Research Article

Antiaging, Stress Resistance, and Neuroprotective Efficacies of Cleistocalyx nervosum var. paniala Fruit Extracts Using Caenorhabditis elegans Model

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1. Introduction

Plants can synthesize many bioactive molecules, better known as “phytochemicals,” which aid in the protection of plants, and can have a huge impact on human health and metabolism [1]. A wide variety of medicinal properties are exhibited by different plants ranging from anti-inflammatory, antioxidant, antitumor and immunomodulatory effects [2], apart from improving cardiovascular ailments [3], treating kidney stones [4] and digestive diseases like inflammatory bowel disease [5], and many more. Another important advantage of using plants for their medicinal properties is that it either can be applied externally or can be consumed as a food or beverage [6–8].
Traditional medicine of countries varies depending upon the species of plants that grow in their vegetation and habitat. In ancient eras, traditional medicinal practices were followed to treat a variety of diseases such as headache, dizziness, cold, wounds, cough, and asthma. However, scientific advancements lead to the identification of bioactive compounds, which can induce these effects. The twentieth century witnessed the advancement of synthetic drugs and antibiotics over plant extracts in curing diseases because of its ease of using and quicker action. However, recently, many side effects of using these drugs surfaced which lead scientists to look back to the traditional way of using plants and their derivatives [1]. Until now, only a small percentage of the existing plant species have been scientifically explored for their bioactivities and possible benefits [9] which opens a wide arena in the field of research.

Cleistocalyx nervosum var. paniula (C. nervosum), an indigenous berry fruit widely grown in the northern parts of Thailand [10], belongs to the family Myrtaceae and is used in traditional medicine as it is known to possess various health benefits [11–15]. Additionally, it is a key ingredient in health drinks and functional foods, because of the characteristic sweet and sour taste along with the natural red color which contains anthocyanins, antioxidants, and phenolics [16]. C. nervosum is one of the richest sources of anthocyanins among various berry fruits [15, 17].

C. nervosum exhibit various medicinal properties and health benefits such as antioxidant and antiaging properties [16, 18], anticarcinogenic properties [11, 12], antiheavy metal toxicity [19], and antimicrobial activities [20, 21]. Our group has previously reported the antioxidant potential and neuroprotective effects of C. nervosum in HT22 cell lines [15]. However, there is no clear idea about the overall health benefits and the in vivo mechanism involved in attaining these effects.

The soil nematode Caenorhabditis elegans is widely used as a model to understand different parameters including aging, development, reproduction, stress resistance, immune enhancement, and neurological disorders [22–24]. C. elegans can be used to understand different neurotoxic disorders such as Alzheimer’s [25], Parkinson’s [26], and dementia [27]. Ease of handling and maintenance, short life cycle and life span, and availability of single-gene mutants make it one of the most preferred models [28]. C. elegans is the first eukaryotic organism to be completely sequenced [29]. Research in C. elegans using various nutraceuticals from plant sources has shed light on the involvement of several genes and pathways along with dietary interventions which can modulate lifespan and healthspan [30].

Many plants or plant derivatives such as green tea [31], tomatidine [32], Streblus asper [33], Paullinia cupana [34], Gengnianchun [35], and mulberry [36] were observed to extend lifespan and healthspan along with improving stress response and antioxidant mechanism in C. elegans. The present study tries to understand the effect of C. nervosum in extending lifespan and healthspan, incorporating neuroprotection along with improving stress resistance in C. elegans.

2. Materials and Methods

2.1. Chemicals, Reagents, and Equipment Used. All the chemicals and reagents used in the study were purchased from Sigma-Aldrich (St. Louis, MO, USA) and HiMedia Laboratories (Mumbai, India). C. elegans were exposed to UV-A for 4 h using a UV transilluminator lamp, SANKYO DENKI (F20T10BL).

2.2. Plant Collection, Extraction, and Detection of In Vitro Antioxidant Potential. Fruit pulp of C. nervosum was collected from ripe fruits from two different locations, Chiang Mai and Lampang, which will be designated as CMK-P and LMK-P, respectively, from now on. The pulp was freeze-dried, and then, 50 g of each powdered pulp was subjected to extraction with ethanol using the Soxhlet extraction method. The extraction was carried out for 2 days. Then, the extracts were concentrated at 50°C using a rotary evaporator, and the crude extract was further made as 100 mg/ml stock solution using dimethyl sulfoxide (DMSO) and stored at -20°C [15].

The in vitro antioxidant activity was monitored through a 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging assay and oxygen radical absorbance capacity (ORAC) assay as described previously [15] by our group, and the results were represented as mg vitamin C (VC)/g dry sample and μmol Trolox (TE)/g dry sample, respectively (n = 3).

2.3. C. elegans Strain Used and Culture Conditions. Wild-type strain N2 (Bristol), daf-16 mutant CF1038, and AB transgenic strain CL2006 were purchased from the Caenorhabditis Genetics Center (University of Minnesota, USA) along with the bacterial food source E. coli OP50. All strains were maintained in nematode growth medium (NGM) at 15°C unless otherwise specified [37]. All the experiments were conducted in age-synchronized young adult worms. Each experiment was done in three independent trials [38].

2.4. Lifespan Assay. The lifespan assay was carried out, as explained previously [38]. The known number (~10) of age-synchronized nematodes (wild type and mutants) was placed in M9 buffer along with E. coli OP50 in a 24-well microtiter plate with different concentrations (1-100 μg/ml for wild type and 10-40 μg/ml for mutants) of C. nervosum fruit extracts dissolved in DMSO. The total number of live worms was counted every 24 h. 5-Fluoro-2′-deoxuryridine (FUDR) was added to prevent the production of progenies inside the experimental setup. Nematodes were considered dead when they do not respond even to a gentle tap or touch with the platinum loop. A parallel vehicle control of DMSO was also used, which was equivalent to the highest concentration of the solvent used. Worms treated only with E. coli OP50 were used as the control group. All the experiments were carried out in biological triplicates.

2.5. Pharyngeal Pumping Assay. The pharyngeal pumping assay was carried out, as explained previously [22]. The known number of young adult stage nematodes (~10) was transferred to NGM plates swabbed with different
concentrations of *C. nervosum* fruit extracts. Pharyngeal pumping was observed once in every 24 h using a stereomicroscope (Motic SMZ-171) for 30 consecutive seconds. Pharyngeal pumping of worms in NGM plates swabbed with *E. coli* OP50 was considered as the control group.

2.6 Lipofuscin Imaging. Accumulation of autofluorescent proteins inside the nematode was done in wild-type nematodes treated with different concentrations of *C. nervosum* (20 and 30 μg/ml) for 5 days. Worms treated only with *E. coli* OP50 were used as the control group. After incubation, the worms were washed thoroughly using M9 buffer and then transferred to a drop of sodium azide in a glass slide. Fluorescent imaging was done in 10 nematodes using a ZEISS LSM 700 confocal microscope using 10x magnification at the objective lens. The images were analyzed using ImageJ software, and the relative fluorescence was represented as arbitrary units (AU).

2.7 Measurement of Extracellular ROS Using DCF. Estimation of extracellular Reactive Oxygen Species (ROS) was done as previously described [38]. Briefly, two sets of wild-type nematodes were exposed to UV-A for 4 h. In the first set, the worms were treated with different concentrations of *C. nervosum* fruit extracts (20 and 30 μg/ml) before exposure. In the second set, the worms were treated with different concentrations of *C. nervosum* fruit extracts (20 and 30 μg/ml) after exposure. In both cases, the fruit extract treatment continued for 5 days and then were washed thoroughly with M9 buffer. After washing, the worms were incubated with 5 μM DCFH-DA for 20 minutes, followed by another wash to remove the excess of DCFH-DA. Further, the worms were transferred to a drop of sodium azide in a glass slide. Fluorescent imaging was done in 10 nematodes using a ZEISS LSM 700 confocal microscope. The images were analyzed using ImageJ software, and the relative fluorescence was represented as arbitrary units (AU). Two controls were used, wherein one set was exposed to UV-A for 4 h and did not receive any extracts (positive control), and the other set had no exposure to UV-A and no extract treatment (negative control).

**Table 1: List of primers used.**

| Gene name | Forward primer | Reverse primer |
|-----------|----------------|----------------|
| daf-2     | TCGAGTCCTCCTCCTACGGTGT | CATCTTGTCCACCACTGTC |
| daf-16    | TGTTGGAATTCATTGCTGAAA | ATGAATATGCTGGCCCTCAG |
| age-1     | ATAGAATCTCAGCGGACCTT | ATAGAATCTCAGCGGACCTT |
| utx-1     | GCCAACAACCAGCTCATCAG | ATCAACGGCATTCTTCTGC |
| col-19    | CACACAAATGCTCCACACAC | CGTGAATTTCTCTGTTCAC |
| egl-8     | GTGTACGTGTCGTTTCTCA | ATGATGACACAGGGGTG |
| egl-30    | TCAGAAAAGCGGAGGTGGGAT | GGGTCTCGTTGTGACACTCG |
| dkg-1     | GTTGGGAAGTGGTGCAAAT | GCGAGCTTGAGGATGAG |
| goa-1     | TGTTCGATGTGGGAGGTCAA | TCGTGCATTCGGTTGTG |
| skn-1     | ATCCATTCGGTAGAGGACCA | GGCCTACGTGCTGATTTTC |
| sir-2.1   | CGGGGGAATGCGAAAGATAA | GAGTGGACCACTCATCAAGA |
| act-2     | ATCGTCTCTCAGCTCGAGGATG | TCACGTCAGCAGAAGTCAG |

**Table 2: In vitro antioxidant potential of *C. nervosum* extracts.**

| Extract used | DPPH (μg VC/g dry sample) | ORAC (μmol TE/g dry sample) |
|--------------|---------------------------|-----------------------------|
| CMK-P        | 72.01 ± 3.32              | 140.17 ± 4.76               |
| LMK-P        | 104.19 ± 5.62             | 164.16 ± 5.45               |

2.8 Total RNA Isolation and Real-Time PCR Analysis. The TRIzol kit (Invitrogen, Carlsbad, CA, USA) was used to isolate total RNA from wild-type nematodes treated with different concentrations of *C. nervosum* fruit extracts (20 and 30 μg/ml). From the total RNA, 1000 ng was converted to cDNA using AccuPower RT Premix (Bioneer, Korea) with oligo dT primers following the manufacturer’s protocol. Real-time PCR was carried out using SYBR Green, Green Star PCR Master Mix (Bioneer, Korea), in the Exicycler Real-Time Quantitative Thermal Block (Bioneer, Daedeok-gu, Korea) with the help of gene-specific primers. The expression data were normalized to the internal control actin and then represented as upregulated or downregulated by normalized with the untreated control. The sequences of the primers are given in Table 1.

2.9 Statistical Analysis. Statistical analysis was carried out using GraphPad Prizm® for Mac version 6.0 h. All the results were represented as the mean ± standard deviation. *p* values lower than 0.05 were considered significant.

### 3. Results

3.1 In Vitro Antioxidant Potential of *C. nervosum* Extracts. The *in vitro* antioxidant potential was analyzed through DPPH and ORAC in *C. nervosum* extracts. The DPPH scavenging activity was observed to be 72.01 ± 3.32 mg VC/g dry sample and 104.19 ± 5.62 mg VC/g dry sample, respectively, for CMK-P and LMK-P extracts. Similarly, the ORAC levels were observed to be 140.17 ± 4.76 μmol TE/g dry sample and 164.16 ± 5.45 μmol TE/g dry sample, respectively, for CMK-P and LMK-P extracts (Table 2) indicating that
LMK-P is with higher *in vitro* antioxidant activity when compared to CMK-P.

3.2. *C. nervosum* could Extend the Median and Maximum Lifespan of *C. elegans*. Both the *C. nervosum* extracts collected were able to extend the median and maximum lifespan of *C. elegans* in all the tested concentrations from 1 to 100 μg/ml. Both CMK-P and LMK-P extracts exhibited an increase in median and maximum lifespan at all the tested concentrations (Figures 1 and 2). However, the higher doses of LMK-P, at 90 and 100 μg/ml, could not increase the lifespan of the nematode; rather, it was similar to that of the control (Figure 2(a)). Doses ranging between 10 and 40 μg/ml in both CMK-P and LMK-P showed maximum significance (*p < 0.05*) in increasing the maximum lifespan which was up to 28, 30, 29, and 28 days, respectively (Figures 1(c) and 2(c)). The worms used as the control, which was fed with laboratory food source *E. coli* OP50 and not treated with any of the extracts, survived up to 22 days (Figures 1 and 2). A parallel vehicle control was also used wherein the worms were treated with the highest dosage of solvent (DMSO) used, which also showed similar lifespan as of the control, indicating that no change was induced by the solvent (Figures 1 and 2).

3.3. *C. nervosum* Could Also Improve the Healthspan of *C. elegans*. Pharyngeal pumping was analyzed in *C. elegans* treated with 20 and 30 μg/ml of both CMK-P and LMK-P extracts. It was observed that both the extracts did not reduce the pharyngeal pumping of the nematodes and were showing a similar pumping rate when compared to the control worms fed with laboratory food source *E. coli* OP50 (Figure 3(a)).

**Figure 1:** CMK-P extract can extend the mean and median lifespan of *C. elegans*. (a) Wild-type nematodes were treated with different concentrations of CMK-P extracts ranging from 1 to 100 μg/ml which could significantly (*p < 0.05*) extend the maximum lifespan of the nematode. Nematodes used as the control which did not receive any extract treatment survived up to 22 days. DMSO was used as a vehicle control which also survived for 22 days. (b) Wild-type nematodes were treated with different concentrations of CMK-P extracts ranging from 1 to 100 μg/ml which could significantly (*p < 0.05*) extend the median lifespan of the nematode. (c) Selective doses which showed maximum extension of lifespan were represented. CMK-P extracts at 10, 20, 30, and 40 μg/ml could extend the lifespan of the nematode up to 28, 30, 29, and 28 days, respectively.

3.3. *C. nervosum* Could Also Improve the Healthspan of *C. elegans*. The level of autofluorescent protein, lipofuscin, which is an indicator of aging, was monitored inside the nematodes treated with 20 and 30 μg/ml of both CMK-P and LMK-P extracts. The LMK-P extract showed a significant (*p < 0.05*) reduction in the levels of lipofuscin in both the doses (Figures 3(g)–3(j)) whereas CMK-P showed significant (*p < 0.05*) reduction in 20 μg/ml concentration when compared to the control (Figures 3(c)–3(f)), indicating that the extract could slow down or reduce the accumulation of this protein.
Further, qPCR analysis of candidate genes that mediate healthspan was monitored. It was observed that the expression of egl-8 and egl-30 was upregulated significantly \((p < 0.05)\) and the expression of col-19, dgk-1, and goa-1 was significantly \((p < 0.05)\) downregulated in worms treated with 20 and 30 \(\mu\)g/ml of both CMK-P and LMK-P extracts when compared to the control (Figure 3(b)).

### 3.4. C. nervosum Mediated Extension of Lifespan, and Healthspan Is Dependent and Independent of DAF-16 Pathway

The qPCR analysis of major players of DAF-16 pathway was monitored in nematodes treated with 20 and 30 \(\mu\)g/ml of both CMK-P and LMK-P extracts. It was observed that the expression of daf-16 was upregulated significantly \((p < 0.05)\) at 30 \(\mu\)g/ml of both the extracts and that of daf-2, age-1, and utx-1 was downregulated significantly \((p < 0.05)\) when compared to the control, which indicated the role of DAF-16 pathway in C. nervosum-mediated extension of lifespan (Figure 4(a)).

In order to analyze the involvement of any other pathways in lifespan extension of C. nervosum extracts, mutants of DAF-16 were treated with 10–40 \(\mu\)g/ml of both CMK-P and LMK-P extracts and the survival level was monitored. A significant \((p < 0.05)\) increase in the median and maximum lifespan was observed at 20 and 30 \(\mu\)g/ml concentration of both CMK-P (Figures 4(b)–4(d)) and LMK-P (Figures 4(e)–4(g)) extracts in the mutant worms. This suggests that some other mechanisms could also mediate the lifespan extension by C. nervosum.

### 3.5. C. nervosum can Activate the Antioxidant Potential inside C. elegans

In order to analyze the antioxidant potential of C. nervosum extracts, C. elegans were induced with oxidative stress by exposing it to UV-A for 4 h [38]. The extracts were analyzed for the protective effects and repair effects by treating the extracts before and after induction of stress individually. There was a significant \((p < 0.05)\) reduction of the oxidative stress level observed, which is directly proportional to the reduction in fluorescence, in both CMK-P and LMK-P extracts (Figure 5).

Further, qPCR analysis of skn-1 and sir-2.1, candidate genes that mediate the antioxidant mechanism in C. elegans, was analyzed after treating with 20 and 30 \(\mu\)g/ml of both CMK-P and LMK-P extracts. It was observed that the expression of both the genes was upregulated significantly \((p < 0.05)\) indicating the activation of the antioxidant mechanism inside the nematode (Figure 6).
3.6. *C. nervosum* can Impart Neuroprotection in Transgenic *C. elegans*. Finally, to analyze the neuroprotective effect of *C. nervosum* extracts, a transgenic strain of *C. elegans*, CL2006, which expresses Aβ1-42 constitutively was treated with 10–40 μg/ml of both CMK-P and LMK-P extracts, and the survival level was monitored. It was observed that both CMK-P (Figures 7(a)–7(c)) and LMK-P (Figures 7(d)–7(f)) at 20 and 30 μg/ml concentration could significantly (*p* < 0.05) extend the median and maximum lifespan of the nematodes suggesting its neuroprotective potential.
4. Discussion

Aging is the process of accumulation of damages to cells, tissues, and organs of an individual which is universal and unique, thereby reducing the overall health of the organism [39, 40]. Even after the advancements in the field of research, the complete mechanism of the aging process is yet unclear. Healthy aging depends on several broad factors such as physiological, biological, nutritional, behavioral, mental, and social factors [41]. From the available scientific knowledge, it is evident that aging can induce stress inside the system in the form of ROS or other stressors, reduce overall
health, and induce age-associated neurological diseases [42–44]. In this regard, the focus is now on medicinal plants and its derivatives, which can exert antiaging potential by various diverse mechanisms including antioxidant, immune-enhancing, and neuroprotective potential with minimum side effects [44–47].

C. nervosum is one such plant that is reported to have immense antioxidant, antimutagenic, anticarcinogenic, and antiaging properties in vitro [11–15]. Our group has recently established this fruit to mediate neuroprotection in HT22 cells majorly based on its antioxidant potential, as it expressed free radical scavenging activity and antioxidant activity, which was evident from DPPH, ORAC, and FRAP assays. Further, cyanidin-3-glucoside was identified as the major anthocyanin, which could have many potential health benefits [15].

Aging is interconnected to lifespan even though both are not equivalent [39]. Lifespan alone can determine the overall survival rate of the organism, although it cannot clearly define the rate of aging [48]. In this regard, it is important

**Figure 5:** C. nervosum extracts could activate the antioxidant potential by reducing the level of ROS in C. elegans. (a) Relative fluorescence intensity comparison of nematodes exposed to UV-A for 4 h to induce stress along with co- and posttreatment with CMK-P and LMK-P extract at 20 and 30 μg/ml showing significant (p < 0.05) reduction in fluorescence when compared to control worms exposed to UV-A without any extract treatment (n = 10). (b) Representative image of the negative control worm which was not exposed to UV-A and did not receive any extract treatment. (c) Representative image of the positive control worm which was exposed to UV-A for 4 h but did not receive any extract treatment. (d) Representative image of the worm which was treated with 20 μg/ml of CMK-P along with UV-A exposure for 4 h (cotreatment). (e) Representative image of the worm which was treated with 30 μg/ml of CMK-P along with UV-A exposure for 4 h (cotreatment). (f) Representative image of the worm which was treated with 20 μg/ml of CMK-P after UV-A exposure for 4 h (posttreatment). (g) Representative image of the worm which was treated with 30 μg/ml of CMK-P after UV-A exposure for 4 h (posttreatment). (h) Representative image of the worm which was treated with 20 μg/ml of LMK-P along with UV-A exposure for 4 h (cotreatment). (i) Representative image of the worm which was treated with 30 μg/ml of LMK-P along with UV-A exposure for 4 h (cotreatment). (j) Representative image of the worm which was treated with 20 μg/ml of LMK-P after UV-A exposure for 4 h (posttreatment). (k) Representative image of the worm which was treated with 30 μg/ml of LMK-P after UV-A exposure for 4 h (posttreatment).
Figure 6: qPCR analysis of candidate genes skn-1 and sir-2.1 that mediate the antioxidant mechanism in C. elegans. Both skn-1 and sir-2.1 expressed significant \( (p < 0.05) \) upregulation in wild-type nematodes when treated with 20 and 30 \( \mu \text{g/ml} \) of CMK-P and LMK-P extracts.

to analyze the lifespan along with healthspan to determine the antiaging properties [39, 48]. In the present study, C. nervosum was able to extend the median and maximum lifespan of C. elegans in all the concentrations tested from 1 to 100 \( \mu \text{g/ml} \) (Figures 1 and 2), except the higher concentrations of LMK-P at 90 and 100 \( \mu \text{g/ml} \), where there was no increase in lifespan and these doses showed similar effects when compared to the control (Figure 2). This suggests that the extracts of C. nervosum are not toxic and can improve the lifespan in C. elegans. Interestingly, selective doses of both the extracts were observed to significantly \( (p < 0.05) \) increase the lifespan (Figures 1 and 2). Many plant extracts and bioactive compounds involved such as Paullinia cupana [34], mulberry anthocyanins [36], Momordica charantia [49], Gastrodia elata [50], Baccharis trimera [51], and Polygonum multiflorum [52] were known to extend lifespan in C. elegans and various other models and express antiaging potential.

Interestingly, in C. elegans, a dietary restriction or calorie restriction process can be activated, which can also extend lifespan, which is interconnected to many other pathways [39, 53, 54]. In order to confirm that the lifespan extension observed was not mediated by dietary restriction, the pyramidal feeding assay was carried out in C. elegans treated with C. nervosum extracts. It was observed that there was no difference in the pyramidal feeding rate in worms fed with C. nervosum extracts when compared to the control (Figure 3(a)). This suggests that there was no dietary restriction mechanism involved in C. elegans when treated with C. nervosum extracts.

Healthspan is another key parameter that has to be monitored to analyze the antiaging potential [39, 48]. In the present study, the level of lipofuscin in the nematode treated with C. nervosum extracts was monitored. Lipofuscin is also known as “age pigment” which is a conserved autofluorescent protein which accumulates over the aging of an organism as it consists of nondegradable, highly oxidized materials [55]. It was observed that the LMK-P extract could significantly \( (p < 0.05) \) reduce the levels of lipofuscin in both the tested doses (Figures 3(g)–3(j)) whereas CMK-P could significantly \( (p < 0.05) \) reduce in 20 \( \mu \text{g/ml} \) concentration when compared to the control (Figures 3(c)–3(f)). Previ-ous reports suggest that the plant extracts which have antiaging potential can reduce the accumulation of lipofuscin in the nematode [52, 56–58]. Further, to confirm the activation of healthspan, qPCR analysis of candidate genes that regulate healthspan was monitored. In C. elegans, col-19 is considered as an adult-specific marker [59, 60] as its expression starts from the late larval stages and increases as it reaches adulthood [22, 61]. In the present study, the expression of col-19 was observed to be downregulated significantly \( (p < 0.05) \) in the nematodes treated with selective doses of both C. nervosum extracts when compared to the control (Figure 3(b)) indicating the antiaging potential of C. nervosum.

The diacylglycerol (DAG) pathway which constitutes the orthologs of Go ligands (egf-8, egl-30, goa-1, and dgk-1) is essential for healthspan [22, 62] including pharyngeal pumping, locomotion, and egg-laying wherein egl-8 and egl-30 are regulating positively [63] and dgk-1 and goa-1 are regulating negatively [64, 65]. Additionally, the serotonin biosynthesis in C. elegans is also mediated by egl-30 and goa-1 [66]. In the present study, the qPCR expression of egl-8 and egl-30 was observed to be upregulated significantly \( (p < 0.05) \) at all doses except 30 \( \mu \text{g/ml} \) of CMK-P extract whereas the expression of dgk-1 and goa-1 was observed to be significantly \( (p < 0.05) \) downregulated (Figure 3(b)). This confirms that C. nervosum extracts can extend the healthspan of the nematode.

Further, to understand the pathway that regulates the extension of lifespan and healthspan mediated by C. nervosum, the role of DAF-16-mediated pathway was investigated. The insulin/IGF-1 signaling (IIS) pathway, commonly known as DAF-16-mediated pathway, is an evolutionarily conserved pathway which is one of the first major pathways to be identified to regulate the aging process in C. elegans. The pathway majorly comprises of daf-2, orthologous to IIS receptor, age-1, orthologous to PI-3-kinase, and daf-16, orthologous to the FOXO (Forkhead Box O) transcription factor. Mutations in daf-2 or age-1 can increase the lifespan, whereas mutations in daf-16 can reduce the lifespan. The pathway is interconnected to many other pathways or transcription regulators such as SKN-1, HSF-1, JNK-1, and mTOR [39, 48, 67]. UTX-1 constitutes to a conserved family of histone demethylases specific for lysine 27 of histone H3 (H3K27me3). RNAi of uts-1 extended the lifespan of the nematode, which was dependent on DAF-16 [68, 69]. It is also crucial for embryonic and postembryonic development of C. elegans [70].

The qPCR expression of daf-2, daf-16, age-1, and uts-1 was analyzed in C. elegans treated with C. nervosum extracts. It was observed that the expression of daf-16 was upregulated significantly \( (p < 0.05) \) at 30 \( \mu \text{g/ml} \) of both the extracts whereas the expression of daf-2, age-1, and uts-1 was downregulated significantly \( (p < 0.05) \) in all the concentrations tested of both C. nervosum extracts (Figure 4(a)). This suggests that the lifespan extension mediated by C. nervosum extracts could be dependent of the DAF-16-mediated pathway. Since the DAF-16-mediated pathway is interconnected to many different pathways and mechanisms [39, 48, 67], the effect of C. nervosum extracts on the lifespan of daf-16
Figure 7: *C. nervosum* extracts could extend the survival of Aβ transgenic strain CL2006. (a) CMK-P at 10, 20, 30, and 40 μg/ml could extend the maximum lifespan of Aβ transgenic strain. (b) Graph showing significant increase in the average maximum survival days of Aβ transgenic strain treated with 10, 20, 30, and 40 μg/ml of CMK-P extracts. (c) Graph showing significant increase in the average median survival days of Aβ transgenic strain treated with 10, 20, 30, and 40 μg/ml of CMK-P extracts. (d) LMK-P at 10, 20, 30, and 40 μg/ml could extend the maximum lifespan of Aβ transgenic strain treated with 10, 20, 30, and 40 μg/ml of LMK-P extracts. (e) Graph showing significant increase in the average median survival days of Aβ transgenic strain treated with 10, 20, 30, and 40 μg/ml of LMK-P extracts. (f) Graph showing significant increase in the average median survival days of Aβ transgenic strain treated with 10, 20, 30, and 40 μg/ml of LMK-P extracts.

mutants was analyzed. Interestingly, it was observed that there was a significant (*p < 0.05*) increase in the median and maximum lifespan of the mutants when treated with both CMK-P (Figures 4(b)–4(d)) and LMK-P (Figures 4(e)–4(g)). This suggests that the lifespan extension induced by *C. nervosum* extracts could be dependent and independent of the DAF-16-mediated pathway.

A previous study suggests that 14-3-3 protein can activate stress resistance in *C. elegans* in a DAF-16-dependent and DAF-16-independent manner [71]. The other transcription factors that regulate longevity in *C. elegans* in both DAF-16-dependent and DAF-16-independent-mediated pathways are mTOR and SKN-1 [39, 67]. The mTOR may regulate longevity either by mediating DAF-16 or via SKN-1 [72]. This suggests the involvement of the transcription factor SKN-1, which is responsible for the antioxidant mechanism in the nematodes treated with *C. nervosum*.

Our group has previously observed the antioxidant properties of *C. nervosum* extract *in vitro* [15]. In this regard, in the present study, the antioxidant activity of *C. nervosum* extracts was validated *in vivo* by monitoring the ability of the extract to reduce the level of oxidative stress formed inside *C. elegans*. Wild-type nematodes were exposed to UV-A for 4 h, which can induce oxidative stress in *C. elegans* [38]. The protective effect and the repair effect of *C. nervosum* extracts were analyzed by treating the extracts to the nematodes during the course and after UV-A exposure, individually. Interestingly, it was observed that in both the cases, there was a significant (*p < 0.05*) reduction in the level of oxidative stress in both CMK-P and LMK-P extract-treated nematodes when compared to those which were exposed to UV-A without any extract treatment which was evident from the reduction in fluorescence that is proportional to the level of oxidative stress inside the nematode (Figure 5). Recent studies report that several plant extracts can activate antioxidant metabolism mediated by *skn-1* to activate antiaging and stress resistance mechanisms [34, 36, 51, 73].

The qPCR expression of two candidate genes which are responsible for antioxidant activity, *skn-1* and *sir-2.1*, was analyzed in wild-type nematodes treated with *C. nervosum* extracts to further confirm the effects. It was observed that both the genes were upregulated significantly (*p < 0.05*) (Figure 6) indicating the activation of the antioxidant mechanism. Orthologous to mammalian sirtuins, *sir-2.1* in *C. elegans* is known to activate the antioxidant mechanism during oxidative stress. Different extracts or compounds such
as green tea [31], black tea [74], resveratrol [75], emodin [76], Polygonum multiflorum [58], and Paulinia cupana [77] were found to induce antioxidant effects in C. elegans via sir-2.1.

The use of antioxidants has recently emerged as a potential treatment option for neurological disorders, since oxidative stress was identified as one of the causes or relative after-effect of neurological disorders [78]. Many plant extracts such as Paulinia cupana [34] and Baccharis trimera [51] were observed to protect the C. elegans model for Alzheimer’s disease from Aβ-mediated toxicity along with antioxidant properties. Our group has also reported the neuroprotective effect of C. nervosum extract from glutamate-induced toxicity in HT22 cell lines [15]. In this regard, in the present study, transgenic strains of C. elegans, which can be used as a model for Alzheimer’s disease, were treated with C. nervosum extracts and observed for their survival. Interestingly, it was observed that both the extracts at 20 and 30 μg/ml concentrations could significantly (p < 0.05) extend the median and maximum lifespan of the worms and keep them in an active state when compared to the control (Figure 7) indicating that the extract could elicit neuroprotective effect in nematodes. Various plant metabolites have been reported to elicit positive effects against neurological diseases by reducing plaque formation, improving memory and learning, reducing Aβ load from the blood-brain barrier, and improving cognitive functions [79].

The activation of SKN-1 by C. nervosum could have played a major role in extending lifespan and healthspan along with improving stress resistance and neuroprotection [80]. Recent reports suggest that plant extracts and its derivatives such as Paulinia cupana [34], rose essential oils [81], and Cratxylum formosum [82] were able to elicit neuroprotection which is mediated via SKN-1-regulated antioxidant response. However, further in-depth molecular studies are required to validate that these effects showed by C. nervosum extracts were dependent on SKN-1.

Additionally, it is also important to note that the two extracts used in the present study were collected from two different provinces in Thailand, Chiang Mai, and Lampang. It was observed from the results that the fruit from Lampang Province had greater in vitro antioxidant potential when compared to that from Chiang Mai (Table 2). In the in vivo experiments using C. elegans, LMK-P at the tested concentrations of 20 and 30 μg/ml was able to significantly reduce the level of lipofuscin accumulation whereas CMK-P was able to express significant reduction only at 20 μg/ml concentrations (Figure 3). Solanum aethiopicum collected from two different locations in Nigeria expressed variations in phenolic profile, polyphenol contents, antioxidant activities, and enzyme inhibitory properties [83]. The in vitro antioxidant activity analyzed in Withania somnifera collected from two different locations in India [84] was also not similar to each other. The differences were mainly observed in the antioxidant activity which could be attributed to the differences in the geographical location and the habitat. However, both the extracts exhibited similar effects in significantly extending the lifespan in wild-type and mutant worms along with mediating the expression of candidate genes that mediate antiaging and stress resistance.

5. Conclusion

Altogether, C. nervosum extracts were found to be nontoxic to C. elegans, and optimum doses were observed to extend lifespan and healthspan significantly. This effect was dependent and independent of DAF-16 which was evident through qPCR and mutant-based analysis. The extract was also able to activate the antioxidant mechanism as it reduced the level of ROS and activated the expression of skn-1 and sir-2.1 which was confirmed through qPCR analysis. The activation of the antioxidant mechanism could have aided in the neuroprotective effect which allowed lifespan extension in the C. elegans model for Alzheimer’s disease. Overall, C. nervosum extracts, which are used in the food industry [85], can be promoted as a major food additive with antiaging, antioxidant, and neuroprotective efficacies.

Data Availability

All the data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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References

[1] E. Nielsen, M. E. E. Temporiti, and R. Cella, “Improvement of phytochemical production by plant cells and organ culture and by genetic engineering,” Plant Cell Reports, vol. 38, no. 10, pp. 1199–1215, 2019.
[2] V. B. Rahimi, F. Ajam, H. Rakhshandeh, and V. R. Askari, “A pharmacological review on Portulaca oleracea L.: focusing on anti-inflammatory, anti-oxidant, immuno-modulatory and antitumor activities,” Journal of Pharmacopuncture Research Fund 2018, CU-56-918-AS, and by the National Research Council of Thailand.

[3] H. Rouhi-Boroujeni, E. Heidarian, H. Rouhi-Boroujeni, F. Deris, and M. Rafieian-Kopaei, “Medicinal plants with multiple effects on cardiovascular diseases: a systematic review,” Current Pharmaceutical Design, vol. 23, no. 7, pp. 999–1015, 2017.
[4] M. Akram and M. Idrees, “Progress and prospects in the management of kidney stones and developments in phytotherapeutic modalities,” International Journal of Immunopharmacology and Pharmacology, vol. 33, 2019.
[5] J. Peng, T. T. Zheng, X. Li et al., “Plant-derived alkaloids: the promising disease-modifying agents for inflammatory bowel disease,” Frontiers in Pharmacology, vol. 10, p. 351, 2019.
A. T. Valduga, I. L. Gonçalves, E. Magri, and J. R. Delalibera Finzer, “Chemistry, pharmacology and new trends in traditional functional and medicinal beverages,” Food Research International, vol. 120, pp. 478–503, 2019.

M. I. Prasanth, B. S. Sivamaruthi, C. Chaiyasut, and T. Tencomnnao, “A review of the role of green tea (Camellia sinensis) in antiphotaging, stress resistance, neuroprotection, and autophagy,” Nutrients, vol. 11, no. 2, p. 474, 2019.

R. Sharma, N. Martins, K. Kuca et al., “Chyawanprash: a traditional Indian bioactive health supplement,” Biomolecules, vol. 9, no. 5, p. 161, 2019.

H. Yuan, Q. Ma, L. Ye, and G. Piao, “R. Wongpoomchai, Kok, 2002.

M. I. Prasanth, B. S. Sivamaruthi, C. Chaiyasut, and R. Wongpoomchai, “Assessment of genotoxicity and antigenotoxicity of an aqueous extract of Cleistocalyx nervosum var. paniala in vitro and in vivo models,” Interdisciplinary Toxicology, vol. 5, pp. 201–206, 2012.

W. Inboot, S. Taya, A. Chailungka, M. Meepowpan, and R. Wongpoomchai, “Genotoxicity and antigenotoxicity of the methanol extract of Cleistocalyx nervosum var. paniala seed using a Salmonella mutation assay and rat liver micronucleus tests,” Molecular & Cellular Toxicology, vol. 8, no. 1, pp. 19–24, 2012.

S. Taya, C. Punvittayagul, W. Inboot, S. Fukushima, and R. Wongpoomchai, “Cleistocalyx nervosum extract ameliorates chemical-induced oxidative stress in early stages of rat hepatocarcinogenesis,” Asian Pacific Journal of Cancer Prevention, vol. 15, no. 6, pp. 2825–2830, 2014.

J. Manosroi, C. Chankhampan, K. Kumguan, W. Manosroi, and A. Manosroi, “In vitro anti-aging activities of extracts from leaves of Ma Kiang (Cleistocalyx nervosum var. paniala),” Pharmaceutical Biology, vol. 53, no. 6, pp. 862–869, 2015.

M. Sukprasansap, P. Channorachote, and T. Tencomnnao, “Cleistocalyx nervosum var. paniala berry fruit protects neurotoxicity against endoplasmic reticulum stress-induced apoptosis,” Food and Chemical Toxicology, vol. 103, pp. 279–288, 2017.

O. Patthamakanokporn, P. Puwastien, A. Nithihamyong, and P. P. Sirichakwal, “Changes of antioxidant activity and total phenolic compounds during storage of selected fruits,” Journal of Food Composition and Analysis, vol. 21, no. 3, pp. 241–248, 2008.

C. Chaiyasut, B. S. Sivamaruthi, N. Pengkumsri et al., “Anthocyanin profile and its antioxidant activity of widely used fruits, vegetables, and flowers in Thailand,” Asian Journal of Pharmaceutical and Clinical Research, vol. 9, no. 6, pp. 218–224, 2016.

S. Taya, C. Punvittayagul, T. Chewonarin, and R. Wongpoomchai, “Effect of aqueous extract from Cleistocalyx nervosum on oxidative status in rat liver,” Thai Journal of Toxicology, vol. 24, no. 2, pp. 101–105, 2009.

W. Poontawe, S. Natakankitkul, and O. Wongmekiat, “Protective effect of Cleistocalyx nervosum var. paniala fruit extract against oxidative renal damage caused by cadmium,” Molecules, vol. 21, no. 2, p. 133, 2016.

S. Tantranat, N. Balmuang, and W. Krusong, “Phenolic enrichment of Ma-Kiang seed extract using absorbent and this enriched extract application for safety control of fresh-cut cantaloupe,” LWT, vol. 106, pp. 105–112, 2019.

B. Sriwanthanha, W. Tressangsri, B. Boriboontrakul, S. Niumvakul, and P. Chavalitumrong, “In vitro effects of Thai medicinal plants on human lymphocyte activity,” Songklanakarin Journal of Science and Technology, vol. 29, Supplement 1, pp. 17–28, 2007.

M. I. Prasanth, G. S. Santoshram, J. P. Bhaskar, and K. Balamurugan, “Ultraviolet-A triggers photoaging in model nematode Caenorhabditis elegans in a DAF-16 dependent pathway,” Age, vol. 38, no. 1, p. 27, 2016.

E. Yanger, M. Safra, M. Levi-Ferber, A. Havig-Chesner, and S. Henis-Korenblit, “Innate immunity mediated longevity and longevity induced by germ cell removal converge on the C-type lectin domain protein IRG-7,” PLoS Genetics, vol. 13, no. 2, article e1006577, 2017.

R. Sharika, P. Subbaiah, and K. Balamurugan, “Studies on reproductive stress caused by candidate Gram positive and Gram negative bacteria using model organism, Caenorhabditis elegans,” Gene, vol. 649, pp. 113–126, 2018.

J. Yang, X. B. Huang, Q. L. Wan et al., “Otophylloside B protects against αβ toxicity in Caenorhabditis elegans models of Alzheimer’s disease,” Natural Products and Bioprospecting, vol. 7, no. 2, pp. 207–214, 2017.

B. A. Martinez, K. A. Caldwell, and G. A. Caldwell, “C. elegans as a model system to accelerate discovery for Parkinson disease,” Current Opinion in Genetics & Development, vol. 44, pp. 102–109, 2017.

O. B. Akinola, “Sweet old memories: a review of the experimental models of the association between diabetes, senility and dementia,” Metabolic Brain Disease, vol. 31, no. 5, pp. 1003–1010, 2016.

H. Qian, X. Xu, and L. E. Niklason, “PCH-2 regulates Caenorhabditis elegans lifespan,” Aging, vol. 7, no. 1, pp. 1–13, 2015.

The C elegans Sequencing Consortium, “Genome sequence of the nematode C. elegans: a platform for investigating biology,” Science, vol. 282, no. 5396, pp. 2012–2018, 1998.

Y. Dong, S. Guha, X. Sun, M. Cao, X. Wang, and S. Zou, “Nutraceutical interventions for promoting healthy aging in invertebrate models,” Oxidative Medicine and Cellular Longevity, vol. 2012, Article ID 718491, 10 pages, 2012.

D. J. Deusing, S. Winter, A. Kler et al., “A catechin-enriched green tea extract prevents glucose-induced survival reduction in Caenorhabditis elegans through sir-2.1 and ubr-1 dependent hormanesis,” Fitoterapia, vol. 102, pp. 163–170, 2015.

E. F. Fang, T. B. Waltz, H. Kassahun et al., “Tomatidine enhances lifespan and healthspan in C. elegans through mitophagy induction via the SKN-1/Nrf2 pathway,” Scientific Reports, vol. 7, no. 1, article 46208, 2017.

A. Prasansuklab, K. Meemon, P. Sobhon, and T. Tencomnnao, “Ethanolic extract of Streblus asper leaves protects against glutamate-induced toxicity in HT22 hippocampal neuronal cells and extends lifespan of Caenorhabditis elegans,” BMC Complementary and Alternative Medicine, vol. 17, no. 1, p. 551, 2017.

P. F. Boasquetis, G. M. M. Silva, F. A. Paiva, R. M. Cavalcanti, C. V. Nunes, and R. de Paula Oliveira, “Guarana (Paullinia cupana) extract protects Caenorhabditis elegans models for Alzheimer disease and Huntington disease through activation
of antioxidant and protein degradation pathways,” *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 9241308, 16 pages, 2018.

[35] F. Meng, J. Li, Y. Rao, W. Wang, and Y. Fu, “Gengnian-chun extends the lifespan of *Caenorhabditis elegans* via the insulin/IGF-1 signalling pathway,” *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 4740739, 10 pages, 2018.

[36] F. Yan, Y. Chen, R. Azat, and X. Zheng, “Mulberry anthocyanin extract ameliorates oxidative damage in HepG2 cells and prolongs the lifespan of *Caenorhabditis elegans* through MAPK and Nrf2 pathways,” *Oxidative Medicine and Cellular Longevity*, vol. 2017, Article ID 7956158, 12 pages, 2017.

[37] S. Brenner, “The genetics of *Caenorhabditis elegans*,” *Genetics*, vol. 77, no. 1, pp. 71–94, 1974.

[38] M. I. Prasanth, D. Venkatesh, D. Murali, J. P. Bhaskar, V. Krishnan, and K. Balamurugan, “Understanding the role of DAF-16 mediated pathway in *Caenorhabditis elegans* during UV-A mediated photaging process,” *Archives of Gerontology and Geriatrics*, vol. 82, pp. 279–285, 2019.

[39] H. A. Tissenbaum, “Genetics, life span, healthspan, and the aging process in *Caenorhabditis elegans*,” *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, vol. 67A, no. 5, pp. 503–510, 2012.

[40] D. H. McDaniel, I. H. Hamzavi, J. A. Zeichner et al., “Total defense + repair: a novel concept in solar protection and skin rejuvenation,” *Journal of Drugs in Dermatology*, vol. 14, no. 7, pp. s3–11, 2015.

[41] J. H. Park and J. J. Park, “A systematic review on factors influencing the healthy aging: a Korean perspective,” *The Journal of Aging Research & Clinical Practice*, vol. 234, 2015.

[42] L. Robert and J. Labat-Robert, “Stress in biology and medicine, role in aging,” *Pathologie Biologique*, vol. 63, no. 4-5, pp. 230–234, 2015.

[43] D. A. Loeffler, “Influence of normal aging on brain autophagy: a complex scenario,” *Frontiers in Aging Neuroscience*, vol. 11, p. 49, 2019.

[44] M. Carocho, I. C. F. R. Ferreira, P. Morales, and M. Soković, “Antioxidants and prooxidants: effects on health and aging 2018,” *Oxidative Medicine and Cellular Longevity*, vol. 2019, Article ID 7971613, 2 pages, 2019.

[45] M. Ayaz, A. Sadiq, M. Junaid, F. Ullah, F. Subhan, and J. Ahmed, “Neuroprotective and anti-aging potentials of essential oils from aromatic and medicinal plants,” *Frontiers in Aging Neuroscience*, vol. 9, p. 168, 2017.

[46] Z. A. M. Yasin, F. Ibrahim, N. N. Rashid, M. F. M. Razif, and R. Yusof, “The importance of some plant extracts as skin anti-aging resources: a review,” *Current Pharmaceutical Biotechnology*, vol. 18, no. 11, pp. 864–876, 2017.

[47] D. P. Xu, Y. Li, X. Meng et al., “Natural antioxidants in foods and medicinal plants: extraction, assessment and resources,” *International Journal of Molecular Sciences*, vol. 18, no. 1, p. 96, 2017.

[48] H. A. Tissenbaum, “Using *C. elegans* for aging research,” *Invertebrate Reproduction & Development*, vol. 59, Supplement 1, pp. 59–63, 2014.

[49] X. Cao, Y. Sun, Y. Lin et al., “Antiaging of cucurbitane glycosides from fruits of *Momordica charantia L.*,” *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 1538632, 10 pages, 2018.

[50] Y. Lin, Y. Sun, Y. Weng, A. Matsuura, L. Xiang, and J. Qi, “Parishin from *Gastrodia elata* extends the lifespan of yeast via regulation of Sir2/Uth1/TOR signaling pathway,” *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 4074690, 11 pages, 2016.

[51] F. Aparecida Paiva, L. de Freitas Bonomo, P. Ferreira Boasques et al., “Carqueja (Baccharis trimera) protects against oxidative stress and β-amyloid-induced toxicity in *Caenorhabditis elegans*,” *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 740162, 15 pages, 2015.

[52] C. Büchler, L. Zhao, S. Havermann et al., “TSG (2,3,5,4′-Tetrahydroxystilbene-2-0-β-D-glucoside) from the Chinese herb *Polygonum multiflorum* increases life span and stress resistance of *Caenorhabditis elegans*,” *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 124357, 12 pages, 2015.

[53] P. Kapahi, M. Kaeberlein, and M. Hansen, “Dietary restriction and lifespan: lessons from invertebrate models,” *Ageing Research Reviews*, vol. 39, pp. 3–14, 2017.

[54] Z. Wu, M. Isik, N. Moroz, M. J. Steinbaugh, P. Zhang, and T. K. Blackwell, “Dietary restriction extends lifespan through metabolic regulation of innate immunity,” *Cell Metabolism*, vol. 29, no. 5, pp. 1192–1205.e8, 2019.

[55] Z. Pincus and F. J. Slack, “Developmental biomarkers of aging in *Caenorhabditis elegans*,” *Developmental Dynamics*, vol. 239, no. 5, pp. 1306–1314, 2010.

[56] L. Rathor, A. Pant, H. Awasthi, D. Mani, and R. Pandey, “An anti-diabetic polyherbal phytomedicine confers stress resistance and extends lifespan in *Caenorhabditis elegans*,” *Biogerontology*, vol. 18, no. 1, pp. 131–147, 2017.

[57] H. Wang, J. Liu, T. Li, and R. H. Liu, “Blueberry extract promotes longevity and stress tolerance via DAF-16 in *Caenorhabditis elegans*,” *Food & Function*, vol. 9, no. 10, pp. 5273–5282, 2018.

[58] C. Saier, C. Büchler, K. Koch, and W. Wätjen, “*Polygonum multiflorum* extract exerts antioxidative effects and increases life span and stress resistance in the model organism *Caenorhabditis elegans* via DAF-16 and SIR-2.1,” *Plants*, vol. 7, no. 3, p. 60, 2018.

[59] M. C. Thein, G. McCormack, A. D. Winter, I. L. Johnstone, C. B. Shoemaker, and A. P. Page, “*Caenorhabditis elegans* exoskeleton collagen COL-19: An adult-specific marker for collagen modification and assembly, and the analysis of organismal morphology,” *Developmental Dynamics*, vol. 226, no. 3, pp. 523–539, 2003.

[60] Y. Li and Y. K. Paik, “A potential role for fatty acid biosynthesis genes during molting and cuticle formation in *Caenorhabditis elegans*,” *BMB Reports*, vol. 44, no. 4, pp. 285–290, 2011.

[61] K. Hada, M. Ashahina, H. Hasegawa, Y. Kanaho, F. J. Slack, and R. Niwa, “The nuclear receptor gene nhr-25 plays multiple roles in the *Caenorhabditis elegans* heterochronic gene network to control the larva-to-adult transition,” *Developmental Biology*, vol. 344, no. 2, pp. 1100–1109, 2010.

[62] A. M. Hofer, “Another dimension to calcium signaling: a look at extracellular calcium,” *Journal of Cell Science*, vol. 118, no. 5, pp. 855–862, 2005.

[63] E. G. Govorunova, M. Moussaiif, A. Kullyev et al., “A homolog of FHM2 is involved in modulation of excitatory neurotransmission by serotonin in *C. elegans*,” *PLoS One*, vol. 5, no. 4, article e10368, 2010.
E. M. Myers, H. Kunitomo, and Y. Iino, “G_{\alpha} \alpha regulates olfactory adaptation by antagonizing G_{\alpha} \alpha-DAG signaling in Caenorhabditis elegans,” Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 4, pp. 1112–1117, 2006.

L. Avery and Y. J. You, “C. elegans Feeding,” WormBook, vol. 21, pp. 1–23, 2012.

E. M. Myers, "G_{\alpha} \alpha, and G_{\alpha} \alpha regulate the expression ofdaf-7, a TGF\beta-like gene, in Caenorhabditis elegans," PLoS One, vol. 7, no. 7, article e40368, 2012.

J. Vandamme and A. E. Salcini, “The genetics of ageing,” Nature, vol. 464, no. 7288, pp. 504–512, 2010.

C. J. Kenyon, “The genetics of ageing,” Nature, vol. 464, no. 7288, pp. 504–512, 2010.

J. T. Maures, E. L. Greer, A. G. Hauswirth, and A. Brunet, “The H3K27 demethylase UTX-1 regulates C. elegans lifespan in a germline-independent, insulin-dependent manner,” Aging Cell, vol. 10, no. 6, pp. 980–990, 2011.

J. Vandamme and A. E. Salcini, "Catalytic-independent roles of UTX-1 in C. elegans development," Worm, vol. 2, no. 2, article e22188, 2013.

C. Araiz, M. T. Château, and S. Galas, "14-3-3 regulates life span by both DAF-16-dependent and -independent mechanisms in Caenorhabditis elegans," Experimental Gerontology, vol. 43, no. 6, pp. 505–519, 2008.

X. Sun, W. D. Chen, and Y. D. Wang, “DAF-16/FOXO transcription factor in aging and longevity,” Frontiers in Pharmacology, vol. 8, p. 548, 2017.

Q. Wang, Y. Huang, C. Qin et al., "Bioactive peptides from Angelica sinensis protein hydrolyzate delay senescence in Caenorhabditis elegans through antioxidant activities," Oxidative Medicine and Cellular Longevity, vol. 2016, Article ID 8956991, 10 pages, 2016.

L. G. Xiong, J. A. Huang, J. Li et al., “Black tea increased survival of Caenorhabditis elegans under stress,” Journal of Agricultural and Food Chemistry, vol. 62, no. 46, pp. 11163–11169, 2014.

J. Lee, G. Kwon, J. Park, J. K. Kim, and Y. H. Lim, “Brief communication: SIR-2.1-dependent lifespan extension of Caenorhabditis elegans by oxyresveratrol and resveratrol,” Experimental Biology and Medicine (Maywood, N.J.), vol. 241, no. 16, pp. 1757–1763, 2016.

X. Zhao, L. Lu, Y. Qi, M. Li, and L. Zhou, "Emodin extends lifespan of Caenorhabditis elegans through insulin/IGF-1 signaling pathway depending on DAF-16 and SIR-2.1," Bioscience, Biotechnology, and Biochemistry, vol. 81, no. 10, pp. 1908–1916, 2017.

L. P. Arantes, M. L. Machado, D. C. Zamberlan et al., "Mechanisms involved in anti-aging effects of guarana (Paullinia cupana) in Caenorhabditis elegans," Brazilian Journal of Medical and Biological Research, vol. 51, no. 9, article e7552, 2018.

F. Pohl and P. Kong Thoo Lin, "The potential use of plant natural products and plant extracts with antioxidant properties for the prevention/treatment of neurodegenerative diseases: in vitro, in vivo and clinical trials," Molecules, vol. 23, no. 12, article 3283, 2018.

B. Dinda, M. Dinda, G. Kulsì, A. Chakraborty, and S. Dinda, "Therapeutic potentials of plant iridoids in Alzheimer’s and Parkinson’s diseases: a review," European Journal of Medicinal Chemistry, vol. 169, pp. 185–199, 2019.

Q. Hu, D. R. D’Amora, L. T. MacNeil, A. J. M. Walhout, and T. J. Kubiszek, "The oxidative stress response in Caenorhabditis elegans requires the GATA transcription factor ELT-3 and SKN-1/Nrf2,” Genetics, vol. 206, no. 4, pp. 1909–1922, 2017.

S. Zhu, H. Li, J. Dong et al., “Rose essential oil delayed Alzheimer’s disease-like symptoms by SKN-1 pathway in C. elegans,” Journal of Agricultural and Food Chemistry, vol. 65, no. 40, pp. 8855–8865, 2017.

R. Keowkase and N. Weerapreeyakul, “Cratoxylum formosum extract protects against amyloid-beta toxicity in a Caenorhabditis elegans model of Alzheimer’s disease,” Planta Medica, vol. 82, no. 6, pp. 516–523, 2016.

E. E. Nwanna, A. A. Adebayo, A. O. Ademosun, and G. Oboh, “Phenolic distribution, antioxidant activity, and enzyme inhibitory properties of eggplant (Solanum aethiopicum) cultivated in two different locations within Nigeria,” Journal of Food Biochemistry, vol. 43, no. 6, article e12797, 2018.

L. Adhikari, R. Kotiyal, M. Pandey, M. Bharkatiya, A. Sematy, and M. Sematy, “Effect of geographical location and type of extract on Total phenol/flavon contents and antioxidant activity of different fruits extracts of Withania somnifera,” Current Drug Discovery Technologies, vol. 15, 2018.

S. Chaisawadi and W. Methawiriyasilp, “Clean production of commercial ‘Makiang’ juice processing for medicinal herbs and health benefits,” Acta Horticulturae, vol. 1, no. 786, pp. 201–208, 2008.
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