

Nucleosides, a Valuable Chemical Marker for Quality Control in Traditional Chinese Medicine *Cordyceps*

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Abstract: *Cordyceps*, a well-known traditional Chinese medicine, is an endoparasitic and/or symbiotic macrofungus in the body of insect and other arthropod, and has received increasing attention worldwide due to its rarity and outstanding curative effects for different diseases. Recent years, however, the counterfeits and mimics of *Cordyceps* are frequently found in markets because of its scarce in nature and high in price. Therefore, quality control of *Cordyceps* and its bioproducts is very important to ensure their safety and efficacy. Nucleoside is recognized as a major active component of *Cordyceps*, and even is used as chemical marker for quality control of *Cordyceps*. In this present review, recent studies and associated patents, with regard to the chemical marker nucleosides for quality control of *Cordyceps* and its bioproducts, including nucleoside components, pharmacological activities, and analytical methods were reviewed and discussed thereof. Also, developing trends in the field have been appraised.

Keywords: *Cordyceps*, nucleoside, bioactivity, chemical marker, quality control.

1. MEDICAL HISTORY AND ITS QUALITY CONTROL STANDARD OF *CORDYCEPS*

The fungi of *Cordyceps*, belonging to the Ascomycota, Pyrenomycetes, Hypocreales, Clavicipitaceae, and the genus *Cordyceps*, are interesting macrofungi because of their characteristic parasitic habit on larvae and pupae of insects, and even on perfect insects. *Cordyceps* is pleomorphism fungi of worldwide distribution, and is particularly abundant and diverse in humid temperate and tropical forests. Within the genus *Cordyceps*, over 400 species have been described so far [1], of which *Cordyceps sinensis* (*Ophiocordyceps sinensis*), also known as “Winter Worm, Summer Grass”, is recognized as the most valuable tonic herb in traditional Chinese medicine (TCM) for centuries. As the story goes, the ancient herdsmen in Himalayan Plateau of Tibet, noticed an incredible phenomenon that their yaks and sheep showed an unusual vigor after grazing where *Cordyceps* grew, and even old yaks also showed signs of being younger with an increasing natural vigor and vitality. Then these herdsmen eventually found the causal agent, a cap-less mushroom. The cap-less mushroom, named as *C. sinensis* by Pier Andrea Saccardo in 1878 (The Latin etymology of *C. sinensis* describes *cord* as “club”, *ceps* as “head”, and *sinensis* as “Chinese”), has been used in TCM ever since. From the historical record, the first written record of the *Cordyceps* mushroom was occurred in Tang Dynasty (AD 618-AD 907) of China. Up until 1757, however, the earliest scientifically depiction of

the medicinal property on *C. sinensis* was written in medical literature Ben-Cao-Cong-Xin (New Compilation of Materia Medica) by Yi-Luo Wu. For the medicinal value of *C. sinensis*, it was used as tonic herb to enhance energy, improve quality of life and promote longevity, and was also used as TCM for the treatment of various medical disorders such as liver disease, immune and respiratory disease, renal dysfunction, heart and lung disease, brain disease, sexual dysfunction, physical fatigue, hyperglycemia and hyperlipidemia [2-8]. Accordingly, due to its rarity, and special efficacy against a variety of diseases, *C. sinensis* has held, and will continue to hold, a highly esteemed position in the vast ranks of Chinese medicines [2]. Perennin, a French Jesuit priest, who stayed at the Chinese Emperor’s court had introduced the knowledge of *C. sinensis* to Western scientific audiences in 1726 [9], however, most Westerners have only come to know of *C. sinensis* within the last 20 years [1,9].

C. sinensis, due to its wonder efficacy against a variety of diseases, has become one of most expensive Chinese herb medicines. Since the 1980s, both the market demand and the price of *C. sinensis* have increased sharply. 2008 price in the major U.S. cities was as high as \$75,000 per kilogram [9]. As we known, *C. sinensis* is a rare species that only distributes in the alpine meadows of Himalayan plateau, with altitudes of 3500 to 4800 meter, and shows strict host specificity (*Hepialus* spp.) and specific environmental requirements (low-temperature and oxygen-deficient environments) for growth. Additionally, overharvesting of *C. sinensis* for gaining more economic benefits has led to its ecological environment destruction and natural resource exhaustion. Based on the above-mentioned two causes, *C. sinensis* keeps a continuously and rapidly decrease in annual yield, at the same time, which also resulted in an incredibly high price. Therefore, the exploitation of *C. sinensis* faces greater challenge

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than other Chinese herbs medicines due to the enormous cost and scarcity of the material at present.

To alleviate the market demand pressure of *C. sinensis*, there have appeared a number of investigations on substitutes of *C. sinensis* since the 1980s [1]. Many members of *Cordyceps* family such as *C. militaris*, *C. ophioglossoides*, *C. sobolifera*, *C. brasiliensis*, and *C. japonica* have been developed for medicinal purposes and used as dietary and health supplements. Furthermore, as studies of different species of *Cordyceps* continue, it has become increasingly apparent that the potential medicinal benefits of *Cordyceps* spp. are not related only to the one species *C. sinensis*. In comparison to other *Cordyceps* species, actually, *C. sinensis* still possesses more abundant total biomass of wild which could create even more opportunities for ancestors' medical practice. Therefore, it is possible that other *Cordyceps* species are able to show the same or even better medicinal properties as *C. sinensis*. Also, recent data suggest that some *Cordyceps* members may exhibit great potentials for the development of drug and function food [2,9]. Furthermore, these *Cordyceps* spp. have also begun to be gradually accepted by the public. Therefore, *Cordyceps* and its products have held, and continue to hold, a highly esteemed position in the vast ranks of TCM.

For many centuries, collecting *Cordyceps* mushrooms in the wild was the only way to obtain them. With the development of modern biotechnology, however, the artificial cultivation of *Cordyceps* mushrooms have reached new heights of sophistication in recent years, where related techniques for producing different *Cordyceps* mushrooms have obtained patents [10-12]. For example, the mycologist cultivated the fruit body by replicating the growing environment of *Cordyceps* mushrooms in the laboratory, and some *Cordyceps* spp. such as *C. militaris*, and *C. pruinosa* have been cultivated commercially [91-92]. More important the large-scale production of mycelium biomass in *Cordyceps* spp. has been also achieved by submerged and/or solid state fermentation techniques over in the last twenty years [10-12]. For nutritional ingredients or bioactive substances, these mycelium biomass obtained by fermentation technique also has higher nutritional value compared to wild *Cordyceps* spp. Furthermore, the culture media in which *Cordyceps* mycelium grows are made of chemically pure and ecologically clean substances, thus these *Cordyceps* products are safe to used as dietary and health supplements. In addition, the production of mycelium biomass of *Cordyceps* by fermentation technique is a standardized production process and only need several days to reach enough biomass yields, while the fruiting body production of *Cordyceps* is a long-term process, taking one to several months for the first fruiting bodies to appear, depending on strains, substrates and environment. Therefore, the mycelium biomass of *Cordyceps* is regarded as a promising alternative for natural *Cordyceps* [1,2].

With the popularity of *Cordyceps* on the rise, and the ever-increasing worldwide demand for *Cordyceps* mushrooms at an all-time high, *Cordyceps* products are abundant and diversity in market. Unfortunately, the increase in supply has given rise to variations in purity and quality, especially a large number of counterfeit, mimic and adulterated *Cordyceps* products are frequently found in market [9,13]. As we

known, most of the Western world prefers their medicine to come in neat little capsules, rather than in the whole caterpillar form, which makes it easier for some suppliers to sell counterfeit with a label of "*Cordyceps*". Therefore, the large variations in quality found in *Cordyceps* products has led many consumers to believe the wild collected variety is medicinally better than the cultivated type, meanwhile the reputation of *Cordyceps* products, especially derived from cultivated *Cordyceps* was severely influenced. Accordingly, a quality control standard of *Cordyceps* products is very important to ensure their safety and efficacy for their long-term development.

The accumulated knowledge about TCM makes their use reasonable and safe, but many problems remain unanswered. Quality control is one of the most important problems for the application and development of TCM [14]. As we known, the efficacy and safety, the cornerstone of the TCM, are closely associated with its chemical compositions. Herein active ingredients are the keypoint and assurance of quality control for TCM raw materials. At present, the quality control of *Cordyceps* has not yet become standardized throughout the world. *Cordyceps* contains all of the essential amino acids, vitamins, polysaccharides, proteins and enzyme, sterols, nucleosides, and trace elements according to previous investigations [9,15,17]. To great extend, it may owe the legend efficacy of *Cordyceps* to its broad range of nutritional and active components. However, it is not possible to analyze all ingredients thereof during the course of quality control of *Cordyceps* products. Currently, several components such as nucleosides, cordycepic acid, and polysaccharides are being assumed to be the rational markers for quality control of *Cordyceps* and its products [15-17].

Nucleosides are considered to be one of the major active components of *Cordyceps* [16,17]. Furthermore, many reports showed that *Cordyceps* mushrooms contain specific nucleosides with potent bioactivities for medicinal purpose. For example, 3'-deoxyadenosine, a naturally occurring nucleoside derivative, was isolated from cultured *C. militaris* in 1950 and named cordycepin [18]. More interestingly, cordycepin is a broad spectrum biocidal compound possessing not only antitumor activity but also antibacteria, antiviral and insecticidal activities [1]. Accordingly, the nucleosides in *Cordyceps* have been a focus since then, and some nucleosides such as adenosine, cordycepin have been authorized as significant markers of *Cordyceps* for quality control. In this article, *Cordyceps*-derived nucleosides, and their bioactivities, analytical methods in the quality control of *Cordyceps* and its products are reviewed and discussed.

2. VARIETIES OF *CORDYCEPS*-DERIVED NUCLEOSIDES AND THEIR PHYSIOLOGICAL ACTIVITIES

To date, approximate twenty varieties of nucleosides and their analogues have been found in *Cordyceps* spp. as listed in Table 1 [17, 19-21], and the major nucleosides and their analogs (see Fig. 1) were described in detail as follows.

Adenosine (1), an important role of biochemical process in organism, is a major nucleoside in *Cordyceps* spp. Furthermore, it is recognized as chemical marker for the quality control of *Cordyceps* products [17]. Also, adenosine is an energy transfer and signal transductant in cells, and can still

Table 1. Nucleoside compounds from *Cordyceps* spp.

Species	Nucleosides	Molecular formula	Bioactivities	References
<i>C. sinensis</i> , <i>C. militaris</i> , <i>C. kyushuensis</i> , <i>C. taii</i> , <i>C. gunnii</i> <i>C. jiangxiensis</i>	Adenosine	C ₁₀ H ₁₃ N ₅ O ₄	immunomodulatory activity, cytoprotections, preventing tissue damage, anti-inflammatory, anticonvulsant	[15,22-26, 100, 95]
<i>C. sinensis</i> , <i>C. militaris</i> , <i>C. jiangxiensis</i>	Adenine	C ₅ H ₅ N ₅	suppressing leucopenia nucleobase of DNA and RNA	[15,21,96-97, 100]
<i>C. militaris</i> , <i>C. kyushuensis</i> , <i>C. jiangxiensis</i>	Cordycepin (3'-deoxyadenosine)	C ₁₀ H ₁₃ N ₅ O ₃	antimicrobial, antiatherosclerosis, antidiabetic, antiischemic damage, antiinflammatory, immunoregulator. antitumor,	[27-36,80, 100]
<i>C. sinensis</i> , <i>C. militaris</i> , <i>C. jiangxiensis</i>	2'-Deoxyadenosine	C ₁₀ H ₁₃ N ₅ O ₃	pharmaceutical intermediate	[20,48]
<i>C. sinensis</i>	2'3'-Dideoxyadenosine	C ₁₀ H ₁₃ N ₅ O ₂	antiviral activity	[9]
<i>C. jiangxiensis</i>	2'-Methoxyadenosine	C ₁₁ H ₁₅ N ₅ O ₄	pharmaceutical intermediate	[51]
<i>C. sinensis</i> , <i>C. pruinosa</i>	N ⁶ -(2-hydroxyethyl)- Adenosine	C ₁₂ H ₁₇ N ₅ O ₅	Ca ²⁺ antagonist	[17,43]
<i>C. militaris</i>	3'-Amino-3'- deoxyadenosine	C ₁₀ H ₁₄ N ₆ O ₃	pharmaceutical intermediate	[96]
<i>C. sinensis</i> , <i>C. kyushuensis</i>	Hypoxanthine	C ₅ H ₄ N ₄ O	pharmaceutical intermediate	[70,79-80]
<i>C. sinensis</i> , <i>C. kyushuensis</i> , <i>C. jiangxiensis</i>	Uracil	C ₄ H ₄ N ₂ O ₂	nucleobase of RNA	[74, 96-97, 100]
<i>C. sinensis</i> , <i>C. kyushuensis</i> , <i>C. militaris</i> , <i>C. taii</i> , <i>C. jiangxiensis</i> , <i>C. gunnii</i>	Uridine	C ₉ H ₁₂ N ₂ O ₆	antidepressant-like activity	[15,59, 79-80, 100]
<i>C. sinensis</i> , <i>C. militaris</i> , <i>C. jiangxiensis</i>	2'-Deoxyuridine	C ₉ H ₁₂ N ₂ O ₅	pharmaceutical intermediate	[19,51,97]
<i>C. jiangxiensis</i>	3'-Methoxyuridine	C ₁₀ H ₁₄ N ₂ O ₆	pharmaceutical intermediate	[21]
<i>C. sinensis</i> , <i>C. kyushuensis</i> , <i>C. militaris</i> , <i>C. taii</i> , <i>C. gunnii</i>	Inosine(Hypoxanthin)	C ₁₀ H ₁₂ N ₄ O ₅	improving the function of nerve system injury	[15,47,70,78]
<i>C. sinensis</i> , <i>C. kyushuensis</i> , <i>C. militaris</i> , <i>C. taii</i> , <i>C. jiangxiensis</i>	Guanosine	C ₁₀ H ₁₃ N ₅ O ₅	immunomodulatory activity	[15,70,76, 100, 95]
<i>C. sinensis</i>	Deoxyguanosine	C ₁₀ H ₁₃ N ₅ O ₄	pharmaceutical intermediate	[88]
<i>C. sinensis</i>	Guanine	C ₅ H ₅ N ₅ O	nucleobase of DNA and RNA	[74,96-97]
<i>C. sinensis</i>	Cytosine	C ₄ H ₅ N ₃ O	nucleobase of RNA and DNA	[96-97]
<i>C. sinensis</i>	Cytidine	C ₉ H ₁₃ N ₃ O ₅	antidepressant-like effect	[61,74,96-97]
<i>C. sinensis</i> , <i>C. jiangxiensis</i>	Thymidine	C ₁₀ H ₁₄ N ₂ O ₅	pharmaceutical intermediate	[20,46,49]
<i>C. sinensis</i> , <i>C. kyushuensis</i>	Thymine	C ₅ H ₆ N ₂ O ₂	component of DNA	[46,74,96-97]

exert a wide spectrum of cytoprotections or preventing tissue damage such as treating chronic heart failure [22], anti-inflammatory properties [23], anticonvulsant activity [24], neuroprotective action under pathological conditions, and inhibitory effects of neurotransmitter release in the central nervous system [25,26]. Therefore, adenosine and its analogues have received so much attention due their various pharmacological effects.

Cordycepin (**2**), namely 3'-deoxyadenosine, the most considerable adenosine analogue from some *Cordyceps*,

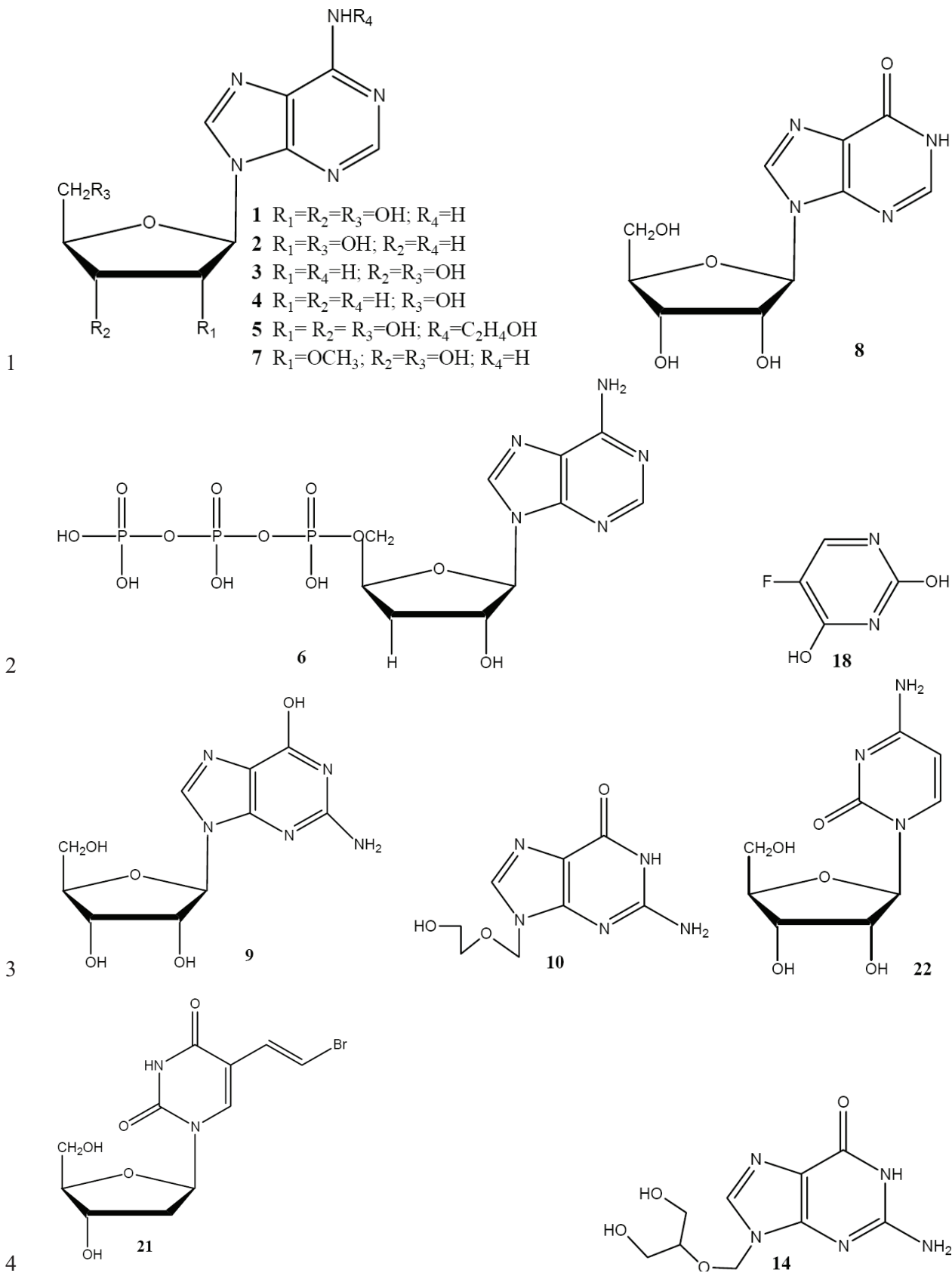
showed various and outstanding pharmacological activities such as antimicrobial, antitumor [27,28]. Some new pharmacological activities of cordycepin and their mechanism of action were discovered in recent years. Cordycepin is able to protect hippocampal neuron against ischemic damage via reducing oxidative damage [29]. Also, it can affect many factors occurrence related to cardiovascular diseases. Chang *et al.* [30] reported that cordycepin could markedly inhibit the activation of matrix metalloproteinases (MMP) 2 and -9, as well as the expression of extracellular MMP inducer in a

dose-dependent manner in collagen type I-activated rat aortic smooth muscle cells (RAoSMCs). Moreover, cordycepin suppressed cyclooxygenase-2 (COX-2) expression related to hyperplasia of RAoSMCs, thus it may induce anti-proliferation in RAoSMCs via the modulation of vessel wall remodeling. Recently, Won *et al.* [31] observed that cordycepin could attenuate the neointima formation of RAoSMCs by inhibiting ROS levels of adenosine receptor-mediated NOS pathways. Cordycepin therefore may be used as a potential anti-atherosclerosis agent to treat restenosis or atherosclerosis. Interestingly, the hypolipidemic and hypoglycemic actions of cordycepin were found. It could prevent high-fat diet (HFD)-induced hyperlipidemia via activation of AMP-activated protein kinase (AMPK), and could also improve insulin sensitivity effectively [32]. Also, cordycepin played an important role on the anti-diabetic effect. Kim's group found that cordycepin inhibited the expression levels of Type 2 diabetes (T2D) regulating genes such as 11β -HSD1 and PPAR γ , as well as expression of co-stimulatory molecules such as ICAM-1 and B7-1/-2 were also decreased [33]. Simultaneously the production of NO and pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α in LPS-activated macrophages via the suppression of nuclear factor- κ B (NF- κ B) activation were decreased [33]. Thus cordycepin suppressed T2D regulating genes through the inactivation of NF- κ B dependent inflammatory responses in activated macrophages. The finding suggests that cordycepin is also anti-inflammatory agent. Indeed, recent data have showed that cordycepin is a good anti-inflammatory action. For example, Noh *et al.* reported that cordycepin is a potent inhibitor of IL-1b-induced chemokine production and MMP expression and strongly blocks the p38/JNK/AP-1 signaling pathway in rheumatoid arthritis synovial fibroblasts, which suggest that it may be a potential candidate to prevent inflammation of rheumatoid arthritis [34]. Moreover, cordycepin suppressed expression, κ B alpha phosphorylation, and translocation of NF- κ B [35]. The expressions of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were also significantly decreased in RAW 264.7 cell after cordycepin treatment [35]. Cordycepin inhibited the production of NO production by down-regulation of iNOS, COX-2 and tumor necrosis factor (TNF- α) genes expression via the suppression of NF- κ B activation, Akt and p38 phosphorylation in LPS-activated RAW 264.7 macrophage cells [35]. Based on the above-mentioned, cordycepin may provide a potential therapeutic approach for inflammation-associated diseases. In addition, cordycepin was also used as an immunoregulator. It intensively regulated the functions of human immune cells *in vitro*, promoted the expression of IL-1 β , IL-6, IL-8, IL-10 and TNF- α of resting cells, and suppressed the PHA-induced expression of IL-2, IL-4, IL-5, IL-12, IFN- γ and TNF- α at the same time [36]. The exploration of different nucleotide derivatives should be valuable in the development of novel modulators for control of the inflammatory process and immune response [36]. More interestingly, Nakamura's group found that cordycepin exhibited an anti-metastatic activity in a haematogenic lung metastasis mouse model [37]. Also, they demonstrated that cordycepin could inhibit the proliferation of metastatic B16-BL6 mouse melanoma and Lewis lung carcinoma cells *in vitro* by stimulating adenosine A3 receptors on the cell membrane, followed by activation of GSK-3 β and cyclin D1 inhibition in

the Wnt signalling pathway [38-40]. Most recently, they found that ADP accelerated hematogenic metastasis, and cordycepin has an inhibitory effect on hematogenic metastasis of B16-F1 mouse melanoma cells via blocking of adenosine-5'-diphosphate-induced platelet aggregation *in vivo* [41]. Additionally, Lee and coworkers [42] reported that cordycepin inhibited TNF- α -induced invasion and migration of cancer cells, which down-regulated the MMP-9 expression due to reducing transcriptional activity of the transcription factors, NF- κ B and activator protein-1 (AP-1).

In the early 1980s, N^6 -(2-hydroxyethyl)-adenosine (**5**), another important active adenosine analogue, which behaves as a Ca^{2+} antagonist and an ionotropic agent, was isolated from cultured *C. pruinosus* [43]. Recent years, other adenosine analogues such as 2'-deoxyadenosine (**3**), 2'3'-dideoxyadenosine (**4**), cordycepin triphosphate (**6**), 3'-amino-3'-deoxyadenosine (**26**) have been also found in *Cordyceps* but not in any other organism in nature [9]. Didanosine, the trade name for 2'3'-dideoxyadenosine, has been used as a drug for the treatment of AIDS in USA [44]. More recently, our group isolated 2'-deoxyadenosine, and 2'-*O*-methyladenosine (**7**) from cultured *C. jiangxiensis* [20,45]. In addition, inosine (**8**), a major biochemical metabolite of adenosine due to oxidative deamination, is also an important nucleoside in *Cordyceps* [19, 46], and can stimulate extensive axon growth in the mature rat corticospinal tract axon growth *in vivo* and may improve functional outcome in patients with central nerve system injury [47], even was also looked as the marker for quality control of *Cordyceps* and its products [46].

Guanosine (**9**) is a purine nucleoside comprising guanine attached to a ribose (ribofuranose) ring via a β - N_9 -glycosidic bond. Also, it is often found in some *Cordyceps* spp. [19,48]. Guanosine and its analogues play important roles in various biochemical processes such as syntheses of nucleic acids and proteins, photosynthesis, muscle contraction and intracellular signal transduction (cGMP). For example, guanosine is required for an RNA splicing reaction in mRNA, when a "self-splicing" intron removes itself from the mRNA message by cutting at both ends, re-ligating, and leaving just the exons on either side to be translated into protein. 2'-Deoxyguanosine (**27**) was found in *C. sinensis* [88], while recent study has showed that 8-hydroxy-2'-deoxyguanosine is a critical biomarker of DNA oxidative damage and carcinogenesis [99]. Aciclovir (chemical name acycloguanosine), a guanosine analog and one of the most commonly used antiviral drugs, was primarily used for the treatment of herpes simplex virus infections, as well as in the treatment of varicella zoster (chickenpox) and herpes zoster (shingles), and was seen as the start of a new era in antiviral therapy due to its extremely selective and low cytotoxicity [49]. However, aciclovir (**10**) and similar acyclic nucleosides suffer from low aqueous solubility and low bioavailability following oral administration. For this reason, valaciclovir (**11**), a valine ester of aciclovir, was developed and was readily metabolized upon oral administration to produce the antiviral nucleoside *in vivo* with a higher biological availability [49]. Subsequently, the field of antiviral therapy was dominated by this and other nucleoside analogues such as penciclovir (**12**), famciclovir (**13**), and ganciclovir (**14**).



(Fig. 1) contd....

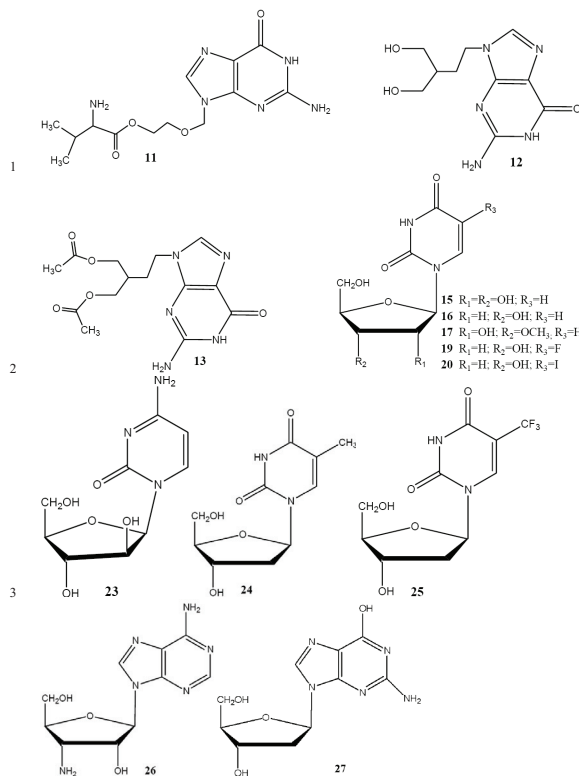


Fig. (1). Chemical structures of major nucleosides and their analogues in *Cordyceps* spp.

Uridine (**15**) and its analogues were also widely found in *Cordyceps* [48, 17, 21]. Uridine is a molecule that is formed when uracil is attached to a ribose ring via a β - N_1 -glycosidic bond, and plays a role in the glycolysis pathway of galactose. Interestingly, uridine can possess a fascinating, naturally occurring self-maintenance system in human brain. Also, uridine rescue could enhance the therapeutic index of 5-fluorouracil (**18**) against mice bearing B16 melanoma or L1210 leukemia [50]. Recently, two uridine analogs such as 2'-deoxyuridine (**16**), and 3'-*O*-methyluridine (**17**), have found in cultured *C. jiangxiensis* by our group [51], but there were no reports about their bioactivity. As we know, some uridine analogues have been used as chemotherapeutic agents for the treatment of cancer diseases. For example, 5-fluorouracil, is an important agent for the treatment of gastrointestinal malignancies. Furthermore, 5-fluorouracil is known to interfere with cellular metabolism through two different pathways [52]. Namely, one involves its conversion to 5-fluorodeoxyuridine 5'-monophosphate (FdUMP), which inhibits the thymidylate synthetase, leading to suppression of DNA synthesis. The second mechanism of action for 5-fluorouracil, via its incorporation as 5-fluorouridine-5'-monophosphate into RNA in place of UMP, can result in a variety of adverse effects on key cellular mechanisms involving RNA function and processing [52]. 5-Fluoro-2'-deoxyuridine (**19**), another uridine analogue, is also frequently used for the treatment of human solid tumors, including hepatic metastases of advanced gastrointestinal adenocarcinomas, renal cell carcinomas, advanced ovarian cancer, advanced breast cancer, and squamous cell carcinoma of the head and neck [53]. However, the rapid catabolism of

5-fluoro-2'-deoxyuridine to the less effective and more toxic nucleobase 5-fluorouracil limits the advantage of using 5-fluoro-2'-deoxyuridine over 5-fluorouracil. Interestingly, 5-(benzyloxybenzyl) barbituric acid acyclonucleoside, an inhibitor of uridine phosphorylase, could enhance the antitumor efficacy of 5-fluoro-2'-deoxyuridine, *in vitro* and *in vivo* [54]. 5-Iodo-2'-deoxyuridine (idoxuridine) (**20**), the first effective antiviral compound, was also the first nucleoside analog to be used as the antiviral drug for the topical treatment of herpes keratitis since 1959 [55]. Subsequently, bromovinyldeoxyuridine (**21**) was widely used as the effective antiviral drug [56]. In addition, researchers at the Massachusetts Institute of Technology found that uridine plus docosahexaenoic acid could substantially increase membrane phosphatide and synaptic protein levels in gerbils brain, which had significant impact on repairing the neurons of gerbils, whose pyrimidine metabolism in gerbils, but not rats, resembles that in humans [57], thus uridine plus docosahexaenoic acid is a potential therapeutic approach for Alzheimer's disease due to fewer and smaller synapses and reduced levels of synaptic proteins, membrane phosphatides in Alzheimer's disease brains. Recently further study showed that dietary supplement consisting of uridine, choline and docosahexaenoic acid could enhance cognitive functions in gerbils [58]. Interestingly, Harvard researchers reported that supplementation in rats with a combination of uridine and EPA/DHA omega-3 fatty acids had antidepressant activity equivalent to that of commonly prescribed antidepressant medications, such as Prozac and other selective serotonin reuptake inhibitors [59].

Cytidine (**22**), consists of cytosine attached to a ribofuranose ring *via* a β - N_1 -glycosidic bond, is a component of RNA, and widely exists dietary sources with high RNA content. Cytidine only was found in some *Cordyceps* spp. [19]. In humans, dietary cytidine is converted into uridine [60], which is probably the compound behind cytidine's metabolic effects. From the standpoint of lipid metabolism, one of the most important of the nucleotide metabolites is cytidine 5'-phosphoric acid, which is a key component of the phospholipid cytidine diphosphate diacylglycerol (CDP-diacylglycerol), a liponucleotide. Therefore, cytidine can increase phospholipid synthesis. Furthermore, it could dramatically decrease immobility in the forced swim test (FST) in rats, a model used in depression research, due to its antidepressant-like effects [61]. Deoxycytidine analogs such as cytarabine (**23**) are extensively used in anticancer [62,63] and antiviral therapies. However, deoxycytidine analogs are prodrugs that require intracellular phosphorylation to become active [64]. For example, deoxycytidine analog monophosphates are deaminated by deoxycytidine monophosphate deaminase to form deoxyuridine analog nucleotides, deoxyuridine analog monophosphates can interact with thymidylate synthase, whereas deoxyuridine analog triphosphates are incorporated into nucleic acids and interfere with polymerases [64].

Thymidine (**24**), more precisely called deoxythymidine or thymine deoxyriboside, is also a frequent nucleoside in *Cordyceps* species [19,20]. In cell biology, thymidine is used to synchronize the cells in S phase, in which it occurs almost exclusively in DNA but also occurs in the T-loop of tRNA. In pharmaceutical industry, thymidine is used to produce azidothymidine (AZT), an antiretroviral drug for the treatment of HIV infection. Trifluorothymidine (TFT) (**25**), is an antiviral derivative of thymidine used mainly in the treatment of primary keratoconjunctivitis and recurrent epithelial keratitis due to herpes simplex virus. For the mechanism of action, TFT is well known to be converted to TFT-monophosphate by thymidine kinase and to inhibit thymidylate synthase. Recently, Suzuki *et al.* have demonstrated the mode of action of TFT, namely TFT-triphosphate (TFT-TP) could be incorporated into DNA to exert DNA-directed cytotoxic effect [65]. Also, TFT showed potent antitumor activity *via* the induction of DNA double-strand breaks, similar to the mechanism of action of 5-fluorouracil and/or 5-fluoro-2'-deoxyuridine [66,67]. TFT, however, might be more effective in colorectal cancer cells to overcome 5-fluorouracil and/or 5-fluoro-2'-deoxyuridine resistance [67]. In addition, TFT is part of the oral fluoropyrimidine formulation TAS-102 and can be activated to its phosphate derivatives, while TAS-102 has been clinically evaluated in phase II studies as an oral chemotherapeutic agent [67].

In summary, nucleosides, as the pharmaceutical intermediates and/or prodrugs, play an important role for the drug development of cancer and infection diseases, and nucleosides and their derivatives have been widely used in anticancer and antiviral therapies [54,68,69]. Up to date, some nucleosides analogues still are used as the first-line clinical chemotherapeutic drugs for the treatments of cancer and virus infection diseases. As the above-mentioned, *Cordyceps* mushrooms contain abundant and diverse active nucleoside compounds. Undoubtedly, it suggests that *Cordyceps* is a

promising biosource for the development of nucleoside drugs.

3. ANALYTICAL METHODS FOR *CORDYCEPS*-DERIVED NUCLEOSIDES

Quality control involving the efficacy and safety is one of the most important problems for the application and development of TCM, while analytical methods are the keypoint of quality control. Recent years, the analytical methods on nucleosides of *Cordyceps* have been systematically developed as listed in Table 2, due to the rapid developments of modern analytical chemistry and characterization techniques.

Thin-layer chromatography (TLC) is a widely used technique for chemical analysis of TCM. TLC has the advantages of many-fold possibilities of detection in analyzing TCM. Furthermore, TLC can be also employed for multiple sample analysis [70]. For each plate, more than 30 spots of samples can be simultaneously analyzed in one time. Therefore, the Pharmacopoeia of the People's Republic of China also uses TLC to provide characteristic fingerprint of active components of nucleosides in *C. sinensis* [16]. Usually, the typical TLC is employed under isocratic elution conditions to detect the specific nucleosides in *C. sinensis*, which has only a low separation ability and selectivity and cannot be applied successfully to analyze complex multi-component systems, *i.e.* simultaneously determine multiple active nucleoside components in *Cordyceps*. For instance, the isocratic TLC gave overlapping spots for inosine and guanosine. Fortunately, TLC associated with the stepwise gradient elution (GE) technique has been developed. In general, the weaker eluent resolves the less retained solutes whereas the stronger eluent resolves the more polar solutes. Thus, a well-chosen gradient condition may separate more components involved. For example, Ma *et al.* [70] successfully employed a TLC method with two-step gradient elution to separate the eight nucleoside compounds commonly presented in *C. sinensis* sample.

HPLC is widely applied to separate and identify the target compounds in TCMs and their preparations. A quantitative method developed by HPLC is simple, stable and durable, and HPLC can be equipped with various detectors, such as a UV detector, photodiode array detector (DAD), evaporative light scattering detector (ELSD), fluorescence detector (FD) and mass spectrometry (MS) [14]. For nucleosides analysis in *Cordyceps*, therefore, HPLC has established its position as sample, reliable and sensitive analytical tool within the last decades years. For example, five nucleosides such as adenosine, cordycepin, 2'-deoxyadenosine, guanosine and uridine in various *Cordyceps* samples were separated and determined at 260 nm using the HPLC-UV method with a C_{18} column and isocratic elution (acetonitrile-water) [48]. Li group developed a HPLC-DAD method for the quantitative determination of purine and pyrimidine bases including adenine, cytosine, guanine, hypoxanthine, thymine, and uracil in natural and cultured *Cordyceps*. The determination was achieved at 254nm on a Zorbax SB-AQ analytical column using the mobile phase including aqueous triethylamine and methanol with a gradient elution system [71]. Furthermore, another new HPLC-DAD method with a Zorbax 300SB C_{18} analytical column was also developed by

Table 2. Analytical methods of nucleosides profile in *Cordyceps* spp.

Sample	Analytical Method	Column/Solid Phase	Mobile Phase	Detection	References
<i>C. sinensis</i> (uracil, uridine, hypoxanthine, inosine, guanosine, adenine, adenosine, cordycepin)	TLC	Silica gel 60 F ₂₅₄	Solvent A (chloroform-ethylacetate-isopropanol-water-triethylamine with a ratio of 10:2:8:0.5:2, V/V); solvent B (chloroform-ethylacetate-isopropanol-water-dimethylformamide with a ratio of 10:2:8:0.5:2, V/V)	UV 256 nm	[70]
<i>C. militaris</i> (cordycepin)	CE	TSP-050375t (Total length 60 cm, effective length, 52 cm, 50µmID×365µm OD uncoated capillary)	20mM sodium borate pH 9.5 containing 28.6% methanol	UV 254 nm	[77]
	HPLC	Mightysil RP-18 (4.6mm×250mm i.d., 5µm)	methanol in 20mM phosphoric acid (15:85, v/v)	UV 260 nm	[77]
<i>C. kyushuensis</i> (adenosine, cordycepin, thymine, uracil, uridine, deoxyuridine, hypoxanthine, guanosine)	CZE	Beckman uncoated fused-silica capillary (Total length 41 cm, effective length 30 cm, 45µm i.d.)	0.025 M borate buffer, pH9.4	DAD 258nm	[79-80]
<i>C. sinensis</i> (adenosine, guanosine, uridine)	CE	Beckman untreated fused-silica capillary (Total length 57 cm, effective length 50 cm, 75µm i.d.)	0.2M boric acid-sodium hydroxide buffer, pH 8.5	UV 254 nm	[76]
<i>C. sinensis</i> (adenine, uracil, adenosine, uridine, guanosine, inosine)	CE	A fused-silica capillary (Total length 56 cm, effective length 48 cm, 75µm i.d.)	500mM boric acid with 12.2% acetonitrile, pH8.6	DAD 254nm	[78]
<i>C. sinensis</i> (adenine, uracil, cytosine, hypoxanthine, thymine, guanine)	HPLC	Zorbax SB-AQ (4.6mm ×250mm i.d., 5µm)	Gradient elution with 5 mmol/l aqueous TEA and methanol	DAD 254 nm	[71]
<i>C. sinensis, C. militaris</i> (adenosine, guanosine, inosine, uridine, cordycepin)	HPLC	Zorbax SB-AQ (4.6mm ×250mm i.d., 5µm)	Gradient elution with 10 mmol/l aqueous TEA and methanol	DAD 254 nm	[93]
<i>C. sinensis, C. militaris</i> (adenine, adenosine, cytosine, cytidine, uracil, uridine, guanine, guanosine, hypoxanthin, inosine, thymine, thymidine, 2'-deoxyuridine, cordycepin)	UPLC	Acquity UPLC BEH C ₁₈ (2.1mm ×50mm i.d., 1.7µm)	Gradient elution with 0.5mM acetic acid and acetonitrile	DAD 254 nm	[19]
<i>C. militaris</i> (adenine, adenosine, cordycepin, uridine, hypoxanthin, guanosine)	HPLC	Zorbax SB-Aq RP18e (4.0mm ×250mm i.d., 5µm)	Gradient elution with methanol and water	DAD 260 nm	[72]
<i>C. militaris</i> (adenosine, cytosine, guanosine, uridine)	HPLC	LiChrospher 100 RP-18 (4.6mm×250 mm i.d., 5µm)	500 mmol/L KH ₂ PO ₄ /H ₃ PO ₄ (pH4.3)	UV 254 nm	[94]
<i>C. sinensis, C. militaris</i> (adenosine, cordycepin, 2'-deoxyadenosine, guanosine, uridine)	HPLC	Daisopak 120-5-ODS-BP (4.6 mm × 250 mm i.d., 5 µm)	Gradient elution with acetonitrile and water	UV 260nm	[48]
<i>C. sinensis</i> (adenosine, cordycepin, cytidine, guanosine, inosine, thymidine, uridine, cytosine, guanine, thymine, uracil)	HPLC	Zorbax 300SB C ₁₈ (4.6mm×250mm i.d., 5µm)	Gradient elution with methanol and water	DAD 260nm	[46]

(Table 2) contd....

Sample	Analytical Method	Column/Solid Phase	Mobile Phase	Detection	References
<i>C. jiangxiensis</i> , <i>C. taii</i> , <i>C. gunnii</i> (uracil, uridine, inosine, guanosine, adenine, adenosine, cordycepin)	HPLC	Shimadzu VP-ODS (4.6mm×250 mm i.d., 5µm)	Gradient elution with methanol and water	DAD 254nm	[15,100]
<i>C. sinensis</i> (adenosine, cordy- cepin, cytidine, guanosine, thymidine, uridine, 2'- deoxyuridine, adenine, cyto- sine, guanine, thymine, uracil)	HPLC	Zorbax NH ₂ (4.6mm ×250mm i.d., 5µm)	Gradient elution with acetonitrile and 10 mmol/L ammonium acetate	DAD 254nm	[97]
<i>C. sinensis</i> , <i>C. militaris</i> (adenine, hypoxanthine, adenosine, cordycepin)	HPLC	Shimadzu VP-ODS (2.0mm×150 mm i.d., 5µm)	water, methanol and formic acid(85:14:1, v/v/v)	DAD-ESI-MS	[98]
<i>C. sinensis</i> (uracil, hypoxan- thine, uridine, thymine, guanine, adenine, adenosine, cordycepin)	HPLC	Shimadzu VP-ODS (2.0mm×150 mm i.d., 5µm)	Gradient elution with ammonium acetate (40 mM, pH 5.2) and metha- nol	DAD-ESI-MS	[74]
<i>C. sinensis</i> (adenosine, guanosine)	HPLC	Zorbax 300SB C ₁₈ (4.6mm×250mm i.d., 5µm)	Gradient elution with aqueous am- monium acetate (8mM) and metha- nol.	DAD-ESI-MS	[95]
<i>C. sinensis</i> , <i>C. militaris</i> (Cytosine, uracil, cytidine, hypoxanthine, guanine, uridine, thymine, adenine, inosine, guanosine, thymidine, adenosine, cordycepin, 3'- Amino-3'-deoxy adenosine, 6-hydroxyethyl-adenosine)	HPLC	Zorbax Eclipse XDB-C ₁₈ (4.6mm× 150mm i.d., 3.5µm)	Gradient elution with aqueous am- monium acetate (5mM) and metha- nol	DAD-ESI- MS/MS	[96]

Note: TLC: thin-layer chromatography; CE: capillary electrophoresis; HPLC: high performance liquid chromatograph; CZE: capillary zone electrophoresis; UPLC: ultra-performance liquid chromatography; MS: mass spectrometry; DAD: diode array detector; ESI-MS: Electrospray ionization tandem mass spectrometry.

Li group, for the simultaneous determination of 11 nucleosides and bases, including adenosine, cordycepin, cytidine, guanosine, inosine, thymidine, uridine, cytosine, guanine, thymine, and uracil in natural and culture *C. sinensis* and cultured *C. militaris*. Determination was performed at 260nm by a gradient elution with the binary mobile phase system (methanol/water) [46]. Yu *et al.* established a HPLC fingerprint method to efficiently identify and distinguish cultured *C. militaris* from different sources, in which six nucleosides such as guanosine, hypoxanthine, adenine, adenosine, uridine and cordycepin were simultaneously determined at 260 nm by HPLC-DAD with a Zorbax SB-AqRP18e reversed-phase column and gradient elution with methanol/water mobile phase [72]. More recently, our research group developed a HPLC-DAD method for the simultaneous and quantitative determination of 7 nucleosides in *C. jiangxiensis*, such as uracil, uridine, inosine, guanosine, adenine, adenosine, and cordycepin. Determination was achieved at 260 nm on a Shimadzu VP-ODS column (4.6 × 250 mm i.d. 5 µm) using a gradient elution with a methanol/water mobile phase [100]. Recent years, to reach a very high-resolution separations in short analysis time for multiple components, the HPLC system is improved through using stationary phases with a particle size of <2µm, which results in a great increase in column efficiencies and high pressure. Accordingly, ultra-performance liquid chromatog-

raphy (UPLC) is designed undertake the high-pressure result from smaller particle size. More interestingly, UPLC is able to take full advantage of chromatographic principle to run separations using shorter columns or higher flow rates without loss of superior resolution and sensitivity [14], and has attracted wide attention of pharmaceutical and biochemical analysts. Li group reported an UPLC method for fast simultaneous determination of 14 nucleosides and nucleobases, including adenine, adenosine, cytosine, cytidine, uracil, uridine, guanine, guanosine, hypoxanthin, inosine, thymine, thymidine, 2'-deoxyuridine and cordycepin. The separation was achieved at 254nm on Waters Acquity UPLC system with Acquity UPLC BEH C₁₈ column, and gradient elution of 0.5mM acetic acid and acetonitrile in 5 min [19].

Generally, HPLC-MS is able to provide a higher selectivity and sensitivity for assaying minor components, isomeric compounds, or compounds without chromophore groups due to specific MS information for target compounds identified. For the quantitative analysis, furthermore, HPLC-MS exerts an excellent potential to get a stable and maximal signal-to-noise through optimizing some factors such as ion sources electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), detective modes (positive or negative ion mode), and the composition of mobile phase. For example, Huang's group developed the quantification of

nucleosides in both *C. sinensis* and *C. militaris* by a LC/ESI-MS in selective ion monitoring (SIM) mode [73,74]. The presence of each nucleoside in *C. sinensis* and *C. militaris* was ascertained by comparison of MS data, UV spectra and retention time with standards. In order to obtain optimum ionizing conditions, both ESI and APCI interface were tested in positive and negative ion mode by scanning between m/z 50 and 350 per second. Then, ESI interface and positive ion mode were chosen. Peaks were detected in both scan and SIM mode, respectively. Due to high selectivity and sensitivity, LC/ESI-MS in SIM mode is proved to be a powerful tool for qualitative analysis and quantitative analysis of nucleosides including uracil, hypoxanthine, uridine, thymine, guanine, adenine, adenosine, and cordycepin [74].

Since the early 1980s, capillary electrophoresis (CE) has evolved into a truly versatile separation technique [75]. CE needs no gradients, and it provides a better separation due to its higher resolution power than HPLC with gradient elution. Moreover, CE requires less maintenance using an uncoated capillary column, short analysis time, small consumption of sample and solvent, and does not need expensive organic solvent. Today, therefore, CE has definitively established a strong position in natural products analysis due to its enormous variability with respect to separation and detection modes, combined with high speed, selectivity and ease of operation [75]. Li and coworkers separated and determined three nucleosides such as adenosine, guanosine, and uridine within 10 min using the calibrated electrophoresis system [76]. Rao and coworker also obtained a good determination for cordycepin in *C. militaris* by the CE method with UV detection at 254 nm [77]. More interestingly, Li's group developed a simple CE method for simultaneous determination of six main nucleosides including adenine, uracil, adenosine, guanosine, uridine and inosine in natural and cultured *C. sinensis* using adenosine monophosphate as an internal standard after optimization of CE conditions, namely a running buffer composed of 500mM boric acid, adjusted pH to 8.6 with sodium hydroxide and 12.2% acetonitrile as modifier was found to be the most appropriate for the separation based on central composite design [78]. As we known, there are multiple separation modes in the CE technique including capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC) and nonaqueous CE (NACE). Ling *et al.* used CZE to simultaneously separate and determine the cordycepin and adenosine in natural and cultured *C. kyushuensis*, and cultured *C. militaris* under the following condition 0.025 M borate buffer, pH9.4, 20 kV, 20°C and 258 nm [79]. Subsequently, the same research group simultaneously quantitatively analyzed eight nucleosides such as cordycepin, thymine, deoxyuridine, uracil, adenosine, hypoxanthine, guanosine, uridine in natural and cultured *C. kyushuensis* under the same condition aforementioned [80]. Though these CE methods have become popular in the fingerprint analysis of nucleosides in *Cordyceps* as an important complementary tool to HPLC for its above-mentioned advantages, standards of nucleosides were necessary. Furthermore, many standards of nucleosides are not able to easily obtain in fact. Fortunately, MS information can be used to identify target compounds due to their different molecular masses. Thus more attention has been paid to the develop-

ment of fingerprint analysis with MS. When CE is coupling with MS, there are two main advantages at least. The first one is that the molecular mass of nucleosides can be temporarily identified by comparing the molecular mass with that in the published literatures and confirmed through other technique such as MS/MS. The second one is that the coexisting effluents from CE separation can be separated by MS if they have different molecular mass. For example, Wang and coworkers developed a CE-MS fingerprint method to for evaluating the urinary nucleosides from 20 bladder cancer patients and 20 healthy volunteers, and obtained three urinary nucleosides as the candidate disease-markers for the diagnosis of bladder cancer [81].

In addition, other methods, such as gas chromatography (GC) [82, 83], ion-pair reverse-phase chromatography (IP-RPC) [84,85], and immunoassays [86,87], have been also developed for the simultaneous determination of nucleosides in biological fluids and herbal materials. However, these methods have disadvantages such as limit of analytes [82,83], long analysis time [84,85], and low sensitivity [86,87]. Of course, it is not validated whether these methods are feasible for the nucleosides analysis of *Cordyceps*. To the best of our knowledge, regrettably, little patent has addressed the methodologies of nucleosides analysis of *Cordyceps* and its products.

4. POTENTIAL QUALITY STANDARDS FOR *CORDYCEPS* AND ITS PRODUCTS

Recent years, it has become increasingly apparent that the potential medicinal benefits of *Cordyceps* spp. are not related only to the one species *C. sinensis*. Many different *Cordyceps* species, have been developed for medicinal purposes and used in health supplements. Due to the rarity and high prices of the wild collected variety, furthermore, with the development of modern biotechnology based cultivation methods, many companies have produced artificially cultivated *Cordyceps* products, both from the mycelium as well as from the fruit bodies, to meet the increase demand of the worldwide market [9]. However, Quality determination in *Cordyceps* for the health supplement industry has been haphazard up until now, because there have been no universally recognized test methods for analyzing this particular supplement [44]. Each company supplying *Cordyceps* product has used different quality standards, some analyze for adenosine, some for cordycepin, some for cordycepic acid, and some for polysaccharide. While all of these tests have some usefulness in determining relative quality, none by itself is in any way meaningful when it comes to whether the product in question will yield good results when used as medicine for humans. Unfortunately, as the above-mentioned, the increase in supply has given rise to variations in purity and quality, creating a situation in which there are a large number of counterfeit and adulterated products being sold, furthermore, which have led many consumers to believe the wild collected *Cordyceps* is medicinally better than the cultivated type [9]. Therefore, a draconic issue has been raised concerning the quality of *Cordyceps*.

As aforementioned, *Cordyceps* spp. contains a broad range of compounds that are considered nutritional, and nu-

cleosides are the major components in both natural and cultured *Cordyceps*. Furthermore, Approximately 20 nucleosides and their related compounds have been found in *Cordyceps* spp. [17,19, 88], especially *Cordyceps* can produced rare nucleosides derivatives with specific functions such as 2',3'-dideoxyadenosine, hydroxyethyladenosine, cordycepin, cordycepin triphosphate, deoxyguanosine, which were not found anywhere else in nature [88]. In addition, cultured *Cordyceps* mycelium contains a higher level of nucleosides than in natural *Cordyceps*. It is generally believed that the sources of nucleosides in natural *Cordyceps* may be different from that of cultured one [17]. The nucleosides of natural *Cordyceps* are changed by many environmental factors. For example, fresh natural *C. sinensis* contains very little amount of nucleosides, as compared to dry and processed one [76]. Furthermore, humidity and heat significantly increased the amount of nucleosides in natural *Cordyceps*. Storage of *Cordyceps* at 75% relative humidity and 40 °C for 10 days, the nucleosides content in natural *Cordyceps* markedly increased to about four-folds. However, the effect of humidity and heat in altering the content of nucleotides could not be revealed in cultured *Cordyceps* mycelia [89].

Based on these reasons above-mentioned, it is still controversial if adenosine is the optimum marker for quality control of *Cordyceps* and its products [48]. For example, adenosine is officially authorized as chemical marker for quality control of natural and cultured *C. sinensis* [16,17]. However, Li's group suggested that adenosine, inosine and cordycepin could be used as markers for discrimination and quality control of different *Cordyceps* [46]. After a thorough review of the literature, the nucleosides, and specifically the deoxy-nucleosides, show the most variation in different samples of natural and cultured *Cordyceps*, in particular many of the deoxy-nucleosides are found in no other organism, or at best, a very limited number [44]. Therefore, Holliday and coworkers have thought that this class of compounds is determined to be the most reliable indicators of medicinal potency for *Cordyceps* products, and have found a new method for assaying the quality of *Cordyceps* products, namely the content of hydroxyl ethyl adenosine analogs (HEAA) including adenosine, cordycepin, dideoxyadenosine, and *N*⁶-(2-hydroxyethyl) adenosine, is recognized as a much more reliable indicator of *Cordyceps* species quality [44, 90]. Recently, our group has isolated two deoxy-nucleosides such as 2'-deoxyuridine, and 2'-deoxyadenosine from cultured *Cordyceps jiangxiensis* [20,51], and the two deoxy-nucleosides were also found in cultured *Cordyceps taii* (data not shown). Interestingly, 3'-*O*-methyluridine, and 2'-*O*-methyladenosine was obtained from cultured *Cordyceps jiangxiensis*, which were also isolated from *Cordyceps* species for the first time [21,51]. In addition, we should pay attention to the fact that only a few *Cordyceps* spp. such as *C. sinensis*, *C. militaris*, and *C. kyushuensis* were systematically investigated according to previous reports (see Tables 1 and 2). Therefore, we are still not able to give the quality control standard concerning nucleoside ingredients for all *Cordyceps* species and their products at present. However, the above-mentioned unique nucleosides, along with HEAA should be used in summation as the quality indicators for comparison of different *Cordyceps* species including *C. sinensis*, *C. militaris*, and *C. kyushuensis*.

CURRENT & FUTURE DEVELOPMENTS

Quality control is the cornerstone of the application and development of TCM. Because the TCM emphasizes the importance of multi-ingredient preparations as being responsible for its pharmacological activities, in contrast to modern chemical drug that often focus on a single chemical entity. Accordingly, the quality control of TCM is more complicating in comparison to chemical drug. Moreover, with the development of modern pharmacology and analytical chemistry, the traditional quality control of TCM has become unadaptable to the requirements of TCM development. For example, the quality control of *Cordyceps* products has promulgated a national standard for decades in China, but it is inadequate that only adenosine is recognized as a major quality index. We should develop more accurate professional standard for the quality control of *Cordyceps* products. Nucleosides are major active ingredients in *Cordyceps* fungi, furthermore, some specific and interesting nucleoside derivatives such as *N*⁶-(2-hydroxyethyl) adenosine, deoxy-nucleosides, methoxyl-nucleosides, were found in *Cordyceps* species but not in any other fungi. Therefore, we should use several specific and valuable nucleosides, even the chromatographic fingerprint of nucleosides as the quality index markers of *Cordyceps* products, but not adenosine only. In addition, especially worth mentioning is, that the related patents on quality control of *Cordyceps* and its bioproduct are only involved in cultivation, preparation, and active components, yet the quality standards patents of *Cordyceps* such as chemical markers, analytical methods, are severe deficiency at present. Accordingly, it suggests that the quality control patents of *Cordyceps* are an important and promising field of research for deep development of *Cordyceps* source in the future.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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