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Beneficial effects of *Paeonia ostii* stamen tea in extending the lifespan and inducing stress resistance on *Caenorhabditis elegans*

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Abstract

Paeonia ostii is an industrial crop with broad prospects, and folks have habit of drinking its stamen tea in China, but its beneficial healthy effects remain largely unclear. Here, we identified its main active components, evaluated its antioxidant activity, and examined its safety property and its beneficial effects in the model animal *Caenorhabditis elegans*. The results showed that *P. ostii* stamen (POS) contained many active components with high antioxidant activity. Moreover, POS tea did not cause lethality, influence growth, locomotion behavior and reproduction, and induce intestinal autofluorescence in *C. elegans*. Furthermore, 1.2 mg·mL^{-1} of POS tea treatment significantly extended the lifespan and improved growth, locomotion behavior and intestinal autofluorescence of *C. elegans*, while its reproduction and *Escherichia coli* OP50 growth were not affected. In addition, POS tea treatment significantly induced stress resistance to extend the lifespan of *C. elegans* under heat stress and oxidative stress conditions. All these suggested that POS tea was safe and had beneficial healthy effects, which could provide a theoretical basis for its production and popularization.

Keywords: *Paeonia ostii*; stamen; tea; lifespan; stress resistance; *Caenorhabditis elegans*.

Practical Application: Function of tree peony stamen tea.

1 Introduction

The genus *Paeonia*, belonging to the family Paeoniaceae, is one of the world's most ancient flowering plant groups (Chen & Li, 1998; Hong et al., 2001; Kubitzki, 2007). It comprises of 35 shrubs and perennial herbs which are extensively grown across five disjunct zones (eastern Asia, central Asia, the western Himalayas, the Mediterranean region, and Pacific North America) of the northern hemisphere (Sang et al., 1997). Members of *Paeonia* are widely cultivated due to its striking ornamental and medicinal values (Kamenetsky & Dole, 2012; Parker et al., 2016). Especially in China, tree peony species are crowned as the "king of the flowers", and are planted as major garden plants with numerous cultivars and hybrids (Kamenetsky & Dole, 2012). Further, many peony species, such as *P. lactiflora*, *P. ostii* and *P. veitchii*, contain chemical compounds with pharmacological activities, such as albiflorin, oxypaeoniflorin, paeoniflorin, and paeonol, that are unique to this genus (Ogawa et al., 2015; Saahene et al., 2018). In traditional Chinese medicine, they are prescribed for women's diseases (dysmenorrhoea or menorrhagia) and for various painful inflammatory conditions such as cholecystitis (Fu et al., 2018).

In China, folks have habit of drinking stamen tea which is made from tree peony stamens. Previous studies showed that the contents of protein, K, Ca, Mg, P, Se, carbohydrate, amino acid, unsaturated fatty acid and tocopherol were high in tree peony stamens, and it possessed high antioxidant activity (Fu et al., 2011). Furthermore, Li et al. (2015) found that water extract of tree peony stamens with high total phenolic and flavonoid contents possessed remarkable antioxidant capacity according to 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Therefore, tree peony stamen tea has important research value as well as its development and potentials. However, whether tree peony stamen tea is non-toxic and its influence on animals have not been reported till now.

In the present study, we used *P. ostii* stamens (POS) as materials. Firstly, we identified the main active components of POS and measured the in vitro antioxidant activities of POS tea. Secondly, we examined the possible safety property of POS tea by using model animal, *Caenorhabditis elegans*, as a in vivo assay system. Thirdly, we investigated the beneficial effects of POS tea in extending the lifespan and inducing stress resistance in *C. elegans*. The results presented here would provide a theoretical basis for the production and popularization of POS tea.

2 Materials and methods

2.1 Plant material and treatment

Plants used in this study were those of about 10-year old *P. ostii* grown the germplasm repository of Horticulture and Plant

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Protection College, Yangzhou University, Jiangsu Province, China (32°39'N, 119°42'E). The stamens were collected in initiating bloom stage, and the filaments were removed. Base on the preliminary experiment, the stamens were fixing-dried with 800 W microwave power for 1 min, and then dried at 80 °C to constant weight. The treated stamens were used for further analysis (Figure 1).

2.2 Identification of main active components in stamens

Main active components including saccharides, proteins, organic acids, flavonoids, phenols, steroids and anthraquinones were identified using reagent color-developing method (Li & Lian, 2012). Content of soluble sugar and soluble protein were evaluated according to the guidelines of reagent kits (Suzhou Comin Biotechnology Co., Ltd., China). Before detection of tea polyphenol content, the samples were pretreated as follow: firstly, 0.2 g stamens were ground to fine powder and extracted with 5 mL 70% methanol solution (70 °C preheat) in 10 mL centrifuge tube. Secondly, the centrifuge tubes were heated in 70 °C water bath, and then centrifuge at 3500 rpm for 10 min, the process was repeat one time for the resulting supernatant. The content of tea polyphenol was calculated using spectroscopy absorbance measurements at 765 nm.

2.3 Preparation of POS tea

A total of 0.6 g treated POS were placed in a 250 mL flask, and 100 mL water at 90 °C was added and left for 20 min, then they were vacuum filtered, and repeated again. The resulting filtrates were combined and subjected to rotary evaporation, and concentrated to a volume of 50 mL. Next, sterile filtration was carried out using a needle filter to obtain 12 $mg{\cdot}mL^{-1}$ POS tea mother liquor, which was diluted with distilled water to 1.2 $mg{\cdot}mL^{-1}$, 1.0 $mg{\cdot}mL^{-1}$, 0.8 $mg{\cdot}mL^{-1}$, 0.6 $mg{\cdot}mL^{-1}$, 0.4 $mg{\cdot}mL^{-1}$ and 0.2 $mg{\cdot}mL^{-1}$ and stored at 4 °C until use.

2.4 In vitro evaluation of antioxidant activities of POS tea

Determination of reducing capacity

POS tea (1 mL) and 0.2 mL phosphate buffer (0.2 M, pH=6.6) were placed in the tube, and 0.5 mL of 1% $K_3Fe(CN)_6$ solution was added and mixed well, the tube was placed in a water bath at 50 °C for 20 min. For the control and blank, $K_3Fe(CN)_6$ solution and stamen tea were replaced by distilled water, respectively. After cooling, 2 mL of 10% trichloroacetic acid was added, and centrifuged at 3000 rpm for 10 min. And then, 2.5 mL distilled water and 0.5 mL of 1% $FeCl_3$ were added to 2.5 mL supernatant. Finally, the absorbance was read at 700 nm with distilled water used as zero adjustment. The reducing capacity was calculated by the following equation: reducing capacity = Abs of sample - Abs of control - Abs of blank.

Determination of hydroxyl free radical (·OH) scavenging activity

POS tea (0.5 mL) was placed in the tube, and 1 mL of 2 mM $FeSO_4$ solution, 1 mL of 8 mM H_2O_2 and 0.5 mL of 6 mM salicylic acid solution were added respectively, the tube was placed in a water bath at 37 °C for 1 h. For the control and blank, H_2O_2 and stamen tea were replaced by distilled water, respectively. After cooling, the absorbance was read at 510 nm with distilled water used as zero adjustment. The ·OH scavenging activity was calculated by the following equation: ·OH scavenging activity (%) = (Abs of blank - Abs of sample - Abs of control) / Abs of blank × 100. Moreover, the antioxidant capacity was expressed as an IC_{50} value.

Determination of DPPH free radical scavenging activity

POS tea (0.5 mL) was placed in the tube, and 2.5 mL of 0.1 mM DPPH solution was added, the tube was placed in a water bath at 28 °C for 1 h. For the control and blank, DPPH solution and stamen tea were replaced by 50% ethanol and distilled water, respectively. After cooling, the absorbance was read at 517 nm with distilled water used as zero adjustment. And DPPH scavenging activity was calculated by the following equation: DPPH scavenging activity (%) = (Abs of blank - Abs of sample - Abs of control) / Abs of blank × 100. Moreover, the antioxidant capacity was expressed as an IC_{50} value.

Determination of ABTS free radical scavenging activity

POS tea (0.5 mL) was placed in the tube, and 3 mL of ABTS working solution was added, the tube was left to react under dark condition at room temperature for 1 h. For the control and blank, ABTS working solution and stamen tea were replaced by distilled water, respectively. The absorbance was read at 734 nm with distilled water used as zero adjustment. And ABTS scavenging activity was calculated by the following equation: ABTS scavenging activity (%) = (Abs of blank - Abs of sample - Abs of control) / Abs of blank × 100. Moreover, the antioxidant capacity was expressed as an IC_{50} value.

2.5 Caenorhabditis elegans strain and culture conditions

Caenorhabditis elegans belongs to the linear animal, has been widely used as a model animal for the screening of antioxidant and aging drugs since the 1980s (Baldi et al., 2009; Sulston et al., 1983). As a model animal, *C. elegans* has the advantages of simple structure, short life history cycle, high reproductive ability, easy culture and observation; in addition, *C. elegans* belongs to hermaphrodites, which can prevent inbreeding from affecting life test, and has a clear genetic background and a genetic sequence that is highly similar to humans (Houthoofd et al., 2003). In present study, wild-type *C. elegans* N2 obtained from Central China Normal University was maintained on *C. elegans* growth medium (NGM) plates seeded with *Escherichia coli* OP50 at 20 °C as described by Brenner (1974). Age synchronous populations of L4-larvae *C. elegans* was obtained as described previously (Donkin & Williams, 1995). Stock stamen tea (12 $mg{\cdot}mL^{-1}$) was added to the NGM plates to a final concentration of 1.2 $mg{\cdot}mL^{-1}$, 1.0 $mg{\cdot}mL^{-1}$, 0.8 $mg{\cdot}mL^{-1}$, 0.6 $mg{\cdot}mL^{-1}$, 0.4 $mg{\cdot}mL^{-1}$ and 0.2 $mg{\cdot}mL^{-1}$. *E. coli* OP50 was spread on the NGM plates as the food for *C. elegans*. For the safety assessment, lifespan, growth, locomotion behavior, reproduction and intestinal autofluorescence assays were used as endpoints.

2.6 Safety evaluation of POS tea on e

POS tea treatment was performed for 24-hr from the stage of L4-larvae for safety evaluation. *C. elegans* was judged to be dead if they did not respond to stimulus using a metal wire, and their body length was measured depending on the flat surface area of *C. elegans* using Olympus SZX2-RFA16. And head thrashes, defined as a change from one direction to another and back again, were counted for 1 min. Body bends, defined as a change in the direction of the part of *C. elegans* corresponding to the posterior bulb of the pharynx along the y axis, assuming that *C. elegans* was traveling along the x axis, were counted for 20 sec. Brood size, defined as the number of offspring at all stages beyond the egg, was counted. Intestinal autofluorescence caused by lysosomal deposits of lipofuscin can accumulate over time in aging *C. elegans* (Shen et al., 2007). For intestinal autofluorescence assay, images were collected for fluorescence in endogenous intestine using a 525-nm bandpass filter and without automatic gain control to preserve relative fluorescence intensity in animals. Fluorescence was recorded and color images were taken for documentation of results with Olympus SZX2-RFA16. Lipofuscin levels were measured using Image J Software (NIH Image, Bethesda, MD, USA) by determining mean pixel intensity in intestines.

2.7 Assays of lifespan, growth, locomotion behavior and intestinal autofluorescence

POS tea treatment was performed throughout the lifespan from the stage of L4-larvae. *C. elegans* was checked every day and scored as dead when they did not respond to stimulus using a metal wire. The mean lifespan of the last 10% *C. elegans* was defined as the maximal lifespan, and their body length, head thrashes, body bends, brood size, intestinal autofluorescence were measured as described above.

2.8 Inhibition evaluation of stamen tea on E. coli OP50

POS tea was added to 100 mL LB liquid medium with a final concentration of 1.2 mg·mL^{-1}. For the control, the corresponding amount of sterile distilled water was added. Subsequently, *E. coli* OP50 was cultured overnight according to 1% inoculum, and then they were inoculated into LB liquid medium. After mixing, the absorbance was measured at 595 nm, and this value was taken as the zero-reference point. LB liquid medium was shaken and cultured at 37 °C in a shaker at a rotation speed of 200 rpm. The absorbance was measured at 595 nm every 1 h and continuously measured for 5 h to draw the growth curve of *E. coli* OP50.

2.9 Assays of heat stress and oxidative stress resistance

POS tea treatment was performed throughout the heat stress and oxidative stress from the stage of L4-larvae. *C. elegans* pre-treated with 1.2 mg·mL^{-1} stamen tea for 3 days was transferred to the 35 °C or 200 μM paraquat. The lifespan, mean lifespan and maximal lifespan were measured as described above. Paraquat was set to induce the oxidative stress in *C. elegans* (Fan et al., 2011).

2.10 Statistical analysis

All the data were means of three replicates with standard deviation. Data were subjected to analysis of variance procedure (ANOVA) using SAS/STAT statistical analysis package (version 6.12, SAS Institute, Cary, NC, USA). Means were separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

3 Results

3.1 Main active components of POS

The main active components of POS include saccharides, proteins, organic acids, flavonoids, phenols, steroids and anthraquinones (Table S1). The contents of soluble sugar, soluble protein, flavonoids and tea polyphenols in stamen were 152.77 mg·g^{-1} DW, 7.52 mg·g^{-1} DW, 18.16 mg·g^{-1} DW and 31.75 mg·g^{-1} DW, respectively (Table S2).

3.2 Antioxidant activities of POS tea

Antioxidant activities of POS tea was evaluated using reducing capacity, ·OH scavenging activity, DPPH scavenging activity and ABTS scavenging activity (Figure 2). Firstly, the reducing capacity was increased gradually with the increase of the stamen tea concentration, and showed significant difference between different concentrations of stamen tea. Similarly, ·OH, DPPH and ABTS scavenging activities also increased continuously with the increasing of stamen tea concentration. Moreover, assay of ·OH, DPPH and ABTS showed that 1.2 mg·mL^{-1} stamen tea effectively scavenged the free radical by 27.43%, 49.26% and 75.77%, respectively. And based on these, IC_{50} value was calculated and their values were 2.38 mg·mL^{-1}, 1.28 mg·mL^{-1} and 0.63 mg·mL^{-1}, respectively.

3.3 Safety evaluation of POS tea on C. elegans

As shown in Figure 3a, a 0.8 mg·mL^{-1}, 1.0 mg·mL^{-1} and 1.2 mg·mL^{-1} stamen tea did not induce *C. elegans* lethality, and the survival of Control and different treatment were all 100%. Moreover, treatment with POS tea did not alter body length, locomotion behavior and the reproduction of *C. elegans*. Furthermore, the head thrashes, body bends and brood size of *C. elegans* were also not significantly different compared to Control. There was no significant difference of intestinal autofluorescence between *C. elegans* treated with 0.8 mg·mL^{-1}, 1.0 mg·mL^{-1} and 1.2 mg·mL^{-1} POS tea and Control (Figure 3b, Table S3).

3.4 POS tea treatment extends the lifespan of C. elegans

The lifespan curve of *C. elegans* with 0.8 mg·mL^{-1}, 1.0 mg·mL^{-1} and 1.2 mg·mL^{-1} POS tea treatment was all shifted to the right as the same as Control, and the trends of the latter two were more significant (Figure 4a). According to the Log-rank (Mantel-Cox) Test analysis, the P-values of the lifespan of *C. elegans* with three concentration stamen tea treatments compared to Control, were 0.6297, 0.0372 and <0.0001, respectively. Moreover, the mean lifespan of *C. elegans* was increased gradually with the increase of stamen tea concentration, and the mean lifespan of *C. elegans* with 1.2 mg·mL^{-1} POS tea treatment was 22.41 days, which was significantly higher than that of Control with 2.85 days.

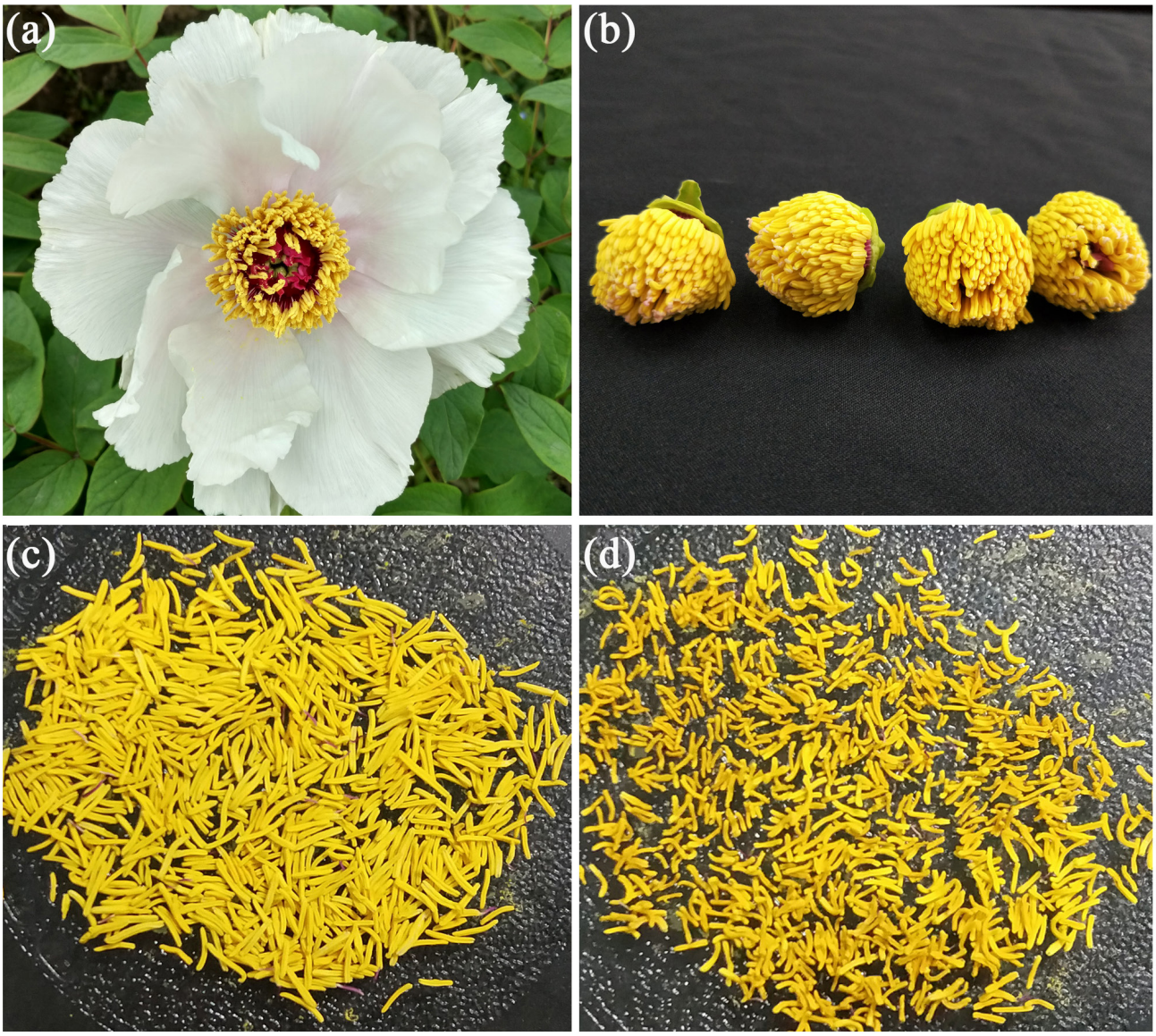

Figure 1. Photo of raw materials of *Paeonia ostii* stamen tea. (a) Blooming flower; (b) Remaining part of flower after removing the petals; (c) Separate stamens; (d) Stamens after aseries of treatments.

When the maximal lifespan of *C. elegans* was concerned, it also increased as the treated concentration of stamen tea increased, and significant difference was obtained between 1.2 mg·mL^{-1} POS tea treatment and Control, and the maximal lifespan of *C. elegans* under 1.2 mg·mL^{-1} POS tea treatment was 29.54 days, which was 3.67 days higher than that of Control (Figure 4b). These results suggested that 1.2 mg·mL^{-1} POS tea was the best treatment to extend the lifespan of *C. elegans*, therefore this concentration would be only used in further study.

3.5 POS tea treatment enhances locomotion behavior of C. elegans

POS tea treatment could enhance locomotion behavior of *C. elegans* (Figure 5). With the increase of *C. elegans* lifespan, head thrashes and body bends all showed a decreasing trend in Control and 1.2 mg·mL^{-1} POS tea treatment. From the fourth day, head thrashes and body bends of *C. elegans* treated with 1.2 mg·mL^{-1} POS tea were significantly higher than that of Control. On the 20th day, head thrashes of *C. elegans* treated with 1.2 mg·mL^{-1} POS tea increased by 96.00% as compared to Control, and its body bends was 1.33 times of Control.

3.6 POS tea treatment decreases intestinal autofluoresence of C. elegans

With regard to intestinal autofluoresence *C. elegans*, Figure 6a showed that it was increased gradually in 1.2 mg·mL^{-1} POS tea treatment and Control with the increase of the lifespan of *C. elegans*. And from the fourth day, the intestinal autofluoresence of *C. elegans* treated with 1.2 mg·mL^{-1} POS tea was always lower than that of Control, and this result was also confirmed by the

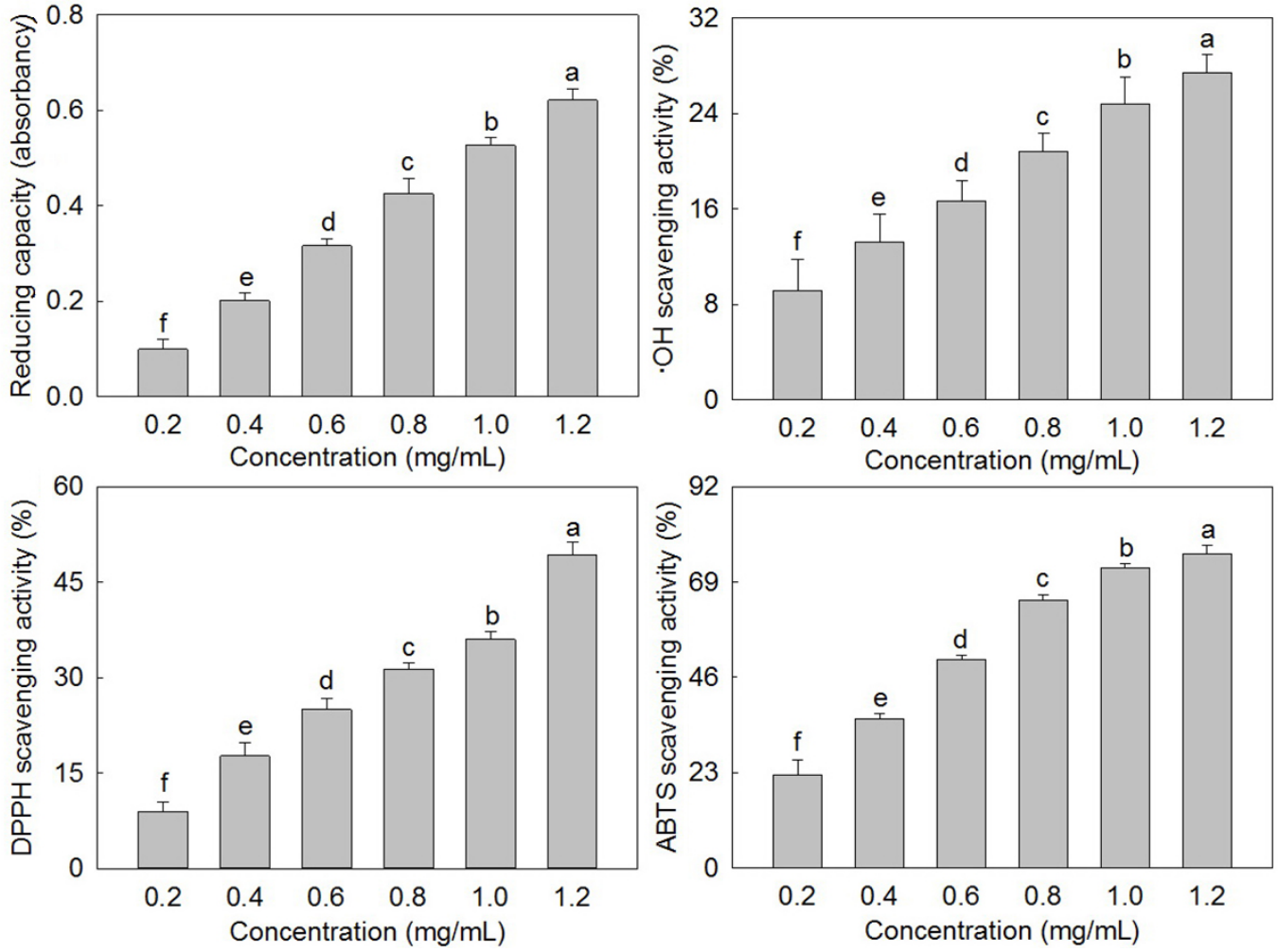

Figure 2. Reducing capacity and free radical scavenging activity of *Paeonia ostii* stamen tea. The values represented the mean ± SD, and different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

fluorescence intensity measurement (Figure 6b). And on the 20th day, the fluorescence intensity of *C. elegans* treated with 1.2 mg·mL^{-1} POS tea was lower than that of Control by 7.32%.

3.7 POS tea treatment does not affect reproduction of C. elegans and growth of E. coli OP50

With the development of *C. elegans*, brood size was increased firstly and then decreased, moreover, the results showed that there was no significant difference in brood size of *C. elegans* treated with 1.2 mg·mL^{-1} POS tea and Control. On the other hand, total brood size was lower in *C. elegans* treated with 1.2 mg·mL^{-1} POS tea compared to Control, but there was no significant difference between them (Figure 6c). Moreover, as shown in Table S4, the growth of *E. coli* OP50 increased gradually with passage of time, and there is no difference between 1.2 mg·mL^{-1} POS tea treatment and Control.

3.8 POS tea treatment induces stress resistance of C. elegans

In order to investigate whether POS tea had stress resistance property, *C. elegans* treated with 1.2 mg·mL^{-1} POS tea and Control were further exposed to heat-stress (35 °C) or 200 μM paraquat. As shown in Figure 7a, 1.2 mg·mL^{-1} POS tea treatment was significantly shift the lifespan curve of *C. elegans* to the right under heat stress, and according to the Log-rank (Mantel-Cox) Test analysis, its P-value was 0.0018 when compared with Control. Under heat stress, mean lifespan and maximal lifespan of *C. elegans* treated with 1.2 mg·mL^{-1} POS tea were significantly higher than those of Control by 14.60% and 13.24%, respectively. Similarly, under oxidative stress, 1.2 mg·mL^{-1} POS tea treatment was also significantly shift lifespan curve of *C. elegans* to the right, and its mean lifespan and maximal lifespan were also significantly higher than those of Control with 18.16% and 20.51%, respectively (Figure 7b).

4 Discussion

Tree peony is well known as a traditional Chinese ornamental and medicinal plant, its roots, seeds and flowers have been well developed, but development and applications of stamens have been long neglected. Fu et al. (2011) found that tree peony stamens were rich in protein, K, Ca, Mg, P, Se, carbohydrate,

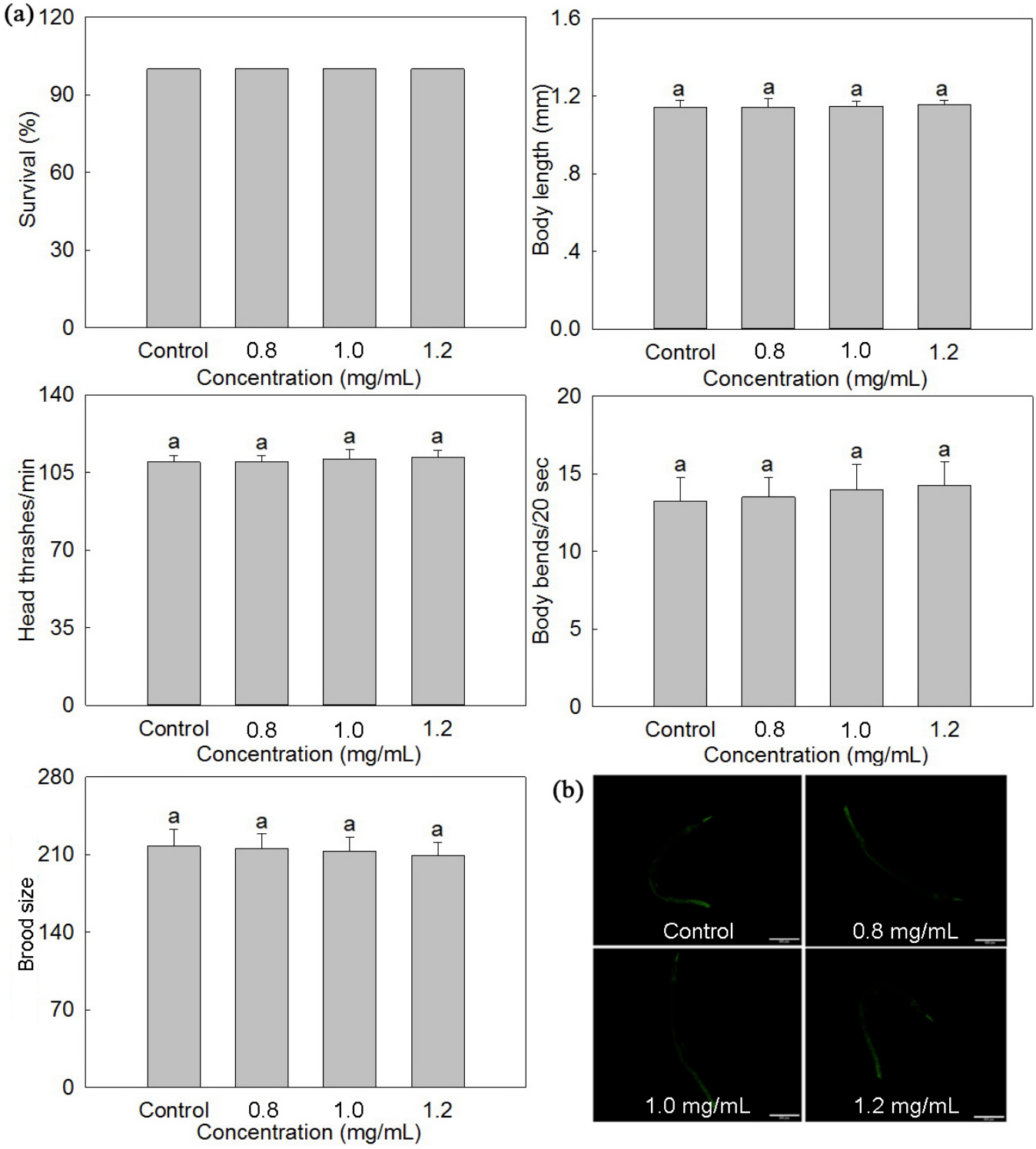

Figure 3. Safety evaluation of *Paeonia ostii* stamen tea on *Caenorhabditis elegans*. (a) Effects of stamen tea treatment on lethality, growth, locomotion behavior and reproduction of nematodes; (b) Effects of stamen tea treatment on intestinal autofluorescence of nematodes. The values represented the mean ± SD, and different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

amino acid, unsaturated fatty acid and tocopherol. In this study, we found that the main active components of *P. ostii* stamens include saccharides, proteins, organic acids, flavonoids, phenols, steroids and anthraquinones. All these results suggested that *P. ostii* stamens had high edible and medicinal values, which could be used for the development of POS tea.

Natural extracts have become an important source of natural antioxidants, because they contain many active components.

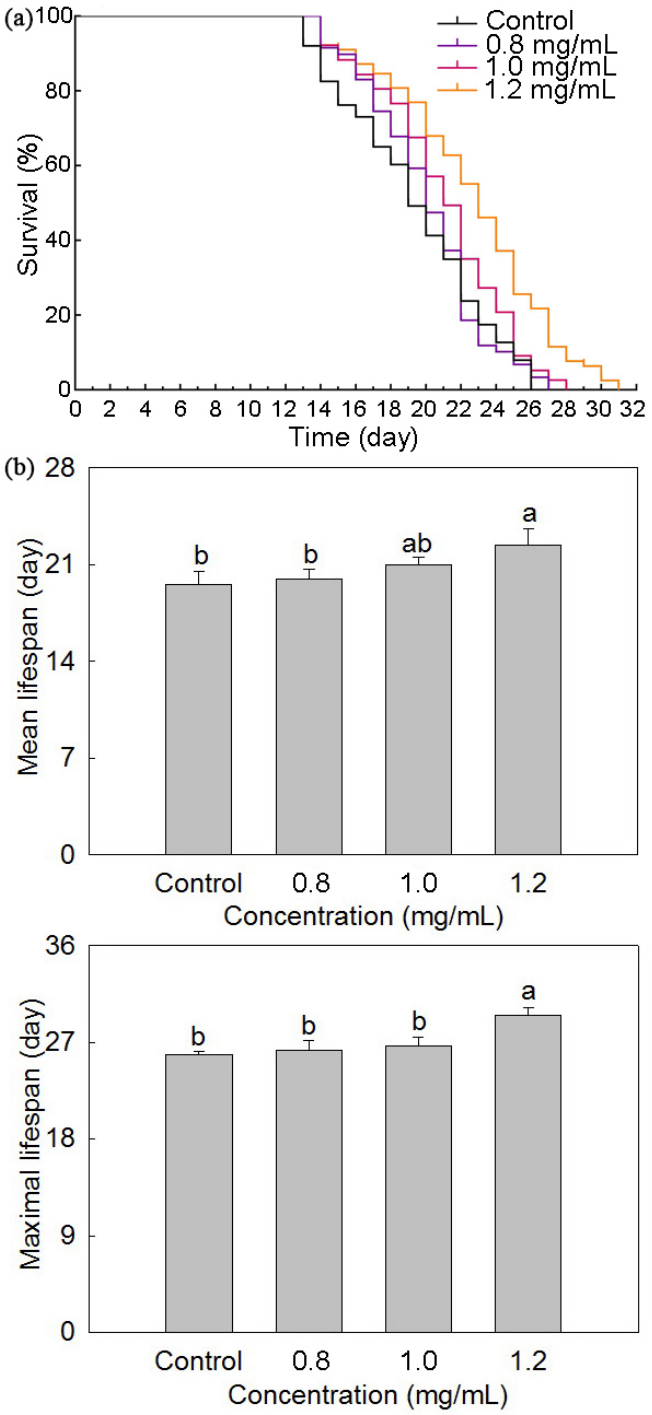

Figure 4. Effects of *Paeonia ostii* stamen tea on lifespan of *Caenorhabditis elegan*. (a) Lifespan curves of nematodes treated with stamen tea; (b) Comparison of mean lifespan and maximal lifespan in nematodes treated with stamen tea. The values represented the mean ± SD, and different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

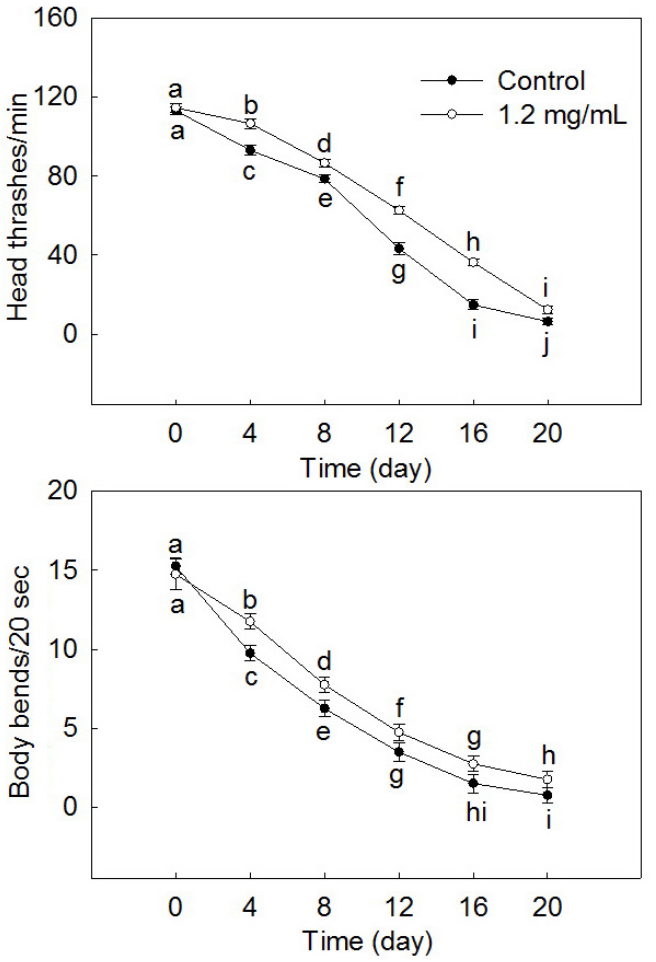

Figure 5. Effects of *Paeonia ostii* stamen tea on locomotion behavior of *Caenorhabditis elegan*. The values represented the mean ± SD, and different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

At present, there are many methods for evaluating antioxidant activity, and they are mainly divided into two categories according to their principles: one is the reducing capacity, and the other is the free radical scavenging activity (Fu et al., 2008). Different antioxidant evaluation methods have different detection principles, which may result in different results for the evaluation of the same antioxidant. In this study, we firstly measured reducing capacity, the higher the absorbance value, the stronger the reducing capacity (Kim et al., 2014). Our results showed that the reducing capacity of POS tea gradually increased with the increase of its concentration, the absorbance reached a maximum of 0.62 at 1.2 mg·mL^{-1} with reducing capacity stronger than that of *Bletilla striata* extract (Song et al., 2017). Moreover, ·OH, DPPH and ABTS scavenging activity were measured in this study, and IC_{50} value was also used to express the free radical scavenging activity. The smaller the IC_{50} value, the higher the free radical scavenging ability and the stronger the antioxidant capacity. Cvetanović et al. (2019) found the IC_{50} value of chamomile extract ·OH was 38.1 mg/mL^{-1}, which was higher than that of POS tea, suggesting that its antioxidant capacity was stronger compared to chamomile extract. Similarly, DPPH and ABTS scavenging activity of POS tea showed a concentration-dependent manner with IC_{50} value of 1.28 mg·mL^{-1} and 0.63 mg·mL^{-1}, respectively, which had stronger antioxidant capacity than that of brinjal

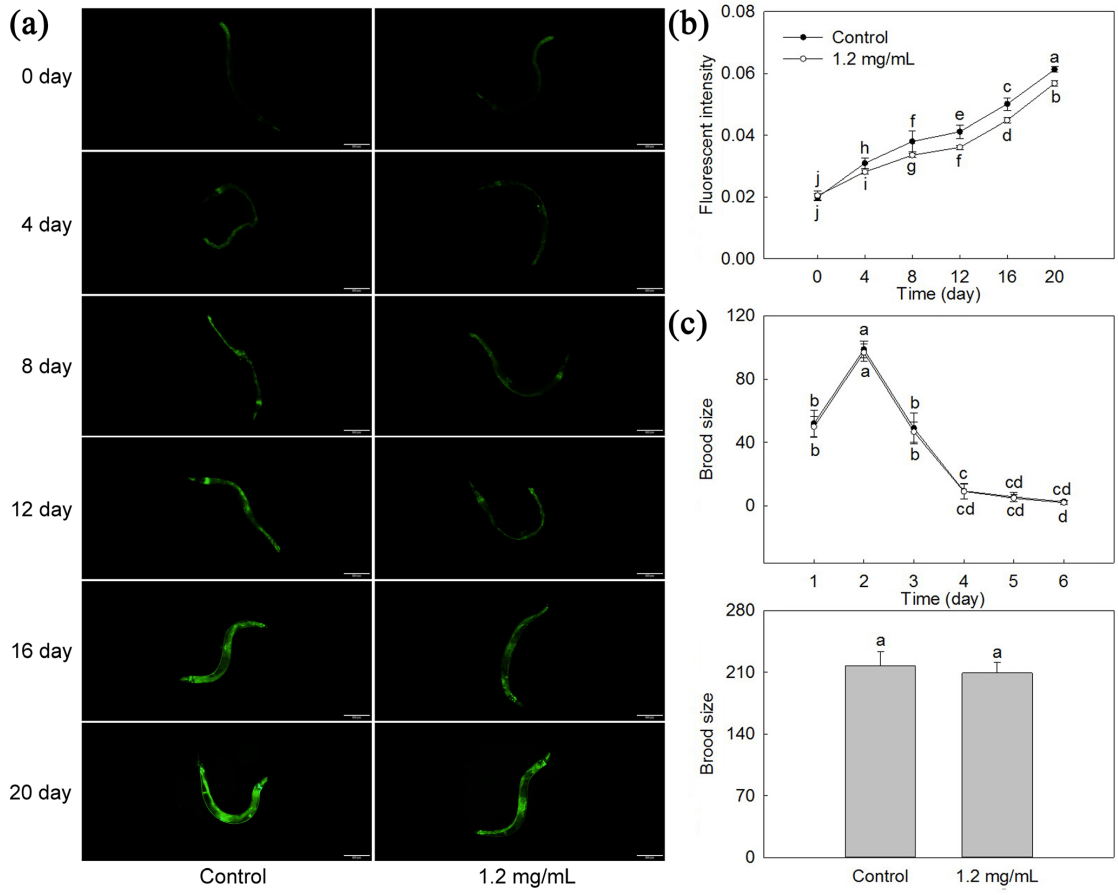

Figure 6. Effects of *Paeonia ostii* stamen tea on intestinal autofluorescence and reproduction of *Caenorhabditis elegan*. (a) Effects of stamen tea treatment on intestinal autofluorescence of nematodes; (b) Fluorescence intensity of intestinal autofluorescence; (c) Effects of stamen tea treatment on reproduction of nematodes. The values represented the mean ± SD, and different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

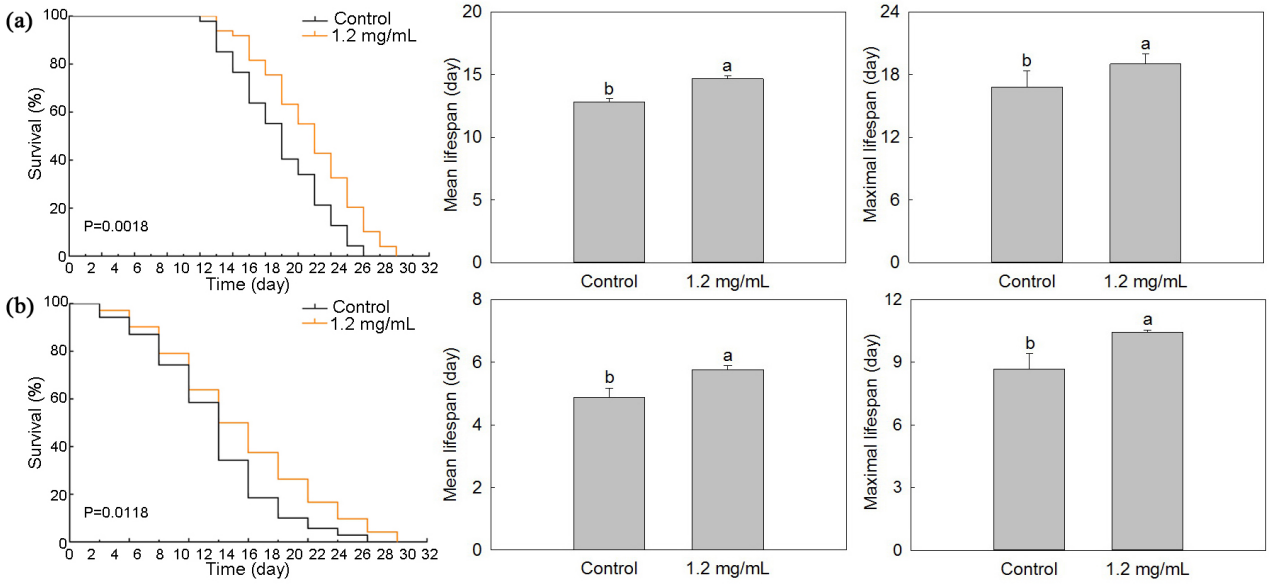

Figure 7. Effects of *Paeonia ostii* stamen tea on lifespan of *Caenorhabditis elegan* under heat stress and oxidative stress. (a) Lifespan curves of nematodes treated with stamen tea and their comparison of mean lifespan and maximal lifespan under heat stress; (b) Lifespan curves of nematodes treated with stamen tea and their comparison of mean lifespan and maximal lifespan under oxidative stress. The values represented the mean ± SD, and different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

extract (Somawathi et al., 2014) and blueberry anthocyanin extracts (Li et al., 2018). These results all indicated that POS tea possessed high antioxidant activity.

In recent years, *C. elegans* has been be used as a model to investigate the beneficial or adverse effects of components (Hirabayashi et al., 2001; Hunt, 2017; Fang et al., 2019; Prasanth et al., 2020; Reigada et al., 2020). A large number of studies have shown that natural products and their active substances can alleviate *C. elegans* aging. For example, Zhang et al. (2013) found wheat gluten hydrolysate could effectively extend the lifespan of *C. elegans*, and Martorell et al. (2016) found a nutritional supplement containing lactoferrin could extend the lifespan, stimulate the immune system and reduce amyloid β peptide toxicity in *C. elegans*. Moreover, the extract of *Lonicera japonica* (Yang et al., 2018), *Glycyrrhizae radix* (Ruan et al., 2016) and *Anacardium occidentale* (Duangjan et al., 2019) also had the similar results, and the lifespan of *C. elegans* had been extended with 21.87%-49.15%.

In the safety evaluation of POS tea on *C. elegans*, it had no significant effect on the lethality, growth, locomotion behavior, reproduction and intestinal autofluorescence of *C. elegans*, which indicated that POS tea has no toxicity. Furthermore, we found that 0.8-1.2 mg·mL^{-1} POS tea could extend the lifespan of *C. elegans* to some extent, and the treated effect of 1.2 mg·mL^{-1} POS tea was the most significant. Moreover, external drug stimulation can have a certain impact on *C. elegans* movement ability, and the observation of locomotion behavior of *C. elegans* was a physiological indicator for evaluating its normal movement (Zhen & Samuel, 2015; Hunt, 2017). This study found that 1.2 mg·mL^{-1} POS tea could significantly improve the locomotion behavior of *C. elegans*, suggesting that POS tea had a positive effect on the muscle activity of *C. elegans*.

In addition, intestinal autofluorescence reflects the deposition of lipofuscin, and its accumulation increases gradually with age, which can be used as an effective biomarker for *C. elegans* aging (Zhang et al., 2013). In this study, 1.2 mg·mL^{-1} POS tea could effectively alleviate the deposition of lipofuscin in *C. elegans*, which indicated that POS tea could delay the aging of *C. elegans* and improve its survival.

Previous studies have shown that the extension of *C. elegans* lifespan is related to the loss or reduction of reproductive capacity (Kenyon, 2010), caloric restriction (Cavallini et al., 2008), environmental stress (Lithgow & Walker, 2002) and other mechanisms. In this study, the mechanism of POS tea extending *C. elegans* lifespan was discussed by observing brood size of *C. elegans*, the growth of *E. coli* OP50 and the lifespan of stressed *C. elegans*. Firstly, previous study showed that there was a "weighing the pros and cons" mechanism between reproduction and lifespan, which suggested that the extension of lifespan was usually at the expense of reducing reproduction (Lu et al., 2012). However, with the deepening of study, it has also been found that the drugs for the extension of lifespan are not necessarily at the expense of reducing reproduction. Wang et al. (2014) found that grape seed proanthocyanidins delayed aging by increasing stress resistance, but did not affect brood size of *C. elegans*. In this study, 1.2 mg·mL^{-1} POS tea did not affect the reproduction of *C. elegans*, indicating that POS tea did not extend the lifespan of *C. elegans* by reducing reproduction, which was consistent with the result of Wang et al. (2014). Moreover, the growth of *E. coli* OP50 was not affected by POS tea treatment, which was not the reason of *C. elegans* lifespan extension.

In addition, the lifespan of longevity *C. elegans* mutant increased with the increase of stress response ability (Hartwig et al., 2009), so the stress response ability of the organism was the main indicator of the body's anti-aging ability under stress conditions. In this study, 1.2 mg·mL^{-1} POS tea significantly extend the lifespan of *C. elegans* under both heat stress and oxidative stress, indicating that POS tea could improve the ability of *C. elegans* to resist heat stress and oxidative stress. This might be one of the mechanisms of anti-aging of POS tea, which was consistent with the result of study in specioside (Asthana et al., 2015).

5 Conclusion

In summary, *P. ostii* stamens contained many active components with high antioxidant activity. Moreover, *C. elegans* was used as the vivo assay system, and systematic evidence suggested the relatively safe property of POS tea. Furthermore, POS tea was capable of extending the lifespan, improving growth, locomotion behavior and intestinal autofluorescence, which might be attributed to its ability to induce stress resistance including heat stress and oxidative. These results would be helpful in understanding the beneficial effects of POS tea and its development as a product.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Availability of data and material

The data used to support the findings of this study are included within the article and supplementary information file.

Author contributions

Tao J. and Zhao D. Q. designed the experiments; Meng J. S. and Cheng M. L. performed experiments; Meng J. S., Cheng M. L. and Zhang K. L. analyzed the data; Meng J. S., Zhao D. Q. and Hadi M. A. M. wrote the paper. All authors read and approved the final manuscript.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Identification of main active components in *Paeonia ostii* stamens

Table S2. Content of main active components in *Paeonia ostii* stamens

Table S3. Effects of *Paeonia ostii* stamen tea on relative fluorescence intensity of intestinal autofluorescence in *Caenorhabditis elegans*

Table S4. Effects of *Paeonia ostii* stamen treatment on the growth of *Escherichia coli OP50*

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