Supporting Information

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Chemical and Biological Investigation of *Ochrosia elliptica* Labill. Cultivated in Egypt

Riham A. El-shiekh [1], Dalia A. Al-Mahdy [1], Mohamed S. Hifnawy [1], Tzvetomira Tzanova [2], Emilie Evain-Bana [2], Stéphanie Philippot [2], Denyse Bagrel [2], Essam A. Abdelsattar [1,*]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Kasr El Aini st., P.B. 11562, Egypt,
[2]Molecular Engineering Laboratory and Pharmacological Biochemistry (LIMBP), Jean Barriol Institute, University of Lorraine, Metz, France

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1 Experimental

1.1 Plant materials

The leaves of *Ochrosia elliptica* Labill. were collected from El-Orman Botanical Garden, Giza, Egypt. The plant was authenticated by Dr. Reem Samir Hamdy, Associate Professor of Taxonomy and Flora, Department of Botany, Faculty of Science, Cairo University and a voucher specimen (no. 28.12.2012) was deposited at the herbarium of the department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

1.2 General Experimental Procedures

EI-MS and ESI-MS were determined on Finnigan Mat SSQ 711 and Ion-trap (Bruker Daltonics, Bremen, Germany) mass spectrometers, respectively. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra of the isolated compounds were recorded on a Bruker High Performance Digital NMR-spectrometer (AC-250), Bremen, Germany in $CDCl_3$, CD_3OD and DMSO-d6 with tetramethylsilane (TMS) as the internal standard. Chemical shifts are given in parts per million (ppm) relative to TMS. Volume of oedema was measured using plythesmometer (7410, Ugo Basile, Comerio, Italy).

1.3 Chemicals and Reagents

All solvents used were of analytical grade and purchased from local market. Column chromatography was performed using silica gel 60 column (270-400 mesh, Merck, Germany), diaion HP-20 column (Sigma-Aldrich chemicals, Germany, sephadex LH-20 column (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and silica gel RP-18 column (70-230 mesh, Sigma-Aldrich chemicals, Germany). Carrageenan was purchased from Sigma-Aldrich, (Darmstadt, Germany). Indomethacin was obtained from EIPICO, Egypt. Isopropyl-thio-*β*-galactoside, 3-*O*-methylfluorescein phosphate, 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) reagent, FRAP reagent, 2{(7-Methoxy-2-oxo-2H-Chromen-yl) Methyl sulfanyl]}Naphtoquinone (SV37) , naphthoquinone and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were purchased from Sigma-Aldrich (Saint-Quentin, Fallavier, France). Doxorubicin was purchased from Sigma, St. Louis, MO, USA.

1.4 Extraction and Isolation

The air dried powdered leaves of *O. elliptica* (1 kg) was exhaustively extracted with 95% ethanol to give 125 g dry residue. The dry residue was suspended in distilled water and partitioned successively with petroleum ether (32 g), dichloromethane (DCM) (11 g) and *n*-butanol (*n*-BuOH) (32.5 g). Based on a preliminary cytotoxic and antioxidant activity testing, the most active DCM and *n*-BuOH fractions were selected for further isolation procedures. The DCM fraction (10 g) was chromatographed on a flash silica gel 60 column and eluted with EtOAc: MeOH: NH_3 starting with (98:2:0.1 v/v/v) and increasing the polarity using 5% MeOH increments up to pure MeOH yielding five collective fractions designated as fractions I-V. Fraction I (380 mg) and II (1 g) were purified on a silica gel 60 column eluted with *n*-hexane to yield compounds **1** (45 mg) and **2** (25 mg), respectively. Fractions III (1 g) and IV (2.3 g) were as well chromatographed on a silica gel 60 column and eluted with *n*-hexane/EtOAc (9.7:0.3 v/v) to afford compounds **3** (30 mg) and **4** (150 mg). Finally, fraction V (3 g) was purified on a silica gel column and eluted with *n*-hexane/EtOAc (8:2 v/v) to yield compound **5** (11 mg). For the *n*-BuOH fraction, 10 grams were fractionated on a diaion HP-20 column and eluted with distilled water followed by 25% methanol increments till 100% pure methanol, yielding five collective fractions. Fraction I was further chromatographed on a sephadex LH-20 column using 100% MeOH as eluent followed by purification on a silica gel RP-18 column using MeOH: H_2O (30:70 v/v) to afford 30 mg of a pure yellow powder (**6**). Finally, fraction V was chromatographed on a sephadex LH-20 column using MeOH: H_2O (50:50 v/v) as eluent to yield compound **7** (7 mg).

Preparation of the Alkaloidal Fraction: One kg of air dried powdered leaves was defatted using *n*-hexane. The plant material was then dried and the crude alkaloidal fraction (3 g) was prepared following the procedure previously described by Nasab [1]. An aliquot of the alkaloidal fraction (2 g) was chromatographed on a flash silica gel 60 column and eluted with 100 % CH_2Cl_2, gradually increasing polarity with 5% increments of MeOH till 15% MeOH, yielding 4 collective fractions designated as IA-IVA. Fraction IIA (800 mg) was purified on a sephadex column and eluted with $CHCl_3$: MeOH (50:50 v/v) to give compound **8** (120 mg). Fraction IVA (500 mg) was purified on a silica gel H60 column and eluted with $CHCl_3$ to give compound **7** (3 mg) again.

1.5 Biological Evaluation

1.5.1 Cell lines and culture conditions

The human breast adenocarcinoma cell line MCF7 (ECACC, Salisbury, UK) [2], its multidrug-resistant counterpart Vcr-R (resistant to adriamycine, vincristine, etoposide) [3] and the metastatic human breast adenocarcinoma cells MDA-MB-231 (ATCC, Manassas, USA) [4] were grown in phenol red-free Roswell Park Memorial Institute (RPMI-1640) medium, supplemented with 10% heat inactivated foetal bovine serum, 10000 U/ml penicillin and 10000 µg/ml streptomycin, 2 mM L-glutamine, 1.25 mM sodium pyruvate, amino acids and vitamins (Eurobio, France). The non-cancerous, but immortalized by telomerase, human breast epithelial cell line hTERT-HME1 (ATCC, Manassas, USA) was grown in a mixture of RPMI-1640 (90%) and mammary epithelial growth medium (10%) (MEGM) [3] (Lonza, Levallois-Perret, France). The cell lines were cultivated at 37°C in a humidified atmosphere containing 5 % CO_2.

1.5.2 Cell viability determination (MTT assay)

Cell survival was detected by the standard 3-(4,5-dimethylthiazol- 2-yl)-2,5 diphenyltetrazolium bromide (MTT) reduction assay [5]. Cells were seeded in 96-well plates at a density of 1 x 104 cells/well and were incubated for 24 h. After 48 exposure to the extracts, the medium with tested samples was removed and fresh media with MTT (final concentration of 0.5 mg/ml) was added. The incubation continued for 2 h at 37 ºC. The formazan crystals were dissolved in DMSO and spectrophotometric determinations were performed at 550 nm. The experiments were repeated four times for each sample.

1.5.3 Evaluation of CDC25s inhibitory potential

Production and Purification of Recombinant Human CDC25 Phosphatases: The CDC25 proteins used in the *in vitro* fluorimetric assays are recombinant human proteins in which CDC25 is linked to a glutathione S-transferase (GST) unit. Recombinant CDC25 proteins of the three isoforms were produced and purified at the Molecular Engineering and Pharmacological Biochemistry Laboratory (LIMBP), Jean Barriol Institute, University of Lorraine, Metz, France, according to the protocol published by [6]. The method requires the production of proteins in *E. coli*, their extraction and purification by an affinity-chromatography process followed by calibration using a fluorimetric assay. Briefly, the bacteria are transformed with a pGex plasmidic vector containing the cDNA GST-CDC25 (A, B or C). The bacteria was then selected on a medium containing ampicillin, allowing selection only of the bacteria that have incorporated the plasmid (wearing an ampicillin resistance gene). Recombinant proteins were expressed by induction with isopropyl-thio-*β*-galactoside (IPTG). Proteins produced are then extracted and purified by affinity chromatography with glutathione (GSH)-agarose which specifically allows the retention of fusion proteins GST-CDC25 via a GSH-GST interaction.

In vitro inhibition of phosphatase assay: CDC25 phosphatase activity was quantified *in vitro* by a fluorimetric assay as previously described by [7]. Measurement of GST-CDC25 enzymatic activity

was performed in 96-well plates in [50 mM Tris–HCl, 50 mM NaCl, 1mM EDTA, and 0.1% BSA (bovine serum albumin), pH 8.1] buffer containing 500 mM 3-*O*-methylfluorescein phosphate as substrate. The GST-CDC25 proteins, diluted in assay buffer, were used at a final concentration of 1mg/well. After 2 h at 30ºC, fluorescent emission of 3-O-methylfluorescein was measured with a CytoFluor® 4000 TC system (Applied/Per Septive Biosystems) with excitation filter: 475nm and emission filter: 510 nm. Naphthoquinone used at a concentration of 10 µM, served as a positive inhibition control. The samples were tested at concentration of 4 mg/1mL. Tests were performed in triplicates. Results are expressed as percent of residual activity i.e. CDC25 activity that still remains after incubation with the eventual inhibitor.

1.5.4 In-vitro antioxidant activity

DPPH scavenging activity measurement: The antioxidant activity of the tested samples was measured by assessing their DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical-scavenging activity [8, 9]. Briefly, DPPH is a stable radical having a violet colour, in the presence of an antioxidant moiety, it is neutralized to a compound with slight yellow colour. This change is proportional to the antioxidant capacity of the sample and is measured by spectrophotometry at 515 nm. Trolox, a cell-permeable, water-soluble derivative of vitamin E with potent antioxidant properties, was used as standard. The degree of discoloration induced by a tested sample is related to that induced by Trolox, allowing its antioxidant potential to be expressed as Trolox equivalents. The stock solution of DPPH is prepared at the concentration of 2 mM and the Trolox solution at the concentration of 2.5 mM. A solution of 10 µL of the test sample (20 mg/mL for the extracts and fractions and 0.08mg/mL for 9-methoxyellipticine) is mixed with 930 µL of methanol and 60 µL of DPPH solution. The final volume is 1 mL. The optical density is read after incubation in the dark for 30 minutes. Each sample was tested twice in triplicate measurements.

Ferric reducing antioxidant power (FRAP): The FRAP assay depends upon the ability of the tested samples to reduce ferric tripyridyl triazine (Fe3+-TPTZ) complex to ferrous tripyridylpyrazine (Fe2+-TPTZ) at low pH [10, 11]. Fe^{2+}–TPTZ has an intense blue color measured at 593 nm. Trolox was used as a reference antioxidant. FRAP reagent was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3$,.$6H_2O$ solution. Freshly prepared FRAP reagent was warmed at 37°C for 30 min and added to the samples every 30 s. Absorbance readings were taken after 30 min every 30 s. Each sample was tested twice with triplicate measurements in each experiment.

1.5.5 Determination of the median lethal dose (LD50)

The LD50 of the total ethanolic extract of the leaves of *O. elliptica*. was determined using the method described by Lorke [12]. Male Albino mice (20-25 g) were divided into groups each of 6 animals. The extract dissolved in distilled water containing few drops of DMSO was administered orally once in doses ranging from 1000 mg/kg b.wt. to 5000 mg/kg b.wt. which was the maximum soluble dose. A control group received the same volumes of distilled water. The animals were monitored for signs of toxicity for the first 4 h and mortality for 24 h following administration.

1.5.6 In-vivo anti-inflammatory activity

The anti-inflammatory study was carried out using the carrageenan induced rat paw oedema model described by Winter, *et al* [13]. Adult male wistar rats were divided into 10 groups (n=6). The rats were handled according to the National Institute of Health for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and approved by the local Research Ethical Committee at the Faculty of Pharmacy, Cairo University (Egypt) and kept under the same hygienic conditions, well balanced diet and water supplied ad-libitum. To minimize the suffering of laboratory animals, procedures including cervical dislocation were done under deep anaesthesia. Control group received 1 mL normal saline solution orally. The other groups received Indomethacin (20 mg/kg), ethanolic, DCM and *n*-BuOH fractions of *O. elliptica* (250 and 500 mg/kg), respectively. One hour later, oedema was induced by a sub-planter injection of 0.1 mL of 1% carrageenan solution in saline in the right hind

paw and 0.1 mL saline in the left hind paw. The paw size was measured using a plethysmometer at 0 h (V_0: before carrageenan injection) and 1, 2, 3 and 4 h intervals later (V_T). The difference between V_T (1, 2, 3 and 4 h) and V0 was taken as the change in oedema volume. The percentages of inhibition were calculated according to the following formula:

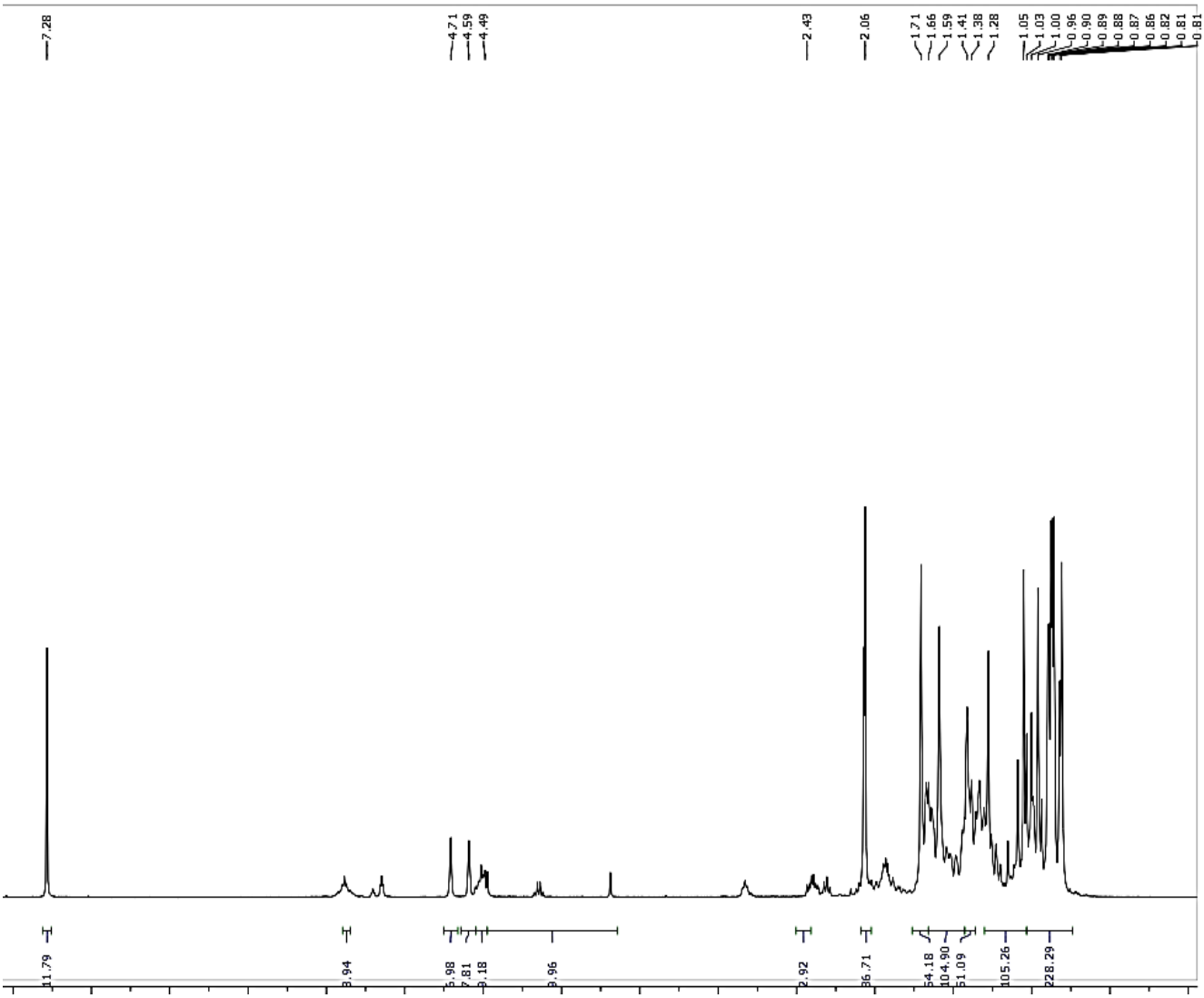
% inhibition = $((V_T - V_0)\text{control} - (V_T - V_0)\text{treated group}) \times 100/(V_T - V_0)\text{control}$

1.5.7 Statistical analysis

Data obtained from animal experiments were expressed as mean ± S.E.M. and as percentage. Results were statistically evaluated by using repeated one way ANOVA test followed by Tukey test for multiple comparisons. The level of significance was set at $P \leq 0.05$.

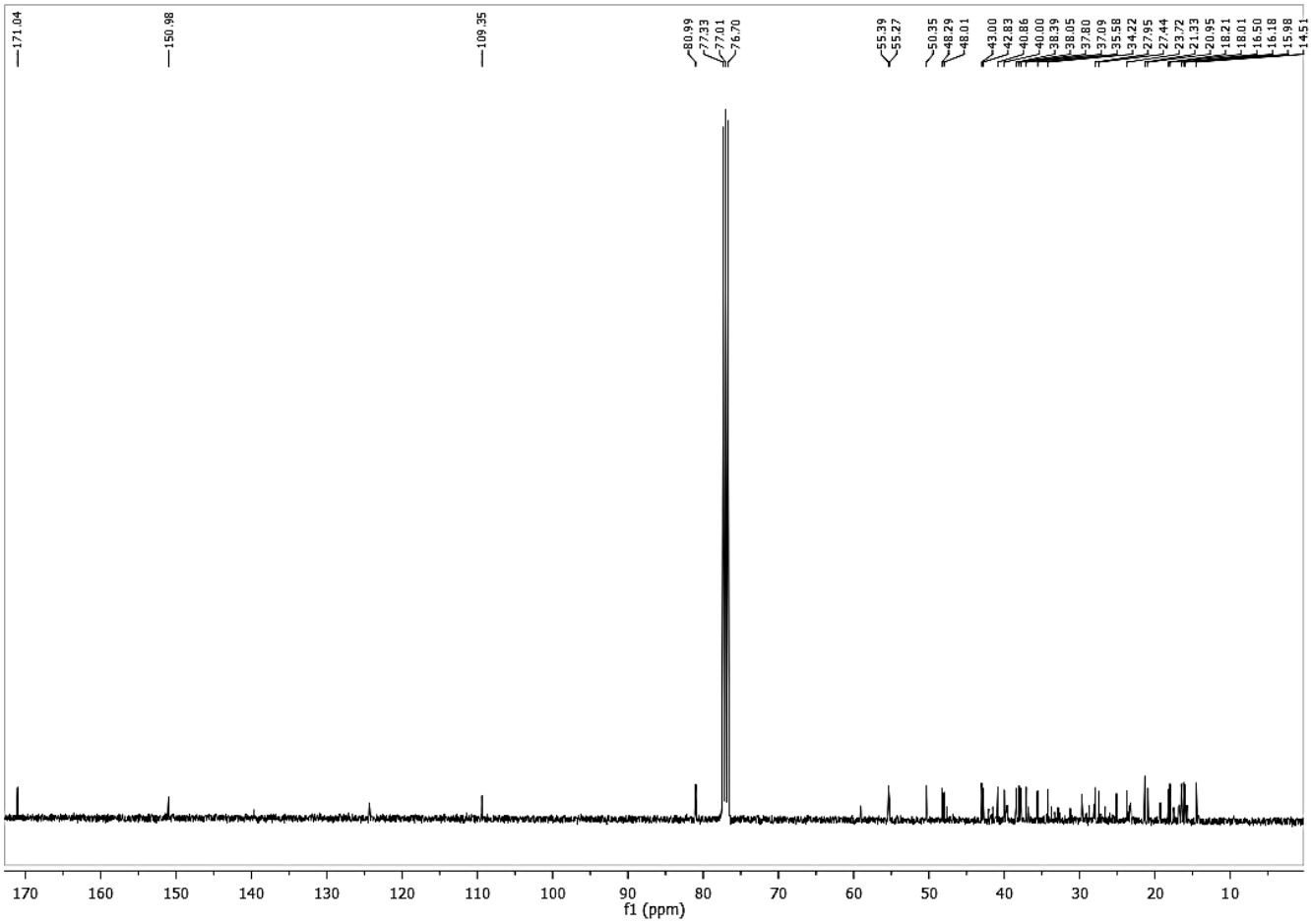
2. Spectroscopic data and characterization

The structures of compounds **(1-8)** were determined by MS, ^{1}H and ^{13}C NMR spectral analysis and was in agreement with previously reported data [14-23]

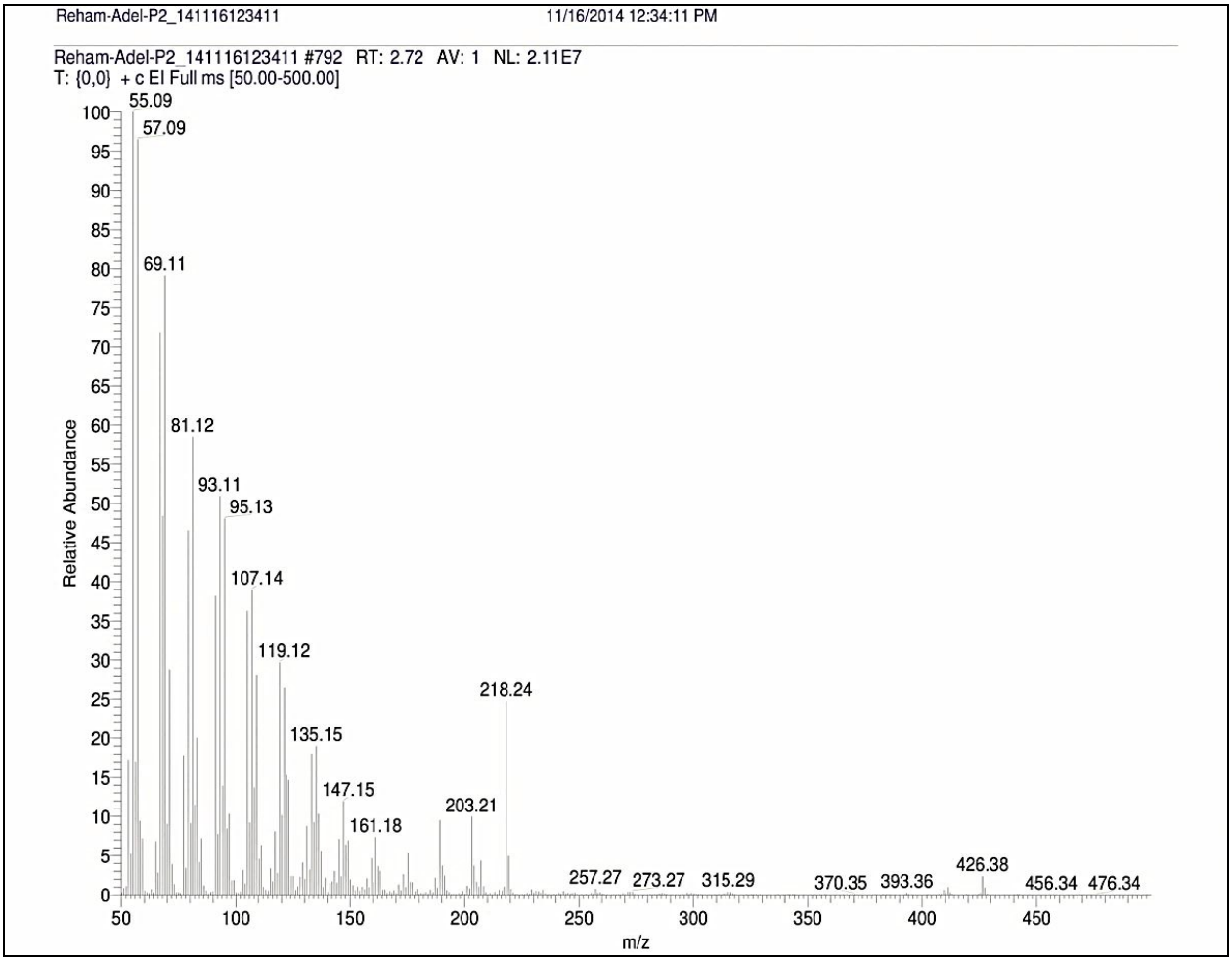


S1: ^{1}H-NMR (400 MHz, $CDCl_3$) Spectrum of Lupeol acetate (**1**)

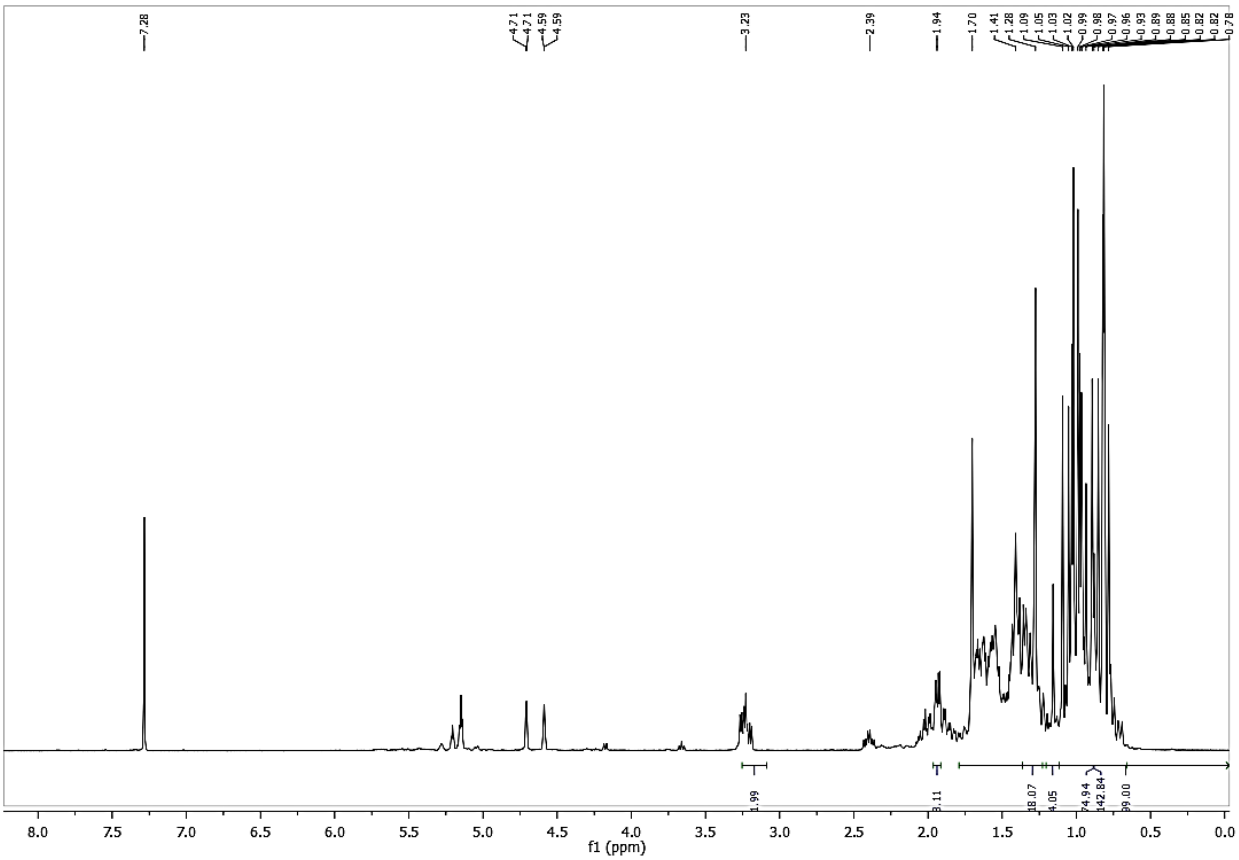
Lupeol acetate **(1)**: White needle crystals, EIMS m/z = 468 for $C_{32}H_{52}O_2$. ^{1}H-NMR ($CDCl_3$, 400 MHz). ^{1}H-NMR ($CDCl_3$, 400 MHz**)**. 4.71 (1H, s, H-29 *b*), 4.59 (1H, s, H-29 *a*), 4.49 (1H, dd, *J*= 12, 4 Hz, H-3), 2.43(1H, m, H-19), 2.06 (3H, s, Me-2`), 1.71 (3H, s, Me-30), 1.66 (2H, m, H-12), 1.05 (3H, s, Me-26), 0.96 (3H, s, Me-27), 0.86 (3H, s, Me-25), 0.82 (3H, s, Me-23), 0.81 (6H, s, Me-24 and Me-28). ^{13}C-NMR ($CDCl_3$, 100 MHz): 38.39 (C-1), 23.72 (C-2), 80.99 (C-3), 37.8 (C-4), 55.39 (C-5), 18.21 (C-6), 34.22 (C-7), 40.86 (C-8), 50.35 (C-9), 37.09 (C-10), 20.95 (C-11), 25.1 (C-12), 38.05 (C-13), 42.83 (C-14), 27.44 (C-15), 35.58 (C-16), 43 (C-17), 48.29 (C-18), 48.01 (C-19), 150.98 (C-20), 29.84 (C-21), 40 (C-22), 27.95 (C-23), 16.5 (C-24), 16.18 (C-25), 15.98 (C-26), 14.51 (C-27), 18.01 (C-28), 109.35 (C-29), 19.29 (C-30), 171.04 (C-1`), 21.33 (C-2`).



S2: ^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of Lupeol acetate (**1**)

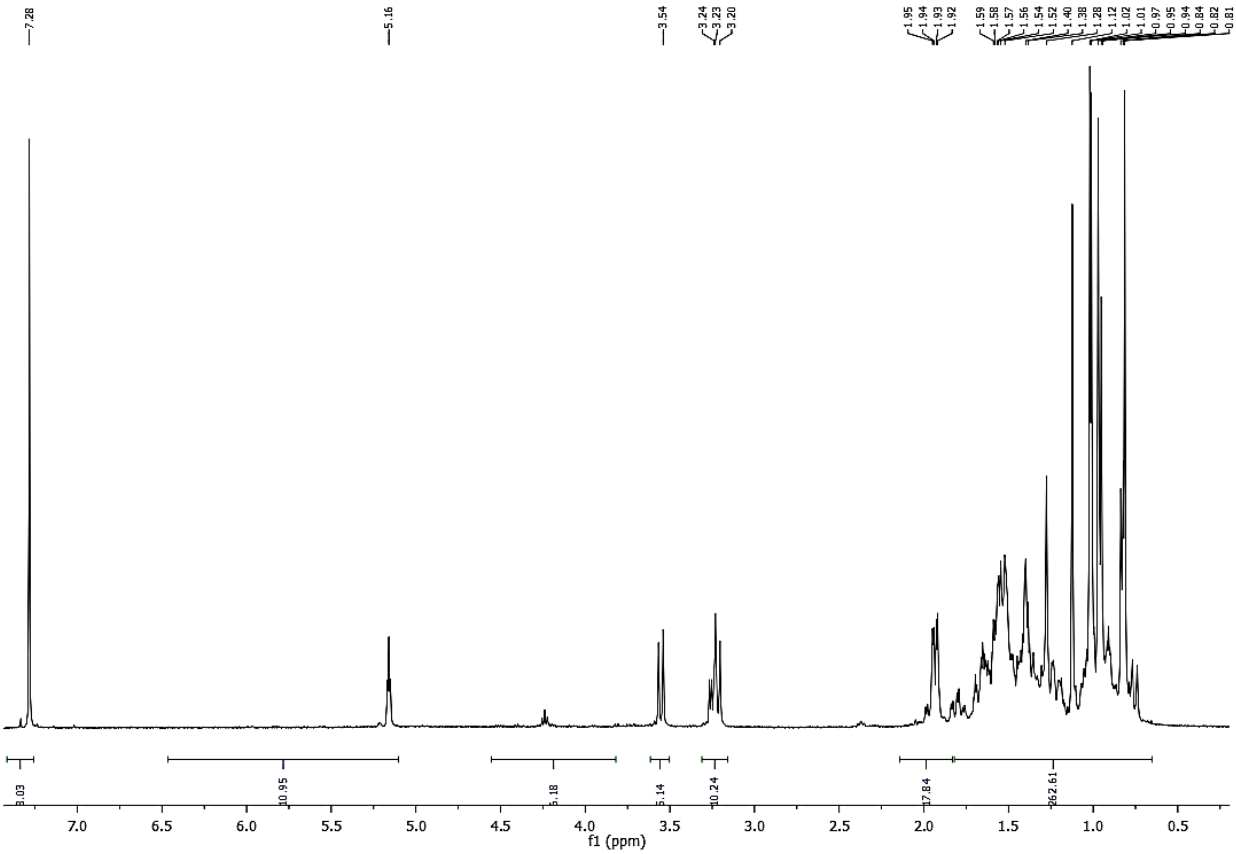


S3: ESI-MS spectrum of Lupeol (**2**)



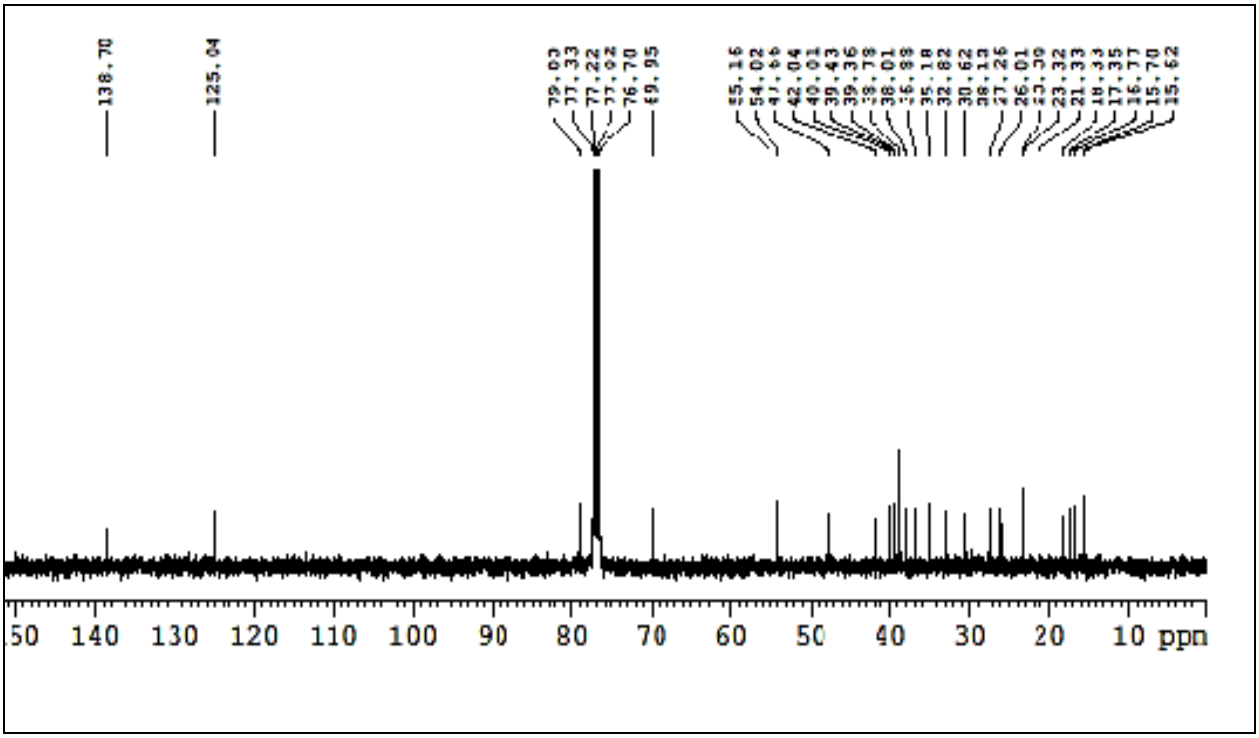
S4: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of Lupeol (**2**)

Lupeol **(2):** White powder, EIMS m/z = 426 for $C_{30}H_{50}O$. ^{1}H-NMR ($CDCl_3$, 400 MHz): 4.71 (1H, d, *J*= 2.04 Hz, H-29 *b*), 4.59 (1H, s, H-29 *a*), 3.23 (1H, dd, *J*= 12, 4 Hz, H-3), 2.39 (1H, m, H-19), 1.70 (3H, s, Me-30), 1.03 (3H, s, Me-26), 0.93 (3H, s, Me-27), 0.85 (3H, s, Me-25), 0.82 (9H, s, Me-23, Me-24, Me-28).

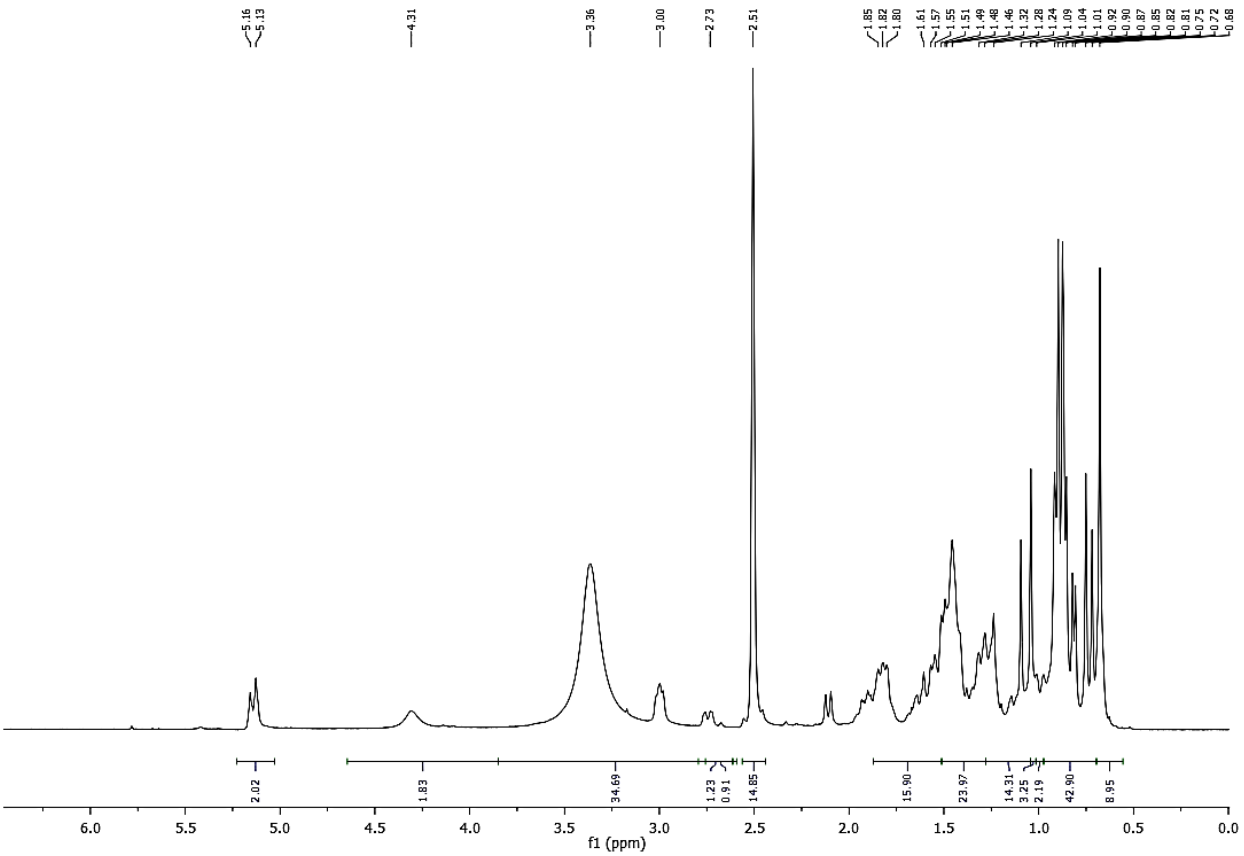


S5: ^{1}H-NMR (100 MHz, $CDCl_3$) spectrum of Uvaol (**3**)

Uvaol **(3)**: White needle crystals, EIMS m/z = 442 for $C_{30}H_{50}O_2$. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.16 (1H, t, *J* = 3.6 Hz, H-12), 3.54 (1H, d, *J*= 11.2 Hz, H 28 *b*), 3.23 (1H, overlapped with H-28 *a*, H-3), 3.20 (1H, d, *J*= 11.2 Hz, H-28 *a*), 1.02 (3H, s, Me-23), 0.97 (3H, s, Me-27), 0.95 (3H, s, Me-26), 0.84 (3H, s, Me-24), 0.81 (3H, d, *J* =3.3 Hz, Me-30), 0.77 (3H, d, *J* =3.4 Hz, Me-29), 0.74 (3H, s, Me-25). ^{13}C-NMR ($CDCl_3$, 100 MHz): 39.36 (C-1), 27.26 (C-2), 79.03 (C-3), 38.78 (C-4), 55.16 (C-5), 18.33 (C-6), 32.82 (C-7), 40.01 (C-8), 47.66 (C-9), 36.88 (C-10), 23.38 (C-11), 125.04 (C-12), 138.70 (C-13), 42.04 (C-14), 26.01 (C-15), 23.38 (C-16), 36.88 (C-17), 54.02 (C-18), 39.43 (C-19), 39.43 (C-20), 30.62 (C-21), 36.38 (C-22), 28.12 (C-23), 15.70 (C-24), 15.62 (C-25), 16.77 (C-26), 23.32 (C-27), 69.95 (C-28), 17.35 (C-29), 21.33 (C-30).

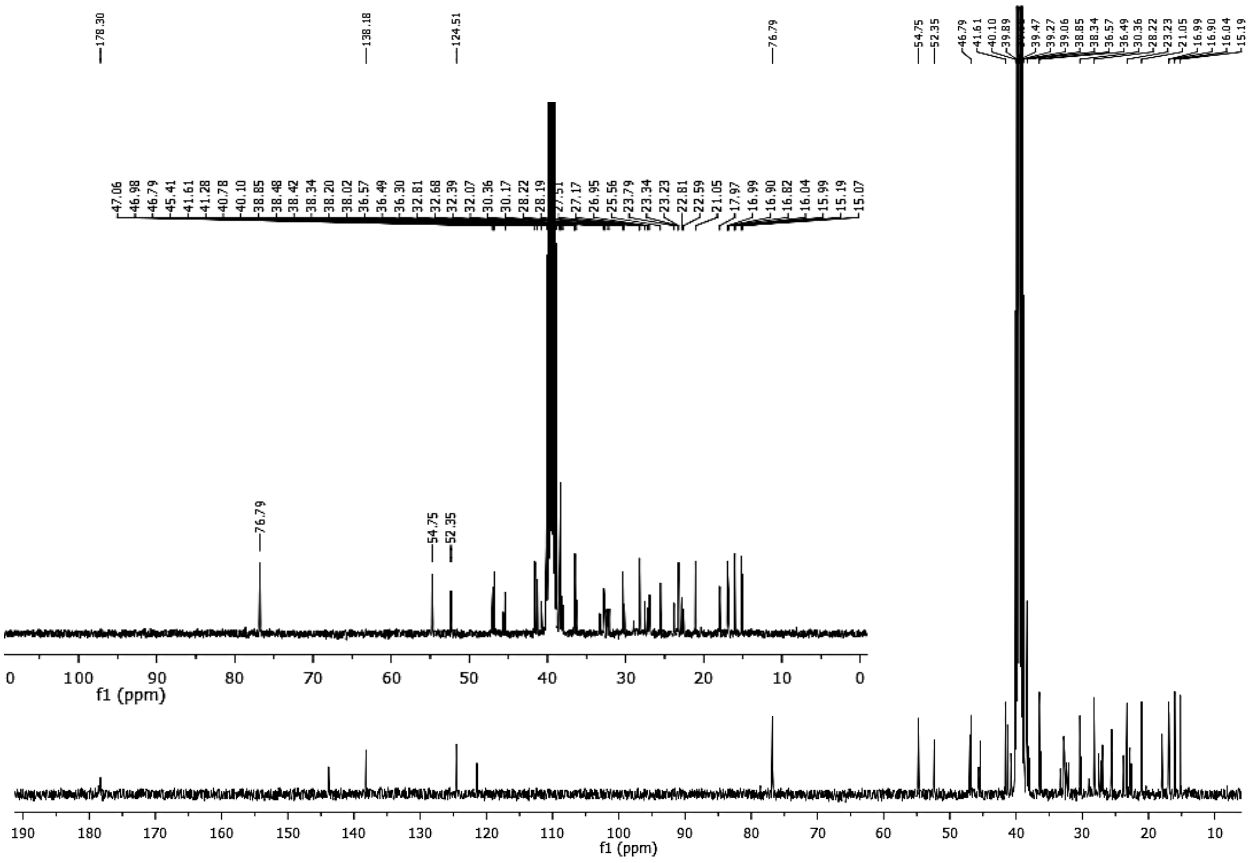


S6: ^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of Uvaol (**3**)

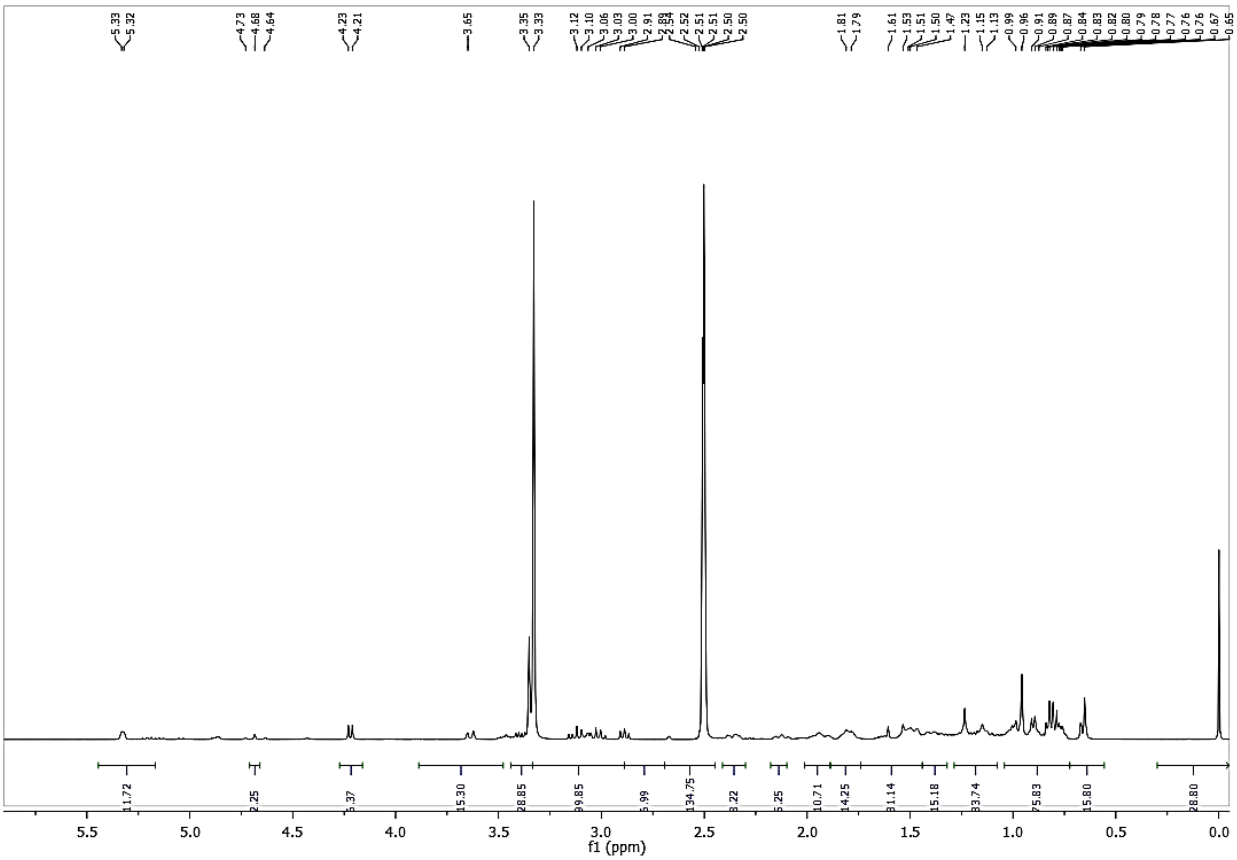


S7: ^{1}H-NMR (400 MHz, DMSO-*d6*) spectrum of Ursolic acid (**4**)

Ursolic acid **(4)**: White powder, EIMS m/z = 456 for $C_{30}H_{48}O_3$. ^{1}H-NMR (DMSO, 400 MHz): 5.16 (1H, br s, H-12), 4.31 (1H, br s, OH), 3.00 (1H, dd, *J*= 12, 6.4 Hz, H-3), 2.73 (1H, d, *J*= 16 Hz, H-18), 1.04 (3H, s, Me-23), 0.92 (3H, s, Me-27), 0.90 (3H, s, Me-26), 0.85 (3H, s, Me-24), 0.82 (3H, d, *J*= 5 Hz, Me-30), 0.75 (3H, d, *J*= 4 Hz, Me-29), 0.72 (3H, s, Me-25). ^{13}C-NMR (DMSO, 100 MHz): 39.47 (C-1), 28.22 (C-2),76.64 (C-3), 38.42 (C-4), 54.75 (C-5), 17.97 (C-6), 32.81 (C-7), 40.10 (C-8), 47.06 (C-9), 36.57 (C-10), 23.23 (C-11), 124.46 (C-12), 138.09 (C-13), 41.61 (C-14), 27.51 (C-15), 23.79 (C-16), 46.79 (C-17), 52.35 (C-18), 39.89 (C-19), 39.89 (C-20), 30.12 (C-21), 36.49 (C-22), 28.22 (C-23), 15.19 (C-24), 15.99 (C-25), 16.90 (C-26), 23.23 (C-27), 178.30 (C-28), 16.99 (C-29), 21.05 (C-30).

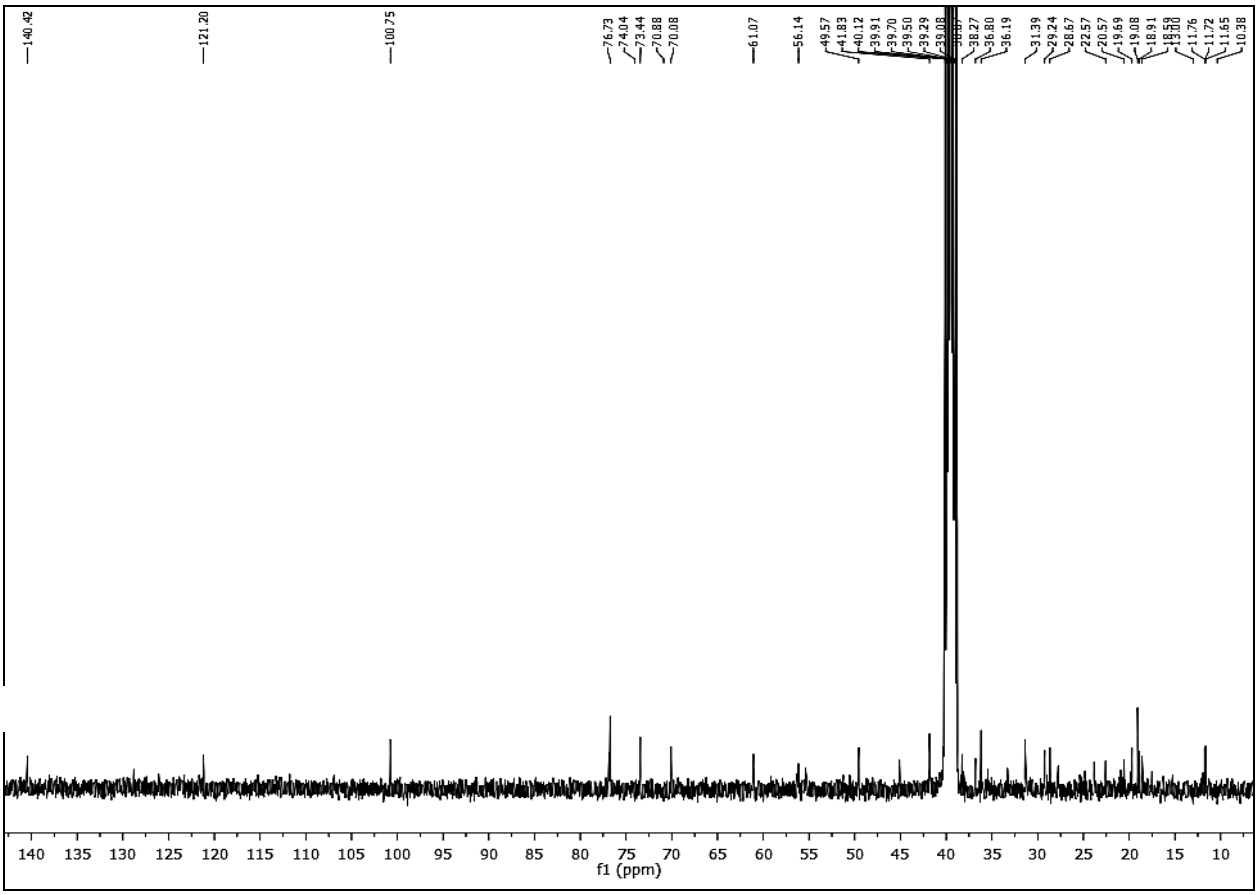


S8: ^{13}C-NMR (100 MHz, DMSO-*d6*) spectrum of Ursolic acid (**4**)

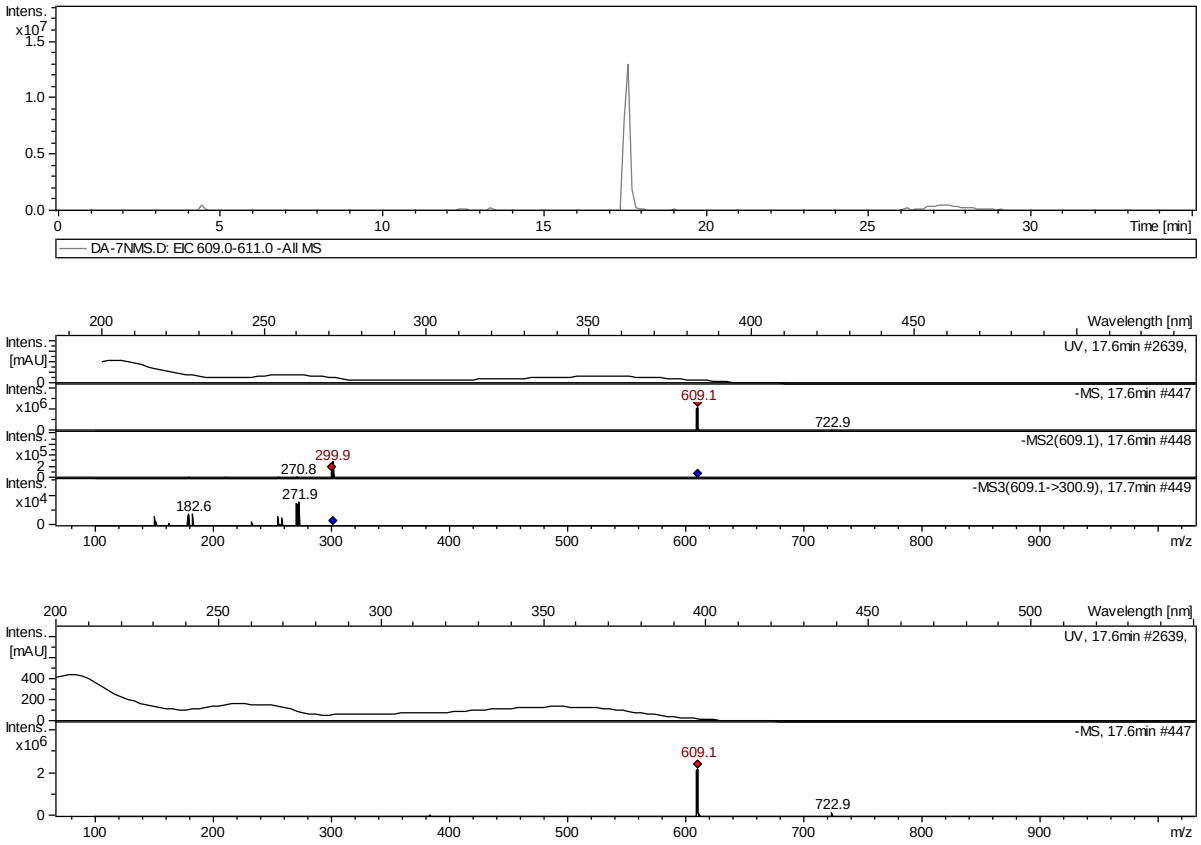


S9: ^{1}H-NMR (400 MHz, DMSO-*d6*) spectrum of *β*-sitosterol glucoside (**5**)

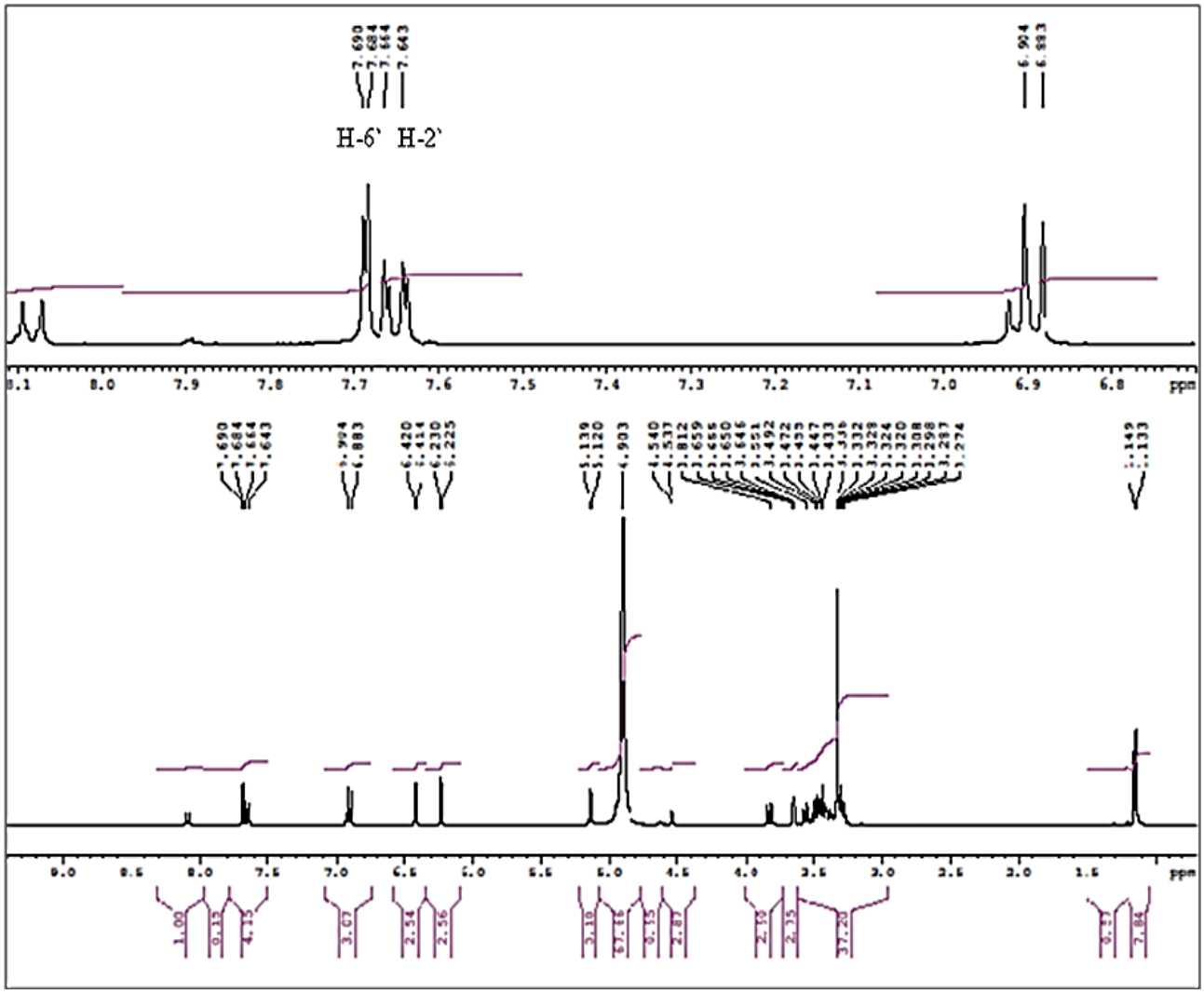
β-sitosterol glucoside **(5)**: White needle crystals, EIMS m/z = 576 for $C_{35}H_{60}O_6$. ^{1}H-NMR (DMSO, 400 MHz): 5.33 (1H, br d, *J*= 4.4 Hz, H-6), 4.21 (1H, d, *J*= 7.8 Hz, H-1`), 3.12 (1H, m, H-3), 0.96 (3H, s, Me-19), 0.84 (3H, d, *J*= 3.08 Hz, Me-21), 0.78 (3H, t, *J*= 5.6 Hz, Me-29), 0.76 (3H, d, *J* = 3 Hz, Me-26), 0.67 (3H, d, *J*= 1.6 Hz, Me-27), 0.65 (3H, s, Me-18). ^{13}C-NMR (DMSO, 100 MHz): 36.80 (C-1), 29.24 (C-2), 76.73 (C-3), 41.83 (C-4), 140.42 (C-5), 121.20 (C-6), 31.39 (C-7), 31.39 (C-8), 49.57 (C-9), 36.19 (C-10), 20.57 (C-11), 38.27 (C-12), 41.83 (C-13), 56.14 (C-14), 23.83 (C-15), 27.76 (C-16), 55.39 (C-17), 11.65 (C-18), 19.08 (C-19), 36.19 (C-20), 18.5 (C-21), 33.34 (C-22), 24.68 (C-23), 45.11 (C-24), 28.67 (C-25), 18.91 (C-26), 19.69 (C-27), 22.57 (C-28), 11.76 (C-29) Sugar carbons: 100.75 (C-1`), 73.44 (C-2`), 76.94 (C-3`), 70.08 (C-4`), 76.73 (C-5`), 61.07 (C-6`).



S10: ^{13}C-NMR (100 MHz, DMSO-*d6*) spectrum of *β*-sitosterol glucoside (**5**)

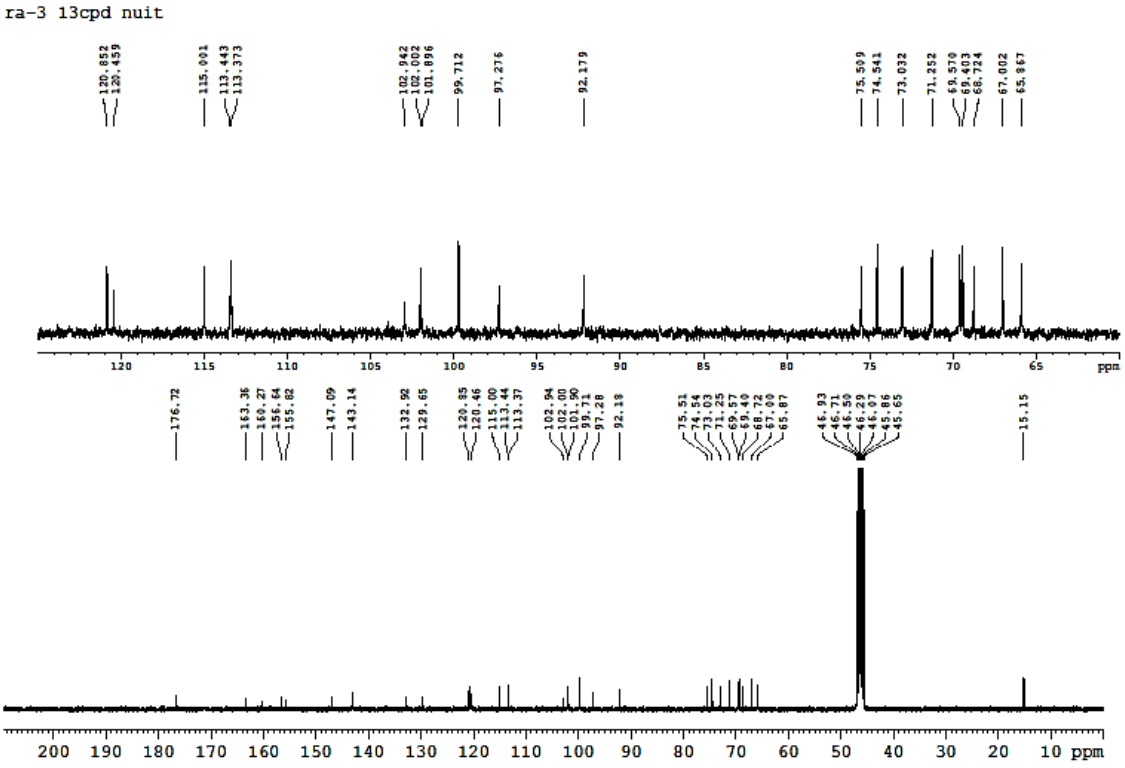


S11: LC-MS spectrum of Quercetin-3-O-α-L-rhamnosyl-β-D-glucopyranoside (Rutin) (**6**)

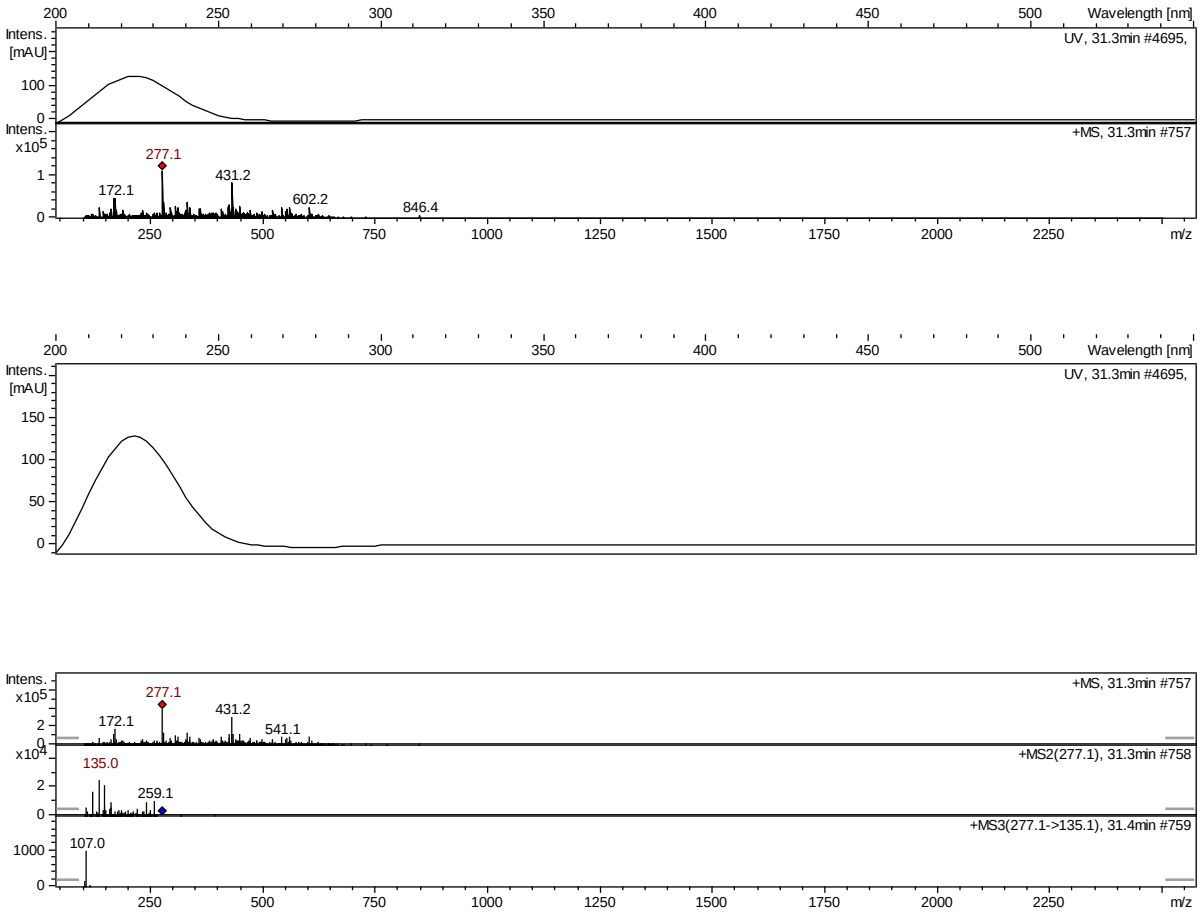


S12: ^{1}H-NMR spectrum of Quercetin-3-O-*α*-L-rhamnosyl-*β*-D-glucopyranoside (Rutin) (**6**)

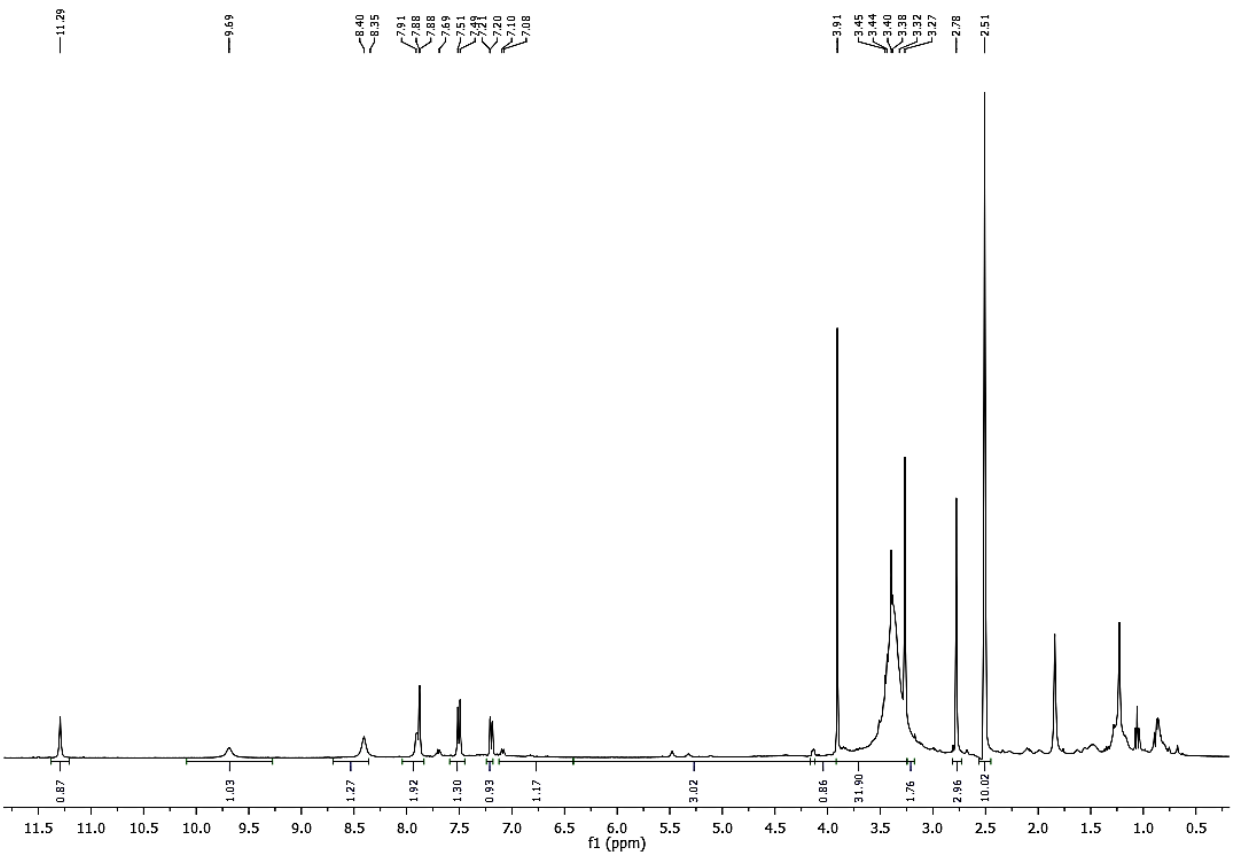
Quercetin-3-O-*α*-L-rhamnosyl-*β*-D-glucopyranoside (Rutin) **(6)**: yellow powder. $C_{27}H_{31}O_{16}$, m/z 609.1 $[M-H]^{-}$. ^{1}H-NMR (CD_3OD, 400MHz): 7.68 (1H, d, *J*= 2.4 Hz, H-2'), 7.64 (1 H, dd, *J*= 8.4, 2.4 Hz, H-6'), 6.88 (1H, d, *J*= 8.4 Hz, H-5'), 6.41 (1 H, d, *J*= 2.4 Hz, H-8), 6.22 (1H, d, *J*= 2.4 Hz, H-6), 5.14 (1H, d, J= 8 Hz, glucose-H-1``), 4.54 (1 H, br s, rhamnose-H-1```), 1.14 (3H, d, J= 6.4 Hz, rhamnose-H-6```).The ^{13}C-NMR (CD_3OD, 100MHz): 155.82 (C-2), 132.92 (C-3), 176.72 (C-4), 160.27 (C-5), 99.71 (C-6), 163.36 (C-7), 97.28 (C-8), 156.64 (C-9), 120.85 (C-1`), 113.43 (C-2`), 143.14 (C-3`), 147.09 (C-4`), 115 (C-5`), 120.85 (C-6`). Sugar carbons: 102 (C-1``), 74.54 (C-2``), 75.51 (C-3``), 71.25 (C-4``), 74.54 (C-5``), 67.00 (C-6``), 101.90 (C-1```), 69.57 (C-2```), 69.40 (C-3```), 73.03 (C-4```), 68.72 (C-5```), 15.15 (C-6```).



S13: ^{13}C-NMR spectrum of Quercetin-3-O-*α*-L-rhamnosyl-*β*-D-glucopyranoside (Rutin) (**6**)

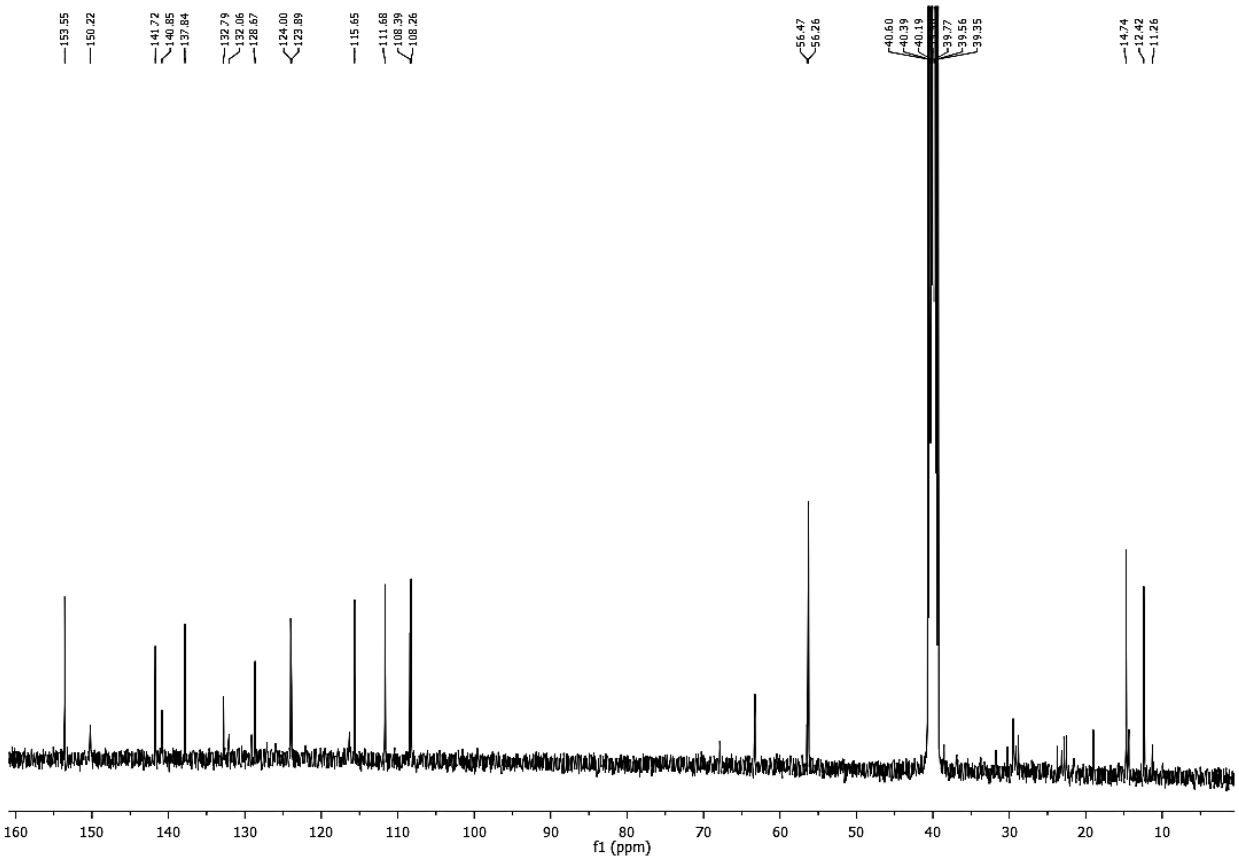


S14: LC-MS spectrum of 8-methoxyellipticine (**7**)

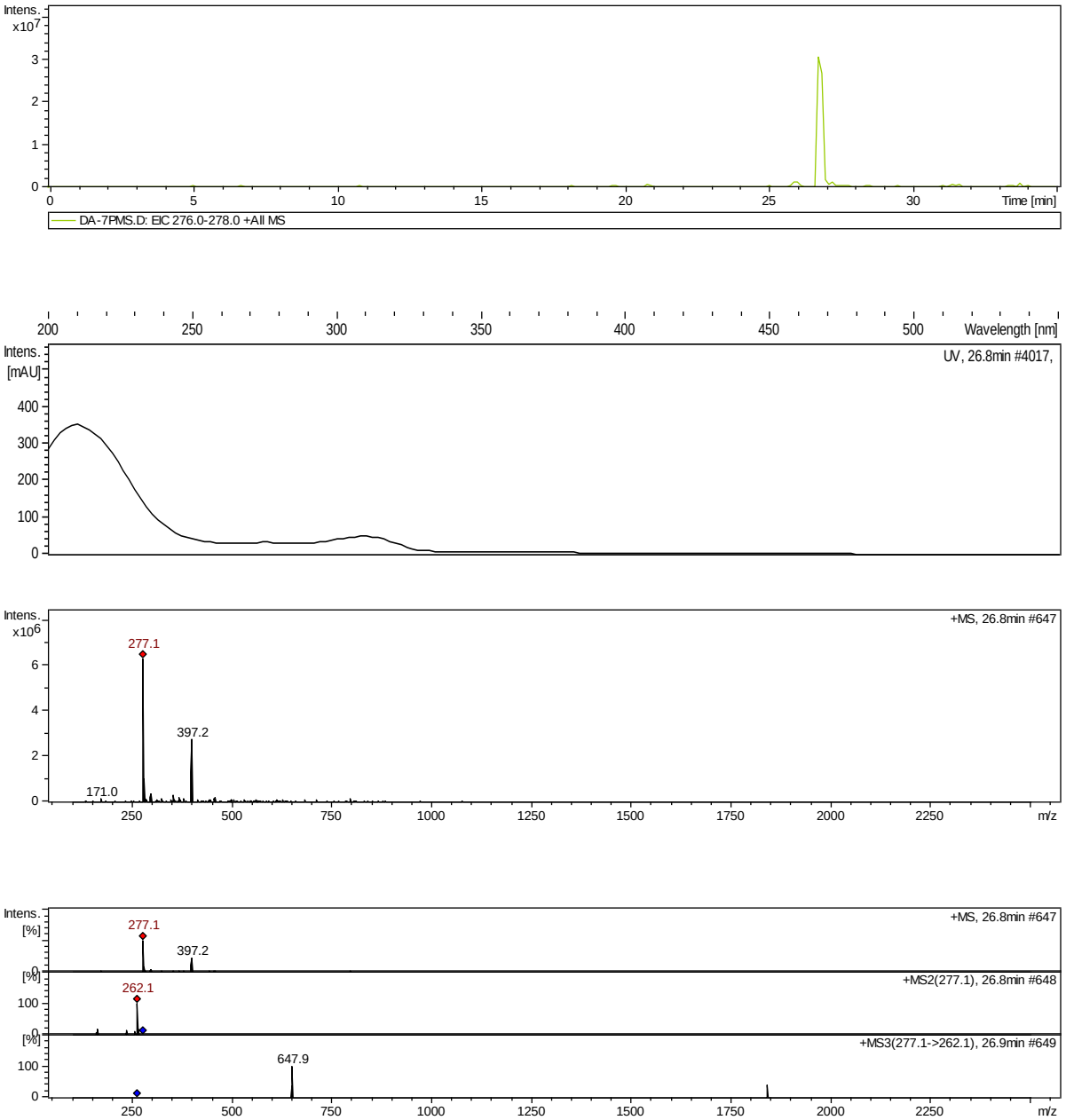


S15: ^{1}H-NMR (400 MHz, CD_3OD) spectrum of 8-methoxyellipticine (**7**)

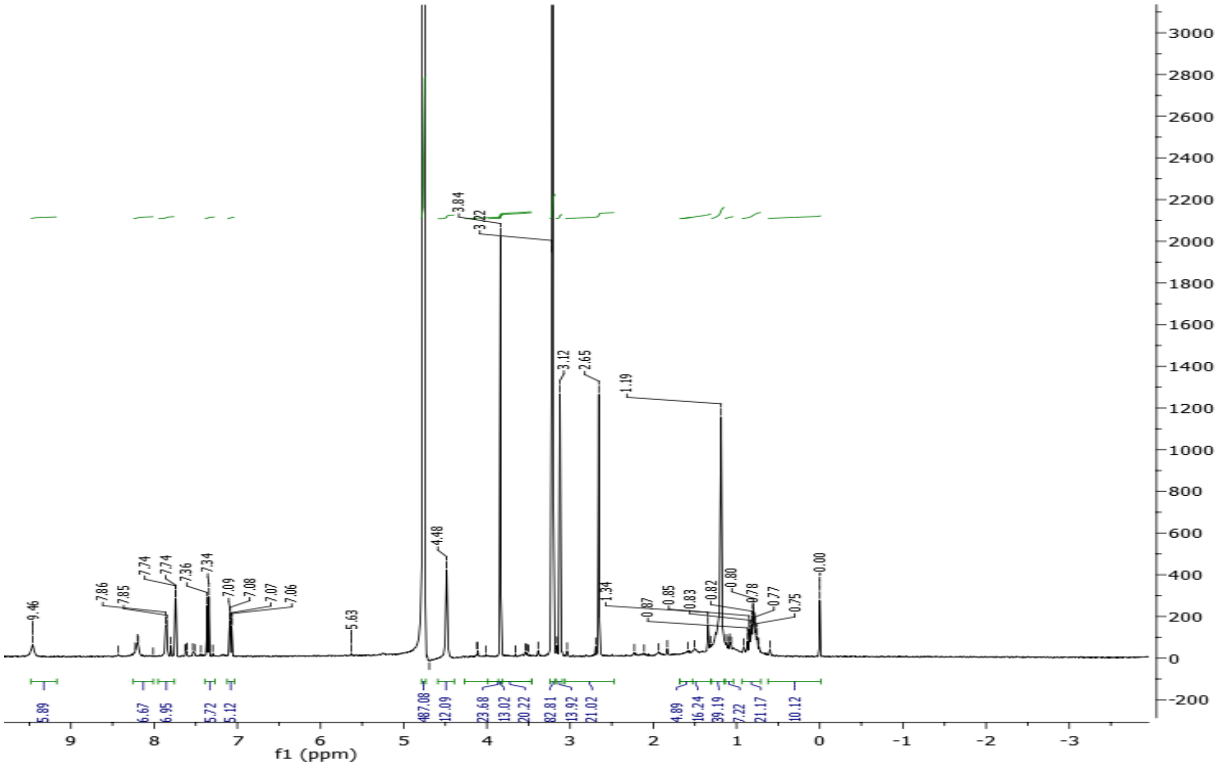
8-methoxyellipticine (**7**): orange powder. $C_{18}H_{17}N_2O$, m/z 277.1 $[M+H]^+$. ^{1}H-NMR (CD_3OD, 400MHz): 11.29 (1 H, br s, NH), 9.69 (1H, br s, H-1), 8.40 (1H, br s, H-3), 7.90 (lH, br d, *J*= 5 Hz, H-4), 7.88 (1H, d, *J*= 8.8 Hz, H-10), 7.50 (1H, d, *J*= 2 Hz, H-7), 7.20 (1H, dd, *J*= 8.8, 2 Hz, H-9), 3.91 (3H, s, MeO-8), 3.27 (3H, s, Me-11), 2.78 (3H, s, Me-5). The ^{13}C-NMR (CD_3OD, 100MHz): 150.22 (C-1), 141.72 (C-3), 115.65 (C-4), 111.68 (C-5), 108.26 (C-7), 153.55 (C-8), 108.39 (C-9), 123.99 (C-10), 128.67 (C-11), 124 (C-12), 137.84 (C-13), 132.06 (C-14), 123.87 (C-15), 140.85 (C-17), 11.26 (Me-5), 14.74 (Me-11), 56.47 (OCH_3).



S16: ^{13}C-NMR (100 MHz, CD_3OD) spectrum of 8-methoxyellipticine (**7**)

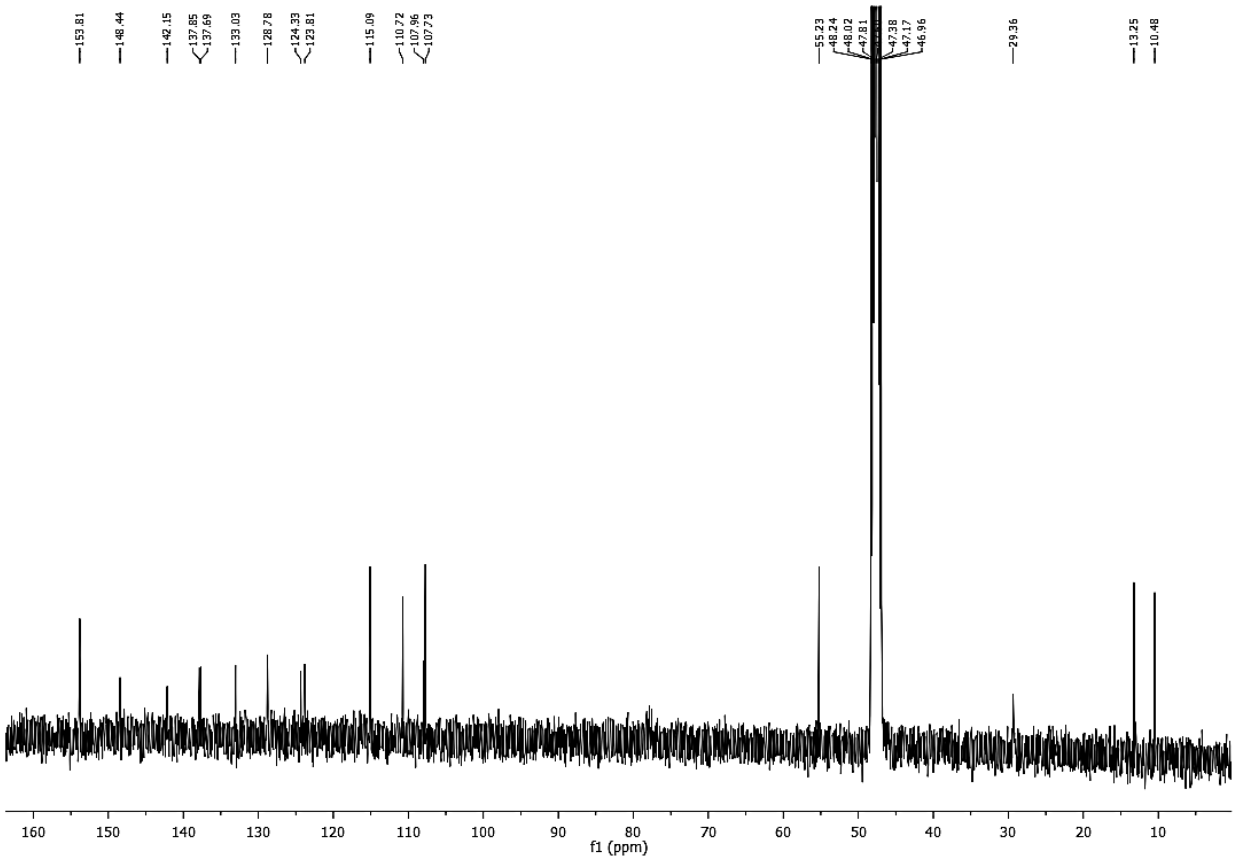


S17: LC-MS spectrum of 9-methoxyellipticine (**8**)

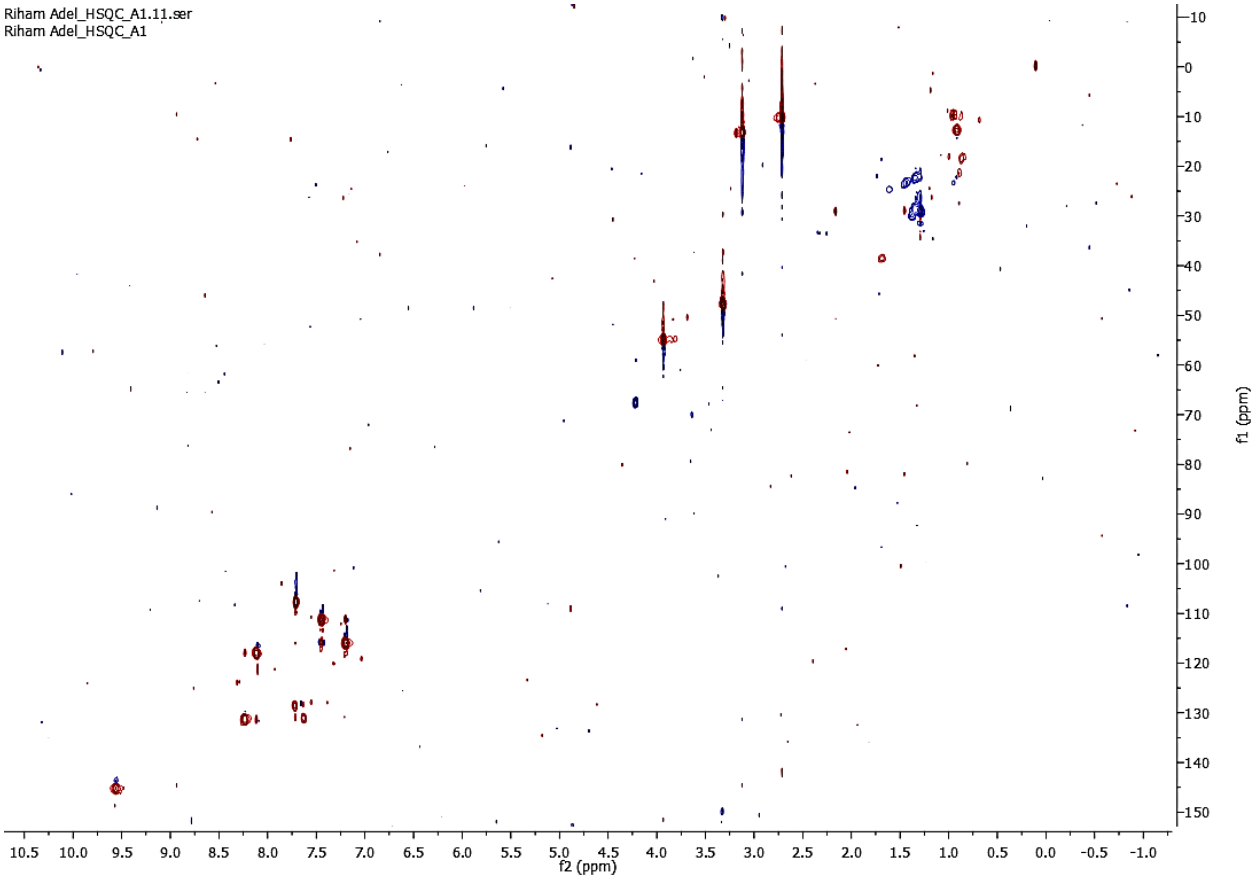


S18: ^{1}H-NMR (400 MHz, CD_3OD) spectrum of 9-methoxyellipticine (**8**)

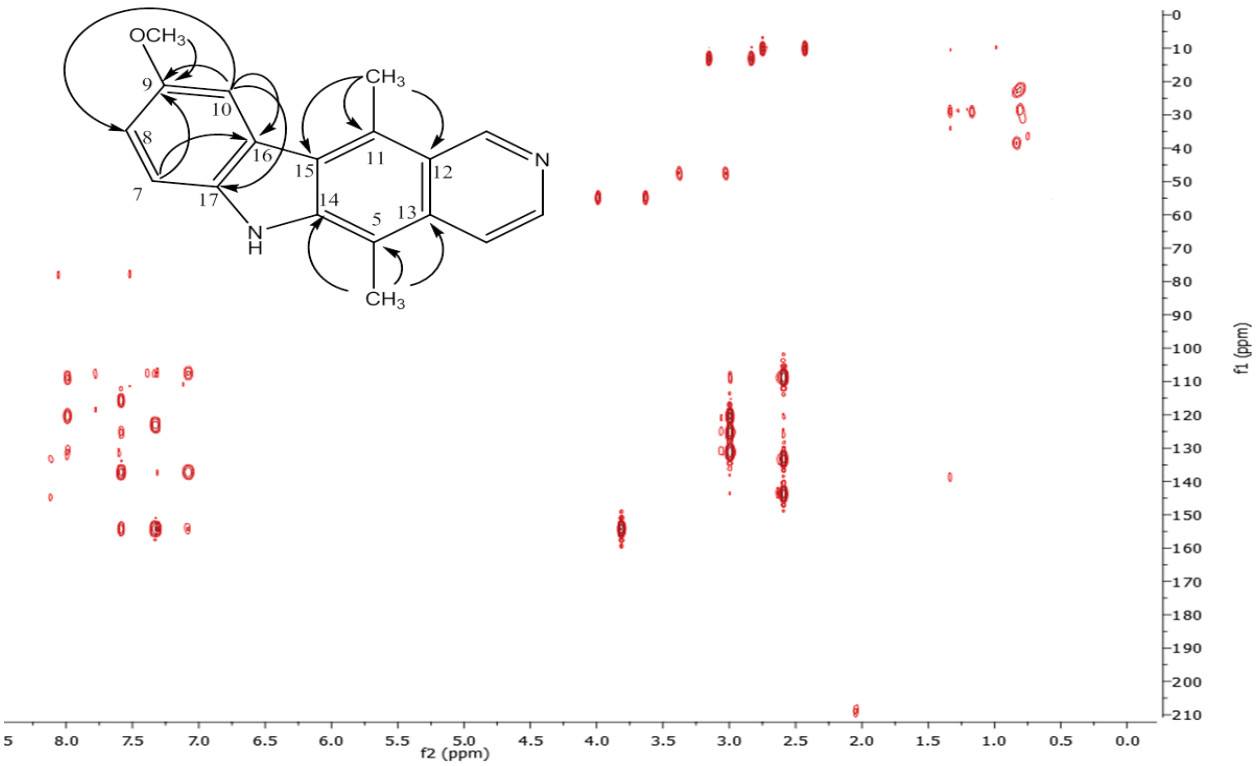
9-methoxyellipticine (**8)**: orange powder. $C_{18}H_{17}N_2O$, m/z 277.1 $[M+H]^+$. ^{1}H-NMR (CD_3OD, 400MHz): 9.46 (1H, br s, H-1), 8.24 (1H, br d, J= 6 Hz, H-3), 7.99 (1H, d, J= 6 Hz , H-4), 7.58 (1H, d, J= 1.9 Hz, H-10), 7.33 (1H, d, J= 8.7 Hz, H-7), 7.08 (1H, dd, J= 8.7, 1.9 Hz, H-8), 3.84 (3H, s, MeO-9), 3.12 (3H, s, Me-11), 2.65 (3H, s, Me-5). The ^{13}C-NMR (CD_3OD, 100MHz): 148.44 (C-1), 142.15 (C-3), 117.9 (C-4), 107.96 (C-5), 107.73 (C-7), 115.09 (C-8), 153.81 (C-9), 110.7 (C-10), 128.76 (C-11), 124.33 (C-12), 142.04 (C-13), 133.03 (C-14), 123.81 (C-15), 125.34 (C-16), 137.69 (C-17), 10.48 (Me-5), 13.25 (Me-11), 55.23 (OCH_3).



S19: ^{13}C-NMR (100 MHz, CD_3OD) spectrum of 9-methoxyellipticine (**8**)



S20: HSQC (500 MHz) spectrum of 9-methoxyellipticine (**8**)



S21: HMBC (500 MHz) spectrum of 9-methoxyellipticine (**8**)

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