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Short communication

Fingerprint analysis of fruiting bodies of cultured *Cordyceps militaris* by high-performance liquid chromatography–photodiode array detection

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Abstract

We have developed and optimized a novel, efficient and accurate fingerprint method using high-performance liquid chromatography–photodiode array detection (HPLC–DAD) for the quality control of cultured *Cordyceps militaris* (L.) Link. The feasibility and advantages of the used chromatographic fingerprint were verified for the evaluation of cultured *C. militaris* by systematically comparing chromatograms with a professional analytical software recommended by State Food and Drug Administration (SFDA) of PR China. The results revealed that the chromatographic fingerprint combining similarity evaluation could efficiently identify and distinguish cultured *C. militaris* from different sources.

Keywords: Quality control; Chromatographic fingerprint; HPLC–DAD; Cultured *Cordyceps militaris*; Traditional Chinese medicines

1. Introduction

The paradigm of traditional Chinese herbal medicine (TCHM) emphasizes the importance of multi-compound, multi-ingredient preparations as being responsible for the activity of the herbal drug, in contrast to modern pharmacology and drug development that often focus on a single chemical entity [1]. The traditional quality control of herbal medicines faces a number of severe challenges in the standardization of TCHM. Chromatographic fingerprint, a comprehensive and quantifiable identification method, is able to reveal chemical information of herbal medicines with chromatogram, spectrograms and other graphs by analytical and chemical techniques [2,3]. State Food and Drug Administration (SFDA) has required that all the injections made from herbal medicines or their raw materials be standardized by chromatographic fingerprinting [4]. Moreover, SFDA has also suggested that all of herbal chromatograms should be evaluated by their similarities, a commonly employed approach based on calculating the correlative coefficient and/or cosine value of vectorial angle of original data [5–7].

The *Cordyceps* species of the traditional Chinese medicinal mushrooms are entomopathogenic fungi. *Cordyceps militaris*, also known as the Chinese caterpillar fungus, possesses pharmacological activities similar to, and according to some studies, more potent than *C. sinensis* (also known as Dong Chong Xia Cao) that is used in certain health food products in Asia [8–10]. Recently, several studies have demonstrated that the extracts and some components of *C. militaris* can exert multiple pharmacological actions, such as anti-inflammatory and humoral-immunity suppressive activities [11–13], ameliorative effects on insulin resistance and insulin secretion [14] and anti-oxidant activity stronger than those of *C. sinensis* and *C. kyushuensis* [15]. Because of the rarity and expensiveness of natural *C. militaris*, scientists have conducted extensive studies on its life cycle with the aim of developing techniques for isolating and therefore harvesting fermentable strains of *C. militaris*. Several strains have been isolated from natural *C. militaris* and produced in high quality by fungus-cultivation technology [16–18]. Our group has undertaken studies on comparing the chemical composition of cultured *C. militaris* with that of the wild *C. militaris* [19]. The products from cultured *C. militaris* have shown to possess similar pharmacological efficacy to that of wild *C. militaris* [20], and cultivated fruiting bodies of *C. militaris* are commonly sold as drug materials and

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health food products in China and South East Asia [21,22]. As a result, it is currently of much interest and importance to develop an effective method for the quality control of cultured *C. militaris*.

In this study, a novel fingerprinting method was first developed using high-performance liquid chromatography–photodiode array detection (HPLC–DAD) for the quality control of cultured *C. militaris*, and similarity evaluation system has also been used to establish the chromatographic fingerprint of the herb.

2. Experimental

2.1. Instrumentation and reagents

HPLC analysis was carried out on a Agilent Series 1100 liquid chromatograph, equipped with a vacuum degasser, a quaternary pump, an auto sampler and a diode array detection (DAD) system, connected to a reversed-phase column (Zorbax SB-Aq RP18e, 5 μm, 250 mm × 4.0 mm i.d., Agilent, USA). Data collection was performed using ChemStation software (Agilent). The water used for all the solutions and dilutions was prepared with a Millipore water purification system. An ultrasonic cleaner (TDL80-2B, Feige, China) was used for extraction. The vacuum concentrator system consisted of a rotary evaporator, a cool ice and a digital bath (EYELA, Japan). Acetonitrile and methanol were of HPLC grade.

2.2. Materials

Eleven fruiting bodies of cultured *C. militaris* collected and cultured by different companies from six provinces of China were examined. All of them were identified by Professor R.M. Yu at Jinan University, China. Six pure nucleosides (guanosine, hypoxanthine, adenine, adenosine, uridine and cordycepin) were used as standards and all of them were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Table 1
The examined mobile phases in optimization of HPLC conditions

Systems	Gradients	Finish time (min)	Peaks
Acetonitrile (A) and water (W)	4–60% A, 96–40% W	10	17
Acetonitrile (A) and buffer solution (B, water–KH_2PO_4, pH 6.86)	4–20% A, 96–80% B	15	23
Methanol (M) and water (W)	5–60% M, 95–40% W	25	22
Methanol (M) and buffer solution (B, water–KH_2PO_4, pH 6.86)	5–50% M, 95–50% B	25	24

Table 2
Optimization standard of the whole chromatographic fingerprint of methanol system

System A: MeOH–H_2O					System B: MeOH–KH_2PO_4				
Peaks	k	S	R	Result	Peaks	k	S	R	Result
1	1				1	1	0.0244	1.81	
2	1.06	0.0148	1.64		2	1.11	0.0255	2.26	
3	1.5	0.0965	11.32		3	1.2	0.0090	0.99	
4	1.76	0.0494	7.64		4	1.24	0.0261	2.42	
5	1.82	0.0108	1.64		5	1.36	0.0268	2.92	
6	2.16	0.0569	6.10		6	1.49	0.0099	1.44	
7	2.28	0.0186	2.19		7	1.54	0.0136	1.90	
8	2.4	0.0180	2.62	0–15 min	8	1.61	0.0151	2.35	0–15 min
9	2.91	0.0698	10.71	$r = 0.00249$	9	1.69	0.0092	1.46	$r = 0.00065$
10	2.99	0.0101	1.39		10	1.74	0.0335	3.53	
11	3.47	0.0567	7.87	15–25 min	11	1.93	0.0200	1.96	15–25 min
12	3.53	0.0067	1.14	$r = 0.52486$	12	2.05	0.0113	1.39	$r = 2.95373$
13	3.72	0.0205	3.53		13	2.12	0.0545	6.01	
14	3.88	0.0167	2.76	0–25 min	14	2.48	0.0400	4.89	0–25 min
15	4.25	0.0365	5.50	$r = 0.001307$	15	2.77	0.0636	7.49	$r = 0.001928$
16	4.44	0.0178	2.27		16	3.35	0.0715	8.91	
17	4.64	0.0181	2.25	R_{min}[a] = 1.14	17	4.02	0.1382	17.70	R_{min}[a] = 0.99
18	4.97	0.0284	3.89		18	5.63	0.0112	1.34	
19	6.97	0.1435	28.50		19	5.78	0.0477	7.49	
20	7.23	0.0160	3.70		20	6.46	0.0224	3.21	
21	8.1	0.0502	9.31		21	6.72	0.0434	5.73	
22	8.53	0.0231	4.40		22	7.42	0.0152	1.86	
					23	7.68	0.0280	4.87	
$\sum$		0.7791	120.37				0.7601	93.93	
$\prod$		1.83×10^{-33}	5.00×10^{12}				1.68×10^{-37}	1.5×10^{11}	

[a] R_{min} represents the lowest resolution in the chromatograms.