Supplementary Materials: An Ecotoxicological Evaluation of Four Fungal Metabolites with Potential Application as Biocides for the Conservation of Cultural Heritage

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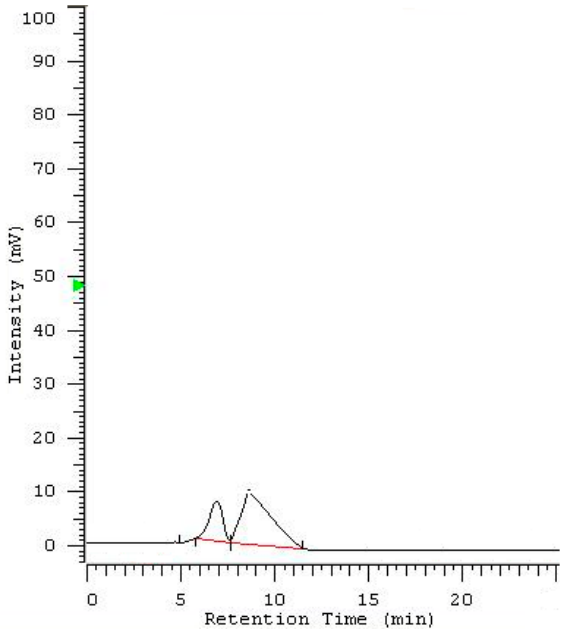

Figure S1. Chromatographic profile of culture medium (ISO medium 2012) 1 μg/mL at 215 nm.

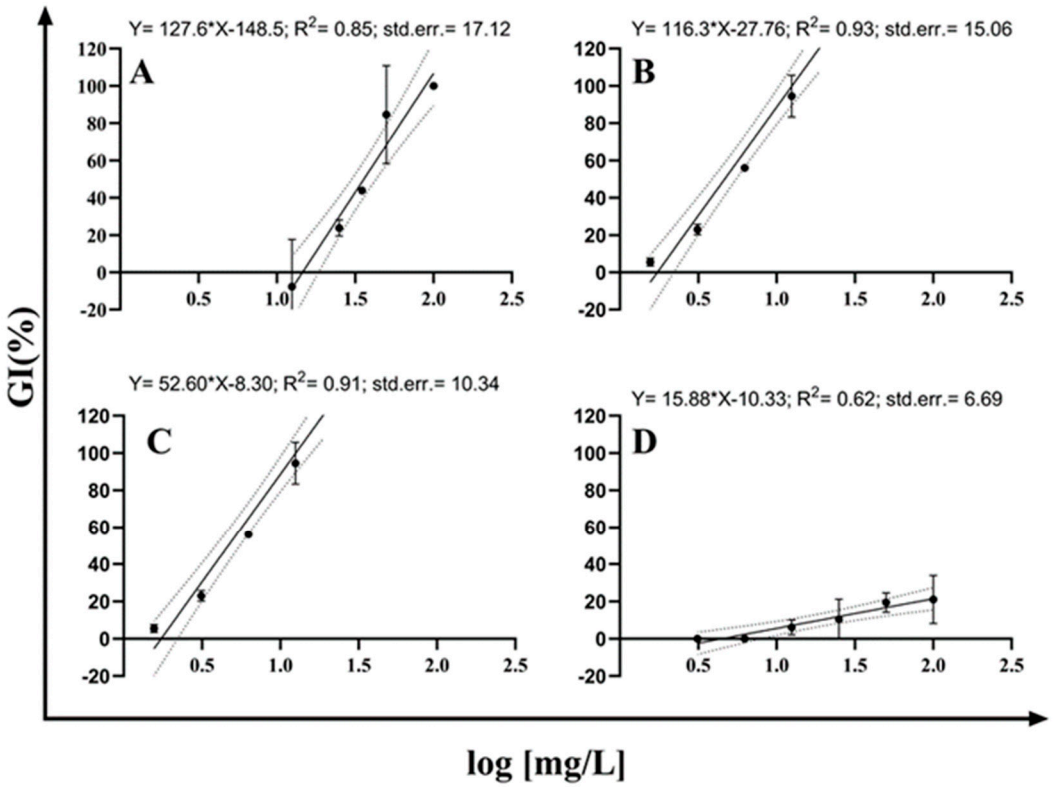

Figure S2. Concentration–response relationship of **1** (A), **2** (B), **3** (C), and **4** (D) exposed to *R. subcapitata*; concentrations in the x-axis are expressed as mg/L: GI = growth inhibition. Limits of the 95% confidence intervals are indicated by the dashed line, fitted log-linear model is indicated by the solid line.

In Figure S2, the results of the GI(%) of *R. subcapitata* are reported for **1**-**4** (Figure S2 A-D). No significant effects were detected at the first lowest exposure concentrations for **1**, **2**, and **3**, and all the concentrations for **4**. Results of *R. subcapitata* showed that the relative toxicity order was **2** > **3**> **1**> **4**. For compound **1**, biostimulation effects were detected at the first lowest exposure concentration. Algae growth impairment occurred for compounds **2** and **3** between 3.125 and 25 mg/L (nominal concentrations), and for compound **1,** between 25 and 50 mg/L. For *R. subcapitata* exposed to **1**, all concentrations provided evidence of concentration–response correlation, and their effects ranged between −8% (12.5 mg/L) and 84% (50 mg/L). For **2**, a significant difference in the concentration–response curves can be observed in Figure S2B, and the maximum detected effect was 94%. For **4**, effects ranged between 6% (12.5 mg/L) and 21% (100 mg/L) (Figure S2C), while for **3**, varied between 21% (at 3.125 mg/L) and 90% (at 50 mg/L) (Figure S2D).

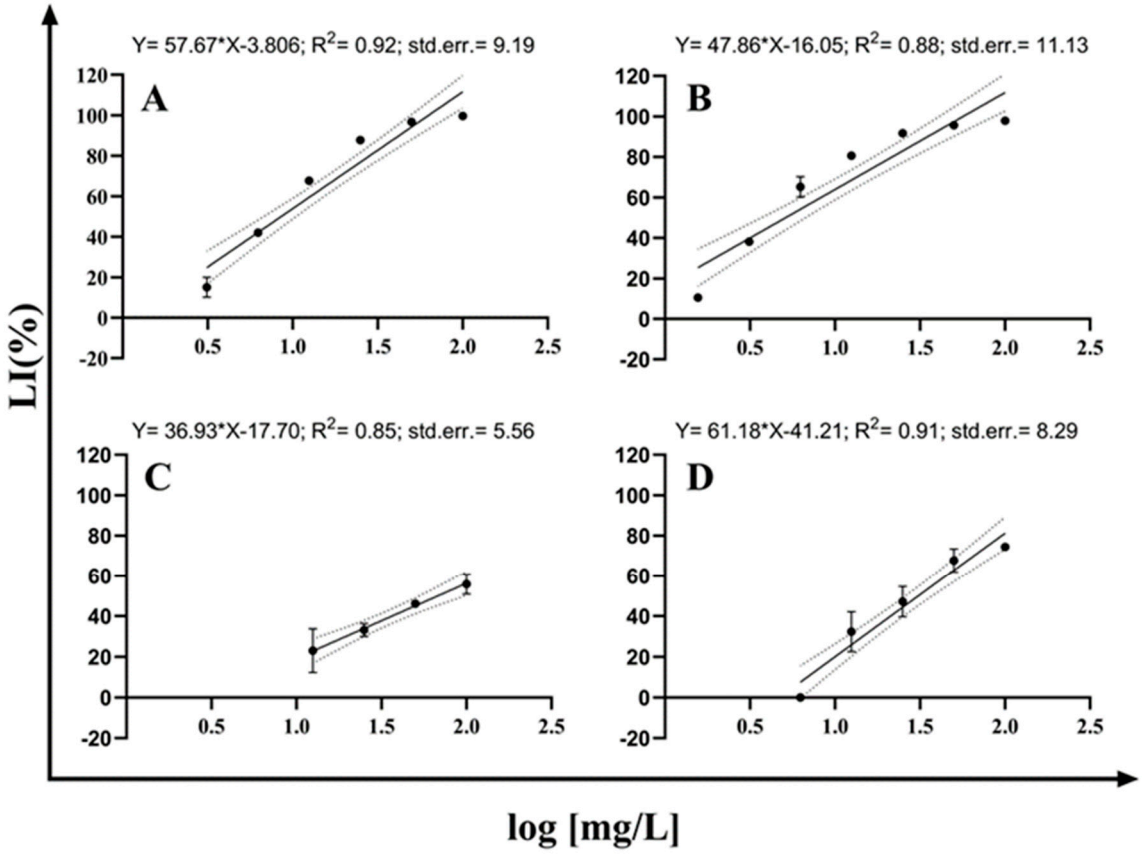

Figure S3. Concentration–response relationship of **1** (A), **2** (B), **3** (C) and **4** (D) exposed to *A. fischeri*; concentrations in the x-axis are expressed as mg/L: LI = luminescence inhibition. Limits of the 95% confidence intervals are indicated by the dashed line, fitted log-linear model is indicated by the solid line. Limits of the 95% confidence intervals are indicated by the dashed line, fitted log-linear model is indicated by the solid line.

In Figure S3, the results of the LI (%) of *A. fischeri* are reported for compounds **1**-**4** (Figure S3 A-D). As a general overview of the obtained results, LI always provided evidence of inhibitory effects at the two highest tested concentrations for the four investigated compounds. Considering the exposure of *A. fischeri* to **1** (Figure S3 A), from 12.5 mg/L to 100 mg/L concentrations, the luminescence was reduced up to 67%. At the two lowest concentrations, the inhibitory effects were considered not harmful (< 40% effect).

The luminescence of *A. fischeri* after *epi*-epoformin exposure significantly ($p < 0.05$) decreased—from 6.25 mg/L to 100 mg/L (Figure S3 B). Effects of **2** were not significantly different from those of the control at 3.12 mg/L. Among the battery of organisms selected for the test, *A. fischeri* was the only one that showed sensitivity towards this compound. The luminescence was reduced up to 75% in the considered concentration range (Figure S3 C). When **4** was tested at the highest concentrations (100 mg/L and 50 mg/L), it showed slightly adverse effects, ranging between 46% and 55%, while the two treatments at the lowest concentration had no effect (Figure S3 D).

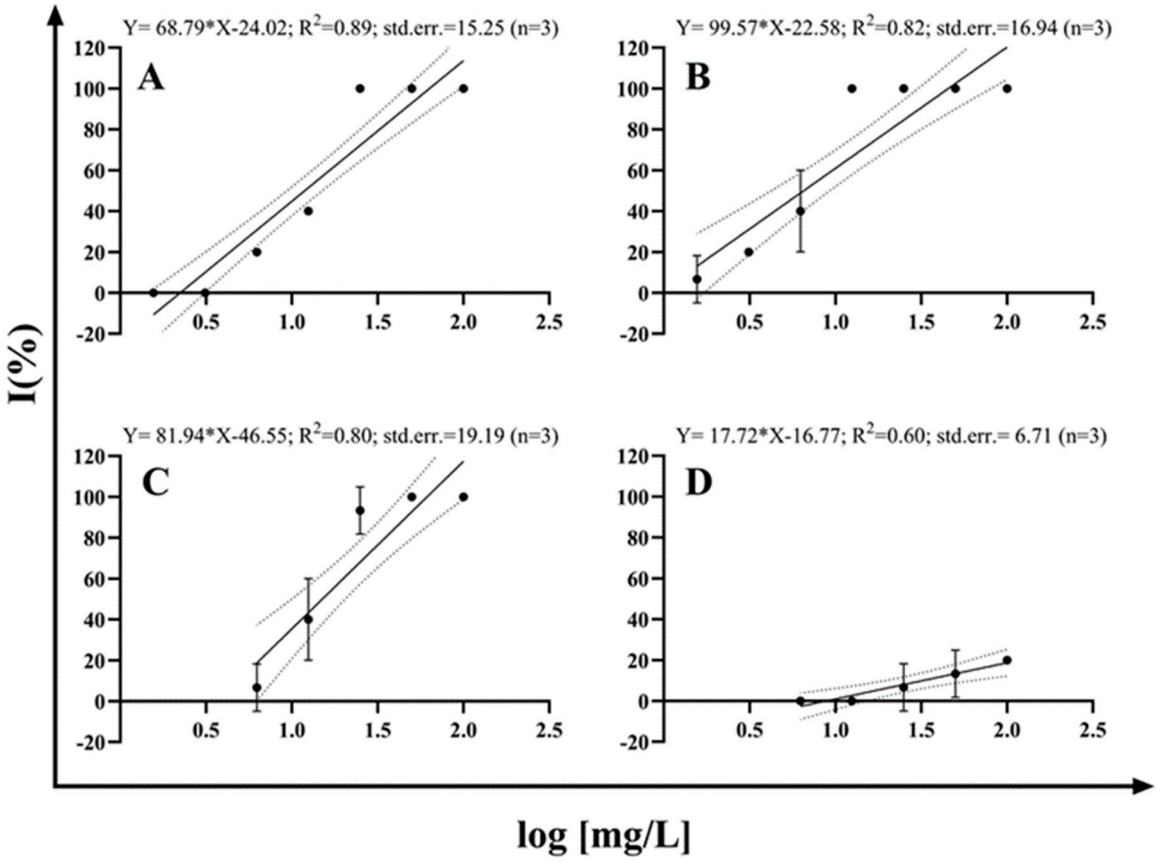

Figure S4. Concentration–response relationship of **1** (A), **2** (B), **3** (C) and **4** (D) exposed to *D. magna*; concentrations in the x-axis are expressed as mg/L: I = immobility. Limits of the 95% confidence intervals are indicated by the dashed line, fitted log-linear model is indicated by the solid line.

The results of the I (%) testing of **1**-**4** on of *D. magna* are reported in Figure S4 (A-D). No significant effects were detected at the first lowest exposure concentrations for **1**-**3**, nor at the other concentrations for **4**. Results with *D. magna* showed that the relative toxicity order was **1**>**2** > **3**> **4**. When daphnids were exposed to **1**, at all exposure concentrations provided evidence of a concentration–response, with significant toxic effect up to 40% at 12.5 mg/L. Indeed. The exposure to **2** showed effects ranged between 6.7% (1.25 mg/L) and 100% (12.5 mg/L). When *D. magna* was exposed to **3**, the effects varied between 6.7% and 100%. For **4**, no significant differences ($p < 0.001$) between concentration were highlighted.

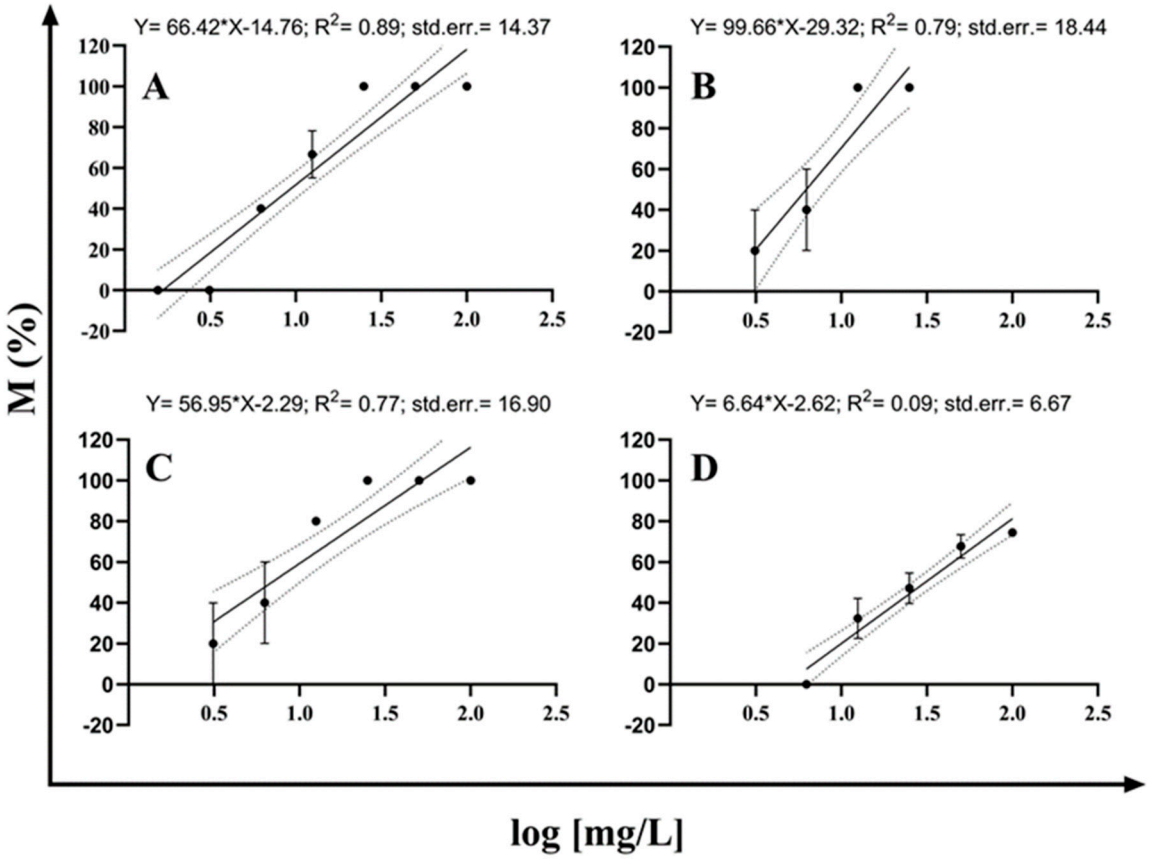

Figure S5. Concentration–response relationship of **1** (A), **2** (B), **3** (C), and **4** (D) exposed to *C. elegans*; concentrations in the x-axis are expressed as mg/L: M = mortality. Limits of the 95% confidence intervals are indicated by the dashed line, fitted log-linear model is indicated by the solid line.

The results about the M (%) of *C. elegans* are reported for **1**-**4** in Figure S5 (A-D). No significant effects were detected at any exposure concentration for **4**. The mortality of nematodes showed a similar toxicity trend for **1** and **3**, but with a higher toxicity level (80%) at 12.5 mg/L for **3**.

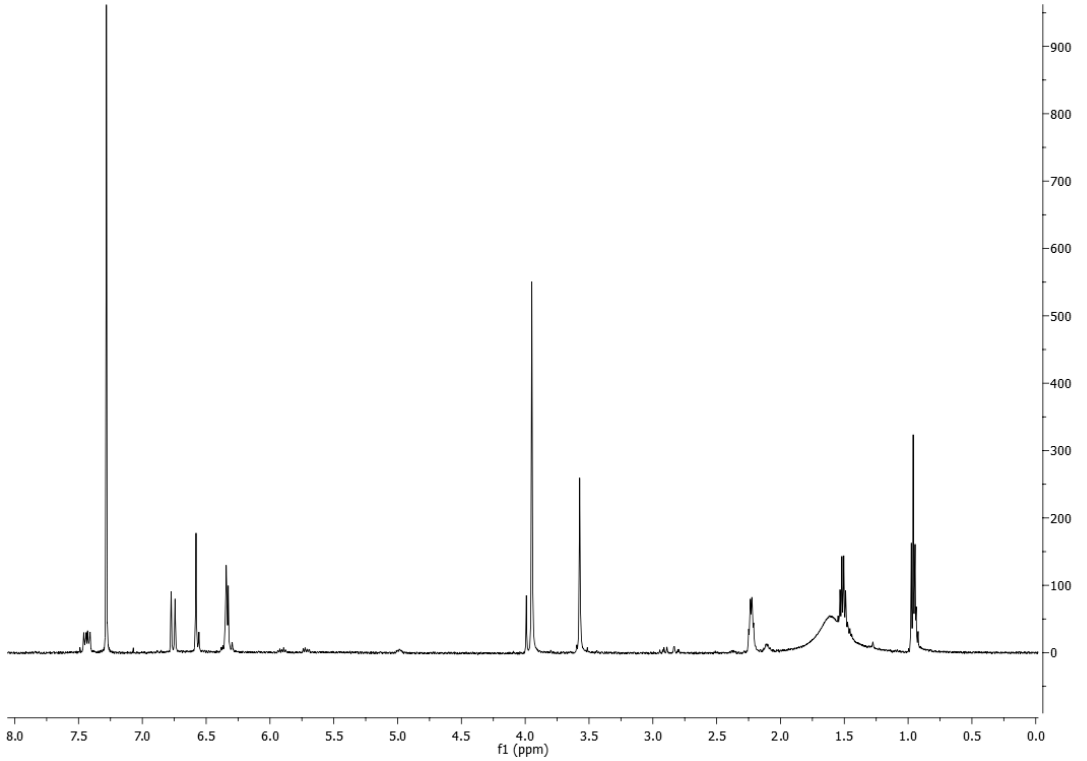

Figure S6. ^{1}H NMR spectrum of cavoxin, **1** ($CDCl_3$, 500 MHz).

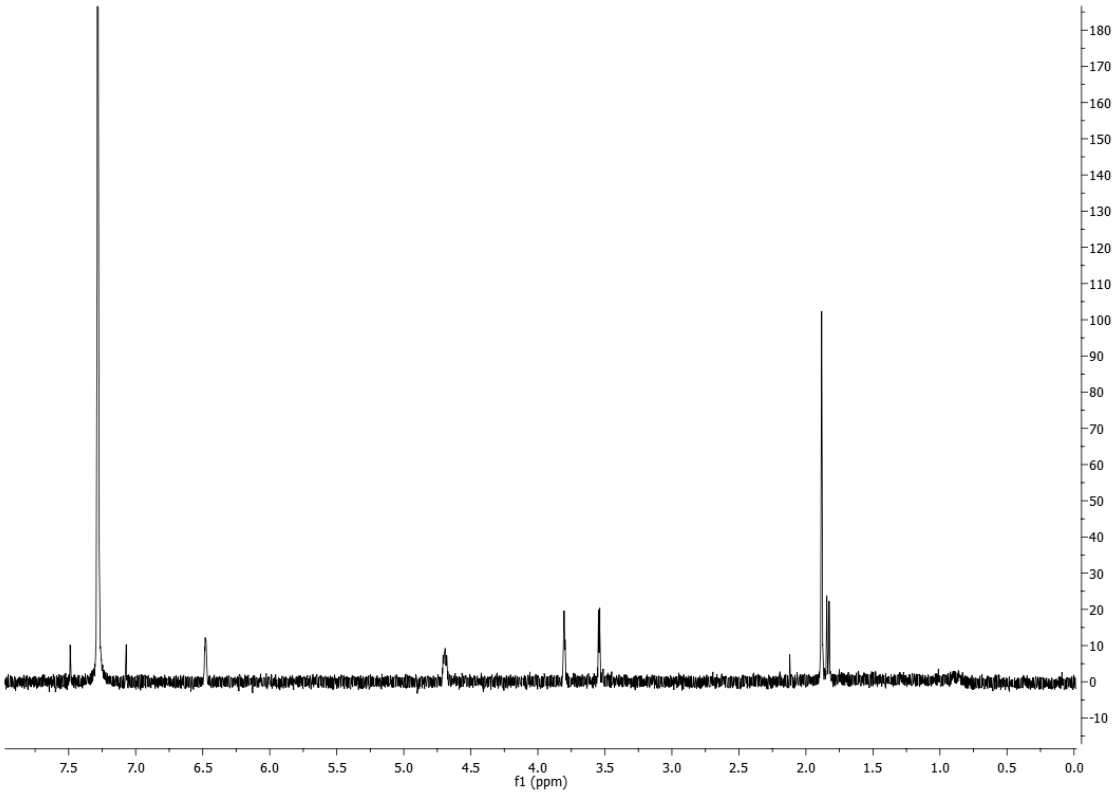

Figure S7. ^{1}H NMR spectrum of *epi*-epoformin, **2** ($CDCl_3$, 500 MHz).

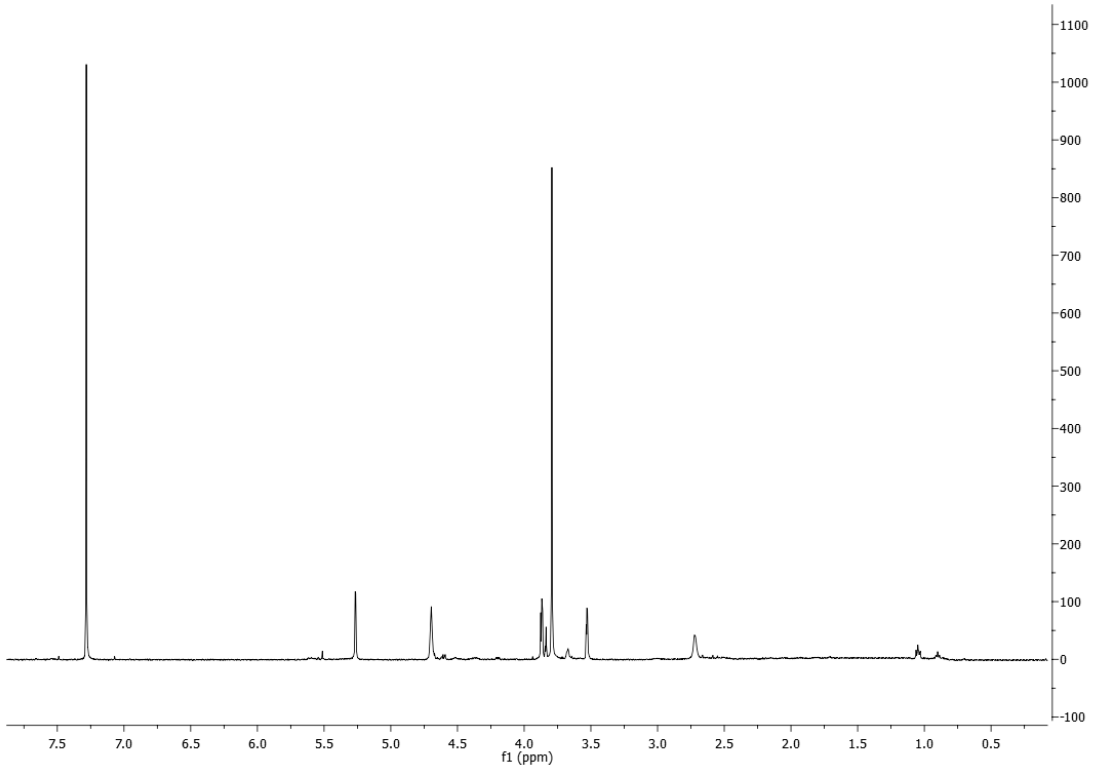

Figure S8. ^{1}H NMR spectrum of sphaeropsidone, **3** ($CDCl_3$, 500 MHz).

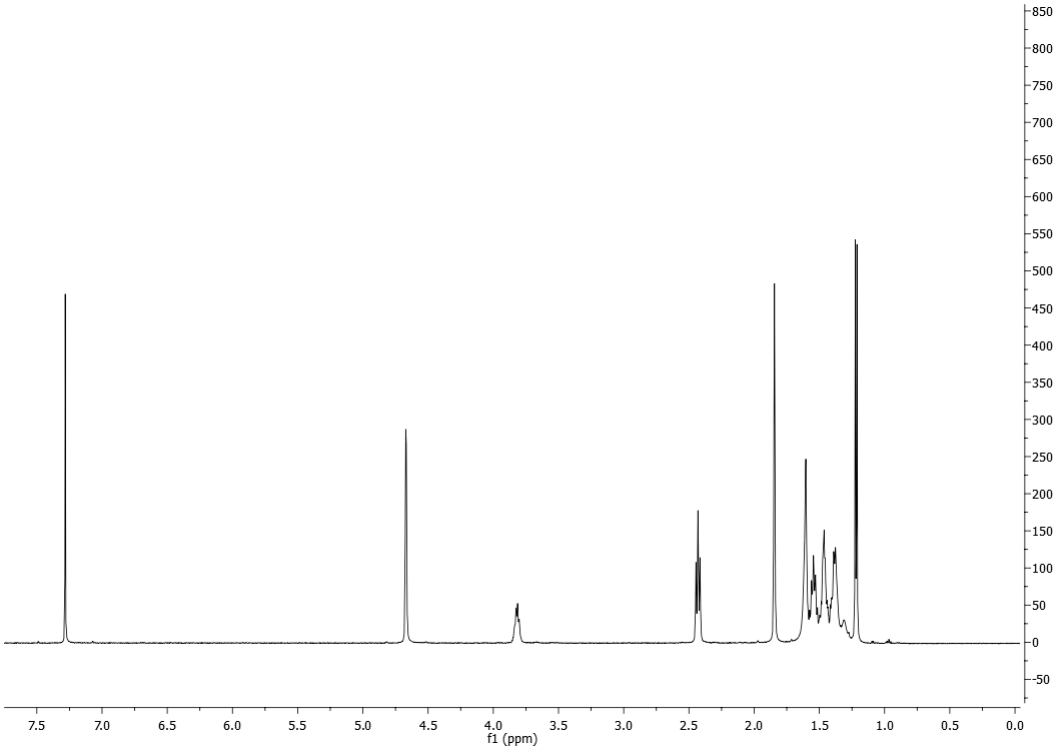

Figure S9. ^{1}H NMR spectrum of seiridin, **4** ($CDCl_3$, 500 MHz).