Anti-aging effect of polysaccharides from the peel of *Zanthoxylum schinifolium* Sieb. et Zucc. on the nematode *Caenorhabditis elegans*

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**ABSTRACT**

*Zanthoxylum schinifolium* Sieb. et Zucc. (green pepper), a well-known traditional Chinese spice, recent studies found that the water extract of *Z. schinifolium* could significantly enhance the innate immune system, and *Z. schinifolium* peel polysaccharide (ZSPP) in the water extract is one of the active ingredients where antioxidant and anti-aging properties of ZSPP is yet to be determined. *Caenorhabditis elegans* was employed to see if ZSPP has anti-aging properties. After medication with ZSPP, the longevity, motility ability, and stress resilience of *C. elegans* were measured. We studied *C. elegans* brood growth and the insulin/insulin-like growth factor (IGF) signalling pathway to learn more about the mechanism of ZSPP’s anti-aging action. According to statistical analysis of the experimental results, ZSPP therapy increased the longevity of *C. elegans*, as well as its motility capacity and stress tolerance. ZSPP was discovered to get a protective effect on UV-irradiated *C. elegans* in specific and ZSPP had no influence on *C. elegans* reproduction. However, after ZSPP therapy, the levels of age-1 and ncf-1 mRNA were lowered. In *C. elegans*, ZSPP has an anti-aging impact via the insulin/IGF signaling system, but it has the potential to evolve into a multifunctional diet in the long term.

**Introduction**

*Zanthoxylum schinifolium* Sieb. et Zucc. (green pepper) is a member of the genus *Zanthoxylum* and an important spice plant mainly distributed in Asian countries, including China. The mature peel of green pepper contains rich volatile oil components. In recent years, domestic and foreign scholars have studied the chemical composition, extraction process and biological activity of green pepper and its volatile oil. Green pepper and its volatile oil have antibacterial (Gao and Pu 2007; Diao et al. 2013), antitumour (Paik et al. 2005; Jun et al. 2007; Li et al. 2013; Wang et al. 2014), antiviral (Chang et al. 1997; Mi and Mi Sook 2014), antioxidant (Chon et al. 2009) and insecticidal activity (Liu et al. 2009; Wang et al. 2011; Liang et al. 2013). After steam distillation of volatile oil from the peel of *Z. schinifolium*, the water extract of its peel is usually regarded as a byproduct and abandoned. Steam distillation is accomplished by flowing dry steam across plant matter, causing the steam volatile chemicals to evaporate, condensate and accumulate in receiver. Low-pressure steam is used in distillation to substitute the volatile chemicals in the whole plant matter. There are fewer reports on the activities of the water extracts of *Z. schinifolium*. Harikrishnan et al. (2010) reported that the adaptive immunity of *Paralichthys olivaceus* might be considerably enhanced by the aqueous extracts of *Z. schinifolium*, which might enhance resistance to disease towards *Uronema marinum*. Organic solvents, such as alkaline or LiCl, are commonly used to decompose the intermolecular hydrogen bonds in polysaccharides, leading in disintegration. The water-soluble polysaccharide in water extract of green pepper peel may be one of the active ingredients responsible for its pharmacological activity. Polysaccharides are immune modulators that activate immune receptors and improve immune function.

Polysaccharides have more than >10 monosaccharide units, while oligosaccharides have 3–10 monosaccharides as a general rule; nevertheless, the exact cutoff varies depending on custom. Polysaccharides are indeed a type of biological polymer that are widely used. The dispersion of the sample between such a mobile phase extractant and a solid phase packing of the columns is the separating principle of HPLC. As a result, the eluted elements of a sample occur at
different times. As a result, the sample ingredients can be separated.

*Caenorhabditis elegans* is a group of microorganisms that has already been utilised intensively in biological processes and toxicology research (Wilkinson et al. 2012). Its accuracy is premised on the reality that the genomes and key communication pathways in *C. elegans* and people are substantially preserved, with 60–90% commonality. Furthermore, aging process of *C. elegans* is comparable to those of humans. In comparison to other commonly used in vitro and in vivo models, *C. elegans* offers a number of clear benefits, along with a limited lifespan, low price, the capacity to modify large amounts of samples, and appropriateness for and in vivo bioactive components and toxicity testing. These outstanding advantages make the study of *C. elegans* more meaningful. In this study, polysaccharides from the water extracts of the green pepper peel were exposed to antioxidant and anti-aging valuations using *C. elegans* as a classical example to provide an experimental basis for the development and utilisation of *Z. schinifolium* peel polysaccharide (ZSPP) in dietary health products.

**Materials and methods**

**Preparation of ZSPP**

ZSPP was prepared in our laboratory using the following method (Tang and Huang 2016). Briefly, 100 g of dried green pepper skin (removed seeds) was heated to reflux with 1 kg of purified water for 3 h, and then rinsed. The polysaccharide was again recovered using a crude product of four volumes of 95% ethanol. The savage technique was used to separate the protein from this sample. Purification of the crude polysaccharide using a DEAE-cellulose column and Sephadex G-100 column chromatography yielded a single peak matching to the pure ZSPP extract. Ethanol is a versatile ingredient that can be found everywhere from wine to whipped cream. It is dependable and produces potent extraction method with little effort. Because ethanol is a good solvent, it quickly reacts with water, dissolving water-soluble compounds such as in photosynthesis. DEAE-Sephadex is a positively charged sludge which will react electrostatically with negative charge molecules, causing them to dissolve slowly than the positive charge components in the sample of interest. This is a common separation method for identifying certain proteins or enzyme inside the body. Sephadex Grade is perfect for creating DNA purification spinning column. Sephadex G-100 is one of the five G-types, which range in size from G-10 for small molecules to G-75 for large compounds. When utilised in a spin-column arrangement, gel filtration polymers will not have a defined exclusion threshold.

**Worm strain maintenance**

Initially derived from the Caenorhabditis Genetic Center, wild-type *C. elegans* N2 and the mutant variant daf-16 were kept on nematode growing medium (NGM) dishes planted with *Escherichia coli* OP50 at 20°C as reported (Sulston and Brenner 1974). NGM is for Nematode Growth Media, and all these plates are designed to allow nematodes such as *C. elegans* to multiply all while making worm harvesting easier. As stated previously, age-synchronised populations of L4 nematode larvae were produced (Donkin and Williams 1995). As feed for the worms, *E. coli* OP50 was placed onto to the NGM plate.

**Safety assay of ZSPP**

Lethality, reproduction and locomotion behaviour were examined to evaluate the safety of ZSPP. With minimal adjustments, these processes were carried out as previously described (Wu et al. 2012). For mortality, reproductive, and locomotor behaviour experiments, L4 larvae were treated with ZSPP for 48 h. Nematodes were reported as deceased in the lethality assay if they did not reply to a tiny platinum wire stimulation. For each experiment, about 100 nematodes were employed. The quantity of offspring recorded at all phases further than the egg stage was used to assess reproductive behaviour. A total of eight duplicates were carried out. From the L4 larval stage, the ZSPP therapy was given for 48 h to test locomotor behaviour. The nematodes were located on the surface of NGM without any *E. coli* OP50 coating. Head thrashes, described as a shift in the orientation of tilting at the mid-body, were recorded for 1 min after a 1-min recovery phase. For 20 s, body bends were tallied, defined as a shift in the orientation of the portion equivalent to the anterior bulb of the pharynx all along y-axis, presuming the nematode was moving down the x-axis. For the CK group, deionised water was used. A total of 16 duplicates were carried out.

**Lifespan assay**

The longevity test was done as described previously (Patananan et al. 2015). From the L4 larval stage forward, the L4 larvae were treated with ZSPP. The nematodes were transplanted every day for first 4 days
of maturity in this study. Every day, the number of surviving nematodes was calculated and recorded. The ones that cannot react to a little gold wire stimulus were deemed dead. For the CK group, deionised water was used. The findings for lifespan are based on at least three experiments.

**Oxidative stress resistance assay**

In a 96-well plate, nematodes were pretreated with 10–80 g/mL ZSPP for 48 h before being moved to media containing paraquat at a maximum concentration of 0.4 mM. To cause peroxidation inside the nematode, paraquat was employed. Reactive oxygen species (ROS) created by the paraquat cyclisation are extremely reactive chemicals that destroy biomolecules, causing lipid oxidation, protein and amino acid destruction, oxidative stress and death of cells. Every day, the nematodes were observed and evaluated as deceased if they did not reply to a tiny platinum wire stimulation. For the CK group, deionised water was used. The findings for longevity are based on at least three experiments.

**Anti-UV characteristics irradiation test**

The anti-UV characteristics (UVC) irradiation test was carried out as previously described (Cai et al. 2012). Anti-cataract agents can be made from compounds with anti-UVC. Coumarin derivatives have been shown to rescue rabbit corneal-derived cells from oxidative stress caused by UVB. L4 stage nematodes pretreated with ZSPP for 48 h were picked onto NGM culture dishes without OP50 and randomly divided into control and irradiation groups. The irradiation group was treated with 100 J/m² UVC in a UV Stratalinker (wavelength 254 nm) and the control group was not irradiated. The Stratalinker UV crosslinker is utilised in cross-link DNA or RNA to membrane made of nylon, methylcellulose or nylon-reinforced methylcellulose. In difference to the old-style process of roasting filters at 80°C for 2 h, the curing process usually takes only 25–50 s. Furthermore, when contrasted to oven-baking, crosslinking has indeed been demonstrated to dramatically boost pollination signal. After irradiation, the nematodes were immediately picked onto NGM culture dishes coated with OP50 and refined in a 20°C biochemical incubator. Each day, the nematodes were observed and evaluated as dead if worms did not reply to a small platinum wire stimulation. For the CK group, distilled water was used. The findings for longevity are based on at least minimum three experiments.

Nematodes pretreated with 10–80 µg/mL ZSPP for 48 h were irradiated with 100 J/m² UVC in a UV Stratalinker. After 7 days of exposure, the morphological changes were observed, and the statistics of alive or deceased worms were counted and recorded to analyse the mortality and lifespan.

**RNA isolation and quantitative real-time polymerase chain reaction**

Total RNA was extracted from worms injected with 40 g/mL ZSPP for 48 h utilising RNAiso Plus (Takara). RNAiso Plus is a complete RNA extraction reagent that can separate RNA in plants or animals tissue and cultivated cells quickly and effectively. The PrimeScript1st strand cdna synthesis kit has been used to reverse-transcribe whole RNA (Takara). A reverse transcriptase (RT) is an enzyme that converts an RNA template into complementary DNA (cDNA), a practice called as RT. This cDNA then can incorporate into the host chromosome, from which additional RNA transcripts can be produced via host-cell synthesis in retroviruses and retrotransposons. The relative production of target genes in relation to the standard act-1 transcript was quantified using quantitative real-time polymerase chain reaction (RT-PCR), and the results have been reported as the expression level ratios (between the target gene and the internal control act-1). The primers used in this study were as follows: Daf-2, forward 5’-CCAACGAAACAGGAGACCT-3’, reverse 5’-CGATAGCCGAAACACGAAC-3’; age-1, forward 5’-AATGGCAAAAGATGCCTG-3’, reverse 5’-GAGGTTCCTCCGGAAA-3’; daf-16, forward 5’-GGAGTTTCGTTCCGATTG-3’, daf-16, forward 5’-CGTTCCTCCGATTMCA-3’, reverse 5’-ATTCCCTTCGTTGC-3’; hcf-1, forward 5’-CGGAAGCCTGGAGTAAC-3’, reverse 5’-ATGGTGCCTGGAGAGAAG-3’; act-1, forward 5’-TGACGGAAAGATCATCACCCG-3’, reverse 5’-CATGGTGTTCTCCCGGAAA-3’.

**Statistical analysis**

Statistical analysis was accomplished by the SPSS 18.0. The SPSS can handle data from practically any form of files to generate tabular synopses, grids and plots of distribution and patterns, descriptive analysis, and advanced statistical analysis. Differences between each group were determined using analysis of variance (ANOVA). ANOVA is a geometrical method that segments an information set’s apparent collective variation into systematic components and random factors. Significance level was determined at the 0.05 and 0.01 probability levels. A two-tailed two-sample t-test was used to quantitatively assess the lifetime information (Minitab Ltd., Coventry, UK).
Results

Safety assay of ZSPP

A safety evaluation of ZSPP in *C. elegans* was carried out. After exposure of L4 nematode larvae *in vivo* for 48 h, in nematode, ZSPP doses of 10–80 g/mL were neither deadly nor impactful (Figure 1(a)). Develop a Zanzibar social protection that enhances the quality of life for all Zanzibaris by gradually lowering poverty, helping Zanzibaris to handle potential costs and social weaknesses, and assuring ubiquitous access to public needs. The rate of survival of nematodes handled with ZSPP was 100%. The reproduction score is indeed a significant consideration when assessing safety. ZSPP seemed to have no impact on the reproductive capacity of *C. elegans*, according to the findings (Figure 1(b)). The effect of ZSPP on *C. elegans* mobility was then investigated. The capacity of nematodes to move was measured using two essential access points: head thrashing and body bend. *C. elegans* administered with ZSPP had considerably improved movement capacity compared with the untreated worms, as shown in Figure 1(c,d), demonstrating that the ZSPP therapy improved *C. elegans* movement behaviour.

Effects of ZSPP on lifespan in *C. elegans*

The effects of varying concentrations of ZSPP on nematode lifetime were investigated. Administration with three concentrations of ZSPP extended the nematode lifetime, as seen in Figure 2. The groups treated with ZSPP at concentrations of 10, 40 and 80 g/mL had mean surviving durations that were considerably longer than the CK group by 3.7, 8.4 and 17.2%, correspondingly, as contrasted to the CK group. These findings revealed a clear dose–effect association between ZSPP concentrations and average nematodes longevity. These findings point to ZSPP’s anti-aging promise as a food products or food supplements. Antioxidants and cell regulators are the two primary classes of compounds that can be employed as anti-aging cream ingredients. Vitamins, tannins and flavonoids are antioxidant that inhibit elastin breakdown by lowering the quantity of FR in the tissue.

Beginning with the L4 larval stage, the worm was given ZSPP therapy for the rest of its life. The mean and standard deviation are represented by bars (** P 0.01, * P 0.05).

Stress resistance properties of ZSPP in *C. elegans*

The ability to withstand stress or ambiguity without being negative towards oneself others or is known as stress

![Figure 1](image-url). (a) Effect of ZSPP on the lethality of *C. elegans*. (b) Effect of ZSPP on the reproduction of *C. elegans*. (c) Effects of ZSPP on the locomotion behavior of *C. elegans* as indicated by head thrash. (d) Effects of ZSPP on the locomotion behavior of *C. elegans* as indicated by body bends.
Stress tolerance is a trait that allows humans to exist or even flourish in high-pressure conditions. In a harsh environment, ZSPP improved the stress resistance of *C. elegans*. ZSPP at doses of 10–80 g/mL could extend the lifetime of nematodes exposed to 40 mM paraquat, a ROS producer, as seen in Figure 3. The group administered with 80 g/mL ZSPP had a much longer mean survival rates than the CK group, by 46%; this difference was statistically significant. The use of ZSPP at a concentration of 40 g/mL increased the mean survival time by 13%, but the change was not statically important. These findings indicated that efficient antioxidant capacity was really only observed at high ZSPP levels. These findings indicated that efficient antioxidant capacity had only been observed at higher ZSPP levels. These findings suggested that ZSPP could have antioxidant characteristics as a food products or nutritional supplements (Figure 4).

**Protective effect of ZSPP on UV-irradiated C. elegans**

Subsequently, the nematodes were preserved with diverse doses of ZSPP ranging from 10 to 80 µg/mL for 48 h and received a 100 J/m² dose of UV radiation, smaller nematodes continued to appear in each treatment group 12–24 h after irradiation associated with the non-irradiated group. The mortality rate was higher in each irradiation group than that in non-irradiated group of 7-day irradiation, but the mortality rate of each dose group of ZSPP was lesser when comparable to the blank group. The death rates of 40 and 80 µg/mL groups were pointedly lower than that of the blank group. A certain dose–effect relationship was observed. This result suggested that ZSPP could protect the nematode from damage caused by UV radiation to a certain extent. It was generally believed that excessive UV radiation could cause cell damage, and the related literature suggested that UVC irradiation could induce ROS production in nematode somatic cells. The protective effect of ZSPP on nematodes may also be due to a certain scavenging effect on ROS.
**ZSPP enhances the lifespan of C. elegans via signalling through the insulin/insulin-like growth factor system**

Several signalling mechanisms control the nematode aging phase (Lapierre and Hansen 2012). The insulin/insulin-like growth factor (IGF) signalling pathway may be the most well-studied of all the routes that govern nematode senescence. Daf-2 and age-1 mutations, which are critical components of the insulin/IGF signalling cascade, have indeed been discovered to lengthen the nematode lifetime, and the transcription factor Daf-16 also increases lifespan directly regulating lifespan meriting cascade, have indeed been discovered to lengthen the nematode lifetime, and the transcription factor Daf-16 also increases lifespan directly regulating lifespan.

The insulin/IGF signalling pathway modulates the tolerance to heat stress, oxidative damage and infections, in addition to its role in lifespan (Evans et al. 2008; McColl et al. 2010). Cell growth, death, development, longevity, metabolic and motility are all mediated by the insulin-like growth factor (IGF) signalling system. Because it plays significant roles in most of the tasks necessary for growth, metabolic and reproduction, the IGF signalling pathway has indeed been linked to aging. Given that ZSPP increases nematode longevity and improves stress tolerance, it is important to know if it does so via the insulin/IGF signalling pathway. We used RT-PCR to look at how the transcription of key components of the insulin/IGF signalling pathway, such as daf-2, age-1, daf-16 and hcf-1, changed following the ZSPP administration.

The expression of age-1 and hcf-1 was down-regulated after the ZSPP therapy, as demonstrated in Figure 5. The transcription of other genes, on either hand, did not alter considerably. These findings suggested that ZSPP was engaged in worm lifespan enhancement by targeting the insulin/IGF signalling system.

**Discussion**

The aging process involves a steady decline of numerous physiological systems, and notwithstanding advancement in the area using biological entities such as yeast, fruit flies and *C. elegans*, still a basic and unresolved issue in science. We showed in this study that a polysaccharide extracted from *Z. schinifolium* peel had anti-aging properties. It greatly prolonged the longevity of *C. elegans*, with a dose of 80 g/mL having the greatest lifespan-extending impact. At the very same time, the CK group’s rate of survival, reproduction indices, and nematode movement behaviour were all regular, confirming its suitability to be used as a functional ingredient. Both under oxidative and UV-irradiated circumstances, nematodes pre-treated with ZSPP had a greater chance of survival than control mice. These findings showed that, in additional to its lifespan-extending benefits in normal circumstances, ZSPP is advantageous to *C. elegans* under stressful conditions.

When worms treated with ZSPP were exposed to high doses of paraquat, they lasted a lot longer than the control nematodes. Therapy with ZSPP at a dosage of 80 g/mL resulted in a 46% increase in mean survival rates. A ROS producer, paraquat, can cause oxidative stress and respiratory distress. ZSPP was able to improve nematode lifespan significantly. These findings suggested that ZSPP’s anti-aging properties were most likely due to its antioxidant capacity and resilience to environmental stresses.

Many species’ lifespans are directly linked to their ability to withstand stress (Chuang et al. 2009). It is well understood that improving survival during abiotic stress, such as extreme heat and ultraviolet light, correlates with increased lifespan. UVC irradiation can significantly induce morphological changes in *C. elegans*, affecting their growth and normal development. The average lifespan of nematode bombarded with a 100 J/m² dose was substantially less than that of the control group, and the fatality rate was significantly higher. However, the nematodes treated with ZSPP exhibited significantly reduced mortality and a prolonged average lifespan compared with the CK group. High-dose UVC irradiation can induce ROS production in nematode...
somatic cells. The outcomes of this testing recommended that the protective effect of ZSPP on nematodes may be derived from the scavenging effect on ROS.

The insulin signalling system has been linked to the modulation of nematode longevity, according to research. Furthermore, the insulin signalling system is implicated in oxidative stress regulation (Sakashita et al. 2010). Antioxidant genes like heat shock protein, peroxidase and superoxide dismutase could be effectively increased if the route is repressed or the message is reduced. It is speculated that after UVC irradiation induces ROS production in nematode somatic cells, ROS may regulate the expression of antioxidant genes and lifespan genes by activating insulin signalling pathways, resulting in a shortened lifespan (Lapiere and Hansen 2012).

Following the ZSPP treatment, we looked at the mRNA levels of important genes in the insulin/IGF signalling pathway. The results showed that age-1 and hcf-1 mRNA levels were much lower than the daf-16 mRNA levels, indicating that ZSPP’s anti-aging impact is mediated via its involvement with the insulin/IGF signalling pathway. ZSPP’s lifespan-extending impact is not due to direct modulation of daf-16 transcription since it does not modify the mRNA level of daf-16. Further research on the influence of ZSPP on the lifespan and survivability of C. elegans models with insulin/IGF signalling pathways would’ve been intriguing.

The study of natural substances with anti-aging properties derived from Chinese traditional medicinal herbs, particularly polysaccharides, has received much interest. In C. elegans, polysaccharides from Epimedium acuminatum Franch and Bletilla striata, for example, have antiaging and antioxidant properties (Xu et al. 2016; Ding et al. 2015). Polysaccharides from Nostoc commune have been reported to not only elevate antioxidant enzyme activities but also decrease malondialdehyde (MDA) content in aging nematodes (Li et al. 2011). Polysaccharides from Dendrobium officinale were reported to significantly prolong the lifespan and significantly improve the tolerance of nematodes (Liu 2016). Polysaccharide from Panax notoginseng can significantly improve the survival rate of C. elegans under oxidative stress and significantly reduce ROS levels (Feng et al. 2018). Owing to their extensive pharmacological properties, natural polysaccharides are expected to be candidates for life control and healthy aging.

Conclusion

Our findings suggest that ZSPP has the power to enhance C. elegans longevity. Furthermore, ZSPP was discovered to strengthen the nematode motility and stress resistance.

In addition, the life-prolonging effect of ZSPP is dependent on the insulin/IGF signalling system and has no influence on nematode fertility. In the meantime, ZSPP showed promise as a radioprotective compound.

Our research would be the one to show that ZSPP has anti-aging characteristics. With nematodes, further investigation of ZSPP’s antioxidant and anti-aging properties could’ve been carried out. To examine the anti-aging effects of ZSPP in mammals and establish ZSPP as a functional diet, further research needs to be done.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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