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Determination of Cordycepin in *Cordyceps kyushuensis* by Capillary Electrophoresis and its Antitumour Activity

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Abstract: A simple, rapid and low-cost method of determination for cordycepin in *Cordyceps kyushuensis* by capillary zone electrophoresis (CZE) was developed. Based on the finding that there is a high concentration of cordycepin in both natural and cultured *Cordyceps kyushuensis*, the *in vitro* antitumor activity of cordycepin and the water extracts of *Cordyceps kyushuensis* has been investigated. This is the first report about the antitumor effect of *Cordyceps kyushuensis.*

Keywords: Cordycepin, *Cordyceps kyushuensis*, capillary zone electrophoresis, cancer cell line, antitumor activity.

Cordyceps kyushuensis Kob. is an insect pathogenic fungus that infects the clanis bclineata walker. This fungus has recently been isolated, identified and cultured in our laboratory. Being interested in the new, local grown species, we have been conducting a series of investigations to explore its chemical constituents and pharmaceutical effects. In our studies, the extracts of *Cordyceps kyushuensis* Kob. exhibit some pharmacological actions such as the antioxidant and antitumour activities *in vitro*.

Cordycepin (3'-deoxyadebosine), the main bioactive component in fungi of the genus *Cordyceps*, was first isolated from the culture filtrates of *Cordyceps militaries*[1]. Reported biological activities of cordycepin include: (a) inhibition of DNA and RNA synthesis[2]; (b) enhancement of cell differentiation[3]; (c) restructuring of cytoskeleton in cells[4]; (d) antitumor activity on leukemia, bladder, colon, lung carcinoma as well as fibroblastoma *in vitro*[5,6] ; (e) inhibition of protein kinase activity[7]; (f) inhibition of the infection and reverse transcriptase activity of human immunodeficiency virus (HIV) type I[8]; (g) antifungal and antibacterial activities[9,10]and *etc*. HPLC and thin-layer chromatography (TLC) methods have been reported to quantify cordycepin in Cordyceps[11,12]. Here, a novel method to determine the content of cordycepin by capillary zone electrophoresis has been developed, and the antitumor activity of the water extract from *Cordyceps kyushuensis* Kob. has been explored.

Natural *Cordyceps kyushuensis* Kob.was collected from Mount Meng in Shandong province and identified by Prof. Ying-lan Guo of the institute of Microbiology, Academia Sinica, Beijing. Natural *Cordyceps sinensis* was purchased from Tibet Pharmaceutiical

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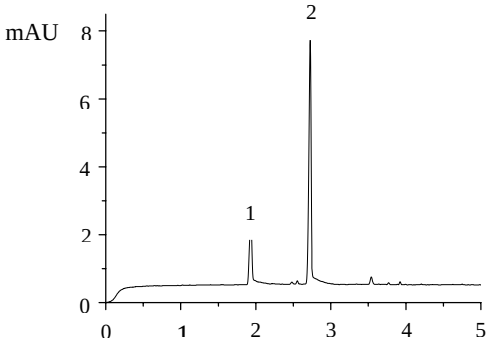
Company, and cultured *Cordyceps kyushuensis* Kob. was obtained from our laboratory. These three species of *Cordyceps* were dried at 60°C, and ground. The dry powder (0.5 g) of the three species of *Cordyceps* was dissolved in 10 mL deionized water and ultrasonicated for 20 min in ice bath. After centrifugation, the supernatant was diluted with deionized water to a specific mark and filtered through 0.45 μm (for CZE) or 0.20 μm (for MTT assay) filters as the sample solution. Cordycepin was first dissolved in deionized water at 0.2 mg/mL as a stock solution and then diluted with buffer to the desired concentration.

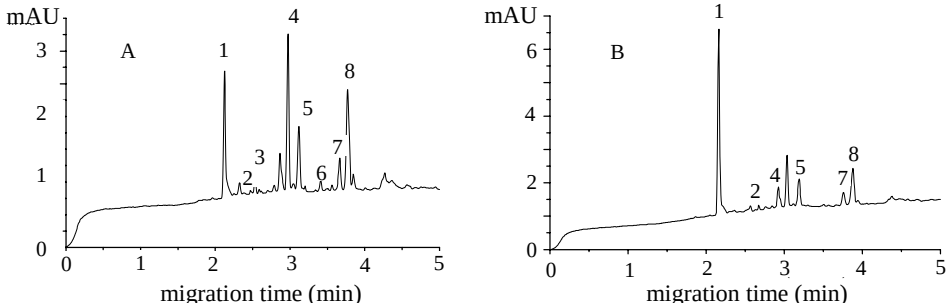
A Beckman Coulter P/ACE MDQ apparatus equipped with a PDA detector and an uncoated fused-silica capillary (45 μm ID ×41 cm, 30 cm effective length) were used for all experiment. The experimental conditions were as follows: the running buffer was borate (adjust to pH 9.4 with sodium hydroxide), applied voltage was 20 kV, operated temperature was 20°C and the detector wavelength was 258 nm. Sample was injected under a 0.5 psi of pressure to simple microvials for 5 sec.

Figure 1 Electropherograms of standard solution



1：Cordycepin; 2：Adenosine

Figure 2 Electropherograms of *C. kyushuensis* Kob. (A) Natural, (B) Cultured



1： Cordycepin 2： Thymine 3： Deoxyuridine 4： Uracil
5： Adenosine 6： Hypoxanthine 7：Guanosine 8： Uridine

Table 1 Cordycepin and adenosine contents in the tested four kinds of *Cordyceps* (mg/g on dried basis)

Simple	Cordycepin	Adenosine
Natural *C. kyushuensis*	1.017	0.531
Cultured *C. Kyushuensis*	2.572	0.446
Natural *C. sinensis*	0.076	0.028

Standard solution (**Figure 1**), containing cordycepin 40 μg/mL and adenosine 60 μg/mL and the extracts of three different *Cordyceps* were monitored and quantitatively analyzed by using the calibrated capillary electrophoresis. Peaks were identified by comparing the migration time of the unknown peaks with those of the standard eluted with the same conditions and by spiking *Cordyceps* sample with stock standard solution of cordycepin. The results show as **Figure 2** and **Table 1**.

In consideration of the high cordycepin content in both natural and cultured *Cordyceps kyushuensis*, we conducted an anticancer experiment *in vitro* of the ultrasonic water extracts of natural *Cordyceps kyushuensis*.(WNC) and cultured *Cordyceps kyushuensis*.(WCC) on the human leukemia cells HL-60, U937, human hapatocarcinoma cells BEL-7402 and human pulmonary carcinoma cells PG, respectively. The cells were treated with various concentrations of the extracts of *Cordyceps kyushuensis* for 48 hours. The cytotoxic effect of the natural and cultured *Cordyceps kyushuensis* on cells was detected by a colorimetric MTT assay. ADM (adriamycin, 12.5 μg/mL) was used as positive control, the growth inhibition rates and IC_{50} values of *Cordyceps kyushuensis* against U937, HL-60, BEL-7402 and PG cell lines were concluded as **Table 2** and **Table 3**.

Table 2 The cytotoxicity of the water extracts of natural and cultured *Cordyceps kyushuensis* on *in vitro* growth of the four cancer cell lines

Concentration mg/mL（crude materials）		Growth inhibition（%）						
		U937		BEL-7402		HL-60		PG
WNC	WCC	WNC	WCC	WNC	WCC	WNC	WNC	WNC
				56.83±2.42				
12.5	12.5	91.41±2.52	71.01±1.34	46.71±1.59	85.89±1.99	29.15±3.32	95.53±2.06	8.83±2.41
6.25	6.25	56.10±1.47	87.30±3.16	31.06±1.53	43.81±0.86	22.54±2.63	59.68±0.77	5.06±1.98
3.125	3.125	54.08±2.14	78.50±0.87	26.71±2.30	25.91±1.51	23.32±1.98	42.24±2.48	0
1.56	1.56	58.60±0.87	71.01±1.55	31.88±2.66	20.26±2.24	28.02±3.65	37.56±1.57	0
0.78	0.78	70.46±3.04	70.93±2.21	25.51±0.93	24.56±3.03	15.23±1.44	22.15±2.63	0
0.39	0.39	75.56±0.85	62.61±1.83	18.86±1.44	6.08±0.52	23.67±1.29	24.29±1.32	0
0.195	0.195	68.45±0.99	59.92±0.76	6.72±0.67	0	26.11±1.87	29.69±1.85	0
0.098	0.098	50.46±1.23	37.34±0.94	0	0	24.46±0.85	18.99±1.63	0
0.049	0.049	58.26±1.72	28.43±2.83	0	0	24.98±0.64	15.15±0.54	0
0.024	0.024	44.37±2.70	17.25±1.73	0	0	23.85±1.93	17.05±2.07	0
0.012	0.012	21.05±1.16	14.05±2.82		0	19.76±2.52	13.21±0.91	0
ADM (12.5 μg/mL)		100	100	100	100	100	100	100
IC_{50}		0.034	0.152	8.280	7.170	4.520		

Table 3 The cytotoxicity of cordycepin on growth of the four cancer cell lines *in vitro*

Cell lines / Concentration(μg/mL)	Growth inhibition（%）			
	U937	BEL-7402	HL-60	PG
250	64.40±1.40	30.47±2.21	55.13±1.27	0
125	56.19±0.83	28.49±1.09	51.38±1.15	0
62.5	63.05±0.57	29.72±1.78	61.90±0.48	0
31.25	61.13±1.32	34.01±1.25	67.34±1.63	0
15.63	59.70±1.57	30.17±1.28	55.12±2.31	0
7.80	49.09±1.96	16.75±0.66	54.45±1.99	0
3.90	54.05±0.54	22.12±1.74	53.28±2.57	0
1.95	49.50±0.85	20.31±0.42	46.64±1.41	0
0.98	38.30±1.75	26.02±2.05	40.45±0.63	0
0.49	19.68±1.22	22.70±1.43	35.72±0.87	0
0.24	11.87±0.63	19.91±2.03	22.70±1.76	0
ADM (12.5μg/mL)	100	100	100	100
IC_{50}	2.160		2.940	

Conclusion

The CZE method is faster, simpler and less expensive than HPLC method, and is therefore suitable for routine assay of cordycepin in *Cordyceps sp*. With a high cordycepin content, *Cordyceps kyushuensis* demonstrated a considerable antitumor activity against some cancer cell lines *in vitro*.

References

1. K. G. Cuningham, W. Manson, E. S. Spring, S. A. Hutchison, *Nature*, **1950**, *166*, 9.
2. F. G. Plagemann, J. Erbe, *Arch. Biochem. Biophy*, **1971**, *144*, 401.
3. P. A. Mathew, L. K. Ells, G. P. Studzinski, *J. Cell Physiol*. **1989**, *140*, 212.
4. G. W. Zieve, E. J. Roemer, *Exp. Cell Res*. **1988**,*177*,18.
5. E. N. Kodama, R. P. McCaffrey, K. Yusa, H. Mitsuya, *Biochem. Pharmac*. **2000**,*59*,273.
6. H. R. Hubbell, D. H. Pequignot, D. H. Willis, C. Lee, R. J. Suhadolink, *Int. J. Cancer*. **1985**, *6*, 389.
7. R. I. Glazer, J. F. Kuo, *Biochem. Pharmac*. **1977**, *26*, 1287.
8. W. E. G. Muller, B. E. Weiler, R. Charubala, L. Plferiderer, *et al*. *Biochem*. **1990**, *30*, 2027.
9. A. M. Sugar, R. P. McCaffrey, Antimicrob. *Agents Chemother*., **1998**, *42*,1424.
10. Y. J. Ahn, S. J. Park, S. G. Lee, *et al*., *J. Agric. Food Chem*., **2000**, *48*, 2744.
11. Y. Sun, N. Bu, JH Liu, *Edible Fungi of China* (in Chinese). **2000**, *18* (6),19.
12. J. M. Liu, D. Liu, L. X. Yang, Y. R. Zhong, S. L. Chui, *J. Chin. Mat. Medica* (in Chinese), **1994**, *19*, 615.

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