Supporting Information

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Melanin synthesis Inhibitors from *Olea europeae*

Amira Elkattan[1,2], Ahmed Gohar[1], Mohamed Amer[1], Zain M. Naeem[1], Ahmed Ashour[1,2] and Kuniyoshi Shimizu[2*]

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

[2]*Department of Agro-environmental Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Nishi-ku, Fukuoka 819-0395, Japan*

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Materials and Methods

Reagents

NaOH and DMSO were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan) The 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) from Sigma (St. Louis, MO), EMEM from Nissui Chemical Co (Osaka, Japan) Other chemicals are of the highest grade commercially available

Plant Material

Olea europeae leaves (variety: Kalamata) were collected from the New Domiat area, Mediterranean coastal region, Egypt on November, 2015. The plant was identified by Prof. Dr. Ibrahim Mashaly, Systematic Botany Department, Faculty of Sciences, Mansoura University. A voucher specimen (No. 565) is kept in Pharmacognosy Department, Faculty of pharmacy, Mansoura University, Egypt.

Extraction and Isolation Procedures

The powdered plant leaves (2.5 kg) were exhausted by maceration in a percolator with MeOH at room temperature. The combined methanol extracts were concentrated to a syrupy consistency under reduced pressure and then allowed to dry in a desiccator over anhydrous $CaCl_2$ to a constant weight (700 g) The residue was suspended in distilled water and then fractionated successively with petroleum ether, methylene chloride and ethyl acetate. The different extracts were evaporated under reduced pressure to obtain petroleum ether fraction (A, 70 g), methylene chloride fraction (B, 120 g) and ethyl acetate fraction (C, 100 g).

Isolation of Compounds

Fraction **A** (70 g) was applied on to the top of a silica gel column (800 g, 70 x 4.0 cm) packed in petroleum ether. The extract was then eluted with petroleum ether containing increased proportions of ethyl acetate till 20% ethyl acetate. The effluent was collected in 250 mL fractions. Each fraction was concentrated to small volume (5 mL) and monitored by TLC using mixture of ethyl acetate - petroleum ether (different proportions) as a developing solvent and vanillin/sulfuric acid spray reagent. Similar fractions were pooled together, solvent evaporated, then subjected to further re-chromatographic separation and purification. Fraction 17-25 (900 mg), eluted with 6% ethyl acetate in petroleum ether, was recrystallized several times from methanol afforded compound **1** (400 mg), Fraction 26-35 (500 mg), eluted with 8% ethyl acetate in petroleum ether, was recrystallized several times from methylene chloride afforded compound **2** (150 mg).

Fraction **B** (30 g) was applied on to the top of a silica gel column (300 g, 52 x 4.0 cm) packed in petroleum ether. The elution was started with petroleum ether, then increased proportions of ethyl acetate till 100% ethyl acetate. The effluent was collected in 250 mL fractions. Each fraction was concentrated to small volume (5 mL) and monitored by TLC using ethyl acetate in petroleum ether (different proportions) as a developing solvent and vanillin/sulfuric acid spray reagent. Similar fractions were pooled together, evaporated to dryness, and then subjected to further chromatographic separation and purification. Fraction 1-23 (1.8 g), eluted with petroleum ether/ethyl acetate (80:20), was purified by re-chromatography on silica gel glass column (60 g, 50 x 2.0 cm) pre-packed in petroleum ether using isocratic elution with 10% ethyl acetate in petroleum ether to obtain compound **3** (25 mg) Fraction 29-35 (2.5 g), eluted with petroleum ether/ethyl acetate (80:20), afforded compound **4** (2.0 g) Fraction 50-54 (700 mg), eluted with petroleum ether/ethyl acetate (70:30), was purified by re-chromatography on silica gel glass column (40 g, 60 x 1.5 cm) pre-packed in petroleum ether. Gradient elution is adopted with different proportions of ethyl acetate in petroleum ether to obtain compound **5** (200 mg) Fraction 60-63 (130 mg), eluted with petroleum ether/ethyl acetate (50:50), was purified by re-chromatography on medium pressure liquid chromatography (MPLC)

Buchi set with UV-ELSD detector using C_{18} silica 40 µm, 40 g prepacked flash column eluted gradiently with H_2O-MeOH (70:30) till 100% MeOH at a flow rate of 40 mL/min to obtain compound **6** (4.1mg) Fraction 76-79 (150 mg), eluted with petroleum ether/ethyl acetate (10:90), was subjected to recrystallization several times from hot methylene chloride to afford compound **7.**

Fraction **C** (50 g) was applied on to the top of a silica gel column (500 g, 70 x 4.5 cm) packed with petroleum ether. Elution was started with 40% ethyl acetate in petroleum ether, then increasing the proportion of ethyl acetate till 100% ethyl acetate followed by ethyl acetate with increased proportions of methanol till 100% MeOH. The effluent was collected in 250 mL fractions. Each fraction was concentrated to small volume (5 mL) and monitored by either normal TLC using different proportions of dichloromethane methanol mixture as developing system or RP-TLC using different proportions of methanol water as developing system and vanillin/sulfuric acid spray reagent. Similar fractions were pooled together, solvent evaporated, and subjected to further chromatographic separation and purification. Fraction 9-20 (600 mg), eluted with petroleum ether/ethyl acetate (50:50), was purified by re-chromatography on MPLC Buchi set with UV-ELSD detector using C_{18} silica 40 g prepacked flash column (silica 40 µm) eluted gradiently with H_2O-MeOH (90:10) till 100% MeOH at a flow rate of 40 mL/min, then it was subjected to further purification over normal preparative TLC using 10% MeOH in DCM as mobile phase to obtain compound **8** (5.7 mg) Fraction 65-90 (8.3 g) was eluted with ethyl acetate/MeOH (98:2) Part of this fraction (4 g) was purified by re-chromatography on MPLC Buchi set with UV-ELSD detector using C_{18} silica 40 g prepacked flash column (silica 40 µm) eluted gradiently with H_2O-MeOH (90:10) till 100% MeOH at a flow rate of 40 mL/min to obtain several compounds. Each one of them was further purified over normal preparative TLC using 20% MeOH in DCM as mobile phase to obtain compound **9** (4.7 mg), compound **10** (48 mg), compound **11**(2.3 mg), compound **12** (2.8 mg), compound **13** (4.6 mg) and compound **14** (4.5 mg) Fraction 106-135 (700 mg) was eluted with ethyl acetate/MeOH (90:10), then purified by re-chromatography on MPLC Buchi set with UV-ELSD detector using C_{18} silica 40 g prepacked flash column (silica 40 µm) eluted gradiently with H_2O-MeOH (90:10) till 100% MeOH at a flow rate of 40 mL/min to obtain three compounds: compound **15** (6 mg), compound **16** (13.9 mg) and compound **17** (6.5 mg).

*α-, β-Amyrin mixture (**1**):* a white amorphous powder; ^{13}C-NMR (150 MHz, $CDCL_3$): δc 38.8 (C-1), 27.3 (C-2), 79.1 (C-3), 38.8 (C-4), 55.2 (C-5), 18.4 (C-6), 32.9 (C-7), 40.0 (C-8), 47.7 (C-9), 36.9 (C-10), 23.4 (C-11), 124.4 (C-12), 139.6 (C-13), 42.1 (C-14), 28.8 (C-15), 26.6 (C-16), 33.8 (C-17), 59.1 (C-18), 39.7 (C-19), 39.6 (C-20), 31.3 (C-21), 41.5 (C-22), 28.1 (C-23), 15.7 (C-24), 15.7 (C-25), 16.9 (C-26), 23.3 (C-27), 28.1 (C-28), 17.5 (C-29), 21.4 (C-30), 38.8 (C-1`), 27.3 (C-2`), 79.1 (C-3`), 38.8 (C-4`), 55.2 (C-5`), 18.4 (C-6`), 32.8 (C-7`), 40.0 (C-8`), 47.7 (C-9`), 36.9 (C-10`), 23.4 (C-11`), 121.7 (C-12`), 145.1 (C-13`), 41.7 (C-14`), 26.6 (C-15`), 27.3 (C-16`), 32.5 (C-17`), 47.3 (C-18`), 46.8 (C-19`), 31.1 (C-20`), 34.8 (C-21`), 37.2 (C-22`), 28.1 (C-23`), 15.6 (C-24`), 15.6 (C-25`), 16.9 (C-26`), 25.8 (C-27`), 28.8 (C-28`), 23.3 (C-29`), 23.4 (C-30`).

*β-sitosterol (**2**):* a white needles ; IR spectrum (KBr, υ_{max} cm^{-1}) showed absorption bands at 3414 cm^{-1} (OH stretching), 2935 cm^{-1} and 2866 cm^{-1} (C-H stretching), 1633 cm^{-1} (C=C stretching), 1461and 1247 cm^{-1} (methylene bending), 1377 cm^{-1} (CH_3 bending), 1050 cm^{-1} (C-O stretching), 962 cm^{-1} (=C-bending)

*Uvaol, Erythrodiol mixture (**3**):* a white needles; ^{13}C-NMR (150 MHz, $CDCL_3$): δc 38.6 (C-1), 27.2 (C-2), 79.0 (C-3), 38.8 (C-4), 55.2 (C-5), 18.4 (C-6), 32.6 (C-7), 39.8 (C-8), 47.6 (C-9), 36.9 (C-10), 23.5 (C-11), 122.4 (C-12), 144.2 (C-13), 41.7 (C-14), 25.5 (C-15), 22.0 (C-16), 36.9 (C-17), 42.3 (C-18), 46.4 (C-19), 31.0 (C-20), 34.1 (C-21), 30.1 (C-22), 28.1 (C-23), 15.5 (C-24), 15.5 (C-25), 16.7 (C-26), 25.9 (C-27), 69.9 (C-28), 33.2 (C-29), 23.6 (C-30), 38.8 (C-1`), 27.3 (C-2`), 79.0 (C-3`), 38.8 (C-4`), 55.2 (C-5`), 18.3 (C-6`), 32.8 (C-7`), 39.3 (C-8`), 47.5 (C-9`), 35.2 (C-10`), 23.3 (C-11`), 125.1 (C-12`), 138.7 (C-13`), 42.0 (C-14`), 26.0 (C-15`), 23.3 (C-16`), 36.9 (C-17`), 54.0 (C-18`), 38.0 (C-19`), 39.4 (C-20`), 30.6 (C-21`), 30.6 (C-22`), 28.0 (C-23`), 15.7 (C-24`), 15.6 (C-25`), 16.8 (C-26`), 23.4 (C-27`), 69.7 (C-28`), 17.4 (C-29`), 21.3 (C-30`).

*Oleanolic acid (**4**):* a white amorphous powder; ^{1}H-NMR (600 MHz, $CDCL_3$): δ_H 3.23 (1H, dd, *J* = 3.6, 10.8 Hz, H-3), 5.28 (1H, br s, H-12), 2.82 (1H, m, H-18), 0.99 (3H, s, H-23), 0.75 (3H, s, H-24), 0.91 (3H, s, H-25), 0.77 (3H, s, H-26), 1.13 (3H, s, H-27), 0.93 (3H, s, H-29), 0.90 (3H, s, H-30); ^{13}C-NMR (150 MHz, $CDCL_3$): δc 38.4 (C-1), 27.2 (C-2), 79.0 (C-3), 38.7 (C-4), 55.2 (C-5), 18.3 (C-6), 32.6 (C-7), 39.3 (C-8), 47.6 (C-9), 37.1 (C-10), 22.9 (C-11), 122.6 (C-12), 143.6 (C-13), 41.6 (C-14), 27.7 (C-15), 23.4 (C-16), 46.5 (C-17), 41.0 (C-18), 45.8 (C-19), 30.7 (C-20), 33.8 (C-21), 32.4 (C-22), 28.1 (C-23), 15.5 (C-24), 15.3 (C-25), 17.1 (C-26), 25.9 (C-27), 182.9 (C-28), 33.1 (C-29), 23.6 (C-30).

*Maslinic acid (**5**):* a white amorphous powder; ^{1}H-NMR (600 MHz, CD_3OD): δ_H 3.62 (1H, m, H-2), 2.91 (1H, d, *J* = 9.6 Hz, H-3), 5.27 (1H, br s, H-12), 1.16 (3H, s, H-23), 1.00 (3H, s, H-24), 0.90 (3H, s, H-25), 0.82 (3H, s, H-26), 1.01 (3H, s, H-27), 0.81 (3H, s, H-29), 0.94 (3H, s, H-30); ^{13}C-NMR (150 MHz, CD_3OD): δc 47.4 (C-1), 69.5 (C-2), 84.5 (C-3), 40.5 (C-4), 56.7 (C-5), 19.6 (C-6), 33.8 (C-7), 40.6 (C-8), 48.1 (C-9), 39.2 (C-10), 24.1 (C-11), 123.4 (C-12), 145.5 (C-13), 42.8 (C-14), 29.3 (C-15), 24.6 (C-16), 47.7 (C-17), 42.9 (C-18), 47.8 (C-19), 31.6 (C-20), 33.9 (C-21), 33.6 (C-22), 30.7 (C-23), 17.8 (C-24), 17.4 (C-25), 17.1 (C-26), 28.8 (C-27), 182.4 (C-28), 34.9 (C-29), 26.4 (C-30).

*Vomifoliol (**6**):* a white amorphous powder; $[\alpha]_D^{25}$ = + 67.7 (1.8, MeOH); UV (MeOH) λ_{max} 240 nm; HR-FAB-MS *m/z* 247.1312 [M+Na$^+$]; (cal. For $C_{13}H_{20}O_3$, 247.1310); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 5.87 (1H, m, H-2), 2.49 (1H, d, *J* =16.8 Hz, H-6a) and 2.17 (1H, d, *J* =16.8 Hz, H-6b), 5.78 (1H, m, H-7), 5.79 (1H, m, H-8), 4.32 (1H, m, H-9), 1.24 (3H, d, *J*=6.6 Hz, H-10), 1.01 (3H, s, H-11), 1.04 (3H, s, H-12), 1.91 (3H, d, *J*=1.2 Hz, H-13); ^{13}C-NMR (150 MHz, CD_3OD): δc 201(C-1), 127.2 (C-2), 167.5 (C-3), 80.0 (C-4), 42.5 (C-5), 50.8 (C-6), 130.0 (C-7), 137.0 (C-8), 68.1 (C-9), 23.5 (C-10), 24.5 (C-11), 23.5 (C-12), 19.6 (C-13).

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This is the first report to describe isolation of vomifoliol from family oleaceae.

*β-sitosterol 3-O-β-D-glucoside (**7**):* a white amorphous powder; IR spectrum (KBr, υ_{max}) showed absorption bands at 3428 cm^{-1} (O-H stretching), 2954 cm^{-1} and 2869 cm^{-1} (-C-H stretching), 1635 cm^{-1} (C=C stretching), 1461 and 1257 cm^{-1} (methylene bending), 1371 cm^{-1} (CH_3 bending), 1166, 1070 and 1022 cm^{-1} (C-O stretching).

*Luteolin (**8**):* a yellow powder; UV (MeOH) λ_{max} 348 and 254 nm; HR-ESI-MS$^+$ *m/z* 287.0780 [M+H]$^+$ while HR-ESI-MS$^-$ m/z 285.0378 [M-H]; (molecular formula $C_{15}H_{10}O_6$); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 6.40 (1H, s, H-3), 6.06 (1H, d, *J* = 1.8 Hz, H-6), 6.26 (1H, d, *J* = 1.2 Hz, H-8), 7.32 (1H, d, *J* = 1.8 Hz, H-2`), 6.85 (1H, d, *J* = 8.4 Hz, H-5`), 7.33 (1H, dd, *J* = 9 and 1.8 Hz, H-6`); $^{13}$C-NMR (150 MHz, $CD_3OD$): δc 165.8 (C-2), 102.9 (C-3), 183.1 (C-4), 162.9 (C-5), 102.4 (C-6), 173.1 (C-7), 96.7 (C-8), 159.9 (C-9), 103.3 (C-10), 122.9 (C-1`), 113.6 (C-2`), 147.7 (C-3`), 152.7 (C-4`), 116.9 (C-5`), 120.2 (C-6`).

*Oleoside dimethylester (**9**):* a brown amorphous powder; $[\alpha]_D^{25}$ = - 2.5 (2.0, MeOH); UV (MeOH) λ_{max} 270 nm; HR-ESI-MS *m/z* 441.1404 [M+Na$^+$]; (molecular formula $C_{18}H_{26}O_{11}$); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 5.92 (1H, s, H-1), 7.52 (1H, s, H-3), 4.00 (1H, m, H-5), 2.47 (1H, dd, *J*=14.4, 9.0 Hz, H-6a), 2.76 (1H, dd, *J*=14.4, 4.8 Hz, H-6b), 6.08 (1H, bq, *J*=7.2 Hz, H-8), 1.74 (3H, bd, *J*=7.2 Hz, H-10), 3.71 (3H, s, -OCH_3), 3.63 (3H, s, -OCH_3), 4.81 (overlapped with solvent peak, H-1`), 3.89 (H-6`a), 3.68 (H-6`b); $^{13}$C-NMR (150 MHz, $CD_3OD$): δc 95.2 (C-1), 155.2 (C-3), 109.5 (C-4), 31.9 (C-5), 41.1 (C-6), 173.6 (C-7), 124.9 (C-8), 130.6 (C-9), 13.6 (C-10), 168.7 (C-11), 51.9 (11-$OCH_3$), 52.2 (7-$OCH_3$), 100.9 (C-1`), 62.8 (C-6`).

*Oleuropein (**10**):* a brown amorphous powder; $[\alpha]_D^{25}$ = - 131.1 (12.6, MeOH); UV (MeOH) λ_{max} 232 nm; ESI-MS$^+$ *m/z* 563 [M+Na$^+$] while ESI-MS$^-$ m/z 539 [M-H]; (molecular formula $C_{25}H_{32}O_{13}$); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 5.90 (1H, s, H-1), 7.50 (1H, s, H-3), 3.98 (1H, m, H-5), 2.46 (1H, dd, *J*=14.4, 9.0 Hz, H-6a), 2.71(1H, dd, *J*=14.4, 4.8 Hz, H-6b), 6.08 (1H, bq, *J*=7.2 Hz, H-8), 1.66 (3H, bd, *J*=6.6 Hz, H-10), 3.71(3H, s, 11-OCH_3), 6.66 (1H, d, *J*=1.8 Hz, H-2`), 6.69 (1H, d, *J*=7.8 Hz, H-

5`), 6.55 (1H, dd, *J*=1.8, 7.8 Hz, H-6`), 2.77 (2H, t, *J*=6.6 Hz, H-7`), 4.21 (1H, dt, *J*=7.2, 10.8 Hz, H-8`a), 4.11 (1H, dt, *J*=7.2, 10.8 Hz, H-8`b), 4.81 (overlapped with the solvent peak, H-1``), 3.89 (H-6``a), 3.67 (H-6``b); ^{13}C-NMR (150 MHz, CD_3OD): δc 95.3 (C-1), 155.2 (C-3), 109.5 (C-4), 31.9 (C-5), 41.3 (C-6), 173.3 (C-7), 124.9 (C-8), 130.8 (C-9), 13.6 (C-10), 168.7 (C-11), 52.0 (11-OCH_3), 130.6 (C-1`), 117.1 (C-2`), 146.3 (C-3`), 145.0 (C-4`), 116.5 (C-5`), 121.4 (C-6`), 35.4 (C-7`), 66.9 (C-8`), 101.0 (C-1``), 62.8 (C-6``).

*Hydroxypinoresinol 1-O-β-D-glucoside (**11**):* a white amorphous powder; $[\alpha]_D^{25}$ = +27 (1.06, MeOH); UV (MeOH) λ_{max} 230, 280 nm; HR-ESI-MS$^+$ *m/z* 559.3138 [M+Na$^+$] and m/z 519.2592 [M+H-H_2O]; (molecular formula $C_{26}H_{32}O_{12}$); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 4.69 (1H, s, H-2), 4.51 (1H, t, *J*=8.4 Hz, H-4a), 3.81 (1H, dd *J*=6.0, 9.0 Hz, H-4b), 3.42 (1H, m, H-5), 4.81 (overlapped with solvent peak, H-6), 4.40 (1H, d, *J*=10.8 Hz, H-8a), 3.95 (1H, d, *J*=10.2 Hz, H-8b), 7.10 (1H, d, *J*=1.2 Hz, H-2`), 6.73 (1H, d, *J*=8.4 Hz, H-5`), 6.87 (1H, dd, *J*=1.8, 8.4 Hz, H-6`), 3.88 (3H, s, -OCH_3), 7.04 (1H, d, *J*=1.8 Hz, H-2``), 6.81 (1H, d, *J*=8.4 Hz, H-5``), 6.89 (1H, dd, *J*=1.8, 8.4 Hz, H-6``), 3.85 (3H, s, -OCH_3), 4.35 (1H, d, *J*=7.8 Hz, H-1```), 3.69 (H-6```a), 3.53 (H-6```b); ^{13}C-NMR (150 MHz, CD_3OD): δc 99.3 (C-1), 90.1 (C-2), 72.2 (C-4), 60.3 (C-5), 86.9 (C-6), 73.3 (C-8), 128.6 (C-1`), 114.1 (C-2`), 148.5 (C-3`), 147.6 (C-4`), 115.3 (C-5`), 122.5 (C-6`), 56.6 (3`-OCH_3), 133.1 (C-1``), 110.8 (C-2``), 149.4 (C-3``), 147.5 (C-4``), 116.4 (C-5``), 120.0 (C-6``), 56.5 (3``- OCH_3), 100.1 (C-1```), 62.5 (C-6```).

*Luteolin 7-O-β-D-glucoside (**12**):* a yellow powder; UV (MeOH) λ_{max} 348 and 254 nm; HR-ESI-MS$^+$ *m/z* 449.1180 [M+H]$^+$ while HR-ESI-MS$^-$ m/z 447.0733 [M-H]; (molecular formula $C_{21}H_{20}O_{11}$); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 6.61 (1H, s, H-3), 6.51 (1H, d, *J* = 2.4 Hz, H-6), 6.81 (1H, d, *J* = 2.4 Hz, H-8), 7.41 (1H, d, *J* = 1.8 Hz, H-2`), 6.91 (1H, d, *J* = 8.4 Hz, H-5`), 7.43 (1H, dd, *J* = 8.4 and 2.4 Hz, H-6`), 5.07 (1H, d, *J* = 7.2 Hz, H-1``), 3.73 (H-6``a) and 3.94 (H-6``b); $^{13}$C-NMR (150 MHz, $CD_3OD$): δc 166.0 (C-2), 104.3 (C-3), 184.0 (C-4), 163.1 (C-5), 101.3 (C-6), 164.9 (C-7), 96.1 (C-8), 159.1 (C-9), 107.3 (C-10), 123.0 (C-1`), 114.3 (C-2`), 147.0 (C-3`), 152.0 (C-4`), 116.9 (C-5`), 120.6 (C-6`), 101.8 (C-1``), 62.6 (C-6``).

*Diosmetin 7-O-β-D-glucoside (**13**):* a yellow powder; UV (MeOH) λ_{max} 348 and 254 nm; HR-ESI-MS$^+$ *m/z* 463.1470 [M+H]$^+$ while HR-ESI-MS$^-$ m/z 461.0948 [M-H]; (molecular formula $C_{22}H_{22}O_{11}$); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 6.55 (1H, s, H-3), 6.47 (1H, d, *J* = 2.4 Hz, H-6), 6.81 (1H, d, *J* = 2.4 Hz, H-8), 7.36 (1H, d, *J* = 2.4 Hz, H-2`), 6.75 (1H, d, *J* = 8.4 Hz, H-5`), 7.50 (1H, dd, *J* = 8.4 and 2.4 Hz, H-6`), 3.88 (3H, s, -OCH_3), 5.07 (1H, d, *J* = 7.8 Hz, H-1``), 3.73 (H-6``a) and 3.94 (H-6``b); $^{13}$C-NMR (150 MHz, $CD_3OD$): δc 168.2 (C-2), 102.1 (C-3), 183.7 (C-4), 162.9 (C-5), 101.8 (C-6), 164.6 (C-7), 96.1 (C-8), 158.9 (C-9), 106.9 (C-10), 117.5 (C-1`), 110.1 (C-2`), 161.5 (C-3`), 152.0 (C-4`), 118.9 (C-5`), 123.2 (C-6`), 56.3 (4`-$OCH_3$), 101.0 (C-1``), 62.5 (C-6``).

*Verbascoside (**14**):* a brown amorphous powder; $[\alpha]_D^{25}$ = -0.83 (2.4, MeOH); UV (MeOH) λ_{max} 270, 340 nm; HR-ESI-MS$^+$ *m/z* 647.2498 [M+Na$^+$] while HR-ESI-MS$^-$ m/z 623.2953 [M-H]; (molecular formula $C_{23}H_{25}O_{10}$); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 7.01 (1H, d, *J*=1.8 Hz, H-2), 6.71 (1H, d, *J*=7.8 Hz, H-5), 6.91 (1H, dd, *J*=1.8,7.8 Hz, H-6), 7.59 (1H, d, *J*=16.2 Hz, H-7), 6.21 (1H, d, *J*=16.2 Hz, H-8), 6.69 (1H, d, *J*=1.8 Hz, H-2`), 6.69 (1H, d, *J*=9.0 Hz, H-5`), 6.56 (1H, dd, *J*=1.8, 8.4 Hz, H-6`), 2.79 (2H, m, H-7`), 3.73 (1H, m, H-8`a), 4.03 (1H, m, H-8`b), 4.38 (1H, d, *J*=7.8 Hz, H-1``), 3.82 (1H, t, *J*=9.6 Hz, H-3``), 4.90 (1H, m, H-4``), 3.63 (H-6``a), 3.53 (H-6``b), 5.18 (1H, brs, H-1```), 1.10 (3H, d, *J*=6.6 Hz, H-6```); ^{13}C-NMR (150 MHz, CD_3OD): δc 126.0 (C-1), 114.3 (C-2), 146.9 (C-3), 149.9 (C-4), 116.7 (C-5), 123.8 (C-6), 148.8 (C-7), 113.0 (C-8), 168.7 (C-9), 131.5 (C-1`), 117.2 (C-2`), 146.3 (C-3`), 144.9 (C-4`), 116.3 (C-5`), 121.2 (C-6`), 36.6 (C-7`), 72.4 (C-8`), 104.3 (C-1``), 81.7 (C-3``), 70.5 (C-4``), 62.4 (C-6``), 103.0 (C-1```), 18.5 (C-6```).

*Oleoside 11-methylester (**15**):* a brown amorphous powder; $[\alpha]_D^{25}$ = -54.2 (2.6, MeOH); UV (MeOH) λ_{max} 242 nm; HR-ESI-MS$^+$ *m/z* 427.1739 [M+Na$^+$] while HR-ESI-MS$^-$ m/z 403.1167 [M-H]; (molecular formula $C_{17}H_{24}O_{11}$); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 6.00 (1H, s, H-1), 7.46 (1H, s, H-3),

4.04 (1H, m, H-5), 2.22 (1H, dd, *J*=13.2, 9.6 Hz, H-6a), 2.64 (1H, dd, *J*=13.2, 4.8 Hz, H-6b), 6.06 (1H, bq, *J*=7.8 Hz, H-8), 1.78 (3H, bd, *J*=7.2 Hz, H-10), 3.71 (3H, s, -OCH_3), 4.79 (1H, d, *J*=7.8 Hz, H-1`), 3.65 (H-6`a), 3.86 (H-6`b); $^{13}$C-NMR (150 MHz, $CD_3OD$): δc 95.5 (C-1), 154.5 (C-3), 111.0 (C-4), 33.0 (C-5), 45.1 (C-6), 179.5 (C-7), 123.9 (C-8), 131.3 (C-9), 13.8 (C-10), 169.1 (C-11), 51.7 (11-$OCH_3$), 100.9 (C-1`), 62.8 (C-6`).

*Secoxyloganin (**16**):* a brown amorphous powder; $[\alpha]_D^{25}$ = -73.0 (8.6, MeOH); UV (MeOH) λ_{max} 234 nm; HR-ESI-MS^+ *m/z* 427.1158 [M+Na^+] while HR-ESI-MS^- m/z 403.1311 [M-H]; (molecular formula $C_{17}H_{24}O_{11}$); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 5.48 (1H, d, *J*=4.2 Hz, H-1), 7.39 (1H, s, H-3), 3.30 (1H, m, H-5), 2.10 (1H, dd, *J*=15.6, 9.6 Hz, H-6a), 2.84 (1H, m, H-6b), 5.69 (1H, m, H-8), 2.82 (1H, m, H-9), 5.26 (1H, d, *J*=17.4 Hz, H-10a), 5.18 (1H, d, *J*=10.2 Hz, H-10b), 3.66 (3H, s, - OCH_3), 4.63 (1H, d, *J*=7.8 Hz, H-1`), 3.71 (H-6`a), 3.87 (H-6`b); $^{13}$C-NMR (150 MHz, $CD_3OD$): δc 97.7 (C-1), 152.8 (C-3), 111.6 (C-4), 30.8 (C-5), 38.5 (C-6), 180.8 (C-7), 135.0 (C-8), 45.4 (C-9), 120.0 (C-10), 169.3 (C-11), 51.6 (11-$OCH_3$), 99.8 (C-1`), 62.8 (C-6`).

*Hydroxytyrosol 8-O-β-D-glucoside, Hydroxytyrosol 4`-O-β-D-glucoside mixture (**17**):* a brown amorphous powder; UV (MeOH) λ_{max} 276 nm; HR-ESI-MS^+ m/z 339.1144 [M+Na^+] while HR-ESI-MS^- *m/z* 315.1005 [M-H]; (molecular formula $C_{14}H_{20}O_8$); ^{1}H-NMR (600 MHz, CD_3OD): δ_H 6.68 (1H, d, *J*=1.8 Hz, H-2), 6.66 (1H, d, *J*=7.8 Hz, H-5), 6.55 (1H, dd, *J*=1.8, 7.8 Hz, H-6), 2.78 (2H, m, H-7), 4.01 (1H, m, H-8a), 3.69 (1H, m, H-8b), 4.29 (1H, d, *J*=7.8 Hz, H-1``), 3.87 (H-6``a), 3.72 (H-6``b), 6.72 (1H, d, *J*=1.8 Hz, H-2`), 7.02 (1H, d, *J*=8.4 Hz, H-5`), 6.63 (1H, dd, *J*=1.8, 8.4 Hz, H-6`), 2.71 (2H, t, *J*=7.2 Hz, H-7`), 3.68 (2H, m, H-8`), 4.69 (1H, d, *J*=7.2 Hz, H-1```), 3.72 (H-6```a), 3.87 (H-6```b); ^{13}C-NMR (150 MHz, CD_3OD): δc 131.5 (C-1), 117.2 (C-2), 146.3 (C-3), 144.8 (C-4), 116.3 (C-5), 121.1 (C-6), 36.6 (C-7), 72.1 (C-8), 104.9 (C-1``), 62.8 (C-6``), 136.2 (C-1`), 117.9 (C-2`), 148.9 (C-3`), 145.5 (C-4`), 119.3 (C-5`), 121.2 (C-6`), 39.8 (C-7`), 64.4 (C-8`), 104.4 (C-1```), 62.5 (C-6```).

Cell Line

A mouse melanoma cell line, B16, was obtained from RIKEN Cell Bank. The cells were maintained in EMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 0.09 mg/mL theophylline. The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2.

B16 Melanoma Cell Line Assay

The cells were placed in two plates of 24-well plastic culture plates (one plate for determining melanin and the other for cell viability) at a density of 1 x 10^5 cells/well and incubated for 24 h in media prior to being treated with the samples. After 24 h, the media were replaced with 998 µL of fresh media and 2 µL of the test sample at different concentrations (n = 3) At the same time, negative control (2 µL DMSO) and positive control; Arbutin at a final concentration of 20 µM in DMSO were tested. The cells were incubated for an additional 48 h, and then the medium was replaced with fresh medium containing each sample. After 24 h, the remaining adherent cells were assayed. To determine the melanin content (for one plate) after removing the medium and washing the cells with PBS, the cell pellet was dissolved in 1.0 mL of 1 N NaOH, Kept overnight in dark, the crude cell extracts were assayed by using a microplate reader at 405 nm to determine the melanin content. The results from the cells treated with the test samples were analyzed as a percentage of the results from the control culture. On the other hand, cell viability was determined by using MTT assay which provides a quantitative measure of the number of viable cells by determining the amount of formazan crystals produced by metabolic activity in samples treated cells versus control cells. So, for the other well plate, 50 µL of MTT reagent in PBS (5 mg/mL) were added to each well. The plates were incubated in a humidified atmosphere of 5% of CO_2 at 37°C for 4 h. After the medium was removed, 1.0 mL isopropyl alcohol (containing 0.04 N HCl) was added, plates were kept overnight in dark and the absorbance was measured at 570 nm.

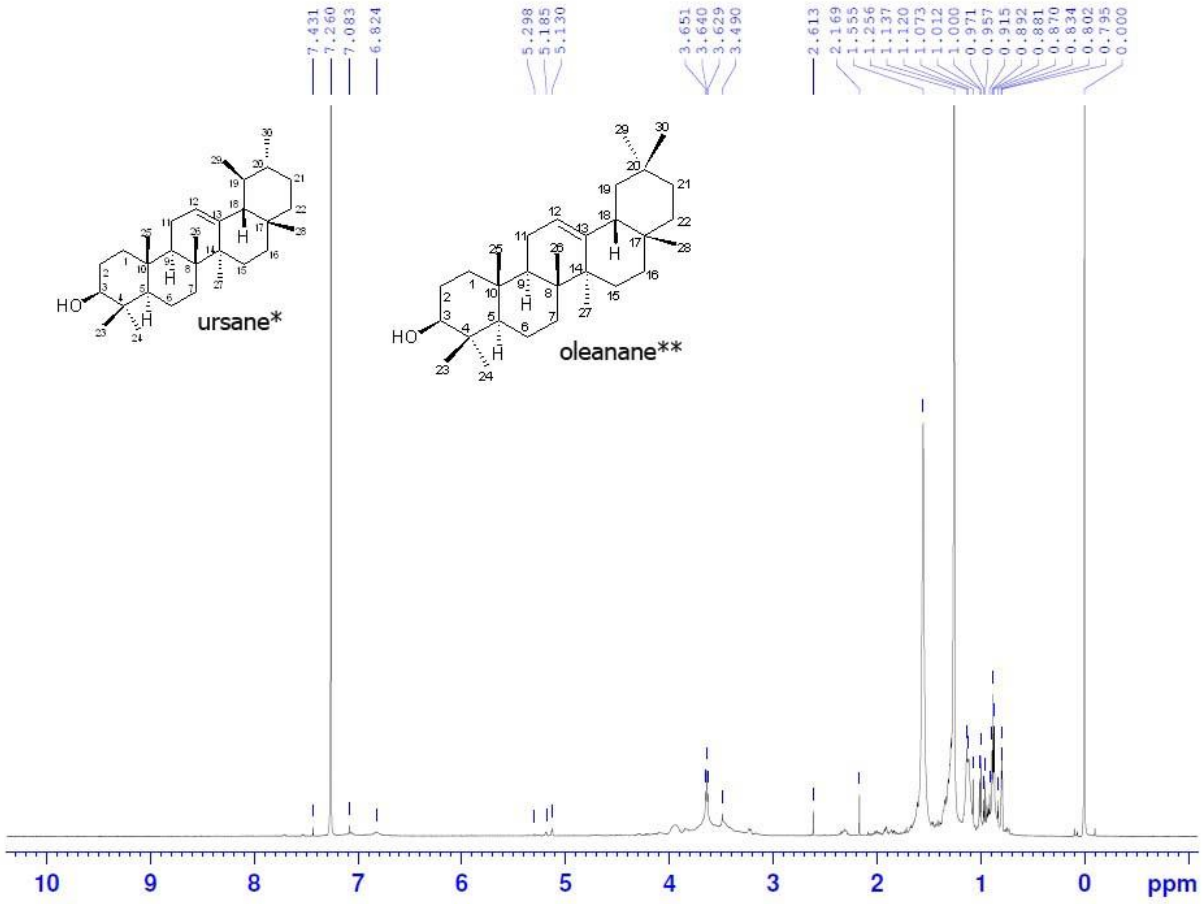

Figure S1 : ^{1}H-NMR spectrum ($CDCL_3$, 600MHZ) of compound (**1**)

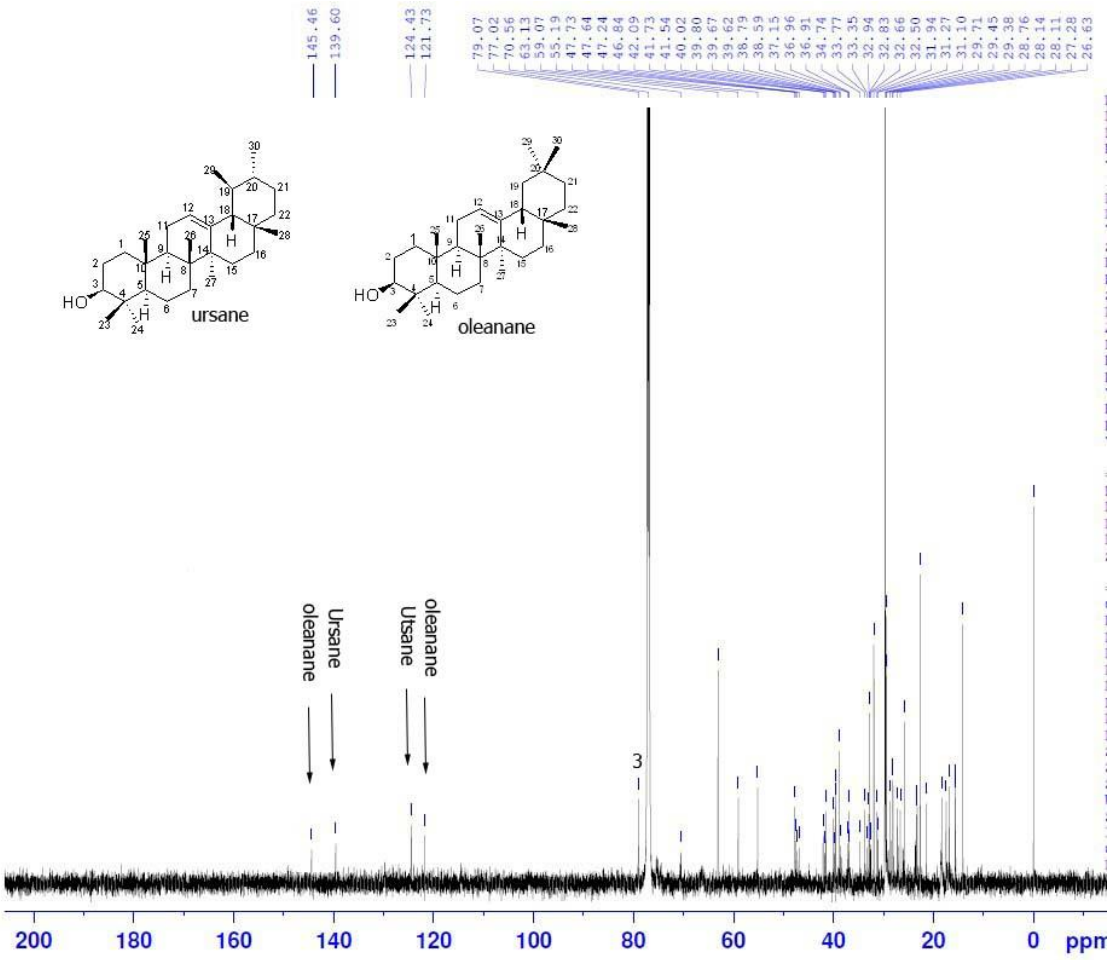

Figure S2 : ^{13}C-NMR spectrum ($CDCL_3$, 150MHZ) of compound (**1**)

Figure S3 : IR (KBr, υ_{max} cm^{-1}) spectrum of compound (**2**)

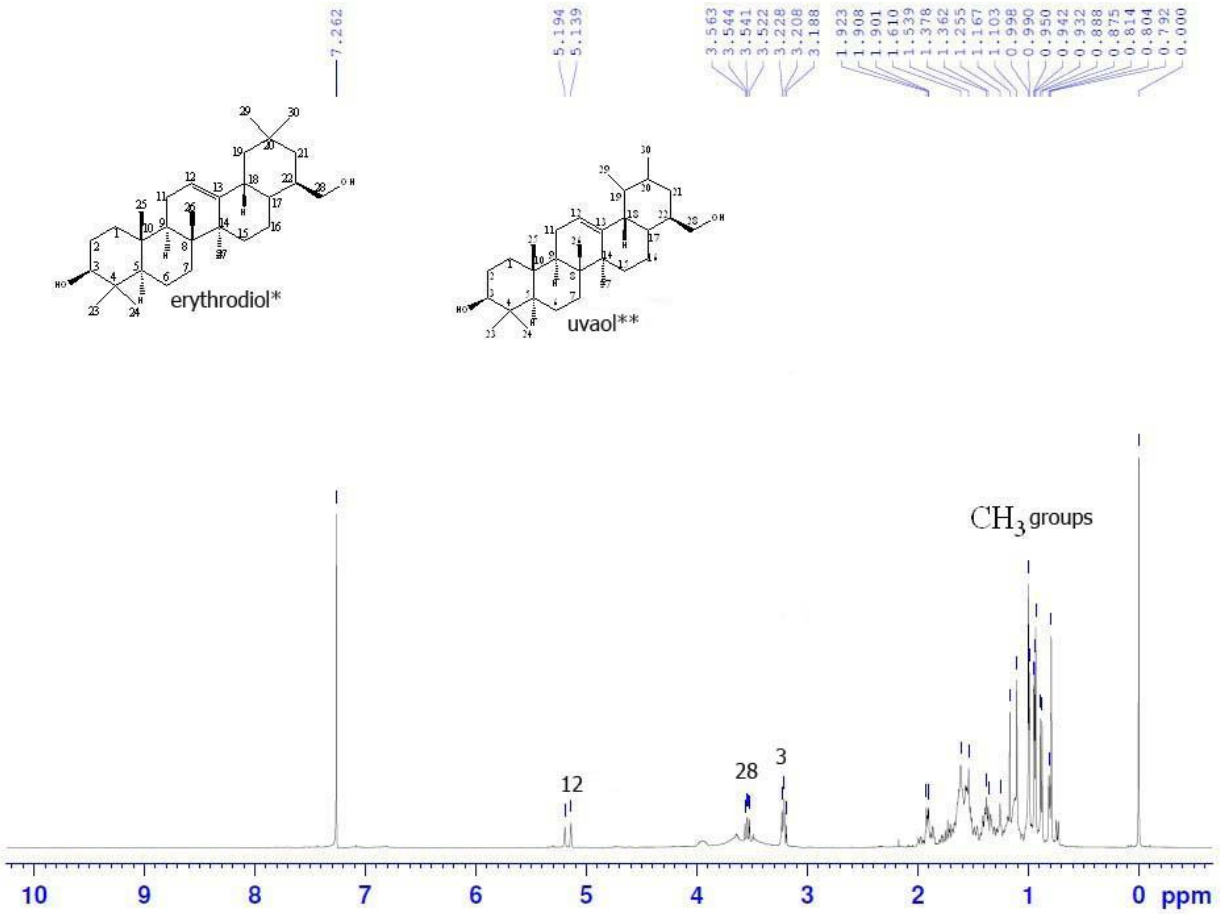

Figure S4 : ^{1}H-NMR spectrum ($CDCL_3$, 600 MHZ) of compound (**3**)

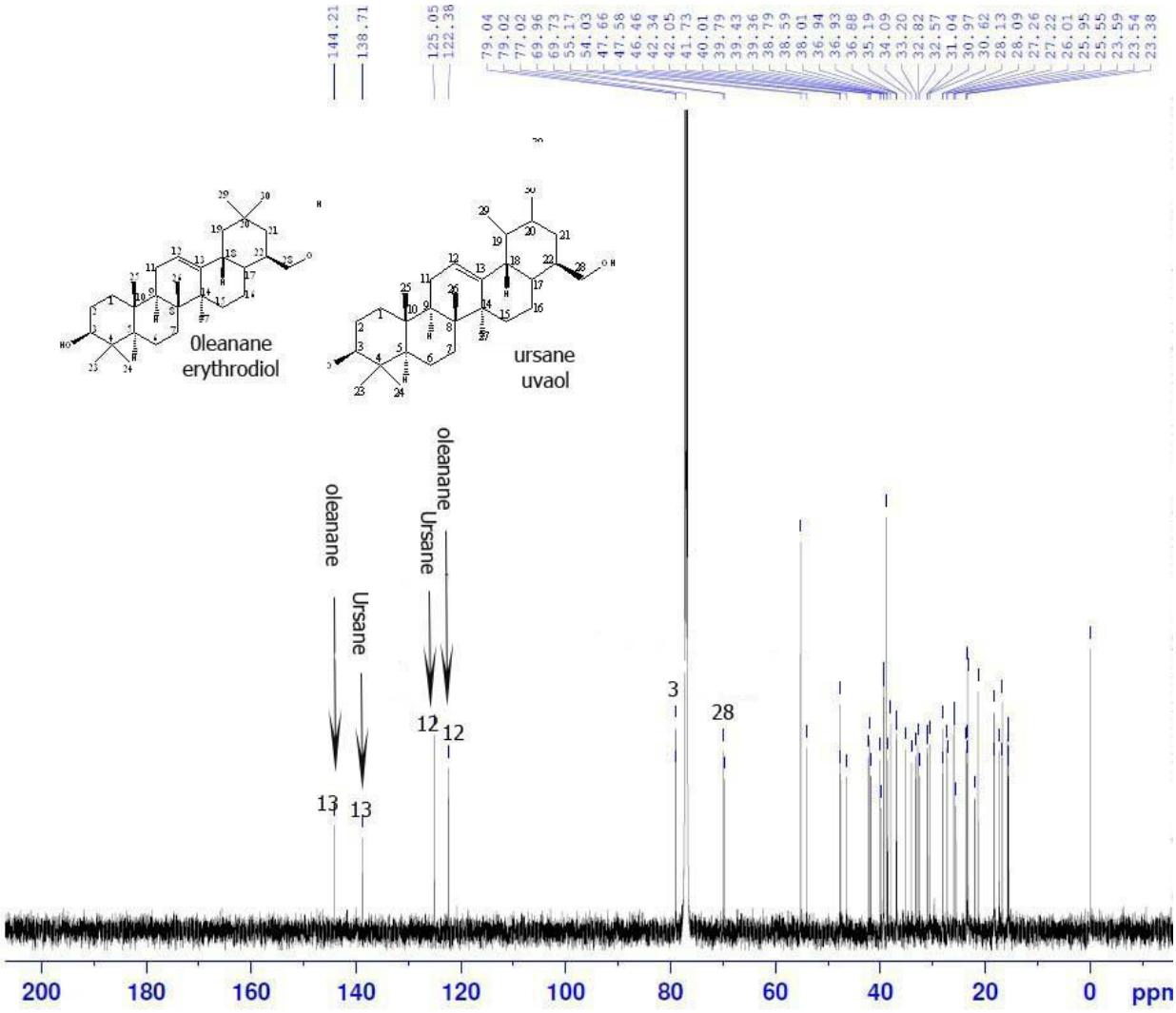

Figure S5 : ^{13}C-NMR spectrum ($CDCL_3$, 150MHZ) of compound (**3**)

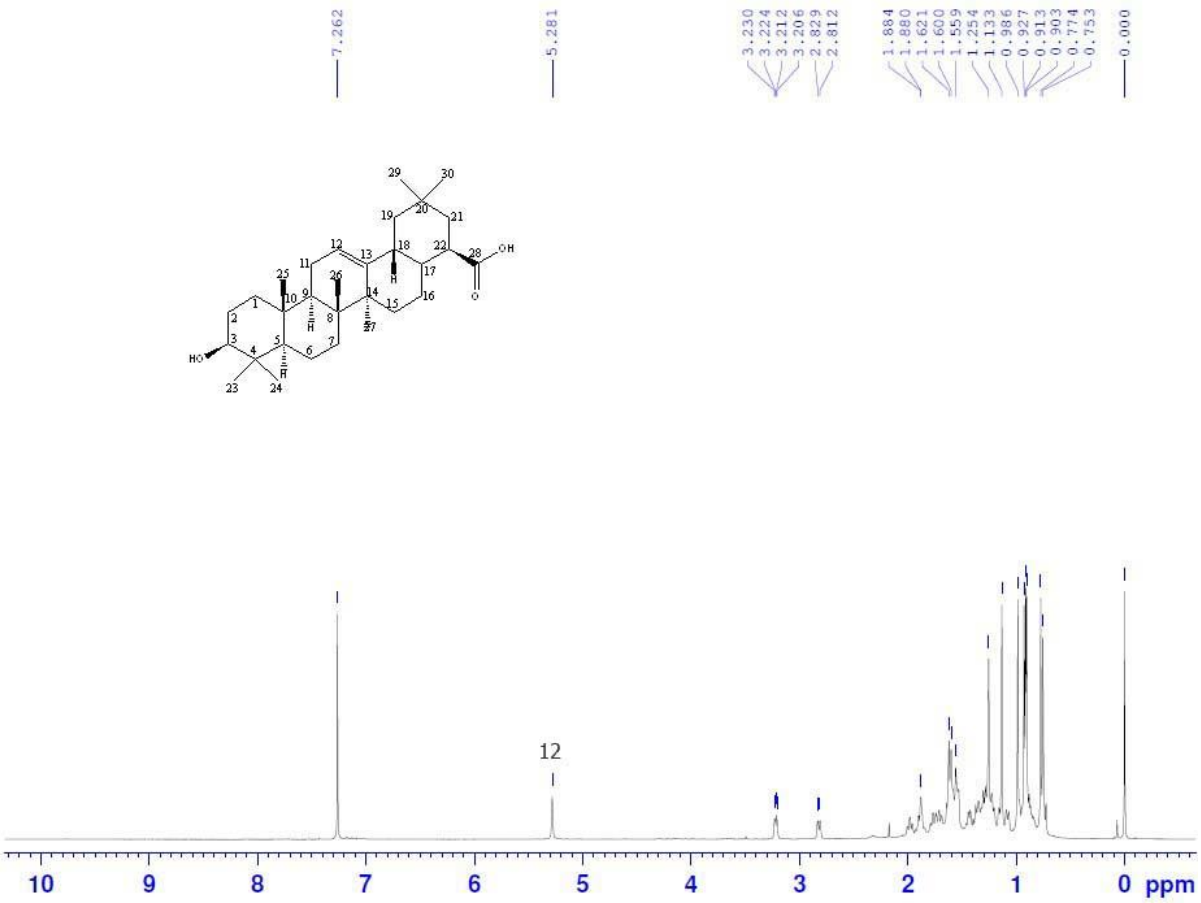

Figure S6 : ^{1}H-NMR ($CDCL_3$, 600 MHZ) spectrum of compound (**4**)

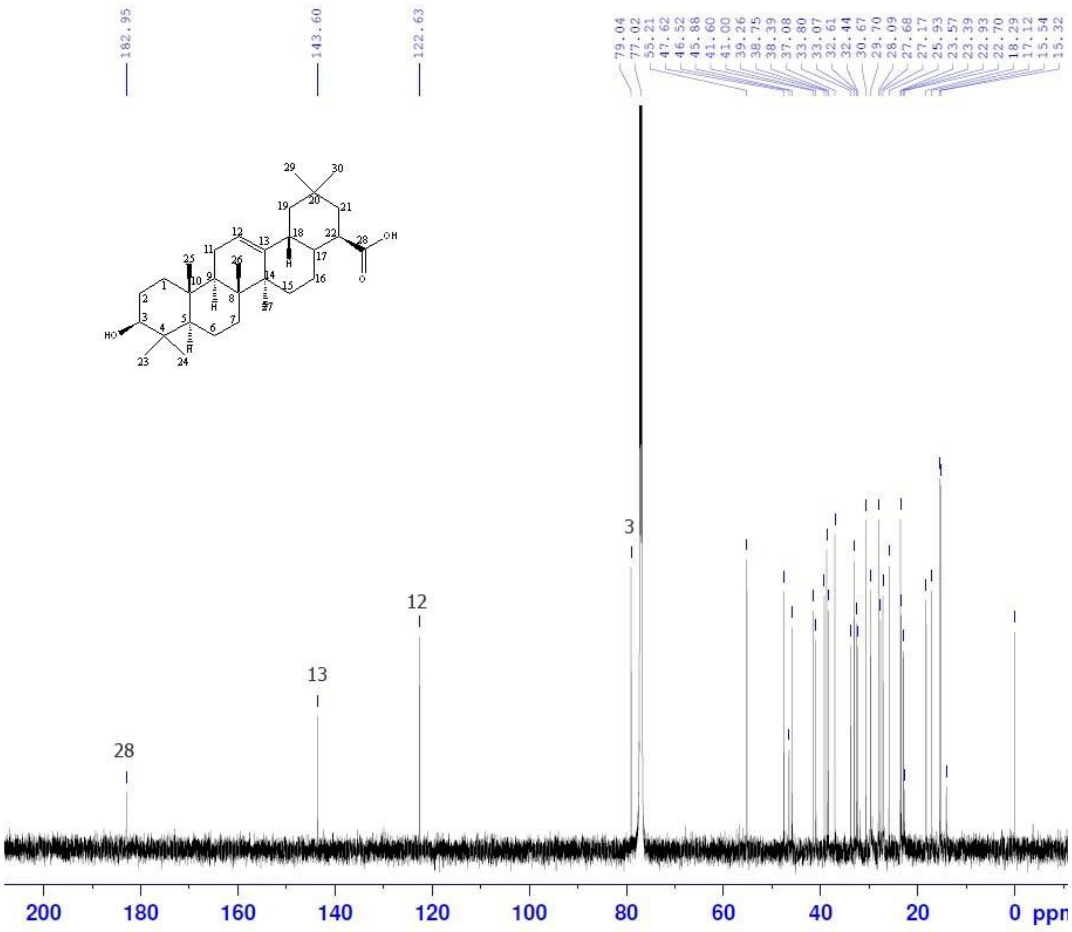

Figure S7 : ^{13}C-NMR spectrum ($CDCL_3$, 150MHZ) of compound (**4**)

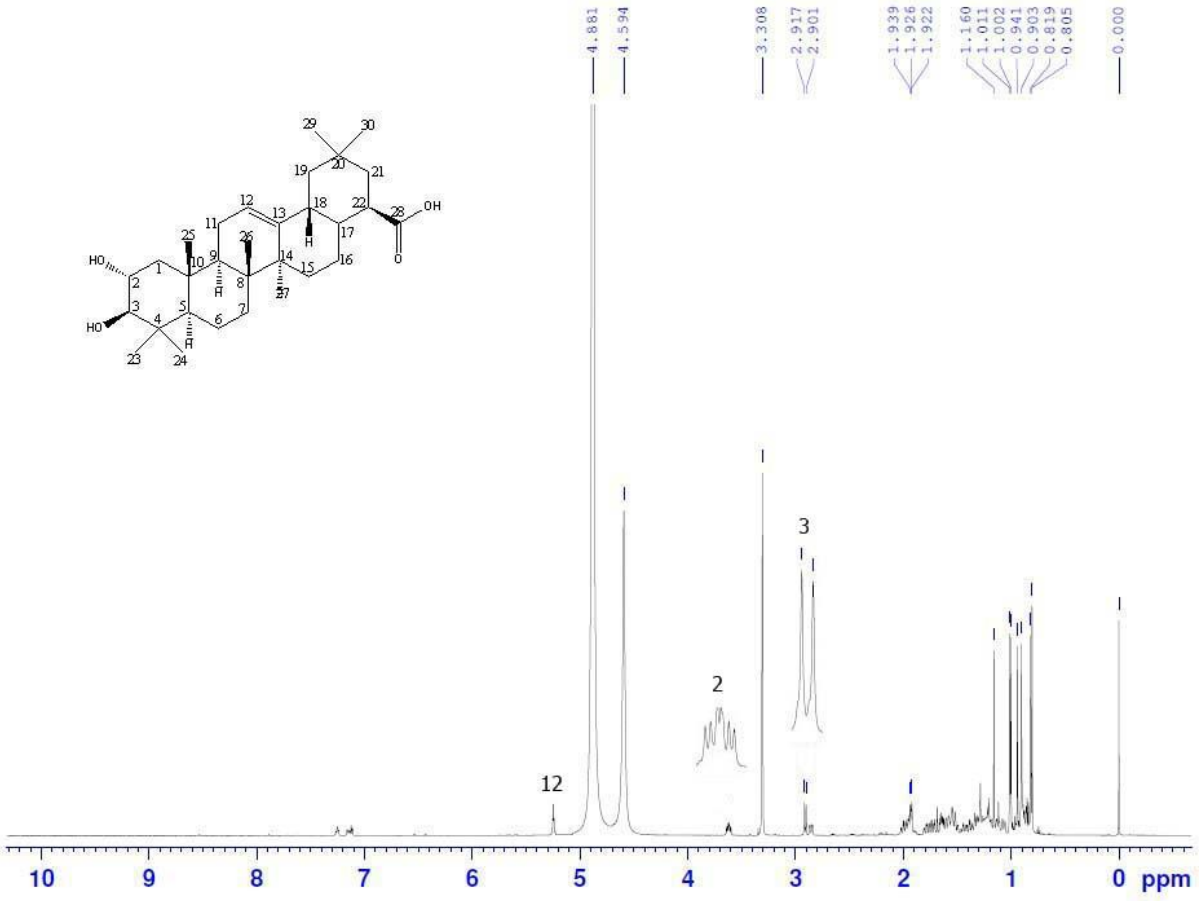

Figure S8 : ^{1}H-NMR (CD_3OD, 600 MHZ) spectrum of compound (**5**)

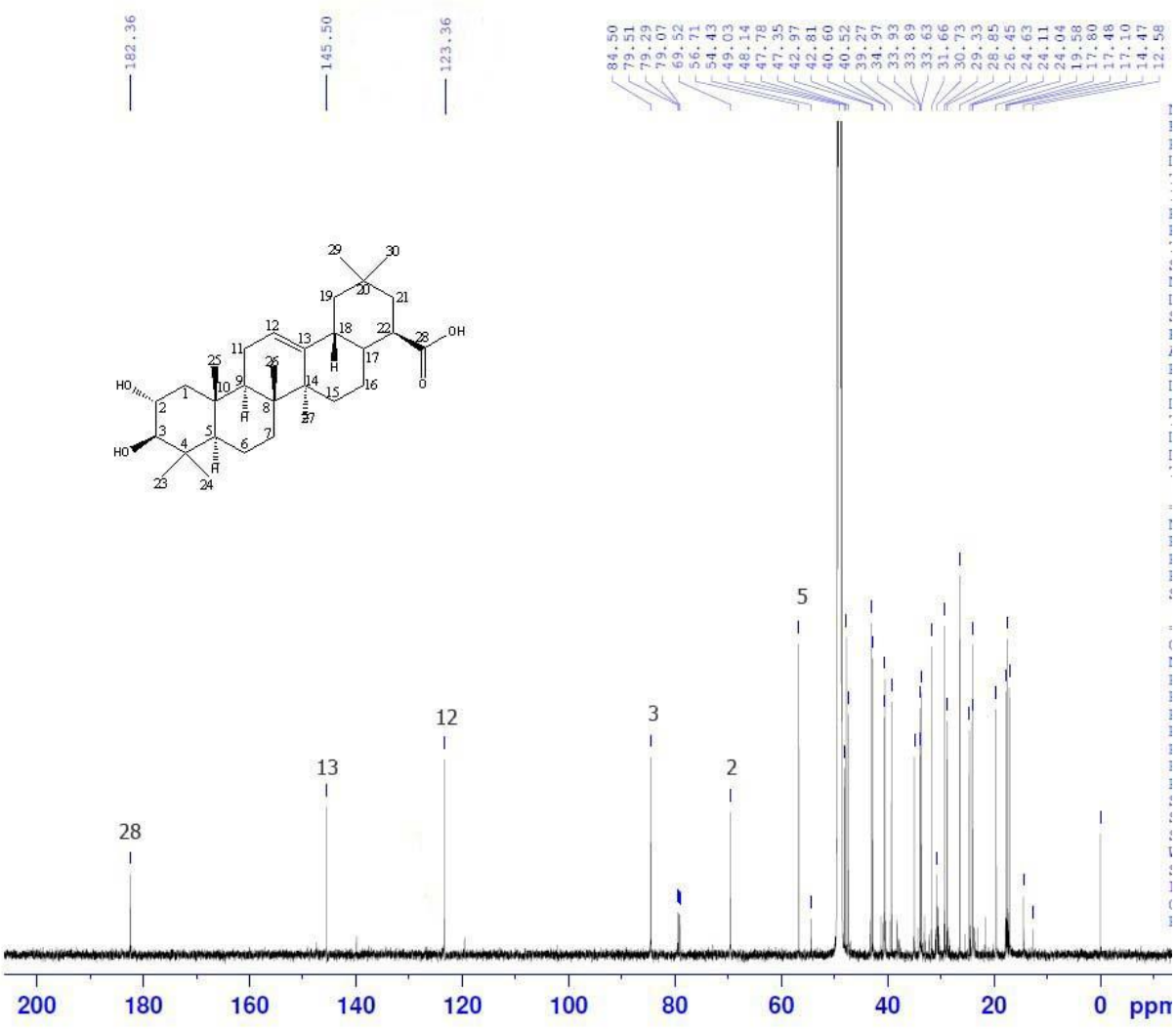

Figure S9 : ^{13}C-NMR spectrum (CD_3OD, 150MHZ) of compound (**5**)

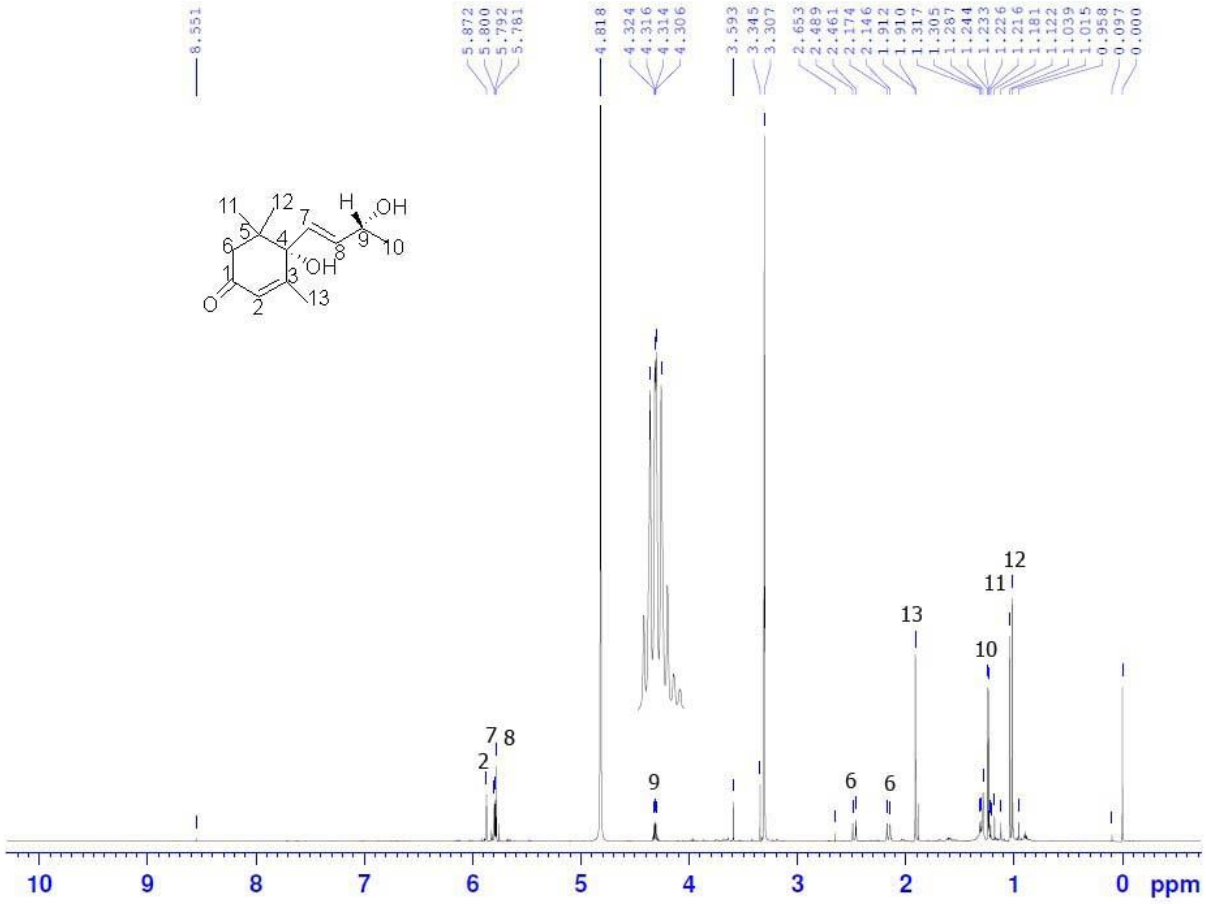

Figure S10 : ^{1}H-NMR spectrum (CD_3OD, 600 MHz) of compound (**6**)

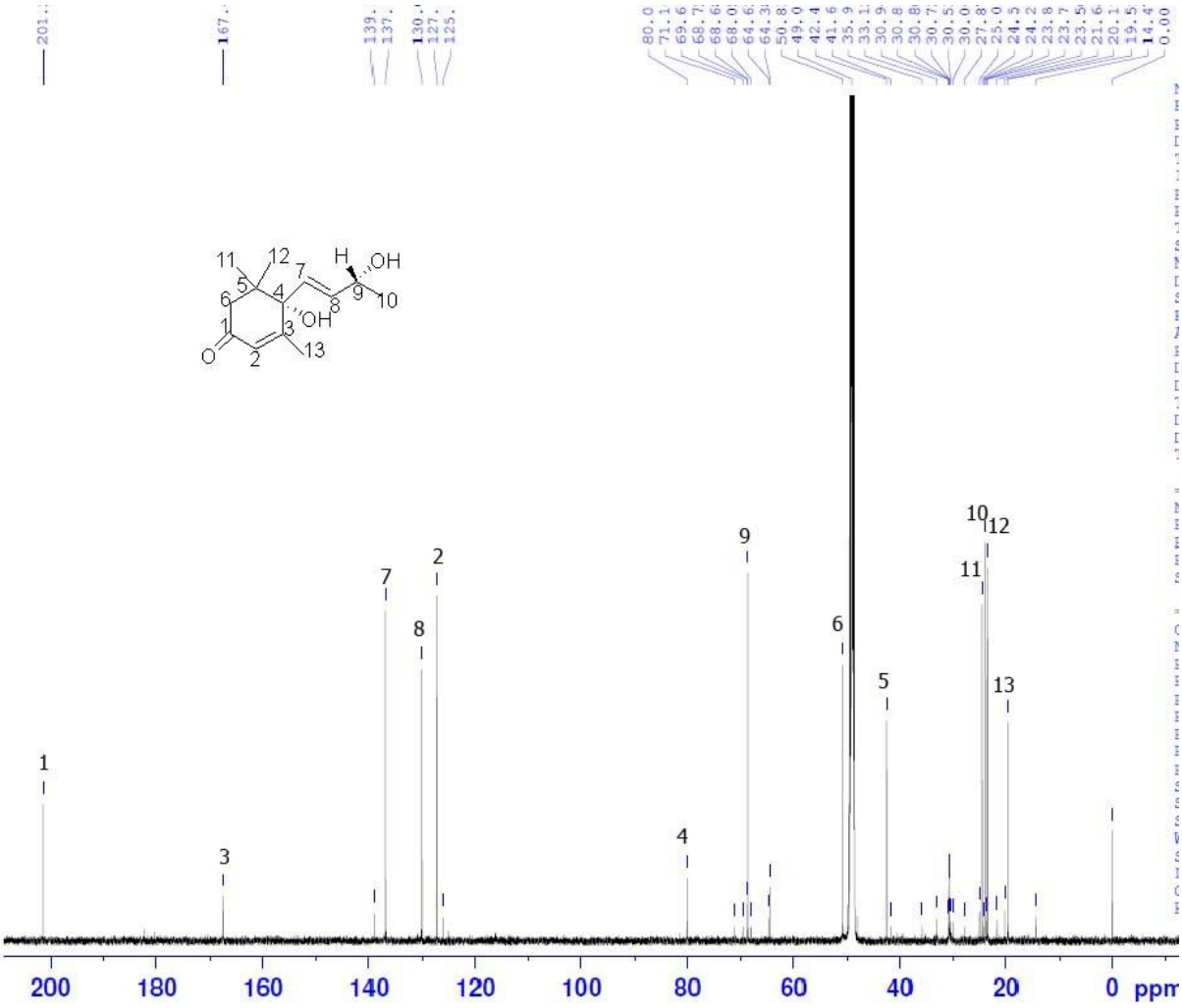

Figure S11 :[13]C-NMR spectrm (CD_3OD, 150MHZ) of compound (**6**)

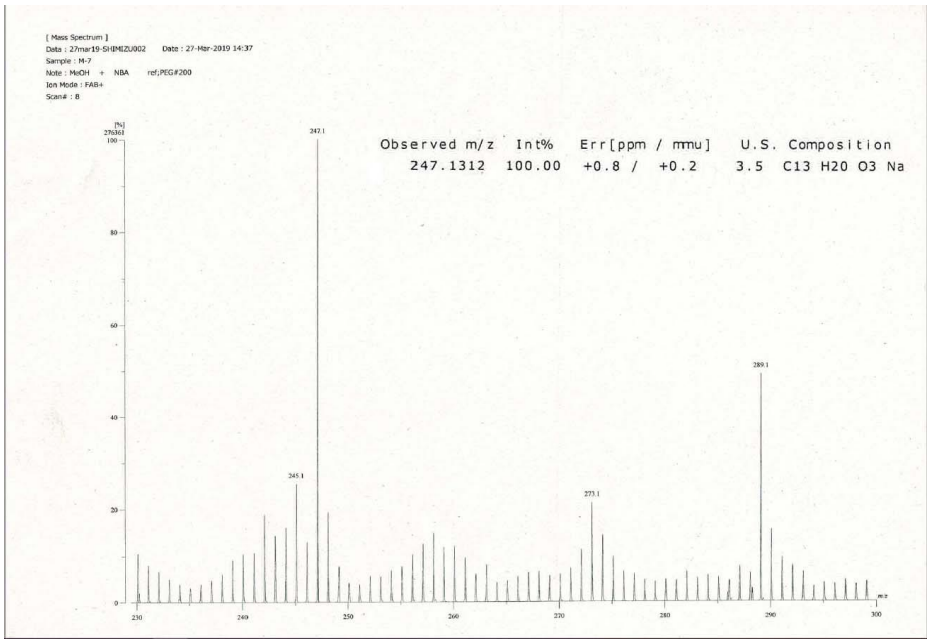

Figure S12 : HR-FAB-MS of compound (**6**)

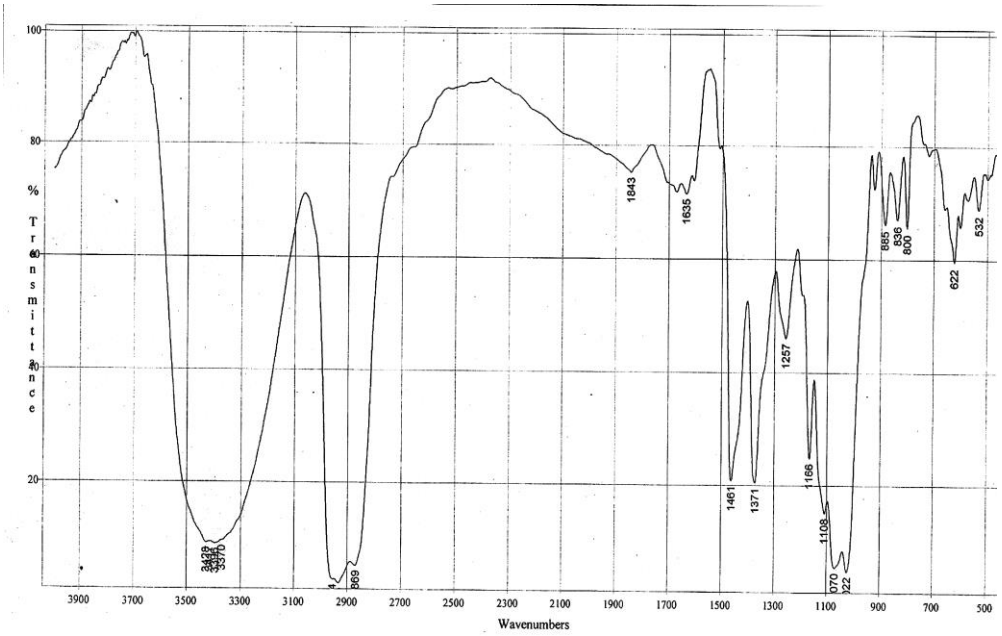

Figure S13 : IR (KBr, υ_{max}) spectrum of compound (**7**)

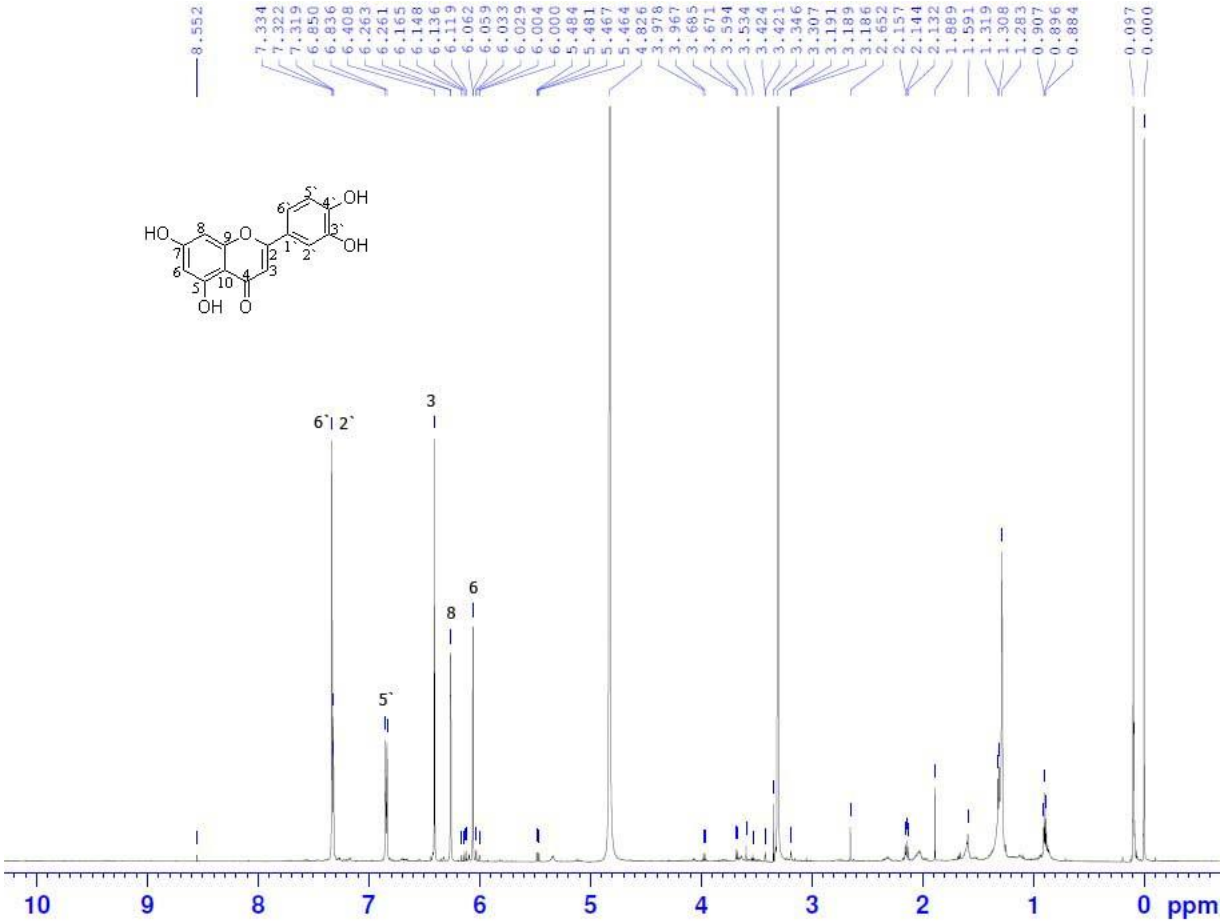

Figure S14 : ^{1}H-NMR spectrum (CD_3OD, 600 MHz) of compound (**8**)

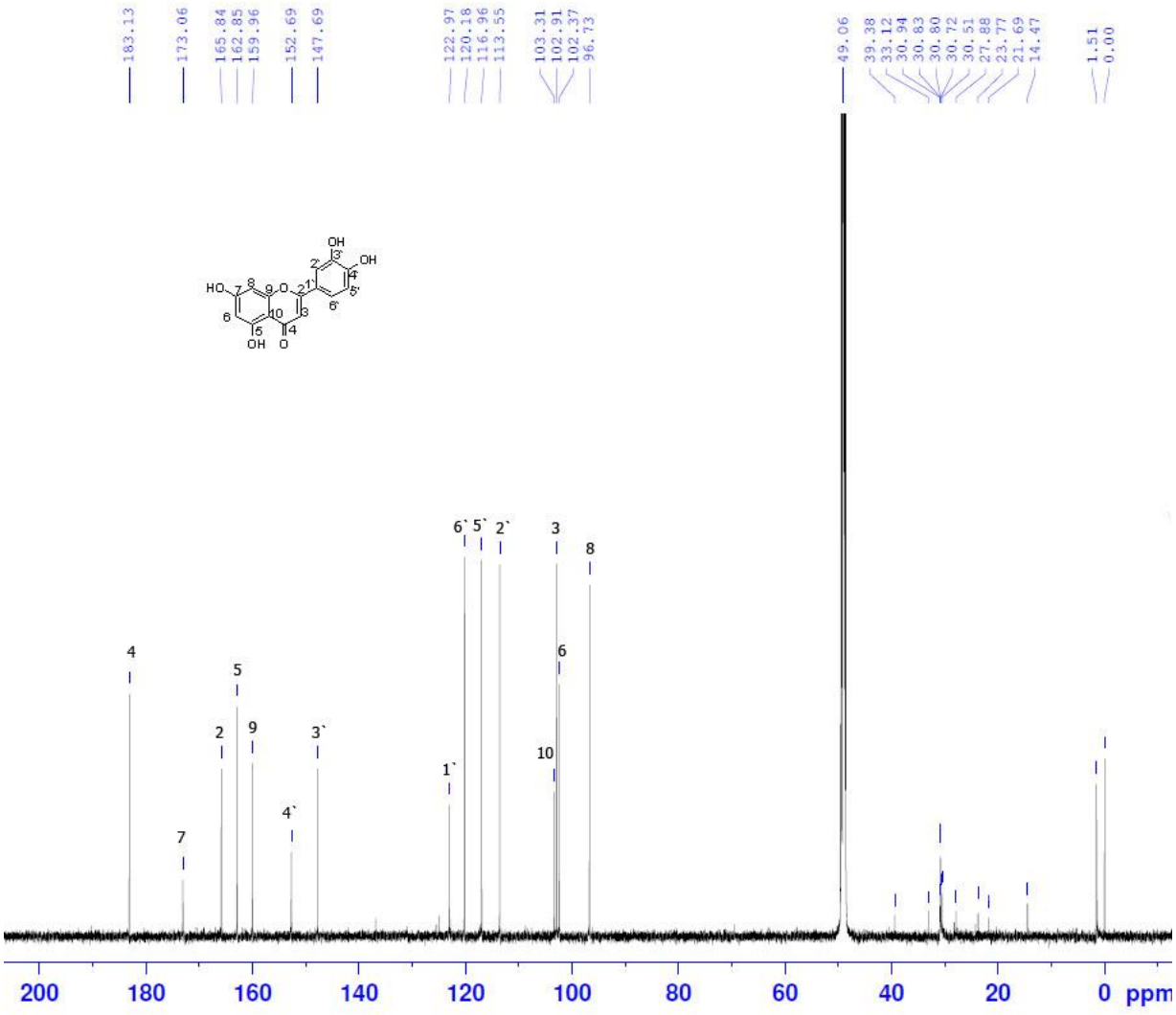

Figure S15 : ^{13}C-NMR spectrm (CD_3OD, 150MHZ) of compound (**8**)

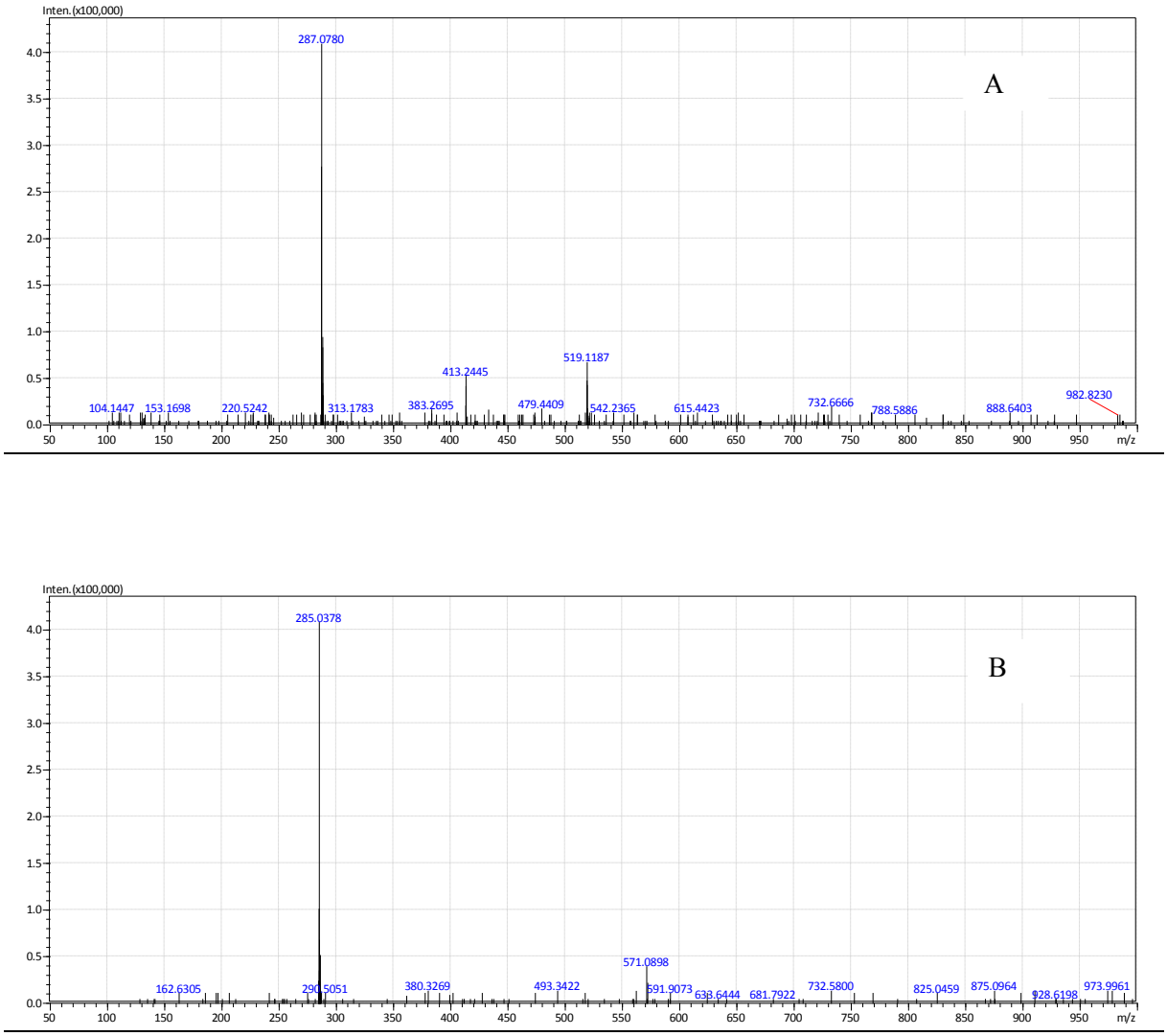

Figure S16 : HR-ESI-MS of compound (**8**)

(A) Positive mode (B) negative mode

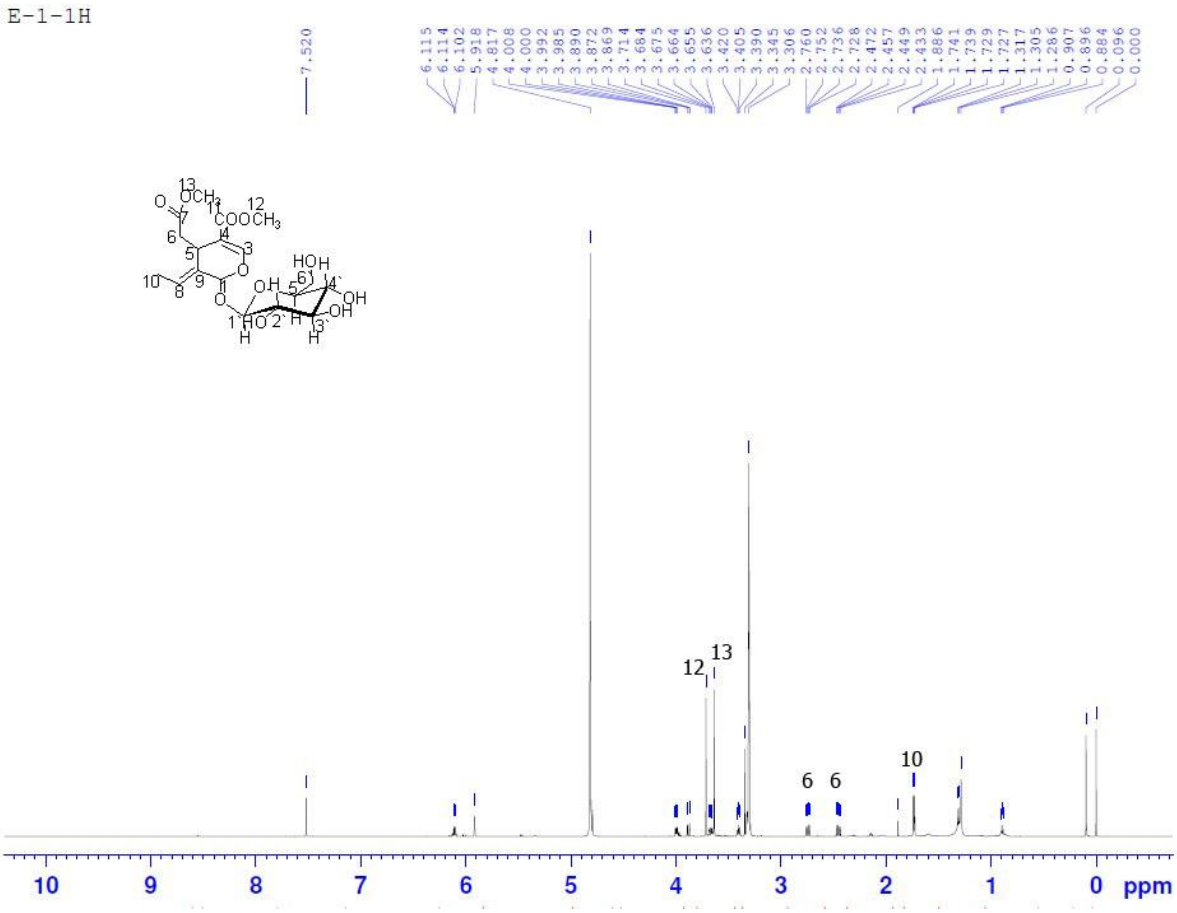

Figure S17 : ^{1}H-NMR spectrum (CD_3OD, 600 MHZ) of compound (**9**)

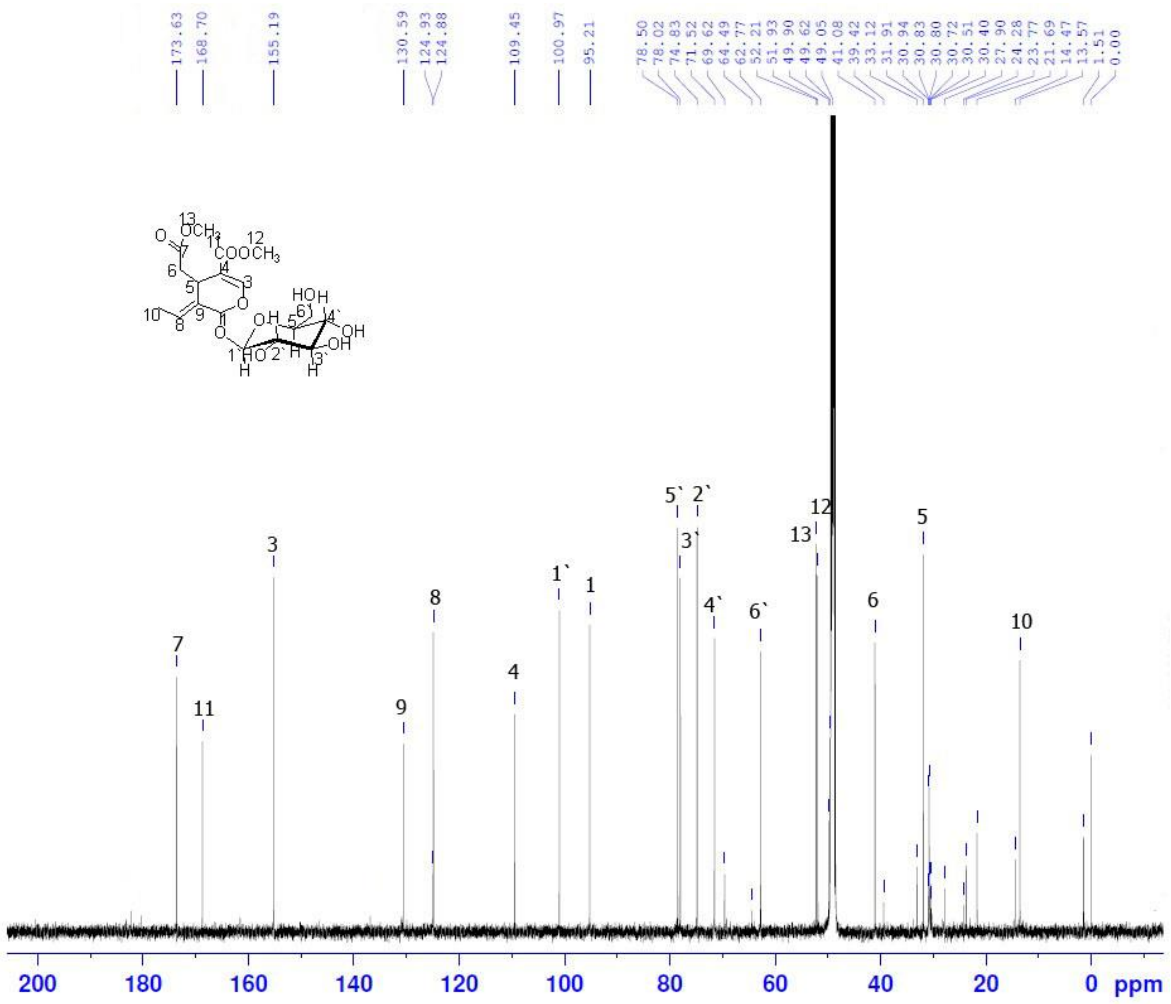

Figure S18 : ^{13}C-NMR spectrum (CD_3OD, 600 MHZ) of compound (**9**)

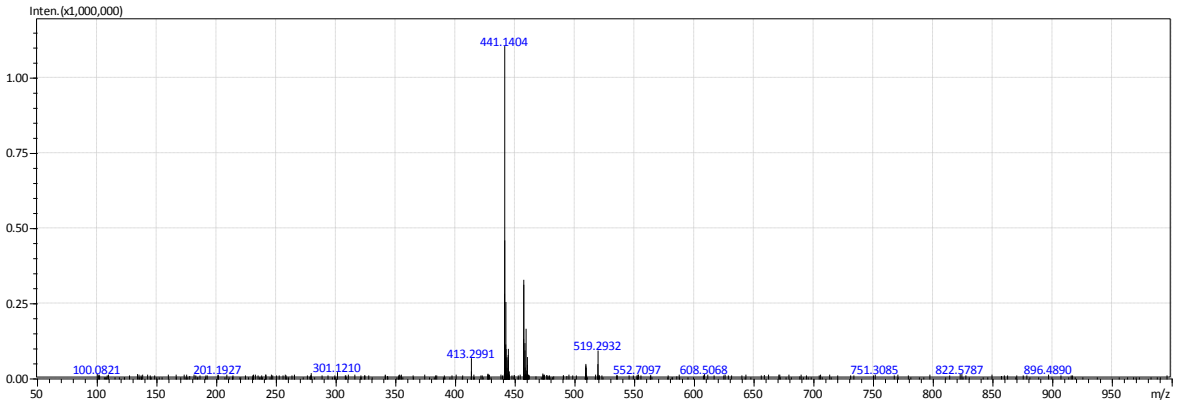

Figure S19 : HR-ESI-MS$^+$ of compound (**9**)

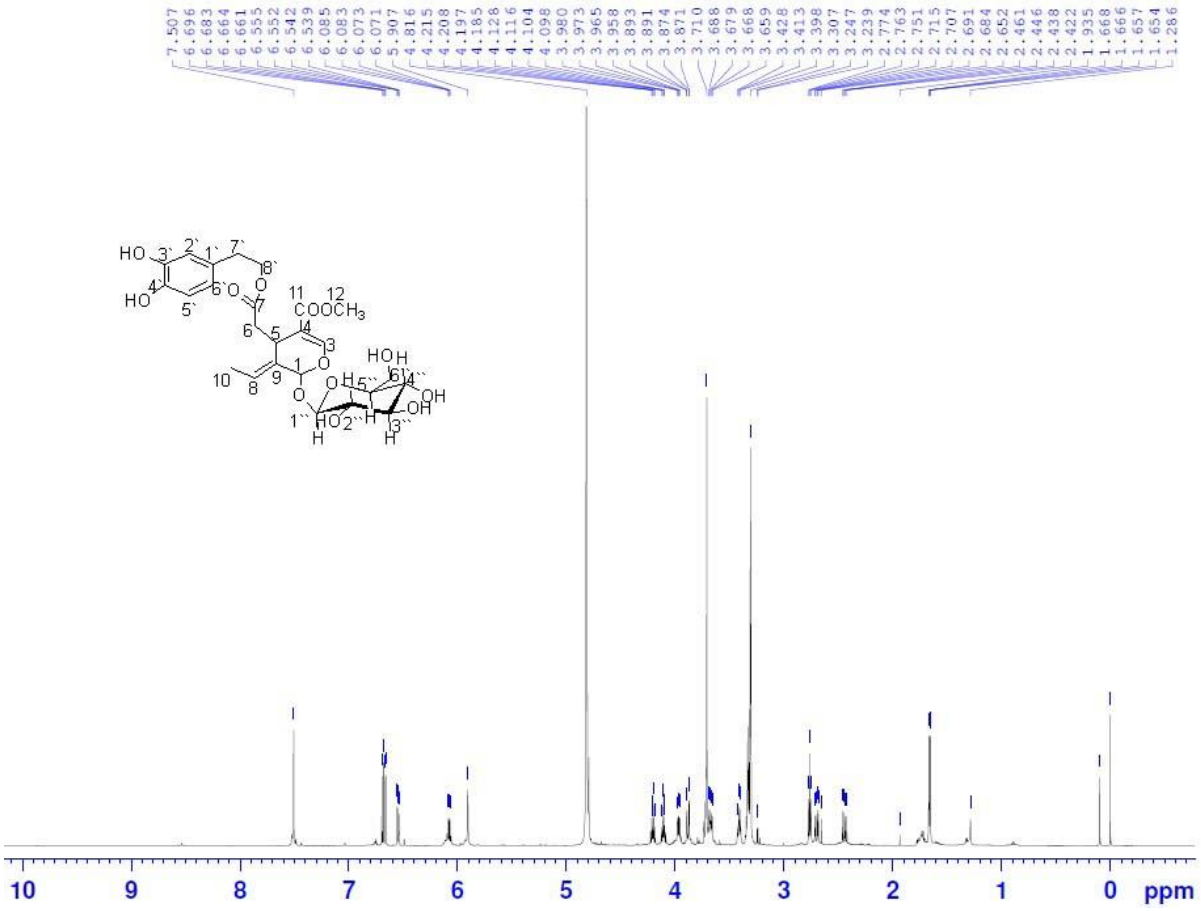

Figure S20 : ^{1}H-NMR spectrum (CD_3OD, 600 MHZ) of compound (**10**)

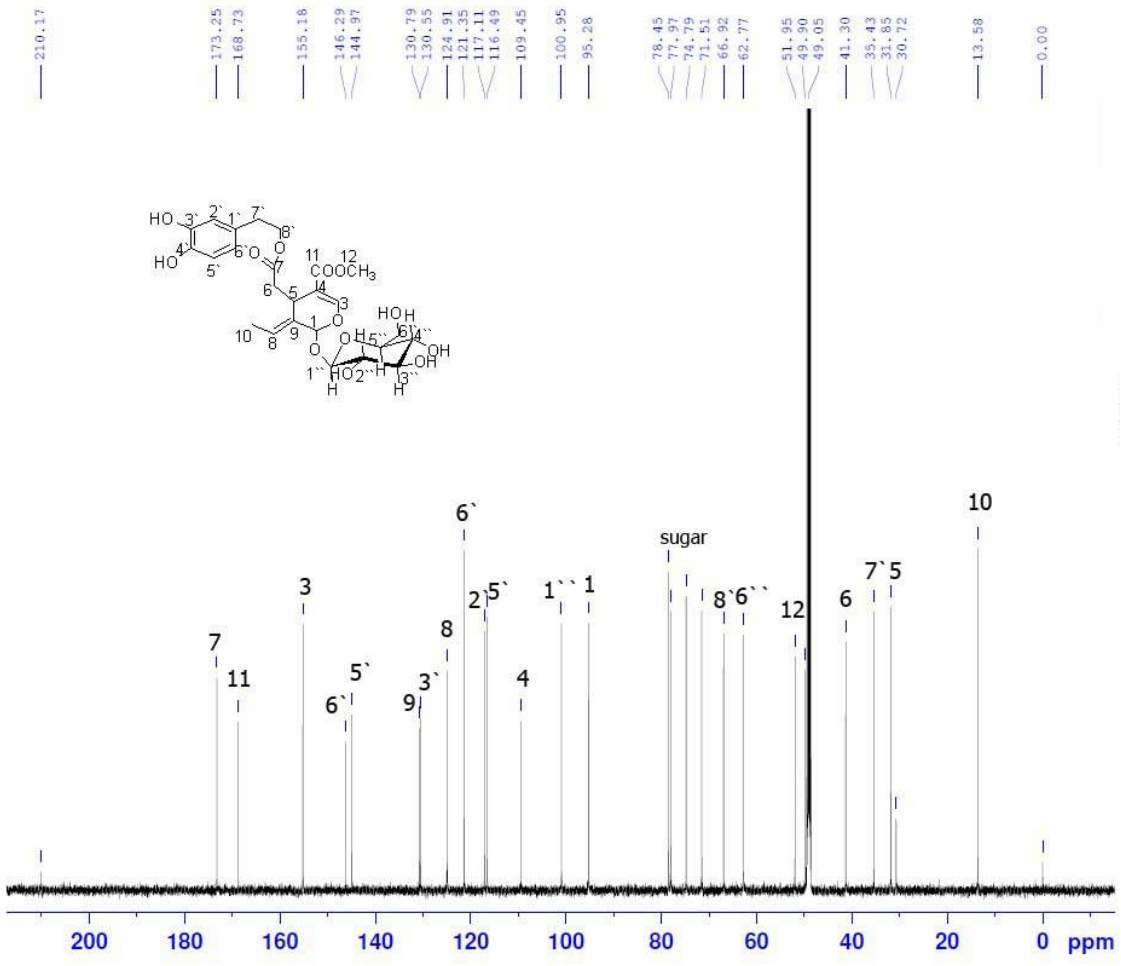

Figure S21 : ^{13}C-NMR spectrum (CD_3OD, 150 MHZ) of compound (**10**)

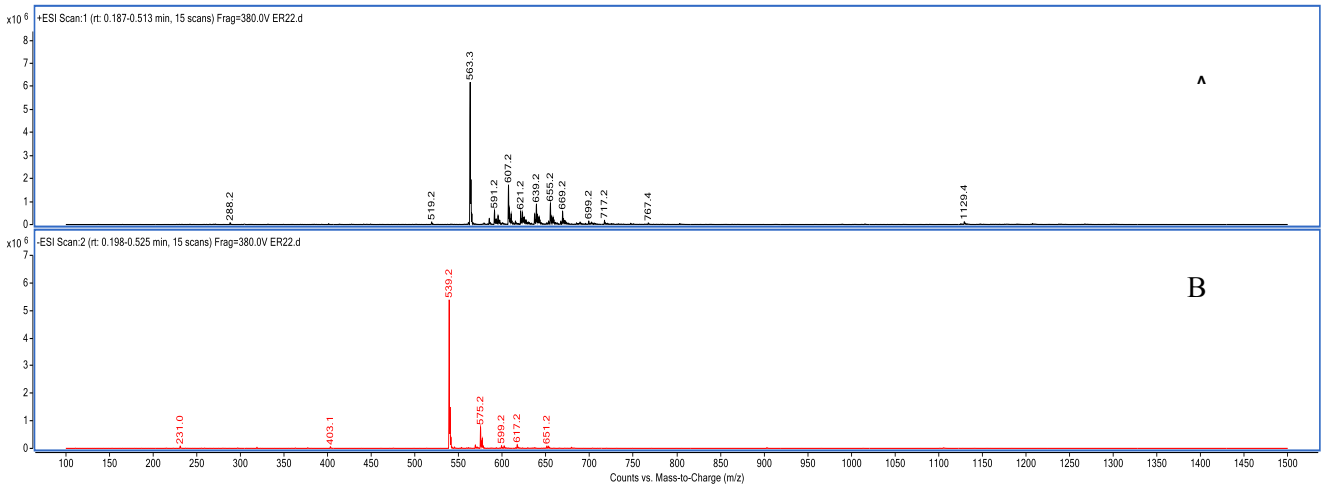

Figure S22 : ESI-MS of compound (**10**)

(A) Positive mode (B) negative mode

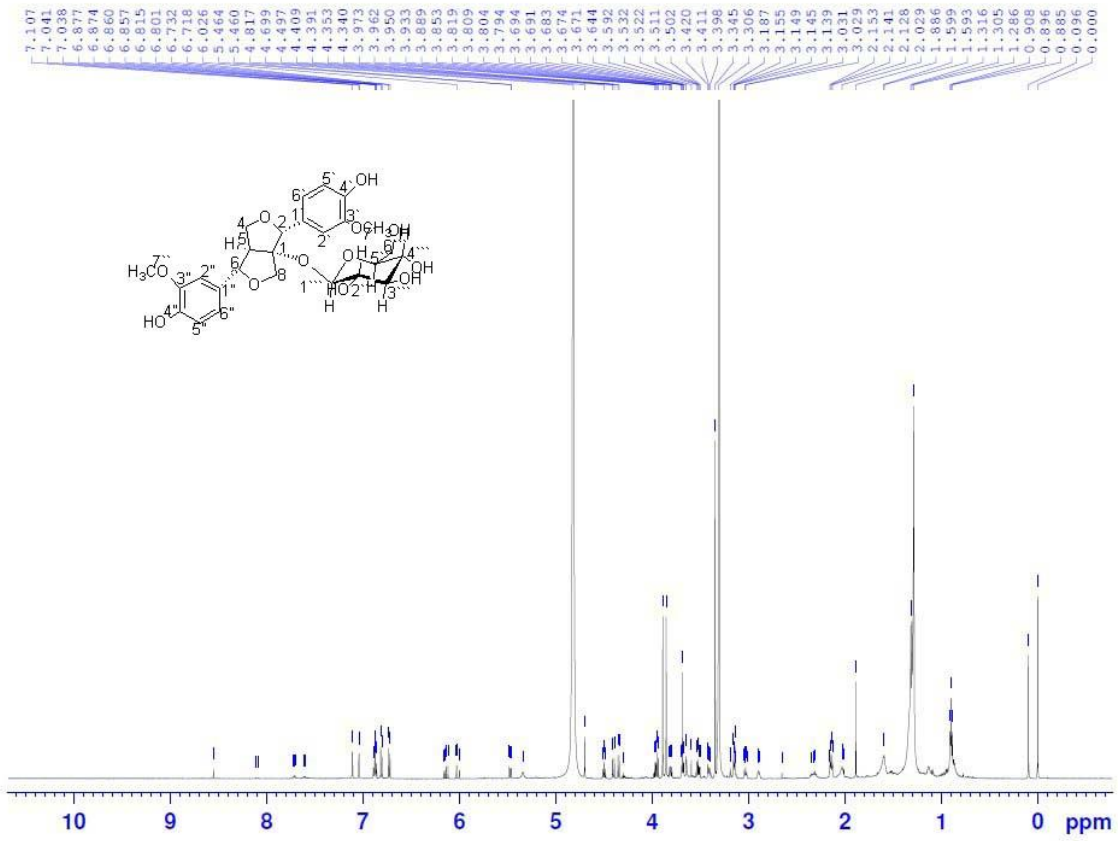

Figure S23 : ^{1}H-NMR spectrum (CD_3OD, 600 MHz) of compound (**11**)

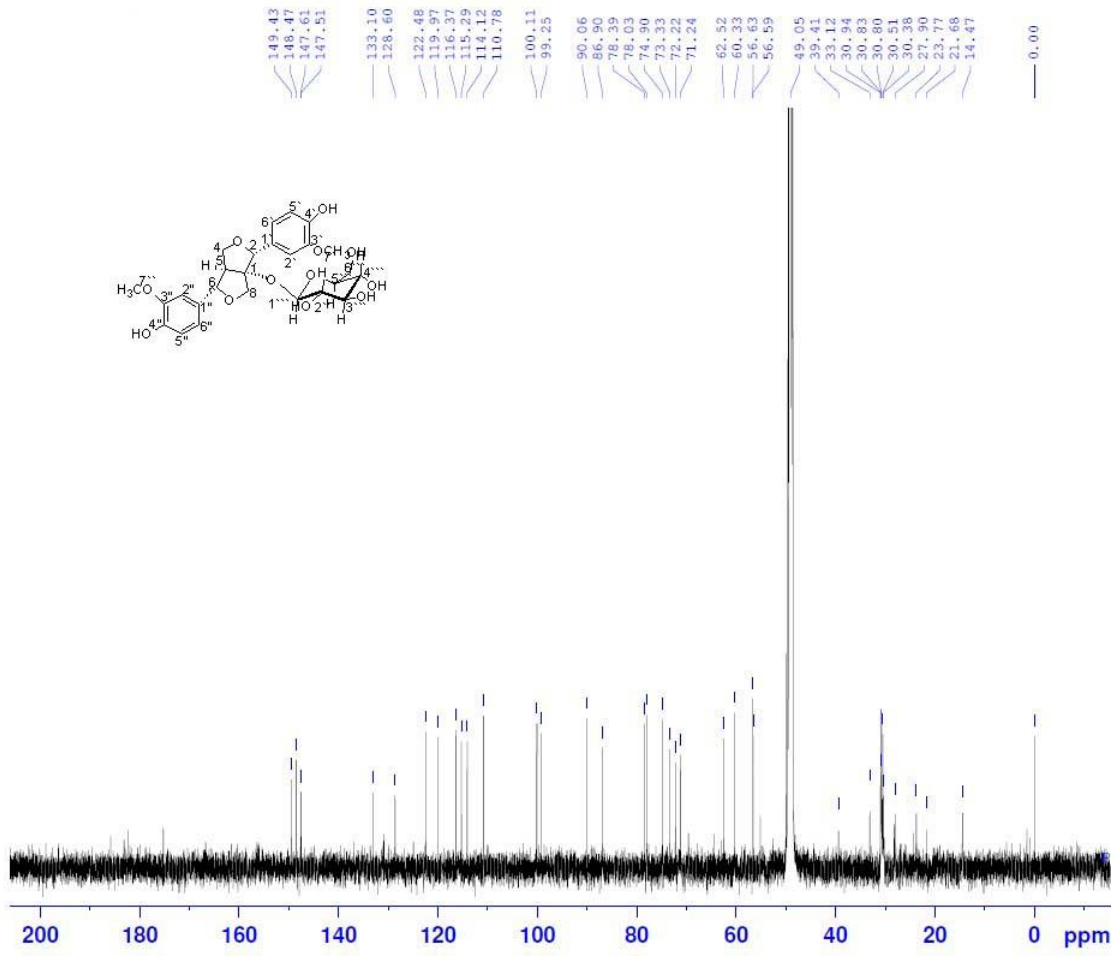

Figure S24 : ^{13}C-NMR spectrum (CD_3OD, 150 MHz) of compound (**11**)

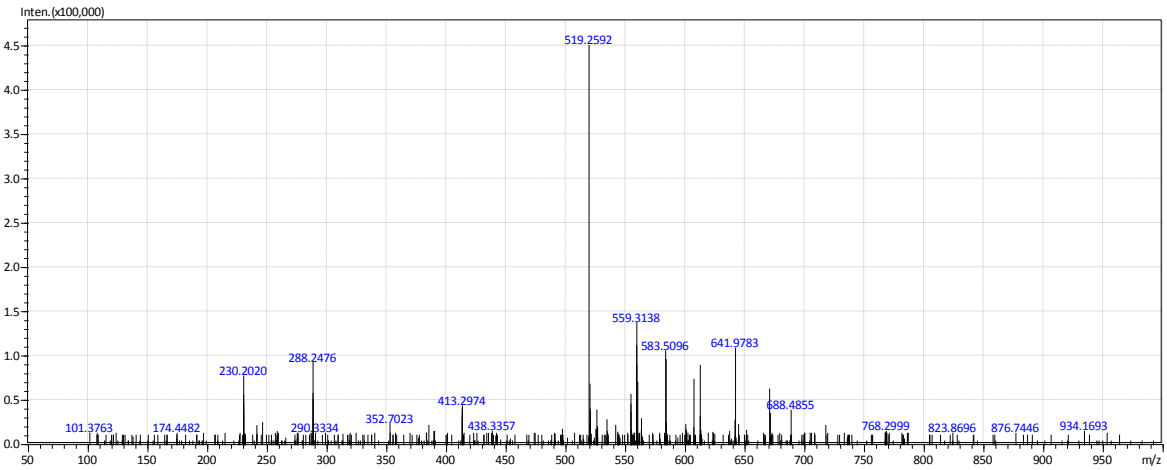

Figure S25 : HR-ESI-MS$^+$ of compound (**11**)

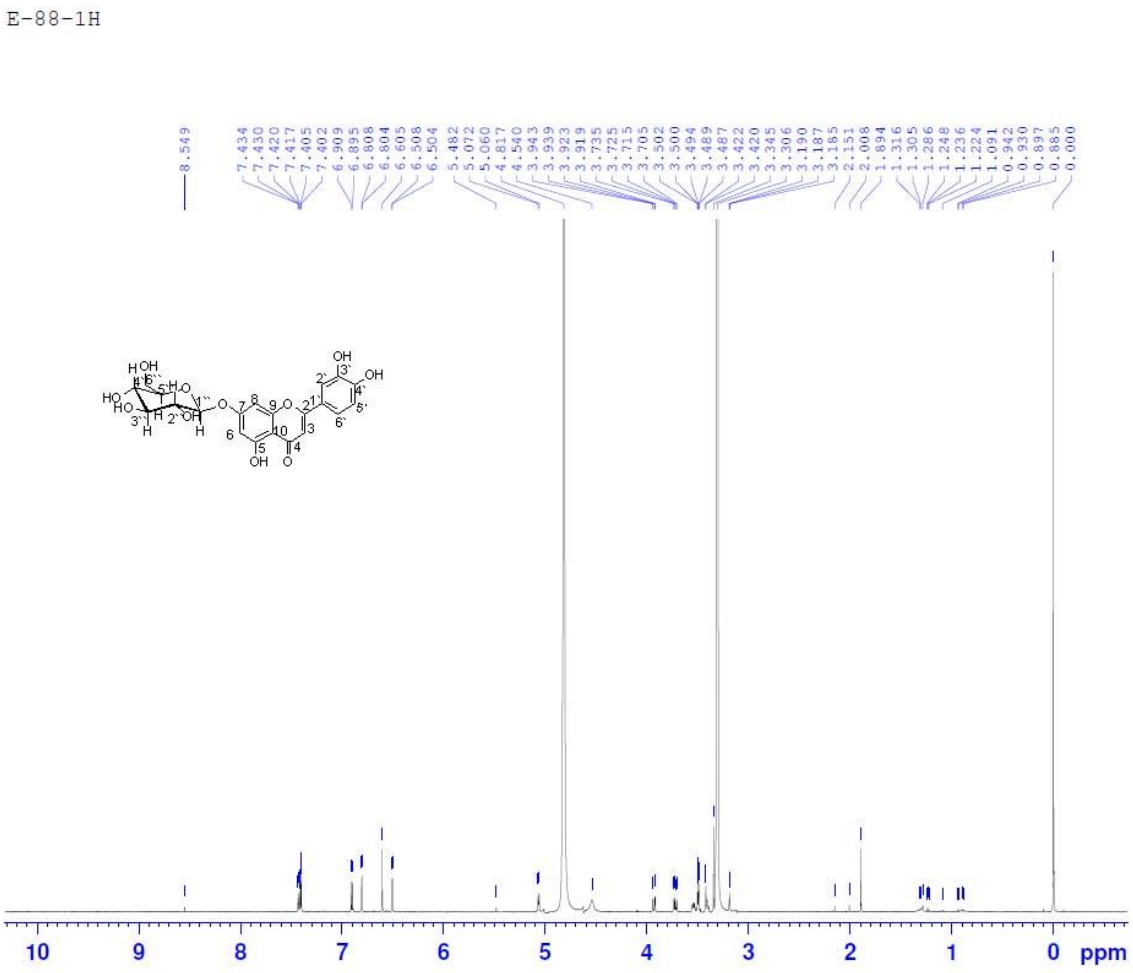

Figure S26 : ^{1}H-NMR spectrum (CD_3OD, 600 MHz) of compound (**12**)

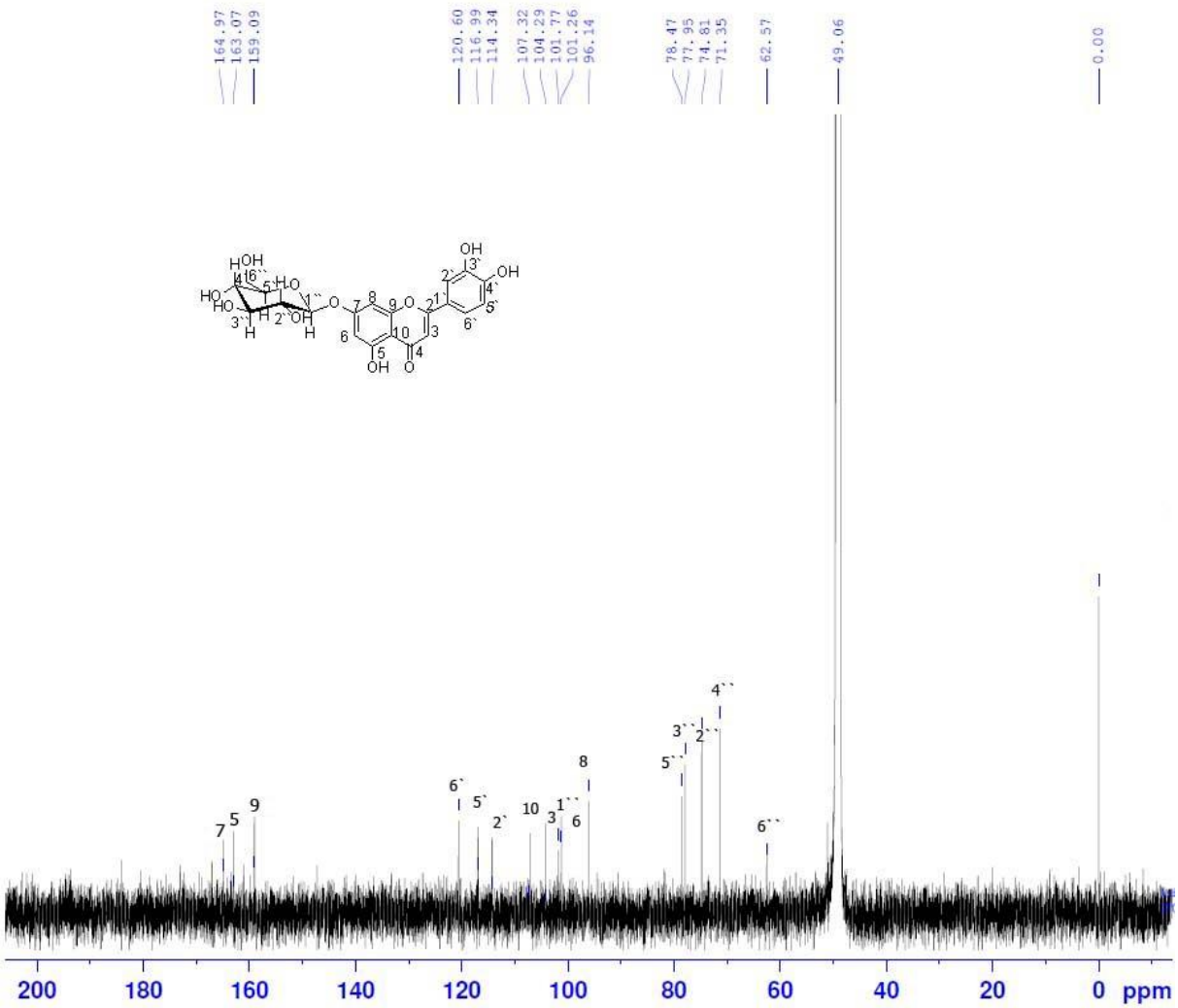

Figure S27 : ^{13}C-NMR spectrum (CD_3OD, 150MHz) of compound (**12**)

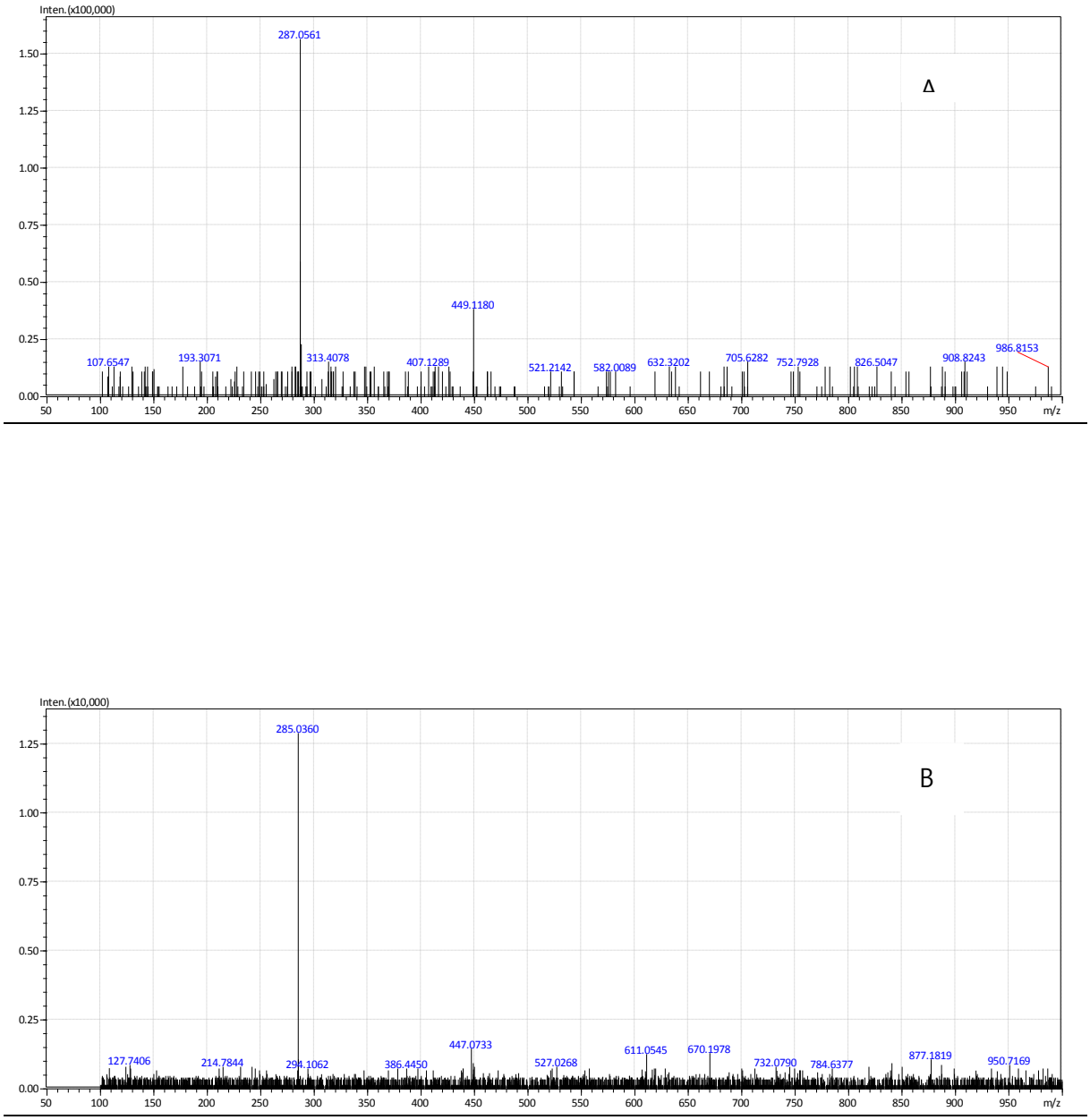

Figure S28 : HR-ESI-MS of compound (**12**)

(A) Positive mode (B) negative mode.

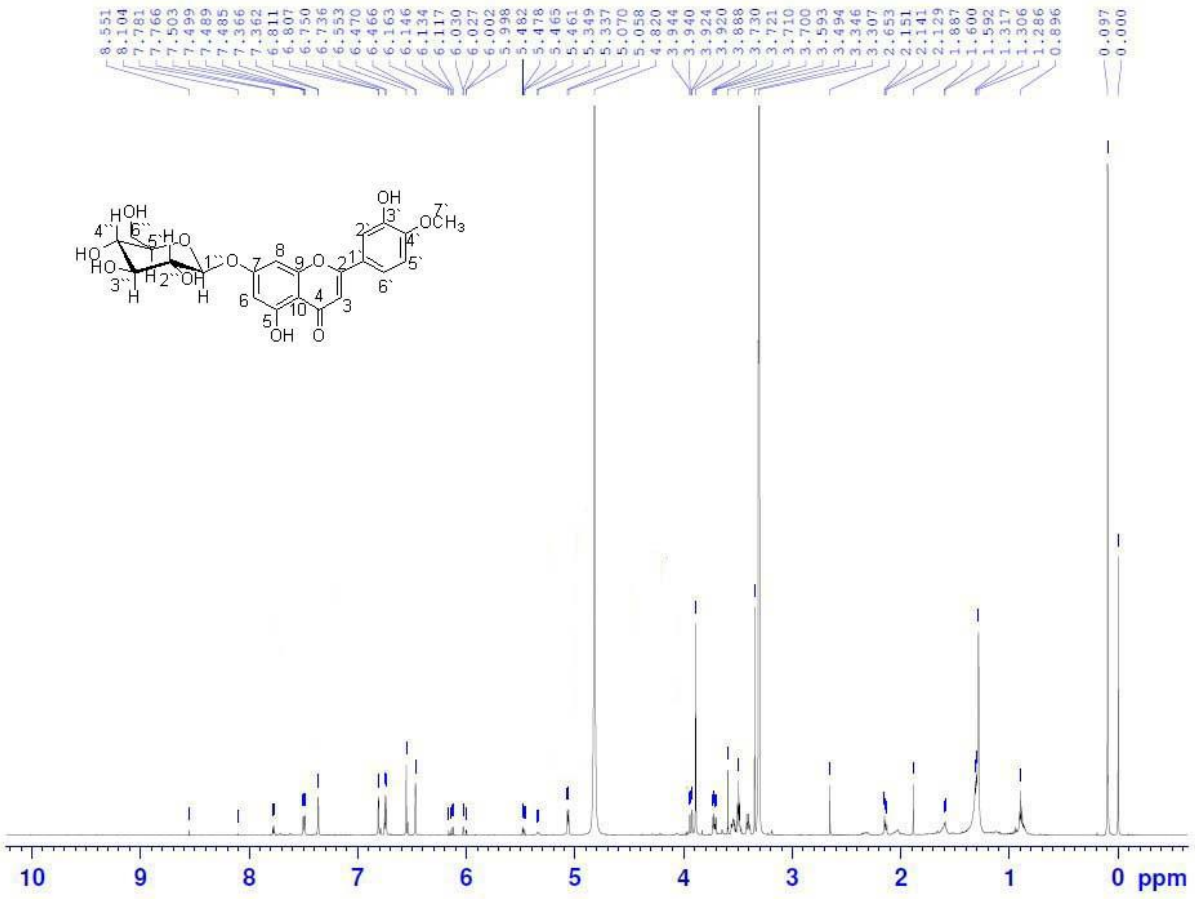

Figure S29 : ^{1}H-NMR spectrum (CD_3OD, 600MHz) of compound (**13**)

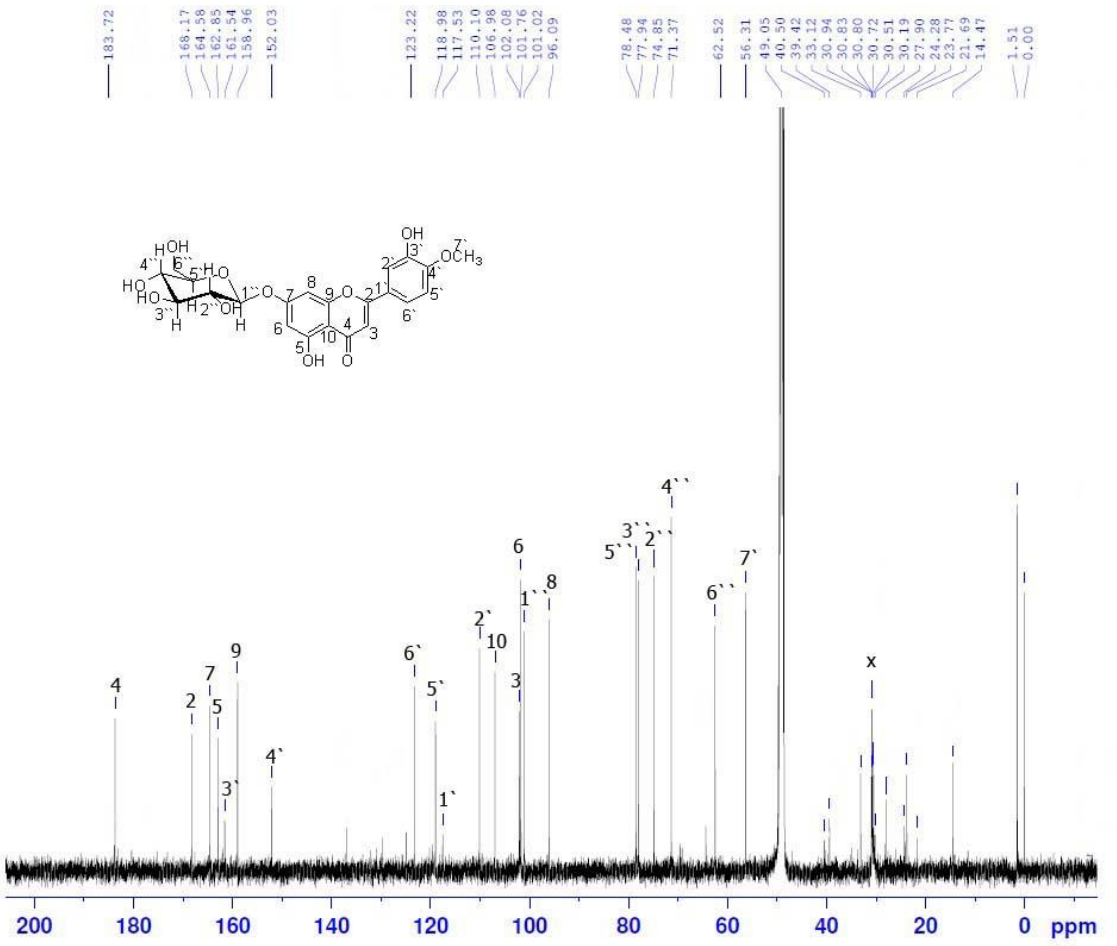

Figure S30 : ^{13}C-NMR spectrum (CD_3OD, 150MHz) of compound (**13**)

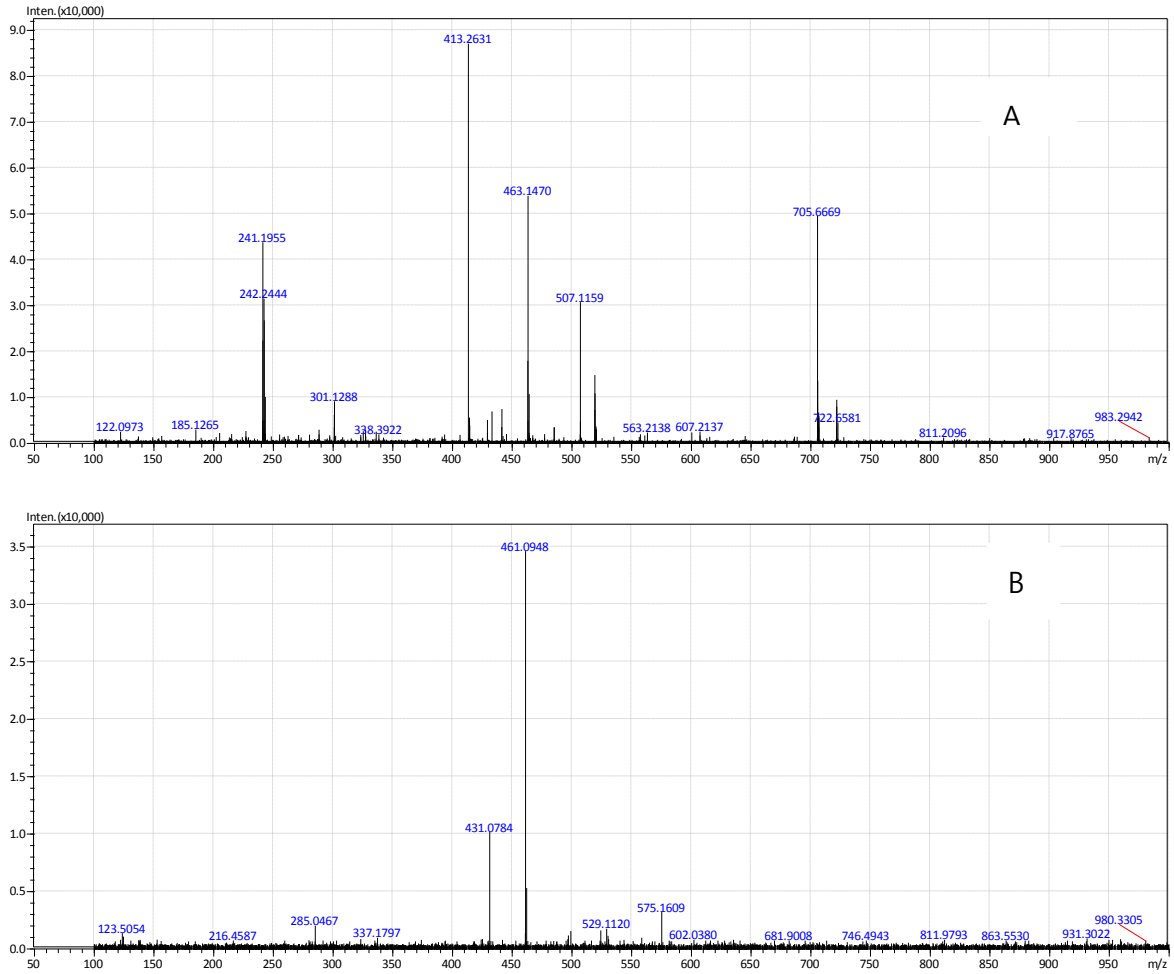

Figure S31 : HR-ESI-MS of compound (**13**)

(A) Positive mode (B) negative mode

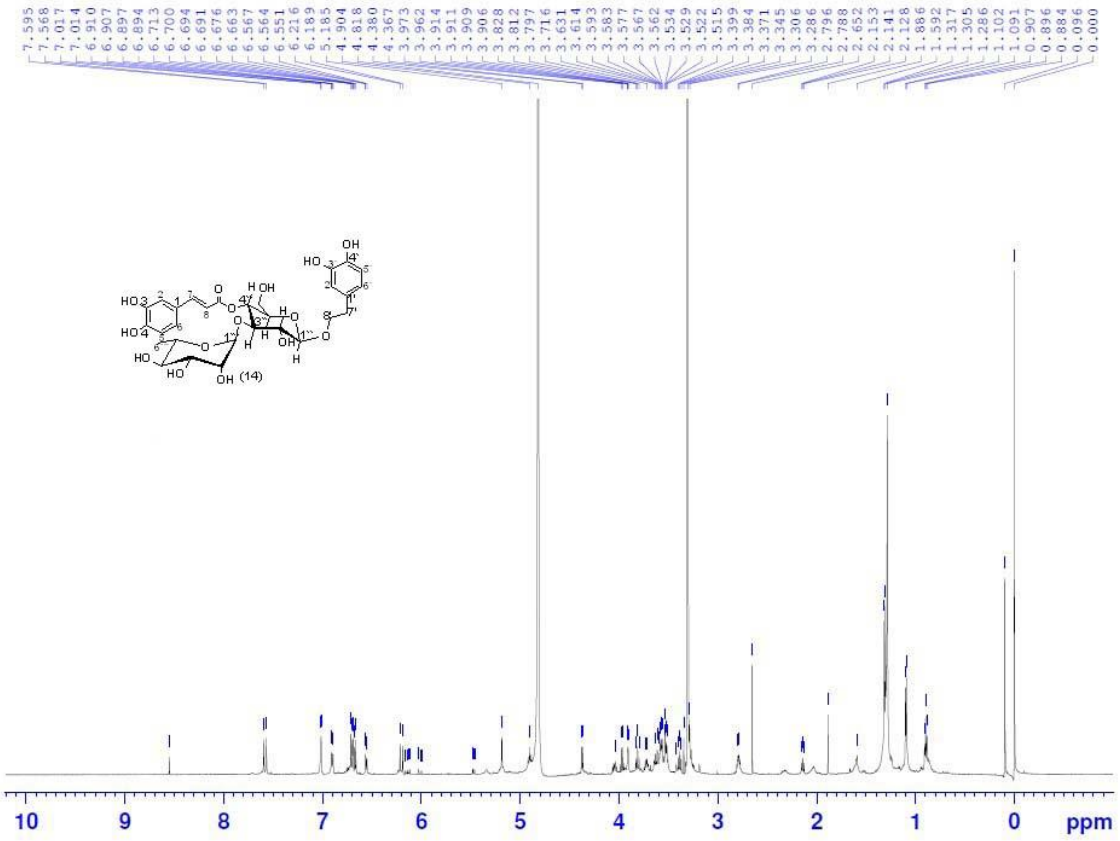

Figure S32 : ^{1}H-NMR spectrum (CD_3OD, 600 MHz) of compound (**14**)

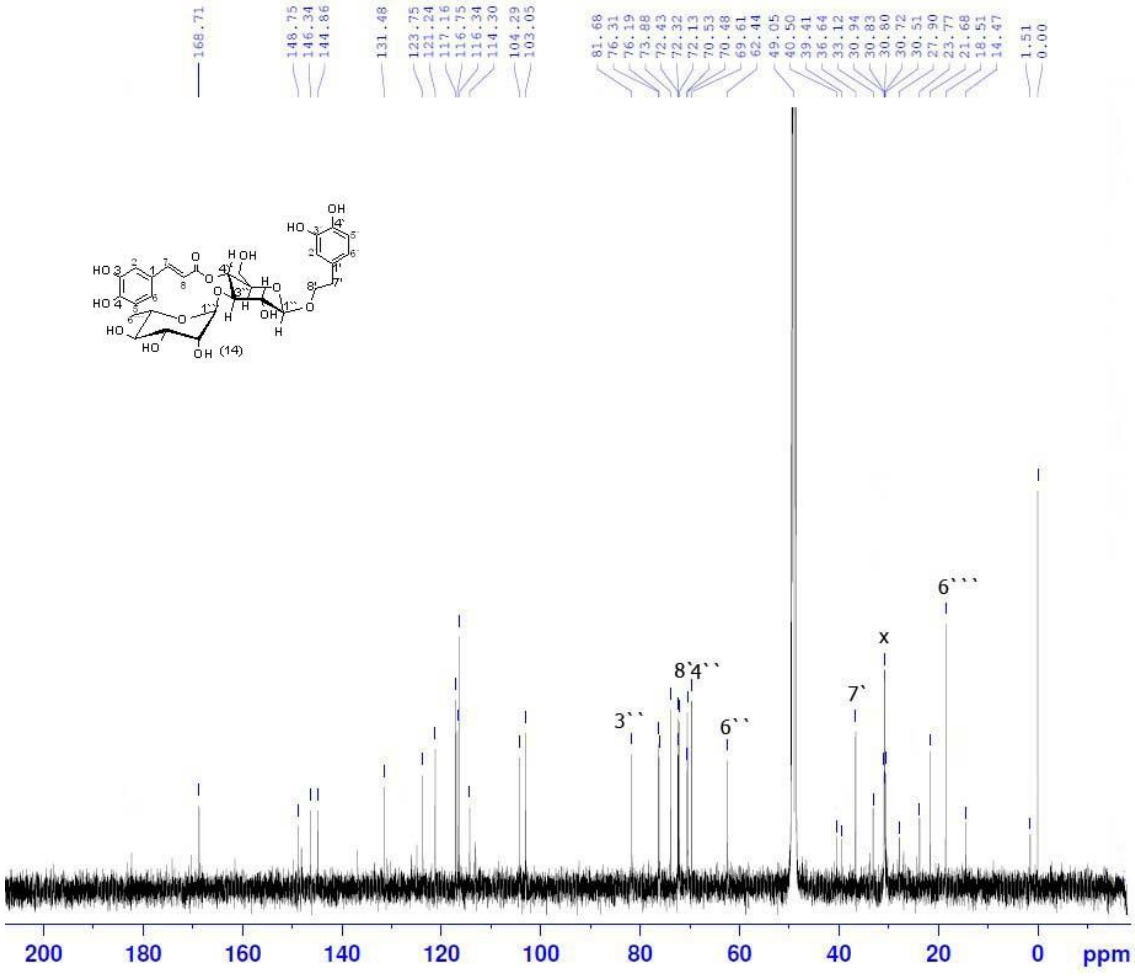

Figure S33 : ^{13}C-NMR spectrum (CD_3OD, 150 MHz) of compound (**14**)

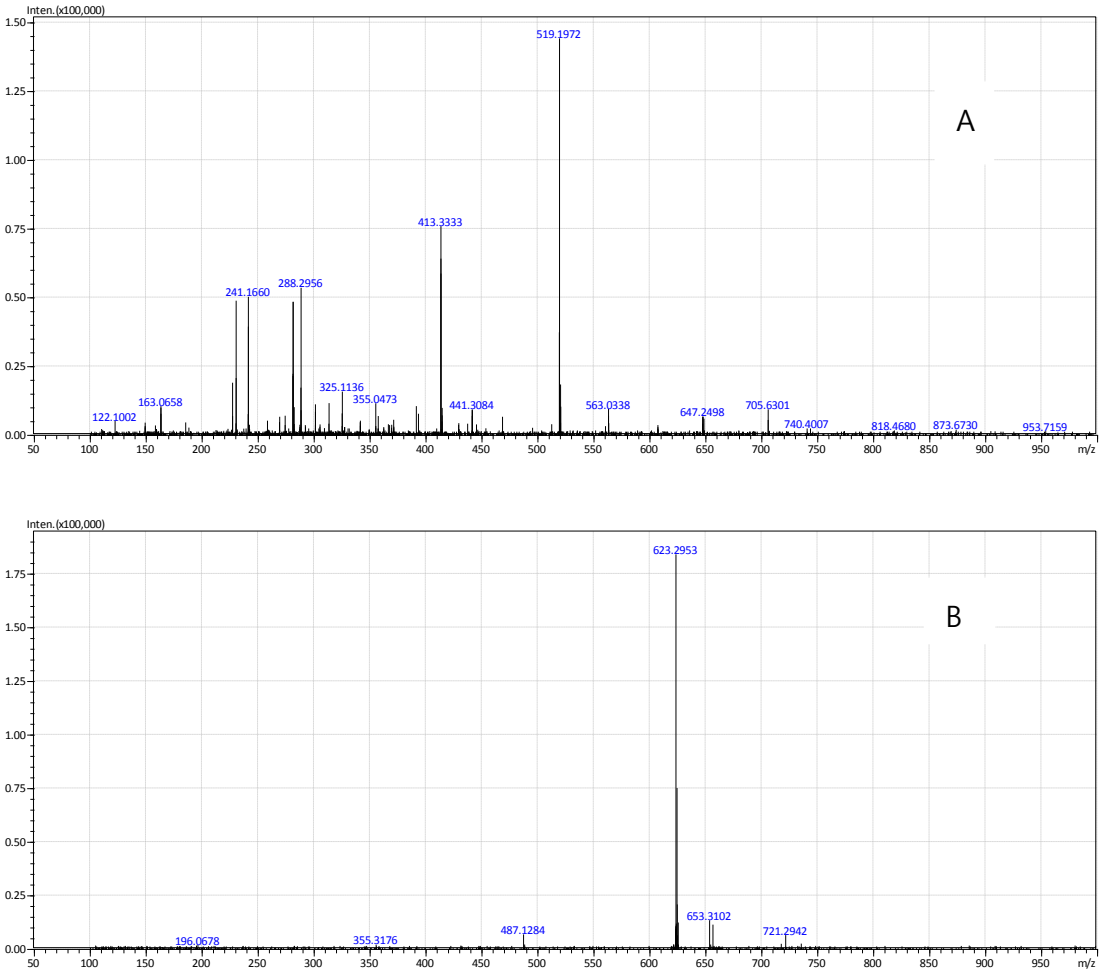

Figure S34 : HR-ESI-MS of compound (**14**)

(A) Positive mode (B) negative mode.

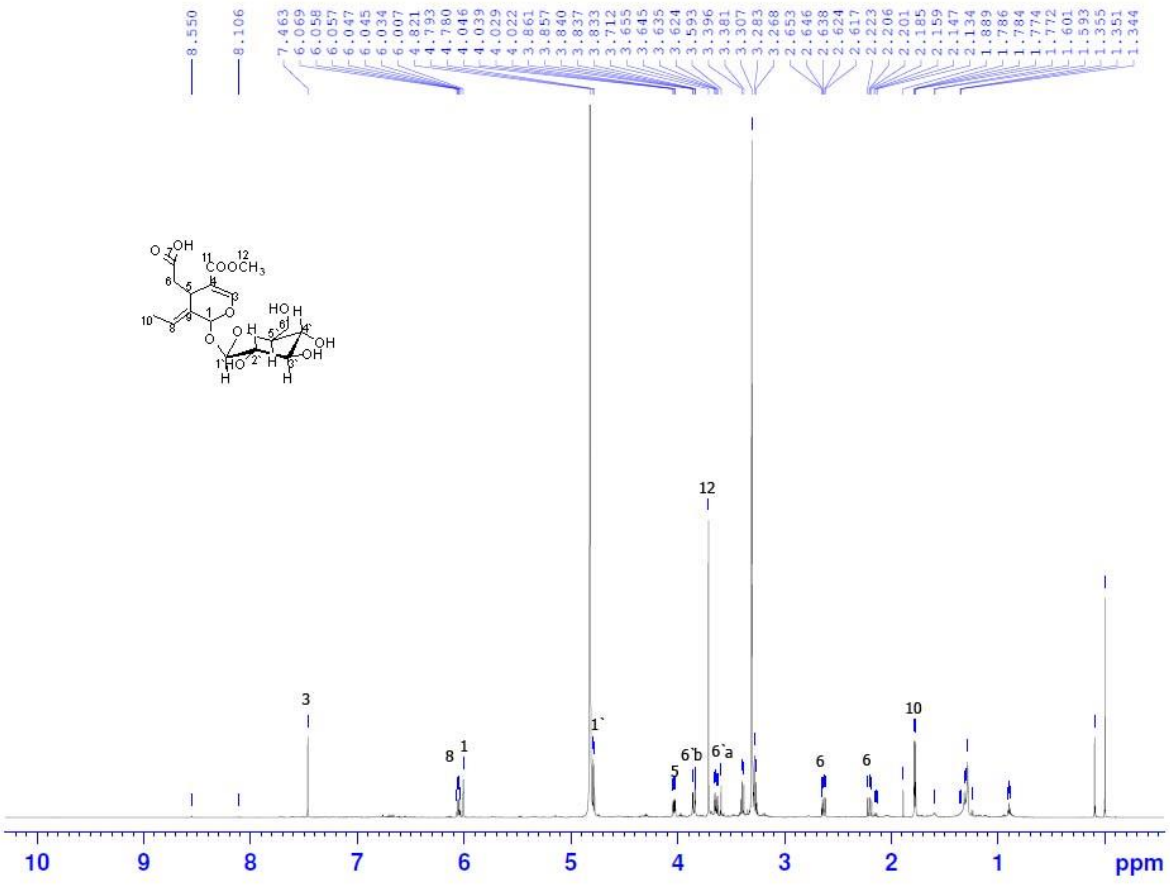

Figure S35 : ^{1}H-NMR spectrum (CD_3OD, 600 MHz) of compound (**15**)

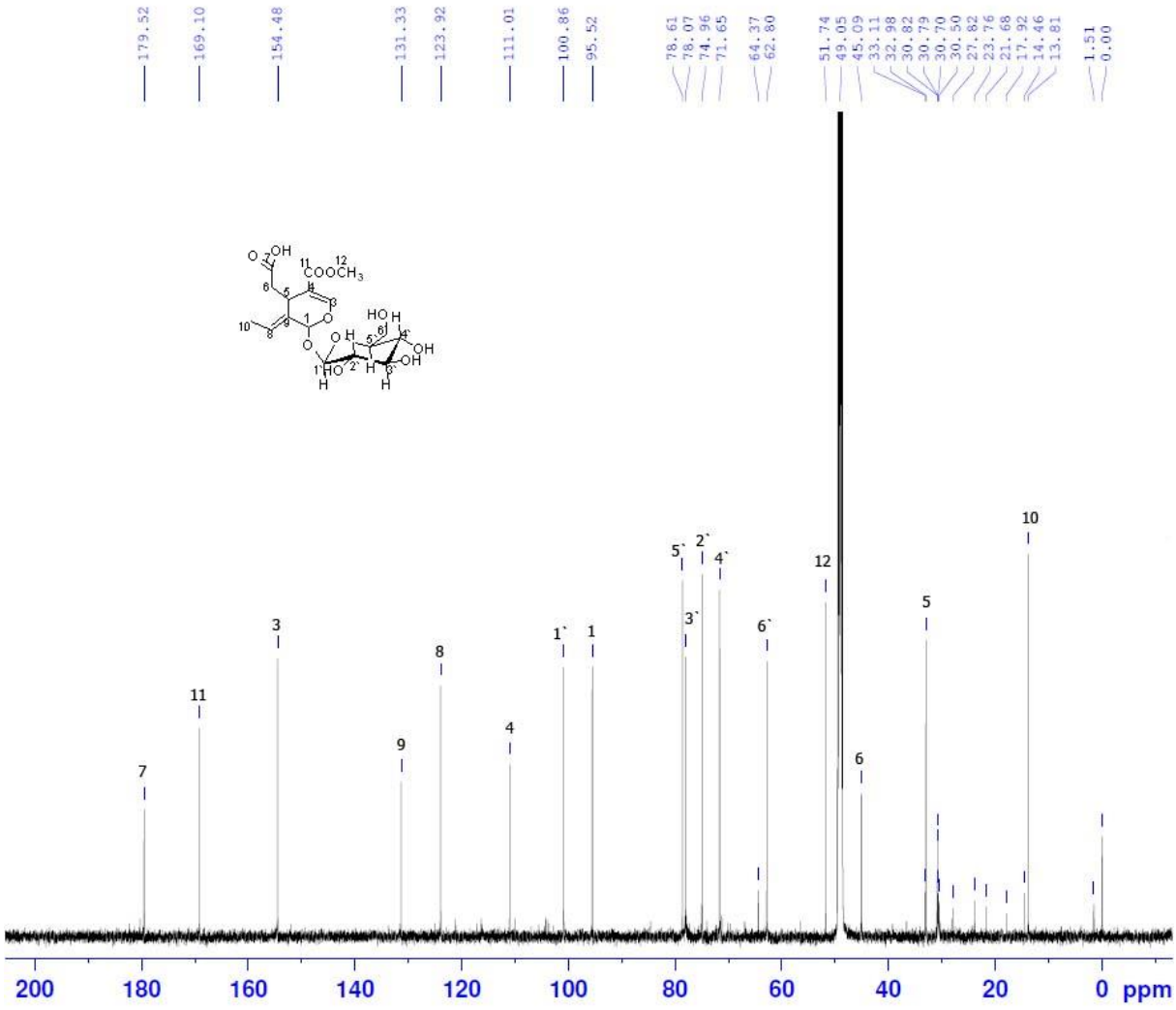

Figure S36 : ^{13}C-NMR spectrum (CD_3OD, 600 MHz) of compound (**15**)

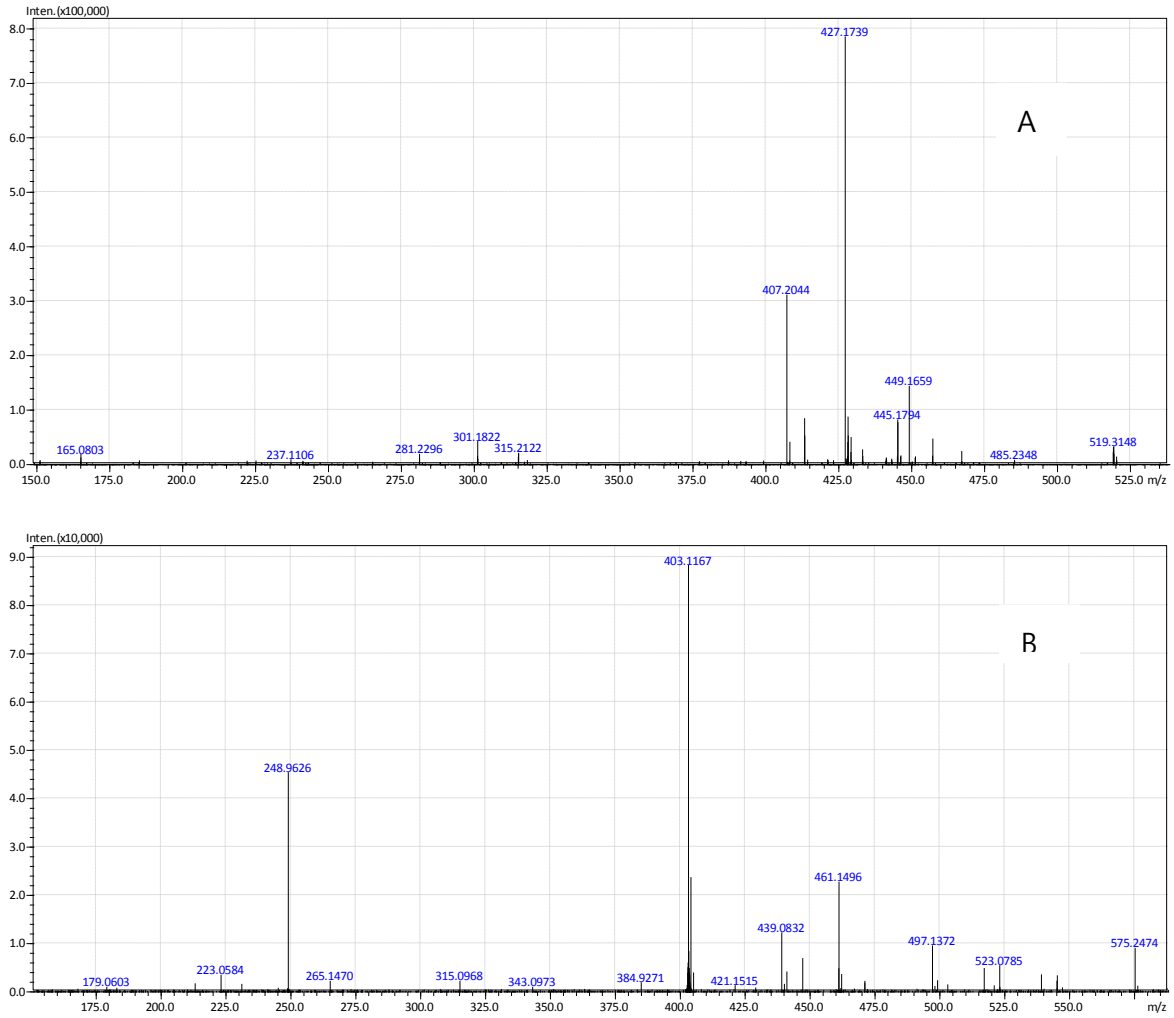

Figure S37 : HR-ESI-MS of compound (**15**)

(A) Positive mode (B) negative mode

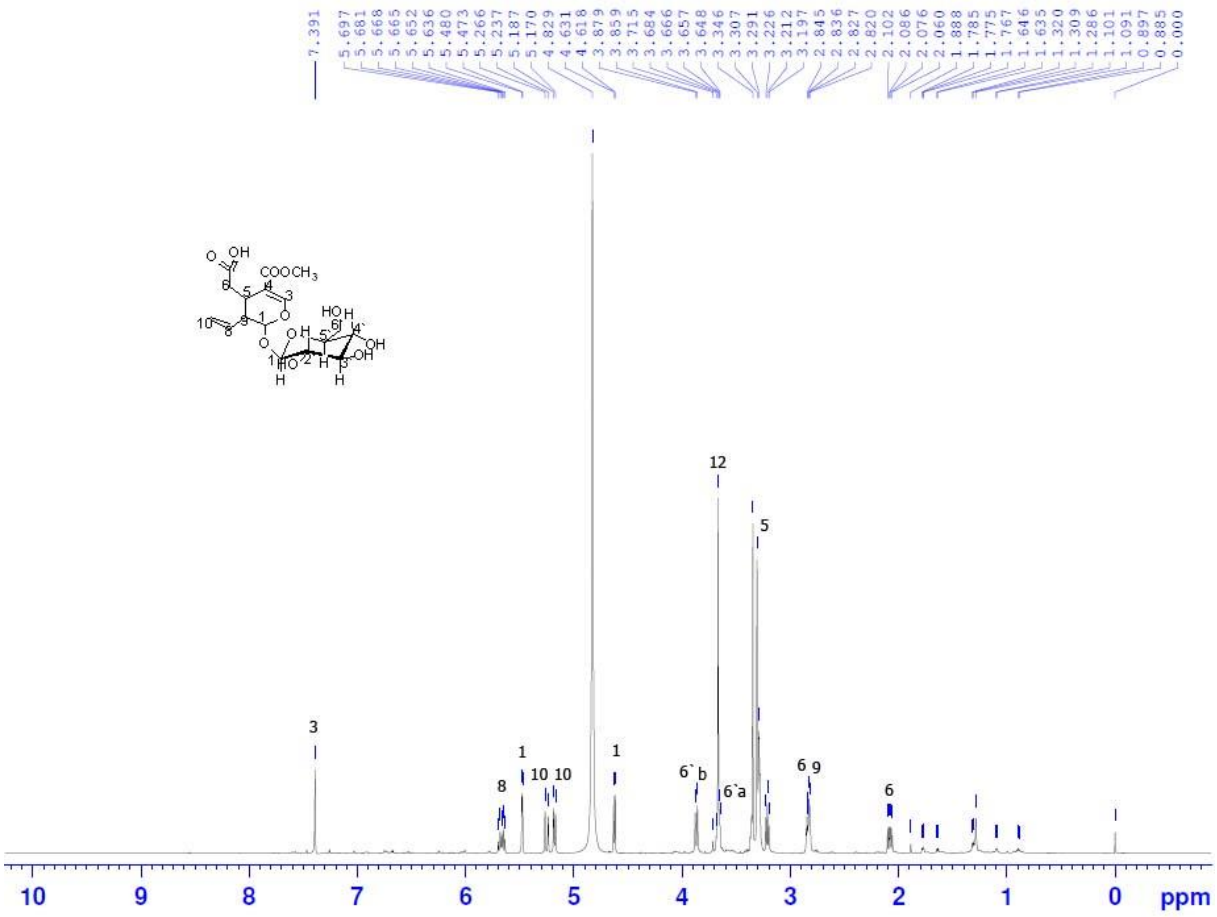

Figure S38 : ^{1}H-NMR spectrum (CD_3OD, 600 MHz) of compound (**16**)

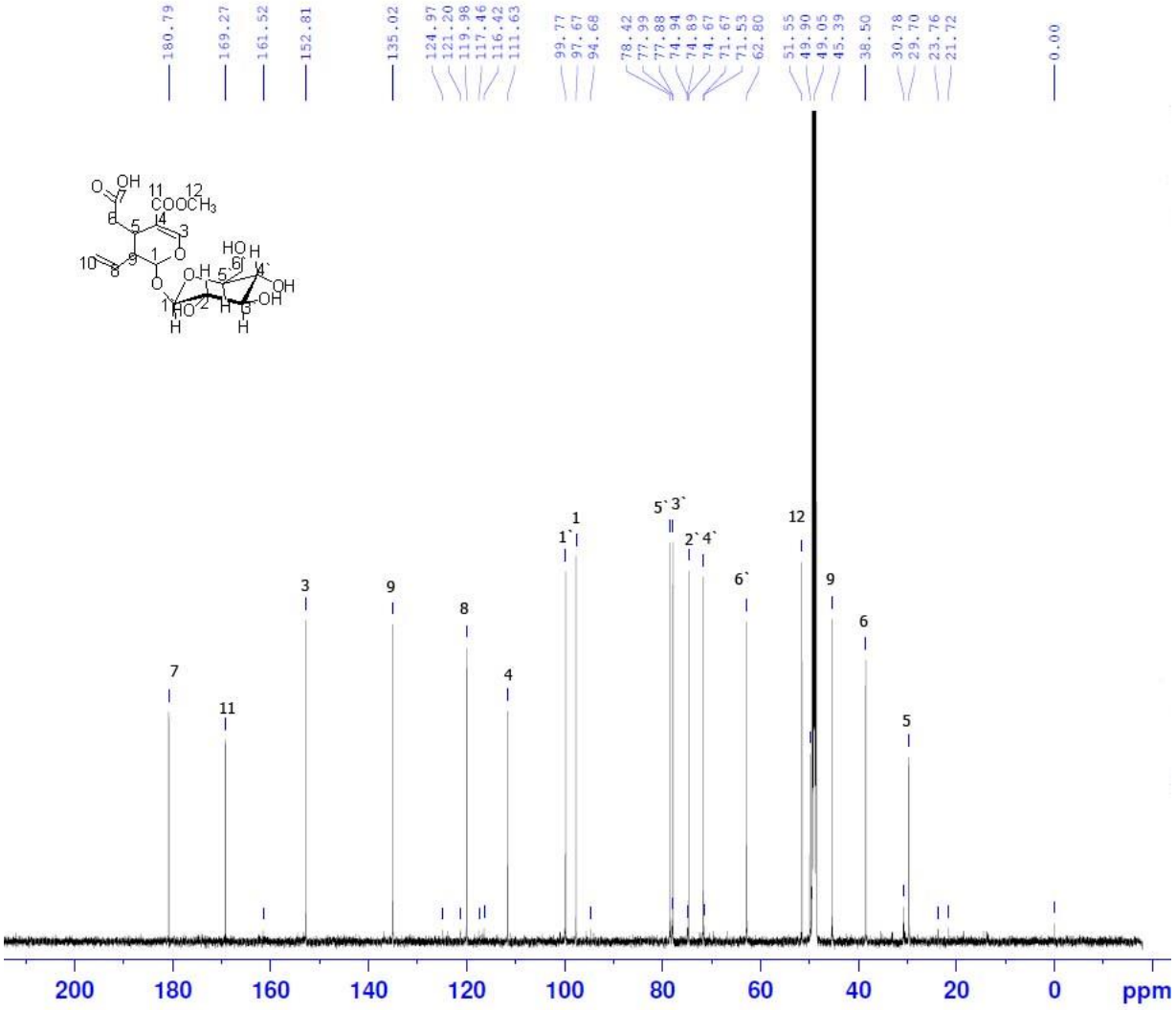

Figure S39 :^{13}C-NMR spectrum (CD_3OD, 150 MHz) of compound (**16**)

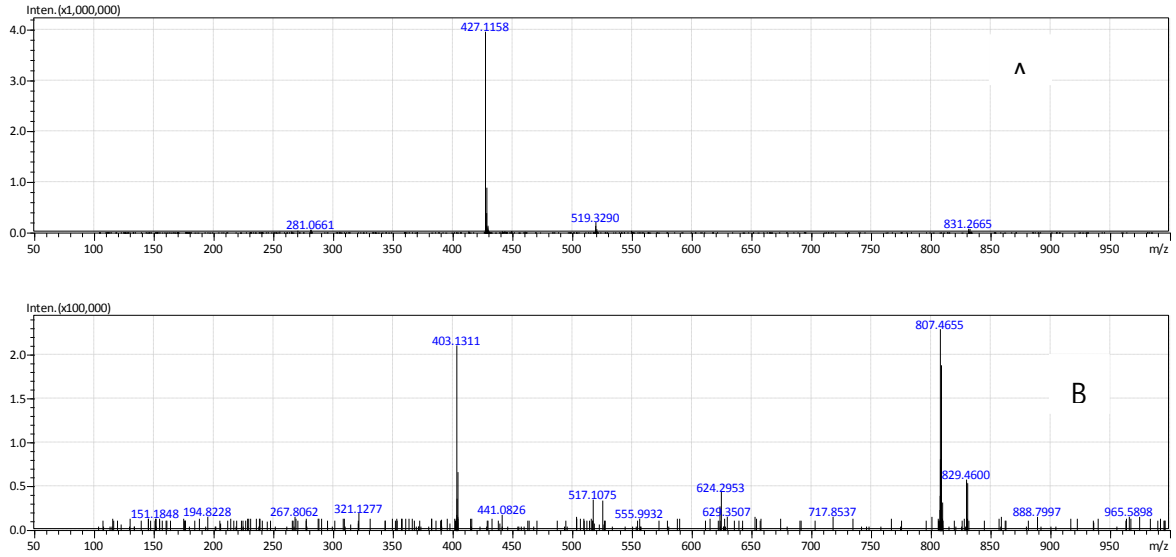

Figure S40 : HR-ESI-MS of compound (**16**)(A) Positive mode (B) negative mode

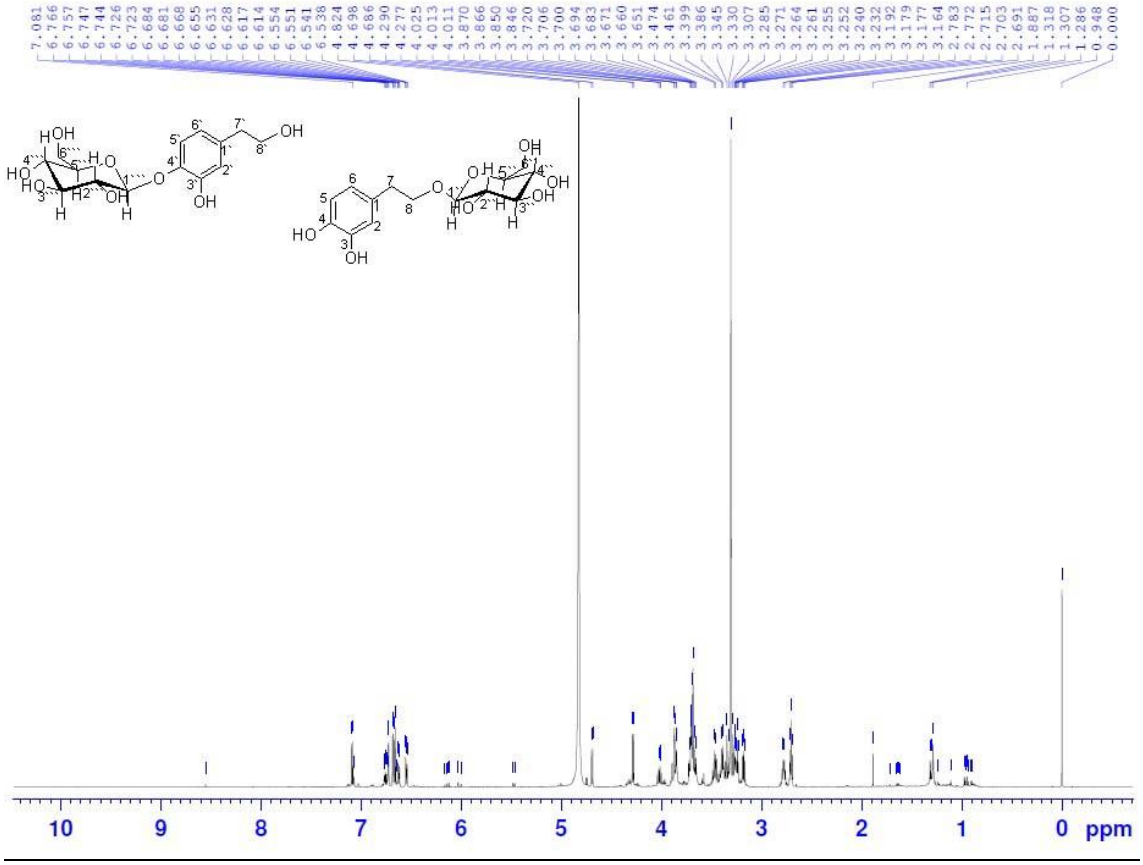

Figure S41 : ^{1}H-NMR spectrum (CD_3OD, 600 MHz) of compound (**17**)

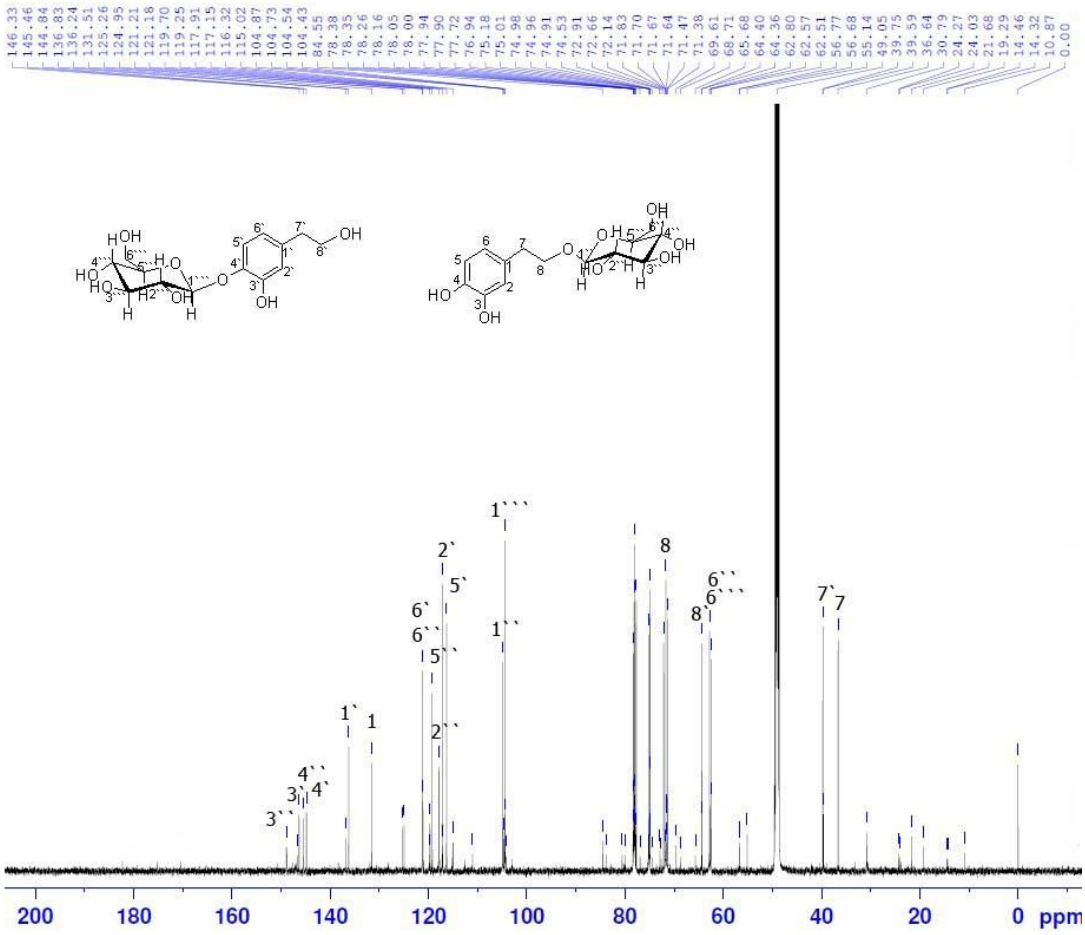

Figure S42 : ^{13}C-NMR spectrum (CD_3OD, 150 MHz) of compound (**17**)

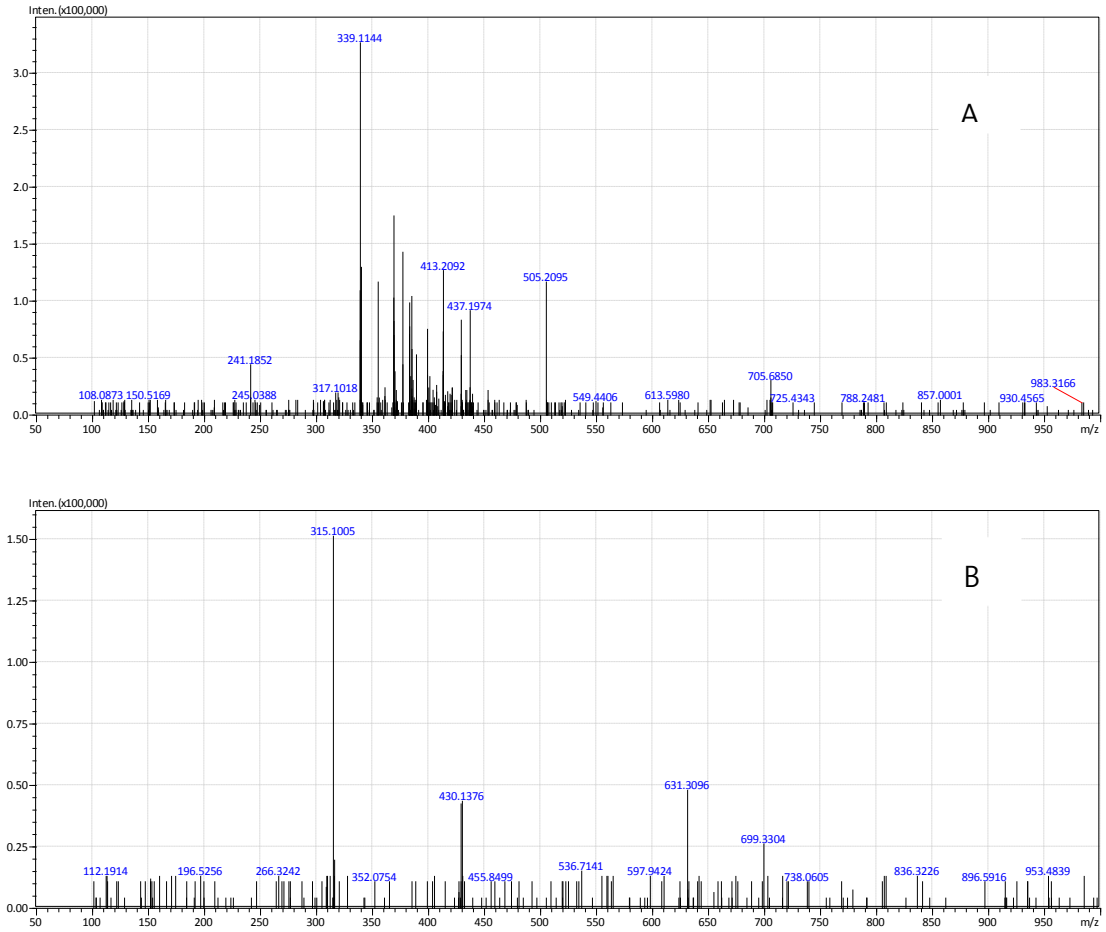

Figure S43 : HR-ESI-MS of compound (**17**)

(A) Positive mode (B) negative mode