



Protective Effects of *Passiflora edulis* Fruit Extract on *Caenorhabditis elegans* under Low and High Oxidative Stress Conditions

Yeşim ÖZKAN^{1*} , Ayşenur ÇELİK¹ 

¹Department of Molecular Biology and Genetics, Faculty of Arts and Science, Ordu University, Ordu

*Correspondence: yozkan52@gmail.com

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Abstract: The worm *Caenorhabditis elegans* has a number of advantages, including lifespan, reactive oxygen species, fecundity, germline cell apoptosis, population growth, and mitochondrial membrane potential, as well as its ability to survive a wide range of environmental conditions, making it a useful model organism for investigating the effects of natural compounds and plants. *Passiflora edulis* Sims *edulis* (purple passion fruit) possesses high antioxidant activity due to its high polyphenol content. Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, is a key factor in cellular dysfunction and degenerative diseases. Natural antioxidants, particularly polyphenolic compounds, have demonstrated potential in mitigating oxidative damage. This study investigates the protective effects of *Passiflora edulis* Sims f. *edulis* (passion fruit) extract (PFE) on the nematode *Caenorhabditis elegans* under low and high oxidative stress conditions. Synchronized L3–L4 nematodes were exposed to 0.5 mmol/L H₂O₂ for 30 minutes (low stress) or 60 minutes (high stress) and subsequently treated with low (28 µL) or high (555 µL) doses of PFE. ROS levels, survival rate, reproduction, and population growth were evaluated. Results indicated that low-dose PFE reduced ROS levels and improved survival, while high-dose PFE increased ROS under both stress and non-stress conditions. Reproductive capacity and population growth were significantly enhanced by PFE, with dose- and stress-dependent effects. These findings suggest that PFE exhibits adaptive hormetic effects at low concentrations and potential protective activity against oxidative stress, whereas high doses may induce oxidative stress under certain conditions.

Keywords: *Caenorhabditis elegans*, *Passiflora edulis*, oxidative stress, ROS, reproduction, survival

INTRODUCTION

Oxidative stress is a fundamental form of cellular stress that arises from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense capacity, playing a central role in the etiology of numerous degenerative pathologies (Spinelli et al., 2024; Remigante et al., 2024). Excessive ROS accumulation disrupts cellular homeostasis by inducing extensive molecular damage, including lipid peroxidation, protein oxidation, and DNA lesions. These molecular perturbations form the biochemical basis for functional decline associated with cardiometabolic diseases,

neurodegenerative syndromes, ischemic injury, and aging (Girotti and Korytowski, 2021; Lv et al., 2025). While ROS at low levels serve critical signaling functions, their overaccumulation triggers cell death pathways, thereby compromising tissue integrity and organismal resilience (Pérez-Torres et al., 2017; Gu et al., 2020). Consequently, oxidative stress represents a pivotal research focus for understanding both physiological regulation and pathophysiological outcomes.

Natural antioxidants, particularly polyphenolic compounds, have garnered increasing scientific interest due to their potential to mitigate the biochemical consequences of oxidative stress. Plant-derived antioxidants exhibit diverse mechanisms, including free radical scavenging, metal ion chelation, and modulation of signaling pathways, making them attractive candidates for both therapeutic interventions and functional food development (Imtiaz et al., 2023). Despite the traditional use of many medicinal plants, the mechanistic basis of their biological effects and their impact at the organismal level remain incompletely understood.

Passiflora edulis Sims f. *edulis* has historically been recognized for its sedative and anxiolytic properties, while recent studies highlight its anti-inflammatory and antioxidant potential (Petry et al., 2001; Dhawan et al., 2004; Coleta et al., 2006; Montanher et al., 2007). The phenolic profile of *Passiflora* species is enriched with flavonoids and other polyphenolic compounds that exhibit strong antioxidant activity (Ferrerres et al., 2007; Zeraik and Yariwake, 2010). However, *in vivo* data on the effects of fruit extracts under oxidative stress in model organisms are limited, particularly regarding how biological resilience is modulated under low versus high oxidative stress conditions.

Caenorhabditis elegans, as a genetically well-characterized model organism, offers unique advantages for investigating oxidative stress biology. Its genome contains orthologs corresponding to approximately 60-80% of human genes and conserves many signaling pathways involved in aging, apoptosis, metabolic regulation, and stress responses (Guarente and Kenyon, 2000; Lant and Storey, 2010). Moreover, its transparent body plan, short life cycle, and low maintenance cost allow real-time *in vivo* monitoring of ROS accumulation and oxidative damage (Kaletta and Hengartner, 2006). These features make *C. elegans* an ideal system for evaluating the biological effects of natural antioxidants. Although flavonoids and other plant-derived compounds have been largely

studied *in vitro* for their modulation of cellular stress mechanisms, the *in vivo* relevance at the whole-organism level remains largely unexplored.

In this study, examining the potential of *Passiflora edulis* fruit extract to enhance organismal resilience under oxidative stress using nematode models can provide valuable insights both mechanistically and for potential functional applications. The aim of the present study is to systematically evaluate the effects of *Passiflora edulis* fruit extract on survival and ROS accumulation in *C. elegans* under low and high oxidative stress conditions. Such analyses are intended to elucidate the modulatory capacity of the fruit extract on oxidative stress tolerance, addressing a significant gap in the literature regarding the biological efficacy of natural antioxidant compounds.

MATERIAL AND METHOD

Material

Caenorhabditis elegans N2 strain (wild-type Bristol) was obtained from İstinye University. All other laboratory materials were sourced from the Department of Molecular Biology and Genetics, Ordu University, and passion fruit (*Passiflora edulis*) was locally procured from Antalya, Turkey.

C. elegans Culture

In this study, the N2 wild-type strain of *C. elegans* was used. Nematodes were maintained on Nematode Growth Medium (NGM) and fed with *Escherichia coli* OP50 under standard culture conditions at a constant temperature of 20°C. Synchronized populations were generated by washing NGM plates containing predominantly gravid adults. To obtain synchronized nematodes, adult worms were treated with a bleaching solution (1 N NaOH and 5% sodium hypochlorite) and gently vortexed every 2 minutes over a total period of 10 minutes. The solution was then centrifuged, and the supernatant containing lysed worm debris was removed, leaving behind an egg pellet. The egg pellet was washed three times and resuspended in M9 buffer. Eggs were incubated with gentle agitation for approximately 12 hours to hatch and establish a synchronized population. Larvae at the L3–L4 stage were subsequently used for all experimental assays.

Preparation of Passion Fruit Extract (PFE)

Prior to extraction, passion fruits were thoroughly washed and crushed together with their seeds using a mortar and pestle. The fruit material was then subjected to

methanol treatment and extracted using a Soxhlet apparatus for approximately 6 hours. Following extraction, the methanolic extract was concentrated under reduced pressure using a rotary evaporator, ensuring complete removal of methanol. This process yielded approximately 3 mL of a semi-solid extract. The obtained extract was stored at -20°C until further use. For experimental applications, the extract was diluted with distilled water to prepare working solutions at two different concentrations: a high-dose group containing 555 µL and a low-dose group containing 28 µL of extract.

Experimental Groups and Stress Application

Experiments were organized into three main groups:

Control groups (CG₁–CG₃): unstressed nematodes, some treated with low or high dose PFE.

Low stress groups (LDS): nematodes exposed to 0.5 mmol/L H₂O₂ for 30 min, followed by PFE treatment.

High stress groups (HDS): nematodes exposed to 0.5 mmol/L H₂O₂ for 60 min, followed by PFE treatment.

After H₂O₂ exposure, nematodes were washed with M9 buffer, and PFE treatment was applied for 24 hours. All groups were incubated at 20°C.

Table 1. Indicating Treatment Regimen Plan for Experimental and Control Groups

Group	Control (no stress)	High Stress (H₂O₂)	Low Stress (H₂O₂)
CG₁	NGM only	HDS	LDS
CG₂	NGM + High Dose PFE (HD PFE)	HDS + HD PFE	LDS + HD PFE
CG₃	NGM + Low Dose PFE (LD PFE)	HDS + LD PFE	LDS + LD PFE

NGM: Nematode Growth Medium, **PFE:** Passion Fruit Extract (*Passiflora edulis*), **HD:** High Dose, **LD:** Low Dose, **HDS:** High Duration Stress (60 min H₂O₂ exposure), **LDS:** Low Duration Stress (30 min H₂O₂ exposure)

Passiflora Extract Treatment Protocol

In the experiments, methanolic extract obtained from fresh *Passiflora edulis* Sims f. *edulis* fruits was used as the therapeutic agent. The extract was applied at two concentrations: 555 µL for the high-dose group and 28 µL for the low-dose group. Hydrogen peroxide (H₂O₂, 1% w/v, 0.5 mmol/L) served as the oxidative stress inducer. Nematodes in the high-stress groups were exposed to H₂O₂ for 60 minutes, whereas

nematodes in the low-stress groups were exposed for 30 minutes. Following exposure, nematodes were carefully washed with M9 buffer to remove residual H₂O₂. Immediately after washing, nematodes in the experimental groups were treated with the passion fruit extract for 24 hours. Control groups were treated with the extract for 24 hours without prior washing. All experimental and control groups were incubated at 20°C throughout the treatment period.

DCFH-DA Assay for Intracellular ROS Levels in C. elegans

Intracellular reactive oxygen species (ROS) levels in *C. elegans* were measured using the molecular probe H₂DCFDA (2',7'-dichlorodihydrofluorescein diacetate), following previously described protocols with minor modifications (Liao et al., 2011; Yang et al., 2013; Yoon et al., 2018). In this assay, non-fluorescent DCFDA permeates the cells and is oxidized by intracellular H₂O₂ to form the fluorescent compound 2',7'-dichlorofluorescein (DCF), which is then quantified using a fluorescence reader. Briefly, adult worms from the experimental groups (exposed to H₂O₂ stress and subsequently treated with passion fruit extract) and control groups were incubated in 6-well plates containing 50 µM H₂DCFDA in M9 buffer at room temperature for 30 minutes in the dark, in triplicate. Following incubation, fluorescence intensity was measured with excitation at 485 nm and emission at 528 nm to assess ROS production.

Visualization of Ros Production in c. elegans Using Fluorescence Microscopy

For fluorescence imaging, worms from both control and experimental groups were incubated in 6-well plates containing 50 µM H₂DCFDA in M9 buffer at 37°C for 1 hour in the dark, with three replicates per group. Following incubation, nematodes were carefully washed with M9 buffer to remove excess dye. Fluorescence imaging was performed using a Nikon Eclipse Ni fluorescence microscope, ensuring consistent exposure time, magnification, and light intensity across all samples. Multiple regions of each nematode were imaged to obtain representative data.

Lethality (Survival Rate) Assay

The survival rate of *C. elegans* was assessed to evaluate the effects of low and high H₂O₂ exposure followed by treatment with passion fruit extract (PFE) (Zhang et al., 2018). For this purpose, synchronized adult nematodes (L4 stage, 5 ± 1 worms per well) were transferred into 24-well plates containing Nematode Growth Medium (NGM). *Escherichia coli* OP50 served as the food source. Nematodes were incubated at 20°C for

7 days. Every 24 hours, each well was examined under a dissection microscope to count live and dead nematodes. Survival was determined based on the worms' motor response to mechanical stimulation (platinum wire touch) (Dhawan et al., 1999). Nematodes that were immobile and unresponsive to the stimulus were considered dead. The survival rate was calculated using the following formula (Zhang et al., 2018):

$$\text{Survival rate of } C. \textit{ elegans} = \left(\frac{\text{Number of surviving nematodes}}{\text{Total number of nematodes}} \right) \times 100$$

Reproduction Assay

Reproductive performance of *C. elegans* was evaluated using a 72-hour assay as described by Dhawan et al. (1999), with minor modifications. Test solutions were prepared from three experimental groups treated with either high or low doses of PFE, alongside a control group. For each well, a single worm from an age-synchronized culture was transferred into 5 μ L of the respective test solution. Nine wells were assigned per concentration, and all treatments were conducted under identical conditions to those used in the lethality assay. After 96 hours, the total number of offspring, including all developmental stages beyond the fifth generation, was recorded. For each experimental condition and control group, the mean number of offspring across the nine wells was calculated, and the entire assay was repeated three times to ensure reproducibility.

Population Growth Assay

The population growth of *C. elegans* was assessed to evaluate the potential restorative effects of PFE following exposure to low and high H₂O₂-induced oxidative stress (Dhawan et al., 1999). Age-synchronized L4 stage nematodes were transferred to 24-well NGM (Nematode Growth Medium) plates at a density of 20 nematodes per well (Corsi et al., 2015). *Escherichia coli* OP50 was used as the food source. Experimental and control groups were subjected to the respective stress and treatment regimens as described in Table 1, with control groups remaining unexposed to H₂O₂. All plates were incubated at 20 \pm 1 $^{\circ}$ C for 5 days. On day 5, the total number of live individuals in each well, including parent nematodes, larvae, and offspring, was counted under a stereomicroscope. Nematodes from each well were collected into 2 mL of M9 buffer, homogenized carefully, and 5 μ L aliquots were taken for counting under a stereomicroscope. Counts were performed separately for each group, and the average across three replicates was calculated. All measurements were conducted on the same day

by the same investigator to minimize variability. Population growth was expressed relative to the initial number of nematodes using the following formula (Zhang et al., 2018).

$$\text{Population Increase (\%)} = \frac{\text{Observed live nematodes} - \text{Initial nematodes}}{\text{Initial nematodes}} \times 100$$

Statistical Analysis

All measurements, including ROS levels and other experimental parameters, were analyzed using IBM SPSS Statistics version 26. Differences between groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test when significant differences were detected. A p-value of <0.05 was considered statistically significant.

RESULTS

Measurement of ROS Levels as an Oxidative Stress Biomarker

In this study, the *in vivo* model organism *Caenorhabditis elegans* was subjected to oxidative stress by exposure to 1% H₂O₂ (0.5 mmol/L) for low (30 min) and high (60 min) durations. The protective potential of *Passiflora edulis* fruit extract (PFE) was subsequently evaluated at both high and low concentrations to assess its capacity to mitigate oxidative stress-induced damage.

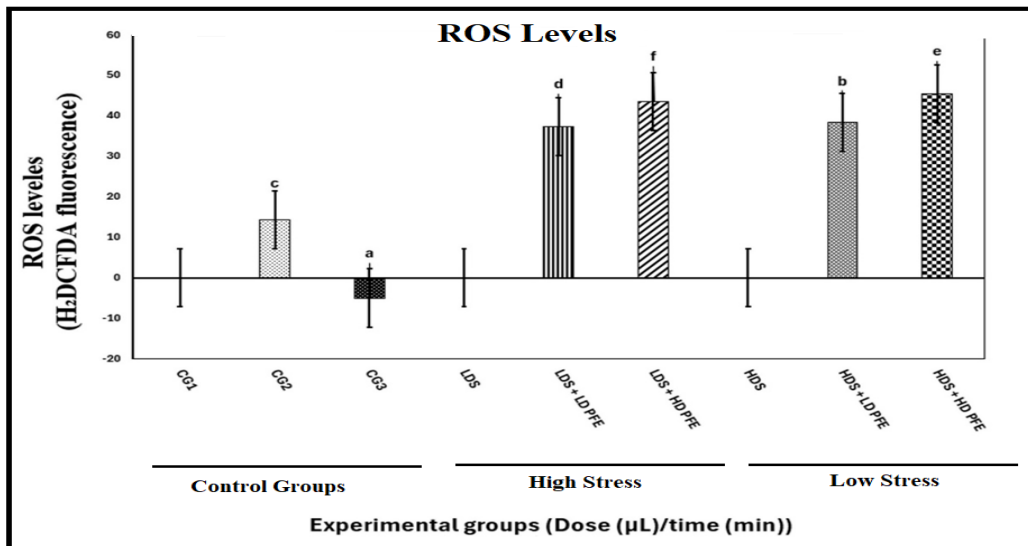


Figure 1. Reactive oxygen species (ROS) levels estimated by the H₂DCFDA assay in wild-type (N2) *Caenorhabditis elegans* strains. The data obtained are the average of three replicate samples. Error bars with different letters indicate that the means are significantly different at the 95% confidence level (P<0.05).

Intracellular ROS levels were measured as a biomarker of oxidative stress, and the results are presented in Fig. 1. Among the three control groups (CG₁, CG₂, and CG₃), which were not exposed to H₂O₂, ROS levels were markedly lower compared to the experimental groups. Within the control groups, the lowest ROS levels were observed in individuals treated with low-dose PFE. Conversely, high-dose extract treatment in CG₁ led to increased ROS levels compared to other control groups, suggesting that PFE may exert a dose-dependent biphasic effect-reducing oxidative stress at low concentrations while inducing stress at higher concentrations. In the experimental groups, different combinations of H₂O₂ exposure and extract dosage were evaluated. The highest ROS levels were observed in nematodes exposed to prolonged H₂O₂ treatment (high stress, 60 min) combined with high-dose extract, followed by worms exposed to short H₂O₂ exposure (low stress, 30 min) with high-dose extract. Notably, the duration of H₂O₂ exposure alone (30 and 60 min) did not significantly affect ROS production, indicating that the high-dose PFE, rather than H₂O₂ exposure duration, was primarily responsible for the observed increase in ROS. In other words, the extract acted as an additional pro-oxidant both in the presence and absence of H₂O₂. ROS levels across groups were ranked as follows: HDS + HD PFE > LDS + HD PFE > HDS + LD PFE > LDS + LD PFE > CG₂ > CG₁ > LDS > HDS > CG₃. Statistical analysis revealed significant differences among all groups, including the controls (P<0.05). These findings indicate that high-dose PFE induces oxidative stress, whereas low-dose extract exerts a protective, stress-reducing effect in *C. elegans* (P<0.05).

Fluorescence Microscopy Visualization of ROS Production

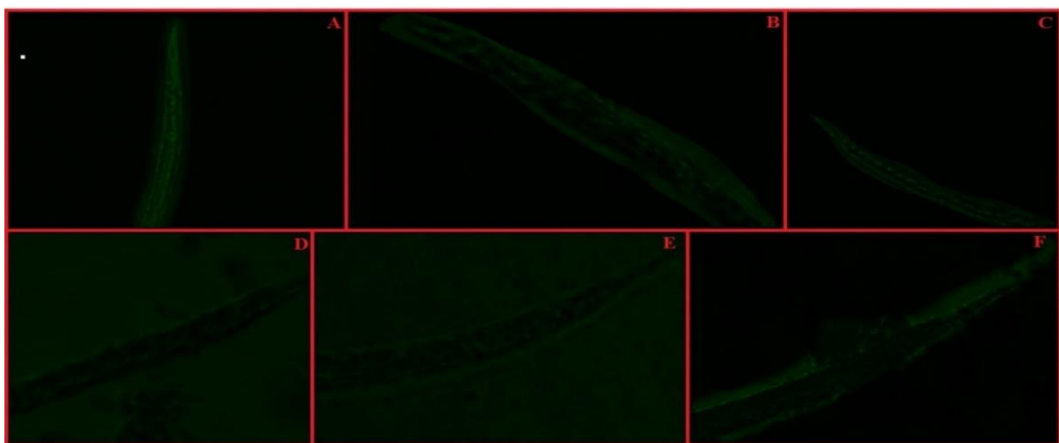


Figure 2. Fluorescence microscope images of the oxidative stress on the *C. elegans* (50 μ m). (A) HDS + HD PFE (B) LDS + HD PFE (C) HDS + LD PFE (D) CG₁ (E) HDS (F) CG₂

Fluorescence microscopy analysis revealed a pattern of ROS production that closely mirrored the quantitative ROS measurements. As shown in Figure 2, except for panels D and E, nematodes exhibited strong green fluorescence, corresponding to the localization of oxidative stress as stained by the H₂DCFDA probe. Visual assessment of fluorescence intensity indicated that the duration of H₂O₂ exposure (short and long) did not produce notable differences in ROS generation. In contrast, nematodes treated with high concentrations of *Passiflora edulis* fruit extract, including those in the control group CG₂, exhibited markedly increased fluorescence intensity, suggesting enhanced ROS accumulation in response to the extract at high doses.

Effect of Extract and H₂O₂ Stress on C. elegans Population Growth

The variation in offspring number in *C. elegans*, depending on the dose of *Passiflora* Fruit Extract (PFE) and the duration of H₂O₂-induced stress, is shown in Figure 3.

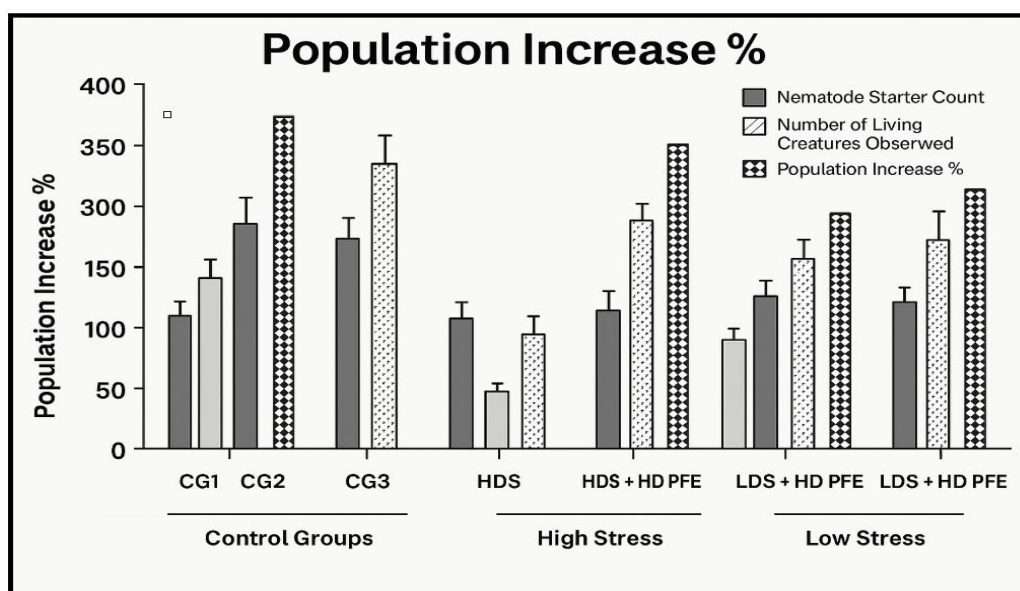


Figure 3. Population growth of *C. elegans* is influenced by *Passiflora* Fruit extract (PFE) concentration and H₂O₂-induced stress. Results from the experiments in which nematodes were incubated for 7 days under H₂O₂ exposure followed by treatment with PFE are presented. Treatment with PFE enhanced nematode reproductive output. Additionally, the extract exhibited a dose-dependent mitigation of oxidative stress. Different letters indicate statistically significant differences between group means as determined by one-way ANOVA (P<0.05) followed by Duncan's Multiple Range Test (DMRT; $\alpha = 0.05$). Error bars represent standard deviations (n=3).

Individuals exhibited significant variability in reproduction based on both the H₂O₂ exposure period and PFE concentration. Both the control groups (without H₂O₂ exposure) and the groups treated with PFE following H₂O₂ stress showed a significant

increase in population size ($P < 0.05$). Population growth was influenced by both the duration of H_2O_2 exposure and the PFE concentration. In groups exposed to H_2O_2 , the number of live nematodes was markedly reduced. However, PFE treatment following H_2O_2 exposure resulted in increased survival. Notably, in groups exposed to prolonged H_2O_2 stress, nematodes treated with a high PFE dose exhibited fewer live individuals compared to those treated with a low PFE dose, indicating that while PFE has a restorative effect, its efficacy decreases at higher concentrations. Specifically, nematodes exposed to prolonged H_2O_2 stress responded better to low-dose PFE treatment than to high-dose treatment. Conversely, in groups exposed to short-term H_2O_2 stress, high-dose PFE treatment resulted in a substantial increase in live nematodes. These results collectively suggest that PFE possesses a strong restorative effect on nematode population growth under oxidative stress conditions.

Effect of Passiflora Fruit Extract on C. elegans Survival Under Oxidative Stress

In the survival analysis of *Caenorhabditis elegans*, exposure to high-duration H_2O_2 stress (HDS) resulted in severe toxicity, markedly reducing survival rates (Figure 4).

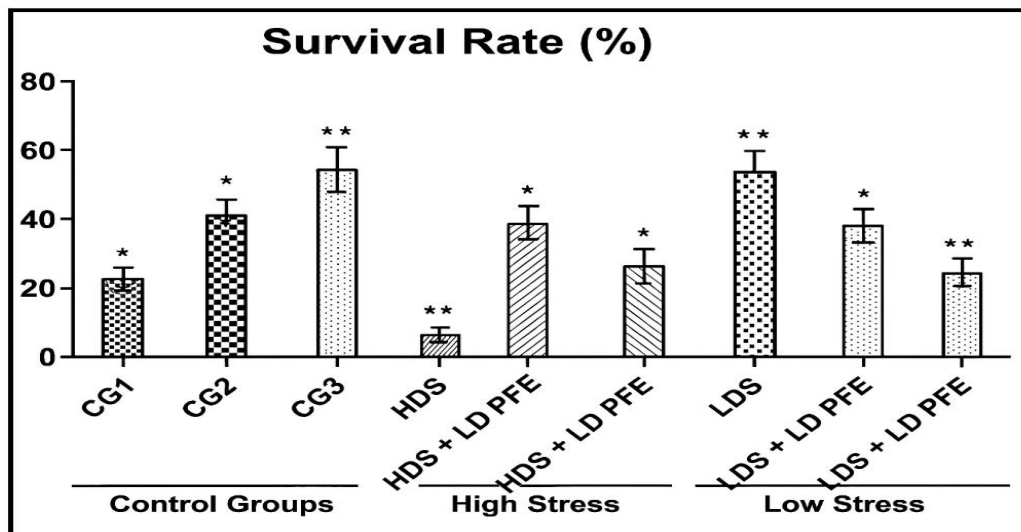


Figure 4. Survival Rate (%) of *Caenorhabditis elegans* after 1% 0.5 mmol/L H_2O_2 -mediated oxidative stress. Different population groups were exposed to low and high duration H_2O_2 before being treated with high and low doses of passiflora fruit extract. Untreated populations were used as controls ($n = 20$). Data are presented as mean survival percentage ($P < 0.05$).

Treatment with PFE at both high and low doses significantly improved survival compared to untreated nematodes under HDS conditions ($P < 0.05$), indicating a protective or anti-stress effect of PFE under severe oxidative stress. Under low-duration stress

(LDS), PFE treatment also enhanced survival at both doses, with the high-dose PFE group exhibiting the highest survival percentage. These findings suggest that PFE may induce an adaptive or mild hormetic response under mild stress conditions. Among the control groups, CG₁ displayed the highest survival, whereas CG₂ and CG₃ showed significantly reduced survival rates, indicating that in the absence of oxidative stress, PFE may exert a mild toxic or nutrient-disturbing effect. Overall, these results demonstrate that PFE exerts a condition-dependent effect: protective under oxidative stress, yet potentially deleterious in stress-free environments.

DISCUSSION

In this study, the biological effects of *Passiflora edulis* Sims f. *edulis* fruit extract (PFE) were systematically investigated in *Caenorhabditis elegans* under low and high oxidative stress conditions. The findings indicate that PFE exerts significant effects on ROS production, survival, and reproduction, depending on both extract concentration and stress duration. ROS measurements and fluorescence microscopy images revealed that high-dose PFE increased ROS levels in nematodes, regardless of whether they were exposed to H₂O₂ stress or not. This suggests that PFE can exhibit pro-oxidant properties at high concentrations, indicating that the biological effects of the extract are dose- and environment-dependent. In contrast, low-dose PFE demonstrated antioxidant activity by reducing ROS levels under both stressed and control conditions. These results are consistent with previous reports in the literature that flavonoids and polyphenolic compounds can exert hormetic effects (Imtiaz et al., 2023). Previous studies support the antioxidative potential of *P. edulis*. Sunitha and Devaki (2009) reported strong radical scavenging activity of *P. edulis* leaf ethanol extracts using DPPH assays. Similarly, methanolic extracts of *P. edulis* fruit and peel reduced neutrophil-mediated ROS production and myeloperoxidase activity, indicating anti-inflammatory and antioxidant effects *in vitro*. *In vivo* studies in rats further showed that consumption of *P. edulis* byproducts enhanced glutathione reductase activity and reduced oxidative damage (da Silva et al., 2014; Cazarin et al., 2015). Additionally, methanolic extracts of *P. edulis* fruits demonstrated broad antioxidant activity (DPPH, ABTS, FRAP, CUPRAC) as well as potential anticancer properties, emphasizing the bioactive potential of the fruit (Badalova et al., 2021).

Our findings in *C. elegans* align with previous studies using this model organism to assess antioxidant activity and stress resistance. For example, the leaf extract of *Garcinia atroviridis* was reported to enhance both heat and oxidative stress resistance and modulate stress-response gene pathways in *C. elegans* (Chuajit et al., 2024). Classical polyphenols, such as curcumin and resveratrol, also demonstrate similar effects in nematodes, reducing ROS accumulation, enhancing survival under oxidative stress, and extending lifespan (Chen et al., 2013; Xu et al., 2023). Furthermore, *Cassia fistula* extracts improved oxidative stress resistance and survival in *C. elegans*, confirming the generalizability of plant-based antioxidant interventions in nematode models (Thabit et al., 2018). Comparing our results with these studies, several parallels emerge. First, similar to curcumin, resveratrol, and *Cassia fistula*, PFE at low doses enhanced ROS scavenging and survival in stressed nematodes. Second, our observation that high-dose PFE can increase oxidative stress is reminiscent of hormetic responses reported for other polyphenols, where excessive doses may paradoxically induce mild stress. Third, PFE treatment also promoted reproductive output and population growth, consistent with the notion that antioxidants can mitigate stress-induced reproductive impairment in *C. elegans*.

Overall, our data corroborate the established antioxidant properties of *P. edulis* and extend these findings by demonstrating its efficacy in a whole-organism *in vivo* model. The differential effects of low and high doses underscore the importance of optimizing extract concentration to maximize protective benefits while avoiding potential pro-oxidant effects. These results contribute to a growing body of evidence supporting the use of natural fruit extracts as modulators of oxidative stress, survival, and reproductive health in model organisms.

CONCLUSION

This study demonstrates that *Passiflora edulis* fruit extract (PFE) exerts dose- and stress-dependent effects on *Caenorhabditis elegans* under oxidative stress. High-dose PFE significantly elevated ROS levels in both stressed and non-stressed nematodes, indicating potential pro-oxidant activity, whereas low-dose PFE consistently reduced ROS, showing strong antioxidant properties. Survival analyses revealed that PFE improved nematode viability under both low and high oxidative stress, with the greatest benefit observed at low stress and moderate extract concentrations. Population and

reproductive assessments further indicated that PFE mitigates the detrimental effects of oxidative stress, although excessively high doses following prolonged stress were less effective, suggesting a hormetic response. Overall, these findings highlight the dual nature of PFE: protective at low doses, yet potentially pro-oxidant at high doses, emphasizing the importance of optimal dosing for achieving maximal antioxidant and restorative effects.

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