

SUPPLEMENTARY INFORMATION FOR:

Anti-Inflammatory and Anti-Arthritic Activities of Glycosylated Flavonoids from *Syzygium jambos* in Edematogenic Agent-Induced Paw Edema in Mice

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Materials and Methods

Cell lines and Culture Conditions

In this study, the cell line RAW 264.7 (ATCC® SC-6003) was used to carry out the tests on the extracts and compounds of *S. jambos*. The cells were cultivated in DMEM (Dulbecco's modified eagle medium, Sigma-Aldrich St. Louis, USA) containing 2 mM L-glutamine (PanReac AppliChem, Barcelona, Spain), supplemented with 10% FBS (Fetal bovine serum, Summit Biotechnology Ft. Collins, CO), 100 U/ml penicillin (Austria) and 100 µg/ml of streptomycin (Fisher Scientific, Pittsburgh, USA) in culture flasks in a CO₂ incubator with a humidified atmosphere containing 5% CO₂ in air at 37°C.

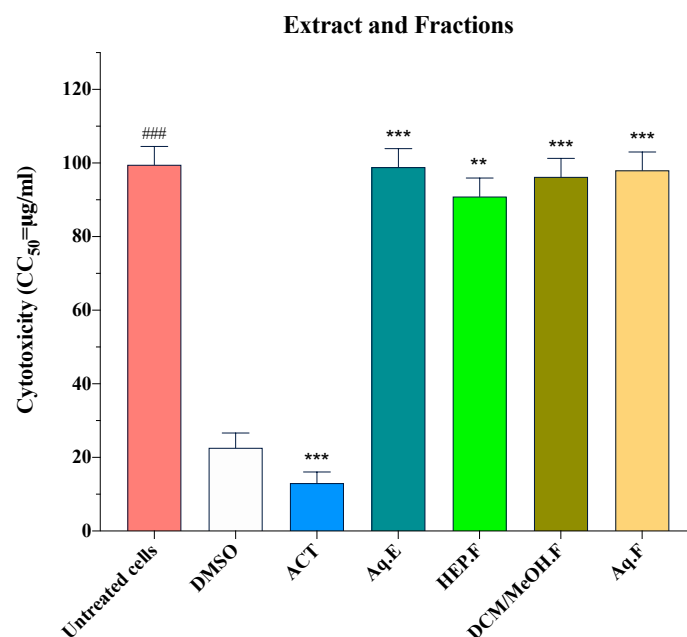
Animals

Male Swiss mice (25-35 g) housed at 22±2 °C and with access to food and water ad libitum were used in these experiments. Experiments were performed during the light phase of the cycle. The animals were allowed to adapt to the laboratory for at least 1 h before testing and were used only once.

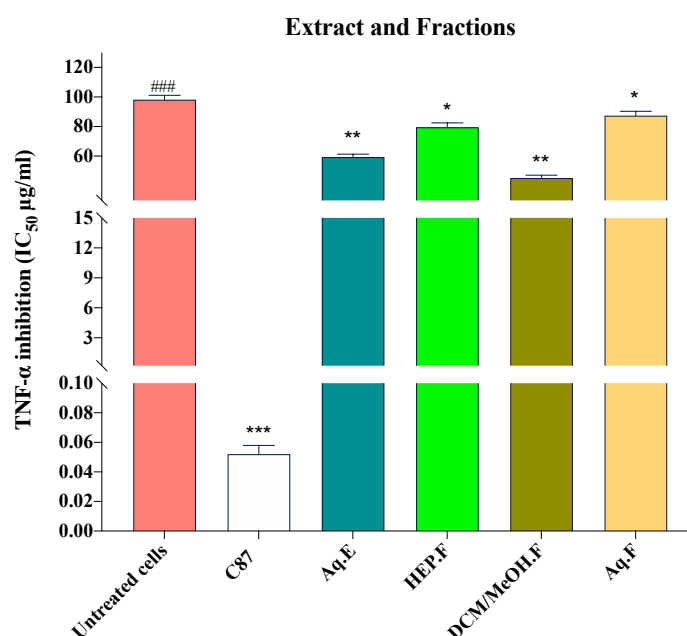
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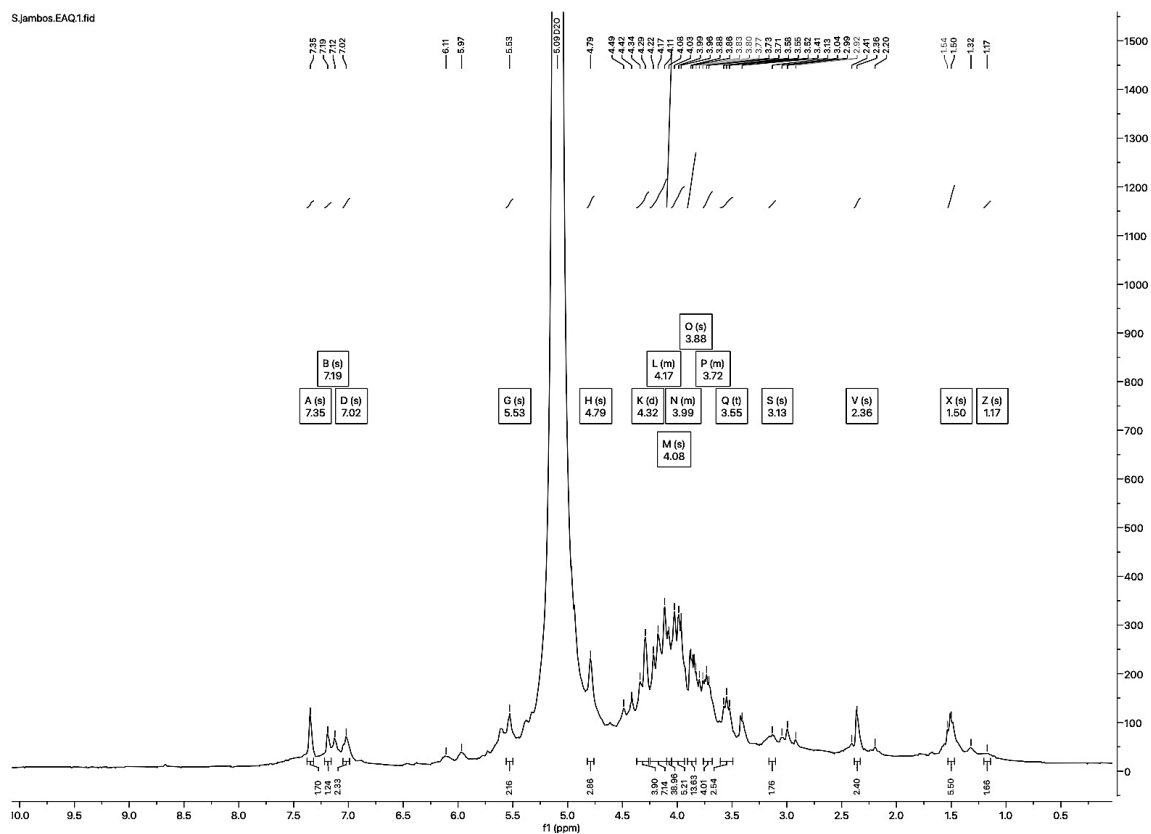
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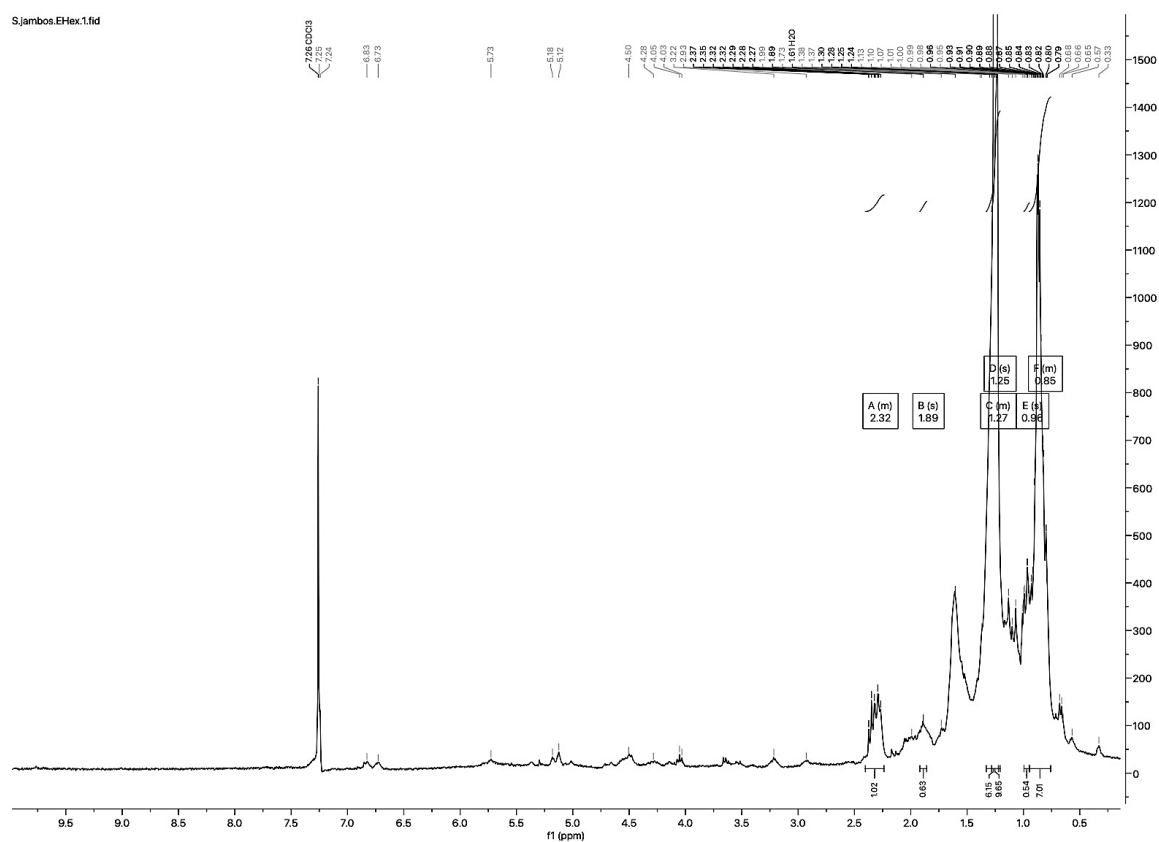
- Figure 1S. CC₅₀s of the LDH (Cytotoxicity) assays calculated for the aqueous extract (Aq.E) and fractions (HEP.F=*n*-C₇H₁₆, DCM/MeOH.F=CH₂Cl₂/MeOH and Aq.F=H₂O) from *Syzygium jambos* was calculated using Prism v9.0.0 (GraphPad Software) using non-linear regression, dose-response curves. CI95%: Confidence interval 95%/Tukey's multiple comparisons test



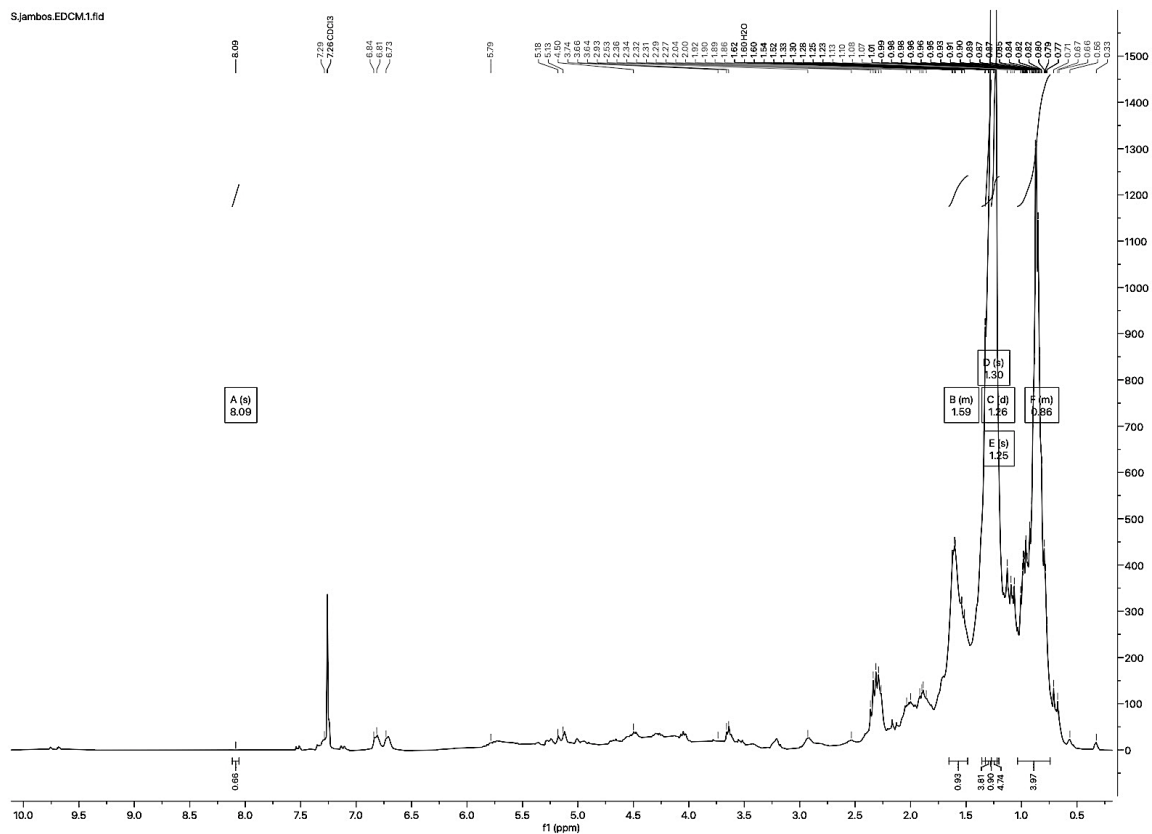
- Figure 2S. Comparative study of the aqueous extract (Aq.E) and fractions (HEP.F=*n*-C₇H₁₆, DCM/MeOH.F=CH₂Cl₂/MeOH and Aq.F=H₂O) from *Syzygium jambos* with the C87 positive control on TNF-α inhibition



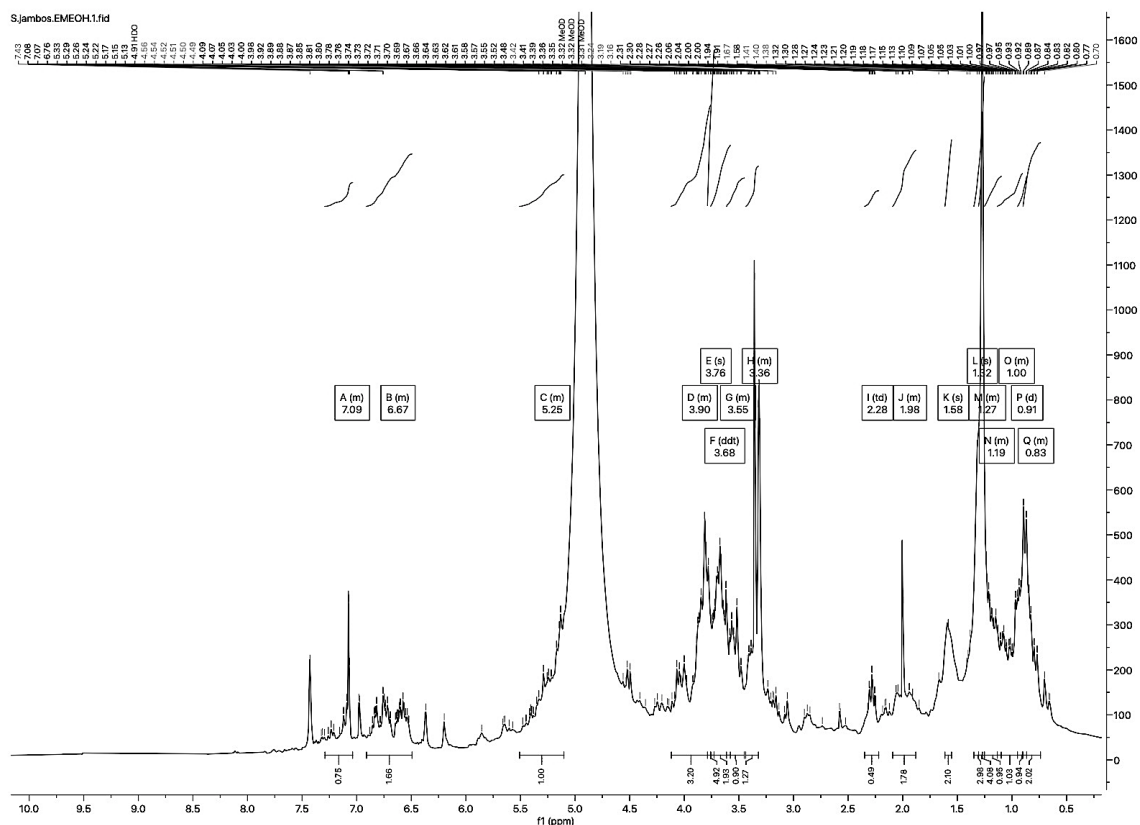
- Figure 3S. ^1H -NMR spectrum of aqueous extract of *Syzygium jambos* in D_2O 300 MHz



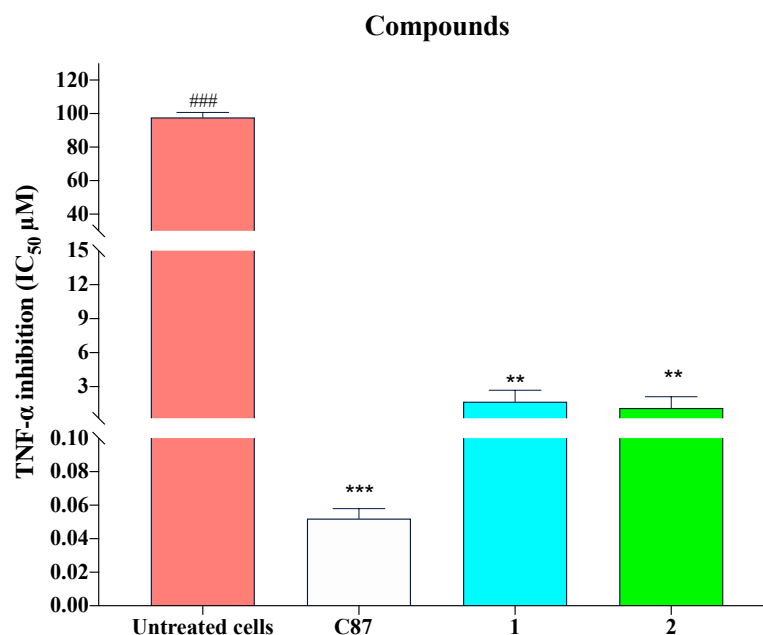
- Figure 4S. ^1H -NMR spectrum of $n\text{-C}_7\text{H}_{16}$ fraction of *Syzygium jambos* in CDCl_3 300 MHz



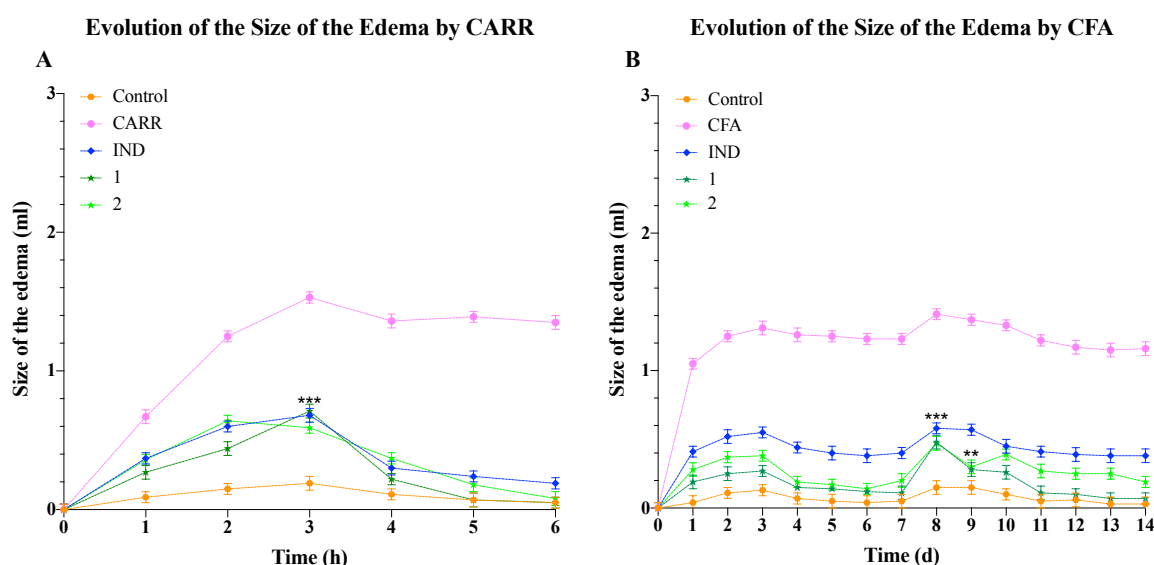
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- Figure 6S. ^1H -NMR spectrum of aqueous fraction of *Syzygium jambos* in MeOD 300 MHz



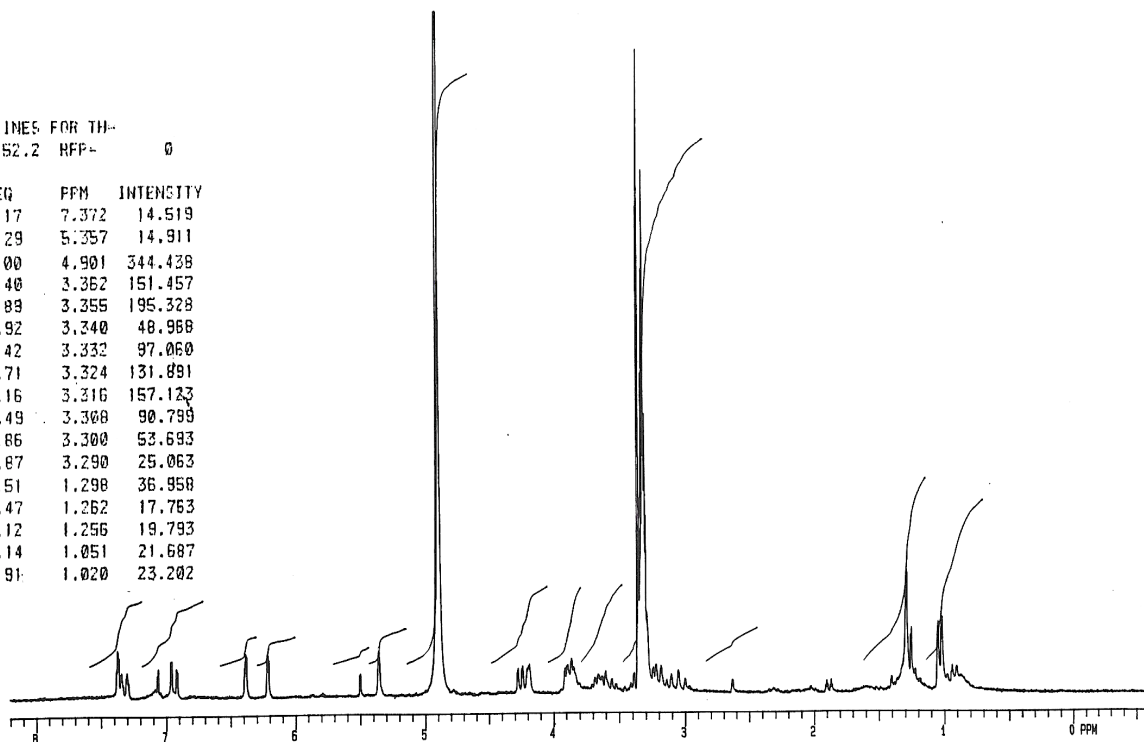
- Figure 7S. Comparative study of the quercetin-3-*O*- β -D-xylofuranosyl-(1 $\rightarrow$ 2)- α -L-rhamnopyranoside (1) and myricetin-3-*O*- β -D-xylofuranosyl-(1 $\rightarrow$ 2)- α -L-rhamnopyranoside (2) compounds from *Syzygium jambos* with the C87 positive control on TNF- α inhibition



- Figure 8S. Edema size evolution over time in the different groups of mice treated with the compounds 1 and 2 from *Syzygium jambos*. Control (untreated control mice). Each value represents the mean $\pm$ SEM of results from six animals. (** p <0.01 and *** p <0.001) highly significant difference in comparison with inflamed mice treated with indomethacin (IND group). Two-way ANOVA followed by Šídák's multiple comparisons test for 10A with a F (1.322, 5.287) and 10B with a F (1.033, 4.133)

SPECTRAL LINES FOR TH-
RFL= 452.2 RFP= 0

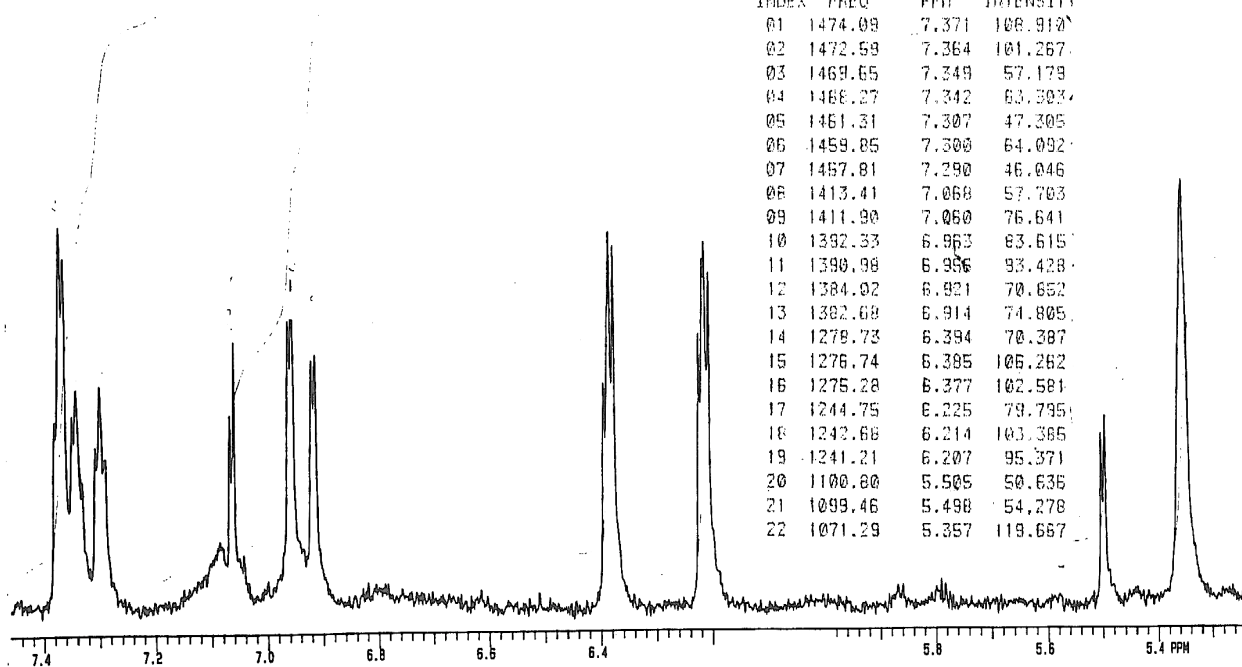
INDEX	FREQ	PPM	INTENSITY
01	1474.17	7.372	14.519
02	1071.29	5.357	14.911
03	980.00	4.901	344.438
04	672.40	3.362	151.457
05	670.89	3.355	195.328
06	667.92	3.340	48.988
07	666.42	3.332	97.060
08	664.71	3.324	131.891
09	663.16	3.316	157.123
10	661.49	3.308	90.799
11	659.86	3.300	53.693
12	657.87	3.290	25.063
13	259.51	1.298	36.958
14	252.47	1.262	17.753
15	251.12	1.256	19.793
16	210.14	1.051	21.687
17	203.91	1.020	23.202



- Figure 9S. ^1H -NMR spectrum of Compound 1 in MeOD 250 MHz

SPECTRAL LINES FOR TH-
RFL= 452.2 RFP= 0

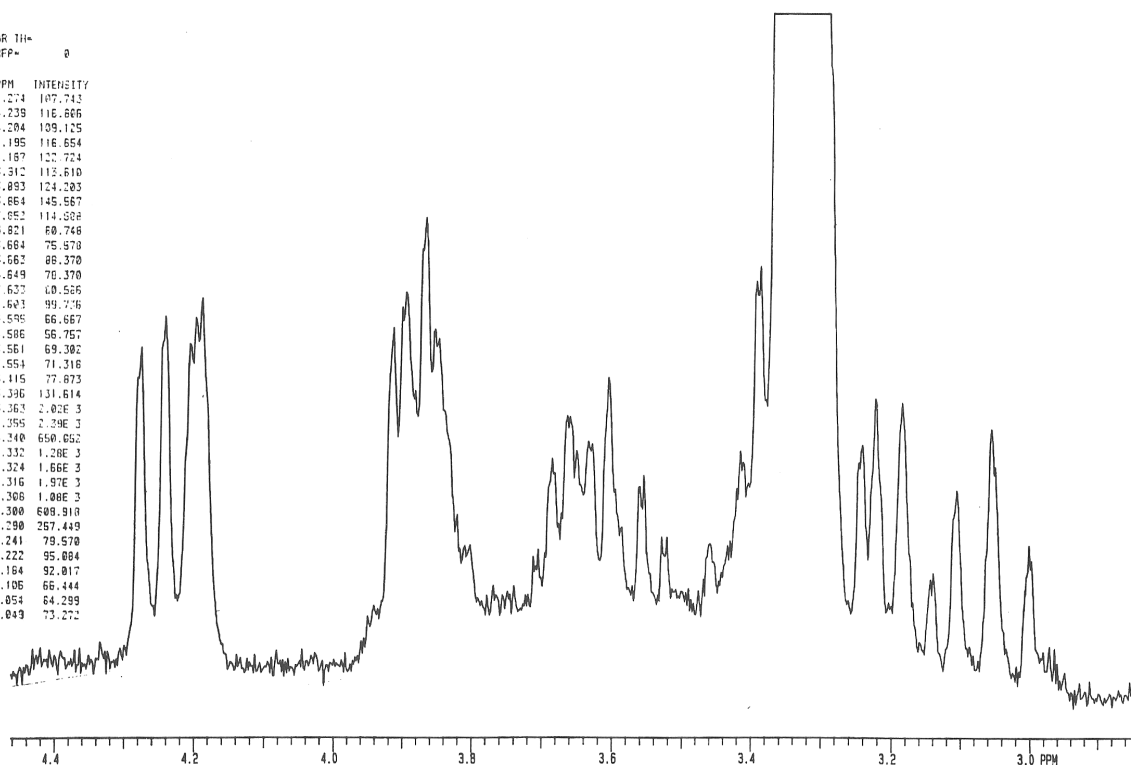
INDEX	FREQ	PPM	INTENSITY
01	1474.09	7.371	108.910
02	1472.59	7.364	101.267
03	1469.65	7.349	57.179
04	1466.27	7.342	63.303
05	1461.31	7.307	47.305
06	1459.85	7.300	64.092
07	1457.81	7.290	46.046
08	1413.41	7.060	57.703
09	1411.90	7.050	76.641
10	1392.33	6.963	83.615
11	1390.98	6.956	93.428
12	1384.02	6.821	70.652
13	1382.68	6.814	74.805
14	1279.73	6.394	70.387
15	1276.74	6.385	106.262
16	1275.28	6.377	102.581
17	1244.75	6.225	79.795
18	1242.66	6.214	103.365
19	1241.21	6.207	95.371
20	1100.80	5.505	50.636
21	1099.46	5.498	54.278
22	1071.29	5.357	119.667



- Figure 10S. ^1H -NMR spectrum of Compound 1 in MeOD 250 MHz (Extension 1)

SPECTRAL LINES FOR TH-
RFL= 452.2 RFP= 0

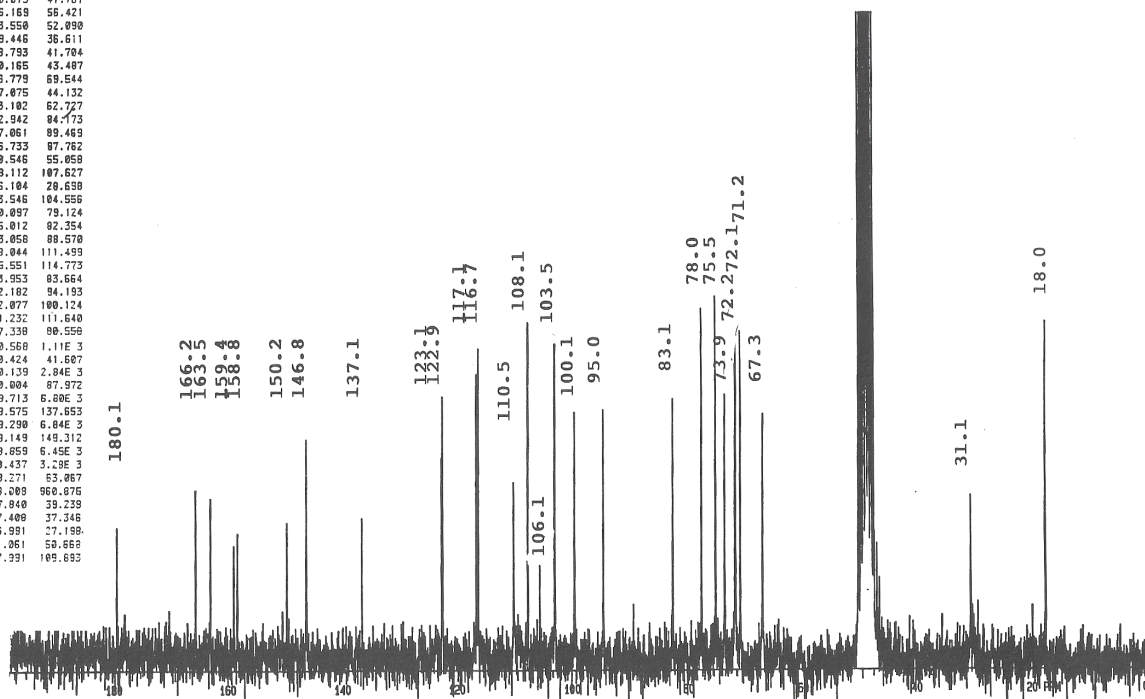
INDEX	FREQ	PPM	INTENSITY
01	854.73	4.274	107.743
02	847.77	4.239	116.666
03	840.73	4.204	109.125
04	838.96	4.195	116.654
05	837.23	4.167	122.724
06	782.25	3.312	113.610
07	778.46	3.893	124.203
08	772.68	3.864	145.567
09	770.28	3.652	114.588
10	764.01	3.821	60.746
11	736.79	3.664	75.570
12	732.47	3.662	88.370
13	729.74	3.649	76.370
14	726.52	3.632	100.566
15	720.58	3.623	99.726
16	710.64	3.595	66.667
17	717.17	3.586	56.757
18	712.08	3.561	69.302
19	710.70	3.554	71.316
20	662.94	3.415	77.873
21	677.08	3.396	131.614
22	672.44	3.363	2.02E 3
23	670.69	3.355	2.39E 3
24	667.92	3.340	660.652
25	666.42	3.332	1.28E 3
26	664.75	3.324	1.66E 3
27	663.16	3.316	1.97E 3
28	661.53	3.308	1.08E 3
29	659.90	3.300	608.918
30	657.91	3.290	257.449
31	648.14	3.241	79.570
32	644.32	3.222	95.084
33	636.79	3.164	92.017
34	621.04	3.106	66.444
35	610.78	3.054	64.299
36	609.68	3.049	73.272



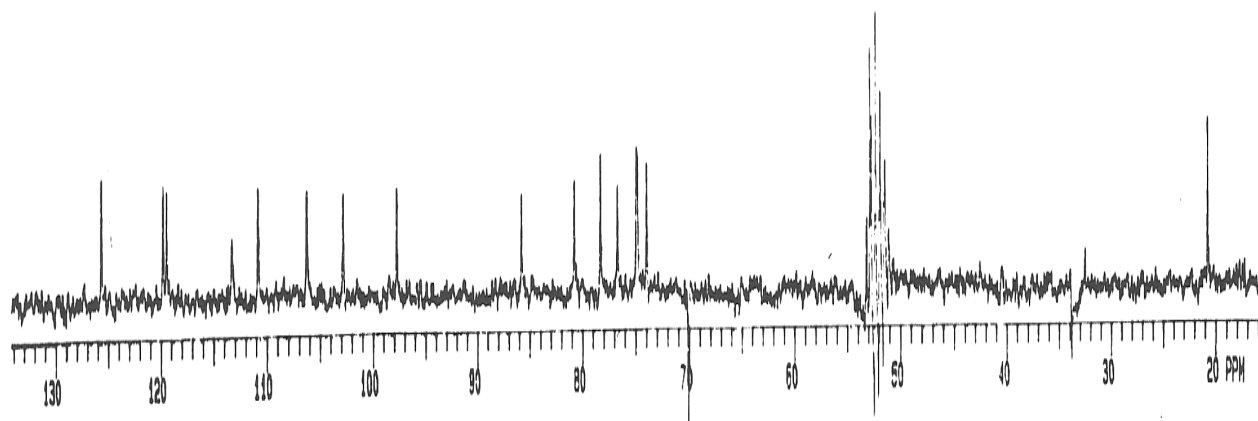
- Figure 11S. ^1H -NMR spectrum of Compound **1** in MeOD 250 MHz (Extension 2)

SPECTRAL LINES FOR TH-
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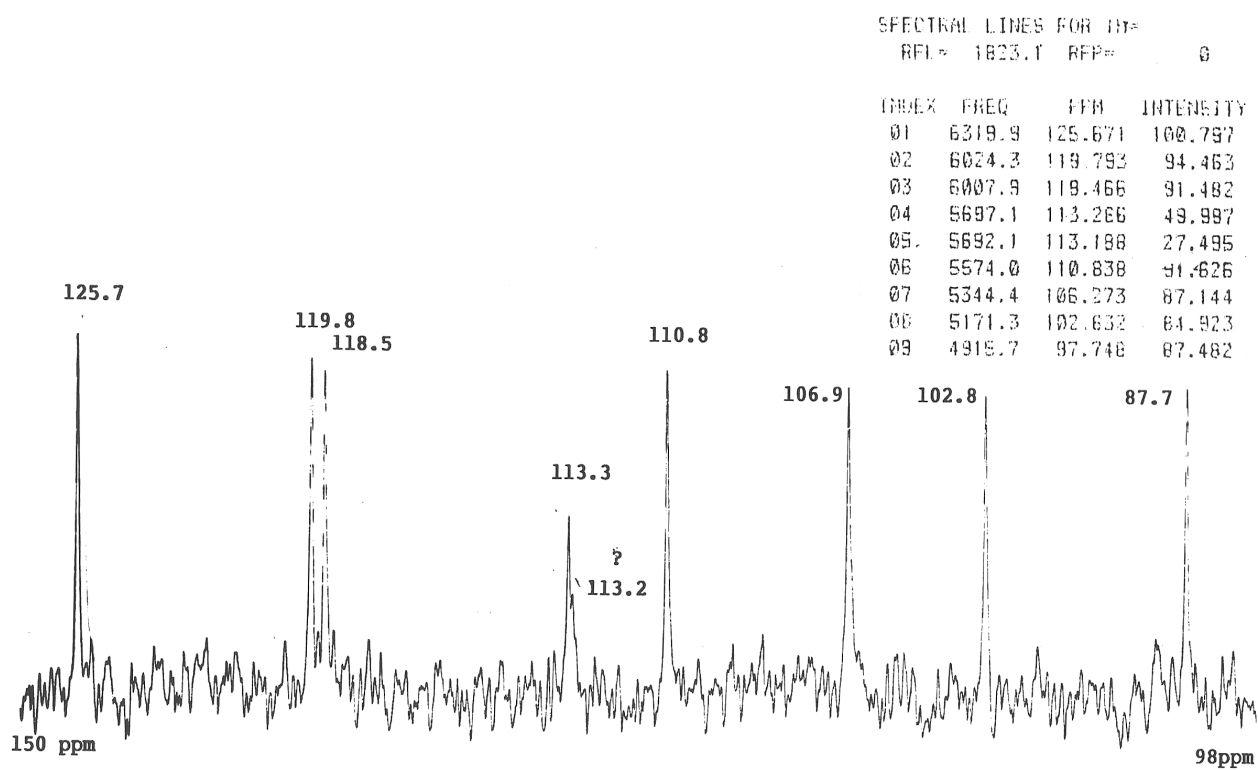
INDEX	FREQ	PPM	INTENSITY
01	9855.0	180.873	41.761
02	8356.5	156.169	56.421
03	8224.8	163.550	52.090
04	8018.4	159.446	36.611
05	7985.6	158.793	41.704
06	7651.7	150.165	43.487
07	7391.4	146.779	59.544
08	6893.4	137.075	44.132
09	6190.7	123.102	62.727
10	6182.7	122.942	84.773
11	5886.9	117.061	89.469
12	5870.4	116.733	87.762
13	5559.3	110.546	55.050
14	5436.9	108.112	107.627
15	5335.9	106.104	28.690
16	5207.3	103.546	104.556
17	5033.8	100.097	79.124
18	4778.1	95.012	82.354
19	4176.9	83.050	98.570
20	3924.8	78.044	111.499
21	3799.4	75.551	114.773
22	3719.1	73.953	83.664
23	3630.0	72.182	94.193
24	3624.7	72.877	100.124
25	3582.2	71.232	111.640
26	3396.4	67.339	98.550
27	2543.0	50.560	1.11E 3
28	2535.0	50.424	41.607
29	2521.5	50.139	2.84E 3
30	2514.7	50.004	87.972
31	2500.0	49.713	6.89E 3
32	2493.1	49.575	137.653
33	2478.0	49.298	6.84E 3
34	2471.7	49.149	149.312
35	2457.1	48.859	6.45E 3
36	2435.9	48.437	3.28E 3
37	2427.5	48.271	63.067
38	2414.3	48.089	960.076
39	2405.9	47.840	39.239
40	2384.1	47.408	37.346
41	2363.2	46.991	27.199
42	1562.0	31.951	50.668
43	304.8	17.291	105.693



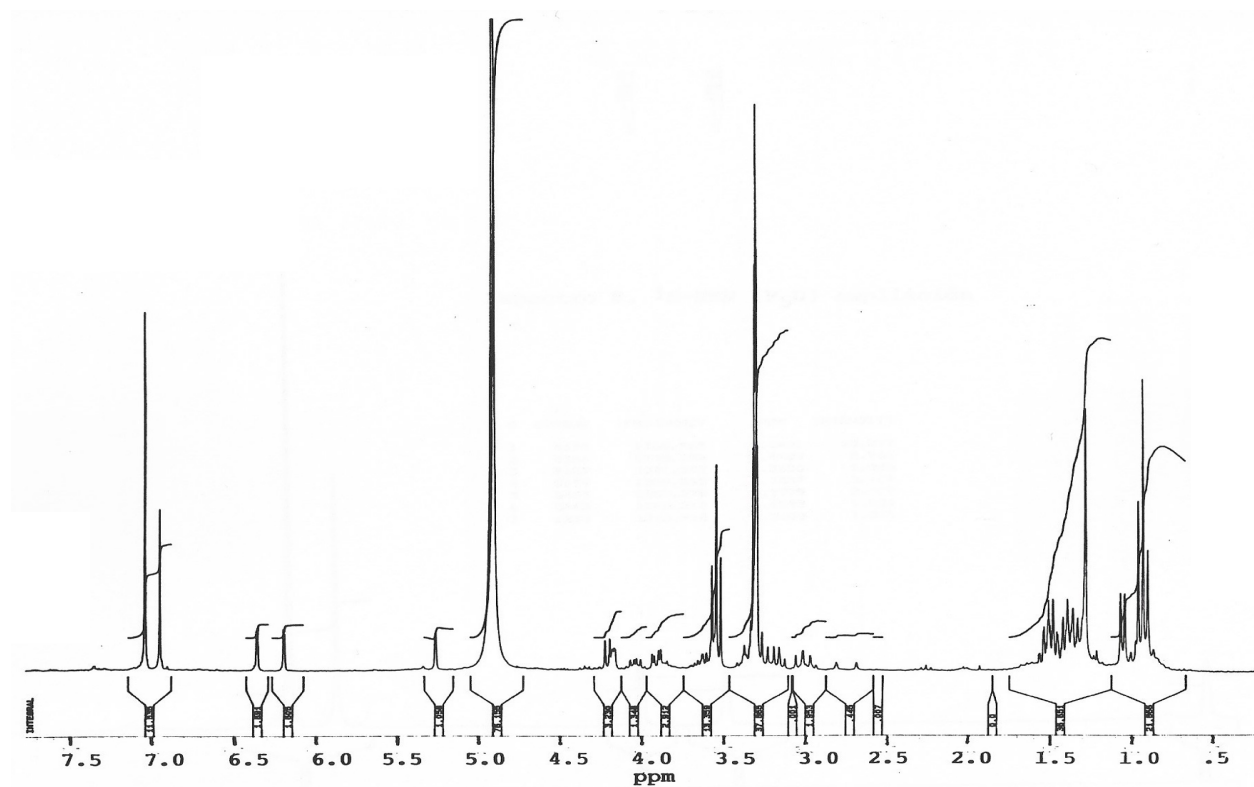
- Figure 12S. ^{13}C -NMR spectrum of Compound **1** in MeOD 75 MHz



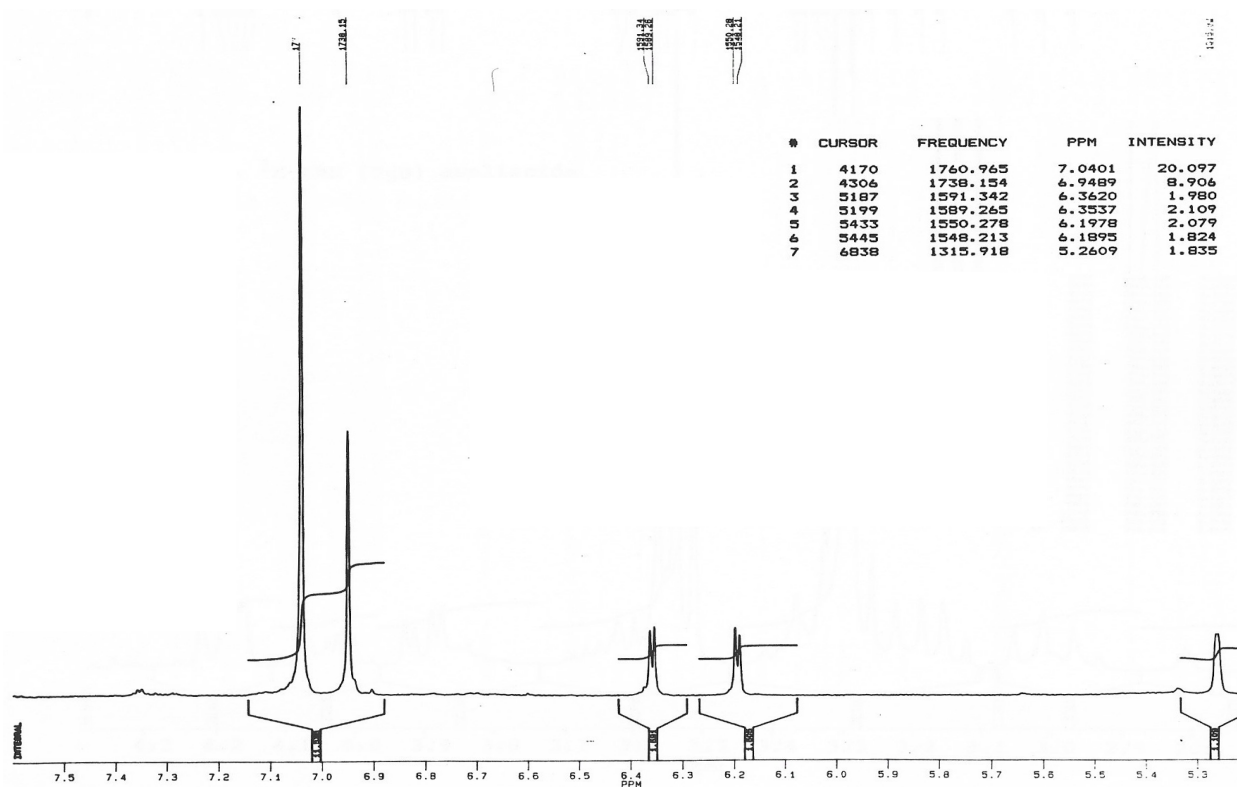
- Figure 13S. DEPT-135 spectrum of Compound **1** in MeOD 75 MHz



- Figure 14S. DEPT-135 spectrum of Compound **1** in MeOD 75 MHz (Extension 1)



- Figure 15S. ^1H -NMR spectrum of Compound **2** in MeOD 250 MHz



- Figure 16S. ^1H -NMR spectrum of Compound **2** in MeOD 250 MHz (Extension 1)

- Table 1S. Assignment of NMR signals of the compounds (**1** and **2**) isolated from *Syzygium jambos* in the Bruker Advance DRX equipment at 250 MHz (^1H)/75 MHz (^{13}C) in $\text{MeOD-}d^4$.

Nº C	Quercetin-3- <i>O</i> - β -D-xylofuranosyl-(1 $\rightarrow$ 2)- α -L-rhamnopyranoside (1) in $\text{MeOD-}d^4$				Myricetin-3- <i>O</i> - β -D-xylofuranosyl-(1 $\rightarrow$ 2)- α -L-rhamnopyranoside (2) in $\text{MeOD-}d^4$			
	δ_{C}	δ_{H}	Mult	J [Hz]	δ_{C}	δ_{H}	Mult	J [Hz]
2	158.8	-	-	-	158.5	-	-	-
3	137.1	-	-	-	137.9	-	-	-
4	180.1	-	-	-	179.8	-	-	-
5	159.4	-	-	-	159.2	-	-	-
6	100.1	6.21	d	2.1	99.8	6.19	d	2
7	166.2	-	-	-	165.9	-	-	-
8	95.0	6.38	d	2.1	94.7	6.35	d	2
9	163.5	-	-	-	163.2	-	-	-
10	106.1	-	-	-	105.8	-	-	-
1'	123.1	-	-	-	122.4	-	-	-
2'	117.1	7.34	d	2.1	110.2	6.95	s	-
3'	146.8	-	-	-	146.3	-	-	-
4'	150.2	-	-	-	139.4	-	-	-
5'	116.7	6.95	d	8.2	146.3	-	-	-
6'	122.9	7.37	dd	2.1, 8.2	110.2	6.95	s	-
Xyl-1	108.1	4.25	dd	7	107.8	4.31	d	7.5
Xyl-2	75.5	3.20	ddt	-	75.3	3.30	ddt	-
Xyl-3	78.0	3.40	mt	-	77.8	3.40	mt	-
Xyl-4	72.1	3.50	mt	-	71.8	3.50	mt	-
Xyl-5	67.3	3.68	mt	-	67.0	3.70	mt	-
		3.05	dd	10.3, 10.3		3.10	dd	10.8, 10.8
Rha-1	103.5	5.45	d	1.5	103.5	5.36	d	1.5
Rha-2	83.1	4.19	dd	1.5, 3.5	82.8	4.27	dd	1.5, 3.5
Rha-3	71.2	3.90	dd	3.5, 9.5	70.8	3.98	dd	3.5, 9.7
Rha-4	73.9	3.40	mt	-	73.8	3.40	ddt	-
Rha-5	72.2	3.90	ddt	-	71.9	4.10	dd	9.3, 6.2
Rha-6	18.0	1.25	d	6	17.8	1.00	d	6.2
Ref.	Data compared with Slowing et al. 1994				Data compared with Slowing et al. 1994			