Supporting Information

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Promising Natural Polyphenols from Olive (*Olea europaea*) Leaves and Seeds: Dual Benefits in the Prevention of Ultraviolet B-induced Fibroblast Skin Damage and Anti Skin Hyperpigmentation

Asih Gayatri[1], Fahd M Abdelkarem[2], Yhiya Amen[1,3], Masako Matsumoto[1], Maki Nagata[1] and Kuniyoshi Shimizu[1*]

[1]*Department of Agro-Environmental Sciences, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Fukuoka 819-0395, Japan*

[2]*Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut 71524, Egypt*

[3]*Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt*

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Table S1: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for compounds **1** and **2**

NO	Compound **1**		Compound **2**	
	δ_C (ppm)	δ_H (ppm) (mult, *J* in Hz, ΣH)	δ_C (ppm)	δ_H (ppm) (mult, *J* in Hz, ΣH)
1	95.2	5.83 (s, 1H, H-1)	97.8	5.48 (d, 4.2 Hz, 1H, H-1)
3	155.2	7.43 (s, 1H, H-3)	153.4	7.49 (d, 1.7 Hz, 1H, H-3)
4	109.4	Cq	110.3	Cq
5	31.8	3.88 (dd, 9.2, 4.4 Hz, 1H, H-5)	29.3	3.24 (m, 1H, H-5)
6	35.4	2.62 (dd, 14.2, 4.5 Hz, 1H, H-6*α*) 2.35 (dd, 14.1, 9.3 Hz, 1H, H-6*β*)	35.7	2.33 (dd, 16.2, 8.7 Hz, 1H, H-6*α*) 2.89 (dd, 16.2, 3.2 Hz, 1H, H-6*β*)
7	173.2	Cq	174.6	Cq
8	124.9	5.99 (q, 8.2, 7.4 Hz, 1H, H-8)	134.7	5.60 (m, 1H, H-8)
9	130.7	Cq	45.6	2.74 (dt, 9.6, 5.0 Hz, 1H, H-9)
10	13.5	1.57 (d, 7.1 Hz, 3H, H-10)	121.6	5.19 (dd, 8.3, 2.4 Hz, 1H, H-10*α*)/5.18 (d, 1.8 Hz, 1H, H-10*β*)
11	168.7	Cq	167.1	Cq
1"	66.9	4.11 (m, 2H, H-1")	67.0	4.22 (t, 6.9 Hz, 2H, H-1")
2"	41.3	2.67 (t, 7.1 Hz, 2H, H-2")	35.7	2.79 (t, 6.9 Hz, 1H, H-2")
3"	130.5	Cq	131.1	Cq
4"	117.1	6.56 (d, 1.9 Hz, 1H, H-4")	117.3	6.69 (d, 1.9 Hz, 1H, H-4")
5"	146.3	Cq	146.5	Cq
6"	144.9	Cq	145.2	Cq
7"	116.4	6.59 (d, 8.0 Hz, 1H, H-7")	116.7	6.57 (dd, 8.0, 1.9 Hz, 1H, H-7")
8"	121.3	6.45 (dd, 8.0 Hz, 1.9 Hz, 1H, H-8")	121.9	6.72 (d, 8.0 Hz, 1H, H-8")
1'	100.9	4.71 (d, 7.9 Hz, 1H, H-1')	100.3	4.68 (d, 7.9 Hz, 1H, H-1')
2'	74.8	3.27 (m,1H, H-2')	74.9	3.22 (m,1H, H-2')
3'	78.4	3.31 (m, 1H, H-3')	78.7	3.32 (m, 1H, H-3')
4'	71.5	3.20 (m, 1H, H-4')	71.8	3.30 (m, 1H, H-4')
5'	77.9	3.79 (m, 1H, H-5')	78.3	3.39 (m, 1H, H-5')
6'	62.7	3.57 (m, 1H, H-6')	63.0	3.92 (bd, H-6')
O-CH_3	51.9	3.62 (s, 3H)	52.0	3.65 (s, 3H)

[a]; dissolved in CD_3OD, ^{1}H NMR (600 MHz and ^{13}C NMR (150 MHz)).
δ values are expressed in ppm and coupling constants (*J*) in Hz
Cq: Quaternary carbon

Compounds Identification

Compound **1** was isolated as a yellow powder, $[\alpha]^{24}_{D}$: −124.68 (c 0.001, MeOH), and its molecular formula was determined as $C_{25}H_{32}O_{13}$ based on the observed peaks at (*m/z* 539.1786 $[M-H]^-$, calcd for 539.1765) and (*m/z* 575.1526 $[M+Cl]^-$, calcd for 575.1547) in the negative HR-ESI-MS, together with 1D and 2D NMR spectroscopy. The ^{1}H-NMR spectrum displayed three aromatic protons at δ_H 6.56 (d, *J* = 1.9 Hz, 1H, H-4") 6.59 (d, *J* = 8.0 Hz, 1H, H-7"), and 6.45 (dd, *J* = 8.0 Hz, *J* = 1.9 Hz, 1H, H-8"). In addition, the presence of a proton signal at δ_H 4.71 (d, *J* = 7.9 Hz, 1H, H-1'), which correlates with a carbon signal at δ_C 100.9 (C-1') trough HSQC, indicates the existence of an anomeric proton. Furthermore, the presence of oxygenated methylenegroup was confirmed by the proton resonance signal observed at δ_H 4.11 (m, 2H, H-1"). This was further corroborated by the carbon spectrum, which displayed a signal at δ_C 66.9, assigned to (C-1", CH_2), analyzed by HSQC data. On the other hand, the olefinic carbon at C-8, CH (124.9) was deduced from the corresponding proton signal observed at δ_H 5.99 (q, *J* = 8.2, 7.4 Hz, 1H, H-8) through detailed analysis of the HSQC. The signal indicates the presence of a singlet proton at 7.43 ppm (H-3) attributed to the vinyl hydrogen and connected to a carbon bearing an oxygen atom, confirmed with HSQC correlation. One singlet methoxyl proton appeared at δ_H 3.62 (OCH_3, s, 3H) and the methoxylated carbon signal at δ_C 51.9. One methyl at δ_H 1.57 (d, *J* = 7.1 Hz, 3H, H-10) and linked to the carbon signal at δ_C 13.5 (C-10, CH_3) based on HSQC evaluation. Then, the sugar protons were determined in the area at δ_H 3.20-4.71.

In the HMBC spectrum, the anomeric proton signal at δ_H 4.71 (d, *J* = 7.9 Hz, 1H, H-1') showed key correlations with the olefinic carbons at δ_C 124.9 (C-8, CH) and δ_C 130.7 (C-9, C) and 95.2 (C-1, CH). These correlations clearly indicated that the glycosidic linkage at C-1' is connected to the elenolic acid moiety, a characteristic secoiridoid structure. Furthermore, the characteristic correlation between oxygenated methylene proton at δ_H 4.11 (m, 2H, H-1") with carbon at δ_C 35.4 (C-6, CH_2), 173.2 (C-7, C) and 130.7 (C-9, C) showed that the hydroxytyrosol ring was connected to elenolic acid. Based on the comprehensive spectroscopic data outline above, compound **1** was identified as oleuropein (Figure 4) in the main text. These findings align well with previously published data [1, 2] confirming the identity of the compound.

Compound **2** was purified as a yellow powder, $[\alpha]^{24}_{D}$: −26.70 (c 0.0005, MeOH). Its molecular formula was $C_{25}H_{32}O_{13}$, deduced from the detected peaks at *m/z* 539.1782 $[M-H]^-$, calcd for 539.1765 and (*m/z* 575.1526 $[M+Cl]^-$, calcd for 575.1545), along with 1D and 2D NMR spectroscopy. The 1D (^{1}H-NMR & ^{13}C-NMR) and 2D (HSQC, HMBC, & ^{1}H-^{1}H COSY) confirmed the existence of hydroxytyrosol, secoiridoid, and sugar moiety. The ^{1}H-NMR data showed the spectral peaks at δ_H 6.57 (dd, *J* = 8.0 Hz, *J* =1.9 Hz, 1H, H-7"), 6.72 (d, *J* = 8.0 Hz, 1H, H-8") and 6.69 (d, *J* = 1.9 Hz, 1H, H-4") which entails the ABX system of ortho and meta substitutions in an aromatic ring. The other two resonances were assigned to the protons of the ethyl chain, with signals observed at δ_H 2.79 (t, *J* = 6.9 Hz, 1H, H-2") and 4.22 (t, *J* = 6.9 Hz, 1H, H-1"), respectively. All the aforementioned spectroscopic data collectively indicated the presence of a hydroxytyrosol moiety within the structure. In addition, the presence of elenolic acid (secoiridoid) ring was detected at δ_H 3.65 (3H, s, 11-OCH_3) as methyl ester group, one methylene at δ_H 2.33 (dd, *J* = 16.2, *J* = 8.7 Hz, 1H, H-6*α*) and 2.89 (dd, *J* = 16.2, *J* = 3.2 Hz, 1H, H-6*β*), two methines at δ_H 3.24 (m, 1H, H-5) and 2.74 (dt, *J* = 9.6, *J* = 5.0 Hz, 1H, H-9), 7.49 (d, *J* = 1.7 Hz, 1H, H-3), and one olefinic proton at δ_H 5.19 (dd, *J* = 8.3, *J* = 2.4 Hz, 1H, H-10*α*) and 5.18 (d, *J* = 1.8 Hz, 1H, H-10*β*). The presence of a vinyl hydrogen was confirmed by the singlet proton at δ_H 7.49 (H-3) and carbon signal at δ_c 153.4 (C-3, CH). In the aliphatic region of the ^{1}H-NMR spectrum, the signal corresponding to the glucosyl moiety was detected at δ_H 3.92-4.68. In combination with the ^{13}C NMR data, it showed for two carbonyl groups at δ_C 174.6 (C-7, C) and 167.1 (C-11, C), successively. In addition, a presence of two oxygenated quaternary carbons at δ_C 146.5 (C-5", C) and 145.2 (C-6", C), respectively. The ^{13}C NMR also exhibited a methoxylated carbon signal at δ_C 52.0.

The presence of olefinic proton signal at δ_H 5.19 (dd, *J* = 8.3 Hz, *J* = 2.4 Hz, 1H, H-10*α*) and 5.18 (d, *J* = 1.8 Hz, 1H, H-10*β*) linked to the δ_C 121.6 (C-10) was assigned by HSQC spectrum. In addition, the correlation between H-2" (2.79, t, 6.9 Hz) with 116.7 (C-7", CH), 121.9 (C-8", CH), and 131.1 (C-

3", C) confirmed that there was a hydroxytyrosol ring. The HMBC correlation of H-1' (4.68, d, J = 7.9 Hz) with C-1, CH (97.8) showed that the sugar moiety was linked to an elenolic acid ring. It was also confirmed with ^{1}H-^{1}H COSY correlation between H-9 and H-1. Furthermore, the linkage between a hydroxytyrosol ring and elenolic acid was evaluated with HMBC correlation of H-4" (6.69, d, J = 1.9 Hz) and C-6, CH_2 (35.7).

Moreover, a subtle distinction between compounds **1** and **2** was observed in the positioning of the olefinic proton on the olenolic acid ring. In the compound **1**, the olefinic carbons was between C-8, CH and C-9, CH proton signal appeared at 5.60 (H-8). Meanwhile, on compound **2**, the spectrum of olefinic proton was terminal between C-8, CH and C-10, CH_2 (Table S2). The observed correlations between H-8/H-9, and H-8/H-10 in the ^{1}H-^{1}H COSY spectrum (Figure S10) confirmed these suggestions. Based on a forementioned data and upon comparison with reported data, compound **2** was identified as oleuroside (Figure 4) [3].

Compound **3** was isolated as a yellow powder, $[\alpha]^{24}_{D}$: + 2.35 (c 0.001, MeOH), and its molecular formula was determined as $C_{20}H_{22}O_6$ by HR-ESI-MS, *m/z* 357.1346 $[M-H]^-$, calcd 357.1338. The ^{1}H NMR (CD_3OD, 400 MHz) δ_H 6.97 (2H, d, J = 1.9 Hz, H-2, H-2'), 6.83 (2H, dd, J = 8.1, J = 1.9 Hz, H-6, H-6'), 6.79 (2H, d, J = 8.1 Hz, H-5, H-5'), 4.73 (2H, d, J = 4.2 Hz, H-7, H-7'), 3.17 (1H, m, H-8), 3.33 (1H, dt, 1.6 Hz, H-8'), 4.25 (2H, dd, J = 6.9, J = 9.0 Hz, H-9, H-9'), 3.88 (6H, s, 3, 3'-OCH_3) attached to C-3, 3'. The ^{13}C NMR (CD_3OD, 100 MHz) δ_C 111.0 (C-2, C-2', CH), 116.1 (C-5, C-5', CH), 120.1 (C-6, C-6', CH), 87.5 (C-7, C-7', CH), 55.4 (C-8, C-8', CH), 72.6 (C-9, C-9', CH_2), 133.8 (C-1, C-1', C), 149.1 (C-3, C-3', C), 147.3 (C-4,C-4', C), and 56.4 (O-CH_3). Compound **3** was identified as pinoresinol (Figure 4) and compared with previous data [4].

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[1]. N. Genc, I. Yildiz, R. Chaoui, R. Erenler, C. Temiz and M. Elmastas (2020). Biosynthesis, characterization and antioxidant activity of oleuropein-mediated silver nanoparticle. *Inorganic and Nano-Metal Chemistry*. **51**, 411-419

[2]. S. Christophoridou, P. Dais, L. I. H. Tseng and M. Spraul (2005). Separation and identification of phenolic compounds in olive oil by coupling high-performance Liquid Chromatography with Postcolumn Solid-Phase Extraction to Nuclear Magnetic Resonance Spectroscopy (LC-SPE-NMR). *Journal of Agricultural and Food Chemistry*. **53**, 4667–4679.

[3]. H. Kuwajima, T. Uemura, T. Takaishi, K. Inoue and H. A. Ino-uye (1988). A secoiridoid glucoside from Olea europaea. *Phytochemistry*. **27**,1757–1759.

[4]. Z. Yue, H. Qin, Y. Li, Y. Sun, Z. Wang, T. Yang, L. Liu, M. Wang, F. Feng and Q. Mei (2013). Chemical constituents of the root of jasminum giraldii. 2013. *Journal of Molecules,* **18**, 4766–4775

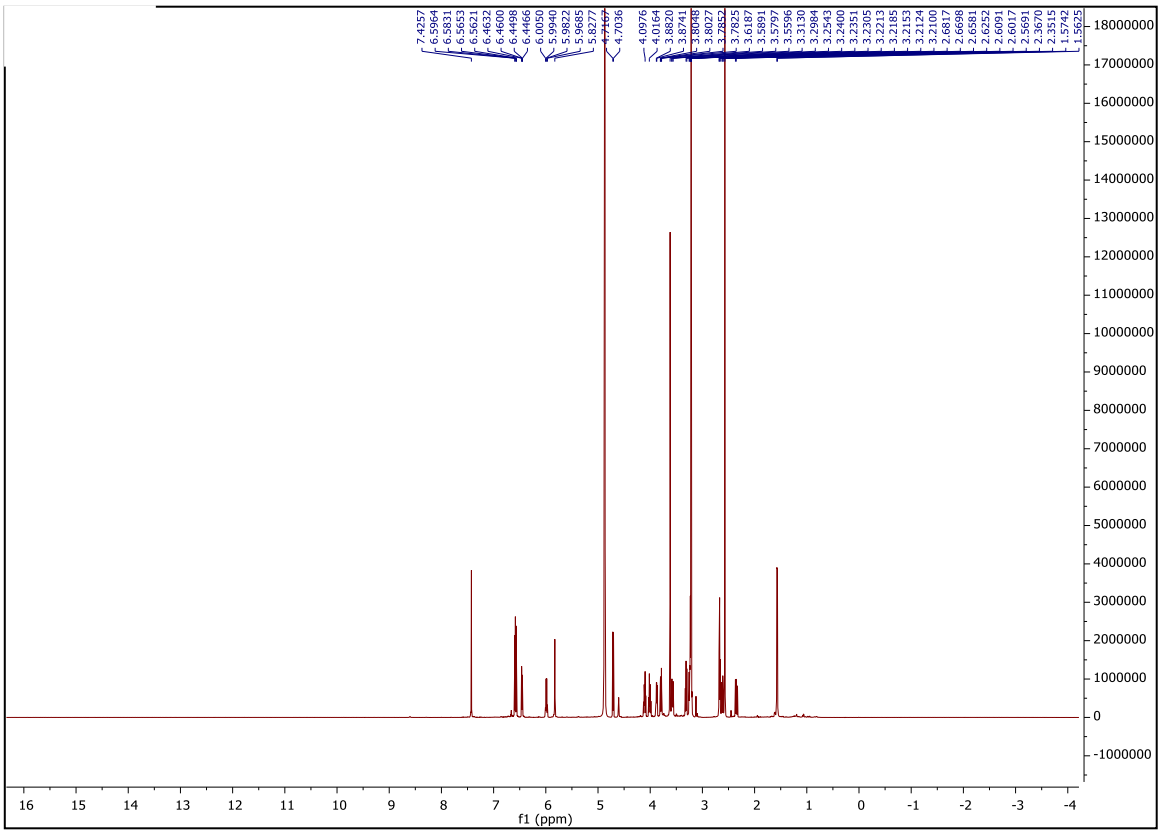

Figure S1: The ^{1}H-NMR spectrum of compound **1** (Oleuropein) in CD_3OD (600 MHz)

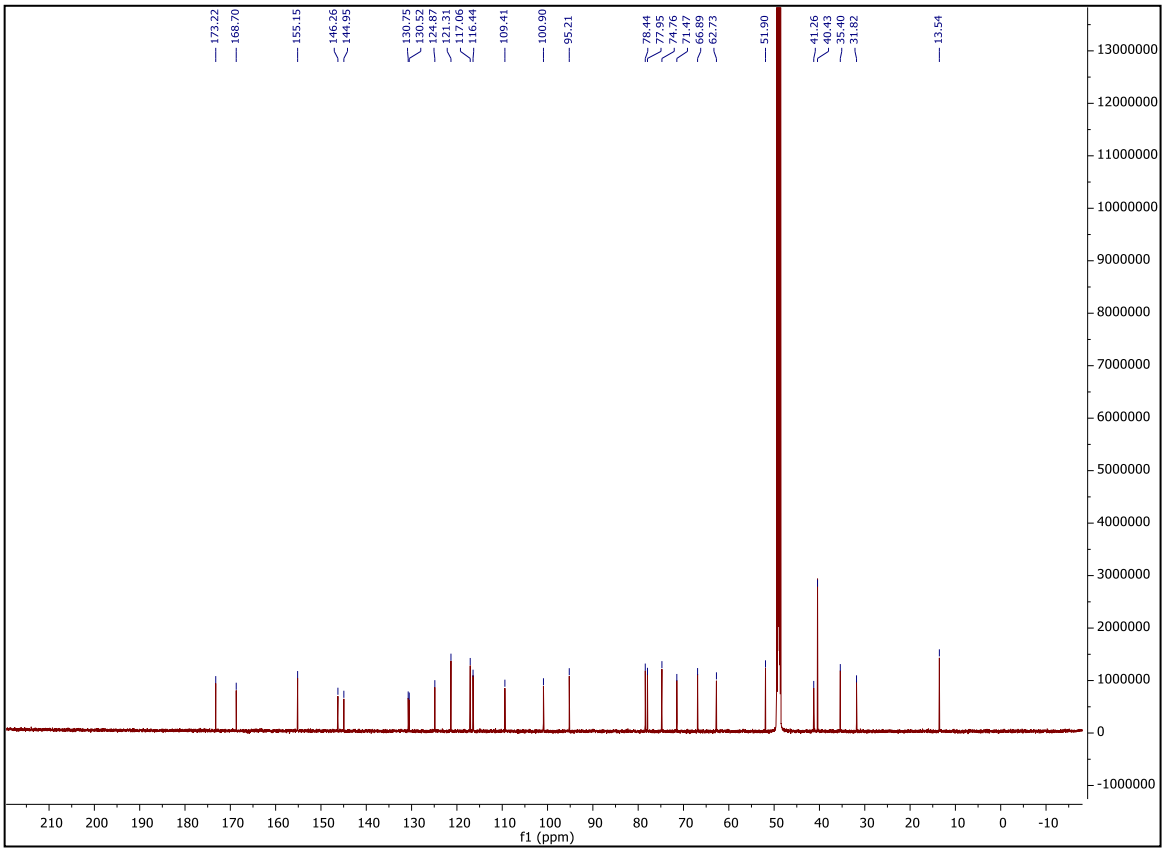

Figure S2: The ^{13}C-NMR spectrum of compound **1** (Oleuropein) in CD_3OD (150 MHz)

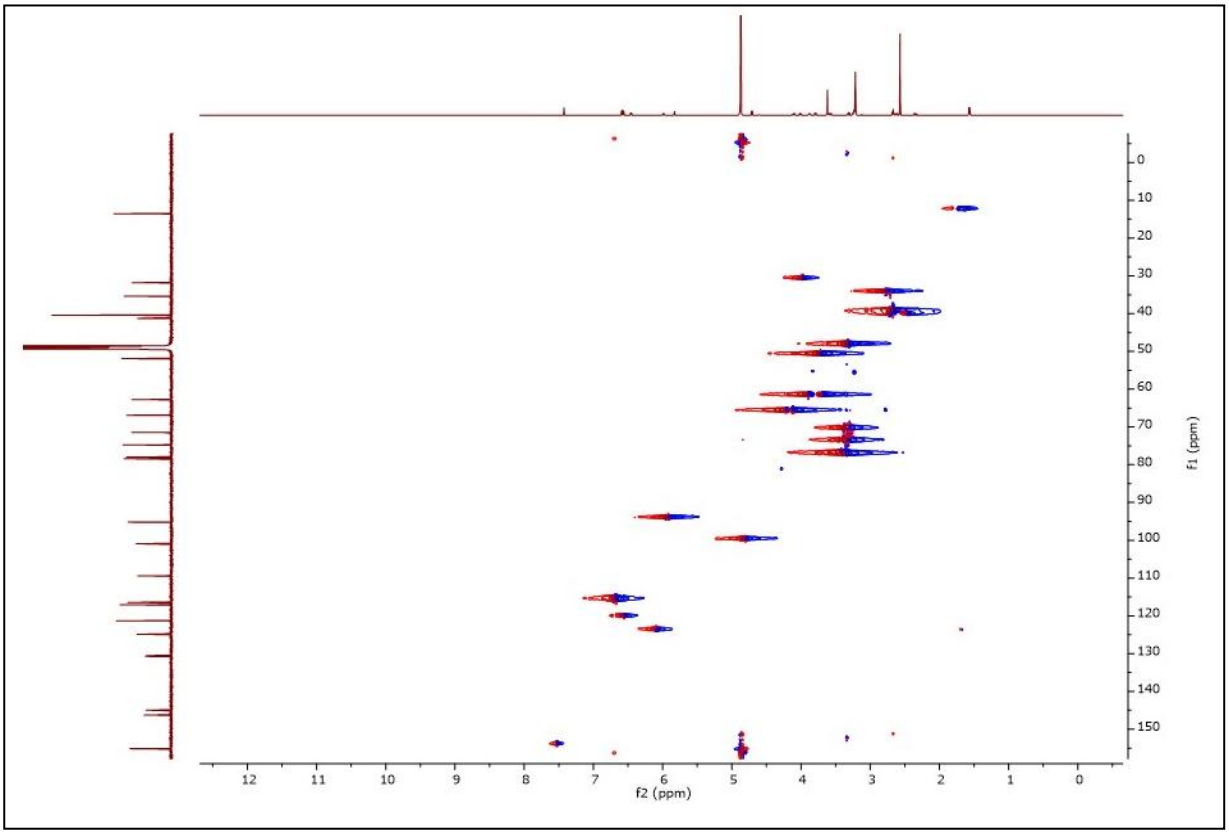

Figure S3: The HSQC spectrum of compound **1** (Oleuropein)

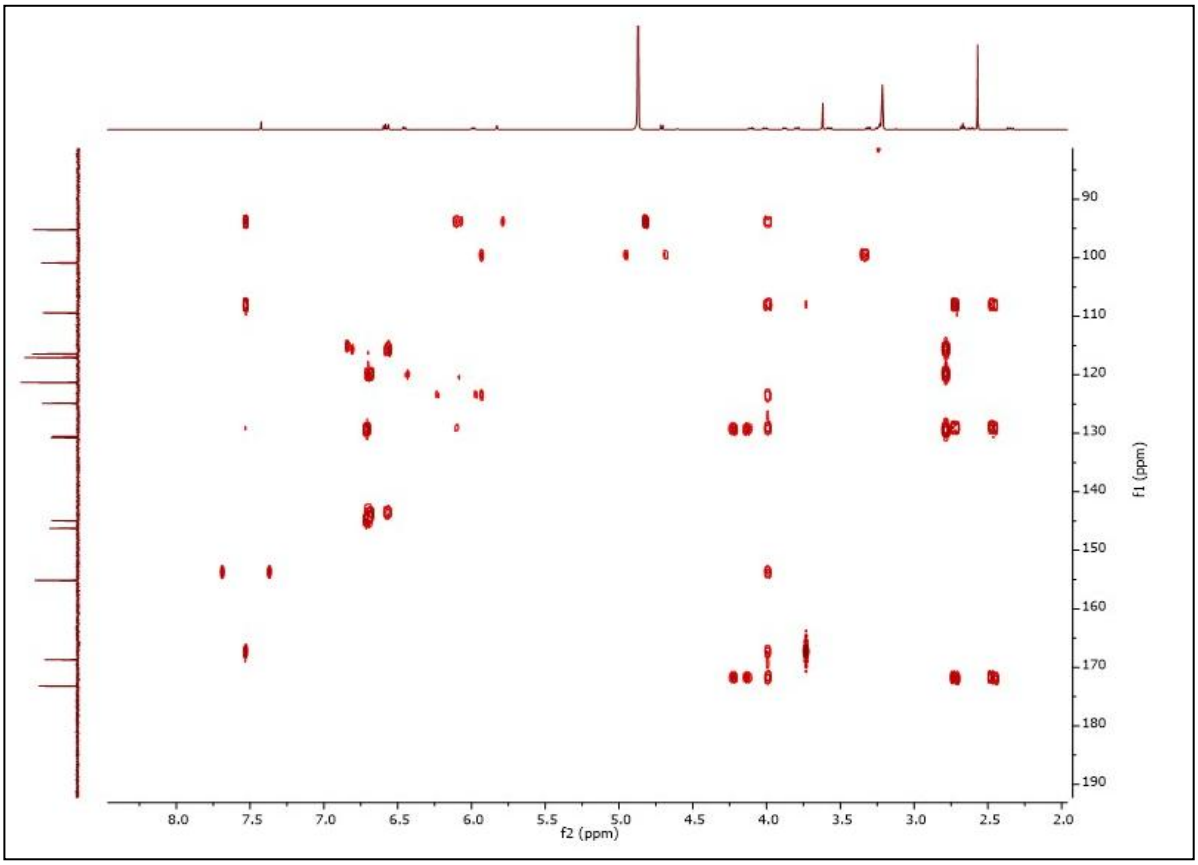

Figure S4: The HMBC spectrum of compound **1** (Oleuropein)

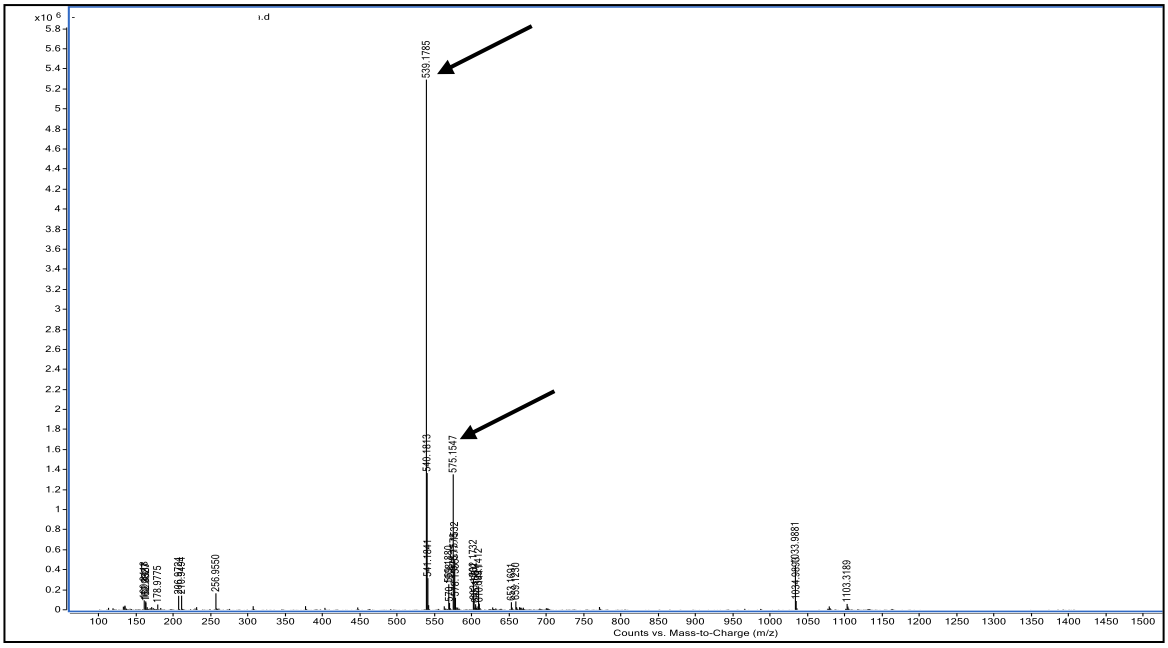

Figure S5: The HRESI-MS data of compound **1** (Oleuropein)

Molecular formula :	Observed :	Calculated :	Adduct Ion:	Error ppm:
$C_{25}H_{32}O_{13}$	539.1786	539.1765	$[M-H]^-$	3.9

Molecular formula :	Observed :	Calculated :	Adduct Ion:	Error ppm:
$C_{25}H_{32}O_{13}$	575.1547	575.1526	$[M+Cl]^-$	3.7

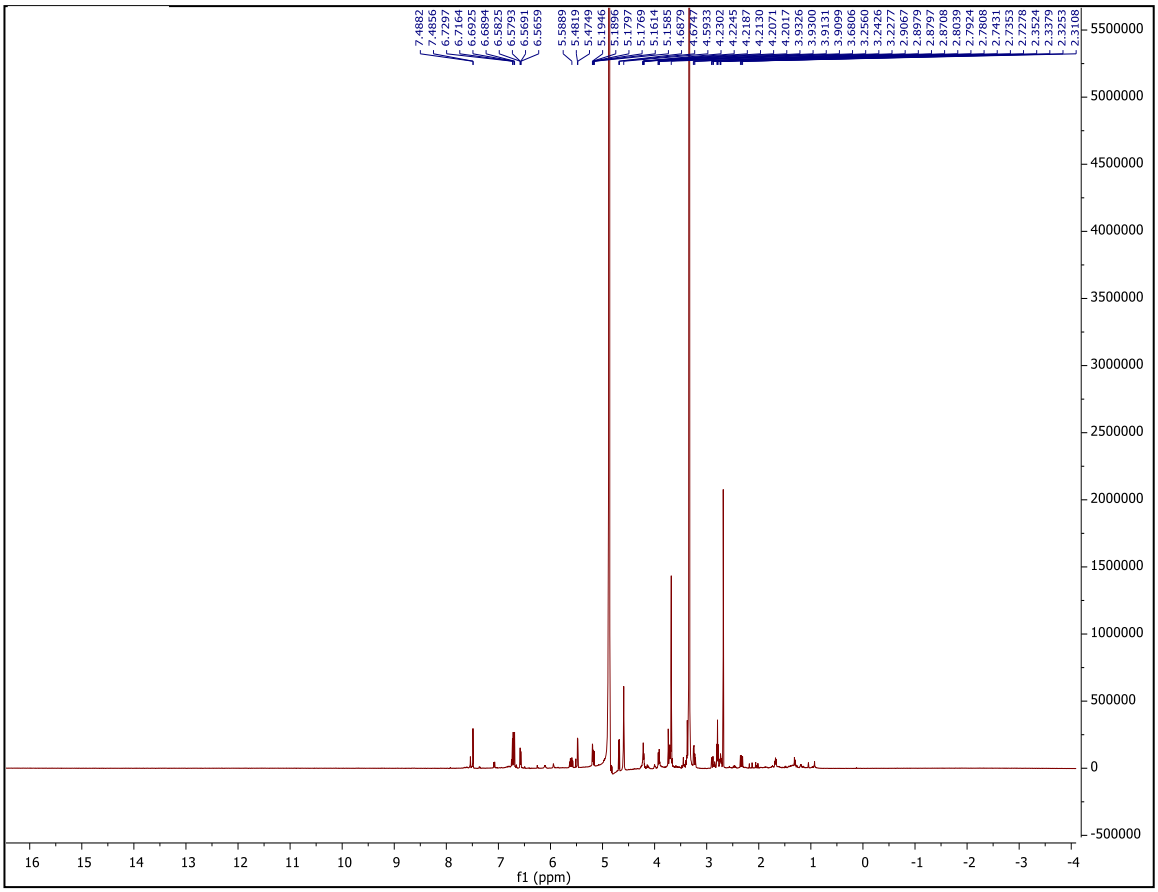

Figure S6: The ^{1}H-NMR spectrum of compound **2** (Oleuroside) in CD_3OD (600 MHz)

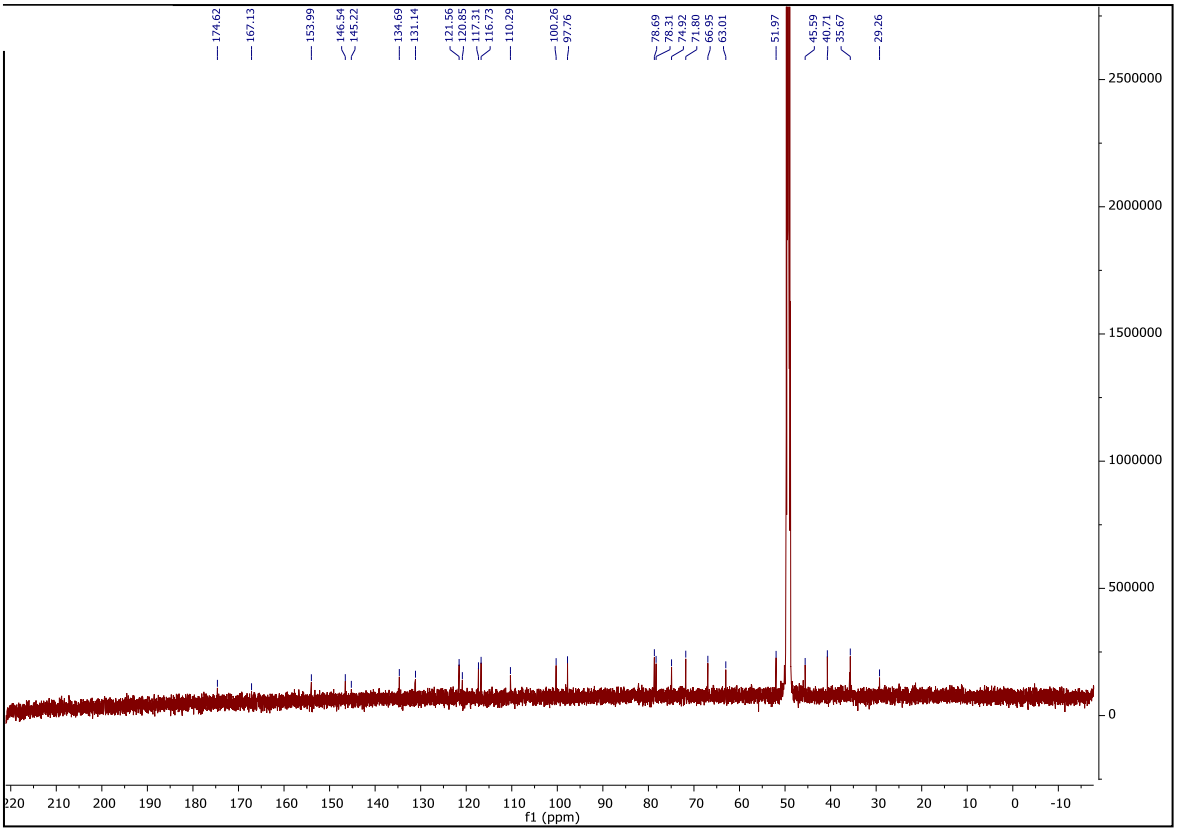

Figure S7: The ^{13}C-NMR spectrum of compound **2** (Oleuroside) in CD_3OD (150 MHz)

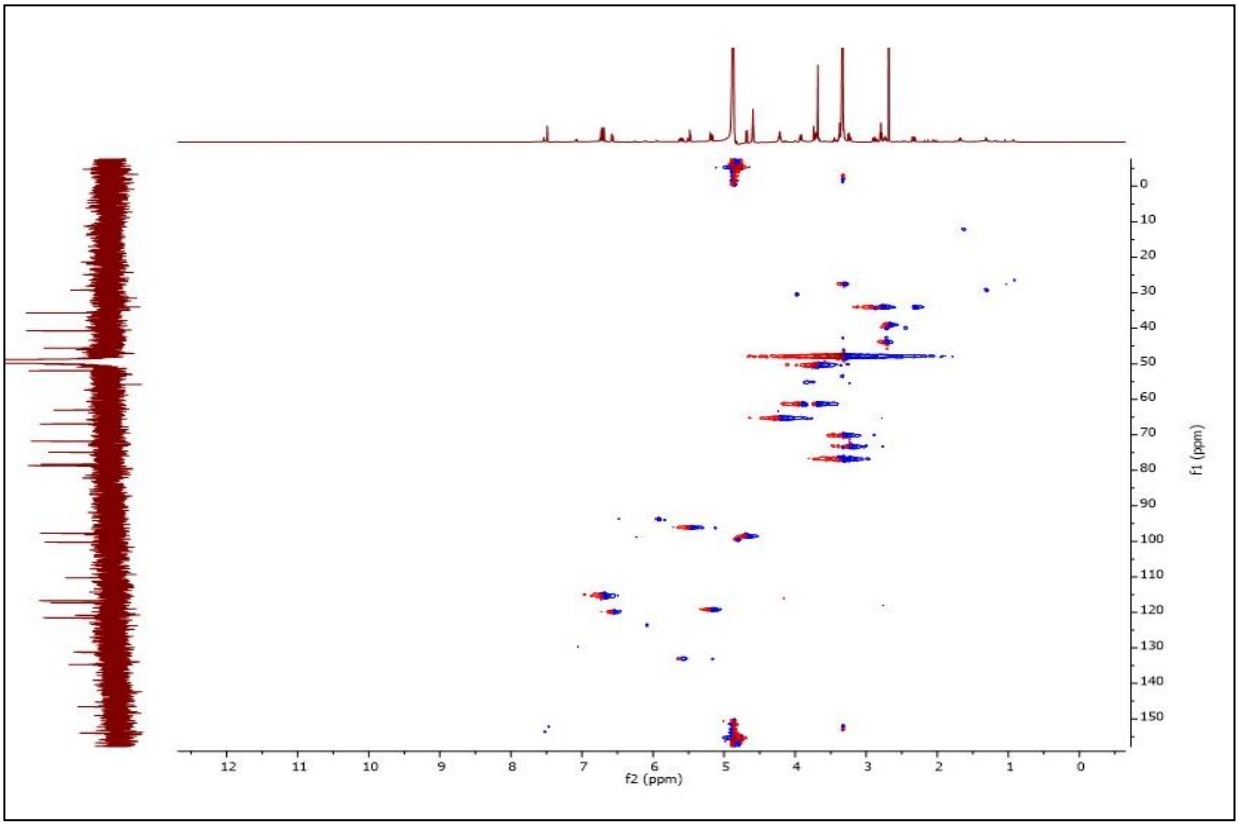

Figure S8: The HSQC spectrum of compound **2** (Oleuroside)

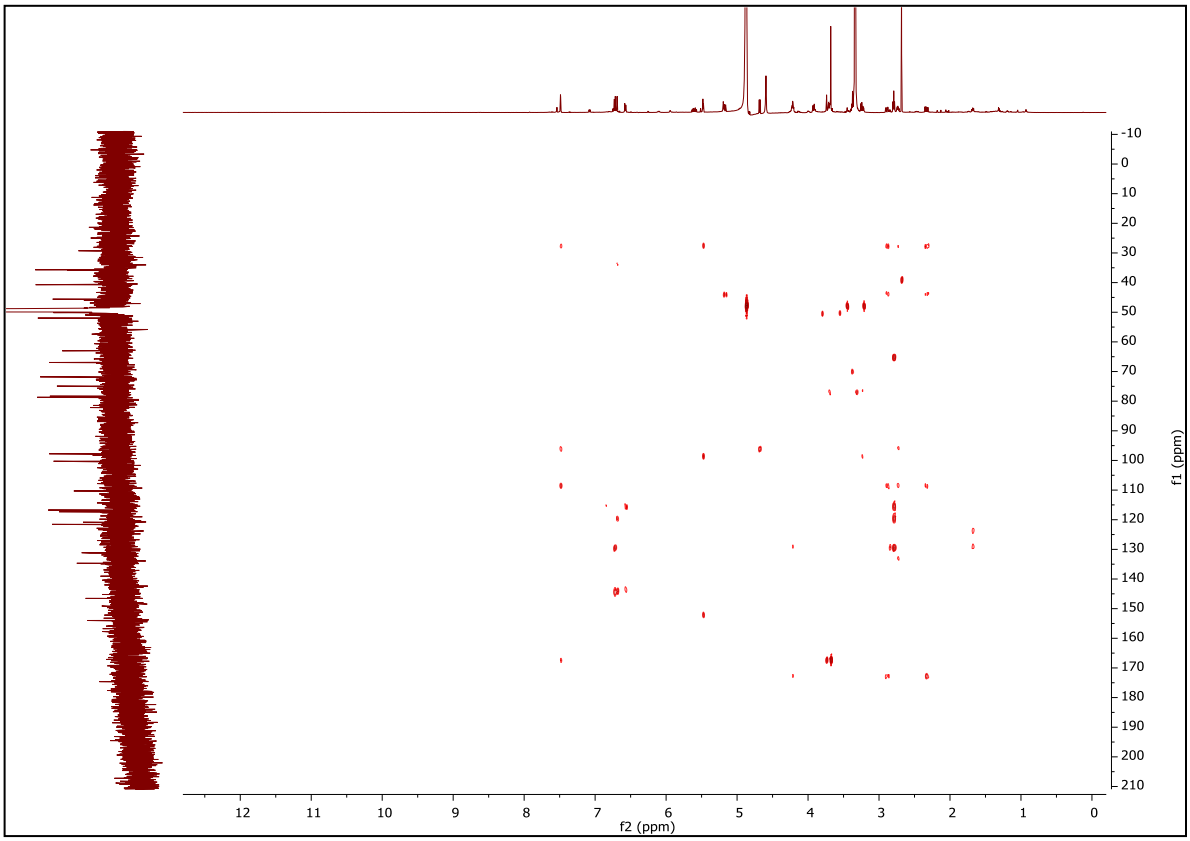

Figure S9: The HMBC spectrum of compound **2** (Oleuroside)

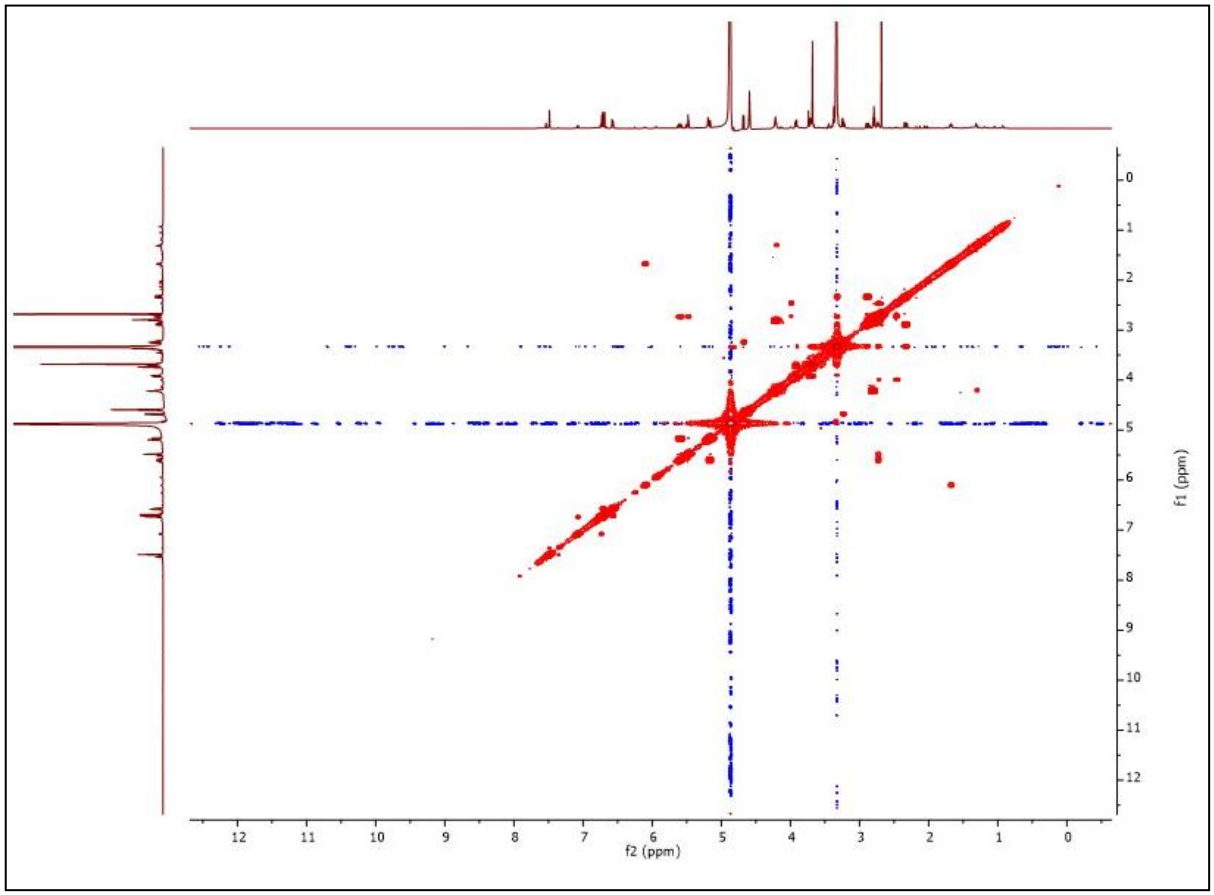

Figure S10: The ^{1}H-^{1}H COSY spectrum of compound **2** (Oleuroside)

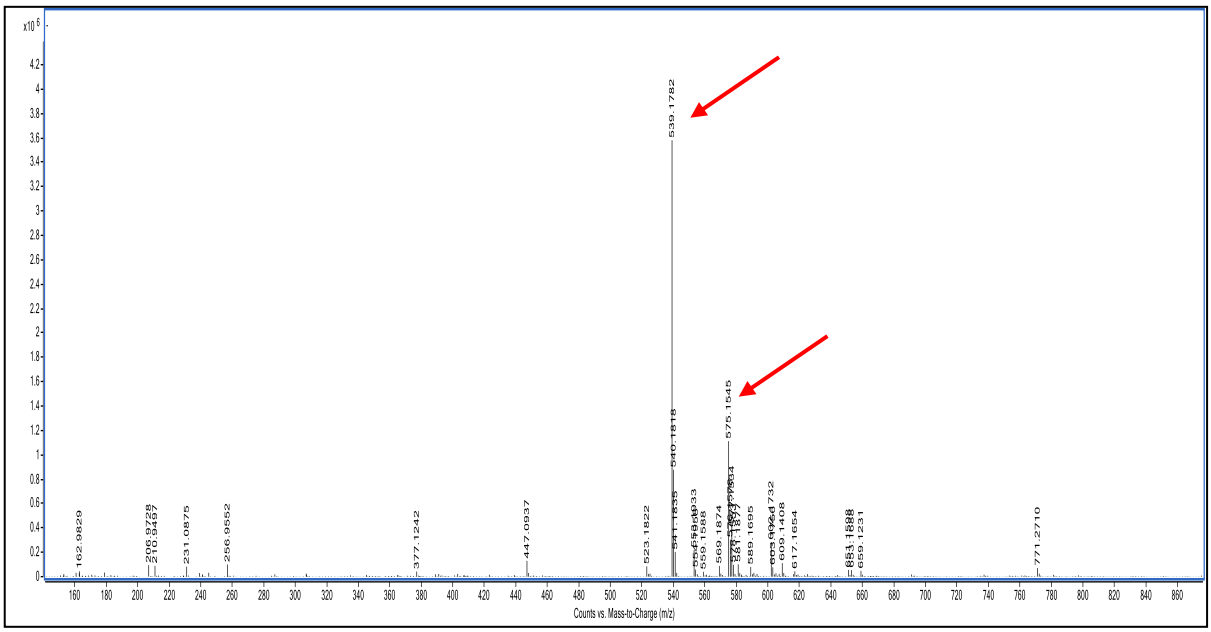

Figure S11: The HRESI-MS data of compound **2**

Molecular formula :	Observed :	Calculated :	Adduct Ion :	Error ppm:
$C_{25}H_{32}O_{13}$	539.1782	539.1765	$[M-H]^-$	3.2

Molecular formula :	Observed :	Calculated :	Adduct Ion:	Error ppm:
$C_{25}H_{32}O_{13}$	575.1545	575.1526	$[M+Cl]^-$	3.3

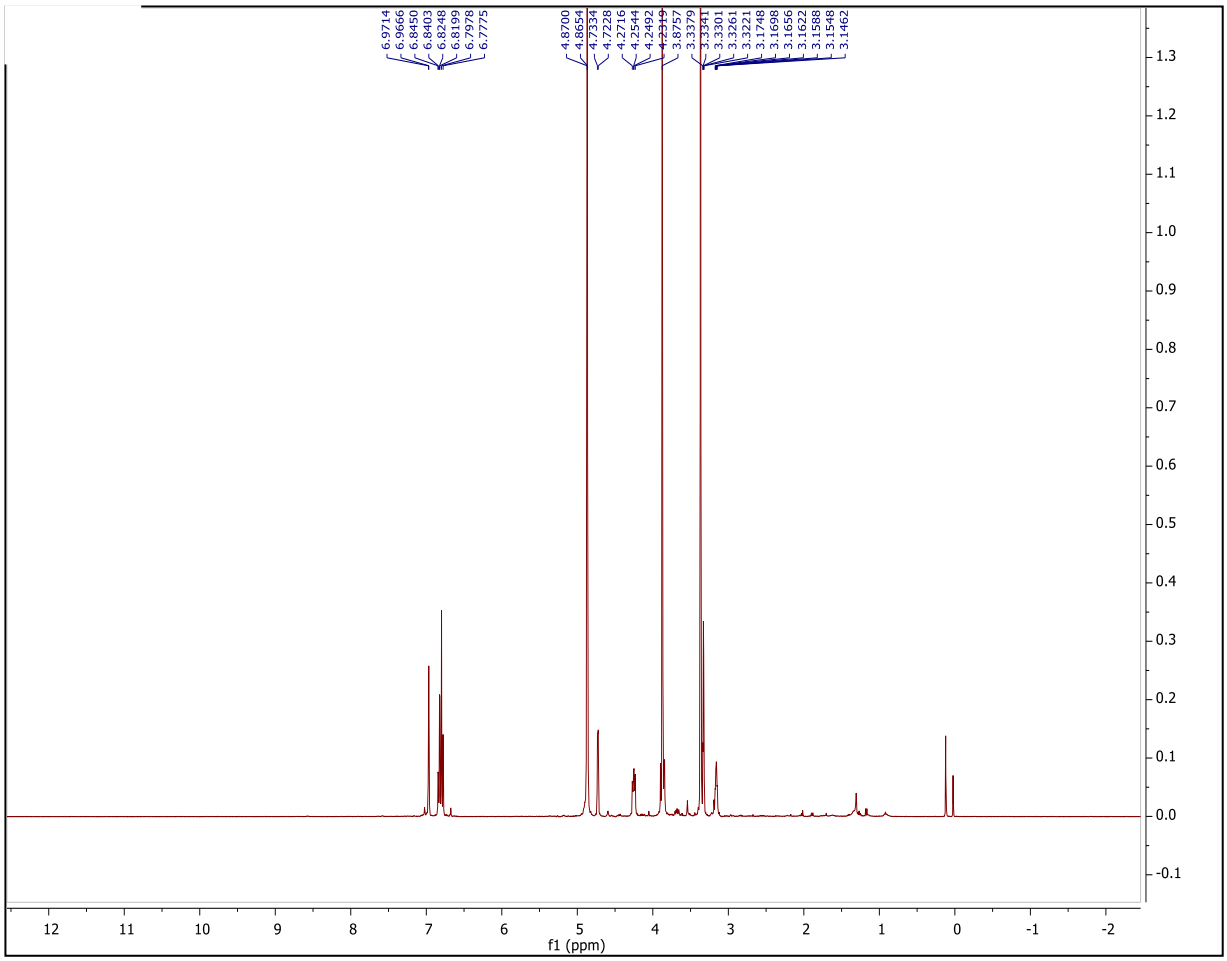

Figure S12: The ^{1}H-NMR spectrum of compound **3** (Pinoresinol) in CD_3OD (400 MHz)

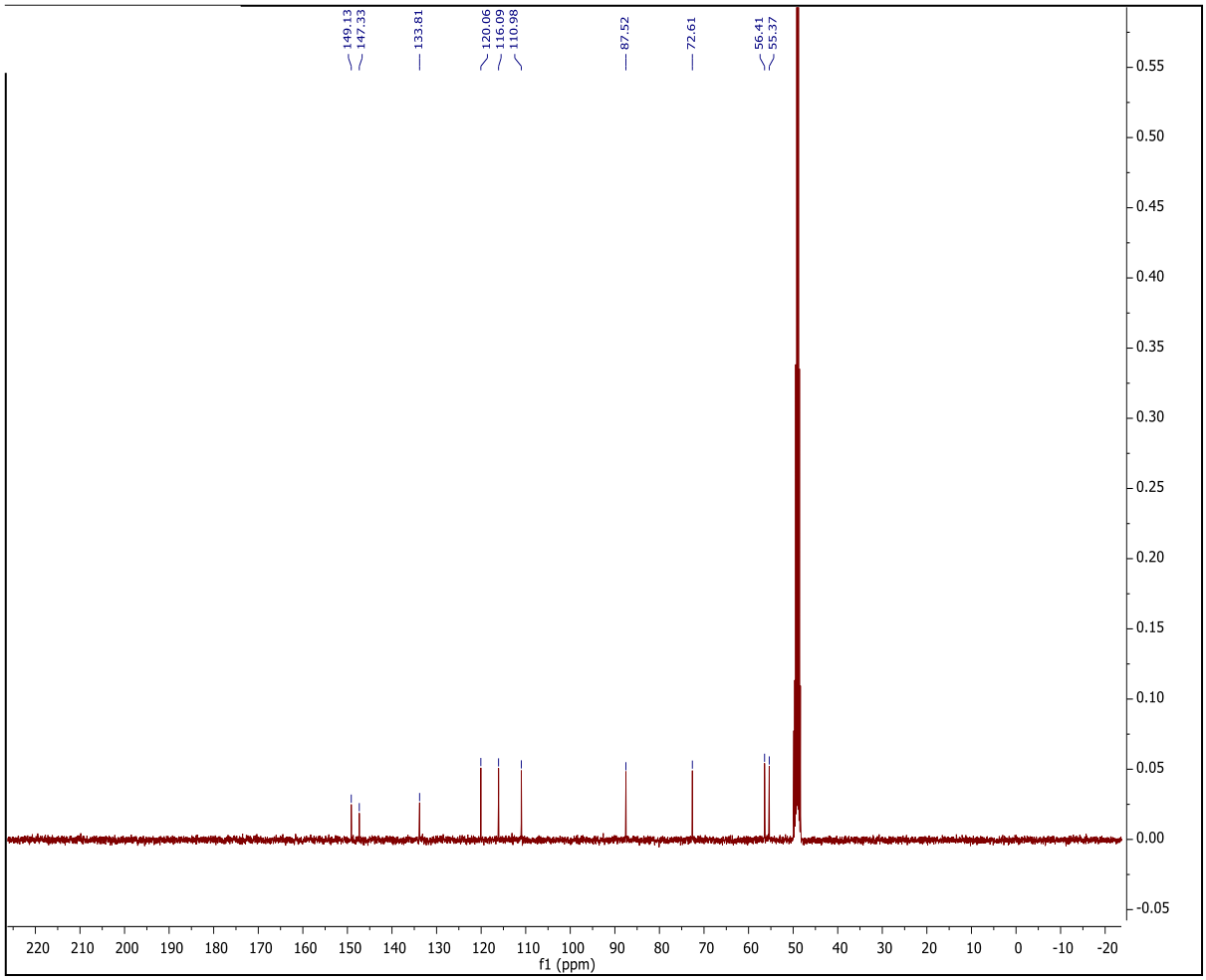

Figure S13: The ^{13}C-NMR spectrum of compound **3** (Pinoresinol) in CD_3OD (100 MHz)

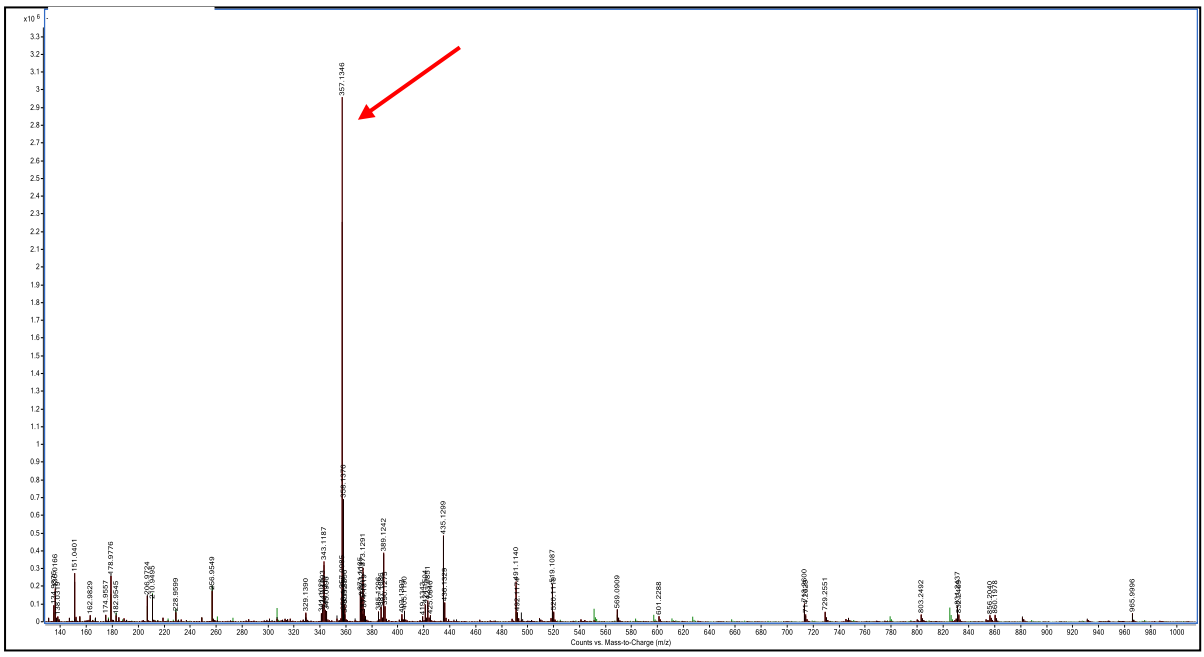

Figure S14: The HRESI-MS data of compound **3** (Pinoresinol)

Molecular formula :	Observed :	Calculated :	Adduct Ion :	Error ppm:
$C_{20}H_{22}O_6$	357.1346	357.1338	$[M-H]^-$	2.2