SUPPLEMENTARY MATERIAL

Chemical and biological studies on *Bridelia ferruginea* grown in Nigeria

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Abstract

Phytochemical investigation of the methanolic extract of dried leaves of *Bridelia ferruginea* led to the isolation and identification of fourteen compounds (**1**–**14**): compound **1** [mixture of palmitic, stearic and oleic acids], stearyl monoester of 2-*O*-*β*-D-glucosylglycerol (**2**), 6*β*-hydroxy-(20*R*)-24-ethylcholest-4-en-3-one (**3a**), 6*β*-hydroxy-(20*R*)-24-ethylcholest-4,22-dien-3-one (**3b**), lutein (**4**), vomifoliol (**5**), corilagin (**6**), kaempferide-3-*O*-*β*-D-glucoside (**7**), myricetin (**8**), isomericitrin (**9**), isoquercetin (**10**), myricitrin (**11**), quercitrin (**12**), rutin (**13**), and *β*-sitosterol glucoside (**14**). The total extract exhibited moderate activity towards CB2 receptor and 90 % inhibition against leishmanial pathogen *Trypanosoma brucei*. Compound **4** exhibited 73 % displacement in CB2 receptor with IC_{50} 56.47 μM, and 93 % inhibition towards *T. brucei* with IC_{50} 4.16 μM. Compound **11** showed 99 % inhibition towards *E. coli* with IC_{50} 1.123 μM.

Keywords

Bridelia ferruginea, flavonoids, cannabinoid receptor, antileishmanial, antibacterial.

Experimental

General experimental procedures

A Bruker model AMX 500 NMR and 400 NMR spectrometers operating on a standard pulse system collected ^{1}H and ^{13}C NMR spectra. The instrument ran at 500 and 400 MHz in ^{1}H and 125 to 100 MHz in ^{13}C. $CDCl_3$, CD_3OD, DMSO-d_6 and C_5D_5N were used as solvents, and TMS was used as an internal standard. FTMS-ESI was done on Thermo Orbitrap Fusion (Thermo Scientific). Sample was analyzed in the negative mode of ionization. Mass was analyzed in Orbitrap (Voltage – 4300, Mass error on the instrument <2 ppm).

Plant material

Bridelia ferruginea leaves were collected at the end of the raining season from Eruwa, Oyo state, Nigeria in November 2016. It was authenticated by botanist Mr. A. Adeyemo from the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state, Nigeria, voucher specimen number: FHI 114711.

Phytochemical studies

Dried leaves of *B. ferruginea* (1.5 kg) were ground in an electric hammer mill. The ground plant material was macerated using methanol (7 L) at room temperature, filtered and concentrated on a rotary evaporator at 40 °C using vacuum to yield 158.01 g of residue.

The crude methanolic extract (140 g) was loaded on a reverse phase column and eluted with H_2O, H_2O-MeOH (1:1, 1:3), and MeOH to yield 4 fractions (**A**-**D**). Normal phase column chromatography of the fraction **D** (10 g) with acetone-hexane gradient yielded 30 fractions (D1-D30). Fractions D4 and D5 were pooled together (600 mg) and loaded onto a silica gel column and eluted with EtOAc-hexane gradient to yield an oily mass (**1**, 5.5 mg, eluted with 10 % EtOAc-hexane), further by GC MS analysis was identified as palmitic acid (53.5 %), stearic acid (16.2 %), and oleic acid (14.3 %) mixture. Fraction D6 (10.2 mg, eluted with 10 % acetone-hexane) showed a single spot on TLC and proved to be steroidal mixture, containing 6*β*-hydroxy-(20*R*)-24-ethylcholest-4-en-3-one, (**3a**), as major and 6*β*-hydroxy-(20*R*)-24-ethylcholest-4,22-dien-3-one (**3b**) as minor components. Fraction D18 (88 mg, eluent of 20 % acetone-hexane) on crystallization from methanol yielded 7.5 mg of a compound **4**, identified as lutein, while fraction D25 (43 mg, eluent of 50 % acetone-hexane) yielded 20 mg of *β*- sitosterol glucoside (**14**).

The fraction **C** (10 g) was partitioned between H_2O / MeOH, EtOAc and DCM to yield three fractions: DCM fraction (**E**), EtOAc fraction (**F**), aqueous fraction (**G**). Fraction **E** (2.5 g) was loaded on to a sorbdex LH-20 column and eluted with 50 % MeOH-DCM to yield 16 fractions (E1-E16). Fractions E6-E8 were pooled together (500 mg) and subjected to silica gel column chromatography using EtOAc-hexane gradient (1:9-1:1) to yield 2.0 mg of vomifoliol (**5**). Fraction E11 was found to be myricetin (**8**, 18.1 mg), while fraction E14 was identified to be fatty acid monoester of 2-*O*-*β*-D-glucosylglycerol (**2**, 6.8mg), the fatty acid was identified using GC-MS to be stearic acid.

The EtOAc fraction (**F**, 1.64 g) was also loaded on to a sorbdex LH-20 column and eluted with 50 % H_2O-MeOH to yield 21 fractions (F1-F21). Fraction F12 (37 mg) was purified on a Hypersep C-18 cartridge column using H_2O-MeOH gradient to yield 1.7 mg of a compound identified to be rutin (**13**). Fractions F13-F21 (150 mg) were also subjected to the Hypersep C-18 cartridge column purification using H_2O-MeOH gradient yielded 1.2 mg of kaempferide-3-*O*-*β*-D-glucoside (**7**), 4.0 mg of corilagin (**6**), 1.8 mg of myricetin-3-*O*-*β*-D-glucoside (isomericitrin, **9**), 3.0 mg of quercetin-3-*O*-*β*-D-glucoside (isoquercetin, **10**), 3.6 mg of myricitrin (**11**) and 1.9 mg of quercitrin (**12**).

Cannabinoid and opioid receptor assays

The affinity of the total extracts, fractions and isolated compounds towards cannabinoid and opioid receptors were carried out according to the published method (Tarawneh et al. 2015).

Antimicrobial, antimalarial and antileishmanial assays

The extracts and isolated compounds were screened for antimicrobial**,** antimalarial and antileishmanial activities at 20 µg/mL concentration using the reported methods (Bharate et al. 2007; Radwan et al. 2007; Ma et al. 2004; Manohar et al., 2014; Jain et al. 2012).

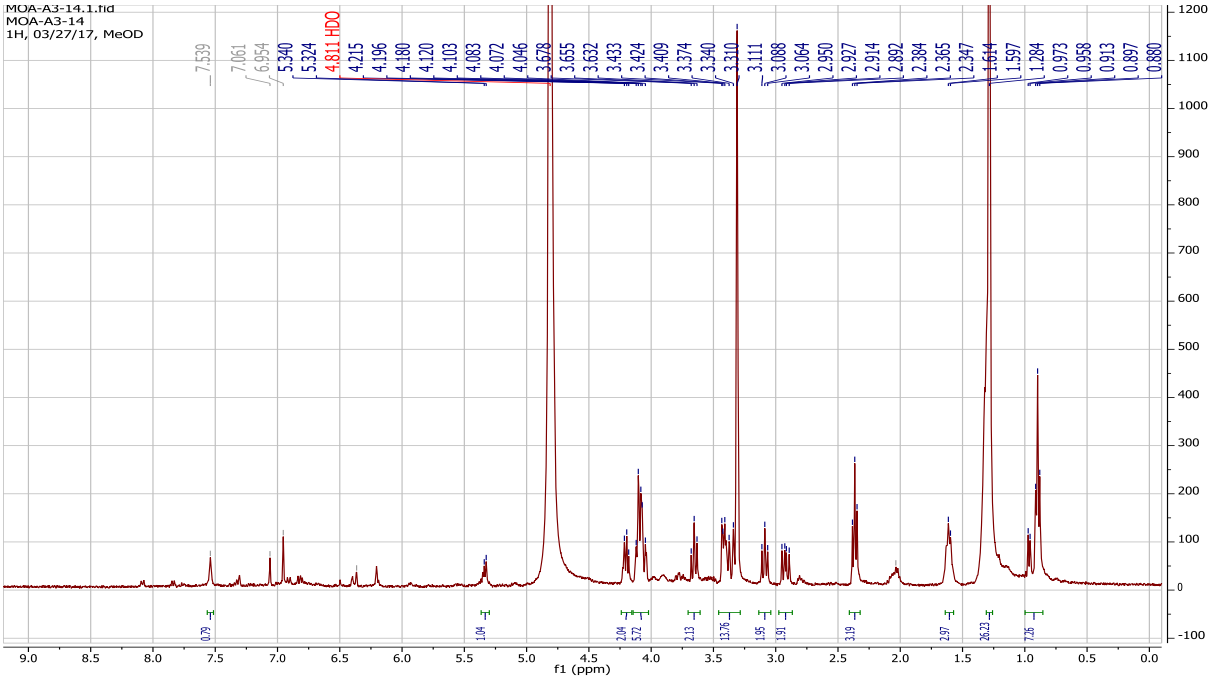

Figure S1: ^{1}H NMR spectrum for fatty acid glyceride (**2**, CD_3OD, 400 MHz).

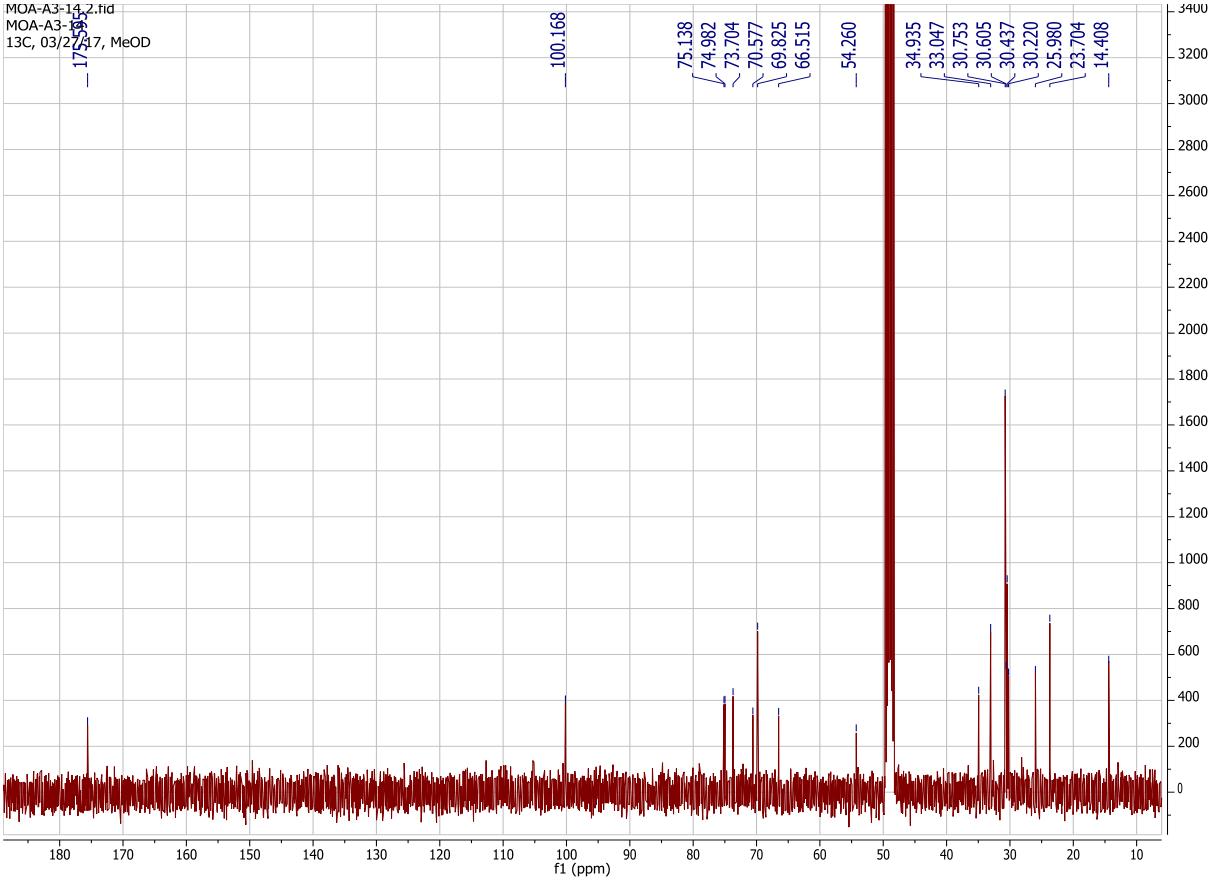

Figure S2: ^{13}C NMR spectrum for fatty acid glyceride (**2**, CD_3OD, 400 MHz).

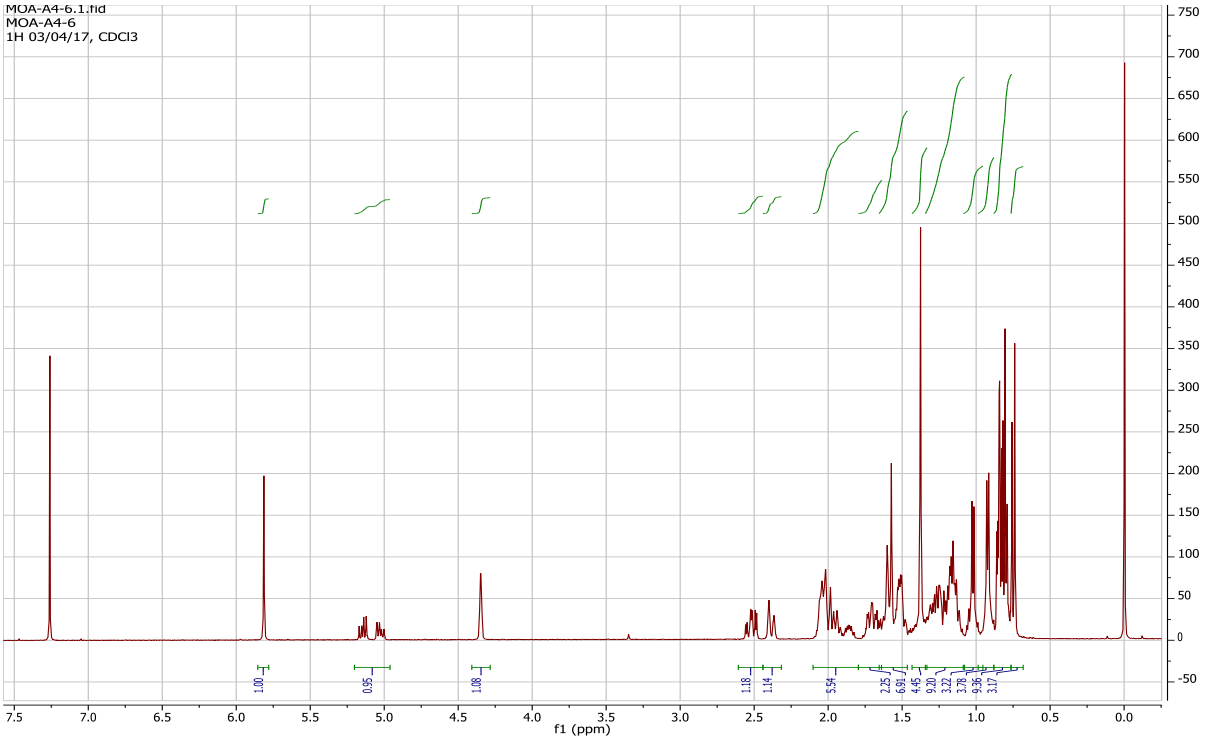

Figure S3: ^{1}H NMR spectrum of **3a** and **3b** ($CDCl_3$, 500 MHz)

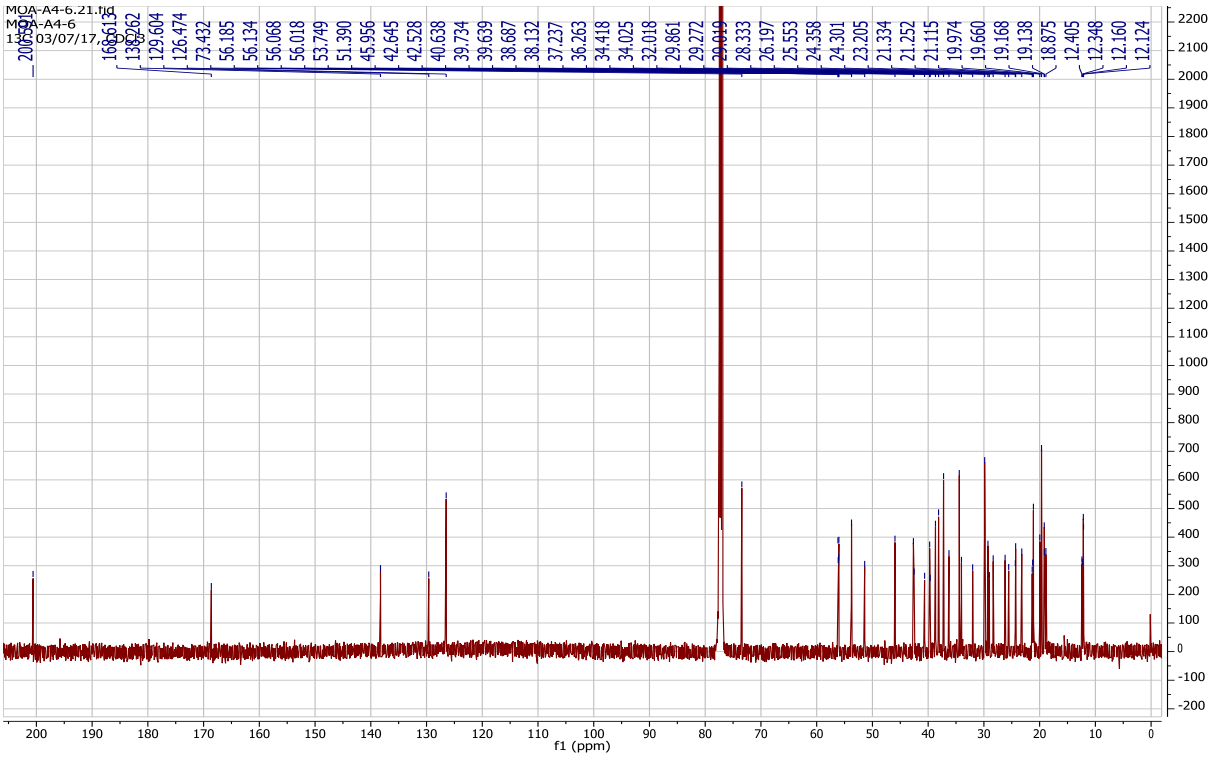

Figure S4: ^{13}C NMR spectrum of **3a** and **3b** ($CDCl_3$, 500 MHz)

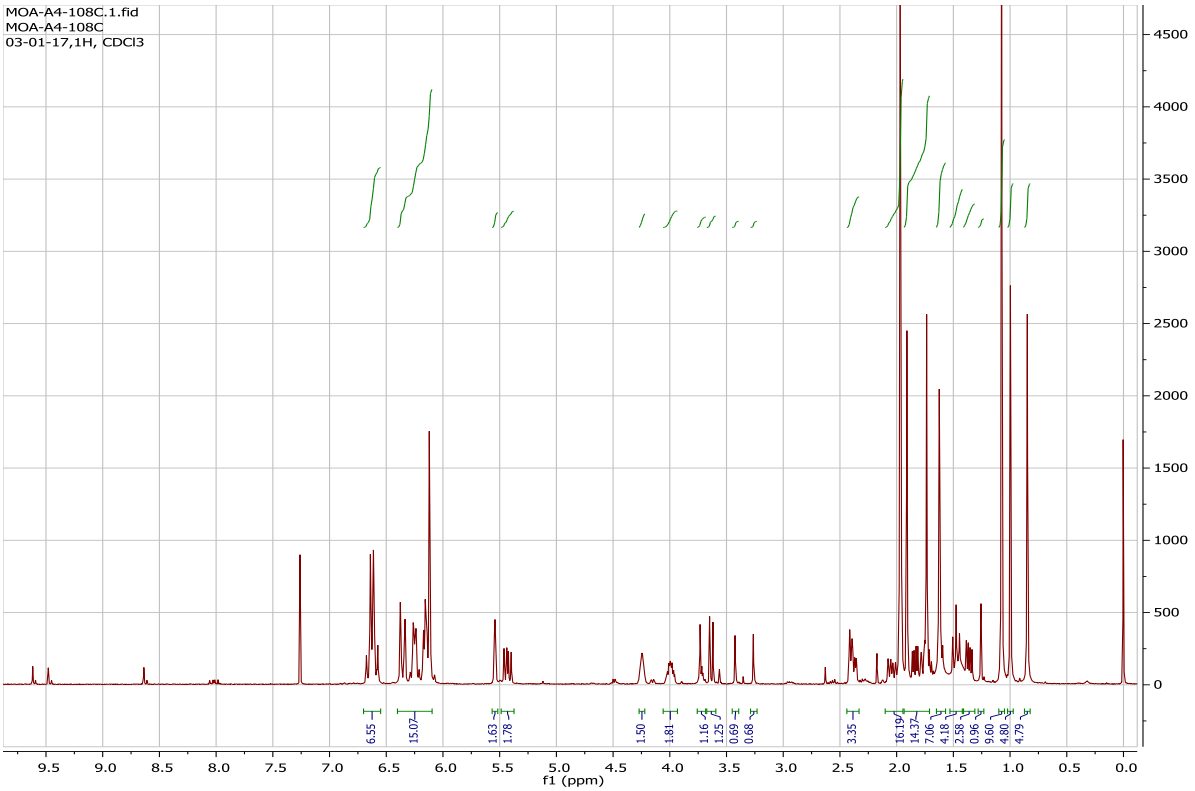

Figure S5: ^{1}H NMR spectrum for lutein (**4**, CDCl3, 400 MHz).

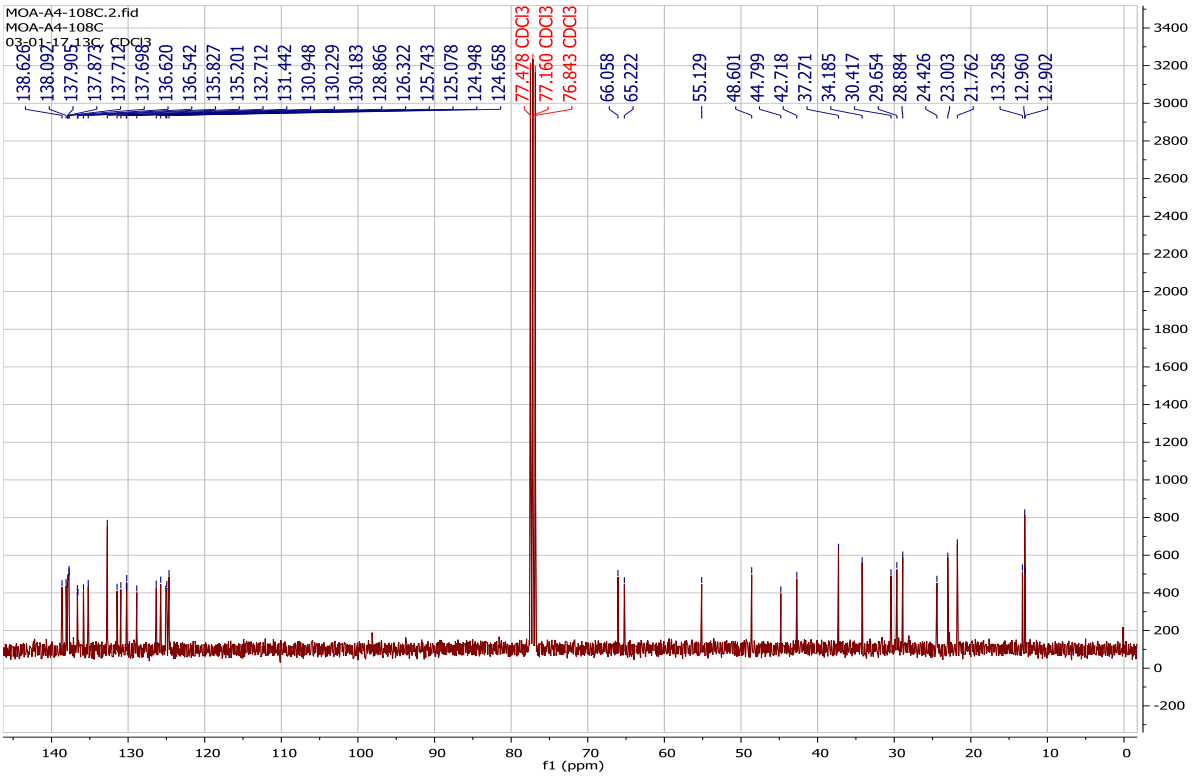

Figure S6:^{13}C NMR spectrum for lutein (**4**, CDCl3, 400 MHz).

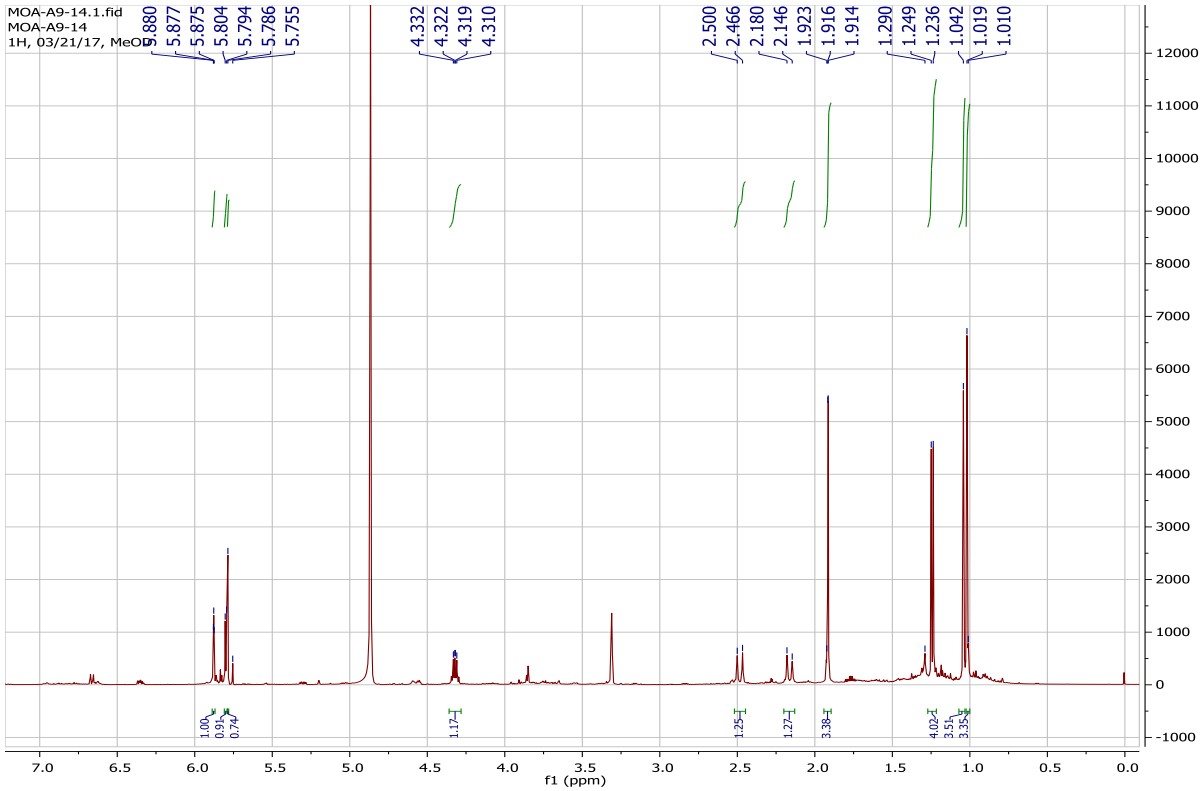

Figure S7: ^{1}H NMR spectrum for vomifoliol (**5**, CD_3OD, 500 MHz).

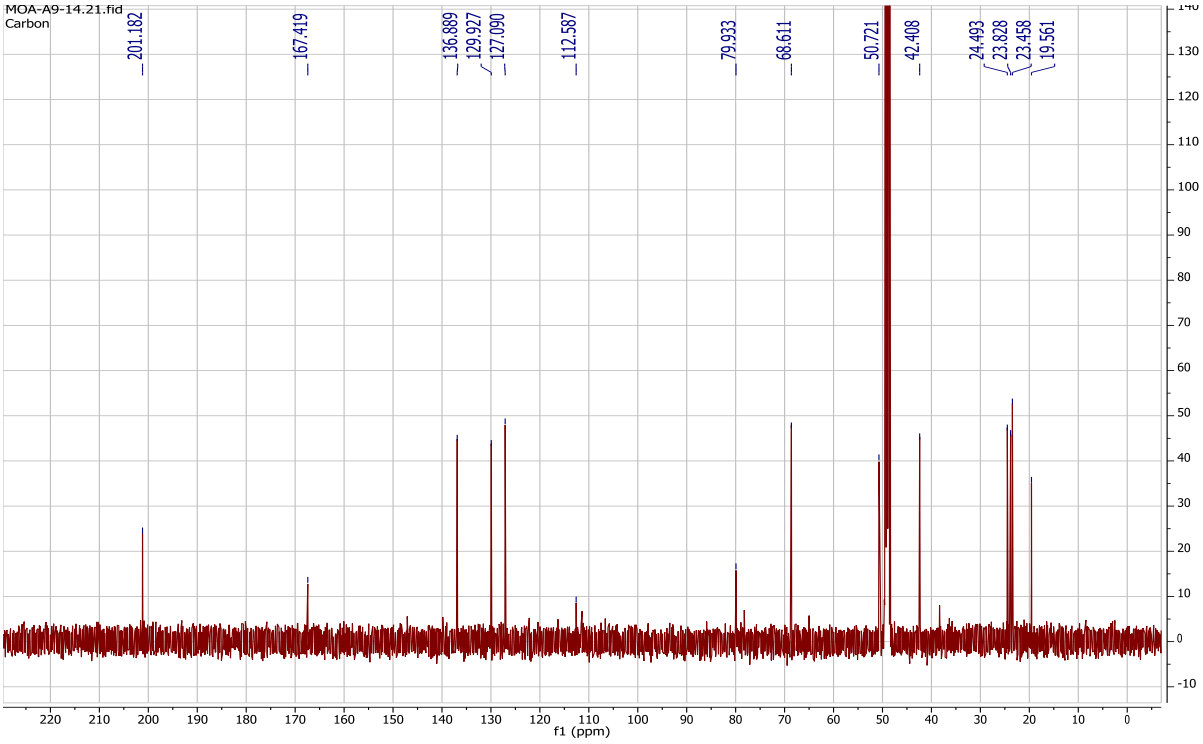

Figure S8: ^{13}C NMR spectrum for vomifoliol (**5**, CD_3OD, 500 MHz).

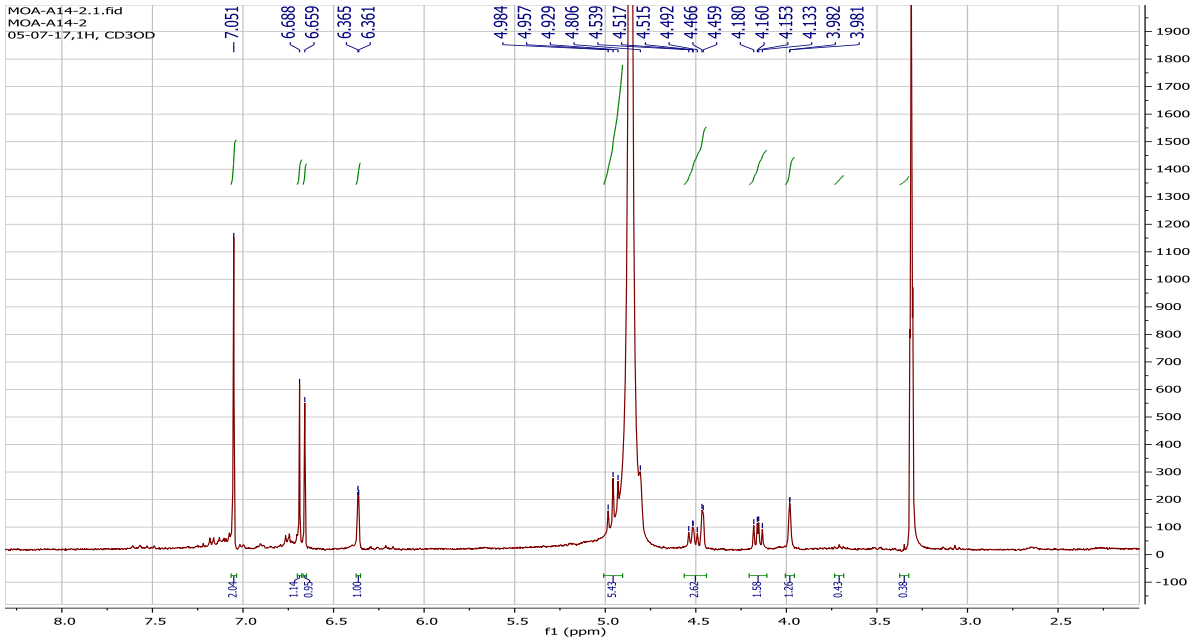

Figure S9: ^{1}H NMR Spectrum for Corilagin (**6**, CD_3OD, 400 MHz).

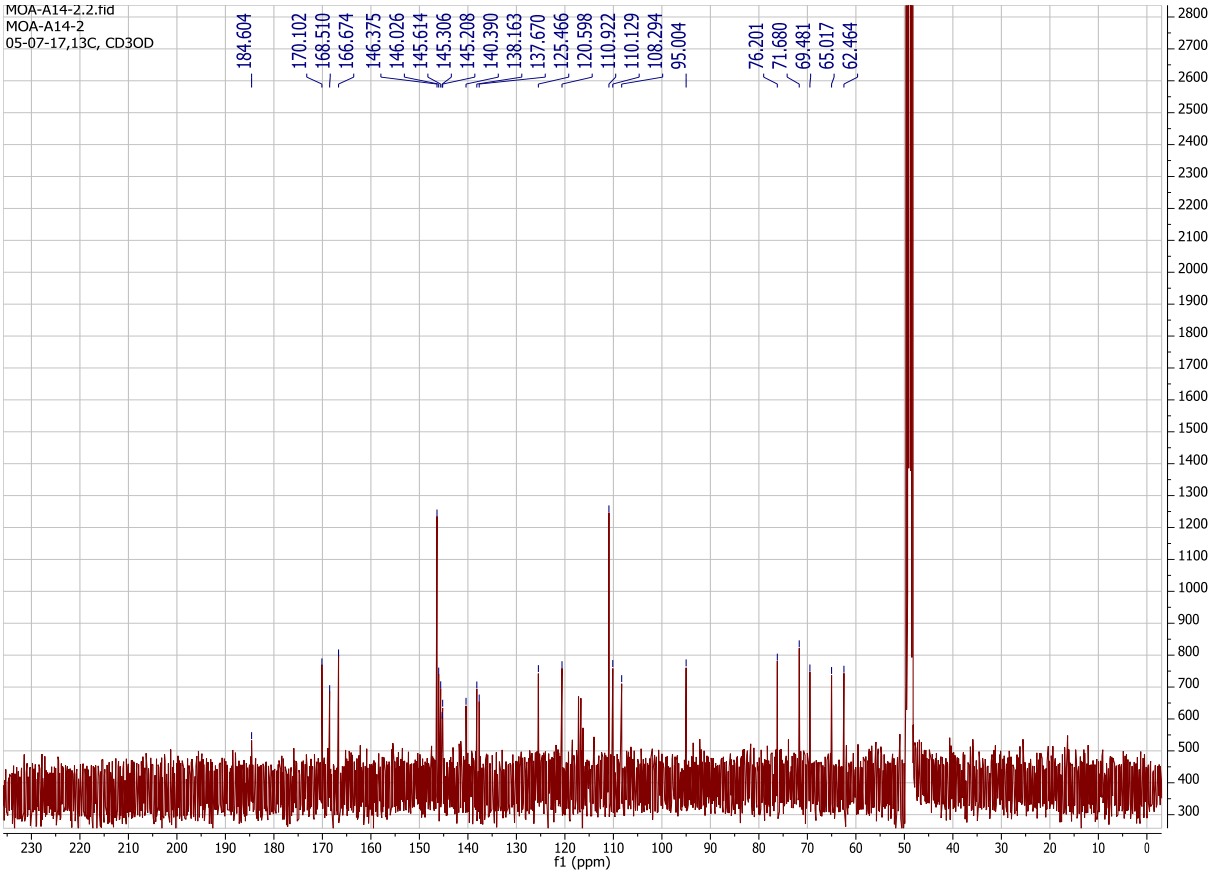

Figure S10: ^{13}C NMR Spectrum for for Corilagin (**6**, CD_3OD, 400 MHz).

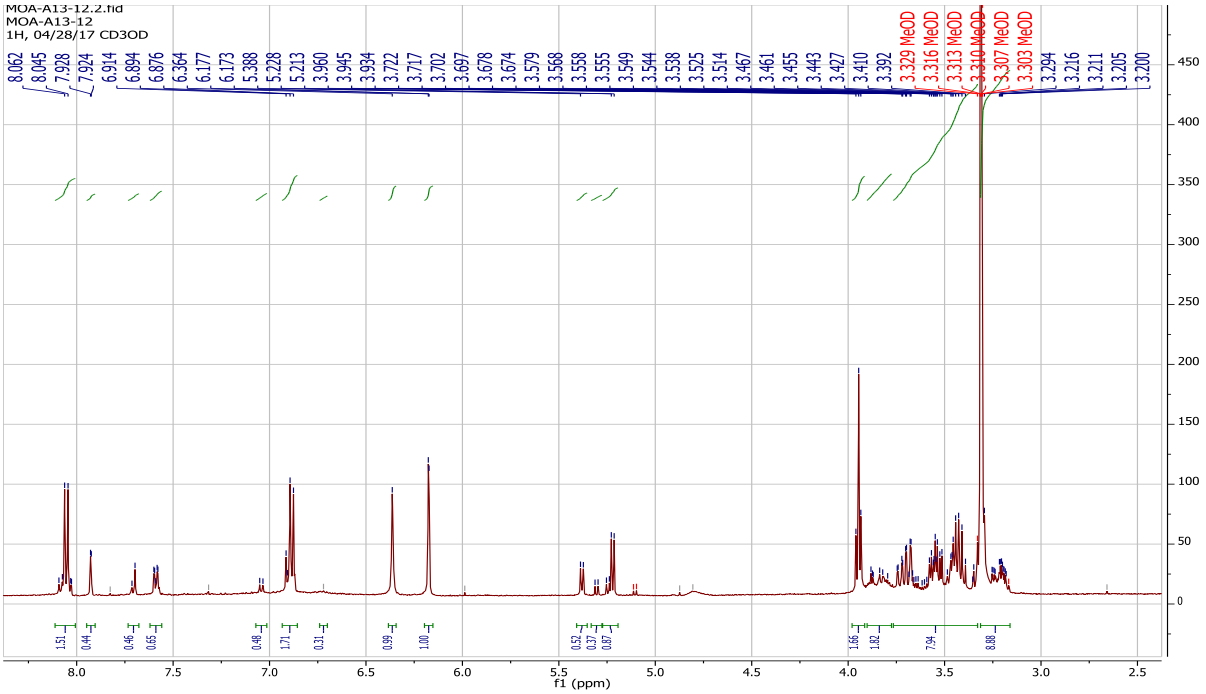

Figure S11: ^{1}H NMR Spectrum for Kaempferide-3-*O*-*β*-glucoside (**7**, CD_3OD, 500 MHz).

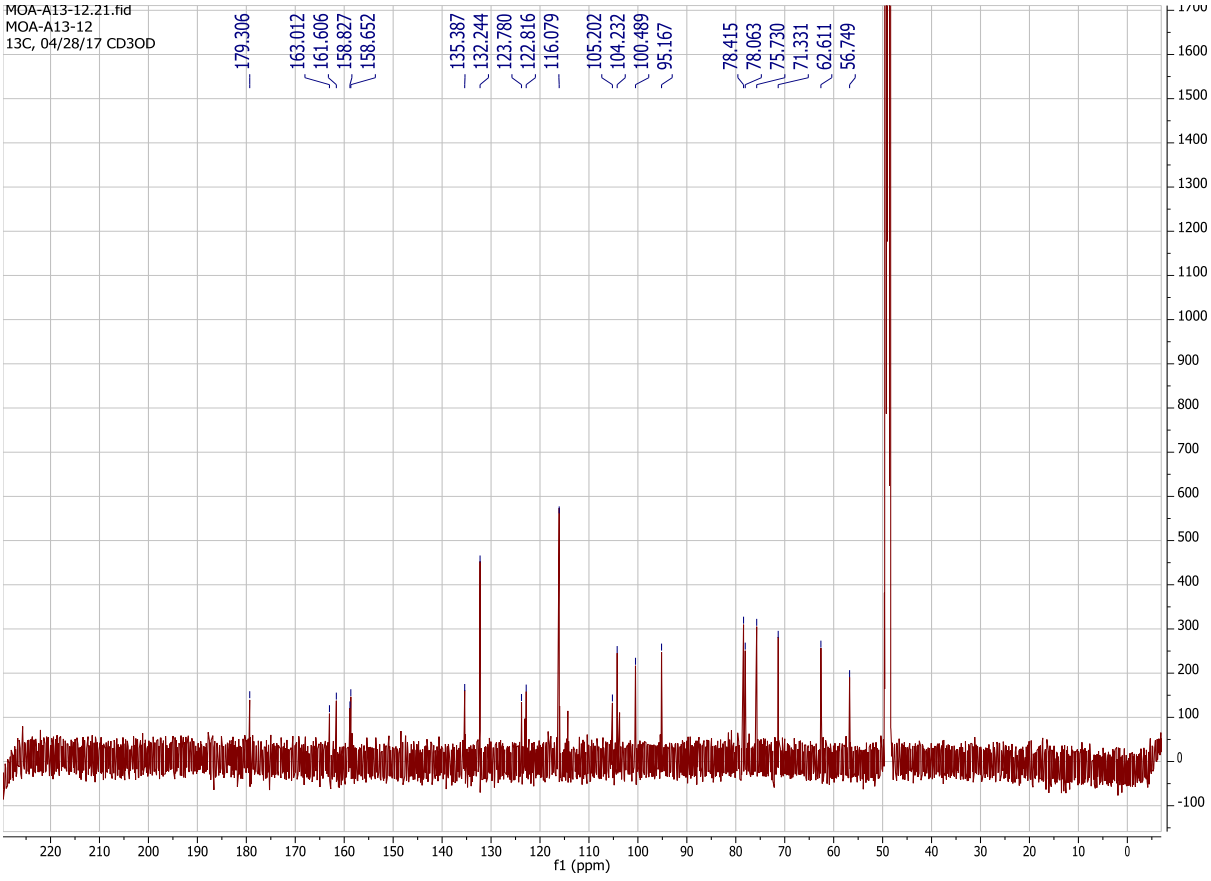

Figure S12: ^{13}C NMR Spectrum for Kaempferide-3-*O*-*β*-glucoside (**7**, CD_3OD, 500 MHz).

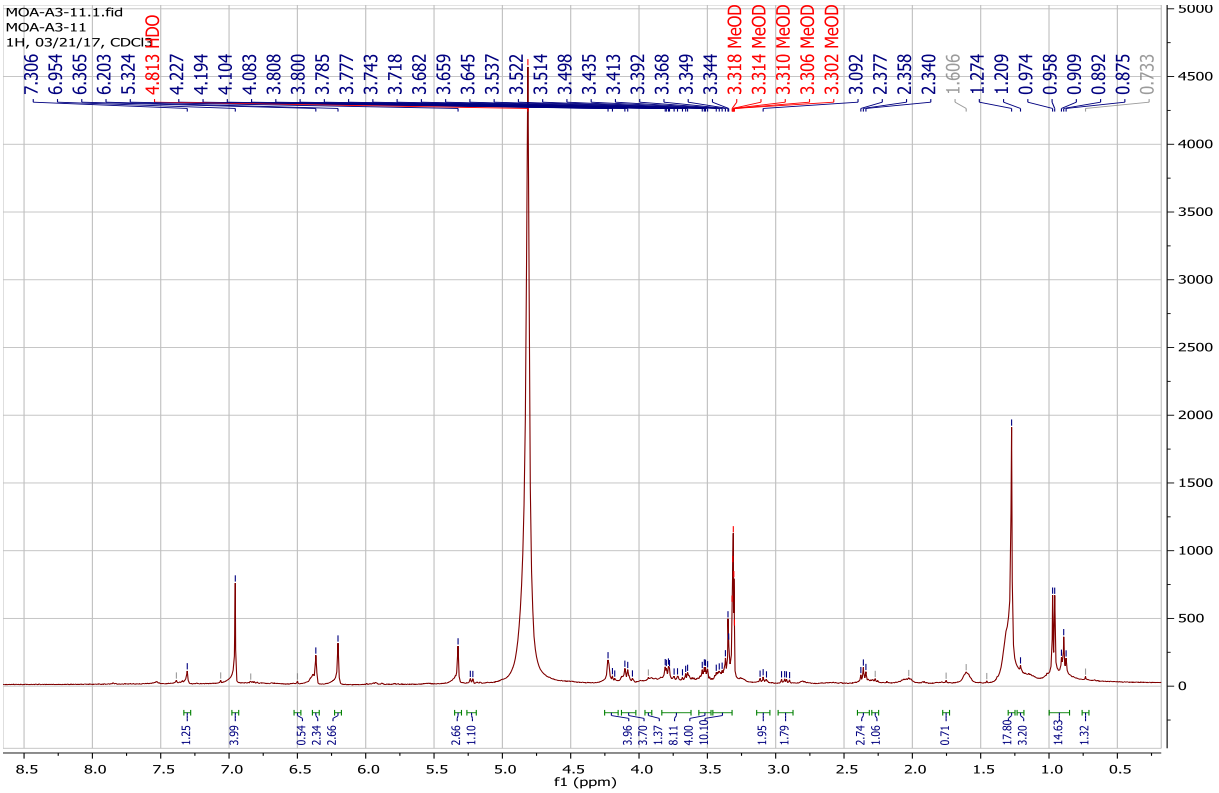

Figure S13: ^{1}H NMR spectrum for myricetin (**8**, CD_3OD, 400 MHz).

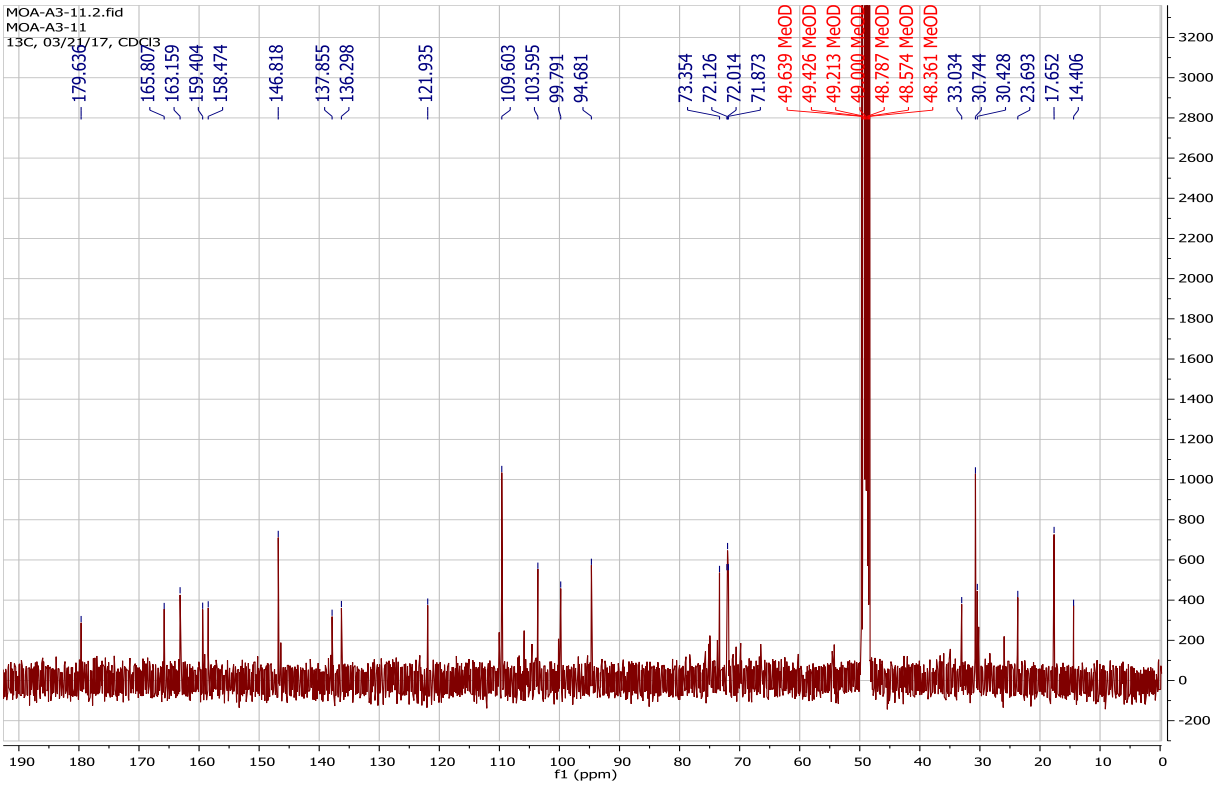

Figure S14: ^{13}C NMR spectrum for myricetin (**8**, CD_3OD, 400 MHz).

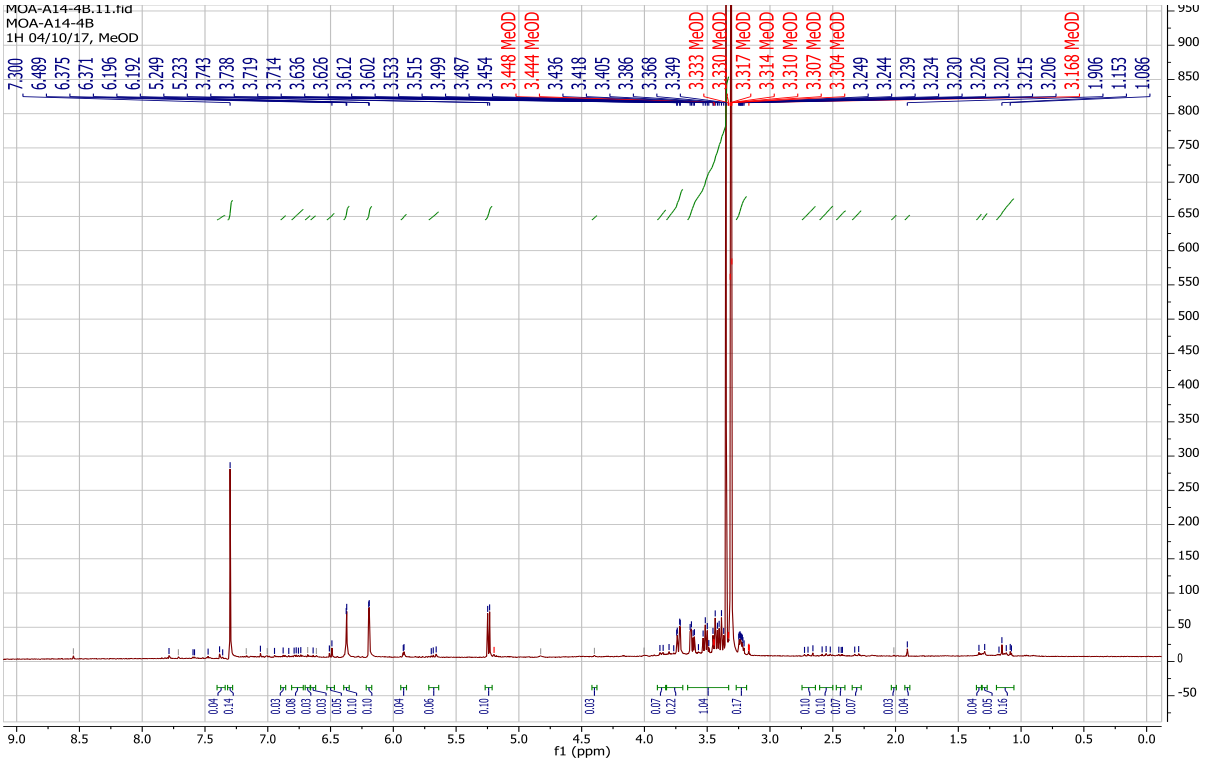

Figure S15: ^{1}H NMR Spectrum for isomericitrin (**9**, CD_3OD, 500 MHz).

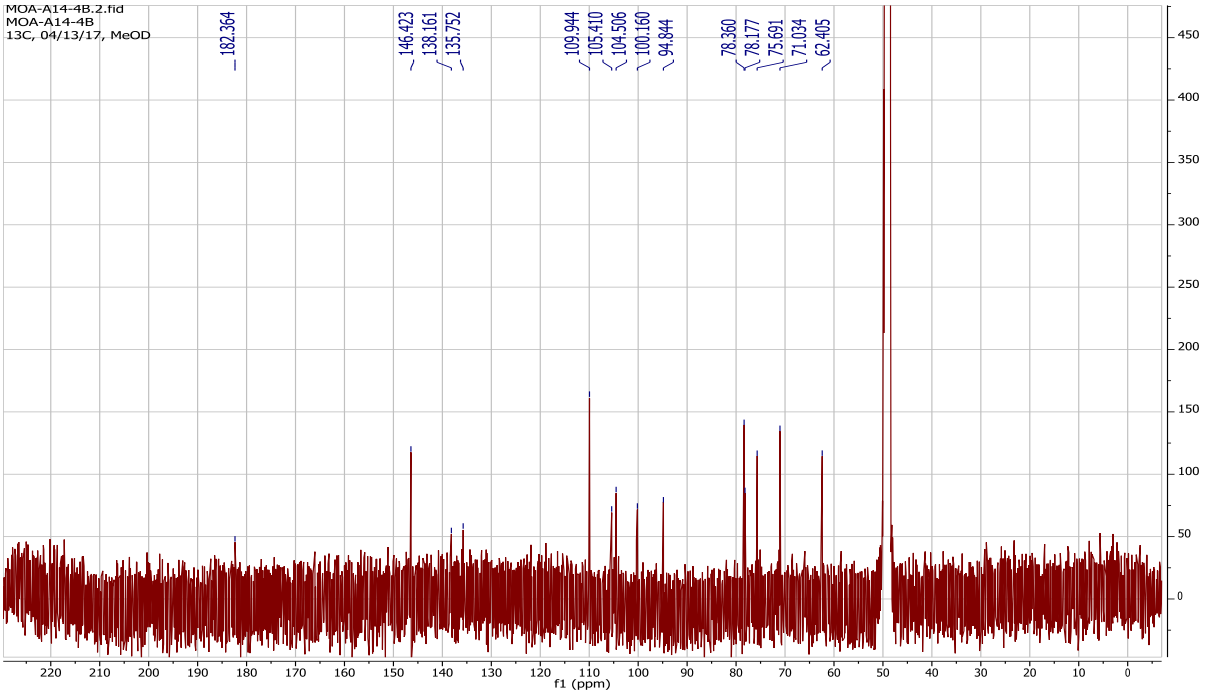

Figure S16: ^{13}C NMR Spectrum isomericitrin (**9**, CD_3OD, 500 MHz).

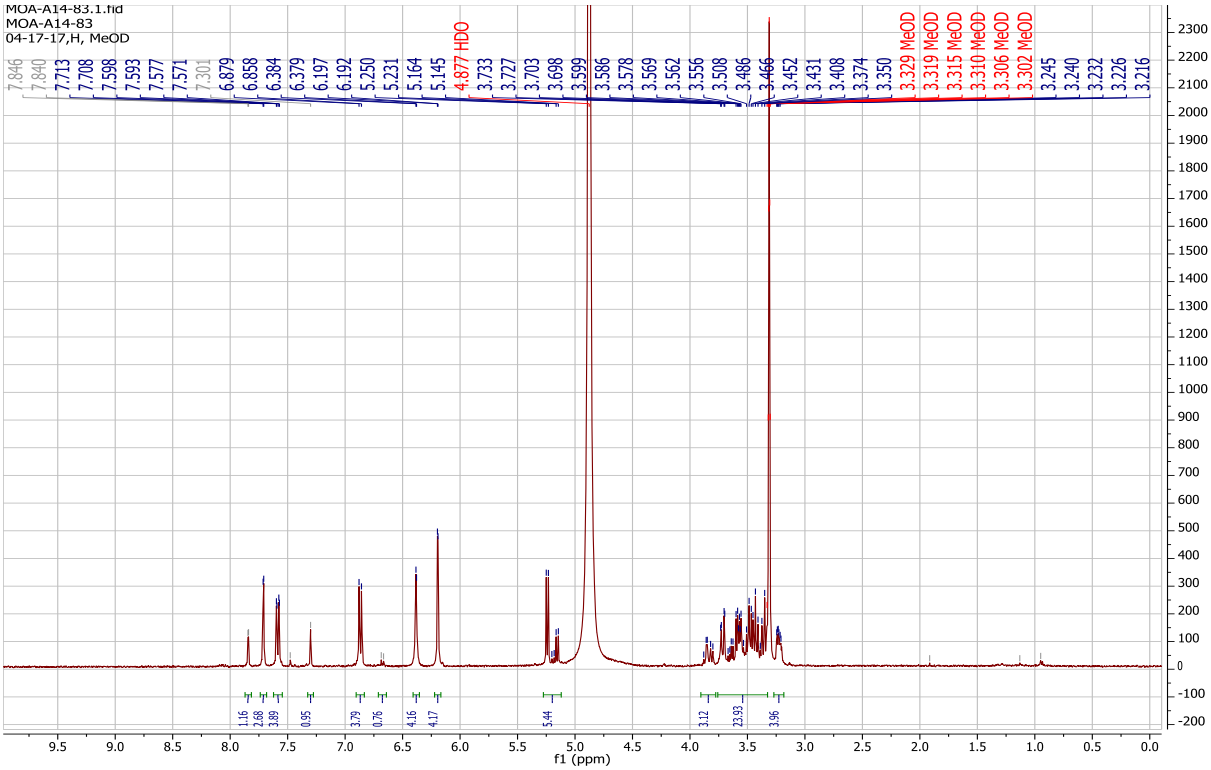

Figure S17: ^{1}H NMR Spectrum for isoquercetin (**10**, CD_3OD, 400 MHz).

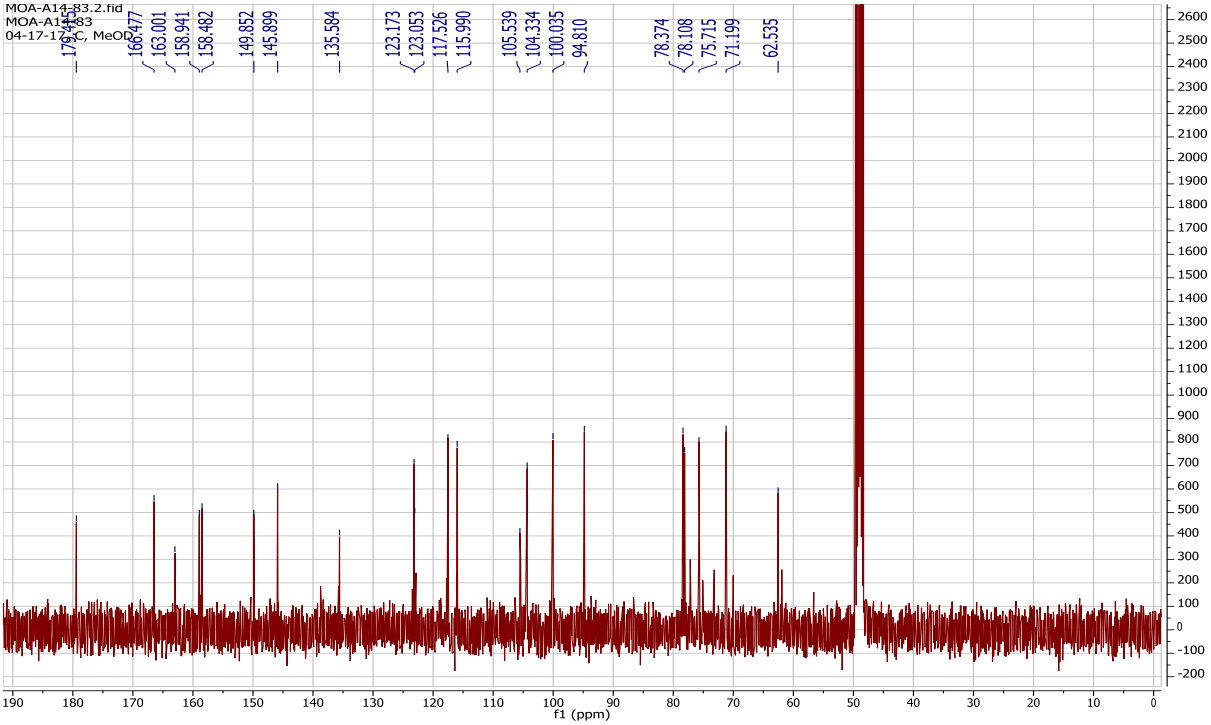

Figure S18: ^{13}C NMR Spectrum for isoquercetin (**10**, CD_3OD, 400 MHz).

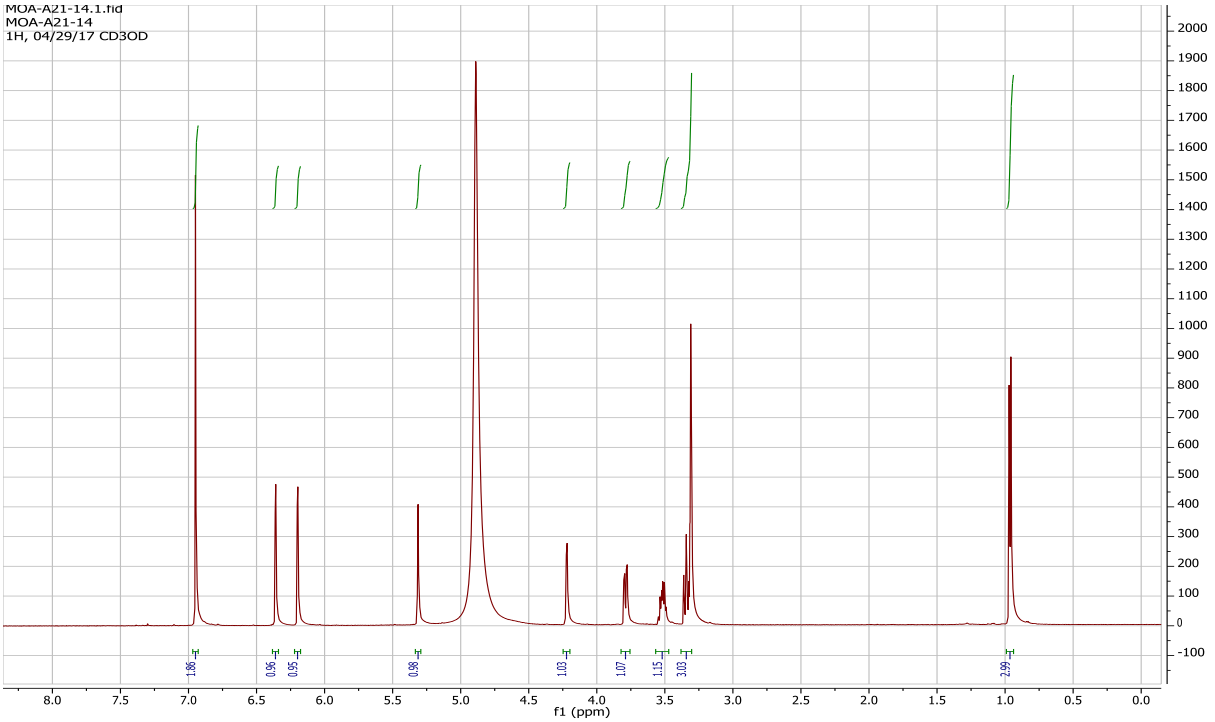

Figure S19: ^{1}H NMR Spectrum for myricitrin (**11**, CD_3OD, 500 MHz).

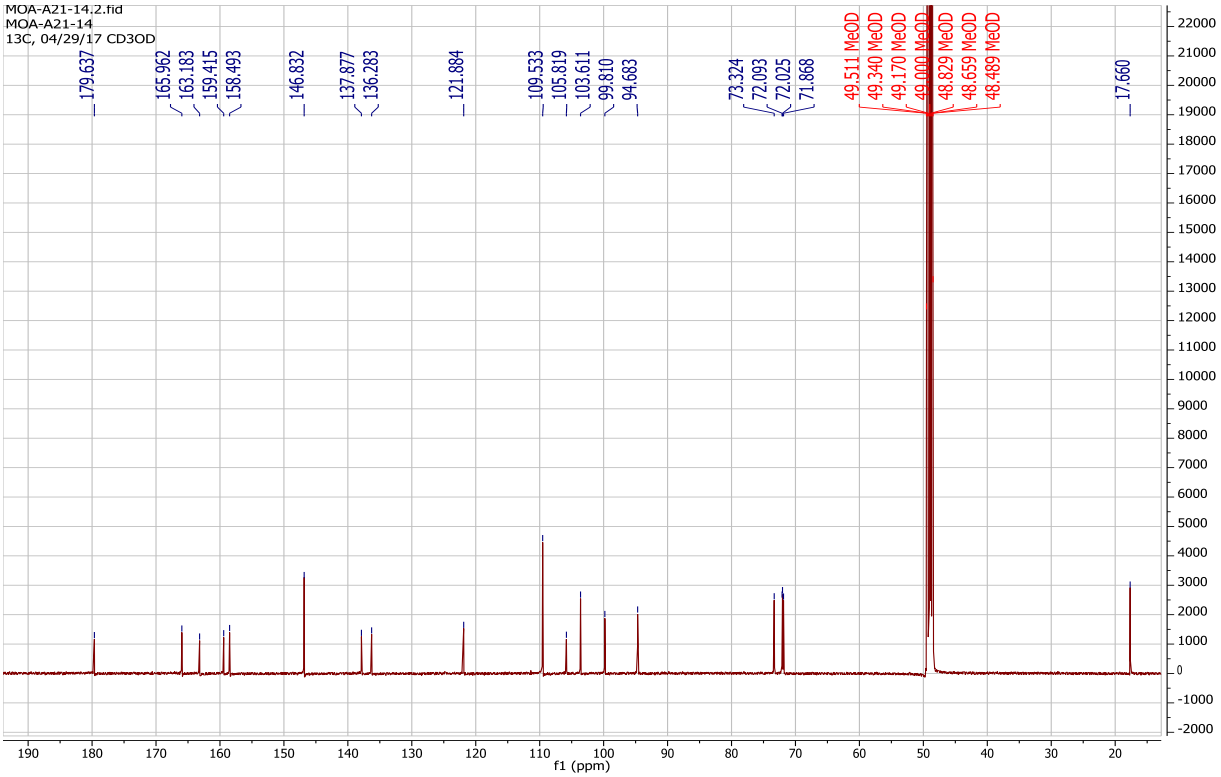

Figure S20: ^{13}C NMR Spectrum for myricitrin (**11**, CD_3OD, 500 MHz).

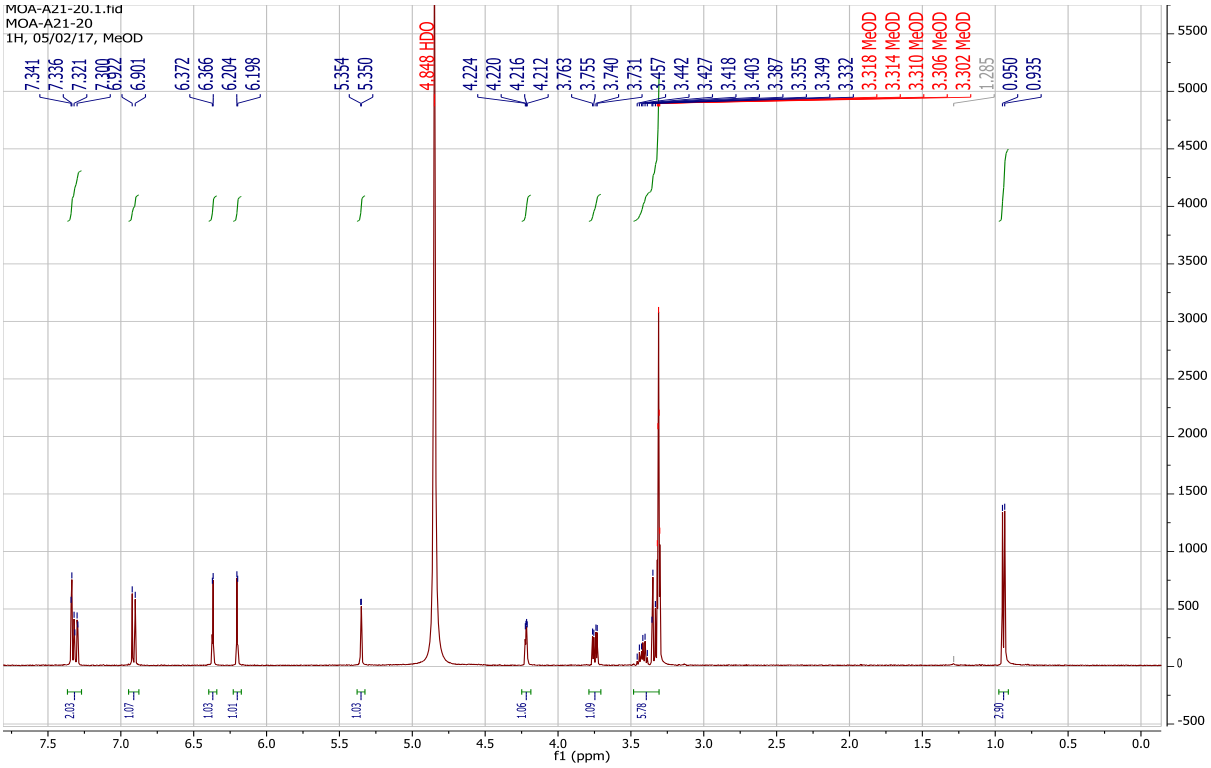

Figure S21: ^{1}H NMR Spectrum for quercitrin (**12**, CD_3OD, 400 MHz).

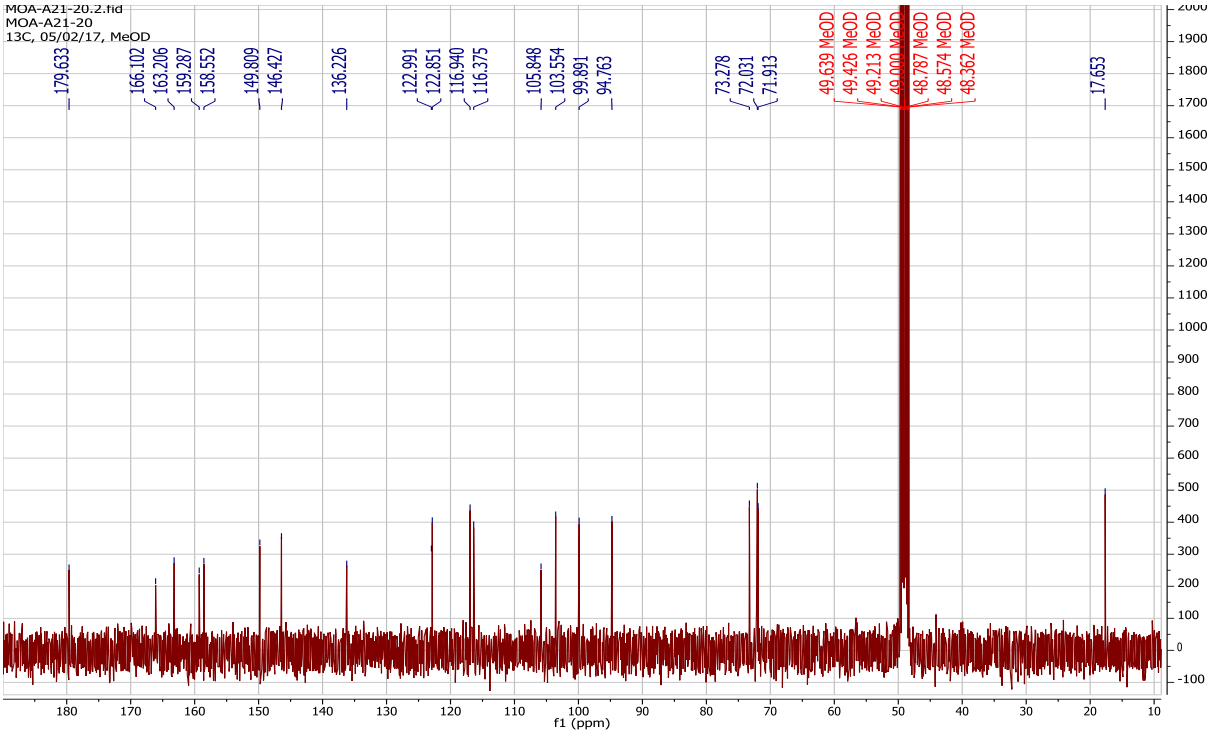

Figure S22: ^{13}C NMR Spectrum for quercitrin (**12**, CD_3OD, 400 MHz).

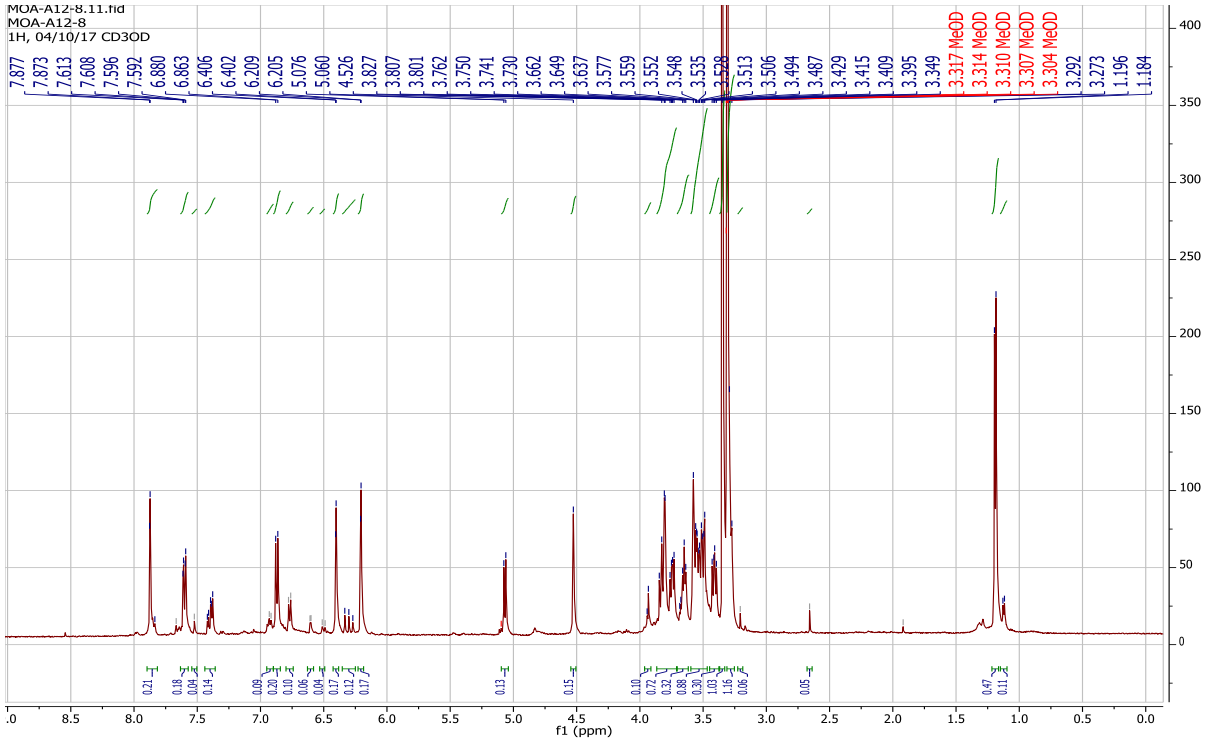

Figure S23: ^{1}H NMR spectrum for rutin (**13**, CD_3OD, 500 MHz).

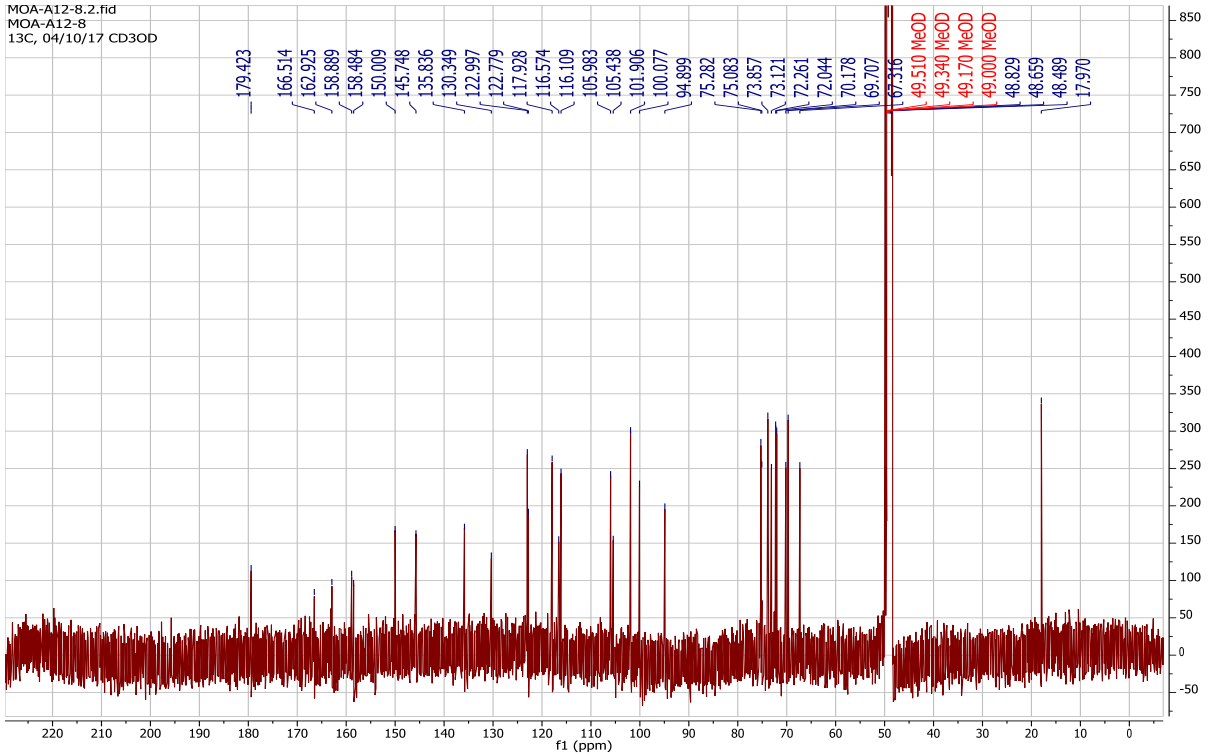

Figure S24: ^{13}C NMR Spectrum for rutin (**13**, CD_3OD, 500 MHz).

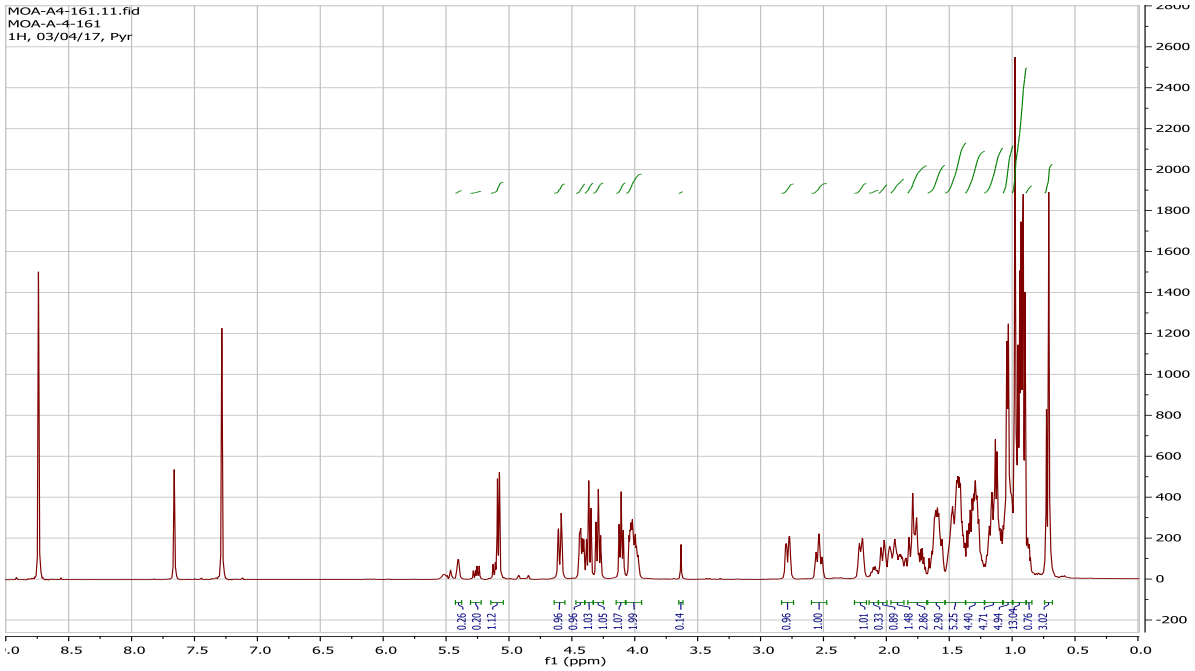

Figure S25: ^{1}H NMR spectrum for β-sitosterol glucoside (**14**, C_5D_5N, 400 MHz).

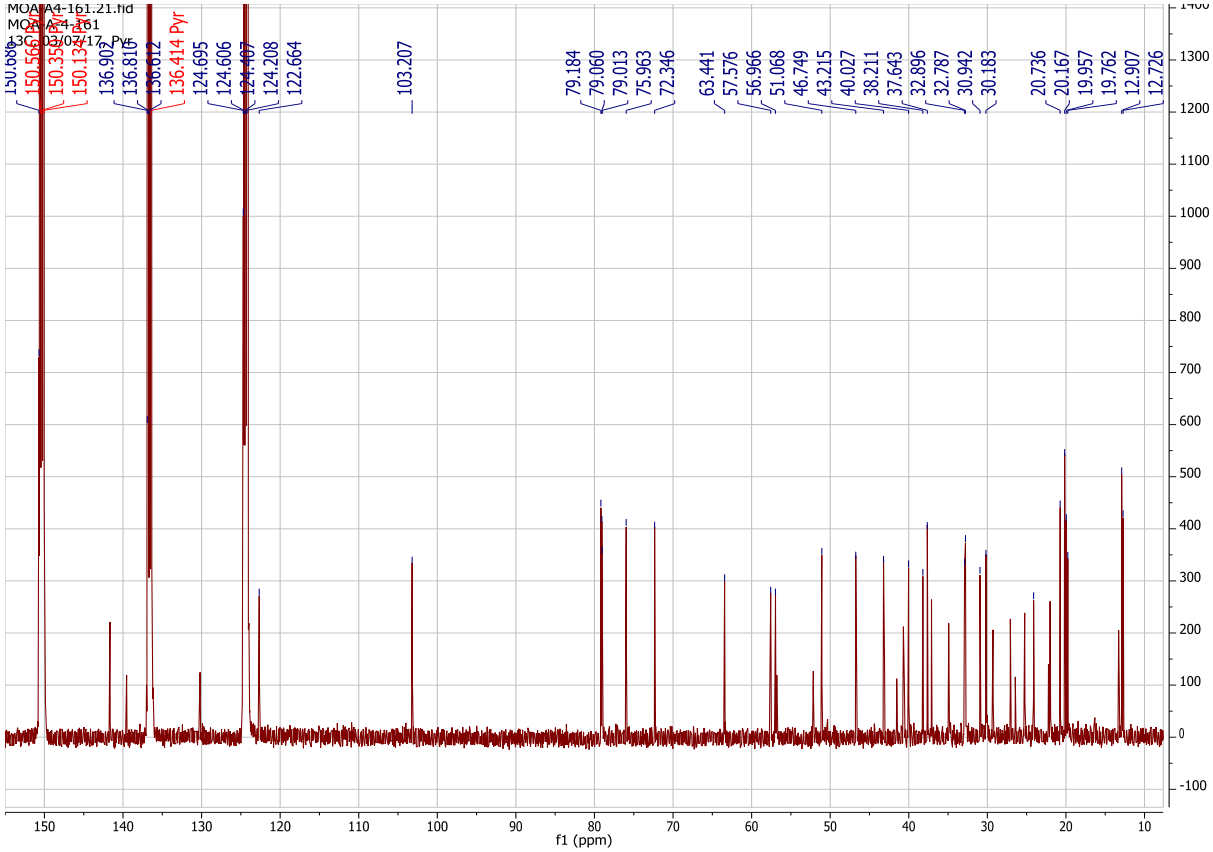

Figure S26: ^{13}C NMR spectrum for β-sitosterol glucoside (**14**, C_5D_5N, 400 MHz).

References

Bharate SB, Khan SI, Yunus NAM, Chauthe SK, Jacob MR, Tekwani BL, Khan IA, Singh IP. 2007. Antiprotozoal and antimicrobial activities of O-alkylated and formylated acylphloroglucinols. Bioorg Med Chem. 15: 87–96.

Jain SK, Sahu R, Walker LA, Tekwani BL. 2012. A parasite rescue and transformation assay for antileishmanial screening against intracellular Leishmania donovani amastigotes in THP1 human acute monocytic leukemia cell line. J Vis Exp. 70: 4054.

Ma G, Khan SI, Jacob MR, Tekwani BL, Li Z, Pasco DS, Walker LA, Khan IA. 2004. Antimicrobial and antileishmanial activities of hypocrellins A and B. Antimicrob Agents Chemother. 48: 4450–4452.

Manohar S, Tripathi M, Rawat DS. 2014. 4-Aminoquinoline based molecular hybrids as antimalarials: An Overview. Curr Top Med Chem. 14: 1706-1733.

Radwan MM, Manly SP, Ross SA. 2007. Two new sulfated sterols from the marine sponge *Lendenfeldia dendyi*. Nat Prod Commun. 2: 901–904.

Tarawneh A, León F, Pettaway S, Elokely KM, Klein ML, Lambert J, Mansoor A, Cutler SJ. 2015. Flavonoids from *Perovskia atriplicifolia* and their *in vitro* displacement of the respective radioligands for human opioid and cannabinoid receptors." J Nat Prod. 78:1461-1465.