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Quality control of *Cordyceps sinensis*, a valued traditional Chinese medicine

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Abstract

Cordyceps sinensis, a well-known and valued traditional Chinese medicine, is also called DongChongXiaCao (winter worm summer grass) in Chinese. It is commonly used to replenish the kidney and soothe the lung for the treatment of fatigue, night sweating, hyposexualities, hyperglycemia, hyperlipidemia, asthemia after severe illness, respiratory disease, renal dysfunction and renal failure, arrhythmias and other heart disease, and liver disease. As the rarity and upstanding curative effects of natural *Cordyceps*, several mycelial strains have been isolated from natural *Cordyceps* and manufactured in large quantities by fermentation technology, and they are commonly sold as health food products in Asia. In addition, some substitutes such as *Cordyceps militaris* also have been used and adulterants also confused the market. Therefore, quality control of *C. sinensis* and its products is very important to ensure their safety and efficacy. Herein, markers and analytical methods for quality control of *Cordyceps* were reviewed and discussed.

Keywords: *Cordyceps*; Quality control; Quality marker; Analytical methods; HPLC; CE

1. Introduction

Cordyceps, one of the well-known traditional Chinese medicines, is a composite consisting of the stromata of the fungus, *Cordyceps sinensis* (Berk.) Sacc. (Family: Hypocreaceae) parasitized on the larva of some species of insects (Family: Hepialidae), and the dead caterpillar. It is also known as "winter worm summer grass" because of its appearance during different seasons (Fig. 1). The parasitic complex of the fungus and the caterpillar is found in the soil of a prairie at an elevation of 3500–5000 m. It mostly distributed in Tibet, Qinghai, Sichuan, Yunnan and Gansu province.

Cordyceps has been known and used in China for medication over 300 years. It was first recorded in "Ben Cao Bei Yao" by Wang Ang in 1694 AD. And was described as: "*Cordyceps* derived from Jiading of Sichuan, shows the highest quality. In winter, it appears as an old silk worm in soil, and moves with hair. In summer, hairs grow out of soil, and turn into grass. They have to be collected in summer, if not they will turn into worm again". *Cordyceps* became known to the Western society during 17th century. In 1878 AD, Italian scholar Saccardo named *Cordyceps* derived from China officially as *Cordyceps sinensis* (Berk.) Sacc., and this nomenclature was adopted until today.

Cordyceps is commonly used in China to replenish the kidney and soothe the lung for the treatment of fatigue, night sweating, hyposexualities, hyperglycemia, hyperlipidemia, asthemia after severe illness, respiratory disease, renal dysfunction and renal failure, arrhythmias and other heart disease, and liver disease [1,2]. Modern pharmacological studies showed that *Cordyceps* was beneficial to several systems, including the circulatory, immune, hematogenic, cardiovascular, respiratory and glandular systems in human body [3]. However, its usage has been limited during the past decades due to the high price and the difficulty of its supply. The growth of *C. sinensis* has a very restricted habitat, and the yield is decreasing every year. In 2001, a total of only a few thousand kg of natural *Cordyceps* were collected with a decrease of over 70% as compared to 1978 in China. Therefore, the isolation of mycelial strain from *Cordyceps* is a trend of many scientists to achieve a large-scale production of

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Fig. 1. The habitat of *Cordyceps* in China: (A) a prairie at an elevation of 3500–5000 m, mainly in the provinces of Qinghai, Tibet, Sichuan, Yunnan and Gansu; (B and C) the parasitic complex of the fungus and the caterpillar is found in the soil; (D) freshly collected *Cordyceps*. Arrowheads in (B and C) indicate the *Cordyceps*.

Cordyceps by fermentation. Besides cultivation of *Cordyceps* by fermentation technology, much effort has also been focus on discovering the alternative species. There are more than 350 types of so called *Cordyceps* or its substitutes have been found worldwide today, such as *Cordyceps militaris* (L.) Link (the most commonly used substitute), *C. martialis* Speg., *C. hawkesii* Gray, *C. liangshanensis* Zang, Liu et Hu, sp. nov., *C. barnesii* Thwaites, *C. cicadicola, C. gracilis* (Grav.) Dur. et Mont., *C. ramose* Teng, *C. ophioglossoides* (Ehrh. Fr) Link and *C. gunnii* (Berk.) Berk etc. In addition, there are counterfeits and mimics such as *Stachys geobombycis* C.Y. Wu, *Stachys sieboldii* Miq. and *Lycopus lucidus* Turcz., etc also emerge on the market [4–8]. Thus, it is a serious problem for authentication and quality control of *Cordyceps* on the market.

Reviews of the clinical usage of *Cordyceps* [1,2], biological and pharmacological properties [9] and its effects on apoptotic homeostasis [10] have been described. Herein, markers and analytical methods for quality control of *Cordyceps* were reviewed and discussed.

2. Significant markers for quality control of *Cordyceps*

2.1. Nucleosides—authentication of Cordyceps

Nucleosides are one of the major components in *Cordyceps*. In 1964, 3′-deoxyadenosine, namely cordycepin, was isolated from cultured *Cordyceps militaris* [11], a related species of *C. sinensis* commonly used as a substitute. Since then, nucleosides in *Cordyceps* have been a focus because cordycepin was shown to have anti-tumor activity. More than 10 nucleosides and its related compounds have been isolated from *Cordyceps* including adenine, adenosine, uracil, uridine, guanidine, guanosine, hypoxanthine, inosine, thymine, thymidine, deoxyuridine [12–17]. However, existence of cordycepin in natural *C. sinensis* is controversial in the past decades. Recently, cordycepin has been identified in natural *C. sinensis* with a very low content in some reports [14,15]. In addition, N^6-(2-hydroxyethyl)-adenosine (Fig. 2), which behaves as a Ca^{2+} antagonist and an iontropic agent, was isolated from cultured mycelia of *Cordyceps* [16]. To date, nucleosides are believed to be the active components in *Cordyceps*, and adenosine has been used as marker for quality control of *C. sinensis* [18]. Indeed, nucleosides are involved in the regulation and modulation of various physiological processes in the central nervous system (CNS). Adenosine is known to depress the excitability of CNS neurons and to inhibit release of various neurotransmitters presynaptically [19,20]. There is growing pharmacological evidence from several animal models of seizure disorder that adenosine possesses anticonvulsant activity [21]. However, fresh natural *C. sinensis* contains very little amount of nucleosides, as compared to dry and processed one [13], and more interestingly cultured *Cordyceps* mycelium contains high level of