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Review Article

Cordyceps fungi: natural products, pharmacological functions and developmental products

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Abstract

Objectives Parasitic Cordyceps fungi, such as *Cordyceps sinensis*, is a parasitic complex of fungus and caterpillar, which has been used for medicinal purposes for centuries particularly in China, Japan and other Asian countries. This article gives a general idea of the latest developments in *C. sinensis* research, with regard to the active chemical components, the pharmacological effects and the research and development of products in recent years.

Key findings The common names for preparations include DongChongXiaCao in Chinese, winter worm summer grass in English. It has many bioactive components, such as 3′-deoxyadenosine, cordycepic acid and Cordyceps polysaccharides. It is commonly used to replenish the kidney and soothe the lung, and for the treatment of fatigue. It also can be used to treat conditions such as night sweating, hyposexuality, hyperglycaemia, hyperlipidaemia, asthenia after severe illness, respiratory disease, renal dysfunction, renal failure, arrhythmias and other heart disease and liver disease. Because of its rarity and outstanding curative effects, several mycelia strains have been isolated from natural Cordyceps and manufactured by fermentation technology, and are commonly sold as health food products. In addition, some substitutes such as *C. militaris* and adulterants also have been used; therefore, quality control of *C. sinensis* and its products is very important to ensure their safety and efficacy.

Summary Recent research advances in the study of Cordyceps, including Cordyceps mushrooms, chemical components, pharmacological functions and developmental products, has been reviewed and discussed. Developing trends in the field have also been appraised.

Keywords chemical components; Cordyceps; development products; pharmacological functions

Introduction

Cordyceps sinensis (Bark) Sacc. Link (Claviceptaceae) (Ascomycetes) is an insect parasitizing fungus. The name Cordyceps comes from the Latin words cord and ceps, meaning ‘club’ and ‘head’, respectively. ‘DongChongXiaCao’ (DCXC), Chinese caterpillar fungus, is the Chinese name given to the complex of larvae and fungi, which is found at high altitudes on the Qinghai-Tibetan plateau. This fungus lives primarily on the head of the larva of one particular species of moth, *Hepialus armoricanus*, but is also occasionally found growing on other moth species.[1] It is generally called ‘DCXC’ in Chinese and ‘Tockukaso’ in Japanese, meaning ‘winter-insect and summer-plant’ because of the growing process: the fungus first parasitizes the larva of some species Hepiaidae, forming a parasitic complex that comprises the remains of the caterpillar and the stroma of the fungus. Because the larvae are infected by fungi in the summer and autumn seasons, and consumed by mycelia and turned into ‘stiff worms’ in winter, they are called ‘winter worms’ (DongChong). In the spring and summer season of the following year, the stroma emerges from the ground, growing from the head of the larva, and is known as ‘summer grass’ (XiaCao).[2,3] *C. sinensis* is an invigorant in Chinese tradition and was honoured as one of the three greatest invigorants together with *Panax* and *Pilose* antlers. It has a history of hundreds of years in medicine. Its name was first seen from ‘Bei Cao Cong Xin’ written by Yiluo Wu in the Qing dynasty, 1757 AD and ‘Ben Cao Gang Mu Shi Yi’ written by

Xueming Zhao in 1765 AD. The French mycology scientist proved that *C. sinensis* is a type of Ascomycetes which parasitizes insect larvae, grows and gradually changes into a fruit body.[4] It is the combination of fungus and larva. However, it is more difficult for authors in the scientific literature to define a formal name. The preparation has been considered to have healing properties for the body and mind; DCXC is still revered by some people. The preparation does indeed contain certain bioactive ingredients (such as 3′-deoxyadenosine, cordycepic acid, cordycepic polysaccharides) that might be beneficial for the prevention and treatment of a variety of ailments, such as respiratory disease, renal dysfunction, hyposexuality and cancers. Many of the related biomedical investigations have been conducted predominantly in China, Japan, Korea and the US, and are extensive.

Modern experimental methods in biochemistry have proved that *C. sinensis* consists of active constituents such as mannitol, nucleosides, ergosterol, aminophenol and trace elements.[5] It has a broad medical effect, and its function of immunity regulation plays an important role in antitumour effects, organ transplantation and the prevention of kidney, liver and heart disease.[6,7] The Chinese Pharmacopoeia (2005) records that the main functions of *C. sinensis* are replenishing the kidney, soothing the lung, staunching bleeding and dispersing phlegm. It can also be used to treat continuous cough caused by fatigue, asthma, haemoptysis, impotence, spermatorrhoea and aches in abdomen and knee.[8] Previous studies mostly focused on aspects such as the resources of *C. sinensis*, its active chemical components and its pharmacological effects. Recently people have paid more attention to protecting the resource and seeking substitutes, and they hope to sustainably utilize this precious medical epiphyte. This article gives a general overview of the latest developments in *C. sinensis* research, with regard to the active chemical components, the pharmacological effects and the research and development of products in recent years.

Related Species and Artificial Cultivation

C. sinensis is a complex of larva and parasitic fungus. Many species in this genus are valuable medicinal fungi with broad development potential. The parasitic complex of the fungus and caterpillar, well known as 'DCXC', is only found in the prairie soil at an elevation of 3500–5000 m. It is mostly distributed in Tibet, Qinghai, Sichuan, Yunnan and Gansu province in China. The fungus and caterpillar complex is limited to *C. sinensis* and *Hepialus armoticamus* Oberthur, while complexes consisting of other species of fungi and insects are called 'ChongCao', not 'DCXC'.[9]

Cordyceps encompasses several *Cordyceps* species, which are widely used for medicinal purpose (e.g., *C. liangshanensis* Zhang, Liu et Hu, *C. taishanensis* Liu et Cao, *C. shanxiensis* Liu, Rong et Jin, *C. gansuensis* Zhang, Wang et Yan, *C. grasspara* Zang, Yang et Li, *C. kangdingensis* Zang et kingjo, *C. guizhouensis* Liu, Liang et Liu, and so on). In addition to *C. militaris*, *C. nutans* Pat. and *C. tricentri* Yasuda, some species such as *C. gunnii* (Berk.) Berk. and C. Roberts (Hook.) Gray, which are usually distributed on the Australian mainland, may be found in China (Zhang, 2003).[9] More than 400 Cordyceps species have been described worldwide and approximately 90 Cordyceps species are distributed in China;[10] *C. militaris* can be used to replace *C. sinensis*. A number of macroscopically similar species have been described, which are either considered as being homogeneous with *C. militaris*, or are simply unnoticed among large collections from a variety of hosts and life-cycle stages.[11–13] Much research into the chemical composition, pharmacological efficiency and clinical application of *C. sinensis* and its substitutes, such as *Hirsutella* spp., *Paecilomyces* spp. and *C. militaris*, is underway.[4] However, in therapeutic practice and literature citations, the term Cordyceps always refers to the species *C. sinensis*.

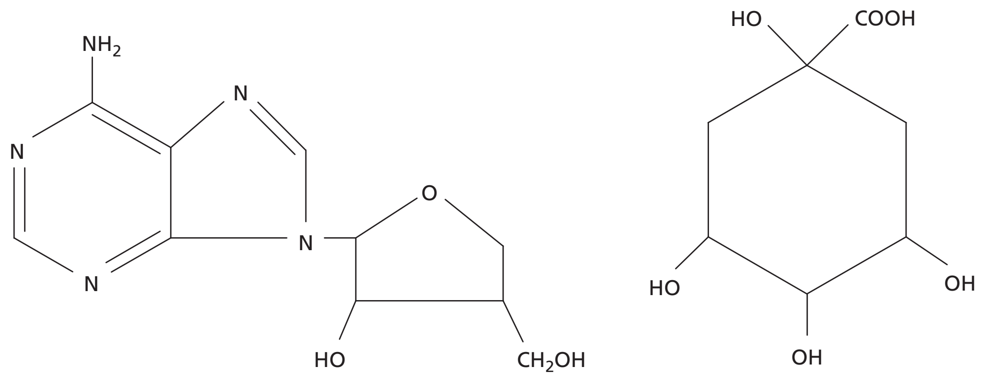
In the early 1980s, many scientific institutes began to study the cultivation of *C. sinensis*. Previous work, over the course of a decade, was mainly focused on the anamorphic fungi related to *C. sinensis*. Shen Nanying made a success of culturing fruit bodies in 1985. The Sichuan TCM Institute also achieved the artificial cultivation of *C. sinensis*, although commercial production has not been carried out because of the high cost and low stability.[14] Up to now, in fact, the artificial cultivation of this valuable fungus has not been successfully achieved; only a product made using a *C. sinensis* anamorph has been made by fermentation methods.[15] Subsequently, production of *C. sinensis*, mostly added to traditional Chinese medicine (TCM) patent prescriptions, has developed rapidly, particularly in China. Nevertheless, only *C. militaris*, producing 3′-deoxyadenosine, has similar pharmacological activity to *C. sinensis*, whose biological characteristics were studied early in the 1950s.[16] Compared with *C. sinensis*, the artificial cultivation of *C. militaris* was easier and successfully achieved in the early 1980s.[17] At that time the multi-product batch manufacture had been achieved; now the mycelia may grow in rice (or kernel) medium and produce fruiting bodies. The process of producing *C. militaris* fruiting bodies is the same as for other cultivated edible mushrooms and can be divided into two major stages. The first involves the preparation of the fruiting culture, stock culture, mother spawn and planting spawn, while the second entails the preparation of growth substrates for mushroom cultivation.[18,19] Currently, the method most widely adopted for commercial production is the cooked rice in bottle procedure (for cultivation details, see Zhang[19], Ren *et al.*,[20] Wen *et al.*[21]).

Natural Products

According to chemical analysis, *C. sinensis* contains crude fats, proteins, fibre, carbohydrate, cordycepin, cordycepic acid, polysaccharide and a series of vitamins, etc. The therapeutic applications of Cordyceps centered primarily on the key effects of increased oxygen utilization and ATP production, and as well the stabilization of blood sugar metabolism. Cordycepin, cordycepic acid and the polysaccharides, vitamins and trace elements may be, at least partially, the cause of these well-known effects.

Cordycepin and Cordycepic Acid

Although the pharmacologically active components of *C. sinensis* are still unresolved, at least two chemical



Cordycepin Cordycepic acid

Figure 1 Chemical structure of compounds in *Cordyceps sinensis.*

constituents, cordycepin and cordycepic acid, have been identified and proposed as important active constituents.[1,22] It is now believed that cordycepic acid is, in fact, D-mannitol, and that cordycepin is 3′-deoxyadenosine (3′-dA), a purine alkaloid, a derivative of the nucleoside adenosine, differing from the latter by the absence of oxygen in the 3′ position of its ribose part (Figure 1). Early in 1950 cordycepin was first isolated from *C. militaris* and its structural formula was confirmed as 3′-deoxyadenosine.[23,24] Subsequently, by means of nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy, cordycepin as a bioactive component was extracted from the fruiting bodies and cultured mycelium of *C. sinensis* and the configuration elucidated. The molecular weight of cordycepin, $C_{10}H_{13}N_5O_3$, is 251. Its melting point is 230–231°C, and maximum absorption is at 259 nm. It can be dissolved in saline, warm alcohol or methanol, but not in benzene, ether or chloroform, so many researchers in laboratories used sterilized saline and phosphate-buffered saline (PBS) as solvent.[25] In general, the content of 3′-dA in the mycelia fermentation preparations (40.8 μg/g)[26] is more than that in the fruiting bodies of *C. sinensis* (< 5.4 μg/g).[27,28]

Cordycepic acid, an isomer of quinic acid, is one of the main active medicinal components. The chemical constituents of *C. sinensis* were first studied in 1957, when a crystalline substance was isolated and the structure was imprecisely concluded to be 1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid.[29] Subsequently, the structure of the crystalline substance named ‘cordycepic acid’ was identified by Sprecher and Sprinson[30] as D-mannitol. Mannitol is a major bio-product with important biological activity, and exists in the wild in the roots, stems and leaves of plants, while more is found in edible fungus, carrot and lichens. In general, the content of cordycepic acid in DCXC is 7–29%, differing in the various growing stages of the fruiting bodies.[31] It is used not only in injections as raw material, but also as a supplement in other medicines.[32] Chemically, mannitol is an alcohol and a sugar, or a polyol; it is similar to xylitol and sorbitol. However, mannitol has a tendency to lose hydrogen ions in aqueous solution, which causes the solution to become acidic. For this reason, it is not uncommon to add a substance to adjust its pH, such as sodium bicarbonate. The chemical formula of mannitol is $C_6H_{14}O_6$, its molecular weight is 182, its melting point is 166°, its relative density is 1.489 (20°C) and its boiling point is 290–295°C (467 kPa). Mannitol, as a functional polyol with notable properties, has been widely used in the medicine and food industries. The content of mannitol varies with the original habitat. In general, there is about 29–85 mg/g in Cordyceps fruiting bodies; the mannitol content in the mycelia of Cordyceps is higher than in fruiting bodies.[33]

Polysaccharides

Cordyceps contains a large amount of polysaccharides, which can be in the range of 3–8% of the total weight,[34,35] and usually comes from the fruiting bodies, the mycelium of solid fermentation submerged cultures and the broth. Cordyceps polysaccharide (CP), one of the main bioactive components, represents a class of structurally diverse biological macromolecules with wide-ranging physiochemical properties. The anti-tumour and immunomodulating activity of the medicinal mushrooms has already been brought to scientific and commercial attention.[36,37] Since 1977, much of scientific investigation in Japan and China has been performed to discover possible functional polysaccharides; many CPs and their components have been isolated and characterized from wild and cultivated Cordyceps species (Table 1).

CP is a multi-branched galactomannan. Based on activity-guided fractionation, a water-soluble protein-containing galactomannan was isolated from the sodium carbonate extract of Cordyceps, and its molecular weight was estimated by gel filtration to be 23 kDa. The isolated compound is composed of D-mannose and D-galactose in a molar ratio of 3 : 5, and contains a small proportion of protein. It is a highly branched structure and composed of (1→6)- and (1→2)-linked α-D-mannopyranosyl residues in the main chain.[56] Cai *et al.*[60] developed a method to study the topography of CP by using an atomic force microscope (AFM). The result showed that CP has a multi-branched structure and a variety of different linkages between adjacent monosaccharides, which make up the small rings and helical structures. However, a neutral mannoglucan with a molar mass of approximately 7700 g/mol was obtained recently from CP, which consisted of Man and Glc units in the molar ratio of 1 : 9. Analysis showed that it had an alpha-D-glucan backbone with (1→4)- and (1→3) linkages, and

Table 1 Cordyceps polysaccharides and their physical and chemical characteristics

No.	Name	Molecular weight	Glycosyl residue composition (molar ratio)	Glycosyl linkage and branches (characteristic signals)	Source
1	P70-1	4.2×10^4	Man:Gal:Glc = 3.12:1.45:1.00	P70-1 has a backbone of 1,6-linked α-D-mannopyranosyl residues with branching points at O-3 composed of 1,4-linked α-D-glucopyranosyl and 1,6-linked β-D-galactopyranosyl residues, and terminated with β-D-galactopyranosyl residues and α-D-glucopyranosyl residues	Fruiting bodies of cultured *C. militaris*[38]
2	P70-2	2.6×10^4	Man:Gal:Glc = 1.75:1.31:1.00		
3	P50-1	5×10^4	Man:Gal:Glc = 2.80:2.62:1.00		
4	SCP-I	1.84×10^5	Glu	SCP-I possesses a backbone of 1,4-linked α-D-glucosyl residues and carries a single (1,6)-linked α-D-glucosyl residue as side chain	Fruit mycelium of *C. sinensis*[39]
5	PC I	[a]3.5×10^5	Man:Gal:Glu = 1:0.65:0.30		Mycelia of *C. sinensis*[40]
6	PCA I	[a]5.56×10^5	Man:Gal = 1:1	PCA I possesses a backbone of 1,4-linked mannopyranosyl residues with branches of O-2, O-3, O-6, etc of mono- or oligosaccharide composed of galactofuranosyl residues	
7	PCA II	[a]1.67×10^3	Man:Gal:Glu = 1:0.71:0.42	PCA II possesses 6 pyranosyl or furanosyl monosaccharide consisting of 3 mannosyl residues, 2 galactosyl residues and 1 glucosyl residue, which is a non-linear linked heterogeneous oligosaccharide	
8	PCB I	[a]6×10^4	Man:Gal = 1:0.73	PCB I possesses a backbone of 1,4-linked mannopyranosyl residues with side chains mainly composed of galactofuranosyl residues and also a few mannopyranosyl and galactopyranosyl residues	
9	PCB II	[a]6×10^4	Man:Gal:Glu = 1:0.51:0.50	PCB II possesses 2 mannosyl residues, 1 galactosyl residue and 1 glucosyl residue connected by a (1,3) linkage	
10	PCC I	[a]5.7×10^4			
11	PCC II				
12		[a]2.01×10^4	Ara:Man:Gal:Glc = 1.86:29.08:25.86:43.05	Composed of a (1,3)-β-D-glucan backbone substituted at O-6 with side chains of (1,6)-β-D-glucopyranosyl units, (1,3)-galactan and (1,2)- mannan	Fermentation broth of *C. dipterigena*[41]
13	CG-1		Glu	Composed of 1,6-linked α-glucopyranosyl residues	Cultured mycelia of *C. gunni*[42]
14	CPS-1	2.3×10^5	Rha:Xyl:Man:Glc:Gla = 1:6.43:25.6:16.0:13.8	Composed of mannose bonded by (1,2) linkage, xylose bonded by (1,4) linkage and rhamnose boned with galactose by (1,2) or (1,3) linkage	Cultured *C. militaris*[43]
15	CPS-2	[a]1.3×10^4	Rha:Glc:Gal = 1:4.46:2.43		Cultured *C. militaris*[44]
16	CPS-3	5×10^3	Glu	Composed of 1,4-linked and 1,6-linked D-glucose with mainly an α-glycoside linkage in a molar ratio of 0.11:0.86 with side chains substituting at the C-6 of every eight glucose residues	
17	CPS-1	2.3×10^4	Rha:Xyl:Man:Glc:Gal = 1:6.43:25.6:16.0:13.8	CPS-1 composed of five kinds of monosaccharide connected by β configuration	Cultured *C. militaris*[45]
18	CPS-2	[a]1.29×10^4	Rha:Glc:Gal = 1:4.46:2.43	CPS-2 composed of three kinds of monosaccharide connected by α configuration	
19	CPS-3	5×10^3	Glu	CPS-3 composed only of glucose connected by α configuration	
20	Ck_1-A	9.27×10^5	A small quantity of uronic acid (GlcA)	Composed of pyranosyl residues connected by α-glycoside linkage	Cultured mycelia of *C. kyushuensis*[46]
21	Ck_3-A	9.25×10^4	May contain a mannose and a little glycuronic acid	Connected by α-glycoside linkage	Mycelia of *C. kyushuensis*[47]
22	CMPS	3×10^4	Rha:Xyl:Man:Glc:Gal = 1.00:0.50:51.99:2.38:36.50	CMPS composed of a backbone of 1,2-linked mannosyl residues, 1,4- or 1,6-linked galactosyl residues, and 1,2-linked galactosyl uronic acid with branches of 1,3-linked glucosyl uronic acid at the C-2 or C-3 of 1,4-linked galactosyl residues in the main chain, mainly bonded by β configuration	Fruiting bodies of *C. militaris*[48]

23	CS-F10	1.5×10^4	Glu	CS-F10 has α-D-glucopyranosyl residues on the terminal of the side-chains and 1,5-linked β-D-galactofuranosyl residues	Mycelia of *C. sinensis*[49]
24	CMB	6×10^4	Gal:Man:Glc = 1.15:1.00:4.79	CMB possesses 1,6-linked mannosyl residues, 1,4-linked galactosyl residues, and even 1,6-linked or 1,4-linked glucosyl residues with branches of 1,4-linked and a few 1,6-linked glucosyl residues substituting at the C-3 of 1,4-linked glucosyl residues with terminal galactosyl or glucosyl residues, mainly connected by α-glycoside linkage	Mycelia of *C. militaris*[50]
25	CS-F3	4.5×10^4	Gal: Glc: Man = 62:28:10		Mycelia of *C. sinensis*[51]
26	CM-I	2.7×10^4	Gal:Man = 6:5	CM-I possesses a backbone of 1,2-linked β-mannosyl residues with branches of 1,6-linked β-galactofuranosyl residues at the C-4 and C-6 of galactosyl residues in the main chian	Culture liquid of *C. militaris*[52]
27	CS-81002	4.3×10^4	Man:Gal:Glc = 10.3:3.6:1	Consists of a backbone of 1,6-linked mannopyranosyl residues with branches of 1,3-linked galactofuranosyl residues, 1,4-linked glucofuranosyl residues and 1,4-linked or 1,2-linked mannopyranosyl at the C-3 and C-2 of mannosyl residues of the main chain in a molar ratio of 6:4	Fermentation medium of *C. sinensis*[53]
28	CI-P	2.5×10^4	Man:Gal = 1:0.85 with traces of D-glucose	CI-P composed of a backbone of 1,6-linked α-D-mannopyranosyl residues substituted at O-2 with single α- or β-D-galactofuranosyl groups, short chains of 1,2-linked β-D-galactofuranosyl residues, or chains of 1,2-linked α-D-mannopyranosyl residues	Insect-body portion of the fungal preparation *C. cicadae*[54]
29	CI-A	2.5×10^4	Man:Gal = 1:0.57 with traces of D-glucose	CI-A possesses nearly same structure as CI-P but fewer and shorter D-galactofuranosyl side-chains and longer 1,2-linked α-D-mannopyranosyl side chains than CI-P	
30	Ch-1	4.3×10^4	Mannose and gaIactose	Both polysaccharides possess backbones of 1,4-linked β-galactosyl and β-mannosyl residues with 1,3-linked and 1,2-linked side chains substituted at O-6	Fruit body of *C. hawkesii*[55]
31	Ch-2	4×10^3			
32	CT-4N	2.3×10^4	Man:Gal = 3:5	Composed of a backbone of 1,6-and 1,2-linked α-D-mannopyranosyl residues with short chains of a large proportion of 1,5-linked β-D-galactofuranosyl residues and a small proportion of 1,6-linked α-D-galactopyranosyl residues at O-2, O-4 and O-6, with the nonreducing terminal groups of a large proportion of α-D-mannopyranosyl groups	*C. sinensis*[56]
33	CO-1	6.32×10^5	Glu	CO-1 composed of a backbone of 1,3-linked β-D-glucopyranosyl residues with a β-D-glucopyranosyl group attached to O-6 of every second D-glucopyranosyl residue of the main chain	The product formed on incubation of the culture filtrate of *C. ophioglossoides*[57]
34	C-3	2.7×10^4	Man:Gal = 4:3	C-3 composed of 1,2-linked and 1,6-linked α-D-mannopyranosyl residues substituted at O-6 and O-2 with terminal β-D-galactofuranosyl and α-D-mannopyranosyl groups, and with short chains of 1,2-linked β-D-galactofuranosyl residues	Ascocarps of *C. cicadae*[58]
35	CS-I		Gal:Man = 1:1	CS composed of a backbone of 1,2-linked D-mannofuranosyl residues with side chains of 1,3-linked 1,5-linked and 1,6-linked D-galactofuranosyl residues and 1,4-linked D-galactopyranosyl residues and with nonreducing terminal groups of D-galactofuranosyl residues and D-mannopyranosyl residues[59]	
36	CS-II				

[a]The component is a proteoglycan or a glycoprotein.

the side chains of alpha-D-(1→6)-mannopyranose (Manp) were attached to the backbone via the O-6 of alpha-(1→3)-glucopyranosyl (Glcp) residues.[61] The study showed that the pharmacological activity of the polysaccharide was correlated with its characteristics such as molecular weight. It has been reported that polyglucans with higher molecular weight (10–1000 kDa) tend to have greater water solubility and therefore have a more effective antitumour activity. The anticancer effects of fungal polysaccharides arise from the enhancement of the body's immune system rather than direct cytocidal effects.[62]

The physicochemical properties of an aqueous solution of crude CP have been analysed, and the result showed some good properties, such as thickening and thixotropic properties, salt and heat resistance and stability over a wide pH range. The dynamic viscosity of CP varies little over the pH range of 4–14. There was a positive relationship between CP oral dosage and its therapeutic effects (the lowest effective dosage of CP is 180 mg per day).[40] In addition it is colourless and odourless and has good solubility and stability in water.[63]

Other Bioactive Components

Nucleotides

Nucleotides (including adenosine, uridine and guanosine) are effective components in Cordyceps mushrooms. Investigations show that guanosine has the highest content of all in natural and artificial Cordyceps.[64] The content of nucleotides in artificial Cordyceps is obviously higher than in natural Cordyceps. This may be related to the rapid metabolism of artificial cultures. A remarkable variation in the adenosine content is found in artificial Cordyceps, with the highest concentration being over six times that of the lowest. The content of adenosine in freshly acquired natural Cordyceps is too low to quantity, but the content of the nucleotide in a preparation stored for a long time is higher. This shows that nucleotides in natural Cordyceps derive from degradation of the nucleoside during the storage process. Subsequent research found that dampness and warmth can significantly increase the nucleotide content in Cordyceps. At a temperature of 40°C and relative humidity of 75%, Cordyceps were stored for 10 days, when the content of nucleotides increased 1–10 fold, while in artificial Cordyceps adenosine, uridine and guanosine showed no obvious change in content. This suggests that the nucleotides in natural and artificial Cordyceps are to some extent different.[5]

Ergosterol

Ergosterol is a sterol unique to fungi and is an important precursor of vitamin D_2, which has important medicinal value. The content of free ergosterol from natural and artificial Cordyceps has been compared. The result showed that the content of free ergosterol in different natural Cordyceps varies significantly, but is evidently higher than than in the mycelia of Cordyceps. The content of ergosterol in the artificial fruit body of *C. militaris* is very high, only lower than that of natural Cordyceps in Tibet, and higher than that in Qinghai and Sichuan. Li *et al.*[65] reported that the content of ergosterol in the mycelia of Cordyceps was 1.44 mg/g, much lower than that in the fruit bodies of Cordyceps (10.68 mg/g).

Crude protein and amino acids

Previous papers have reported that the content of crude protein was in the range 29.1–33%.[27,33,66] The protein was composed of 18 amino acids, including aspartic acid, threonine, serine, glutamate, proline, glycine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, cystine, cysteine and tryptophan. The content of amino acids after hydrolysis is mostly reported as 20–25%, the lowest being 5.53%, the highest being 39.22%. The highest contents are glutamate, arginine and aspartic acid, and the major pharmacological components are arginine, glutamate, tryptophan and tyrosine.[67] The content of amino acids in the commercial preparation of Cordyceps is significantly higher than in the mycelia of *C. sinensis*, which is similar to the content in the fruit body of *C. sinensis*. The content of amino acids in *C. sinensis* and related products are shown as in Table 2.

Fatty acids and metal elements

Fatty acids are composed of carbon, hydrogen and oxygen and are the major component of lipids, phospholipids and glycolipids; they can be classified as saturated or unsaturated fatty acids. In Cordyceps, the unsaturated fatty acid content reaches 57.84%, including $C_{16:1}$, $C_{17:1}$, $C_{18:1}$ and $C_{18:2}$. The linoleic acid content is the highest at 38.44%, followed by the oleic acid, which is 17.94%. The saturated fatty acid content is 42.16%, including C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{20} and C_{22}. The palmitic acid and the octadeca acid contents are the highest, and are 21.86% and 15.78%, respectively. Unsaturated fatty acid is an effective physiologically active component, which has the unique function of decreasing blood lipids and protecting against cardiovascular disease. The content of unsaturated fatty acid is higher than that of saturated fatty acid in commercial preparations of Cordyceps, and the linoleic acid and oleic acid contents are significantly higher than others.

Table 2 Amino acid levels in *Cordyceps sinensis* mycelia fermentation preparations and two samples of fruit body

Amino acid composition	Mycelium powders	Mycelia	Fruit body A	Fruit body B
Asp	2.47	1.05	1.67	1.84
Thr	0.95	0.65	1.47	0.83
Ser	0.82	0.49	1.51	0.78
Glu	3.55	1.12	3.44	2.66
Pro	0.90	0.72	0.96	0.95
Gly	1.19	0.58	1.25	0.73
Ala	1.21	0.75	1.40	0.95
Cys	0.25		0.43	
Val	1.42	0.63	1.13	0.80
Met	0.47	0.18	0.25	0.18
Ile	1.14	0.44	0.62	0.53
Leu	1.84	0.69	1.20	0.95
Tyr	0.89	0.31	0.88	0.67
Phe	1.84	0.51	0.71	0.61
Lys	1.27	0.81	1.66	1.15
His	0.58	0.28	1.71	1.13
Arg	1.47	0.04	2.88	1.6
Total content	21.59	9.23	23.15	16.40

The amino acid level in the *C. sinensis* fermentation preparations were more than those in the *C. sinensis* fruit body.

Investigations show that Cordyceps contains many metal elements, and the content significantly depends on different species and metal elements (Table 3).[26,68,69] Many metal elements, Zn, Mg, Mn and so on, are of great significance to the development and maintenance of function of the gonads. Zn is effective in the kidney at preventing cadmium from damaging the nephrons.[70] High contents of Zn, Mg, Mn play an important role in warming and tonifying the kidney yang effectively, aiding kidney recovery and improving the essence of life.[71]

Pharmacological Function

Fungi are an important source of material in TCM. Medicinal mushrooms have an established history of use in traditional oriental therapies. Extracts from about 200 species of fungi have been shown to stimulate immunoreactivity and inhibit the growth of different kinds of tumours.[72–75] According to the theory of TCM, the main effects of Cordyceps are enriching the lung yin and yang, which includes treating chronic lower back pain, sensitivity to cold, over abundance of mucus and tears, chronic cough and wheezing, blood in phlegm from consumption due to kidney yang (shenyangxu), etc. Based on Western medicine, Cordyceps has an antibacterial function, and reduces asthma, lowers blood pressure and strengthens the heart beat. In the public domain, people often add it into hen or duck soup to strengthen the body and 'renew' sick people.[3,76] Although there are 300 species of Cordyceps, the research into its pharmacological effect has focused only on two species, *C. sinensis* and *C. militaris*.

Antitumour effects

Tumour diseases are one of the main causes of death in the world. Antitumour activity is the most significant therapeutic interest associated with these mushrooms. The ability to inhibit the growth of different tumours was detected in many genera of Cordyceps species. Cordyceps species and their extracts have been recognized as a traditional remedy, used in TCM for the prevention and treatment of cancer and several other diseases.[77]

The bioactive components in Cordyceps that have antitumour activity are mainly the polysaccharides, sterols and adenosine. The antitumour action of CP has been proved extensively,[40,59,78,79] and that of sterols and adenosine is a hot research topic in Cordyceps.[80,81] New antitumour compounds have been continually isolated, and the effects of Cordyceps extract on tumour growth have been evaluated.[82,83] For instance, Zhang *et al.*[84] compared the antitumour effects and chemical components of the extracts of cultivated *C. sinensis* with natural *C. sinensis*. The Cordyceps was extracted with petroleum ether (PE), ethyl acetate (EtOAc), ethanol (EtOH) or water. The cytotoxicity of all the extracts was observed with MTT assay on a breast cancer cell line (MCF-7), a mouse melanoma cell line (B16), a human premyelocytic leukaemia cell line (HL-60) and a human hepatocellular carcinoma cell line (Hep G2). The chemical constituents in the extracts were analysed by HPLC. The results showed that all of the extracts from the Cordyceps mushroom had much stronger cytotoxicity on the B16 cell line and the EtOAc extract had the most potent activity; the chemical components of all the extracts were analysed and ergosterol and adenosine were found to be potential compounds.[84] We still do not know the mechanism by which Cordyceps inhibits the growth of various cancer cells; inhibition might occur by one of several means, summarized as follows: (1) enhancing immunological function and non-specific immunity;[85] (2) selectively inhibiting RNA synthesis, thereby affecting the protein synthesis;[86] (3) restricting the sprouting of blood vessels;[87] (4) inducing tumour cell apoptosis;[88] (5) regulation of signal pathways;[89] (6) antioxidation and anti-free radical activity;[90] (7) anti-mutation effect; (8) interfering with the replication of tumour-inducing viruses; (9) inducing nucleic acid methylation.[91]

Immunomodulating effects

The possibility that extracts and isolated components from mushrooms stimulate or suppress specific components of the immune system has been studied in recent years. Immunomodulators can be effective agents for treating or preventing diseases and illnesses that stem from certain immunodeficiencies and other depressed states of immunity.[92] There is an extensive body of research focused on the immuno-enhancing and immunosuppressive properties of various Cordyceps species.[93,94] As previously stated, several major substances with immunomodulatory activity have been isolated from Cordyceps.

Table 3 Amount of metal elements in mycelial fermentation preparations and fruit body of *Cordyceps sinensis*

Metal element	Mycelium powder (mg/kg)	Fruit body (mg/kg)	Metal element	Mycelium powder (mg/kg)	Fruit body (mg/kg)
K	11500a	3975	Fe	205	3136
P	3600	3671	Cu	8.86[a]	2.8
Na	310	547	Zn	152[a]	13.9
Mg	2700[a]	1813	Mn	45.6[a]	39.2
Ca	800	1656	Si	54.3[a]	n.t.
Al	169	7664	Co	0.21	1.097
B	15.4[a]	7.79[a]	Ni	2.05	3.76
Mo	0.3[a]	n.d.	Se	0.52[a]	0.34
Cd	0.22[a]	0.051	V	1.10	11.82
Pb	0.2	0.36	Cr	0.97	4.42

[a]Metal levels in *Cordyceps sinensis* mycelium preparation were more than those in fruit body; n.d., not detected; n.t., not tested.

The major immunomodulating effects of the active substances derived from these mushrooms include mitogenicity and activation of immune cells, such as the lymph proliferative response, natural killer (NK) cell activity and phytohaemagglutinin (PHA)-stimulated interleukin-2 (IL-2) and tumour necrosis factor-α (TNF-α) production on human mononuclear cells (HMNC),[6] resulting in the production of cytokines. The therapeutic effects of the mushrooms, such as suppression of autoimmune diseases and allergy, have been associated in many cases with their immunomodulating effects.[95–97]

While it is known that Cordyceps extracts have immunomodulatory activity, the standard approach has been to isolate, characterize and administer the pure active constituents. However, different components in a Cordyceps species extract may have synergistic activity. There are several reports of mushrooms containing more than one polysaccharide with immunomodulatory activity. For example, Yu *et al.*[98] used components I, II, III and IV of polysaccharide groups and a negative control group. Administration of *C. sinensis* polysaccharides to NIH mice lasted for five days at a dose of 35 mg/kg by intraperitoneal and subcutaneous injection. They investigated the pharmacodynamics of immune activity of different components, measured the weight of thymus and spleen of mice injected intraperitoneally, and then calculated the viscera index for those injected subcutaneously. The results indicated that different components of *C. sinensis* polysaccharides could enhance the immune response, spleen index, thymus index and the phagocytic function of monocyte-macrophages.[98] The response to different polysaccharides is likely to be mediated by different cell surface receptors, which may be present only on specific subsets of cells and may trigger distinct downstream responses. A combination of such responses involving different cell subsets could conceivably provide greater tumour inhibition than that which could be induced by a single polysaccharide.[78] On the other hand, latest reports show that Cordyceps has an anti-rejection effect in the experimental research of organ transplantation. For example, Guan and Yu[99] studied the prevention of rejection of transplanted kidney by artificial cultured *Cordyceps* in rats; the results showed that Cordyceps powder can markedly prolong rat renal allograft survival.

Protective effects on human organs

Protection of liver

Liver disease is one of the major diseases that is seriously harmful to human health and affects quality of life. The incidence of primary liver cancer is continuously rising in the world, and about 50 000 people die of this disease every year, of which about 40% are in China, which may be related to hepatitis virus infection. Thus effort has concentrated on preventing liver disease using TCM, and Cordyceps is one of the various materials of TCM.

Animal tests and clinical research data show that Cordyceps has a protective effect in liver patients, including those with viral hepatitis A, chronic hepatitis B, chronic hepatitis C, hepatic fibrosis, etc. It enhances organic cell immunological function, reverts HBeAg-positive to HBeAg-negative, improves liver function, inhibits hepatic fibrosis, and so on.[100] In recent years, significant progress has been made in the prevention of liver disease. For instance, to find an effective drug to cure patients with chronic hepatitis B, Gong *et al.*[101] have treated 25 patients with *C. sinensis*. T lymphocyte subsets (CD4, CD2), hyaluronic acid (HC) and precollagen type III (PC III), were observed before and after treatment. After 3 months of treatment, CD4 and CD4/CD2 ratio increased significantly ($P < 0.05$), while HA and PC III decreased significantly ($P < 0.05$) compared with the control. These results suggested that beneficial effects might be obtained by using *C. sinensis* to adjust the T lymphocyte subset levels and to treat hepatic fibrosis in patient with chronic hepatitis B.[101]

Bioactive components of cordyceps for liver protection are mostly CPs. The CPs can improve the immunological functions of organic cells, removing harmful components and thus reducing the injury to liver cells. However the content and efficacy of CPs vary with the species.[102,103] The effects of CPs in protecting the liver were presented as follows:[104] (1) protective effect on immune liver injury; (2) effect on patients with chronic hepatitis B; (3) effect on patients with hepatocirrhosis after hepatitis;[105] (4) protective effect on liver fibrosis.[101]

The key to treating liver disease is to inhibit and clear the virus from the liver of patients, regulate the immunological function of the organism, and reverse the developing process of liver fibrosis, etc. *C. sinensis* and its related products obviously act to hasten macrophage phagocytosis, and enhance the immune function of organisms and possess a strong capacity to clear the virus.[106]

Protection of kidney

Nephrosis is a kidney disease, always considered to be serious, which is harmful to the health of the urinary system. It can recur easily and acutely, while it is difficult to cure. The modern method of treatment still lacks a satisfactory curative effect. The medical treatment for the disease mainly uses glucocorticoids and an immunodepressant. The Chinese native medicinal treatment for nephrosis has a rapid effect, and the side effects are also very small, so it is given prominence.[107]

In DCXC, there are about 19 amino acids, alcoholic components, nucleotides, trace elements and vitamins as previously stated. The effects in protecting the kidney are mainly presented as three aspects: (1) a therapeutic effect on toxic kidney injury; (2) protecting against chronic renal function failure; (3) reversing the effect of glomerulonephritis in an animal model.[7] These have been proved by a series of experiments.

Protection of heart and antihypertensive effects

C. sinensis extracts were tested on myocardial ischaemia/reperfusion injury in rat isolated hearts. Xu *et al.*[108] reported that the alcoholic extracts had a protective effect on myocardial injury induced by adriamycin in rats. The mechanism was suggested to be that mannitol, amino acids and polysaccharides in the extracts played an important role; all the substances had the effect of nourishing the myocardium and enhancing its anti-injury capacity.[108] With the same experimental methods, Gu *et al.*[109] reported that

Lungfit (*C. sinensis* compounds) could be beneficial for myocardial ischaemia/reperfusion injury as well. However, both alcohol and water extracts had obviously protective effects on the rat isolated heart.

On the other hand, *C. sinensis* has an inhibitory effect on arrhythmias induced by aconitine, barium chloride and adrenaline, and can increase nutritional myocardial blood flow, thereby improving myocardial ischaemia.[110] Some other papers reported similar findings, but isolates came from different extraction methods.[111] Considering antihypertensive effects, Wu *et al*.[112,113] observed the effect of *C. sinensis* on blood pressure in renal hypertensive rats. The results showed that the renal hypertension could be prevented significantly by treatment with *C. sinensis* since the cardiac hypertrophy and vascular remodelling were reversed. Therefore, they came to the conclusion that *C. sinensis* has a curative role in renal hypertension.[112,113]

In short, *C. sinensis* has many effects on the cardiovascular system, such as having a negative frequency, reducing myocardial oxygen consumption, improving myocardial ischaemia, anti-platelet aggregation and anti-arrhythmic effects, etc. On the haematopoietic system, *C. sinensis* has a protective effect on mice platelet reduction and platelet ultrastructural damage.

Protection of other organs

C. sinensis also has obvious effects on other organ systems.[114–116] For example, on the central nervous system, *C. sinensis* has sedative, anticonvulsant and cooling effects. On the respiratory system, *C. sinensis* has a significant relaxant role in the bronchi, markedly increases adrenaline secretion from the adrenal glands and also has a role in tracheal contraction caused by histamine; it also has an antitussive, expectorant and anti-asthmatic action and prevents pulmonary emphysema. On the endocrine system, *C. sinensis* has effects as a male hormone; CP can increase plasma corticosterone levels, etc.

Developmental Products

In recent years, functional foods, also known as nutraceuticals, medical foods or nutritional foods, are driving food markets around the world and are expected to be one of the emerging trends for the food industry in the new millennium. Cordyceps are important raw sources. As the prices of Cordyceps are rising fast, many people harvest them. Meanwhile animal husbandry is developing rapidly, which destroys the ecological equilibrium, reducing the wild Cordyceps resources. To increase the yields of Cordyceps and meet medicinal demand, researchers have tried to obtain the purified mycelium of Cordyceps from the artificial fermentation of the stroma of Cordyceps since the 1970s. Due to their similarity in biological activity and chemical composition, there will be a trend towards replacing natural Cordyceps resources. So far many fungi have been studied further, including *Cephalosporium sinensis*, *Paecilomyces hepiali*, *P. sinensis*, *Scydalium* spp. and *C. militaris*, and many different improved productions have appeared on the market.[13] At present, the development of Cordyceps and its substitutes is mainly focused on the following three areas.

Dietetic Chinese drugs

Dietetic Chinese drugs are also known as ‘edible Chinese drugs’, ‘dietetic material medicine’ or ‘medicinal food’, although the word ‘drug’ could be misinterpreted in the West. The basic theories of the Chinese medicated diet (CMD) arose from the main theories of herbal medicine in TCM.[117] Accordingly, the homologous culture of medicine and food are an important part of TCM theory; there is a saying: ‘food is your medicine, and medicine is your food’. Much Chinese herbal medicine not only involves the treatment of disease, but also includes very good food, such as daily vegetables and fruits, which have the dual role of being food and drug at the same time. *C. militaris*, as healthy green food, could be used in stir-fries or be added to stewed chicken or duck, cooking soup, hot pot and tea. Especially, cooking soup with Cordyceps is very popular. Now the Guangdong Academy of Agricultural Sciences Institute of Sericulture and Insects has developed a series of tea herbs. What’s more, the Chengdu JinCao Company has prepared food with *C. militaris* which is aimed for mass consumption, such as Cordyceps-Saussurea, Cordyceps-Aloe and Cordyceps-Dictyophora soup.[118]

Health food

Health food, also called ‘functional food’, is used widely and designed as foods or food components that help to modulate the human body and cure or prevent diseases. Many other terms, such as ‘medical foods’, ‘nutraceuticals’, ‘nutritional foods’ and ‘designed foods’, as well as their definitions, have also emerged. Health foods need to be approved by the SFDA (State Food and Drug Administration) in China and before marketing. Therefore, developing health food is another important way to develop Cordyceps products directed to different people and different physiological states with different demands of nutrition and health. There has been some *C. militaris* health food on the market, such as oral liquid Cordyceps tonic, tonic Cordyceps wine for kidney reinforcing,[119] Cordyceps health beer, Cordyceps capsules (such as AiMei soft capsule, using the tradename ‘Xinkeqi’, which has the unique function of immunomodulation and delaying senility, produced by Jilin Xinkeqi Health Food Co., Ltd), and so on.[120] These have been used mainly by the following groups: young people, the elderly, pregnant women, people with nutritional imbalance and so on. Focused design like this not only influences physiological regulation, but also regulates the nutrition and senses.

It is worth noting that, in recent years, as increasing attention is given to the function of *C. militaris*, foreign manufacturers have shown an interest, and export volumes are increasing. Presently Japan, Korea, Malaysia and other countries are increasing their research and development of Cordyceps functional food.[10]

Developing the ‘drugs’

The most bioactive components extracted from Cordyceps, such as cordycepin, cordycepic acid and CP, which have anti-aging and sleep regulating effects, have been authorized as new medicinal resources by the SFDA of China. Therefore, using Cordyceps (including its extracts) and adding other TCM materials or other nutrient resources, we can develop many products through modern technology. These products have many functions that are mainly focused on the following aspects: enhancing physique, anti-aging, protecting the heart, improving sleep, increasing appetite, increasing immunity, etc. For example, '*C. militaris* mycelia powder and the capsule of *C. militaris* mycelia powder' had been authorized as a Chinese national drug in April 2003; Jilin Northeast Tiger Pharmaceutical Co., Ltd reported to the State Ministry of Health to declare classes of new drugs, which have been approved and called Xinkeqi Capsules. Meanwhile, '*C. sinensis* polysaccharide liposomal oral liquid' (Guangzhou Xingqun Pharmaceutical Co., Ltd),[121] and 'Bailing Capsules' (Hangzhou Eastchina Pharmaceutical Co., Ltd),[122] have also been approved for entry into the market. So far, there are approximately twenty types of TCM preparation related to Cordyceps, many of which are polysaccharide products.[123]

Concluding Remarks

Cordyceps, one of the well-known TCMs, consists of the dried fungus *C. sinensis* growing on caterpillar larvae. These larvae include about 20 species of five genera, *Hepialus*, *Hepialiscas*, *Napialus*, *Forkalus* and *Bipectilus*, which are classified as Hepialidae. Among them, *Hepialus armoricanus*, *H. obilfurcus*, *H. baimaensis* and *H. renzhiensis* are predominant hosts of *C. sinensis*.[124] The parasitic complex of the fungus and the caterpillar is found in the soil of the prairie at an elevation of 3500–5000 m.[122] It is commonly used in China to replenish the kidney and soothe the lung, for the treatment of fatigue, night sweating, hyposexuality, hyperglycaemia, hyperlipidaemia, asthenia after severe illness, respiratory disease, renal dysfunction and renal failure, arrhythmias and other heart disease and liver disease. The natural Cordyceps (wild *C. sinensis*) is rare and expensive in the local market; researchers in China have extensively observed its life cycle with the aim of developing techniques for isolating fermentable strains of *C. sinensis*. Several mycelial strains have been isolated from natural Cordyceps and manufactured in large quantity by fermentation technology. At present, cultured Cordyceps mycelia are commonly sold as health-food products in South East Asia.[125]

According to incomplete statistics, at present there are about 100 scientific research institutes working on the research and development of medicinal fungi. More than 200 factories are engaged in producing fungus preparations. In China, there are more than 30 kinds of medicinal fungus that are used for obtaining their active component or mycelium and fermentation production by solid-state fermenting or deep-seated fermenting. This provides abundant materials for manufacturing medicine. By now, there are 20–30 types medicament products and approximately 100 types of health-care products.[126]

In order to develop, utilize and protect this rare medicinal resource, the Chinese government has bestowed it special support and sponsored much research by the national research foundations over recent years. For instance, during the seventh five-year plan (1986–1990), many research institutes were engaged in the research and development of Cordyceps in Shanghai, Shanxi, Jiangxi and Qinghai provinces, after the national science committee put research into *C. sinensis* in the Spark Plan Project. Subsequently, the research and development of Cordyceps has been listed as a key project to be pursued in the science and technology area for three continuous five-year plans (1986–2000), from the seventh five-year plan (1986–1990) to the ninth five-year plan (1996–2000). For example, 'wild cultivation, research and development of *C. sinensis* in the Naqu District of Tibet', China Key Science Program in the national ninth five-year plan, strongly supports the cultivation of *C. sinensis* by simulating its naturally growing environment, and exploiting its production in the north Tibet altiplano.[4] 'The R&D of cordycepin polysaccharide' (No. 96-C03-01-03), which was listed in the key project of the ninth five-year plan, is not only a breakthrough in the theoretical research of Cordyceps, but it also has an active significance for the development of Cordyceps products.[127] During recent decades, as the findings of new uses for Cordyceps and development of Cordyceps preparations continues, some correlative products have rapidly appeared one after another, such as Cordyceps capsule, Cordyceps oral liquid, Cordyceps drink and so on. The development of the curative and health-care products from Cordyceps is more and more favourable among people in China. Meanwhile, we are facing an arduous task. *C. sinensis*, with its long history and remarkable efficacy, cannot continue to serve for the health of human beings, so we must be clearly aware of this and make efforts to discover a substitute for *C. sinensis*. It is delightful that the eleventh five-year-plan annual emphasis item of 2007 in the medicine technology field of 863 Program (Hi-tech research and development program of China) also strongly supports the research on *C. militaris* as a substitute for *C. sinensis*. It is credible that a substitute having the same efficacy as *C. sinensis* will appear in the near future and well serve the human being's health project.

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Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Zhu JS *et al.* The scientific rediscovery of an ancient Chinese herbal medicine: Cordyceps sinensis: part I. *J Altern Complement Med* 1998; 4: 289–303.
2. Pegler DN *et al.* The Chinese 'caterpillar fungus'. *Mycologist* 1994; 8: 3–5.
3. Mizuno T. Medicinal effects and utilization of Cordyceps (Fr.) Link (Ascomycetes) and Isaria Fr. (Mitosporic Fungi) Chinese caterpillar fungi, 'Tochukaso' (review). *Int J Med Mushrooms* 1999; 1: 251–261.

4. Gu DX *et al.* A review and prospect on the studies of *Cordyceps sinensis* (Berk) Sacc. *J Chin Inst Food Sci Technol* 2006; 6: 137–141.
5. Wei L *et al.* Research advanced in functional composition content of Cordyceps. *West Chin J Pharm Sci* 2003; 18: 359–360.
6. Kuo YC *et al.* Cordyceps sinensis as an immunomodulatory agent. *Am J Chin Med* 1996; 24: 111–125.
7. Shi YX. Update on researches of pharmacological effects of *Cordyceps*. *Chin Pharm* 2005; 14: 72–74.
8. The State Pharmacopoeia Committee of People Republic of China. *Pharmacopoeia of the People's Republic of China, Part I.* Beijing: Chemical Industry Publishing House, 2005.
9. Zhang P. Advances on Cordyceps genus fungi research. *J Biol* 2003; 20: 43–45.
10. Liang ZQ. Current situation and ponderation of Cordyceps Fr. research and exploitation in China. *Acta Edulis Fungi* 2001; 8: 53–62.
11. Liang ZQ *et al.* Some entomogenous fungi from Wuyishan and Zhangjiajie nature reserves I. Cordyceps. *Mycosystema* 2002; 21: 162–166.
12. Sung GH, Spatafora JW. Cordyceps cardinalis sp. nov., a new species of Cordyceps with an east Asian-eastern North American distribution. *Mycologia* 2004; 96: 658–666.
13. Lei WS *et al*. General situation of research on Cordyceps sinensis. *J Navy Med* 2006; 27: 262–269.
14. Guo HC *et al.* Research and development for Cordyceps sinensis. *J Microbiol* 2003; 23: 50–55.
15. Jiang Y, Yao YJ. Anamorphic fungi related to Cordyceps sinensis. *Mycosystema* 2003; 22: 161–176.
16. Wu W *et al.* Review on studies and applications of Cordyceps militaris. *Acta Agric Shanghai* 2000; 16: 99–104.
17. Gu HS, Liang MY. Study on the manual cultivation of Cordyceps militaris. *Med Inf Lett* 1987; 5: 51–52.
18. Wang JF, Yang CQ. Research advancement of *Cordyceps militaris* on product development and artificial cultivation. *Lishizhen Med Mat Med Res* 2006; 17: 268–269.
19. Zhang HF. Study on conditions of culture on fruiting body of Cordyceps militaris. *J Jilin Med Coll* 2005; 26: 192–194.
20. Ren GL *et al.* A study on indoor cultivation technique of Cordyceps militaris. *J Henan Agric Univ* 2006; 40: 66–68.
21. Wen L *et al.* Optimization of Cordyceps militaris cultivation for high production of cordycepin and adenosine. *Jiangsu J Agric Sci* 2005; 21: 359–363.
22. Huang LF *et al.* Simultaneous separation and determination of active components in Cordyceps sinensis and Cordyceps militaris by LC/ESI-MS. *J Pharm Biomed Anal* 2003; 33: 1155–1162.
23. Cunningham KG *et al.* Cordycepin, a metabolic product from cultures of cordyceps militaries (Linn.) link. Part I. Isolation and characterization. *J Chem Soc* 1951; 43: 2299–2300.
24. Kaczka EA *et al.* Identification of cordycepin, a metabolite of Cordyceps militaris, as 3′-deoxyadenosine. *Biochem Biophys Res Commun* 1964; 14: 456–457.
25. Li J, Jiang HY. Progression on study of Cordycepin. *US Chin Health Hyg J* 2005; 8: 27–30.
26. Wang ZS *et al.* Chemical components of Cordyceps sinensis mycelial fermentation preparations in solid media. *Nat Prod Res Dev* 2005; 17: 331–336.
27. Hsu TH *et al.* A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom DongChongXiaCao, its counterfeit and mimic, and fermented mycelium of Cordyceps sinensis. *Food Chem* 2002; 78: 463–469.
28. Lu P *et al.* Capillary zone electrophoresis determination of cordycepin in Cordyceps spp. extracted by using ultrasonic. *Mycosystema* 2002; 21: 394–399.
29. Chatterjee R *et al.* Cordyceps sinesis (Berkeley) Saccardo: structure of cordycepic acid. *J Am Pharm Assoc* 1957; 46: 114–118.
30. Sprecher M, Sprinson DB. A reinvestigation of the structure of 'cordycepic acid'. *J Org Chem* 1963; 28: 2490–2491.
31. Jiang P. Pharmacology constituent and function of Cordyceps sinensis. *J Westnorthern Med* 1987; 2: 43–44.
32. Zhan TR, Song JM. Progress in studies of mannitol in medicinal application. *Chin J Marine Drugs* 2003; 22: 57–61.
33. Xu JT. *China Medicinal and Edible Fungi*. Beijing: Beijing Medical University and China Xiehe Medical University Press, 1997.
34. Li SP *et al.* Anti-oxidation activity of different types of natural Cordyceps sinensis and cultured Cordyceps mycelia. *Phytomedicine* 2001; 8: 207–212.
35. Li SP *et al.* The fruiting body and its host of Cordyceps sinensis show close resemblance in main constituents and anti-oxidation activity. *Phytomedicine* 2002; 9: 319–324.
36. Wang JF *et al.* Research progress on polysaccharides from *Cordyceps sinensis*. *Chin Trad Herbal Drugs* 2006; 37: 6–8.
37. Koh JH *et al.* Antifatigue and antistress effect of the hot-water fraction from mycelia of Cordyceps sinensis. *Biol Pharm Bull* 2003; 26: 691–694.
38. Yu RM *et al.* Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured Cordyceps militaris. *Carbohydr Polym* 2007; 70: 430–436.
39. Wu YL *et al.* Studies on isolation and structural features of a polysaccharide from the mycelium of a Chinese edible fungus (Cordyceps sinensis). *Carbohydr Polym* 2006; 63: 251–256.
40. Yuan JG *et al.* Studies on the components and pharmacological action of polysaccharide from Cordyceps sinensis. *Food Drug* 2005; 7: 45–48.
41. Methacanon P *et al.* Structural elucidation of bioactive fungi-derived polymers. *Carbohydr Polym* 2005; 60: 199–203.
42. Zhang YM *et al.* Research situation and development trends of Cordyceps gunnii. *Guizhou Agric Sci* 2006; 34: 121–123.
43. Yu RM *et al.* Isolation and biological properties of polysaccharide CPS-1 from cultured Cordyceps militaris. *Fitoterapia* 2004; 75: 465–472.
44. Yu RM *et al.* Isolation, purification and identification of polysaccharides from cultured Cordyceps militaris. *Fitoterapia* 2004; 75: 662–666.
45. Wang L *et al.* Separation, purification and structural analysis of polysaccharides in cultured Cordyceps militaris L. Link. *Chin J Biochem Pharm* 2003; 24: 23–25.
46. Wang YB *et al.* Purification and properties of polysaccharide Ck_1-A from cultured mycelia of Cordyceps kyushuensis. *Mycosystema* 2003; 22: 452–456.
47. Wang YB, Zhang CK. Isolation, purification and properties of a polysaccharide Ck3-A from Cordyceps kyushuensis cultured mycelium. *Food Ferment Ind* 2003; 29: 25–27.
48. Bin W *et al.* Studies on polysaccharide in the tissue cultures of Cordyceps militaris. *J ShenYang Pharm Univ* 2000; 17: 361–364.
49. Kiho T *et al.* Structural features and hypoglycemic activity of a polysaccharide (CS-F10) from the cultured mycelium of Cordyceps sinensis. *Biol Pharm Bull* 1999; 22: 966–970.
50. Wang BJ *et al.* Studies on structure and properties of water soluble polysaccharide from fruiting body of Cordyceps militaris (L.) Link. *Chem Res Chin Univ* 2003; 19: 37–40.
51. Kiho T *et al.* Polysaccharides in fungi. XXXII. Hypoglycemic activity and chemical properties of a polysaccharide from the cultural mycelium of Cordyceps sinensis. *Biol Pharm Bull* 1993; 16: 1291–1293.
52. Gai XJ, Zhang YS. Study of extracellular polysaccharide from Cordyceps militaris (L.) Link I. purification and structural study of Galactomannan (CM-I). *Mycosystema* 1992; 11: 300–307.
53. Gong M *et al.* Molecular structure and immunoactivity of the polysaccharide from Cordyceps sinensis (Berk) Sacc. *Chin J Biochem Mol Biol* 1990; 6: 486–492.

54. Kiho T *et al.* Minor, protein-containing galactomannans from the insect-body portion of the fungal preparation Chan hua (Cordyceps cicadae). *Carbohydr Res* 1988; 181: 207–215.
55. Chen AP, Wu DR. Studies on fungi polysaccharide I. isolation and some properties of water-soluble polysaccharide of Cordyceps hawkesii. *J Anhui Univ (Nat Sci)* 1987; 11: 70–75.
56. Kiho T *et al.* A minor, protein-containing galactomannan from a sodium carbonate extract of Cordyceps sinensis. *Carbohydr Res* 1986; 156: 189–197.
57. Yamada H *et al.* Structure and antitumor activity of an alkali-soluble polysaccharide from Cordyceps ophioglossoides. *Carbohydr Res* 1984; 125: 107–115.
58. Ukai S *et al.* Structure of a new galactomannan from the ascocarps of Cordyceps cicadae Shing. *Carbohydr Res* 1982; 101: 109–116.
59. Miyazaki T *et al.* Studies on fungal polysaccharides XX. galactomannan of Cordyceps sinesis. *Chem Pharm Bull* 1977; 25: 3325–3328.
60. Cai LT *et al.* Observation of the structure morphology of Cordyceps polysaccharide by atomic force microscope. *J Chin Electron Microsc Soc* 1999; 18: 103–105.
61. Wu Y *et al.* Isolation and characterization of a mannoglucan from edible Cordyceps sinensis mycelium. *Carbohydr Res* 2007; 342: 870–875.
62. Zhou XW *et al.* Ganodermataceae: natural products and their related pharmacological functions. *Am J Chin Med* 2007; 35: 559–574.
63. Li X, Xu L. Studies on expolysaccharide (EPS) fermentation by Cordyceps militaris, and on its physical and chemical properties and antioxidation. *J Microbiol* 1997; 17: 13–17.
64. Li SP *et al.* The nucleosides contents and their variation in natural Cordyceps sinensis and cultured Cordyceps mycelia. *J. Chin. Pharm. Sci.* 2001; 10: 175–179.
65. Li SP *et al.* RP-HPLC determination of ergosterol in natural and cultured Cordyceps. *Chin J Mod Appl Pharm* 2001; 18: 297–299.
66. Crisan EV, Sands A. Nutrition value. In: Chang ST, Hayes WA, eds. *The Biology and Cultivation of Edible Mushrooms.* New York: Academic Press, 1978: 137–165.
67. Ji S *et al.* Introduction of the research status in chemical constituents of Cordyceps. *J Fujian Coll Trad Chin Med* 1999; 9: 46–47.
68. Ma BR *et al.* Compared of chemical composition between Cordyceps militaris and Cordyceps sinensis. *Chin Edible Fungi* 1993; 13: 34–40.
69. Li DS *et al.* Contrast analysis of mainly chemical ingredient of Cordyceps menshannsis and Cordyceps sinensis. *Edible Fungi China* 2002; 21: 35–37.
70. Chen Q. *Microelement and Health*. Beijing: Beijing University Press, 1989.
71. Zhu J, Wang ZG. Several metal tests in Cordyceps sinesis and its mycelium. *J Jining Med Coll* 1993; 12: 52–54.
72. Lull C *et al.* Antiinflammatory and immunomodulating properties of fungal metabolites. *Mediators Inflamm* 2005; 2: 63–80.
73. Wojcikowski K *et al*. Herbs or natural substances as complementary therapies for chronic kidney disease: ideas for future studies. *J Lab Clin Med* 2006; 147: 160–166.
74. Zhang QX, Wu JY. Cordyceps sinensis mycelium extract induces human premyelocytic leukemia cell apoptosis through mitochondrion pathway. *Exp Biol Med* 2007; 232: 52–57.
75. Wang HX *et al.* A polysaccharide-peptide complex from cultured mycelia of the mushroom Tricholoma mongolicum with immunoenhancing and antitumor activities. *Biochem Cell Biol* 1996; 74: 95–100.
76. Ng TB, Wang HX. Pharmacological actions of Cordyceps, a prized folk medicine. *J Pharm Pharmacol* 2005; 57: 1509–1519.
77. He JC, Zhang YH. Advances in studies on the anti-tumor activity of Cordyceps sinensis. *Chin J Trad West Med* 2006; 7: 2144–2147.
78. Ohno N *et al.* Antitumor activity of a beta-1,3,3-glucan obtained from liquid cultured mycelium of Grifola frondosa. *J Pharmacobiodyn* 1986; 9: 861–864.
79. Cheng WR *et al.* Progress of study on polysaccharide from *Cordyceps sinensis. Mod Food Sci. Technol* 2006; 22: 284–286, 289.
80. Jagger DV *et al.* Inhibition of Ehrlich mouse ascites tumor growth by cordycepin. *Cancer Res* 1961; 21: 216–220.
81. Huang H *et al.* Inhibitory effects of Cordyceps extract on growth of colon cancer cells. *J Chin Med Mater* 2007; 30: 310–313.
82. Bok JW *et al.* Antitumor sterols from the mycelia of Cordyceps sinensis. *Phytochemistry* 1999; 51: 891–898.
83. Ohta Y *et al. In vivo* anti-influenza virus activity of an immunomodulatory acidic polysaccharide isolated from Cordyceps militaris grown on germinated soybeans. *J Agric Food Chem* 2007; 55: 10194–10199.
84. Zhang QX *et al.* Comparison of antitumor effect of extracts in cultivated Cordyceps sinensis fungus HK-1 and natural Cordyceps sinensis. *Chin Tradit Herbal Drugs* 2005; 36: 1346–1349.
85. Sun Y *et al.* Experimental study on fruiting bodies of *Cordyceps militaris* in inhibiting the growth of carcinoma in mice with hepatocarcinoma and promoting the activity of IL-2 and NK cells. *Chin Pharm* 2002; 11: 39–40.
86. Wu GQ *et al.* Study progress in research tumour with aweto. *Guiding J TCM* 2005; 11: 80–82.
87. Yoo HS *et al.* Effects of Cordyceps militaris extract on angiogenesis and tumor growth. *Acta Pharm Sin* 2004; 25: 657–665.
88. Koc Y *et al.* Induction of apoptosis by cordycepin in ADA-inhibited TdT-positive leukemia cells. *Leukemia* 1996; 10: 1019–1024.
89. Fuller BB *et al.* Alpha-melanocyte-stimulating hormone regulation of tyrosinase in Cloudman S-91 mouse melanoma cell cultures. *J Biol Chem* 1987; 262: 4024–4033.
90. Li SP *et al.* A polysaccharide isolated from Cordyceps sinensis, a traditional Chinese medicine, protects PC12 cells against hydrogen peroxide-induced injury. *Life Sci*. 2003; 73: 2503–2513.
91. Ji PY *et al.* On activity and mechanism of anti-tumor of *Cordyceps militaris. J Beihua Univ (Nat Sci)* 2005; 6: 324–329.
92. Chirigos MA. Immunomodulators: current and future development and application. *Thy Res* 1992; 19: S7–S20.
93. Koh JH *et al.* Activation of macrophages and the intestinal immune system by an orally administered decoction from cultured medium of *Corydyceps sinensis. Biosci Biotechnol Biochem* 2002; 66: 407–411.
94. Ko KM, Leung HY. Enhancement of ATP generation capacity, antioxidant activity and immunomodulatory activities by Chinese Yang and Yin tonifying herbs. *Chin Med* 2007; 2: 1–3.
95. Zhao KR *et al.* Pharmacological effects of *Cordyceps sinensis*. *World Phytomed* 2006; 21: 105–108.
96. Shin KH *et al.* Anti-tumour and immunostimulating activities of the fruiting bodies of Paecilomyces japonica, a new type of Cordyceps spp. *Phytother Res* 2003; 17: 830–833.
97. Shin KH *et al*. Antioxidant and immunostimulating activities of the fruiting bodies of *Paecilomyces japonica*, a new type of *Cordyceps* sp. *Ann NY Acad Sci* 2001; 928: 261–273.
98. Yu LX *et al.* Effects of different components of Cordyceps sinensis polysaccharide on immune function. *J Zhejiang Coll Tradit Chin Med* 2004; 28: 49–50.
99. Guan DL, Yu HY. Experimental study of cultured *Cordyceps* mycelium in inhibiting the rejection of kidney transplantation in rat. *Chin J Urol* 1991; 12: 332–334.

100. Liu YK, Shen W. Inhibitive effect of Cordyceps sinensis on experimental hepatic fibrosis and its possible mechanism. *World J Gastroenterol* 2003; 9: 529–533.
101. Gong HY *et al.* Effects of *Cordyceps sinensis* on T lymphocyte subsets and hepatofibrosis in patients with chronic hepatitis B. *Bull Hunan Med Univ* 2000; 25: 248–250.
102. Ma DY *et al.* Research progress in pharmacological actions of *Cordyceps sinensis* and its mycelia. *J Chin Med Mat* 2001; 24: 455–458.
103. Fan MZ *et al.* Evaluation for the protective effect of Cordyceps polysaccharides on immunological liver injury in mice. *Acta Univ Med Anhui* 1999; 34: 173–175.
104. Chen HX, Jia XB. Research progress on pharmacological actions of cordyceps polysaccharides from *Cordyceps militaris*. *J JiangsSu Univ (Med)* 2005; 15: 74–77.
105. Qiu DK *et al. Cordyceps sinensis* liposome oral liquid on the immunomodulation of T cells in treatment of patients with chronic hepatitis B. *Chin J Digestion* 1995; 15: 265–267.
106. Liu JL, Zhou YC. Effect of Cordyceps taii on macrophage phagocytosis and uviofast protection. *Nat Prod Res Dev* 2001; 13: 20–22.
107. Xie HH. Nephrosis Chinese native medicine treatment research progress. *World Health Digest (Medical Periodical)* 2007; 4: 46–48.
108. Xu HY *et al.* The protective effect of *Cordyceps sinensis* on adriamycin-induced myocardial damage. *Acta Chin Med Pharmacol* 2000; 28: 64–65.
109. Gu ZL *et al.* The protective effect of lung fit on myocardial ischemia/reperfusion injury in isolated rat heart. *Chin J Hemorheol* 2002; 12: 279–282.
110. Guo HP, Yang ZM. Study progress in pharmacologic action of *Cordyceps sinensis*. *Chin Trad Herb Drugs* 1999; 30: 231–233.
111. Yu Z *et al.* The effect of *Cordyceps sinensis* on the injury of cultured neonatal rat myocardial cells due to anoxia-reoxygenation. *J First Military Med Univ* 1998; 18: 108–109.
112. Wu XX *et al.* Effect of *Cordyceps sinensis* on cardiac and vascular function in renal hypertensive rats. *Chin J Integr Med Cardio-/cerebro Vascular Dis* 2005; 3: 137–138.
113. Wu XX *et al.* Effect of *Cordyceps sinensis* on blood pressure in renal hypertensive rats. *J Jinzhou Med Coll* 2001; 22: 10–11.
114. Guo TF, Guo TY. Study on pharmacological action and clinical application of *Cordyceps sinensis*. *J Jindongnan Teachers Coll* 2000; 21: 70–73.
115. Xu CD. Advances of researches on *Cordyceps sinensis*. *J Fungal Res* 2006; 4: 60–64.
116. Zhang CK, Yuan SR. Recent advance in studies on immuno pharmacological activities of Cordyceps sinensis and its submerge-cultured mycelia. J Capital Univ Med Sci 1997; 18: 37–38.
117. Xu Y. Perspectives on the 21st century development of functio nal foods: bridging Chinese medicated diet and functional foods. *Int J Food Sci Technol* 2001; 36: 229–242.
118. Du XF *et al.* Research progress of Cordyceps militaris. *Liaoning Agric Sci* 2003; 4: 26–28.
119. Li N *et al.* Research and development of manual cultivation technique and health food. *J Changchun Normal Univ* 2001a; 20: 36–37.
120. Wang X *et al.* Manufacture of north aweto beverage. *Acad Periodical Farm Prod Process* 2006; 5: 43–45.
121. Feng ZZ *et al.* Quality standards for the *Cordyceps sinensis* Sacc polysaccharide liposomal oral liquid. *J ShengYang Pharm Univ* 2005; 22: 203–206.
122. Ma CJ *et al.* Extract and content measuring of cordycepic polyascchamide in Bailing Capsule. *J Zhejiang Coll Trad Chin Med* 2004; 7: 81–82.
123. Hu QX *et al.* Countermeasures for protecting, developing and utilizing Chinese caterpillar fungus and their resources in China. *Chin J Agric Resour Regional Planning* 2005; 26: 43–47.
124. Gao HC *et al.* Research and development for Cordyceps sinensis. *J Microbiol* 2003; 23: 50–55.
125. Li SP *et al.* The contents and their change of nucleosides from natural Cordyceps sinensis and cultured Cordyceps mycelia. *Acta Pharm Sin* 2001; 36: 436–439.
126. Yang LH *et al.* Chinese medicinal fungus and research & development of their production. *Contemporary Eco-Agric* 2003; 1: 30–33.
127. Yuan JG *et al.* R & D of Cordycepin polysaccharide. Fine Specialty Chem 2002; 10: 15–17.