AMB Express

ORIGINAL ARTICLE

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Metabolites and novel compounds with anti-microbial or antiaging activities from *Cordyceps fumosorosea*

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Abstract

High-resolution mass spectrometry (HRMS) and nuclear magnetic resonance (NMR) analysis revealed that there are 20 main components in spores and mycelia extract of *Cordyceps fumosorosea* strain RCEF 6672 including mannitol (**1**), uridine (**2**), adenine (**3**). N^6-(2-hydroxyethyl)-adenosine (**4**). N^6-(2-hydroxyethylacetate)-adenosine (**5**), fumosoroseanoside A (**6**) and B (**7**), ovalicin-4α-alcohol (**8**), 1-linoleoyl-sn-glycero-3-phosphocholine (**9**) and its isomer (**10**), fumosoroseain A (**11**) and its isomer (**12**), 5 non-ribosomal peptides (**13** to **17**) and 3 fatty acids (**18** to **20**). The compounds **5**, **6**, **7**, **9** and **11** were prepared with preparative and semi-preparative HPLC and identified with 1D and 2D NMR. Compounds **4** and **5** were the first time identified from *C. fumosorosea*. Compounds **6**, **7** and **11** are novel compounds. Compounds **6** and **7** showed antibacterial and antifungal activities, and **11** showed antiaging activity. All the secondary metabolites (**4** to **8** and **11** to **17**) have strong bioactivities indicating that the metabolites have pharmaceutical development potentiality.

Keypoints

Clarified small molecular metabolites of *C. fumosorosea* for the first time.

Identified three novel compounds with antimicrobial or antiaging activities.

The fungus has development potentiality for rich in bioactive metabolites.

Keywords: *Cordyceps fumosorosea*, Fumosoroseanosides, Fumosoroseain, Antibacterial, Antifungal, Antiaging

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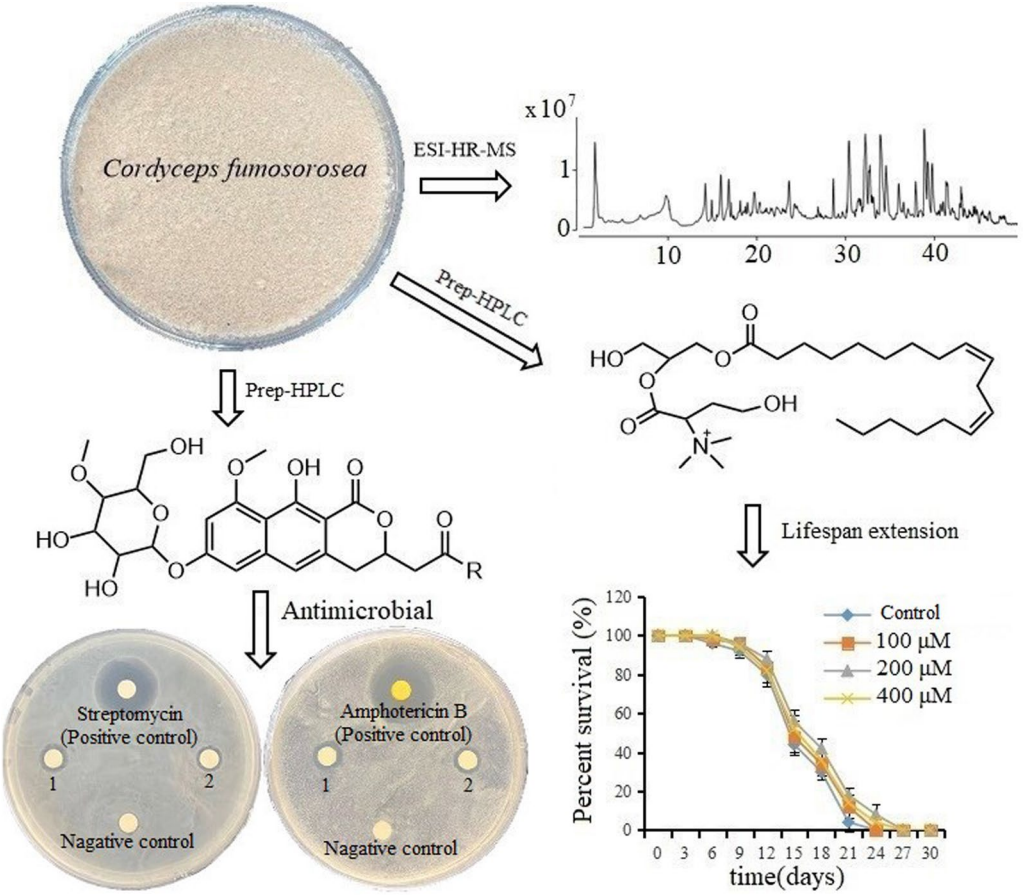
Graphical Abstract



Introduction

Cordyceps are highly valued traditional medicines or healthy foods in Asian countries for a long history (Xiao et al. 2009; Liu et al. 2015, 2020; Lou et al. 2020). Some fungi of *Cordyceps* are also widely used in agriculture for pest biocontrol (Zhang et al. 2019; Kumar et al. 2021). Because long term coevolution with insects, the fungi of *Cordyceps* can synthesize a variety of bioactive metabolites such as cordycepin, militarinone, myriocin, cyclosporin, destruxins and enniatins etc. (Mularczyk et al. 2020; Hu and Li 2007; Isaka et al. 2000; Schmidt et al. 2002; Bagli et al. 1973; Wartburg and Traber 1988;Meca et al. 2010; Lu et al. 2013; Lu et al. 2014). As a well-known entomopathogenic fungi, *C. fumosorosea* can not only kill many kinds of pests but also synthesize more than 10 bioactive compounds, such as beauvericins, beauverolides, cepharosporolides, trichocaranes and fumosorinone A etc. (Hu and Li 2007; Hugo et al. 1983; Masahiko et al. 2005; Chen et al. 2018; Buchter et al. 2020). However, there is no metabolites reported from spores of the fungus yet. Meanwhile, our primary gene prediction based on the genome sequences (GCA-003025305.1) (Shang et al. 2016) with antiSMASH revealed that there were 40 secondary metabolic gene clusters in *C. fumosorosea*, including 28 non-ribosomal peptides (NRPs), 3 poly-ketone, 3 terpene, 1 siderophore, 5 NRPs or polyketone gene clusters. Our primary HPLC-HRMS analysis showed that *C. fumosorosea* had complicated metabolites including several possible unknown compounds. To make clear the structure and bioactivity of the unknown metabolites will be able to not only reveal the utilization value of the fungus, but also help us to evaluate chemical ecological safety of the fungal pesticide. Therefore, we launched a systematic analysis and identification of the metabolites of *C. fumosorosea*. In this study we will focus on the unknown metabolites. Through HPLC-HRMS guided isolation we can prepare the unknown compounds directly without the disturbance of the known

metabolites. Because that *Cordyceps* do not rot easily in the soil and are usually used as tonic medicine or food, they are presumed to contain antibiotics and antiaging compounds (Ji et al. 2008; Schmidt et al. 2003; Olatunji et al. 2018; He et al. 2018), therefore, the isolated novel compounds of this study will be submitted to antimicrobial and antiaging tests. In this study *Escherichia coli* and *Candida albicans* will be used to test the antimicrobial activities, and *Caenorhabditis elegans* will be used for the antiaging assays. Nematode is widely used to study the biological effects of aging and human diseases because that its genome contains more than 18, 000 genes, of which 60 to 80% are homologous to human genes, meanwhile, it has highly conserved metabolic pathways and easy-to-maintain (Moliner et al. 2018; Calvo et al. 2016; Gruber et al. 2009).

Materials and methods

Chemicals

All solvents used for extraction were of analytical grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). HPLC grade methanol and formic acid were from Tedia Company of China (Shanghai, China). Deuterated NMR solvents were purchased from Cambridge Isotope Laboratory (Andover, MA, USA).

Instrumentation

Preparative HPLC was performed using Agilent modules consisting of an autosampler PS410, two pumps M400, a UV detector 1260VWD and an automatic fraction collector 440FC. Semi-preparative HPLC was performed using Shimadzu modules consisting of an autosampler SIL-20A, two pumps LC-20AD and a PDA detector SPD-M20A. ESI-HR-MS data were obtained using an Agilent 1100 HPLC tandem 6510QTOF MS spectrometer. NMR experiments were recorded using a Bruker Advance 600 MHz spectrometer. Chemical shifts were referenced to the residual $(CD_3)_2SO$ signal (δ_H 2.50, 3.33, δC 40.80) or CD_3OD signal (δ_H 4.87, 3.31, δ_C 49.15) as internal standards for 1D NMR and 2D NMR.

Fungal material

The fungal strain was isolated from *C. fumosorosea* infected lepidopterous larva collected in Anhui Province (China) and grown at 25 °C on PDA plates. The isolated strain was identified by morphology and sequence analysis of the ITS region of the rDNA. It has been catalogued RCEF 6672 and deposited in the Research Center for Entomogenous Fungi (RCEF, WDCM1031), Anhui Agricultural University.

Fermentation, extraction and isolation

After 7 days cultivation on PDA plate, the spores of the fungus were harvested with a 0.05% Tween 80 solution to make a spore suspension (6.8×10^6). 300 μL of the suspension was inoculated on a 150 mm PDA plate, and 2000 of the plates were inoculated for mass cultivation. After 10 days cultivation the spores and surface mycelia were scraped off the plates and freeze dried. The dried spores and mycelia were extracted with methanol and ethyl acetate mixture (1:1) under 40 kHz ultrasonication for three times in 30 min, and the total solid–liquid ratio is 1: 4 (w/v). The extract was evaporated under reduced pressure, and 10.5 g pasty crude extract was obtained. With the assistance of 40 kHz ultrasonication, 8 g of crude extract was redissolved in 10 ml of methanol. The turbid liquid was centrifuged at 10,000 *g* for 10 min, and the supernatant was separated by preparative HPLC with an Agilent Prep-C18 (21.2 mm × 250 mm, 10 μm) column for primary isolation, and semi-preparative HPLC with an Agilent SB-C18 (9.4 mm × 250 mm, 5 μm) column for final purification. The conditions for preparative HPLC are as follows: injection volume 150 μL; flow rate 15 mL/min; elution gradient: 0–15 min, 30% to 100% methanol, 15–20 min, 100% methanol, 20—22 min, 100% to 30%. The effluents were collected every one minute for one tube and tested by HPLC-HRMS. The tube contained unknown compounds was condensed under reduced pressure and submitted to semi-preparative HPLC. The conditions for semi-preparative HPLC are as follows: injection volume 30 μL; flow rate 3 mL/min; elution gradient: 0–1 min, 40% methanol, 1–15 min, 40% to 100% methanol, 15–18 min, 100% methanol, 18–22 min, 100% to 40% methanol, 22–25 min, 40% methanol. The effluents of the unknown compounds were collected according to the m/z of the unknown compounds detected by HRMS through a 10:1 splitter.

HPLC-HRMS analysis

The crude extract and prepared pure compounds were analyzed with an Agilent Poroshell 120 EC-C18 (2.7 μm, 3.0 × 100 mm) column, and the LC parameters were set as follows: injection volume, 5 μL; column temperature, 25 °C; and flow rate, 0.4 mL/min. The mobile phase was composed of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile, and a gradient elution was carried out: 0–40 min, 5–100% B, 40–45 min, 100% B, 45–55 min, 100–5% B. The eluates were monitored with a PDA performing a full wavelength scan from 200 to 600 nm, and a TOF MS with following parameter settings: gas temperature, 350 °C; drying gas, 10 L/min;

nebulizer pressure, 45 psi; capillary voltage, 4000 V in positive mode and 3500 V in negative mode; fragmentor voltage, 215 V in positive mode and 170 V in negative mode; skimmer voltage, 60 V. Data acquisition was performed in the m/z range of 50–1700 Da. The effluents of the preparative HPLC were detected by HPLC-HRMS without chromatographic column.

Metabolite identification

The molecular formulas of the metabolites were calculated by Mass Hunter based on accurate mass and isotopic pattern recognition. Compounds were putatively identified by searching the molecular formulas against the in-house entomopathogenic fungi database and the *Dictionary of Natural Products* (http://dnp.chemnetbase.com/). The known compounds were confirmed by UV/visible spectra whenever possible, and verified by their elution order (polarity) and structure characteristics. Some common metabolites of entomopathogenic fungi such as mannitol, adenosine and beauvericin A were confirmed with standards. Molecular formulas without corresponding compounds in the database were labeled as unknown compounds and submitted to identification with 1D and 2D NMR.

Antimicrobial activity

The antimicrobial activities of the metabolites were evaluated by the disc diffusion assay according to the method reported by Wu et al. (Wu et al. 2018). Streptomycin was used as positive control for antibacterial tests against *E. coli* (ATCC 25922). Amphotericin B was used as positive control for the antifungal bioassays against *C. albicans* strain CMCC98001 (from China General Microbiological Culture Collection Center). All samples were prepared to a final concentration of 1, 2 and 3 mg/mL in 1.5% DMSO, and 10 μL were loaded on the paper discs (6.0 diameters). The width of the transparent antimicrobial zone was measured with a vernier caliper. Each experiment was repeated three times.

Determination of Antiaging activity

Antiaging activity tests included lifespan test, reproduction assay, locomotion assay and thermotolerance assay. *C. elegans* (bought from Caenorhabditis Genetics Center, University of Minnesota) was fed with *E. coli* strain OP50 according to the method reported by Onken and Driscoll (2010). The nematodes were treated with a solution of sodium hypochlorite and sodium hydroxide to make the nematode eggs hatching and growing synchronously according to the reported method (Rathor et al. 2017).

Lifespan was measured according to the method of Liu et al. (2016). The synchronized L4 larvae (3 days old, 50 per plate) were cultivated on NGM plate (containing *E. coli* strain OP50) at 20 °C with different content of compounds (100, 200, 400 μM) or 0.1% DMSO as control. The nematodes were transferred to new plates every two days to ensure adequate food, and the number of dead worms were recoded simultaneously.

Reproduction assay was carried out according to the method reported by Chen et al. (2014). Each plate contained only one L4 larva which was transferred to fresh plate every 24 h until the nematode no longer laid eggs. The number of offspring produced by each nematode in the whole breeding period were calculated and summed to obtain the total number of offspring.

Locomotion assay was performed according to the method of Wilson et al. (2006). 50 *C. elegans* were cultivated and treated with compounds as described in lifespan assays. On the 5th, 8th, 12th and 16th day of adulthood the nematodes were counted and classified into three classes: motion A nematodes moved constantly, motion B nematodes only moved when prodded and motion C nematodes sway only head or tail if prodded.

Thermotolerance assay was performed according to the Wilson's method (Wilson et al. 2006). 50 nematodes were cultivated and treated with compounds as described in lifespan assays. After four days of culture, the nematodes were exposed to 35 °C, and the dead nematodes were monitored every 1 h until all the nematodes died.

Results

Primary analysis of the main metabolites of *C. fumosorosea*

HPLC-HRMS analysis showed that there are 20 main metabolites in the extract from mycelia and spores of *C. fumosorosea* (Fig. 1). Database query according to the high-resolution (HR) mass and UV spectra (Fig. 1) and standards comparison found there were 13 known compounds (Abraham et al. 2015; Daniela et al. 2020; Manrico et al. 1975), and 7 possible unknown compounds, which were not found in the *Dictionary of Natural Products* database (Table 1).

Among the 7 possible unknown compounds the **9** and **10**, **11** and **12** have the same mass and ultraviolet spectrum respectively, indicating that they are two pairs of stereoisomers. Five of the unknown compounds were prepared by HRMS guided isolation with preparative HPLC and semi-preparative HPLC according to the above-mentioned methods. We obtained 11.3 mg compound **5**, 17.6 mg compound **6**, 5.3 mg compound **7**, 9.5 mg compound **9** and 16.9 mg compound **11**. Compound **5** is white powder. Compounds **6** and **7** are light-yellow amorphous powder, and the compound **9** and **11** are colorless oil. The purity and molecular formulas of the prepared compounds was confirmed by HPLC-HRMS (Fig. 1) and submitted to NMR analysis.

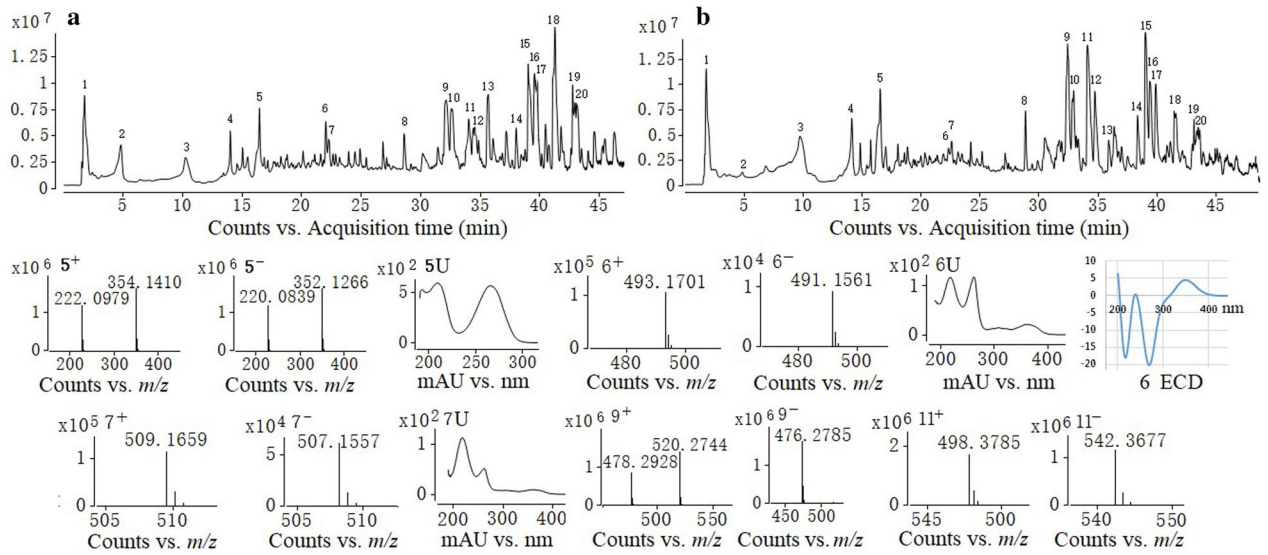


Fig. 1 HPLC-HRMS analysis of the extract form spores and mycelia of *Cordyceps fumosorosea*. **A** is the base peak chromatogram of the crude extract in positive model; **B** is the base peak chromatogram of the crude extract in negative model; number 5, 6, 7, 9 and 11 are the five possible unknown metabolites, and '+', '−', 'U' and 'ECD' mean cation, anion, ultraviolet spectrum and circular dichroism of the metabolites respectively

Structure elucidation of the isolated compounds

Compound **5**: The molecular formula was calculated as $C_{14}H_{19}N_5O_6$ based on its ESI-HRMS (Fig. 1). The characteristic UV absorptions (λ_{max}) are at 209 and 265 nm (Fig. 1). The ^{1}H- and ^{13}C NMR spectra of **5** (Additional file 1: Fig. S1) showed characteristic signals for adenosine, a major constituent of Cordyceps (Hu and Li 2007). There are two methylene signals at δ_H 3.84 (2H, m) and 4.27 (2H, t, J = 5.4 Hz) and δc 39.1 and 62.7, which were very similar to those of N^6-(2-hydroxyethyl) adenosine (Furuya et al. 1983). However, additional signals for an acetyl moiety were observed at δ_H 2.05 (3H, s), δ_C 19.4 and 171.5 in the HMBC spectrum, which suggested **5** as an acetylated form of N^6 -(2-hydroxyethyl) adenosine. The position of an acetyl moiety was determined from the HMBC correlation between δ_C 171.5 and δ_H 4.27 (H-2′). Taken together, the structure of **5** was determined as N^6-(2-hydroxyethylacetate) adenosine (Fig. 2). The NMR signals assignment was list in Table 2. Literature inquiry showed that N^6-(2-hydroxyethylacetate) adenosine is the known compound cordyrrole B which can significantly inhibit adipocyte differentiation and pancreatic lipase (Kim et al. 2014).

Compound **6**: The molecular formula was calculated as $C_{24}H_{28}O_{11}$ based on its ESI-HRMS (Fig. 1). The characteristic UV absorptions (λ_{max}) are at 260, 310, and 360 nm (Fig. 1). ^{13}C and DEPT135 (Additional file 1: Fig. S2) showed that compound **6** has 24 carbon atoms including 9 quaternary carbons, 9 tertiary carbons, 3 secondary carbons and 3 primary carbons which connected 24 hydrogen atoms. The other 4 hydrogens in the molecule are probably active hydrogens. The chemical shifts of ^{1}H and ^{13}C and coupling of C–H obtained by 1D and 2D NMR (Fig. 2; Additional file 1: Fig S1) spectra are shown in Table 3. The carbons at δ101.2, δ79.4, δ76.5, δ76.2, δ74.0, δ60.6 and δ60.0, and coupling relationships between the hydrogens connected to the carbons (Fig. 2) according well with a 4′-methoxyl-glucose moiety which existed commonly in metabolites of entomopathogenic fungus (Hu et al. 2002, 2009). The chemical shifts and coupling relationships between aromatic carbons and hydrogens (Fig. 2) showed that the aglycone is a derivative of 1H-naphtho[2,3-c] pyran-1-ones which is consistent with the report (Ayer et al. 1991). The chemical shift at 205.5 indicated the existence of ketone. The long-range coupling between the hydrogens of the two methoxy groups and carbons at δ 79.4 and 161.6 (Fig. 2) confirmed that the methoxy groups are at the position of Glu-C4' and the position 9 respectively. The terminal hydrogen at δ 4.99 has a double peak on the ^{1}H spectrum with 7.2 Hz coupling constant, indicating that the glucosyl group is β configuration. The ECD spectrum (Fig. 1) showed the

Table 1 Main metabolites of extract form spores and mycelia of *Cordyceps fumosorosea*

Retention time (min) (Number)	Mass (m/z) and erro (mDa)	Uvλmax (nm)	Molecular formula	Name
1.66 (1)	$[M-H]^-$181.0729, 1.1	200	$C_6H_{14}O_6$	Mannitol[a]
4.83 (2)	$[M-H]^-$ 243.0625, 0.2; $[2\ M-H]^-$ 487.1321, 0.3 $[M+H]^+$ 245.0763, 0.5; $[M\text{-}C_5H_7O_4]^+$113.0349, 0.3	206, 261	$C_9H_{12}N_2O_6$	Uridine[a]
9.74 (3)	$[M\text{-}H]^-$ 266.0899, 0.4; $[M\text{-}C_5H_9O_4]^-$ 134.0476, 0.4 $[M+H]^+$ 268.1045, 0.5; $[M\text{-}C_5H_7O_4]^+$136.0822, 0.6	205, 258	$C_{10}H_{13}N_5O_4$	Adenine[a]
14.13 (4)	$[M-H]^-$ 310.1155, 0.2; $[M\text{-}C_5H_9O_4]^-$ 178.0731, 0.3 $[M+H]^+$ 312.1305, 0.3; $[M\text{-}C_5H_7O_4]^+$180.0876, 0.4	209, 265	$C_{12}H_{17}N_5O_5$	N^6-(2-Hydroxyethyl)-Adenosine[a]
16.41 (5)	$[M-H]^-$ 352.1266, 0.3; $[M\text{-}C_5H_9O_4]^-$ 220.0839, 0.1 $[M+H]^+$ 354.1410, 0.2; $[M\text{-}C_5H_7O_4]^+$ 222.0979, 0.7	209, 265	$C_{14}H_{19}N_5O_6$	Unknown
22.24 (6)	$[M-H]^-$ 491.1565, 0.6; $[M+H]^+$ 493.1699, 0.5	262, 361	$C_{24}H_{28}O_{11}$	Unknown
22.43 (7)	$[M-H]^-$ 507.1513, 0.5; $[M+H]^+$ 509.1658, 0.4	262, 361	$C_{24}H_{28}O_{12}$	Unknown
28.85 (8)	$[M-H]^-$ 297.1718, 1.1; $[M+H]^+$ 299.1859, 0.6; $[M+Na]^+$ 321.1680, 0.8	200	$C_{16}H_{26}O_5$	Ovalicin-4α-alcohol
32.41 (9) 32.88 (10)	$[M-H]^-$ 476.2785, 0.2; $[M+H]^+$ 478.2928, 0; $[M+Na]^+$ 500.2744, 0.4	200	$C_{23}H_{44}NO_7P$	Unknown
34.28 (11) 34.83 (12)	$[M+COOH]^-$ 542.3695, 0.3; $[M+H]^+$ 498.3791, 0.2; $[M\text{-}C_{18}H_{29}O]^+$ 236.1495, 0.3	200	$C_{28}H_{51}NO_6$	Unknown
35.94 (13)	$[M-H]^-$ 486.2863, 0.5; $[M+H]^+$ 488.3010, 0.7	215	$C_{27}H_{41}N_3O_5$	Beauveriolide I[b]
38.36 (14)	$[M-H]^-$ 734.4031, 0.9; $[M+H]^+$ 736.4173, 0.5; $[M+NH_4]^+$ 753.4438, 0.5; $[M+Na]^+$ 758.3985, 0.2	210	$C_{41}H_{57}N_3O_9$	Beauveriolide E
39.06 (15)	$[M\text{-}H]^-$ 782.4039, 0.7; $[M+H]^+$ 784.4171, 0.3; $[M+NH_4]^+$ 801.4439, 0.6; $[M+Na]^+$ 806.3991, 0.4	255	$C_{45}H_{57}N_3O_9$	Beauvericin[b]
39.45 (16)	$[M\text{-}H]^-$ 804.3469, 0.8; $[M+H]^+$ 806.3612, 0.5	225, 280	$C_{41}H_{51}N_5O_{12}$	Conoideocrellide D
39.92 (17)	$[M-H]^-$ 796.4185, 0.6; $[M+H]^+$ 798.4331, 0.7; $[M+NH_4]^+$ 815.465, 0.6; $[M+Na]^+$ 820.4138, 0.8	260	$C_{46}H_{59}N_3O_9$	Beauvericin A[a]
41.69 (18)	$[M-H]^-$ 279.2332, 0.2; $[M+H]^+$ 281.2469, 0.6	200	$C_{18}H_{32}O_2$	Linoleic acid
43.21 (19)	$[M-H]^-$ 255.2334, 0.4; $[M+H]^+$ 257.2473, 0.2	200	$C_{16}H_{32}O_2$	Palmitic acid
43.54 (20)	$[M-H]^-$ 281.2481, 0.5; $[M+H]^+$ 283.2629, 0.3	200	$C_{18}H_{34}O_2$	Oleic acid

[a] Confirmed with standards

[b] Have been reported from *Cordyceps fumosorosea*, *Isaria fumosorosea* or *Paecilomyces fumosoroseus* [33–35]

compound is a R configuration. Based on above analysis the structure of the new compound (**6**) was depicted in Fig. 2 and named as fumosoroseanoside A.

Compound **7**: The molecular formula was assigned as $C_{24}H_{28}O_{12}$ based on its ESI-HRMS (Fig. 1). Compound **7** has just one more oxygen atom than compound **6**, and they have the same UV absorptions (Additional file 1: Fig. S2). Obviously, compound **7** is possibly an oxo-derivate of fumosoroseanoside A, and the extra oxygen atom is not in the conjugated system of the molecule. The ^1H and ^{13}C NMR spectra (Additional file 1: Fig. S2) of compound **7** are shown in Table 3. By comparing the ^1H and ^{13}C NMR between compound **7** and compound **6**, the methyl signals of compound 7 (δ_H 2.14, δ_C 30.6) were disappeared, while one more methoxy group (δ_H 3.63, δ_C 52.0) appeared. Meanwhile, the keto carbon at δ_C 205.5 shifted to a higher field δ_C 170.5. Obviously, the methyl group in compound **6** which combined to the carbonyl carbon of the ketone group was oxidized and formed a methyl ester group. Based on above analysis the structure of the new compound (**7**) was shown in Fig. 2 and named as fumosoroseanoside B.

Compound **9**: The molecular formula was calculated as $C_{23}H_{44}NO_7P$ based on its ESI-HRMS (Fig. 1). ^{31}P-NMR confirmed the existence of phosphorus atom. ^{13}C and DEPT (Additional file 1: Fig. S4) showed a typical linoleic acid group with a carbonyl carbon (δ_C 173.7), 4 unsaturated carbons (δ_C 129.07, 129.13, 130. 90, 130.97), 12 methylene carbon (δ_C 23 to 34) and a methyl carbon (δ_C 14.4). The chemical shifts of the other 5 carbons including 4 methylenes (δ_C 63.2, 66.2, 67.8, 41.8) and 1 methine carbons (δ_C 69.9) suggested the existence of a glycerol and an ethanolamine group. Carbons δ_C 69.9, 67.8, 63.2 and 41.8 were split with J = 7.5, 5.7, 6.6 and 5.2 Hz respectively suggesting that they had two or three bonds coupling with the phosphorus atom of phosphate group. The coupling constants between the phosphorus atom and the second carbon (J = 7.5) and the third carbon

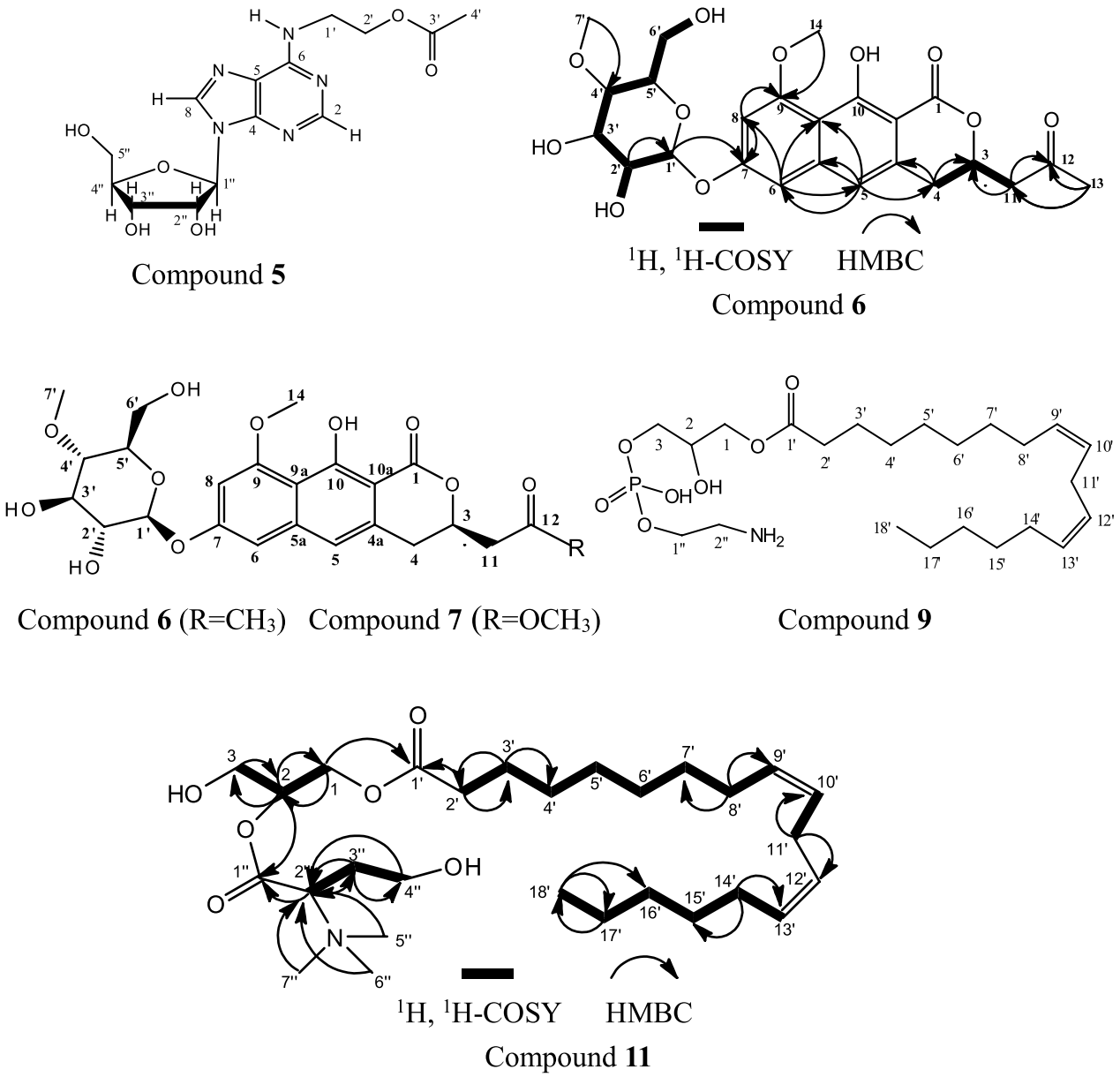


Fig. 2 Structures of compounds **5**, **6**, **7**, **9** and **11**, and HMBC and ^{1}H-^{1}H COSY correlations of the compounds **6**, **7** and **11**

(J = 5.7) of the glycerol moiety suggested that the esterification position is the third carbon of the glycerol group. Therefore, compound 9 is 1-linoleoyl-sn-glycero-3-phosphocholine (Fig. 2). The structure was confirmed with ^{1}H, COSY, HMQC and HMBC (Additional file 1: Fig. S4) and the signals assignment was list in Table 4. Despite that this compound has been reported several times (He et al. 2018; Luo et al. 2013) it is the first time identified with one- and two-dimension NMR from microorganism.

Compound **11**: The molecular formula was assigned as $C_{28}H_{52}NO_6$ based on its ESI-HRMS (Fig. 1). The UV absorptions (λ_{max}) are at 231 nm (Fig. 1). ^{13}C, DEPT90 and DEPT135 (Additional file 1: Fig. S5) showed a typical linoleic acid group with a carbonyl carbon (δ_C 174.1), 4 unsaturated carbons (δ_C 129.96, 129.87, 128.13, 128.06), 12 methylene carbon (δ_C 25 to 34) and a methyl carbon (δ_C 13.48). The three methyl groups with the same chemical shift values (δ_C 51.41, δ_H 3.20) are consistent

Table 2 1D and 2D NMR spectral data (δ in ppm) of compound **5**

Position	^{13}C (δ)	DEPT, HMQC	δ_H, mult (J in Hz)	$^{1}H,^{1}H$-COSY (δ)	HMBC (δ)
2	152.1	CH	8.19, br		
4	148.0	C			
5	120.2	C			
6	155.0	C			
8	140.3	CH	8.23, s		
1′	39.1	CH2	3.84, m	4.27	
2′	62.7	CH2	4.27, t (5.4)		
3′	171.5	C			
4′	19.4	CH3	2.00, s		171.5
1″	89.9	CH	5.93, d (6.4)	4.71	74.1, 140.3, 148.0
2″	74.1	CH	4.71, dd (6.4, 5.1)	4.30, 5.93	89.9
3″	71.3	CH	4.30, dd (5.1, 2.7)	4.71, 4.14	62.2, 89.9
4″	86.9	CH	4.14, q (2.6)	3.71, 3.85, 4.30	71.3
5″	62.2	CH2	3.71, dd (12.5, 2.7); 3.85 dd (12.5, 2.7)	3.85, 4.14; 3.71, 4.14	71.3, 86.9

with the characteristics of trimethylamine. Long-range coupling between the hydrogen (δ_H 3.75) of the trimethylamine connected methine and the carbonyl carbon (δ_C 170.75) and an ethanol group (Fig. 2) suggested the existence of a 2-trimethylamino-4-hydroxybutyrate moieties. Long-range coupling between the glycerol hydrogen and δ_C 174.10, δ_C 170.75 revealed that the linoleic acid and 2-trimethylamino-4-hydroxybutyrate moieties were connected to C_1 and C_2 of the glycerol moiety, respectively. Therefore, compound **11** is 1-linoleoyl-sn-glycero-2-(2-trimethylamino-4-hydroxybutyric ester). Its structure and hydrocarbon attributes are shown in Fig. 2 and Table 5. This compound was found to be a new compound by database and literatures enquiry and named as fumosoroseain A.

Bioactivities determination

Bioactivities of N^6-(2-hydroxyethylacetate) adenosine and linoleoyl-glycero-phosphocholine have been reported already (Kim et al. 2014; Harrison et al. 2020; Papandreou et al. 2021; Azarcoya-Barrera et al. 2021), therefore we just submitted the 3 novel compounds for bioactivity studies. Because *Cordyceps* are presumed to contain antibiotics and antiaging compounds (Ji et al. 2008; Schmidt et al. 2003; Olatunji et al. 2018; He et al. 2018), the isolated novel compounds were submitted to antimicrobial and antiaging tests.

Antimicrobial activity

Compound **6** and **7** showed significant ($P < 0.05$) antibacterial and antifungal activities compared with the negative control (the solvent) at the experimental doses from 10 to 30 μg per paper disk (Fig. 3). Compound **6** had slightly stronger inhibition ability on microbial than compound **7**, while compound **11** did not show detectable inhibitory effect against bacteria or fungi at the experimental doses.

Antiaging activity

Because compound **6** and **7** can inhibit *E. coli* and make nematodes starve to death, only compound **11** was submitted to nematodes antiaging model.

Lifespan was the main antiaging indicator of *C. elegans*. As shown in Fig. 4, compound **11** could extend the lifespan of the nematodes at the concentration from 100 μM to 400 μM, and at 200 μM the compound has the most significant activity ($P < 0.05$), and the average lifespan was extended by 11.3% at 200 μM (Fig. 4a). Results of the reproduction tests (Fig. 4b) showed that the number of eggs per day of 100 μg and 200 μg treatment group were 368 ± 35 and 450.61 ± 52.21, which is significantly higher than that of the control group ($P < 0.05$). But the 400 μM treatment group had no significant effect on promoting reproduction ($P > 0.05$). Results of the locomotion tests (Fig. 4c) showed that with the aging of the nematodes,

Table 3 1D and 2D NMR spectral data (δ in ppm) of compound **6**, and ^{1}H and ^{13}C NMR spectral data of compound **7**

Compound 6						Compound 7	
Position	^{13}C (δ)	DEPT135, HMQC	δ_H, mult (J in Hz)	$^{1}H,^{1}H$-COSY (δ)	HMBC (δ)	^{13}C (δ)	δ_H, mult (J in Hz)
1	170.4	C				170.5	
3	75.3	CH	4.97, m	2.96, 3.04; 2.91, 2.98		75.1	4.86, m
4	32.6	CH_2	2.96, m; 3.04, m	2.96, 3.04, 4.97	134.7	33.1	3.00, m; 3.03, m
4a	134.7	C				134.6	
5	115.6	CH	6.97, s		100.9, 110.3, 32.6, 140.9	115.5	6.88, s
5a	140.9	C				140.9	
6	100.9	CH	6.84, d (2.3)	6.74	110.3, 115.6, 102.0, 161.6	100.9	6.82, d (2.3)
7	158.1	C				158.1	
8	102	CH	6.74, d(2.2)	6.84	110.3, 161.6, 158.1, 101.2	102	6.72, d (2.2)
9	161.6	C				161.6	
9a	110.3	C				110.3	
10	163.4	C				163.4	
10a	101.3	C				101.3	
11	47.7	CH_2	2.91, dd(17.1, 5.2); 2.98, dd (17.2, 7.3)	2.91, 2.98, 4.97	205.5, 75.3	40.6	2.79, dd (16.1, 8.1), 2.88, dd (16.1, 4.3)
12	205.5	C				170.4	
13(R)	30.6	CH_3	2.14, s		205.5, 47.7	52.2	3.63, s
14	56.0	CH_3	3.83, s			56.1	3.80, s
1′	101.2	CH	4.99, d (7.2)	3.38	158.1	101.2	4.99, d (7.2)
2′	74	CH	3.38, m	4.99, 5.23, 3.42	101.2	74	3.38, m
3′	76.2	CH	3.42, m	3.02, 3.38		76.2	3.42, m
4′	79.4	CH	3.02, m	3.41, 3.42	76.2, 76.5	79.4	3.02, m
5′	76.5	CH	3.41, m	3.02		76.5	3.41, m
6′	60.6	CH_2	3.48, m; 3.62, m	3.48, 3.62, 3.42, 4.71		60.6	3.48, m; 3.62, m
7′	60.0	CH_3	3.43, s		79.4	60.0	3.43, s
		OH	3.3				
		OH	4.71	3.48, 3.62			
		OH	5.23	3.38			
		OH	8.35				

the constantly moved nematodes (Motion A) gradually decreased, but the addition of compound **11** could significantly delay the decline of the nematode's motion ability ($P < 0.05$). In the heat shock experiment (Fig. 4d), the survival rate of nematodes treated with compound **11** was increased, especially after 5 h treatment, the survival rate of the treated group significantly higher than that of the control group ($P < 0.05$).

Results of above tests showed that compound **11** can significantly prolong lifespan, increase fertility and enhance stress tolerance. This indicated that compound **11** had significant antiaging activity at the tested concentrations. Compound **11** (fumosoroseain A) is a kind of lecithin derivative which usually possess many bioactivities including anti-aging bioactivity at a suitable concentration (Papandreou et al. 2021; Azarcoya-Barrera et al. 2021; Derbyshire and Obeid 2020; Zhang et al. 2021), but how the compound 11 plays anti-aging bioactivity needs to be further studied.

Discussions

Above analysis showed that there are 20 main small molecular metabolites in mycelia and spores extract of *C. fumosorosea* including 8 primary metabolites and 12 secondary metabolites. The primary metabolites included 1 nucleobase (**2**), 1 nucleoside (**3**), 3 fatty acids (**18** to **20**) and 2 phospholipids (**9** and **10**). Among them adenosine (**3**) is one of the main healthy constituents of Cordyceps

Table 4 1D and 2D NMR spectral data (δ in ppm) of compound **9**

Position	^{13}C (δ)	DEPT, HMQC	δ_H, mult (J in Hz)	COSY (δ)	HMBC (δ)
1	66.23	CH_2	4.13, dd (11.3, 6.1); 4.20, dd (11.3, 4.6)	4.20; 4.00 4.13; 4.00	175.41, 69.9
2	69.90 (d, J = 7.5)	CH	4.00, quint (5.3)	3.92	67.80, 66.23
3	67.80 (d, J = 5.7)	CH_2	3.92, m	4.0	68.54
1′	175.41	C			
2′	34.93	CH_2	2.38, t (7.5)	1.65	26.0
3′	26.0	CH_2	1.65, m	2.38; 1.36	34.93
4′	30.23	CH_2	1.34–1.37	1.65	26.0–30.48
5′	30.24	CH_2			
6′	30.32	CH_2			
7′	30.48	CH_2			
8′	28.17	CH_2	2.09, dd (13.17, 6.37)	1.39; 5.39	130.87
9′	130.87	CH	5.39, m	2.09	28.17
10′	129.13	CH	5.33, m	2.80	26.56
11′	26.56	CH_2	2.80, t (6.3)	5.33	129.07, 129.13
12′	129.07	CH	5.33, m	2.76	25.56
13′	130.96	CH	5.39, m	2.09	27.17
14′	28.17	CH_2	2.09, dd (13.17, 6.37)	1.39; 5.39	130.96
15′	30.74	CH_2	1.34–1.37		
16′	32.64	CH_2			
17′	23.60	CH_2	1.33, m	0.93	14.45
18′	14.45	CH_3	0.93, t (6.7)	1.33	23.60
1″	63.23 (d, J = 5.2)	CH_2	4.08, dt (7.4, 5.0)	3.17	41.78
2″	41.78 (d, J = 6.6)	CH_2	3.17, t (5.0)	4.08	63.23

(Hu and Li 2007; He et al. 2018; Luo et al. 2013, and phospholipids (**9** and **10**) are nutrients with many bioactivities (Azarcoya-Barrera et al. 2021; Derbyshire and Obeid 2020; Zhang et al. 2021). Mannitol (**1**) is a common secondary metabolite of Cordyceps with heathy functions, and related to fungal sporulation and environmental adaption (Hu and Li 2007; He et al. 2018; Velez et al. 2007). Among the other 11 secondary metabolites there are 5 NRPs (**13** to **17**), 2 glycosides (**6** and **7**), 2 alkaloids (**11** and **12**), 2 nucleosides (**4** and **5**) and 1 terpene derivate (**8**).

NRPs (compound **13** to **17**) are existed in most cordyceps. Among them the beauveriolides (**13** and **14**) have anti-aging, beta-amyloid lowering and anti-atherogenic activities, while beauvericins (**15** and **17**) have insecticidal, induction of cell apoptosis and ionophoric property, but they have also cytotoxicity (Chen et al. 2020). N^6-(2-Hydroxyethyl)-adenosine (**4**) was

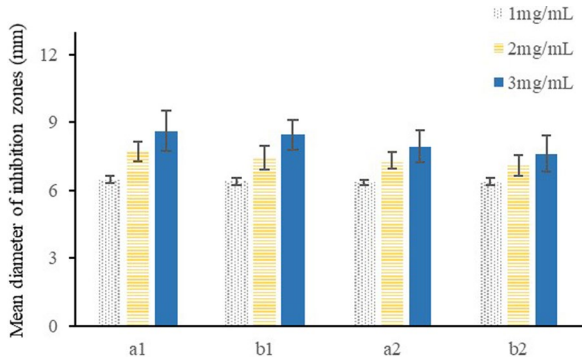


Fig. 3 Antibacterial and antifungal activities of compounds **6** and **7**. The mean diameter of inhibition zones resulting from the antimicrobial activities of compounds 6 and 7. **a1** Results of compound 6 against *C. albicans*. **b1** Results of compound 6 against *E. coli*. **a2** Results of compound 7 against *C. albicans*. **b2** Results of compound 7 against *E. coli*

Table 5 1D and 2D NMR spectral data (δ in ppm) of compound **11**

Position	^{13}C (δ)	DEPT, HMQC	δ_H, mult (J in Hz)	COSY (δ)	HMBC (δ)
1	65.52	CH_2	4.06, dd (11.5, 6.0);	4.15; 3.94	68.54; 174.31
			4.15, dd (11.5, 4.7)	4.06; 3.94	
2	68.54	CH	3.94, quint (5.4)	3.46; 3.51	65.52; 170.75
3	72.35	CH_2	3.46, dd (10.5, 4.6)	3.51; 3.94	68.54
			3.51, dd (10.5, 5.6)	3.46; 3.94	
1′	174.31	C			
2′	33.94	CH_2	2.34, t (7.5)	1.61	25.0; 174.31
3′	25.0	CH_2	1.61, m	2.34; 1.32	33.94; 29.70
4′	29.16	CH_2	1.30–1.35	1.35–1.29; 1.61; 2.05	25.0–29.70
5′	29.19	CH_2			
6′	29.29	CH_2			
7′	29.7	CH_2			
8′	27.17	CH_2	2.05, m	1.35; 5.35	129.87/129.96; 29.16/29.19
9′	129.96	CH	5.35, m	2.05	27.17
10′	128.13	CH	5.31, m	2.76	25.56
11′	25.56	CH_2	2.76, t (6.6)	5.31	128.06/128.13
12′	128.06	CH	5.31, m	2.76	25.56
13′	129.87	CH	5.35, m	2.05	27.17
14′	27.17	CH_2	2.05, m	1.35; 5.35	129.87/129.96; 29.16/29.19
15′	29.46	CH_2	1.30–1.35	1.35–1.29; 1.61; 2.05	25.0–29.70
16′	31.64	CH_2			
17′	22.6	CH_2	1.31, m	0.9	13.48
18′	13.48	CH_3	0.90, t (6.5)	1.31	22.60; 31.64
1″	170.75	C			
2″	76.67	CH	3.75, dd (11.2, 2.6)	2.07	170.75; 28.03; 51.04
3″	28.03	CH_2	2.07, m;	2.23; 3.75	76.67; 67.55
			2.23, m	2.07; 3.55; 3.65	
4″	67.55	CH_2	3.55, m;	3.65; 2.23; 2.07	76.67
			3.65, m	3.55; 2.23; 2.07	
5–7″	51.41	$3CH_3$	3.20, s		76.67

the first time detected from *C. fumosorosea*. It exists in several other cordyceps and has Ca^{2+} antagonists, anticancer, inotropic and renal protection activities, but it also induces oxidative stress (Kim et al. 2014; Chen et al. 2020). The known compound N^6-(2-hydroxyethylacetate) (**5**) adenosine is an analogue of N^6-(2-hydroxyethyl) adenosine and was identified from *C. fumosorosea* for the first time. Ovalicin-4α-alcohol (**8**) which was originally reported from *Metarhizium anisopliae* is a arterialisation inhibitor, a vascularisation inhibitor and an antipsoriatic agent (Yamaguchi and Hayashi 2010).

The compounds **6**, **7** and **11** are novel compounds and identified as fumosoroseanoside A (**6**), B (**7**) and fumosoroseain A (**11**). Bioactivity tests showed that fumosoroseanoside A and B had antibacterial and antifungal activities which may contribute to the niche competition of *C. fumosorosea* against other bacteria and fungi which would live on the killed pests. Fumosoroseain A (**11**) shows anti-aging activity, suggesting that this compound has potential to be developed as antiaging agent (Adrien et al. 2017; Gems and Partridge 2008; Vayndorf et al. 2013). Apart from the new compounds, the known secondary metabolites N^6-(2-hydroxyethyl)-adenosine, N^6-(2-hydroxyethylacetate)-adenosine, ovalicin-4α-alcohol, beauveriolide I, and E have also healthy or medicinal bioactivities (Hu and Li 2007; Furuya et al. 1983; Kim et al. 2014). Obviously, the metabolites of *C. fumosorosea* have pharmaceutical potentiality. This study established metabolic development basis for *C. fumosorosea*.

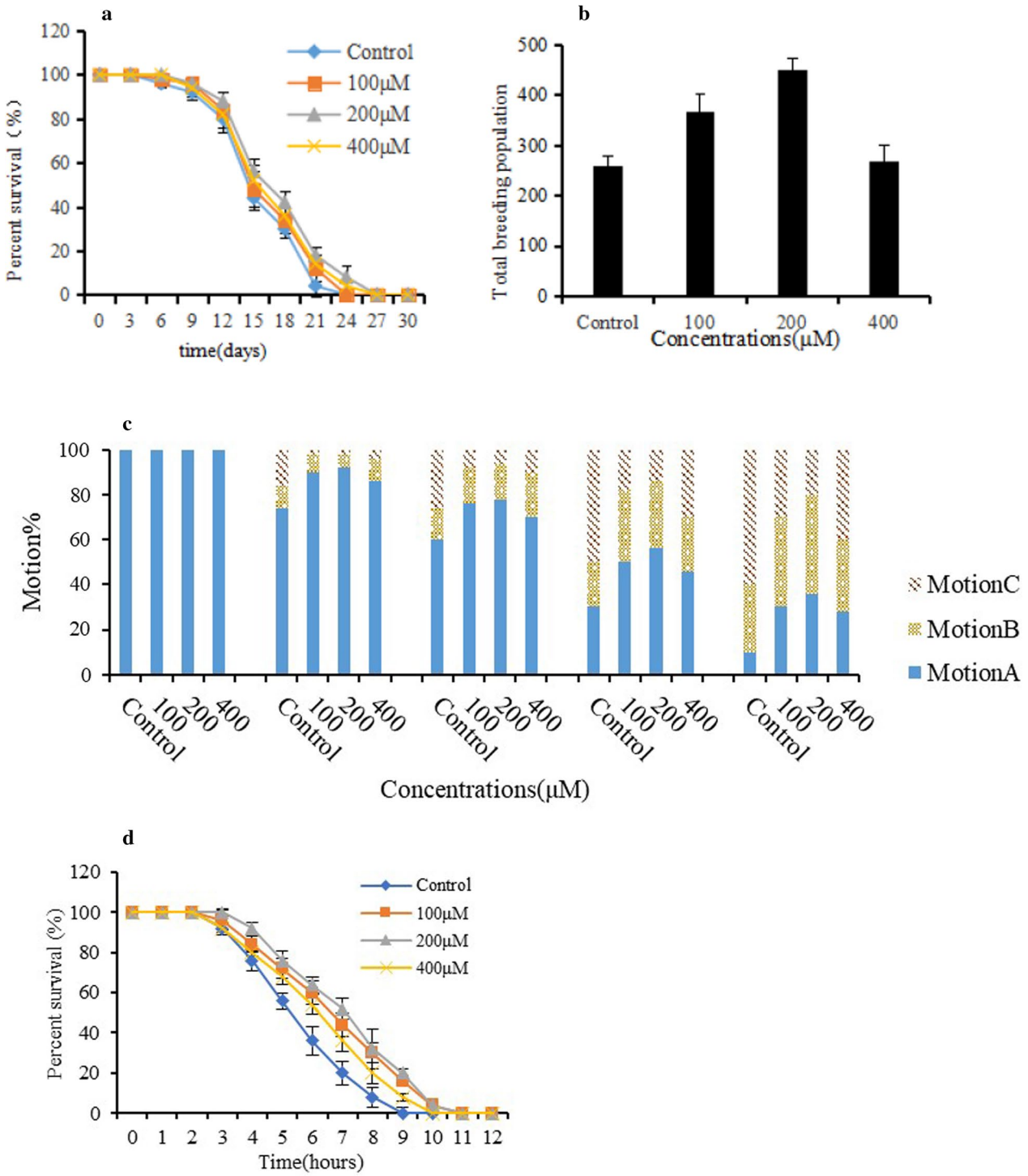


Fig. 4 Antiaging activities of compound **11. a** Effect of compound 11 on lifespan of *C. elegans* (Control, equal volume solvent). **b** Effect of compound 11 on reproduction of *C. elegans* (Control, equal volume solvent). **c** Effect of compound 11 on locomotion (Control, equal volume solvent). **d** Effect of compound 11 on heat stress tolerance of *C. elegans* (Control, equal volume solvent)

Abbreviations
RCEF: Research Center on Entomogenous Fungi; μM: Micro mole per liter; 1D: One dimension; 2D: Two dimensions; NMR: Nuclear magnetic resonance; ESI: Electric spray ionization; HR: High resolution; MS: Mass spectrum; HPLC: High performance liquid chromatography; DMSO: Dimethyl sulfoxide; TOF: Time of flight; DEPT: Distortionless enhancement by polarization transfer; HMQC: Heteronuclear multiple quantum coherence; HMBC: Heteronuclear multiple-bond correlation; COSY: ^{1}H-^{1}H correlation spectroscopy; DNP: Dictionary of Natural Products.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13568-022-01379-w.

Additional file 1: Figure S1. 1D NMR and 2D NMR spectrometry of Compound **5** in CD3OD. **Figure S2.** 1D NMR and 2D NMR spectrometry of Compound **6** in (CD3)2SO. **Figure S3.** ^{1}H and ^{13}C NMR spectrometry of Compound **7** in (CD3)2SO. **Figure S4.** 1D NMR and 2D NMR spectrometry of Compound **9** in CD3OD. **Figure S5.** 1D NMR and 2D NMR spectrometry of Compound **11** in CD3OD.

Acknowledgements
Thanks are dure to Prof. Dr. H. Zhou, Naval Postgraduate School, United States, for grammatical modification of the manuscript.

Authors' contributions
JW and XZ contribute equally to the paper. FH and RL conceived and designed the research. JW and XZ conducted experiments, analyzed data and wrote the manuscript. MD, LY and CZ helped in the experiments and data analysis. GB revised the manuscript. All the authors have read and approved the final manuscript.

Funding
This work was funded by the Natural Science Foundation of China (grant no. 31570024) and the Natural Science Foundation of Anhui Provincial Department of Education (KJ2015ZD21).

Availability of data and materials
The Supporting Information can be found with this article. It includes 1D and 2D NMR spectra of Compound 5 (Additional file 1: Fig. S1), 1D and 2D NMR spectra of Compound 6 (Additional file 1: Fig. S2), ^{1}H and ^{13}C NMR spectra of Compound 7 (Additional file 1: Fig. S3), 1D and 2D NMR spectra of Compound 9 (Additional file 1: Fig. S4), 1D and 2D NMR spectra of Compound 11 (Additional file 1: Fig. S5).

Declarations

Ethics approval and consent to participate
This article does not contain any studies with animals performed by any of the authors.

Consent for publication
All the authors listed have approved the publication of this manuscript.

Competing interests
All the authors declare that they have no conflict of interest.

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Received: 12 February 2022 Accepted: 15 March 2022
Published online: 02 April 2022

References

Abraham MM, Martin GA, Conchita T, Hortensia NB, Rachel M (2015) Potent anti-calmodulin activity of cyclotetradepsipeptides isolated from *Isaria fumosorosea* using a newly designed piosensor. Nat Prod Commun 10:113–116. https://doi.org/10.1177/1934578X1501000128

Adrien M, Geoffroy B, Vincent F, Andy M, Juliana AJ, Philippe N, Bronikowski AM, Morgan TJ, Theodore G, Carter PA (2017) Age-Related Changes in Locomotor Performance Reveal a Similar Pattern for *Caenorhabditis elegans*, *Mus domesticus*, *Canis familiaris*, *Equus caballus*, and *Homo sapiens*. J Gerontol A Biol Med 72:455–463. https://doi.org/10.1093/gerona/glw136

Ayer W, Craw P, Nozawa K (1991) Two 1 H-naphtho [2,3-c] pyran-1-one metabolites from the fungus *Paecilomyces variotii*. Can J Chem 69:189–191. https://doi.org/10.1002/chin.199127272

Azarcoya-Barrera J, Field CJ, Goruk S, Makarowski A, Curtis JM, Pouliot Y, Jacobs RL, Richard C (2021) Buttermilk: an important source of lipid soluble forms of choline that influences the immune system development in Sprague-Dawley rat offspring. Eur J Nutr 60:2807–2818. https://doi.org/10.1007/s00394-020-02462-3

Bagli J, KluepfelSt.-Jacques DM (1973) Elucidation of structure and stereochemistry of myriocin. A novel antifungal antibiotic. J Org Chem 38:1253–1260. https://doi.org/10.1021/jo00947a001

Buchter C, Koch K, Freyer M, Baier S, Saier C, Honnen S, Watjen W (2020) The mycotoxin beauvericin impairs development, fertility and life span in the nematode *Caenorhabditis elegans* accompanied by increased germ cell apoptosis and lipofuscin accumulation. Toxicol Lett 334:102–109. https://doi.org/10.1016/j.toxlet.2020.09.016

Calvo DR, Martorell P, Genovés S, Gosálbez L (2016) Development of novel functional ingredients: need for testing systems and solutions with *Caenorhabditis elegans*. Trends Food Sci Technol 54:197–203. https://doi.org/10.1016/j.tifs.2016.05.006

Chen Y, Onken B, Chen H, Xiao S, Liu X, Driscoll M, Cao Y, Huang Q (2014) Mechanism of longevity extension of *caenorhabditis elegans* induced by pentagalloyl glucose isolated from *eucalyptus* leaves. J Agric Food Chem 62:3422–3431. https://doi.org/10.1021/jf500210p

Chen C, Xue T, Fan P, Meng L, Wei J, Luo D (2018) Cytotoxic activity of Shp2 inhibitor fumosorinone in human cancer cells. Oncol Lett 15:10055–10062. https://doi.org/10.3892/ol.2018.8593

Chen B, Sun Y, Luo F, Wang C (2020) Bioactive metabolites and potential mycotoxins produced by *Cordyceps* fungi: a review of safety. Toxins 12:410. https://doi.org/10.3390/toxins12060410

Daniela Š, Petr T, Alexandr J, Petr H (2020) Rapid methods for the separation of natural mixtures of beauverolides, cholesterol acyltransferase inhibitors, isolated from the fungus *Isaria fumosorosea*. J Sep Sci 43:962–969. https://doi.org/10.1002/jssc.201901084

Derbyshire E, Obeid R (2020) Choline, neurological development and brain function: a systematic review focusing on the first 1000 days. Nutrients 12:1731. https://doi.org/10.3390/nu12061731

Furuya T, Mirotani M, Masuzawa M (1983) N^{6}-(2-hydroxyethyl) adenosine a biologically active compound from cultured mycelia of Cordyceps and *Isaria* species. Phytochemisrry 22:2509–2512. https://doi.org/10.1016/0031-9422(83)80150-2

Gems D, Partridge L (2008) Stress-response hormesis and aging: "that which does not kill us makes us stronger." Cell Metab 7:200–203. https://doi.org/10.1016/j.cmet.2008.01.001

Gruber J, Ng LF, Poovathingal SK, Halliwell B (2009) Deceptively simple but simply deceptive—*Caenorhabditis elegans* lifespan studies: considerations for aging and antioxidant effects. FEBS Lett 21:3377–3387. https://doi.org/10.1016/j.febslet.2009.09.051

Harrison K, Foster-Brown C, Kelaiditis C, Mavrommatis Y, Pilic L (2020) The associations between phosphatidylethanolamine N-methyltransferase gene, dietary choline intake and anxiety and depression in healthy UK adults. Proc Nutr Soc 79:E784. https://doi.org/10.1017/S0029665120007703

He YQ, Zhang WC, Peng F, Lu RL, Zhou H, Bao GH, Wang B, Huang B, Li ZZ, Hu FL (2018) Metabolomic variation in wild and cultured Cordyceps and mycelia of *Isaria cicadae*. Biomed Chromatogr 33:1–13. https://doi.org/10.1002/bmc.4478

Hu FL, Schmidt K, Stoyanova S, Li ZZ, Grafe U, Hamburger M (2002) Radical scavengers from the entomogenous deuteromycete *Beauveria amorpha*. Planta Med 68:64–65. https://doi.org/10.1055/s-2002-19868

Hu FL, He YQ, Huang B, Li C, Fan M, Li ZZ (2009) Secondary metabolites in a soybean fermentation broth of *Paecilomyces militaris*. Food Chem 116:198–201. https://doi.org/10.1016/j.foodchem.2009.02.055
Hugo P, Rainer Z, Norbert M, Horst K (1983) Incorporation of radioactive precursors into beauvericin produced by *Paecilomyces fumosoroseus*. Pergamon 22:1719–1720. https://doi.org/10.1016/S0031-9422(00)80257-5
HuLI FLZZ (2007) Secondary metabolites and their bioactivities of *Cordyceps* and its related fungi. Mycosystema 26:607–632. https://doi.org/10.3969/j.issn.1672-6472.2007.04.021
Isaka M, Tanticharoen M, Thebtaranonth Y (2000) ChemInform abstract: cordyanhydrides a and b. two unique anhydrides from the insect pathogenic fungus *Cordyceps pseudomilitaris* BCC 1620. J Cheminform 41:1657–1660. https://doi.org/10.1016/S0040-4039(00)00008-3
Ji DB, Jia Y, Li CL, Wang YH, Cai SQ (2008) Antiaging effect of *Cordyceps sinensis* extract. Phytother Res 23:116–122. https://doi.org/10.1002/ptr.2576
Kim SB, Ahn B, Kim M, Ji HJ, Shin SK, Hong IP, Kim CY, Hwang BY, Lee MK (2014) Effect of *Cordyceps militaris* extract and active constituents on metabolic parameters of obesity induced by high-fat diet in C58BL/6J mice. J Ethnopharmacol 151:478–484. https://doi.org/10.1016/j.jep.2013.10.064
Kumar C, Jacob TK, Devasahayam S, Geethu C, Hariharan V (2021) Characterization and biocontrol potential of a naturally occurring isolate of *Metarhizium pingshaense* infecting *Conogethes punctiferalis*. Microbiol Res 243:126645. https://doi.org/10.1016/j.micres.2020.126645
Liu Y, Wang J, Wang W, Zhang H, Zhang X, Han C (2015) The chemical constituents and pharmacological actions of *Cordyceps sinensis*. J Evid Based Complement Altern Med 2015:575063. https://doi.org/10.1155/2015/575063
Liu X, Luo Q, Rakariyatham K, Cao Y, Goulette T, Liu X, Xiao H (2016) Antioxidation and anti-ageing activities of different stereoisomeric astaxanthin in vitro and in vivo. J Funct Foods 25:50–61. https://doi.org/10.1016/j.jff.2016.05.009
Liu YN, Liu BY, Ma YC, Yang HL, Liu GQ (2020) Analysis of reference genes stability and histidine kinase expression under cold stress in *Cordyceps militaris*. PLoS One 15:e0236898. https://doi.org/10.1371/journal.pone.0236898
Lou H, Zhao W, Chen BX, Yu YH, Guo LQ (2020) Cmfhp gene mediates fruiting body development and carotenoid production in *Cordyceps militaris*. Biomolecules 10:410. https://doi.org/10.3390/biom10030410
Lu R, Luo F, Hu FL, Huang B, Li C, Bao G (2013) Identification and production of a novel natural pigment, cordycepoid A, from *Cordyceps bifusispora*. Appl Microbiol Biotechnol 97(14):6241–6249. https://doi.org/10.1007/s00253-013-4966-y
Lu R, Liu X, Gao S (2014) New tyrosinase inhibitors from *Paecilomyces gunnii*. J Agric Food Chem 62:11917–11923. https://doi.org/10.1021/jf504128c
Luo FF, Lu RL, Zhou H, Hu FL, Bao GH, Huang B, Li ZZ (2013) Metabolic effect of an exogenous gene on transgenic *Beauveria bassiana* using liquid chromatography-mass spectrometry-based metabolomics. J Agric Food Chem 61:7008–7017. https://doi.org/10.1021/jf401703b
Manrico B, Aristide C, Giovanni P, Beniamino S (1975) Isolation of beauvericin from *Paecilomyces fumosoroseus*. Phytochemistry 14:1865–1866. https://doi.org/10.1016/0031-9422(75)85311-8
Masahiko I, Prasat K, Kanyawim K, Nigel LH-J, Yodhathai T (2005) Bioactive substances from insect pathogenic fungi. Acc Chem Res 38:813–823. https://doi.org/10.1002/chin.200607264
Meca G, Soriano JM, Gaspari A, Ritieni A, Moretti A, ManEs J (2010) Antifungal effects of the bioactive compounds enniatins A, A(1), B, B(1). Toxicon 56:480–485. https://doi.org/10.1016/j.toxicon.2010.04.013
Moliner C, Barros L, Dias MI, López V, Langa E (2018) Edible flowers of tagetes erecta l. as functional ingredients: phenolic composition, antioxidant and protective effects on Caenorhabditis elegans. Nutrients 10:2002. https://doi.org/10.3390/nu10122002
Mularczyk M, Michalak I, Marycz K (2020) Astaxanthin and other nutrients from *Haematococcus pluvialis*-multifunctional applications. Mar Drugs 18:459. https://doi.org/10.3390/md18090459
Olatunji OJ, Tang J, Tola A, Auberon F, Oluwaniyi O, Ouyang Z (2018) The genus Cordyceps: an extensive review of its traditional uses, phytochemistry and pharmacology. Fitoterapia 129:293–316. https://doi.org/10.1016/j.fitote.2018.05.010
Onken B, Driscoll M (2010) Metformin induces a dietary restriction–like state and the oxidative stress response to extend *C. elegans* Healthspan via AMPK, LKB1, and SKN-1. PLoS One 5:e8758. https://doi.org/10.1371/journal.pone.0008758
Papandreou C, Bulló M, Hernández-Alonso P, Ruiz-Canela M, Li J, Guasch-Ferré M, Toledo E, Clish C, Corella D, Estruch R (2021) Choline metabolism and risk of atrial fibrillation and heart failure in the PREDIMED Study. Clin Chem 67:288–297. https://doi.org/10.1093/clinchem/hvaa224
Rathor L, Pant A, Awasthi H, Mani D, Pandey R (2017) An antidiabetic polyherbal phytomedicine confers stress resistance and extends lifespan in *Caenorhabditis elegans*. Biogerontology 18:131–147. https://doi.org/10.1007/s10522-016-9668-2
Schmidt K, Stoyanova S, Schubert B, Li Z, Hamburger M (2002) Militarinone A, a neurotrophic pyridone alkaloid from *Paecilomyces militaris*. Org Lett 4:197–199. https://doi.org/10.1021/ol016920j
Schmidt K, Li ZZ, Schubert B, Huang B, Stoyanova S, Hamburger M (2003) Screening of entomopathogenic deuteromycetes for activities on targets involved in degenerative diseases of the central nervous system. J Ethnopharmacol 89:251–260. https://doi.org/10.1016/j.jep.2003.08.009
Shang Y, Xiao G, Zheng P, Cen K, Zhan S, Wang C (2016) Divergent and convergent evolution of fungal pathogenicity. Genome Biol Evol 8:1374–1387. https://doi.org/10.1093/gbe/evw082
Vayndorf EM, Lee SS, Liu RH (2013) Whole apple extracts increase lifespan, healthspan and resistance to stress in *Caenorhabditis elegans*. J Funct Foods 5:1235–1243. https://doi.org/10.1016/j.jff.2013.04.006
Velez H, Glassbrook NJ, Daub ME (2007) Mannitol metabolism in the phytopathogenic fungus *Alternaria alternata*. Fungal Genet Biol 44:258–268. https://doi.org/10.1016/j.fgb.2006.09.008
Wartburg AV, Traber R (1988) Cyclosporins, fungal metabolites with immunosuppressive activities. Prog Med Chem 25:1–33. https://doi.org/10.1016/S0079-6468(08)70276-5
Wilson MA, Shukitt-Hale B, Kalt W, Ingram DK, Joseph JA, Wolko CA (2006) Blueberry polyphenols increase lifespan and thermotolerance in *Caenorhabditis elegans*. Aging Cell 5:59–68. https://doi.org/10.1111/j.1474-9726.2006.00192.x
Wu YZ, Zhang HW, Sun ZH, Dai JG, Lin S (2018) Byssopectin A, an unusual octaketide dimer and the precursor derivatives from the endophytic fungus *Byssochlamys spectabilis* IMM0002 and their biological activities. Eur J Med Chem 145:717–725. https://doi.org/10.1016/j.ejmech.2018.01.030
Xiao JH, Xiao DM, Sun ZH, Xiong Q, Zhong JJ (2009) Chemical compositions and antimicrobial property of three edible and medicinal *Cordyceps* species. J Food Agric Environ 7:91–100. https://doi.org/10.3168/jds.2009-92-7-3543
Yamaguchi J, Hayashi Y (2010) Syntheses of fumagillin and ovalicin. Chem Eur J 16:3884–3901. https://doi.org/10.1002/chem.200902433
Zhang J, Liu SS, Yuan WY, Wei JJ, Zhao YX, Luo DQ (2019) Carotane-type sesquiterpenes from cultures of the insect pathogenic fungus *Isaria fumosorosea*. J Asian Nat Prod Res 21:234–240. https://doi.org/10.1080/10286020.2017.1410143
Zhang K, Lyu W, Gao Y, Zhang X, Sun Y, Huang B (2021) Choline-Mediated lipid reprogramming as a dominant salt tolerance mechanism in grass species lacking glycine betaine. Plant Cell Physiol 61:2018–2030. https://doi.org/10.1093/pcp/pcaa116

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