# SUPPLEMENTARY MATERIAL

# Two amide glycosides from *Portulaca oleracea* L. and its bioactivities

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## Abstract

Two novel amide glycosides, named oleraciamide E (**1**) and oleraciamide F (**2**), were isolated from the *Portulaca oleracea* L. Their structures were determined by means of 1D and 2D NMR spectroscopic and UHPLC-ESI-TOF-MS methods. Oleraciamide E (**1**) exhibited anticholinesterase activity with $IC_{50}$ values of 52.43 ± 0.33 μM, and presented scavenging activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical quenching assay, with the $IC_{50}$ values of 24.64 ± 0.33 μM.

**Keywords**: *Portulaca oleracea* L.; amide glycosides; anticholinesterase activity; antioxidant activity

### Experimental section

#### *General experimental procedures*

UV spectra and its absorbance data were measured with a HITACHI U-3010 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The IR spectrum was recorded by using an IR200 spectrophotometer (Thermo Electron Corporation, Waltham, MA). The NMR spectra in DMSO-$d_6$ was obtained from an AVANCE 600 MHz instrument with cryogenic probe (Bruker Corporation, Switzerland). Relative molecular mass was obtained from a 6520 quadrupole-time-of-flight mass spectrometer (Agilent, Palo Alto, CA). A Shimadzu Nexera X2 UHPLC LC-30A system (Shimadzu, Kyoto, Japan), with a solvent delivery pump (LC-30AD), a vacuum degasser (DGU-20A), a UV spectrophotometric detector (SPD-20A) and LabStation software (Shimadzu), was used for separation and purity. The column of analysis was Kromasil C18 column

(150mm × 4.6 mm, 5 μm, Dalian Johnsson Separation Science and Technology Corporation). The 96-well microplate reader (HBS-1096A) was provided from Nanjing Detie Experimental Equipment Co., Ltd. (Nanjing, China). In the separation process, silica gel (100-200 and 200-300 mesh, Qingdao Marine Chemical Co., Qingdao, China), AB-8 macroporous resin (Donghong Chemical Co., Ltd., China), ODS (20-40 and 40-70 μm) and Sephadex LH-20 (GE Healthcare, Marlborough, MA) were used. TLC was conducted on TLC plates precoated with silica gel GF254 (Qingdao Marine Chemical Co., Qingdao, China).

***Plant materials and chemicals***

The whole herbs of *P. oleracea* L. were obtained from Shijiazhuang (Hebei, China) in June 2017, and identified by Prof. Xixiang Ying. The voucher specimen (No. 20171001) was deposited at Liaoning University of Traditional Chinese Medicine. $Na_2HPO_4$ and $NaH_2PO_4$, which were used for preparing the phosphate buffer saline (PBS), were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) (purity ≥ 99%). 5,5-dithiobis-2-nitrobenzoic acid (DTNB) (purity ≥ 99%) was provided by Shanghai Jinshui Biotechnology Co., Ltd. (Shanghai, China). Acetylthiocholine iodide (ATCI) (purity ≥ 99%) and acetylcholinesterase (AChE) (vitality ≥ 200 units/mg protein) were provided by Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) (purity ≥ 99%) was provided by Sigma Co. (USA). Eserine (purity ≥ 98%) was provided by Shanghai Hanxiang Biotechnology Co., Ltd. (Shanghai, China). Butylated hydroxylanisole (BHA) (purity ≥ 98%) was provided by Shanghai Xiangrui Biological Technology

Co., Ltd. (Shanghai, China). The HPLC grade of methanol and formic acid were purchased from Damao Chemical Reagent Plant (Tianjin, China, purity ≥ 99.9%). Other reagents of analytical grade were purchased from Jinfeng Chemical Factory (Tianjin, China), and the water was WAHAHA purified water (Shenyang, China).

***Isolation and identification***

A sample of the air-dried aerial segments *P. oleracea* (250 kg) were refluxed twice times with 10-fold amount water for 2 h each time, and then the water extract was concentrated, affording a crude extract (21 kg). And subjected to chromatography (61 × 55 cm, approximately, 150 kg) on a 100-200 mesh silica-gel column eluted with gradients of ethyl acetate and ethanol (1:0, 1:1, 1:2, v/v), affording three fractions evaporated (2 kg). These fractions were subjected to the AB-8 macroporous resin column chromatography, eluting with ethanol and water (30:70, 50:50, 70:30, 100:0, v/v) as the gradient eluant to obtain five fractions (15 L each). The fraction of 30% extracting was concentrated under reduced pressure to obtain the extract of 400 g, which was subjected to a 200-300 mesh silica-gel column (120 × 8 cm, approximately, 1.2 kg) for further partitioned with ethyl acetate, ethyl acetate and methanol (5:1, 2:1, 1:2, 1:4, v/v) as the gradient eluant to obtain five fractions (fraction 1-fraction 5, 10 L each). The ethyl acetate fraction (fraction 1, 120 g) displayed orange when sprayed with Dragendorff's reagent, which were subjected to the 40-70 μm octadecylsilyl (ODS) column chromatography (25 × 3 cm, approximately, 150 g, Ultimate XB-C18) with methanol and water (40:60, 60:40, 100:0, v/v) as the gradient eluant under medium pressure, acquiring three fractions (fraction 1.1-fraction 1.3, 500 mL each).

Fraction 1.1 (77 g) displayed orange when repeatedly sprayed with Dragendorff's reagent, which were applied to the 20-40 μm ODS column chromatography (25 × 3 cm, 150 g, Ultimate XB-C18) with methanol and water (40:60, 60:40, 100:0, v/v) as the gradient eluant under medium pressure, obtaining three fractions (fraction 1.1.1-fraction 1.1.3, 300 mL each). Fraction 1.1.2 (28 g) was turned to orange when repeatedly exposed to Dragendorff's reagent, and further separated by a Sephadex LH-20 column (100 g, 2 × 150 cm) using methanol as eluant to obtain six fractions (fraction 1.1.2.1-fraction 1.1.2.6, 100 mL each). Fraction 1.1.2.3 (8 g) was turned to orange when repeatedly exposed to Dragendorff's reagent, and further purified by UHPLC, and eluted with acetonitrile and 0.1% formic acid (27:73, v/v, 1.0 mL/min), obtained compound **1** (1 mg, purity of > 99% with UHPLC, $t_R$ 15.490 min) and compound **2** (0.5 mg, purity of > 99% with UHPLC, $t_R$ 20.284 min).

***Acetylcholinesterase activity assay***

Ellman's colorimetric method (Ellman GL et al. 1961) with some modifications was used to determine the acetylcholinesterase activity. The 140 μL PBS (0.1 M, pH = 8.0, containing 0.1 mol/L $NaH_2PO_4$ and $Na_2HPO_4$), 10 μL DTNB (15 mmol/L), 15 μL AChE (0.2 U/mL) and 20 μL sample solution in five concentrations (1, 5, 10, 20, 40 μM) were mixed and incubated on the 96-well plates at 37°C for 10 min, and then added 10 μL ATCI (15 mmol/L) along with 10 μL DTNB (15 mmol/L), incubated for 20 min at 37 °C. The absorbance was measured at 405 nm, and methanol was added to instead of the samples for the blank control, and eserine was used and prepared into the same concentrations with the samples for the positive control. The percent

inhibition rate of AChE was obtained using the following equation, in which $A_{sample}$ was the absorbance of the test compound, and $A_{blank}$ was the absorbance of the blank control. The anticholinesterase activity was evaluated by the value of $IC_{50}$, and the values were expressed as the means ± SD for n=5.

Anticholinesterase activity (%) = $[(A_{blank} – A_{sample})/A_{blank}] \times 100\%$

***Assay of scavenging DPPH radicals***

DPPH radical scavenging activity test was determined by the modified method described formerly (Gulcin et al. 2007). Briefly, 1 mL sample solution in five concentrations (1, 2.5, 10, 20, 40 μM) and 1 mL DPPH solution (80 μM) were mixed at room temperature for 10 min. The absorbance was measured at 517 nm. Equal amounts of methanol and DPPH were prepared to be the blank group, and the sample solution mixed with methanol served as the control group. Butylated hydroxylanisole (BHA) was tested as a positive control, and prepared into the same concentrations with other samples. The percent DPPH radical scavenging was calculated according to the following equation, in which $A_{control}$ was the absorbance of the control group, $A_{sample}$ was the absorbance of the test compound, and $A_{blank}$ was the absorbance of the blank control. The antioxidant capacity was evaluated by the value of $IC_{50}$, and the values were expressed as the means ± SD for n = 5.

DPPH radical scavenging effect (%) = $[1 - (A_{sample} – A_{control})/A_{blank}] \times 100\%$

**References**

Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. 1961. A new and rapid

colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7:88-95.

Gulcin I, Elmastas M and Aboul-Enein HY. 2007. Determination of antioxidant and radical scavenging activity of Basil (*Ocimum basilicum* L. Family Lamiaceae) assayed by different methodologies. Phytother Res. 21:354-361.

## Supporting information

Supplementary material relating to this article is available online, alongside, Tables S1-S4, Figure S1-S20 and Experimental section.

Figure S13. IR (KBr) spectrum of oleraciamide E

Figure S14. Anticholinesterase effect of oleraciamide E and eserine (n = 5).

Figure S15. Antioxidant activities of oleraciamide E and BHA (n = 5).

Figure S16. Key $^{1}$H-$^{1}$H COSY and ROESY correlations of oleraciamide F

Figure S17. $^{1}$H NMR (600 MHz) spectrum of oleraciamide F in DMSO-$d_6$

Figure S18. $^{1}$H-$^{1}$H COSY spectrum of oleraciamide F in DMSO-$d_6$

Figure S19. ROESY spectrum of oleraciamide F in DMSO-$d_6$

Figure S20. UHPLC-ESI-TOF-MS of oleraciamide F $[M\text{-}C_6H_{12}O_6+H]^+$

Table S1. $^{1}$H-NMR (600 MHz) and $^{13}$C-NMR (150MHz) data of oleraciamide E in DMSO-$d_6$

| Position | δC | Type | δH mult. (J in Hz) | COSY | HMBC | NOE |
|---|---|---|---|---|---|---|
| 1 | 126.2 | C | | | | |
| 2/6 | 129.4 | CH | 7.41 d (9.36) | 3/5, 2′ | 4, 1′ | 3/5, 2′ |
| 3/5 | 115.7 | CH | 6.78 d (8.28) | 2/6 | 1, 4 | 6, 3′ |
| 4 | 159.1 | C | | | | |
| 1′ | 163.9 | C | | | | |
| 2′ | 118.0 | CH | 6.68 d (15.24) | 3′, 2/6 | 1, 1′, 2″ | 6, 3′ |
| 3′ | 139.9 | CH | 7.39 d (15.50) | 2′ | 2, 6, 1′, 2′ | 3/5, 2′ |
| 1″ | | N | | | | |
| 2″ | 33.5 | $CH_2$ | 3.07 d (16.98) | | 2‴, 1‴, 5‴ | 6‴ |
| 1‴ | 126.1 | C | | | | |
| 2‴ | 143.6 | C | | | | |
| 3‴ | 108.6 | CH | 8.12 s | | 1‴, 2‴, 4‴, 5‴ | 2⁗ |
| 4‴ | 143.2 | C | | | | |
| 5‴ | 136.1 | C | | | | |
| 6‴ | 111.7 | CH | 6.64 s | | 2″, 4‴, 5‴ | 2″ |
| 1⁗ | | O | | | | |
| 2⁗ | 104.2 | CH | 4.53 d (6.84) | 3⁗, 4⁗, 5⁗ | 4‴, 3⁗, 4⁗, 5⁗ | 3‴ |
| 3⁗ | 73.5 | CH | 3.27 m | 2⁗, 4⁗, 5⁗, 6⁗ | 4⁗, 5⁗ | |
| 4⁗ | 76.0 | CH | 3.27 m | 2⁗, 3⁗, 5⁗, 6⁗ | 5⁗ | |

| | | | | | |
|---|---|---|---|---|---|
| 5‴′ | 69.2 | CH | 3.27 m | 2‴′, 3‴′, 4‴′, 6‴′ | 4‴′ |
| 6‴′ | 77.0 | CH | 3.23 m | 3‴′, 4‴′, 5‴′ | 3‴′ |
| 7‴′ | 62.6 | $CH_2$ | 3.32 m | 3‴′, 4‴′, 5‴′, 6‴′ | 5‴′ |

Table S2. $^1$H-NMR (600MHz), COSY and ROESY data of oleraciamide F in DMSO-$d_6$

| Position | $\delta_H$ mult. (*J* in Hz) | COSY | ROESY |
|---|---|---|---|
| 2 | 7.16 s | | 2′, 3′, MeO-$C_3$ |
| 5 | 7.02 d (7.9) | 6 | |
| 6 | 6.79 d (7.9) | 5 | |
| 2′ | 6.73 d (15.5) | 3′ | 2 |
| 3′ | 7.39 d (15.5) | 2′ | 2 |
| 2″ | a: 3.09 d (16.0) | 2″b | 6‴′ |
| | b: 3.28 m | 2″a | |
| 3‴′ | 8.12 s | | 2‴′ |
| 6‴′ | 6.64 s | | 2″a |
| 2‴′ | 4.52 d (6.4) | 3‴′ | 3‴′, 6‴′ |
| 3‴′ | 3.27 m | 2‴′ | |
| 4‴′ | 3.272 or 3.273 m | HO-$C_{4‴′}$ | |
| 5‴′ | 3.272 or 3.273 m | HO-$C_{5‴′}$ | |
| 6‴′ | 3.22 m | 7‴′a | 2‴′, 7‴′a, 7‴′b |
| 7‴′ | a: 3.59 m | 6‴′, 7‴′b, HO-$C_{7‴′}$ | 6‴′, 7‴′b, HO-$C_{7‴′}$ |
| | b: 3.72 m | 7‴′a, HO-$C_{7‴′}$ | 6‴′, 7‴′b, HO-$C_{7‴′}$ |
| MeO-$C_3$ | 3.81 s | | 2 |
| HO-$C_{4‴′}$ | 4.98 d (2.9) or 5.13 d (3.0) | 4‴′ | |
| HO-$C_{5‴′}$ | 4.98 d (2.9) or 5.13 d (3.0) | 5‴′ | |
| HO-$C_{7‴′}$ | 4.50 t (5.9) | 7‴′a, 7‴′b | 7‴′a, 7‴′b |

Table S3. $IC_{50}$ (μM) for anticholinesterase effect of oleraciamide E (*n* = 5)

| Compound and standard inhibitor | AChE $IC_{50}$ (μM) |
|---|---|
| Oleraciamide E | 52.43 ± 0.33 |
| Eserine | 32.11 ± 0.10 |

Eserine is the positive control and values are expressed as the means ± SD for *n* = 5.

Table S4. $IC_{50}$ (μM) for antioxidant activity of oleraciamide E (n = 5)

| Compound and standard inhibitor | DPPH $IC_{50}$ (μM) |
|---|---|
| Oleraciamide E | 24.64 ± 0.33 |
| BHA | 56.39 ± 0.53 |

BHA is the positive control and values are expressed as the means ± SD for *n* = 5.

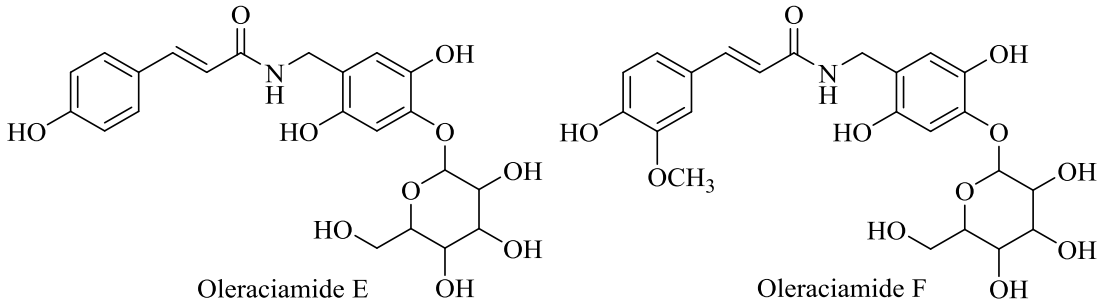


Figure S1. Structures of oleraciamide E and oleraciamide F

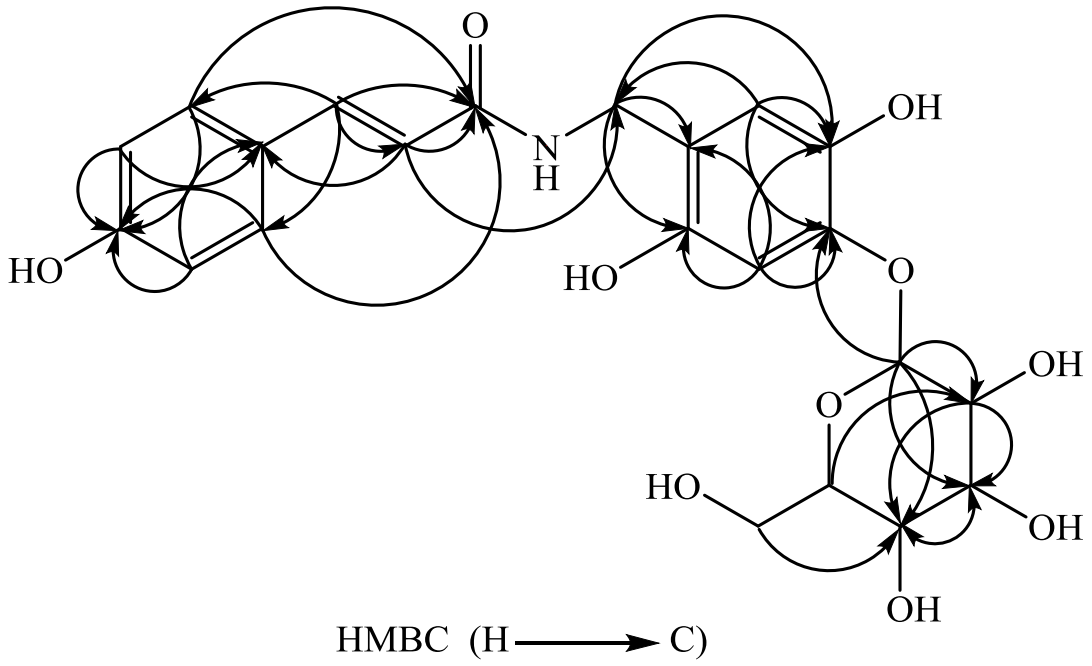


Figure S2. Key HMBC correlations of oleraciamide E

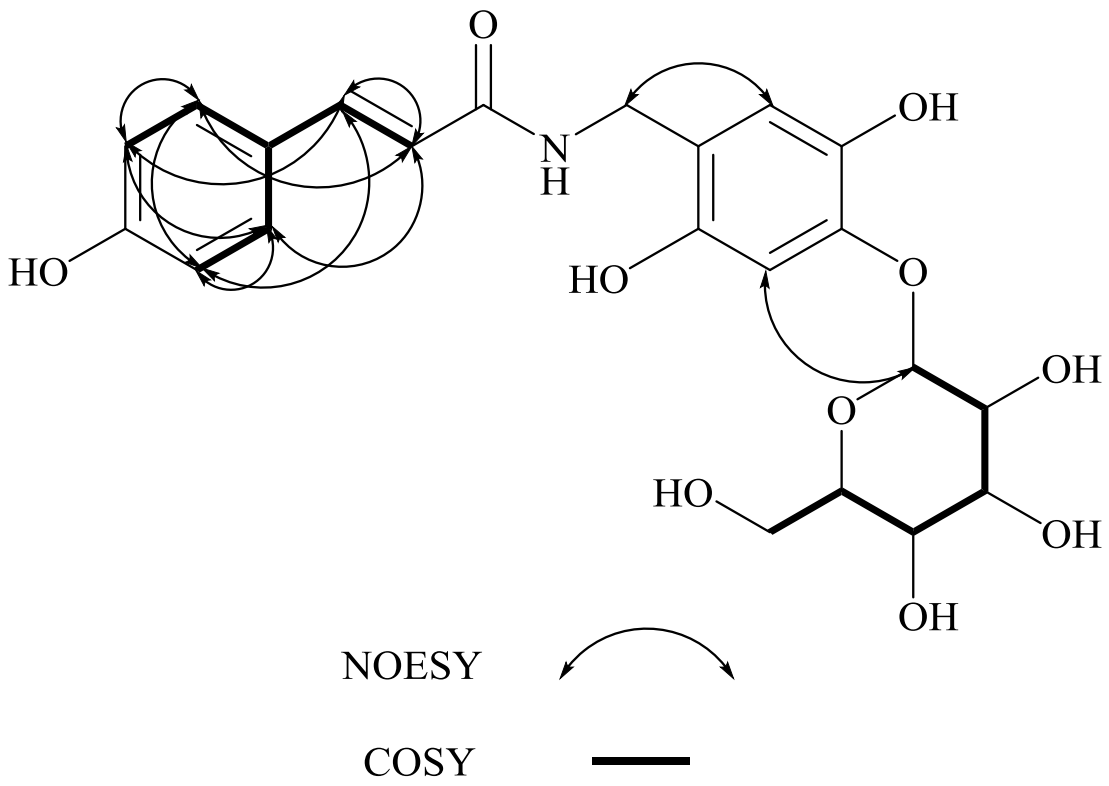


Figure S3. Key $^{1}$H-$^{1}$H COSY and NOESY correlations of oleraciamide E

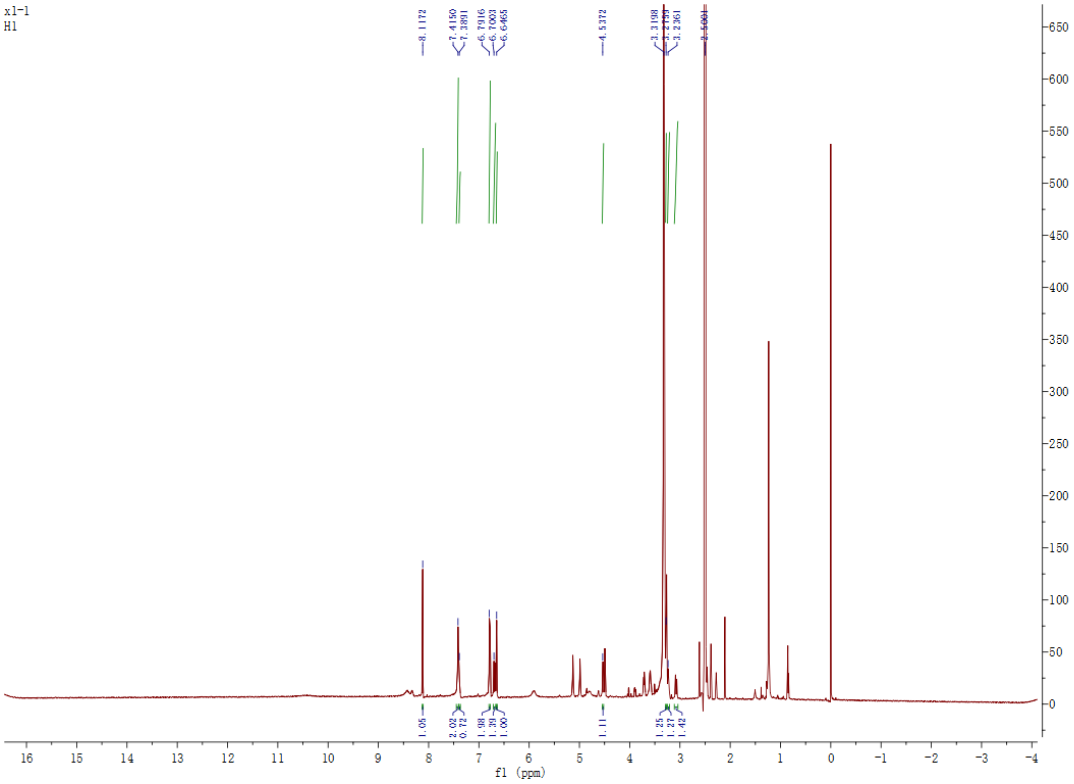


Figure S4. $^{1}$H NMR (600 MHz) spectrum of oleraciamide E in DMSO-$d_6$

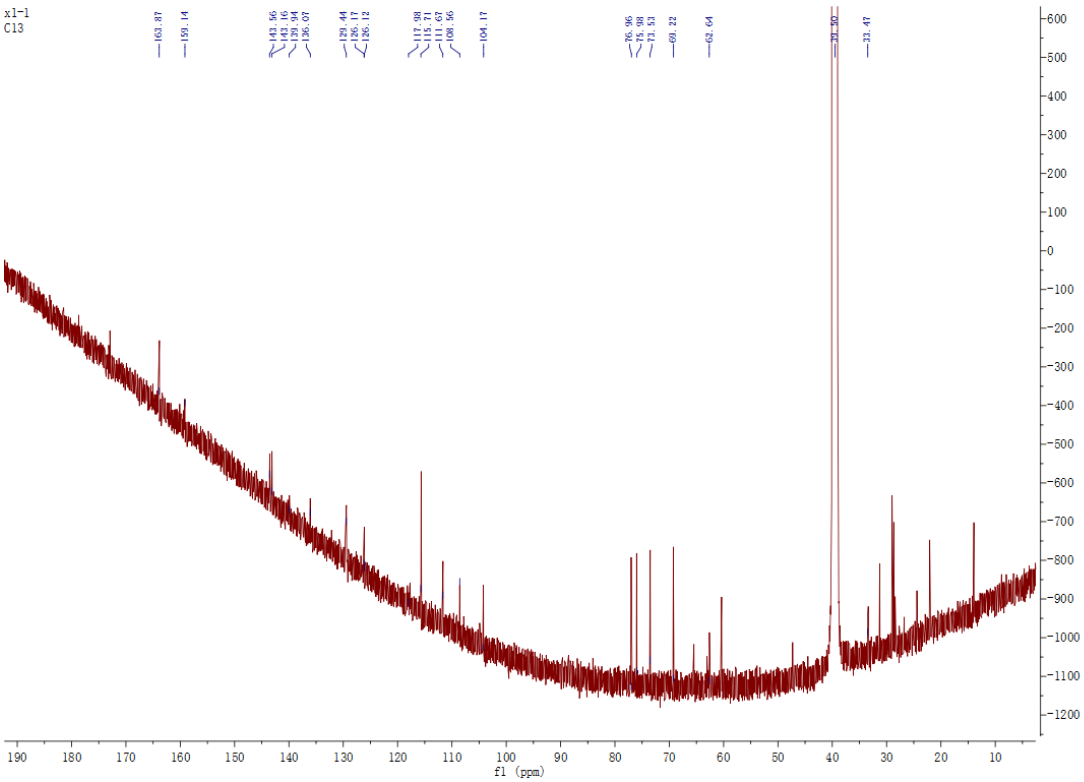


Figure S5. $^{13}$C NMR (150 MHz) spectrum of oleraciamide E in DMSO-$d_6$

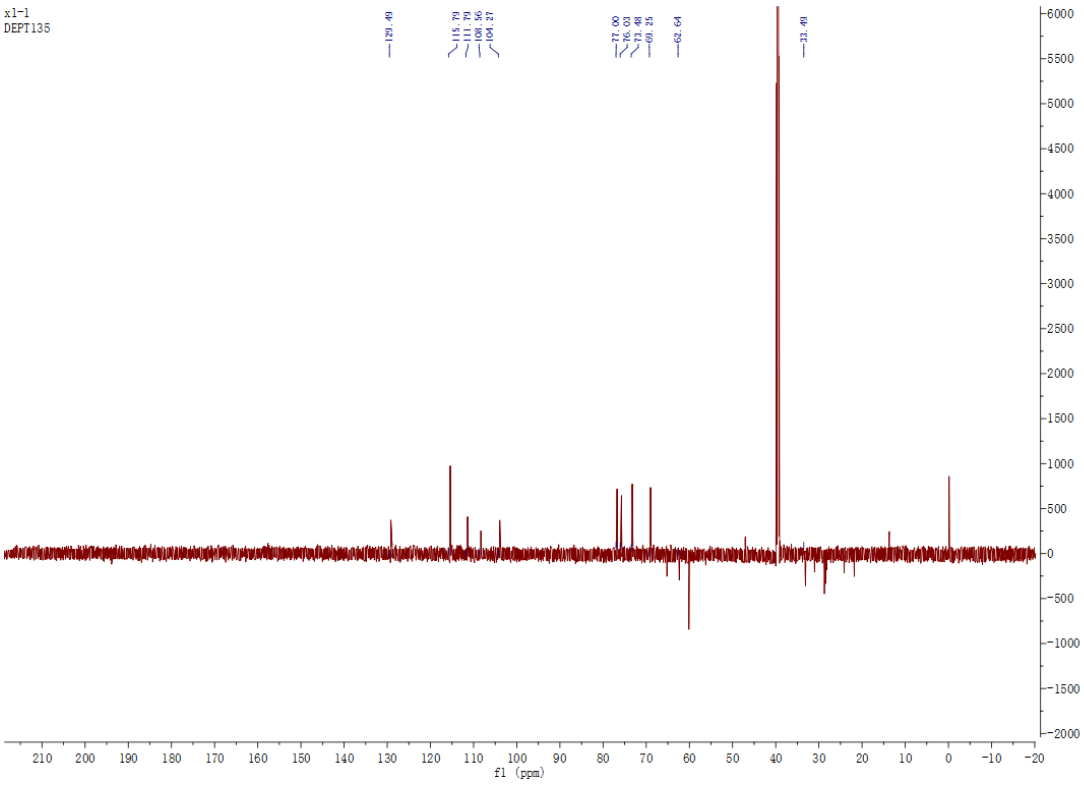


Figure S6. DEPT spectrum of oleraciamide E in DMSO-$d_6$

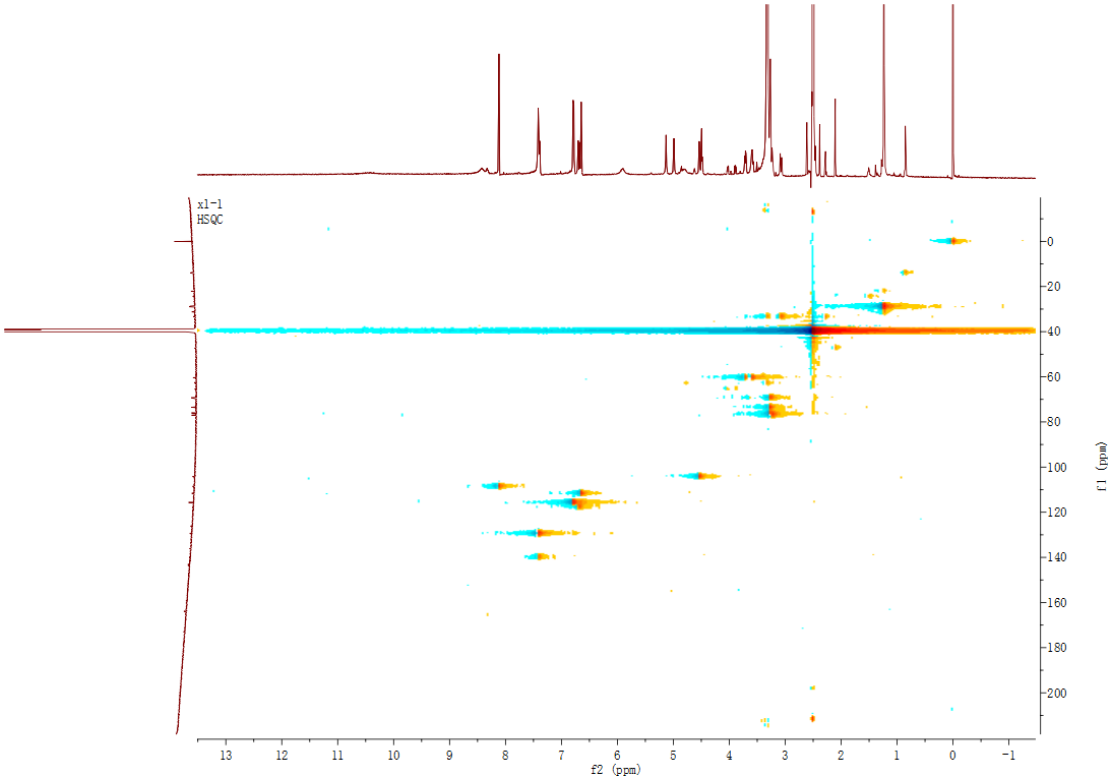


Figure S7. HSQC spectrum of oleraciamide E in DMSO-$d_6$

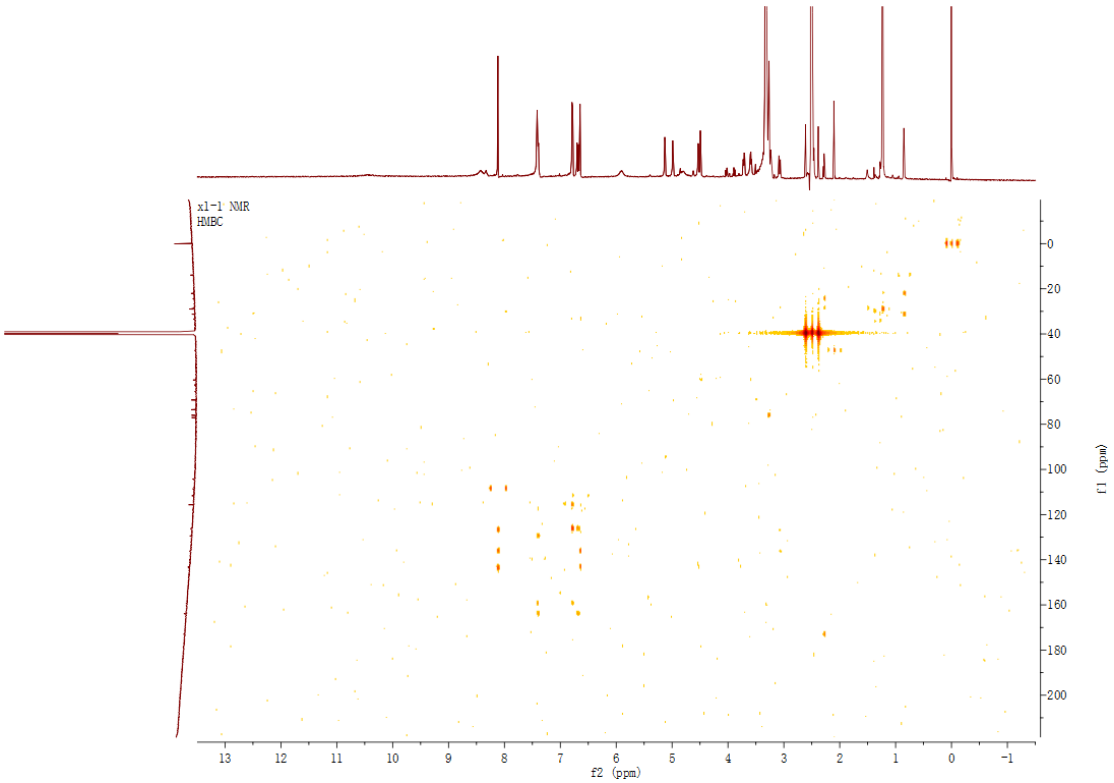


Figure S8. HMBC spectrum of oleraciamide E in DMSO-$d_6$

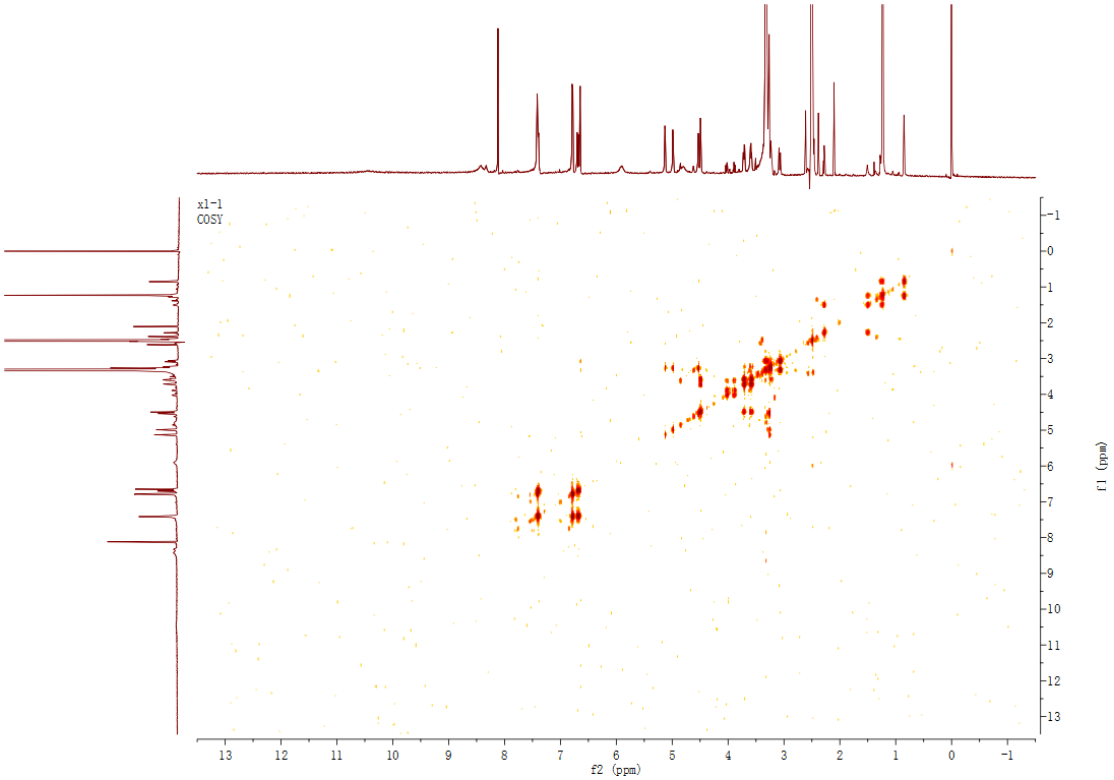


Figure S9. $^{1}$H-$^{1}$H COSY spectrum of oleraciamide E in DMSO-$d_6$

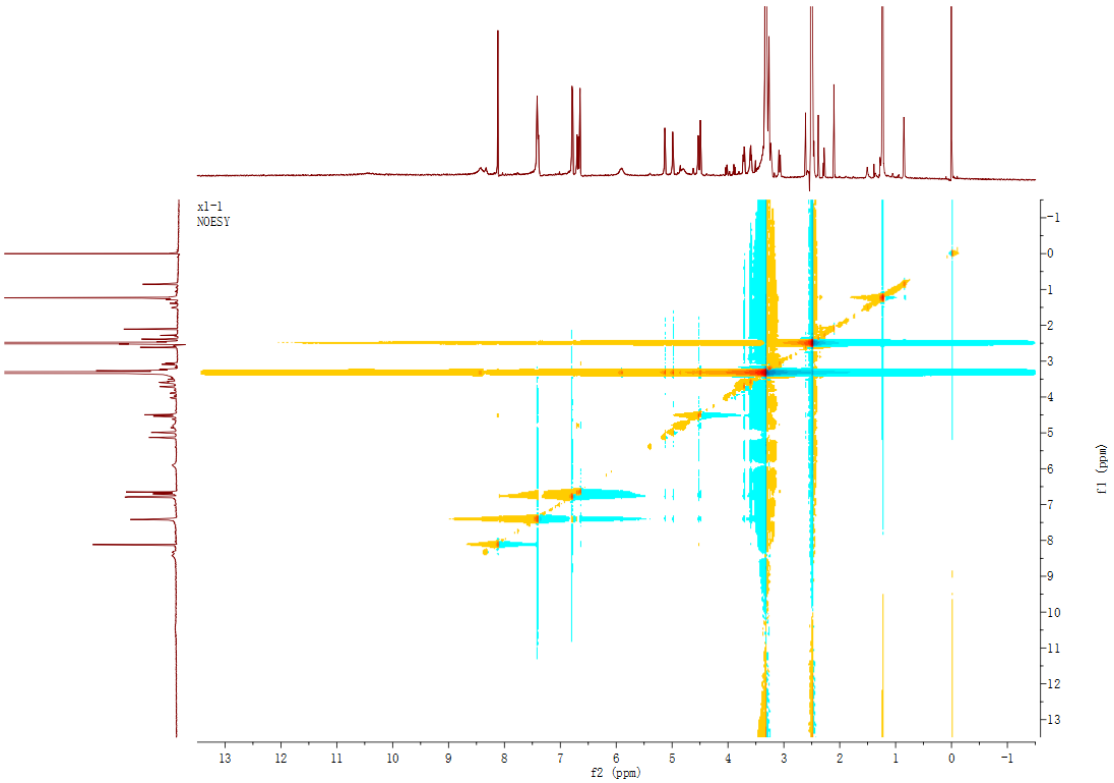


Figure S10. NOESY spectrum of oleraciamide E in DMSO-$d_6$

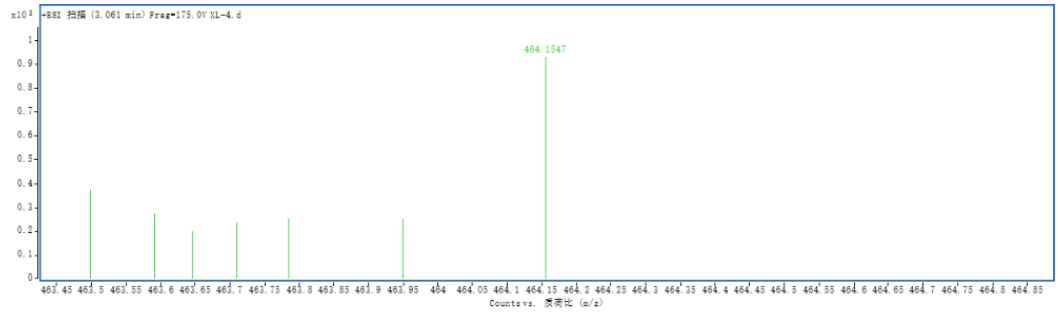


Figure S11. UHPLC-ESI-TOF-MS of oleraciamide E

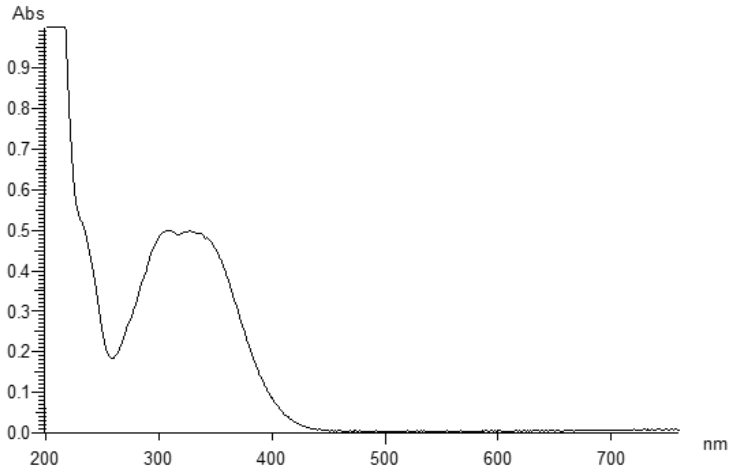


Figure S12. UV spectrum of oleraciamide E in methanol

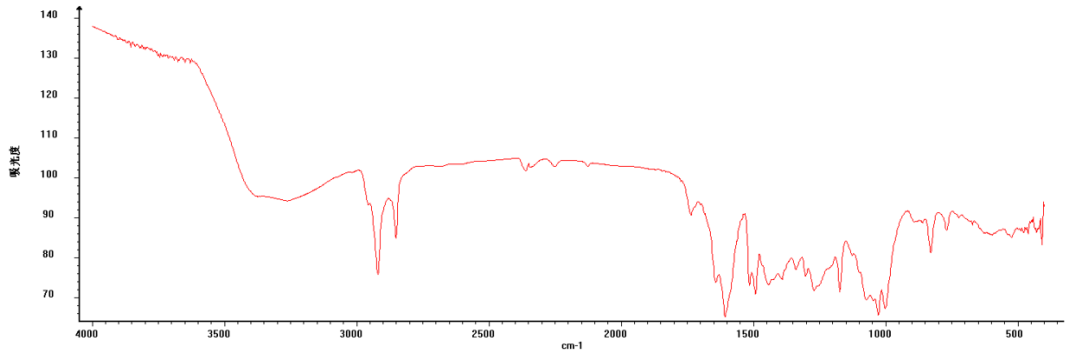


Figure S13. IR (KBr) spectrum of oleraciamide E

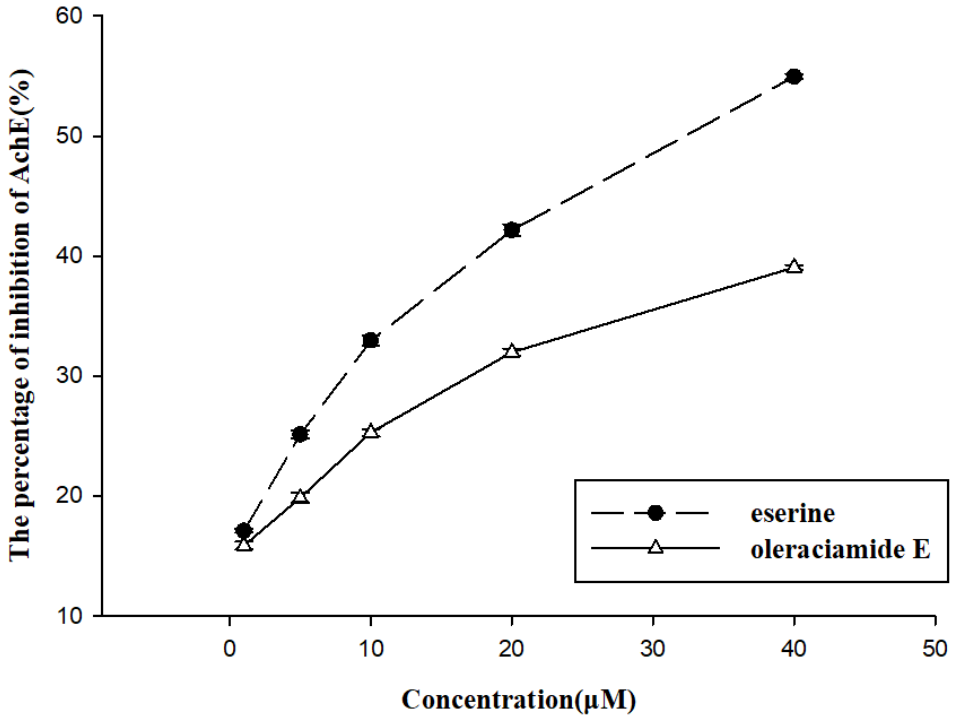


Figure S14. Anticholinesterase effect of oleraciamide E and eserine (n = 5).

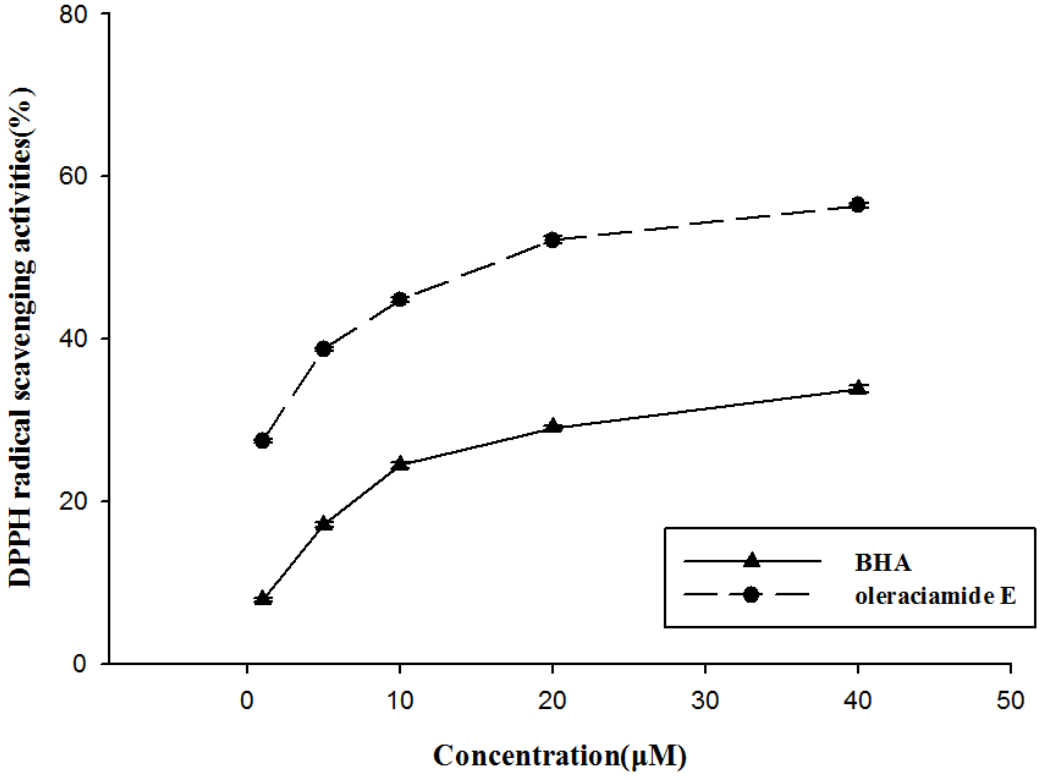


Figure S15. Antioxidant activities of oleraciamide E and BHA (n = 5).

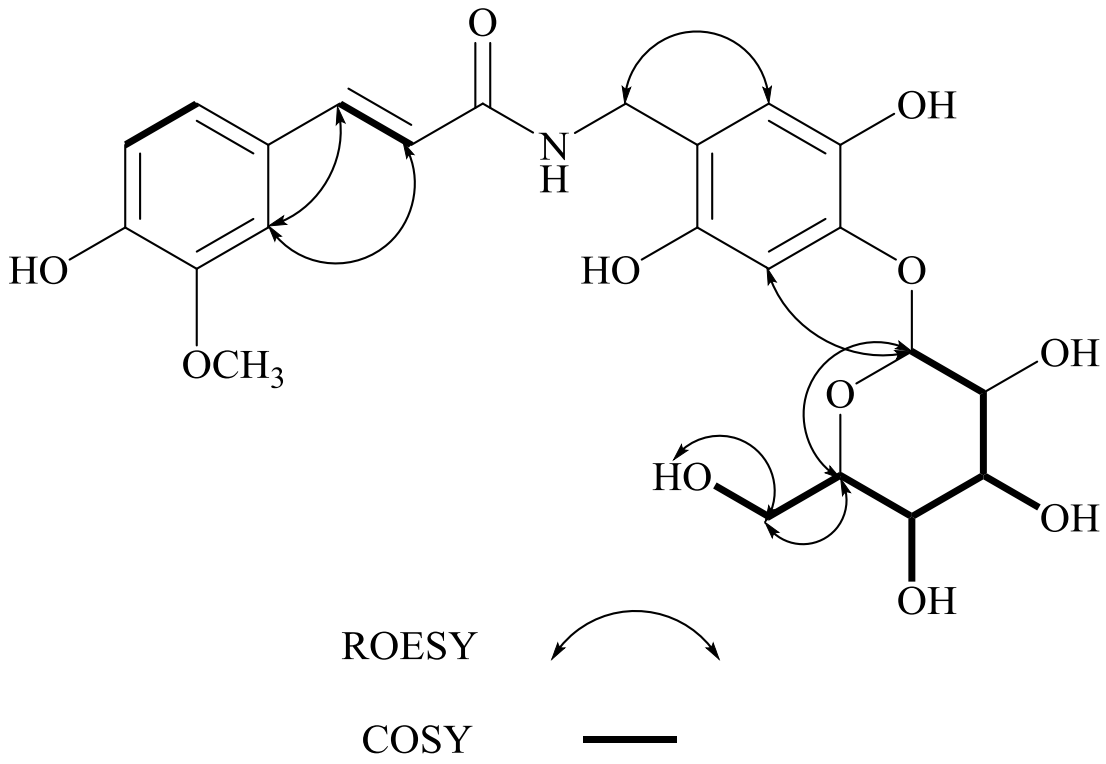


Figure S16. Key $^{1}$H-$^{1}$H COSY and ROESY correlations of oleraciamide F

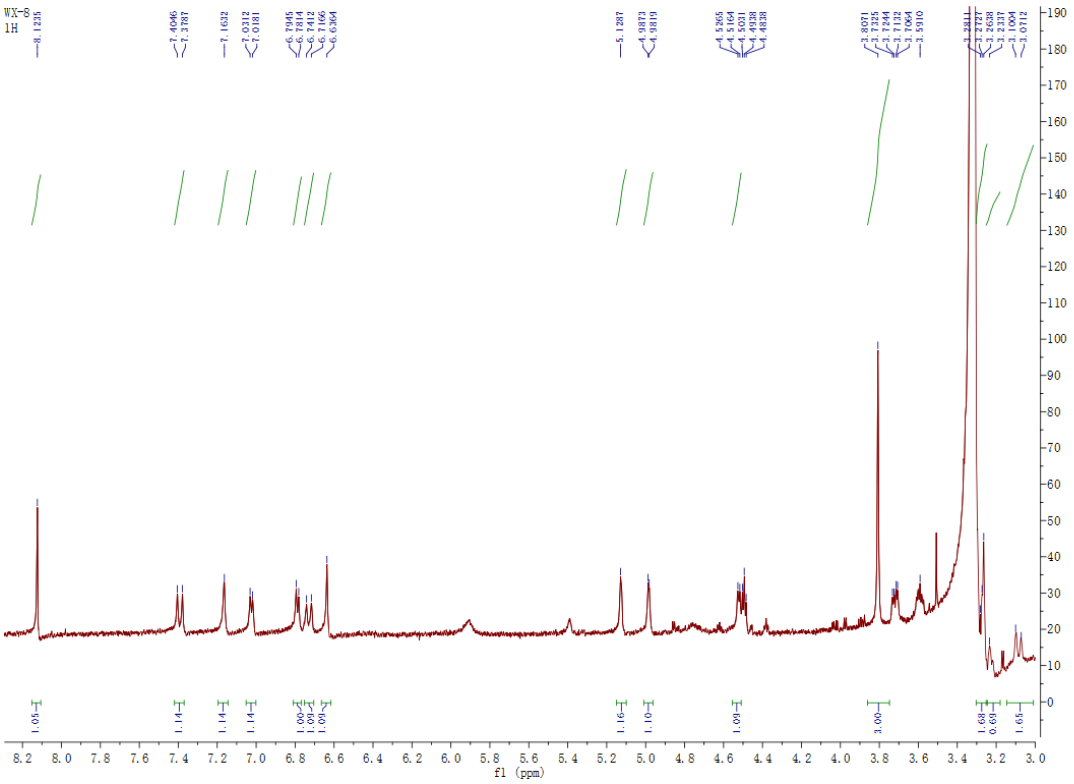


Figure S17. $^{1}$H NMR (600 MHz) spectrum of oleraciamide F in DMSO-$d_6$

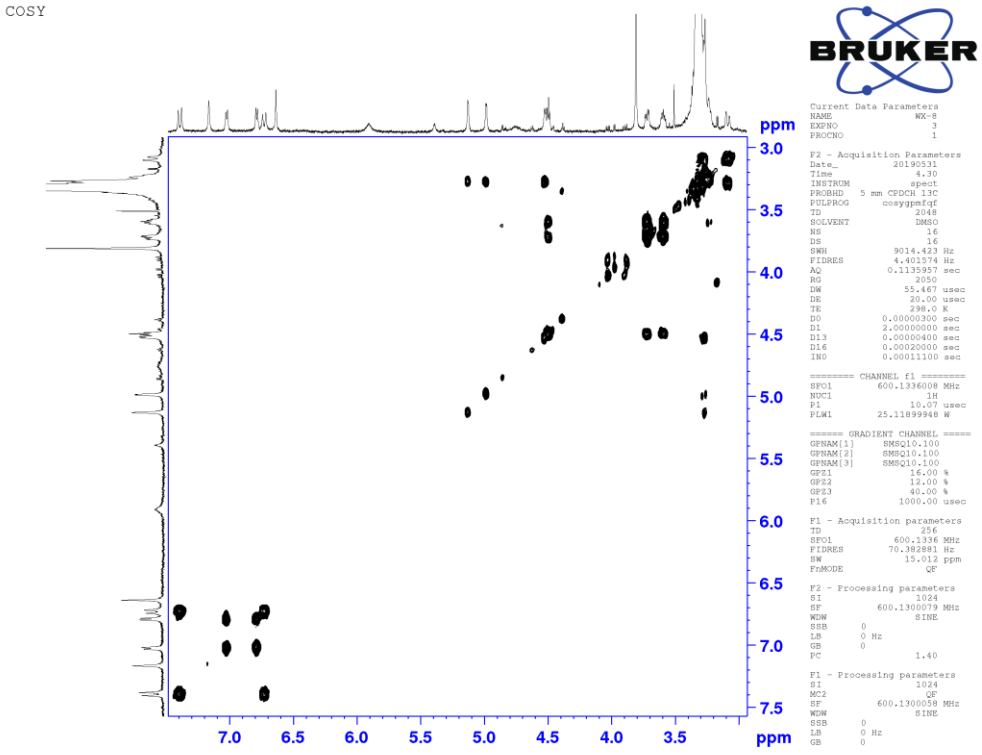


Figure S18. $^{1}$H-$^{1}$H COSY spectrum of oleraciamide F in DMSO-$d_6$

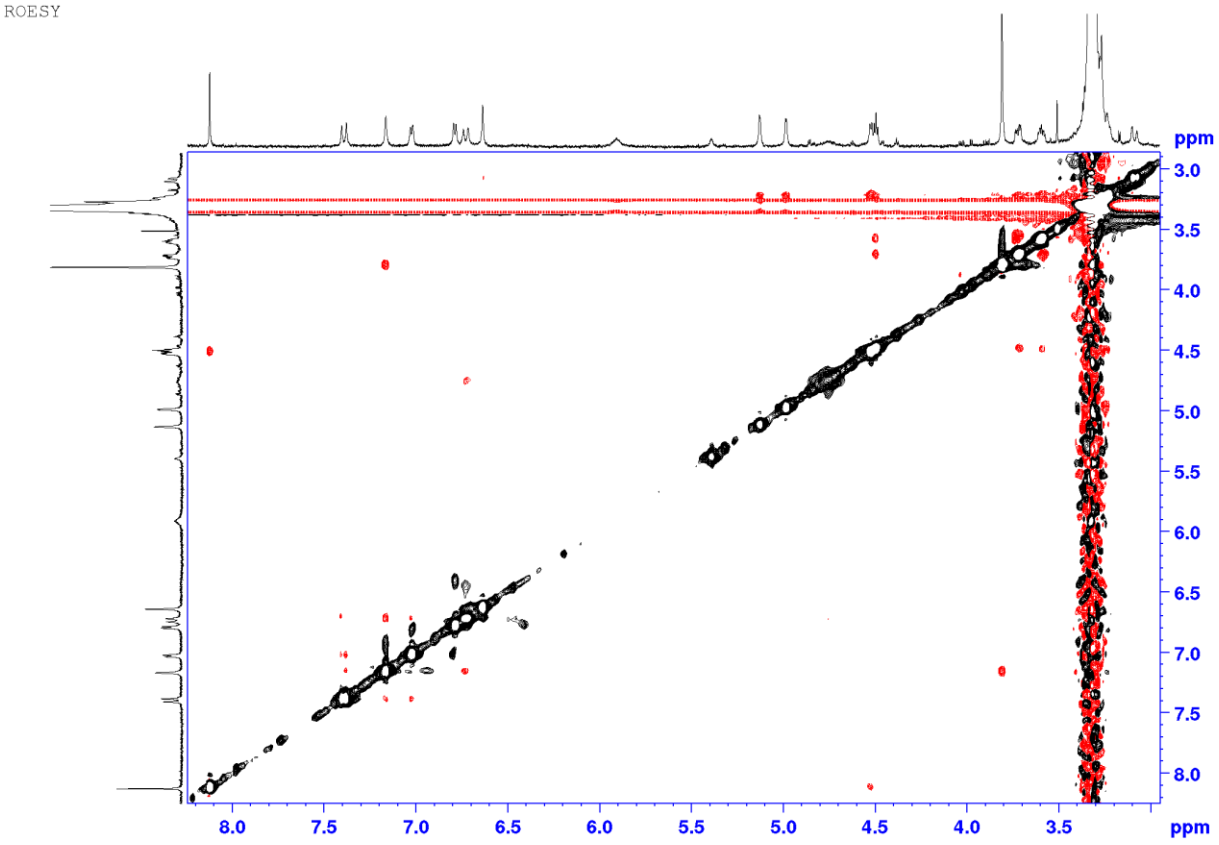


Figure S19. ROESY spectrum of oleraciamide F in DMSO-$d_6$

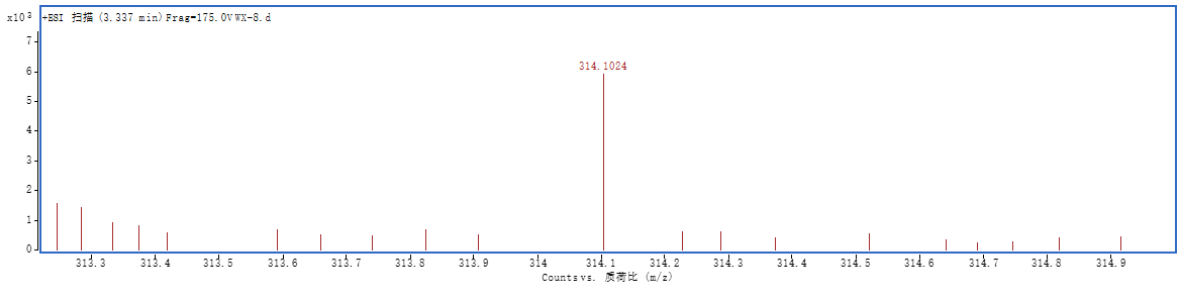


Figure S20. UHPLC-ESI-TOF-MS of oleraciamide F $[M\text{-}C_6H_{12}O_6+H]^+$