

Supplementary material

Chemical Evaluation, Antioxidant, Antiproliferative, Anti-Inflammatory and Antibacterial Activities of Organic Extract and Semi-purified Fractions of the Adriatic Sea Fan, *Eunicella cavolini*

Dario Matulja¹, Petra Grbčić¹, Krunoslav Bojanić², Natalija Topić-Popović², Rozelindra Čož-Rakovac², Sylvain Laclef³, Tomislav Šmuc², Ozren Jović², Dean Marković^{1*}, Sandra Kraljević Pavelić^{4*}

- 1 Department of Biotechnology, University of Rijeka, Radmile Matejčić 2, 51000 Rijeka, Croatia; dario.matulja@biotech.uniri.hr (Dario Matulja); petra.grbic@biotech.uniri.hr (P.G.); dean.markovic@biotech.uniri.hr (D.M.)
- 2 Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia; Krunoslav.Bojanic@irb.hr (K.B.); Natalija.Topic.Popovic@irb.hr (N.T.P.); Rozelindra.Coz-Rakovac@irb.hr (R.Č.-R.); tomislav.smuc@irb.hr (T.Š.); ozren.jovic@irb.hr (O.J.)
- 3 Laboratoire de Glycochimie, des Antimicrobiens et des Agroressources (LG2A) UMR CNRS 7378 - Institut de Chimie de Picardie FR 3085, Université de Picardie Jules Verne, 33 rue Saint Leu, 80039 Amiens CEDEX, France; sylvain.laclef@u-picardie.fr (S.L.)
- 4 Faculty of Health Studies, University of Rijeka, Viktora Cara Emina 5, Rijeka, 51000, Croatia; sandrakp@uniri.hr
- * Correspondence: sandrakp@uniri.hr (S.K.P.); dean.markovic@biotech.uniri.hr (D.M.); +385-51- 688 266 (S.K.P.); +385-91-500-8676 (D.M.)

The HRMS and MS/MS were performed with different samples which spectra are available online at www.mdpi.com/xxx/s1. Figures S1-S5: HRMS spectra of OE, F1-F4, respectively. Figures S6-S20: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compounds 1-15 from Table 3, respectively.

Mass Spectroscopy of the different fractions:

HRMS of the different fractions were performed using a SYNAPT G2-Si Q-TOF hybrid quadrupole time-of-flight instrument (Waters-Micromass, Manchester, UK), equipped with an electrospray (ESI) ionisation source (Z-spray) and an additional sprayer for the reference compound (Lock Spray). The samples were prepared by dissolving 1 mg of the different fraction in MeOH. The sample (1 µL of injection volume) were loaded on a KINETEX C₁₈ (1.3 µm, 50 x 2.1 mm) column (Phenomenex, Torrance, CA, USA) heated at 50 °C. The effluent was fully directed toward the ESI source of the Q-TOF instrument. ESI-HRMS data were recorded in the positive ion modes. The source and desolvation temperatures were 120 and 450 °C, respectively. Nitrogen was used as a drying and nebulizing gas at flow rates of 50 and 900 L/h, respectively. Typically, the capillary voltage was 3 kV, the sampling cone voltage 20 V and the source offset 20 V. Lock mass corrections using [M+H]⁺, [M-H]⁻ ions at *m/z* 556.2771 and 554.2615 of a leucine-enkephalin solution (50 pg µL⁻¹ in 50:50 acetonitrile/water + 0.1% formic acid) were applied for accurate mass measurements (elemental composition determination). The mass range was 50–1150 Da and spectra were recorded at 0.2 s/scan in the centroid mode at a resolution of 20,000 (FWHM) in the sensitivity mode. Data acquisition and processing were performed with MassLynx 4.1 software.

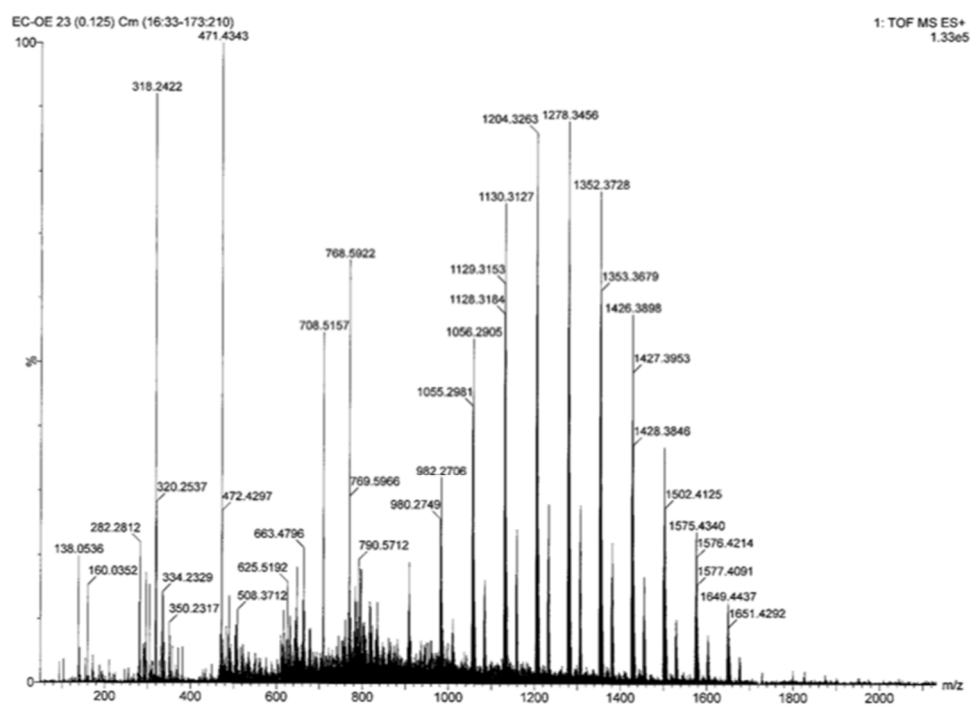


Figure S1. HRMS spectrum of OE.

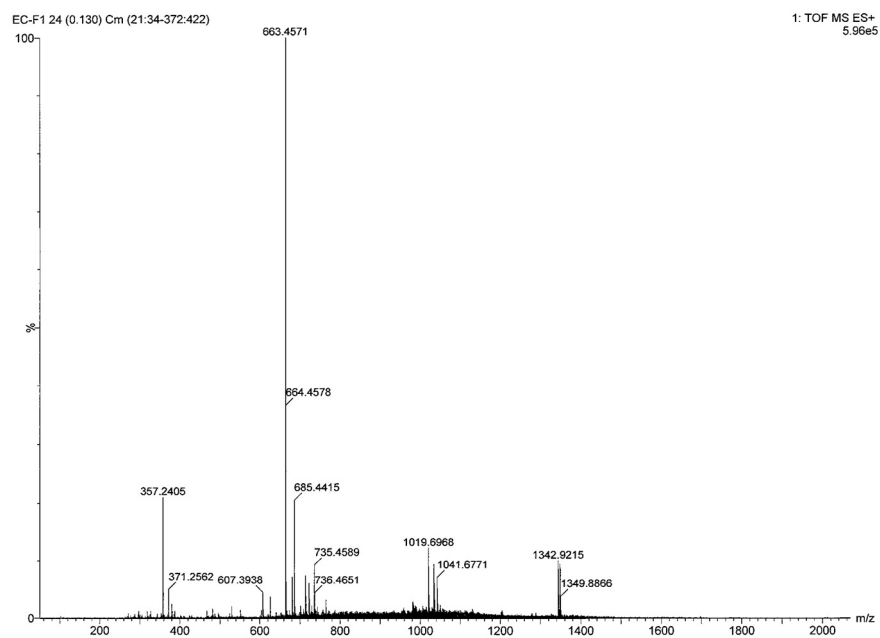


Figure S2. HRMS spectrum of F1.

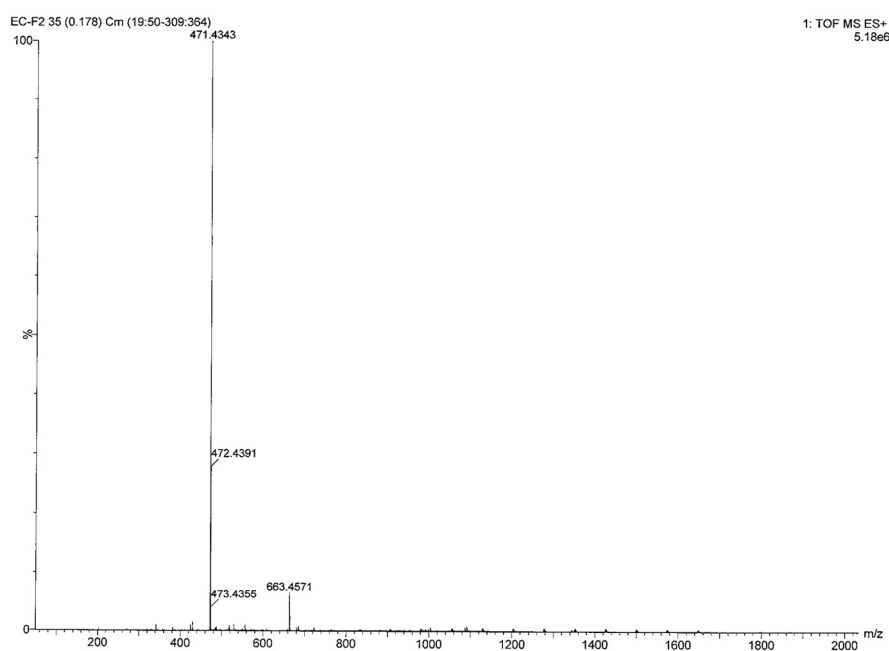


Figure S3. HRMS spectrum of F2.

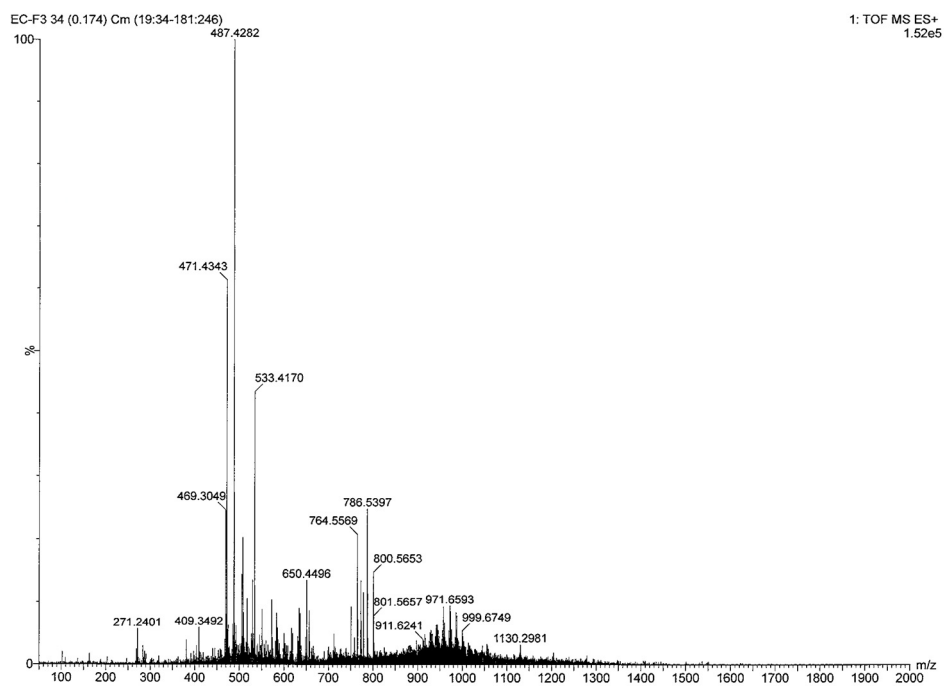


Figure S4. HRMS spectrum of **F3**.

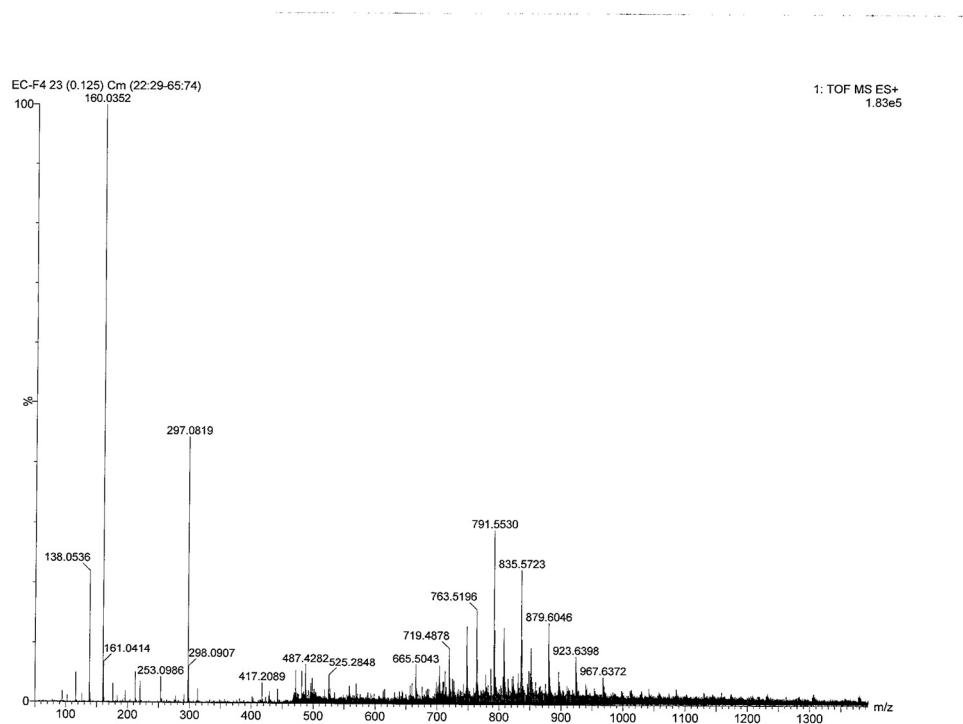


Figure S5. HRMS spectrum of **F4**.

GNPS dereplication

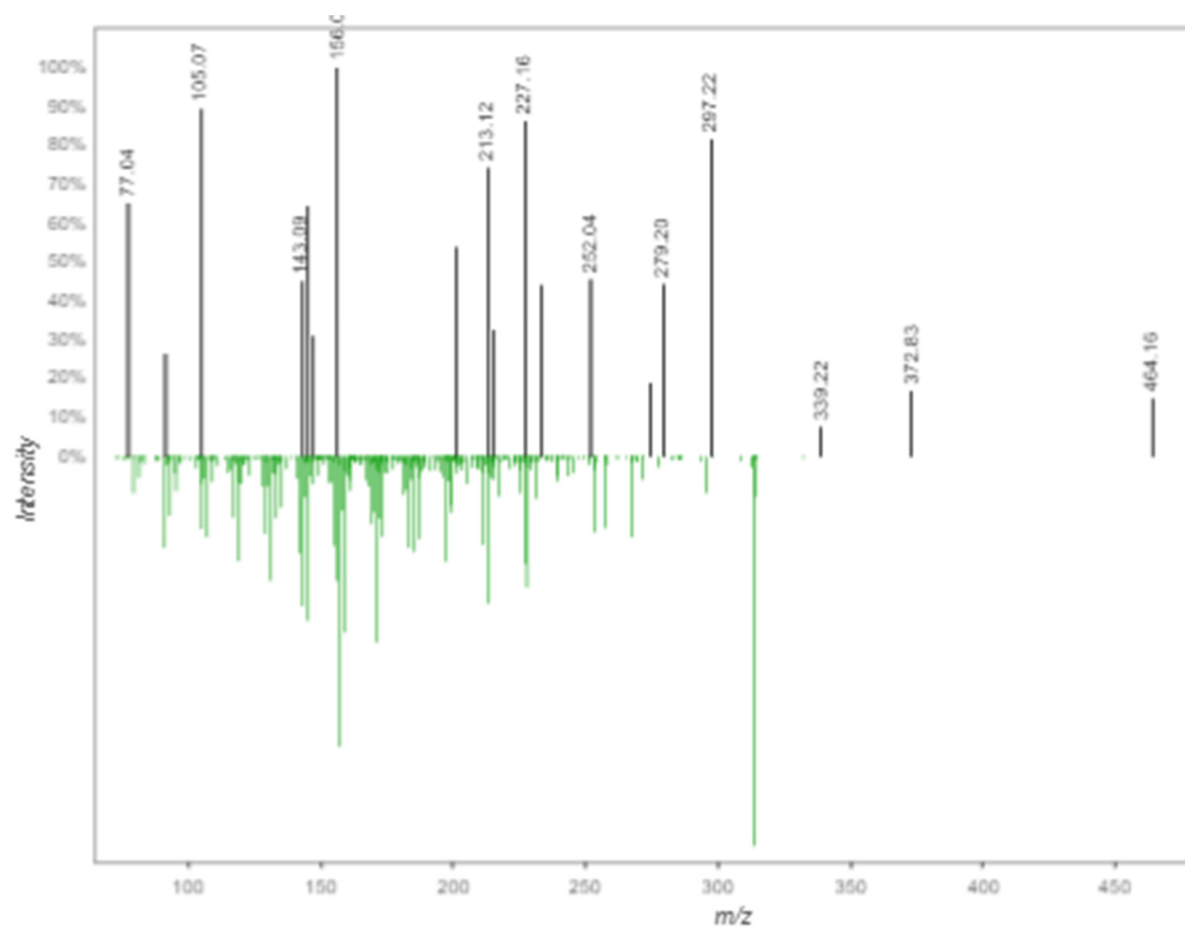


Figure S6: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 1.

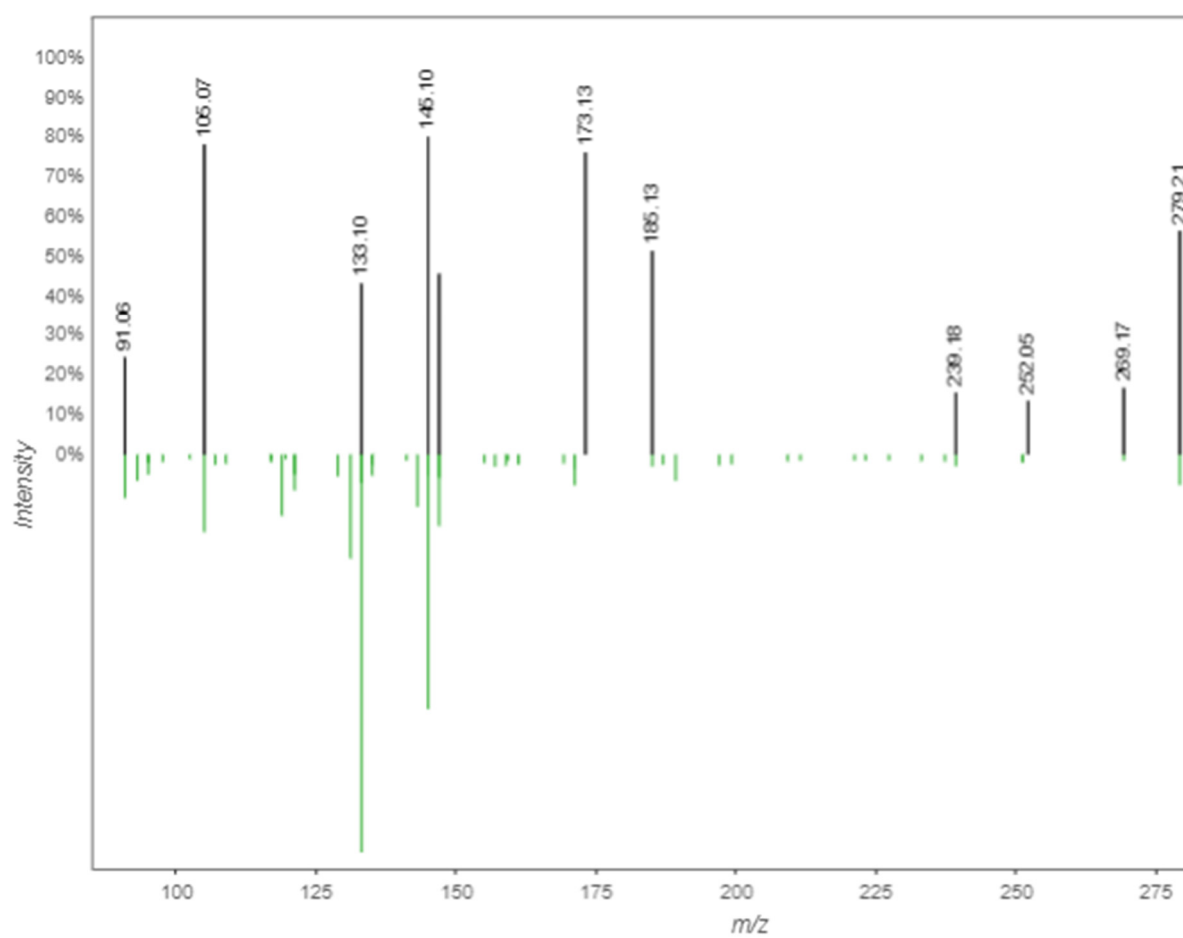


Figure S7: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 2.

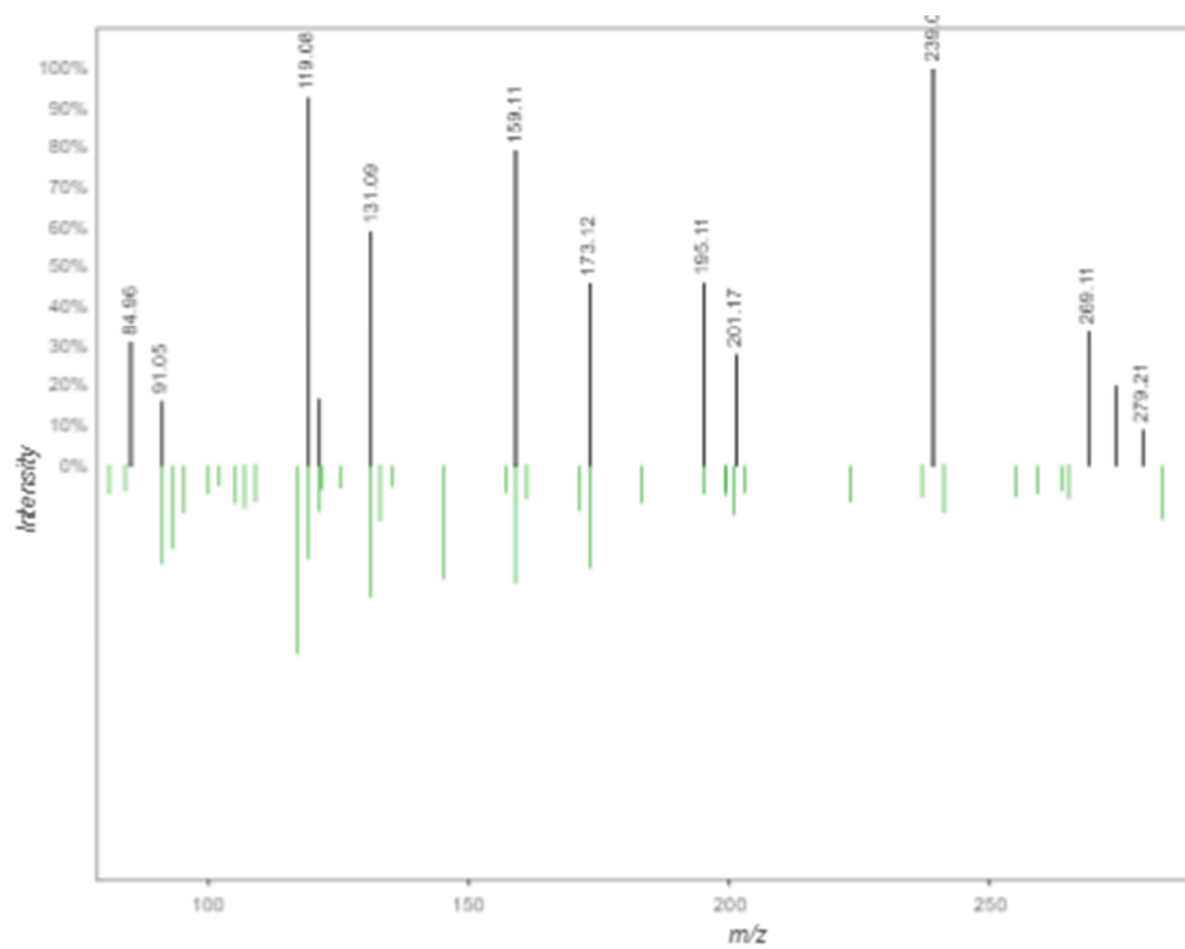


Figure S8: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 3.

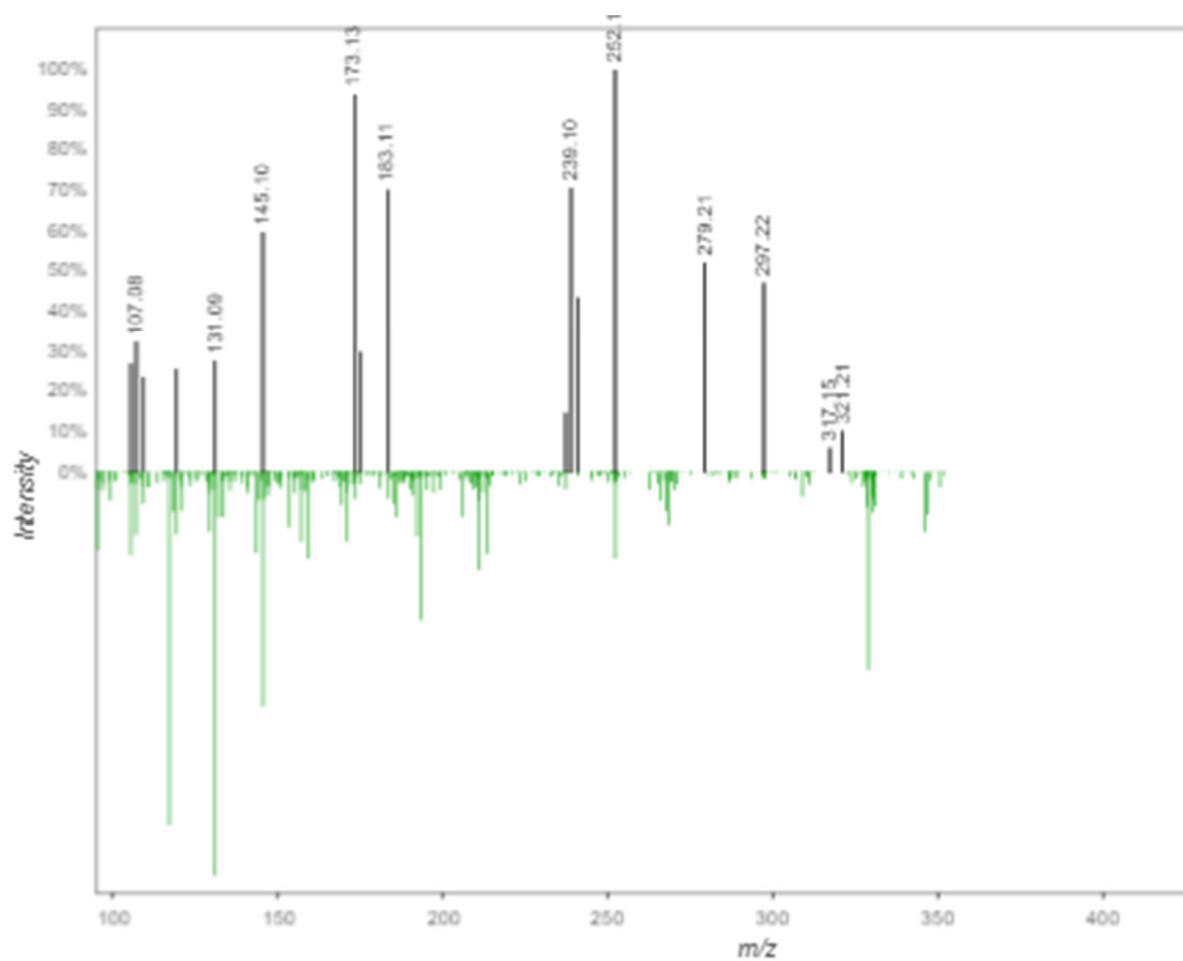


Figure S9: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 4.

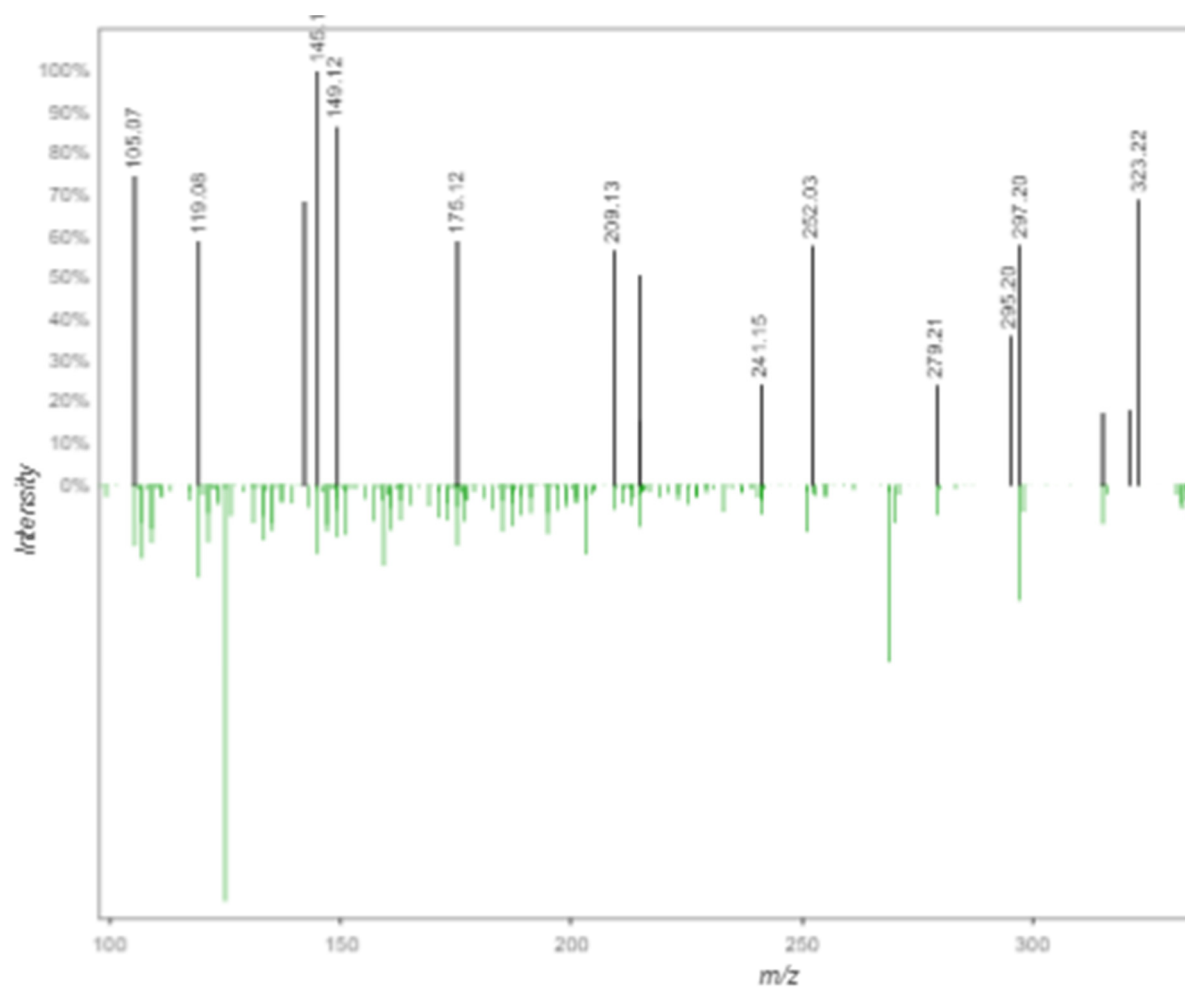


Figure S10: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 5.

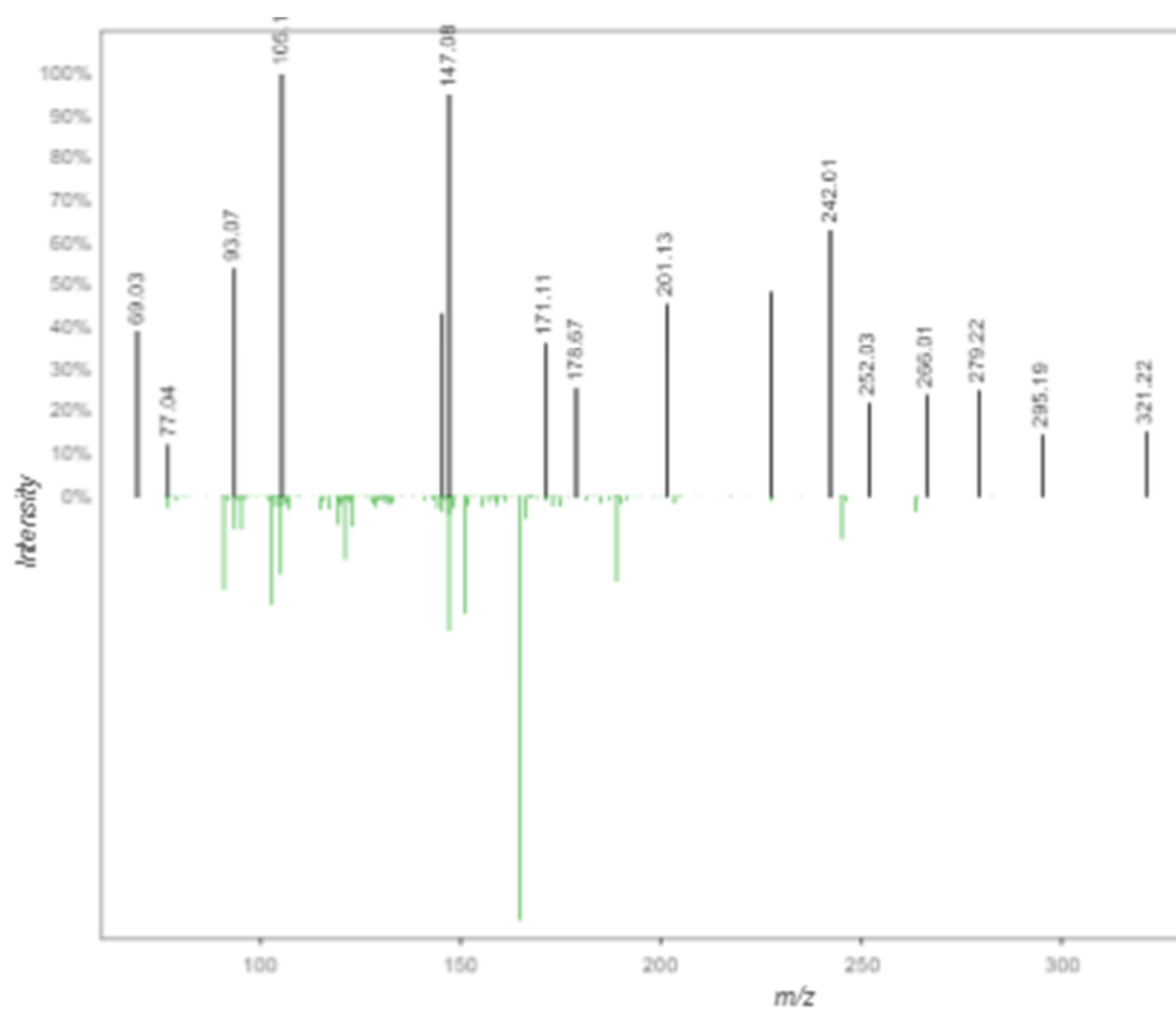


Figure S11: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 6.

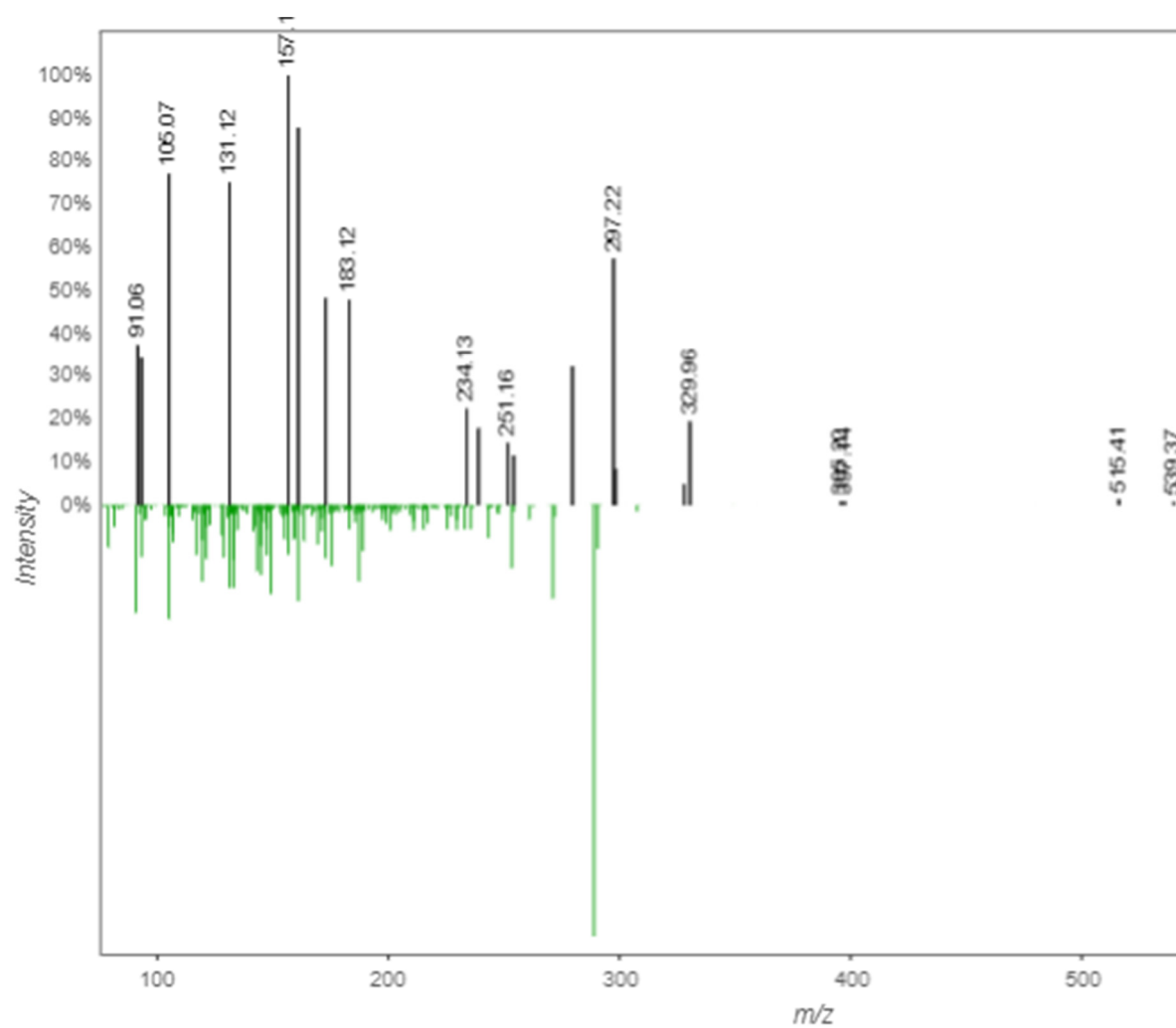


Figure S12: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 7.

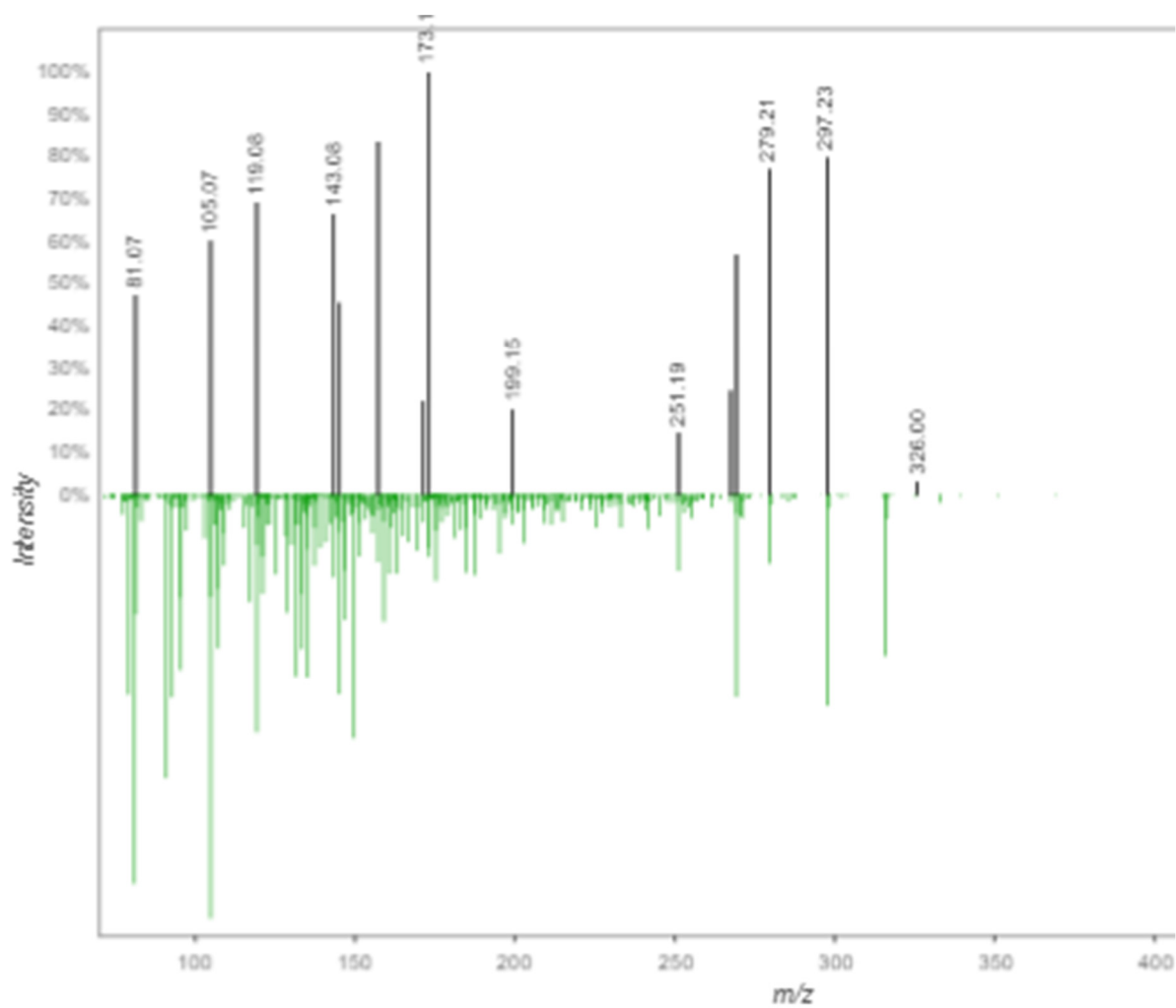


Figure S13: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 8.

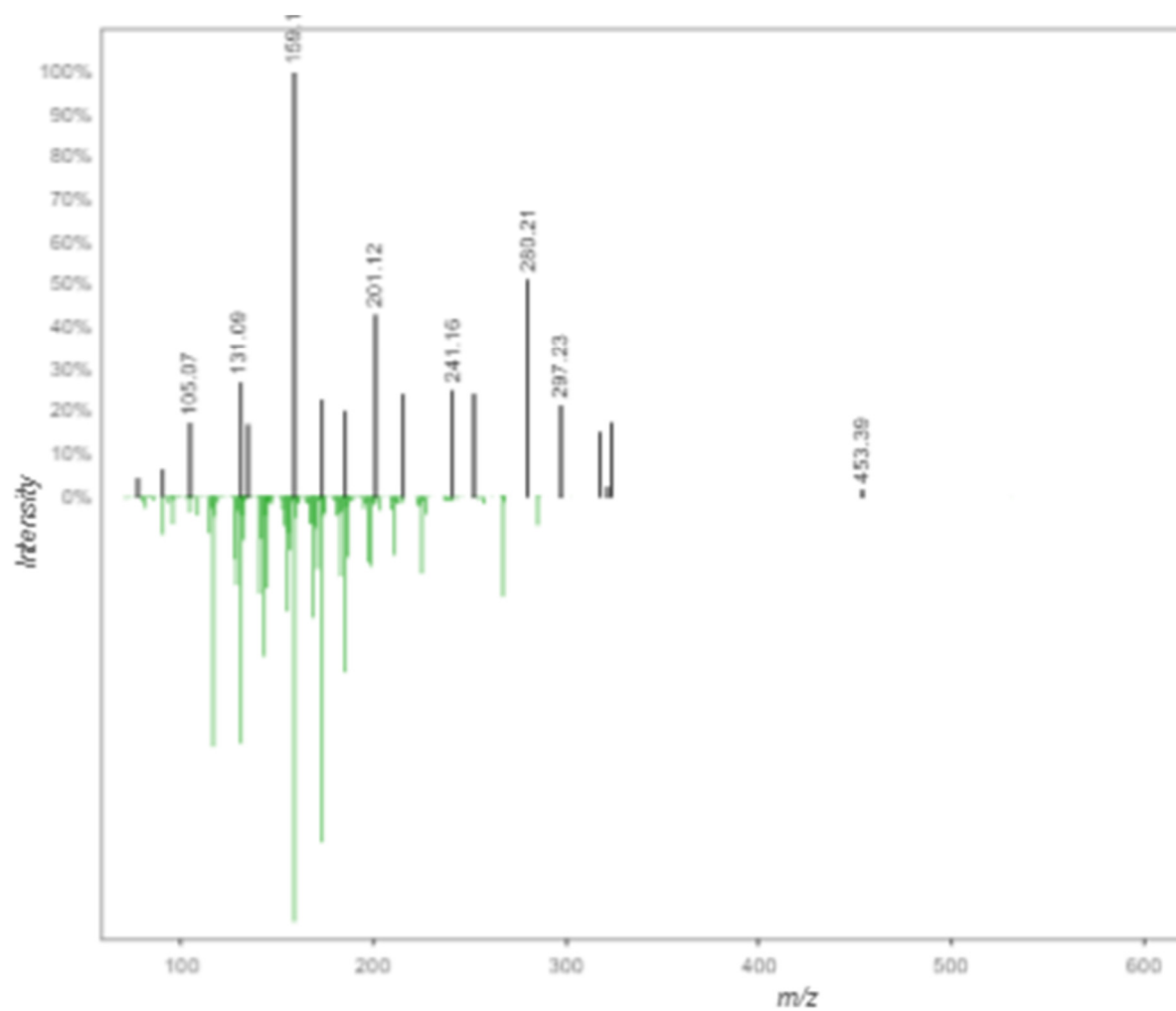


Figure S14: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 9.

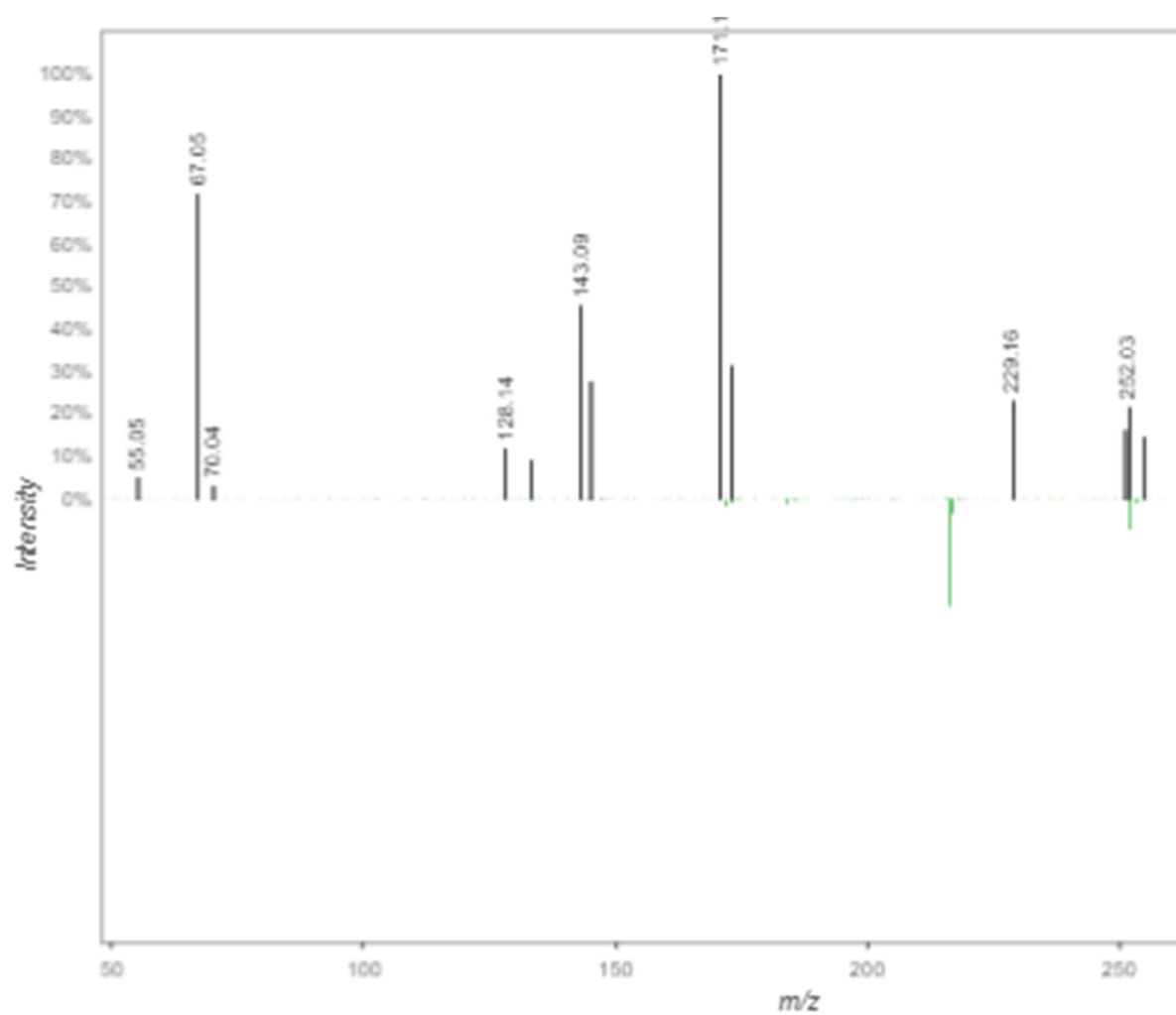


Figure S15: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 10.

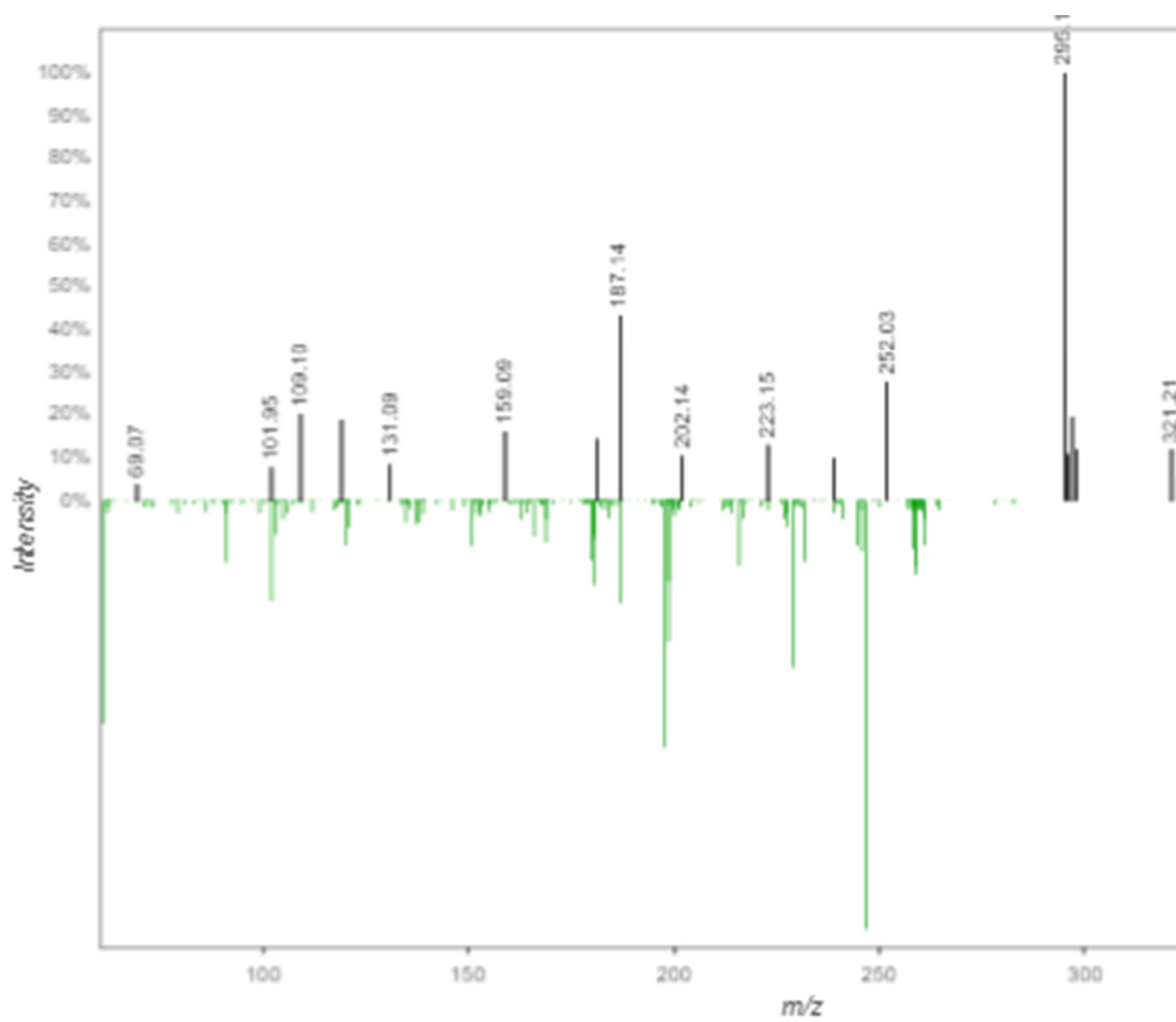


Figure S16: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 11.

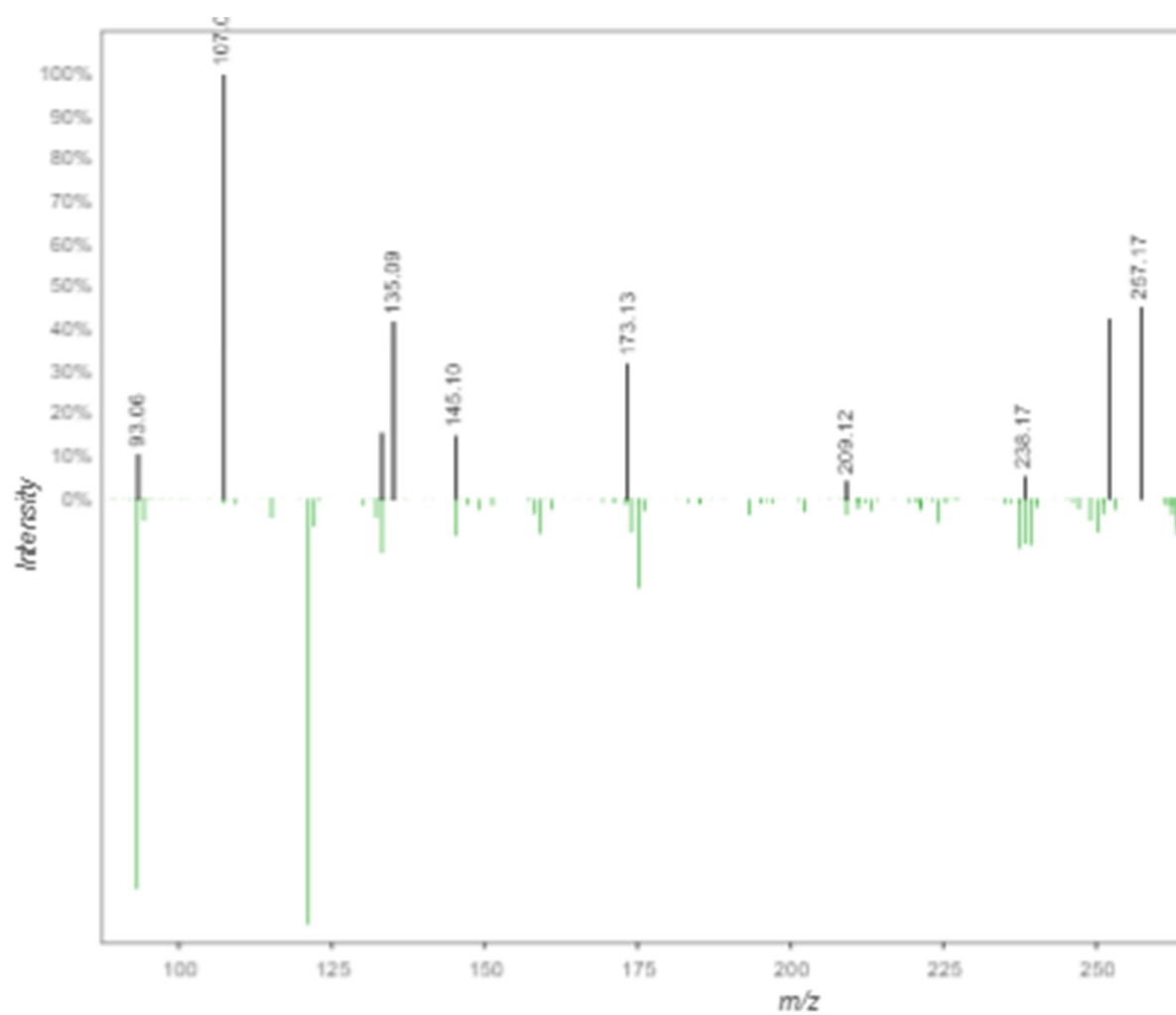


Figure S17: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 12.

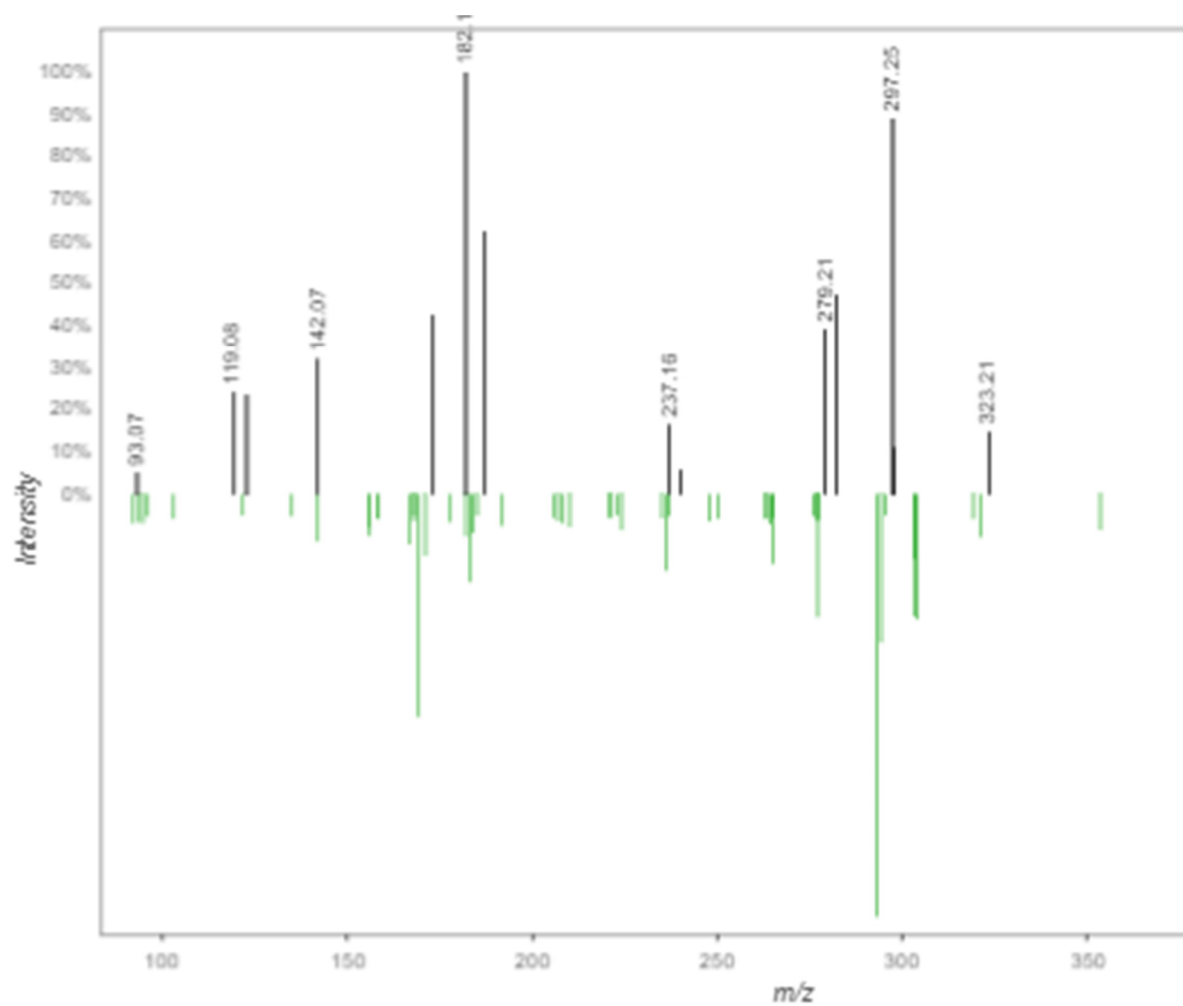


Figure S18: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 13.

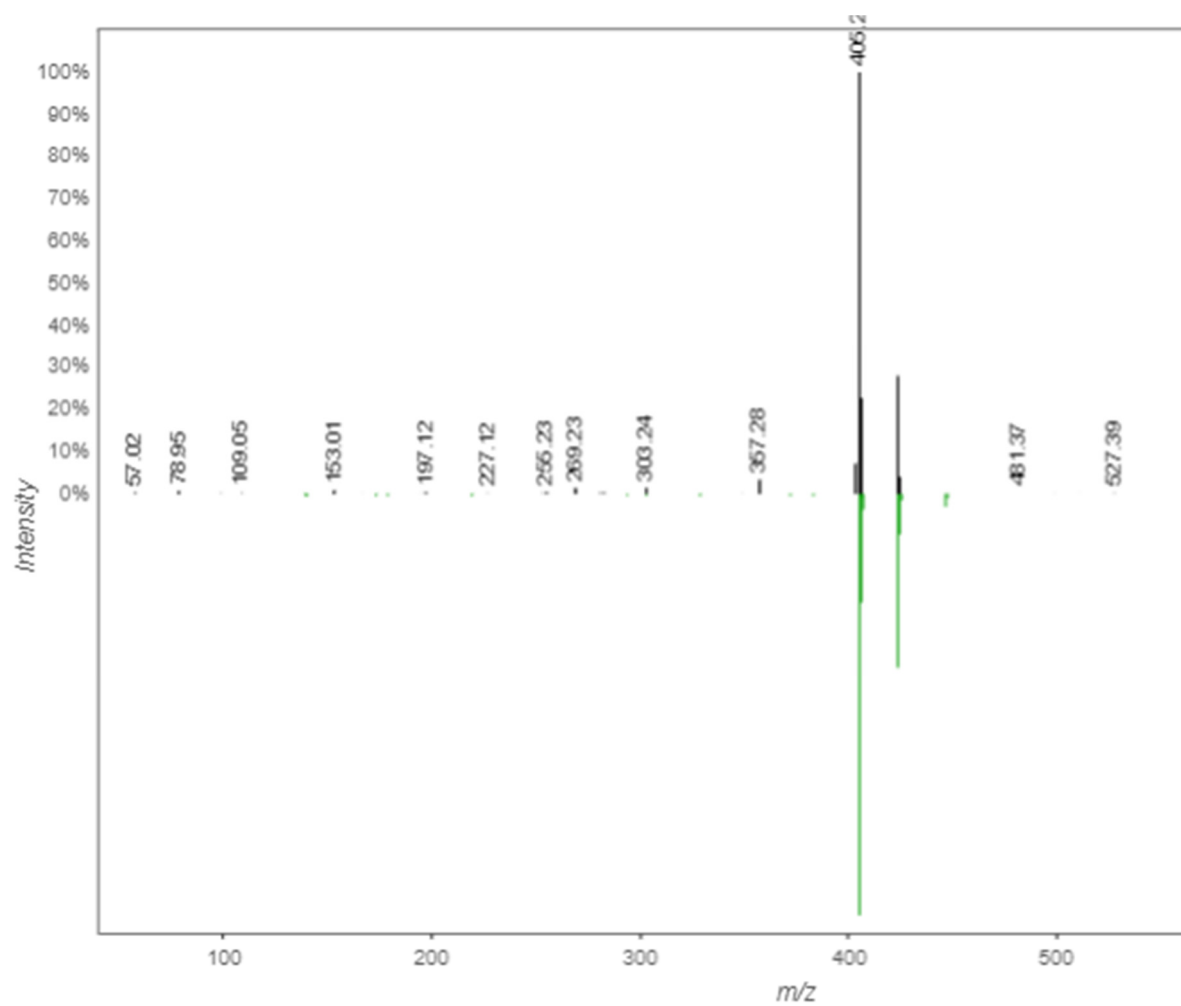


Figure S19: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 14.

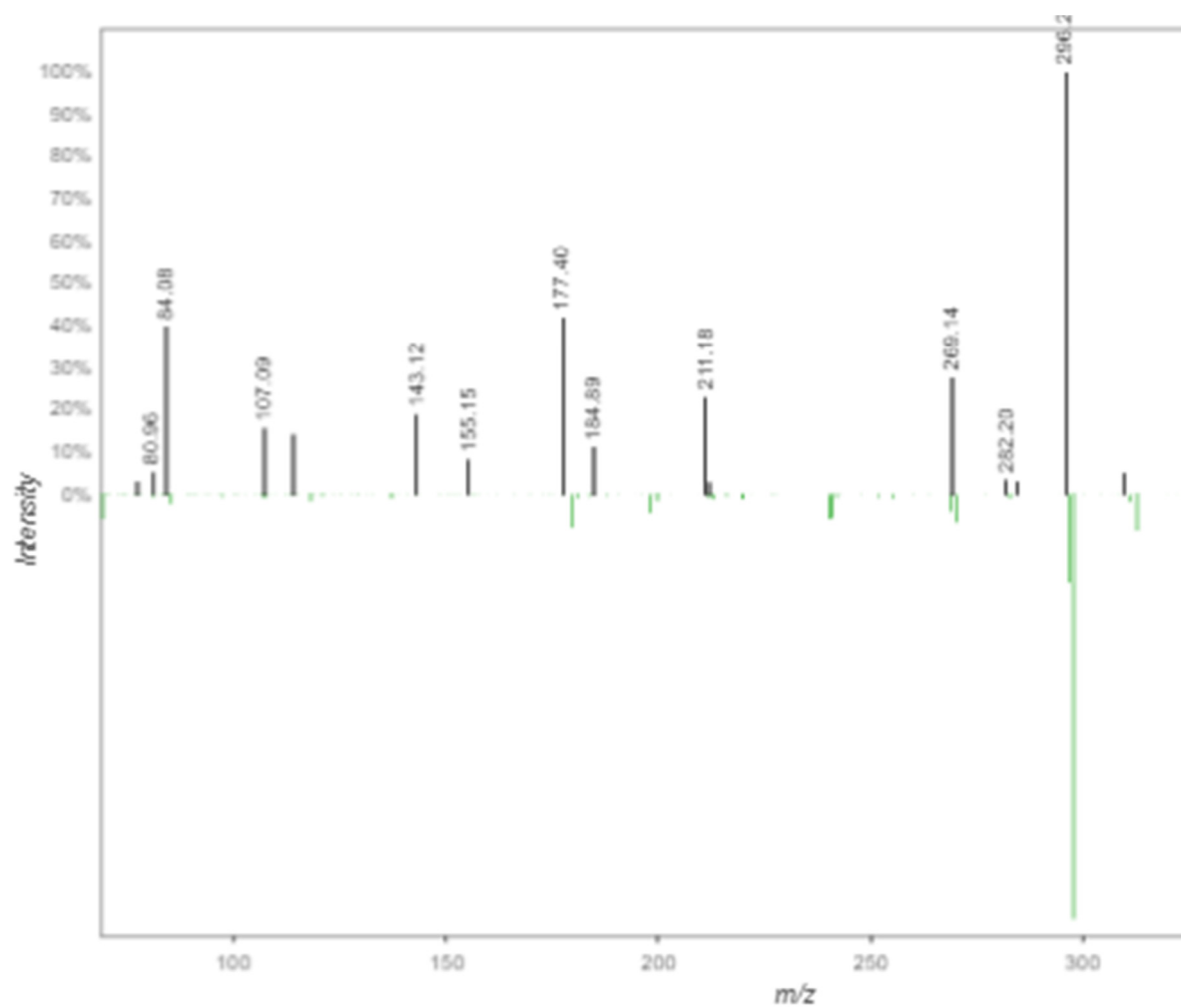


Figure S20: MS/MS match between GNPS database (green) and *Eunicella cavolini* sample (black) for compound 15.