SUPPLEMENTARY MATERIAL

Phytochemical constituents from the roots and lignotubers of *Rhoicissus tridentata* and their *in vitro* uterotonic activity

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Abstract

Rhoicissus tridentata is one of the most frequently used plants in preparing *Isihlambezo*, a herbal drink consumed by many South African women to induce labour and tone the uterus in pregnancy. This study aimed to identify the uteroactive compounds in this plant. Chromatographic purification of the methanol and water extracts from the roots yielded eight compounds, i.e. morin 3-*O*-α-L-rhamnopyranoside, *trans*-resveratrol 3-*O*-β-glucopyranoside, a mixture of asiatic and arjunolic acids, quercetin 3-*O*-rhamnopyranosideside, catechin, β-sitosterol, and linoleic acid. All compounds were evaluated for their uterotonic effects using uterine smooth muscle isolated from stilboestrol-primed Sprague-Dawley rats. The mixture of asiatic and arjunolic acids showed the highest activity with EC_{50} of 0.02129 µg/mL for amplitude. These results validate the use of *R. tridentata* in ethnomedicine to facilitate labour in childbirth. Morin 3-O-α-L-rhamnopyranoside and *trans*-resveratrol 3-*O*-β-glucopyranoside caused a relaxation of the uterine muscle, which suggests that some compounds in *R. tridentata* possess opposing activities.

Keywords: *Rhoicissus*; uterotonic activity; asiatic acid; arjunolic acid; *trans*-resveratrol

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1. Experimental

1.1 General Experimental

TLC plates (Kieselgel 60 F_{254}, 0.25 mm) were used for analysis of the chemical profile of extracts and pure compounds. Compounds were detected as spots when viewing the TLC plate under UV radiation (254 or 365 nm) or by staining the plate with *p*-anisaldehyde/H_2SO_4, followed by heating. Columns packed with silica gel 60 and centrifugal chromatography (Chromatotron™ model 7924, Harrison Research) were used for the purification of compounds. NMR spectra were recorded on Bruker Avance III spectrometers (400 MHz or 500 MHz) at 30 °C. All chemical shifts were expressed in parts per million (ppm). HPLC-PDA analysis was conducted using a Shimadzu HPLC system, which included a controller, solvent supply unit, photodiode-array detector, an autosampler, and a Phenomenex Kinetex C18 column (100 x 4.60 mm) with 2.6 µm particle size. The analysis was performed at a temperature of 40 °C using a mobile phase comprising 1% acetic acid (A) and methanol (B). Elution commenced with 30% of B, and a linear gradient was employed to achieve 90% at 20 min, 100% at 25 min, and then returning to 30% at 30 min. The injection volume was 4.00 µL, and the flow rate was set at 0.400 µL/min. UV detection was carried out at 340 nm and the UV-Vis peak spectra was recorded in the range of 210-450 nm. Mass spectra were recorded on a time-of-flight Waters LCT Premier mass spectrometer using electrospray ionisation (ESI) in the positive or negative mode.

1.2 Plant material

The roots and lignotubers of *Rhoicissus tridentata* were collected in April from the vendors in the Warwick Triangle (Durban, South Africa). The plant was identified by Ms Alison Young (Curator, University of KwaZulu-Natal) and the voucher specimen (van Heerden 20) was deposited in the Bews Herbarium, University of KwaZulu-Natal, Pietermaritzburg. The roots were washed, sliced into small pieces, and oven-dried at 30 °C for 3 days. The dried sample was grounded into a fine

powder before solvent extraction.

1.3 Extraction and Isolation

The powdered roots and lignotubers (2.5 kg) were extracted sequentially at room temperature with dichloromethane (DCM, 2 x 4.0 L), methanol (MeOH, 2 x 4.0 L), and water (2 x 4.0 L) for 48 h. The solvents were evaporated under vacuum using a rotary evaporator at 40 °C to give 13 g of DCM extract, 72 g of MeOH extract, and 8.0 g of aqueous crude extract. The chemical profiles of the MeOH and aqueous extracts were similar (based on TLC), and these extracts were combined. The combined MeOH-water extract (80 g) was separated by vacuum liquid chromatography on silica gel 60 using DCM (2.5 L), acetone (2.0 L), and MeOH (2.0 L) as eluents. Each fraction was dried under vacuum using a rotary evaporator at 40 °C to yield F1 (0.90 g), F2 (9.96 g), and F3 (61.2 g). The % yield of the fractions (% w/w of the starting mass of the crude extract) was determined as 1.1% F1, 12.5% F2, and 76.6% F3. Findings from the uterotonic activity assay using the rat uterine smooth muscle strips revealed that F2 was the most uteroactive fraction, hence, this fraction was selected for further isolation of compounds.

F2 (9.96 g) was subjected to column chromatography on silica gel 60 using 1:1 DCM-ethyl acetate (EtOAc) to yield 5 fractions of 200 mL each (A1–A5). Purification of A3 (1.34 g) by centrifugal chromatography using diethyl ether (DEE)–MeOH (9:1, 2 x 200 mL) afforded compound **1** (13.6 mg, 0.017% w/w) as a yellow solid (R_f = 0.69, DCM-MeOH, 7:3), and compound **5** (21.8 mg, 0.027% w/w) as a pale-yellow solid (R_f = 0.62, DCM-MeOH, 7:3). Purification of A4 (0.76 g) on a Chromatotron™ eluting with DCM-MeOH (8.5:1.5, 2 x 100 mL) gave 61.0 mg (0.076% w/w) of compound **2** (R_f = 0.10, DCM-MeOH, 8.5:1.5). Fraction A2 (0.63 g) was purified by centrifugal chromatography eluting with DCM–EtOAc (1:1, 2 x 100 mL) to afford an inseparable mixture of compound **3** and **4** (26.7 mg, 0.033% w/w) as a light-yellow solid

(R_f = 0.38, DEE- MeOH, 9.5:0.5), and compound **6** (2.0 mg, 0.0025% w/w) as a light-brown solid (R_f = 0.31, DCM-MeOH, 9:1). A1 showed a chemical profile similar to F1 on TLC and these fractions were combined. Further purification of these fractions (3.42 g) by column chromatography using DCM-EtOAc (8:2) afforded compound **7** (293 mg, 0.37% w/w) as white crystals (R_f = 0.38, DCM-EtOAc, 9:1) and compound **8** (3.0 mg, 0.0038% w/w) as an off-white solid (R_f = 0.75, DCM–EtOAc, 1:1). The structures of pure compounds were characterised using NMR, HPLC-PDA, and LCMS/HRMS.

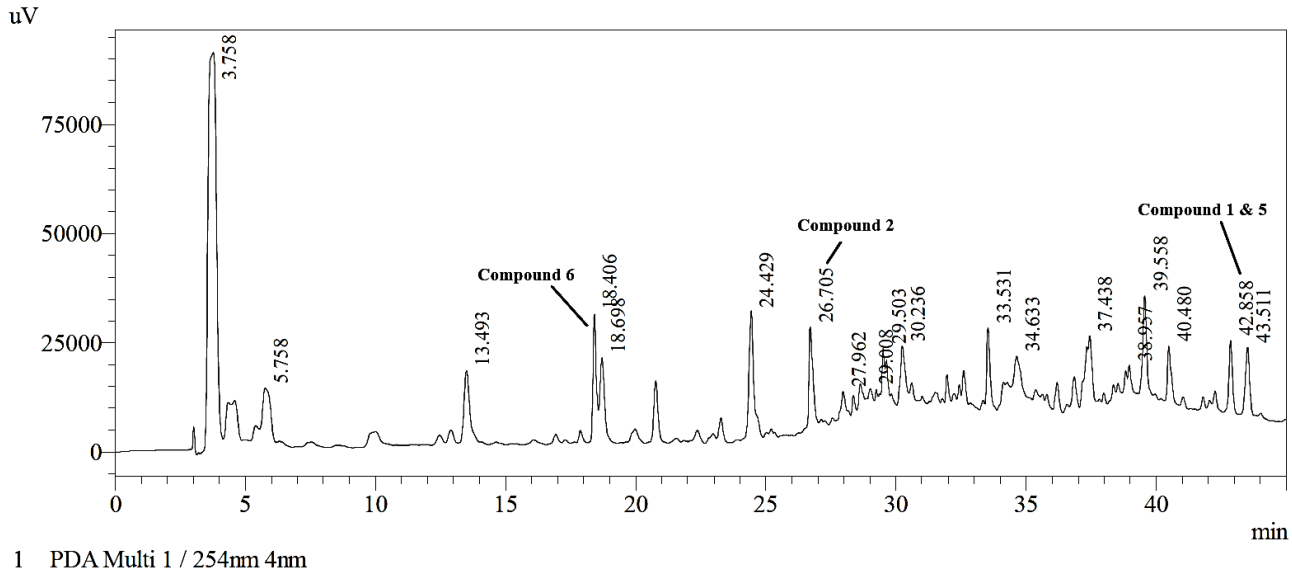

Figure S1. HPLC-PDA chromatogram of the crude methanol extract from the roots and lignotubers of *R. tridentata*.

1.4 Physical data of the isolated compounds

Morin 3-*O*-α-L-rhamnopyranoside (1) was isolated as a light yellow fine powder, mp 271-276 °C. ^{1}H NMR (400 MHz, CD_3OD) δ_H 0.92 (3H, d, 6.2, 6"-Me), 3.50 (1H, dd, 6.2, 6.03, H-5"), 4.25 (1H, m, H-2"), 3.83 (1H, dd, 9.6, 3.7, H-3"), 3.29 (1H, m, H-4"), 6.22 (1H, d, 2.0, H-8), 6.08 (1H, d, 2.0, H-6), 5.29 (1H, d, 1.7, H-1"), 6.73 (d, 8.2, H-6'), 7.13 (1H, d, 2.0, H-3'), 7.21 (dd, 8.2, 2.0, H-5') (Figure S5); ^{13}C NMR (125 MHz, CD_3OD) δ_C 178.8 (C, C-4), 162.7 (C, C-7), 159.1 (C, C-

5), 153.2 (C, C-2), 159.0 (C, C-8a), 121.9 (C, C-3'), 156.7 (C, C-4'), 135.3 (C, C-3), 121.1 (C, C-1'), 159.9 (C, C-2'), 109.2 (CH, C-6') 109.5 (CH, C-5'), 103.8 (C, C-4a), 103.4 (CH, C-1"), 102.0 (CH, C-6), 96.5 (CH, C-8), 73.4 (CH, C-4"), 72.1 (CH, C-3"), 72.0 (CH, C-2"), 72.1 (CH, C-5"), 17.7 (CH_3, 6"-Me) (Figure S6). HRESIMS (positive ionization mode) m/z 417.3710 $[M+Na]^+$ (Calcd for $C_{21}H_{20}O_{11}Na^+$, 417.3712); $[\alpha]_D$28 -22.1° (c 0.1), CH_3OH) (lit., $[\alpha]_D$ -20.8, c 0.003); λ_{max} 257 and 352 nm

***Trans*-resveratrol glucoside (2)** was isolated as a brown solid, mp 223-225 °C. ^1H NMR (500 MHz, CD_3OD): δ_H 3.42-3.73 (11H, m, H-2", H-3", H-4", and H-5"), 3.76 (1H, dd, 11.9, 2.3, H-6b"), 3.95 (1H, dd, 11.9, 2.3, H-6a"), 4.91 (2H, d, 7.5, H-1"), 6.47 (1H, m, H-4), 6.64 (1H, m, 1.70, H-6), 6.79 (2H, d, 8.7, H-3', 5'), 6.80 (1H, m, 1.7, H-2), 6.87 (1H, d, 16.3, H-7), 7.04 (1H, d, 16.3, H-8), 7.39 (2H, d, 8.7, H-2', 6') (Figure S7); ^{13}C NMR (125 MHz, CD_3OD) δ_C 62.6 (CH_2, C-6"), 71.5 (CH, C-4"), 73.9 (CH, C-2"), 78.1 (CH, C-3"), 78.3 (CH, C-5"), 102.5 (CH, C-1"), 104.1 (CH, C-4), 107.1 (CH, C-2), 108.4 (CH, C-6), 116.5 (CH, C-3',5'), 126.7 (CH, C-7), 128.9 (CH, C-2', 6'), 130.0 (CH, C-8), 131.4 (C, C-1'), 141.4 (C, C-1), 158.5 (C, C-4'), 159.6 (C, C-5), 160.5 (C, C-3) (Figure S8). LRESIMS (positive ionization mode) m/z 413.1199 $[M+Na]^+$ (Calcd for $C_{20}H_{22}O_8Na^+$, 413.1212); λ_{max} 310 nm

Asiatic (3) and arjunolic (4) acid mixture was isolated as light-yellow solid. ^1H NMR and ^{13}C NMR (Figure S9-S10) (125 MHz, CD_3OD) data is shown in Table S1 below. HRESIMS (positive ionization mode) m/z 511.3396 $[M+Na]^+$ (Calcd for $C_{30}H_{48}O_5Na^+$, 511.3399).

Quercetin 3-rhamnoside (5) was isolated as a light yellow fine powder, mp 178-182 °C. ^1H NMR (400 MHz, CD_3OD) δ_H 0.96 (3H, d, 5.9, 6"-Me), 3.44 (1H, dd, 5.9, 5.9, H-5"), 4.24 (1H, m, H-2"), 3.77 (1H, dd, 9.4, 3.4, H-3"), 3.37 (1H, m, H-4"), 6.40 (1H, d, 2.3, H-8), 6.23 (1H, d, 2.3, H-6), 5.37 (1H, d, 1.7, H-1"), 6.94 (1H, d, 8.2, H-5'), 7.33 (1H, dd, 8.2, 2.26, H-6'), 7.36 (d, 2.3, H-2') (Figure S11); ^{13}C NMR (125 MHz, CD_3OD) δ_C 179.7 (C, C-4), 166.0 (C, C-7), 163.4 (C, C-5), 159.3 (C, C-2), 158.6 (C, C-8a), 149.8 (C, C-3'), 146.4 (C, C-4'), 136.2 (C, C-3), 123.0 (C, C-1'), 122.9 (CH, C-6'), 116.97 (CH, C-2') 116.4 (CH, C-5'), 105.9 (C, C-4a), 103.6 (CH, C-1"), 99.9 (CH, C-6), 94.7 (CH, C-8), 73.3 (CH, C-4"), 72.2 (CH, C-3"), 72.0 (CH, C-2"), 71.9 (CH, C-5"), 17.7 (CH_3, 6"-Me) (Figure S12). HRESIMS (positive ionization mode) m/z 471.3709 $[M+Na]^+$ (Calcd for $C_{21}H_{20}O_{11}Na^+$, 471.3712); λ_{max} 257 and 352 nm.

Catechin (6) was isolated as a light brown solid, mp 175-177 °C. ^{1}H NMR (400 MHz, CD_3OD): δ_H 2.53 (1H, dd, 8.2, H-4β), 2.86 (1H, dd, 15.9, 5.6, H-4α), 3.99 (1H, m, H-3), 4.59 (1H, d, 7.3, H-2), 5.88 (1H, d, 2.3, H-8), 5.95 (1H, d, 2.3, H-6), 6.75 (1H, dd, 8.2, 1.9, H-6'), 6.79 (1H, d, 8.2, H-5'), 6.86 (1H, d, 1.9, H-2') (Figure S13); ^{13}C NMR (100 MHz, CD_3OD) δ_C 28.5 (CH_2, C-4), 68.8 (CH, C-3), 82.9 (CH, C-2), 95.5 (CH, C-8), 96.3 (CH, C-6), 100.8 (C, C-4a), 115.3 (CH, C-2'), 116.1 (CH, C-5'), 120.0 (CH, C-6'), 132.2 (C, C-1'), 146.2 (C, C-3'), 146.3 (C, C-4'), 156.9 (C, C-8a), 157.6 (C, C-5), 157.8 (C, C-7) (Figure S14). HRESI-MS (positive ionization mode) m/z 313.2611 $[M+Na]^+$ (Calcd for $C_{15}H_{14}O_6Na^+$, 313.2620); λ_{max} 278 nm

β-sitosterol (7) was isolated as white crystals (recrystallized from methanol), mp 139-140 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.68 (3H, br.s, Me-18), 0.83 (10H, m, Me-26, Me-27 & Me-29, H-24), 0.92 (5H, d, 6.5, H-21 & H-9), 1.00 (4H, br.s, Me-19, H-17), 1.22 (14H, m, H-1, H-12, H-14, H-15, H-16, H-20, H-22, H-23 & H-28), 1.57 (9H, m, H-8, H-11, H-15b, H-22b, H-25), 1.84 (3H, m, H-1b, H-2 & H-16b), 1.99 (2H, m, H-7 & H-12b), 2.22 (2H, m, H-4), 3.51 (1H, m, H-3), 5.35 (1H, d, 4.9, H-6) (Figure S15); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 11.7 (CH_3, C-18), 11.9 (CH_3, C-29), 18.8 (CH_3, C-21), 19.0 (CH_3, C-19), 19.4 (CH_3, C-27), 19.8 (CH_3, C-26), 21.1 (CH_2, C-11), 23.1 (CH_2, C-28), 24.3 (CH_2, C-15), 26.1 (CH_2, C-23), 28.3 (CH_2, C-16) , 29.2 (CH, C-25), 31.6 (CH_2, C-2), 31.7 (CH, C-8), 31.9 (CH_2, C-7), 33.9 (CH_2, C-22), 36.2 (CH, C-20), 36.5 (C, C-10), 37.3 (CH_2, C-1), 39.8 (CH_2, C-12), 42.33 (CH_2, C-4), 42.34 (C, C-13), 45.9 (CH, C-24), 50.2 (CH, C-9), 55.9 (CH, C-17), 56.8 (CH, C-14), 71.8 (CH, C-3), 121.7 (CH, C-6), 140.8 (C, C-5) (Figure S16). LRESIMS (negative ionization mode, m/z 413.2742 $[M-H]^-$.

Linoleic acid (8) was isolated as off-white sticky solid, ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.89 (3H, t, 6.8, H-18), 1.29 (14H, m, H-4, H-5, H-6, H-7, H-15, H-16, H-17), 1.63 (3H, m, H-3), 2.04 (4H, m, H-8, H-14), 2.35 (3H, t, 7.4, H-2), 2.78 (2H, m, H-11), 5.35 (4H, m, H-9, H-10, H-12, H-13) (Figure S17); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 179.9 (C, C-1), 130.2 (CH, C-13), 130.0 (CH, C-9), 128.1 (CH, C-10), 127.9 (CH, C-12), 34.0 (CH_2, C-2), 31.5 (CH_2, C-16), 29.6 (CH_2, C-7), 29.3 (CH_2, C-15), 29.1 (CH_2, C-5), 29.1 (CH_2, C-6), 29.0 (CH_2, C-4), 27.2 (CH_2, C-8), 27.2 (CH_2, C-14), 25.6 (CH_2, C-11), 24.7 (CH_2, C-3), 22.6 (CH_2, C-17), 14.0 (CH_3, C-18) (Figure S18). LRESIMS (negative ionization mode, m/z 281.2530 $[M-H]^-$.

Table S1. NMR spectroscopic data (400 MHz, CD_3OD) for asiatic (**3**) and arjunolic acids (**4**).

	Asiatic acid (3)		**Arjunolic acid (4)**	
Position	**δC**	**δH (J in Hz)**	**δC**	**δH (*J* in Hz)**
1a	47.3	1.68, m	47.3	1.68, m
1b		1.68, m		1.68, m
2	69.7	3.72, m	69.7	3.72, m
3	78.3	3.38, dd (9.6, 1.7)	78.3	3.38, dd (9.6, 1.7)
4	44.1		44.1	
5	47.8	3.32	47.8	3.32
6a	19	1.44, m	19	1.44, m
6b		1.44, m		1.44, m
7a	33.7	1.54, m	33.6	1.54, m
7b		1.75, m		1.75, m
8	40.8		40.4	
9	48	1.29, m	48	1.29, m
10	38.9		38.9	
11	23.9	1.11, m	24.1	1.96
12	126.7	5.26, m	126.7	5.26, m
13	138.6		138.6	
14	43		43	
15a & 15b	29.2	1.09, m & 1.96, m	29.2	1.09, m & 1.96, m
16a & 16b	25.3	1.64, m & 1.64, m	25.3	1.64, m & 1.64, m
17	48		48	
18	54.4	2.22, d (11.0)	42.8	2.87, dd (14.3, 3.7)
19a & 19b	40.4	0.98, m	47.9	0.92, m & 1.96, m
20	40.4	0.98, m	31.8	
21a	31.6	1.34	31.6	1.34
21b		1.51		1.51
22a	38.1	1.67, m	38.1	1.67, m
22b		1.34, m		1.34, m
23	66.4	3.52, d (11.1); 3.29, d (11.1)	66.4	3.52, d (11.1); 3.29, d (11.1)
24	13.9	0.72, s	13.8	0.72, s
25	17.8	1.07	17.5	1.06
26	17.9	0.83	17.7	0.83
27	24	1.15, s	26.5	1.20, s
28	181.7		181.7	
29	17.6	0.92, d (6.4)	33.4	0.93, s
30	23.7	0.96, m	23.8	0.96, s

1.5 Assay procedure for uterotonic activity

Non-pregnant female Sprague-Dawley rats (200-250 g) were bred in the Biomedical Research Unit of the University of KwaZulu-Natal, Westville campus (Dube 2014). The oestrous state in the rats was induced by a single intraperitoneal injection of stilboestrol (12 mg/kg, i.p.) one day before the experiments to ensure regular uterine muscle contractility. The animals were sacrificed by decapitation, and the uterine muscle strips of approximately 2-3 cm long were removed and placed in De Jalon's solution at room temperature. One rat was sacrificed for each crude extract or pure compound, and the uterine muscle strip from each animal was divided into six portions of similar length, which were used for the six different concentrations prepared and tested for each extract or compound. De Jalon's solution was continuously aerated with 5% carbon dioxide in oxygen. Each uterine muscle strip was subjected to an applied resting tension of 1.0 g and allowed to equilibrate for 45-60 min. The tissue was incubated in a fresh De Jalon's solution for 10 min and contractions were recorded (negative control). The tissue was allowed to stabilise for another 10 min before the addition of each drug (oxytocin, crude extracts, or pure compounds).

Oxytocin (1.00-6.00 mg/mL) was used as a positive control. Graded concentration of the crude extracts (0.13-62.08 mg/mL), and isolated compounds (0.01-57.10 μg/mL) were each administered. The tissue was allowed to stabilise for another 10 min in the drug before recording contractions. After each treatment, the tissue was washed three times with De Jalon's solution and allowed to stabilise for 10 min before the next drug challenge. The assay was repeated three times for each dose (rinsing well before each test), and fresh uterine strips were used for each dose range. Contraction amplitude and frequency were recorded using LabChart of a Powerlab system (AD Instruments, Bella Vista, Australia). Data obtained was presented as a percentage change in contraction with reference to the non-treated control.

1.6 Statistical analysis

All data were expressed as mean ± SEM (n= 6 for each sample, where n refers to the number of experiments/sample). Statistical analysis was performed on Graph Pad Prism (version 5.00, Graph Pad Software, Inc., San Diego, California, USA) using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. A value of $P \leq 0.05$ was considered statistically significant. The uterine responses in the presence of extracts and pure compounds were analysed by non-linear regression analysis using the variable Hill slope and EC_{50}. The area under the curve was calculated from Graph Pad Prism using the negative control as a baseline.

Table S2. Effects of the crude extracts and secondary metabolites from *R. tridentata* on the mean force and frequency of uterine contractions.

Extract or drug	Extract or drug concentration	*% Mean force ± SEM*	*% Mean frequency ± SEM*
methanol-water extract	31.0 mg/mL	85.00% ± 0.081	23.00% ± 2.618
Acetone fraction	2.13 mg/mL	74.30% ± 0.535	33.20% ± 5.766
Methanol fraction	5.00 mg/mL	57.00% ± 2.260	29.70% ± 4.331
Asiatic-arjunolic acid	6.14 µg/mL	13.42% ± 0.084	12.04% ± 3.128
β-sitosterol	57.1 µg/mL	42.00% ± 0.145	19.00% ± 4.164
Oxytocin	6.00 mg/mL	81.30% ± 0.231	64.10% ± 7.823

n= 6 observations, SEM= standard error of the mean.

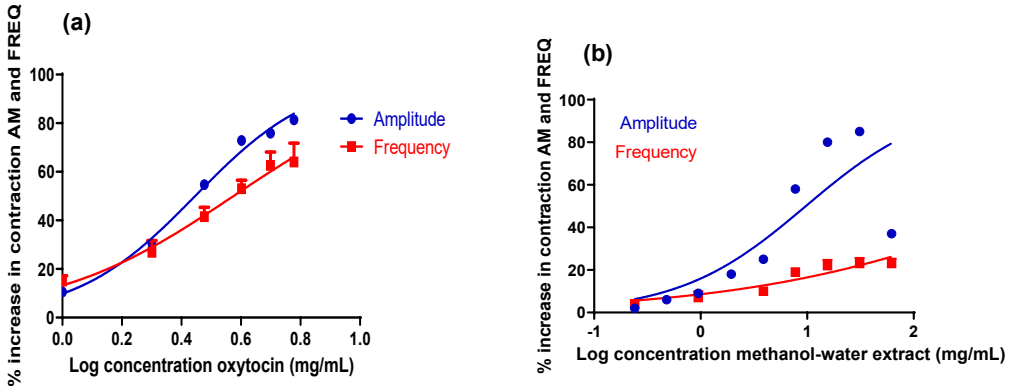

Figure S2. Rat uterine muscle response after addition of (a) oxytocin and (b) methanol-water extract of *R. tridentata*.

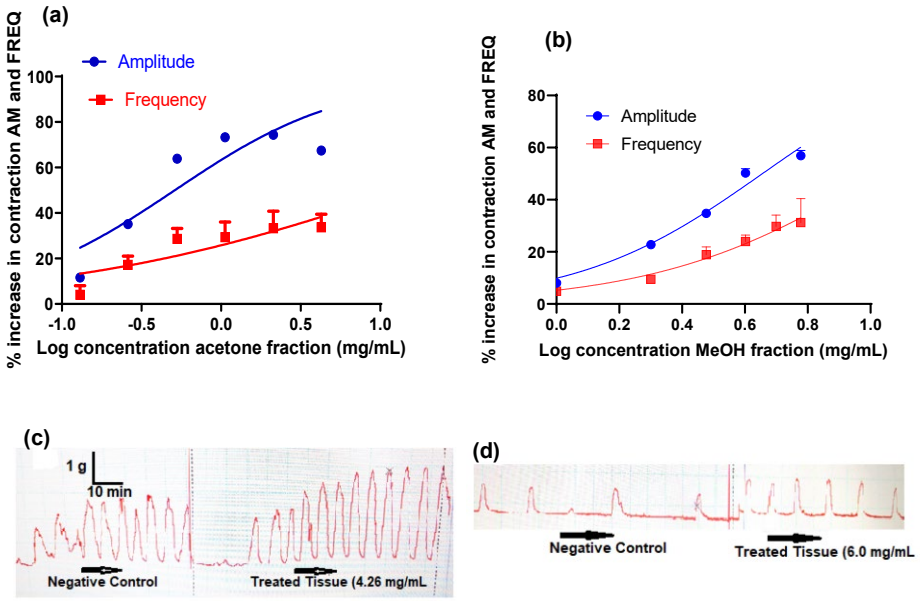

Figure S3. Rat uterine muscle response when subjected to (a) acetone fraction; (b) methanol fraction from the roots and lignotubers of *R. tridentata*. (c) Representative tracing of the uterine response after the addition of F2 (4.26 mg); (d) Response after the addition of F3 (6.00 mg).

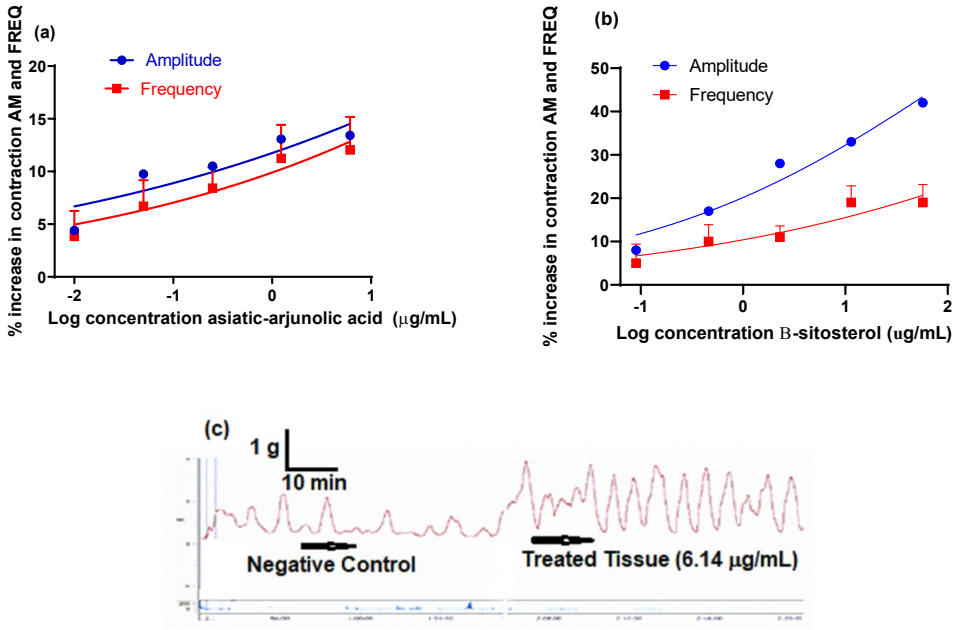

Figure S4. Rat uterine muscle response after addition of (a) a mixture of asiatic acid (**3**) and arjunolic acid (**4**); and (b) β-sitosterol (**7**). (c) Representative tracing of the uterine response to cumulative addition of a mixture of **3** and **4**.

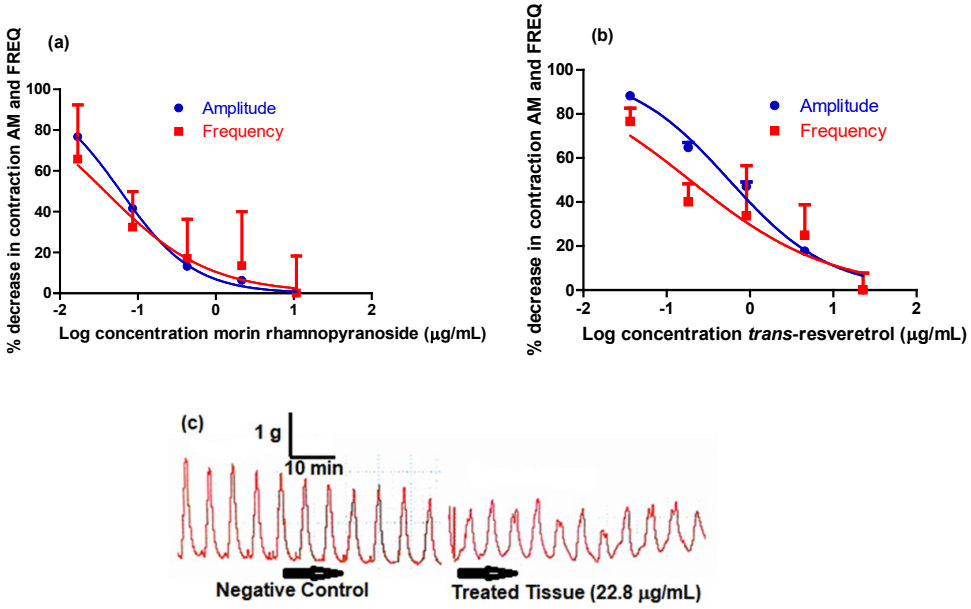

Figure S5. Rat uterine muscle response after addition of (a) morin 3-*O*-α-L-rhamnopyranoside (**1**), (b) *trans*-resveratrol glucoside (**2**). (c) Representative tracing of the uterine response to cumulative addition of **2**.

2. NMR SPECTRA

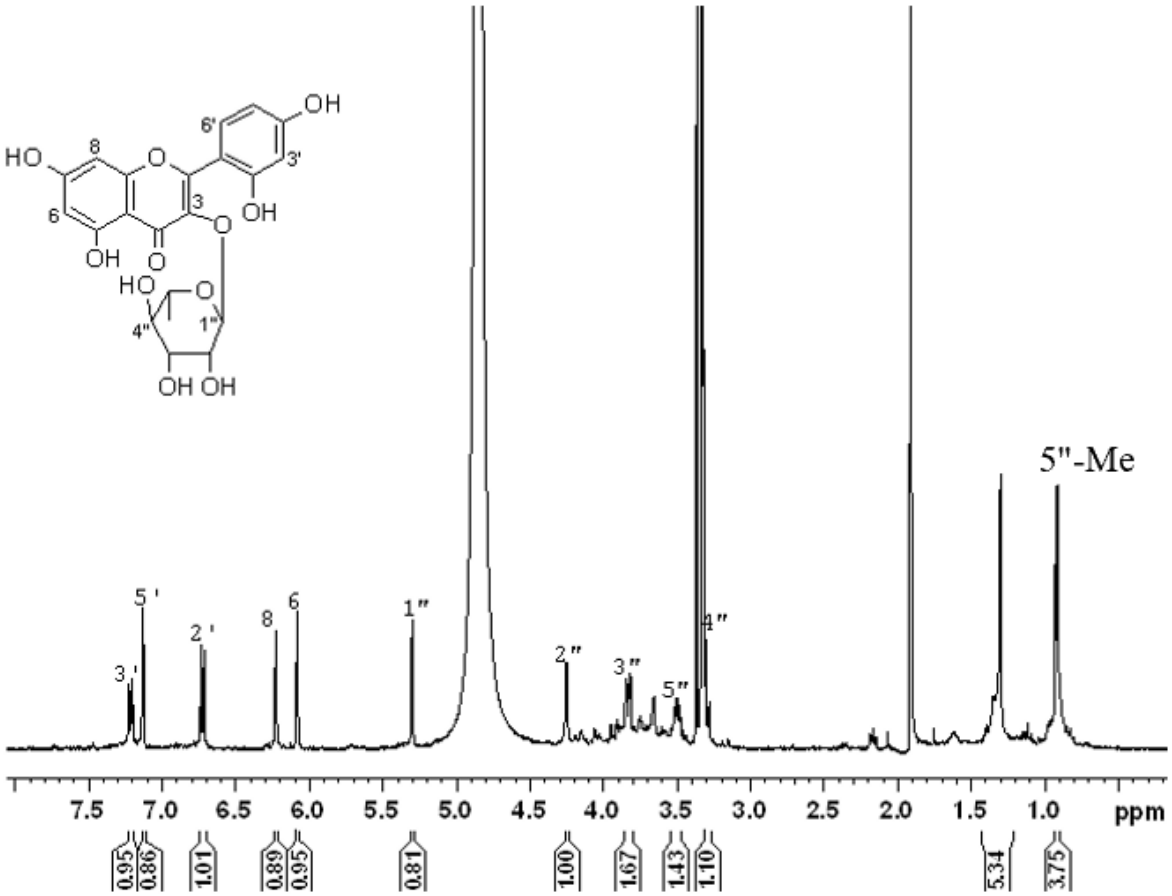

Figure S6: ^{1}H NMR spectrum of morin 3-*O*-α-L-rhamnopyranoside (**1**) in CD_3OD

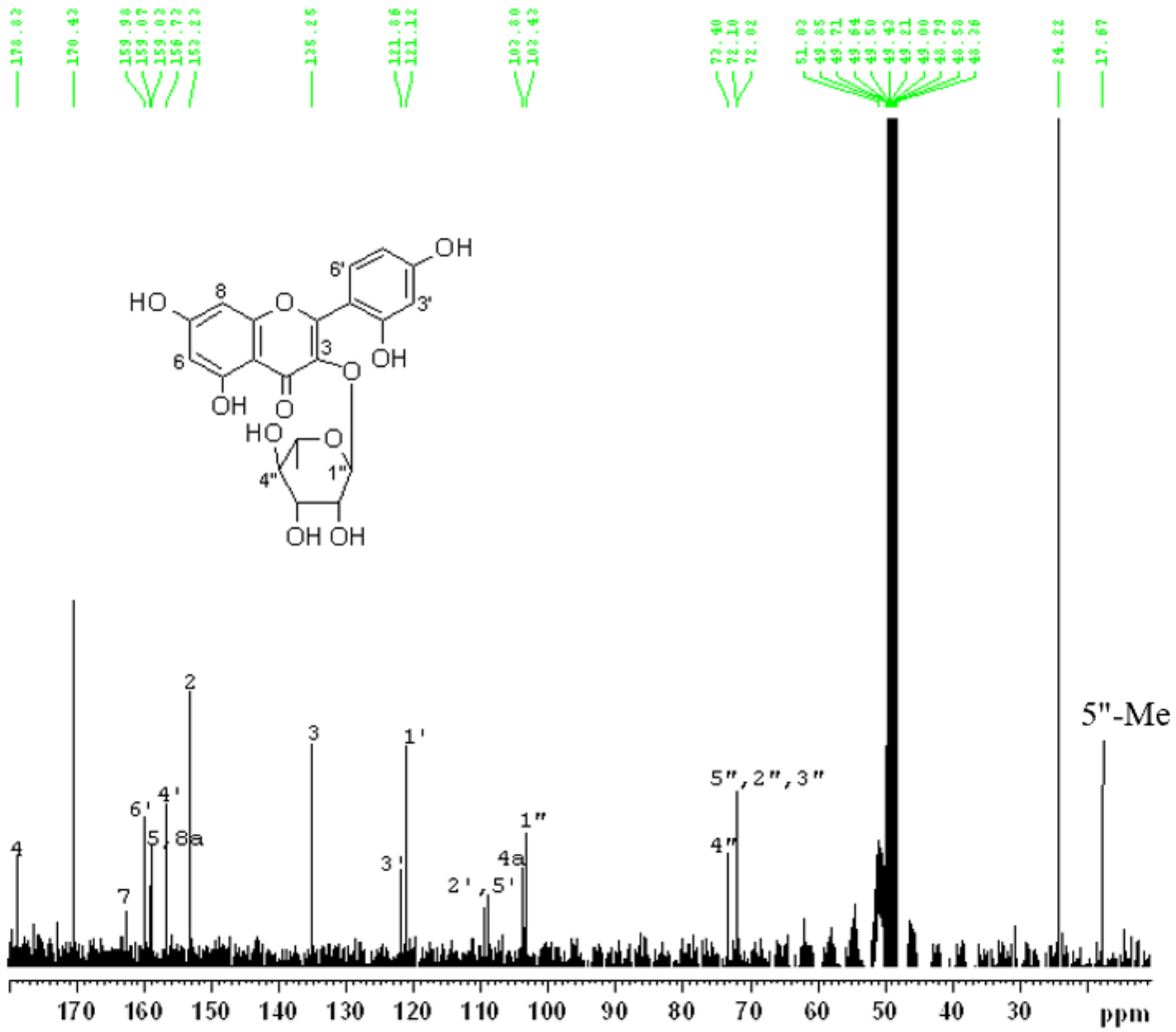

Figure S7: ^{13}C NMR spectrum of morin 3-*O*-α-L-rhamnopyranoside (**1**) in CD_3OD

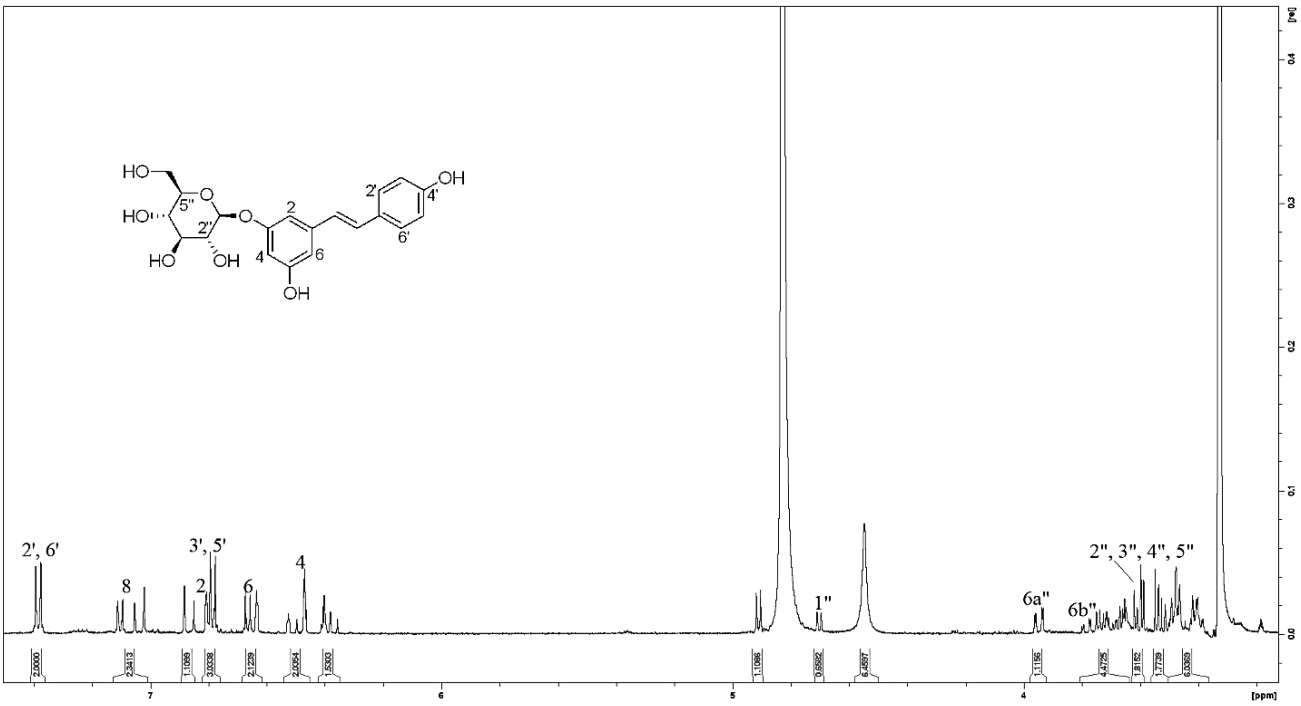

Figure S8: ^{1}H NMR spectrum of *trans*-resveratrol glucoside (**2**) in CD_3OD

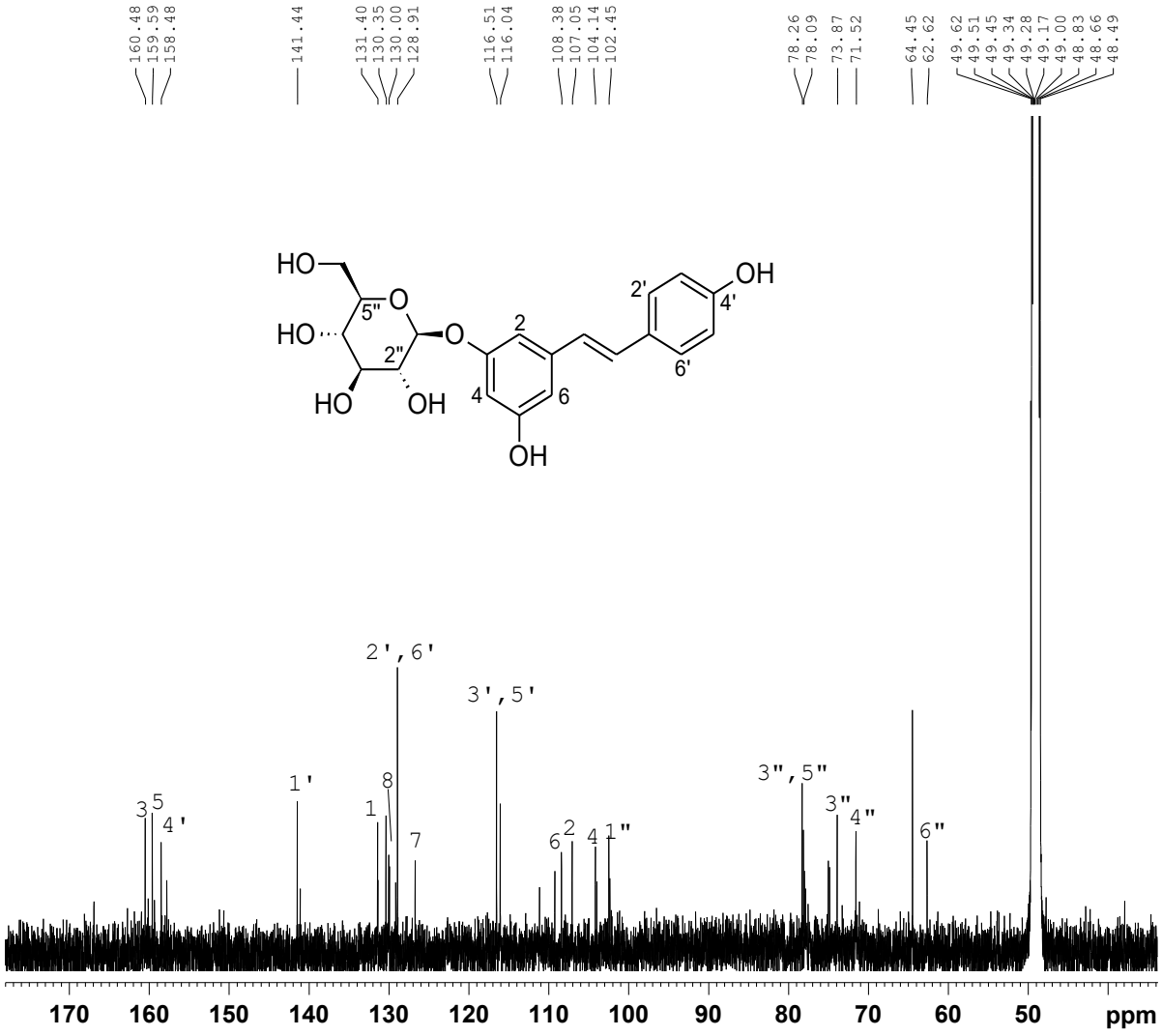

Figure S9: ^{13}C NMR spectrum of *trans*-resveratrol glucoside (**2**) in CD_3OD

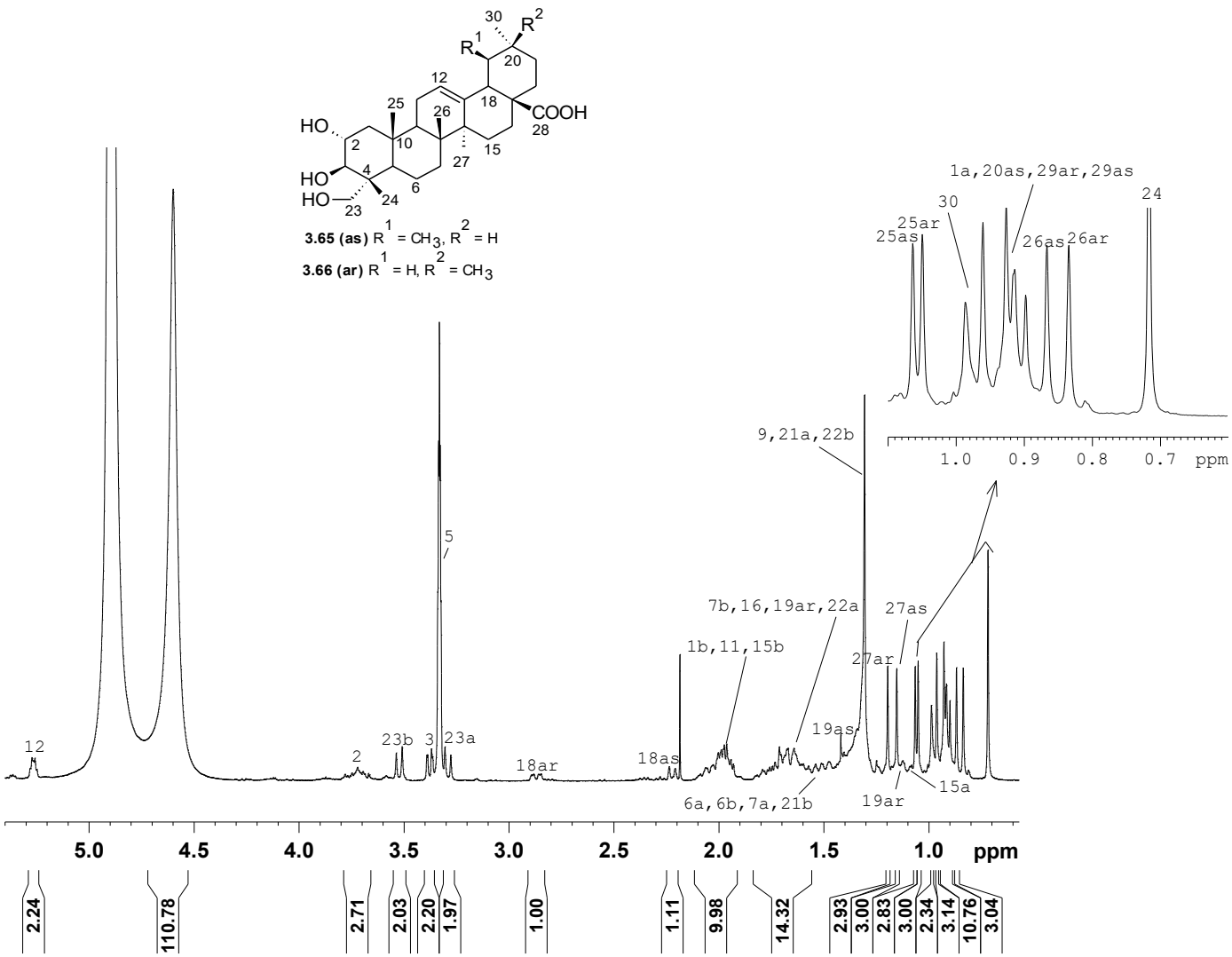

Figure S10: ^{1}H NMR spectrum of asiatic acid-arjunolic acid mixture (**3** & **4**) in CD_3OD

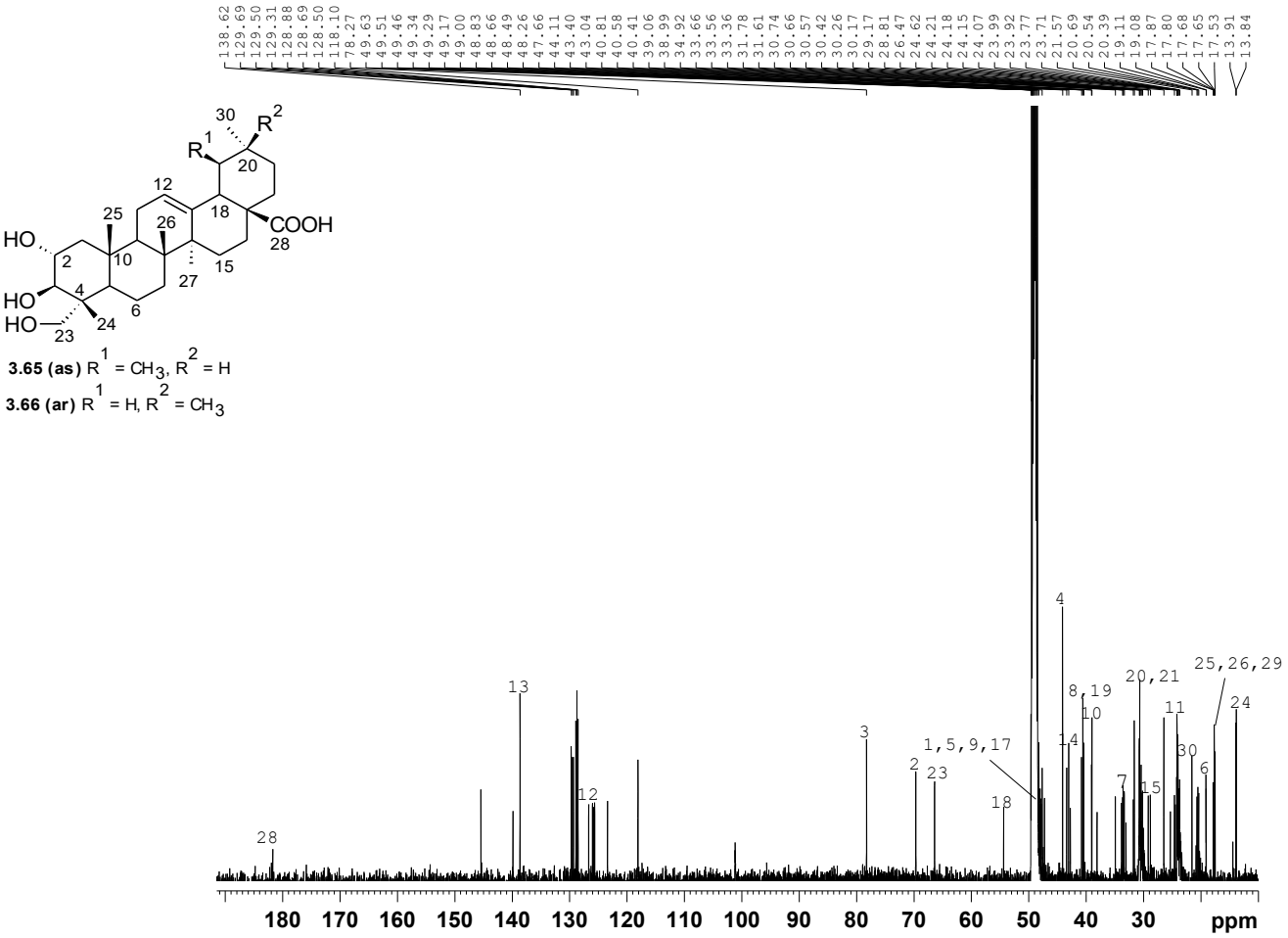

Figure S11: ^{13}C NMR spectrum of asiatic acid-arjunolic acid (**3** & **4**) mixture in CD_3OD

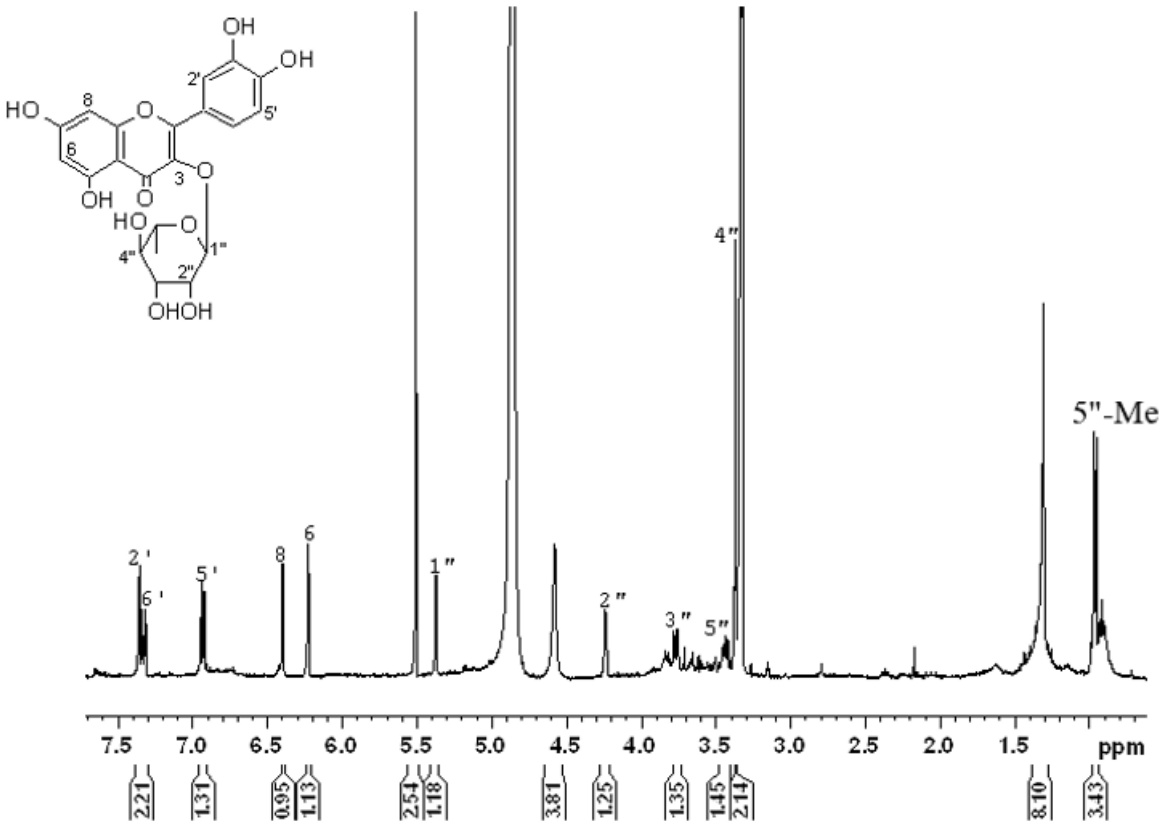

Figure S12: ^{1}H NMR spectrum of quercetin 3-rhamnoside (**5**) in CD_3OD

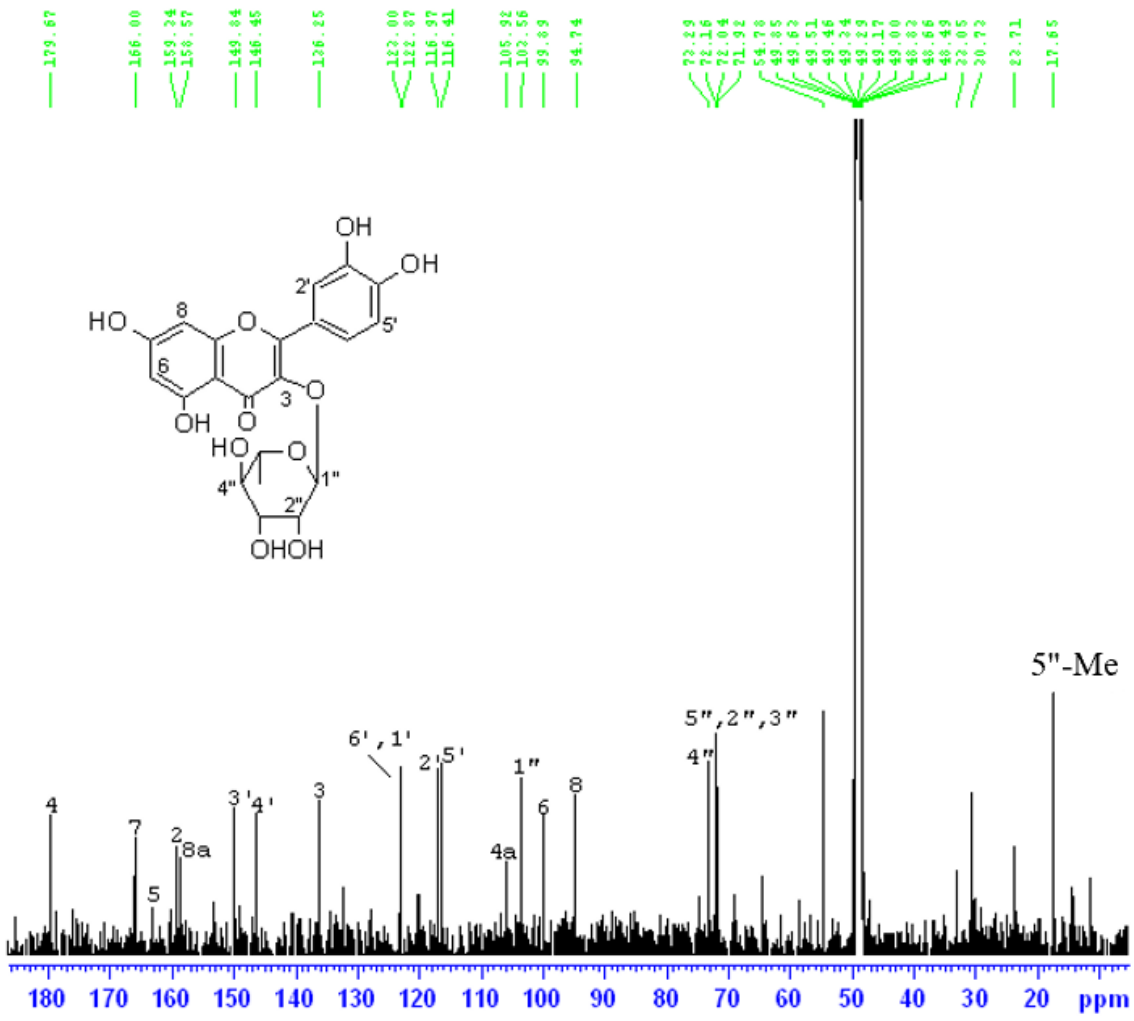

Figure S13: ^{13}C NMR spectrum of quercetin 3-rhamnoside (**5**) in CD_3OD

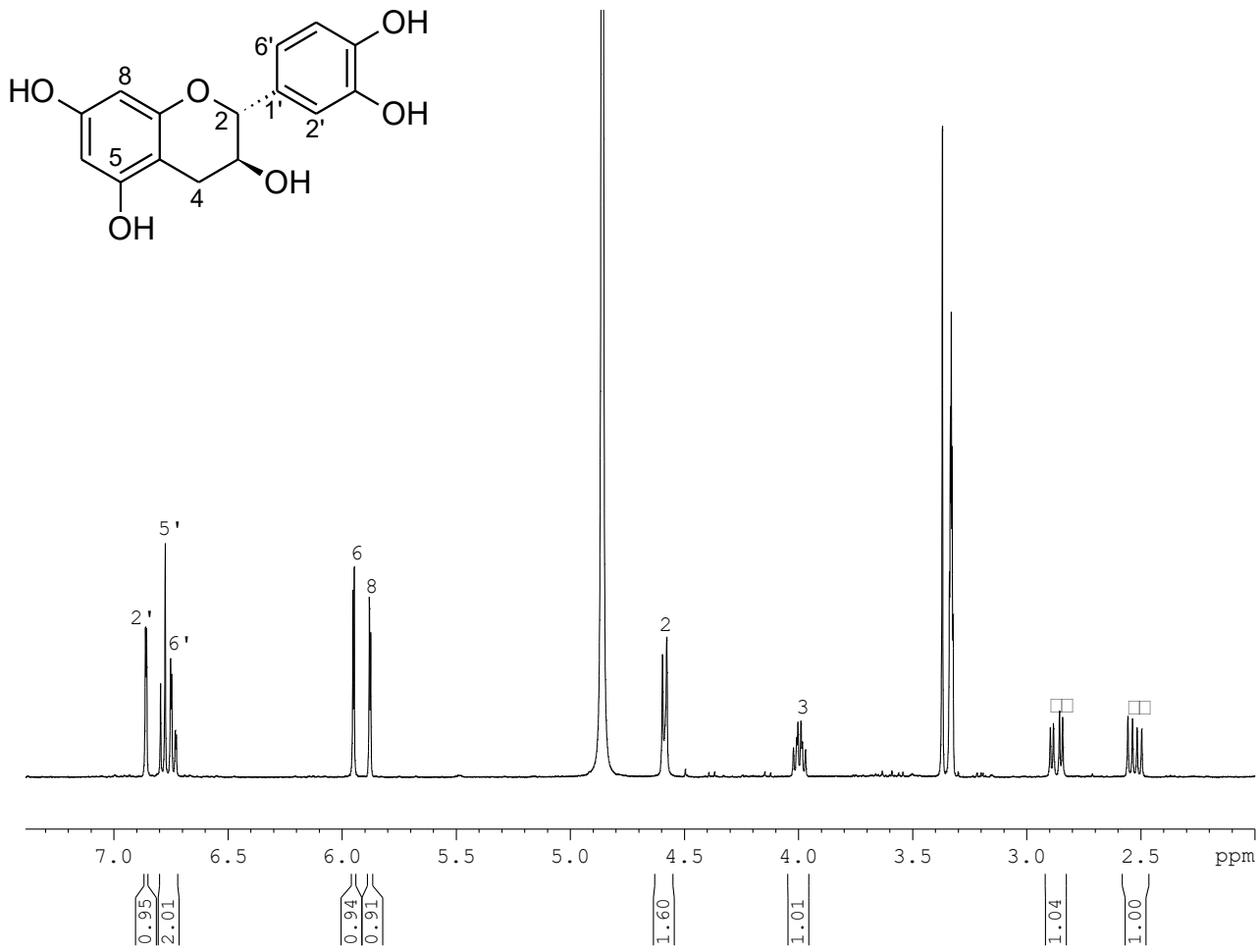

Figure S14: ^{1}H NMR spectrum of catechin (**6**) in CD_3OD

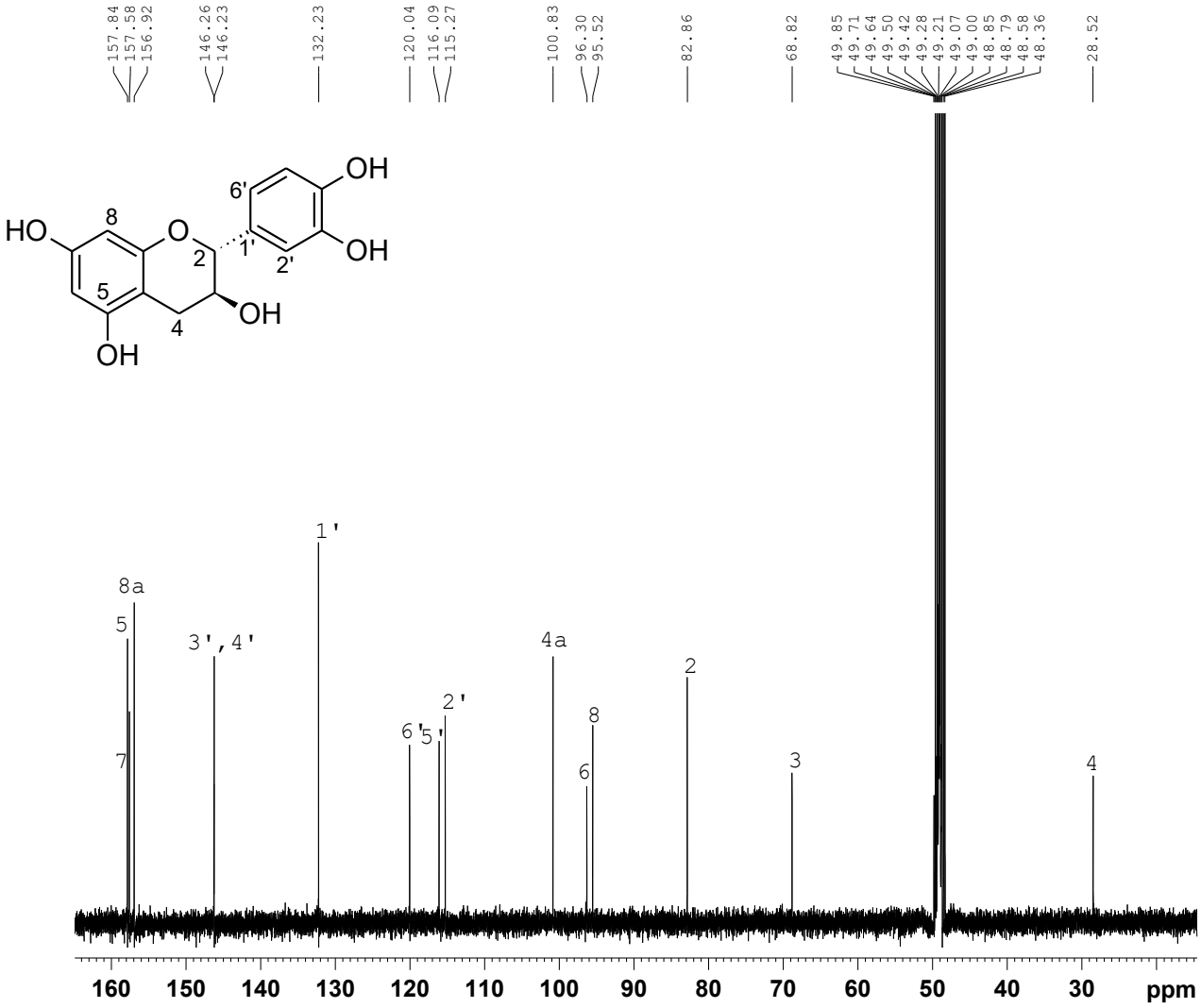

Figure S15: ^{13}C NMR spectrum of catechin (**6**) in CD_3OD

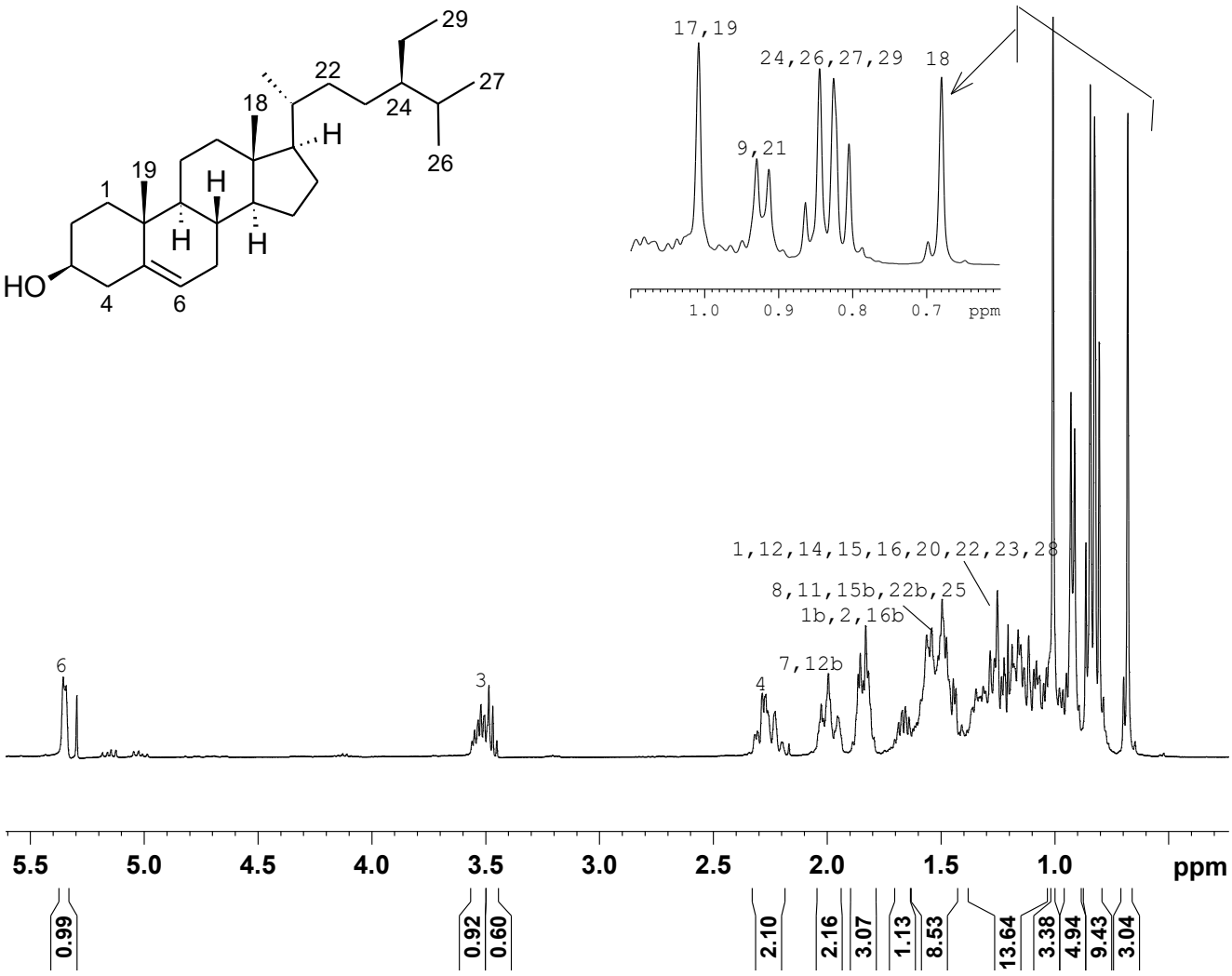

Figure S16: ^{1}H NMR spectrum of β-sitosterol (**7**) in $CDCl_3$

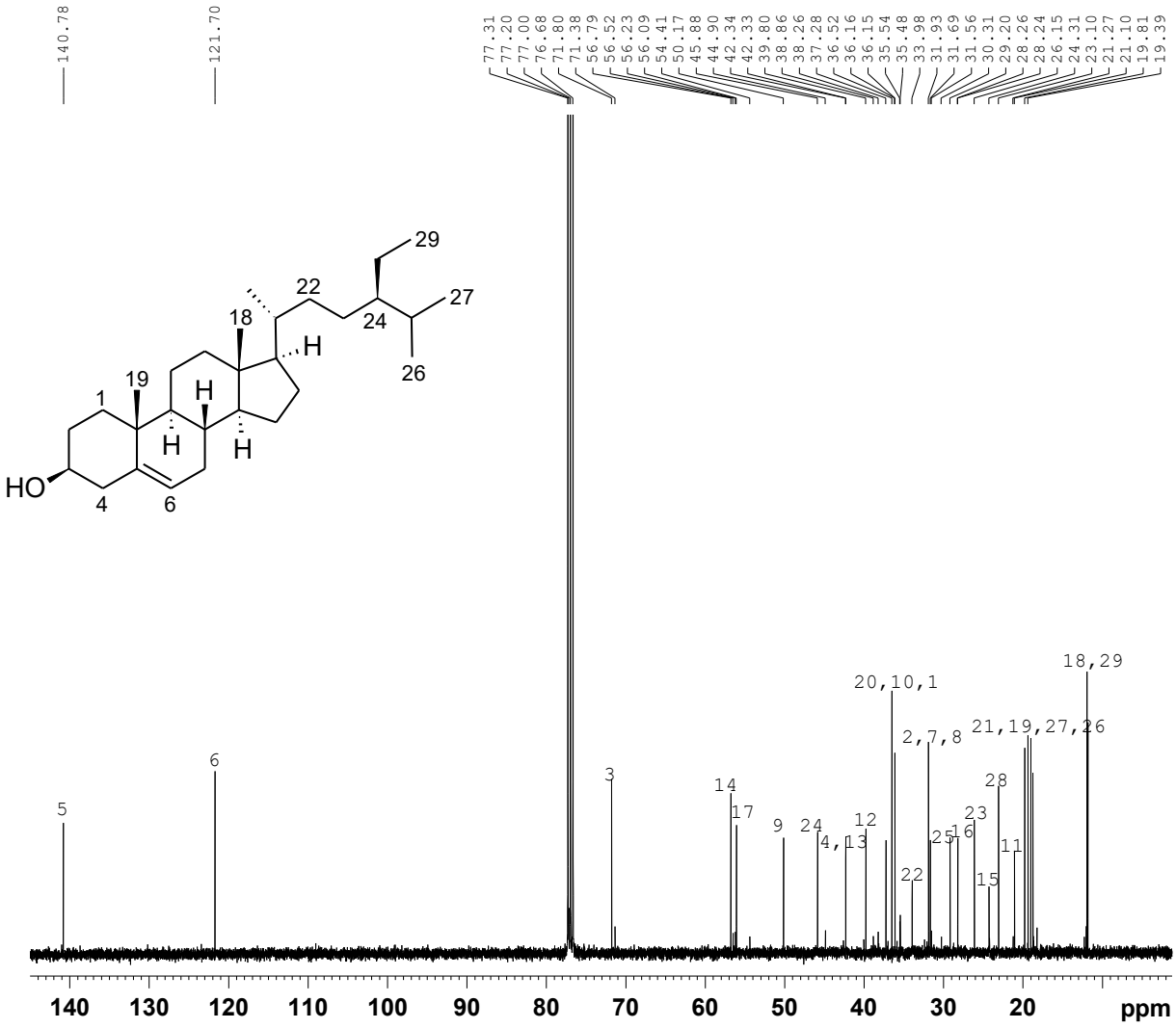

Figure S17: ^{13}C NMR spectrum of β-sitosterol (**7**) in $CDCl_3$

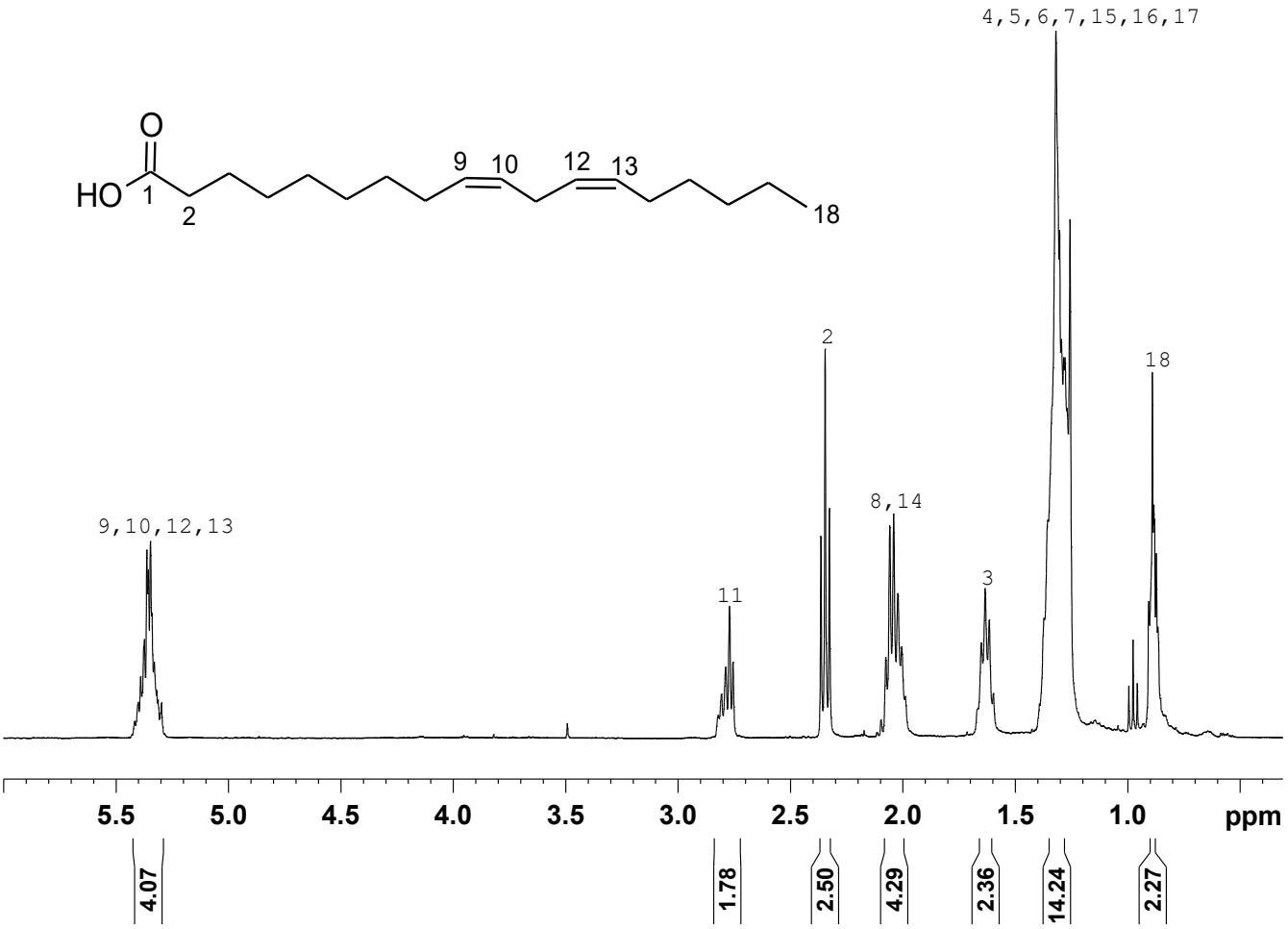

Figure S18: ^{1}H NMR spectrum of linoleic acid (**8**) in $CDCl_3$

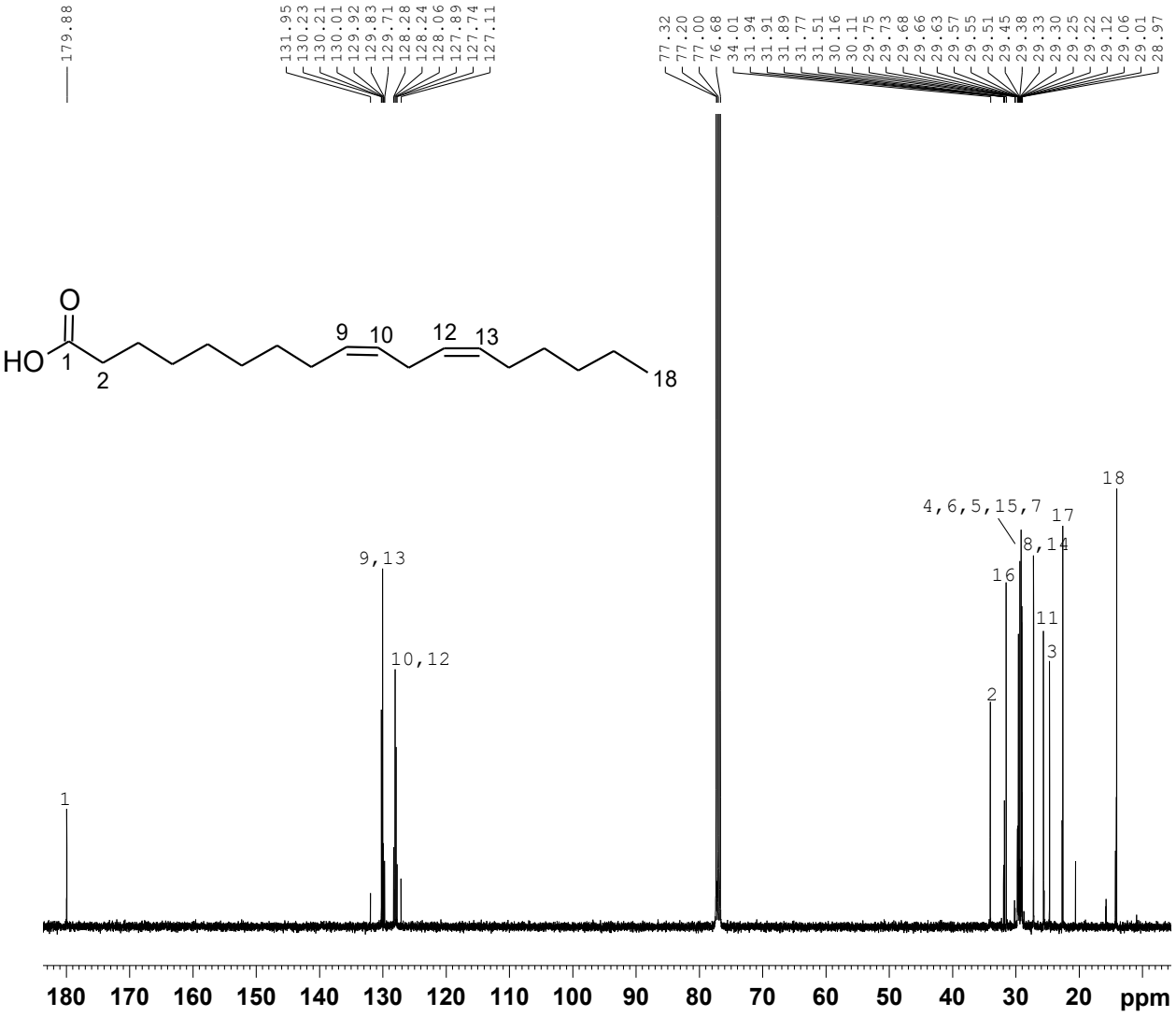

Figure S19: ^{13}C NMR spectrum of linoleic acid (**8**) in $CDCl_3$

Dube SC. 2014. Contractile effects of Gunnera perpensa and *Rhoicissus tridentata* bioactive extracts in isolated rat uterine muscles [Master's Thesis). Durban: University of KwaZulu-Natal.