or combination therapy for HBV and HCV, the adverse reactions caused by TCMs have not been discussed in-depth. A few studies have reported that some TCMs attenuate the adverse effects of chemotherapy or radiotherapy and produce fewer or no adverse reactions, but other studies have suggested that TCMs may cause obvious adverse reactions (*65-67*). Kansui, the root of *Euphorbia kansui T.N. Liou ex T.P. Wang*, has been used as a remedy for edema, ascites, and asthma. According to one study, however, kansui affected cardiac and hepatic function and it affected the histomorphology of the heart, liver, and kidneys in rats (*68*). The authors of a review concluded that the major adverse reactions to TCMs were gastrointestinal symptoms including abdominal bloating or pain, epigastric discomfort, and stomach disorder, followed by diarrhea, headaches, nausea, breast distension or pain, abnormal vaginal bleeding, and dizziness (*69*). In a randomized controlled trial, Li *et al*. reported that Tongxinluo Capsules, a TCM used to treat coronary disease and angina pectoris, caused an adverse reaction in the form of stomachaches in the treatment group (*70*). Berberine, an active compound extracted from the herb *Berberis*, may induce the onset of competitive junctional rhythm, causing a loss of atrioventricular synchronization, and reduce chronotropic competence with the onset of symptoms upon exertion (*71*). In fact, the present evidence of adverse reactions and toxicity is insufficient. There are a few obstacles to evaluating adverse reactions: *i*) experimental data is limited to pharmacokinetics and inaccurate clinical research, *ii*) only a few studies have documented target organ toxicity, and *iii*) the evidence is not sufficient to elucidate the biochemical mechanisms responsible for the biological activities of TCMs. Thus, further studies are needed to study the pharmacokinetics and features of TCMs and their active compounds, and comprehensive and accurate data should be collected through clinical trials. Furthermore, mechanisms causing adverse reactions to TCMs need to be explored in depth.

7. Conclusion

Hepatitis is a severe liver disease leading to chronic hepatitis, acute liver failure, or hepatocellular carcinoma. Two pathogens that predominantly cause hepatitis are HBV and HCV. Conventional medications cause adverse effects and are costly, so TCMs and related bioactive compounds are garnering attention as CAM. This review has summarized the TCMs and active compounds used to treat HBV and HCV. Several points have become evident from this review: *i*) HepG2.2.15 is a generally accepted cell line for anti-HBV research *in vitro*; *ii*) ducks are used as animal model for DHBV infection to study anti-HBV activity *in vivo*; *iii*) Hun7.5 is a cell line that is often used in anti-HCV research *in vitro*; *iv*) there are few animal models for anti-HCV research *in vivo*; *v*) in addition to

TCMs and compounds derived from herbs, compounds derived from animals are also used to inhibit HCV infection and have yielded desired results; *vi*) a few therapies combining compounds and medications have yielded satisfactory results; *vii*) thus far, few clinical trials have been conducted on active compounds; *viii*) limited basic studies thus far have yet to elucidate the mechanisms of antiviral action of TCMs; *ix*) several chemically modified bioactive compounds have displayed more effective antivirus activity than natural products. Several steps need to be taken in future work on treatments for HBV and HCV infections. More bioactive compounds from TCMs need to be explored, new animal models need to be created for anti-HCV research, novel chemically modified bioactive compounds need to be developed, clinical trials need to be conducted, and the molecular mechanisms of action of TCMs need to be elucidated.

Acknowledgements

This work was supported by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan.

References

1. Yoshio S, Kanto T. Host-virus interactions in hepatitis B and hepatitis C infection. J Gastroenterol. 2016; 51:409-420.
2. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012; 142:1264-1273.
3. Nishida N, Kudo M. Clinical features of vascular disorders associated with chronic hepatitis virus infection. Dig Dis. 2014; 32:786-790.
4. Kovari H, Sabin CA, Ledergerber B, Ryom L, Worm SW, Smith C, Phillips A, Reiss P, Fontas E, Petoumenos K, De Wit S, Morlat P, Lundgren JD, Weber R. Antiretroviral drug-related liver mortality among HIV-positive persons in the absence of hepatitis B or C virus coinfection: The data collection on adverse events of anti-HIV drugs study. Clin Infect Dis. 2013; 56:870-879.
5. Lu J, Zhang S, Liu Y, Du X, Ren S, Zhang H, Ma L, Chen Y, Chen X, Shen C. Effect of Peg-interferon alpha-2a combined with Adefovir in HBV postpartum women with normal levels of ALT and high levels of HBV DNA. Liver Int. 2015; 35:1692-1699.
6. Palacios-Alvarez I, Roman-Curto C, Mir-Bonafe JM, Canueto J, Usero-Barcena T, Fernandez-Lopez E. Autoimmune response as a side effect of treatment with interferon-alpha in melanoma: Does this have prognostic implications? Int J Dermatol. 2015; 54:e91-93.
7. Gualdesi MS, Brinon MC, Quevedo MA. Intestinal permeability of lamivudine (3TC) and two novel 3TC prodrugs. Experimental and theoretical analyses. Eur J Pharm Sci. 2012; 47:965-978.
8. Zhang Y, Lu H, Ji H, Li Y. Inflammatory pseudotumor of the liver: A case report and literature review. Intractable Rare Dis Res. 2015; 4:155-158.
9. Ali AH, Carey EJ, Lindor KD. Current research on the treatment of primary sclerosing cholangitis. Intractable Rare Dis Res. 2015; 4:1-6.
10. Takayama S, Iwasaki K. Systematic review of traditional Chinese medicine for geriatrics. Geriatr Gerontol Int. 2016; doi: 10.1111/ggi.12803.
11. Tsai DS, Huang MH, Chang YS, Li TC, Peng WH. The use of Chinese herbal medicines associated with reduced mortality in chronic hepatitis B patients receiving lamivudine treatment. J Ethnopharmacol. 2015; 174:161-167.
12. Song PP, Gao JJ, Kokudo N, Tang W. Standardization of traditional Chinese medicine and evaluation of evidence from its clinical practice. Drug Discov Ther. 2011; 5:261-265.
13. Kennedy J. Herb and supplement use in the US adult population. Clin Ther. 2005; 27:1847-1858.
14. Xia JF, Gao JJ, Inagaki Y, Kokudo N, Nakata M, Tang W. Flavonoids as potential anti-hepatocellular carcinoma agents: Recent approaches using HepG2 cell line. Drug Discov Ther. 2013; 7:1-8.
15. Larson EC, Hathaway LB, Lamb JG, Pond CD, Rai PP, Matainaho TK, Piskaut P, Barrows LR, Franklin MR. Interactions of Papua New Guinea medicinal plant extracts with antiretroviral therapy. J Ethnopharmacol. 2014; 155:1433-1440.
16. Wei J, Lin L, Su X, Qin S, Xu Q, Tang Z, Deng Y, Zhou Y, He S. Anti-hepatitis B virus activity of leaf extracts in human HepG2.2.15 cells. Biomed Rep. 2014; 2:147-151.
17. Peng W, Qin R, Li X, Zhou H. Botany, phytochemistry, pharmacology, and potential application of *Polygonum cuspidatum Sieb.et* Zucc.: A review. J Ethnopharmacol. 2013; 148:729-745.
18. Ma L, Song J, Shi Y, Wang C, Chen B, Xie D, Jia X. Anti-hepatitis B virus activity of chickweed [*Stellaria media* (L.) Vill.] extracts in HepG2.2.15 cells. Molecules. 2012; 17:8633-8646.
19. Shi SY, Zhou HH, Huang KL, Li HB, Liu SQ, Zhao Y. Application of high-speed counter-current chromatography for the isolation of antiviral eremophilenolides from *Ligularia atroviolacea*. Biomed Chromatogr. 2008; 22:985-991.
20. Tang LL, Sheng JF, Xu CH, Liu KZ. Clinical and experimental effectiveness of Astragali compound in the treatment of chronic viral hepatitis B. J Int Med Res. 2009; 37:662-667.
21. Han YQ, Huang ZM, Yang XB, Liu HZ, Wu GX. *In vivo* and *in vitro* anti-hepatitis B virus activity of total phenolics from *Oenanthe javanica*. J Ethnopharmacol. 2008; 118:148-153.
22. Tseng YP, Wu YC, Leu YL, Yeh SF, Chou CK. *Scutellariae radix* suppresses hepatitis B virus production in human hepatoma cells. Front Biosci (Elite Ed). 2010; 2:1538-1547.
23. Zhu YF, Gu XB, Guo XY, Yan ZH, Pu YC, Tu KW, Hua Z, Pei H. Effect of compound qizhu granule on cellular immunity of chronic hepatitis B patients. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2014; 34:1178-1181. (in Chinese)
24. He J, Zhou D, Tong G, Xing Y, Chen Y, Zhang X, Zhan B, Gao H, Zhou X, Xiong Y, Liu X, Peng L, Qiu M, Zheng Y. Efficacy and safety of a chinese herbal formula (invigorating kidney and strengthening spleen) in chronic hepatitis B virus carrier: Results from a multicenter, randomized, double-blind, and placebo-controlled trial. Evid Based Complement Alternat Med. 2013;

2013:961926.
25. Cui X, Inagaki Y, Xu H, Wang D, Qi F, Kokudo N, Fang D, Tang W. Anti-hepatitis B virus activities of cinobufacini and its active components bufalin and cinobufagin in HepG2.2.15 cells. Biol Pharm Bull. 2010; 33:1728-1732.
26. Yu Q, Xi Q. Observation on the effect of cinobufacini and IFN-α 2b treating chronic hepatitis B. Capital Medicine. 2011; 16:48-49.
27. Zhao XL, Han JX. The connotation of the Quantum Traditional Chinese Medicine and the exploration of its experimental technology system for diagnosis. Drug Discov Ther. 2013; 7:225-232.
28. Zhou G, He YP. Problems in quality standard research of new traditional Chinese medicine compound. Zhongguo Zhong Yao Za Zhi. 2014; 39:3389-3391. (in Chinese)
29. Li L, Bonneton F, Chen XY, Laudet V. Botanical compounds and their regulation of nuclear receptor action: The case of traditional Chinese medicine. Mol Cell Endocrinol. 2015; 401:221-237.
30. Huang H, Peng X, Zhong C. Idiopathic pulmonary fibrosis: The current status of its epidemiology, diagnosis, and treatment in China. Intractable Rare Dis Res. 2013; 2:88-93.
31. Dai JJ, Tao HM, Min QX, Zhu QH. Anti-hepatitis B virus activities of friedelolactones from *Viola diffusa* Ging. Phytomedicine. 2015; 22:724-729.
32. Xu HB, Ma YB, Huang XY, Geng CA, Wang H, Zhao Y, Yang TH, Chen XL, Yang CY, Zhang XM, Chen JJ. Bioactivity-guided isolation of anti-hepatitis B virus active sesquiterpenoids from the traditional Chinese medicine: Rhizomes of *Cyperus rotundus*. J Ethnopharmacol. 2015; 171:131-140.
33. Wang HL, Geng CA, Ma YB, Zhang XM, Chen JJ. Three new secoiridoids, swermacrolactones A-C and anti-hepatitis B virus activity from *Swertia macrosperma*. Fitoterapia. 2013; 89:183-187.
34. Jiang ZY, Liu WF, Zhang XM, Luo J, Ma YB, Chen JJ. Anti-HBV active constituents from *Piper longum*. Bioorg Med Chem Lett. 2013; 23:2123-2127.
35. Wang HL, He K, Geng CA, Zhang XM, Ma YB, Luo J, Chen JJ. Gentiocrucines A-E, five unusual lactonic enamino Ketones from *Swertia macrosperma* and *Swertia angustifolia*. Planta Medica. 2012; 78:1867-1872.
36. Xiang YF, Ju HQ, Li S, Zhang YJ, Yang CR, Wang YF. Effects of 1,2,4,6-tetra-O-galloyl-beta-D-glucose from *P. emblica* on HBsAg and HBeAg secretion in HepG2.2.15 cell culture. Virol Sin. 2010; 25:375-380.
37. Nakata M, Kawaguchi S, Oikawa A, Inamura A, Nomoto S, Miyai H, Nonaka T, Ichimi S, Fujita-Yamaguchi Y, Luo C, Gao B, Tang W. An aqueous extract from toad skin prevents gelatinase activities derived from fetal serum albumin and serum-free culture medium of human breast carcinoma MDA-MB-231 cells. Drug Discov Ther. 2015; 9:417-421.
38. Cao TW, Geng CA, Ma YB, He K, Zhou NJ, Zhou J, Zhang XM, Chen JJ. Chemical constituents of *Swertia delavayi* and their anti-hepatitis B virus activity. Zhongguo Zhong Yao Za Zhi. 2015; 40:897-902. (in Chinese)
39. Chen H, Ma YB, Huang XY, Geng CA, Zhao Y, Wang LJ, Guo RH, Liang WJ, Zhang XM, Chen JJ. Synthesis, structure-activity relationships and biological evaluation of dehydroandrographolide and andrographolide derivatives as novel anti-hepatitis B virus agents. Bioorg Med Chem Lett. 2014; 24:2353-2359.
40. Geng CA, Huang XY, Lei LG, Zhang XM, Chen JJ. Chemical constituents of *Saniculiphyllum guangxiense*. Chem Biodivers. 2012; 9:1508-1516.
41. Li Z, Li LJ, Sun Y, Li J. Identification of natural compounds with anti-hepatitis B virus activity from *Rheum palmatum* L. ethanol extract. Chemotherapy. 2007; 53:320-326.
42. Guo Q, Zhao L, You Q, Yang Y, Gu H, Song G, Lu N, Xin J. Anti-hepatitis B virus activity of wogonin *in vitro* and *in vivo*. Antiviral Res. 2007; 74:16-24.
43. Matsunaga H, Kamisuki S, Kaneko M, Yamaguchi Y, Takeuchi T, Watashi K, Sugawara F. Isolation and structure of vanitaracin A, a novel anti-hepatitis B virus compound from *Talaromyces sp*. Bioorg Med Chem Lett. 2015; 25:4325-4328.
44. Kaneko M, Watashi K, Kamisuki S, *et al*. A novel tricyclic polyketide, vanitaracin A, specifically inhibits the entry of hepatitis B and D viruses by targeting sodium taurocholate cotransporting polypeptide. J Virol. 2015; 89:11945-11953.
45. Huang Q, Zhang S, Huang R, Wei L, Chen Y, Lv S, Liang C, Tan S, Liang S, Zhuo L, Lin X. Isolation and identification of an anti-hepatitis B virus compound from *Hydrocotyle sibthorpioides Lam*. J Ethnopharmacol. 2013; 150:568-575.
46. Zhou YB, Wang YF, Zhang Y, Zheng LY, Yang XA, Wang N, Jiang JH, Ma F, Yin DT, Sun CY, Wang QD. *In vitro* activity of cepharanthine hydrochloride against clinical wild-type and lamivudine-resistant hepatitis B virus isolates. Eur J Pharmacol. 2012; 683:10-15.
47. Gao LM, Han YX, Wang YP, Li YH, Shan YQ, Li X, Peng ZG, Bi CW, Zhang T, Du NN, Jiang JD, Song DQ. Design and synthesis of oxymatrine analogues overcoming drug resistance in hepatitis B virus through targeting host heat stress cognate 70. J Med Chem. 2011; 54:869-876.
48. Bao LD, Ren XH, Ma RL, Wang Y, Yuan HW, Lv HJ. Efficacy of Artemisia annua polysaccharides as an adjuvant to hepatitis C vaccination. Genet Mol Res. 2015; 14:4957-4965.
49. Duan D, Li Z, Luo H, Zhang W, Chen L, Xu X. Antiviral compounds from traditional Chinese medicines *Galla Chinese* as inhibitors of HCV NS3 protease. Bioorg Med Chem Lett. 2004; 14:6041-6044.
50. Padilla MA, Rodrigues RA, Bastos JC, Martini MC, Barnabe AC, Kohn LK, Uetanabaro AP, Bomfim GF, Afonso RS, Fantinatti-Garboggini F, Arns CW. Actinobacteria from Termite Mounds Show Antiviral Activity against Bovine Viral Diarrhea Virus, a Surrogate Model for Hepatitis C Virus. Evid Based Complement Alternat Med. 2015; 2015:745754.
51. Deng G, Kurtz RC, Vickers A, Lau N, Yeung KS, Shia J, Cassileth B. A single arm phase II study of a Far-Eastern traditional herbal formulation (sho-sai-ko-to or xiao-chai-hu-tang) in chronic hepatitis C patients. J Ethnopharmacol. 2011; 136:83-87.
52. Lyu J, Imachi H, Fukunaga K, Yoshimoto T, Zhang H, Murao K. Roles of lipoprotein receptors in the entry of hepatitis C virus. World J Hepatol. 2015; 7:2535-2542.
53. Elsebai MF, Koutsoudakis G, Saludes V, Perez-Vilaro G, Turpeinen A, Mattila S, Pirttila AM, Fontaine-Vive F, Mehiri M, Meyerhans A, Diez J. Pan-genotypic Hepatitis C Virus Inhibition by Natural Products Derived from the Wild Egyptian Artichoke. J Virol. 2016; 90:1918-1930.
54. Calland N, Sahuc ME, Belouzard S, *et al*. Polyphenols

Inhibit Hepatitis C Virus Entry by a New Mechanism of Action. J Virol. 2015; 89:10053-10063.
55. Lin LT, Chung CY, Hsu WC, Chang SP, Hung TC, Shields J, Russell RS, Lin CC, Li CF, Yen MH, Tyrrell DL, Richardson CD. Saikosaponin b2 is a naturally occurring terpenoid that efficiently inhibits hepatitis C virus entry. J Hepatol. 2015; 62:541-548.
56. Yu F, Wang Q, Zhang Z, *et al*. Development of oleanane-type triterpenes as a new class of HCV entry inhibitors. J Med Chem. 2013; 56:4300-4319.
57. Zhu SL, Wang L, Cao ZY, Wang J, Jing MZ, Xia ZC, Ao F, Ye LB, Liu S, Zhu Y. Inducible CYP4F12 enhances Hepatitis C virus infection *via* association with viral nonstructural protein 5B. Biochem Biophys Res Commun. 2016; 471:95-102.
58. Wozniak L, Skapska S, Marszalek K. Ursolic Acid--A Pentacyclic Triterpenoid with a Wide Spectrum of Pharmacological Activities. Molecules. 2015; 20:20614-20641.
59. Kong L, Li S, Han X, Xiang Z, Fang X, Li B, Wang W, Zhong H, Gao J, Ye L. Inhibition of HCV RNA-dependent RNA polymerase activity by aqueous extract from *Fructus Ligustri Lucidi*. Virus Res. 2007; 128:9-17.
60. Lee S, Yoon KD, Lee M, *et al*. Identification of a resveratrol tetramer as a potent inhibitor of hepatitis C virus helicase. Br J Pharmacol. 2016; 173:191-211.
61. Ashfaq UA, Jalil A, Ul Qamar MT. Antiviral phytochemicals identification from *Azadirachta indica* leaves against HCV NS3 protease: An in silico approach. Nat Prod Res. 2015;1-4.
62. Lan KH, Wang YW, Lee WP, Lan KL, Tseng SH, Hung LR, Yen SH, Lin HC, Lee SD. Multiple effects of Honokiol on the life cycle of hepatitis C virus. Liver Int. 2012; 32:989-997.
63. Ratnoglik SL, Aoki C, Sudarmono P, Komoto M, Deng L, Shoji I, Fuchino H, Kawahara N, Hotta H. Antiviral activity of extracts from *Morinda citrifolia* leaves and chlorophyll catabolites, pheophorbide a and pyropheophorbide a, against hepatitis C virus. Microbiology and Immunology. 2014; 58:188-194.
64. Althagafy HS, Graf TN, Sy-Cordero AA, Gufford BT, Paine MF, Wagoner J, Polyak SJ, Croatt MP, Oberlies NH. Semisynthesis, cytotoxicity, antiviral activity, and drug interaction liability of 7-O-methylated analogues of flavonolignans from milk thistle. Bioorg Med Chem. 2013; 21:3919-3926.
65. Yu M, Zhao Y. Cantharis by photosynthetic bacteria biotransformation: Reduced toxicity and improved antitumor efficacy. J Ethnopharmacol. 2016; 186:151-158.
66. Wang Z, Li J, Ji Y, An P, Zhang S, Li Z. Traditional herbal medicine: A review of potential of inhibitory hepatocellular carcinoma in basic research and clinical trial. Evid Based Complement Alternat Med. 2013; 2013:268963.
67. Li D, Lu L, Zhang J, Wang X, Xing Y, Wu H, Yang X, Shi Z, Zhao M, Fan S, Meng A. Mitigating the effects of Xuebijing injection on hematopoietic cell injury induced by total body irradiation with gamma rays by decreasing reactive oxygen species levels. Int J Mol Sci. 2014; 15:10541-10553.
68. Shen J, Wang J, Shang EX, Tang YP, Kai J, Cao YJ, Zhou GS, Tao WW, Kang A, Su SL, Zhang L, Qian DW, Duan JA. The dosage-toxicity-efficacy relationship of kansui and licorice in malignant pleural effusion rats based on factor analysis. J Ethnopharmacol. 2016; 186:251-256.
69. Xu LW, Jia M, Salchow R, Kentsch M, Cui XJ, Deng HY, Sun ZJ, Kluwe L. Efficacy and side effects of chinese herbal medicine for menopausal symptoms: A critical review. Evid Based Complement Alternat Med. 2012; 2012:568106.
70. Li S, Qi T. 36 Cases report of the tongxinluo capsule combined with western medicine on CSX. Yunan Tradit Chin Med. 2009; 30:15-16.
71. Cannillo M, Frea S, Fornengo C, Toso E, Mercurio G, Battista S, Gaita F. Berberine behind the thriller of marked symptomatic bradycardia. World J Cardiol. 2013; 5:261-264.

(Received June 9, 2016; Revised June 20, 2016; Accepted June 21, 2016)