Extraction of L-quebrachitol from sea buckthorn leaves and its anti-aging effects

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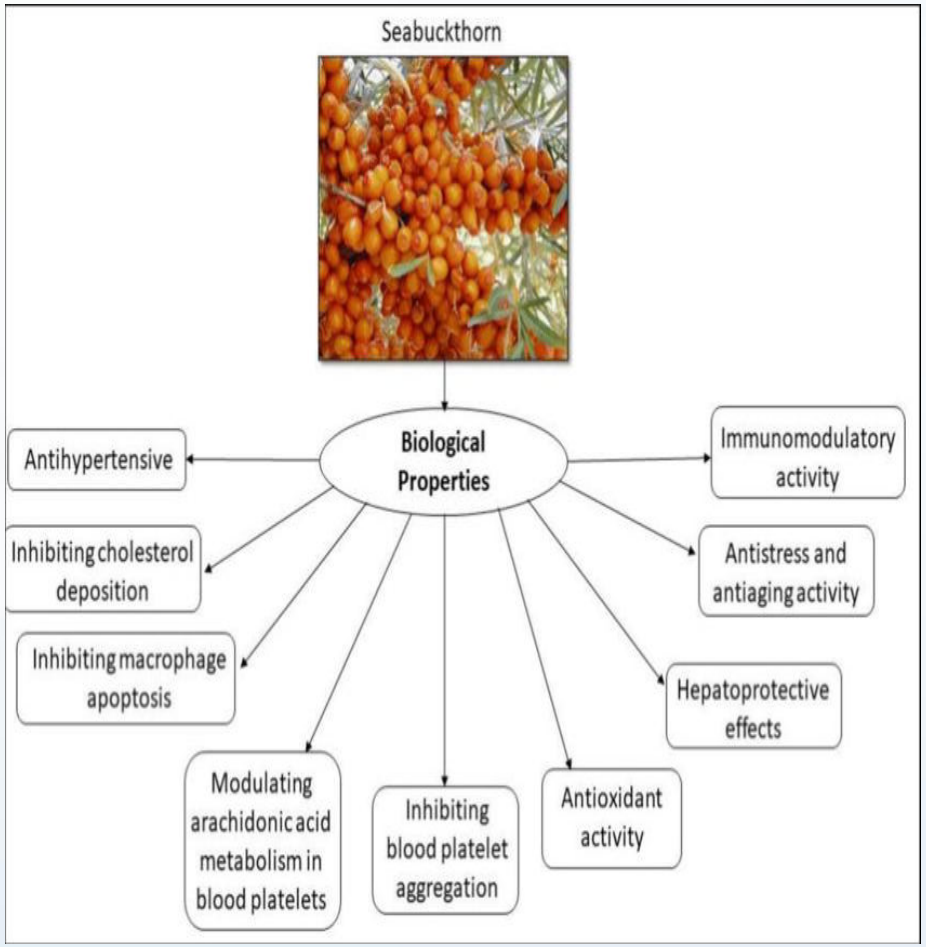
Outline

1. Background
2. Extraction, purification, identification
3. Effects on aging
4. Relevant mechanisms
5. Conclusions and outlook

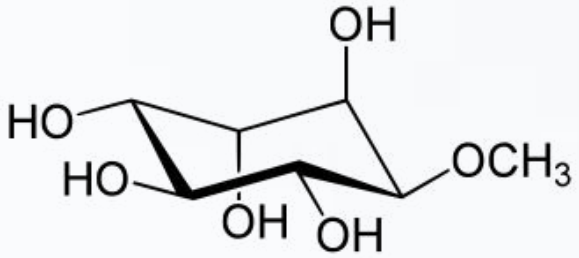
Bioactive compounds in sea buckthorn

- ✓ Sea buckthorn contains many types of nutritious and bioactive compounds, such as polyphenols (especially flavonoids), ascorbic acid, vitamins, carotenoids, etc.
- ✓ These active ingredients have a variety of biological functions, such as antioxidant, anti-tumor, anti-inflammatory, anti-aging and so on
- ✓ L-quebrachitol (QBC) in sea buckthorn was first discovered by researchers in Uzbekistan in 1976, recognized by Prof. Yang Baoru as having the highest content in Chinese sea buckthorn (*Hippophae rhamnoides* subsp. *sinensis* Rousi)



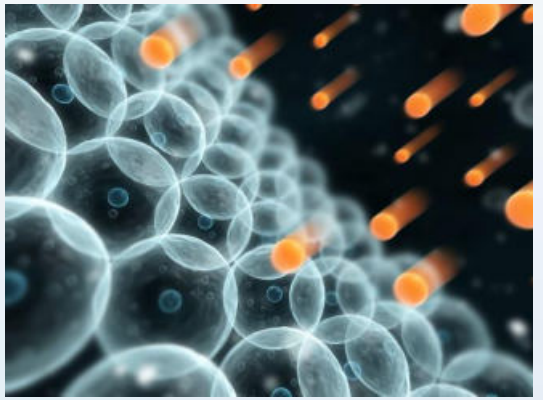
L-quebrachitol

- ✓ L-quebrachitol (QBC) is a derivative of inositol, scientifically known as 2-O-methyl-L-inositol

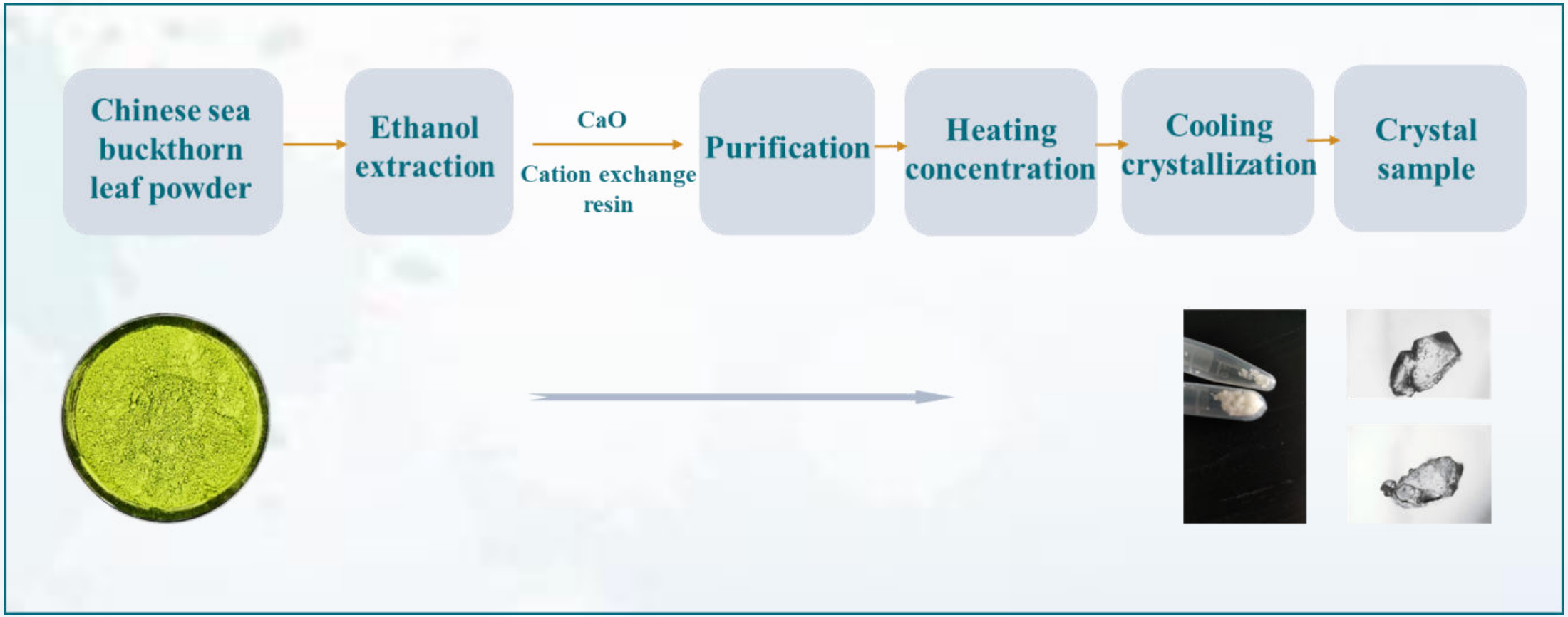


- ✓ Large variations in content between organs in Chinese sea buckthorn, with the highest content in leaves (59.73 mg/g DW), followed by pulp (2.19 mg/g FW) and seeds (0.39 mg/g FW)

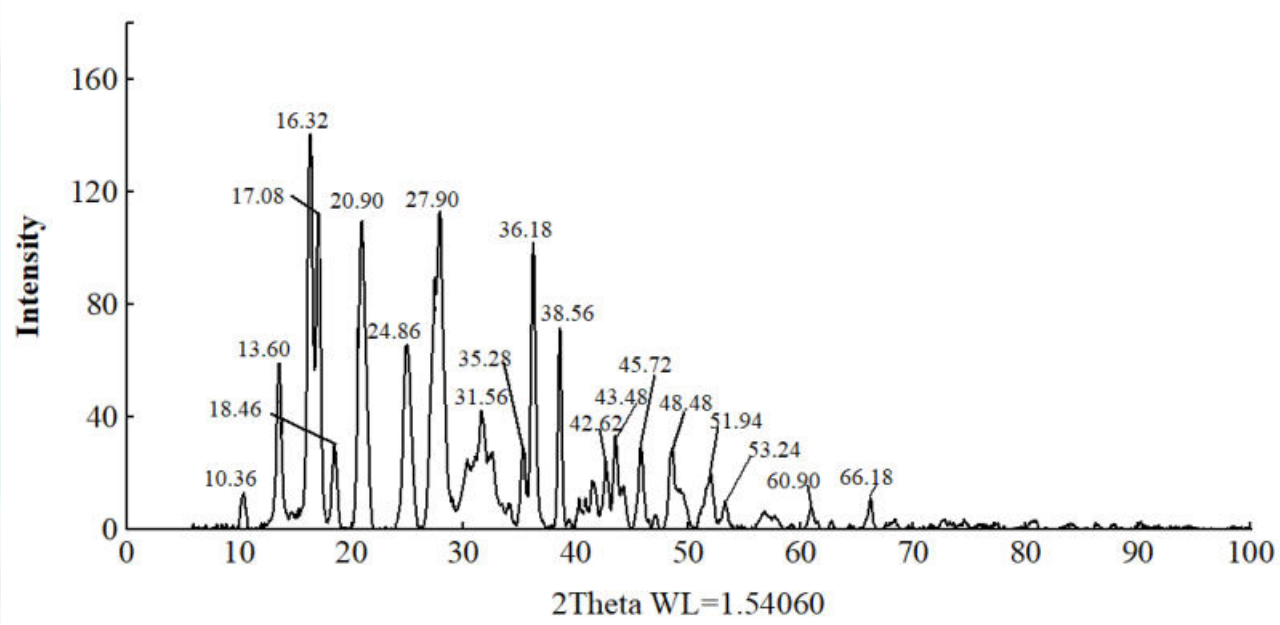
- ✓ QBC can treat diabetes by maintaining blood sugar balance, scavenging reactive oxygen species and fighting acute gastric lesions. However, its anti-aging effects and mechanisms have not been reported



QBC extraction from Chinese sea buckthorn

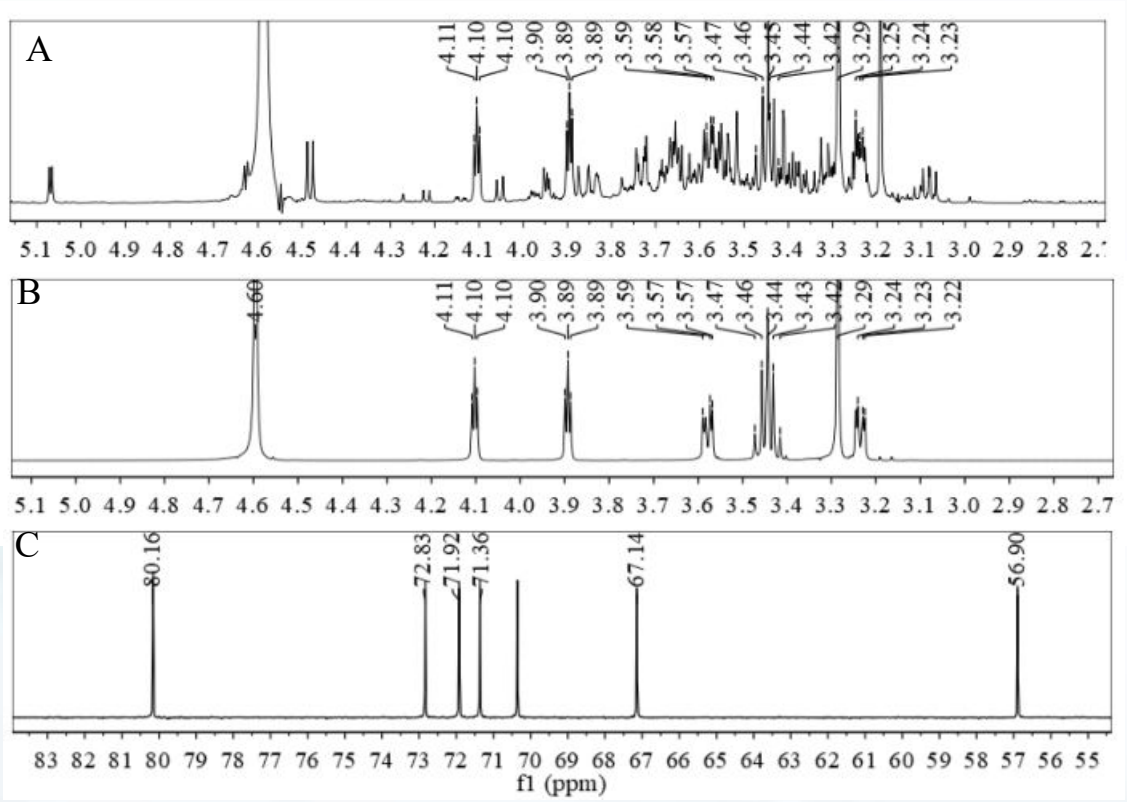


XRD analysis of crystal samples



- ✓ X-ray diffraction (XRD) testing is used to determine the physical phase and crystal structure of the material
- ✓ XRD analysis indicated that the angles of the strongest diffraction peaks were 16.32◦, 20.90◦, 27.90◦, 36.18◦, 17.08◦ and 38.56◦, confirming that the samples have a crystal structure

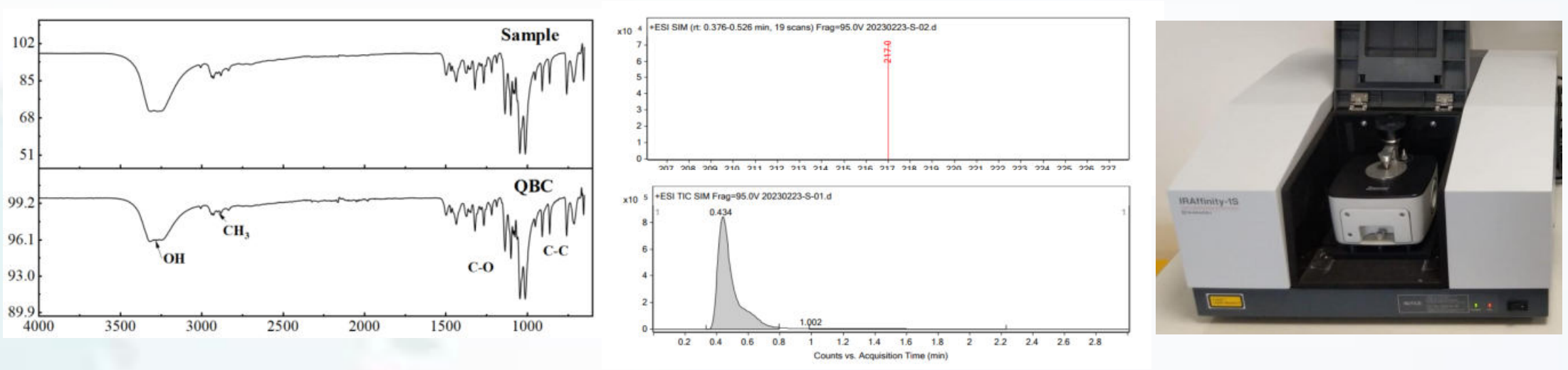
NMR Analysis



A: Methanol precipitate ^{1}H-NMR spectrum; B: Crystal samples ^{1}H-NMR spectrum; C: ^{13}C-NMR spectrum

- ✓ Nuclear magnetic resonance (NMR) scans are used to determine the structure and type of substances
- ✓ NMR analysis showed that the spectral data of the crystalline samples were the same as the spectral data of stardard QBC

FTIR and HPLC-MS analysis

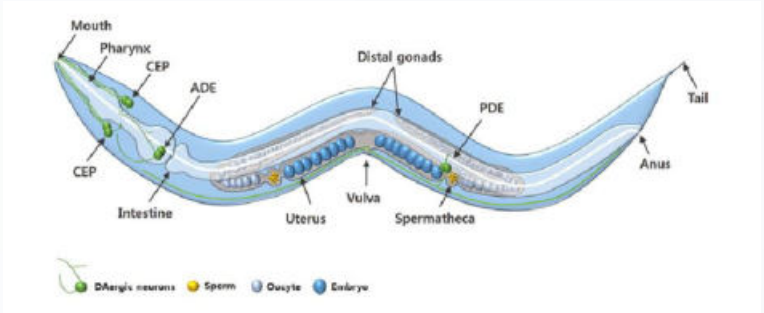


FTIR spectra, HPLC-MS mass spectra of crystalline samples and molecular formula of QBG

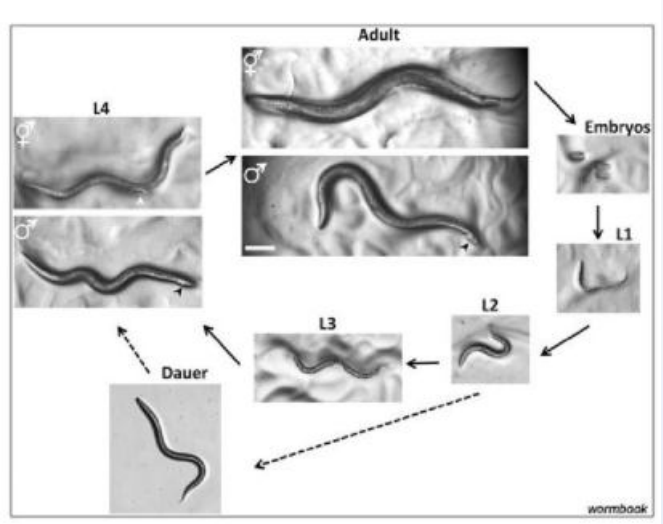
- ✓ Fourier Transform Infrared Spectroscopy (FTIR) is used to identify functional groups and deduce the structure of ingredients; HPLC-MS is used to determine relative molecular mass and molecular formula
- ✓ FTIR analysis showed that the main peaks appeared at 3291, 2910, 1439, 1325, 1139, 1042, 910, 756cm^{-1}, which were identical to the characteristic peaks of the control product of QBC
- ✓ HPLC-MS analysis gives a *m/z* 217. 0［M+Na］$^{+}$quasi-molecular ion peak with a crystal relative molecular mass of 194. 0
- ✓ The purity of the samples was calculated to be 90.20-94.15% by external standard quantification

Nematode (*Caenorhabditis elegans*)

- ✓ A model of choice for aging research
- ✓ Due to the short life cycle, a large number of synchronized nematodes can be obtained
- ✓ Genetic signaling pathways associated with aging have been identified, including the insulin/IGF-1 signaling pathway, the AMPK signaling pathway, the target of rapamycin signaling pathway, caloric restriction and mitochondrial function

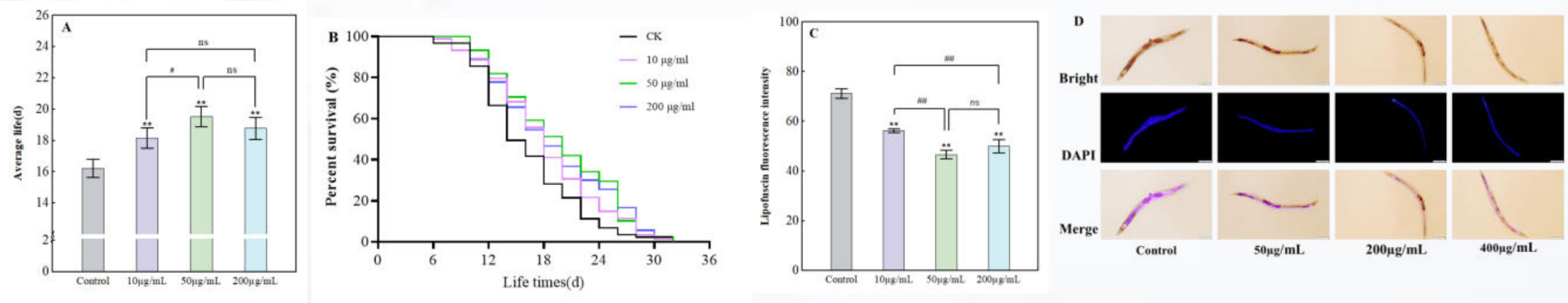


C. elegans anatomy



The growth cycle of *C. elegans*

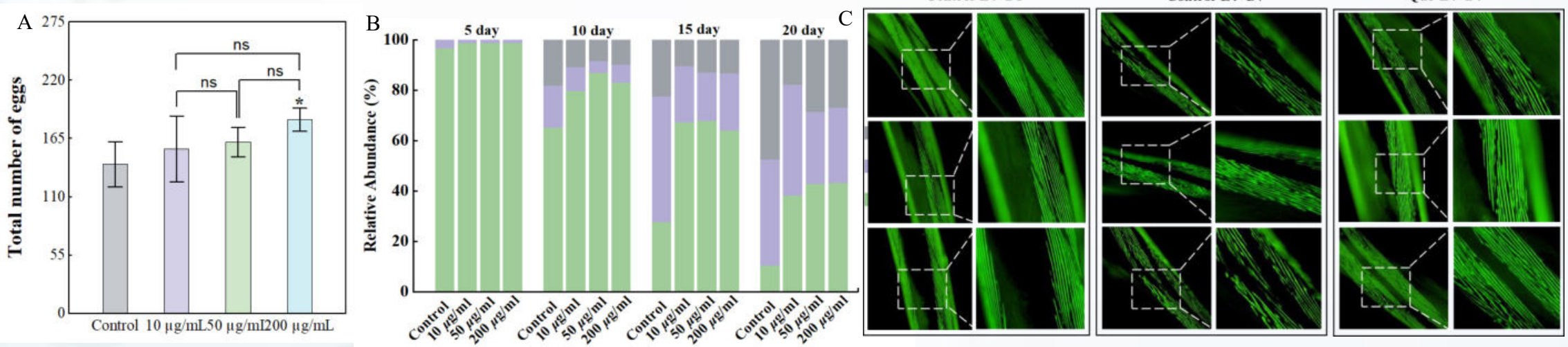
QBC prolongs the life of nematodes



A and B: Mean life span of nematodes in different treatment groups;
C: Quantification of lipofuscin fluorescence intensity in nematodes; D: Comparative fluorescence of lipofuscin in nematodes (200μm)

- ✓ Lipofuscin is an oxidative by-product of lysosomal degradation, and its accumulation is positively correlated with aging
- ✓ QBC significantly prolongs nematode life and reduces lipofuscin accumulation

QBC improves the health parameters of the organism



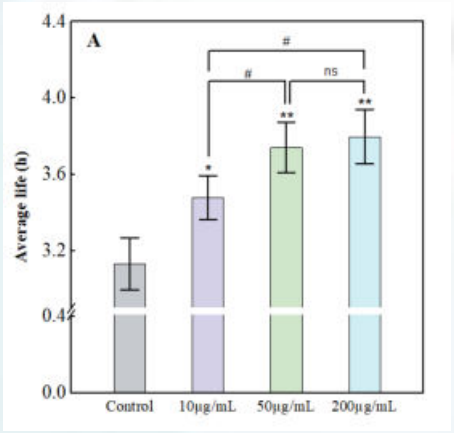
Total number of eggs

Nematode motility

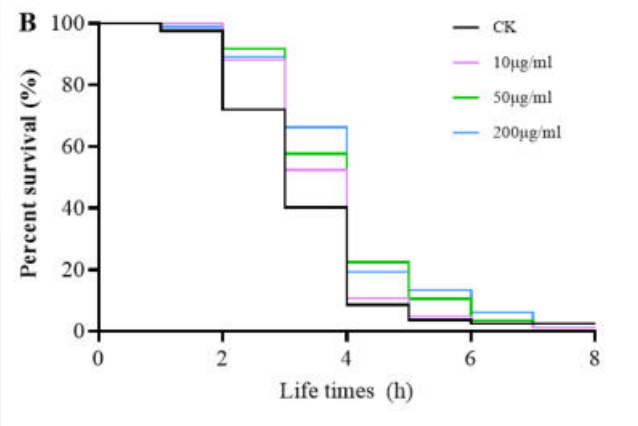
Muscle tissue status

- ✓ Low concentrations of QBC had no effect on nematode egg production, while high concentrations significantly enhanced nematode reproduction
- ✓ QBC improves nematode motility and significantly protects against nematode muscle morphology defects
- ✓ 50 μg/mL is the optimal concentration of QBC for anti-aging

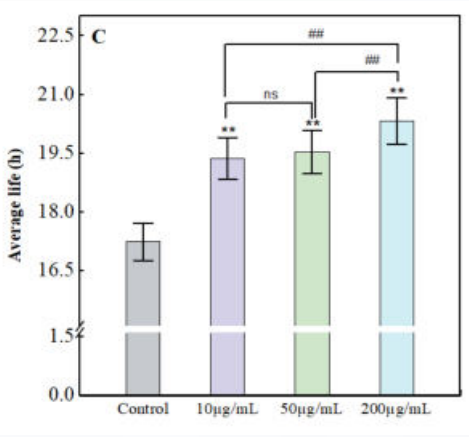
QGC improves nematode resistance to stress



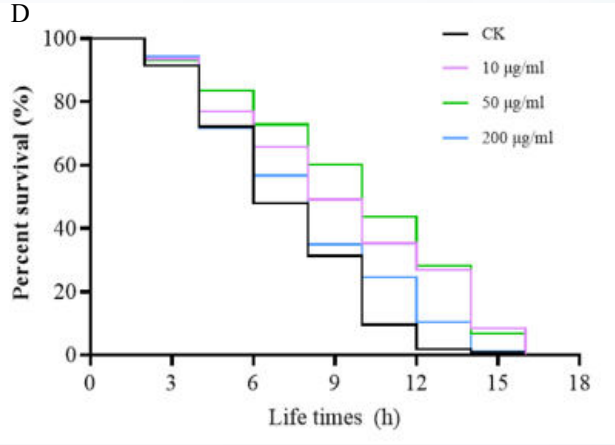
Average nematode lifespan under oxidative stress



Life curves of nematodes under oxidative stress



Average life span of nematodes under stress

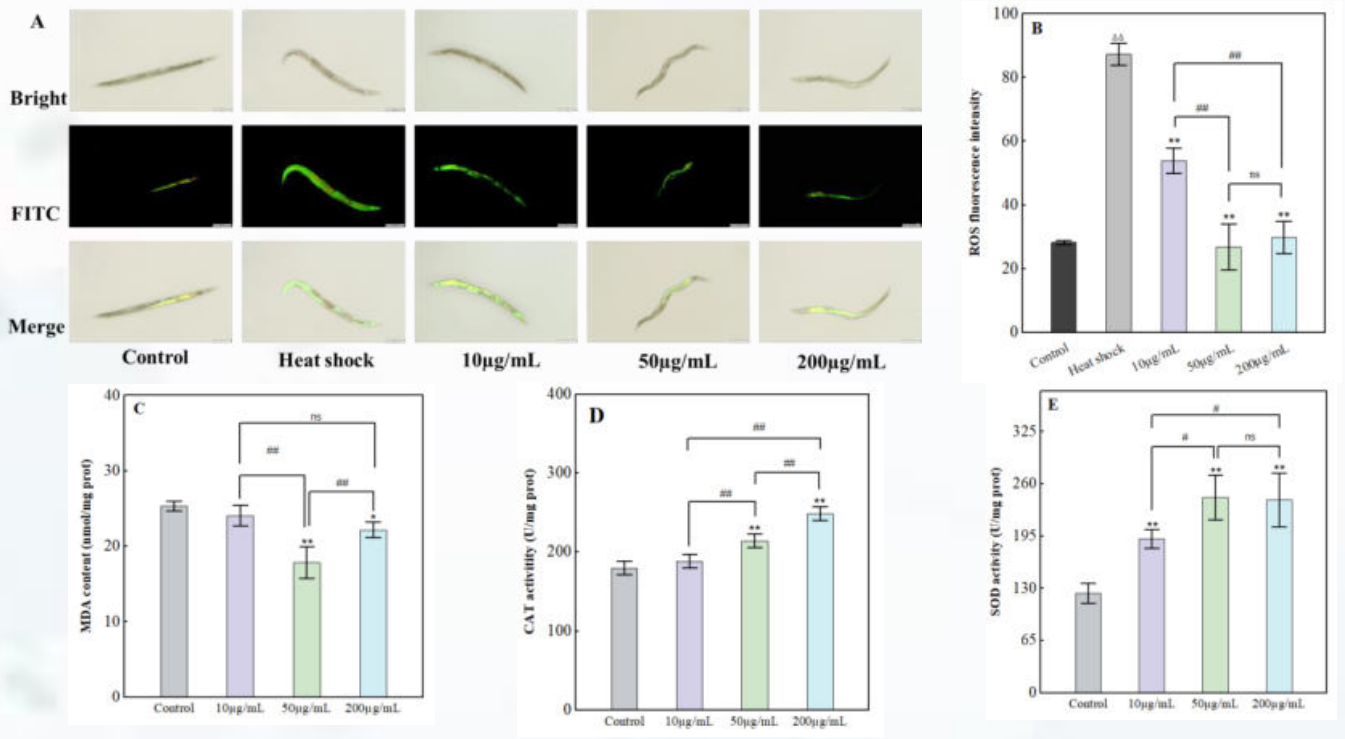


Life curves of nematodes under heat stress

✓Stress capacity in response to specific environmental stimuli is often used to characterize health in aging

✓QBC effectively improves nematode resistance to H_2O_2-induced oxidative stress and 36 oC heat stress

QBC improves antioxidant capacity in nematodes

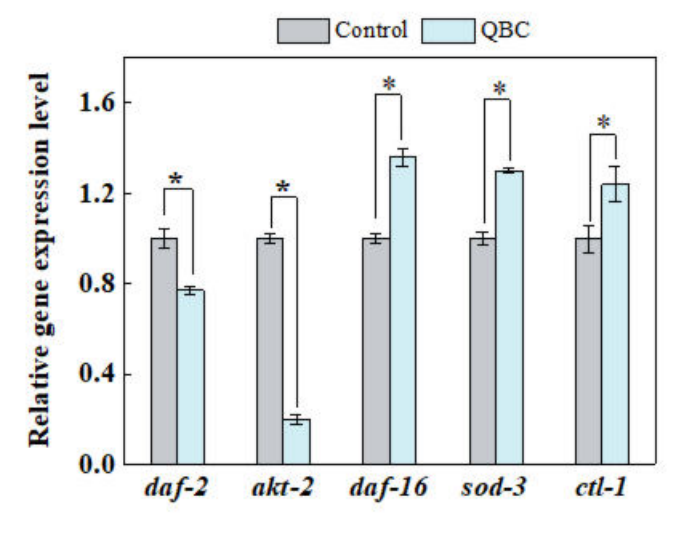


A: Comparison of fluorescence levels of ROS in nematodes (200 μm) (200μm); B: Quantification of ROS fluorescence intensity in nematodes; C: MDA levels; D: CAT activity; E: SOD activity

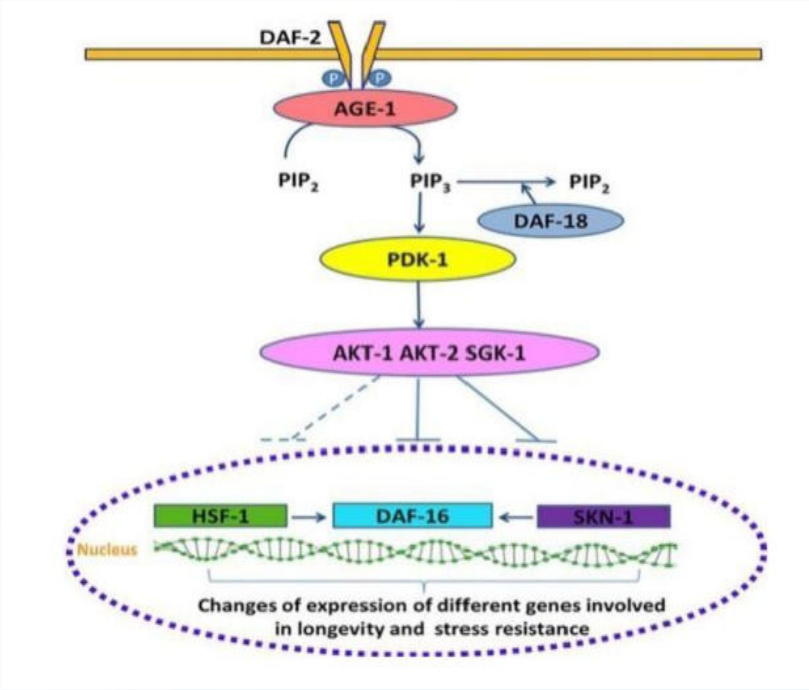
✓QBC increases catalase (CAT) and superoxide dismutase (SOD) activities in nematodes under stress conditions

✓QBC effectively reduces reactive oxygen species (ROS) and malondialdehyde (MDA) levels in nematodes

QBC modulates insulin signaling pathway



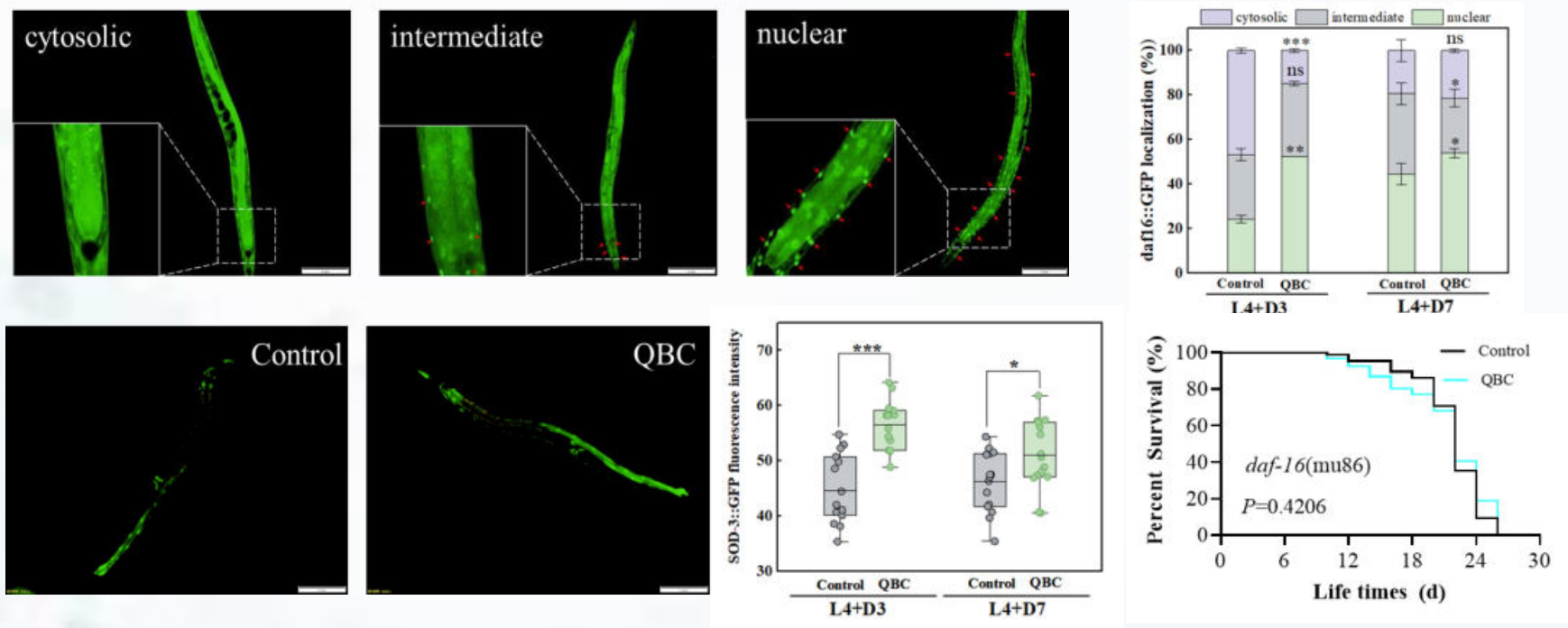
Effect of QBC on gene expression in the IIS pathway



IIS pathway

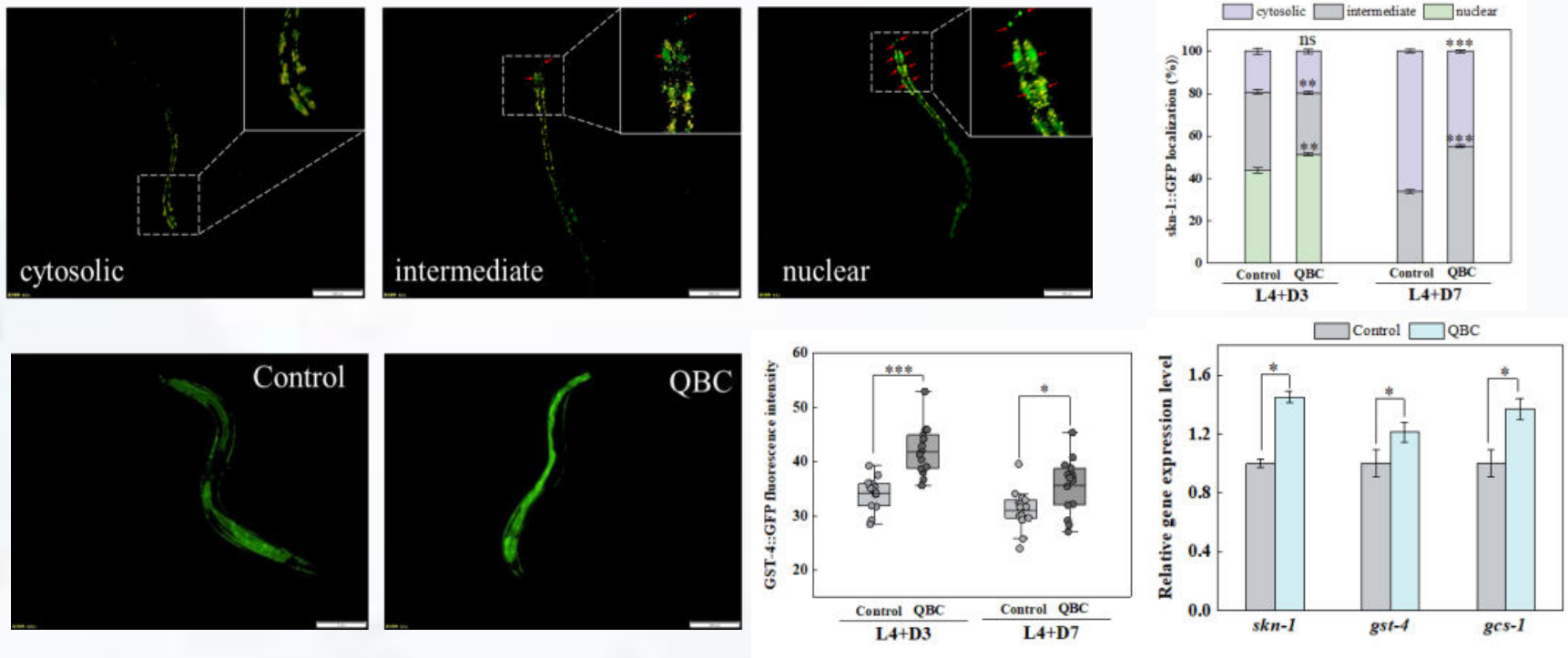
- ✓ Insulin/IGF-1 signaling pathway (II) plays an important role in anti-aging
- ✓ DAF-16, SKN-1 and HSF-1 are three important transcription factors in the IIS signaling pathway
- ✓ QBC down-regulated the expression of *daf-2* and *akt-2* and up-regulated the expression of *daf-16* and its target genes *sod-3* and *ctl-1*, suggesting that the IIS pathway is involved in the anti-aging process

QBC activates transcription factor DAF-16 for IIS pathway



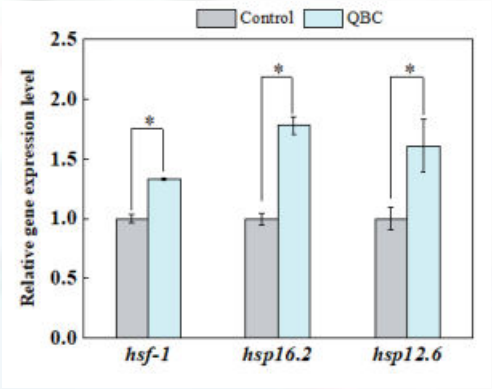
- ✓ QBC activates DAF-16, promotes its nuclear translocation and upregulates the expression of downstream genes sod-3 (SOD gene) and ctl-1 (CAT gene)
- ✓ The absence of DAF-16 prevented the lifespan-extending effect of QBC on the nematode, suggesting that the anti-aging activity dependent on the DAF-16

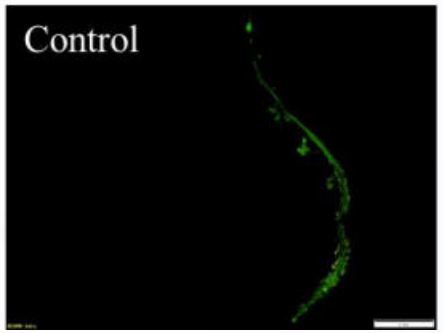
QBC activates transcription factor SKN-1 for IIS pathway

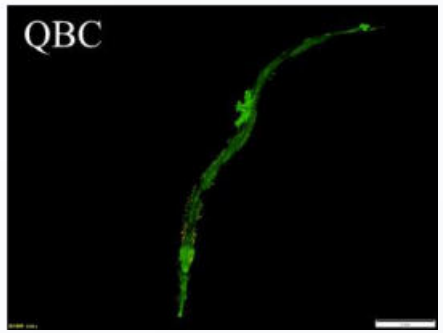


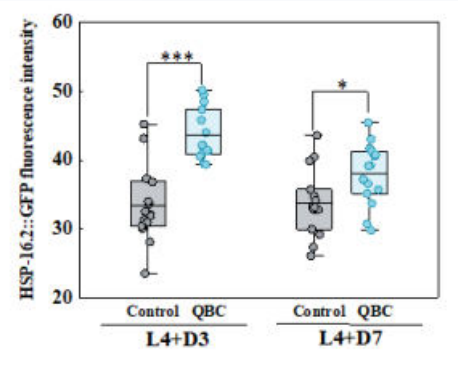
- ✓ QBC activated SKN-1, promoted its nuclear translocation and upregulated the expression of downstream genes *gst-4* (glutathione S-transferase gene) and *gsc-1* (γ-glutamylcysteine synthetase gene)
- ✓ This result was verified using CL2166 transgenic nematodes (GST-4::GFP), where treatment upregulated the expression level of GST-4 protein

QBC activates transcription factor HSF-1 for IIS pathway



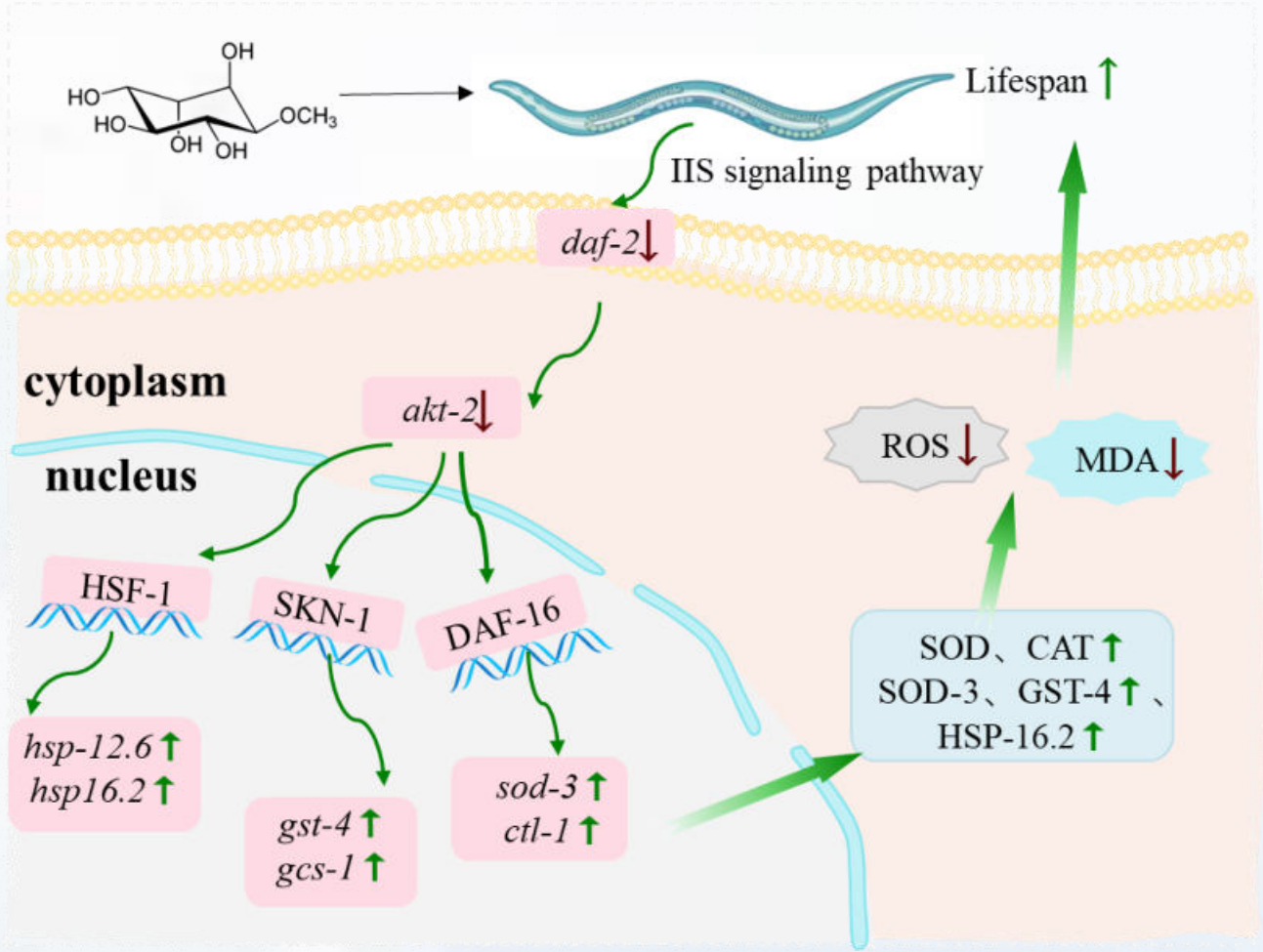




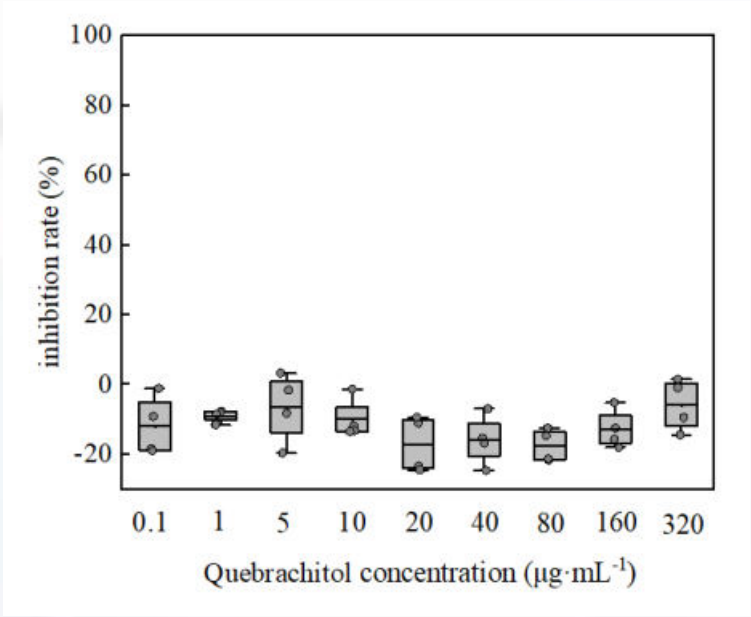


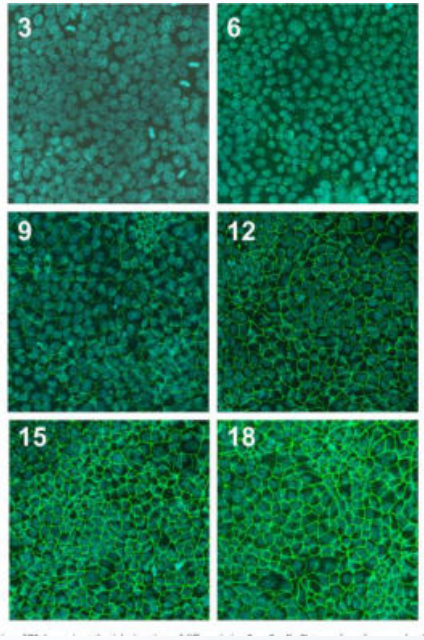
- ✓ QBC upregulated the expression of *hsf-1* and its downstream genes *hsp16.2* (small heat shock protein gene) and hsp*12.6*
- ✓ QBC upregulated the expression of HSP-16.2::GFP protein

QBC's potential lifespan-prolonging mechanism



QBC safety assessment





Effect of QBC on the inhibition rate of Caco-2 cells

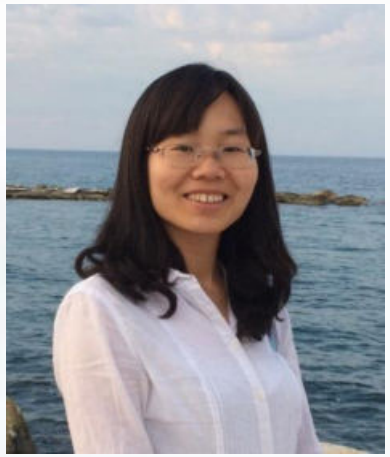
- ✓ Caco-2 cell model as an *in vitro* method for assessing drug toxicity
- ✓ The cell inhibition rate did not fluctuate much when the treatment concentration was 0.1-320 μg/mL, indicating that QBC did not show toxicity to Caco-2 cells

Conclusions and outlook

- ✓ Sea buckthorn leaf is a good source of natural QBC, and sea buckthorn leaf powder can be extracted, separated and purified to provide high purity QBC crystals
- ✓ QBC in sea buckthorn leaves significantly prolongs the average and maximum lifespan of nematodes, improves several health indicators during the ageing process, and is non-toxic to Caco-2 cells
- ✓ QBC promotes longevity in nematodes mainly by increasing antioxidant capacity and modulating the insulin signaling pathway
- ✓ To further conduct clinical trials to validate and develop related products

Acknowledgement

Dr. Zhao Jinmei



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