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ARTICLE

Extraction of Polyphenols from Herbaceous Peony Petals and Evaluation of *In Vitro* and *In Vivo* Antioxidant Activity

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ABSTRACT: Herbaceous peony (*Paeonia lactiflora* Pall.) is a traditional Chinese flower with high ornamental value. However, the antioxidant value of *P. lactiflora* petals has not been fully utilized, and the petals are often discarded as waste after flowering. Plant petals are typically rich in polyphenolic compounds with potent antioxidant properties, but research on the antioxidant properties of *P. lactiflora* petals remains insufficient. To investigate the antioxidant effects of *P. lactiflora* petal polyphenol extract (PPP), this study first determined and compared the PPP content of seven *P. lactiflora* cultivars, and then evaluated their antioxidant effects through *in vitro* and *in vivo* assays. Among the seven cultivars, the petals of *P. lactiflora* 'Dan Feng' exhibited the highest polyphenol content, reaching 81.38 mg/g, which was significantly higher than that of the other cultivars. PPP exhibited strong antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals, with scavenging rates of 84.31% and 92.40% at 100 μg/mL, respectively. In contrast, its hydroxyl free radical (·OH) scavenging activity was relatively weaker, with a scavenging rate of 48.02% at 1 mg/mL, significantly lower than that of vitamin C. Furthermore, the *in vivo* antioxidant effects of PPP were evaluated using *Caenorhabditis elegans*. The results showed that PPP enhanced locomotion, improved resistance to heat and oxidative stress, increased antioxidant enzyme activity, reduced intestinal lipofuscin accumulation and the levels of reactive oxygen species (ROS), and extended lifespan without significantly affecting reproduction. These results indicate that PPP possesses strong antioxidant activity, providing a theoretical foundation for the development and utilization of *P. lactiflora* petals.

KEYWORDS: *Paeonia lactiflora*; petal; polyphenol extract; *Caenorhabditis elegans*; antioxidant activity

1 Introduction

Paeonia lactiflora Pall. is a renowned plant species native to China. It holds significant value in horticulture, landscaping, and the cut flower industry. Current research on *P. lactiflora* focuses primarily on ornamental value, stress tolerance, genetic breeding, chemical composition, and pharmacological activity [1]. Meanwhile, the exploration and utilization of bioactive compounds in *P. lactiflora* have mainly centered on the roots. For example, Sun et al. isolated and identified over 50 monoterpene glycosides from *Radix Paeoniae Rubra*, among which paeoniflorin was the most abundant [2]. However, as petals are also rich in bioactive compounds, it is frequently observed that *P. lactiflora* petals are often discarded as waste after flowering during production, which leads to resource wastage. Plant petals are rich in active substances, such as polyphenolic compounds, lipophilic carotenoids, and terpenoids [3]. Research indicates that *P. lactiflora* petals are rich in diverse chemical constituents, including phenolic acids, flavonoids, fatty acids, volatile compounds, and other components such as paeonol and benzoic acid. These components confer

notable medicinal benefits, comprising antioxidative, antibacterial, anti-inflammatory, anti-hyperuricemic, and anti-aging activities [4]. Nevertheless, existing investigations into the antioxidant activity of *P. lactiflora* petals is mostly limited to *in vitro* chemical assays. There has been no systematic comparison of polyphenol content differences among different varieties, and studies evaluating *in vivo* antioxidant effects using live models such as *Caenorhabditis elegans* are lacking.

Polyphenolic compounds in *P. lactiflora* are among the secondary metabolites produced during its growth and development, exhibiting potent antioxidant effects [5]. Polyphenols mainly include phenolic acids, flavonoids, stilbenoids, and lignans [6]. Their composition and concentration vary among different plants, and there are even significant differences between different organs of the same plant [7]. Polyphenols are widely distributed in the flowers, fruits, leaves, seeds, bark, and roots of plants, with particularly high concentrations in tissues that are brightly colored or possess defensive functions, such as petals, fruits peels, tea leaves, and grape seeds [8–11]. Polyphenols exhibit antioxidant, anti-inflammatory, anti-obesity, neuroprotective, and cardioprotective activities. Among these, their reducing properties enable reactive oxygen species (ROS) scavenging [12]. Processes such as cellular damage, and even organismal aging and stress adaptation, are influenced by ROS levels. Therefore, identifying natural antioxidants to protect organisms from damage induced by ROS has been a research focus. When ROS production exceeds the scavenging capacity of the endogenous antioxidant system, oxidative stress occurs, leading to cellular damage. Due to their powerful antioxidant activity, plant polyphenols have garnered significant attention. Research indicates that plant petals are rich in polyphenolic compounds. These compounds can effectively scavenge excess free radicals in the body. As reported by Kanth et al. [13], pink *Camellia japonica* L. petals contain abundant phenolic compounds that exhibit significant ROS-scavenging and antioxidant activities. This provides a valuable reference for further investigating the antioxidative properties of polyphenolic compounds in *P. lactiflora* petals.

C. elegans is a eukaryotic model organism that feeds on microorganisms in its environment. Its advantages, including a short life cycle, small size, optical transparency, ease of cultivation and low cost, make it an excellent *in vivo* system for assessing polyphenolic antioxidant activity [14]. A large body of research has employed *C. elegans* to evaluate the antioxidative potential of polyphenols from various sources [15]. Hou et al. [16] found that polyphenols from *Eucommia ulmoides* Oliv. prolonged its survival time and enhanced oxidative defense of *C. elegans*. Similarly, polyphenolic compounds isolated from *Rosa roxburghii* Tratt. pomace were shown to improve the survival rates and reduce ROS level of *C. elegans* [17].

In this study, we first evaluated the *in vitro* radical scavenging efficiency of polyphenols extracted from petals of *P. lactiflora* against hydroxyl, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radicals. Then, we utilized the *C. elegans* to look into the antioxidant activity of *P. lactiflora* petal polyphenol extract (PPP) by assesssing its effects on *C. elegans* lifespan, reproduction, locomotion behavior, heat stress resistance, oxidative stress resistance, intestinal autofluorescence, the levels of ROS, and antioxidant enzyme activity. This work evaluated the antioxidant function of PPP, and laid a basis for applying the antioxidant value of polyphenolic compounds in *P. lactiflora* petals.

2 Materials and Methods

2.1 Materials and Chemicals

The plant materials were *P. lactiflora* cultivars ‘Yangfei Chuyu’, ‘Da Fugui’, ‘Qiao Ling’, ‘Zi Fengyu’, ‘Sha La’, ‘Yali Shanda’, and ‘Dan Feng’. These plants were four-year-old and cultivated in the peony germplasm resource garden of Yangzhou University, China (32°39′ N, 119°2′ E). These cultivars were widely

grown in Yangzhou, encompassing major flower colors (white, pink, and purple-red) and diverse floral forms (single, semi-double, and double), with distinct differences in their morphological characteristics. All cultivars were managed under standard field practices, including timely irrigation, intertillage weeding, and pre-flowering topdressing with compound fertilizer. At full bloom, flowers were collected, and only the petals were retained. The petals were dried at 65°C, and then stored in resealable bags in a cool and dark place. *Escherichia coli* OP50 and *C. elegans* wild-type strain N2 were preserved in the laboratory of Floriculture Physiology and Molecular Biology, Yangzhou University (Yangzhou, China). Folin-Ciocalteu reagent, 5-fluoro-2′-deoxyuridine (FUDR) were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). ABTS and DPPH were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Juglone was purchased from Beijing Coolaber Technology Co., Ltd. (Beijing, China). Levamisole hydrochloride and 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). The catalase (CAT) assay kit and the total superoxide dismutase (SOD) assay kit were obtained from Suzhou Tecmin Biotechnology Co., Ltd. (Suzhou, China). All other chemical reagents were of analytical grade.

2.2 Extraction of Polyphenols from Petals of Different P. lactiflora Cultivars

1 g of dried *P. lactiflora* petals from different cultivars was weighed and ground. The *P. lactiflora* petals were extracted with 43% (v/v) aqueous ethanol at a solid-to-liquid ratio of 1:46 (w/v), ultrasonically extracted at 40°C and 200 W for 31 min. This procedure was performed in triplicate, and the pooled filtrates were concentrated to ethanol-free solution under vacuum in a rotary evaporator at 40°C [18], then lyophilized to yield powdered PPP, which was stored in a dry environment.

2.3 Determination of Polyphenol Content

An appropriate dilution of the PPP solution (1 mL) was combined with 2.5 mL of Folin-Ciocalteu reagent. Following a 5-min incubation, 2.0 mL of 7.5% Na_2CO_3 solution and 4.5 mL of water were introduced. Absorbance at 760 nm was recorded following a 60-min incubation in the dark at 25°C. Prepare standard gallic acid solutions spanning 1 to 6 μg/mL, process them simultaneously under the same conditions as above, measure their absorbance, and plot a standard curve. Polyphenol content was given in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW). The above procedure was repeated three times.

2.4 Hydroxyl Free Radical (·OH) Scavenging Activity

The ·OH scavenging activity was assessed with slight modifications to methods by Ai et al. [19]. The stock solution of PPP (100 mg/mL) was obtained by dissolving 1 g of PPP powder in a small amount of anhydrous ethanol and then diluting to 10 mL with distilled water. Gradient concentrations of sample solutions were obtained by taking 20, 40, 60, 80, and 100 μL of the stock solution and diluting each to 10 mL with distilled water, respectively. Using distilled water as solvent, 6 mM solutions of $FeSO_4$ (168 mg/100 mL), H_2O_2 (615 μL 30% stock/1 L), and salicylic acid (83 mg/100 mL) were prepared. All three reagent solutions were prepared immediately before use. To 1 mL of sample solution, 1 mL each of 6 mM $FeSO_4$, 6 mM H_2O_2, and 6 mM salicylic acid were added. The mixture was maintained at 37°C for 30 min, followed by 25 min at room temperature. Absorbance was read at 510 nm, using distilled water as the blank. The reference standard was vitamin C (Vc), which was prepared in ultrapure water at the same concentration range as the samples. The above procedure was repeated three times. Eq. (1) was used to calculate the ·OH scavenging activity:

$$\cdot OH \text{ Scavenging activity } (\%) = [1 - (A_1 - A_3)/A_2] \times 100 \quad (1)$$

In this equation, A_1, A_2, A_3 were the absorbance of the sample group, the distilled water substituted the sample group, the distilled water substituted the H_2O_2 group, respectively. The IC_{50} value was used to express the antioxidant capacity.

2.5 DPPH Free Radical Scavenging Activity

The DPPH scavenging activity was measured with slight modifications to previously described methods by Cao [20] and Wang et al. [21]. The sample stock solution was prepared according to Section 2.4. Gradient concentrations of sample solutions were obtained by taking 2, 4, 6, 8, and 10 μL of the stock solution and diluting each to 10 mL with distilled water, respectively. To obtain a 0.1 mM DPPH working solution, 16 mg of DPPH powder was dissolved in 200 mL of anhydrous ethanol and then diluted to 400 mL with distilled water. A reaction mixture was prepared by combining the sample solution (0.5 mL) with 0.1 mM DPPH solution (2.5 mL). The reaction proceeded in the dark at room temperature for 30 min. Absorbance was read at 517 nm, using distilled water as the blank. The positive control was Vc, which was prepared in ultrapure water at the same gradient concentrations as the sample solutions. The above procedure was repeated three times. Eq. (2) was used to calculate the DPPH scavenging activity:

$$\text{DPPH Scavenging activity } (\%) = [1 - (A_1 - A_3)/A_2] \times 100 \quad (2)$$

In this equation, A_1, A_2, A_3 were the absorbance of the sample group, the distilled water substituted the sample group, the 50% ethanol substituted the DPPH ethanol solution group, respectively. The IC_{50} value was used to express the antioxidant capacity.

2.6 ABTS Free Radical Scavenging Activity

ABTS free radical scavenging activity was measured following a slightly modified version of the procedure reported by Meng et al. [22]. The sample stock solution and gradient concentrations of sample solutions were made following the procedure in Section 2.5. The ABTS working solution was obtained fresh by keeping 5 mL of 7 mM ABTS and 88 μL of 140 mM potassium persulfate (both dissolved in distilled water) at room temperature in darkness for 14 h, subsequently diluting with distilled water to an A_{734} of 0.70 ± 0.02. A reaction system was obtained by combining the sample (0.5 mL) with 7 mM ABTS working solution (3 mL). The reaction proceeded in the dark at room temperature for 1 h. Absorbance was measured at 734 nm relative to a distilled water blank. The positive control was Vc, which was prepared in ultrapure water at the same gradient concentrations as the sample solutions. The above procedure was repeated three times. Eq. (3) was used to calculate the ABTS scavenging activity:

$$\text{ABTS Scavenging activity } (\%) = [1 - (A_1 - A_3)/A_2] \times 100 \quad (3)$$

In this equation, A_1, A_2, A_3 were the absorbance of the sample solution group, the distilled water substituted the sample group, the distilled water substituted the ABTS working solution group, respectively. The IC_{50} value was used to express the antioxidant capacity.

2.7 Culture and Synchronization of C. elegans

Wild-type *C. elegans* strain N2 was cultured at 20°C on nematode growth medium (NGM) plates inoculated with *E. coli* OP50 as a food source. The bleaching method was used to obtain nematodes at a uniform developmental stage. Pregnant nematodes were harvested from plates using M9 buffer and exposed to 1 mL bleach solution for 5 min to lyse adult bodies and release eggs. After three washes with M9 buffer, the collected eggs were placed onto fresh NGM plates inoculated with OP50 and incubated at 20°C. After approximately 2 d, *C. elegans* reached the L4 stage.

2.8 Safety Evaluation of PPP on C. elegans

L4-stage synchronized nematodes were placed onto NGM plates supplemented with 0.1, 0.5, and 1 mg/mL PPP. The control group (CK) received no PPP. For each concentration, 10 worms were randomly chosen for testing, with each nematode considered as one biological replicate. After incubation for 24 h at 20°C, survival rate, body length, head thrashes frequency, and body bending frequency were measured. Nematode death was defined as the absence of motor response when touched with a platinum wire pick [23]. For body length measurement, an inverted microscope equipped with a micrometer was used to observe nematodes mounted on 2% agarose pads. A head thrash referred to a complete side-to-side swing of the head across the body midline and back. A body bend referred to a full-body wave along the longitudinal axis completing one wavelength amplitude [24]. Both were counted for 20 s.

2.9 Effects of PPP on the Lifespan of C. elegans

L4-stage synchronized nematodes were placed onto NGM plates supplemented with PPP (0.1, 0.5, 1 mg/mL) or control plates, with 30 nematodes per concentration and 3 biological replicates. All plates were supplemented with 50 μM FUDR to inhibit nematode reproduction [25]. The *C. elegans* were cultured at 20°C and moved to fresh corresponding plates daily, and survival was assessed daily until no survivors remained. The maximal lifespan was the average lifespan of the last 10% *C. elegans* [26].

2.10 Effects of PPP on the Reproduction of C. elegans

L4-stage synchronized nematodes were placed onto NGM plates supplemented with PPP (0.1, 0.5, 1 mg/mL) or control plates, with 1 nematode per concentration and 5 biological replicates. Nematodes were moved to new corresponding plates daily. The original plates were incubated at 20°C for 2~3 d to enable egg hatching. The number of larvae on each plate was enumerated. Daily counts were summed to obtain the brood size [27].

2.11 Effects of PPP on the Locomotion Behavior of C. elegans

L4-stage synchronized nematodes were cultured on NGM plates supplemented with PPP (0.1, 0.5, 1 mg/mL) or control plates at 20°C, transferred daily. After 5 d, 10 nematodes were selected from each group randomly, with each nematode considered as one biological replicate. They were placed on blank NGM plates, which were overlaid with 500 μL of M9 buffer. After allowing them to settle for 1 min, head thrashes and body bends were enumerated within 20 s.

2.12 Effects of PPP on the Intestinal Autofluorescence of C. elegans

L4-stage synchronized nematodes were grown on NGM plates supplemented with PPP (0.1, 0.5, 1 mg/mL) or control plates at 20°C, transferred daily. After 5 d, 3 nematodes were randomly selected from

each group and moved to a 2% agarose pad, anesthetized with 5 μM levamisole hydrochloride, and covered with a coverslip. Each nematode was considered a single biological replicate, resulting in a total of three biological replicates. The intestinal autofluorescence was observed with a fluorescence microscope. ImageJ software was employed for quantification of the fluorescence signal [28].

2.13 Effects of PPP on the Heat Stress Resistance of C. elegans

L4-stage synchronized nematodes were grown on NGM plates containing PPP (0.1, 0.5, 1 mg/mL) or control plates, with 30 nematodes per concentration and 3 biological replicates. Each group was cultured at 20°C for 3 d, transferred daily. Nematodes were then relocated onto blank NGM plates and placed under 35°C conditions. Survival rates were recorded every hour until all individuals had perished [29].

2.14 Effects of PPP on the Oxidative Stress Resistance of C. elegans

After 3 d of PPP exposure as described in Section 2.13, nematodes were moved to NGM plates containing 400 μM juglone, with 30 nematodes per concentration and 3 biological replicates. Plates were kept at 20°C, and survival was recorded every hour until all individuals had perished [30].

2.15 Effects of PPP on the ROS Levels of C. elegans

L4-stage synchronized nematodes were grown on NGM plates supplemented with PPP (0.1, 0.5, 1 mg/mL) or control plates at 20°C, with 60 nematodes per group, and transferred daily. After 3 d, the *C. elegans* were rinsed with M9 buffer, collected into microcentrifuge tubes. Once the nematodes had settled, the liquid phase was discarded, and the sediment was reconstituted in M9 buffer to 100 μL. Then, 1 μL of 10 mM H_2DCFDA was introduced and mixed thoroughly. The mixtures were incubated for 30 min at 37°C under dark conditions, centrifuged at 3000 rpm for 3 min to remove the supernatant [31]. After three washes with M9 buffer, the nematodes were placed onto a 2% agarose pad, immobilized with 5 μM levamisole hydrochloride, and covered with a coverslip. 3 nematodes were selected from each group for observation randomly. Each nematode was considered a single biological replicate, resulting in a total of three biological replicates. The fluorescence images were observed with a fluorescence microscope. ImageJ software was employed for quantification of the fluorescence signal.

2.16 Effects of PPP on the Antioxidant Enzyme Activity of C. elegans

L4-stage synchronized nematodes were grown on NGM plates supplemented with PPP (0.1, 0.5, 1 mg/mL) or control plates at 20°C, with approximately 5000 nematodes per group. After 3 d, the nematodes were harvested using M9 buffer, pelleted by gravity in microcentrifuge tubes, and left to sediment. The supernatants were removed [32]. The levels of SOD and CAT in the nematodes were determined using the commercial kits, following the manufacturer's protocols. The experiment was performed with 3 biological replicates.

2.17 Statistical Analysis

All data are presented as the mean ± standard deviation (SD) of at least three biological replicates. Graphs and statistical analyses were performed using the GraphPad Prism 10.1 software (GraphPad software, San Diego, CA, USA). Prior to statistical comparisons, the Shapiro-Wilk test was performed to check normality, and the Brown-Forsythe test to verify variance homogeneity. Groups that passed both tests were compared using one-way analysis of variance (ANOVA) with Tukey's test. Those that failed normality were

analyzed by the Kruskal-Wallis H test with Dunn's test for multiple comparisons. Statistical significance was set at $p < 0.05$.

3 Results

3.1 Determination of Polyphenol Content

The absorbance values were assayed by the Folin-Ciocalteu reagent method, with a calibration curve for gallic acid generated as y = 0.1337x + 0.0144 (R^2 = 0.9977).

The polyphenol content varied among different *P. lactiflora* cultivars (Table 1). The order of content from highest to lowest was: 'Dan Feng', 'Da Fugui', 'Zi Fengyu', 'Yali Shanda', 'Qiao Ling', 'Sha La', and 'Yangfei Chuyu'. Specifically, the lowest polyphenol content was found in 'Yangfei Chuyu' petals at 39.42 mg GAE/g. The highest was found in 'Dan Feng' petals at 81.38 mg GAE/g. 'Da Fugui' ranked second highest at 76.74 mg GAE/g. Significant differences existed among the tested cultivars, with the highest value being approximately twice that of the lowest. These results indicated that the cultivar was one of the important factors affecting PPP content.

Table 1: Polyphenol content in petals of different *P. lactiflora* cultivars.

Cultivars	Polyphenol Content (mg GAE/g)
'Yangfei Chuyu'	39.42 ± 0.06^{g}
'Da Fugui'	76.74 ± 0.18^{b}
'Qiao Ling'	60.28 ± 2.56^{e}
'Zi Fengyu'	71.92 ± 0.97^{c}
'Sha La'	50.96 ± 0.30^{f}
'Yali Shanda'	64.92 ± 1.22^{d}
'Dan Feng'	81.38 ± 0.21^{a}

Values represented mean ± SD (n = 3 biological extraction replicates). Distinct superscripts reflect statistical significance based on one-way ANOVA with Tukey's test ($p < 0.05$).

3.2 In Vitro Antioxidant Activities of PPP

Plant extracts rich in antioxidants such as phenolics can effectively scavenge free radicals and exert significant regulatory effects on signaling pathways associated with oxidative stress [33]. Therefore, the free radical scavenging ability of PPP was further examined by ·OH, DPPH, and ABTS radical scavenging assays. Vc served as the reference standard for comparing antioxidative efficacy. Specifically, as the concentration increased from 0.2 to 1.0 mg/mL, the ·OH scavenging activity increased from 22.96% to 48.02% (Fig. 1a). Similarly, when the concentration increased from 20 to 100 μg/mL, the DPPH and ABTS scavenging activity increased from 58.17% and 23.2% to 84.31% and 92.40%, respectively (Fig. 1b,c). Meanwhile, Vc always displayed markedly higher radical scavenging capacities than PPP at equivalent concentrations as a positive control. On this basis, the IC_{50} values of PPP for ·OH, DPPH, and ABTS radicals were calculated to be 1.16 mg/mL, 12.9 μg/mL and 38.38 μg/mL, in the order named. These results confirmed that PPP exhibited potent DPPH and ABTS scavenging activity, while ·OH scavenging activity was relatively weaker. Moreover, the scavenging abilities of PPP showed concentration-dependent scavenging activity. PPP exhibited concentration-dependent scavenging effects in all three free radicals.

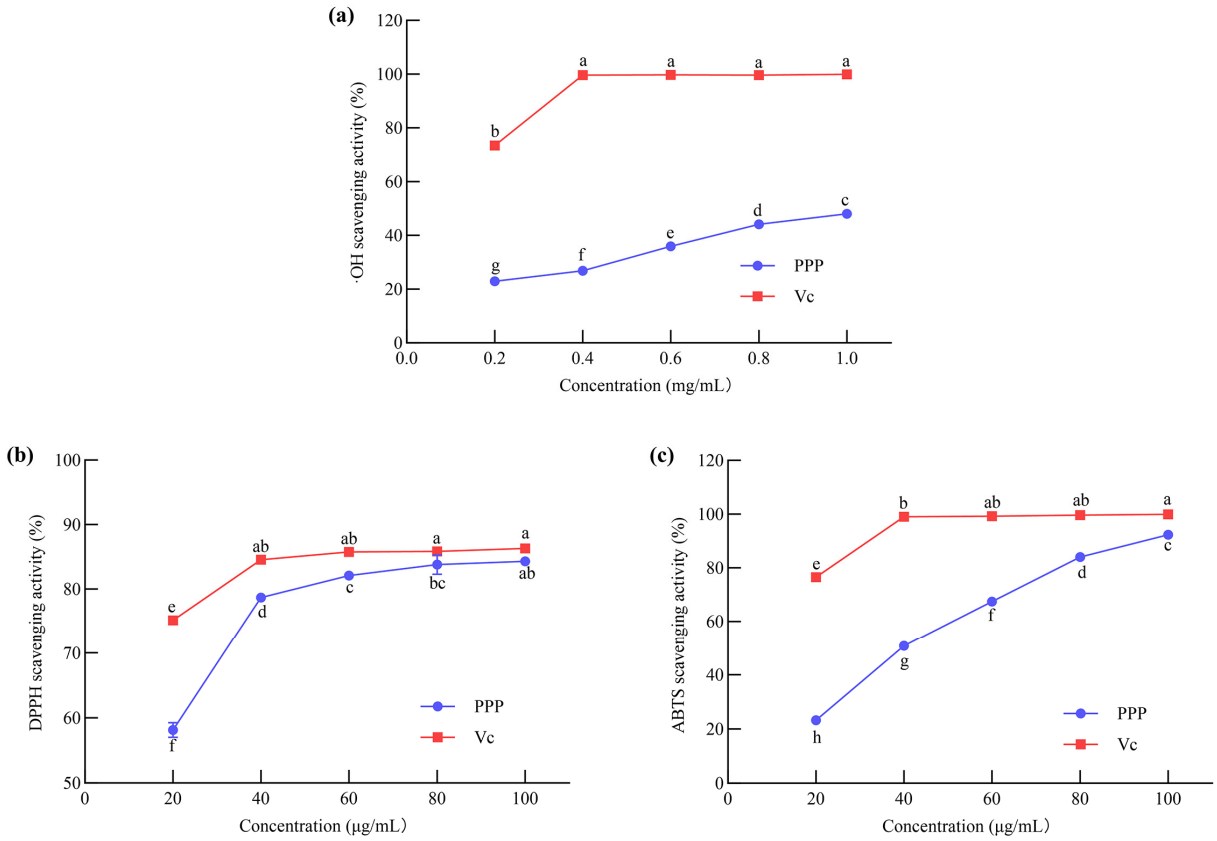

Figure 1: Antioxidant activities of *P. lactiflora* petal polyphenol extract *in vitro*. (**a**) ·OH radical scavenging activity, (**b**) DPPH radical scavenging activity, (**c**) ABTS radical scavenging activity. PPP, *P. lactiflora* petal polyphenol extract; Vc, vitamin C. Values represented mean ± SD (n = 3 biological replicates). Distinct superscripts reflect statistical significance based on one-way ANOVA with Tukey's test ($p < 0.05$).

3.3 PPP Toxicity Assessment of C. elegans

To assess the potential toxicity of PPP towards *C. elegans*, the survival rate, body length, head thrashes, and body bends were measured on NGM plates containing graded concentrations of PPP. The survival remained 100% across all groups (Fig. 2a). Relative to the control group, body length of PPP-treated *C. elegans* showed no significant change (Fig. 2b). Although head thrashes and body bends decreased as PPP levels rose from 0.1 to 1.0 mg/mL, these differences were not statistically significant (Fig. 2c,d). As such, PPP exhibited no toxic effects on *C. elegans* across the concentration range of 0.1–1.0 mg/mL, making it suitable for subsequent antioxidant studies.

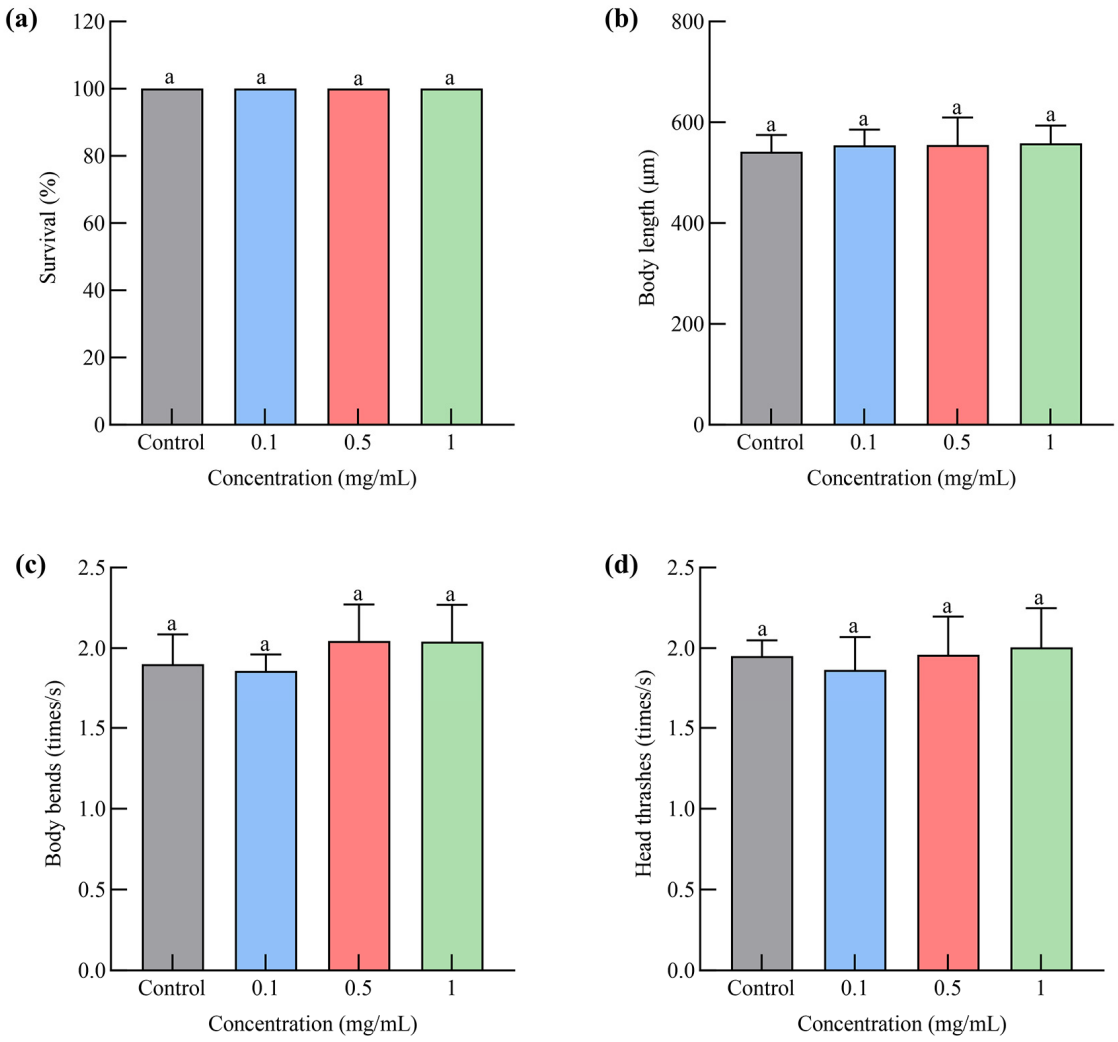

Figure 2: Influences of *P. lactiflora* petal polyphenol extract on the toxicity profile of *C. elegans*. (**a**) survival rate, (**b**) body length, (**c**) body bends, (**d**) head thrashes. Values represented mean ± SD (n = 10 per group). Distinct superscripts reflect statistical significance based on one-way ANOVA with Tukey's test (**a**,**c**) or Kruskal-Wallis H test with Dunn's test (**b**,**d**) ($p < 0.05$).

3.4 PPP on Lifespan of C. elegans

All three PPP concentrations (0.1, 0.5, and 1.0 mg/mL) shifted the survival curve of *C. elegans* to the right, indicating an extension of both mean and maximal lifespans (Fig. 3a). The mean lifespan increased with PPP concentration, reaching 13.96 and 14.84 d under 0.5 and 1.0 mg/mL treatments, both markedly surpassing the control group (Fig. 3b). A similar concentration-dependent trend was observed for maximal lifespan. The maximal lifespan of *C. elegans* exposed to 0.5 and 1.0 mg/mL PPP was markedly prolonged relative to the control. Notably, the longest maximal lifespan, 21.56 d, was observed in the 1.0 mg/mL PPP treatment group, which was approximately 3.23 d longer than that of the control (Fig. 3c). Such findings strongly suggested that PPP effectively promoted lifespan extension in *C. elegans*.

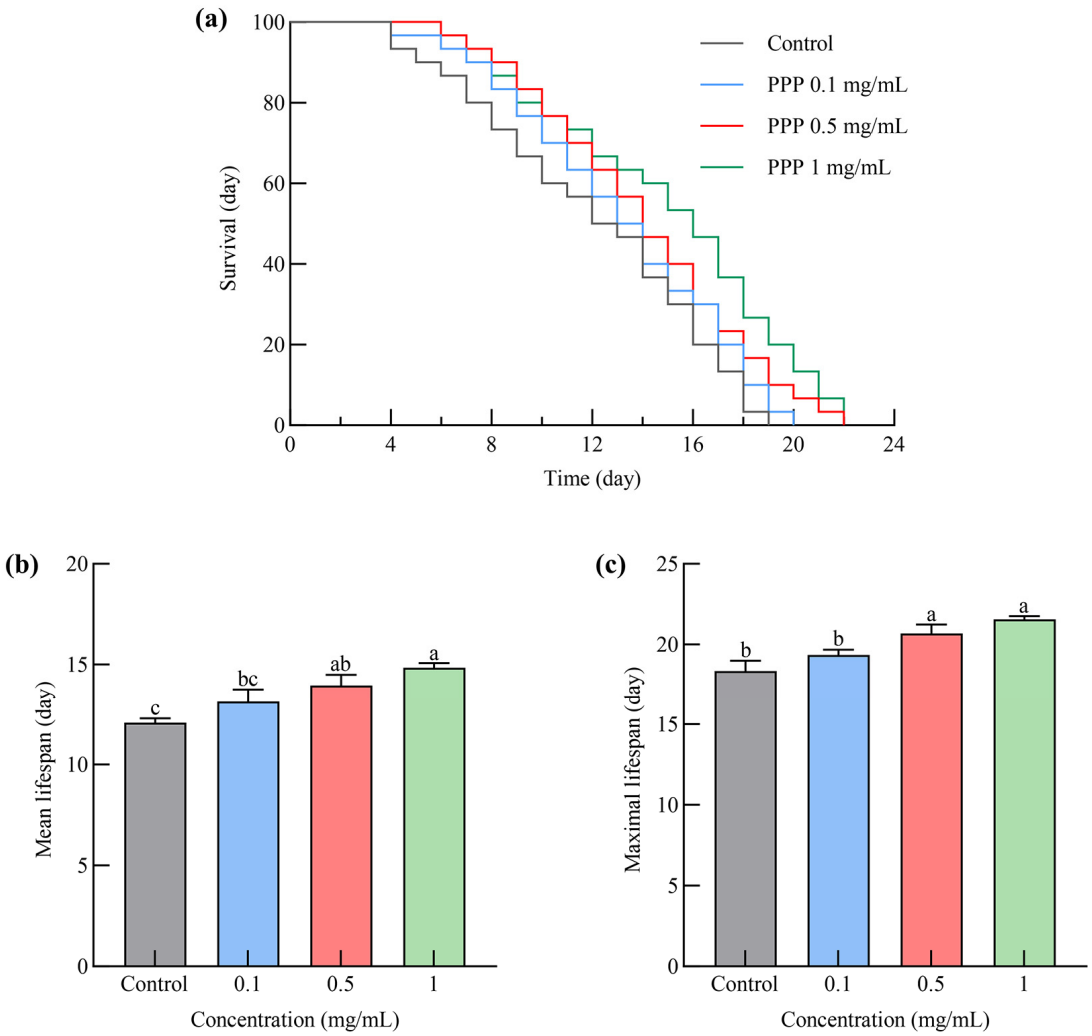

Figure 3: Influences of *P. lactiflora* petal polyphenol extract on lifespan of *C. elegans*. (**a**) survival curves, (**b**) mean lifespan, (**c**) maximal lifespan. Values represented mean ± SD (n = 30 per group, 3 biological replicates). Distinct superscripts reflect statistical significance based on one-way ANOVA with Tukey's test ($p < 0.05$).

3.5 PPP on Locomotion Behavior of C. elegans

Locomotion ability is an important indicator of organism aging [34]. The impacts of PPP on the locomotion behavior of *C. elegans* were assessed by quantified head thrashes and body bends. Both parameters displayed a dose-responsive increase following PPP treatment. Relative to the control group, PPP at a concentration of 1.0 mg/mL significantly enhanced the locomotion ability. Specifically, head thrashing frequency rose from 1.45 times/s in control to 1.80 times/s, while body bending frequency increased from 1.44 times/s to 1.76 times/s, respectively (Fig. 4). These results indicated that PPP significantly improved the locomotion ability of *C. elegans*.

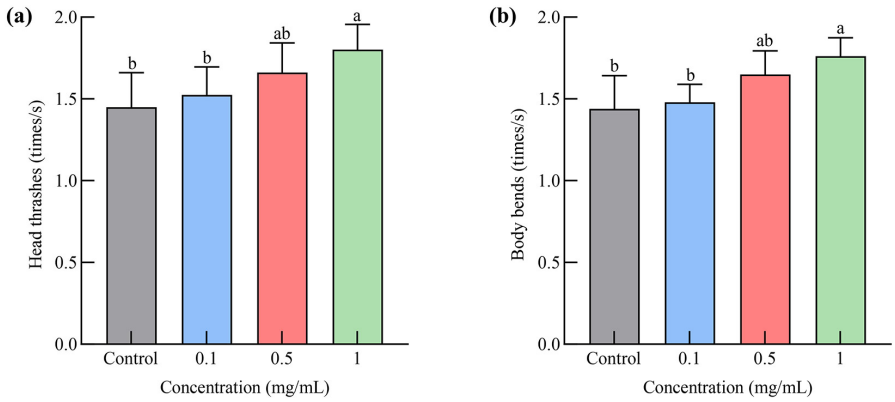

Figure 4: Influences of *P. lactiflora* petal polyphenol extract on locomotion behavior of *C. elegans*. (**a**) head thrashes, (**b**) body bends. Values represented mean ± SD (n = 10 per group). Distinct superscripts reflect statistical significance based on Kruskal-Wallis H test with Dunn's test ($p < 0.05$).

3.6 PPP on Intestinal Autofluorescence of C. elegans

Lipofuscin is an age-associated autofluorescent pigment accumulating over time [35]. In order to determine PPP's anti-aging properties, the intestinal autofluorescence of nematodes was assessed. The intestinal autofluorescence of nematodes treated with PPP was visibly lower than that of nematodes without PPP (Fig. 5a). Quantitative analysis revealed that at 0.5 and 1.0 mg/mL PPP concentrations, the mean fluorescence intensities were 21.64 and 17.80 arbitrary units (a.u.), significantly reduced by 14.70% and 29.84%, respectively, relative to the control (Fig. 5b). These results demonstrated that PPP delayed aging and enhanced antioxidant function by reducing lipofuscin accumulation in *C. elegans*.

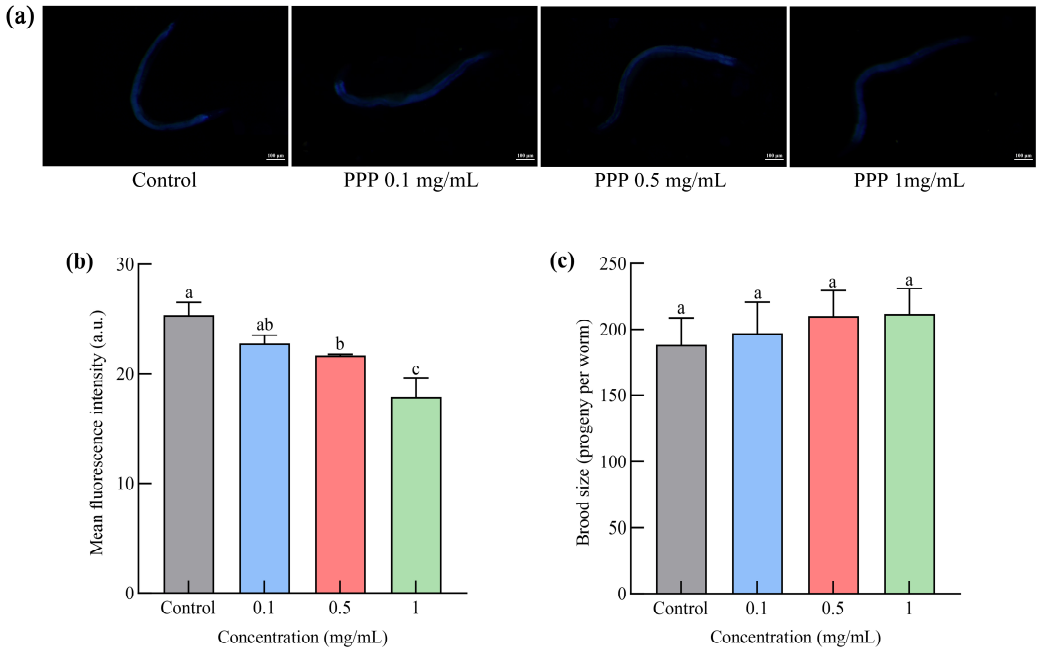

Figure 5: Influences of *P. lactiflora* petal polyphenol extract on intestinal autofluorescence and reproduction of *C. elegans*. (**a**) intestinal autofluorescence, (**b**) mean fluorescence intensity (n = 3 per group), (**c**) brood size (n = 5 per group). a.u., arbitrary units. Values represented mean ± SD. Distinct superscripts reflect statistical significance based on one-way ANOVA with Tukey's test ($p < 0.05$).

3.7 PPP on Reproduction of C. elegans

To assess whether the lifespan-extending influence of PPP was associated with changes in reproduction, the brood size of *C. elegans* was measured following PPP treatment. The brood size showed a slight increasing trend with increasing PPP concentrations, reaching 188.40 ± 20.74, 197.20 ± 23.74, 210.40 ± 19.13 and 211.80 ± 19.45 progeny per worm in the control and 0.1, 0.5, and 1.0 mg/mL PPP treatment groups, respectively (Fig. 5c). Nevertheless, these differences were not statistically notable. These results demonstrated that the lifespan-extending effect of PPP was not associated with changes in reproduction.

3.8 PPP on Stress Resistance of C. elegans

Thermotolerance assessment of *C. elegans* was conducted by exposing control and PPP-treated worms to 35°C, and their survival rate, mean lifespan, and maximal lifespan were assessed. Relative to the control, the 1.0 mg/mL PPP group exhibited a marked rightward shift in its survival curve, indicating enhanced heat stress resistance. Moreover, the average survival time of *C. elegans* receiving 1.0 mg/mL PPP reached 7.24 h, representing a significant 28.60% increase over the control group. Likewise, the maximal lifespan of *C. elegans* exposed to 1.0 mg/mL PPP group reached 11.89 h, an increase of 21.59% relative to the control (Fig. 6a).

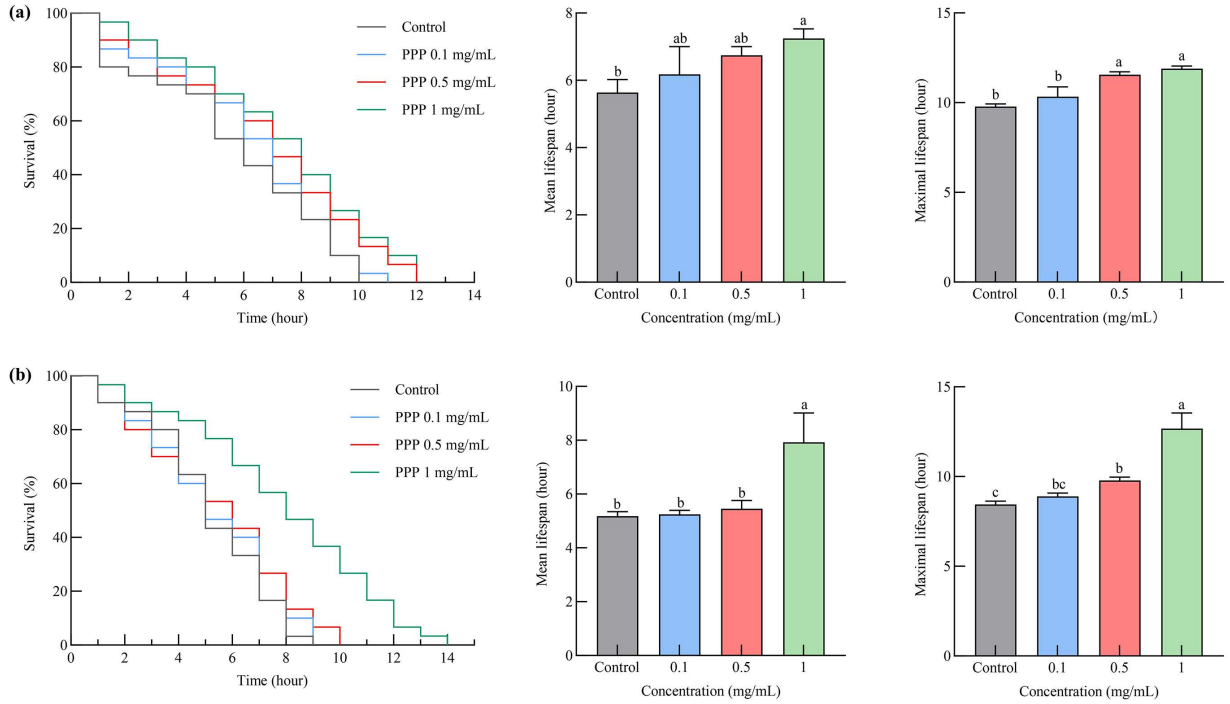

Figure 6: Influences of *P. lactiflora* petal polyphenol extract on stress resistance of *C. elegans*. (**a**) survival curves, mean lifespan, and maximal lifespan under heat stress, (**b**) survival curves, mean lifespan, and maximal lifespan under oxidative stress. Values represented mean ± SD (n = 30 per group, 3 biological replicates). Distinct superscripts reflect statistical significance based on one-way ANOVA with Tukey's test ($p < 0.05$).

Similarly, *C. elegans* from PPP groups with different concentrations were incubated in a medium supplemented with 400 μM juglone to examine whether PPP protected against oxidative damage. 1.0 mg/mL PPP group exhibited a marked rightward shift in its survival curve under oxidative stress, which deviated markedly from that of control. The mean lifespan of *C. elegans* in 1.0 mg/mL PPP group under oxidative

stress reached 7.92 h, representing a significant 53.01% increase over controls. Additionally, the maximal lifespan of *C. elegans* in the 1.0 mg/mL PPP group was 12.67 h, corresponding to a significant 50.00% improvement over the control (Fig. 6b). These results indicated that PPP effectively boosted the tolerance of *C. elegans* to various environmental stresses.

3.9 PPP on the ROS Levels and Antioxidant Enzyme Activity of C. elegans

Treatment with PPP induced a concentration-dependent decrease in ROS mean fluorescence intensity (Fig. 7a,b), with 0.5 and 1.0 mg/mL groups showing statistically significant differences from the control, corresponding to reductions of 27.69% and 42.13%, respectively. In addition, SOD and CAT activities rose steadily with increasing PPP concentrations, both peaking at 1.0 mg/mL, which were significantly elevated over the control corresponding to increases of 169% and 44.36%, respectively (Fig. 7c,d). These observations indicated that PPP not only directly scavenged excess ROS but also upregulated the endogenous antioxidant enzymes, thus effectively ameliorating oxidative stress injury in nematodes. The observed concentration-response relationship implied that PPP may exert its antioxidative action by stimulating the endogenous enzymatic defenses.

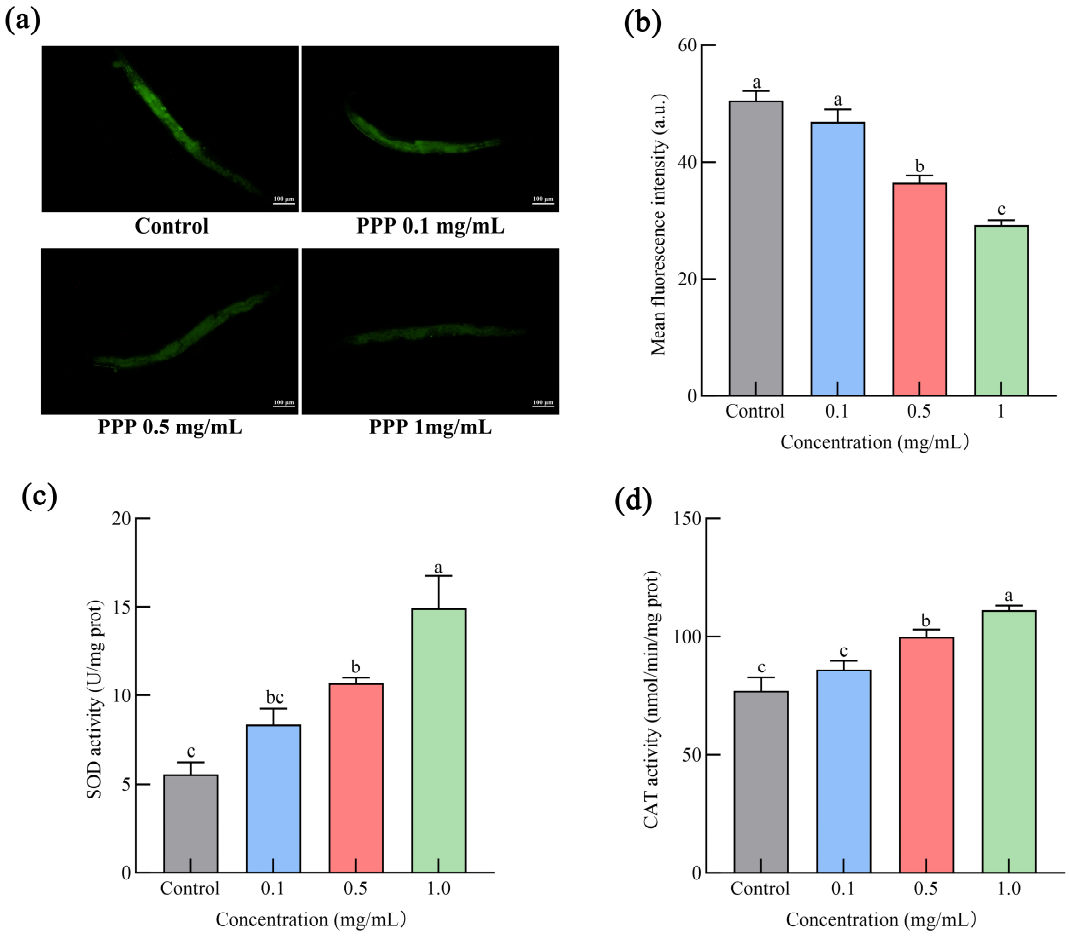

Figure 7: Modulation of intracellular ROS and enzymatic antioxidant activities by *P. lactiflora* petal polyphenol extract in *C. elegans*. (**a**) ROS fluorescence images, (**b**) ROS mean fluorescence intensity, (**c**) SOD activity, (**d**) CAT activity. Values represented mean ± SD (n = 3 biological replicates). Distinct superscripts reflect statistical significance based on one-way ANOVA with Tukey's test ($p < 0.05$).

4 Discussion

Natural polyphenols represent a widespread class of phytochemicals that function as secondary plant metabolites. They not only enhance plants' resistance to external stressors but also contribute substantially to human well-being. In recent years, over 8000 types of polyphenols have been identified, and their biological and pharmacological activities have been extensively studied [36]. A large body of research has demonstrated that plant polyphenols exhibit antioxidant, anti-tumor, anti-coagulant, and other effects [37]. In this study, polyphenols were isolated from peony petals using ultrasound-assisted extraction, and the *in vitro* antioxidant activity of PPP was evaluated by three free radical scavenging assays. The ·OH is a highly reactive oxygen species with strong oxidizing properties, second only to fluorine in nature, and can react with most organic compounds [38]. DPPH is a nitrogen-centered radical and remains stable at ambient temperature [39]. ABTS is a water-soluble organic radical that commonly exists as a cation [40]. This study found that PPP exhibited strong scavenging capacity against DPPH and ABTS radicals, while also demonstrating moderate ·OH radical scavenging ability. These results indicate that PPP possesses good *in vitro* radical scavenging capacity. Polyphenols isolated from *Pueraria lobata* (Willd.) Ohwi root have been demonstrated to effectively scavenge DPPH and ·OH radicals [41]. Research by Li et al. [42] indicated that polyphenolic compounds from eucalyptus leaf also displayed substantial antioxidant activity. Consequently, *P. lactiflora* petals represent a potential source of natural polyphenols and offer new options for antioxidant development.

A series of physiological parameters in *C. elegans* were measured to evaluate the *in vivo* antioxidant efficacy of PPP in the present work. To assess the safety of PPP, the optimal concentration range for administration was determined by using three indicators: survival rate, motility and body length in the worm model. According to the experimental data, PPP had no significant effects on these indicators within the range of 0.1–1 mg/mL, indicating no toxicity to *C. elegans* within this concentration range.

With its small body size, short life cycle, and a lifespan of only 15–22 days at 25°C, *C. elegans* proves to be a valuable organism for longevity research. Compared to other model organisms like mice and *Drosophila melanogaster*, the short lifespan of *C. elegans* enables rapid completion of longevity assays and accelerates the evaluation of anti-aging interventions. In this study, tested concentrations of PPP all extended worm longevity to some extent, with 1.0 mg/mL PPP showing the most significant effect. These results indicate that PPP possesses lifespan-prolonging effects, which are consistent with previous findings on polyphenol extracts from *Phyllanthus emblica* L. fruit [43]. Some scholars have suggested that there is a compromise between fertility and lifespan, meaning that a longer lifespan often reduces reproductive capacity. However, as research has advanced, it has become evident that some natural products do not adhere to this mechanism when extending lifespan. This study showed that PPP prolonged *C. elegans* lifespan while not changing the number of its offspring, which was consistent with the effects of polyphenols from Finger citron on reproductive function in *C. elegans* [44]. Therefore, the role of PPP in prolonging lifespan of *C. elegans* is not mediated by the suppression of reproductive capacity.

As a key phenotypic marker of physiological function, the locomotion behavior of *C. elegans* reflects their nervous system integrity and demonstrates a direct correlation with lifespan [45]. Herein, 1.0 mg/mL PPP significantly improved the motility of *C. elegans*, indicating that PPP positively regulates the nervous system, thereby enhancing their spontaneous locomotor activity. Lipofuscin is a brownish-yellow, autofluorescent substance that builds up progressively during aging and cannot be degraded effectively. Consequently, the deposition of lipofuscin serves as a key biomarker of organismal aging [46]. In this study, 0.5 and 1.0 mg/mL PPP markedly lowered intestinal autofluorescence of *C. elegans*, suggesting that PPP may delay aging and improve the physiological status. Studies have indicated that *C. elegans* enhances

their survival and longevity under stress by modulating the antioxidant defense system [47]. Therefore, the oxidative stress response level serves as a core indicator of an organism's antioxidant and anti-aging defense capacity under stress conditions. We observed that 1.0 mg/mL PPP significantly prolonged the mean lifespan of *C. elegans* under heat and oxidative stress, suggesting that PPP enhances stress tolerance. Previous articles have reported that polyphenolic compounds derived from hempseed [48] and mulberry leaves [49] can enhance the nematode's ability to withstand external stressors and prolong its lifecycle. Our biochemical experiments showed that PPP treatment led to a concentration-associated fall in intracellular ROS, along with a corresponding rise in SOD and CAT activities. These findings firmly establish that PPP reinforces the endogenous enzymatic antioxidant barrier.

Collectively, the above results indicated that PPP exhibited favorable antioxidant activity in *C. elegans*. However, this study did not purify PPP, nor did it employ chromatographic methods to separate and quantify individual phenolic components. Consequently, the key phenolic constituents responsible for the effects have not yet been identified. Despite the lack of chemical characterization data, the bioactivity confirmed from the present work offers a reliable basis for subsequent work on isolating bioactive substances and analyzing active components by chromatographic techniques. In addition, although *C. elegans* is applied broadly in research on antioxidant mechanisms, it has inherent limitations. As an invertebrate, *C. elegans* is distinct from mammals and humans in metabolic routes, antioxidant defense systems, intestinal structure, and gut microbiota composition [50–52]. For example, the intestines of *C. elegans* serve as the primary site for absorption and metabolism, lacking the complex gut microbiota interaction network present in mammals, which may result in differences in the biotransformation pathways and bioactive forms of polyphenolic compounds *in vivo* [53]. Therefore, although the antioxidant effects of PPP observed in *C. elegans* provide important clues for mechanistic exploration, care should be exercised when directly applying these observations to humans. Future studies should employ mammalian models and human cell-based experiments to further validate the antioxidant activity and molecular mechanisms of PPP in higher organisms.

5 Conclusion

In summary, PPP exhibited strong free radical scavenging activity and considerable *in vitro* antioxidant activity. In *C. elegans*, PPP significantly improved locomotion behavior, enhanced stress resistance, reduced lipofuscin accumulation and the levels of ROS, extended the lifespan, and increased antioxidant enzyme activity. These findings suggest that PPP has potential as a natural antioxidant and anti-aging resource, providing a theoretical basis for its further development and utilization. Nevertheless, given the complexity of the components of PPP, further research should focus on the extraction, purification, and characterization of its active ingredients.

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Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

PPP	*P. lactiflora* petal polyphenol extract
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)
ROS	Reactive oxygen species
Vc	Vitamin C
·OH	Hydroxyl free radical
NGM	Nematode growth medium
CK	The control group
FUDR	5-fluoro-2′-deoxyuridine
H_2DCFDA	2′,7′-dichlorodihydrofluorescein diacetate
CAT	Catalase
SOD	Superoxide dismutase
SD	Standard deviation
ANOVA	Analysis of variance
a.u.	Arbitrary units

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