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Review

Niuchangchih (*Antrodia camphorata*) and its potential in treating liver diseases

Zong-Hua Ao[a], Zheng-Hong Xu[a,*], Zhen-Ming Lu[b], Hong-Yu Xu[a], Xiao-Mei Zhang[a], Wen-Fang Dou[a]

[a] *Laboratory of Pharmaceutical Engineering, School of Medicine and Pharmaceutics, Jiangnan University, No. 1800 Lihu Road, Wuxi 214122, PR China*
[b] *The Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, Wuxi 214122, PR China*

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ABSTRACT

Niuchangchih (*Antrodia camphorata* (M. Zang & C.H. Su) Sheng H. Wu, Ryvarden & T.T. Chang) is a basidiomycete endemic to Taiwan. It is well known as a Traditional Chinese Medicine (TCM), and Taiwanese aborigines used this species to treat liver diseases and food and drug intoxication. The compounds identified in *Niuchangchih* are predominantly polysaccharides, triterpenoids, steroids, benzenoids and maleic/succinic acid derivatives. Recent research has revealed that *Niuchangchih* possesses extensive biological activity, such as hepatoprotective, antihypertensive, anti-hyperlipidemic, immuno-modulatory, anticancer, anti-inflammatory and antioxidant activities. The fruiting bodies and fermented products of *Niuchangchih* have been reported to exhibit activity when treating liver diseases, such as preventing ethanol-, CCl_4- and cytokine-induced liver injury, inhibiting the hepatitis B virus, ameliorating fatty liver and liver fibrosis, and inhibiting liver cancer cells. This review will address the protective effects of *Niuchangchih* on the pathological development of liver diseases, and the underlying mechanisms of action are also discussed.

Contents

* Corresponding author. Tel.: +86 510 85918206; fax: +86 510 85918206.
E-mail address: zhenghxu@jiangnan.edu.cn (Z.-H. Xu).

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1. Introduction

The liver is the most important organ in terms of biochemical activity in the human body. The liver has great capacity to detoxify and synthesize useful substances, and therefore, damage to the liver inflicted by hepatotoxic agents has grave consequences (Achliya et al., 2004). Many risk factors, including hepatic viruses, alcohol consumption and chemical agents, have significant impact on the etiologies of liver diseases. Environmental pollution, bad dietary habits, and hepatic viruses have been considered to be the main factors that cause liver diseases (Day and Yeaman, 1994; Szabo, 2003). There are several characteristic pathologies in the livers of patients with liver disease, including fatty liver, hepatitis, liver fibrosis, hepatocirrhosis and liver cancer. Liver fibrosis is the common end stage of most chronic liver diseases regardless of the etiology (Bataller and Brenner, 2005), and its progression leads to liver cirrhosis and liver cancer. Currently, it is believed that the early stage of liver fibrosis can be reversed, while liver cirrhosis cannot. Therefore, preventing and eliminating the bad factors, and ameliorating fatty liver and liver fibrosis, are the most effective methods to prevent the liver from ultimately deteriorating (Freidman, 1993; Brenner et al., 2000). Much progress has been made in the understanding of the pathogenesis of liver diseases, resulting in improved prevention and therapy with promising prospects for even more effective treatments. In view of the severe undesirable side effects of synthetic agents, there is a growing focus on following systematic research methodology and evaluating the scientific basis of traditional herbal medicines that claim to possess hepatoprotective activity (Shahani, 1999; Achliya et al., 2004).

Niuchangchih, also named *Antrodia camphorata* (M. Zang & C.H. Su) Sheng H. Wu, Ryvarden & T.T. Chang, is a fungus that only grows on the brown heartwood of *Cinnamomum kanehirae* Hayata (Lauraceae) in Taiwan (Fig. 1) (Wu et al., 1997). *Niuchangchih* is also called "Niu-chang-ku", or "Chang-chih", in China. "Niu-chang" is the Chinese common name for *Cinnamomum kanehirae* (Bull camphor tree); "ku" in Chinese means mushroom; and "chih" means *Ganoderma*-like fungus. Being a local species, *Niuchangchih* was historically only used in Taiwan by the aborigines as a traditional prescription for the discomforts caused by alcohol drinking or exhaustion. The fruiting bodies of *Niuchangchih* are also used as a Chinese folk medicine for the treatment of liver diseases, food and drug intoxication, diarrhea, abdominal pain, hypertension, itchy skin and tumorigenic diseases (Tsai and Liaw, 1985; Chen et al., 2001a). However, in 1990, *Niuchangchih* was first reported as a new species. In the years since the initial report, *Niuchangchih* has received tremendous attention from the public. Primary investigations have revealed that *Niuchangchih* has extensive biological activities, such as hepatoprotective effects, anti-hepatitis B virus effects, anticancer activity, and antioxidant and anti-inflammation activities (Liu et al., 2007a; Rao et al., 2007).

Fig. 1. *Niuchangchih* fruiting bodies.

Niuchangchih grows at altitudes between 450 and 2000 m in the mountain ranges, and in the counties of Taoyuan, Miaoli, Nantou, Kaohsiung and Taitung. The trophophase of *Niuchangchih* occurs from June to October (Chen et al., 2001b). The fruiting bodies of *Niuchangchih* assume different shapes like the plate-type, the horse's hoof, or the tower shape, which is morphologically similar to *Antrodia salmonea* (vernacularly called Shiang-Shan-Chih), a brown heart rot basidiomata in the empty rotten trunk of *Cunninghamia konishii* Hayata (Lauraceae) (Chang and Chou, 2004). The red to light cinnamon fruiting bodies of *Niuchangchih* are bitter and have a mild camphor scent like the host woods (Chang and Chou, 1995). Chemical ingredients found in *Niuchangchih* include polysaccharides, triterpenoids, sesquiterpene lactones, steroids, phenol compounds, adenosine, cordycepin, ergosterol, etc. (Chang et al., 2005; Lu et al., 2006) The mycelia isolated from the fruiting bodies of *Niuchangchih* form orange red and orange brown to light cinnamon-colored colonies (Chang and Chou, 1995).

In the wild, the fruiting bodies of *Niuchangchih* grow slowly and are hardly noticeable until the host tree falls down. In order to harvest *Niuchangchih* more easily, some people illegally fall the host trees. This illegal felling has severely threatened *Cinnamomum kanehirae* (Chang et al., 2005), and the trees are currently protected by the Taiwan government. However, the wild fruiting bodies of *Niuchangchih* are in great demand and have been sold for about U.S. $15,000 per kg (Wang et al., 2005) due to the efficacies of this fungus. Thus, artificial cultivation was developed as a substitute. Currently, *Niuchangchih* is commercially available in Taiwan in the form of fermented wine or pure cultures in powdered, tablet and capsule form (Cheng et al., 2005b). The mycelia produced by liquid fermentation are innocuous (Lin et al., 2001).

In Chinese folk medicine, the fruiting bodies of *Niuchangchih* are considered to be a potent hepatoprotective remedy. The fruiting bodies and mycelia of *Niuchangchih* have been reported to exhibit the activities of preventing and ameliorating liver diseases, such as preventing ethanol- and CCl_4-induced liver injury, inhibiting the hepatitis B virus, ameliorating fatty liver and liver fibrosis, and inhibiting liver cancer cell growth. This review will address the protective effects of *Niuchangchih* on the pathological development of liver diseases. Most of the data presented here are from in vitro and animal studies, because the efficacy of *Niuchangchih* in preclinical liver diseases is not well documented.

2. Taxonomy

In past years, the taxonomy of *Niuchangchih* has been identified a few times, and several latin names have been suggested to stand as the correct name for the fungus. In 1990, *Niuchangchih* was first identified as a new *Ganoderma* species, *Ganoderma camphoratum*, due to their similar characteristics (Zang and Su, 1990). The generic name, however, was based on a mistake as the type was contaminated by spores of a *Ganoderma* species. Then, Chang and Chou (1995) described the species as *Antrodia cinnamomea* due to its dimitic hyphal system with clamped generative hyphae and ability to cause brown rot. After studying the both types of *Ganoderma comphoratum* and *Antrodia cinnamomea*, these fungi were found to be conspecific. Thus, a new combination, *Antrodia camphorata*, was thought to be more appropriate (Wu et al., 1997). However, given that the host tree of this latter species is *Cinnamomum kanehirae* rather than *Cinnamomum camphora* (L.) Presl., *Antrodia cinnamomea* was suggested again to stand as the correct name for the fungus associated with *Cinnamomum kanehirae* (Chang and Chou, 2004). In 2004, a phylogenetic analysis based on sequence data

derived from large ribosomal subunit (LSU) sequences of ribosomal RNA genes (rDNA) indicated that *Niuchangchih* is distantly related to other species in *Antrodia* and, consequently, the fungus was transferred to the new genus *Taiwanofungus* (Wu et al., 2004). However, using polymorphism analysis of internal transcribed spacer (ITS) regions of the ribosomal RNA gene, *Niuchangchih* was reconsidered as an *Antrodia* species (Chiu, 2007). The current taxonomic position of *Niuchangchih* is as follows (Hawksworth et al., 1995): Fungi, Basidiomycota, Homobasidiomycetes, Aphyllophorales, Polyporacea. Clearly, however, the nomenclature and exact taxonomy (genus and species) of *Niuchangchih* is still the subject of debate and needs further research. In this article, we have chosen to use the traditional name, *Niuchangchih*, to describe this unique Formosan fungus.

3. Ethnomedicine

Niuchangchih has a long history of medicinal use in Taiwan. It had been popularly used as a folkloric medicine long before 1773 (Su, 2002) for the treatment of twisted tendons and muscle damage, terrified mental state, influenza, cold, headache, fever and many internally affiliated diseases (Peng et al., 2007). In 1773, a famous doctor in the Traditional Chinese Medicine arena named Wu-Sha, who re-located to Taiwan from the Fujian province of China, found that Taiwan aborigines had discomfort caused by excess alcohol or exhaustion because of lifestyle (Su, 2002). The locals often chewed the fruiting bodies of *Niuchangchih* or took the decoction of the fruiting bodies, and noticed that this mushroom worked well for alcoholic hangover relief. Dr. Wu studied the effects of *Niuchangchih* based on the locals' experiences, and began to use it to treat diarrhea, abdominal pain, hypertension, itchy skin, viral infection, stomachitis, diabetes mellitus, nephritis, proteinuria, liver cirrhosis, hepatoma, influenza, car sickness, calenture and motion-sickness (Tsai and Liaw, 1985; Chiu and Zhang, 2001; Su, 2002; Chen, 2008). After being used for years, the mushroom is now believed to be one of the most potent liver-protecting herbs in Taiwan. However, little primary ethnomedical data describing its liver-protecting activity was recorded in the ancient literature. Recently, many studies have indicated that its medicinal applications go far beyond the original usage. It has been reported that many chemical components of *Niuchangchih* have functional properties like antioxidant, anticancer, antiviral, and antibiotic properties. Therefore, demand for the fruiting bodies of *Niuchangchih* has far exceeded the supply, and it is now considered among the most expensive herbal medicines on the market.

4. Chemical constituents

A series of publications have appeared on the structural characterization of the secondary metabolites of the fungus. Most of the investigators studied the fruiting bodies, though there are a few publications on the constituents of the mycelia of *Niuchangchih* in submerged cultures. The compounds identified from *Niuchangchih* are predominantly polysaccharides (Chen et al., 2005; Lin and Chen, 2007; Wu et al., 2007c), benzenoids, diterpenes, triterpenoids, steroids and maleic/succinic acid derivatives. These are summarized, with their structures and bioactivities, in Table 1.

5. Pharmacological studies

Several researchers have reported on the different biological activities of *Niuchangchih* in various in vitro and in vivo test models. As summarized in Table 2, different extracts and compounds of this species have been found to exhibit hepatoprotective, neuroprotective, antihypertensive, anti-hyperlipidemic, anti-genotoxic, anti-angiogenic, antimicrobial, depigment, immuno-modulatory, anticancer, anti-inflammatory, and antioxidant activities. The protective effects of *Niuchangchih* on the pathological development of liver diseases will be described in greater detail in the following sections.

5.1. Hepatoprotective activity of Niuchangchih

5.1.1. Effect on ethanol-induced acute liver injury

Bibulosity is one of the 10 factors leading to illness and death in the world. In western countries, the rate of death induced by alcohol toxicosis is similar to cancer and coronary heart disease. The major organ which metabolizes alcohol is the liver (Domschke et al., 1974). The liver disease induced by drinking is the alcohol liver diseases (ALD), which remains one of the most common causes of chronic liver diseases in the world. Alcohol consumption produces a spectrum of histologic abnormalities in the liver, including steatosis (fatty liver), steatohepatitis (alcoholic hepatitis) and cirrhosis. The three lesions of liver may occur alone, at the same time or sequentially (Day and Yeaman, 1994). Except abstinence and the treatment of the illness, there is a lack of effective drugs to cure alcohol addiction and interrupt the course of ALD.

There are three main pathways for the metabolism of ethanol, each located in a different subcellular compartment: the alcohol dehydrogenase (ADH) pathway in the cytosol, the microsomal ethanol-oxidizing system (MEOS) located in the endoplasmic reticula and catalase in the peroxisomes (Jiménéz-López et al., 2002). Among these pathways, the alcohol dehydrogenase pathway is the major metabolic pathway during the early stage of chronic alcohol liver injury. In the ADH-mediated oxidation of ethanol, hydrogen is transferred from the substrate to the cofactor nicotinamide adenine dinucleotide (NAD), resulting in excess conversion to its reduced form (NADH) with the production of acetaldehyde (Cronholm, 1985; Cronholm et al., 1988; French, 2000). The excess production of NADH alters the redox state in the liver and, in turn, leads to a variety of metabolic abnormalities. The elevated ratio of NADH to NAD increases the concentration of α-glycerophosphate and suppresses the citric acid cycle, which favors accumulation of hepatic triacylglycerols by trapping fatty acids.

Niuchangchih has been used to cure the discomfort caused by excess alcohol for many years. Recently, scientific research showed that both the fruiting bodies and mycelia of *Niuchangchih* possessed protective activity against liver hepatitis and fatty liver induced by acute hepatotoxicity of alcohol (Dai et al., 2003). Using acute ethanol-intoxicated rats as an experimental model, we compared the hepatoprotective effects of *Niuchangchih* and Liangjun (*Armillariella tabescens*), a traditional Chinese fungi drug for liver diseases, on liver injury induced by ethanol (Lu et al., 2007b). Treatment with *Niuchangchih* notably prevented the ethanol-induced elevation of levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin to an extent that was comparable to the standard drug silymarin. Meanwhile, the results from histological studies also supported the above parameters. Currently, the isolation and testing of constituents likely to be responsible for the hepatoprotective activities of *Niuchangchih* against alcoholic liver diseases is under investigation in our lab.

5.1.2. Effect on hepatitis induced by CCl_4

CCl_4-treated animals are frequently used to evaluate the hepatoprotective effect of tested samples. It is well established that hepatotoxicity by CCl_4 is due to enzymatic activation that releases CCl_3 radicals in the free state, which in turn disrupts the structure

Table 1
Compounds isolated from *Niuchangchih*.

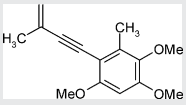
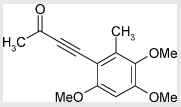
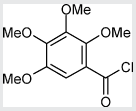
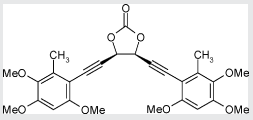
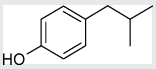
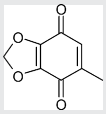
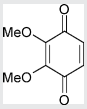
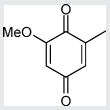
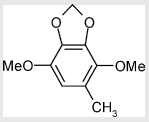
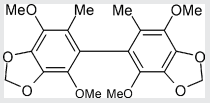
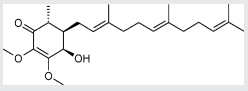
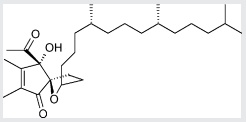
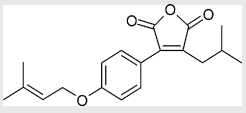
No.	Chemical class	Compound name	Compound structure	Sources	Bioactivity	References
1	Fatty acids	Methyl oleate		F		Wu and Chiang (1995)
2	Benzenoids	Antrocamphin A		F	Anti-inflammatory activity, in vitro	Chen et al. (2007b)
3	Benzenoids	Antrocamphin B		F		Chen et al. (2007b)
4	Benzenoids	2,3,4,5-Tetramethoxybenzoyl chloride		F		Chen et al. (2007b)
5	Benzenoids	Antrodioxolanone		F		Chen et al. (2007b)
6	Benzenoids	Isobutylphenol		M		Wu et al. (2007b)
7	Benzoquinone	5-Methyl-benzo[1,3]dioxole-4,7-dione		M		Wu et al. (2007b)
8	Benzoquinone derivative	2,3-Dimethoxy-5-methyl[1,4]benzoquinone		M		Wu et al. (2007b)
9	Benzoquinone derivative	2-Methoxy-5-methyl[1,4]benzoquinone		M	Antioxidant, in vitro	Wu et al. (2007b)
10	Phenyl methanoids	4,7-Dimethoxy-5-methyl-1,3-benzodioxole		F		Chiang et al. (1995), Chen et al. (2007b)
11	Biphenyl methanoids	2,2′,5,5′-Tetramethoxy-3,4,3′,4′-dimethylenedioxy-6,6′-dimethyl biphenyl		F	Anti-HBV, in vitro	Chiang et al. (1995), Shen et al. (2003a), Chen et al. (2007b)
12	Ubiquinone derivatives	Antroquinonol		M, F	Antitumor, in vitro; anti-HBV, in vitro	Lee et al. (2007), Liu et al. (2008)
13	Tocopherols	α-Tocospiro B		F		Chen et al. (2007b)
14	Maleic anhydrides	Camphorataanhydride A		m		Nakamura et al. (2004), Cheng et al. (2008a,b)

Table 1 (*Continued*)

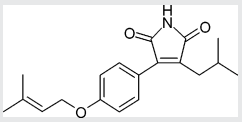
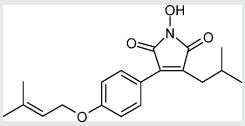
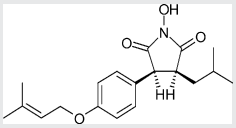
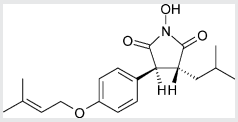
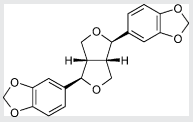
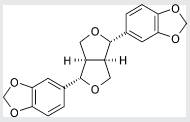
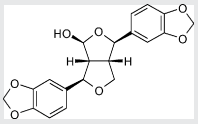
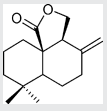
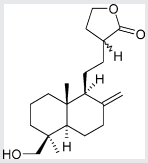
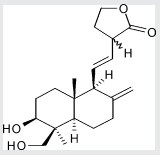
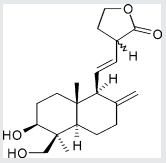
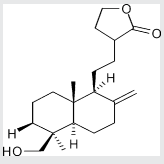
No.	Chemical class	Compound name	Compound structure	Sources	Bioactivity	References
15	Maleimides	Camphorataimide B		M, B	Anti-HBV, in vitro; cytotoxic effect, in vitro	Nakamura et al. (2004), Shen et al. (2005), Cheng et al. (2008a,b)
16	Maleimides	Camphorataimide C		M	Cytotoxic effect, in vitro	Nakamura et al. (2004), Cheng et al. (2008a,b)
17	Succinic acid derivatives	Camphorataimide D		M		Nakamura et al. (2004), Cheng et al. (2008a,b)
18	Succinic acid derivatives	Camphorataimide E		M		Nakamura et al. (2004), Cheng et al. (2008a,b)
19	Lignans	(+)-Sesamin		F		Wu and Chiang (1995)
20	Lignans	(−)-Sesamin		F		Chen et al. (2007b)
21	Lignans	4-Hydroxysesamin		F		Wu and Chiang (1995)
22	Sesquiterpene lactones	Antrocin		F		Chiang et al. (1995)
23	Diterpenes	19-Hydroxylabda-8(17)-en-16,15-olide		F		Chen et al. (2006)
24	Diterpenes	3β,19-Dihydroxylabda-8(17),11*E*-dien-16,15-olide		F		Chen et al. (2006)
25	Diterpenes	13-*epi*-3β,19-Dihydroxylabda-8(17),11*E*-dien-16,15-olide		F		Chen et al. (2006)
26	Diterpenes	19-Hydroxylabda-8(17),13-dien-16,15-olide		F		Chen et al. (2006)

Table 1 (*Continued*)

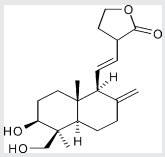
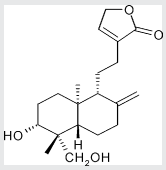
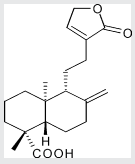
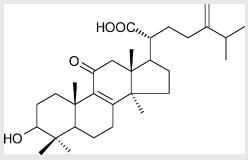
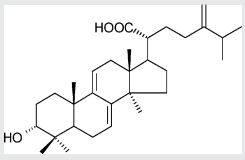
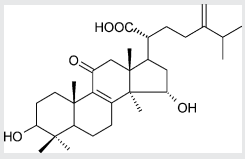
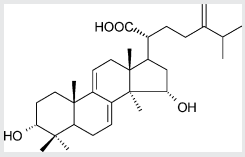
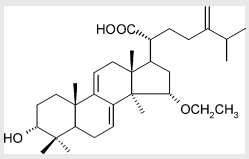
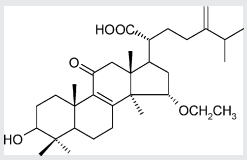
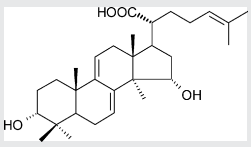
No.	Chemical class	Compound name	Compound structure	Sources	Bioactivity	References
27	Diterpenes	14-Deoxy-11,12-didehydroandrographolide		F		Chen et al. (2006)
28	Diterpenes	14-Deoxyandrographolide		F		Chen et al. (2006)
29	Diterpenes	Pinusolidic acid		F		Chen et al. (2006)
30	Triterpenoids	Eburicoic acid		F		Shen et al. (2003a)
31	Triterpenoids	Dehydroeburicoic acid		F	Anti-inflammatory activity, in vitro	Cherng et al. (1995), Yang et al. (1996), Shen et al. (2003a), Chen et al. (2007b)
32	Triterpenoids	Sulphurenic acid		F		Shen et al. (2003a)
33	Triterpenoids	Dehydrosulphurenic acid		F		Yang et al. (1996), Shen et al. (2003a)
34	Triterpenoids	15α-Acetyl-dehydrosulphurenic acid		F		Yang et al. (1996), Shen et al. (2003a)
35	Triterpenoids	Versisponic acid D		F		Shen et al. (2003a)
36	Triterpenoids	3β,15α-Dihydroxylanosta-7,9(11),24-trien-21-oic acid		F		Shen et al. (2003a)

Table 1 (*Continued*)

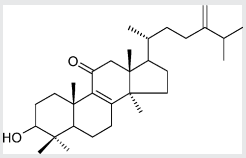
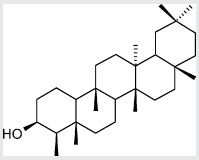
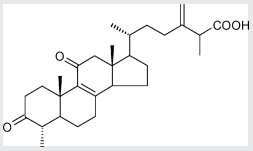
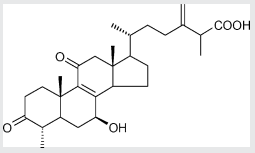
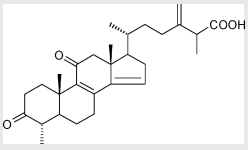
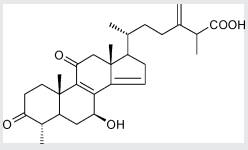
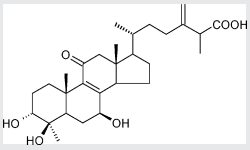
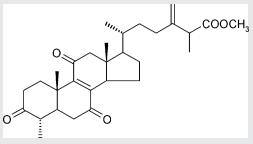
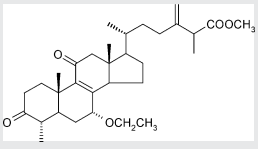
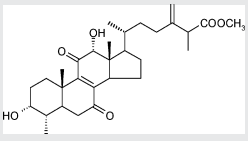
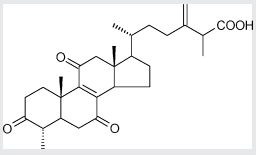
No.	Chemical class	Compound name	Compound structure	Sources	Bioactivity	References
37	Triterpenoids	24-Methylenedihyd-rolanosterol		F		Cherng et al. (1995)
38	Triterpenoids	*epi*-Friedelinol		F		Chen et al. (2007b)
39	Steroids	Antcin A		F	Anti-inflammatory activity, in vitro	Cherng et al. (1995), Chen et al. (2007b)
40	Steroids	Antcin C		F		Cherng et al. (1995)
41	Steroids	Antcin E		F		Cherng et al. (1996)
42	Steroids	Antcin F		F		Cherng et al. (1996)
43	Steroids	Antcin K		F		Shen et al. (2003a)
44	Steroids	Methyl antcinate B		F		Shen et al. (2003a)
45	Steroids	Methyl antcinate G		F		Cherng et al. (1996)
46	Steroids	Methyl antcinate H		F		Cherng et al. (1996), Shen et al. (2003a)
47	Steroids	Zhankuic acid A (Antcin B)		F	Cytotoxic effect, in vitro; anti-inflammatory activity, in vitro	Cherng et al. (1995), Chen et al. (1995, 2007b), Shen et al. (2003a)

Table 1 (*Continued*)

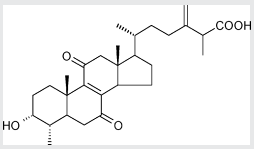
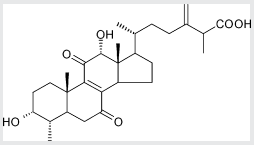
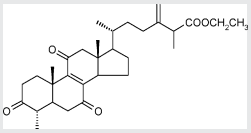
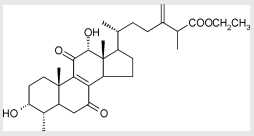
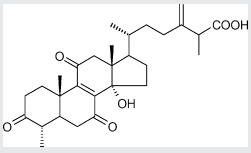
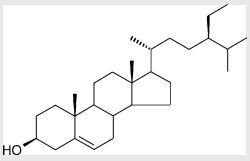
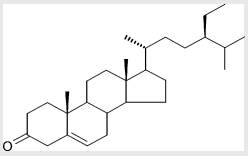
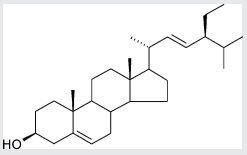
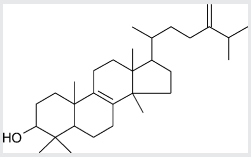
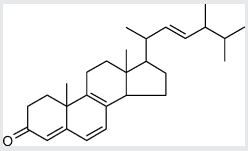
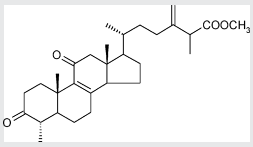
No.	Chemical class	Compound name	Compound structure	Sources	Bioactivity	References
48	Steroids	Zhankuic acid B		F	Anticholinergic and antiserotonergic activities, in vitro	Chen et al. (1995), Shen et al. (2003a)
49	Steroids	Zhankuic acid C (Antcin H)		F	Cytotoxic effect, in vitro	Chen et al. (1995), Shen et al. (2003a)
50	Steroids	Zhankuic acid D		F		Yang et al. (1996)
51	Steroids	Zhankuic acid E		F		Yang et al. (1996)
52	Steroids	Zhankuic acid F (Antcin D)		F		Shen et al. (1997), Cherng et al. (1996)
53	Steroids	β-Sitosterol		F		Wu and Chiang (1995), Chen et al. (2007b)
54	Steroids	β-Sitostenone		F		Chen et al. (2007b)
55	Steroids	Stigmasterol		F		Chen et al. (2007b)
56	Steroids	Eburicol		F		Wu and Chiang (1995), Chen et al. (2007b)
57	Steroids	Ergosta-4,6,8(14),22-tetraen-3-one		F		Chen et al. (2007b)

Table 1 (*Continued*)

No.	Chemical class	Compound name	Compound structure	Sources	Bioactivity	References
58	Steroids	Methyl-3,11-dioxo-4α-methylergost-8,24(28)-dien-26-oate		F		Wu and Chiang (1995)

Note: F: fruiting bodies; M: mycelia; B: culture broth; HBV: hepatic B virus

and function of lipids and protein macromolecules in the membrane of the cell organelles (Mujumdar et al., 1998).

The dry matter of fermented filtrate (DMF) from submerged cultures of *Niuchangchih* and aqueous extracts from *Niuchangchih* fruiting bodies have been reported to possess hepatoprotective activity against liver diseases induced by CCl_4 (Hsiao et al., 2003; Song and Yen, 2003). Both of them could reduce glutathione (GSH)-dependent enzymes (glutathione peroxidase, glutathione reductase, and glutathione S-transferase), and the GSH/GSSG ratio was significantly improved by the oral pretreatment of rats with DMF ($P < 0.01$). Histopathological evaluation of the rat liver revealed that the DMF reduced the incidence of liver lesions, including neutrophil infiltration, hydropic swelling and necrosis induced by CCl_4. Recently, Huang et al. (2006) reformulated the filtrate of *Niuchangchih* with extracts from some TCM, such as *Astragalus membranaceus*, as well as *Salvia miltiorrhiza* and *Lycimm chinense*, and found that the new formula showed significant inhibitory activity against the elevated ALT level in CCl_4-treated animals to an extent that was even better than when the filtrate was used alone.

5.1.3. Effect on Propionibacterium acnes- and lipopolysaccharide-induced hepatitis

The injection of *Propionibacterium acnes* (*P. acnes*) followed by a low dose of lipopolysaccharide (LPS) can induce fulminant hepatitis in mice, and this model has been used for the analysis of liver injury. Han et al. (2006) have separated and purified a neutral polysaccharide named *ACN2a* from the hot water extract of the mycelium of *Niuchangchih* that has hepatoprotective activity. The hepatoprotective effect of *ACN2a* was evaluated using the mouse model of liver injury that was induced by *Propionibacterium acnes*-LPS. The administration of *ACN2a* (0.4 or 0.8 g/(kg d)) significantly prevented an increase of the activities of AST and ALT in the serum of mice treated with *Propionibacterium acnes*-LPS, indicating hepatoprotective activity in vivo. Using the same cytokine-induced fulminant hepatitis animal model, Dr. Hattori isolated five maleic and succinic acid derivatives (Table 1; compound 14–18), namely Hepasim®, as the active compound(s) responsible for the anti-hepatitis activity of mycelia of *Niuchangchih* (Hattori and Sheu, 2006).

5.1.4. Possible mechanisms underlying the hepatoprotective effect of Niuchangchih

Currently available data show that *Niuchangchih* may exert its protective effects on liver injury though different mechanisms, such as scavenging free radicals responsible for cell damage, enhancing the enzymes responsible for antioxidant activity, inhibiting the inflammatory mediators and/or induction of the regeneration of the liver cells.

Chemical agents used in the in vivo research, including ethanol, CCl_4, and *Propionibacterium acnes*-LPS, not only induced liver injury, but also initiated oxidative stress and increased the level of reactive oxygen species (ROS), as manifested by an elevated level of MDA and an altered activity of SOD. Active oxygen molecules such as superoxide radicals ($O_2^{\bullet -}$) and hydroxyl radicals ($OH^{\bullet}$) have been shown to modify and damage proteins, carbohydrates, and DNA in both in vitro and in vivo models (Halliwell and Gutteridge, 1990). It is well known that free radicals derived from oxygen and other chemicals are important factors related to injury of the liver (Poli, 1993). Accumulating data have shown that *Niuchangchih* is a potent direct free radical scavenger (Huang et al., 1999; Song and Yen, 2002; Hsiao et al., 2003; Mau et al., 2004; Shu and Lung, 2008). It is therefore possible that prevention against liver injury by *Niuchangchih* is partially due to its antioxidant activity. The stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) tests provided direct evidence that the *Niuchangchih* extract acted as a direct free radical scavenger (Huang et al., 1999; Hsiao et al., 2003; Mau et al., 2004). The antioxidant activities of the filtrate and mycelia extracts of *Niuchangchih* were correlated with the presence of total polyphenols, crude triterpenoids, and the protein/polysaccharide ratio of the crude polysaccharides (Song and Yen, 2002). Aqueous extracts of *Niuchangchih* inhibited nonenzymatic iron-induced lipid peroxidation in rat brain homogenates with an IC_{50} value of about 3.1 mg/ml (Hsiao et al., 2003). These results suggest that *Niuchangchih* exerts effective protection against chemical-induced hepatic injury in vivo by free radical scavenging activities.

Although the precise underlying mechanisms are not fully understood, *Niuchangchih* may act as an enzyme modulator in a way that is unrelated to its antioxidant properties. The DMF may play a role in preventing oxidative damage in living systems by upregulating hepatic GSH-dependent enzymes to preserve the normal GSH/GSSH ratio during CCl_4 metabolism (Song and Yen, 2003). The DMF showed the strongest inhibition of lipid peroxidation as a function of its concentration, and its effects were comparable to the antioxidant activity of BHA at the same concentration of 0.2 mg/ml (Song and Yen, 2002). When Hep G_2 cells were pretreated with the DMF at a concentration of 0.1 mg/ml for 4 h and then induced by 1 h of treatment with H_2O_2 (100 μM), lipid peroxidation was significantly ($P < 0.05$) decreased as measured by the formation of malondialdehyde. In another study, different solvent extracts (water, ethanol or ethyl acetate) from the fermented filtrate of *Niuchangchih* could increase hepatic SOD activity and the expression of CYP 1A1 in rat liver (Chen, 2003).

Based on our own studies and those by other groups, we believe that there are multiple potent mechanisms underlying the hepatoprotective effects of *Niuchangchih*. Further studies to fully elucidate the exact mechanism of the effects modulated by *Niuchangchih* are necessary.

5.2. Inhibition of hepatitis B virus (HBV) replication

HBV infection is known to cause acute and chronic hepatitis. Some chronic hepatitis patients subsequently suffer from cirrhosis and liver failure, and some may eventually develop hepatocellular carcinoma (HCC) (Szmuness, 1978; Beasley et al., 1981). Viral hepatitis is the most common cause of HCC worldwide, followed by alcoholic liver diseases (Lodato et al., 2006). Despite the availability of an effective preventive vaccine in recent years, about 300 million existing chronic carriers still urgently need therapy.

Table 2
A summary of the studies conducted on the pharmacological activities of *Niuchangchih*.

Study pertaining	Sample preparation, dosage and route	Model, study design	Observations	References
Hepatoprotective effect	Aqueous extract of *Niuchangchih* (250, 750, and 1250 mg/kg per day, respectively, p.o., 4 days per week)	Male ICR mice, in vivo	*Niuchangchih* showed protection against chronic CCl_4-induced hepatic injury by mediating antioxidative and free radical scavenging activities	Hsiao et al. (2003)
	Dry matter of fermented filtrate (DMF) from *Niuchangchih* (0.25 and 0.50 g/kg, p.o., daily)	Male Sprague–Dawley rats, in vivo	DMF inhibited CCl_4-induced hepatotoxicity by upregulating hepatic GSH-dependent enzymes to preserve the normal GSH/GSSH ratio and free radical scavenging effect	Song and Yen (2003)
	Mycelia or fruiting bodies of *Niuchangchih* (0.5 and 1.0 g/kg, p.o., daily)	Male Sprague-Dawley rats, in vivo	*Niuchangchih* showed protective effect on acute ethanol-induced liver injury due to its potent antioxidant ability	Dai et al. (2003)
	Mycelia of *Niuchangchih* (0.5 and 1.0 g/kg, i.g., daily)	Male Sprague-Dawley rats, in vivo	*Niuchangchih* prevented ethanol-induced elevation of serum levels of AST, ALT, ALP, and bilirubin comparable with silymarin	Lu et al. (2007b)
	Neutral polysaccharide isolated from the mycelia of *Niuchangchih* (0.4 and 0.8 g/kg, p.o., daily)	Male ICR mice, in vivo	*Niuchangchih* significantly prevented increases in AST and ALT enzyme activities in mice treated with *Propionibacterium acnes* and Lipopolysaccharide	Han et al. (2006)
	Filtrate of fermented mycelia from *Niuchangchih* (0.5 and 1.0 g/kg, p.o., daily)	Male Wistar rats, in vivo	*Niuchangchih* retarded the progression of liver fibrosis in CCl_4-treated rats possibly by scavenging free radicals formed in the liver	Lin et al. (2006)
	Fermented filtrate of *Niuchangchih*	CCl_4-induced hepatic injury in mouse, in vivo	Hepatoprotective effect of *Niuchangchih* on CCl_4-treated mouse was enhanced by extracts from Chinese traditional medicines, such as *Astragalus membranaceus*, *Salvia miltiorrhiza*, *Lycimm chinense*	Huang et al. (2006)
Anti-HBV activity	Polysaccharides from mycelia of five *Niuchangchih* strains (50 μg/ml)	HBV-producing cell line MS-G2, in vitro	All mycelia polysaccharide preparations exhibited anti-hepatitis B virus activity	Lee et al. (2002)
	Ten compounds isolated from fruiting bodies of *Niuchangchih* (5–50 μM)	Wild-type HBV producing cell line ES2, lamivudine-resistant HBV DNA integrated HCC cell line M33, in vitro	Bioassay-guided fractionation resulted in the isolation of an anti-HBV biphenyl	Huang et al. (2003)
	Culture broth of *Niuchangchih* (5–50 μM)	HBV-producing cell line MS-G2, in vitro	Bioassay-guided fractionation resulted in the isolation of an anti-HBV pyrroledione	Shen et al. (2005)
Neuroprotective effect	Extract of *Niuchangchih* mycelia (1–1000 μg/ml)	Neuronal-like rat PC12 cells, in vitro	Serum deprivation-induced PC12 cell apoptosis was prevented	Huang et al. (2005)
	Adenosine from the extract of *Niuchangchih* mycelia (1–1000 μg/ml)	Neuronal-like rat PC12 cells, in vitro	Adenosine prevented rat PC12 cells from serum deprivation-induced apoptosis through the activation of adenosine A2A receptors	Lu et al. (2006)
	Extract of *Niuchangchih* mycelia (1–1000 μg/ml)	Neuronal-like rat PC12 cells, in vitro	*Niuchangchih* prevented serum-deprived PC12 cell apoptosis through a PKA-dependent pathway and by suppression of JNK and p38 activities	Lu et al. (2008)
Antihypertensive effect	Mycelia extracts from five *Niuchangchih* strains (80–400 μg/ml)	Isolated aortic rings from Sprague-Dawley rats, in vitro	*Niuchangchih* induced an elevation in $[Ca^{2+}]_i$ mainly due to Ca^{2+} influx in endothelial cells, increased NO release and activated cGMP system activities which caused endothelium-dependent relaxation	Wang et al. (2003)

Table 2 (*Continued*)

Study pertaining	Sample preparation, dosage and route	Model, study design	Observations	References
	Methanolic extract from wild and solid-state cultures of *Niuchangchih* (10 mg/kg)	Spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats, in vivo	The systolic blood pressure and diastolic blood pressure of SHR were lowered, but not of WKY rats	Liu et al. (2007b)
Anti-hyperlipidemic effect	Fruiting bodies of *Niuchangchih*	Sprague-Dawley rats, in vivo	*Niuchangchih* decreased the triglyceride and glucose levels, but could not affect the cholesterol level in rat fed with high cholesterol diet	Yen (2006)
Anti-genotoxic activity	Aqueous extracts from *Niuchangchih* mycelia (3 g/kg)	Human TK6 cells, in vitro; pregnant mice, in vivo	*Niuchangchih* possess the chemopreventive effects with no observable cytotoxicity in male mice, pregnant mice and their fetuses	Chiang et al. (2004)
Anti-angiogenic activity	Polysaccharide from mycelia of *Niuchangchih* (1 μg/ml)	Bovine aortic endothelial cells, in vitro	Polysaccharides inhibited cyclin D1 expression through inhibition of VEGF receptor signaling, leading to the suppression of angiogenesis	Cheng et al. (2005a)
Antimicrobial activity	Water and 95% ethanolic extract of mycelia of *Niuchangchih* in solid-state culture (5–10 mg)	*Bacillus cereus*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Lactobacillus lactis*, *Staphylococcus aureus*, *Staphylococcus faecalis*, *Enterobacter aerogenes*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *Shigella sonnei*, in vitro	Both water and ethanolic extract of *Niuchangchih* mycelia showed antimicrobial activity	Wu and Liang (2005)
Depigment effect	Water and ethanol extracts of *Niuchangchih* after fermented with *Scutellaria baicalensis*	B16-F10 cell, in vitro	Both water and ethanol extracts could protect cell from UV damage. However, ethanol extract had distinguished inhibition on tyrosinase in the cell after cell irradiation, but water extract did not	Chen (2007)
Immuno-modulatory activity	Compounds from fruiting bodies of *Niuchangchih* (50 μM)	Peripheral human neutrophils (PMN) and mononuclear cells (MNC), in vitro	Compounds from *Niuchangchih* exhibited leukocyte modulating activity by inhibiting ROS production in human PMN and MNC with no significant cytotoxic effect	Shen et al. (2003b)
	Polysaccharide from *Niuchangchih* mycelia (2.5 mg/d, i.g., 6 weeks)	Male BALB/c mice, *Schistosoma mansoni*, in vivo	*Niuchangchih* modulated the expression of CD8α and the MHC class II molecule I-A/I-E in splenic DCs and macrophages, the $CD4^+/CD8^+$ ratio of T cells and the number of B lymphocytes, also inhibited *Schistosoma mansoni* infection in vivo	Chen et al. (2008)
	Polysaccharide from *Niuchangchih* mycelia (1.0 and 2.5 mg/d, i.g., 6 weeks)	Male BALB/c or C57BL/6 mice, T1/T2 doubly transgenic mice, *Schistosoma mansoni*, in vivo	*Niuchangchih* modulated the expression of Type 1 cytokines on splenocytes as well as the percentages of $CD4^+$ T cells and B lymphocytes, induced these immune cells towards Type 1 differentiation, reduced the infection rate of *Schistosoma mansoni* in vivo	Cheng et al. (2008a,b)
Anticancer activity	Ethylacetate extract from fruiting bodies of *Niuchangchih* (10–100 μg/ml)	Human liver cancer cell lines Hep G2 and PLC/PRF/5, in vitro	Mechanisms of anticancer activity of *Niuchangchih* were involved to (1) induction of apoptosis, (2) initiation of Fas/Fas ligand pathway in Hep G2 cells, (3) trigger of mitochondrial pathway, (4) modulation of Bcl-2 family protein and (5) inhibition of NF-κB signaling pathway	Hsu et al. (2005)

Table 2 (*Continued*)

Study pertaining	Sample preparation, dosage and route	Model, study design	Observations	References
	Methanolic extracts of mycelia (MEM) from *Niuchangchih* (10–200 μg/ml)	Human hepatoma HepG2 cells, in vitro	MEM induced HepG2 apoptosis through inhibition of cell growth and up-regulation of Fas/FasL to activate the pathway of caspase-3 and -8 cascades	Song et al. (2005a)
	Methanolic extracts of mycelia (MEM) from *Niuchangchih* (10–50 μg/ml)	Chang liver cells, HepG2 and Hep3B cells, primary hepatocytes isolated from Sprague-Dawley rats, in vitro	MEM-induced apoptosis pathway in hepatoma cells were through activation of caspase-8 and -3 cascades and regulation of the cell cycle progression	Song et al. (2005b)
	Ethylacetate extract from fruiting bodies of *Niuchangchih* (30–120 μg/ml)	Human liver cancer cell line Hep 3B, in vitro	*Niuchangchih* induced apoptosis of Hep 3B cells through calcium and calpain-dependent pathways	Kuo et al. (2006)
	Ethylacetate extract from fruiting bodies of *Niuchangchih* (10–40 μg/ml)	Human liver cancer cell line PLC/PRF/5, in vitro	*Niuchangchih* inhibited invasion of cancer cells by down-regulation of invasion-related factors through NF-κB inhibition	Hsu et al. (2007)
	Extracts from *Niuchangchih* mycelia in liquid/solid-state culture	Human hepatoma cell lines C3A and PLC/PRF/5, in vitro; ICR nude mice inoculated with C3A or PLC/PRF/5, in vivo	*Niuchangchih* extract, when combined with anti-tumor agents, showed adjuvant antiproliferative effects on hepatoma cells (in vitro) and on xenografted cells in tumor-implanted nude mice (in vivo)	Chang et al. (2008)
	Fermented culture broth of *Niuchangchih* (25–150 μg/ml)	Human breast cancer cell line MCF-7, human healthy breast cell line HBL100, in vitro	*Niuchangchih* exhibited antiproliferative effect by induction of apoptosis that is associated with cytochrome c translocation, caspase 3 activation, PARP degradation, and dysregulation of Bcl-2 and Bax in MCF-7 cells	Yang et al. (2006a)
	Fermented culture broth of *Niuchangchih* (40–240 g/ml)	Human breast cancer cell line MDA-MB-231 and the MCF-7, in vitro	*Niuchangchih* exhibited antiproliferative effect by induction of apoptosis that is associated with COX-2 inhibition in MDA-MB-231 cells	Hseu et al. (2007)
	Fermented culture broth of *Niuchangchih* (40–240 g/ml)	Human breast cancer cells MDA-MB-231, in vitro; Nude mice inoculated with MDA-MB-231 cells, in vivo	*Niuchangchih* treatment induced cell cycle arrest and apoptosis of human breast cancer cells both in vitro and in vivo	Hseu et al. (2008a)
	Extract from fruiting bodies of *Niuchangchih* (10–150 μg/ml)	Human urinary bladder cancer cell line T24, in vitro	*Niuchangchih* was effective in inducing phase G_2M arrest, acting as an anti-proliferative, and an anti-metastatic agent against bladder cancer cell T24 cells	Peng et al. (2006)
	Extract from fruiting bodies of *Niuchangchih* (10–200 μg/ml)	Human urinary bladder cancer cell lines, RT4, T24 (HTB-4) and TSGH-8301, in vitro	*Niuchangchih* extract showed different significant inhibitory effects on the growth and proliferation of TCC cell lines through different mechanisms	Peng et al. (2007)
	Fermented culture broth of *Niuchangchih* (25–150 μg/ml)	Human premyelocytic leukemia HL-60, in vitro	*Niuchangchih* exerted antiproliferative action and growth inhibition through apoptosis induction	Hseu et al. (2004)
	Polysaccharide from *Niuchangchih* mycelia in submerged culture (200 μg/ml, in vitro; 100 and 200 mg/kg, in vivo)	Human leukemic U937 cells, in vitro; sarcoma 180-bearing mice, in vivo	Polysaccharides in *Niuchangchih* possessed antitumor effect through the activation of host immune response	Liu et al. (2004)
	Mycelia powder of *Niuchangchih* in submerged culture (100–200 μg/ml)	Human MG63 osteosarcoma cells, in vitro	*Niuchangchih* exerted multiple effects on viability and $[Ca^{2+}]_i$, and evoked apoptosis via inhibiting ERK and MAPK phosphorylation	Lu et al. (2007a)

Table 2 (*Continued*)

Study pertaining	Sample preparation, dosage and route	Model, study design	Observations	References
	Ethanolic extract of fruiting bodies of *Niuchangchih* (50–200 μg/ml)	Human prostate adenocarcinoma cell lines LNCaP and PC-3, in vitro	*Niuchangchih* showed anti-cancer activity against both PC-3 and LNCaP cells by modulating cell cycle regulatory proteins through different signaling pathways	Chen et al. (2007c)
	Ethanolic extract from mycelia of *Niuchangchih* in solid-state culture (0.2–2%)	Human non-small cell lung carcinoma A549 cell and primary human fetal lung fibroblast MRC-5, in vitro	Several tumor-associated genes that were changed in their expression by *Niuchangchih* were located, including human calpain small subunit, galectin-1, eukaryotic translation initiation factor 5A, annexin V and Rho GDP dissociation inhibitor α	Wu et al. (2006)
Anti-inflammatory activity	Methanolic extract from cultured mycelia of *Niuchangchih* (1–50 μg/ml)	Peripheral human neutrophils (PMN) or mononuclear cells (MNC), in vitro	The extracellular ROS production in PMN or MNC were prevented	Shen et al. (2004a)
	Zhankuic acids A, B, C, and antcin K from fruiting bodies of *Niuchangchih* (1–25 μM)	Peripheral human neutrophils, in vitro	Zhankuic acids and antcin K exhibited leukocytes modulating activity by inhibiting ROS production and firm adhesion by neutrophils with no significant cytotoxic effect	Shen et al. (2004b)
	Fermented culture broth of *Niuchangchih* (25–100 μg/ml)	Murine macrophage cell line RAW264.7, in vitro	*Niuchangchih* inhibited the production of cytokines and the degradation of IκB-α in LPS-stimulated macrophages, by down-regulation of iNOS and COX-2 expression via the suppression of NF-κB activation	Hseu et al. (2005)
	Lipopolysaccharide from mycelia of *Niuchangchih* (0.1–50 μg/ml)	Bovine aortic endothelial cells, in vitro	Bacterial LPS-induced intercellular adhersion molecule-1 and monocyte adhesion were reversed	Cheng et al. (2005b)
	Polysaccharide fractions from *Niuchangchih* mycelia (50–200 μg/ml)	Murine macrophage cell line RAW 264.7, in vitro	The lipopolysaccharide-induced NO production and the protein expression by the iNOS gene were inhibited	Chen et al. (2007a)
	Methanolic extracts from wild fruiting body, liquid-state fermentation, and solid-state culture of *Niuchangchih* (50 μg/ml)	Mouse microglia cell line EOC13.31, Female Balb/c mice, in vitro	*Niuchangchih* significantly inhibited iNOS, COX-2, and TNF-α expression in LPS/IFNγ- or β-amyloid-activated microglia	Liu et al. (2007a)
	$CHCl_3$ and methanolic extracts from fruiting bodies of *Niuchangchih* (3.125–50 μg/ml)	Mouse peritoneal excluded macrophages, in vitro	Anti-inflammatory effect of *Niuchangchih* is due to the inhibition of macrophage-mediated inflammatory mediators (NO, TNF-α and IL-12) and cell cycle arrest in G0/G1 phase in LPS/IFN-γ activated mouse peritoneal macrophages	Rao et al. (2007)
	Compounds isolated from fruiting bodies of *Niuchangchih* (100 μmol/L)	Neutrophils isolated from the venous blood of consenting healthy volunteers	Antrocamphin A, antcin A and antcin B exhibited potent inhibition against fMLP-induced superoxide production with IC_{50} values less than 10 μM	Chen et al. (2007b)
	Fractionated polysaccharides from mycelia powder of *Niuchangchih* (50–200 μg/ml)	Murine macrophage cell line RAW 264.7, in vitro	*Niuchangchih* inhibited the production of IL-6, IL-10, MPC-5, RANTES, and NO in LPS-stimulated mouse macrophages, by transcriptional down regulation of IL-6, IL-10, and iNOS genes	Wu et al. (2007c)
Antioxidant effect	Fresh and air-dried fruiting bodies and air-dried mycelia of *Niuchangchih* (2.5–10 mg/ml)	Antioxidant activity, reducing power, DPPH scavenging effect, chelating activity, in vitro	*Niuchangchih* acted as antioxidant and oxygen scavenger	Huang et al. (1999)

Table 2 (*Continued*)

Study pertaining	Sample preparation, dosage and route	Model, study design	Observations	References
	Dry matter of cultural medium, filtrate, and different solvent extracts of mycelia from *Niuchangchih* in submerged culture (0.2 mg/ml)	Antioxidant activities and free radical scavenging effects, in vitro	Dry matter of filtrate of *Niuchangchih* showed excellent antioxidant activities in different model systems	Song and Yen (2002)
	Aqueous extract from *Niuchangchih* mycelia (12.5–50 μl)	Normal human erythrocytes, HL-60 leukemic cells, in vitro	*Niuchangchih* reduced AAPH-induced erythrocyte hemolysis, lipid/protein peroxidation, and cell damage	Hseu et al. (2002)
	Methanolic extracts from red or white mycelia of *Niuchangchih* (0.5–10 mg/ml)	Antioxidant activity, reducing power, scavenging abilities on DPPH and hydroxyl radicals and chelating ability, in vitro	Antioxidants contents in mycelia were found in the order of total phenols > tocopherols > ascorbic acid > β-carotene. The antioxidant properties were good for mycelia at days 10–16	Mau et al. (2003)
	Methanolic extracts from red or white mycelia of *Niuchangchih* (0.5–10 mg/ml)	Antioxidant activity, reducing power, scavenging abilities on DPPH and hydroxyl radicals and chelating ability, in vitro	Both mycelia extracts showed potent antioxidant activities	Mau et al. (2004)
	Fermented culture broth of *Niuchangchih* (25–100 μg/ml) and aqueous extracts of mycelia from *Niuchangchih* (50–200 μg/ml)	Human low-density lipoproteins (LDL), human umbilical vein endothelial cells, in vitro	$CuSO_4$- or AAPH-induced oxidative modification of LDL was reduced; The endothelial cells were protected from the damaging effects of the $CuSO_4$-oxidized LDL	Yang et al. (2006b)
	Methanolic extract of mycelia of *Niuchangchih* irradiated with γ-rays (0.5–10 mg/ml)	Antioxidant activity, reducing power, scavenging abilities on DPPH and hydroxyl radicals and chelating ability, in vitro	γ-Irradiation enhanced the antioxidant properties of *Niuchangchih* mycelia	Huang and Mau (2007)
	Water-soluble polysaccharides from *Niuchangchih* in submerged culture (200 μg/ml)	Hydrogen peroxide-induced cytotoxicity and DNA damage in Chang liver cells, in vitro	Polysaccharides in *Niuchangchih* showed antioxidant properties by up-regulation of GST activity, maintenance of normal GSH/GSSG ratio, and scavenging of ROS	Tsai et al. (2007)
	Fermented culture broth of *Niuchangchih* (25–100 μg/ml) and aqueous extracts of mycelia from *Niuchangchih* (50–200 μg/ml)	Human umbilical vein endothelial cells, in vitro	The oxidative cell damage induced by AAPH was reduced, as evidence by reduced DNA fragmentation, cytochrome c release, caspase-3 activation, and dysregulation of Bcl-2 and Bax	Hseu et al. (2008b)

HBV is known to consist of 42-nm Dane particles and 22-nm subviral particles (containing both spherical and filamentous shells), both having the surface envelope. Serological markers are used routinely as diagnostic and prognostic indicators of acute and chronic HBV infections. The presence of hepatitis B surface antigen (HBsAg) is the most common marker of HBV infection, whereas hepatitis B e antigen (HBeAg) is used as an ancillary marker, primarily to indicate active HBV replication and associated progressive liver disease (Chisari, 2000).

Currently available data show that both fruiting bodies and liquid fermentation products (include mycelia and culture) of *Niuchangchih* possess anti-HBV activity. Polysaccharides, biphenyl, malaeimide and cyclohexenone are considered to be the bioactive components responsible for the anti-HBV activity of *Niuchangchih*.

It was reported that extracts from the mycelia of *Niuchangchih* have high inhibitory activities of HBV in a dose-dependent manner and show no cytotoxicity. In vivo HBV tests showed that mycelia of *Niuchangchih* had anti-HBV function and healing abilities for hepatitis (Chen et al., 2003). In another study, the anti-HBV effects of the polysaccharides from cultured mycelia of five *Niuchangchih* strains were evaluated in vitro. At a dosage of 50 μg/ml, polysaccharides from strain B86 showed the highest level of anti-HBsAg activity, which was higher than α-interferon at a dosage of 1000 U/ml. Furthermore, none of the polysaccharides exhibited cytotoxic effects (Lee et al., 2002).

Huang et al. also found that the ethanol extract of *Niuchangchih* displayed anti-HBV effects on both wild-type and lamivudine-resistant HBV mutants (Huang et al., 2003). Therefore, Huang's group further investigated the anti-viral activities of 10 pure compounds (Shen et al., 2003a) isolated from *Niuchangchih*, which included one biphenyl, four ergostane- and five lanostane derivatives. Among the 10 compounds, the biphenyl, namely 2,2′,5,5′-tetramethoxy-3,4,3′,4′-bis(methlylene-dioxy)-6,6′-dimethyl-biphenyl (Table 1; compound 11), was positively identified as the single active compound responsible for the anti-HBV effect of *Niuchangchih* on both wild-type and lamivudine-resistant HBV mutants. When compared to positive control interferon α-2a, the effect of compound 11 on wild-type HBV cell line ES2 was equal to that of interferon α-2a at a concentration of 1000 U/ml. When applied to lamivudine-resistant HBV cell line M33, the effect of compound 11 was less than that of α-2a at a concentration of 1000 U/ml, but equal to that of interferon α-2a at a concentration of 250 U/ml (Huang et al., 2003). More recently, bioassay-guided fractionation resulted in the isolation of an anti-HBV maleimide, namely Camphorataimide B (Table 1; compound 15), from the culture broth of *Niuchangchih*. Compound 15 suppressed both HBsAg and HBeAg expression with the

moderate inhibition percentages of 35.2 and 12.8%, respectively, at the non-cytotoxic concentration of 50 μM (Shen et al., 2005). Antroquinonol (Table 1; compound 12), a cyclohexenone isolated from mycelia and the fruiting bodies of *Niuchangchih*, was recently reported to be potent in inhibiting the synthesis of HBsAg and HBeAg to achieve the goal of HBV inhibition (Liu et al., 2008).

In conclusion, the ability of *Niuchangchih* to inhibit the replication of HBV in vivo and in vitro may be one additional reason for considering this fungus as a potential therapeutic for HBV infection. On the other hand, anti-HBV activity has not been reported for polysaccharides from any other mushroom (Lee et al., 2002). Thus, further studies on the relationship between specific polysaccharide fraction and their biological activities are also required.

5.3. Niuchangchih and liver fibrosis

Liver fibrosis is the common end stage of most chronic liver diseases, regardless of the etiology (Bataller and Brenner, 2005), and its progression leads to liver cirrhosis and liver cancer. Currently, it is regarded that the early phase of liver fibrosis can be reversed, while liver cirrhosis cannot. Thus, the key challenges in curing liver cirrhosis are how to diagnose the liver fibrosis at an early phase and developing new drugs against it (Freidman, 1993).

CCl_4-treated rats are frequently used as an experimental model to study liver fibrosis. Using this experimental model, Lin et al. (2006) have assayed the effectiveness of the filtrate of fermented mycelia from *Antrodia camphorata* (FMAC) in the preventive and curative treatment of liver fibrosis. Small fibrotic nodules were present in CCl_4-treated rats as evidenced from morphological analysis, and this was reversed when treated with FMAC. Post-treatment with FMAC to CCl_4-administered rats clearly accelerated the reversal of fibrosis and lowered the elevated mRNA levels of hepatic collagen I, transforming growth factor (TGF)-β1 and tissue inhibitors of matrix metalloproteinase (TIMP)-1.

Although the exact mechanisms of pathogenesis in liver cirrhosis are still obscure, the role of free radicals and lipid peroxides has attracted considerable attention (Gebhardt, 2002). It has been found that the metabolism of CCl_4 involves the production of free radicals through its activation by drug metabolizing enzymes located in the endoplasmic reticulum (Basu, 2003). CCl_4 is capable of causing liver lipid peroxidation, resulting in liver fibrosis (Comporti et al., 2005). Lin et al. (2006) confirmed that hepatic lipid peroxidation is increased during hepatic fibrogenesis. By treatment with FMAC, hepatic malondialdehyde (MDA) and hydroxyproline (HP) contents in curative groups were remarkably restored, indicating that *Niuchangchih* retards the progression of liver fibrosis, possibly by scavenging free radicals formed in the liver. Previous studies have reported that *Niuchangchih* plays a role in preventing oxidative damage in living systems by mediating the activities of hepatic antioxidative enzymes and scavenging free radicals formed during CCl_4 metabolism (Hsiao et al., 2003; Song and Yen, 2003). In another study, however, FMAC was effective in reversing liver fibrosis induced by dimethylnitrosamine (DMN), while the lowered activities of antioxidative enzymes (SOD, catalase and GSH-Px) in the liver were not restored by FMAC (Guo, 2002). Therefore, more in vivo studies and randomized controlled clinical studies should be performed to further elucidate the mechanisms of action of *Niuchangchih*.

5.4. Effect on liver cancer

Liver cancer is one of the most common malignancies in the world, and also one of the four most prevalent malignant diseases of adults in China, Korea and Sub-Sahara Africa (Marrero, 2006; Motola-Kuba et al., 2006). Ninety percent of liver cancers develop in the context of chronic liver diseases, and mainly in patients with cirrhosis. Although chemotherapeutic agents are the main approach for liver cancer, they are relatively ineffective. Accordingly, screening compounds for potential use as effective therapeutic agents for liver cancer is an important undertaking.

Both the fruiting bodies and mycelia of *Niuchangchih* have potent antiproliferative activity against liver cancer in vitro and in vivo. It was indicated that there were multiple potent mechanisms underlying the anticancerous effects of *Niuchangchih*.

Many studies have shown that the antiproliferative activity of *Niuchangchih* was related to cell cycle arrest and the induction of apoptosis. Song et al. (2005b) investigated the effect of the methanol extract of mycelia (MEM) from *Niuchangchih* on the inhibition of cell viability and the mechanism of MEM-induced cytotoxic in hepatoma cells. The IC_{50} of MEM on the cytotoxicity of Hep G2 (wild type p53) and Hep 3B (delete p53) was 49.5 and 62.7 μg/ml, respectively, after 48 h of incubation. Cell and nuclear morphological changes of the human hepatoma cells (Hep 3B and Hep G2) were suggestive of apoptosis. Cell cycle analysis revealed that MEM treatment induced apoptosis on Hep G2 via G_0/G_1 cell cycle arrest. The results also indicated that MEM-induced Hep G2 apoptosis through activation of the caspase-3 and -8 cascades, and regulation of the cell cycle progression to inhibit hepatoma cell proliferation. According to the results, Song and his colleagues hypothesized that the death receptor (DR)-regulated pathway may be the major mechanism of MEM-mediated apoptosis in Hep G2 cells. Thus, the involvement of the Fas/Fas ligand (FasL) death-receptor pathway in the MEM-induced apoptosis of Hep G2 cells was investigated. The results demonstrated that MEM-induced Hep G2 apoptosis through the inhibition of cell growth and the upregulation of Fas/FasL to activate the caspase-3 and -8 cascades (Song et al., 2005a). In another study, the inhibition of cell proliferation and the apoptosis induction resulting from *Niuchangchih* exposure was also confirmed (Hsu et al., 2005). The ethyl acetate extract from *Antrodia camphorata* (EAC) inhibited cell growth in two liver cancer cells, Hep G2 and PLC/PRF/5 cells, in a dose-dependent manner. In Fas/APO-1 positive-Hep G2 cells, EAC increased the expression level of Fas/APO-1 and its two forms of ligands, membrane-bound Fas ligand (mFasL) and soluble Fas ligand (sFasL), in a p53-independent manner. In addition, EAC also initiated the mitochondrial apoptotic pathway through regulation of Bcl-2 family protein expression, release of cytochrome c, and activation of caspase-9, both in Hep G2 and PLC/PRF/5 cells. Furthermore, EAC also inhibited cell survival signaling by enhancing the amount of IκBα in the cytoplasm and reducing the level and activity of NF-κB in the nucleus, which subsequently attenuated the expression of Bcl-X_L in Hep G2 and PLC/PRF/5 cells. Treatment with EAC also caused another human liver cancer cell line, Hep 3B, to undergo apoptotic cell death by way of the calcium–calpain–mitochondria signaling pathway (Kuo et al., 2006).

The activation of the immune response of the host was considered to be another mechanism by which *Niuchangchih* treated liver cancer (Meng, 2005). C57BL/6 mice bearing hepatic H22 tumors were used to investigate the anticancer and immuno-modulatory activity of the fruiting bodies of *Niuchangchih*. After 5 weeks, the inhibition rate of the high dose group (1000 mg/(kg d)) for H22 tumors was 74.24%. The macrophage phagocytic function of the high dose group was increased significantly compared with the other groups ($P < 0.01$). The proliferation activity of T cells and the ability to generate antibodies of B cells were improved significantly in the high dose group ($P < 0.01$), and the $CD4^+/CD8^+$ of mice bearing hepatic H22 tumors was recovered from inverted to normal. Compared with negative and normal control groups, the cell toxic activity of NK and LAK cells was improved significantly, and the serum concentration of interleukin-2 (IL-2), IL-12

and tumor necrosis factor-alpha (TNF-α) were increased significantly.

A recent study reported that EAC could inhibit the invasiveness and metastasis of liver cancer cells through the inhibition of angiogenesis (Hsu et al., 2007). Tumor growth inhibition was most evident in mice treated with EAC at 300 mg/(kg d), while about a 50% reduction in tumor size was observed compared to mice treated with the vehicle. EAC treatment inhibited the expression of VEGF, MMP-2 and MMP-9, and increased the expression of TIMP-1 and TIMP-2, thereby resulting in cancer invasion inhibition. Further analysis revealed that EAC suppressed constitutive and inducible NF-κB together with a reduction in MMP-9 and VEGF protein expression, MMP-9 activity and inducible cancer invasion.

In summary, the anticancer effects of *Niuchangchih* have been investigated in many studies, which have demonstrated the possibility of using *Niuchangchih* in the treatment of human liver cancer. However, as mentioned above, the anticancer activity of *Niuchangchih* is not the result of one mechanism of action only, but rather several mechanisms, including the induction of apoptosis, initiation of the calcium–calpain-dependent pathway, inhibition of angiogenesis and activation of the immune response. To further elucidate the mechanisms of action of *Niuchangchih*, more in vitro and in vivo studies should be carried out. On the other hand, *Niuchangchih* is an herbal medicine that possesses various types of active compounds, and the exact compounds responsible for the anticancer activity of *Niuchangchih* should also be further screened.

6. Effects of strains and culture conditions on its bioactivities

The fruiting bodies of *Niuchangchih* are in great demand in Taiwan due to host specificity, rarity in nature, and the difficulty of artificial cultivation. At present, solid-state culture and liquid fermentation of *Niuchangchih* have been used to obtain fruiting bodies and mycelia of *Niuchangchih*. However, it usually takes several months to cultivate the fruiting body of mushrooms, and it is difficult to control the product quality during soil cultivation (Chang and Wang, 2005; Zhong and Tang, 2004). It is generally recognized that growing mushroom mycelia in a defined medium by submerged fermentation is a rapid and alternative method to obtain fungal biomass of consistent quality (Yang and Liau, 1998). The large-scale production of the mycelia of *Niuchangchih* by submerged culture has been established, while the red color, aromatic smell and bitter taste of the harvested mycelia are similar to the fruiting bodies (Ao et al., 2003; Wu et al., 2007a).

On the other hand, marked effects have been observed on the relationship between the selected strains or culture conditions and the bioactivities of mycelia. Different culture medium and culture conditions could affect the formation of the red color in the fermented products, as the inhibitory activity against liver cancer cells was relative to the red color of the culture medium filtrate (Huang et al., 2002). Compositional analysis of polysaccharides and lipopolysaccharide showed differences in the gel profiles and carbohydrate components among different *Niuchangchih* strains (Lee et al., 2002; Cheng et al., 2005b). Polysaccharides from *Niuchangchih* strains (B71, B85, B86, BCRC35396 and BCRC35398) appeared to show varying levels of activity against the anti-HBV (Lee et al., 2002). In a study aimed at examining the effects of mycelial extracts from five different *Niuchangchih* strains on vascular tension, Wang et al. (2003) discovered that strain B85 produced the strongest vasorelaxation of the aorta among the five strains of *Niuchangchih* tested.

Aside from the strains, the pH values of the culture medium also affect the antioxidant properties and production and molecular weight distribution of exopolysaccharides from *Niuchangchih* in submerged cultures (Shu and Lung, 2004, 2008). While using the same strain, red or white mycelia of *Niuchangchih* were obtained due to different fermentation operations (Mau et al., 2004). Using the conjugated diene method, the antioxidant activity of the methanol extract of white mycelia was better than that of red mycelia (EC_{50} 3.11 vs. 19.8 mg/ml). Both mycelia were efficient in terms of the reducing power and scavenging effect on DPPH radicals, but white mycelia showed significantly lower EC_{50} values (1.56 and 1.70 mg/ml) (Mau et al., 2004). The difference was also observed in a few *Niuchangchih* mycelia cultured in different mediums on their scavenging activity against reactive oxygen species (Shen et al., 2004a).

As marked effects of different strains or culture conditions have been observed on the bioactivities of fermented products, the consensus strain should be selected and the optimum conditions for cultivating the mycelia and bioactive components warrants further investigation.

7. Conclusions

The Baisdomycete, *Niuchangchih*, has been shown to possess activity to prevent chemical and biological liver damage, avoid fatty liver and inhibit the hepatic B virus. Also, it ameliorates liver fibrosis and inhibits the growth of hepatoma cells. Thus, *Niuchangchih* is a useful substance to use in treating liver diseases due to its integrated bioactivities.

At present, solid-state culture and liquid fermentation are used to obtain fruiting bodies and mycelia of *Niuchangchih*, as well as useful metabolites for human requirements. However, very little data on the difference between the wild fruiting bodies and artificial products of *Niuchangchih* exists with regard to product components, biological function and medical effectiveness. Considering that many factors, such as the media and culture conditions, can affect the yield and bioactivity of *Niuchangchih* products, the metabolism of the fungus deserves to be extensively examined in order to obtain more metabolites possessing bioactive effects.

Niuchangchih is a medicinal fungus that possesses various types of active compounds (Table 1). Another traditional Chinese medicine, *Ganoderma lucidum*, has been considered as a therapeutic fungal biofactory, in that numerous compounds have been reported from the fungus (Paterson, 2006). Could *Niuchangchih* be the next one? Notably, the compounds isolated from the wild fruiting bodies are completely different from those isolated from the mycelia and culture broth (Table 1). The former are the majority because the previous research has focused on the isolation and identification of compounds from *Niuchangchih* fruiting bodies. Analysis of other compounds in the artificial products of *Niuchangchih* would be desirable, and it may be possible to isolate more novel compounds.

With regard to the potential of *Niuchangchih* for treating liver diseases, such as preventing chemical liver diseases, ameliorating liver fibrosis and inhibiting liver cancer, little information is available about the exact bioactive compounds of the fungus. Various extracts of *Niuchangchih* were applied in the literature to evaluate the bioactivities (Table 2). However, the significance of tests on extracts is reduced compared to those on pure compounds. Otherwise, the interaction between the different active compounds of *Niuchangchih*, whether they act synergistically or independently to elicit their protective activity against liver diseases, is not clear. Therefore, systematic research is needed to elucidate its bioactivities and to screen for the compounds responsible for such bioactivities. Furthermore, to further elucidate the mechanisms of action of *Niuchangchih*, more in vivo tests and randomized controlled clinical trials should be carried out, and the molecular mechanisms should be studied intensively.

Being the subject of debate, the nomenclature and taxonomy of *Niuchangchih* is still confusing. Different strains of *Niuchangchih* result in diverse bioactivities of the fermented cultures. Therefore, more molecular studies, such as amplified fragment length polymorphism (AFLP)-based DNA fingerprinting (Vos et al., 1995), should be used to establish the consensus strain, distinguish and select standardized stains for experiments, and determine the taxonomy of *Niuchangchih* and the geographic origins of the source material.

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References

Achliya, G.S., Wadodkar, S.G., Dorle, A.K., 2004. Evaluation of hepatoprotective effect of *Amalkadi Ghrita* against carbon tetrachloride-induced hepatic damage in rats. Journal of Ethnopharmacology 90, 229–232.

Ao, Z.H., Fu, H.Z., Zou, X.L., 2003. Large Scale Zhangzhi Fungus Deep Fermenting Process. CN 1456661-2003-11-19.

Basu, S., 2003. Carbon tetrachloride-induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant nutrients. Toxicology 189, 113–127.

Bataller, R., Brenner, D.A., 2005. Liver fibrosis. Journal of Clinical Investigation 115, 209–218.

Beasley, R.P., Hwang, L.Y., Lin, C.C., Chien, C.S., 1981. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22707 men in Taiwan. Lancet 2, 1129–1133.

Brenner, D.A., Waterboer, T., Chio, S.K., Lindquist, J.N., Stefanovic, B., Burchardt, E., Yamauchi, M., Gillan, A., Rippe, R.A., 2000. New aspects of hepatic fibrosis. Journal of Hepatology 32 (Suppl. 1), 32–38.

Chang, T.T., Chou, W.W., 1995. *Antrodia cinnamomea* sp. nov. on *Cinnamomum kanehirai* in Taiwan. Mycological Research 99, 756–758.

Chang, T.T., Chou, W.W., 2004. *Antrodia cinnamomea* reconsidered and *A. salmonea* sp. nov. on *Cunninghamia konishii* in Taiwan. Botanical Bulletin of Academia Sinica 45, 347–352.

Chang, T.T., Wang, W.R., 2005. Basidiomatal formation of *Antrodia cinnamomea* on artificial agra media. Botanical Bulletin of Academia Sinica 46, 151–154.

Chang, C.Y., Lue, M.Y., Pan, T.M., 2005. Determination of adenosine, cordycepin and ergosterol contents in cultivated *Antrodia camphorata* by HPLC method. Journal of Food and Drug Analysis 13, 338–342.

Chang, C.Y., Huang, Z.N., Yu, H.H., Chang, L.H., Li, S.L., Chen, Y.P., Lee, K.Y., Chuu, J.J., 2008. The adjuvant effects of *Antrodia Camphorata* extracts combined with anti-tumor agents on multidrug resistant human hepatoma cells. Journal of Ethnopharmacology 118, 387–395.

Chen, X.H., 2003. Effects of the organic solvent extracts of the fermented mycelia extracts of *Antrodia camphorata* on the hepatic antioxidation and drug-metabolism systems of rats. Master Thesis. Chung Shan Medical University, Taiwan, China.

Chen, P.C., 2007. The depigment effect on B16-F10 melanoma cell by herb fermentation products with *Antrodia camphorata*. Master Thesis. Southern Taiwan University of Technology, Taiwan, China.

Chen, J.C., 2008. King of *Ganoderma*: *Antrodia camphorata* in Taiwan, 2nd ed. YuenChiJai Book Publishing Co., Taipei, Taiwan.

Chen, C.H., Yang, S.W., Shen, Y.C., 1995. New steroid acids from *Antrodia cinnamomea*, a fungal parasite of *Cinnamomum micranthum*. Journal of Natural Products 58, 1655–1661.

Chen, C.J., Su, C.H., Lan, M.H., 2001a. Study on solid cultivation and bioactivity of *Antrodia camphorata*. Fungal Sciences 16, 65–72.

Chen, J.C., Lin, W.H., Chen, C.N., Sheu, S.J., Huang, S.J., Chen, Y.L., 2001b. Development of *Antrodia camphorata* mycelium with submerged culture. Fungal Sciences 16, 7–22.

Chen, J.C., Chen, C.N., Sheu, S.J., Hu, M.L., Tsai, C.C., Dai, Y.Y., Sio, H.M., Chuang, C.H., 2003. Liver-caring medicine containing *Antrodia camphorata*, US 0113297-2003-6-19.

Chen, S.C., Lu, M.K., Cheng, J.J., Wang, D.L., 2005. Antiangiogenic activities of polysaccharides isolated from medicinal fungi. FEMS Microbiology Letters 249, 247–254.

Chen, C.C., Shiao, Y.J., Lin, R.D., Shao, Y.Y., Lai, M.N., Lin, C.C., Ng, L.T., Kuo, Y.H., 2006. Neuroprotective diterpenes from the fruiting body of *Antrodia camphorata*. Journal of Natural Products 69, 689–691.

Chen, C.C., Liu, Y.W., Ker, Y.B., Wu, Y.Y., Lai, E.Y., Chyau, C.C., Hseu, T.H., Peng, R.Y., 2007a. Chemical characterization and anti-inflammatory effect of polysaccharides fractionated from submerge-cultured *Antrodia camphorata* mycelia. Journal of Agricultural and Food Chemistry 55, 5007–5012.

Chen, J.J., Lin, W.J., Liao, C.H., Shieh, P.C., 2007b. Anti-inflammatory benzenoids from *Antrodia camphorata*. Journal of Natural Products 70, 989–992.

Chen, K.C., Peng, C.C., Peng, R.Y., Su, C.H., Chiang, H.S., Yan, J.H., Hsieh-Li, H.M., 2007c. Unique formosan mushroom *Antrodia camphorata* differentially inhibits androgen-responsive LNCaP and -independent PC-3 prostate cancer cells. Nutrition and Cancer—An International Journal 57, 111–121.

Chen, Y.J., Cheng, P.C., Lin, C.N., Liao, H.F., Chen, Y.Y., Chen, C.C., Lee, K.M., 2008. Polysaccharides from *Antrodia camphorata* mycelia extracts possess immunomodulatory activity and inhibits infection of *Schistosoma mansoni*. International Immunopharmacology 8, 458–467.

Cheng, J.J., Huang, N.K., Chang, T.T., Wang, D.L., Lu, M.K., 2005a. Study for anti-angiogenic activities of polysaccharides isolated from *Antrodia cinnamomea* in endothelial cells. Life Sciences 76, 3029–3042.

Cheng, J.J., Yang, C.J., Cheng, C.H., Wang, Y.T., Huang, N.K., Lu, M.K., 2005b. Characterization and functional study of *Antrodia camphorata* lipopolysaccharide. Journal of Agricultural and Food Chemistry 53, 469–474.

Cheng, C.F., Lai, Z.C., Lee, Y.J., 2008a. Total synthesis of (±)-camphorataimides and (±)-himanimides by $NaBH_4/Ni(OAc)_2$ or Zn/AcOH stereoselective reduction. Tetrahedron 64, 4347–4354.

Cheng, P.C., Hsu, C.Y., Chen, C.C., Lee, K.M., 2008b. In vivo immunomodulatory effects of *Antrodia camphorata* polysaccharides in a T1/T2 doubly transgenic mouse model for inhibiting infection of *Schistosoma mansoni*. Toxicology and Applied Pharmacology 227, 291–298.

Cherng, I.H., Chiang, H.C., Cheng, M.C., Wang, Y., 1995. Three new triterpenoids from *Antrodia cinnamomea*. Journal of Natural Products 58, 365–371.

Cherng, I.H., Wu, D.P., Chiang, H.C., 1996. Triterpenoids from *Antrodia cinnamomea*. Phytochemistry 41, 263–267.

Chiang, H.C., Wu, D.P., Cherng, I.W., Ueng, C.H., 1995. A sesquiterpene lactone, phenyl and biphenyl compounds from *Antrodia cinnamomea*. Phytochemistry 39, 613–616.

Chiang, S.Y., Hsieh, C.L., Chang, L.L., Pei, S.Y., Lin, M.Y., Kao, S.T., 2004. The safety and anti-genotoxic effects of *Antrodia camphorata* in vitro, in pregnant mice and their fetuses. Toxicology and Applied Pharmacology 197, 350–351.

Chisari, F.V., 2000. Viruses, immunity, and cancer: lessons from hepatitis B. American Journal of Pathology 156, 1118–1132.

Chiu, H.H., 2007. Phylogenetic analysis of *Antrodia* species and *Antrodia camphorata* inferred from internal transcribed spacer region. Antonie van Leeuwenhoek 91, 267–276.

Chiu, N.Y., Zhang, G.X., 2001. The Illustrated Medicinal Plant in Taiwan, vol. 6. SMC Publishing Inc., Taibei.

Comporti, M., Arezzini, B., Signorini, C., Sgherri, C., Monaco, B., Gardi, C., 2005. F2-isoprostanes stimulate collagen synthesis in activated hepatic stellate cells: a link with liver fibrosis? Laboratory Investigation 85, 1381–1391.

Cronholm, T., 1985. Hydrogen transfer between ethanol molecules during oxidoreduction in vivo. Biochemical Journal 229, 315–322.

Cronholm, T., Jones, A.W., Skagerberg, S., 1988. Mechanism and regulation of ethanol elimination in humans: intermolecular hydrogen transfer and oxidoreduction in vivo. Alcoholism, Clinical and Experimental Research 12, 683–686.

Dai, Y.Y., Chuang, C.H., Tsai, C.C., Sio, H.M., Huang, S.C., Chen, J.C., Hu, M.L., 2003. The protection of *Antrodia camphorata* against acute hepatotoxicity of alcohol in rats. Journal of Food and Drug Analysis 11, 177–185.

Day, C.P., Yeaman, S.J., 1994. The biochemistry of alcohol-induced fatty liver. Biochemica et Biophysica Acta 1215, 33–48.

Domschke, S., Domschke, W., Lieber, C.S., 1974. Hepatic redox state: attenuation of the acute effects of ethanol induced by chronic ethanol consumption. Life Sciences 15, 1327–1334.

Freidman, S., 1993. The cellular basis of hepatic fibrosis mechanism and treatment strategies. New England Journal of Medicine 328, 1828–1835.

French, S.W., 2000. Mechanisms of alcoholic liver injury. Canadian Journal of Gastroenterology 14, 327–332.

Gebhardt, R., 2002. Inhibition of cholesterol biosynthesis in HepG2 cells by artichoke extracts is reinforced by glucosidase pretreatment. Phytotherapy Research 16, 368–372.

Guo, S.Q., 2002. Ameliorative effects of *Antrodia camphorata* on liver fibrosis and gastrointestinal functions in rats. Master Thesis. China Medical College, Taiwan, China.

Halliwell, B., Gutteridge, J.M., 1990. Role of free radicals and catalytic metal ions in human disease: an overview. Methods in Enzymology 186, 1–85.

Han, H.F., Nakamura, N., Zuo, F., Hirakawa, A., Yokozawa, T., Hattori, M., 2006. Protective effects of a neutral polysaccharide isolated from the mycelium of *Antrodia cinnamomea* on *Propionibacterium acnes* and lipopolysaccharide induced hepatic injury in mice. Chemical & Pharmaceutical Bulletin 54, 496–500.

Hattori, M., Sheu, C.C., 2006. Compounds from *Antrodia camphorata* having anti-inflammatory and anti-tumor activity, US 7109232-2006-9-19.

Hawksworth, D.L., Kirk, P.M., Sutton, B.C., Pegler, D.N., 1995. Ainsworth and Bisby's Dictionary of the Fungi, 8th ed. International Mycological Institute, Egham, United Kingdom.

Hseu, Y.C., Chang, W.C., Hseu, Y.T., Lee, C.Y., Yech, Y.J., Chen, P.C., Chen, J.Y., Yang, H.L., 2002. Protection of oxidative damage by aqueous extract from *Antrodia camphorata* mycelia in normal human erythrocytes. Life Sciences 71, 469–482.

Hseu, Y.C., Yang, H.L., Lai, Y.C., Lin, J.G., Chen, G.W., Chang, Y.H., 2004. Induction of apoptosis by *Antrodia camphorata* in human premyelocytic leukemia HL-60 cells. Nutrition and Cancer-an International Journal 48, 189–197.

Hseu, Y.C., Wu, F.Y., Wu, J.J., Chen, J.Y., Chang, W.H., Lu, F.J., Lai, Y.C., Yang, H.L., 2005. Anti-inflammatory potential of *Antrodia camphorata* through inhibition of NOS,

COX-2 and cytokines via the NF-kappa B pathway. International Immunopharmacology 5, 1914–1925.
Hseu, Y.C., Chen, S.C., Tsai, P.C., Chen, C.S., Lu, F.J., Chang, N.W., Yang, H.L., 2007. Inhibition of cyclooxygenase-2 and induction of apoptosis in estrogen-nonresponsive breast cancer cells by *Antrodia camphorata*. Food and Chemical Toxicology 45, 1107–1115.
Hseu, Y.C., Chen, S.C., Chen, H.C., Liao, J.W., Yang, H.L., 2008a. *Antrodia camphorata* inhibits proliferation of human breast cancer cells in vitro and in vivo. Food and Chemical Toxicology 46, 2680–2688.
Hseu, Y.C., Chen, S.C., Yech, Y.J., Wang, L., Yang, H.L., 2008b. Antioxidant activity of *Antrodia camphorata* on free radical-induced endothelial cell damage. Journal of Ethnopharmacology 118, 237–245.
Hsiao, G., Shen, M.Y., Lin, K.H., Lan, M.H., Wu, L.Y., Chou, D.S., Lin, C.H., Su, C.H., Sheu, J.R., 2003. Antioxidant and hepatoprotective effective of *Antrodia camphorata* extract. Journal of Agricultural and Food Chemistry 51, 3302–3308.
Hsu, Y.L., Kuo, Y.C., Kuo, P.L., Ng, L.T., Kuo, Y.H., Lin, C.C., 2005. Apoptotic effects of extract from *Antrodia camphorata* fruiting bodies in human hepatocellular carcinoma cell lines. Cancer Letters 221, 77–89.
Hsu, Y.L., Kuo, P.L., Cho, C.Y., Ni, W.C., Tzeng, T.F., Ng, L.T., Kuo, Y.H., Lin, C.C., 2007. *Antrodia cinnamomea* fruiting bodies extract suppresses the invasive potential of human liver cancer cell line PLC/PRF/5 through inhibition of nuclear factor κB pathway. Food and Chemical Toxicology 45, 1249–1257.
Huang, S.J., Mau, J.L., 2007. Antioxidant properties of methanolic extracts from *Antrodia camphorata* with various doses of gamma-irradiation. Food Chemistry 105, 1702–1710.
Huang, L.C., Huang, S.J., Chen, C.C., Mau, J.L., 1999. Antioxidant properties of *Antrodia camphorata*. In: Proceedings of the 3rd International Conference on Mushroom Biology and Mushroom Products, Sydney, Australia, pp. 275–283.
Huang, R.C., Chen, J.C., Wang, B.C., 2002. Isolate of *Antrodia camphorata* process for producing a culture of the same and product obtained thereby. US 6391615-2002, 5–21.
Huang, R.L., Huang, Q., Chen, C.F., Chang, T.T., Chou, C.J., 2003. Anti-viral effects of active compounds from *Antrodia camphorata* on wild-type and lamivudine-resistant mutant HBV. The Chinese Pharmaceutical Journal 55, 371–379.
Huang, N.K., Cheng, J.J., Lai, W.L., Lu, M.K., 2005. *Antrodia camphorata* prevents rat pheochromo cells from serum deprivation-induced apoptosis. FEMS Microbiology Letters 244, 213–219.
Huang, J.S., Chang, H.C., Li, E.I.C., Huang, T.M., Su, Y.H., Wang, K.C., 2006. Enhancement of hepatoprotective efficacy of *Antrodia camphorata* by Chinese tradition medicine. Journal of Gastroenterology and Hepatology 21 (Suppl. 2), A234.
Jiménéz-López, J.M., Carrasco, M.P., Segovia, J.L., Marco, C., 2002. Resistance of HepG2 cells against the adverse effects of ethanol related to neutral lipid and phospholipid metabolism. Biochemical Pharmacology 63, 1485–1490.
Kuo, P.L., Hsu, Y.L., Cho, C.Y., Ng, L.T., Kuo, Y.H., Lin, C.C., 2006. Apoptotic effects of *Antrodia cinnamomea* fruiting bodies extract are mediated through calcium and calpain-dependent pathways in Hep 3B cells. Food and Chemical Toxicology 44, 1316–1326.
Lee, I.H., Huang, R.L., Chen, C.T., Chen, H.C., Hsu, W.C., Lu, M.K., 2002. *Antrodia camphorata* polysaccharides exhibit anti-hepatitis B virus effects. FEMS Microbiology Letters 209, 63–67.
Lee, T.H., Lee, C.K., Tsou, W.L., Liu, S.Y., Kuo, M.T., Wen, W.C., 2007. A new cytotoxic agent from solid-state fermented mycelium of *Antrodia camphorata*. Planta Medica 63, 86–88.
Lin, E.S., Chen, Y.H., 2007. Factors affecting mycelial biomass and exopolysaccharide production in submerged cultivation of *Antrodia cinnamomea* using complex media. Bioresource Technology 98, 2511–2517.
Lin, W.C., Kuo, S.C., Wu, Y.W., 2001. Effects of 28-days' repeated oral administration of the fermented extract of mycelia of *Antrodia comphorata* (CCRC 93032) on rats. Journal of Chinese Medicine 12, 293–303.
Lin, W.C., Kuo, S.C., Lin, W.L., Fang, H.L., Wang, B.C., 2006. Filtrate of fermented mycelia from *Antrodia camphorata* reduces liver fibrosis induced carbon tetrachloride in rats. World Journal of Gastroenterology 12, 2369–2374.
Liu, J.J., Huang, T.S., Hsu, M.L., Chen, C.C., Lin, W.S., Lu, F.J., Chang, W.H., 2004. Antitumor effects of the partially purified polysaccharides from *Antrodia camphorata* and the mechanism of its action. Toxicology and Applied Pharmacology 201, 186–193.
Liu, D.Z., Liang, H.J., Chen, C.H., Su, C.H., Lee, Z.H., Huang, C.T., Hou, W.C., Lin, S.Y., Zhong, W.B., Lin, P.J., Hung, L.F., Liang, Y.C., 2007a. Comparative anti-inflammatory characterization of wild fruiting body, liquid-state fermentation, and solid-state culture of *Taiwanofungus camphoratus* in microglia and the mechanism of its action. Journal of Ethnopharmacology 113, 45–53.
Liu, D.Z., Liang, Y.C., Lin, S.Y., Lin, Y.S., Wu, W.C., Hou, W.C., Su, C.H., 2007b. Antihypertensive activities of a solid-state culture of *Taiwanofungus camphoratus* (Chang-Chih) in spontaneously hypertensive rats. Bioscience Biotechnology and Biochemistry 71, 23–30.
Liu, S.Y., Kuo, M.T., Wen, W.C., 2008. Inhibition of hepatitis B virus by cyclohexenone compounds from *Antrodia camphorata*. US 7411003.
Lodato, F., Mazzella, G., Festi, D., Azzaroli, F., Colecchia, A., Roda, E., 2006. Hepatocellular carcinoma prevention: a worldwide emergence between the opulence of developed countries and the economic constraints of developing nations. World Journal of Gastroenterology 12, 7239–7249.
Lu, M.K., Cheng, J.J., Lai, W.L., Lin, Y.R., Huang, N.K., 2006. Adenosine as an active component of *Antrodia cinnamomea* that prevents rat PC12 cells from serum deprivation-induced apoptosis through the activation of adenosine A_{2A} receptors. Life Sciences 79, 252–258.
Lu, Y.C., Huang, C.C., Huang, C.J., Chu, S.T., Chi, C.C., Su, H.H., Hsu, S.S., Wang, J.L., Chen, I.S., Liu, S.I., Huang, J.K., Ho, C.M., Kuo, S.J., Jan, C.R., 2007a. Effects of *Antrodia camphorata* on viability, apoptosis, $[Ca^{2+}]_i$, and MAPKs phosphorylation in MG63 human osteosarcoma cells. Drug Development Research 68, 71–78.
Lu, Z.M., Tao, W.Y., Zou, X.L., Fu, H.Z., Ao, Z.H., 2007b. Protective effects of mycelia of *Antrodia camphorata* and *Armillariella tabescens* in submerged culture against ethanol-induced hepatic toxicity in rats. Journal of Ethnopharmcology 110, 160–164.
Lu, M.K., Cheng, J.J., Lai, W.L., Lin, Y.J., Huang, N.K., 2008. Fermented *Antrodia cinnamomea* extract protects rat PC12 cells from serum deprivation-induced apoptosis: the role of the MAPK family. Journal of Agricultural and Food Chemistry 56, 865–874.
Marrero, J.A., 2006. Hepatocellular carcinoma. Current Opinion in Gastroenterology 22, 248–253.
Mau, J.L., Huang, P.N., Huang, S.J., Chen, C.C., 2003. Time course for antioxidants production by *Antrodia camphorata* in submerged culture. Fungal Sciences 18, 59–71.
Mau, J.L., Huang, P.N., Huang, S.J., Chen, C.C., 2004. Antioxidant properties of methanol extracts from two kinds of *Antrodia camphorata* mycelia. Food Chemistry 86, 25–31.
Meng, F.Y., 2005. The antineoplastic property of *Antrodia camphorata* and its effect on the immune function of the tumor bearing mouse. Master Thesis. Harbin Medical University, China.
Motola-Kuba, D., Zamora-Valdes, D., Uribe, M., Mendez-Sanchez, N., 2006. Hepatocellular carcinoma. An overview. Annals of Hepatology 5, 16–24.
Mujumdar, A.M., Upadhye, A.S., Pradhan, A.M., 1998. Effect of *Azadirachta indica* leaf extract on CCl_4 induced hepatic damage in albino rats. Indian Journal of Pharmaceutical Sciences 60, 363–367.
Nakamura, N., Hirakawa, A., Gao, J.J., Kakuda, H., Shiro, M., Komatsu, Y., Sheu, C.C., Hattori, M., 2004. Five new maleic and succinic acid derivatives from the mycelium of *Antrodia camphorata* and their cytotoxic effects on LLC tumor cell line. Journal of Natural Products 67, 46–48.
Paterson, R.R.M., 2006. *Ganoderma*—a therapeutic fungal biofactory. Phytochemistry 67, 1985–2001.
Peng, C.C., Chen, K.C., Peng, R.Y., Su, C.H., Hsieh-Li, H.M., 2006. Human urinary bladder cancer T24 cells are susceptible to the *Antrodia camphorata* extracts. Cancer Letters 243, 109–119.
Peng, C.C., Chen, K.C., Peng, R.Y., Chyau, C.C., Su, C.H., Hsieh-Li, H.M., 2007. *Antrodia camphorata* extract induces replicative senescence in superficial TCC, and inhibits the absolute migration capability in invasive bladder carcinoma cells. Journal of Ethnopharmacology 109, 93–103.
Poli, G., 1993. Liver damage due to free radicals. British Medical Bulletin 49, 604–620.
Rao, Y.K., Fang, S.H., Tzeng, Y.M., 2007. Evaluation of the anti-inflammatory and anti-proliferation tumoral cells activities of *Antrodia camphorata*, *Cordyceps sinensis*, and *Cinnamomum osmophloeum* bark extracts. Journal of Ethnopharmacology 114, 78–85.
Shahani, S., 1999. Evaluation of hepatoprotective efficacy of APCL-A polyherbal formulation in vivo in rats. Indian Drugs 36, 628–631.
Shen, Y.C., Yang, S.W., Lin, C.S., Chen, C.H., Kuo, Y.H., Chen, C.F., 1997. Zhankuic acid F: a new metabolite from a Formosan fungus *Antrodia cinnamomea*. Planta Medica 73, 1412–1415.
Shen, C.C., Kuo, Y.C., Huang, R.L., Lin, L.C., Don, M.J., Chang, T.T., Chou, C.J., 2003a. New ergostane and lanostane from *Antrodia camphorata*. Journal of Chinese Medicine 14, 247–258.
Shen, Y.C., Chen, C.F., Wang, Y.H., Chang, T.T., Chou, C.J., 2003b. Evaluation of the immuno-modulating activity of some active principles isolated from the fruiting bodies of *Antrodia camphorata*. The Chinese Pharmaceutical Journal 55, 313–318.
Shen, Y.C., Chou, C.J., Wang, Y.H., Chen, C.F., Chou, Y.C., Lu, M.K., 2004a. Anti-inflammatory activity of the extracts from mycelia of *Antrodia camphorata* cultured with water-soluble fractions from five different *Cinnamomum* species. FEMS Microbiology Letters 231, 137–143.
Shen, Y.C., Wang, Y.H., Chou, Y.C., Chen, C.F., Lin, L.C., Chang, T.T., Tien, J.H., Chou, C.J., 2004b. Evaluation of the anti-inflammatory activity of zhankuic acids isolated from the fruiting bodies of *Antrodia camphorata*. Planta Medica 70, 310–314.
Shen, C.C., Yang, H.C., Huang, R.L., Chen, J.C., Chen, C.C., 2005. Anti-HBV principle from the culture broth of *Antrodia camphorata* (strain # CCRC-35396). Journal of Chinese Medicine 16, 57–61.
Shu, C.H., Lung, M.Y., 2004. Effect of pH on the production and molecular weight distribution of exopolysaccharide by *Antrodia camphorata* in batch cultures. Process Biochemistry 39, 931–937.
Shu, C.H., Lung, M.Y., 2008. Effect of culture pH on the antioxidant properties of *Antrodia camphorata* in submerged culture. Journal of the Chinese Institute of Chemical Engineers 39, 1–8.
Song, T.Y., Yen, G.C., 2002. Antioxidant properties of *Antrodia camphorata* in submerged culture. Journal of Agricultural and Food Chemistry 50, 3322–3327.
Song, T.Y., Yen, G.C., 2003. Protective effects of fermented filtrate from *Antrodia camphorata* in submerged culture against CCl_4-induced hepatic toxicity in rats. Journal of Agricultural and Food Chemistry 51, 1571–1577.
Song, T.Y., Hsu, S.L., Yeh, C.T., Yen, G.C., 2005a. Mycelia from *Antrodia camphorata* in submerged culture induced apoptosis of human heptoma HepG2 cells possibility through regulation of Fas pathway. Journal of Agricultural and Food Chemistry 53, 5559–5564.

Song, T.Y., Hsu, S.L., Yen, G.C., 2005b. Induction of apoptosis in human heptoma cells by mycelium of *Antrodia camphorata* in submerged culture. Journal of Ethnopharmacology 100, 158–167.

Su, C.H., 2002. Health Guardian Angel: *Antrodia camphorata*, 1st ed. EKS Book Publishing Co., Taipei, Taiwan.

Szabo, G., 2003. Pathogenic interactions between alcohol and hepatitis C. Current Gastroenterology Reports 5, 86–92.

Szmuness, W., 1978. Hepatocellular carcinoma and hepatitis B virus: evidence for a causal association. Progress in Medical Virology 24, 40–69.

Tsai, Z.T., Liaw, S.L., 1985. The Use and the Effect of Ganoderma. San Yun Press, Taiwan, pp. 116–117.

Tsai, M.C., Song, T.Y., Shih, P.H., Yen, G.C., 2007. Antioxidant properties of water-soluble polysaccharides from *Antrodia cinnamomea* in submerged culture. Food Chemistry 104, 1115–1122.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van De Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kulper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23, 4407–4414.

Wang, G.J., Tseng, H.W., Chou, C.J., Tsai, T.H., Chen, C.T., Lu, M.K., 2003. The vasorelaxation of *Antrodia camphorata* mycelia: involvement of endothelial Ca^{2+}-NO-cGMP pathway. Life Sciences 73, 2769–2783.

Wang, W.M., Wu, R.Y., Ko, W.H., 2005. Variation and segregation following nuclear transplantation in *Antrodia cinnamomea*. Botanical Bulletin of Academia Sinica 46, 217–222.

Wu, D.P., Chiang, H.C., 1995. Constituents of *Antrodia cinnamomea*. Journal of the Chinese Chemical Society 42, 797–800.

Wu, C.Y., Liang, Z.C., 2005. Antimicrobial activity of extract from *Antrodia camphorata* cultured on pearl barley by solid-state fermentation. Taiwanese Journal of Agricultural Chemistry and Food Science 43, 295–303.

Wu, S.H., Ryvarden, L., Chang, T.T., 1997. *Antrodia camphorata* ("niu-chang-chih"), new combination of a medicinal fungus in Taiwan. Botanical Bulletin of Academia Sinica 38, 273–275.

Wu, S.H., Yu, Z.H., Dai, Y.C., Chen, C.T., Su, C.H., Chen, L.C., Hsu, W.C., Hwang, G.Y., 2004. *Taiwanofungus*, a polypore new genus. Fungal Sciences 19, 109–116.

Wu, H., Pan, C.L., Yao, Y.C., Chang, S.S., Li, S.L., Wu, T.F., 2006. Proteomic analysis of the effect of *Antrodia camphorata* extract on human lung cancer A549 cell. Proteomics 6, 826–835.

Wu, M.C., Lin, S.J., Wang, B.C., 2007a. Process for producing a culture of *Antrodia camphorata* and product obtained thereby. US 7157090.

Wu, M.D., Cheng, M.J., Wang, B.C., Wang, W.Y., Lai, J.T., Yuan, G.F., 2007b. Chemical constituents from the mycelia of *Antrodia camphorata*. Journal of the Chilean Chemical Society 52, 1338–1340.

Wu, Y.Y., Chen, C.C., Chyau, C.C., Chung, S.Y., Liu, Y.W., 2007c. Modulation of inflammation-related genes of polysaccharides fractionated from mycelia of medicinal basidiomycete *Antrodia camphorata*. Acta Pharmacologica Sinica 28, 258–267.

Yang, F.C., Liau, C.B., 1998. Effects of cultivating conditions on the mycelial growth of *Ganoderma lucidum* in submerged flask cultures. Bioprocess Engineering 19, 233–236.

Yang, S.W., Shen, Y.C., Chen, C.H., 1996. Steroids and triterpenoids of *Antrodia cinnamomea*—a fungus parasitic on *Cinnamomum micranthum*. Phytochemistry 41, 1389–1392.

Yang, H.L., Chen, C.S., Chang, W.H., Lu, F.J., Lai, Y.C., Chen, C.C., Hseu, T.H., Kuo, C.T., Hseu, Y.C., 2006a. Growth inhibition and induction of apoptosis in MCF-7 breast cancer cells by *Antrodia camphorata*. Cancer Letters 231, 215–227.

Yang, H.L., Hseu, Y.C., Chen, J.Y., Yech, Y.J., Lu, F.J., Wang, H.H., Lint, P.S., Wang, B.C., 2006b. *Antrodia camphorata* in submerged culture protects low density lipoproteins against oxidative modification. American Journal of Chinese Medicine 34, 217–231.

Yen, S.J., 2006. Study on the hypotriglyceridemic effect of *Antrodia camphorata* in rats with high-cholesterol diet. Master Thesis. Taibei Medical University, Taiwan, China.

Zang, M., Su, Q.H., 1990. *Ganoderma comphoratum*, a new taxon in genus *Ganoderma* from Taiwan, China. Acta Botanica Yunnanica 12, 395–396.

Zhong, J.J., Tang, Y.J., 2004. Submerged cultivation of medicinal mushrooms for production of valuable bioactive metabolites. Advances in Biochemical Engineering, Biotechnology 87, 25–59.