

Supplementary material for:

Piper tectoniifolium Kunth: a new natural source of the neolignan (-)-grandisin with potential vascular effects

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Supplementary material:

Quantification of (-)-grandisin in different vegetative parts and extracts of *Piper tectoniifolium*.

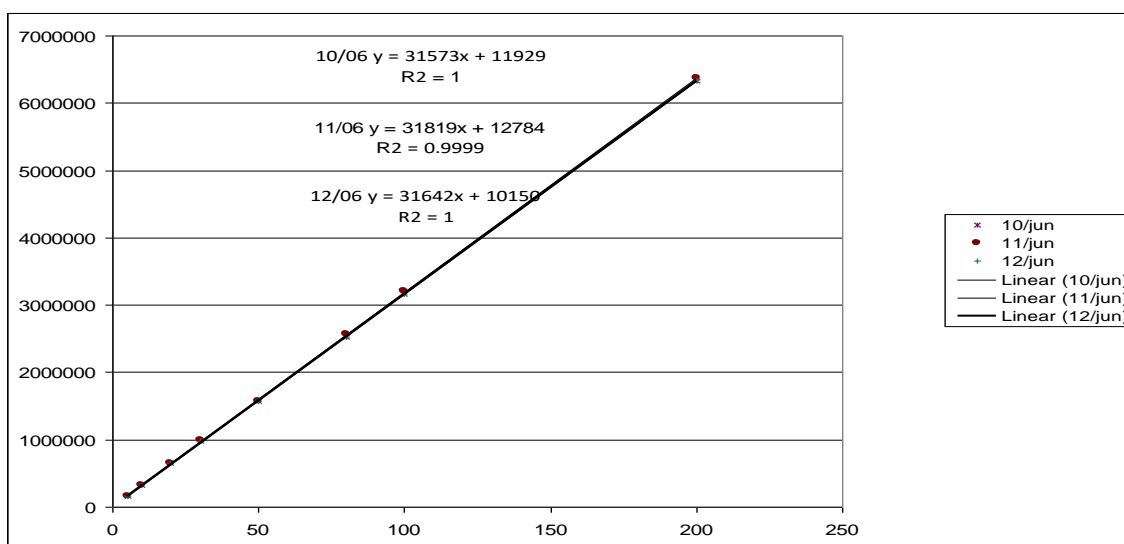


Figure S1 - Analytical curves obtained in three different days in the range of 5 to 200 µg/ mL.

Table S1 - Intra-day and inter-day precision. Values of absorbance in mAU.

Intra-day*	AVR	Intra-day*	AVR	Inter-day*	AVR	Inter-day*	AVR
15 µg/mL		150 µg/mL		15 µg/mL		150 µg/mL	
	490913		4943103		492539		4943103
SD	2020	SD	20698	SD	2808	SD	20698
RSD %	0.41	RSD %	0.42	RSD %	0.57	RSD%	0.42

* six repetition of the same concentration in the same day (intra-day) or in different days (inter-days); AVR = average; SD = standard deviation; RSD % = relative standard deviation.

Table S2 - Accuracy test in the range 5 to 200 µg/mL of standard solution of (-)-grandisin.

Nominal [] µg/mL	Abs 1	Abs 2	Abs 3	Abs Average	[] calc	Δµg	Δ% µg	Δ%
200	6331978	6371797	6347893	6350556	200.11	0.11	0.05	100.05
100	3167927	3204472	3166183	3179527	100.00	0.00	0.00	100.00
80	2528831	2570371	2524594	2541266	79.85	-0.15	-0.18	99.82
50	1568422	1569367	1580622	1572804	49.28	-0.72	-1.43	98.57
30	983332	990435	984319	986028.9	30.76	0.76	2.53	102.53
20	652740	653226	649271	651745.4	20.21	0.21	1.04	101.04
10	327377	327542	326729	327216	9.96	-0.04	-0.37	99.63
5	163593.3	165258	164138	164329.7	4.82	-0.18	-3.59	96.41

[] = concentration; [] calc = concentration of (-)-grandisin calculated from de analytical curve;
Abs = absorbance in mAU; D = difference.

Table S3 - Robustness parameters.

	Nominal		Variation		number
ACN in Mobile Phase %	65	A	66	a	1
Flow Rate	1	B	0.9	b	2
pH	3.0	C	3.5	c	3
Temperature Oven	50	D	45	d	4
Acid Type	acetic	E	phosphoric	e	5

ACN = acetonitrile (HPLC grade, Tedia, Brazil); acetic and phosphoric acids from Sigma-Aldrich (Brazil).

Table S4 – Robustness experimental tests for 15 and 150 µg/mL.

Parameters	0	1	2	3	4	5
ACN in Mobile Phase %	A	a	A	A	A	A
Flow Rate	B	B	b	B	B	B
pH	C	C	C	c	C	C
Temperatura oven	D	D	D	D	d	D
Acid type	E	E	E	E	E	e
150 µg/mL	4958537	4968870	5151483	5234026	5063866	5208143
	4977901	4990776	5137569	5283034	5058509	5203008
	4977078	4983942	5149535	5044924	5016598	5168147
Average	4971172	4981196	5146196	5187328	5046324	5193099
SD	10950	11208	7534	125736	25883	21761
RSD%	0.22	0.23	0.15	2.42	0.51	0.42
15 µg/mL	501268	499852	502577	502890	496497	509927

	501032	492796	504001	516983	491126	500105
	493051	493813	504557	505974	493126	500957
Average	498450	495487	503712	508616	493583	503663
SD	4677	3814	1021	7409	2715	5441
RSD%	0.94	0.77	0.20	1.46	0.55	1.08

ACN = acetonitrile (HPLC grade, Tedia, Brazil); acetic and phosphoric acids from Sigma-Aldrich (Brasil). 0 = nominal condition (acetonitrile 65%; flow rate 1 mL⁻¹; pH = 3.0; temperature oven 50 °C; acid type = acetic); 1 = (**acetonitrile 66%**; flow rate 1 mL⁻¹; pH = 3.0; temperature oven 50 °C; acid type = acetic); 2 = (acetonitrile 65%; **flow rate 0.9 mL⁻¹**; pH = 3.0; temperature oven 50 °C; acid type = acetic); 3 = (acetonitrile 65%; flow rate 1 mL⁻¹; **pH = 3.5**; temperature oven 45 °C; acid type = acetic); 4 = (acetonitrile 65%; flow rate 1 mL⁻¹; pH = 3.0; **temperature oven 45 °C**; acid type = acetic); 5 = (acetonitrile 65%; flow rate 1 mL⁻¹; pH = 3.0; temperature oven 50 °C; **acid type = phosphoric**).

Table S5 – Robustness experimental tests for 15 and 150 µg/mL.

Sample	Average	SD	100%	SD	Recovery %
Methanol leaf extract (10 mg/mL) + grandisin (60 µg/mL)	4209300	3788	4210260	2105	99.98
Methanol leaf extract (10 mg/mL) + grandisin (30 µg/mL)	3239765	5831	3241688	3890	99.94

Supplementary material:

Chemical characterization of (-)-grandisin isolated from *Piper tectoniifolium*.

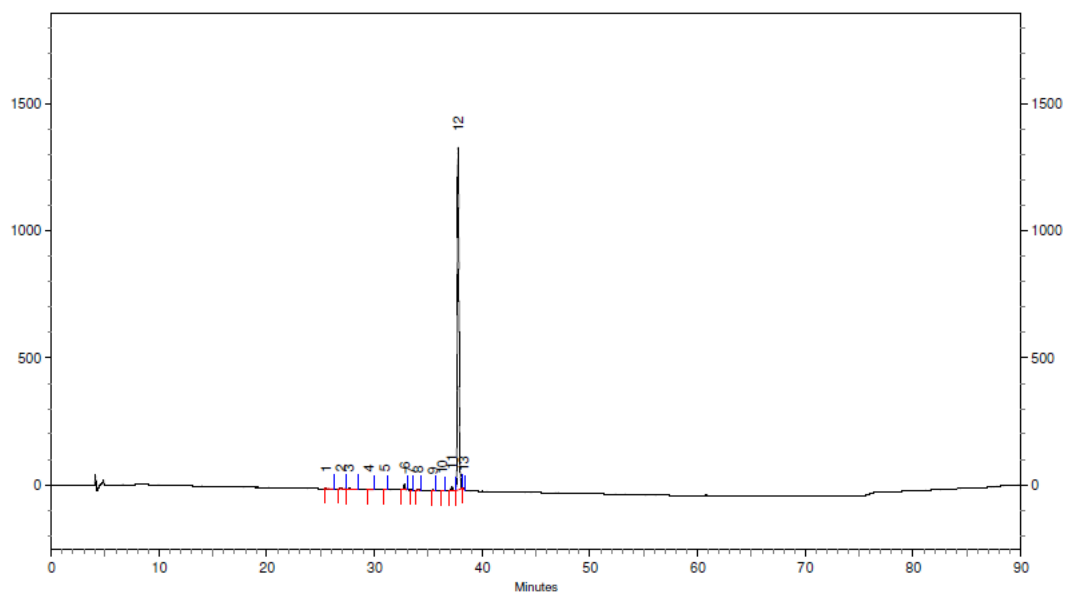


Figure S2 - HPLC chromatogram for the sample grandisin obtained from the dichloromethane extract of *P. tectoniifolium* leaves.

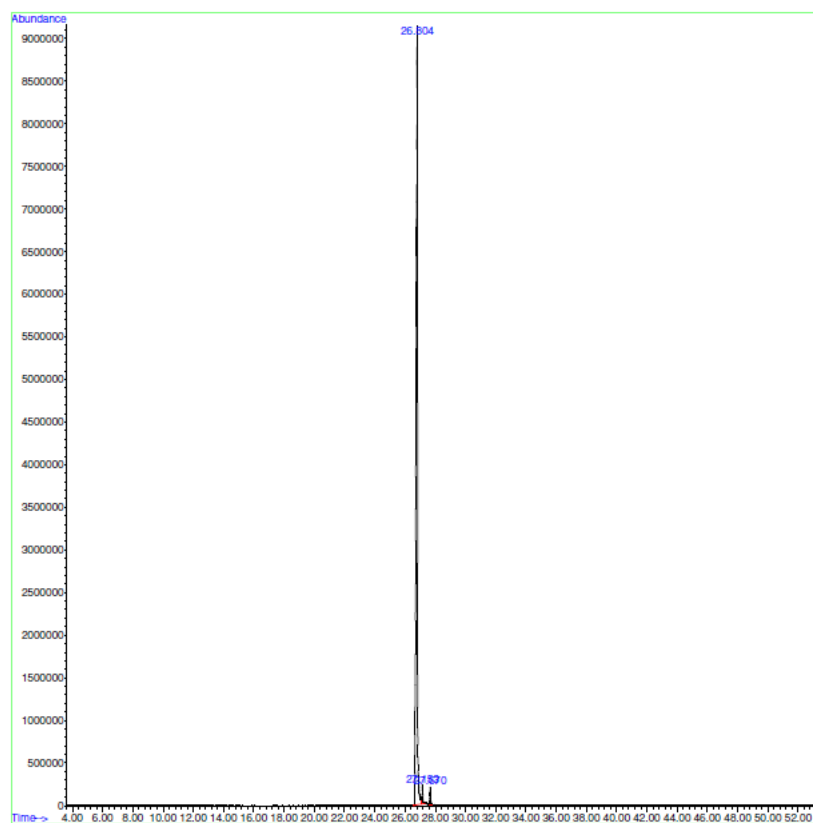


Figure S3 - GC-MS chromatogram for the sample grandisin, obtained from *P. tectoniifolium* leaves extract.

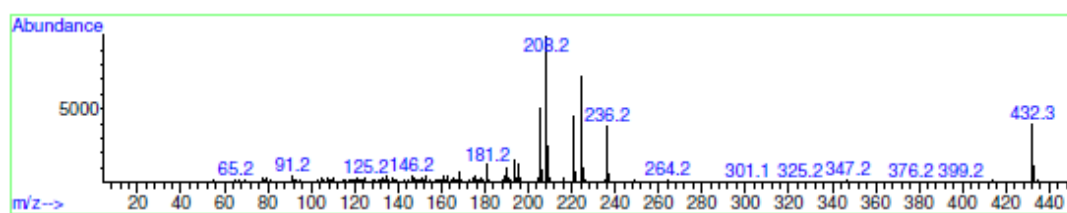


Figure S4 - Mass profile of the grandisin.

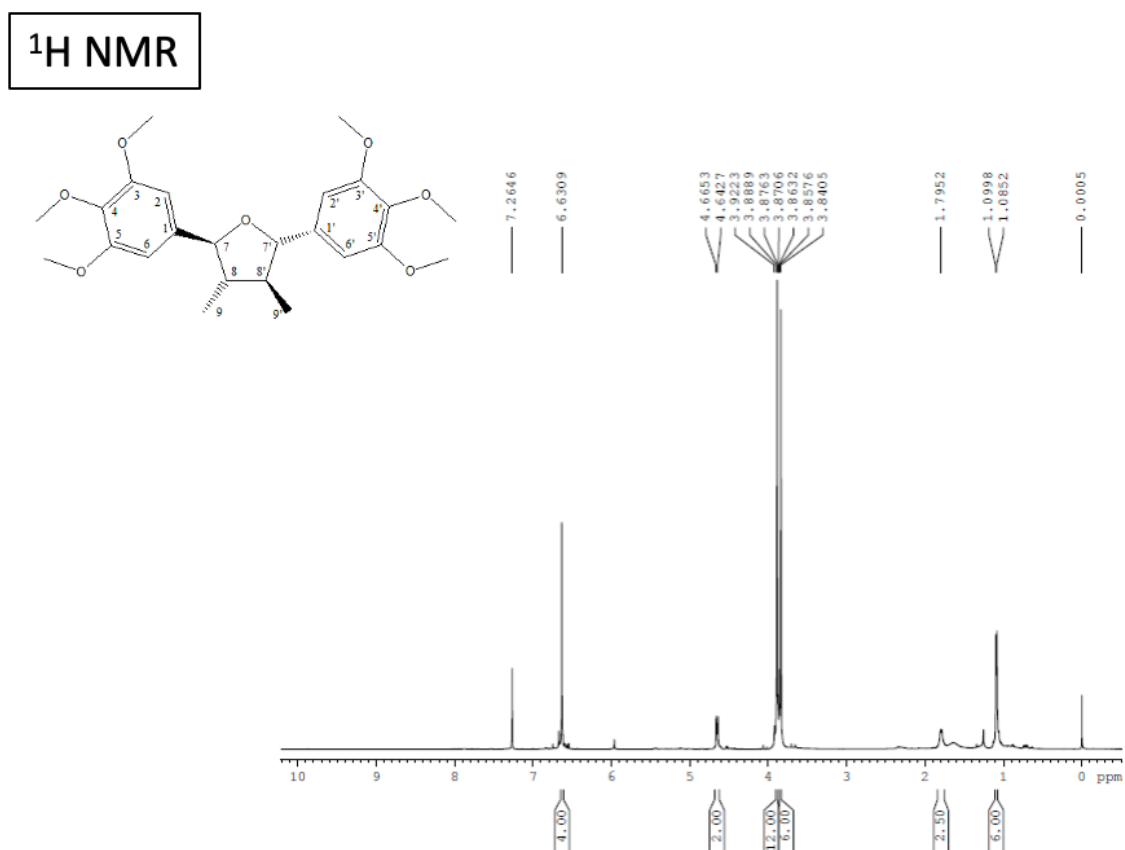


Figure S5 - ¹H NMR spectrum of grandisin neolignan.

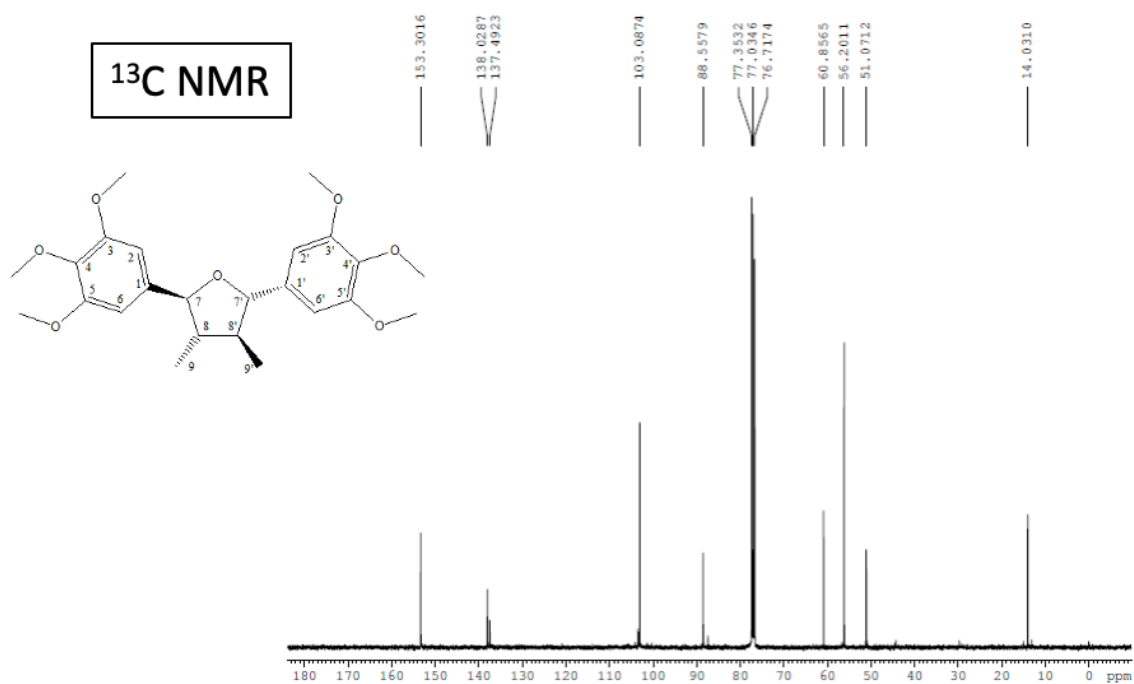


Figure S6 - ^{13}C NMR spectrum of grandisin neolignan.

Table S6- Data referring to ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra in CDCl_3 of grandisin neolignan isolated from *Piper tectoniifolium*.

Position	^1H , δ Grandisin	^{13}C , δ Grandisin
1 e 1'	-	137.5
2 e 2'	6.63, <i>s</i>	103.1
3 e 3'	-	153.3
4 e 4'	-	138.0
5 e 5'	-	153.3
6 e 6'	6.63, <i>s</i>	103.1
7 e 7'	4.67, <i>d</i>	88.6
8 e 8'	1.80, <i>m</i>	51.1
9 e 9'	1.10, <i>d</i>	14.0
MeO-3 e MeO-3'	3.89, <i>s</i>	56.2
MeO-4 e MeO-4'	3.84, <i>s</i>	60.9
MeO-5 e MeO-5'	3.89, <i>s</i>	56.2

* δH multiplicity (J in Hz); *s* = singlet, *d* = doublet, *m* = multiplet.

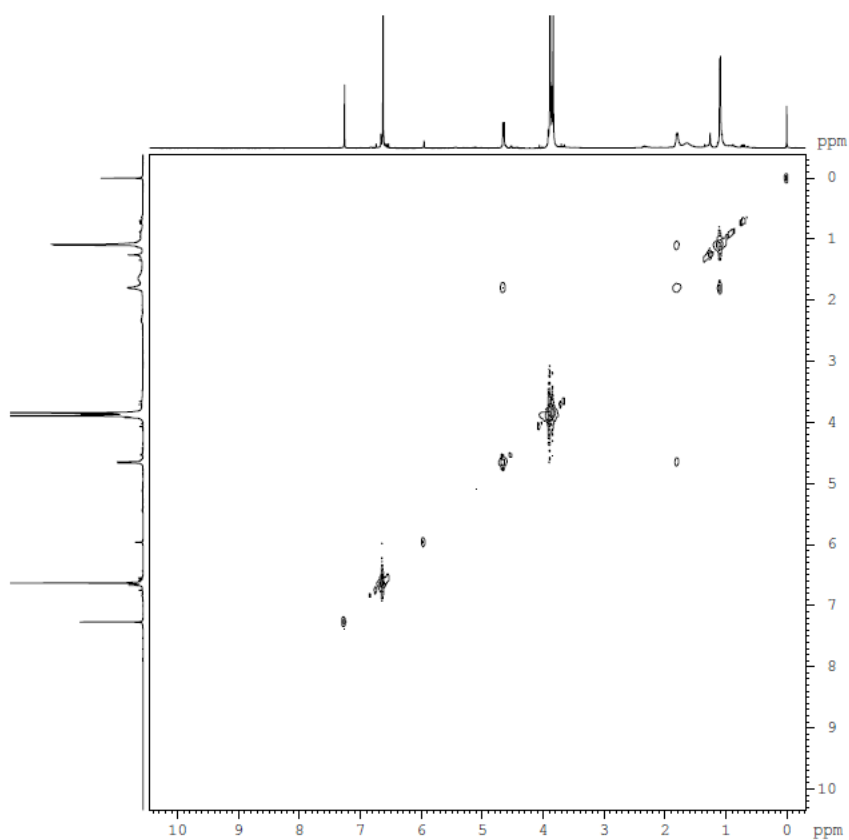


Figure S7 - COSY $^1\text{H} \times ^1\text{H}$ Homonuclear Correlation Spectrum for grandisin neolignan.

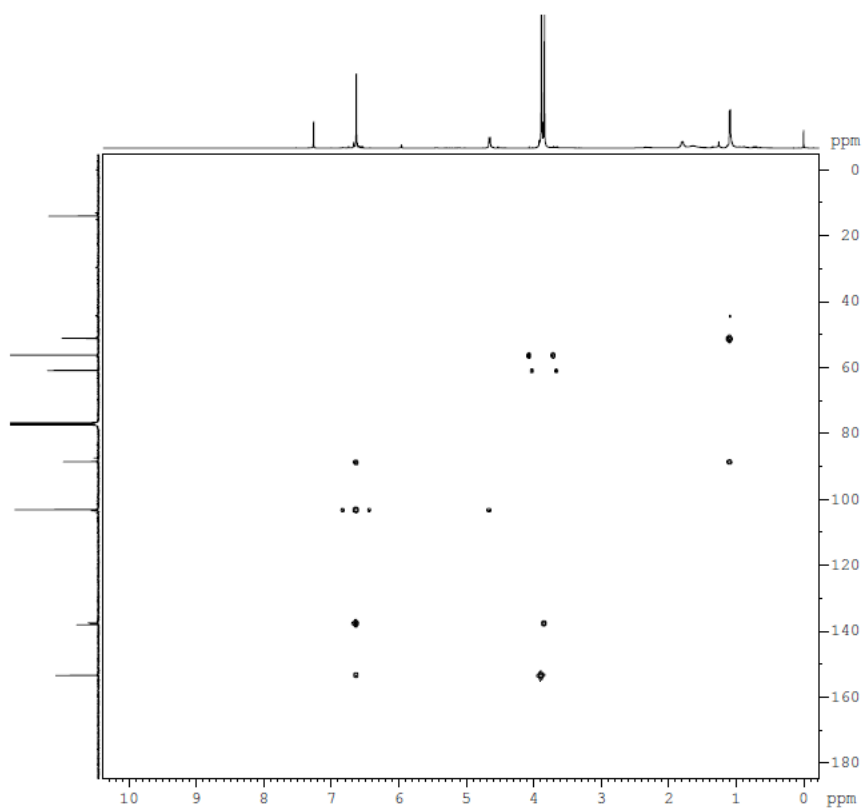


Figure S8 - HMBC $^1\text{H} \times ^{13}\text{C}$ Heteronuclear Multiple Bond Correlation Spectrum for the grandisin neolignan.

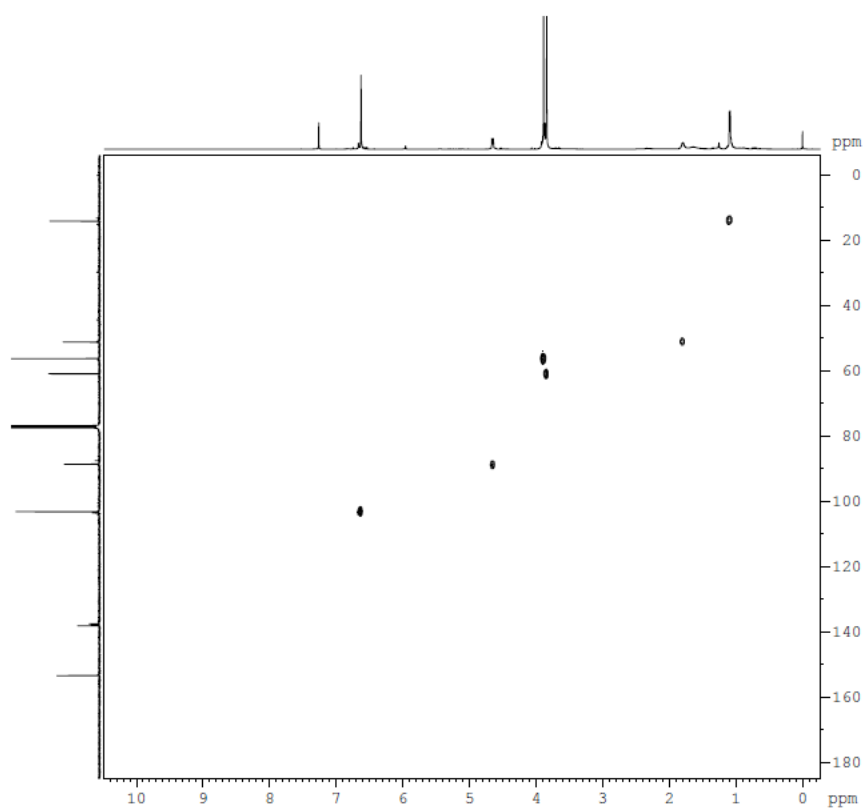


Figure S9 - HSQC $^1\text{H} \times ^{13}\text{C}$ Heteronuclear Correlation Spectrum for the grandisin neolignan.