AkiNik

Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2013; 2 (4): 43-45
© 2013 AkiNik Publications
Received: 13-9-2013
Accepted: 18-9-2013

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Simultaneous Extraction, determination and analysis of Adenosine, Cordycepin and other derivatives of *Cordyceps sinensis* of Nepal by new validated HPLC method

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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. Parasitic Cordyceps fungi, such as *Cordyceps sinensis*, are a parasitic complex of fungus and caterpillar, which has been used for medicinal purposes for centuries particularly in China, Japan and other Asian countries. It is commonly used to replenish the kidney and soothe the lung for the treatment of fatigue, night sweating, hyposexualities, hyperglycemia, hyperlipidemia, asthenia after severe illness, respiratory disease, renal dysfunction and renal failure, arrhythmias and other heart disease, and liver disease. Therefore, quality control of *C. sinensis* and its products is very important to ensure their safety and efficacy. A new HPLC method was developed to determine the content of the Cordycepin and Adenosine in caterpillar fungus. HPLC method was performed on a protoSIL C18 reversed-phase column (250×4.6mm, 5μm) .All of the reference substances and sample were separated with the mobile phase of methanol: water (20:80) under isocratic elution for 25min, flow rate was 1.0mL/min, the detection wavelength was 254nm, and the column temperature was 30 ℃.The content of the Cordycepin and Adenosine in the standardized extract of caterpillar fruiting body of fungus were 0.288 mg/g and 0.346 mg/g. The average recovery rate were about 101.1 % (n=9, RSD=1.33%) in the caterpillar fruiting body. This developed method, which is simple and with high sensitivity as well as selectivity, can be used for quality evaluation of the Cordyceps.

Keywords: *Cordyceps sinensis*, Adenosine

1. Introduction

Cordyceps includes several Cordyceps species, which are widely used for medicinal purpose or food additives. Among them, *Cordyceps sinensis*, (Fig 4).

Fig 4: *Cordyceps sinensis*

DongChong-XiaCao in Chinese, is a complex of larva corpus of Hepialus armoricanus. For several centuries in China, *C. sinensis* was widely used as a kind of tonic food and herbal medicine for preventing or curing various diseases. For example, *C. sinensis* plays an important role in the treatment of respiratory and cerebrovascular diseases, enhancement of body immunomodulator function and regulation of liver and renal metabolism [1].

Due to the limited distribution, high price, over-exploitation and difficulty in artificial culture to obtain mycelium, the resource of *C. sinensis* has been endangered [2]. HPLC is one of the main means in quality monitoring of food and medicine products. It has been widely used in variety determination. For the moment, it is acknowledged that the major bioactive components in cordyceps are adenosine and Cordycepin. Hitherto, there are various HPLC methods that had been widely used in the determination of adenosine and Cordycepin from *C. sinensis* [2, 3 ,4].

The main purpose in the present research paper was to detect the contents of bioactive compounds and establish a standard of quality control for *C. sinensis* through an easy standardized HPLC method. This study further established foundation for pharmacology experiments.

2. Materials and Methods

2.1 Preparation of *Cordyceps sinensis* Extract:

Dried *Cordyceps sinensis* fruiting body on caterpillar was supplied by Gyan herbal products Nepal. The raw material is made to coarse powder and different extracts were made using water and alcohols. The extracts were dried and kept for analysis.

2.2 Determination of the bioactive components

All the HPLC analysis was carried out on a waters 486 photo-diode array detector (Millipore, USA) a manual injector and a C18 protoSIL reversed-phase column (250×4.6 mm, 5 µm). Standards of Cordycepin and adenosine were purchased from Sigma Chemical Corporation (St. Louis, MO, USA). The standard Adenosine and Cordycepin solvent was consecutively injected five times to draw calibration curves. The injection volumes are 3,5,10, 15 and 20 µl, respectively. The determination condition of the samples was set as follows: the mobile phase adopted in the analysis consists of methanol and water was in the ratio of 20:80. The separation was conducted in isocratic elution with a flow rate of 1.0ml/min with a detection wave length of 254 nm and injection volume is 10 µl.

3. Result and Discussion:

Optimization of chromatographic conditions in this study, the effect of chromatographic condition on the separation was investigated; especially flow rate and mobile phase. The separation was compared when using different solvent as mobile phase, such as methanol, Acetonitrile, ethanol and buffer salt solution. The results shown the best separation was obtained under a specific concentrations of methanol and water. Based on the flow rate of mobile phase, the elution time was determined. It was found that 1.0 ml/min was a proper flow rate. As Cordycepin and adenosine are polarity organic matters, with the increase of percentage of methanol in mobile phase, the difference of retention time of Cordycepin and adenosine become small which resulted in a poor separation. When using methanol and water in the ration of 20:80 as mobile phase the retention time is less than 15 minutes and a good separation of peaks were achieved. To understand for more detectable molecules in the extracts we used elution time of 30 min. Alcoholic extracts has shown more amount of Adenosine and Cordycepin 0.288 mg/g and 0.346 mg/g when compared to pure water extract 0.0918 mg/g and 0.102 mg/g as in fig 1.

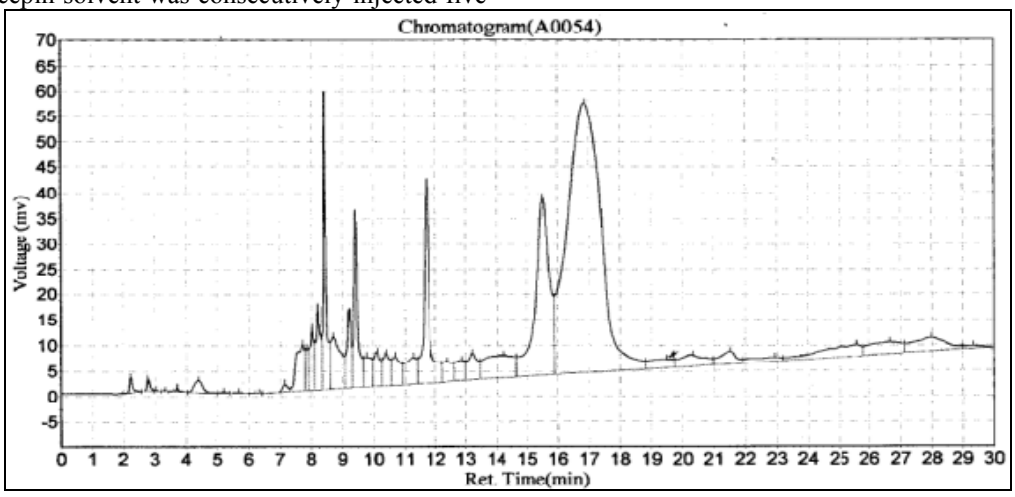

Fig 1: Water extract chromatogram

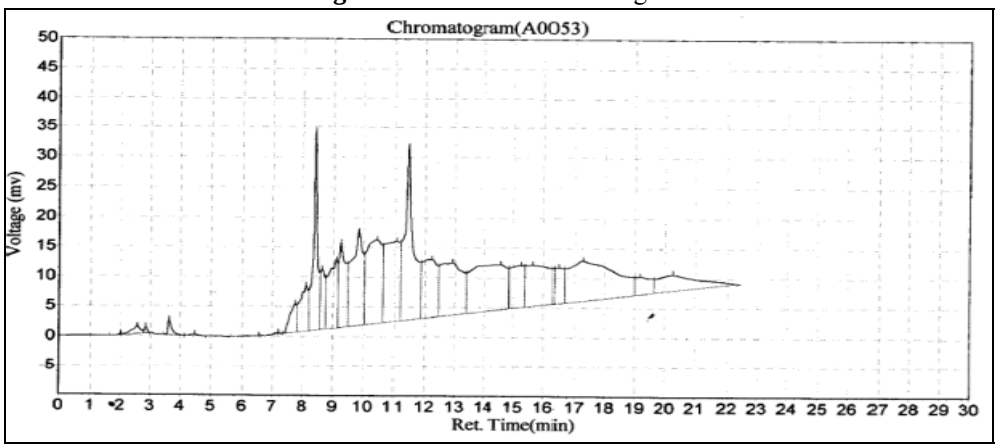

Fig 2: Standardized alcoholic extract chromatogram when compared to standards fig 3.

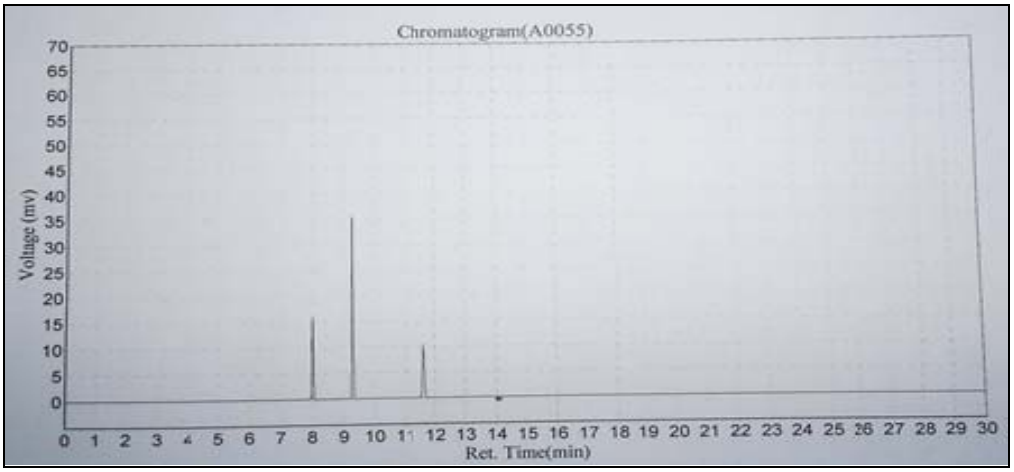

Fig 3: Chromatogram of standards Adenosine and Cordycepin

In this study, we established an optimized chromatographic condition with a mobile phase methanol-water (20:80, V/V), flow rate 1.0 ml/min and detection wavelength 254 nm, which had good separation on adenosine and Cordycepin in cordyceps products within 15 min.

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