American Journal of Biochemistry and Biotechnology

Research Article

Purification, Structural Characterization and Anti-Aging and Stress Activities of Cordyceps Mycelium Polysaccharides from *Paecilomyces Hepiali*

Xiaokang Hu, Huixiu Ma, Guicai Chen, Dehui Dai and Weilian Hu

Zhejiang University of Science and Technology, School of Biological and Chemical Engineering, Hangzhou, China

Article history
Received: 20-12-2024
Revised: 20-12-2024
Accepted: 15-07-2025

Corresponding Author:
Weilian Hu
Zhejiang University of Science and Technology, School of Biological and Chemical Engineering, Hangzhou, China
E-mail: weilian89@126.com

Abstract: This study identified and characterized the bioactive polysaccharide components from *Cordyceps* mycelium powder (CMP) derived from *Paecilomyces hepiali*, evaluating their anti-aging and stress-protective activities. Initial screening using *Drosophila melanogaster* revealed that crude *Cordyceps* polysaccharides (CCP) exhibited comparable effects to CMP and *Cordyceps* aqueous extracts (CAE) on natural lifespan and UV stress resistance, suggesting CCP as the primary bioactive component. CCP was purified using ion exchange and size-exclusion chromatography, yielding two homogeneous fractions (CCP-1-1 and CCP-2-1). Structural characterization by HPLC monosaccharide analysis and infrared spectroscopy revealed distinct monosaccharide compositions and functional groups between fractions. *In vitro* antioxidant assays demonstrated both fractions effectively scavenged hydroxyl radicals (·OH), DPPH radicals, and superoxide anions (O_2^-), while exhibiting strong reducing power. Using *Caenorhabditis elegans* as a model organism, both CCP-1-1 and CCP-2-1 significantly extended mean lifespan under normal conditions, heavy metal stress, and UV exposure ($p < 0.05$). Additionally, CCP-2-1 enhanced survival under heat stress ($p < 0.05$). Biochemical analysis revealed that both fractions significantly increased antioxidant enzyme activities (superoxide dismutase [SOD] and catalase [CAT]) while reducing malondialdehyde (MDA) levels ($p < 0.05$), indicating enhanced cellular antioxidant defense. These findings demonstrate that CCP represents the principal anti-aging component of CMP, with purified fractions CCP-1-1 and CCP-2-1 exhibiting potent lifespan-extending and stress-protective effects through modulation of antioxidant enzyme systems. This research provides a foundation for developing *Cordyceps*-derived polysaccharides as functional ingredients for healthy aging applications.

Keywords: *Cordyceps* Polysaccharides, *Paecilomyces hepiali*, Anti-Aging, Oxidative Stress, *Caenorhabditis elegans*, Antioxidant Enzymes, Stress Resistance, Structural Characterization

Introduction

Cordyceps is a traditional and valuable Chinese medicine in China (Liu et al., 2024). Recent studies demonstrate its diverse pharmacological activities, making it suitable for treating diseases like diabetes, acute liver injury, and colitis (Liu et al., 2024). Due to its good disease prevention and health care functions, Cordyceps has been actively explored and used since ancient times (Xu et al., 2016). However, because of the strict growth environment, large market demand and low fertility rate the natural environment has been severely damaged (Zhou et al., 2009; Lu et al., 2020). Therefore, some strains had been isolated and produced in lieu of natural Cordyceps (Zhang et al., 2013). *P. hepiali* was extracted from fresh Cordyceps using advanced biotechnological methods on Hepialus armoricanus larvae. This strain was identified as Paecilomyces Bain, a fungus in the Moniliaceae family (Hu et al., 2015). The National Health Commission of China stipulated that only two kinds of mycelium could be used in dietary supplements. One of them is produced by *P. hepiali* (Dan et al., 2021). Previous research had shown that, Cordyceps is a complex of larval

SCIENCE Publications

corpses and fungal stroma mainly formed by the *P. hepiali* infesting Hepialus armoricanus (Zuo et al., 2024). Artificial fermentation powder produced by *P. hepiali* has extremely similar biological activities to natural Cordyceps samples. The State Food and Drug Administration of China approved it as a health food.

Mycelium of *P. hepiali* had many biological activities including hypoglycemic effect, antioxidant effect, anti-tumor effect, immunomodulatory effect, and protecting kidney effect (Dan et al., 2021). (Chen et al., 2022). demonstrated its immunomodulatory function, which enhances the survival of lupus mice (Chen et al., 1999). *P. hepiali* could regulate the central nervous system, thus to treat palpitations and insomnia by reducing parasympathetic excitability (Wu et al., 2015). Furthermore, it is able to produce obvious effects about tumor-inhibition (Jiang et al., 2010). In addition, *P. hepiali* had the ability to help patients improve left ventricular diastolic function who have coronary artery disease and hypercardiosis (Cai et al., 2002). Due to the convenience and controllable conditions with liquid fermentation, the mycelium obtained by liquid fermentation of *P. hepiali* has significant commercial prospects. Although the quality of fermentation powder is evaluated by the content of adenosine in the Chinese Pharmacopoeia (2020 edition), the active ingredient the anti-aging and anti-acute damage roles of fermentation powder from *P. hepiali* was still unknown.

Previous research has shown that Cordyceps crude polysaccharides and homogeneous polysaccharides could effectively scavenge free radicals and improve the antioxidant system (Wang et al., 2022). Excess of free radicals would accelerate aging, and oxidative stress would lead to an excess of free radicals and a deficiency of antioxidants. Therefore, Cordyceps polysaccharides may be an uppermost active ingredient in anti-aging and anti-acute damage roles (Zhang et al., 2015).

In this paper, the biological activity of CMP, CAE and CCP from *P. hepiali* was compared by examining the natural lifespan and lifespan under UV stress of Drosophila melanogaster, aimed to screening the uppermost active ingredient of Cordyceps mycelium powder. Furthermore, the molecular structures, anti-aging and acute stress protection effects and mechanism of the homogeneous polysaccharides purified from CCP were measured and compared. These findings may serve as a foundation for advancing and applying *P. hepiali*.

Materials and Methods

Materials

P. hepiali was extracted from the stromata of fresh Cordyceps in Qinghai Province, China.Ascorbic Acid, 5-Fluorouracil, Salicylic Acid, Potassium Ferricyanide, Ferric Chloride, Pyrogallol, and 1,1-Diphenyl-2-Picrylhydrazyl were sourced from Sinopharm Chemical Reagent Co., Ltd. Mannose, Glucuronic Acid, Rhamnose, Galacturonic Acid, Glucose, Galactose, Arabinose, Fucose, Trifluoroacetic Acid, and 1-Phenyl-3-Methyl-5-Pyrazolone were obtained from Shanghai McLean Biochemical Science and Technology Co., Ltd. Cupric chloride and other analytical-grade reagents were acquired from Shanghai Lingfeng Chemical Reagent Co., Ltd. Malondialdehyde, Catalase, and Superoxide Dismutase assay kits were procured from Nanjing Jiancheng Bio-engineering Institute. *C. elegans* and *D. melanogaster* were purchased from Shanghai Model Organisms, Shanghai, China.

Extraction of Polysaccharides

The CMP were obtained through liquid fermentation. The fermentation medium contained 20 g/L Soya Bean Powder, 20 g/L, 20 g/L Sucrose,5g/L,67g/L Yeast Extract Powder, 1.5 g/L KH2PO4, 0.375 g/L MgSO4 and 50 mL Water. CAE and CCP were prepared following the method outlined by Chen et al. (2022). The CMP was incubated at a liquid-solid ratio of 20:1 (mL/g) at 90 °C for 3 hours, followed by centrifugation at 8000 rpm to collect the supernatant. Then CAE was acquired by freeze-drying of the supernatant. Introduce alcohol to the supernatant until reaching an 80% final concentration, allow to stand overnight at 4 °C, centrifue at 8000 rpm, and collect the precipitate. CCP were acquired by freeze-drying of the precipitate. Polysaccharide content was assessed using the phenol-sulfuric acid method (Liu et al., 2022). By measuring, each gram of CAE was equal to 4.45g of the CMP crude drug, and each gram of CCP was equal to 6.67g of the CMP crude drug.

Effects of CMP, CAE and CCP on D. Melanogaster for Natural Lifespan and UV Stress

D. Melanogaster Cultures and Treatment

D. melanogaster were maintained on Drosophila Growth Medium (DGM) tubes composed of 110 g/L corn flour, 82 g/L sucrose, 20 g/L Yeast Extract Powder, 10 g/L Bacteriological Agar, and 10 mmol/L Propanoic Acid.Unmated adult flies were placed in DGM tubes to lay eggs for 24 hours at 25 °C, ensuring a synchronized population.Removed the parents, after 7 d, all chrysalises became the adult flies(F_1).The fourth-generation flies (F_4), obtained by repeating the operation three times, were used as the baseline for experiments comparing CMP, CAE, and CCP (Ye et al., 2022).

Effects of CMP, CAE and CCP on D. Melanogaster for Natural Lifespan

To screen the uppermost active ingredient about anti-aging and anti-acute damage of *P. hepiali* fermentation

powder, concentrations of CMP, CAE and CCP were determined based on the same CMP crude drug level.The Lifespan assay was conducted with minor modifications as outlined by Zhang et al. (2020). A total of 1200 unmated F4 flies, comprising equal numbers of males and females, were collected within 8 hours of eclosion and randomly assigned to ten groups of 120 flies each.The control group received the basal diet, while the experimental groups were administered varying doses of CMP (10, 20, and 40 g/L), CAE (2.25, 4.5, and 9 g/L), and CCP (1.5, 3, and 6 g/L). All groups were kept at 25 ± 0.5 °C with a 12-hour light/dark cycle. Flies were moved to new medium biweekly.Lifespan was recorded daily until all flies were dead, separately for males and females.The mean lifespan and its standard error were determined.

Effects of CMP, CAE and CCP on D. Melanogaster for UV Stress

The UV stress assay was conducted with minor modifications based on the method outlined by (Wang et al., 2014). The grouping method mirrored that used in the natural lifespan experiment.A total of 1200 F4 flies, comprising equal numbers of males and females, were randomly assigned to ten groups of 120 each.These groups were fed a basal diet and low, medium, and high doses of CMP, CAE and CCP respectively. After 20 days of feeding, the flies were isolated, starved for 2 hours, and then placed in culture tubes.The hungry flies were maintained at 25 ± 0.5 °C under 10 W UV light positioned 30 cm away. Survival was monitored hourly, and the average survival time was calculated separately for males and females (Mikhail et al., 2013).

Preparation of Homogeneous Polysaccharide of P. Hepiali

Referring to the method of polysaccharide purification (Huang et al., 2016). The CCP was dissolved in deionized water and purified using a DEAE-52 cellulose column.The eluate was collected in 5 mL increments per tube after elution with a 0-1 mol/L NaCl solution at a flow rate of 1 mL/min for 360 minutes. The phenol-sulfuric acid method was used to detect the collected solution and plot the elution curve. The polysaccharides that have the same peak were concentrated, dialyzed, and freeze-dried.Then the polysaccharides were purified through a Sephadex G-100 column and eluted with deionized water (0.2 mL/min) respectively. The elution curve was plotted, and the homogeneous polysaccharides were concentrated, dialyzed, and freeze-dried.

Monosaccharide Composition Analysis

The determination of monosaccharide composition was carried out using the modified PMP pre-column HPLC method (Liu et al., 2024). In brief, the CCP-1-1 and CCP-2-1 were dissolved in 2 mol/L TFA respectively and degraded at a temperature of 90°C for 12 h. After drying, deionized water was added to re-dissolve and obtain the samples. Samples and monosaccharide standards were combined with 0.2 mol/L NaOH and PMP solution in methanol, then incubated at 70°C for 80 minutes. The reaction was stopped by adding 0.5 mol/L HCl after cooling to room temperature. The solution was dissolved in 1 mL of chloroform. Following vigorous shaking and centrifugation, the organic phase beneath the aqueous layer was meticulously discarded, and surplus reagents were eliminated.Perform the extraction procedure three times. The water layer was diluted with 150 μL of water, filtered through a 0.22 μm membrane, and analyzed using HPLC. The chromatography utilized a Diamonsil C18 column (4.6 mm × 250 mm, 5 μm) with mobile phases consisting of solvent A (0.1 mol/L PBS, pH adjusted to 7.0) and solvent B (acetonitrile). The proportion of phase A to phase B was adjusted to 86:14. The column was kept at 30°C with a flow rate of 1.0 mL/min. A 20 μL injection volume was utilized. And the result was detected at 245 nm.

FT-IR Determination

Fourier transform infrared spectra of CCP-1-1 and CCP-2-1 were determined by the KBr-pellets method on an FT-IR spectrometer (Zhao et al., 2023).

Antioxidant Activity Assay in vitro

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of CCP-1-1 and CCP-2-1 was tested using a modified procedure based on previous research methodology (Tan et al., 2018). A 1 mL sample solution (1-5 g/L) was combined with 1 mL each of $FeSO_4$ (9 mmol/L), H_2O_2 (5 mmol/L), and salicylic acid solution (9 mmol/L in ethanol). The mixture was thoroughly combined and incubated at 37°C for 1 hour, using ascorbic acid as a positive control. The solution's absorbance was recorded at 510 nm. The percentage of hydroxyl radicals was determined using Equation (1).

$$Scavenging\ efficiency\ (\%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100 \qquad (1)$$

A_1, A_2, and A_0 are the absorbance values of the sample, a control reaction with 100 % ethanol instead of a salicylic acid/ethanol solution, and a blank control, respectively.

DPPH• Scavenging Activity

The DPPH• scavenging activity was evaluated using the method outlined by Hu (Hu et al., 2022).In summary, 1 mL of sample solution (1-5 g/L) was combined with 1 mL of a 0.2 mmol/L DPPH• ethanol solution. The reaction mixture

was vigorously shaken to ensure uniform mixing and then incubated for 30 minutes in the absence of light.Ascorbic acid served as a positive control.The solution's absorbance was recorded at 517 nm. The DPPH• scavenging ability was determined using Equation (2):

$$Scavenging\ efficiency\ (\%) = \left[1 - \left(\frac{B_1 - B_2}{B_0}\right) \times 100\right] \quad (2)$$

B_1, B_2, and B_0 are the absorbance values of the DPPH• solution alone, the sample/DPPH• mixture, and a control reaction with water instead of the DPPH• solution, respectively.

Superoxide Anion Radical-Scavenging Activity

The super anion radical scavenging activities were evaluated using the established method (Shi et al., 2023). A 4.5 mL solution of 0.05 mol/L Tris-HCl buffer (pH 8.2) was held at 25 °C for 20 minutes.Subsequently, 1 mL of the polysaccharide sample solutions was combined with 0.4 mL of 25 mmol/L Pyrogallol and incubated at 25 °C for 5 minutes. The reaction was halted by the rapid addition of 1 mL of 8 mol/L HCl. Ascorbic acid served as a positive contrast. Absorbance was recorded at 318 nm. The superoxide anion scavenging activity was determined using Equation (3)

$$Superoxide\ anion\ cavenging\ effect\ (\%) = 1 - \frac{C_2 - C_1}{C_0} \quad (3)$$

C_1, C_2, and C_0 represent the absorbance of the samples, the absorbance using ultrapure water in place of the o-cresol solution, and the absorbance using ultrapure water instead of the sample solution, respectively.

Ferrous Ion Chelating Activity

The ferrous ion chelating activity was determined following the method described with slight modifications (Celep et al., 2012). A mixture was prepared by combining 2 mL of a sample solution (1-5 g/L) with 7.4 mL of 55% ethanol, 0.2 mL of 2 mM ferrous chloride solution, and 0.4 mL of 5 mmol/L ferrozine solution. The mixture was thoroughly combined and left at room temperature for 20 minutes. The mixture's absorbance was measured at a wavelength of 562 nm. Ascorbic acid served as a positive control. The ferrous ion-chelating activity was determined using Equation (4).

$$Ferrous\ ion\ chelating\ activity = \frac{D_0 - D_1}{D_0} \quad (4)$$

D_1 and D_0 represent the absorbance values of the samples and distilled water, respectively.

Cultivation and Treatment of C. Elegans

The *C. elegans* were cultured on Nematode Growth Medium (NGM) plates containing3g/L NaCl,2.5g/L peptone, 17 g/L agar,5g/L cholesterol, 1mmol/L CaCl2,1 mmol/L MgSO4 and 25 mmol/L potassium phosphate buffer at pH 6.0 (Tamagno et al., 2022). Escherichia coli OP50 was seeded onto the plates and incubated at 20 °C. Adult worms were placed on NGM plates to lay eggs for 6 hours at 20°C, achieving a synchronized population. All worms reached the L4 stage after 72 hours, serving as the baseline for all experiments.

Effects of CCP-1-1 and CCP-2-1 on C. Elegans for Natural Lifespan

The natural lifespan assay was conducted following the method outlined by Ma et al. (2021), with minor adjustments. CCP-1-1 and CCP-2-1 were incorporated into the basic NGM at concentrations of 1, 2, and 4 g/L, representing low, medium, and high doses for each group, respectively, with the basic NGM serving as the control group. Specifically, in the constant temperature incubator, L4 stage worms were placed into the medium, each group contained 20 worms. All groups were kept at 20 ± 0.5 °C with a 12-hour light/dark cycle. The worms were relocated to new medium daily.Lifespan was recorded daily until all worms were dead. The mean lifespan and its standard error were determined.Each experiment was conducted independently on three separate occasions.

Effects of CCP-1-1 and CCP-2-1 on C. Elegans for Cu^{2+} Stress

The Cu^{2+} stress assay was conducted with minor modifications based on the method outlined by Ma et al. (2024). A total of 140L4 stage worms were collected and randomly assigned to seven groups, each containing 20 worms. The control group received the basal diet, while the experimental groups were administered varying doses of CCP-1-1 and CCP-2-1 at 1, 2, and 4g/L. A 3 mmol/L Cu2+ solution was prepared by dissolving it in K-medium, which contains 3.2 g/L NaCl and 2.4 g/L KCl, for subsequent use. A 24-well plate was used, 1 mL of Cu2+ and K-medium solution were added to each well. Next, each well was added 20 worms collected from respective groups.All groups were kept at 20 ± 0.5 °C in a stable temperature incubator. Subsequently, lifespan was recorded every 2 h until all worms were dead. The mean lifespan and its standard error were determined. Each experiment was conducted independently on three separate occasions.

Effects of CCPs on C. Elegans for UV Stress

The UV stress assay was conducted with minor modifications as outlined by Ma et al. (2024). A total of 140 L4 stage worms were collected and randomly assigned to seven groups, each containing 20 worms.The control group received the basal diet, while the experimental groups were administered varying doses of

CCP-1-1 and CCP-2-1 at 1, 2, and 4 g/L.A 24-well plate was used, with each well containing 1 mL K-medium solution and 20 worms collected from respective groups.Control and experimental worms were kept at 20 °C, under UV light (10 W) at a distance of 30 cm.Subsequently, lifespan was recorded every 10 min until all worms were dead.The mean lifespan and its standard error were determined.Each experiment was conducted independently on three separate occasions.

Effects of CCPs on C. Elegans for Heat Stress

A heat stress assay was conducted with minor modifications as outlined by Ma et al. (2024). A total of 140 L4 stage worms were collected and randomly assigned to seven groups, each containing 20 worms. The control group received the basal diet, while the experimental groups were administered varying doses of CCP-1-1 and CCP-2-1 at 1, 2, and 4 g/L. A 24-well plate was used, with each well containing 1mL K-medium solution and 20 worms collected from respective groups. Control and experimental worms were kept at 31 °C, under natural light.Subsequently, lifespan was recorded every 1h until all worms were dead. The average lifespan and its standard error were determined. Each experiment was conducted independently on three separate occasions.

Antioxidant Index Detection in Vivo

Enzyme activity was examined by collecting synchronized L4 stage worms from various groups through centrifugation at 8000 rpm for 5 minutes. Subsequently, they underwent washing and cleaning. Worms were sonicated on ice for 4 minutes. The mixture was centrifuged at 3000 rpm and 4°C for 10 minutes, then collected for analysis.

The content of Malondialdehyde (MDA), the activity of Catalase (CAT) and the activity of Super Oxide Dismutase (SOD) were determined by the kits' instructions.

Statistical Analyses

Data are expressed as mean ± standard error of the mean. IBM SPSS Statistics 26 was used for statistical analyses. Group differences were assessed using ANOVA, followed by Tukey's post-hoc tests. A significance threshold of 0.05 was used for statistical analysis.

Results and Discussion

Effect of CMP, CAE and CCP for Natural Lifespan

The introduction of CMP, CAE, and CCP resulted in rightward shifts in the survival curves compared to the control group (Fig. 1). It indicated that CMP, CAE and CCP all extend the natural lifespan of female and male *D. melanogaster*.

As shown in Tab.CMP, CAE, and CCP significantly extended the natural lifespan of flies of both sexes compared to the control group (p<0.05). The natural lifespan improved with higher concentrations of additives. At high doses (40g/L CMP, 9g/L CAE and 6g/L CCP), average lifespan growth rates of CMP, CAE and CCP were 32.9 %, 36.2 and 32.6 % in females, and were 59.9, 63.8 % and 60.7 % in males, separately. The polysaccharides from Phyllanthus emblica were found to increase natural lifespan, and there was a gender difference in the protective effect in Drosophila melanogaster (Li et al., 2023).

This is consistent with our findings. CMP, CAE, and CCP showed no significant differences (p>0.05). The results suggested that the main ingredient with an anti-aging role was the CCP in fermentation products.

Groups with different lowercase letters show significant differences (p < 0.05), while those with the same letters do not (p>0.05). The same convention applies here and hereafter.

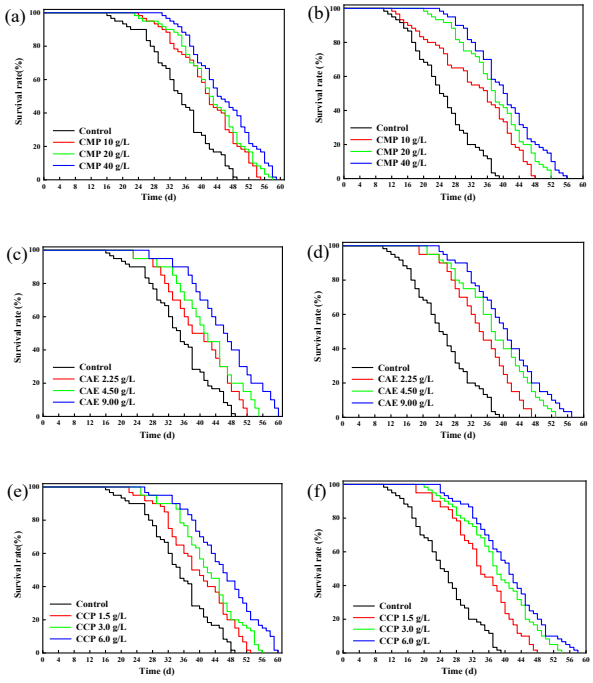


Fig. 1: Effect of CMP, CAE, and CCP on the natural lifespan of *D. melanogaster*.Survival curves for flies with added compounds: (a) females with CMP, (b) males with CMP, (c) females with CAE, (d) males with CAE, (e) females with CCP, and (f) males with CCP

Table.1: Effect of CMP, CAE and CCP on the Natural Lifespan of *D.* melanogaster

Group		Female Flies		Male Flies	
		Average Lifespan/d	**Growth rate of Lifespan/%**	**Average Lifespan/d**	**Growth rate of Lifespan/%**
Control		34.0±11.9 ^{b}	—	25.2±12.0 ^{c}	—
CMP (g/L)	10.0	40.8±15.1 ^{ab}	20.0	34.5±20.1 ^{b}	36.9
	20.0	41.9±13.8 ^{ab}	23.2	36.8±15.2 ^{b}	46.0
	40.0	45.2±12.8 ^{a}	32.9	40.3±16.4 ^{a}	59.9
CAE (g/L)	2.25	39.8±13.4 ^{ab}	17.1	33.5±16.1 ^{b}	32.9
	4.50	40.7±16.8 ^{ab}	19.7	35.3±12.2 ^{b}	40.1
	9.00	46.3±13.6 ^{a}	36.2	41.3±17.5 ^{a}	63.8
CCP (g/L)	1.50	39.6±11.4 ^{ab}	16.5	31.5±18.1 ^{bc}	25.0
	3.00	43.7±14.8 ^{a}	28.5	35.8±14.2 ^{b}	42.1
	6.00	45.1±7.60 ^{a}	32.6	40.5±12.5 ^{a}	60.7

Table 2: Effect of CMP, CAE and CCP for Lifespan of *D. melanogaster* with UV stress

Group		Female Flies		Male Flies	
		Average Lifespan/d	**Growth rate of Lifespan/%**	**Average Lifespan/d**	**Growth rate of Lifespan/%**
Control		11.4±5.94 ^{b}	—	11.0±2.72 ^{b}	—
CMP (g/L)	10.0	17.3±5.84 ^{a}	51.8	12.2±4.02 ^{b}	10.9
	20.0	18.0±6.16 ^{a}	57.9	15.4±4.56 ^{a}	40.0
	40.0	19.6±6.64 ^{a}	71.9	16.4±5.09 ^{a}	49.1
CAE (g/L)	2.25	18.2±5.48 ^{a}	59.6	11.4±4.08 ^{b}	3.64
	4.50	19.3±6.02 ^{a}	69.2	15.5±4.18 ^{a}	40.9
	9.00	20.4±6.25 ^{a}	78.9	16.6±4.84 ^{a}	63.8
CCP (g/L)	1.50	17.7±5.16 ^{a}	55.3	12.7±3.85 ^{b}	15.5
	3.00	19.1±5.64 ^{a}	67.5	14.3±3.84 ^{a}	30.0
	6.00	20.2±6.22 ^{a}	77.2	17.2±4.56 ^{a}	56.4

Effect of CMP, CAE and CCP on UV Stress

UV is a major DNA-damaging environmental stress for most, UV induces DNA lesions and produces that damage other cellular macromolecules (Park et al., 2017). Therefore, UV caused acute damage in *D. melanogaster*. The introduction of CMP, CAE, and CCP resulted in rightward shifts in the survival curves compared to the control group (Fig. 2). The study demonstrated that both male and female flies exhibited an increased lifespan.

All doses of CMP, CAE, and CCP significantly extended the average lifespan of female flies compared to the control group ($p<0.05$).

Medium and high doses of CMP, CAE, and CCP significantly extended the average lifespan of male flies ($p<0.05$). Xu's prior research demonstrated that Mosla chinensis extracts significantly improve survival following exposure to various stress factors ($p<0.05$). The average survival time under stress conditions was significantly influenced by sex ($p<0.001$), with females exhibiting greater stress resistance than males (Xu et al., 2024).

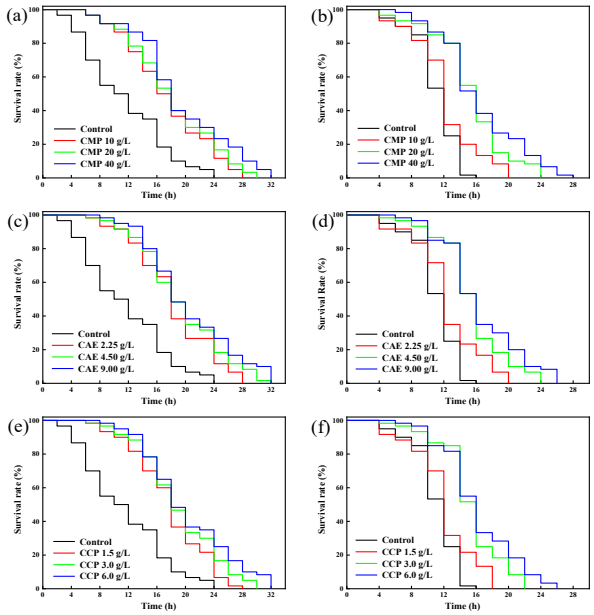


Fig. 2: Effect of CMP, CAE and CCP for lifespan of *D. melanogaster* with UV stress. Survival curves for flies with added compounds: (a) females with CMP, (b) males with CMP, (c) females with CAE, (d) males with CAE, (e) females with CCP, and (f) males with CCP.

This is consistent with our findings. No significant difference was observed among CMP, CAE, and CCP at the same dosage level (p>0.05). In summary, CCP was the main ingredient that repaired the UV acute damage. The finding showed that the water-soluble polysaccharide was possibly the main ingredient with anti-UV stress

The active ingredients of CMP mainly included polysaccharides, nucleosides, sterols and mannitol.And the active ingredients of CAE mainly included polysaccharides and nucleosides (Krishna et al., 2024). Among them, polysaccharides had the effects of immunomodulation and anti-aging; nucleosides had a significant effect on cancer therapy; sterols had the effects of anti-tumor and regulating apoptosis; and mannitol had the effects of inhibiting germs, tonifying the lungs and benefiting the kidneys (Hu et al., 2024). The results of our study found that CMP, CAE and CCP didn't show significant differences in anti-aging and anti-UV stress. Therefore, it was supported that CCP may be the main ingredient with anti-aging and anti-UV acute damage effects in fermentation powder form *P. hepiali*.

DEAE-52 cellulose was used as the stationary phase and solution containing specific ions was used as the mobile phase to separate the mixture components. Two completely separated peaks (CCP-1 and CCP-2) were obtained by purification of CCP with DEAE-52 cellulose column chromatography (Fig. 3).

Chromatographic column. Good symmetry was shown in Where, CCP-1 was the elution fraction of pure water and CCP-2 was the elution fraction of NaCl (30 -50 %) solution.

Therefore, CCP-1 was a neutral polysaccharide and CCP-2 was an acidic polysaccharide. Previous research reports indicated that there always both have neutral and acidic polysaccharides from Cordyceps polysaccharides with different strains what were purified with DEAE-52 cellulose column chromatography (Wang et al., 2024). This is consistent with our findings.

Purification of CCP-1 and CCP-2 with Sephadex G-100 chromatography

Different molecules had different shapes and sizes; they were separated and purified depending on the different movement speeds in the Sephadex chromatographic column. Good symmetry was shown in both elution peaks; therefore, the two purified elution peaks were collected and named CCP-1-1 (Fig.4a) and CCP-2-1 (Fig.4b) separately.

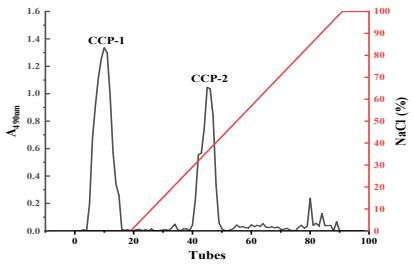


Fig.3: The elution profiles of CCP using the DEAE-52 cellulose column

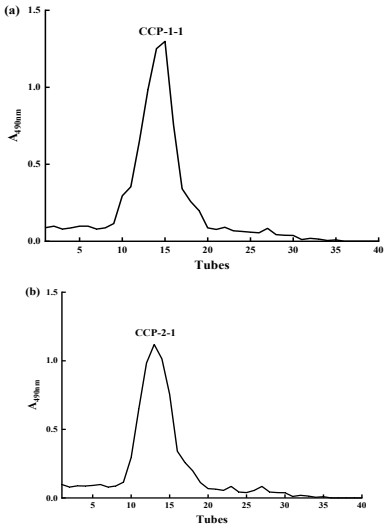


Fig.4: The elution curve of CCP-1 and CCP-2 with Sephadex G-100 chromatography. (a) Purification curve of CCP-1; (b) Purification curve of CCP-2

Monosaccharide Composition Analysis of CCP-1-1 and CCP-2-1

Accurate monosaccharide composition analysis is essential for understanding polysaccharide properties and structures, aiding in the exploration of structure-activity relationships. The monosaccharide composition of CCP-1-1 included mannose, glucose, and galactose in a molar ratio of 1:1:0.5, while CCP-2-1 comprised mannose, glucuronic acid, glucose, and galactose in a molar ratio of 1:0.27:0.16:3.11 (see Fig. 5, Table 3).

The monosaccharide composition and molar ratio of the two homogeneous polysaccharides differed significantly.

A prior study demonstrated the purification of two homogeneous polysaccharides, PHPS-D and PHPS-W, from the mycelium of *P. hepiali* through submerged fermentation.According to Wang et al. (2022), PHPS-D is primarily composed of glucuronic acid (22.5 ± 0.4%) and glucose (40.3 ± 0.5%), while PHPS-W predominantly consists of glucose (30.1 ± 0.4%) and galactose (20.6 ± 0.5%). The differing results may be due to variations in the source, variety, and extraction methods of polysaccharides, leading to differences in monosaccharide composition.Previous research indicates that polysaccharides with diverse monosaccharide compositions demonstrate varying bioactivities, with a greater variety of monosaccharide types associated with enhanced antioxidant activities (Li et al., 2020).

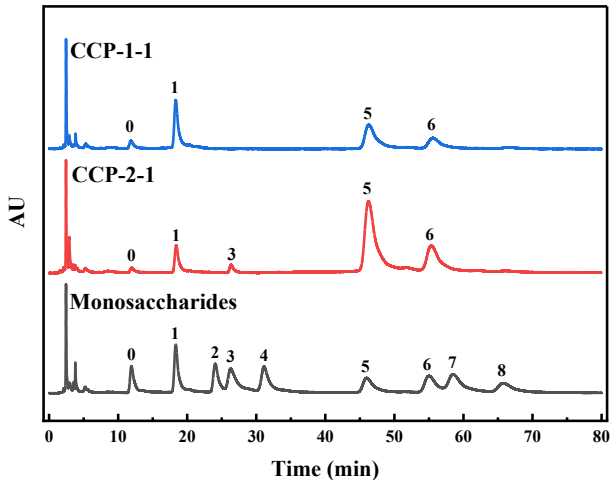


Fig.5: Monosaccharide composition analysis of CCP-1-1 and CCP-2-1. 0: PMP, 1: Mannose, 2: Rhamnose, 3: Glucuronic acid, 4: Galacturonic acid, 5: Glucose, 6: Galactose, 7: Arabinose, 8: Fucose.

Analysis using Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR analysis effectively identifies polysaccharide absorption peaks, aiding in structural determination. The spectra of CCP-1-1 and CCP-2-1 were examined within the 4000-400 cm^{-1} range. CCP-1-1 and CCP-2-1 exhibit absorption peaks around 3400 cm-1 and 1630 cm^{-1}, attributed to the stretching vibrations of hydroxyl groups in polysaccharide glycosides and carbonyl (C=O) groups.Furthermore, absorption peaks around 2935 cm^{-1} are observed, corresponding to the stretching vibrations of methyl groups (C-H). The absorption peaks between 1000-1200 cm^{-1} are attributed to the stretching vibration of C-O-C bonds in the pyran ring (Zhang et al., 2019). CCP-2-1 exhibited absorption peaks at approximately 1405 and 505 cm^{-1}, attributed to the stretching vibration of C-H bonds and the in-plane bending vibration of C-C=O bonds, respectively, in contrast to CCP-1-1. It is probably because CCP-2-1 contained small amounts of Glca. Infrared spectroscopy analysis of *P. hepiali* fermentation broth polysaccharides identified absorption peaks associated with O-H and C=O bond stretching vibrations.

Table. 3: Monosaccharide composition analysis of CCP-1-1 and CCP-2-1

Monosaccharides	Mole ratio			
	Man	Glca	Glc	Gal
CCP-1-1	1		1	0.5
CCP-2-1	1	0.27	6.16	3.11

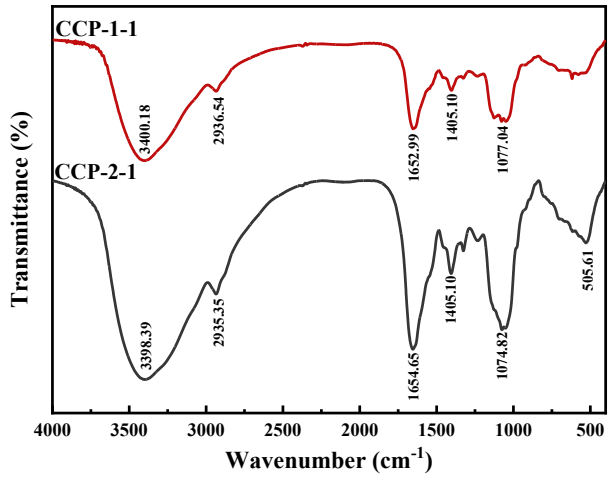


Fig. 6: FT-IR spectra of CCP-1-1 and CCP-2-1

Antioxidant Activity Analysis of CCP-1-1 and CCP-2-1 in vitro

The scavenging effects of the CCP-1-1 and CCP-2-1 on hydroxyl radicals are illustrated in Fig. 7a.In all test concentrations, CCP-2-1 hydroxyl radical scavenging activity showed a higher level than CCP-1-1.The scavenging activities of CCP-1-1 and CCP-2-1 increased with higher concentrations.At a concentration of 5 g/L, the scavenging abilities of CCP-1-1 and CCP-2-1 increased to 24.06 and 39.66%, respectively.The superior hydroxyl radical scavenging ability of CCP-2-1 may be

because CCP-2-1 contains a small amount of glucuronic acid (Ma et al., 2024).

The scavenging effects of the CCP-1-1 and CCP-2-1 on superoxide radicals are shown in Fig. 7b. The scavenging activities of CCP-1-1 and CCP-2-1 increased with higher concentrations. At 5 g/L concentration, CCP-1-1 and CCP-2-1 exhibited scavenging activities of 63.98 and 73.05%, respectively. The findings indicate that CCP-2-1 is the most effective at scavenging superoxide anion radicals.

The scavenging effects of CCP-1-1 and CCP-2-1 on DPPH• are shown in Fig. 7c. Polysaccharides exhibited enhanced scavenging activity as their concentration increased. At a concentration of 5 g/L, CCP-1-1 exhibited a scavenging activity of 45.55%, while CCP-2-1 demonstrated a higher activity of 58.05%. The findings suggest that CCP-1-1 and CCP-2-1 could function as electron or hydrogen donors to neutralize DPPH•. As concentration increased, CCP-2-1 exhibited greater DPPH• scavenging activity compared to CCP-1-1.

The capacity to chelate iron ions is demonstrated in Fig. 7d. The chelating capacities of iron ions with a 5 g/L concentration of CCP-1-1 and CCP-2-1 were 25.82 and 25.90 %. In addition, CCP-2-1 exhibited a higher ability to chelate iron ions than CCP-1-1 with a 3 g/L concentration ($p > 0.05$). The chelating capacity of CCP-1-1 and CCP-2-1 all increased with higher concentrations. It suggested that CCP-1-1 and CCP-2-1 both could chelate iron ions.

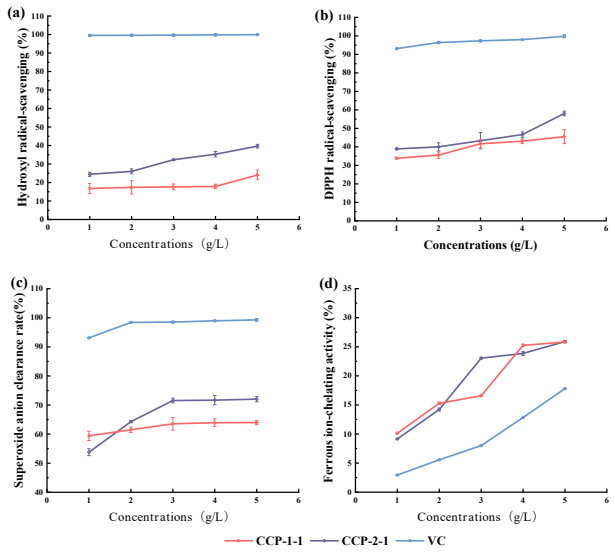


Fig. 7: In vitro antioxidant properties of CCP-1-1 and CCP-2- (b) Scavenging activities of superoxide anion radicals; (c) Scavenging activities of DPPH•; (d) Ferrous ion-chelating capacity

Eliminating harmful free radicals is essential for the antioxidant protection of cells or food systems. The chelating properties and antioxidant activities of polysaccharides can be influenced by their monosaccharide composition, molecular weight, and protein content. In vitro assays demonstrated that homogeneous polysaccharides from CCP exhibit strong antioxidant activity, aligning with previous research findings.

Impact of CCP-1-1 and CCP-2-1 on C. Elegans Longevity

Based on the fast growth rate and small feeding dose, *C. elegans* has been selected as a common model animal in recent years (Almotayri et al., 2024). Unlike CMP, CCP could be completely dissolved in water to feed *C. elegans*. Consequently, in vivo bioactivity experiments on homogeneous polysaccharides were conducted using *C. elegans* as a model organism.

The natural lifespan experiment is the most direct way to assess the anti-aging ability of polysaccharides (Pan et al.,2023). The addition of CCP-1-1 and CCP-2-1 extended the natural lifespan of *C. elegans*, as evidenced by the rightward shift in the survival curve (Fig. 8A). Both homogeneous polysaccharides significantly extended the average lifespan of *C. elegans* compared to the control group ($p<0.05$) (Fig.8B). These results suggested that CCP-1-1 and CCP-2-1 play a protective role against aging in organisms.

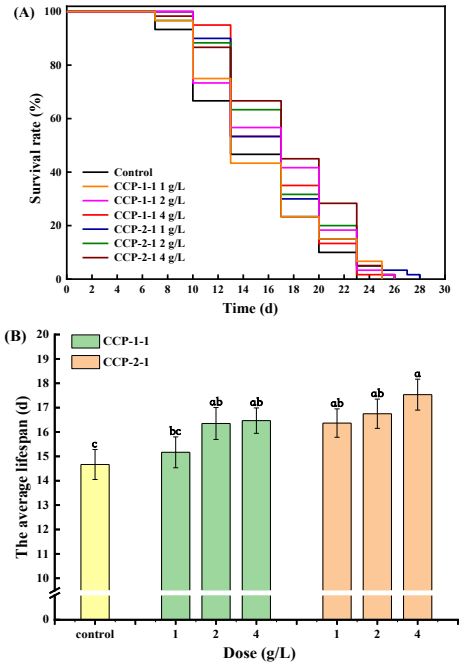


Fig. 8: Impact of CCP-1-1 and CCP-2-1 on C. elegans' natural lifespan. (A) Survival curve of C. elegans; (B) Average natural lifespan of C. elegans.

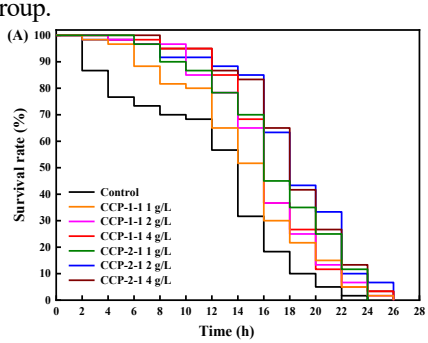
Previous research showed that the rating polysaccharides P50, P60 and P70 from Cordyceps Militaries would significantly increase the lifespan of *D. melanogaster* respectively (p<0.05) (Zhang et al., 2016).

Effect of CCP-1-1 and CCP-2-1 on C. Elegans for Cu^{2+} Stress

Industrial effluent and emission discharges lead to the accumulation of heavy metal ions in the human body via the food chain, potentially causing diseases like heart, respiratory, and neurodegenerative disorders (Hsu et al., 2018). The introduction of CCP-1-1 and CCP-2-1 resulted in a significant rightward shift of the survival curve (Fig.9A). It indicates that the lifespan of the *C. Elegans* was extended under heavy metal Cu^{2+} stress. All doses of CCP-1-1 and CCP-2-1 significantly extended the average lifespan of worms following Cu^{2+} intoxication compared to the control group (p<0.05) (Fig.9B). And the anti-heavy metal activities of CCP-1-1 and CCP-2-1 increased with higher concentrations. CCP-2-1 significantly improves lifespan compared to CCP-1-1 (p<0.05). At a high dose of 4 g/L, the average lifespan of CCP-1-1 and CCP-2-1 increased by 37.25 and 51.67%, respectively, compared to the control group. Previous studies suggest that polysaccharides can mitigate Cu^{2+} toxicity by decreasing its bioaccumulation in tissues, modulating neurotransmitters, lowering lipid accumulation, and enhancing growth performance (Jia et al., 2023). Polysaccharides from Agaricus blazei Murill were observed to mitigate histopathological damage and partially alleviate Cu^{2+} toxicity in chickens during dietary exposure(Hu et al., 2017).

Effect of CCP-1-1 and CCP-2-1 on C. Elegans for UV Stress

The introduction of CCPs resulted in rightward shifts of the survival curves for CCP-1-1 and CCP-2-1, suggesting an increased lifespan of the worms under UV stress (Fig 10A). All doses of CCP-2-1 and the high dose of CCP-1-1 (4 g/L) significantly extended the average lifespan of *C. elegans* compared to the control group (p<0.05) (Fig 10B). Moreover, the anti-UV activities of CCP-1-1 and CCP-2-1 increased with higher doses. At a high dose, the average lifespan of CCP-1-1 and CCP-2-1 increases by 7.81 and 10.35%, respectively, compared to the control group.



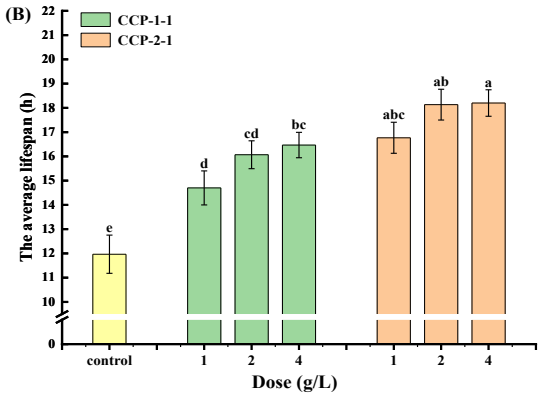
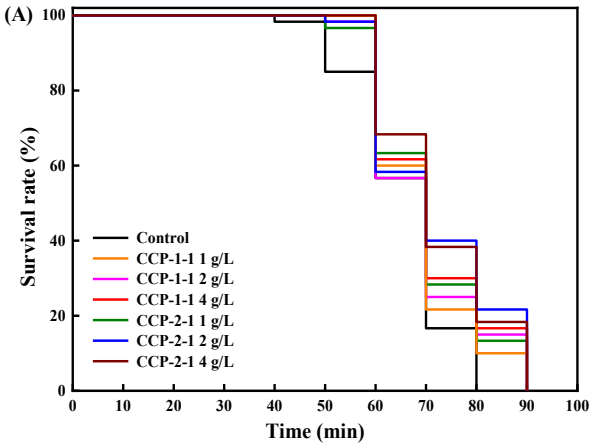


Fig. 9: Effect of CCP-1-1 and CCP-2-1 on C. elegans lifespan under Cu^{2+} exposure. (A) Survival curves and (B) average lifespan of C. elegans subjected to Cu^{2+} exposure



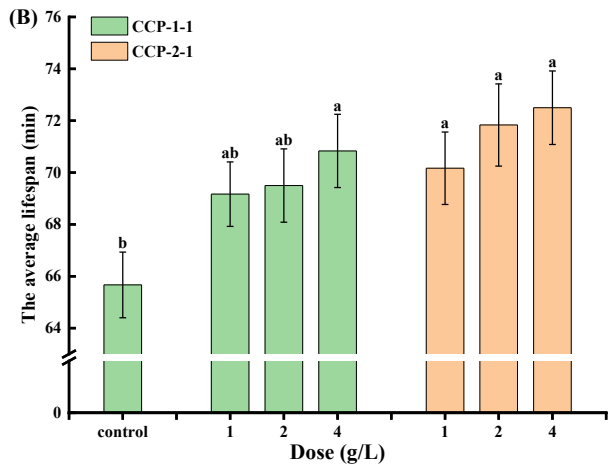


Fig. 10: Impact of CCP-1-1 and CCP-2-1 on the longevity of C. elegans when exposed to UV stress.(A) Survival curves and (B) average lifespan of C. elegans subjected to UV exposure

Previous studies indicate that polysaccharides effectively mitigate UV stress through mechanisms like enhancing SOD enzyme activity and decreasing reactive oxygen levels. Polysaccharides extracted from Dimocarpus longan Lour

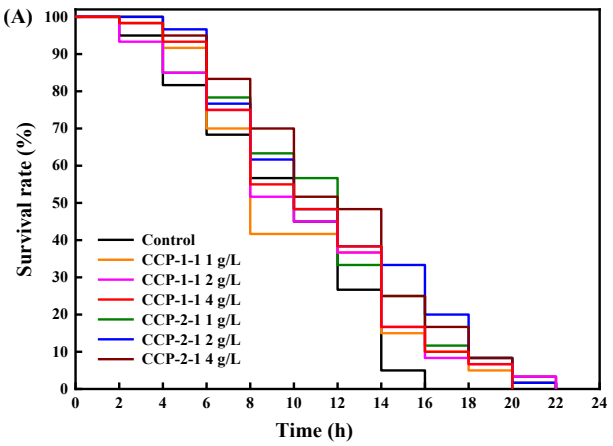
have been shown to mitigate UV-induced damage and prolong the lifespan of *C. elegans* under UV stress conditions (Ma et al., 2021).

Effect of CCP-1-1 and CCP-2-1 on C. Elegans for Heat Stress

Elevated temperatures disrupt cellular homeostasis, hinder growth and development, and can lead to animal mortality (Li et al., 2021). Previous research showed that heat stress can produce a series of physiological damage to the body effect, but heat stress pretreatment can improve the body's tolerance to withstand adverse stimulus threshold (Wang et al., 2023). The introduction of CCP-1-1 and CCP-2-1 resulted in rightward-shifted survival curves, suggesting an increased lifespan of *C. elegans* under heat stress conditions (Fig. 11A). Medium and high doses of CCP-2-1 (2 and 4 g/L) notably extended the average lifespan of *C. elegans* compared to the control group. It increased by 22.3 and 29.84 % respectively. Xu et al. (2017) demonstrated that polysaccharides from Atractylodes macrocephala Koidz alleviate heat stress-induced immune dysfunction in chicken spleens by mitigating oxidative stress, improving mitochondrial function, and inhibiting apoptosis. Polysaccharides from *Gynostemma pentaphyllum* could alleviate heat stress in mice by decreasing MDA levels and enhancing CAT, SOD, and GSH-Px content (Zhang et al.,2020).

Effect of CCP-1-1 and CCP-2-1 on the Antioxidant Index of C. Elegans

One of the key contributors to aging is reactive oxygen radicals, and their excessive buildup leads to oxidative stress, speeding up the aging process (Wang et al., 2019). CAT and SOD are the main antioxidant enzyme systems in *C. elegans* and increasing the vitality of antioxidant enzymes can scavenge excess free radicals in *C. elegans* body and improve the survival rate of *C. Elegans* (Jin et al., 2019).



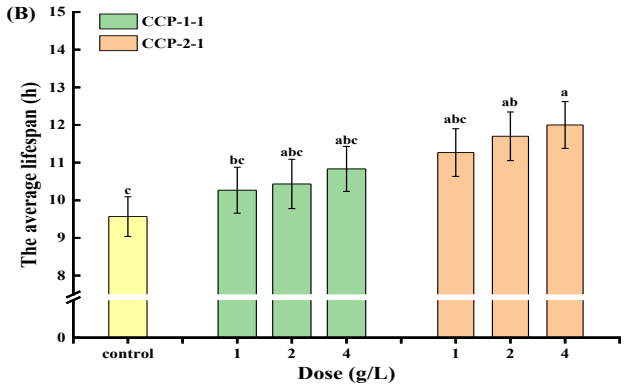


Fig. 11: Impact of CCP-1-1 and CCP-2-1 on C. elegans lifespan during heat stress. (A) Survival curves and (B) average lifespan of C. elegans subjected to heat stress

MDA is a measure of oxidative damage and responds to the degree of oxidation. Exposure to UV, heat stress, and heavy metal ions can lead to the formation of free radicals and lipid peroxidation, resulting in the production of cytotoxic MDA. This affects the mitochondrial respiratory chain complex and key enzymes, thereby influencing the organism's lifespan (Tsikas et al., 2017).

Figure 12A illustrates that CAT activities in the CCP-1-1 and CCP-2-1 groups significantly exceeded those of the control group ($p<0.05$), with further increases observed at higher doses.At high doses (4 g/L), the CAT activities in CCP-1-1 and CCP-2-1 groups increased by 143.08 and 186.45 % respectively.CCP-2-1 demonstrated greater effectiveness compared to CCP-1-1 ($p < 0.05$).

Figure 12B illustrates that SOD activities in the CCP-1-1 and CCP-2-1 groups significantly exceeded those of the control group ($p<0.05$), with further increases observed at higher doses.At high a dose (4 g/L), the SOD activities in CCP-1-1 and CCP-2-1 groups increased by 201.77 and 355.01 % respectively. CCP-2-1 demonstrated greater efficacy than CCP-1-1 ($p<0.05$).

Figure 12C illustrates that MDA levels in the CCP-1-1 and CCP-2-1 groups were significantly lower than those in the control group ($p<0.05$), with a further decrease observed at higher concentrations. At a high dose (4 g/L), MDA contents in both CCP-1-1 and CCP-2-1 groups decreased by approximately 74% compared to the control group, with no significant difference between the two groups ($p>0.05$).

These results demonstrated that CCP-1-1 and CCP-2-1 had significant antioxidant effects in vivo. They could increase SOD and CAT enzyme activities, while reducing MDA content. CCP-2-1 had greater antioxidant activities, which may be related to differences in molecular structure. Wang et al. (2019) demonstrated that an overabundance of reactive oxygen species leads to oxidative stress, thereby hastening aging. Pu observed that polysaccharides had antioxidant effects in clearing free radicals to improve oxidative stress and inhibit lipid

peroxidation (Pu et al., 2015). Our findings are consistent with previous research.CCP-1-1 and CCP-2-1 notably increase lifespan and improve stress resistance in *C. elegans*, potentially due to their strong antioxidant properties.

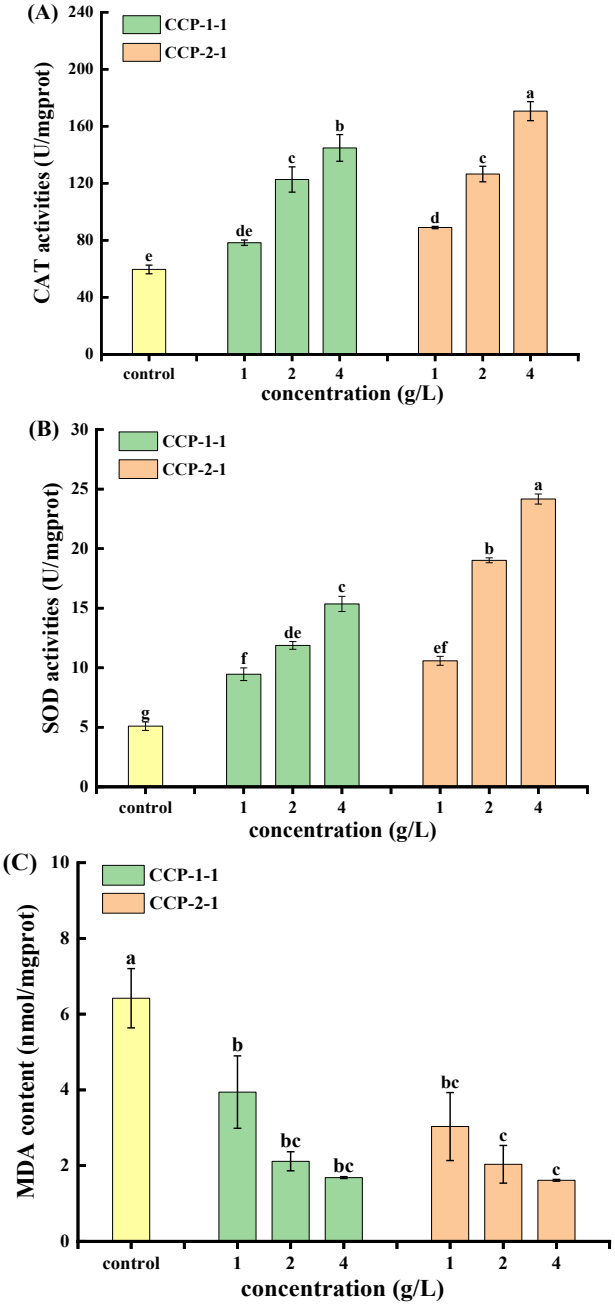


Fig.12: MDA content, along with SOD and CAT activities, were assessed for Control, CCP-1-1, and CCP-2-1.(A) Catalase (CAT) enzyme activities; (B) Superoxide dismutase (SOD) enzyme activities; (C) Malondialdehyde (MDA) content.Values are expressed as means ± SE with a sample size of n = 3

Further research is required to elucidate the detailed antioxidant mechanisms of CCPs.

Conclusion

In this paper, polysaccharides were supposed to be the uppermost active ingredient in Cordyceps mycelium powder among CMP, CAE and CCP, comparing the anti-aging and anti-acute damage roles in *D.melanogaster*. Two homogeneous poly-saccharide fractions (CCP-1-1 and CCP-2-1) were obtained from CCP using ion exchange and molecular sieve chromatography successively. They were different in molecular structures by pre-column derivatization HPLC and infrared spectroscopy. In vitro antioxidant activities were assessed by evaluating the scavenging abilities of hydroxyl (OH), 2,2-diphenyl-1-picrylhydrazyl (DPPH•), and superoxide (O^{2-}) radicals, along with measuring the reducing power. Moreover, they can significantly improve the natural lifespan and alleviate lifespan reduction of *C. elegans* caused by acute damage under UV, heat, and heavy metal stress. It may be attributed that two homogeneous polysaccharide fractions effectively reduce the free radical content by adjusting the antioxidant index *in vivo*. The biological activities of CCP-2-1 were more effective than those of CCP-1-1. These results offer a theoretical foundation for advancing and applying Cordyceps polysaccharides.

Acknowledgment

The authors wish to acknowledge Wei Li, laboratory animal center of Zhejiang University, for her help. We appreciate the publishers' assistance with this research work. We appreciate the editing team's efforts in publishing the content. We appreciate the publishers for providing the resources and platforms for disseminating the findings of our study.

Funding Information

This study was supported by the Technologies Research and Development program of Zhejiang province (LGN22C170006).

Author's Contributions

Xiaokang Hu was involved in the entire experimental process, including design, execution, analysis, and manuscript writing.
Huixiu Ma, Guicai Chen, **Dehui Da and Weilian Hu**: Participate in the part of the process of experimental design, experimental process, and result analysis.

Ethics

This article is original and contains unpublished material. All of the authors have read and approved the manuscript and no ethical issues are involved.

References

Almotayri, A., Thomas, J., Munasinghe, M., & Jois, M. (2021). The Effect of Mianserin on Lifespan of Caenorhabditis elegan is Abolished by Glucose. *Current Aging Science*, *14*(2), 118–123. https://doi.org/10.2174/1874609813999210104203614

Cai, J., Ren, X., Fan, Z., Sun, Z., Zao, X., Sun, Y., & Zhang, H. (2002). Effects of Astragalus and Cordyceps sinensis on Left Ventricular Diastolic Function and Blood Lipids in Heart Disease. *Chinese Journal of Integrated Traditional and Western Medicine in Intensive and Critical Care*, *009*(003), 174–175.

Celep, E., Aydın, A., & Yesilada, E. (2012). A comparative study on the in vitro antioxidant potentials of three edible fruits: Cornelian cherry, Japanese persimmon and cherry laurel. *Food and Chemical Toxicology*, *50*(9), 3329–3335. https://doi.org/10.1016/j.fct.2012.06.010

Chen, S., Guan, X., Yong, T., Gao, X., Xiao, C., Xie, Y., Chen, D., Hu, H., & Wu, Q. (2022). Structural characterization and hepatoprotective activity of an acidic polysaccharide from Ganoderma lucidum. *Food Chemistry: X*, *13*, 100204. https://doi.org/10.1016/j.fochx.2022.100204

Chen, Y., Zhang, Y.-P., Yang, Y., & Yang, D. (1999). Genetic Diversity and Taxonomic Implication of Cordyceps sinensis as Revealed by RAPD Markers. *Biochemical Genetics*, *37*(June 1999), 201–213. https://doi.org/10.1023/a:1018738706123

Dan, A., Hu, Y., Chen, R., Lin, X., Tian, Y., & Wang, S. (2021). Advances in research on chemical constituents and pharmacological effects of Paecilomyces hepiali. *Food Science and Human Wellness*, *10*(4), 401–407. https://doi.org/10.1016/j.fshw.2021.04.002

Hsu, H.-W., Bondy, S. C., & Kitazawa, M. (2018). Environmental and Dietary Exposure to Copper and Its Cellular Mechanisms Linking to Alzheimer's Disease. *Toxicological Sciences*, *163*(2), 338–345. https://doi.org/10.1093/toxsci/kfy025

Hu, X., Huang, X., Wang, B., Yue, Y., Zhou, F., Li, C., & Huang, F. (2015). Research progress on anti-tumour and immunomodulatory effects of Cordyceps sinensis. *Drug Evaluation Research*, *38*(04), 448–452.

Hu, X., Pan, Q., Ji, B., Aan, Y., Qi, Y., La, Z., Jin, M., Ge, S., Lang, J., De, Q., Ren, K., & Wang, S. (2024). Advances in Compositional Analysis of Cordyceps sinensis. *Chemical Analysis and Meterage*, *33*(07), 124–129.

Hu, X., Zhang, R., Xie, Y., Wang, H., & Ge, M. (2017). The Protective Effects of Polysaccharides from Agaricus blazei Murill Against Cadmium-Induced Oxidant Stress and Inflammatory Damage in Chicken Livers. In *Biological Trace Element Research* (Vol. 178, pp. 117–126). https://doi.org/10.1007/s12011-016-0905-y

Hu, Y., Wang, S.-X., Wu, F.-Y., Wu, K.-J., Shi, R.-P., Qin, L.-H., Lu, C.-F., Wang, S.-Q., Wang, F.-F., & Zhou, S. (2022). Effects and Mechanism of Ganoderma lucidum Polysaccharides in the Treatment of Diabetic Nephropathy in Streptozotocin-Induced Diabetic Rats. In *BioMed Research International* (Vol. 2022, p. 4314415). https://doi.org/10.1155/2022/4314415

Huang, Y. (2016). *Study on the Isolation, Purification, Structural Identification and Antitumor Activity of Polysaccharides from Ganoderma capense [D*.

Jia, D., Tang, Y., Qin, F., Liu, B., Hu, T., & Chen, W. (2023). Ganoderma lucidum polysaccharide alleviates Cd toxicity in common carp (Cyprinus carpio): Neuropeptide, growth performance and lipid accumulation. In *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* (Vol. 271, p. 109663). https://doi.org/10.1016/j.cbpc.2023.109663

Jiang, L., Bao, H., & Yang, M. (2010). Antitumor Activity of the Petroleum Ether Extract from the Mycelium of Paecilomyces hepiali. *Acta Edulis Fungi*, *17*(04), 58-60 10 16488 1005. https://doi.org/10.16488/j.cnki.1005-9873.2010.04.001

Jin, S. (2019). *Study on the Mechanism and Stability of Sanqi Powder in Delaying Aging of Caenorhabditis elegans.Wuhan:Hubei University of Chinese Medicine*.

Krishna, K. V., Ulhas, R. S., & Malaviya, A. (2024). Bioactive compounds from Cordyceps and their therapeutic potential. *Critical Reviews in Biotechnology*, *44*(5), 753–773. https://doi.org/10.1080/07388551.2023.2231139

Li, L., Hu, X., Huang, Y., Jia, W., Wang, X., & Guo, X. (2023). The Protective Role of Aqueous Extract of Phyllanthus emblica on the Lifespan and Climbing Capacity of Drosophila melanogaster. *Carcinogenesis,Teratogenesis & Mutagenesis*, *36*(02), 143–149.

Li, S., Liu, D., Liu, Y., Liu, B., & Chen, X. (2020). Quercetin and Its Mixture Increase the Stress Resistance of Caenorhabditis elegans to UV-B. *International Journal of Environmental Research and Public Health*, *17*(5), 1572. https://doi.org/10.3390/ijerph17051572

Li, Z., & Howell, S. H. (2021). Heat Stress Responses and Thermotolerance in Maize. *International Journal of Molecular Sciences*, *22*(2), 948. https://doi.org/10.3390/ijms22020948

Liu, J., Zhen, Y., Liu, W., Yuan, T., & Yan, J. (2024). An Accurate Method for the Determination of Fructose Content in Polysaccharides of Rhizoma Polygonati. *Food Research and Development*, *45*(21), 164–171.

Liu, L., Feng, J., Gao, K., Zhou, S., Yan, M., Tang, C., Zhou, J., Liu, Y., & Zhang, J. (2022). Influence of carbon and nitrogen sources on structural features and immunomodulatory activity of exopolysaccharides from Ganoderma lucidum. In *Process Biochemistry* (Vol. 119, pp. 96–105). https://doi.org/10.1016/j.procbio.2022.05.016

Lu, J., He, R., Sun, P., Zhang, F., Linhardt, R. J., & Zhang, A. (2020). Molecular mechanisms of bioactive polysaccharides from Ganoderma lucidum (Lingzhi), a review. *International Journal of Biological Macromolecules*, *150*, 765–774. https://doi.org/10.1016/j.ijbiomac.2020.02.035

Ma, H., Dai, D., Chen, G., & Hu, W. (2024). Protective Role of *Ganoderma lucidum* Polysaccharides Against Stress Induced by Heavy Metals in *Caenorhabditis elegans American Journal of Biochemistry and Biotechnology*, *20*(2), 112–125. https://doi.org/10.3844/ajbbsp.2024.112.125

Ma, Y. (2021). *The Mechanism of Action of Longan Extract in Stress Response Studied by Applying the Caenorhabditis elegans Model*.

Ni, Z., Li, J., Qian, X., Yong, Y., Zhang, P., Geng, Y., Wang, Y., Chen, A., & Shao, Y. (2024). Optimization of the conditions for microwave-assisted hot-water extraction of polysaccharides from Cordyceps militaris and analysis of their hypoglycemic activity. *Journal of Food Measurement and Characterization*, *18*(7), 5766–5778. https://doi.org/10.1007/s11694-024-02607-6

Park, H.-E. H., Jung, Y., & Lee, S.-J. V. (2017). Survival assays using Caenorhabditis elegans. *Molecules and Cells*, *40*(2), 90–99. https://doi.org/10.14348/molcells.2017.0017

Pu, X., Fan, W., Yu, S., Li, Y., Ma, X., Liu, L., Ren, J., & Zhang, W. (2015). Polysaccharides from Angelica and Astragalus exert hepatoprotective effects against carbon-tetrachloride-induced intoxication in mice. In *Canadian Journal of Physiology and Pharmacology* (Vol. 93, Issue 1, pp. 39–43). https://doi.org/10.1139/cjpp-2014-0331

Shi, Y., Xie, H., Zheng, S., Yu, G., Wang, F., Li, L., Zhang, B., Li, Y., & Luo, S. (2024). Analysis of Biochemical Characteristics of Local Tea Tree Germplasm in Wuyishan and the Activity of Tea Polysaccharides in Scavenging Superoxide Anion Radicals. *Journal of Agricultural Science and Technology*, *26*(05), 65–76. https://doi.org/10.13304/j.nykjdb.2023.0622

Tamagno, W. A., Santini, W., Santos, A., Alves, C., Bilibio, D., Sutorillo, N. T., Zamberlan, D. C., Kaizer, R. R., & Barcellos, L. J. G. (2022). Pitaya fruit extract ameliorates the healthspan on copper-induced toxicity of Caenorhabditis elegans. In *Journal of Food Biochemistry* (Vol. 46, Issue 3, p. e14050). https://doi.org/10.1111/jfbc.14050

Tan, X., Sun, J., Xu, Z., Li, H., Hu, J., Ning, H., Qin, Z., Pei, H., Sun, T., & Zhang, X. (2018). Effect of heat stress on production and in-vitro antioxidant activity of polysaccharides in Ganoderma lucidum. In *Bioprocess and Biosystems Engineering* (Vol. 41, pp. 135–141). https://doi.org/10.1007/s00449-017-1850-7

Tsikas, D. (2017). Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Analytical Biochemistry*, *524*, 13–30. https://doi.org/10.1016/j.ab.2016.10.021

Wang, C., An, J., Bai, Y., Li, H., Chen, H., Ou, D., & Liu, Y. (2019). Tris(1,3-dichloro-2-propyl) phosphate accelerated the aging process induced by the 4-hydroxynon-2-enal response to reactive oxidative species in Caenorhabditis elegans. *Environmental Pollution*, *246*, 904–913. https://doi.org/10.1016/j.envpol.2018.12.082

Wang, J. (2017). *Characterization of the Chemical Composition and Fine Structure of Polysaccharides in Natural Cordyceps sinensis and Fermented Cordyceps militaris Powder*.

Wang, L.-J., Zhou, L.-J., Zhu, Z.-H., Ma, W.-H., & Lei, C.-L. (2014). Differential Temporal Expression Profiles of*Heat Shock Protein*Genes in*Drosophila melanogaster* (Diptera: Drosophilidae) Under Ultraviolet A Radiation Stress. *Environmental Entomology*, *43*(5), 1427–1434. https://doi.org/10.1603/en13240

Wang, Y. (2011). *Effects of heat stress on emotion, behavior, learning and memory in mice*.

Wang, Y., Xu, F., Cheng, J., Wu, X., Xu, J., Li, C., Li, W., Xie, N., Wang, Y., & He, L. (2022). Natural Deep Eutectic Solvent-Assisted Extraction, Structural Characterization, and Immunomodulatory Activity of Polysaccharides from Paecilomyces hepiali. *Molecules*, *27*(22), 8020. https://doi.org/10.3390/molecules27228020

Wu, X., Ma, H., & Liu, N. (2009). Comparison of the Medicinal Value between Cordyceps militaris and Cordyceps sinensis. *Edible and Medicinal Mushrooms*, *17*(5), 12–14.

Wu, Z. (2015). Effect of different drying methods on chemical composition and bioactivity of finger citron polysaccharides. *International Journal of Biological Macromolecules*, *76*, 218–223. https://doi.org/10.1016/j.ijbiomac.2015.02.043

Xu, D., Li, B., Cao, N., Li, W., Tian, Y., & Huang, Y. (2017). The protective effects of polysaccharide of *Atractylodes macrocephala* Koidz (PAMK) on the chicken spleen under heat stress via antagonizing apoptosis and restoring the immune function. *Oncotarget*, *8*(41), 70394–70405. https://doi.org/10.18632/oncotarget.19709

Xu, H., Zhen., X., C., Z., & Liu, F. (2000). he Protective Effect of Cordyceps Sinensis on Doxorubicin-Induced Cardiac Injury. *Acta Chinese Medicine and Pharmacology*, *03*, 64–65. https://doi.org/10.19664/j.cnki.1002-2392.2000.03.063

Xu, J., Huang, Y., Chen, X.-X., Zheng, S.-C., Chen, P., & Mo, M.-H. (2016). The Mechanisms of Pharmacological Activities of*Ophiocordyceps sinensis*Fungi. *Phytotherapy Research*, *30*(10), 1572–1583. https://doi.org/10.1002/ptr.5673

Xu, M., Zhu, Y., Su, D., Song, Y., Zhang, W., Xu, L., Ma, Q., Liu, Z., & Guo, S. (2024). The Effects of the Seed Oil of Mosla chinensis "Jiangxiangru" on the Sleep, Olfactory Capacity and Antioxidant Indexes of Drosophila melanogaster. *Acta Laboratorium Animalis Scientia Sinica*, *32*(09), 1182–1190.

Ye, M., Qiu, T., Peng, W., Chen, W., Ye, Y., & Lin, Y. R. (2011). Purification, characterization and hypoglycemic activity of extracellular polysaccharides from Lachnum calyculiforme. In *Carbohydrate Polymers* (Vol. 86, Issue 1, pp. 285–290). https://doi.org/10.1016/j.carbpol.2011.04.051

Ye, W., Zhang, L., Wang, D., He, Y., Li, N., Jiang, J., & Ma, Y. (2022). Effects of Viscum coloratum (Kom.) Nakai f. Lutescens Kitag polysaccharide on fertility, longevity and antioxidant capacity of Drosophila melanogaster. *Food Science and Technology*, *42*, 1–10. https://doi.org/10.1590/fst.09721

Zhang, C., Fu, L., Yang, X., Li, K., & Xin, J. (2015). The Research Advances on the Relationship between Reactive Oxygen Free Radicals and Cell Senescence. *Guangzhou Chemical Industry*, *43*(19), 5–7.

Zhang, J., Zhao, P., Li, B., Huang, C., & Tan, H. (2013). Animal Experimental Study on the Immune Enhancement Function of Paecilomyces hepiali. *Journal of Guangxi Medical University*, *30*(04), 520–522. https://doi.org/10.16190/j.cnki.45-1211/r.2013.04.008

Zhang, S., He, F., Chen, X., & Ding, K. (2019). Isolation and structural characterization of a pectin from Lycium ruthenicum Murr and its anti-pancreatic ductal adenocarcinoma cell activity. *Carbohydrate Polymers*, *223*, 115104. https://doi.org/10.1016/j.carbpol.2019.115104

Zhang, W., Gong, T., Han, D., Wang, D., & Zhan, J. (2016). A Comparative Study on the Components of Artificial Cordyceps and Cordyceps Sinensis. *Beijing Journal Of Traditional Chinese Medicine*, *35*(01), 87–91.

Zhang, W., Zhi, H., & Sun, H. (2020). Effects of Heavy Metal Pollution on Fitness and Swimming Performance of Bufo raddei Tadpole. In *Bulletin of Environmental Contamination and Toxicology* (Vol. 105, pp. 387–392). https://doi.org/10.1007/s00128-020-02953-3

Zhao, Y., Li, Q., Wang, M., Wang, Y., Piao, C., Yu, H., Liu, J., & Li, Z. (2023). Structural characterization of polysaccharides after fermentation from Ganoderma lucidum and its antioxidant activity in HepG2 cells induced by H2O2. In *Food Chemistry: X* (Vol. 18, p. 100682). https://doi.org/10.1016/j.fochx.2023.100682

Zhou, Q., Xiao, X., & Lin, R. (2009). The Research Status of Liquid Deep Fermentation Mycelia Related Preparations of Cordyceps Sinensis. *Chinese Journal of Pharmaceutical Analysis*, *29*(04), 680–687.

Zuo, S., Liu, L., Wang, Y., & Tao, J. (2024). Patent Analysis on the Artificial Cultivation and Processing of Cordyceps Sinensis in China. *Edible and Medicinal Mushrooms*, *32*(05), 316–324.