

1 **Electronic supplementary information (ESI)**

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3 **Healthy lifespan extension mediated by Oenothrin B isolated from *Eucalyptus***

4 ***grandis* × *Eucalyptus urophylla* GL9 in *Caenorhabditis elegans***

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9 **1. The properties of the mutants used in the study were provided as followed:**

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11 (1) The *daf-16(mgDf50)* null mutant strain lacks the fork- head family transcription

12 factor that is regulated by the IIS pathway;

13 (2) Long-lived *age-1(hx546)* mutant is deficient in PI-3 kinase signaling which is

14 regulated by the IIS pathway;

15 (3) CF1553 {muIs84 [pAD76 (*sod-3::gfp*)]} is the transgenic GFP reporter strain

16 which can reflect the level of *sod-3* encodes a manganese superoxide dismutase in *C.*

17 *elegans*;

18 (4) Mutant *eat-2(ad1116)* is a long-lived strain and a genetic model for dietary

19 restriction due to decreased pharyngeal pumping;

20 (5) Mutant *sir-2.1(ok434)* lacks the gene encodes a histone deacetylase-like protein

21 that integrates metabolic situation with lifespan;

22 (6) *Mev-1(kn1)* mutant is the strain having defect in the succinate dehydrogenase

23 cytochrome b large subunit in the complex II of the mitochondrial electron transport

24 chain;

25 (7) The mutant *isp-1(qm150)* has low oxygen consumption, insensitivity to ROS, and  
26 long longevity. The *isp-1* gene encodes a component of mitochondrial complex III,  
27 which accepts electrons and protons from mitochondrial complexes I and II;

28 (8) The long-lived *clk-1(qm30)* mutant is deficient in the endogenous form of  
29 coenzyme Q<sub>10</sub>, which is responsible for carrying electrons and protons from  
30 mitochondrial complexes I and II to complex III.

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## 32 **2. Supplementary Figure Captions**

33 **Fig. S1:** Reversed Phase-HPLC chromatogram of OEB. HPLC was performed by a  
34 Shimadzu LC-10AT HPLC system with an ultraviolet detector (SPD-10A Detector)  
35 (Shimadzu, Kyoto, Japan). The analytical conditions were as follows: column,  
36 Diamonsil reversed-phase columns C18 (250 × 4.6 mm, 5 μm) (Dikma Technologies  
37 Inc. Beijing, China); Mobile phase, solvent A was 0.2% formic acid and solvent B was  
38 methanol (MeOH) (0-60 min, 10%-90% B in A); Flow rate, 1.0 mL/min; Detection  
39 wavelength, 270 nm.

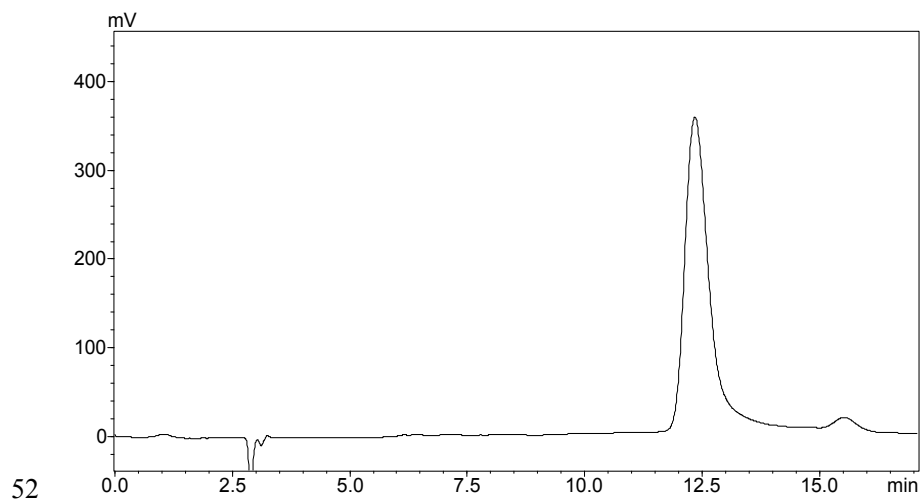
40 **Fig. S2:** <sup>1</sup>H-NMR of Oenothlein B. The compound was identified by <sup>1</sup>H spectroscopy.  
41 And the <sup>1</sup>H spectra was carried out on Varian INOVA instruments (600MHz) in  
42 CD<sub>3</sub>OD.

43 **Fig. S3:** High-resolution mass spectrometer of OEB (HRMS) (ESI, negative mode)  
44 (A) MS<sub>1</sub>; (B) MS<sub>2</sub>, and (C) MS<sub>3</sub>.

45 **Fig. S4:** The growth curves of *Escherichia coli* strain OP50 incubated with or without  
46 160 μM OEB.

47 **Fig. S5:** Effects of OEB on ROS scavenging in wild-type worms. Worms were  
48 cultured with or without different concentrations of OEB at 20 °C for 4 days. Different  
49 letters above the column denote significant differences ( $p < 0.05$ ).

50 **Fig. S6:** Colonization of OP50 in *C. elegans* intestine. The results represent average  
51 Log colony-forming units (CFU) from 10 worms of three independent experiments.

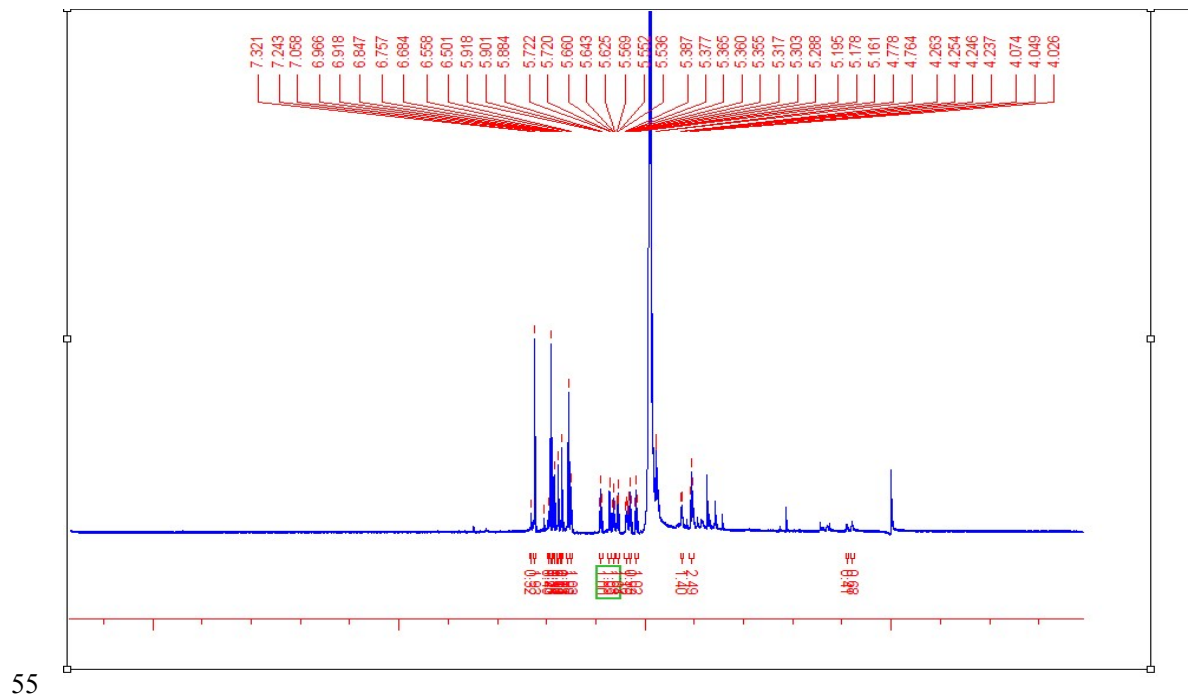


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**Fig. S1**

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55  
56 **Fig. S2**

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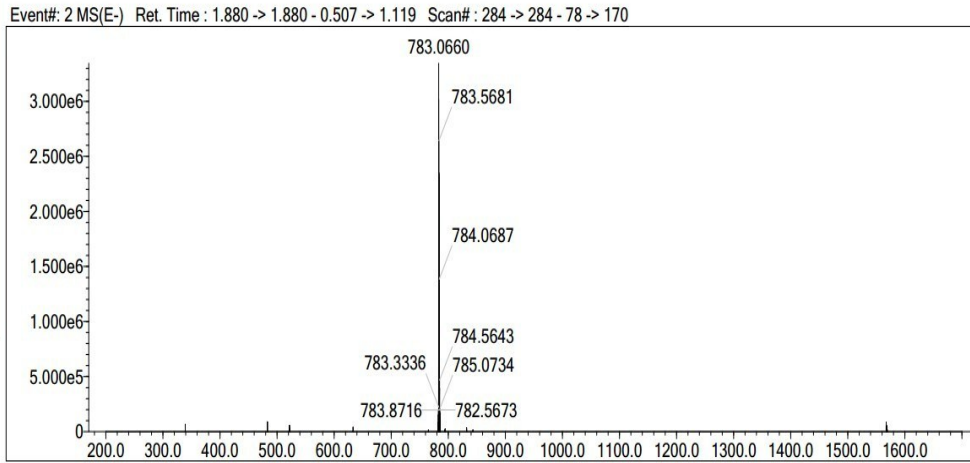
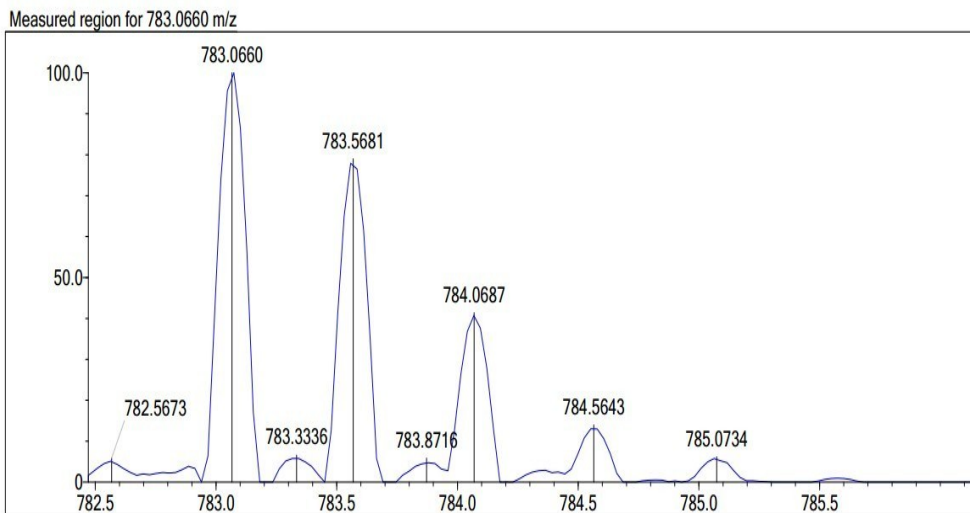
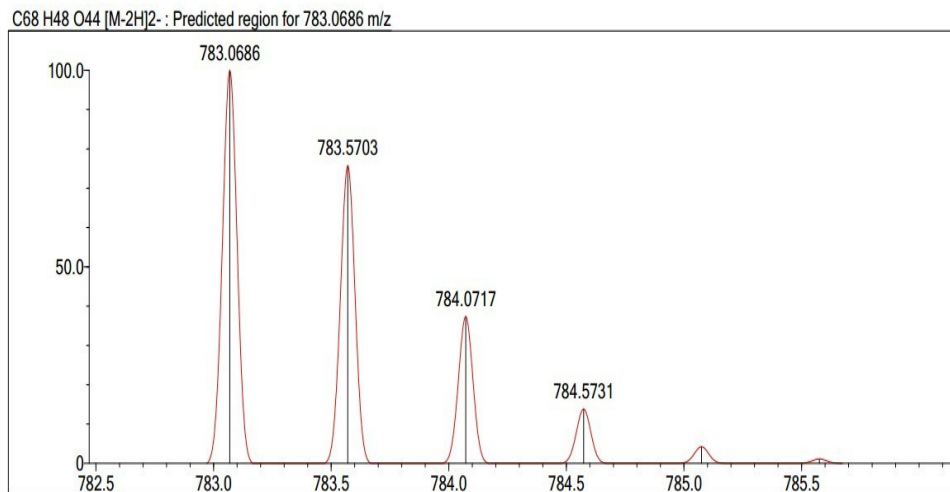
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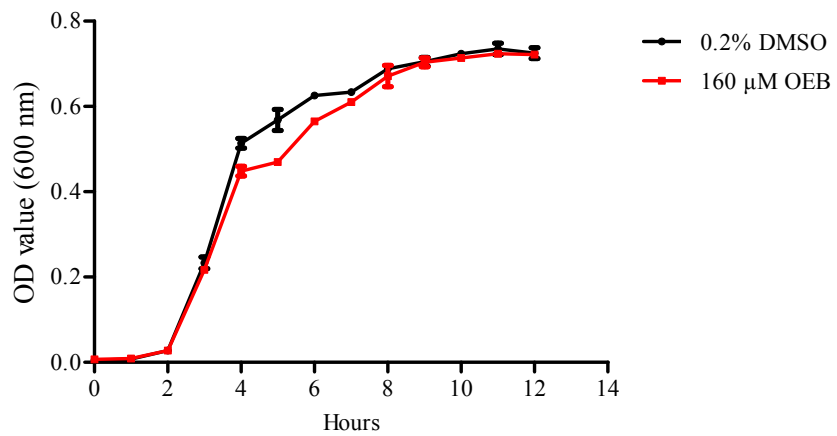
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**A****B****C**

| Rank | Score | Formula (M) | Ion      | Meas. m/z | Pred. m/z | Df. (mDa) | Df. (ppm) | Iso    | DBE  |
|------|-------|-------------|----------|-----------|-----------|-----------|-----------|--------|------|
| 1    | 94.20 | C68 H48 O44 | [M-2H]2- | 783.0660  | 783.0686  | -2.6      | -3.32     | 100.00 | 45.0 |

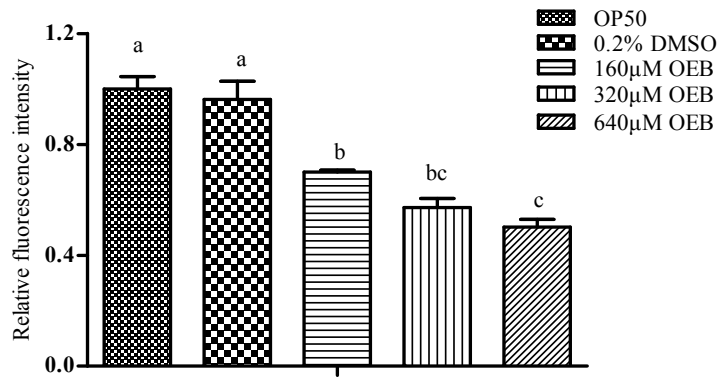
65 **Fig. S3**



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67 **Fig. S4**

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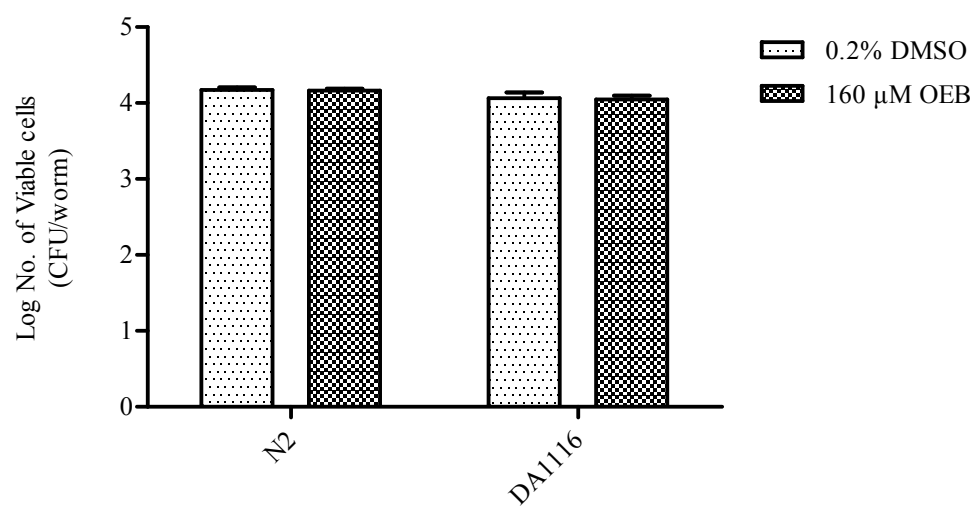


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70 **Fig. S5**

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73 **Fig. S6**

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