7 Cultivation, Bioactive Metabolites, and Application of Caterpillar Mushroom *Cordyceps militaris*: Current State, Issues, and Perspectives

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Abstract

Cordyceps militaris is a valuable mushroom with wide use in food and medicine. This fungal product usage in many countries, especially in Southeast Asia, has become widespread. There is a growing realization that *C. militaris* can be used as a succedaneum for Chinese cordyceps (*Ophiocordyceps sinensis*) due to their similar chemical characteristics and therapeutic properties. In nature, the complicated life cycle of *C. militaris* consists of teleomorph stage, anamorph stage, and the lifespan of the host insects. The fruiting bodies propagated by inoculation on cereal substrates and silkworm pupae have been successfully mass-produced. A battery of active components such as cordycepin, adenosine, N6-(2-hydroxyethyl)-adenosine, carotenoid, and polysaccharide have been extracted from fruiting body. Evidence shows that *C. militaris* has various bioactivities such as immunomodulatory, anti-inflammatory, antitumor, antimicrobial, insecticidal, anti-fibrotic, liver protection, kidney protection, and pneumonia protection. This fungus finds can be found in functional food, healthcare

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T. Satyanarayana, S. K. Deshmukh (eds.), *Fungi and Fungal Products in Human Welfare and Biotechnology*, https://doi.org/10.1007/978-981-19-8853-0_7

fields, as well as skin care products in East Asian countries represented by China, Japan, and Korea. Full elucidation of the production capacities of different metabolites and the quality control of the products are critically needed in the future. This review will be helpful for the future research and application of this fungus.

Keywords

Cordyceps militaris · Life cycle · Fruiting body cultivation · Cordycepin · Pharmaceutical and therapeutic potential · Industry and application

7.1 Introduction

Caterpillar mushroom, *Cordyceps militaris* (L.) Fr., (Cordycipitaceae, Ascomycetes), is one of the popular edible and medicinal fungi. It is the type species of the modified genus *Cordyceps* of the family Cordycipitaceae [1]. *C. militaris* is more and more regarded as a succedaneum for Chinese cordyceps (*Ophiocordyceps sinensis*) in traditional Chinese medicine on account of similar chemical and medicinal properties [2–4].

There are several resemblances between *C. militaris* and *O. sinensis*; however, they differ in their hosts, appearance, and geographical distribution. The hosts of *C. militaris* are Lepidopteran pupae or larvae, and their fruiting bodies are yellow or orange in color (Fig. 7.1a, b), while the hosts of *O. sinensis* are larvae of *Hepialus*, and the color of their fruiting bodies is dark brown (Fig. 7.1c, d). The natural resource of Chinese cordyceps fungus is scarce, which is only found on the Tibetan Plateau. Being different from the Chinese cordyceps fungus, *C. militaris* is distributed worldwide, including North and South America, Europe, and Asia, but with a low population density in nature.

Cordyceps militaris is well-known because of its radio-protection [5], anti-influenza virus [6], anti-inflammatory, and antitumor [7] activities. A series of components, including cordycepin, adenosine, N6-(2-hydroxyethyl)-adenosine, and carotenoids, have been extracted from the *C. militaris*. The use of *Cordyceps militaris* product in medicinal treatment and health products has been well received in several countries, especially in Southeast Asia. In 2009, the Ministry of Health of the People's Republic of China officially recognized *C. militaris* as a novel food [http://www.moh.gov.cn/publicfiles/business/htmlfiles/zwgkzt/pgg/200903/39591.htm].

Large-scale fermentation and substrate culture have been widely carried out in China to meet the great demand for medicinal and edible products [8, 9]. At present, the fruiting body has been successfully cultivated commercially, which is not only used in medicine and healthcare products but also can be sold directly in supermarkets as edible fungi.

This review will summarize the research progress including the life cycle and fruiting body cultivation, metabolites and pharmacological activities,

Fig. 7.1 Fruiting body of *Cordyceps militaris* and *Ophiocordyceps sinensis* in the wild: (**a**) fruiting body of *C. militaris* out of the ground; (**b**) fruiting body of *C. militaris* and the pupae; (**c**) fruiting body of *O. sinensis* out of the ground; (**d**). fruiting body of *O. sinensis* and its host

pharmaceutical and therapeutic potential, safety and toxicity, as well as industry and applications. The issue of making this prized fungus beneficial to all mankind is critically discussed. This review summarizes the existing research results and lays the foundation for the future research.

7.2 Life Cycle and Fruiting Body Cultivation

7.2.1 Life Cycle of *Cordyceps militaris*

In nature, the complicated life cycle of *C. militaris* comprises of teleomorph stage and anamorph stage as well as the lifespan of the host insects (Fig. 7.2). The hosts are holometabolous insects (Fig. 7.2). The insects could be infected by conidia, ascospores, and hyphae. Under the specific environmental conditions, the ascospores could be ejected and then diffused by wind or water to infect the insects. *C. militaris*

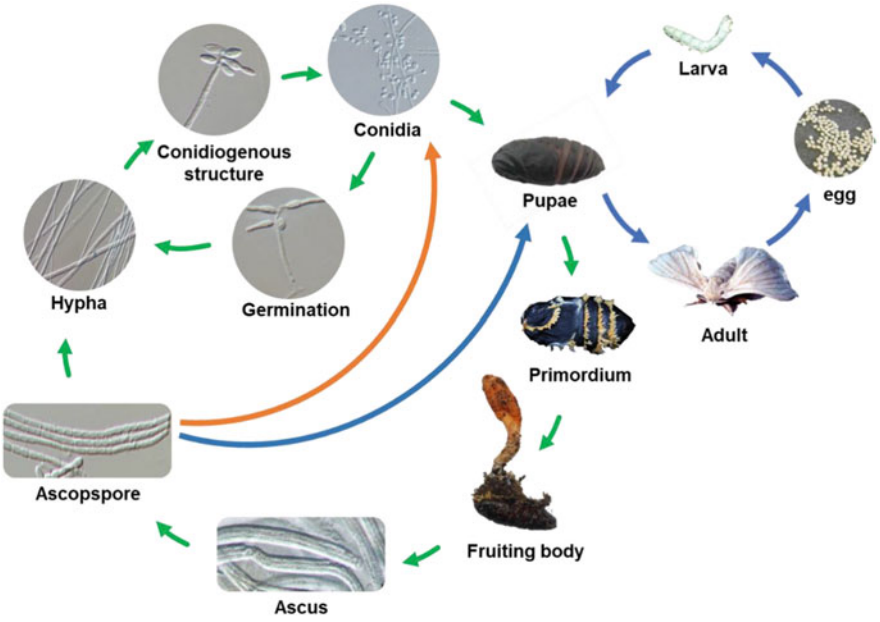

Fig. 7.2 Life cycle of *Cordyceps militaris*

colonizes the living host insects, and when the insects are eventually dead, it switches to necrotrophy. Being different from the *O. sinensis* which is homothallic, *C. militaris* is sexually heterothallic. Interestingly, single mating-type strain could also develop fruiting body without mating and meiosis, but sexual perithecia can't be generated [10].

7.2.2 The Isolates of *Cordyceps militaris*

C. militaris can be isolated from spores and tissues. For spore isolation, ascospores ejected from mature stromata are collected and then spread in sterile Potato Dextrose Agar (PDA) medium. The ascospores can germinate after 2–3 days. For isolation of tissue culture, the fruiting body was sterilized with 70% ethanol, and a piece of tissue was cut and put in sterile PDA medium under aseptic conditions.

7.2.3 Cultivation of Fruiting Bodies of *Cordyceps Militaris* on Cereal Substrates

Fruiting body cultivation on cereal substrates was divided into six stages: spawn production, mycelial growth under dark, coloring after light irradiation, primordium differentiation, fruiting body development, and fruiting body harvest. In a petri plate,

the strain was cultured on Potato Dextrose Agar (PDA), and a sterilizing cutter is used to make five to eight circles with a diameter of 5 mm from the agar plate medium and transfer them to the seed medium, that is, potato dextrose liquid medium. The liquid spawn was cultured in a 500 mL flask with 150 mL of potato dextrose liquid medium in a rotary shaker incubator. Wheat or rice medium was prepared by mixing 30 g of cereal and 45 mL of distilled water in 500 mL glass bottles and was then sterilized. The inoculated cultivation was cultured at 20 °C for 7–10 days under dark condition and then under 12 h light and 12 h dark conditions, with relative humidity greater than 80%. The primordium was induced at temperature of 18–22 °C, high humidity of 70–80% under a light intensity of 400–500 lx. After 60–70 days of culture, fresh fruiting bodies were harvested. The by-products produced in large quantities (mainly culture substrates) are mostly used as animal feed.

7.2.4 Cultivation of Fruiting Bodies of *Cordyceps militaris* on Pupae

We can obtain the complete products similar to the natural products of *C. militaris* by cultivation on pupae. The fruiting bodies of pupae are displayed in Fig. 7.3b; *Bombyx mori* silkworm pupae can be raised for fruiting body cultivation. In order to remove entangled hyphae, the liquid spawn was prepared as mentioned above and then filtered through sterilized gauze. A syringe is used to inject liquid spawn (0.1 mL) into each pupa. The injected pupae were incubated in containers at 20 °C until fungal growth caused their bodies to become stiff and mummified after injection inoculation.

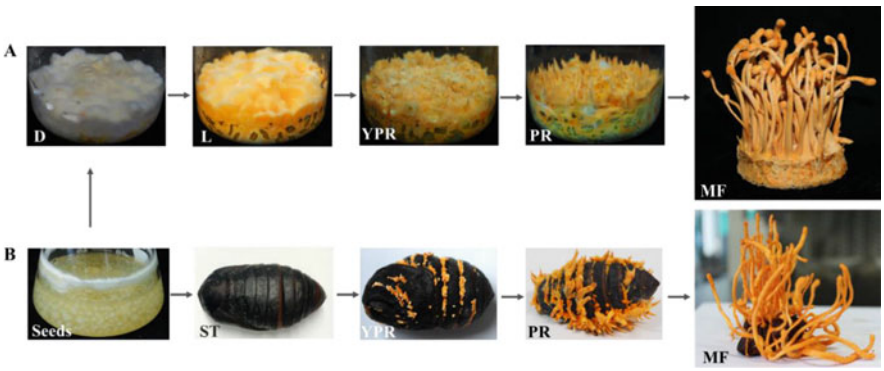

Fig. 7.3 *Cordyceps militaris* fruiting bodies cultivated on wheat medium (**a**) and on pupae (**b**): D (dark); L (light irradiation for 4 d); ST (sclerotium); YPR (young primordium); PR (primordium); MF (mature fruiting body)

7.2.5 Strain Degeneration of *Cordyceps militaris* and Strain Preservation

During the industrial cultivation, the degeneration of *C. militaris* strain frequently results in a decline in growth rate, fruiting body output, and secondary metabolite content, which can cause significant financial losses [11, 12]. The degeneration of *C. militaris* is influenced by environmental factors (such as culture medium, preservation techniques, oxidative stress, and subculture) as well as genetic variation (such as DNA methylation and degeneration-related genes). The primary factor for the degeneration of *C. militaris* strains is genetic variation [13].

Degradation can be avoided or delayed by proper preservation methods. Lyophilization allows *C. militaris* strains to preserve their natural properties and avoid degradation, which is appropriate for long-term preservation (at least 4 years). The characteristics of *C. militaris* strains stored in sterile water are similar to that of −196 °C cryopreservation in liquid nitrogen for at least 1 year. Preservation in sterile water is an effective method for the preservation of *C. militaris* strains, which can be used in factory production. For this fungus, −80 °C cryogenic freezing is not applicable [14].

7.3 Metabolites and Biological Activities

Multiple compounds have been extracted, detected, and purified from *C. militaris*, including cordycepin, cordycepic acid, carotenoids, ergosterol, polysaccharides, nucleosides, and other compounds.

7.3.1 Nucleosides and Analogues

Cordycepin (3′-deoxyadenosine, $C_{10}H_{13}N_5O_3$) is the most important nucleoside analogue which was first purified from *C. militaris* [15, 16], and it has demonstrated a wide range of pharmacological activities. A ribose moiety is attached to a purine (adenine) nucleoside molecule via a β-N9-glycosidic bond in the structure of cordycepin (Fig. 7.4). Most of the ^{14}C-labeled substances (adenosine, adenine, etc.) and ^{3}H -labeled ribose were determined to be potential cordycepin precursors [17, 18]. Therefore, it was assumed that cordycepin was synthesized via purine nucleotide pathway. However, a comprehensive description of cordycepin production was not proposed and confirmed until 2017 [19]. It was demonstrated that adenosine served as the starting point for the sequential reactions of phosphorylation, dephosphorylation, and reduction, which were performed by the Cns1/Cns2 complex (Fig. 7.4).

Various pharmacological activities of cordycepin including antioxidant, anticancer, anti-inflammatory, antidiabetic, and antimicrobial activities and inhibition of platelet aggregation have been reported (Table 7.1). Cordycepin induces both extrinsic apoptotic and caspase-mediated intrinsic regulation [20–23], as well as

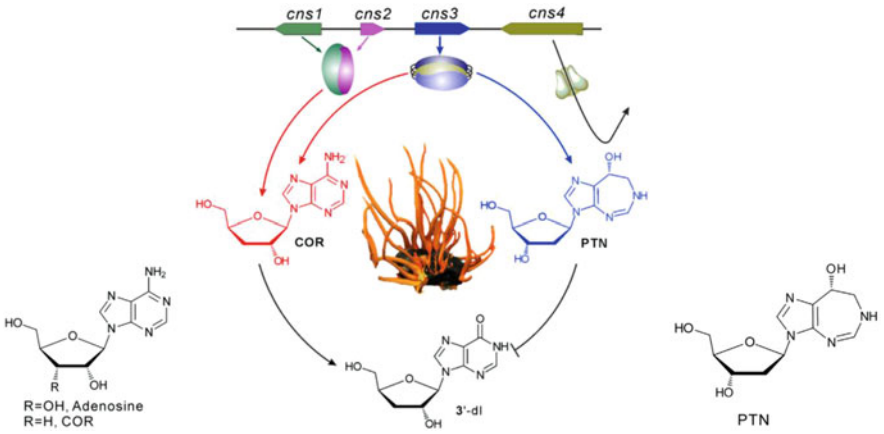

Fig. 7.4 Chemical structures and synthesis pathway of cordycepin and pentostatin [19]: COR, cordycepin; PTN, pentostatin; 3'dI, 3′-deoxyinosine

Table 7.1 Biological activities of metabolites from *Cordyceps militaris*

Metabolites	Biological activities	References
Adenosine	Regulate energy homeostasis, affect the function of various organs and central nervous system activity, and modulate irregular heartbeat, delay recovery of synaptic transmission following spreading depolarization; tumor immunity	[40] [43] [42] [39] [41]
Cordycepin	Anti-inflammatory, antioxidant, antitumor, antidiabetic, antibacterial, platelet aggregation inhibition, hypolipidemic, analgesia, immunomodulatory, antithrombotic effects, and neuroprotective effects	[89] [90] [23] [91] [92] [93] [94] [95] [96]
Cordycepic acid	Anti-lung cancer	[71]
Polysaccharides	Elevating TNF-α and IL-1β secretion in macrophage, increasing nitric oxide production in macrophage, enhancing T lymphocyte proliferation and IL-2 secretion, promoting macrophage phagocytotic and acid phosphatase activities, anti-influenza, stimulating dendritic cell maturation	[97] [98] [99] [100] [101]
Pentostatin	Treatment of several forms of leukemia	[102]
Ergosterol	Anti-inflammatory activity, diuretic activity, anticancer activity, antioxidant, antitumor, inhibition of bladder carcinogenesis promotion	[65] [103] [104] [68]

resistant cancer metastasis [22, 24], and proliferation via MAPK [25, 26], GSK-3β [23, 27], and ERK-JNK pathways [26, 28] found that cordycepin may reduce non-alcoholic fatty liver in ob/ob mice, and the potential mechanism may be mediated by reduced expression of genes involved in inflammation and lipid formation. Cordycepin could also modulate the central nervous system by suppressing excitatory synaptic transmission via a presynaptic mechanism in rat hippocampal slices [29]. Besides, cordycepin could shield dopamine neurons from rotenone-induced apoptosis by effectively improving mitochondrial dysfunction [30]. Cordycepin could stimulate Wnt/β-catenin signaling pathway through the activation of adenosine receptors, which may accelerate the process of tissue remodeling, showing its potential in the treatment of skin wounds. In brief, cordycepin showed important therapeutic properties in various ailments, as well as nutraceuticals for chronic disease prevention [31].

Another adenosine analogue, pentostatin (2′-deoxycoformycin, $C_{11}H_{16}N_4O_4$), was first isolated from *Streptomyces antibioticus* in the 1970s [32], which is an adenosine deaminase irreversible inhibitor preventing the deamination of cordycepin to 3′-deoxyinosine (Fig. 7.4). It was confirmed that an individual gene cluster can generate both cordycepin and pentostatin [19]. Pentostatin helps to suppress deamination of cordycepin to 3′-deoxyinosine in vivo, probably by blocking adenosine deaminase in *C. militaris*.

These two adenosine analogues produced in tandem follow a bacterial-like protector-protégé strategy of purine metabolism. Pentostatin, in combination with cordycepin, has been reported in clinical trials of refractory TdT-positive leukemia [http://clinicaltrials.gov/show/NCT00709215].

Pentostatin, a purine analogue, was authorized for use in the United States in 1991 to treat hairy cell leukemia and T cell lymphomas [33]. Pentostatin, as a strong adenosine deaminase (ADA) inhibitor, has also been investigated for use in preventing GVHD (graft-versus-host disease) [34–36]. The combination of cordycepin and pentostatin showed reduction of epimastigotes and trypomastigotes in vitro, but no therapeutic effect infected by *Trypanosoma cruzi* in mice [37]. Pentostatin could also have common adverse effects, including fever, headache, nausea, fatigue, infection, anorexia, vomiting, and rash [33].

Additional nucleosides were isolated from *C. militaris* such as adenine, adenosine, uracil, uridine, guanidine, guanosine, hypoxanthine, and N6-(2-Hydroxyethyl)-adenosine.

Adenosine ($C_{10}H_{13}N_5O_4$, Fig. 7.5) is involved in various physiological and pathophysiological regulatory mechanisms [38] and has been used as quality standard of some cordyceps products. Adenosine serves as a signaling substance in addition to being a significant component of ribonucleic acids and adenine nucleotides. It can regulate energy homeostasis, affect the function of various organs and central nervous system activity, and modulate irregular heartbeat (Table 7.1). Extracellular adenosine acts as a signaling molecule by binding to adenosine receptors, which could trigger multiple signaling pathways to regulate energy homeostasis and affect the operation of many organs [39]. Adenosine can be used for a variety of important therapies, including regulating central nervous system

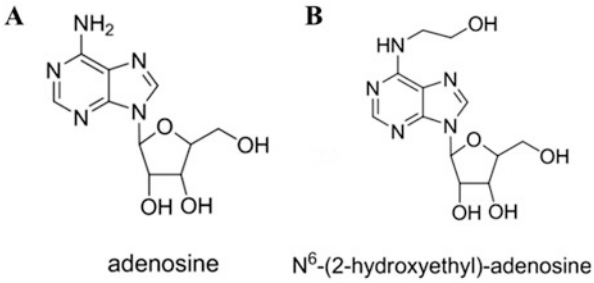

Fig. 7.5 Chemical structures of adenosine (**a**) and N6-(2-hydroxyethyl)-adenosine (**b**) [46]

(CNS) function and modulating irregular heartbeat (arrhythmias) [40, 41]. The accumulation of extracellular adenosine in neural tissue resulted in a delay in the synaptic recovery process after spreading depolarization [42]. Leone and Emens described the function of adenosine signaling in regulating tumor immunity, emphasizing underlying therapeutic targets in the pathway [43]. Besides, behavioral and physiological symptoms produced by insufficient adenosine were consistent with many comorbidities of autism in rodents [44], and adenosine might be a novel therapeutic target for autism in rodents and humans [45].

The first calcium antagonist of biological origin, N6-(2-hydroxyethyl)-adenosine ($C_{12}H_{17}N_5O_5$, HEA, Fig. 7.5), is also an inotropic agent [46]. Among the over 500 species of *Cordyceps* sensu lato, HEA could be produce by only a few species such as *C. pruinosa*, *C. militaris*, and *I. tenuipes* [47]. Recent studies have shown that HEA has a variety of biological functions. In pharmacological studies, cerebral and coronary circulation could be regulated by HEA which acts as a sedative [48]. It can prevent K562 erythroleukemia cells from proliferating in vitro [49]. The analgesic mechanism of HEA differs from that of opioids, a widely used analgesic drug. More importantly, the benefit of HEA over opioids is that it is neither addictive nor pepsin-affected [50]. Peng et al. reported that HEA shielded mice from renal ischemia reperfusion damage [51]. Additionally, HEA reduced the pro-inflammatory reactions brought on by lipopolysaccharide through inhibiting the toll-like receptor 4-mediated nuclear factor-κB signalling pathway [52]. A recent study showed that HEA targeting an adenosine receptor showed insecticidal activity to *Plutella xylostella* [53], suggesting an environmentally friendly pesticide [54].

7.3.2 Polysaccharide

One of the major biologically active elements of *C. militaris* is polysaccharide. The healthcare and pharmacological activities of *C. militaris* were confirmed by a mass of animal experiments and clinical experiments (Table 7.1). *Cordyceps militaris* contains various polysaccharides, which mainly composed of mannose, glucose, and galactose in varying molar ratios [55–58]. In addition, several isolated polysaccharides contained monosaccharides such as xylose, rhamnose, and arabinose [59, 60]. The change of monosaccharide molar ratio of polysaccharides may be

related to raw materials, separation and purification techniques, etc. Polysaccharides had antiviral, antioxidative, anti-inflammatory, antitumor, neuroprotective, antihypertensive, and immunomodulatory biological effects [6, 61, 62]. The biological functions and healthcare functions of polysaccharides were reviewed by Zhang et al. [63].

7.3.3 Ergosterol

Ergosterol ($C_{28}H_{44}O$, Fig. 7.6) is an important sterol component of *C. militaris* which is an indispensable source of fat-soluble vitamin D and can be determined by HPLC method [64]. Ergosterol was first isolated from *Claviceps purpurea* (Fr.) Tul in 1898; subsequently, it was discovered in a variety of edible and medicinal fungi. As an important component of fungal cell membranes, it plays a significant role in ensuring cell viability, membrane integrity, and completion of the cell cycle.

Ergosterol safeguards the phospholipids in cell membranes from oxidation by operating as an antioxidant in a manner akin to vitamin E [65]. Ergosterol significantly improved the anticancer effect [66]. By regulating the TGF-β1/Smad2 signaling pathway, ergosterol decreased the proliferation of mesangial cells and the subsequent deposition of ECM [67]. Both oral administration and intraperitoneal administration of ergosterol inhibited the promotion of bladder carcinogenesis with saccharin sodium, and the last one showed a stronger effect [68].

7.3.4 Cordycepic Acid

Cordycepic acid ($C_6H_{14}O_6$) also known as D-mannitol was first determined from *O. sinensis* which was reported to be a quinic acid isomer [69]. As a tetrahydroxy monobasic acid, it forms a crystalline tetra-acetate, a mono-methyl ester, and a tetrabenzoate in *O. sinensis* [69]. The structure and chemical composition of mannitol between *O. sinensis* and *C. militaris* are substantially uniform. Cordycepic acid can

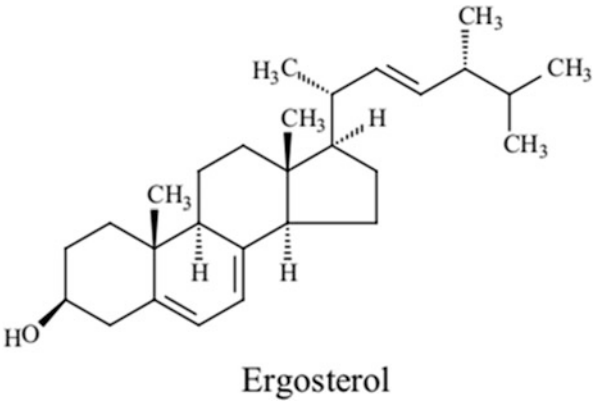

Fig. 7.6 Chemical structure of ergosterol [64]

improve LPS-induced hepatic stem cell inflammation phenotype and TGFB1-induced hepatic stem cell fibrosis response [70]. By regulating Nrf-2/HO-1/NLRP3/NF-κB signal in nude mice, cordycepic acid has a significant antitumor effect on carcinoma of the lung [71].

7.3.5 Carotenoids

Carotenoids are a class of pigmented chemicals produced by plants and microbes but not by animals [72]. All carotenoids possess a polyisoprenoid structure [73]. Under the illumination condition, the colony of *C. militaris* exhibited the color of orange with high carotenoid content in mycelia [74]. Carotenoids isolated from the fruiting body of *C. militaris* include lutein, zeaxanthin, and four cordyxanthins (Fig. 7.7) [75, 76]. *C. militaris* is rich in carotenoids, with a greater amount than other known mushrooms. It was proposed that carotenoid concentration should be used as a quality criteria for commercial products of this valuable fungus [77].

As a family of natural organic pigment compounds, carotenoid could decrease oxidative stress, which is related to the progression [78], and the progression of age-related diseases [79]. Cell-mediated immune function and sexual attraction changed with the carotenoid content of the diet in male zebra finches [80]. Carotenoid had also characteristic functions such as cell cycle inhibition, improvement of gap-junctional communication, and induction of cell differentiation and apoptosis [81].

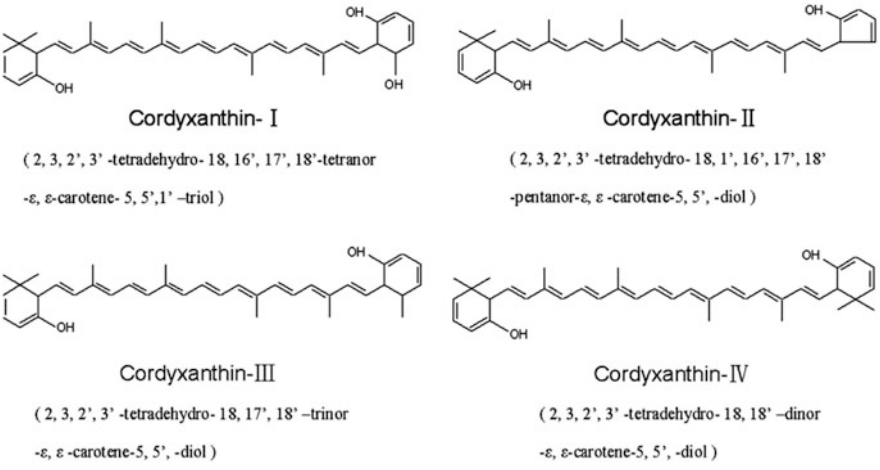

Fig. 7.7 Chemical structure of four cordyxanthins [75]

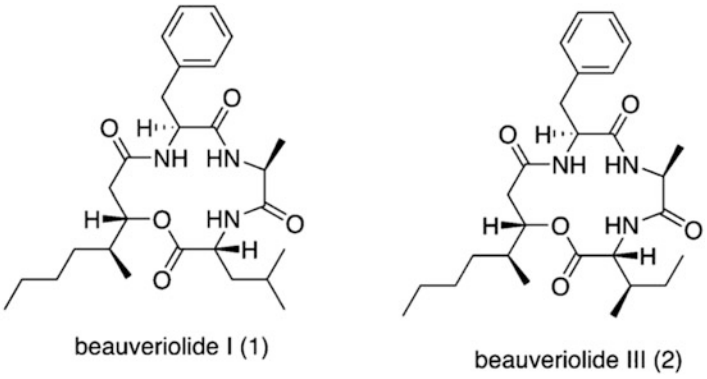

Fig. 7.8 Chemical structure of beauveriolides [88]

7.3.6 Other Compounds

5,5'-Dibuthoxy-2,2′-bifuran is initially found from plants. It was extracted for the first time from *C. militaris* that showed antibacterial activity against *Bacillus subtilis* and *Escherichia coli* [82].

In addition to the above compounds, superoxide dismutase (SOD) is an active protease that can eliminate oxidative stress to protect cells from damage of aerobic metabolite [83]. Cephalosporolides C, E, and F, ten-membered macrolides ($C_{10}H_{14}O_4$), 2-carboxymethyl-4-(3′-hydroxybutyl) furan, and pyridine-2, 6-dicarboxylic acid ($C_7H_5NO_4$) were also extracted from *C. militaris* [84]. *C. militaris* lectin showed no hemagglutination activity in human ABO erythrocytes but had agglutination activity in mouse and rat erythrocytes [85]. Cerebrosides with anti-inflammatory and anti-PTP1B activities were isolated in *C. militaris* [86, 87]. Recently, *C. militaris* genome mining revealed a possible gene cluster for acyl-CoA: cholesterol acyltransferase inhibitor beauveriolides (Fig. 7.8) [88].

Apart from the metabolites described above, genomic analysis of *C. militaris* identified secondary metabolite clusters of seven polyketide synthases (PKS), five NRPS-PKS, eight non-ribosomal peptide synthetases (NRPS), and four terpene synthases (TS). Many more metabolites should be expected to be dug out.

7.4 Pharmaceutical and Therapeutic Potential of *Cordyceps militaris*

Cordyceps has long been used as a tonifying kidney and lung health medicine, as well as to treat asthma, tuberculosis, chronic bronchitis, and other respiratory illnesses. Evidence showed that *C. militaris* are beneficial to act as anti-angiogenic, anti-inflammatory, antitumor, antioxidant, anti-aging, immunomodulatory, antimicrobial, antiviral, anti-protozoal, insecticidal, anti-fibrotic, hypolipidemic, liver

Table 7.2 List of pharmacological effects of *Cordyceps militaris*

Pharmacological effects	Samples of *Cordyceps militaris*	References
Anti-angiogenic	*C. militaris* extract	[105]
Antitumor/anti-proliferatory	*C. militaris* protein	[106, 107]
	Aqueous extract	
	BuOH extracts	[108]
	Crude polysaccharide	[109]
	Alcohol extract (anti-non-small cell lung cancer)	[110]
Induce apoptosis	Aqueous extract	[111]
		[106, 107]
	Alcohol extract	[110]
Immunomodulatory	Purified cordycepin	
	Homogeneous polysaccharide (CMP-III)	[112]
Anti-inflammatory	Water extract	[110]
	Constituents isolated from *C. militaris*	[113]
Hypoglycemic	*C. militaris* extract	[114]
	Exopolysaccharide	[109]
Anti-fibrotic	EPC from *C. militaris*	[115]
Improves chronic kidney disease	Cordycepin	[87]
Anti-PTP1B (protein tyrosine phosphatase 1B)	A new cerebroside	[116]
Antioxidant	*C. militaris* extracts-mediated nanoemulsion	[117]
Against liver dysfunction and obesity	Fermented *C. militaris* extract	[118]
Attenuated doxorubicin-induced cytotoxic effects in chemotherapy	Polysaccharides	[119]
Against bisphenol A-induced reproductive damage	*C. militaris* extract	[23]
Inhibit RANKL-induced osteoclast differentiation	*C. militaris* mushroom and cordycepin	[120]
Lipid-lowering	Two novel polysaccharides, CM1 and CMS	[121]
Prevent Pb^{2+}-induced liver and kidney toxicity	Extracellular polysaccharide	[122]
Alleviated allergic rhinitis	Ethanol extract prepared from silkworm pupa-cultivated *C. militaris* fruiting bodies	[123]
Anti-atherosclerotic	Alkaline-extracted polysaccharides were obtained from the fruiting body	[124]
Antidepressant	Water extract	[125]

protection, and lung protection. The pharmaceutical and therapeutic potential of *C. militaris* and the samples used were listed in Table 7.2.

7.5 Safety and Toxicity

Mycotoxin contamination in food and feed has long posed a threat to human and animal health [126]. *C. militaris* has been consumed for many years, and its safety has also been demonstrated because the genome lacks genes known to be responsible for human mycotoxins [127]. The sub-chronic toxicity experiments of submerged mycelial of *C. militaris* in rats for 90 days showed that the threshold of no adverse reaction of *C. militaris* hyphae to male and female SD rats was 4000 mg per kg BW per day [128]. *C. militaris* extract has a good effect on mild liver dysfunction by inhibiting lipid accumulation and slowing down the progression of fatty liver [129]. It was proved that cordycepin is a non-toxic and non-mutagenic compound by test and in rat model. The hematological, blood chemistry, and tissue microanatomical characteristics of rats fed cordycepin daily for 30 consecutive days were not significantly different from those of normal rats [130].

However, the production of highly effective cordycepin and penstatin is a key criterion for the selection of strains for mass production. Similar to the side effects of pentostatin and adenosine analogues, there have been anecdotal reports of the adverse effects of nausea and diarrhea after ingestion of products with high levels of cordycepin/pentostatin [66].

7.6 Industry and Application

In traditional Chinese medicine, *Cordyceps* fungi have been employed for centuries. For various biological and pharmacological activities of *C. militaris*, there are many applications in functional food, healthcare fields, as well as skin care product in China, Japan, Korea, and other East Asian countries. Mass production of *C. militaris* inoculated on cereal substrates and silkworm pupae has long been successful. Currently, cordyceps have turned to be a big industry. Without taking into account the yield of *C. militaris* in South Korea, Japan, Vietnam, and Thailand, yearly output amounts to 90, 559 tons in 2018 and annual value of production was about 10 billion RMB in China.

Fruiting bodies have been commercially cultivated on a large scale, not only for medicine and healthcare products but also for direct consumption in supermarkets as edible fungi. There are a great number of products of dry or fresh fruiting bodies of *C. militaris* on the market (Fig. 7.9). The famous cordyceps recipe is made with duck. *C. militaris* chicken soup is also popular (Fig. 7.10).

In 2009, the Ministry of Health of the People's Republic of China officially recognized *C. militaris* as a novel food. The fruiting bodies of *C. militaris* have also been processed into a variety of different products, including cordyceps noodle, drink, tea, and so on (Fig. 7.11). In addition, 36 officially healthy foods are made from *C. militaris*. Most of them were used for improving the immunity. *C. militaris* is used in cosmetic, and there are products of mask, lotion, moisturizer, and so on (Fig. 7.12). In recent years, it has been reported that the solid-based residues (SBRs) of *Cordyceps* mushroom fruiting body can be used as a potential source of crude

Fig. 7.9 Fresh and dried fruiting bodies of *Cordyceps militaris* in the market. (**a**) products of fresh fruiting body; (**b**) products of dry fruiting body

Fig. 7.10 Dishes cooking with *Cordyceps militaris*

Fig. 7.11 Processed foods of *Cordyceps militaris*. (**a**) noodle; (**b**) beverage; (**c**) tea; (**d**) health food; (**e**) candy; (**f**) health products with high cordycepin content

Fig. 7.12 Cosmetics of *Cordyceps militaris*

extracts for cosmetics and can be further used as multifunctional ingredients in cosmetics and cosmeceuticals [131]. Nowadays, an enormous industry building on *C. militaris* has been flourished in China.

C. militaris mycelia powder (Z20030034) and capsule (Z20030035) (Fig. 7.13) which are manufactured by Jilin Zhong sheng Pharmaceutical Co., Ltd. have been approved by the Ministry of Health of the People's Republic of China. The pharmacological effects of the medicines are shown in the drug instruction.

Fig. 7.13 Capsule (Z20030035) manufactured by Jilin Zhong sheng Pharmaceutical Co., Ltd., China

7.7 Perspective

7.7.1 *Cordyceps militaris* and the Other Species of *Cordyceps* s.l.

Cordyceps s.l. include three families of Hypocrealean fungi, i.e., Cordycipitaceae, Ophiocordycipitaceae, and Clavicipitaceae. Only a few fungi of cordyceps have been employed in traditional medicine, such as *Ophiocordyceps sinensis*, *C. militaris*, and *C. cicadae* (syn. *Isaria cicadae*). Among them, *C. militaris*, *C. cicadae*, and *O. guangdongensis* have been approved as novel foods by the Ministry of Health of the People's Republic of China. All the related fungi were called as *Cordyceps* fungi. Here, we called on that the accurate species name should be used both in the scientific research and industrial production in case of confusion.

7.7.2 In-Depth Investigations of the Secondary Metabolites of *Cordyceps militaris*

Except cordycepin, the polysaccharides of other pharmacologically active compounds need to be identified and elucidated in relation to their structure and function. In contrast to the few known metabolites, *C. militaris* genome encodes a series of biosynthetic gene clusters with the potential to generate a large number of unknown compounds. Full elucidation of the production capacities of different secondary metabolites is an urgent need in the future.

7.7.3 Quality Control for *Cordyceps militaris* Products

Although *C. militaris* has become a massive industry and various products have been in market, there are no special active components for the products except the common ones such as polysaccharides and adenosine. One of the major problems limiting development in modern industry is the lack of quality control over medicinal fungi and other traditional Chinese medicines. Chemical (secondary metabolites) and protein fingerprinting should be the choice for the quality control in industry.

7.8 Conclusions

Cordyceps militaris has attracted considerable research and commercial interest because it contains active compounds beneficial to human health, and the fruiting bodies are relatively easy to grow with a short growth period. We believe the fruiting body and the products of *C. militaris* will benefit mankind more and more in the future.

References

1. Sung G, Hywel-Jones NL, Sung JM, Luangsa-ard JJ, Shrestha B et al (2007) Phylogenetic classification of *Cordyceps* and the *Clavicipitaceous* fungi. Stud Mycol 57:5–59
2. Dong JZ, Lei C, Ai XR, Wang Y (2012) Selenium enrichment on *Cordyceps militaris* link and analysis on its main active components. Appl Biochem Biotechnol 166:1215–1224
3. Gao SY, Wang FZ (2008) Research of commercialized cultivation technology on *cordyceps militaris*. North Hortic 9:212–215. (in Chinese)
4. Yue GGL, Lau CBS, Fung KP, Leung PC, Ko WH (2008) Effects of Cordyceps sinensis, *Cordyceps militaris* and their isolated compounds on ion transport in Calu-3 human airway epithelial cells. J Ethnopharmacol 117:92–101
5. Jeong MH, Park YS, Jeong DH, Lee CG, Kim JS et al (2014) In vitro evaluation of *Cordyceps militaris* as a potential radioprotective agent. Int J Mol Med 34:1349–1357
6. Lee HH, Park H, Sung GH, Lee K, Lee T et al (2014) Anti-influenza effect of *Cordyceps militaris* through immunomodulation in a DBA/2 mouse model. Mikrobiol Zh 52:696–701
7. Liu F, Zhu ZY, Sun XL, Gao H, Zhang YM (2017a) The preparation of three selenium-containing *Cordyceps militaris* polysaccharides: characterization and anti-tumor activities. Int J Biol Macromol 99:196–204
8. Dai JJ, Fan T, Wu CH, Xiao LZ, Tian SF (2007) Summarization of the study on the artificial cultivation of *Cordyceps militaris* link. J Anhui Agric Sci 35:5469–5471. (in Chinese)
9. Gu YX, Wang ZS, Li SX, Yuan QS (2007) Effect of multiple factors on accumulation of nucleosides and bases in *Cordyceps militaris*. Food Chem 102:1304–1309
10. Lu YZ, Xia YL, Luo FF, Dong CH, Wang CS (2016) Functional convergence and divergence of mating-type genes fulfilling in *Cordyceps militaris*. Fungal Genet Biol 2016(88):35–43
11. Xiong CH, Xia YL, Zheng P, Wang CS (2013) Increasing oxidative stress tolerance and subculturing stability of *cordyceps militaris* by overexpression of a glutathione peroxidase gene. Appl Microbiol Biotechnol 97:2009–2015
12. Yin J, Xin XD, Weng YJ, Gui ZZ (2017) Transcriptome-wide analysis reveals the progress of *cordyceps militaris* subculture degeneration. PLoS One 12:e0186279
13. Lou HW, Lin JF, Guo LQ, Wang XW, Tian SQ et al (2019) Advances in research on *Cordyceps militaris* degeneration. Appl Microbiol Biotechnol 103:7835–7841
14. Liu Q, Wang F, Liu KB, Dong CH (2018) Influence of strain preservation methods on fruiting body growth and metabolite production by the medicinal mushroom *Cordyceps militaris* (ascomycetes). Int J Med Mushrooms 20:1003–1011
15. Ahn YJ, Park SJ, Lee SG, Shin SC, Choi DH (2000) Cordycepin: selective growth inhibitor derived from liquid culture of *Cordyceps militaris* against *Clostridium* spp. J Agric Food Chem 48:2744–2748
16. Cunningham KG, Manson W, Spring FS, Hutchinson SA (1950) Cordycepin, a metabolic product isolated from cultures of *Cordyceps militaris* (Linn.) link. Nature 166:949–949
17. Kredich NM, Guarino AJ (1961) Homocitrullylaminoadenosine, a nucleoside isolated form *Cordyceps militaris*. J Biol Chem 236:3300–3302

18. Lennon MB, Suhadolnik RJ (1976) Biosynthesis of 3'-Deoxydenosine by cordyceps-militaris-mechanism of reduction. Biochim Biophys Acta-Biomembr 425:532–536
19. Xia YL, Luo FF, Shang YF, Chen PL, Lu YZ, Wang CS (2017) Fungal cordycepin biosynthesis is coupled with the production of the safeguard molecule pentostatin. Cell Chem Biol 24:1479–1489
20. Shao LW, Huang LH, Yan S, Jin JD, Ren SY (2016) Cordycepin induces apoptosis in human liver cancer HepG2 cells through extrinsic and intrinsic signaling pathways. Oncol Lett 12: 995–1000
21. Tian T, Song L, Zheng Q, Hu XJ, Yu RM (2014) Induction of apoptosis by *Cordyceps militaris* fraction in human chronic myeloid leukemia K562 cells involved with mitochondrial dysfunction. Pharmacogn Mag 10:325–331
22. Tao XD, Ning Y, Zhao XW, Pan T (2016) The effects of cordycepin on the cell proliferation, migration and apoptosis in human lung cancer cell lines A549 and NCI-H460. J Pharm Pharmacol 68:901–911
23. Wang J, Chen C, Jiang ZH, Wang M, Jiang H, Zhang XY (2016) Protective effect of *Cordyceps militaris* extract against bisphenol an induced reproductive damage. Syst Biol Reprod Med 62:249–257
24. Robinson DR, Wu YM, Lonigro RJ, Vats P, Cobain E et al (2017) Integrative clinical genomics of metastatic cancer. Nature 548:297–303
25. Baik JS, Mun SW, Kim KS, Park SJ, Yoon HK et al (2016) Apoptotic effects of Cordycepin through the extrinsic pathway and p38 MAPK activation in human glioblastoma U87MG cells. J Microbiol Biotechnol 26:309–314
26. Hwang JH, Joo JC, Kim DJ, Jo E, Jang IS (2016) Cordycepin promotes apoptosis by modulating the ERK-JNK signaling pathway via DUSP5 in renal cancer cells. Am J Cancer Res 6:1758–1771
27. Fishman P, Bar-Yehuda S, Liang BT, Jacobson KA (2012) Pharmacological and therapeutic effects of A3 adenosine receptor agonists. Drug Discov 17:359–366
28. Zhong L, Zhao L, Yang F, Yang W, Sun Y et al (2017) Evaluation of anti-fatigue property of the extruded product of cereal grains mixed with *Cordyceps militaris* on mice. J Int Soc Sport Nutr 14:15
29. Yao LH, Huang JN, Li CH, Li HH, Yan WW et al (2013) Cordycepin suppresses excitatory synaptic transmission in rat hippocampal slices via a presynaptic mechanism. CNS Neurosci Ther 19:216–221
30. Jiang X, Tang PC, Chen Q, Zhang X, Fan YY et al (2019) Cordycepin exerts neuroprotective effects via an anti-apoptotic mechanism based on the mitochondrial pathway in a rotenone-induced parkinsonism rat model. CNS Neurol Disord Drug Targets 18:609–620
31. Devi A, Chennakesavulu S, Suresh C, Reddy ABM (2018) In: Rani V, Yadav UCS (eds) Nutraceuticals and their role in human health and disease. Functional food and human health. Springer Nature, Singapore, pp 375–403
32. Woo PWK, Dion HW, Lange SM, Dahl LF & Durham LJ (1975) Cheminform abstract: a novel adenosine and Ara-A, deaminase inhibitor, (R)-3-(2-deoxy-beta-D-erythropento-furanosyl)-3, 6, 7, 8-tetrahydroimidazo (4, 5-D) (1, 3) diazepin-8-OL. Chem. Inform. 6: Abstract 446
33. Lamanna N, Kay NE (2009) Pentostatin treatment combinations in chronic lymphocytic leukemia. Clin Adv Hematol Oncol 7:386–392
34. Bolanos MJ, Jacobsohn DA, Margolis J, Ogden A, Wientjes MG et al (2005) Pentostatin in steroid-refractory acute graft-versus-host disease. J Clin Oncol 23:2661–2668
35. Jacobsohn DA, Chen AR, Zahurak M, Piantadosi S, Anders V, Bolanos MJ (2007) Phase II study of Pentostatin in patients with corticosteroid-refractory chronic graft-versus-host disease. J Clin Oncol 25:4255–4261
36. Parmar S, Andersson BS, Couriel D, Munsell MF, Fernandez-Vina M et al (2011) Prophylaxis of graft-versus-host disease in unrelated donor transplantation with pentostatin, tacrolimus,

and mini-methotrexate: a phase I/II controlled, adaptively randomized study. J Clin Oncol 29: 294–302
37. do Carmo GM, de Sa Ma F, Grando TH, Gressler LT, Baldissera MD et al (2019) Cordycepin (3′-deoxyadenosine) and pentostatin (deoxycoformycin) against *Trypanosoma cruzi*. Exp Parasitol 199:47–51
38. Jacobson KA, Hoffmann C, Cattabeni F, Abbracchio MP (1999) Adenosine-induced cell death: evidence for receptor-mediated signalling. Apoptosis 4:197–211
39. Novotný J (2015) Adenosine and its role in physiology. Cesk Fysiol 64:35–44
40. Choudhury H, Chellappan DK, Sengupta P, Pandey M, Gorain B (2019) Adenosine receptors in modulation of central nervous system disorders. Curr Pharm Des 25:2808–2827
41. Nattel S (2012) Adenosine and atrial arrhythmias: exploring electrophysiological mechanisms in vivo. PACE-Pacing Clin Electrophysiol 35:553–555
42. Lindquist BE, Shuttleworth CW (2017) Evidence that adenosine contributes to Leao's spreading depression in vivo. J Cereb Blood Flow Metab 37:1656–1669
43. Leone RD, Emens LA (2018) Targeting adenosine for cancer immunotherapy. J Immunother Cancer 6:57
44. Mcpartland J, Volkmar FR (2012) Autism and related disorders. Handb Clin Neurol 106:407
45. Masino SA, Kawamura M Jr, Cote JL, Williams RB, Ruskin DN (2013) Adenosine and autism: a spectrum of opportunities. Neuropharmacology 68:116–121
46. Furuya T, Hirotani M, Matsuzawa M (1983) N6-(2-hydroxyethyl) adenosine, a biologically active compound from cultured mycelia of *Cordyceps* and *Isaria* species. Phytochemistry 22: 2509–2512
47. Liu K, Wang F, Wang WZ, Dong CH (2017b) *Beauveria bassiana*: a new N6-(2-hydroxyethyl)-adenosine-producing fungus. Mycology 8:259–266
48. Wang DM, Liu XH, Guo H, Huang JH, Wang L (2013) Design, synthesis and biological activity evaluation of adenosine analogues. Acta Pharm Sin 48:881–886. (in Chinese)
49. Zhu LN, Xue JJ, Liu YF, Zhou S, Tang QJ (2013) Isolation, purification and anti-tumor activity of N6-(2-hydroxyethyl)-adenosine from the fruiting body *Cordyceps militaris* cultured. Acta Edulis Fungi 20:62–65. (in Chinese)
50. Chai YQ, Wei ZM, Chen ZA, Li XL, Liu YG et al., (2004) N6-(2-hydroxyethyl)-adenosine' application in the preparation of analgesic drugs. China, ZL200410094511.0 (in Chinese)
51. Peng XX, Chai YQ, Zhu BC, Jin YW, Li XL et al (2015) The protective effects of N~6-(2-hydroxyethyl)-adenosine extracted from *Ophiocordyceps sobolifera* on renal ischemia reperfusion injury (IRI) in mice. Mycosystema 34:311–320
52. Lu MY, Chen CC, Lee LY, Lin TW, Kuo CF et al (2015) N6-(2-hydroxyethyl) adenosine in the medicinal mushroom *Cordyceps cicadae* attenuates lipopolysaccharide-stimulated pro-inflammatory responses by suppressing TLR4-mediated NF-κB signaling pathways. J Nat Prod 78:2452–2460
53. Fang M, Chai YQ, Chen GV, Wang HD, Huang B (2016) N-6-(2-hydroxyethyl)-adenosine exhibits insecticidal activity against *Plutella xylostella* via adenosine receptors. PLoS One 11: e0162859
54. Chai YQ, Chen GJ, Jin YW, Liu YG, Li XL et al., (2015) Use of N6-(2-hydroxyethyl)-adenosine in preparation of crop pesticide. USA, US20140256669 A1
55. Lee JS, Kwon JS, Won DP, Lee KE, Shin WC et al (2010a) Study on macrophage activation and structural characteristics of purified polysaccharide from the liquid culture broth of *Cordyceps militaris*. Carbohydr Polym 82:982–988
56. Lee JS, Kwon JS, Yun JS, Pahk JW, Shin WC et al (2010b) Structural characterization of immunostimulating polysaccharide from cultured mycelia of *Cordyceps militaris*. Carbohydr Polym 80:1011–1017
57. Smiderle FR, Sassaki GL, Griensven LV, Iacomini M (2013) Isolation and chemical characterization of a glucogalactomannan of the medicinal mushroom Cordyceps militaris. Carbohydr Polym 97:74–80

58. Yu RM, Yang W, Song LY, Yan CY, Zhang Z et al (2007) Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordyceps militaris*. Carbohydr Polym 70:430–436
59. Chen R, Jin CG, Li HP, Liu ZQ, Lu J et al (2014) Ultrahigh pressure extraction of polysaccharides from *Cordyceps militaris* and evaluation of antioxidant activity. Sep Purif Technol 134:90–99
60. Dong CH, Guo SP, Wang WF, Liu XZ (2015) Cordyceps industry in China. Mycology 6:121–129
61. Rodrigues C, Sousa C, Lopes JA, Novais A, Peixe L (2020) A front line on klebsiella pneumoniae capsular polysaccharide knowledge: fourier transform infrared spectroscopy as an accurate and fast typing tool. Msystems 5:e00386–e00319
62. Sims IM, Frese SA, Walter J, Loach D, Wilson M et al (2011) Structure and functions of exopolysaccharide produced by gut commensal *Lactobacillus reuteri* 100-23. ISME J 5:1115–1124
63. Zhang J, Wen C, Gu J, Ji C, Duan Y et al (2019) Effects of subcritical water extraction microenvironment on the structure and biological activities of polysaccharides from *Lentinus edodes*. Int J Biol Macromol 123:1002–1011
64. Li SP, Li P, Lai CM, Gong YX, Kan KKW et al (2004) Simultaneous determination of ergosterol, nucleosides and their bases from natural and cultured *Cordyceps* by pressurized liquid extraction and high-performance liquid chromatography. J Chromatogr A 1036:239–243
65. Dupont S, Lemetais G, Ferreira T, Cayot P, Gervais P et al (2012) Ergosterol biosynthesis: a fungal pathway for life on land? Evolution 66:2961–2968
66. Chen YJ, Wu YQ, Li SL, Du SM, Hao XM et al (2021) Large-scale isolation and antitumor mechanism evaluation of compounds from the traditional Chinese medicine *Cordyceps militaris*. Eur J Med Chem 212:113142
67. Dong ZH, Sun YY, Wei GG, Li SY, Zhao ZX (2019) Ergosterol ameliorates diabetic nephropathy by attenuating mesangial cell proliferation and extracellular matrix deposition via the TGF-β1/Smad2 signaling pathway. Nutrients 11:483–500
68. Yazawa Y, Ikarashi N, Hoshino M, Kikkawa H, Sakuma F (2020) Inhibitory effect of ergosterol on bladder carcinogenesis is due to androgen signaling inhibition by brassicasterol, a metabolite of ergosterol. J Nat Med 74:680–688
69. Chatterjee R, Srinivasan KS, Maiti PC (1957) *Cordyceps sinensis* (Berkeley) saccardo: structure of cordycepic acid. J Am Pharm Assoc 46:114–118
70. Ouyang YY, Zhang Z, Cao YR, Zhang YQ, Tao YY et al (2013) Effects of cordyceps acid and cordycepin on the inflammatory and fibrogenic response of hepatic stellate cells. Chin J Hepatol 21:275–278
71. Wang Y, Yang ZS, Bao DP, Li B, Yin X et al (2021) Improving hypoxia adaption causes distinct effects on growth and bioactive compounds synthesis in an entomopathogenic fungus *cordyceps militaris*. Front Microbiol 12:698436
72. Rao AV, Rao LG (2007) Carotenoids and human health. Hawaii Med J 55:207–216
73. Britton G (1995) Structure and properties of carotenoids in relation to function. FASEB J 9:1551–1558
74. Yang Y, Bu N, Wang S, Zhang J, Wang Y et al (2020) Carotenoid production by caterpillar medicinal mushrooms, *Cordyceps militaris* (Ascomycetes), under different culture conditions. Int J Med Mushrooms 22:1191–1201
75. Dong JZ, Wang SH, Ai XR, Yao L, Sun ZW, Can L (2013) Composition and characterization of cordyxanthins from *cordyceps militaris* fruit bodies. J Funct Food 5:1450–1455
76. Yan XT, Bao HY, Bau T (2010) Isolation and identification of one natural pigment from cultured *Cordyceps militaris*. Mygosystema 29:777–781
77. Yang T, Sun JD, Lian TT, Wang WZ, Dong CH (2014) Process optimization for extraction of carotenoids from medicinal caterpillar fungus, *Cordyceps militaris* (Ascomycetes). Int J Med Mushrooms 16:125–135

78. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153:1194–1217
79. Tan BL, Norhaizan ME, Liew WPP, Rahman HS (2018) Antioxidant and oxidative stress: a mutual interplay in age-related diseases. Front Pharmacol 9:1162
80. Blount JD, Metcalfe NB, Birkhead TR, Surai PF (2003) Carotenoid modulation of immune function and sexual attractiveness in zebra finches. Science 300:125–127
81. Nagao A (2009) Absorption and function of dietary carotenoids. Forum Nutr 61:55–63
82. Yan XH, Qi QY, Wang SH, Wang CS (2016) Isolation and identification of bioactive compounds from *Cordyceps militaris*. Mycosystema 2016(35):605–610
83. Wang Z, He Z, Li S, Yuan Q (2005) Purification and partial characterization of cu, Zn containing superoxide dismutase from entomogenous fungal species *cordyceps militaris*. Enzym Microb Technol 36:862–869
84. Rukachaisirikul V, Pramjit S, Pakawatchai C, Isaka M, Supothina S (2004) 10-membered macrolides from the insect pathogenic fungus *Cordyceps militaris* BCC 2816. J Nat Prod 67: 1953–1955
85. Jung EC, Kim KD, Bae CH, Kim JC, Kim DK et al (2007) A mushroom lectin from ascomycete *Cordyceps militaris*. Biochim Biophys Acta Gen Subj 1770:833–838
86. Chiu CP, Liu SC, Tang CH, Chan Y, Mohamed ES et al (2016) Anti-inflammatory cerebrosides from cultivated *Cordyceps militaris*. J Agric Food Chem 64:1540–1548
87. Sun JB, Xu J, Wang S, Hou ZD, Lu XC et al (2019a) A new cerebroside from *Cordyceps militaris* with anti-PTP1B activity. Fitoterapia 138:104342
88. Wang J, Chen HL, Li WR, Shan LL (2020) Cordyceps acid alleviates lung cancer in nude mice. J Biochem Mol Toxicol 35:e22670
89. Elkhateeb WA, Daba GM, Thomas P, Wen TC (2019) Medicinal mushrooms as a new source of natural therapeutic bioactive compounds. Egypt Pharm J 18:88–101
90. Huenga DY, Hsieh CH, Cheng YC, Tsai WC, Chen Y (2017) Cordycepin inhibits migration of human glioblastoma cells by affecting lysosomal degradation and protein phosphatase activation. J Nutr Biochem 41:109–116
91. Bawadekji A, Ali KA, Ali MA (2016) A review of the bioactive compound and medicinal value of *Cordyceps militaris*. North J Appl For 1:69–76
92. Liu Y, Wang JH, Wang W, Zhang HY, Zhang XL et al (2015) The chemical constituents and pharmacological actions of *Cordyceps sinensis*. Evid Based Complement Altern Med 2015:1–12
93. Kwon HW, Shin JH, Lim DH, Ok WJ, Nam GS et al (2016) Antiplatelet and antithrombotic effects of cordycepin-enriched WIB-801CE from *Cordyceps militaris* ex vivo, in vivo, and in vitro. BMC Complement Altern Med 16:508
94. Cui JD (2015) Biotechnological production and applications of *Cordyceps militaris*, a valued traditional Chinese medicine. Crit Rev Biotechnol 35:475–484
95. Liao YH, Ling JY, Zhang GY, Liu FJ, Tao S et al (2015) Cordycepin induces cell cycle arrest and apoptosis by inducing DNA damage and up-regulation of p53 in Leukemia cells. Cell Cycle 14:761–771
96. Wu WD, Hu ZM, Shang MJ, Zhao DJ, Huang DS (2014) Cordycepin down-regulates multiple drug resistant (MDR)/HIF-1α through regulating AMPK/mTORC1 signaling in GBC-SD gallbladder cancer cells. Int J Mol Sci 15:12778–12790
97. Lee SK, Lee JH, Kim HR, Chun Y, Lee JH et al (2019) Improved cordycepin production by *Cordyceps militaris* KYL05 using casein hydrolysate in submerged conditions. Biomol Ther 9:461
98. Lee JS, Kwon DS, Lee KR, Park JM, Ha SJ et al (2015) Mechanism of macrophage activation induced by polysaccharide from *cordyceps militaris* culture broth. Carbohydr Polym 120:29–37
99. Kim HS, Kim JY, Kang JS, Kim HM, Kim YO et al (2010) Cordlan polysaccharide isolated from mushroom *Cordyceps militaris* induces dendritic cell maturation through toll-like receptor 4 signalings. Food Chem Toxicol 48:1926–1933

100. Kwon JS, Lee JS, Shin WC, Lee KE, Hong EK (2009) Optimization of culture conditions and medium components for the production of mycelial biomass and exo-polysaccharides with *Cordyceps militaris* in liquid culture. Biotechnol Bioprocess Eng 14:756–762
101. Cheung JH, Li J, Cheung AWH, Yue Z, Zheng KYZ et al (2009) Cordysinocan, a polysaccharide isolated from cultured Cordyceps, activates immune responses in cultured T-lymphocytes and macrophages: Signaling cascade and induction of cytokines. J Ethnopharmacol 124:61–68
102. Else M, Dearden CE, Matutes E, Forconi F, Lauria F et al (2011) Rituximab with pentostatin or cladribine: an effective combination treatment for hairy cell leukemia after disease recurrence. Leuk Lymphoma 52:75–78
103. Li XG, Wu QP, Xie YZ, Ding YR, Du WW et al (2015) Ergosterol purified from medicinal mushroom Amauroderma rude inhibits cancer growth in vitro and in vivo by up-regulating multiple tumor suppressors. Oncotarget 6:17832–17846
104. Yasukawa K, Aoki T, Takido M, Ikekawa T, Saito H et al (1994) Inhibitory effects of ergosterol isolated from the edible mushroom hypsizigus marmoreus on tpa-induced inflammatory ear edema and tumor promotion I mice. Phytother Res 8:10–13
105. Yoo HS, Shin JW, Cho JH, Son CG, Lee YW et al (2004) Effects of *Cordyceps militaris* extract on angiogenesis and tumor growth. Acta Pharmacol Sin 25:657–665
106. Park BT, Na KH, Jung EC, Park JW, Kim HH (2009a) Antifungal and anticancer activities of a protein from the mushroom *Cordyceps militaris*. Korean J Physiol Pharmacol 13:49–54
107. Park SE, Kim J, Lee YW, Yoo HS, Cho CK (2009b) Antitumor activity of water extracts from *Cordyceps militaris* in NCI-H460 cell xenografted nude mice. J Acupunct Meridian Stud 2: 294–300
108. Mollah ML, Dong KP, Park HJ (2012) *Cordyceps militaris* grown on germinated soybean induces G2/M cell cycle arrest through downregulation of cyclin B1 and Cdc25c in human colon cancer HT-29 cells. Evid Based Complement Altern Med 2012:249217
109. Shang XL, Pan LC, Tang Y, Luo Y, Zhu ZY et al (2020) H-1 NMR-based metabonomics of the hypoglycemic effect of polysaccharides from *Cordyceps militaris* on streptozotocin-induced diabetes in mice. Nat Prod Res 34:1366–1372
110. Jo WS, Choi YJ, Kim HJ, Lee JY, Nam BH, Lee JD (2010) The anti-inflammatory effects of water extract from *cordyceps militaris* in murine macrophage. Mycobiology 38:46–51
111. Jin C, Kim G, Choi Y (2008) Induction of apoptosis by aqueous extract of cordyceps militaris through activation of caspases and inactivation of Akt in human breast cancer MDA-MB-231 cells. J Microbiol Biotechnol 18:1997–2003
112. He BL, Zheng QW, Guo LQ, Huang JY, Yun F et al (2020) Structural characterization and immune-enhancing activity of a novel high-molecular-weight polysaccharide from *Cordyceps militaris*. Int J Biol Macromol 145:11–20
113. Rao YK, Fang SH, Wu WS, Tzeng YM (2010) Constituents isolated from *cordyceps militaris* suppress enhanced inflammatory mediator's production and human cancer cell proliferation. J Ethnopharmacol 131:363–367
114. Chu HL, Chien JC, Duh PD (2011) Protective effect of *Cordyceps militaris* against high glucose-induced oxidative stress in human umbilical vein endothelial cells. Food Chem 129: 871–876
115. Nan JX, Park EJ, Yang BK, Song CH, Ko G et al (2001) Antifibrotic effect of extracellular biopolymer from submerged mycelial cultures of *Cordyceps militaris* on liver fibrosis induced by bile duct ligation and scission in rats. Arch Pharm Res 24:327
116. Sun T, Dong W, Jiang G, Yang J, Liu J et al (2019b) *Cordyceps militaris* improves chronic kidney disease by affecting TLR4/NF-κB redox signaling pathway. Oxidative Med Cell Longev 2019:7850863
117. Rupa EJ, Li JF, Arif MH, Yaxi H, Puja AM et al (2020) *Cordyceps militaris* fungus extracts-mediated nanoemulsion for improvement antioxidant, antimicrobial, and anti-inflammatory activities. Molecules 25:5733

118. Tran NKS, Kim GT, Park SH, Lee D, Shim SM et al (2019) Fermented *Cordyceps militaris* extract prevents hepatosteatosis and adipocyte hypertrophy in high fat diet-fed mice. Nutrients 13:50
119. Lin RK, Choong CY, Hsu WH, Tai CJ, Tai CJ (2019) Polysaccharides obtained from mycelia of *Cordyceps militaris* attenuated doxorubicin-induced cytotoxic effects in chemotherapy. Afr Health Sci 19:2156–2163
120. Kim J, Lee H, Kang KS, Chun KH, Hwang GS (2015) *Cordyceps militaris* mushroom and Cordycepin inhibit rankl-induced osteoclast differentiation. J Med Food 18:446–452
121. Hu SM, Wang J, Li FH, Hou PB, Yin JY, Yang ZX (2019) Structural characterisation and cholesterol efflux improving capacity of the novel polysaccharides from *Cordyceps militaris*. Int J Biol Macromol 131:264–272
122. Song QY, Zhu ZY (2020) Using *Cordyceps militaris* extracellular polysaccharides to prevent Pb^{2+}-induced liver and kidney toxicity by activating Nrf2 signals and modulating gut microbiota. Food Funct 11:9226–9239
123. Wu TF, Shi WY, Chiu YC, Chan YY (2021) Investigation of the molecular mechanism underlying the inhibitory activities of ethanol extract of Bombyx mori pupa-incubated *Cordyceps militaris* fruiting bodies toward allergic rhinitis. Biomed Pharmacother 135:111248
124. Yang XQ, Lin P, Wang J, Liu N, Yin F et al (2021) Purification, characterization and anti-atherosclerotic effects of the polysaccharides from the fruiting body of *Cordyceps militaris*. Int J Biol Macromol 181:890–904
125. Lin YE, Chen YC, Lu KH, Huang YJ, Panyod S et al (2021) Antidepressant-like effects of water extract of *Cordyceps militaris* (Linn.) link by modulation of ROCK2/PTEN/Akt signaling in an unpredictable chronic mild stress-induced animal model. J Ethnopharmacol 276: 114194
126. Gallo A, Ferrara M, Perrone G (2013) Phylogenetic study of polyketide synthases and nonribosomal peptide synthetases involved in the biosynthesis of mycotoxins. Toxins 5: 717–742
127. Zheng P, Xia YL, Xiao GH, Xiong CH, Hu X et al (2011) Genome sequence of the insect pathogenic fungus *Cordyceps militaris*, a valued traditional Chinese medicine. Genome Biol 35:475–484
128. Jhou BY, Fang WC, Chen YL, Chen CC (2018) A 90-day subchronic toxicity study of submerged mycelial culture of *Cordyceps militaris* in rats. Toxicol Res 7:977–986
129. Heo JY, Baik HW, Kim HJ, Lee JM, Kim HW et al (2015) The efficacy and safety of *Cordyceps militaris* in Korean adults who have mild liver dysfunction. J Clin Nutr 7:81–86
130. Aramwit P, Porasuphatana S, Srichana T, Nakpheng T (2015) Toxicity evaluation of cordycepin and its delivery system for sustained in vitro anti-lung cancer activity. Nanoscale Res Lett 10:152–161
131. Pintathong P, Chomnunti P, Sangthong S, Jirarat A, Chaiwut P (2021) The feasibility of utilizing cultured *Cordyceps militaris* residues in cosmetics: biological activity assessment of their crude extracts. J Fungi 7:973