Constituent and effects of polysaccharides isolated from *Sophora moorcroftiana* seeds on lifespan, reproduction, stress resistance, and antimicrobial capacity in *Caenorhabditis elegans*

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[ABSTRACT] *Sophora moorcroftiana* (*S. moorcroftiana*) is an endemic leguminous dwarf shrub in Tibet, China. Decoctions of the seeds have been used in Chinese folk medicine for dephlogistication, detoxication, and infectious diseases. The present study aimed to investigate the constituent and biological effects of polysaccharides from *S. moorcroftiana* seeds in *Caenorhabditis elegans* (*C. elegans*). Polysaccharides from *S. moorcroftiana* seeds (SMpol) were extracted with 60% ethanol and constituent was analyzed by GC-MS. SMpol was composed of glucose, galactose and inositol in the molar ratio of 35.7 : 1.3 : 17.0. Synchronized worms were treated with SMpol and then lifespan, motility, reproduction, stress resistance and antimicrobial activity were examined. Compared with the control group, the lifespan was increased to the average of 27.3 days and the number of laying eggs showed a 1.3-fold increase in nematodes treated with SMpol (4 mg mL⁻¹). In SMpol (4 mg mL⁻¹) treated worms, there was a 1.1-fold increase in 24-h survival of acute heat stress and a 1.6-fold increase in 2-h survival of oxidative stress. The colonization of the bacteria in the SMpol treated nematode was significantly lower than that of the untreated group by 68.3%. *In vivo* studies showed SMpol significantly extended the lifespan, improved reproduction, increased stress resistance and antimicrobial capacity of *C. elegans*. In conclusion, those results indicated that the polysaccharides from *S. moorcroftiana* seeds were involved in a variety of biological activities leading to its modulatory effects on *C. elegans* which may be developed as a natural supplement agent.

[KEY WORDS] *Sophora moorcroftiana*; Polysaccharide; *Caenorhabditis elegans*; Anti-infection; Reproduction; Stress resistance

[Introduction] *Sophora moorcroftiana* (*S. moorcroftiana*), an endemic shrub in Tibet, China, is found in the wide valleys and on semi-arid mountain slopes from 2 800 to 4 400 m above sea level along the middle reaches of the Yarlung Zangbo River, and has played an important role in vegetation restoration and medicinal properties [¹]. According to historical records, the ripe seeds from August to October are used as medicine. In particular, the decoction of *S. moorcroftiana* seeds has been used in Chinese folk medicine for treatment of infectious diseases, detoxication, and dephlogistication [¹].

In our previous studies, we have examined the antimicrobial, antitumor and protoscolicidal activities of various extracts from *S. moorcroftiana* seeds with chloroform, 95% alcohol and water, respectively. Chloroform extract from *S. moorcroftiana* seeds shows antimicrobial, protoscolicidal, anti-inflammatory activities [²-³], and induces apoptosis of human gastric cancer cell line *in vitro* [³-⁴]. Meanwhile, the crude extract of 95% ethanol can also induce apoptosis in human gastric cancer cells [³-⁴] and show a weak inhibiting effect on S180 sarcoma development *in vivo* [¹-⁴]. The aqueous extracts of *S. moorcroftiana* seeds show a promoting proliferation effect on lymphocytes and phagocytes in suppressed
mice\textsuperscript{[5]} and S\textsubscript{180}-tumor bearing mice\textsuperscript{[6]}. However, as a magical medicinal plant in Chinese Tibet, which has not been fully studied, decoctions of the seeds used in Chinese folk medicine may have more unknown values \textit{in vivo}.

Interestingly, we have found that the 95\% ethanolic extracts from \textit{S. moorcroftiana} seeds at high concentration (≥ 0.4 mg·mL\textsuperscript{-1}) shows a potential protoscolicidal effect and at low concentration (≤ 0.1 mg·mL\textsuperscript{-1}) shows a lifespan extending effect \textit{in Caenorhabditis elegans (C. elegans)}\textsuperscript{[8]}. There is no clear mechanism for this dose-dependent of ethanolic extract. Because the decoction of seeds is used in a conventional way to treat diseases\textsuperscript{[1]}, we extracted water-soluble polysaccharides from \textit{S. moorcroftiana} seeds with 60\% ethanol. The biological effects of polysaccharides from \textit{S. moorcroftiana} seeds on toxicity, lifespan, motility, reproduction, stress resistance and antimicrobial activity in \textit{C. elegans} were examined and constituent of polysaccharides was analyzed by Gas Chromatography-Mass Spectrometer (GC-MS) in the present study.

\textit{C. elegans} has been commonly used as a model in recent years. A number of key findings relevant to mammals have been reported in studies with the \textit{C. elegans}\textsuperscript{[8-9]}. The conservation of biological principles between \textit{C. elegans} and mammals shows about 60\%–80\% of human gene homologues which has been discovered in \textit{C. elegans}\textsuperscript{[10-12]}. Because \textit{C. elegans} has a short life span (average 14–20 days at 20 °C) and is easy to culture\textsuperscript{[8-9]}, it has been used extensively to study the effect of natural product extracts, including some Chinese medicinal herbs\textsuperscript{[12]}. Many physiological and physiological indices of \textit{C. elegans}, such as life span, reproduction, behavior response, stress and antimicrobial capacity, can be used to evaluate the effect of the natural product extracts\textsuperscript{[13-15]}. In the present study, we purified polysaccharides from \textit{S. moorcroftiana} seeds and then investigated the biological efficacy on nematodes. Our results indicated that the polysaccharides intervention led to benefits to overall health status in \textit{C. elegans}, which might have an implication in a future use of \textit{S. moorcroftiana} seeds in humans.

\textbf{Materials and Methods}

\textit{Extraction and purification of polysaccharides from \textit{S. moorcroftiana} seeds}

The seeds of \textit{S. moorcroftiana} were collected from the Bank of Yalu Tsangbo River, Tibet altiplano, and air dried at room temperature for 3 weeks. The identity of the seed was confirmed by Professor LI Hong-Yu, School of Pharmacy, Lanzhou University, China. A herbarium voucher specimen of seeds (M2001-1005) was deposited in the Department of Immunology, School of Basic Medical Sciences, Lanzhou University, Lanzhou, China. Polysaccharides from the \textit{S. moorcroftiana} seeds were prepared as previously described with slight modifications\textsuperscript{[6]}. Briefly, the powdered seeds (200 g) of \textit{S. moorcroftiana} were heated to reflux with 60\% ethanol (4 L) for 4 h at 80 °C and filtrated through filter paper (Whatman No. 2), and the filtrate was collected. The supernatant of ethanol extracts (SMeth) was concentrated in vacuum (400 mL) and was adjusted to acid-stage (pH 4) with 12 mol·L\textsuperscript{-1} of HCl. The acidulated fluid was centrifuged and the non-water-soluble precipitation fraction was removed. The supernatant was adjusted pH to 12 with 10 mol·L\textsuperscript{-1} of NaOH and centrifuged and the non-water-soluble precipitation fraction removed. The supernatant was extracted with chloroform at the volume ratio of 1 : 1 for removing a mixture of the alkaloids, lipids and proteins (SMchl) and the water solution was concentrated in vacuum to obtain the polysaccharide from \textit{S. moorcroftiana} seeds (SMpol, yield 8.8\%) for the present study. Total sugar content of the polysaccharide was determined by the phenol-sulfuric acid method, using glucose as the standard\textsuperscript{[16]}.

\textit{Constituent analysis of polysaccharides}

The pigmentum of SMpol was degraded with H\textsubscript{2}O\textsubscript{2} (30\%)\textsuperscript{[17-18]} . For monosaccharide analysis of SMpol, the SMpol (15 mg) was dissolved in 10 mL of trifluoroacetic acid (2 mol·L\textsuperscript{-1}) and hydrolyzed at 120 °C for 3 h in a sealed glass tube. The hydrolysates of SMpol were performed with aldononitrile acetates derivatization according to conventional protocols and analyzed by GC-MS on an Agilent GC6890/MSD5973 (Agilent Technologies, Palo Alto, CA, USA)\textsuperscript{[19]}. The acetylation of SMpol was mixed with 50 g·L\textsuperscript{-1} of hydroxylamine hydrochloride and with 0.5 mL of pyridine and then heated in a water bath at 90 °C for 40 min. After the reaction, the mixture was cooled at room temperature. After 5 mL of acetic anhydride was added, the tube was sealed and incubated in a water bath at 90 °C for additional 40 min. Authentic standard monosaccharides (D-ribose, L-thannose, L-arabinose, D-xylose, D-mannose, D-glucose and D-galactose) were prepared with myo-inositol (2 mg) in the same way. A DB-1701 capillary column (30 m × 0.25 mm × 0.25 µm) (Agilent, USA) was used for the analysis. The chromatography was performed under the following conditions: N\textsubscript{2}, 30 mL·min\textsuperscript{-1}; air, 400 mL·min\textsuperscript{-1}; injection temperature, 270 °C; and detector temperature, 270 °C.

\textit{C. elegans stains, maintenance and synchronization}

The \textit{C. elegans} wild-type (N2) strains and its food source \textit{Escherichia coli} OP50 strain were kindly provided by Professor LI Hong-Yu, who obtained these from Caenorhabditis Genetic Center at the University of Minnesota (USA). \textit{C. elegans} cultivation media supplies were purchased from Solarbio (Beijing, China). All the nematodes were grown on Nematode Growth Medium (NGM) agar plates with \textit{E. coli} OP50 as food source at 20 °C\textsuperscript{[20]}. The L4 stage worms were prepared for synchronization, and hermaphrodites were decomposed in freshly prepared bleaching solution 20\% bleach, 0.5 mol·L\textsuperscript{-1} NaOH, with agitation (150 r·min\textsuperscript{-1}) until eggs was released. Briefly, eggs were incubated in M9 buffer (M9: KH\textsubscript{2}PO\textsubscript{4} 3.0 g, Na\textsubscript{2}HPO\textsubscript{4} 6.0 g, NaCl 5.0 g, 1 mol·L\textsuperscript{-1} MgSO\textsubscript{4} 1 mL, and dH\textsubscript{2}O 1 000 mL) without food at 20 °C overnight (12 h), which would make larva hatch and prevent nematode
developing owing to starvation \textsuperscript{[17]}. All the L1 stage larvae were placed on the NGM agar plates (60 mm across) with enriched OP50 and grown at 20 °C.

\textbf{Survival assays in \textit{C. elegans}}

According to the standard protocol recently described\textsuperscript{[21]}, the experiments were performed in 96-well microtitre plates with 200 μL of M9 buffer composed of extracts decoctions of \textit{S. moorcroftiana} seeds (SMdec), SMeth, SMchl and SMpol, respectively) at the concentrations of 5 and 2.5 mg mL\textsuperscript{−1}, respectively (Fig. 1). M9 buffer solution was used as the control. 60 h later, the synchronized L4 worms were moved to their respective experimental plates. In each plate, 15 worms were moved to each well. 15 worms were randomly transferred with a platinum wire in each plate. All worms were grown in a biochemical incubator at 20 °C for 3 days. In each day, survivals were measured as the presence of pharyngeal pumping after stimulus and survival rate was then calculated. The assays were conducted in duplicate.

\textbf{Fig. 1 A scheme of the intervention with SMpol in \textit{Caenorhabditis elegans}}

\textbf{Life span assay of \textit{C. elegans}}

We performed the life span tests according to the standard protocol recently described by Swain \textit{et al.}\textsuperscript{[22]} 12 h later the synchronized L1 larvae were moved into NGM agar plates which were supplied with SMdec and SMpol in proportion to the required concentration and kept at 20 °C until larvae growth into L4 period (after 60 h) (Fig. 1). These animals were then transferred into the NGM agar plates without polysaccharides and fed with OP50 for life span assays. The experiment was performed at 20 °C by checking the nematodes everyday on a fixed time-point until all the nematodes died. The nematodes who escaped from the Petri dishes were excluded from this assay. Day 1 was defined as the first day when L4 animals were transferred into the NGM agar plates without polysaccharides treatment. Each experiment was administered in triplicate and three biological repeats.

\textbf{Reproduction assay of \textit{C. elegans}}

We performed the reproduction assay according to the standard protocol recently described \textsuperscript{[23]}. Briefly, synchronized L1 larva were moved into NGM medium agar plates with SMpol and SMchl and kept at 20 °C until larvae grew into L4 (Fig. 1). Afterward, these animals were transferred into the NGM agar plates and fed with OP50. To keep from egg laying at the border of the NGM agar plates and possible escape of the worms, palmitic acid (78 mmol L\textsuperscript{−1}) was placed at the edge of each agar plate as described by Harada \textit{et al.}\textsuperscript{[21-22]}. These hermaphroditicuses were cultured at 20 °C for 6 days. Every day, the animals were transferred to a NGM agar without treatment. The number of eggs from the animals was recorded every day until they stopped laying eggs.

\textbf{Motility assay of \textit{C. elegans}}

The behavioral assays were conducted according to the standard protocol described by Tsalik and Hobert \textsuperscript{[24]}. In this experiment, the assay conditions were the same as that described above for the life span assay (Fig. 1). Moreover, nematodes were transferred into the NGM covered with enough OP50 for agents tested. Subsequently thrashes assay per 20 s, body bends frequency per 20 s and pharyngeal pumping per 20 s were taken count under a zoom stereo microscope (Phenix Optical Co., Ltd., Shangrao, China). Each experiment was performed in triplicate.

\textbf{Stress resistance assays of \textit{C. elegans}}

The feeding procedures were the same as described above for the the life span assay. For the acute heat resistance test \textsuperscript{[25]}, the L4 stage worms treated with or without SMpol were transferred into 96-well microtitre plates with 200 μL of M9 buffer and incubated at 35 °C. The death number of worms was recorded every hour until all worms in the control plates died. For the oxidative resistance test \textsuperscript{[26]}, the resistance to oxidative stress was examined by exposing animals to H\textsubscript{2}O\textsubscript{2}. Ten L4 stage adult worms treated with or without SMpol were transferred into 96-well microtitre plates with 200 μL of M9 buffer and 40 nmol H\textsubscript{2}O\textsubscript{2} and incubated at 20 °C. The death number of worms was recorded every half-hour until all worms in the control plates died. All the experiments were performed in triplicate.
**Antibacterial assay of polysaccharides in vitro**

*Pseudomonas aeruginosa* (Pa1991235) was provided by the laboratory of medical immunology at Lanzhou University. The disk diffusion method was used for the determination of the antibacterial activity of polysaccharides \[^2\]. Briefly, a suspension of the tested Pa1991235 (0.1 mL of \(10^8\) CFUs mL\(^{-1}\)) was spread on the solid LB plates in 90-mm Petri dishes. Sterile filter-paper disks (6 mm in diameter) were impregnated with 10 µL per disk of the polysaccharides (1–10 mg mL\(^{-1}\)) and placed onto plates. These plates were incubated at 37 °C for 24 h to develop bacteria. The diameters of the inhibition zones were measured in mm using a vernier caliper. Each test was performed in triplicate and repeated three times.

**Pseudomonas aeruginosa killing assay in C. elegans**

For the killing assay \[^27\-28\], synchronized L1 larva was grown on NGM agar plates with SMpol in the medium from L1 stage to L4 stage (Fig. 1). Before transferring, the Pa1991235 bacterial lawn on the NGM plates was prepared. The L4 stage worms treated with or without SMpol were then transferred onto the NGM agar plates with Pa1991235 bacterial lawn for 8 h. Afterward, the worms were washed with M9 buffer thrice and transferred to new NGM agar plates with OP50 lawn. All the plates were kept at 20 °C and the live worms were counted every day under a dissection microscope at a certain time until all worms in the control plates died \[^28\]. The nematodes who escaped from the Petri dishes were excluded from this assay. All experiments were performed in triplicate.

**Determination of bacterial load in C. elegans**

Pathogen infection assays were performed as described as the aforementioned killing assay \[^28\]. A colony-forming unit (CFU) was used to measured PA strains numbers *in vivo*. Worms were washed thrice with M9 buffer to remove bacteria covered the surface of nematodes. Briefly, from Day 0 to Day 5, the nematodes were washed with M9 buffer and then crushed with glass homogenizers (500 µL of M9 buffer). The worm tissue homogenate (50 µL) was diluted with sterilization M9 buffer (450 µL). A serial dilution of tissue homogenate was made with a range from 10\(^1\) to 10\(^4\). 10 µL of tissue homogenate was spread on LB culture-medium and incubated at 37 °C for 12 h \[^29\]. Each experiment was repeated at least three times.

**Statistical analysis**

The data were analyzed using SPSS 18.0 for Windows software (SPSS, Inc., Chicago, IL, USA). The results are shown as means ± standard deviation (SD). Statistical significance was determined using one-way ANOVA, T tests, and Log-rank test as appropriate.

**Results**

**Constituent of polysaccharides**

The total sugar content of SMpol was determined with phenol-sulfuric acid method. The average value was 82.23%, indicating that obtained polysaccharides from *S. moorcroftiana* could be conducted for its preliminary activity on *C. elegans*. The SMpol with molecular weight of 900 kDa was analyzed by acetylated and GC-MS for the composition. The results indicated that SMpol was mainly composed of glucose, galactose and inositol in a molar ratio of 35.7 : 1.3 : 17.0.

**Survival rate of C. elegans**

Four kinds of extracts from *S. moorcroftiana* seeds, SMeth, SMdec, SMchl and SMpol, were tested in survival assays in *C. elegans* at concentrations from 2.5 to 5.0 mg mL\(^{-1}\) (Fig. 2). The survival rates of nematodes in SMpol and SMdec treatment groups showed no significantly difference in 72 h, compared with the control. Meanwhile, the survival rates of nematodes in SMeth and SMchl treatment groups were 58.3% and 70.6% at the concentration of 5 mg mL\(^{-1}\), and 42.5% and 55.1% at the concentration of 2.5 mg mL\(^{-1}\) in 72 h, respectively, which showed a potential protoscolicidal effect. But SMpol and SMdec did not display a toxic effect in nematodes at 2.5–5.0 mg mL\(^{-1}\).

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(13.9 days) of untreated N2 nematodes (control group), the SMpol (4 mg·mL⁻¹) significantly prolonged the life of wild-type N2 nematodes up to the average of 27.3 days. Meanwhile, compared with the control group, SMdec treatment did not increase the life span of C. elegans (P > 0.05).

**Effects on reproductive capacity of C. elegans**

Compared to the controls, the reproductive capacity and offspring production of adult’s C. elegans treated with SMpol were improved in dose-dependent manner (Table 1 and Fig. 3). Moreover, compared with the controls, the 4 and 2 mg·mL⁻¹ SMpol treatment not only increased the number of laying eggs by 1.3-fold and 1.2-fold, respectively, but also extended the time of oviposition by 32.7% and 31.3%, respectively.

**Table 1 Reproductive and life-cycle parameters of C. elegans supplied with SMpol and SMapo. Values in the table are means ± SD, n = 35**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Eggs laid per adult worm over 6 day</th>
<th>Mean span of oviposition (day)</th>
<th>Mean life span (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMpol 4 mg·mL⁻¹</td>
<td>481.8 ± 91.2**</td>
<td>8.0 ± 1.8</td>
<td>27.3 ± 3.1**</td>
</tr>
<tr>
<td>SMpol 2 mg·mL⁻¹</td>
<td>486.4 ± 91.3**</td>
<td>7.9 ± 1.9</td>
<td>23.2 ± 4.9**</td>
</tr>
<tr>
<td>SMpol 1 mg·mL⁻¹</td>
<td>230.0 ± 73.5</td>
<td>6.2 ± 1.0</td>
<td>17.0 ± 3.6</td>
</tr>
<tr>
<td>SMdec 4 mg·mL⁻¹</td>
<td>254.0 ± 80.0</td>
<td>6.0 ± 1.6</td>
<td>16.5 ± 3.9</td>
</tr>
<tr>
<td>Control</td>
<td>237.7 ± 85.0</td>
<td>6.0 ± 1.2</td>
<td>13.9 ± 3.8</td>
</tr>
</tbody>
</table>

The experiment was performed on NGM agar. P value was calculated using Student’s t-test. *P < 0.05, **P < 0.01 vs the control group

**Fig. 3  Eggs laid per adult over 6 days with a dose-dependent manner. SMpol (4 and 2 mg·mL⁻¹) treatment promoted C. elegans reproduction and prolong the days of reproductive capacity. The P value of egg number was calculated using one-way ANOVA (P = 0.002). The data are expressed as means ± SD, n = 15**

**No significant effects on motility of C. elegans**

Locomotory behavior plays an important role on assessing the excitability of nematode nervous system. As shown in Fig. 4, neither SMpol nor SMdec had obvious influence on the behavior response of the nematodes, compared with the control group (P > 0.05).

**Effects on the resistance of C.elegans to stress**

According to the results of two kinds of stress experiments, SMpol treatment prolonged life span on N2 nematodes under lethal conditions, rather than simply improving survival on old age. The results of the stress assays (Fig. 5) revealed that the resistance to both thermal and oxidative stresses was increased with SMpol treatment. The treatment with SMpol showed a 1.1-fold increase in 24-h survival at 35 °C, compared with the controls (Fig. 5A). Meanwhile, the resistance to oxidative stress showed a 1.6-fold increase in 2-h survival, compared with the controls (Fig. 5B).

**No inhibition on Pa1991235 proliferation in vitro**

There were no zones appearing of inhibition on Pa1991235 proliferation at the concentration of SMpol from 1 to 10 mg·mL⁻¹, indicating that the polysaccharides from S. moorcroftiana seeds did not have a direct activity against the tested bacteria.

**Effects on antimicrobial capacity of C. elegans**

After treatment with SMpol, the N2 nematodes infected with Pa1991235 showed a higher survival rate than that of the controls (Fig. 6). Specifically, all the worms without treated died in 5 days, SMpol protected up to 138% of the worms against the lethal effect of Pa1991235 infection (P = 0.008), indicating that the treatment with SMpol as a food supplement improved survival in dose-dependent manner.

**Effects on bacterial loads in C.elegans**

After infected, the bacillary loads in N2 nematodes were determined (Fig. 7). The SMpol provided a strong protective effect for nematodes. With time prolonged, the increasing of Pa1991235 CFU in untreated C.elegans reached to a peak at third day and then began to decline, but the trend was still at a high level. During the period of 6 days, the CFU of Pa1991235 in SMpol treated worms was different from each
other. Compared with the control group, the colonization of the bacteria in the SMpol treated nematode reached a peak at the second day, which was significantly lower than that of the untreated group by 68.3% ($P = 0.013$). On the sixth day, the worms treated with SMpol (4 mg·mL$^{-1}$) almost had removed all of the bacteria in the body.

![Fig. 4](image)

**Fig. 4** Motility of *C. elegans* treated with SMpol. The body bends, pharyngeal pumping and thrashes are the main indicator measures of the motility of *C. elegans*. $P$ values were calculated using one-way ANOVA, and were $P = 0.324$ (A), $P = 0.862$ (B) and $P = 0.507$ (C), respectively.

![Fig. 5](image)

**Fig. 5** SMpol treatment enhanced stress tolerance in *C. elegans*. (A) SMpol treatment-enhanced thermo stress tolerance N2 animals with and without SMpol were transferred to 35 °C for 24 h. And also SMpol can prolong nematodes lifespan exceeded 20h better than SMapo (19 h). The data was passed log-rank test with $P = 0.007$. (B) SMpol treatment-enhanced oxidative stress tolerance of SMpol-treated and untreated N2 animals exposed to 40 nmol hydrogen peroxide for 2 h at 20 °C. SMpol (4 mg·mL$^{-1}$) appeared a strong resistance to poision which were not only prolong life but also the vitality of *C. elegans*. The data was analyzed by log-rank test $t$ ($P = 0.015$)
Fig. 6 Fraction survival of *C. elegans* treated with SMpol. Untreated worms were all died in 5 days. Obviously, SMpol prolong the lifespan of Pa-infected worms in dose-dependent manner. The data was passed Log-rank test with $P = 0.013$

Discussion

*S. moorcroftiana* is an endemic shrub in Tibet, China. The seeds of *S. moorcroftiana* are used to treat infectious diseases, detoxication and dephlogistication in Chinese folk medicine [1]. Up to date, we have already employed a lot of laboratorial methods to demonstrate that chloroform, 95% alcohol and water extracts of *S. moorcroftiana* seeds have antimicrobial, antitumor and protoscolicidal activities [1-6]. However, the polysaccharides from *S. moorcroftiana* seeds and their biological efficacy were firstly reported in the present study. In general, polysaccharides were extracted with water at 80–100 °C and precipitated by ethanol. We extracted water-soluble polysaccharides from *S. moorcroftiana* seeds with 60% ethanol, which was an untraditional extraction method. Gas chromatography analysis showed that SMpol was composed of glucose, galactose and inositol in a molar ratio of 35.7 : 1.3 : 17.0.

Recent studies suggest that polysaccharides from traditional medicinal plants have great potentials in promoting health in animals. For instance, polysaccharides from *Epimedium acuminatum* Franch and *Bletilla striata* show anti-aging and antioxidant activities in *C. elegans* [30-31]. Supplementation of blueberry extracts prolongs life span of *C. elegans* and *Drosophila* [32-33]. In the present study, we used a non-traditional extraction method to obtain SMpol, which showed an unusual activity in *C. elegans*. The fractions of SMeth and SMchl showed a potential protoscolicidal effect, which may have an important role in the treatment of verminosis. Interestingly, water-soluble polysaccharides from *S. moorcroftiana* seeds with 60% ethanol had beneficial effects on the nematodes. The effect of SMpol on the longevity of the nematodes was significantly better than that of SMdec. At the same time, the SMpol promoted the reproduction of nematodes, including prolong the spawning time and increasing the quantity of offspring. However, we found that neither SMpol nor SMdec could affect the behavior response of nematodes.

There are multiple examples in the literature suggesting that life span-extending interventions are effective in resisting environmental stress and many nature products are conferring both increased resistance to stress and extended lifespan [25-26, 32-35]. Numerous conditions like environmental induction such as heat stress, oxidative stress and signals from the reproductive system, and supplement nutrition can increase the life span and reproduction of *C. elegans* [25-26, 32-35]. Longevity, reproduction and stress resistance are interrelated phenomenon, which seem to be a response to mediate life span extensions [34]. *S. moorcroftiana* is originated in the altiplano areas of Tibet, where there is plenty of sunshine and UV irradiation [1]. Untreated *S. moorcroftiana* seeds germinated and propagated in the altiplano areas [1]. We found that polysaccharides from *S. moorcroftiana* seeds showed resistance to stresses of heat shock and oxidative damage, which is required for extended lifespan and reproduction in *C. elegans* in the present study. Thus, SMpol may be one of an important fraction of *S. moorcroftiana* seeds for developing in altiplano areas, with the valuable activity of prolonging life span and reproduction.
capacity in *C. elegans* under stress and laboratory conditions. *Pseudomonas aeruginosa* killing assay has been used to identify novel antimicrobial compounds and innate immune mechanisms [27-28, 36]. Using the established *C. elegans/P. aeruginosa* killing assay model, we tested the ability of SMpol to induce immune responses and antibacterial capacity of the Pa1991235-infected *C. elegans*. The survival of infected *C. elegans* relies on the colonization and subsequently proliferation of pathogenic bacteria within the worm gut [27-29, 36]. We observed that SMpol significantly elevated the percentage of survival rate in infected worms. Compared to the control worms, the survival span in worms treated with SMpol was significantly prolonged in dose-dependent manner. Meanwhile, we also observed a considerable decrease of bacterial loads in worms treated with SMpol as compared to controls. In addition, we excluded the possibility of direct killing of Pa1991235 by SMpol as the reason for improved animal survival. The nematode pharynx is a neuromuscular tube relying on pumping mechanisms to trap and concentrate bacteria for transportation into the intestine during feeding [36]. Our results showed that SMpol did not diminish *C. elegans* pharyngeal pumping activities. Those results indicated that the prospect of SMpol possesses compounds capable of attenuating bacterial virulence without affecting bacterial viability. This aspect is desired for searching new agents against pathogenic bacteria as the selective pressure responsible for the development of antibiotic resistance in bacteria is reduced.

**Conclusion**

SMpol, polysaccharides with molecular weight of 900 kDa from *Sophora moorcroftiana*, significantly enhanced various physiological functions of *C. elegans*, such as lifespan, anti-infection, resistance to stress, and reproduction. The seeds of *S. moorcroftiana* may offer more value in vivo to humans. However, further study will be needed to reveal the molecular mechanisms of activities seen in the nematodes treated with polysaccharides.

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