Supporting information

Comparison of cordycepin and isocordycepin in ambient temperature water extraction and boiling water extraction

Chemicals. Cordycepin (> 98%) was purchased from Winherb (Shanghai, China). Isocordycepin (99.87%) and 2′-deoxyadenosine 5′-di-phosphate sodium salt (dADP, 2Na, 98.24%) was purchased from Aladdin (Shanghai, China). 2′-deoxyadenosine-5′-monophosphate (dAMP, 99.6%), 2′-deoxyinosine (99.5%) and 2′-Deoxyadenosine-5′-triphosphate disodium salt（dATP, 2Na, 98%） were purchased from Shanghai yuanye Bio-Technology Co., Ltd (Shanghai, China).

Preparation of standard solution. Reference solution, including cordycepin (0.2036 μg/mL), isocordycepin (0.1996 μg/mL), dAMP (4.076 μg/mL), dADP (98.42 μg/mL), dATP (40.52μg/mL) and 2′-deoxyinosine (41.12 μg/mL), was prepared by deionized water.

Preparation of sample solutions. *Ambient temperature water extraction (ATWE):* The sample extraction was carried out according to the method described in a literature with minor changes [12]. The sample powder (0.1g) was accurately weighed into a conical flask equipped with a stopper. 25 mL water was added into it and then equilibrated for 1 h. After that, the ultrasonic extraction (33 kHz, 250 W) was carried out for 30 min. The sample solution was filtered by 0.22 μm membrane before LC-

MS/MS analysis.

Boiling water extraction (BWE): The sample extraction was carried out according to the method described in a literature with minor changes [19]. The sample powder (0.1g) was accurately weighed into an conical flask equipped with a stopper and 10.0 mL boiling water (95 ℃–97 ℃) was subsequently added. The flask was weighed. Then ultrasonic extraction (33 kHz, 250 W) at 75 ℃ was carried out for 30 min. After cooling, the solution was adjusted to the original weight with water and filtered by 0.22 μm membrane.

LC-MS/MS condition. The LC separation was performed on an Agilent poroshell 120 SB-Aq C18 column (3.0x150 mm, 2.7 μm). 10 mM ammonium acetate aqueous solution (A) and methanol (B) were used as the mobile phases. The gradient elution was performed as follows: 0–10 min, 0–2% B; 10–15 min, 2–10% B; 15–50 min, 10–20% B. The flow rate was 0.3 mL min^{-1} and the column temperature was 30 ℃. The injection volume was 1 μL. The MRM transitions (precursor ion → product ion), fragmentor voltages, and collision energy (CE) values selected for each compound were given in Table S1.

Table S1 MRM transitions, fragmentor voltages, and CE values for monitored compounds

Name	MRM transitions (m/z)	Dwell time (ms)	Fragmentor voltages (V)	CE (V)
cordycepin	252 > 136	100	70	20
isocordycepin	252 > 236	100	70	20
2′-deoxyinosine	253 > 137	100	140	20
dAMP	332 > 136	100	110	30
dADP	412 > 136	100	110	30
dATP	492 > 136	100	110	30

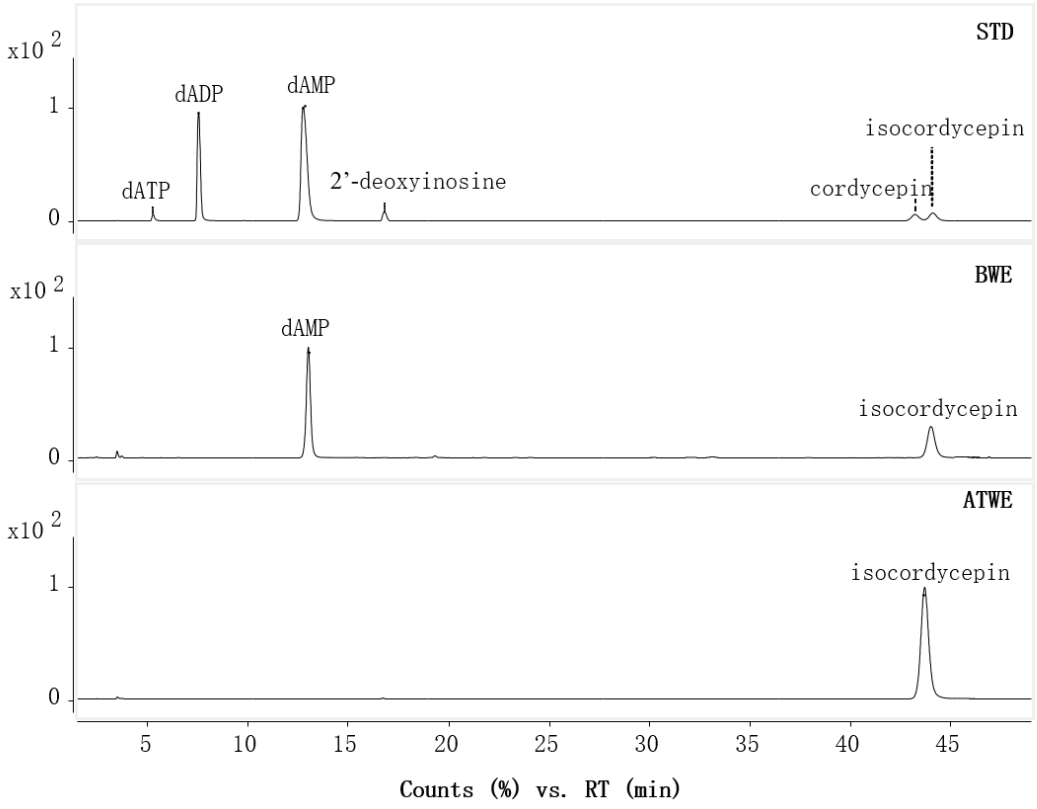

Figure S1. Chromatograms of standard solution (STD), *Cordyceps sinensis* BWE solution and *Cordyceps sinensis* ATWE solution