

Available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/jff

Bioactive principles from *Cordyceps sinensis*: A potent food supplement – A review

M.G. Shashidhar^{a,b}, P. Giridhar^c, K. Udaya Sankar^b, B. Manohar^{a,b,*}

^aAcademy of Scientific and Innovative Research, Council of Scientific and Industrial research, New Delhi, India

^bDepartment of Food Engineering, CSIR-Central Food Technological Research Institute, Mysore 570020, India

^cDepartment of Plant Cell Biotechnology, CSIR-Central Food Technological Research Institute, Mysore 570020, India

ARTICLE INFO

Article history:

Received 14 February 2013

Received in revised form

18 April 2013

Accepted 19 April 2013

Available online 24 May 2013

Keywords:

Cordyceps sinensis

Bioactives

Extraction

Health benefits

ABSTRACT

Cordyceps sinensis (CS) is a well-known entomophagus fungus, naturally distributed in the Tibetan Plateau of Asia and Himalayas. Recently this synonym is transferred to *Ophiocordyceps* by both scientific and non-scientific communities. It is widely used as a tonic and medicinal food in traditional Chinese medicine (TCM), as it possess wonderful health benefits. To support its functional attributes, various investigations have been carried out to find out its adaptogenic, aphrodisiac, anti-oxidant, anti-aging, neuroprotective, nootropic, immunomodulatory, anti-cancer and hepatoprotective role. Its fruiting portion as well as the larvae possesses potent bio-active fractions and their composition almost found to be similar in both. The bioactive principles are nucleosides, exo-polysaccharides, sterols and, proteins, among others. Among nucleosides, adenosine and cordycepin are the major biochemical markers. Further, different types of solvent extracts and their mixtures exhibit wide range of pharmacological activities, while the water and methanol extracts with the richest sources of nucleosides and polysaccharides also show wide range of pharmacological activities. This review gives a panoramic view of potential health benefits of various classes of bio-active fractions along with the need for sustainable management of CS for human wellness.

© 2013 Elsevier Ltd. All rights reserved.

Contents

1. Introduction	1014
2. Economics	1015
3. Artificial cultivation	1015
3.1. Submerged fermentation	1015
3.2. Solid state fermentation	1015
4. Bioactive principles and their structures	1015
4.1. Polysaccharides	1016
4.2. Nucleosides	1018
4.3. Sterols	1018

* Corresponding author at: Department of Food Engineering, CSIR-Central Food Technological Research Institute, Mysore 570020, India.
Fax: +91 821 2517233.

E-mail addresses: manoharmysore@yahoo.co.in, manoharb@cftri.res.in (B. Manohar).

1756-4646/\$ - see front matter © 2013 Elsevier Ltd. All rights reserved.

<http://dx.doi.org/10.1016/j.jff.2013.04.018>

4.4. Other components	1018
5. Extraction	1019
5.1. Water extraction	1019
5.2. Alcoholic extraction	1019
5.3. Ethyl acetate extraction	1020
5.4. Supercritical CO ₂ extraction	1020
6. Health benefits	1020
6.1. Adaptogenic activity	1020
6.2. Immunomodulatory effects	1020
6.3. Antioxidant activity	1022
6.4. Anti-cancer	1023
6.5. Anti-diabetes activity	1023
6.6. Anti-inflammatory activity	1023
6.7. Kidney protection	1024
6.8. Lung and liver protection	1024
6.9. Apoptosis	1025
6.10. Aphrodisiac activity	1025
6.11. Anti-fatigue and improves stamina	1025
7. Future prospects	1025
8. Conclusions	1025
Acknowledgements	1026
References	1026

1. Introduction

Cordyceps sinensis (CS) a fungus belongs to the family “Clavicipitaceae” which is a rare and exotic medicinal mushroom well known in China and has been used in traditional Chinese medicine (TCM) for centuries. It is widely distributed in China, Tibetan Plateau, Bhutan, Nepal and north east regions of India at an altitude of 3500–5000 metres above sea level. In Chinese, it is called as *Dong Chong Xia Cao* which means “winter worm summer grass” (Li et al., 2006a; Li, Yang, & Tsim, 2006b). Traditionally it is known as herbal Viagra. It is also called as caterpillar fungus as it parasitizes *Lepidopteran* larvae, deposits spores, grows through the insect and eventually mummify it. Following overwintering, the fungus ruptures the host body and forms the stroma (fruiting body) that are connected to the dead larva as shown in Fig. 1. At present, there are

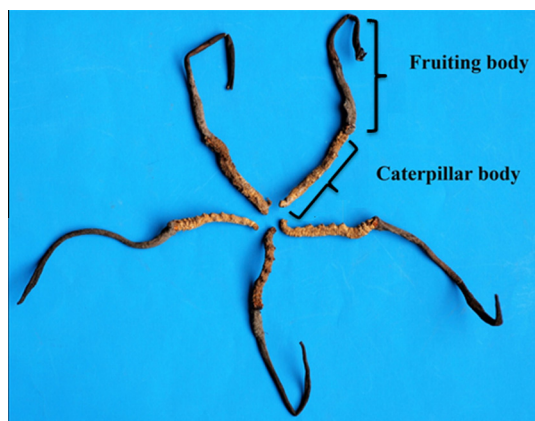


Fig. 1 – Wild *Cordyceps sinensis* emerging out of the head of a dead caterpillar.

more than 680 species coming under the genus *Cordyceps* (Holliday, Cleaver, & Wasser, 2005) but usually *Cordyceps* refers precisely to the species *C. sinensis* (Holliday & Cleaver, 2008; Mizuno, 1999). Both dead larva and fruiting body have been used as a traditional medicine and health food for hundreds of years for “lung invigoration and kidney nourishment” in China (Dong & Yao, 2007; Kuo et al., 1994). Many aspects pertaining to the research on CS is well reviewed (Halperin, 1999).

Cordyceps and its products containing major active ingredients are available in China and many Western countries; however, established scientific evidences on its health effects are lacking. In Chinese Pharmacopoeia it has been officially classified as a drug since 1964. Further, its usage has been increased due to Severe Acute Respiratory Syndrome (SARS) in China in 2003 and recently claimed treatments against nervous system, cardiovascular diseases, tumors, aging, respiratory, renal, liver, hypo-sexuality and hyperlipidemia (Chen et al., 2006; Kuo, Sua, Yang, Huang, & Chen, 2006; Wang & Shiao, 2000). CS is marketed as a dietary supplement under considerations by the FDA and hence, there is a great market demand for *cordyceps* increasing in many countries (Dong & Yao, 2007). In TCM, *cordyceps* has always been revered. The increase in its popularity has led to over-harvesting and subsequent scarcity of wild species (Holliday, Cleaver, Megan, & Patel, 2004; Hsu, Shiao, Hsieh, & Chang, 2002). Convention on International Trade in Endangered Species (CITES) Management Authority of China has officially classified this fungus as an endangered species (CITES Management Authority of China, 2012). Further, researchers looked forward to cultivate this species on artificial medium and by mid-1990’s, artificially cultivated CS are marketed all over the world (Holliday et al., 2004; Miz-

uno, 1999). Promising methods like bioreactor cultivating technology have been followed to cultivate isolated living strains from natural CS to meet human needs and to reduce the pressure on natural resources of the species (Dong & Yao, 2007). Cultured and fermented mycelial products with similar pharmacologically active components are now used in clinical practice (Zhu et al., 1998a, 1998b).

This review explores the therapeutic bio-factory of CS, extraction methods, techniques used for identification of each active principle and possible link to their potential health benefits.

2. Economics

During the last 8–10 years CS trade activities have established the huge market demand particularly in China, Tibet, Nepal and Himalayan region. In rural Tibet, CS collection has become important source of income and contributed 40% to local households, 8.5% to the GDP in 2004. The annual production in the Tibetan Plateau was estimated in 2009 at 80–175 tonnes and 1 kg of caterpillars traded for US\$ 3000 (lowest quality) to over US\$ 18,000 (best quality, largest larvae) in 2008 (Winkler, 2009). The value of 1 kg caterpillars was estimated at about 30,000–60,000 Nepali rupees in Nepal, and about Rs. 100,000 in India (Sharma, 2004). During the year 2011 the value of 1 kg of caterpillars increased from 350,000 to 450,000 Nepali rupees in Nepal. According to a recent report, in north Indian villages a single fungus was worth Rs. 150 (about £2 or \$3), which is more than the daily wage of a manual laborer (Jeffrey, 2012).

Over harvesting of CS is perceived to pose great threat to environment and cause serious ecological imbalance. The increased pressure of harvesting may lead to complete disappearance of this species in future. Hence regulatory policies, practices and alternative strategies for large scale production are needed to save CS species. More research must be carried out to devise effective management strategies to secure long term existence of CS.

3. Artificial cultivation

In view of the recent demand for the CS, there are many attempts in cultivating the CS artificially. An achievement of large scale production of CS by fermentation is the major goal for many researchers so that fungal strains can be easily isolated from natural CS, manufactured in large quantities by fermentation technology (Wang, 1995; Yin & Tang, 1995; Zhu, Halpern, & Jones, 1998a, 1998b). Initial attempts to develop an efficient technology for cultivation of fruiting bodies became futile. A preliminary study on alternate generation of CS was conducted. The infestation of hepialus larvae by CS, growth and reproduction of hypha body in the hemolymph of host larvae, growth of stroma, maturity of hymenium and the abjection and germination of ascospores were observed (Li, Zeng, Yin, & Huang, 1998). But solid state and submerged fermentations remained widely used for the production of CS mycelial biomass and components. Liquid or submerged fermentation is a preferred system for efficient production of desired bio-active compounds by mycelial cul-

tures because of the ease with which the conditions can be manipulated and optimized with high mycelial production as demonstrated for various fungi. Generally CS grows poorly at a temperature above 21 °C (Dong & Yao, 2010). Various studies have focused on optimization of growth conditions for submerged and solid state cultivation.

3.1. Submerged fermentation

A study conducted to optimize the nutritional requirements (17 carbohydrates, 16 nitrogen compounds, 9 vitamins, 4 macro-elements, 4 trace-elements and 8 ratios of carbon to nitrogen) for the successful submerged cultivation of CS wherein a maximum of 22 g/l of mycelial biomass could be obtained in submerged cultures after 40 days (Dong & Yao, 2005). Further growth conditions and nutritional factors were optimized to maximize the production of polysaccharides (Hsieh, Tsai, Hsu, Chang, & Lo, 2005; Leung, Zhang, & Wu, 2005), exo-polysaccharides and cordycepin (Kim & Yun, 2005). Attempt to compare growth characteristics of CS with other endophytic fungi of Nepal Himalaya (Bikash & Jyoti, 2012) revealed that growth rate of the CS was very slow as compared to other associated fungi. Leung and Wu (2007) studied the effects of ammonium feeding on the production of cordycepin (3'-deoxyadenosine, a nucleoside analog) and exopolysaccharides (EPS) in mycelial culture of a new CS (Cs-HK1) (Leung & Wu, 2007).

3.2. Solid state fermentation

Cultivation on the solid-medium method is adopted by many manufacturers in Japan and United States of America. In this method, the substrate comprises of grains or a mixed bag of cereals on which mycelium is allowed to grow. The grain is usually rice, wheat or rye although many different sorts of grains are used. Although this methodology harvests the mycelium with maximum bio-actives recovery and this can be well-known low price technique but disadvantage is, mycelia contains high content of grain matter than actual CS substance. Similarly Cleaver, Holliday, and Powers (2011) studied artificial cultivation of CS on substrate (Cleaver et al., 2011) and also reported a novel method for hybridizing different strains of CS for the purpose of obtaining strains having the medicinal, health stimulating properties comparable or improved relative to strains of CS grown in the wild (Cleaver, Holliday, & Powers, 2005). Solid fermentation conditions were optimized through single factor test to recover maximum polysaccharides where soybean meal and rice bran (1:2 w/w) as substrates and arrived at optimum amount of inoculation (20%), fermentation temperature (26 °C), water content of medium (60%), air relative humidity (60%) and fermentation time (7 days) (Wu, Chen, & Hao, 2009).

4. Bioactive principles and their structures

Smith, Rowan, and Tan (2000) reported the usage of medicinal mushrooms for health benefits most probably in the form of extracts, concentrates, or powders now termed mushroom nutraceuticals (Smith et al., 2000). CS is rich source of novel

Table 2 – List of various classes of compounds and their pharmacological activities reported in *Cordyceps sinensis*.

Constituents	Pharmacological functions	References
Polysaccharides		
–	Antioxidant activity	Li, Li, Dong, and Tsim (2001a), Yu et al. (2006a, 2006b) and Zhang et al. (2011)
CS-F30 and CS-F10	Hypoglycemic and hypolipidemic effect	Kiho, Ookubo, Usui, Ukai, and Hirano (1999), Kiho et al. (1996)
Polysaccharide fraction from CS (PSCS)	Antileukemic	Chen et al. (1997)
Mannoglucan	Cytotoxic activity	Wu et al. (2007b)
Exo-polysaccharides	Immunomodulatory and antitumour effects	Yang et al. (2005), Zhang, Yang, Chen, Hou, and Han (2005) and Zhang, Li, Qiu, Chen, and Zheng (2008)
Acid polysaccharide fraction (APSF)	Immunomodulatory effect	Chen, Zhang, Wenbin Shen, and Wang (2010) and Chen et al. (2012)
Heteropolysaccharides (PS-A)	Hypercholesterolemia	Kim (2010)
Nucleosides		
Adenosine	Immunomodulatory & pharmacokinetic effects, cardioprotection	Kim (2010) and Yu, Zhao, Zhu, and Li (2007)
Guanosine Cordycepin	Immunomodulatory effects Anti-cancer effects, pharmacokinetic effect, immunomodulatory effect, antileukemic	Kim (2010) Kodama et al. (2000), Nakamura et al. (2006), Pao et al. (2012), Tsai, Lin, and Tsai (2010) Yoshikawa et al. (2011) and Zhou et al. (2008)
Sterols		
Ergosterol	Anti-cancer effects	Matsuda et al. (2009) and Wu et al. (2007a)
β -Sitosterol	Anti-cancer effects	Matsuda et al. (2009) and Wu et al. (2007a)
5 α ,8 α -Epidioxy-24(R)-methylcholesta-6,22-dien-3 β -D-glucopyranoside	Anti-cancer effects	Bok et al. (1999)
5,6-Epoxy-24(R)-methylcholesta-7,22-dien-3 β -ol	Anti-cancer effects	Bok et al. (1999)
5 α ,8 α -Epidioxy-22E-ergosta-6,22-dien-3 β -ol	Anti-cancer effects	Matsuda et al. (2009)
5 α ,8 α -Epidioxy-22E-ergosta-6,9(11),22-trien-3 β -ol	Anti-cancer effects	Matsuda et al. (2009)
5 α ,6 α -Epoxy-5-ergosta-7,22-dien-3 β -ol	Anti-cancer effects	Matsuda et al. (2009)
Protein constituents	Hypotensive and vasorelaxant activities	Chiou et al. (2000)
Proteins		
CSAP	Anti-bacterial activity	Zheng et al. (2006)
Cordymin (peptide)	Anti-inflammatory and anti-nociceptive activities	Qian et al. (2012)
Cordyceptide A (cyclopeptide)	Cytotoxic activities	Jing et al. (2005)
Cordyceamides A and B	Cytotoxic activities	Jia et al. (2009)

macro molecular mass. Guan, Zhao, Feng, Hu, and Li (2011) attempted qualitative analysis and characterization of polysaccharides by a method called “Saccharide mapping” (Guan & Li, 2010) and reported a range of monomers that build a macromolecular mass of polysaccharide containing major (1 \rightarrow 4)- β , (1 \rightarrow 4)- α , (1 \rightarrow 6)- α -glucosidic and 1 \rightarrow 4- β -D-mannosi-

dic linkages, etc. (Guan et al., 2011). Further, content of carbohydrates extracted by pressurized liquid extraction (PLE) extraction in both natural and cultured CS compared and 10 monosaccharides (rhamnose, ribose, arabinose, xylose, mannose, glucose, galactose, mannitol, fructose and sorbose) were determined by GC-MS (Guan, Yang, & Li, 2010).

Methylation, Smith degradation, acetolysis, NMR spectroscopy (^1H , ^{13}C , ^{13}C - ^1H 2D-COSY) and acid hydrolysis studies were conducted to determine the structural features of D-glucan (backbone composed of (1 \rightarrow 4)-D-glucosyl residues and carried a single (1 \rightarrow 6)-linked D-glucosyl residue), which exhibits immune stimulating properties (Yalin, Cuirong, & Yuanjiang, 2006) and in another study the structural analysis of a neutral (1 \rightarrow 3), (1 \rightarrow 4)- β -D-glucan was done by NMR and IR spectroscopic measurements (Yalin, Cuirong, & Yuanjiang, 2005). Mannitol (sugar alcohol) is another compound that found to have anti-oxidant activity extracted from natural (32.22 ± 1.5 g/100 g of extract) and cultured mycelia (21.77 ± 0.73 g/100 g of extract) of CS (Dong & Yao, 2007). A neutral mannoglucan (Man and Glc units in the molar ratio of 1:9) was characterized using chemical analysis and NMR and IR spectroscopy, and its structure reveals α -D-glucan backbone with (1 \rightarrow 4) and (1 \rightarrow 3)-linkages, and the side chains of α -D-(1 \rightarrow 6)-Manp were attached to the back bone via O-6 of α -(1 \rightarrow 3)-Glc p residues (Wu, Hu, Pan, Zhou, & Zhou, 2007b). From ascocarps of CS, by hot water extraction and ethanol fractionation galactomannan was purified from CS-I and its structure was elucidated using periodate oxidation, Smith degradation, methylation analysis, partial acid hydrolysis, and ^{13}C NMR spectrometry, revealed that it contains mannan core as (1 \rightarrow 2)- α -linked-D-mannopyranosyl residues and galactosyl as branch contains (1 \rightarrow 3), (1 \rightarrow 5), and (1 \rightarrow 6)-linked-D-galactofuranosyl, (1 \rightarrow 4)-linked-D-galactopyranosyl residues (Toshio, Naoko, & Haruki, 1977) and in another study a minor protein containing galactomannan from 5% sodium carbonate extract of CS was found (Kiho, Tabata, & Ukai, 1986). Nie et al. (2011) studied the structural features of hydrophilic polysaccharide (CBHP) using methylation analysis and 2D NMR spectroscopy. According to them, the polysaccharide structure is composed mainly of glucose (95.19%) that constitutes backbone where Glc p units were linked through α -1,4 linkage (65.7%), followed by t-Glc p (20.7%), 1,2,3,6-Glc p (4.1%), 1,2,4,6-Glc p (3.0%), 1,3,6-Glc p (2.0%), 1,4,6-Glc p (1.6%), and 1,2-Manp (1.9%) and 1,3-Galp (1.0%) and the branching points are located at O-2 or O-6 of Glc p with α -terminal-D-Glc p as side chain. The trace amounts of 1,2-Manp and 1,3-Galp linkages are probably located randomly in the side chains (Nie et al., 2011).

4.2. Nucleosides

Nucleosides are believed to be one of the key active components in CS (Li, Li, Dong, & Tsim, 2001b), in that adenosine and cordycepin are being used as a marker for quality control. The nucleosides content differs among natural and cultured CS (Li et al., 2001c). Many other nucleosides have been found including uridine, several distinct structures of deoxyuridines, adenosine, 2'-3'-dideoxyadenosine, hydroxyethyladenosine, cordycepin triphosphate, guanidine, deoxyguanidine, which were not found anywhere else in nature. Adenosine and Cordycepin (3'-deoxyadenosine) are pharmaceutically active components exhibits multiple pharmacological actions such as immunomodulatory, anti-oxidant etc. Cordycepin was originally extracted from *Cordyceps militaris*. Chen and Chu (1996) characterized cordycepin and 2'-deoxyadenosine, using nuclear magnetic resonance (NMR) and infrared spectroscopy

(IR) in an extract of CS (Chen & Chu, 1996). In a quality assessment study of CS, Li et al. (2006a, 2006b) determined the presence of cordycepin in CS by analytical methods like capillary electrophoresis, HPLC and also reported it as an active principle (Li et al., 2006a, 2006b). Moreover, it is required to make sure the presence of cordycepin for authenticity of genuine CS. Several other analytical methods and identification techniques studied like RP-HPLC (Shiao, Wang, Lin, Lien, & Wang, 1994; Yu, Wang, Huang, & Duh, 2006a; Yu et al., 2006b), HPLC-ESI-MS (Huang, Guo, Liang, & Chen, 2004), and HPLC-DAD (Jiang, Liu, Li, & Jin, 2008) have been used for the determination of cordycepin. Other than adenosine and cordycepin few other nucleosides and nucleobases were reported in water extract of stroma and caterpillar of CS like cytosine, uracil, cytidine, hypoxanthine, uridine, thymine, adenine, inosine, guanosine and thymidine (Yuan, Zhao, Wang, Kuang, & Liu, 2008).

4.3. Sterols

Ergosterol, a principal sterol determined in CS by HPLC (Li & Li, 1991; Li et al., 2004) and present in two forms, as free ergosterol and esterified ergosterol, which have different physiological functions (Yuan, Wang, Liu, Kuang, & Zhao, 2007). In a study two sterols isolated from methanol extract found to exhibit anti-tumor activity and its structure was established by 1D and 2D NMR spectroscopy (Bok, Lerner, Chilton, Klingeman, & Towers, 1999). Cholesterol, campesterol and β -sitosterol including ergosterol in natural (wild) CS were determined using pressurized liquid extraction (PLE), trimethylsilyl (TMS) derivatization and GC-MS analysis (Yang, Feng, Zhao, & Li, 2009). These phytosterols play important role in treating colon, prostate, breast cancer and their bioactivities are helpful in elucidating therapeutic indications of CS.

4.4. Other components

In addition to above main components, CS also contains proteins, peptides, polyamines, all essential amino acids and some uncommon cyclic dipeptides, including cyclo-[Gly-Pro], cyclo-[Leu-Pro], cyclo-[Val-Pro], cyclo-[Ala-Leu], cyclo-[Ala-Val], and cyclo-[Thr-Leu]. Some of these are found to show potent anti-tumor, antiviral, and antibiotic activities. Cyclic dipeptides like cyclo-(Leu-Pro) and cyclo-(Phe-Pro) found to exhibit antimicrobial effects and have anti-mutagenic properties against growth of vancomycin-resistant enterococci (VRE) and pathogenic yeasts (Rhee, 2004). A study reveals the inhibitory action of cyclic dipeptides (produced by bacterium) against aflatoxin production (Yan et al., 2004). The content of protein in dead larvae (29.1%), fruiting body (30.4%) and in fermented mycelia (14.8%) varies. Amino acid profile lists three major principle amino acid (glutamic acid, aspartic acid and arginine) in the larvae-fruiting body (Hsu et al., 2002). Cordymin, a peptide purified from the medicinal mushroom of CS exhibits anti-inflammatory and anti-nociceptive activities (Qian, Pan, & Guo, 2012). Hypotensive and vasorelaxant activities were contributed by protein constituents of CS (Chiou, Chang, Chou, & Chen, 2000). For the first time an anti-bacterial protein (CSAP) from cultured mycelia was purified and characterized by chromatography and SDS-PAGE.

The CSAP exhibits anti-microbial activity (60%) against Gram-positive and Gram negative bacteria and improved temperature stability but did not show haemagglutinating activity (Zheng et al., 2006). A new cyclodipeptide named as Cordyce-dipeptide A and modified dipeptides named as Cordyce-amides A and B, were isolated from the culture liquid of CS and structures were elucidated by 1D and 2D NMR techniques. It is found to show cytotoxic activities towards L-929, A375, and Hela cells (Jia, Tao, & Feng, 2009; Jing, Xiao, Chun, Li, & Gao, 2005).

Some other compounds like polyamines (1,3-diamino propane, cadaverine, spermidine, spermine and putrescine), free fatty acids (lauric acid, myristic acid, pentadecanoic acid, palmitoleic acid, palmitic acid, linoleic acid, oleic acid, stearic acid, docosanoic acid and lignoceric acid) have also been identified (Mishra & Upadhyay, 2011).

5. Extraction

Several extraction methods use solvents employed for isolation of selective bio-active compounds (Chen, Wanga, Nieb, & Marcone, 2013). Each extract exhibits potent biological

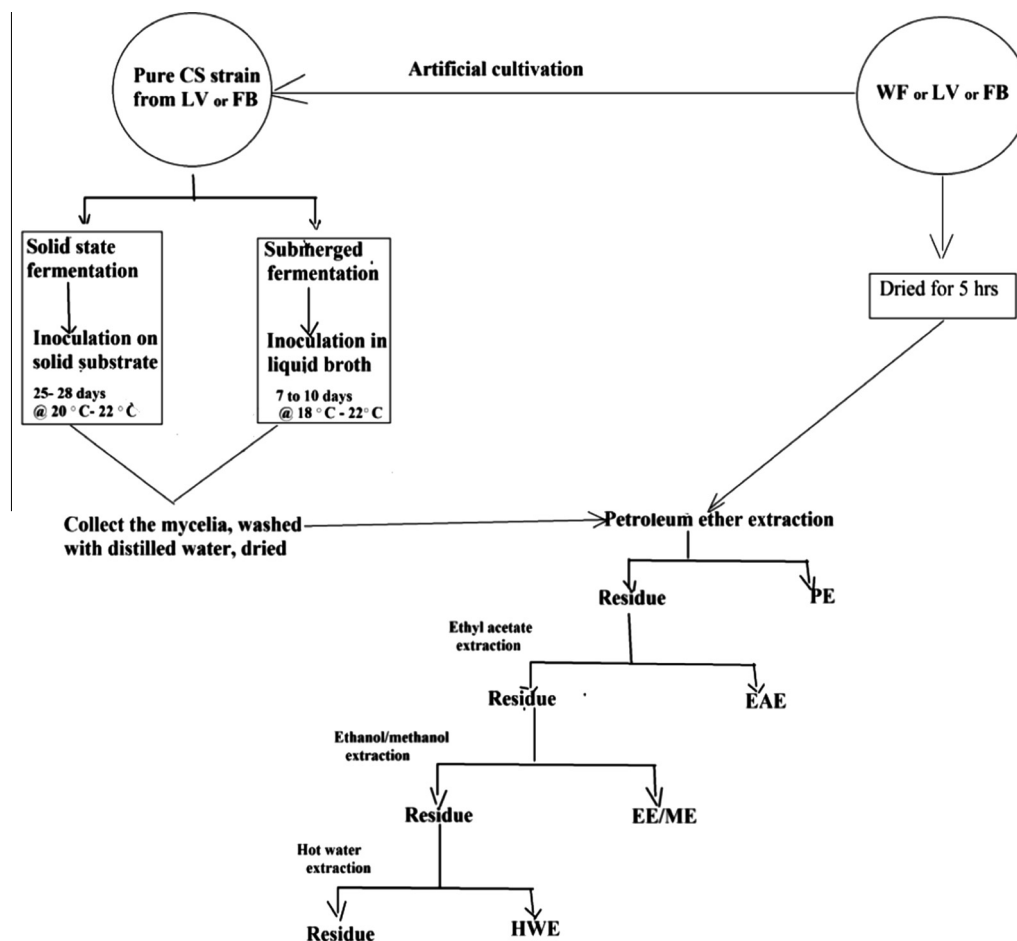
activities. Few studies on solvent extraction of CS are briefly discussed below and flow chart for processing of bio-actives from CS is represented in Fig. 2.

5.1. Water extraction

Water being a high polar molecule, extracts polar compounds like nucleosides and polysaccharides of CS and the ratio of water to material was studied to obtain maximum polysaccharide fraction. A study reported best extraction conditions: water: CS powder = 2.5:1, pH 7.5–8.0 and extraction time 24 h (Sun, Zhang, & Lei, 2003). Hot water extraction yield may vary in the range 25–30% and possesses potential health benefits like anti-oxidant activities (Li et al., 2003; Yamaguchi, Kagota, Nakamura, Shinozuka, & Kunitomo, 2000b).

5.2. Alcoholic extraction

Methanol and ethanol and/or aqueous methanol and ethanol are also good solvents for the extraction of bio-active principles from CS. These alcoholic extracts are rich in very potent bio-actives like nucleosides, polysaccharides, proteins. Etha-



WF-Whole fungus; LV- Larvae; FB- Fruiting body; PE- Petroleum extract; EAE- Ethyl acetate extract; EE- Ethanol extract; ME- Methanol extract; HWE- Hot water extract.

Fig. 2 – Flow chart of the processing of bio-active compounds from *Cordyceps sinensis*.

nolic extract shows strong anti-oxidant activity (Yamaguchi et al., 2000b), preserve β -cell function and offer renoprotection (Kan et al., 2012). Methanolic extract obtained from culture liquid of CS also showed cytotoxic effect against cancer cell lines (Jia et al., 2009). More about pharmacological effects of these extracts as such or purified fractions of extracts are discussed in later section.

5.3. Ethyl acetate extraction

Ethyl acetate extract of CS contains a group of compounds that are different from usually found in water and alcoholic extract. Though the yield in this method is low, it contains the carbohydrates, adenosine, ergosterol and trace amount of cordycepin, of which ergosterol and related compounds were identified as a major class of active constituents contributing to the *in vitro* cytotoxicity. It induces apoptosis in human pre myelocytic leukemia HL60 cells with an ED $50 \pm 25 \mu\text{g/ml}$ for 2 days treatment (Zhang, Wu, Hu, & Li, 2004) and inhibits the proliferation of cancer cell lines (Wu, Zhang, & Leung, 2007a). Further research is used to understand structural features and efficacy of active compounds in ethyl acetate extract. Petroleum ether extract reported to show strong anti-oxidant activities, cellular and humoral immune response and immunomodulatory effects (Wu, Sun, Qin, Pan, & Sun, 2006; Wu et al., 2007a).

5.4. Supercritical CO₂ extraction

In recent years, extraction using supercritical CO₂ has emerged as an alternate technology having wide applications in chemical and food industries. The process does not involve any toxic organic solvents for extraction and is carried out at milder conditions making it the best solvent for extraction of bio-active compounds (particularly non-polar) in its purest form. There are enormous amount of literature available dealing with fundamental and applications of supercritical fluid extraction in various fields (Camila & Meireles, 2010). Ethanolic extract of CS was fractionated by using supercritical CO₂ as an elution solvent and these fractions showed strong scavenging ability and selectively inhibited the growth of colorectal and hepatocellular cancer cells by the process of apoptosis (Wang, Won, Yu, & Su, 2005).

6. Health benefits

Many studies *in vitro* and *in vivo* support that CS has diverse biological activities and pharmacological potential. CS in its natural form and fermented mycelial products exhibit wide spectrum of pharmacological actions in renal, immunologic, hepatic, nervous systems and cardiovascular as well as anti-cancer activity (Yin & Tang, 1995; Zhu et al., 1998a, 1998b). Primarily polysaccharides, nucleosides and its derivatives (or modified), and cyclosporine like secondary metabolites are active fractions, exhibits potent health benefits. Aqueous extracts (mainly contains polysaccharide containing) and alcohol extracts of CS are most commonly used for *in vitro* and *in vivo* studies.

6.1. Adaptogenic activity

Basically adaptogens belongs to a class of metabolic regulators which increase the ability of an organism to adapt to environmental factors and to avoid damage from such factors. Studies showed CS also possesses a potential remedy for harmful factors like stresses, aging etc. In an experiment to study the anti-aging effect of hot water extract of CS, mice aged by D-galactose and castrated rats were treated. Similar study reported the anti-aging properties of CS (Chen & Li, 1993). Study indicated that CS extract improved the activity of superoxide dismutase, glutathione peroxidase and catalase and lowered the level of lipid peroxidation and monoamine oxidase activity in the aged mice (Ji et al., 2009). Further, hot water extract of mycelium ((150 mg/kg/days) showed significant inhibition against increase in total cholesterol and the decrease in alkaline phosphatase levels in mice orally administered with CS extract (Koh, Kim, Kim, Song, & Suh, 2003).

6.2. Immunomodulatory effects

This Chinese herb is useful in preventing and treating many age related diseases. Kuo, Tsai, Shiao, Chen, and Lin (1996) reported that CS contains immunosuppressive ingredients that are not cytotoxic on human mononuclear cells. Various fractions (column fractions) of MeOH extract, were tested for their production of lymphoproliferative response, natural killer (NK) cell activity, and phytohemagglutinin (PHA) stimulated interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF- α) on human mononuclear cells (HMNC). Out of the 15 column fractions, two fractions (CS-36-39 and CS-48-51) significantly inhibited the blastogenesis response ($IC_{50} = 71.0 \pm 3.0$ and $21.7 \pm 2.0 \mu\text{g/ml}$, respectively), NK cell activity ($IC_{50} = 25.0 \pm 2.5$ and $12.9 \pm 5.8 \mu\text{g/ml}$, respectively), IL-2 production of HMNC stimulated by PHA ($IC_{50} = 9.6 \pm 2.3$ and $5.5 \pm 1.6 \mu\text{g/ml}$, respectively) and obstruction of TNF- α production in HMNC cultures ($IC_{50} = 2.7 \pm 1.0$ and $12.5 \pm 3 \mu\text{g/ml}$, respectively) (Kuo et al., 1996). And the same study group conducted human clinical trial (five male subjects; mean age 35 years) and reported that methanolic extract from the fruiting bodies of CS contains immunomodulating agents, causes regulation of bronchoalveolar lavage fluids (BALFs) cell function. In this study, lipopolysaccharide (LPS) induced bronchoalveolar lavage fluids (BALFs) cells proliferation, inflammatory cytokines production, and genes expression were evaluated after treatment with CS MeOH extract. It showed suppressory effect (92.6% at 200 mg/ml) on BALF cells proliferation with stimulated with LPS. Further, CS-19-22 among 15 sub fractions, showed better activities: (1) suppressed BALF cells proliferation (dose dependent), (2) reduced the IL-1b, IL-6, IL-8, IL-10 and TNF- α production in LPS activated BALF cell cultures, (3) enhanced production of IL-12 and IFN-g production in activated BALF cells (Kuo et al., 2001).

In a study to find out the potential of some chinese herbs viz. *Atractylodes ovata*, *Anqelica sinensis*, *C. sinensis*, *Liqustrum lucidum*, *Codonopsis pilosula* and *Homo sapiens* on systemic lupus erythematosus (SLE) which is an important autoimmune disease. CS improved survival and inhibited anti-ds DNA antibody production in lupus mice (NZB/NZW F1) and also im-

proved the defective IL-2 production (Chen et al., 1993). Liu, Lu, and Ji (1992) suggested that CS could be utilized as an approach of biological responsive modifier therapy (BRMT) in the treatment of leukemia. Similarly another study reported that CS could augment the NK cell activity and improves the CD16 marker expression on lymphocytes and the binding capacity to K562 cells (Liu et al., 1992). Further, acid polysaccharide fraction (APSF), from an anamorph of CS has stimulating activity on macrophages where increased expressions of TNF- α , IL-12 and iNOS, and reduced the expression of IL-10 of Ana-1 cells were observed (Chen, Yuan, Wang, Song, & Zhang, 2012). On the other hand CS extract was found to enhance the MHC class II antigen expression on HA22T/VGH cells of human hepatoma cell line HA22T/VGH. Immunostaining of monoclonal antibody (MAb) L243 against the HLA DR region of MHC class II antigens was analyzed using cytofluorimetry where CS extract down regulated the MHC class II antigen expression making immune surveillance of host more effective against tumor cells (Chiu et al., 1998).

A pure compound (H1-A) isolated from CS reported to show good immunomodulatory effect by improving the survival of lupus mice reducing anti-ds-DNA production. In clinical presentation, the treated group had a reduction in lymphadenopathy, a delayed progression of proteinuria, and an improvement in kidney function and revealed that there was no significant change in immune complex deposition (Lin et al., 1999; Yang, Chen, Kuo, & Lin, 1999). Immunosuppressant effect of CS was demonstrated wherein CS showed inhibition effect for immune reaction viz. phagocytic function of peripheral blood leucocytes, mitogenic response of spleen lymphocytes to Con A, and mixed lymphocyte culture and LPS-induced IL-1 release of macrophages (Zhu & Yu, 1990). In another study the inhibitory effect of a CS preparation (CS-1) on the immune response responsible for the organ transplant rejection has been studied. Moreover, CS-1 can prolong the survival of grafted heart without causing infection, and it did not exert detrimental effects on vital organ (Zhang & Xia, 1990).

The exo-polysaccharides obtained from submerged fermentation of CS contributes to immunomodulatory activity by the way of enhancing cytokine synthesis, CD11b expression, and phagocytosis (Kuo, Chang, Cheng, & Wu, 2007). Especially the exo-polysaccharides (Fr. A) are responsible for production of TNF- α , IL-6, and IL-10 dose-dependently, augmentation of surface expression of CD11b in monocytes and polymorphonuclear (PMN) neutrophils, and also elevated phagocytosis in monocytes and PMN. On the other hand intracellular polysaccharides (Fr. B) moderately induced TNF- α release, CD11b expression and phagocytosis.

Several human trials have been conducted to test efficacy of CS on declining immune function against chronic renal failure (CRF) patients (study group) and the results showed decrease of OKT3, OKT4, and OKT4/OKT8 in CRF, proportional occurrence of OKT4 and OKT4/OKT8 to plasma albumin and Hb levels. It also improved renal function and increased the OKT4 and OKT4/OKT8 ratio (Guan, Hu, & Hou, 1992).

Crystallized preparation of CS (Cs-Cr) could cause significant elevation of T helper cells and Lyt-1/Lyt-2 (T helper to T suppressor cell) ratio both in peripheral blood and the treated mice spleen and also could provide protection against immunosuppressive effects of prednisolone acetate and cyclophosphamide to T helper cells (Chen, Chen, Sun, Hsieh, & Henshall, 1991). In view of this there is a possibility of using CS for treatment against immunodeficient or immunosuppressed patients (Guan et al., 1992).

Alcoholic extract of CS plays an important protective role against viral-induced murine myocarditis by inducing IFN- γ and regulating T lymphocyte (Li et al., 2006a, 2006b) and similarly air-pouch bacterial inoculation model was used to investigate protective efficacy of CS mycelium extract against Group A streptococcus (GAS) infection (Kuo et al., 2005). On the other hand water extract showed immunomodulating effect cum anti-inflammatory effect wherein CS acts as an activator and inducer of immature Dendritic cells (DC) by stimulating surface expression of CD11c, CD205, CD40, CD80, CD83, CD86, and MHC-II causing immature DC to ma-

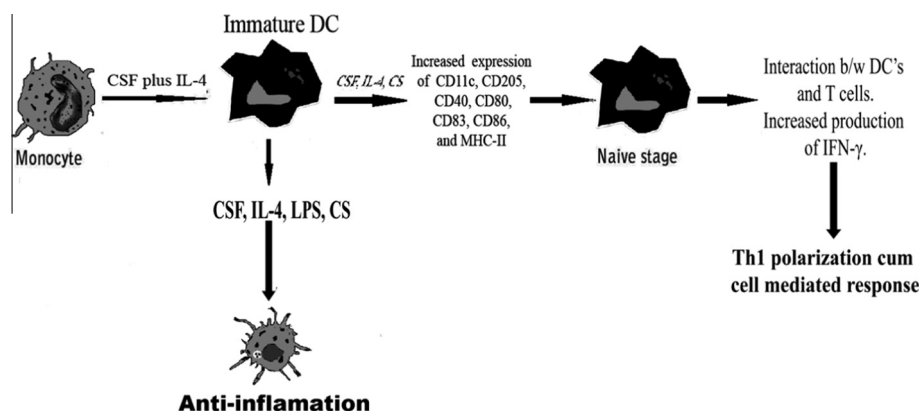


Fig. 3 – Immunomodulatory effects of *Cordyceps sinensis* on dendritic cells in two different physiological stages: naive and LPS-induced inflammatory. A study demonstrates *Cordyceps sinensis* acts as an activator and maturation inducer of immature DCs inducing T cells proliferation, priming DCs towards Th1 immunity cum cell mediated response. On the other hand CS regulates LPS induced inflammation. CSF, colony stimulating factor; DC, dendritic cells; CS, water extract of *Cordyceps sinensis* (Adapted from: Li et al., 2009).

ture with decreased endocytic activity, promotes the Th1 polarization. This indicates that CS can potentially modulate DCs toward cell-mediated immunity (Li et al., 2009). The mechanism can be well depicted in Fig. 3. Few more studies providing information on immunomodulatory effect of CS are available (Chen, 1983, 1985; Zhang, 1985).

6.3. Antioxidant activity

CS attracted many research interests for its antioxidant activity. Li, Su, Dong, and Tsim (2002) reported that extracts of mycelia obtained from sclerotia and stroma of CS showed similar antioxidant actions like lipid peroxidation, xanthine oxidase, and induction of hemolysis assays (Li et al., 2002). Various solvent extracts of CS are effective in scavenging hy-

droxyl radicals (Cai, Zhang, Chen, & Yin, 2004), superoxide anion radicals (Zhang, Pu, Yin, & Zhong, 2003). Both water and ethanol extract of CS showed scavenging effect on reactive oxygen species (ROS) of by inhibiting malondialdehyde formation by the peroxy-nitrite generator SIN-1 (Yamaguchi, Kagota, Nakamura, Shinozuka, & Kunitomo, 2000a; Yamaguchi et al., 2000b). Similarly anti-oxidative effects of the hot water extracts from natural and cultured mycelia of CS using six *in vitro* assays was investigated. Extracts showed the superior effect on the inhibition of linoleic peroxidation with an inhibition rate over 90%, slightly lower scavenging activities on superoxide anion and hydroxyl radicals than that of BHT, DPPH scavenging activities over 80% inhibition and moderate reducing power and ferrous ion chelating activity (Dong & Yao, 2007) (Fig.4).

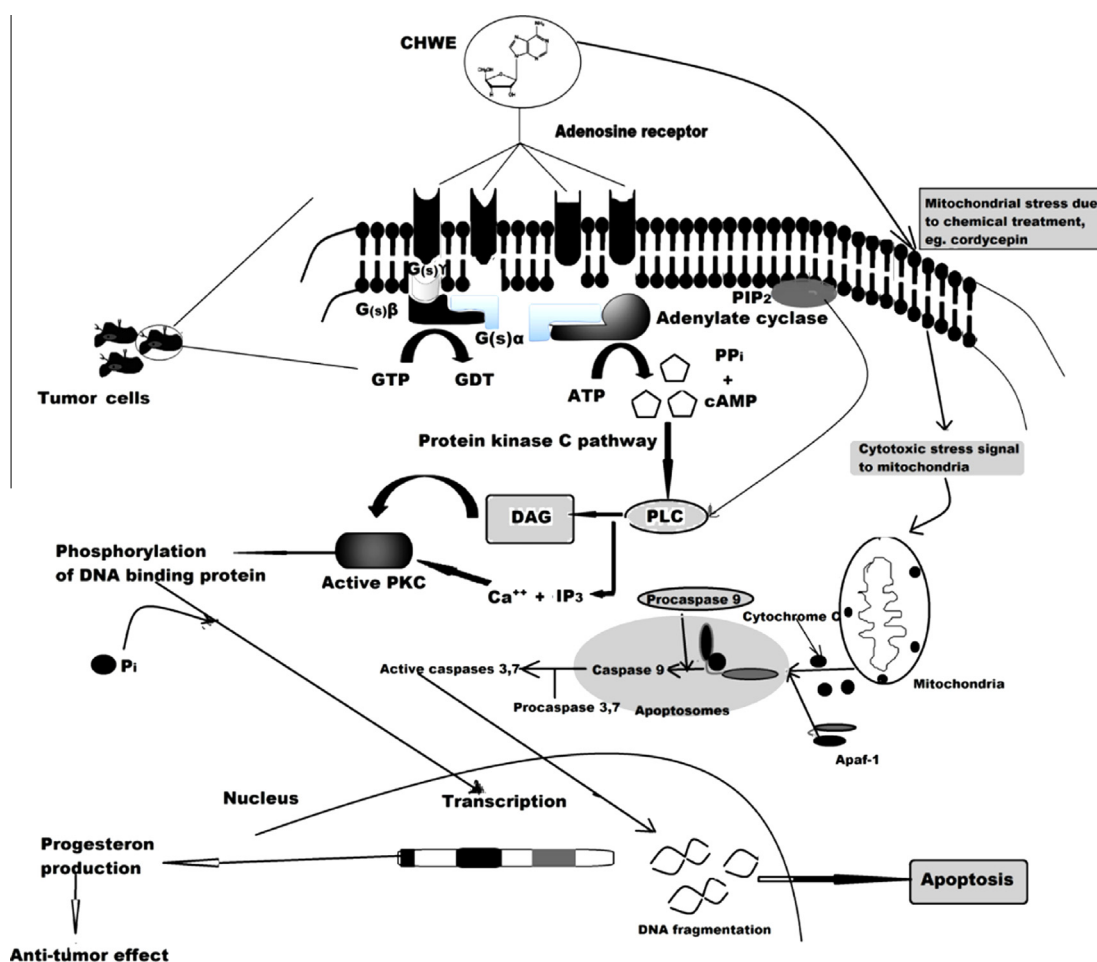


Fig. 4 – The simplified experimental scheme describing cordycepin induced anti-tumor and apoptosis processes. Cordycepin in hot water extract of *Cordyceps sinensis* shows inhibitory actions by stimulating adenosine receptors in turn activates protein kinase C pathway which can be activated through DAG, PLC and Ca^{2+} . This signaling pathway stimulates steroidogenesis which results in progesterone production that brings about anti-tumor effect. On other hand cordycepin induces cytotoxic stress signal to mitochondria activating caspase 9, 3,7 cascade pathway via apoptosomes. This cascade caspases pathway brings about DNA fragmentation, plasma blebbing which is characteristic of apoptosis (Adapted from: Huang, Chuang, Chen, & Leu, 2000; Huang, Hsu, Tsai, Sheu, & Leu, 2001; Jen et al., 2011; Leu, Poon, Pao, & Huang, 2011; Pao, Pan, Leu, & Huang, 2012; Yoshikawa et al., 2011). CHWE – Cordycepin content in hot water extract of *Cordyceps sinensis*; PLC – Phospholipase C; PKC – Protein kinase C; Apaf-1 – Apoptotic peptidase activating factor 1; PIP2 – phosphatidylinositol – bisphosphate; DAG – Diacylglycerol.

6.4. Anti-cancer

Medicinal mushrooms are most promising for cancer therapy and *Cordyceps* has been effectively used as an anti-tumor herb in Chinese medicines (Ji, 1999). Several previous studies reported anti-tumor activities of natural and cultivated CS (Yamaguchi et al., 1990). Basically water or alcohol extracts contain polar compounds that contribute to anti-cancer effects. Similar anti-cancer effect of warm water extract of dried CS was studied, where Ehrlich ascites carcinoma (EAC), allogeneic to ICR mice, Meth A fibrosarcoma (Meth A), syngeneic to BALB/c mice were used as the target tumor cell lines and reported that antitumor effect of extract may be mediated through its immunomodulating action (Yoshida et al., 1989). Nakamura et al. (1999) investigated the effect of the water extract of CS (WECS) on liver metastasis of Lewis lung carcinoma (LLC) and B16 melanoma (B16) cells in mice. In both LLC and B16 experiments, water extract showed strong cytotoxic effect, not because of cordycepin but just because of other components (Nakamura et al., 1999) and further, it was reported that cordycepin exerts inhibitory effect on the growth of mouse melanoma and lung carcinoma cells by stimulating adenosine A3 receptors on tumor cells (Nakamura et al., 2006). In recent studies, water extract from dried fruiting bodies of cultured CS, is evaluated for its anti-cancer effect, where cordycepin which is a effective ingredient in water extract and found to show anti-cancer effect on B16–BL6 cells, LLC cells, HT1080 human fibrosarcoma (HT1080) cells and human colon carcinoma (CW-2) cells by stimulating adenosine A3 receptor (A3-R) (Yoshikawa et al., 2011) and which is also followed by Glycogen Synthase Kinase-3 β activation and Cyclin D1 suppression by using radioligand binding assay (Yoshikawa et al., 2008).

The methanolic extract from the fruiting bodies of CS showed inhibitory effect on tumor cell lines (K562, Vero, Wish, Calu-1, and Raji cells) (Kuo et al., 2006) and it is reported that low-molecular-weight tumor cell growth inhibitors, other than cordycepin and polysaccharides in CS are responsible for this effect. Role of ethanolic extract of CS (CS-II) as an immunopotentiating agent in treating cancer and immune deficient patients has been reported (Xu, Peng, Chen, & Chen, 1992).

Two antitumor sterols (ergosterols) isolated from the methanolic extract of cultured mycelia of CS, found to inhibit (glycosylated form of ergosterol peroxide) the proliferation of K562, Jurkat, WM-1341, HL-60 and RPMI-8226 tumor cell lines more potently (Kodama, Mc Caffrey, Yusa, and Mitsuya (2000)). Furthermore, chloroform and n-butanol fractions of methanol extract from CS were investigated for *in vitro* tumor cell growth inhibitory activities. Tested extracts showed dose dependent inhibition of enhance production TNF- α and IL-12 in LPS/IFN- γ activated murine peritoneal macrophages. On the other hand, these extracts were also evaluated for their tumor-cell proliferation activities in different type of cancer cell lines such as Jurkat, HepG2, PC 3, Colon 205, and MCF 7 as well as normal PBMCs and showed growth inhibition on these cancer cells but no cytotoxic effect towards normal PBMCs up to the concentration range studied (0–150 μ g/ml) (Rao, Fang, & Tzeng, 2007). An inhibitory effect of CS is re-

ported by some other researchers also (Chen, Shiao, Lee, & Wang, 1997; Du, 1986; Lin, 1984; Zhang, 1987).

6.5. Anti-diabetes activity

Diabetes is a serious widespread health issue especially among populations that rely on the Western diet. A large body of research has been conducted on the beneficial effects of CS on the blood glucose metabolism and on its potential as a blood sugar regulation agent.

In an *in vivo* pharmacology study, Cordy Max™ Cs-4, a mycelial fermentation product of CS, used to check its efficacy for improving glucose metabolism and insulin sensitivity. Study interventions were defined for 17 days to adult Wistar rats (male and female) and fasting blood glucose, fasting plasma insulin, glucose-insulin index, and oral glucose tolerance were measured. It caused: (1) reduction in fasting blood glucose, (2) 37% decrease in fasting plasma insulin in the high dose treatment groups, (3) rise in glucose-insulin index, (4) improved glucose tolerance at 0.5, 1.0, and 2.0 h after oral administration of a bolus of glucose (Zhao et al., 2002).

In a study of comparison of anti-hyperglycemic activity of natural and fermented CS, male Wistar rats were induced diabetes by nicotinamide and streptozotocin. These rats were orally administered a placebo (STZ group), fruiting bodies (FB group, 1 g/day), fermented mycelia (MCS group, 1 g/day), fermented broth (BCS group, 1 g/day), or fermented mycelia plus broth (XCS group, 0.5 g/day of each) of CS. Blood glucose concentration and the serum concentrations of fructosamine were significantly lower in the FB, MCS, BCS, and XCS groups than in the STZ group. This indicates that, the fermented products of CS could be developed as potential anti-diabetic agents or functional foods for persons with a high risk of diabetes mellitus (Lo, Hsu, Tu, & Lin, 2006). Similar clinical study on patients with hyperglycemic disease when treated daily with CS (3 g showed improvement in their blood sugar measurement in 95% while those that received mainstream medical treatment showed only 54% improvement (http://www.advancedalternativescenter.com/Marvlix_with_Cordyceps_Sinensis_p/marvlix.ht). *Cordyceps* improves blood glucose metabolism and increases insulin sensitivity in normal animals (Zhao et al., 2002).

6.6. Anti-inflammatory activity

Anti-inflammatory processes are considered to be a risk or trigger factor for human stroke. Many effective pharmacological agents are employed for the treatment of inflammatory diseases and new pharmacological targets continue to be explored which is an important area for translational medicine. Recently CS is found to be effective against inflammatory reactions.

In one study crude extracts and partial purified fractions of mycelia of CS were tested for their inhibitory effects on superoxide anion generation and elastase release by human neutrophils in response to N-formyl-methionyl-leucyl phenylalanine/cytochalasin B (FMLP/CB). For the first time, Cordysinins A–E (1–5) five constituents have been identified and found to display significant inhibition (Yang, Kuo, Hwang, & Wu, 2011). Cordymin, a purified peptide from this fungus

found to display their action against cytokines (IL-1 β and TNF- α) to decrease the severity of the inflammatory reaction. Cordymin-2 and cordymin-4 showed 53 and 73% inhibition, respectively (Qian et al., 2012). Liu, Li, Zhao, Tang, and Guo (2011) demonstrated the neuroprotective potential of CS mycelium inhibition through anti-inflammation in a rat model of ischaemia-reperfusion (Liu et al., 2011). Chloroform and n-butanol fractions of methanol extract from CS were investigated for their anti-inflammatory activity. Tested extracts showed dose dependent inhibition of enhanced production of inflammatory mediators such as nitric oxide (NO) through reducing inducible NO synthase expression (Rao et al., 2007).

6.7. Kidney protection

For a long time in the treatment of nephritis, CS was found to deliver major contributions and has been used successively. An *in vitro* model aimed at solving problem of pathogenesis of immunoglobulin a nephropathy (IgAN) due to harmful action of cytokines and growth factors released by mesangial cells upon stimulation by nephritogenic IgA immune complexes (IgAIC). Therefore human mesangial cells (HMC) activating mode was established and incubated with IL-1 and IL-6. Crude MeOH extract obtained from fruiting bodies of CS, further subjected to fractionation by silica gel column chromatography to have 6 fractions. Fraction (F-2), one among them found to be effective against IL-1 and IL-6. Further in IgAN animal model where hematuria and proteinuria was induced by R36A (Pneumococcal C-polysaccharide purified from *Streptococcus pneumoniae*) as antigen and anti-R36A IgA monoclonal antibody. The mice in the IgAN model fed with 1% F-2 in diet had significant reduction of hematuria and proteinuria together with histopathologic improvement. H1-A, a purified compound can suppress the activated HMC and alleviate IgAN (Berger's disease) with clinical and histologic improvement (Ding et al., 2011).

Due to severe side effects, high doses of cyclosporin A (CsA) cannot be used in the long term treatment of kidney allograft recipients. When renal transplant patients were treated with CS it caused: (1) least incidences of complications in the treatment group, (2) no significant difference in the serum level of IL-2 in two groups and the serum level of IL-10 in treatment group. The study proposed that CS may be used in combination with a low dose of CsA in the long term treatment of kidney transplant patients (Ding et al., 2009, 2011) and also in similar acute and chronic experiments CS showed to protect the kidney from cyclosporine A nephrotoxicity (CsA-Nx) and ameliorate the glomerular and interstitial injuries (Zhao & Li, 1993). In another study, effect of CS on aminoglycoside (AG) induced nephrotoxicity was evaluated. Rats with renal tubular injury showed healing improvements after treatment which was evidenced by less prominent increment of BUN, SCr, sodium excretion, urinary NAGase and less severity of histopathological changes as compared with control (Li, Zheng, & Liu, 1996). In a similar study, the protective effect on aminoglycoside nephrotoxicity by CS in the old patients was also observed (Bao, Wu, & Zheng, 1994; Zhu & Rippe, 2004).

The curative effects of CS on chronic kidney disease has been demonstrated (Zhong et al., 2011). CS was tested on rats

with 5/6 nephrectomy and at 4 and 8 weeks after 5/6 nephrectomy, rats were sacrificed the kidneys, serum and urine collected for ^1H NMR spectral analysis. The curative effect of CS was also confirmed by metabolomics results.

Many human trials have been conducted to test the efficacy of CS to prevent lung and kidney damage. Zhou et al. (2008) worked on renal health enhancing potential of CS (http://www.advancedalternativescenter.com/Marvlix_with_Cordyceps_Sinensis_p/marvlix.ht). They discovered that this effect may come from significant increased production of 17-hydroxy-corticosteroid and 17-ketosteroid levels (Hobbs, 1995). Guan et al. (1992) conducted a trial on 51 patients with chronic renal failure, which is a life-threatening condition that often affects the elderly. When these patients were given the drug (CS at 3–5 g/day), it showed significant improvement in kidney function (Guan et al., 1992). Sometimes kidneys failure is often linked to problems occurring in other organs and systems such as hypertension, proteinuria and anemia. A study involving subjects suffering from above said ailments, were treated with CS. CS decreased blood pressure (15%) after one month, significantly reduced urinary protein and increased superoxide dismutase enzymes. The study further suggests that an increase in the free radical-scavenging capability of oxygen in the bloodstream, which would result in reduced oxidative cellular damage. Further, 57 patients with gentamicin-induced kidney damage were treated with 4.5 g of *Cordyceps* per day. CS improved normal kidney function (89%) as compared to control group (45%) after six days, indicating CS effectively shortens the time to recovery significantly (Zhu & Rippe, 2004).

Hong, Zhang, and Fan (2012), in one of their intervention protocol for review discussed about common uses of CS as an adjuvant immunosuppressive agent in both initial long term maintenance treatment for kidney transplant recipients in China (Hong et al., 2012) and some other study has been conducted that provide essential information on CS, a key to kidney health (Xu, Huang, Jiang, Xu, & Mi, 1995).

6.8. Lung and liver protection

Many respiratory illnesses like asthma, COPD and bronchitis have been treated by CS, which has been used as a promoter of respiratory health in China for more than a thousand years. Recently many clinical trials have been undertaken to prove *Cordyceps*' ability to enhance oxygen utilization and cellular oxygen uptake. Mice were treated with CS to look for their survival status in low oxygen environments. Mice were survived up three times longer than those untreated accepting scientific fact that CS has ability to utilize oxygen efficiently (Zhu & Rippe, 2004) and improves respiratory function (Han, 1995; Zheng & Deng, 1995).

Wang, Leeb, Chenc, Yud, and Duh (2012) investigated the hepatoprotective actions of water extracts of both CS and *Cordyceps militaries* and compared among them. *Cordyceps militaries* extract (CME) and CS extract (CSE) at 500 $\mu\text{g/ml}$ were treated with t-butyl hydroperoxide (t-BHP) induced HepG2 cells. Extracts showed no significance difference in their activity against reactive oxygen species (ROS) generation, glutathione stimulating hormone (GSH) content, TBARS formation and Bcl-2/Bax ratio. But CME showed better inhibitory

effect on caspase 3 activity and CSE exhibited superior activity against mitochondrial membrane potential (Wang et al., 2012).

6.9. Apoptosis

Apoptosis is a programmed cell death induced either via extrinsic factors through activation of Caspases 8 pathway or intrinsic factors through mitochondrial pathway. Caspases have great role in apoptotic signaling pathway and contribute to overall apoptotic morphology by causing irreversible damages to cellular components.

Sterols in methanol and ethyl acetate extract of cultured mycelium of CS reported to show a potent apoptotic effect on pro-myelocytic leukemia HL-60 cells, causing DNA fragmentation which is a characteristic of apoptotic cells (Matsuda et al., 2009). Similar work demonstrates, cordycepin in CS extract induced apoptosis of MA-10 Mouse Leydig Tumor Cell through the activation of caspase-9 and -3 and -7 pathway. The cordycepin treatment resulted in rounding-up of cell, plasma membrane blebbing and DNA fragmentation by arresting pre-mitosis synthesis phases. The study revealed mechanism of apoptotic mediated anticancer effect of cordycepin (Jen, Lin, Huang, & Leu, 2011) Fig. 4. Similar study reported the apoptosis of HL-60 cells by CS mycelia extract (Zhang & Wu, 2007; Zhang et al., 2004) and anti-apoptotic properties of CS (Buenz, Weaver, Bauer, Chalpin, & Badley, 2004).

6.10. Aphrodisiac activity

Recently aphrodisiac activity has been reported due to suspected testosterone like metabolites and libido-promoting activity in CS. Wang et al. reported that CS contains a factor that stimulates corticosteroid production in the animal model. In the study, a water-soluble extract of CS was used to investigate its pharmacological function on primary rat adrenal cell cultures and the signaling pathway involved. But authors are not sure about the mechanism of CS induced steroidogenesis, whether it acts directly on the adrenal glands or indirectly via the hypothalamus–pituitary axis (Wang, Lee, Lin, & Chang, 1998). A study reported that hot water extract has a mild beneficial effect on sexual function in castrated rats (Ji et al., 2009).

A clinical study reported that CS supplement to 22 males, showed increased sperm count (33%) and decreased incidence of sperm malformations (29%) (Guo, 1986). In another study involving both men and women of 189 patients with decreased libido and desire showed improvement of symptoms and desire of 66% upon treatment with CS (Wan, Guo, & Deng, 1988). Further, after CS supplement caused prevention and improvement of adrenal glands and thymus hormones, and infertile sperm count improved by 300% (Huang et al., 1987), improvement of libido and desire at 86% in woman (Dong & Yao, 2007).

6.11. Anti-fatigue and improves stamina

Many studies were conducted to prove that CS reduces fatigue and boosts stamina for athletes. Hot water (HW) fraction of mycelium of CS mainly contains carbohydrate (78.9%) was or-

ally administered to mice to determine the swimming endurance capacity using an adjustable current swimming pool. CS was found to prolong swimming time (75–90 min) of test groups as compared to the control indicating HWs extract has effect on recovery from exhaustion with lessening of fatigue (Koh et al., 2003). And in similar studies, mice demonstrated their improved swimming capabilities after 6 weeks of CS supplementation compared with a control group (Xiao et al., 1999). In an *in vivo* pharmacology study, effects of CordyMax Cs-4, a mycelial fermentation product of CS, on energy metabolism was evaluated. In mice administered with CS-4, they found an increased level of β adenosine triphosphate (ATP) in liver suggesting a higher hepatic bioenergy status, suggesting clinical effectiveness of CordyMax in alleviating fatigue and improving physical endurance, especially in elderly subjects (Dai, 2001).

Unbelievable performance by Chinese women athletes at Chinese National Games in Beijing in September 1993 astounded the world of international track and field. This has attracted international attention to caterpillar fungus (Steinkraus, 1994).

7. Future prospects

C. sinensis is a medicinal edible mushroom which can undoubtedly be supplemented in ordinary foods, health foods, functional foods or as nutraceuticals. But a thorough scientific research on compositional analysis for judging the nutritional quality and bio-activity of CS is necessary. There is a need for *in vivo* validation to demonstrate its health potential and to identify, characterize new biomolecules to link their biological action for value addition. Efficient artificial cultivation methods for CS are needed to be followed to obtain wet or dry biomass for large scale extraction of bio-active principles. A thorough research on established principles of science is necessary on efficient downstream processing of CS in a cost effective manner and for sustainable production using *in vitro* technologies. Possible enhanced role of CS as such or its extracts in food and pharmaceutical industries to develop health, functional foods and nutraceuticals with unquestionable scientific evidence is the need of the hour. Furthermore studies on encapsulation of extract of CS to formulate in deliverable forms such as liposomes and food emulsions to engineer sustainable drug delivery systems.

8. Conclusions

C. sinensis is one of the miracle traditional Chinese medicines growing in popularity because of its attributed extraordinary health benefits like enhanced physical stamina for superior performance, anti-cancer and protection for lungs and kidneys. This mushroom because of its outstanding curative properties has huge demand as health care product and fetching huge financial gains for the collectors. Its popularity and demand has further confirmed the great importance of Eastern system of medicines.

The mechanisms of action of bio-active compounds of CS still elude scientific inquiry and too many other problems of the biochemical pathways need to be unraveled. There is an

urgent need to devise methods of effective cultivation methods and to ensure effective process technologies for utmost recovery of bio-active principles. Further, crude extracts of CS needs to be well characterized chemically rather than extrapolating *in vitro* information just to imply therapeutic importance. Moreover, bio guided fractionation and linking the active components to various bio-activities in the CS offers a lot of scope for research. Though it shows potent pharmacological actions but mechanism behind these effects at molecular level interaction in the human system is unclear and these must be a thorough scientific scrutiny to answer every aspects of CS.

Acknowledgements

The author (S.M.G.) acknowledges the award of senior research fellowship from Council of Scientific and Industrial Research, New Delhi, India. The authors also acknowledge the Director, CSIR-Central Food Technological Research Institute, Mysore, India for the encouragement given.

REFERENCES

- Bao, Z. D., Wu, Z. G., & Zheng, F. (1994). Amelioration of aminoglycoside nephrotoxicity by *Cordyceps sinensis* in old patients. *Chinese Journal of Integrated Traditional and Western Medicines*, 14(271–3), 259.
- Bikash, B., & Jyoti, M. (2012). *In-vitro* culture of *Ophicordyceps sinensis* (Yarsagumba) and their associated endophytic fungi of Nepal Himalaya. *Scientific World*, 10, 38–42.
- Bok, J. W., Lerner, L., Chilton, J., Klingeman, H. G., & Towers, G. H. (1999). Antitumor sterols from the mycelia of *Cordyceps sinensis*. *Phytochemistry*, 51, 891–898.
- Buenz, E. J., Weaver, J. G., Bauer, B. A., Chalpin, S. D., & Badley, A. D. (2004). *Cordyceps sinensis* extracts do not prevent Fas-receptor and hydro-gen peroxide-induced T-cell apoptosis. *Journal of Ethnopharmacology*, 90, 57–62.
- Cai, G. Z., Zhang, Y., Chen, S., & Yin, D. (2004). Scavenging effect of *Cordyceps* growing in different environment on hydroxyl radical. *Chinese Traditional and Herbal Drugs*, 35, 57–59 [in Chinese].
- Camila, G. P., Pereira, M., Angela, A., & Meireles, M. A. A. (2010). Supercritical fluid extraction of bioactive compounds: Fundamentals, applications and economics perspectives. *Food Bioprocess Technology*, 3, 340–372.
- Chen, Y. P. (1983). Studies on immunological actions of *Cordyceps sinensis*. I. Effect on cellular immunity. *Bulletin of Chinese Materia Medica*, 8, 33–35.
- Chen, D. M. (1985). The effect of natural *Cordyceps sinensis* and its cultured mycelia on murine immuno-organs and function of the mononuclear macrophage system. *Chinese Journal of Modern Developments in Traditional Medicine*, 5, 42–44.
- Chen, G. Z., Chen, G. L., Sun, T., Hsieh, G. C., & Henshall, J. M. (1991). Effects of *Cordyceps sinensis* on murine T lymphocyte subsets. *Chinese Medical Journal (English)*, 104, 4–8.
- Chen, S., & Chu, J. (1996). NMR and IR studies on the characterization of cordycepin and 2'-deoxyadenosine. *Chinese Journal of Natibiotics*, 21, 9–12.
- Chen, K., & Li, C. (1993). Recent advances in studies on traditional Chinese anti-aging materia medica. *Journal of Traditional Chinese Medicine*, 13, 223–226.
- Chen, Y. J., Shiao, M. S., Lee, S. S., & Wang, S. Y. (1997). Effect of *Cordyceps sinensis* on the proliferation and differentiation of human leukemic U937 cells. *Life Science*, 60, 2349–2359.
- Chen, P. X., Wang, S., Nieb, S., & Marcone, M. (2013). Properties of *Cordyceps sinensis*: A review. *Journal of Functional Foods*, 5, 550–569.
- Chen, J. R., Yen, J. H., Lin, C. C., Tsai, W. J., Liu, W. J., Tsai, J. J., Lin, S. F., & Liu, H. W. (1993). The effects of Chinese herbs on improving survival and inhibiting anti-DNA antibody production in lupus mice. *The American Journal of Chinese Medicine*, 21, 257–262.
- Chen, W., Yuan, F., Wang, K., Song, D., & Zhang, W. (2012). Modulatory effects of the acid polysaccharide fraction from one of anamorph of *Cordyceps sinensis* on Ana-1 cells. *Journal of Ethnopharmacology*, 142, 739–745.
- Chen, J., Zhang, W., Lu, T., Li, J., Zheng, Y., & Kong, L. (2006). Morphological and genetic characterization of a cultivated *Cordyceps sinensis* fungus and its polysaccharide component possessing antioxidant property in H22 tumor-bearing mice. *Life Science*, 78, 2742–2748.
- Chen, W., Zhang, W., Wenbin Shen, W., & Wang, K. (2010). Effects of the acid polysaccharide fraction isolated from a cultivated *Cordyceps sinensis* on macrophages *in vitro*. *Cellular Immunology*, 262, 69–74.
- Chiou, W. F., Chang, P. C., Chou, C. J., & Chen, C. F. (2000). Protein constituent contributes to the hypotensive and vasorelaxant activities of *Codyceps sinensis*. *Life Science*, 66, 1369–1376.
- Chiu, J. H., Ju, C. H., Wu, L. H., Lui, W. Y., Wu, C. W., Shiao, M. S., & Hong, C. Y. (1998). *Cordyceps sinensis* increases the expression of major histocompatibility complex class II antigens on human hepatoma cell line HA22T/VGH cells. *American Journal of Chinese Medicines*, 26, 159–170.
- CITES Management Authority of China. (2012). Announcement of adjustment for catalogue of exporting and importing wild animals and plants (in Chinese). In *Proceedings of the meeting held on 24–26 May 2012*, Thimphu, Bhutan (CoP16 Inf. 49).
- Cleaver, P., Holliday, J., & Powers, M. (2005). US Patent No. US, 7,407,795 B2.
- Cleaver, P., Holliday, J., & Powers, M. (2011). US Patent No. US 8,008,060 B2.
- Dai, G. B. (2001). CordyMax Cs-4 improves steady-state bioenergy status in mouse liver. *Journal of Alternative and Complementary Medicine*, 7, 231–240.
- Ding, C., Tian, P., Jia, L., Li, Y., Ding, X., Xiang, H., Xue, W., & Zhao, Y. (2009). The synergistic effects of *Cordyceps sinensis* with Cs A in preventing allograft rejection. *Frontiers in Bioscience*, 14, 3864–3871.
- Ding, C., Tian, P. X., Xue, W., Ding, X., Yan, H., Pan, X., Feng, X., Xiang, H., Hou, J., & Tian, X. (2011). Efficacy of *Cordyceps sinensis* in long term treatment of renal transplant patients. *Frontiers in Bioscience (Elite Edition)*, 1, 301–307.
- Dong, C.-H., & Yao, Y.-J. (2005). Nutritional requirements of mycelial growth of *Cordyceps sinensis* in submerged culture. *Journal of Applied Microbiology*, 99, 483–492.
- Dong, C.-H., & Yao, Y.-J. (2007). *In vitro* evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of *Cordyceps sinensis*. *LWT – Food Science and Technology*, 41, 669–677.
- Dong, C.-H., & Yao, Y.-J. (2010). On the reliability of fungal materials used in studies on *Ophicordyceps sinensis*. *Journal of Industrial Microbiology and Biotechnology*, 38, 1027–1035.
- Du, D. J. (1986). Antitumor activity of *Cordyceps sinensis* and cultured *Cordyceps* mycelia. *Bulletin of Chinese Materia Medica*, 11, 51–54.
- Guan, Y. J., Hu, Z., & Hou, M. (1992). Effect of *Cordyceps sinensis* on T-lymphocyte subsets in chronic renal failure. *Chinese Journal of Integrated Traditional and Western Medicines*, 12(338–339), 323.

- Guan, J., & Li, S. P. (2010). Discrimination of polysaccharides from traditional Chinese medicines using saccharide mapping—Enzymatic digestion followed by chromatographic analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 51, 590–598.
- Guan, J., Yang, F. Q., & Li, S. (2010). Evaluation of carbohydrates in natural and cultured *Cordyceps* by pressurized liquid extraction and gas chromatography coupled with mass spectrometry. *Molecules*, 15, 4227–4241.
- Guan, J., Zhao, J., Feng, K., Hu, D.-J., & Li, S. P. (2011). Comparison and characterization of polysaccharides from natural and cultured *Cordyceps* using saccharide mapping. *Analytical and Bioanalytical Chemistry*, 399, 3465–3474.
- Guo, Y. (1986). Medicinal chemistry, pharmacology and clinical applications of fermented mycelia of *Cordyceps sinensis* and JinShuBao capsule. *Journal of Modern Diagnostics Therapeutics*, 1, 60–65.
- Halperin, G. (1999). *Cordyceps: China's healing mushroom*. USA: Avery Publishing.
- Han, S. (1995). Experiences in treating patients of chronic bronchitis and pulmonary diseases with Cs-4 capsule (JinShuiBao). *Journal of Administration of Traditional Chinese Medicine*, 5, 33–34.
- Hobbs, C. (1995). *Medicinal mushrooms: An exploration of tradition, healing, and culture*. Santa Cruz, CA: Botanica Press.
- Holliday, J. C., & Cleaver, M. (2008). Medicinal value of the caterpillar fungi species of the genus *Cordyceps* (Fr.) Link (Ascomycetes). A review. *International Journal of Medicinal Mushrooms*, 10, 219–234.
- Holliday, J., Cleaver, P., Megan, L. P., & Patel, D. (2004). Analysis of quality and techniques for hybridization of medicinal fungus *Cordyceps sinensis* (Berk.) Sacc. (Ascomycetes). *International Journal of Medicinal Mushrooms*, 6, 151–164.
- Holliday, J., Cleaver, M., & Wasser, S. P. (2005). *Cordyceps* part 1. In *Encyclopedia of dietary supplements* (pp. 1–13). USA: Dekker Encyclopedias, Taylor and Francis Publishing.
- Hong, T., Zhang, M., & Fan, J. (2012). *Cordyceps sinensis* (a traditional Chinese medicine) for kidney transplant recipients. *Cochrane Database of Systematic Reviews*, 1–11.
- Hsieh, C., Tsai, M.-J., Hsu, T.-H., Chang, D.-M., & Lo, C.-T. (2005). Medium optimization for polysaccharide production of *Cordyceps sinensis*. *Applied Biochemistry and Biotechnology*, 120, 145–157.
- Hsu, T. H., Shiao, L. H., Hsieh, C., & Chang, D. M. (2002). A comparison of the chemical composition and bio-active ingredients of the Chinese medicinal mushroom *Dong Chong Xia Cao*, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. *Food Chemistry*, 78, 463–469.
- Huang, B. M., Chuang, Y. M., Chen, C. F., & Leu, S. F. (2000). Effects of extracted *Cordyceps sinensis* on steroidogenesis in MA-10 mouse Leydig tumor cells. *Biological and Pharmaceutical Bulletin*, 23, 1532–1535.
- Huang, L., Guo, F., Liang, Y., & Chen, B. (2004). Determination of adenosine and cordycepin in *Cordyceps sinensis* and *C. militaris* with HPLC–ESI-MS. *China Journal of Chinese Materia Medica*, 29, 762–764.
- Huang, B. M., Hsu, C. C., Tsai, S. J., Sheu, C. C., & Leu, S. F. (2001). Effects of *Cordyceps* on testosterone production in normal mouse Leydig cells. *Life Science*, 69, 2593–2602.
- Huang, Y., Lu, J., Zhu, B., Wen, Q., Jia, F., Zeng, S., Chen, T., Li, Y., Xheng, G., & Yi, Z. (1987). Prevention and improvement of adrenal glands and thymus hormones, and infertile sperm count improve by 300% after *Cordyceps* supplement. *Chinese Medicinal Preparation Research*, 10, 24–25.
- Jeffrey, C. (2012). The 'Viagra' transforming local economies in India. BBC News. Retrieved 09.07.2012.
- Jen, C. Y., Lin, C. Y., Huang, B. M., & Leu, S. F. (2011). Cordycepin induced MA-10 mouse Leydig tumor cell apoptosis through caspase-9 pathway. *Evidence-Based Complementary and Alternative Medicine*, 1–11.
- Ji, Y. B. (1999). *Pharmacological action and application of anticancer traditional Chinese medicine (150 Cordyceps sinensis (Berk) Sacc.)* p. 94. Ha'erbin, China: Heilongjiang Science and Technology Press (in Chinese).
- Ji, D. B., Ye, J., Li, C. L., Wang, Y. H., Zhao, J., & Cai, S. Q. (2009). Antiaging effect of *Cordyceps sinensis* extract. *Phytotherapy Research*, 23, 116–122.
- Jia, J., Tao, H., & Feng, B. (2009). Cordyceamides A and B from the Culture Liquid of *Cordyceps sinensis* (BERK.) SACC. *Chemical and Pharmaceutical Bulletin*, 57, 99–101.
- Jiang, -F., Liu, C.-Q., Li, D.-J., & Jin, B.-Q. (2008). Determination of cordycepin from cultured *Cordyceps sinensis* by HPLC-DAD. *Food Science*, 29, 352–354.
- Jing, M., Xiao, C., Chun, F. W., Li, J. W., & Gao, H. (2005). Cordycedipeptide A, a new cyclodipeptide from the culture liquid of *Cordyceps sinensis* (BERK.) SACC.. *Chemical and Pharmaceutical Bulletin*, 53, 582–583.
- Kan, W.-C., Wang, H.-Y., Chien, C.-C., Li, S.-L., Chen, Y.-C., Chang, L.-H., Cheng, C.-H., Tsai, W.-C., Hwang, J.-C., Su, S.-B., Huang, L.-H., & Chuu, J.-J. (2012). Effects of extract from solid-state fermented *Cordyceps sinensis* on Type 2 diabetes mellitus. *Evidence-Based Complementary and Alternative Medicines*, 2, 1–10.
- Kiho, T., Ookubo, K., Usui, S., Ukai, S., & Hirano, K. (1999). Structural features and hypoglycemic activity of a polysaccharide (CS-F10) from the cultured mycelium of *Cordyceps sinensis*. *Biological and Pharmaceutical Bulletin*, 22, 966–970.
- Kiho, T., Tabata, H., & Ukai, S. (1986). A minor, protein containing galactomannan from asodium carbonate extract of *Cordyceps sinensis*. *Carbohydrate Research*, 156, 189–197.
- Kiho, T., Yamane, A., Hui, J., Usui, S., & Ukai, S. (1996). Polysaccharides in fungi. XXXVI. Hypoglycemic activity of a polysaccharide (CS-F30) from the cultural mycelium of *Cordyceps sinensis* and its effect on glucose metabolism in mouse liver. *Biological and Pharmaceutical Bulletin*, 19, 294–296.
- Kim, S. D. (2010). Isolation, structure and cholesterol esterase inhibitory activity of a polysaccharide, PS-A, from *Cordyceps sinensis*. *Journal of the Korean Society for Applied Biological Chemistry*, 53, 784–789.
- Kim, H., & Yun, J. (2005). A comparative study on the production of exopolysaccharides between two entomopathogenic fungi *Cordyceps militaris* and *Cordyceps sinensis* in submerged mycelial cultures. *Journal of Applied Microbiology*, 99, 728–738.
- Kodama, E. N., Mc Caffrey, R. P., Yusa, K., & Mitsuya, H. (2000). Antileukemic activity and mechanism of action of cordycepin against terminal deoxynucleotidyl transferase-positive (TdT+) leukemic cells. *Biochemical Pharmacology*, 59, 273–281.
- Koh, J. H., Kim, K. M., Kim, J. M., Song, J. C., & Suh, H. J. (2003). Antifatigue and antistress effect of the hot-water fraction from mycelia of *Cordyceps sinensis*. *Biological and Pharmaceutical Bulletin*, 26, 691–694.
- Kuo, M. C., Chang, C. Y., Cheng, T. L., & Wu, M. J. (2007). Immunomodulatory effect of exo-polysaccharides from submerged cultured *Cordyceps sinensis*: Enhancement of cytokine synthesis, CD11b expression, and phagocytosis. *Applied Microbiology and Biotechnology*, 75, 769–775.
- Kuo, C. F., Chen, C. C., Luo, Y. H., Huang, R. Y., Chuang, W. J., Sheu, C. C., & Lin, Y. S. (2005). *Cordyceps sinensis* mycelium protects mice from group A streptococcal infection. *Journal of Medical Microbiology*, 54, 795–802.
- Kuo, Y. C., Lin, C. Y., Tsai, W. J., Wu, C. L., Chen, C. F., & Shiao, M. S. (1994). Growth inhibitors against tumor cells in *Cordyceps sinensis* other than cordycepin and polysaccharides. *Cancer Investigation*, 12, 611–615.

- Kuo, H. C., Sua, Y. L., Yang, H. L., Huang, I. C., & Chen, T. Y. (2006). Differentiation of *Cordyceps sinensis* by a PCR-single-stranded conformation polymorphism-based method and characterization of the fermented products in Taiwan. *Food Biotechnology*, 20, 161–170.
- Kuo, Y. C., Tsai, W. J., Shiao, M. S., Chen, C. F., & Lin, C. Y. (1996). *Cordyceps sinensis* as an immunomodulatory agent. *American Journal of Chinese Medicines*, 24, 111–125.
- Kuo, Y. C., Tsai, W. J., Wang, J. Y., Chang, S. C., Lin, C. Y., & Shiao, M. S. (2001). Regulation of bronchoalveolar lavage fluids cell function by the immunomodulatory agents from *Cordyceps sinensis*. *Life Science*, 68, 1067–1082.
- Leu, S. F., Poon, S. L., Pao, H. Y., & Huang, B. M. (2011). The *in vivo* and *in vitro* stimulatory effects of cordycepin on mouse Leydig cell steroidogenesis. *Bioscience, Biotechnology, Biochemistry*, 75, 723–731.
- Leung, P., & Wu, J. (2007). Effects of ammonium feeding on the production of bio-active metabolites (cordycepin and exopolysaccharides) in mycelial culture of a *Cordyceps sinensis* fungus. *Journal of Applied Microbiology*, 103, 1942–1949.
- Leung, P., Zhang, Q., & Wu, J. (2005). Mycelium cultivation, chemical composition and antitumour activity of a *Toypocladium* sp. fungus isolated from wild *Cordyceps sinensis*. *Journal of Applied Microbiology*, 101, 275–283.
- Li, C., Chiang, C. S., Tsai, M. L., Hseu, R., Shu, W., Chuang, C., Sun, Y., Chang, Y., Lin, J., Chen, C., Huang, C., & Hsu, I. (2009). Two-sided effect of *Cordyceps sinensis* on dendritic cells in different physiological stages. *Journal of Leukocyte Biology*, 85, 987–995.
- Li, F., Gao, X. Y., Rao, B. F., Liu, L., Dong, B., & Cui, L. Q. (2006a). Effects of *Cordyceps sinensis* alcohol extractive on serum interferon-gamma level and splenic T lymphocyte subset in mice with viral myocarditis. *Chinese Journal of Cellular and Molecular Immunology*, 22, 321–323.
- Li, Y. H., & Li, X. L. (1991). Determination of ergosterol in *Cordyceps sinensis* and *Cordyceps* black-bone chicken capsules by HPLC. *Acta Pharmaceutica Sinica*, 26, 768–771.
- Li, S. P., Li, P., Dong, T. T., & Tsim, K. W. (2001a). Anti-oxidation activity of different types of natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia. *Phytomedicines*, 8, 207–212.
- Li, S. P., Li, P., Dong, T. T., & Tsim, K. W. (2001b). Determination of nucleosides in natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia by capillary electrophoresis. *Electrophoresis*, 22, 144–150.
- Li, S. P., Li, P., Ji, H., Zhang, P., Dong, T. T., & Tsim, K. W. (2001c). The contents and their change of nucleosides from natural *Cordyceps sinensis* and cultured *Cordyceps* mycelia. *Acta Pharmaceutica Sinica*, 36, 436–439.
- Li, S. P., Li, P., Lai, C. M., Gong, Y. X., Kan, K. K., Dong, T. T., Tsim, K. W., & Wang, Y. T. (2004). Simultaneous determination of ergosterol, nucleosides and their bases from natural and cultured *Cordyceps* by pressurised liquid extraction and high-performance liquid chromatography. *Journal of Chromatography*, 1036, 239–243.
- Li, S.-P., Su, Z.-R., Dong, T. T., & Tsim, K. W. (2002). The fruiting body and its caterpillar host of *Cordyceps sinensis* show close resemblance in main constituents and anti-oxidation activity. *Phytomedicines*, 9, 319–324.
- Li, S. P., Yang, F. Q., & Tsim, K. W. K. (2006b). Quality control of *Cordyceps sinensis*, a valued traditional Chinese medicine. *Journal of Pharmaceutical and Biomedical Analysis*, 41, 1571–1584.
- Li, Q., Zeng, W., Yin, D., & Huang, T. (1998). A preliminary study on alternation of generations of *Cordyceps sinensis* (Berkey) Sacc. *China Journal of Chinese Materia Medica*, 23(210–2), 254.
- Li, S. P., Zhao, K. J., Ji, Z. N., Song, Z. H., Dong, T. T., Lo, C. K., Cheung, J. K. H., Zhu, S. Q., & Tsim, K. W. (2003). A polysaccharide isolated from *Cordyceps sinensis*, a traditional Chinese medicine, protect PC12 cells against hydrogen peroxide-induced injury. *Life Science*, 73, 2503–2513.
- Li, L. S., Zheng, F., & Liu, Z. H. (1996). Experimental study on effect of *Cordyceps sinensis* in ameliorating aminoglycoside induced nephrotoxicity. *Chinese Journal of Integrated Traditional and Western Medicine*, 16, 733–737.
- Lin, P. Z. (1984). Inhibitory effect of *Cordyceps* on carcinogenesis of the fore stomach in mice. *Chinese Journal of Oncology*, 6, 335–337.
- Lin, C. Y., Ku, F. M., Kuo, Y. C., Chen, C. F., Chen, W. P., Chen, A., & Shiao, M. S. (1999). Inhibition of activated human mesangial cell proliferation by the natural product of *Cordyceps sinensis* (H1-A): An implication for treatment of IgA mesangial nephropathy. *Journal of Laboratory and Clinical Medicine*, 133, 55–63.
- Liu, Z., Li, P., Zhao, D., Tang, H., & Guo, J. (2011). Anti-inflammation effects of *Cordyceps sinensis* mycelium in focal cerebral ischemic injury rats. *Inflammation*, 34, 639–644.
- Liu, C., Lu, S., & Ji, M. R. (1992). Effects of *Cordyceps sinensis* (CS) on *in vitro* natural killer cells. *Chinese Journal of Integrated Traditional and Western Medicine*, 12, 267–269.
- Lo, H.-C., Hsu, T.-H., Tu, S.-T., & Lin, K.-C. (2006). Anti-hyperglycemic activity of natural and fermented *Cordyceps sinensis* in rats with diabetes induced by nicotinamide and streptozotocin. *American Journal of Chinese Medicines*, 34, 819.
- Matsuda, H., Akaki, J., Nakamura, S., Okazaki, Y., Kojima, H., Tamesada, M., & Yoshikawa, M. (2009). Apoptosis-inducing effects of sterols from the dried powder of cultured mycelium of *Cordyceps sinensis*. *Chemical and Pharmaceutical Bulletin (Tokyo)*, 57, 411–414.
- Mishra, R. N., & Upadhyay, Y. (2011). *Cordiceps sinensis*: The Chinese Rasayan-current research scenario. *International Journal of Research in Pharmaceutical and Biomedical Science*, 2, 1503–1519.
- Mizuno, T. (1999). Medicinal effects and utilization of *Cordyceps* (Fr.) Link (Ascomycetes) and *Isaria* Fr. (Mitosporicfungi) Chinese caterpillar fungi, “Tochukaso” (review). *International Journal of Medicinal Mushrooms*, 1, 251–262.
- Nakamura, K., Yamaguchi, Y., Kagota, S., Kwon, Y. M., Shinozuka, K., & Kunitomo, M. (1999). Inhibitory effect of *Cordyceps sinensis* on spontaneous liver metastasis of Lewis lung carcinoma and B16 melanoma cells in syngeneic mice. *Japanese Journal of Pharmacology*, 79, 335–341.
- Nakamura, K., Yoshikawa, N., Yamaguchi, Y., Kagota, S., Shinozuka, K., & Kunitomo, M. (2006). Antitumor effect of cordycepin (3'-deoxyadenosine) on mouse melanoma and lung carcinoma cells involves adenosine A3 receptor stimulation. *Anticancer Research*, 26, 43–48.
- Nie, S.-P., Guia, S. W., Phillips, A. O., Xie, M.-Y., Phillips, G. O., Assaf, S. A., & Zhang, X. (2011). Elucidation of the structure of a bio-active hydrophilic polysaccharide from *Cordyceps sinensis* by methylation analysis and NMR spectroscopy. *Carbohydrate Polymers*, 84, 894–899.
- Pao, H., Pan, B., Leu, S., & Huang, B. (2012). Cordycepin stimulated steroidogenesis in MA-10 mouse Leydig tumor cells through the protein kinase C pathway. *Journal of Agricultural and Food Chemistry*, 60, 4905–4913.
- Qian, G., Pan, G.-F., & Guo, J.-Y. (2012). Anti-inflammatory and antinociceptive effects of cordymin, a peptide purified from the medicinal mushroom *Cordyceps sinensis*. *Natural Product Research*, 1–5.
- 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.
- Rhee, K. H. (2004). Cyclic dipeptides exhibit synergistic, broad spectrum antimicrobial effects and have anti-mutagenic properties. *International Journal of Antimicrobial Agents*, 24, 423–427.

- Sharma, S. (2004). Trade of *Cordyceps sinensis* from high altitudes of the Indian Himalaya: Conservation and biotechnological priorities. *Current Science*, 86, 1614–1619.
- Shiao, M., Wang, Z., Lin, L., Lien, J., & Wang, J. (1994). Profiles of nucleosides and nitrogen bases in Chinese medicinal fungus *Cordyceps sinensis* and related species. *Botanical Bulletin of Academia Sinica*, 35, 261–267.
- Smith, J. E., Rowan, N. J., & Tan, K. K. (2000). Functional food science and the medicinal mushrooms. *International Journal Medicinal Mushrooms*, 2, 277–285.
- Steinkraus D. C. W. (1994). Chinese caterpillar fungus and world record runners. United State of America.
- Sun, Y., Zhang, X., & Lei, P. (2003). Study on character for separation and extraction of polysaccharide from hyphae of *Cordyceps sinensis*. *Acta Chinese Medicine and Pharmacology*, 2, 3.
- Toshio, M., Naoko, O., & Haruki, Y. (1977). Studies on fungal polysaccharides. XX. Galactomannan of *Cordyceps sinensis*. *Chemical and Pharmaceutical Bulletin*, 25, 3324–3328.
- Tsai, Y., Lin, L., & Tsai, T. (2010). Pharmacokinetics of adenosine and cordycepin, a bio-active constituent of *Cordyceps sinensis* in rat. *Journal of Agricultural and Food Chemistry*, 58, 4638–4643.
- Wan, F., Guo, Y., & Deng, X. (1988). Research on animal studies shows *Cordyceps* increases natural sex hormones. *Chinese Traditional Patented Medicine*, 9, 29–31.
- Wang, G.-D. (1995). *Cordyceps* species: Ecology, cultivation and application. Beijing, China: Science and Technology Reference Press (in Chinese).
- Wang, S. M., Lee, L. J., Lin, W. W., & Chang, C. M. (1998). Effects of a water-soluble extract of *Cordyceps sinensis* on steroidogenesis and capsular morphology of lipid droplets in cultured rat adrenocortical cells. *Journal of Cellular Biochemistry*, 69, 483–489.
- Wang, B. S., Leeb, C. P., Chenc, Z. T., Yud, H. M., & Duh, P. D. (2012). Comparison of the hepatoprotective activity between cultured *Cordyceps militaris* and natural *Cordyceps sinensis*. *Journal of Functional Foods*, 4, 489–495.
- Wang, S. Y., & Shiao, M. S. (2000). Pharmacological functions of Chinese medicinal fungus *Cordyceps sinensis* and related species. *Journal of Food and Drug Analysis*, 8, 248–257.
- Wang, B., Won, S., Yu, Z., & Su, C. (2005). Free radical scavenging and apoptotic effects of *Cordyceps sinensis* fractionated by supercritical carbon dioxide. *Food and Chemical Toxicology*, 43, 543–552.
- Winkler, D. (2009). Caterpillar fungus (*Ophiocordyceps sinensis*) production and sustainability on the Tibetan Plateau and in the Himalayas. *Asian Medicines*, 5, 291–316.
- Wu, C.-Q., Chen, Y., & Hao, Y. (2009). Solid fermentation production of *Cordyceps sinensis* polysaccharide. *Food Science*, 30, 186–189.
- Wu, Y., Hu, N., Pan, Y., Zhou, L., & Zhou, X. (2007b). Isolation and characterization of a mannoglucan from edible *Cordyceps sinensis* mycelium. *Carbohydrate Research*, 342, 870–875.
- Wu, Y., Sun, H., Qin, F., Pan, Y., & Sun, C. (2006). Effect of various extracts and a polysaccharide from the edible mycelia of *Cordyceps sinensis* on cellular and humoral immune response against ovalbumin in mice. *Phytotherapy Research*, 20, 646–652.
- Wu, J., Zhang, Q., & Leung, P. (2007a). Inhibitory effects of ethyl acetate extract of *Cordyceps sinensis* mycelium on various cancer cells in culture and B16 melanoma in C57BL/6 mice. *Phytomedicines*, 14, 43–49.
- Xiao, Y., Huang, X. Z., Chen, G., Wang M. B., Zhu, J. S., & Cooper, C. B. (1999). Increased aerobic capacity in healthy elderly humans given fermented *Cordyceps Cs-4*: A placebo controlled trial. *Annual Meeting American College of Sports Medicine*. Seattle, WA.
- Xu, F., Huang, J. B., Jiang, L., Xu, J., & Mi, J. (1995). Amelioration of cyclosporin nephrotoxicity by *Cordyceps sinensis* in kidney transplanted recipients. *Nephrology Dialysis Transplantation*, 10, 142–143.
- Xu, R. H., Peng, X. E., Chen, G. Z., & Chen, G. L. (1992). Effects of *Cordyceps sinensis* on natural killer activity and colony formation of B16 melanoma. *Chinese Medical Journal*, 105, 97–101.
- Yalin, W., Cuirong, S., & Yuanjiang, P. (2005). Structural analysis of a neutral (1–3), (1–4)- α -D-glucan from the mycelia of *Cordyceps sinensis*. *Journal of Natural Products*, 68, 812–814.
- Yalin, W., Cuirong, S., & Yuanjiang, P. (2006). Studies on isolation and structural features of a polysaccharide from the mycelium of an Chinese edible fungus (*Cordyceps sinensis*). *Carbohydrate Polymers*, 63, 51–256.
- Yamaguchi, Y., Kagota, S., Nakamura, K., Shinozuka, K., & Kunitomo, M. (2000a). Inhibitory effects of water extracts from fruiting bodies of cultured *Cordyceps sinensis* on raised serum lipid peroxide levels and aortic cholesterol deposition in atherosclerotic mice. *Phytotherapy Research*, 14, 650–652.
- Yamaguchi, Y., Kagota, S., Nakamura, K., Shinozuka, K., & Kunitomo, M. (2000b). Antioxidant activity of the extracts from fruiting bodies of cultured *Cordyceps sinensis*. *Phytotherapy Research*, 14, 647–649.
- Yamaguchi, N., Yoshida, J., Ren, L. J., Chen, H., Miyazawa, Y., Fujii, Y., Huang, Y. X., & Takamura, S. (1990). Augmentation of various immune reactivities of tumor-bearing hosts with an extract of *Cordyceps sinensis*. *Biotherapy*, 2, 199–205.
- Yan, P. S., Song, Y., Sakuno, E., Nakajima, H., Nakagawa, H., & Yabe, K. (2004). Cyclo (L-leucyl-L-prolyl) produced by *Achromobacter xylosoxidans* inhibits aflatoxin production by *Aspergillus parasiticus*. *Applied and Environmental Microbiology*, 70, 7466–7473.
- Yang, L. Y., Chen, A., Kuo, Y. C., & Lin, C. Y. (1999). Efficacy of a pure compound H1-A extracted from *Cordyceps sinensis* on autoimmune disease of MRL lpr/lpr mice. *Journal of Laboratory and Clinical Medicine*, 134, 492–500.
- Yang, F., Feng, K., Zhao, J., & Li, S. (2009). Analysis of sterols and fatty acids in natural and cultured *Cordyceps* by one-step derivatization followed with gas chromatography–mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 49, 1172–1178.
- Yang, M., Kuo, P., Hwang, T., & Wu, T. S. (2011). Anti-inflammatory principles from *Cordyceps sinensis*. *Journal of Natural Products*, 74, 1996–2000.
- Yang, J., Zhang, W., Shi, P., Chen, J., Han, X., & Wang, Y. (2005). Effects of exopoly saccharidefraction (EPSF) from a cultivated *Cordyceps sinensis* fungus on c-Myc, c-Fos, and VEGF expression in B16 melanoma-bearing mice. *Pathology – Research and Practice*, 201, 745–750.
- Yin, D. H., & Tang, X. M. (1995). Advances in the study on artificial cultivation of *Cordyceps sinensis*. *China Journal of Chinese Materia Medica*, 20, 707–709.
- Yoshida, J., Takamura, S., Yamaguchi, N., Ren, L. J., Chen, H., Koshimura, S., & Suzuki, S. (1989). Antitumor activity of an extract of *Cordyceps sinensis* (Berk.) Sacc. against murine tumor cell lines. *The Japanese Journal of Experimental Medicine*, 59, 157–161.
- Yoshikawa, N., Nishiuchi, A., Kubo, E., Yamaguchi, Y., Kunitomo, M., Kagota, S., Shinozuka, K., & Nakamura, K. (2011). *Cordyceps sinensis* acts as an adenosine A3 receptor agonist on mouse melanoma and lung carcinoma cells, and human fibrosarcoma and colon carcinoma cells. *Pharmacology & Pharmacy*, 2, 266–270.
- Yoshikawa, N., Yamada, S., Takeuchi, C., Kagota, S., Shinozuka, K., Kunitomo, M., & Nakamura, K. (2008). Cordycepin (3'-deoxyadenosine) inhibits the growth of B16–BL6 mouse melanoma cells through the stimulation of adenosine A3 receptor followed by glycogen synthase kinase-3beta activation and cyclin D1 suppression. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 377, 591–595.

- Yu, H., Wang, B., Huang, S., & Duh, P. (2006a). Comparison of protective effects between cultured *Cordyceps militaris* and natural *Cordyceps sinensis* against oxidative damage. *Journal of Agricultural and Food Chemistry*, 54, 3132–3138.
- Yu, L., Zhao, J., Li, S. P., Fan, H., Hong, M., Wang, Y., & Zhu, Q. (2006b). Quality evaluation of *Cordyceps* through simultaneous determination of eleven nucleosides and bases by RP-HPLC. *Journal of Separation Science*, 2, 953–958.
- Yu, L., Zhao, J., Zhu, Q., & Li, S. (2007). Macrophage biospecific extraction and high performance liquid chromatography for hypothesis of immunological active components in *Cordyceps sinensis*. *Journal of Pharmaceutical and Biomedical Analysis*, 44, 439–443.
- Yuan, J., Wang, J., Liu, X., Kuang, H., & Zhao, S. (2007). Simultaneous determination of free ergosterol and ergosterol esters in *Cordyceps sinensis* by HPLC. *Food Chemistry*, 105, 1755–1759.
- Yuan, J.-P., Zhao, S.-Y., Wang, J.-H., Kuang, H.-C., & Liu, X. (2008). Distribution of nucleosides and nucleobases in edible fungi. *Journal of Agricultural and Food Chemistry*, 56, 809–815.
- Zhang, S. L. (1985). Activation of murine peritoneal macrophages by natural *Cordyceps sinensis* and its cultured mycelia. *Chinese Journal of Modern Developments Traditional Medicines*, 5, 45–47.
- Zhang, S. L. (1987). Lewis lung cancer of mice treated with *Cordyceps sinensis* and its artificial cultured mycelia. *Bulletin of Chinese Materia Medica*, 12, 53–54.
- Zhang, W., Li, J., Qiu, S., Chen, J., & Zheng, Y. (2008). Effects of the exopolysaccharide fraction (EPSF) from a cultivated *Cordyceps sinensis* on immunocytes of H22 tumor bearing mice. *Phytotherapy*, 79, 168–173.
- Zhang, X. Q., Pu, Y. P., Yin, L. H., & Zhong, W. J. (2003). Studies on the scavenging effect on superoxide anion free radical and hydroxyl free radical of *Cordyceps sinensis*. *Chinese Journal of Gerontology*, 23, 773–775 (in Chinese).
- Zhang, Q., & Wu, J. (2007). *Cordyceps sinensis* mycelium extract induces human premyelocytic leukemia cell apoptosis through mitochondrion pathway. *Experimental Biology and Medicine*, 232, 52–57.
- Zhang, Q., Wu, J., Hu, Z., & Li, D. (2004). Induction of HL-60 apoptosis by ethyl acetate extract of *Cordyceps sinensis* fungal mycelium. *Life Science*, 75, 2911–2919.
- Zhang, Z., & Xia, S. S. (1990). *Cordyceps Sinensis*-I as an immunosuppressant in heterotopic. *Journal of Tongji Medical University*, 10, 100–103.
- Zhang, W., Yang, J., Chen, J., Hou, Y., & Han, X. (2005). Immunomodulatory and antitumour effects of an exopolysaccharide fraction from cultivated *Cordyceps sinensis* (Chinese caterpillar fungus) on tumour-bearing mice. *Biotechnology and Applied Biochemistry*, 42, 9–15.
- Zhang, J., Yu, Y., Zhang, Z., Ding, Y., Dai, X., & Li, Y. (2011). Effect of polysaccharide from cultured *Cordyceps sinensis* on immune function and anti-oxidation activity of mice exposed to 60Co. *International Immunopharmacology*, 11, 2251–2257.
- Zhao, X., & Li, L. (1993). *Cordyceps sinensis* in protection of the kidney from cyclosporine A nephrotoxicity. *Zhonghua Yi Xue Za Zhi*, 73(410–2), 447.
- Zhao, C. S., Yin, W. T., Wang, J. Y., Zhang, Y., Yu, H., Cooper, R., Smidt, C., & Zhu, J. S. (2002). CordyMax™ Cs-4 improves glucose metabolism and increases insulin sensitivity in normal rats. *The Journal of Alternative and Complementary Medicines*, 8, 309–314.
- Zheng, L., & Deng, W. (1995). The clinical efficacy of *Cordyceps sinensis* Cs-4 capsule in treating chronic bronchitis and its effect on pulmonary function. *Journal of Administration of Traditional Chinese Medicine*, 5((Suppl.)), 9–11.
- Zheng, H., Maoqing, Y., XIA Liqiu, X., Wenjuan, T., Liang, L., & Guolin, Z. (2006). Purification and characterization of an antibacterial protein from the cultured mycelia of *Cordyceps sinensis*. *Wuhan University Journal of Natural Science*, 11, 709–714.
- Zhong, F., Liu, X., Zhou, Q., Hao, X., Lu, Y., Guo, S., Wang, W., Lin, D., & Chen, N. (2011). ¹H NMR spectroscopy analysis of metabolites in the kidneys provides new insight into pathophysiological mechanisms: Applications for treatment with *Cordyceps sinensis*. *Nephrology Dialysis Transplantation*, 1–9.
- Zhou, X., Luo, L., Dressel, W., Shadier, G., Krumbiegel, D., Schmidtke, P., Zepp, F., & Meyer, C. U. (2008). Cordycepin is an immunoregulatory active ingredient of *Cordyceps sinensis*. *The American Journal of Chinese Medicines*, 36, 967–980.
- Zhu, J.S., & Rippe, J. (2004). In *Proceedings of the American physiological society's (APS) annual scientific conference, experimental biology*. Washington, DC.
- Zhu, J. S., Halpern, G. M., & Jones, K. (1998a). The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis*: Part I. *Journal of Alternative & Complementary Medicines*, 4, 289–303.
- Zhu, J. S., Halpern, G. M., & Jones, K. (1998b). The scientific rediscovery of a precious ancient Chinese herbal regimen: *Cordyceps sinensis*: Part II. *Journal of Alternative & Complementary Medicines*, 4, 429–457.
- Zhu, X. Y., & Yu, H. Y. (1990). Immunosuppressive effect of cultured *Cordyceps sinensis* on cellular immune responses. *Chinese Journal of Modern Developments Traditional Medicines*, 10, 485–487.