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Research Article

CYANIDIN-3-GLUCOSIDE PROMOTES LONGEVITY AND TOLERANCE AGAINST UVA AND OXIDATIVE STRESS IN *CAENORHABDITIS ELEGANS*

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ABSTRACT: Crude anthocyanins extracted from different fruits and vegetables extend the lifespan of various organisms. However, the effects of pure anthocyanin compounds on the lifespan are still unknown. In this paper, we examined the effects of cyanidin-3-glucoside (C3G) on the lifespan and health span of *Caenorhabditis elegans* (*C. elegans*). We used egg-laying and pharyngeal pumping rates as indicators of their health. We also exposed the nematode to various stressors to determine whether C3G could protect them against heat, UVA, and H_2O_2. We found out that 25 µM of C3G extended the lifespan of *C. elegans* by 14% and enhanced the pharyngeal pumping by 6.3% ($p \leq 0.05$) without stress. This same amount of C3G improved the pharyngeal pumping rate of the nematode by 6% under UVA stress ($p \leq 0.05$). In the presence of exogenous H_2O_2, 25 µM of C3G prolonged the lifespan of *C. elegans* by 62% and augmented the pharyngeal pumping by 6.2% ($p \leq 0.05$). Overall, we observed that C3G extended the lifespan of *C. elegans* in the presence and absence of oxidative stress and improved the pharyngeal pumping even with or without UVA and oxidative stress. These findings have potential implications for the use of C3G in mitigating the effects of aging and oxidative stress. However, further studies are needed to elucidate the mechanisms underlying these effects.

Key words: Cyanidin-3-glucoside, Lifespan, Health span, Oxidative stress, UVA.

INTRODUCTION

Parts of medicinal plants are used in healthcare purposes since very ancient days. Various phytoconstituents of the medicinal plant parts play active roles in the system together (Pattanayak 2021, Patel *et al.* 2022, Paul and Sujatha 2022). Anthocyanins are flavonoid plant pigments that can be found in various fruits and vegetables. They possess strong antioxidant properties due to their high radical scavenging activity, which can prevent lipid peroxidation (Wang *et al.* 1997). Most anthocyanins exhibit potent antioxidant activity comparable to that of alpha-tocopherol, catechin, and quercitin (Kähkönen and Heinonen 2003). Anthocyanins have also been shown to affect telomere length and mitochondrial activity in various in vitro studies (Symonds *et al.* 2013, Yao and Vieira 2007). However, *in vivo*, studies using crude extracts of anthocyanins mainly focus on their effects on learning, memory, and anti-inflammatory activities (Andres-Lacueva *et al.* 2005, Varadinova *et al.* 2009, Hou *et al.* 2010).

Anthocyanin extracted from purple wheat, mulberry, blueberry, and acai berry have all demonstrated the potential in extending lifespan and improving stress resistance in *C. elegans* (Chen *et al.* 2013, Yan *et al.* 2017, Peixoto *et al.* 2016). However, the lifespan extension observed from different plant extracts varies. For instance, blueberry polyphenols only improve thermotolerance in *C. elegans*, but not against oxidative stress (Wilson *et al.* 2006). On the other hand, anthocyanins extracted from acai berries protect *C. elegans* from oxidative stress (Peixoto *et al.* 2016). We believe that this variation could be attributed to the presence of different classes of anthocyanin compounds, varying amounts of anthocyanins, and possible synergistic

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or antagonistic effects among the compounds present in the crude extract. Thus, isolating and characterizing the pure anthocyanin compound is crucial in determining its effects on longevity.

There are six different classes of anthocyanins: cyanidin, delphinidin, peonidin, petunidin, pelargonidin, and malvidin. To the best of our knowledge, only peonidin has been reported to extend lifespan and enhance the health of an *in vivo* model (Nas *et al.* 2021a). Studies on cyanidin and delphinidin have shown that they can protect the host from bacterial infection (Nas *et al.* 2021b, Nas *et al.* 2019). This suggests that pure anthocyanin compounds alone can deliver the beneficial effects previously shown by crude anthocyanin extracts. Therefore, we investigated the effects of cyanidin-3-glucoside on the lifespan and health of *C. elegans* in the absence or presence of heat, UV, and oxidative stress.

MATERIALS AND METHODS

Procurement and storage of chemicals

Cyanidin-3-glucoside was procured from ASPolyphenols, Norway. The positive control, coenzyme Q10 (coQ10), was purchased from ApexBio, USA. The compounds were reconstituted with water and stored in darkness at < -20°C. Freshly prepared solutions were made daily from the thawed stock solution. The freshly prepared solutions were placed in an amber vial and kept at 4°C before usage.

Procurement and maintenance of *C. elegans*

Wild type N2 was ordered from the Caenorhabditis Genetic Center (CGC) at the University of Minnesota, USA. *C. elegans* were grown in a nematode growth medium (NGM) seeded with OP50 *E. coli*. The storage condition of the NGM plates was maintained at 20°C throughout the experiment.

Measurement of lifespan

Each NGM plate was seeded with OP50 *E. coli* containing the following treatments: negative control (distilled water), positive control (174 µM coQ10), and C3G treatments (25, 12.5, and 6.25 µM C3G in distilled water). Thirty age-synchronized (±4 hours) worms starting from the L1 stage were exposed to these treatments and transferred to a fresh NGM plate every day. The number of dead, alive, and missing worms was counted daily under a stereomicroscope until all the worms were dead. Nematodes were scored as alive if they were motile or if they responded to a light stroke with a platinum wire. This assay was performed three times using a different set of nematodes.

Measurement of egg-laying ability

The eggs laid by the nematodes in each NGM plate from the previous set-up were counted through a photograph captured by an Amscope MD500 camera (7.5 fps, 35 mm, 1080p HD) starting on the first day of their adulthood. The egg-laying assay was performed thrice on a different set of worms until no more eggs were observed.

Measurement of the pharyngeal pumping rate

The pharyngeal pumping rate of each treated age-synchronized nematode was measured starting on day 1 of adulthood using a camera (Amscope MD500). One complete cycle of synchronous contraction and relaxation of the corpus and the terminal bulb was considered the pharyngeal pump. The pharyngeal pumping rate was measured by counting the number of pumps per minute (ppm). Pharyngeal pumping was measured thrice on a different set of worms until all were deceased.

Heat stress assay

Thirty age-synchronized L4 nematodes in each treatment were exposed to 30°C for 30 minutes daily until all individuals were deceased. The measurement of lifespan, egg-laying, and pharyngeal pumping was performed the day after each exposure following the previously mentioned protocol. This experiment was performed in three trials.

UVA stress assay

NGM plates containing 30 age-synchronized L4 *C. elegans* were exposed to ultraviolet light (UVA 365nm, 1300 µW/cm2) for 2 min daily. The handheld UV lamp (UV-GL-58) was placed 3 inches above the uncovered NGM plates. The measurement of lifespan, egg-laying, and pharyngeal pumping was performed the day after each exposure following the previously mentioned protocol (Nas *et al.* 2021a). This experiment was performed in three trials.

Oxidative stress assay

We dispensed 100 µM of freshly prepared hydrogen peroxide (H_2O_2) solution to the head of each nematode daily. They were immediately washed with distilled water before transferring to freshly prepared NGM plates. The measurement of lifespan, egg-laying, and pharyngeal pumping was performed the day after each exposure following the previously mentioned protocol (Nas *et al.* 2021a). This experiment was performed in three trials.

Statistical analysis

All data were presented as mean ± standard error. The result of all experiments involving the survival rate was analyzed using a log-rank test through OASIS version 2 (South Korea) (Nas *et al.* 2021a). Data obtained from egg-laying and pharyngeal pumping were analyzed using ANOVA through Graph Pad Prism version 8 (USA). Comparisons within and among the treatment groups were made using Tukey's multiple comparison test (Nas *et al.* 2021a). Statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effects of C3G on the lifespan and the health of *C. elegans* without stress

We found out that 25 µM of C3G was able to significantly extend the lifespan and enhance the pharyngeal pumping of *C. elegans* ($p \leq 0.05$), as shown in Fig. 1. However, the positive control, coQ10 and C3G were not able to improve the egg-laying ability of the nematode. In Fig. 1A, 25 µM of C3G increased the mean lifespan of *C. elegans* by 2.55 days compared to coQ10, which extended the lifespan of the nematode by about 3.7 days. The lifespan extension observed in C3G-treated worms was comparable with those fed 100 µg/mL purple wheat extract (Chen *et al.* 2013). It is interesting to note that the purple wheat extract rich in containing 42.6% C3G also has other kinds of anthocyanins (*i.e.*, peonidin-3-glucoside and malvidin-3-galactoside). This suggests that the higher amount of C3G or combination with other kinds of anthocyanins may not be additive on lifespan extension.

The absence of improvement in egg-laying ability suggests that C3G does not affect the aging pathways associated with the deterioration of egg-laying muscles. Our findings corroborate the previous report on the effect of acai berry extract on the brood size of *C. elegans* (Peixoto *et al.* 2016).

The pharyngeal pumping rate of *C. elegans* also improved by 13.6 pumps/min on average after exposure to 25 µM C3G, as shown in Fig. 1C. coQ10 also significantly enhanced the pharyngeal pumping of untreated *C. elegans* ($p \leq 0.05$), albeit not significantly higher than C3G ($p > 0.05$). This observed rate is comparable with recently published data on cyanidin-rich acai berry extract (Peixoto *et al.* 2016). The high pharyngeal pumping rate indicates the active feeding behavior of the worms. It implies that age-related muscle deterioration may have been impeded by C3G supplementation. This also suggests that the lifespan extension effects of C3G may be independent of caloric restriction.

Effects of C3G on the lifespan and the health of *C. elegans* with heat stress

We observed that the lifespan of untreated worms after heat stress, 30°C for 30 minutes daily, significantly dropped by 15% ($p \leq 0.05$). No observable change in the egg-laying ability was observed after heat stress. However, an 8.5% reduction in pharyngeal pumping rate was observed in untreated nematodes that were exposed to chronic heat stress ($p \leq 0.05$). When we compared the lifespan, egg-laying ability, and pharyngeal pumping of the worms treated with C3G and coQ10 to the untreated, they were all comparable ($p > 0.05$), as shown in Fig. 2. In a recent study, red cabbage extract containing high amounts of various kinds of cyanidin extended the survival of *C. elegans* against acute heat stress (35°C for 4 hours) by 31.64% (Zhang *et al.* 2021). Unlike their study, we exposed the nematodes to chronic heat stress, which may explain the disparity in our data. We speculate that cyanidin may be beneficial for acute heat stress but not

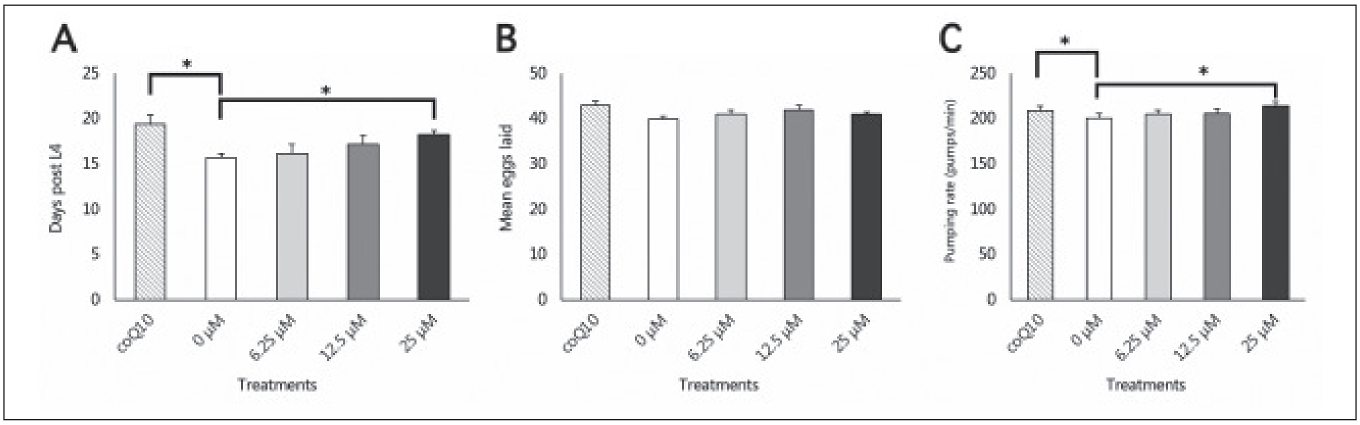

Fig. 1. Effects of C3G on the lifespan and healthspan of *C. elegans* without stress.
The nematodes given with varying concentrations of C3G (6.25, 12, and 25 µM) were examined for (A) mean lifespan, (B) average number of eggs laid, and (C) average daily pharyngeal pumping rate. The * denotes significance at $p \leq 0.05$.

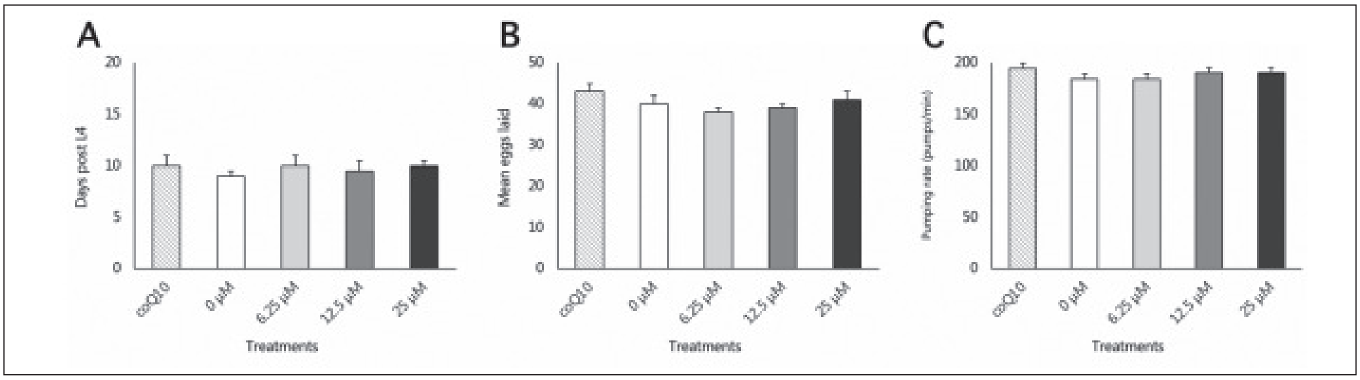

Fig. 2. Effects of C3G on the lifespan and healthspan of *C. elegans* with heat stress.
The nematodes given with varying concentrations of C3G (6.25, 12, and 25 µM) were incubated to 30°C for 30 minutes daily before observed for (A) mean lifespan, (B) average number of eggs laid, and (C) average daily pharyngeal pumping rate. The * denotes significance at p≤0.05.

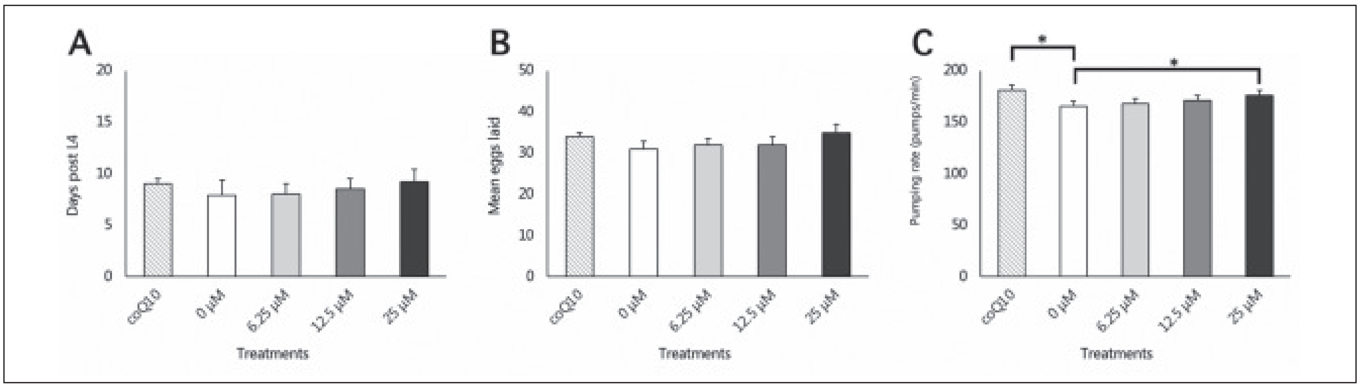

Fig. 3. Effects of C3G on the lifespan and healthspan of *C. elegans* with UVA stress.
The nematodes given with varying concentrations of C3G (6.25, 12, and 25 µM) were exposed to 365 nm UVA light for 2 minutes daily and observed for (A) mean lifespan, (B) average number of eggs laid, and (C) average daily pharyngeal pumping rate. The * denotes significance at p≤0.05.

against chronic heat stress. It is also important to note that the lack of significant effects observed in this study could be due to the specific concentration and treatment duration used. Further studies are needed to determine the optimal dosages and treatment durations of C3G and coQ10 for heat stress protection.

Effects of C3G on the lifespan and the health of *C. elegans* with UVA stress

After we exposed the nematodes to 365 nm UVA light for 2 minutes daily, we observed that the mean lifespan of the untreated worms was reduced by 49.7% (p≤0.05). Both the egg-laying and pharyngeal pumping of the UVA-exposed untreated worms decreased significantly (p≤0.05) by 22.5% and 17.6%, respectively. However, C3G treatment does not significantly increase the mean lifespan and average eggs laid by *C. elegans* (p>0.05) despite the significant improvement in the pharyngeal pumping (p≤0.05), as shown in Fig. 3. Both CoQ10 and C3G treated nematodes displayed 9.7% and 6% higher average pharyngeal pumping rates when compared with the untreated worms, as shown in Fig. 3C. We compared our findings with the data from acute UV exposure of *C. elegans* treated with black rice extract rich in cyanidin (Li *et al.* 2023). They reported a 36.3% increase in mean lifespan after 4-hour exposure (Li *et al.* 2023). Similar to our speculation in heat stress assay, cyanidin may have protected *C. elegans* from acute UV stress but not against chronic exposure. Although, we observed that the deterioration of the pharyngeal pumping muscle may have been slowed down by cyanidin in chronic UV exposure.

The results of this study suggest that exposure to UVA light can have detrimental effects on the lifespan, egg-laying, and pharyngeal pumping rate of *C. elegans*. These findings are consistent with previous studies that have shown that exposure to UVA radiation can induce

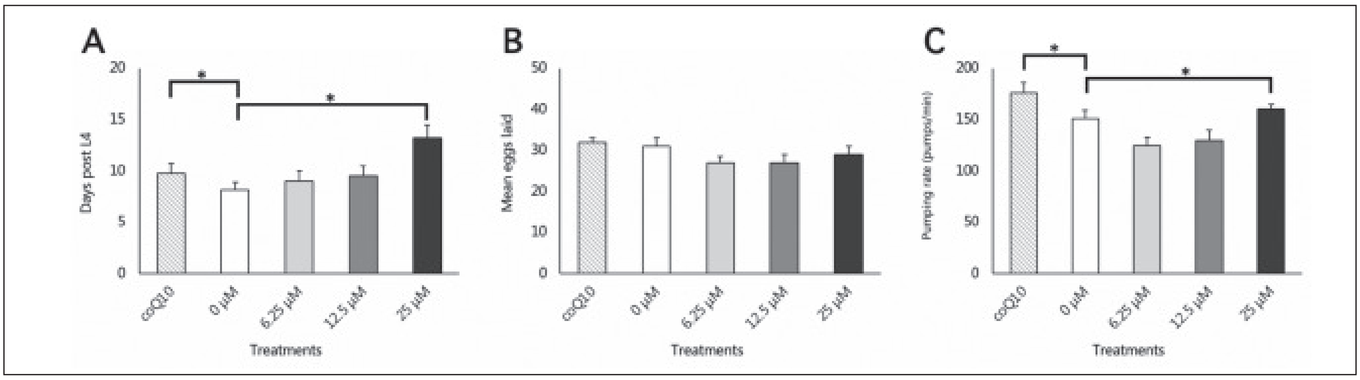

Fig. 4. Effects of C3G on the lifespan and healthspan of *C. elegans* with oxidative stress.
The nematodes given with varying concentrations of C3G (6.25, 12, and 25 µM) were also fed with 100 µM of H_2O_2. Their (A) mean lifespan, (B) average number of eggs laid, and (C) average daily pharyngeal pumping rate were examined daily after each exposure. The * denotes significance at $p \leq 0.05$.

oxidative stress, damage DNA, and disrupt normal cellular processes, leading to premature aging and reduced lifespan (Panich *et al.* 2016). Interestingly, treatment with C3G did not significantly increase the lifespan or egg-laying of UVA-exposed nematodes, although it did improve pharyngeal pumping. This may suggest that C3G has specific effects on certain physiological processes in *C. elegans* that are not related to the lifespan or reproductive output.

On the other hand, treatment with CoQ10 resulted in a significant increase in pharyngeal pumping rate, suggesting that CoQ10 may have a protective effect against UVA-induced damage. CoQ10 is a potent antioxidant that can scavenge free radicals and prevent oxidative damage to cellular structures, including mitochondria (Hseu *et al.* 2019). Since UVA radiation is known to induce the production of reactive oxygen species (ROS), CoQ10 supplementation may help to mitigate the effects of UVA-induced oxidative stress in *C. elegans*.

Effects of C3G on the lifespan and the health of *C. elegans* with oxidative stress

The nematodes that received no treatment aside from 100 µM of H_2O_2 were observed to have a 47% shorter lifespan, 22.5% reduced egg-laying, and 24.9% lower pharyngeal pumping rate. Both coQ10 and C3G significantly increased the mean lifespan and pharyngeal pumping ($p \leq 0.05$), as shown in Fig. 4. C3G extended the mean lifespan of the H_2O_2-treated nematodes by 5.06 days, which is also significantly higher than the 19.7% increase brought by coQ10 supplementation ($p \leq 0.05$). However, coQ10 enhanced the pharyngeal pumping of *C. elegans* by 25.32 pumps/min on average, 2.5-folds higher than 25 µM of C3G ($p \leq 0.05$).

The mechanisms by which coQ10 and C3G exert their beneficial effects are not fully understood. However, it has been suggested that coQ10 may enhance mitochondrial function and reduce oxidative stress by acting as an electron carrier in the electron transport chain (Xie *et al.* 2020). On the other hand, C3G has been shown to activate the Nrf2 pathway, which regulates the expression of genes involved in cellular defense against oxidative stress (Rhaman *et al.* 2021). The present study has some limitations. The mechanisms by which coQ10 and C3G exert their effects were not directly examined. Further studies are needed to determine the specific molecular pathways involved.

CONCLUSION

In this study, the effects of cyanidin-3-glucoside (C3G) on the lifespan, muscle deterioration, reproductive output, and stress resistance of *Caenorhabditis elegans* were investigated. Results showed that C3G supplementation significantly extended the lifespan of *C. elegans*, which was comparable to the effect of coQ10. This extension in lifespan was accompanied by an improvement in the pharyngeal pumping rate, indicating that C3G may prevent age-related muscle deterioration. However, C3G did not have a significant effect on egg-laying ability or protection against heat stress. When tested on UVA-exposed nematodes, C3G did not increase the lifespan or egg-laying, but it did improve pharyngeal pumping. This suggests that C3G may have specific effects on certain physiological processes in *C. elegans* that are not related to lifespan or reproductive output. On the other hand, C3G was found to significantly increase the mean lifespan and pharyngeal pumping rate of H_2O_2-treated nematodes, indicating its potential as an anti-aging and anti-oxidant agent. Overall, these findings suggest that C3G may hold the potential in extending the lifespan and improving the health span of organisms, including humans, through its antioxidant property. However, further research is needed to investigate its potential as an anti-aging supplement.

REFERENCES

Andres-Lacueva C, Shukitt-Hale B, Galli RL, Jauregui O, Lamuela-Raventos RM *et al.* (2005) Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. Nutr Neurosci 8(2): 111-120.

Chen W, Muller D, Richling E, Wink M (2013) Anthocyanin-rich purple wheat prolongs the life span of *Caenorhabditis elegans* probably by activating the DAF-16/FOXO transcription factor. J Agric Food Chem 61(12): 3047-3053.

Hou Z, Qin P, Ren G (2010) Effect of anthocyanin-rich extract from black rice (*Oryza sativa* L. Japonica) on chronically alcohol-induced liver damage in rats. J Agric Food Chem 58(5): 3191-3196.

Hseu YC, Ho YG, Mathew DC, Yen HR, Chen XZ *et al.* (2019) The *in vitro* and *in vivo* depigmenting activity of Coenzyme Q10 through the down-regulation of ∝-MSH signaling pathways and induction of Nrf2/ARE-mediated antioxidant genes in UVA-irradiated skin keratinocytes. Biochem Pharmacol 164: 299-310.

Kähkönen MP, Heinonen M (2003) Antioxidant activity of anthocyanins and their aglycons. J Agric Food Chem 51(3): 628-633.

Li X, Wang X, Wang K, Yang X, Liu X *et al.* (2023) Black rice anthocyanin extract enhances the antioxidant capacity in PC12 cells and improves the lifespan by activating IIS pathway in *Caenorhabditis elegans*.Toxicol Pharmacol 265: 109533.

Nas JS, Roxas CK, Acero RR, Gamit AL, Kim JP *et al.* (2019) *Solanum melongena* (Egg plant) crude anthocyanin extract and delphinidin-3-glucoside protects *Caenorhabditis elegans* against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Phil J Health Res Dev 23(4):17-24.

Nas JS, Manalo RV, Medina PM (2021a) Peonidin-3-glucoside extends the lifespan of *Caenorhabditis elegans* and enhances its tolerance to heat, UV, and oxidative stresses. Science Asia 47(4): 457.

Nas JS, Sanchez A, Bullago JC, Fatalla JK, Gellecanao Jr F (2021b) molecular interactions of cyanidin-3-glucoside with bacterial proteins modulate the virulence of selected pathogens in *Caenorhabditis elegans.* Asian J Biol Life Sci 10(1): 151.

Panich U, Sittithumcharee G, Rathviboon N, Jirawatnotai S (2016) Ultraviolet radiation-induced skin aging: the role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging. Stem Cell Int 2016: 7370642.

Patel A, Shah H, Gandhi T (2022) Saponin rich fraction of *Bauhinia variegata* Linn. ameliorates kidney stone formation in Rats. Explor Anim Med Res 12(1): 74-84. DOI: 10.52635/eamr/12.1.74-84.

Pattanayak S (2021) Plants in healthcare: past, present and future. Explor Anim Med Res 11(2): 140-144. DOI: 10.52635/eamr/11.2.140-144.

Paul A, Sujatha K (2022) Concurrent effect of *Linum usitatissimum* and *Emblica officinalis* on lead induced oxidative stress and histomorphological changes in uterus of female Wistar rats. Explor Anim Med Res 12(2): 264-272. DOI: 10.52635/eamr/12.2.264-272.

Peixoto H, Roxo M, Krstin S, Rohrig T, Richling E *et al.* (2016) An anthocyanin-rich extract of acai (*Euterpe precatoria* Mart.) increases stress resistance and retards aging-related markers in *Caenorhabditis elegans*. J Agric Food Chem 64(6): 1283-1290.

Rahman S, Mathew S, Nair P, Ramadan WS, Vazhappilly CG (2021) Health benefits of cyanidin-3-glucoside as a potent modulator of Nrf2-mediated oxidative stress. Inflammopharmacology 29: 907-923.

Symonds EL, Konczak I, Fenech M (2013) The Australian fruit Illawarra plum (*Podocarpus elatus* Endl., Podocarpaceae) inhibits telomerase, increases histone deacetylase activity and decreases proliferation of colon cancer cells. Brit J Nutr 109(12): 2117-2125.

Varadinova MG, Docheva-Drenska DI, Boyadjieva NI (2009) Effects of anthocyanins on learning and memory of ovariectomized rats. Menopause 16(2): 345-349.

Wang H, Nair MG, Iezzoni AF, Strasburg GM, Booren AM *et al.* (1997) Quantification and characterization of anthocyanins in Balaton tart cherries. J Agric Food Chem 45(7): 2556-2560.

Wilson MA, Shukitt Hale B, Kalt W, Ingram DK, Joseph JA *et al.* (2006) Blueberry polyphenols increase lifespan and thermo-tolerance in *Caenorhabditis elegans*. Aging cell 5(1): 59-68.

Xie T, Wang C, Jin Y, Meng Q, Liu Q *et al.* (2020) CoenzymeQ10-induced activation of AMPK-YAP-OPA1 pathway alleviates atherosclerosis by improving mitochondrial function, inhibiting oxidative stress and promoting energy metabolism. Front Pharmacol 11: 1034, https://doi.org/10.3389/fphar.2020.01034.

Yan F, Chen Y, Azat R, Zheng X (2017) Mulberry anthocyanin extract ameliorates oxidative damage in HepG2 cells and prolongs the lifespan of *Caenorhabditis elegans* through MAPK and Nrf2 pathways. Oxid Med Cell Longev 2017: 7956158.

Yao Y, Vieira A (2007) Protective activities of Vaccinium antioxidants with potential relevance to mitochondrial dysfunction and neurotoxicity. Neurotoxicol 28(1): 93-100.

Zhang N, Jiao S, Jing P (2021) Red cabbage rather than green cabbage increases stress resistance and extends the lifespan of *Caenorhabditis elegans*. Antioxidants 10(6): 930.

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