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Article

Association of Euterpe oleracea, Bixa orellana, Myciaria dubia, and Astrocaryum aculeatum (The Terasen® nutraceutical) Increases the Lifespan of Caenorhabditis Elegans

Ester L. de Melo [1, 2], Bruno A. M. Tavares [1], Nayara N. D. Colares [1], Aline L. do Nascimento [1, 3], Helison de O. Carvalho [1], Andrés N. Castro [4], Arlindo C. M. Pereira [5], Carlos E. Winter [6], Iracirema da S. Sena [1], Luiz F. Moreira [1] and José C. T. Carvalho [1, 2, 3]*

[1] Drug Research Laboratory, Department of Biological and Health Sciences, Federal University of Amapá, Macapá, Amapá, Brazil
[2] Postgraduate Program in Tropical Biodiversity, Department of Biological and Health Sciences, Federal University of Amapá, Macapá, Amapá, Brazil
[3] Postgraduate Program in Pharmaceutical Innovation, Department of Biological Sciences and Health, Federal University of Amapá, Macapá, Amapá, Brazil
[4] Laboratory of Natural Product Pharmacology, Department of Pharmacy, Faculty of Chemistry, National Autonomous University of Mexico, University City, Coyoacán, Zip Code 04510 Mexico City, Mexico
[5] Faculty of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Ribeirão Preto, Sao Paulo, Brazil
[6] Laboratory of Molecular Biology of Nematodes, Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, São Paulo, Brazil
* Correspondence: farmacos@unifap.br

Abstract: Aging is a complex process associated with tissue degeneration and an increased risk of age-related diseases. This study aimed to evaluate the impact of Terasen®, a nutraceutical containing standardized extracts of *Euterpe oleracea*, *Myrciaria dubia*, and purified oil of *Bixa orellana* and *Astrocaryum aculeatum* on the lifespan of *Caenorhabditis elegans*, a widely used model organism for aging research. The findings demonstrated that Terasen® exhibited significant antioxidant activity and influenced the feeding behavior of *C. elegans*, leading to a reduced pharyngeal pumping rate and a decreased number of offspring produced by treated individuals. Notably, Terasen® also displayed a remarkable ability to extend the lifespan of *C. elegans*. These findings suggest that Terasen® may possess promising anti-aging effects in vivo, warranting further investigation.

Keywords: longevity; healthspan; lifespan; nutraceuticals; *C. elegans*

1. Introduction

Aging is a natural process during an individual's lifetime that leads to a decline in natural body functions [1,2]. The functional changes associated with aging have been identified as risk factors for various health problems, including hypertension, osteoporosis, diabetes, cataracts, heart failure, and neurodegenerative diseases [3].

According to the World Health Organization (WHO), global life expectancy is increasing, and it is projected that the elderly population will reach 1.4 billion people by 2030 and exceed 2.1 billion by 2050 [4]. With this demographic shift, it is imperative to seek solutions that address the detrimental effects of aging, ensuring that individuals can maintain their independence and experience healthy aging [2,5].

Pharmacological interventions aimed at prolonging human longevity and preventing age-related diseases are under investigation. The nematode *Caenorhabditis elegans* has emerged as a promising model for studying the determinants of longevity. *C. elegans* has a short lifespan, compact size, and conserved genetic pathways that regulate aging, such as insulin signaling, oxidative stress response, and longevity-related genes [6].

In this study, we employed the *C. elegans* model to investigate the effects of Terasen®, nutraceutical formulation composed of standardized extracts of *Euterpe oleracea*, *Myrciaria dubia*, and

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purified oil of *Bixa orellana* and *Astrocaryum aculeatum*. The phytochemical markers of Terasen® include anthocyanins, quercetin, ellagic acid, gallic acid, carotenoids, geranylgeraniol, and tocotrienols, which have been extensively studied for their pharmacological properties and their potential impact on longevity.

2. Materials and Methods

2.1. Product

The nutraceutical product in the form of encapsulated granules, Terasen®, was provided by Ages Bioactive Compounds (São Paulo, SP, Brazil), batch URU201101.

2.2. Evaluation of Antioxidant Activity

The Terasen® sample was extracted using DMSO and vortex agitation for the antioxidant activity analysis. At the end of the procedure, all samples were prepared at a final concentration of 1 mg/ml. Gallic acid and DMSO were used as the standard and negative control, respectively. The results were expressed as the percentage of radical scavenging using the following equation: Inhibition (%) = [(Ac - As) / Ac] × 100, where Ac is the absorbance of the negative control (DMSO) and as is the absorbance of the sample. All analyses were performed in triplicates.

2.2.1. DPPH Radical Scavenging Activity

The method described by [7], with some modifications, was used to determine antioxidant activity using DPPH. The DPPH solution (2,2-diphenyl-1-picrylhydrazyl) was prepared by dissolving 1 mg of DPPH in 12 ml of absolute ethanol. Then, 270 µL of this solution and 30 µL of ethanol were added to the microplate, adjusting the solution with ethanol to achieve an absorbance of 1.00 ± 0.1 nm. For the test, 30 µL of the sample and 270 µL of the DPPH solution were added to the plate, and it was incubated in the dark for 30 minutes. After this time, the absorbance was measured at 517 nm using an ELISA reader (Kasuaki, Dr-200Bn-Bi).

2.2.2. ABTS Radical Scavenging Assay

The antioxidant activity was evaluated using the ABTS method described by [8] with some modifications. The ABTS solution (7 mM) (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) was mixed with a potassium persulfate solution (2.45 mM) and incubated at room temperature in the dark for 16 hours. Then, 270 µL of the ABTS solution was mixed with 30 µL of the sample. The plate was incubated in the dark for 30 minutes, and the absorbance was measured at 630 nm using an ELISA reader (Kasuaki, Dr-200Bn-Bi).

2.3. Caenorhabditis Elegans Strains

The N2 Bristol (wild-type) strains of *Caenorhabditis elegans* used in this study were obtained from the Department of Parasitology/ICB/USP (ICB, University of São Paulo, SP). All nematodes were incubated at 20 °C and grown on NGM (nematode growth medium) seeded with *Escherichia coli* OP50 as a food source.

2.3.1. Reproduction Assay

The reproduction assay was conducted following the method described by [9]. Synchronized L4 nematodes (n=5) were daily transferred until the end of the reproductive period to NGM plates with only *E. coli* OP50 in LB medium (negative control) or *E. coli* in LB medium plus different concentrations of Terasen® solubilized in the medium (250, 500, or 1000 µg/mL), and the eggs were counted. This assay was performed in triplicate, and the results were presented as the mean number of offspring.

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2.3.2. Pharyngeal Pumping Rate

The pharyngeal pumping rate was evaluated using the methodology proposed by [10]. Synchronized L4 nematodes were raised on NGM plates and treated with only *E. coli* OP50 in LB medium (negative control) or *E. coli* in LB medium plus different concentrations of Terasen® solubilized in the medium (250, 500, or 1000 µg/mL). On the 3rd, 6th, and 9th day of adulthood, 10 worms were randomly selected, and the number of pharyngeal contractions during a 60-second interval was quantified. The experiment was performed in triplicate.

2.3.3. Growth Alteration Assay

The growth alteration assay was performed following the method described by [11]. The animals were raised from the L1 stage on NGM plates receiving only *E. coli* OP50 in LB medium (negative control) or *E. coli* in LB medium plus different concentrations of Terasen® solubilized in the medium (250, 500, or 1000 µg/mL). On the 4th and 8th day of adulthood, the animals were photographed using a stereomicroscope and camera (Luxeo 4D, Labomed, CA, USA), and their body length was measured from head to tail using ImageJ software (v1.53u, Massachusetts, USA). The experiment was performed in triplicate.

2.3.4. Locomotion Analysis Assay

The locomotion analysis assay followed the protocol described by [12]. Initially, synchronized N2 nematodes were transferred to NGM plates and treated only *E. coli* OP50 in LB medium (negative control) or *E. coli* in LB medium plus different concentrations of Terasen® solubilized in the medium (250, 500, or 1000 µg/mL). After 4 and 8 days of treatment, each nematode was placed on a glass dish with 100 µL of M9 buffer. After 1 minute of recovery, the total number of body bends was counted over 20 seconds using a stereomicroscope.

2.3.5. Lifespan Assessment

The lifespan of nematodes was assessed following the method of [10]. Synchronized L4 stage nematodes (n=20) were transferred to NGM plates and treated with only *E. coli* OP50 in LB medium (negative control) or *E. coli* in LB medium plus different concentrations of Terasen® solubilized in the medium (250, 500, or 1000 µg/mL). The nematodes were transferred daily to fresh NGM plates with their respective treatments for the first six days, followed by transfers every two days afterward. Daily worm counts were conducted until the death of all nematodes, with worms unresponsive to a gentle touch with a platinum wire being marked as dead and excluded from the plates. The results were reported as the percentage of survival, mean lifespan, and median lifespan. The lifespan tests were conducted in triplicate at a constant temperature of 20°C.

2.4. Statistical Analysis

Statistical analysis was conducted using GraphPad Prism software (version 5.03). The results were presented as mean ± SEM. Two-Way ANOVA was used to compare data (factors: treatment; days), followed by multiple Tukey's posthoc tests in case of statistical difference. One-Way ANOVA followed by Tukey's test was employed to compare AUCs. The log-rank Mantel-Cox test was used to compare survival curves. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Radical Scavenging Assessment

In the DPPH and ABTS radical inhibition assays, the values obtained with Terasen® were 20.85 ± 2.99% and 69.95 ± 5.39% inhibition relative to DMSO, respectively, compared to 75.35 ± 4.42% and 95.82 ± 0.39% inhibition relative to DMSO in the gallic acid control. It is observed that the inhibition percentage was significantly higher in the ABTS assay, and this result is directly related to the

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phenolic compound content present in Terasen®. Phenolic compounds have a higher affinity for scavenging the ABTS radical due to differences in their chemical structures and the properties of free radicals [13,14].

3.2. Assessment of Pharyngeal Pumping Rate

In order to investigate whether exposure to Terasen® could affect the pharyngeal pumping rate, a crucial measure for evaluating feeding behavior in *C. elegans* [11], an investigation was conducted in *C. elegans* exposed to different concentrations of Terasen® (250, 500, or 1000 µg/mL), which revealed statistically significant results. Compared to the control group, there was a statistically significant reduction in pharyngeal pumping rate on days 3, 6, and 9 of adult life in *C. elegans* exposed to Terasen® ($p < 0.05$; Figure 1). These findings suggest that exposure to Terasen® may reduce the feeding behavior of these organisms.

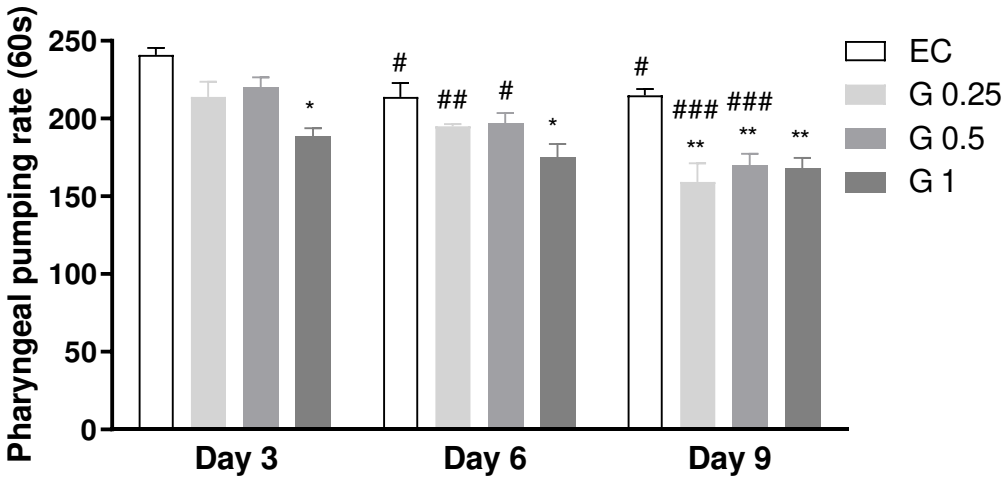

Figure 1. Effect of treatment with Terasen® 250 µg/mL (G 0.25), 500 µg/mL (G 0.5), or 1000 µg/mL (G1) and Escherichia coli on Pharyngeal Pumping Rate of *C. elegans* in the treated groups. Results are expressed as mean ± SEM, where * indicates a significant difference vs. the control group on the same day and # indicates a significant difference vs. the same group on day 3. Treatment factor: $p < 0.0001$; Day factor: $p < 0.0001$; Interaction: ns ($p < 0.05$; Two-Way ANOVA followed by the posthoc Tukey's test).

3.3. Reproduction Assessment

To investigate the impact of Terasen® on *C. elegans* reproduction, egg-laying was evaluated in animals treated with different concentrations of the formulation (250, 500, or 1000 µg/mL). The results revealed a significant reduction in egg production in animals exposed to Terasen® compared to the control group ($p < 0.001$; Figure 2). However, it is observed that this drastic decrease tends to mitigate from the second day on and could be explained by possible initial stress due to an exogenous compound in the medium. Still, mainly due to the initial decrease, there was a statistical difference in overall values given by the AUC. Among the treated groups, there was a concentration-dependent increase in the progeny.

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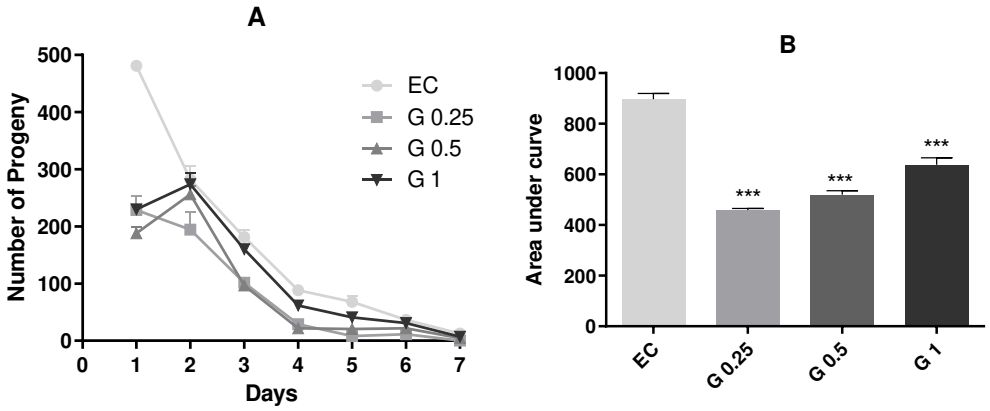

Figure 2. Effect of treatment with Terasen® 250 µg/mL (G 0.25), 500 µg/mL (G 0.5), or 1000 µg/mL (G1) and Escherichia coli on *C. elegans* reproduction. (A) Represents the results with mean and SEM of the groups according to the days evaluated; (B) Expresses the area under the curve, ***statistically significant results ($p < 0.001$) vs. EC group (One-Way ANOVA followed by Tukey posthoc test).

3.4. Locomotion Assessment

The movement capacity of *C. elegans* was evaluated in control animals and animals treated with different concentrations of Terasen® (250, 500, or 1000 µg/mL). The results demonstrate that Terasen® did not promote decline or improvement in nematode motility in the young adult phase (day 4). During the aging phase (day 8), nematode motility was reduced in animals treated with different concentrations of Terasen® (Figure 3). However, considering the data from all days, the difference was insignificant considering treatment ($p = 0.0952$; Two-Way ANOVA).

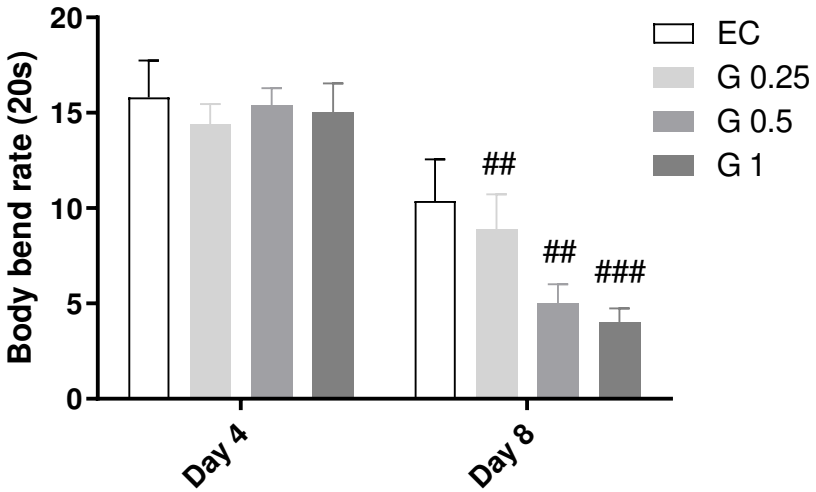

Figure 3. Effect of treatment with Terasen® at 250 µg/mL (G 0.25), 500 µg/mL (G 0.5), or 1000 µg/mL (G1) and Escherichia coli on the locomotion of *C. elegans*. Results are expressed as mean and SEM, where # indicates a significant difference vs. the same group on day 4. Treatment factor: ns; Day factor: $p < 0.0001$; Interaction: ns ($p < 0.05$; Two-Way ANOVA followed by the posthoc Tukey's test).

3.5. Size Evaluation

The results demonstrated a reduction in body length with increasing Terasen® concentration compared to the control group on day 4 (Figure 4). However, on the 8th day of adulthood, with increased exposure time, the length of the animals treated with Terasen® and the control group did

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not show a noticeable variation when compared to each other. Considering data from all days, however, it is observed a significant effect of the treatment ($p < 0.05$; Two-Way ANOVA) that was dependent on the day (Interaction: $p < 0.05$).

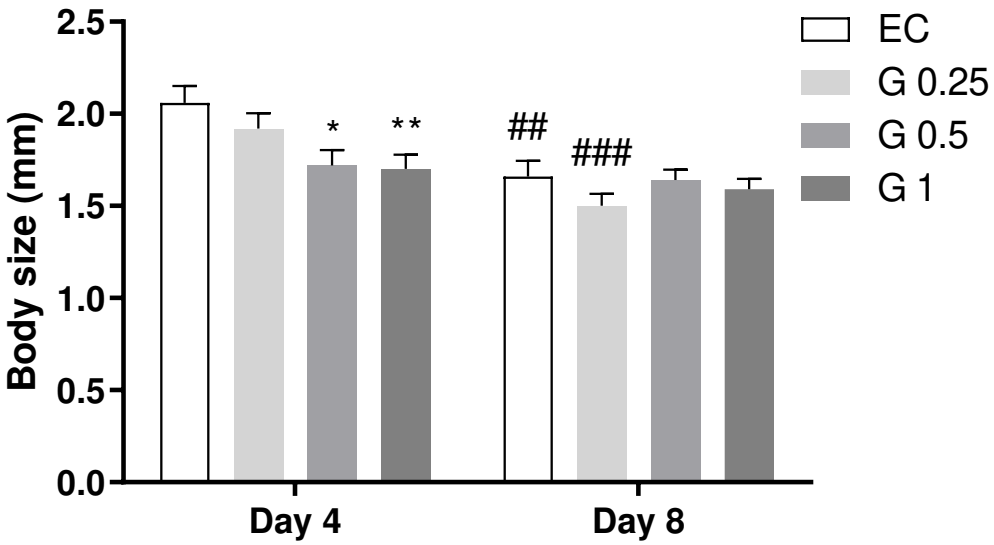

Figure 4. Effect of treatment with Terasen® at 250 µg/mL (G 0.25), 500 µg/mL (G 0.5), or 1000 µg/mL (G1) and Escherichia coli on the size of *C. elegans*. Results are expressed as mean and SEM, where * indicates a significant difference vs. the control group on the same day and # indicates a significant difference vs. the same group on day 4. Treatment factor: $p < 0.05$; Day factor: $p < 0.0001$; Interaction: $p < 0.05$ ($p < 0.05$; Two-Way ANOVA followed by the posthoc Tukey's test).

3.6. Lifespan Evaluation

The potential of Terasen® on longevity was assessed. The results showed that, compared to the control group, the average lifespan of *C. elegans* was significantly ($p < 0.05$) prolonged after exposure to Terasen® treatment (Figure 5). The median survival (days) was 6 for the control, 6 for Terasen® 250 µg/mL, 7 for 500 µg/mL, and 9 for 1000 µg/mL. The maximum lifespan of wild-type *C. elegans* increased by 2 days (22.22%) after treatment with 500 µg/mL of Terasen® ($p<0.005$) and 5 days (55.56%) after treatment with 1000 µg/mL compared to the control group. Based on the results, the use of Terasen® showed a concentration-dependent boost in the lifespan of nematodes.

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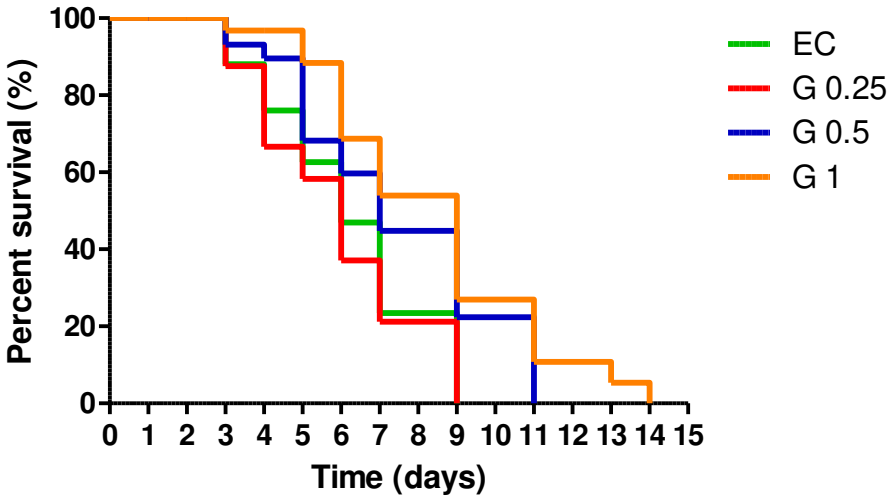

Figure 5. Effect of treatment with Terasen® at 250 µg/mL (G 0.25), 500 µg/mL (G 0.5), or 1000 µg/mL (G1) and Escherichia coli on the lifespan of *C. elegans*. The log-rank test using Mantel-Cox survival analysis demonstrated the statistical significance ($p<0.05$) of the difference between the curves.

4. Discussion

With aging, there is an imbalance of reactive species. Hence, we assessed the radical scavenging of Terasen® through DPPH and ABTS methods. Although the radical scavenging in the DPPH assay was low compared to the positive control gallic acid, it was high in the ABTS assay. This could be explained by the presence of the phenolic compounds from the formulation with a higher affinity for radical scavenging of ABST [13,14].

Then, the potential of Terasen® on longevity was evaluated in vivo using *C. elegans* as a model organism. This tiny nematode, which measures only 1 mm in length as an adult, is cultured in Petri dishes using solid and liquid culture media, and its primary food source is Escherichia coli. With its two types of sex, hermaphrodite (XX) and male (XO), the hermaphrodite can generate 300 to 1400 offspring, facilitating the creation of a uniform genetic lineage [15].

Despite its simple anatomy with few tissues and organs, the nervous system, gastrointestinal tract, gonads, and muscles of *C. elegans* are comparable to those of more complex animals [16]. Although it lacks adipose tissue or a liver, its intestine can perform similar functions, such as lipid synthesis and lipoprotein secretion [17]. The genetic sequence of *C. elegans* is well known, and genomic research has revealed that its genome has 60 to 80% of genes similar to those of human diseases and metabolic pathways [18].

C. elegans has proven to be a valuable organism for studying aging, as it undergoes noticeable changes over time, such as alterations in body movement, pharyngeal pumping, egg-laying posture, and body size, among others [19,20].

The pharynx of *C. elegans* has rhythmic contractions that occur at high frequencies in young adults, reaching 200-300 contractions per minute. However, with aging, there is a progressive decrease in the frequency of pharyngeal contractions [21]. This study observed that the pharyngeal pumping rate of Terasen®-treated groups was decreased compared to the control group, indicating that Terasen® likely decreased the nematode's feeding behavior.

Caloric restriction has been widely studied and is associated with increased longevity in various organisms, including *C. elegans*, because reducing calorie intake promotes metabolic changes that stimulate stress resistance and cellular protection [22]. This was observed in the study conducted by (Lakowski and Hekimi, 1998), where the authors reported that caloric restriction extended the lifespan of *C. elegans*.

During this study, the production of eggs in *C. elegans* was monitored daily, revealing a decrease in the number of progenies in animals treated with Terasen® compared to the control group.

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Monitoring reproductive capacity is essential to age research, as several interventions that prolong adult lifespan impact progeny production. Dietary restrictions also have been associated with reductions in progeny production during the early life phase of animals and a reduction in the total number of progenies produced by self-fertile hermaphrodites [22].

The results showed that after 4 days of Terasen® treatment, there were significant changes in the body length of the nematodes compared to the control, although this difference mitigates on the eighth day. As for the body movements, there were no statistically significant changes in the animals treated ($p > 0.05$; Two-Way ANOVA), although a trend was observed of decreased movement. Usually, young adult hermaphrodites exhibit fluid and synchronized body movement. However, their motor coordination deteriorates as they age, becoming increasingly uncoordinated and unpredictable until they stop moving [19].

Finally, we observed that the treatment increased the average and maximum lifespan of the nematodes. As observed, other effects were seen, including decreased pharyngeal contractions, reduced body length, and decreased laid eggs. Notably, all these effects are also observed during caloric restriction [24]. Hence, it appears that Terasen® may activate the same pathways triggered by caloric restriction, even if food is normally provided.

Caloric restriction is a non-genetic intervention that can increase the lifespan and decrease the incidence of age-related diseases. Hence, a body of research has been aiming to test the mechanisms beneath this process to achieve healthy aging. Some of the core components involved in the beneficial effects of caloric restriction are the mTOR [25,26] and the insulin/insulin-like growth factor-1 signaling (IIS) pathway in humans [27,28]. Accordingly, in *C. elegans*, the IGF-1 receptor is codified by the gene daf-2 in worms (igf1 in humans); reduction of this pathway will cause activation of DAF-16 (orthologue of the human FOXO), a transcription factor that will synthesize products involved in longevity and stress resilience [9,29]. The gene daf-16 is related to increased life expectancy in *C. elegans* [9,30]. Another important player involved in life expectancy in *C. elegans* is SKN-1 (orthologue of the human Nrf2), which is involved in the resistance to oxidative stress (Figure 6) [31].

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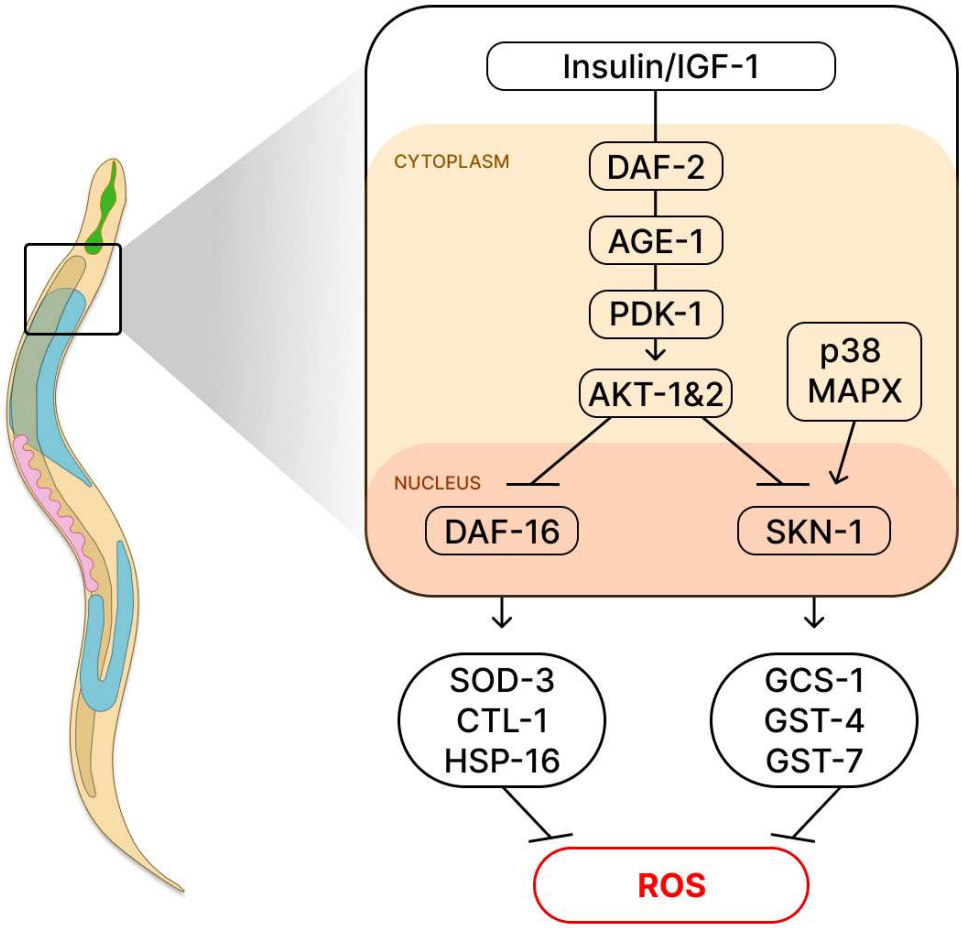

Figure 6. The insulin/IGF-1 (insulin/insulin-like growth factor-1) signaling pathway and its correlation with the SKN-1 transcription factor is an area of study that warrants a formal overview. The IGF receptor in *C. elegans*, which is encoded by the DAF-2 gene, initiates a cascade of phosphorylation events involving AGE-1/PI3K, PDK-1, AKT-1/2. By inhibiting the activity of DAF-2, the transcription factor DAF-16 is activated, which in turn activates genes such as superoxide dismutase-3 (sod-3), catalase-1 (ctl-1), and small heat shock protein-16.2 (hsp-16.2), thus providing protection against oxidative stress. The regulation of life expectancy, which involves the transcription factor SKN-1, acts through the activation of p38 mitogens (MAPK). SKN-1 plays a crucial role in regulating antioxidant genes, cellular detoxification, and longevity. The activation of SKN-1 regulates target genes in the nucleus, including GCS-1, GST-4, and GST-7.

Finally, we observed that the treatment increased the average and maximum lifespan of the nematodes. As observed, other effects were seen, including decreased pharyngeal contractions, reduced body length, and decreased laid eggs. Notably, all these effects are also observed during caloric restriction [24]. Hence, it appears that Terasen® may activate the same pathways triggered by caloric restriction, even if food is normally provided.

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SKN-1 (orthologue of the human Nrf2), which is involved in the resistance to oxidative stress (Figure 6) [31,33].

Studies in the literature report that some plant secondary metabolites can aid in longevity by acting in these pathways. In line with the results observed here, some phytochemical compounds present in Terasen® have been reported to increase *C. elegan*'s lifespan, including anthocyanins, which are found in *E. oleracea*, quercetin, which is a marker from *M. dubia*, and tocotrienols, abundantly found in *B. orellana*.

[29] reported that the extract of black rice, which is a rich source of anthocyanins (representing 43% of the extract), extended the lifespan, enhanced stress resistance, increased antioxidant enzymes activity, and reduced the accumulation of lipofuscin, ROS, and MDA. The treatment downregulated the expression of age-1 and daf-2 mRNA while upregulated daf-16 mRNA and upregulated protein expression of SOD-3, CTL-1, and GST. The major anthocyanins found were cyanidin-3-O-glucoside (76.15 %) and peonidin-3-O-glucoside (22.11 %).

The increased lifespan and stress resistance in *C. elegans* induced by cyanidin-3-O-glucoside (the most abundant anthocyanin from *E. oleracea*) was also reported by [34]. Other anthocyanin-rich sources that were reported to increase *C. elegans*' lifespan were *Prunus cerasus* [35], *Paeonia suffruticosa* [36], *Eugenia uniflora* [37], and *Euterpe precatoria* [38]. *E. oleracea* also increased longevity in *Drosophila melanogaster* [39].

M. dubia has quercetin as marker. [40] reported that quercetin increased the mean lifespan of *C. elegans* by 15%, and increased the translocation of DAF-16 to the nucleus, a mechanism correlated with stress response and longevity. This was also reported by [41], who also showed that quercetin is safer than its glycosylated forms.

The phytochemical markers of *A. aculeatum* include gallic acid, ellagic acid, and carotenoids. Cocoa beans, derived from *Theobroma cacao*, are commonly used to produce chocolate and cocoa powder. A single serving of cocoa powder (about 2 tablespoons) contains high levels of gallic acid [42]. In a study conducted by [43], the effects of cocoa powder enriched with polyphenols were evaluated on resistance to oxidative stress in biological models such as the yeast *Saccharomyces cerevisiae* and the nematode *C. elegans*. The results showed that cocoa enriched with polyphenols increased resistance to oxidative stress in both models, as well as life expectancy in the nematode. These effects were attributed to the presence of polyphenols in cocoa and the sirtuins Hst3 and SIR-2.1, in addition to the insulin/IGF-1 signaling pathway in the nematode.

A recent study [44] delved into the impact of extracts from *Glochidion zeylanicum* leaves on *C. elegans*, specifically examining their anti-aging and oxidative stress resistance properties. The study employed HPLC analysis to identify oxyresveratrol and quercetin, as well as high levels of gallic acid and catechin within the extracts. Findings revealed that these leaf extracts from *G. zeylanicum* provided protection against oxidative stress, regulated stress response genes like SOD-3 and GST-4, and involved transcription factors DAF-16/FoxO and SKN-1/Nrf-2 in the oxidative stress resistance properties. Additionally, the extracts improved pharyngeal pumping function and increased the life expectancy of worms, suggesting anti-aging benefits.

The impact of raspberry extracts (RE), which consist of various phytochemical compounds such as ellagic acid, salicylic acid, chlorogenic acid, p-coumaric acid, quercitrin, catechin, and luteolin, on the lifespan of *C. elegans* and the underlying mechanisms were studied [33]. The findings demonstrate that RE enhances resistance to oxidative stress, increases the activity of antioxidant enzymes, and decreases the excessive production of reactive oxygen species. Nevertheless, in certain mutants, the administration of RE had disparate effects, indicating that the SKN-1/Nrf2 pathway is vital in regulating RE-induced longevity and response to oxidative stress.

A study by [45] evaluated the effects of ß-carotene and fucoxanthin on the lifespans of *D. melanogaster* and *C. elegans*. The findings indicated that both carotenoids extended the lifespan of fruit flies, while only fucoxanthin exhibited beneficial effects on nematodes. The activation of the FOXO transcription factor by carotenoids initiated a series of events that triggered the activation of stress response genes, culminating in heightened resistance to stressors and an increase in lifespan.

According to research conducted by [46], mamey carotenoids were analyzed for their antioxidant properties on nematodes. The study found that both pure β-carotene and mamey pulp extract (MPCE) enhanced resistance to oxidative stress and increased the survival rate of the nematodes. However, it was observed that mamey skin extract (MSCE) had adverse effects due to the degradation of β-carotene. Even though high concentrations of MSCE were toxic, they still managed to improve survival, likely due to antioxidant mechanisms and the phenomenon of hormesis.

Finally, Terasen® has the oil of *B. orellana* in its composition, a rich source of tocotrienols. Tocotrienols are another group of compounds that potentially increase lifespan. [47] showed that *C. elegans* subjected to oxidative stress with hydrogen peroxide had decreased lifespan and increased lipofuscin accumulation. On the other hand, animals treated with a tocotrienols-rich fraction had restored lifespan and reduced lipofuscin accumulation. [48] reported that the lipophilic extract of *B. orellana* increased the median and maximum lifespan by 35% and 27%, increasing oxidative and thermal stress without affecting the fertility (which is different from what was observed here). According to the authors, the effect depended on the insulin/insulin growth factor-1 pathway.

5. Conclusions

Based on this research evaluating the potential of Terasen® in longevity, using *C. elegans* as a study model, it can be concluded that Terasen® was effective in significantly extending the median and maximum lifespan of the worms compared to untreated animals. Terasen® also affected the feeding behavior of the nematode, resulting in a reduction in the pharyngeal pumping rate. The effects observed here are similar to those of caloric restriction, including decreased progeny and size, even if food is typically provided. These results are consistent with other research showing that secondary metabolites found in Terasen® can also prolong life in *C. elegans*.

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