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In vitro evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of *Cordyceps sinensis*

C.-H. Dong[a,b], Y.-J. Yao[a,*]

[a]*Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China*
[b]*Graduate School of the Chinese Academy of Sciences, Beijing 10049, China*

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Abstract

Cordyceps sinensis, one of the best known traditional Chinese medicines and health foods, has been highly valued for the treatment of a wide range of diseases and reported to have antioxidant properties. In the present study, the antioxidant activities of hot-water extracts from natural and cultured mycelia of *C. sinensis* were investigated and evaluated using six in vitro assays, including inhibition of linoleic acid peroxidation; scavenging abilities on DPPH•, hydroxyl and superoxide anion radicals; the reducing power and the chelating ability on ferrous ions. Among these assays, the extracts showed the best effect on the inhibition of linoleic peroxidation with the lowest IC50 values and with an inhibition rate over 90% at concentration of 0.8–1.6 mg/ml, more stable than that of α-tocopherol, a recognised natural antioxidant. The scavenging activities on superoxide anion and hydroxyl radicals of the two extracts were slightly lower than that of butylated hydroxytoluene. DPPH• scavenging activities of both extracts reached over 80% inhibition at 4–8 mg/ml. Both extracts showed moderate reducing power and ferrous ion chelating activity. The IC50 value of the extract from cultured mycelia in all the tests, except for linoleic acid peroxidation, was significantly lower than that of natural mycelia. There was no evident correlation between the antioxidant activity and the content of protein, polysaccharides and mannitol of extracts from *C. sinensis*; the antioxidant activity may be due to a combined effect of these or some other compounds. These results suggested that both the extracts from cultured and natural mycelia have direct and potent antioxidant activities and that the cultured mycelia of the fungus could be used for the antioxidant activity to reduce the human demands on the natural resources of the fungus, an endangered species.

Keywords: *Cordyceps sinensis*; Reactive oxygen species; Aqueous extracts; Tonic

1. Introduction

Cordyceps sinensis (Berk.) Sacc., an entomogenous fungus, is one of the best known traditional Chinese medicines and health foods. The fungus parasitises larvae of moths (*Lepidoptera*), especially *Hepialus armoricanus* Oberthür, and converts each larva into a sclerotium, from which the stroma and fruit-body grows (Pegler, Yao, & Li, 1994). The complex including the fungal stroma and the sclerotium, which appears as larva body owing to the intact exoskeleton of the insect, has been used as a health food and traditional medicine to invigorate the lung and nourish the kidney in China for hundreds of years, at least from the 17th century (Wang, 1694; Zhao, 1765).

C. sinensis is believed to have several effects on the human body and is mainly used as a tonic to strengthen the body, especially after a serious illness. However, recent studies have demonstrated that *C. sinensis* can be used to treat a wide range of conditions, including respiratory, renal, liver, nervous system and cardiovascular diseases; tumour; aging; and also hyposexuality and hyperlipidemia (reviewed in Zhu, Halpern, & Jones, 1998). It has been officially classified as a drug in the Chinese Pharmacopoeia since 1964 (Committee of Pharmacopoeia, Chinese Ministry of Health, 1964, 2005). The use of the fungal products in medicinal treatment and in health foods has become very popular since 1990s and more so since the outbreak of the Severe Acute Respiratory Syndrome (SARS) in China in

*Corresponding author. Tel.: +86 10 64807496; fax: +86 10 64807518.
E-mail address: yaoyj@sun.im.ac.cn (Y.-J. Yao).

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2003. The market demand for *C. sinensis* is growing sharply in many countries, especially in Asia, in recent years (Sharma, 2004; Vinning & Tobgay, 2004).

C. sinensis is endemic to the Tibetan Plateau and may be found only from above 3000 m in altitude (Jiang & Yao, 2002; Wang, 1995; Yao, 2004) and the natural resources of the fungus are very limited due to its confined geographic distribution and over exploitation in recent years (Yao, 2004). The price of natural products of *C. sinensis* is now over US$12,000/kg (for average quality in 2006) in the market and is still increasing. As substitution to the natural products, living strains have been isolated from natural *C. sinensis* and cultivated in large quantity by fermentation technology (Wang, 1995; Zhu et al., 1998). The fermentation of mycelia has been proved as a promising method to meet the needs of human consumption and to reduce the pressure on natural resources of the species which is in danger (It has been officially classified as an endanger species by CITES Management Authority of China and China Customers, 2000).

There are increasing evidences that degenerative or pathological events, such as senescence, asthma and cancer, are associated with accumulation of an excess of reactive oxygen species (ROS), which results in oxidative damage to DNA, proteins and other macromolecules (Balaban, Nemoto, & Finkel, 2005; Klaunig & Kamendulis, 2004; Stevenson, Koch, & Britton, 2006). Antioxidants, which scavenge free radicals, are known to play important roles in preventing the diseases induced by ROS (Park et al., 2004; Willcox, Ash, & Catignani, 2004).

As a valued traditional Chinese medicine for the treatment of a wide range of diseases, *C. sinensis* has attracted many research interests in recent years for the antioxidant activity. Li, Su, Dong, and Tsim (2002) compared the antioxidant activities of extracts using phosphate buffered saline (PBS) at 37 °C from stroma and sclerotium of natural *C. sinensis*, and the results showed that mycelia from both the stroma and the sclerotium had similar potency in their antioxidant activities in xanthine oxidase, induction of hemolysis and lipid peroxidation assays. It was reported that aqueous extracts from natural *C. sinensis* could scavenge hydroxyl radicals (Cai, Chen, Yin, & Zhang, 2004) and that methanol extracts could do the same on hydroxyl and superoxide anion radicals (Zhang, Pu, Yin, & Zhong, 2003).

There are also some studies on the antioxidant activity of *C. sinensis* using unauthenticated material. A polysaccharide which can protect PC12 cells against hydrogen peroxide-induced neuronal cell toxicity was isolated by Li et al. (2003), but the *Cordyceps* mycelia used from Wan Fong Pharmaceutical Factory (Zhejiang, China) were derived from a fungal strain named *Cephalosporium sinensis* Chen sp. nov. [sic! A nomenclaturally illegitimate fungal name], which raised doubts whether it is truly a strain of *C. sinensis* (Jiang & Yao, 2003) and, indeed, was later proved to be a different species (Ke, 2005). Yamaguchi, Kagota, Nakamura, Shinozuka, and Kunitomo (2000a, 2000b) reported that both water and ethanol extracts from artificial cultivated fruit-bodies of *C. sinensis* from the Xinhui Xinhan Artificial *Cordyceps* Factory (Guangdong, China) could scavenge ROS by inhibiting malondialdehyde formation by the peroxynitrite generator SIN-1. Although the results have been referred to in many subsequent investigations (e.g. Buenz, Bauer, Osmundson, & Motley, 2004, 2005; Li et al., 2003), there is, however, a doubt that the fungal material used was authentic *C. sinensis*, due to the fact that reports exist of cultivation of fruit-bodies of this fungus not being repeatable and that the manufacturer is, in fact, selling products of *C. militaris*. Li, Li, Dong, and Tsim (2001) compared the antioxidant activities of natural *C. sinensis* and cultured *Cordyceps* mycelia from different sources by three tests (xanthine oxidase, induction of hemolysis and lipid peroxidation assays) using PBS at 37 °C for 12 h to extract the compounds from samples, and were able to show the similar effects of the cultured mycelia to the natural products. However, the cultured material used by Li et al. (2001) was derived from a wide range of strains, of which some are apparently not from a true *C. sinensis*, e.g. products from Chinese Medicine Factory of Jiangxi and from Hebei Boding Pharmaceutical Factory (see Jiang & Yao, 2003). Further, the traditional Chinese medicines are boiled in water for medications or as health foods and soaked in alcohol as drink. The extraction using different solvents and temperatures may have resulted in different compounds.

A direct correlation between antioxidant activity and metal chelating and also reducing power has been reported for extracts from some traditional Chinese medicines (Jung, Seog, Choi, Park, & Cho, 2006; Mau, Tsai, Tseng, & Huang, 2005), but there has been no report on metal chelating and reducing power of extracts from *C. sinensis* so far. Due to the increasing interest in the relationship between antioxidants and diseases, there is a need to get an overall measure of the antioxidant activity of extracts from *C. sinensis* using reliable fungal material.

In the present study, the antioxidant activities of the hot water extracts from natural and cultured mycelia of *C. sinensis* were investigated in vitro and evaluated by inhibition of linoleic acid peroxidation; scavenging abilities on DPPH•, hydroxyl and superoxide anion radicals; and the reducing power and the chelating ability on ferrous ions. The contents of protein, polysaccharides and mannitol of extracts from both natural and cultivated *C. sinensis* were also determined to reveal their correlation with antioxidant activity.

2. Materials and methods

2.1. Chemicals

Linoleic acid, ferrozine, 2-deoxy-*o*-ribose and 1,1-diphenyl-2-picrylhydrazyl (DPPH•) were purchased from Sigma-Aldrich