# *Supplementary materials*

## Secondary metabolites with ecologic and medicinal implications in *Anthemis cretica* subsp. *petraea* from Majella National Park.

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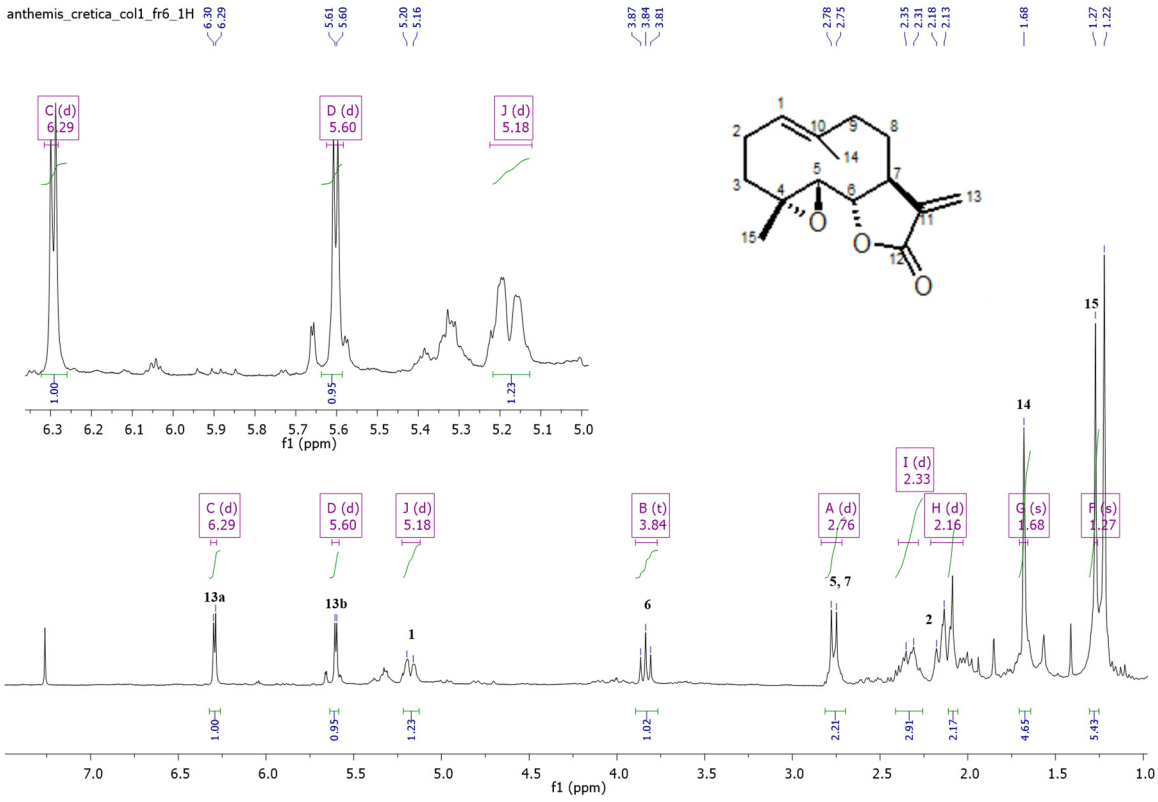
*Anthemis cretica* subsp. *petraea* (Ten.) Greuter is a plant belonging to the Asteraceae family and *Anthemis cretica* subsp. *petraea* (Ten.) Greuter is a plant belonging to the Asteraceae family and endemic of central Italy. In this paper, the first analysis of the ethanolic fraction of a sample collected in the Majella National Park is reported. Seven compounds were isolated and identified namely **parthenolide** (**1**), **9α-acetoxyparthenolide** (**2**), **tamarixetin** (**3**), **7-hydroxycoumarin** (**4**), **4'-hydroxyacetophenone** (**5**), **leucanthemitol** (**conduritol F)** (**6**) and ***proto*-quercitol** (**7**). This result was achieved by means of Column Chromatography (CC) for what concerns the isolation part and spectroscopic and spectrometric techniques for what concerns the identification one. The presence of these compounds is of high relevance. Compounds **1** and **2** are the chemosystematic markers of the family, thus confirming the correct botanical classification of the species. On the other side, compounds **3**, **5** and **7** were identified for the first time in the species and, instead, confirm the tendency of endemic entities to develop characteristic metabolite patterns in respect with cosmopolite species. Moreover, the presence of compounds **6** and **7** has ecologic implications and may be linked to the adaption of this taxon to dry environments. The production of these osmolytes may, in fact, represent the reason why this species is able to survive in extreme conditions of aridity. Lastly, from the medicinal standpoint, the isolated compounds are all endowed with interesting biological activities and may justify, on a molecular base, the large traditional uses of *Anthemis* species and also state that the subspecies *petraea* may be used as well.

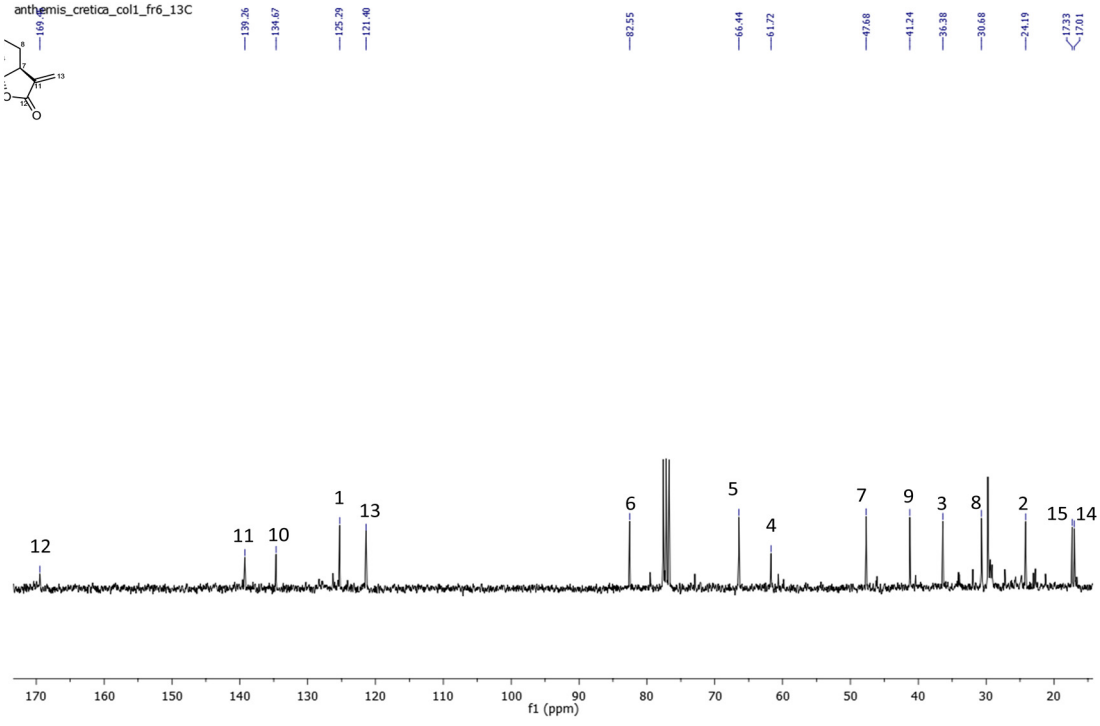
**Keywords:** Adaptation ; Secondary metabolites; Diterpenoids; Flavonoids; Cyclitols; Abiotic stress; Traditional Uses; Chemotaxonomy.

## 1. Original NMR and MS spectra of the identified compounds:

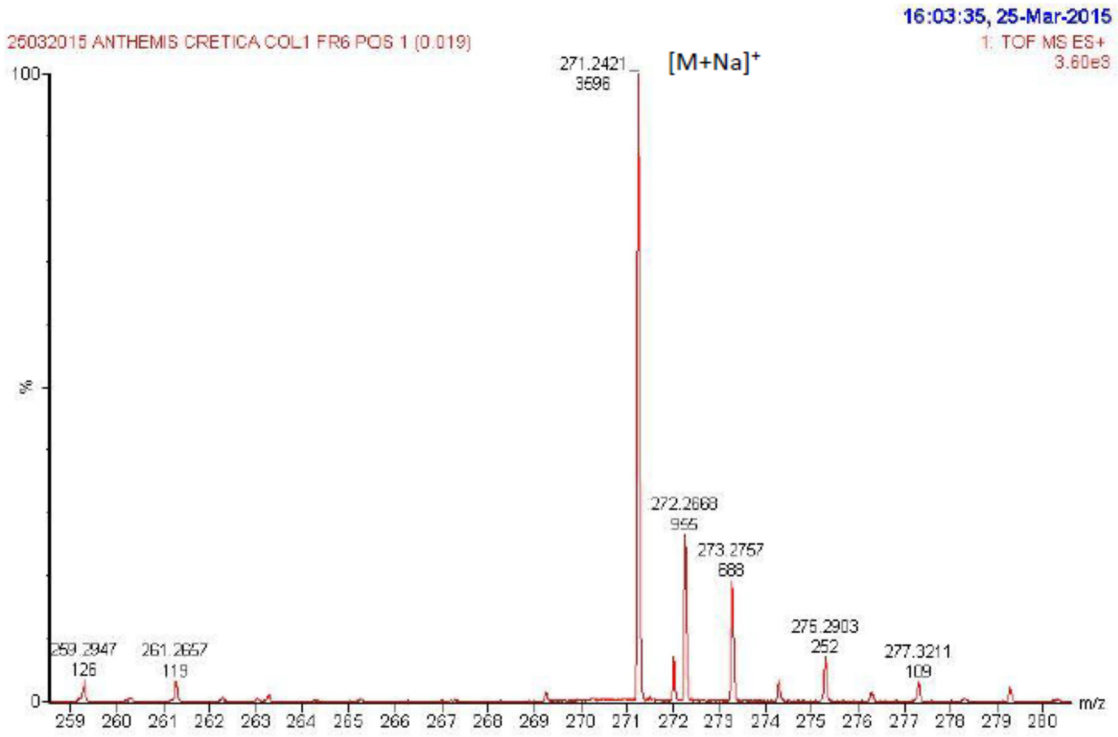
### Parthenolide (**1**):



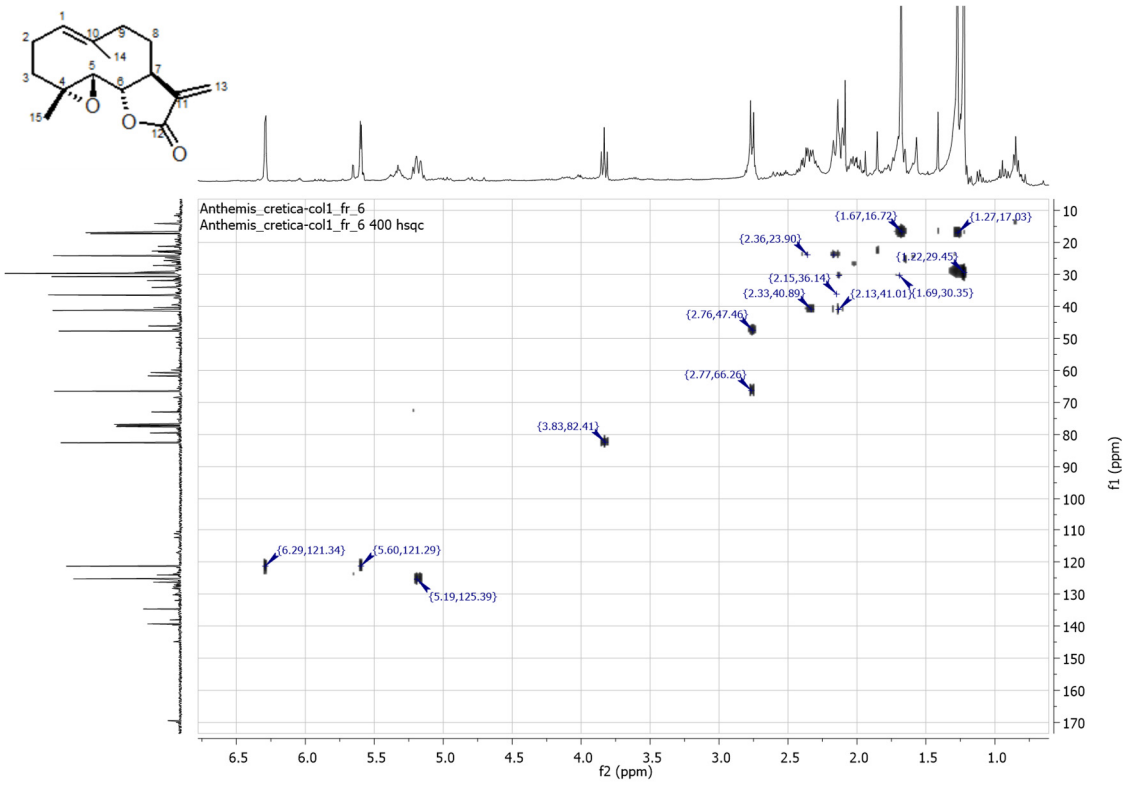

Supplemetary Figure 1: $^{1}$H NMR of parthenolide (**1**).




Supplemetary Figure 2: $^{13}$C NMR of parthenolide (**1**).

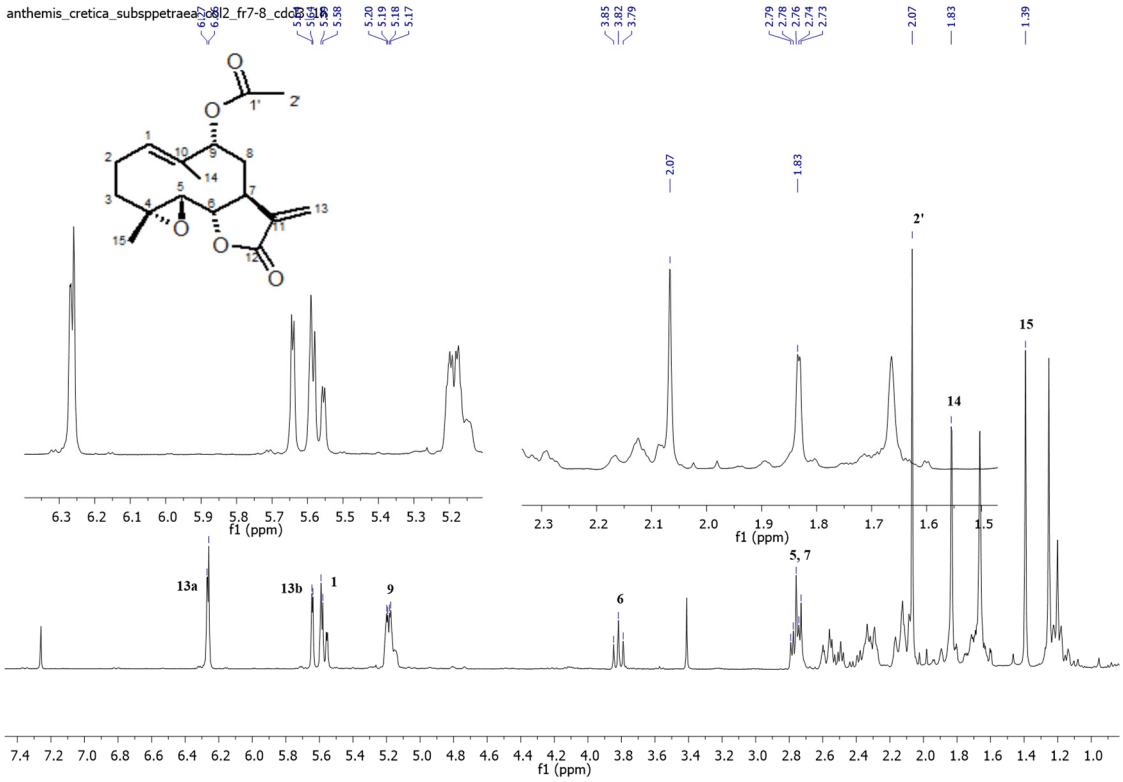



Supplemetary Figure 3: ESI-MS spectrum of parthenolide (**1**).

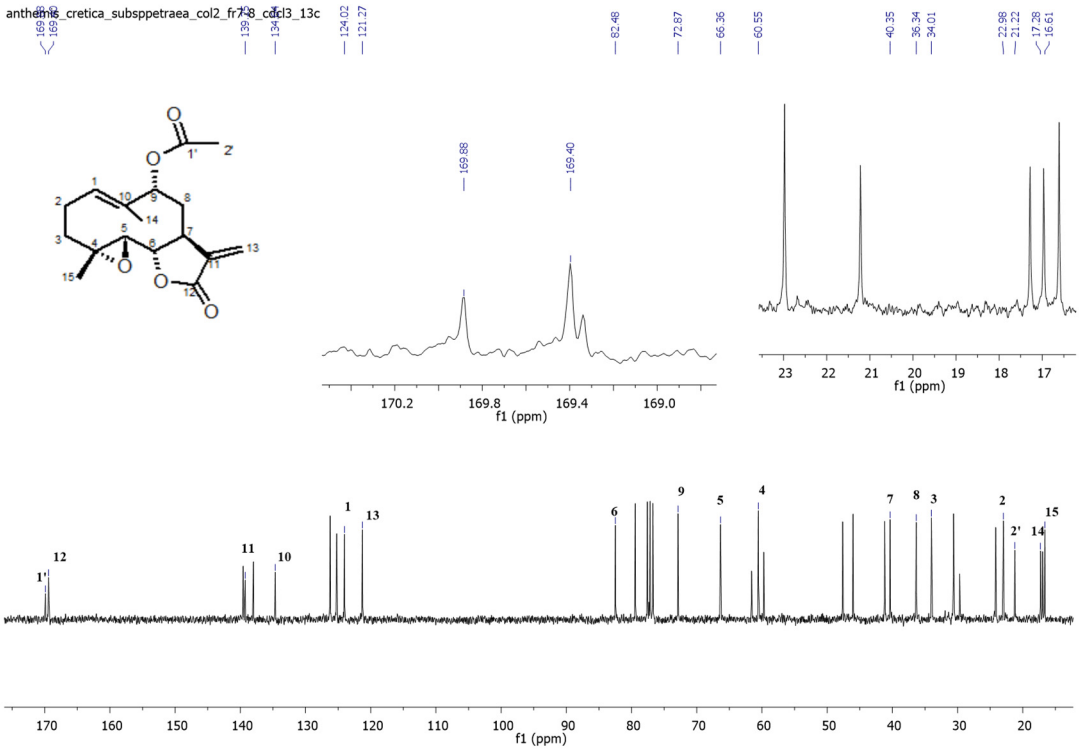



Supplemetary Figure 4: HSQC spectrum of parthenolide (**1**).

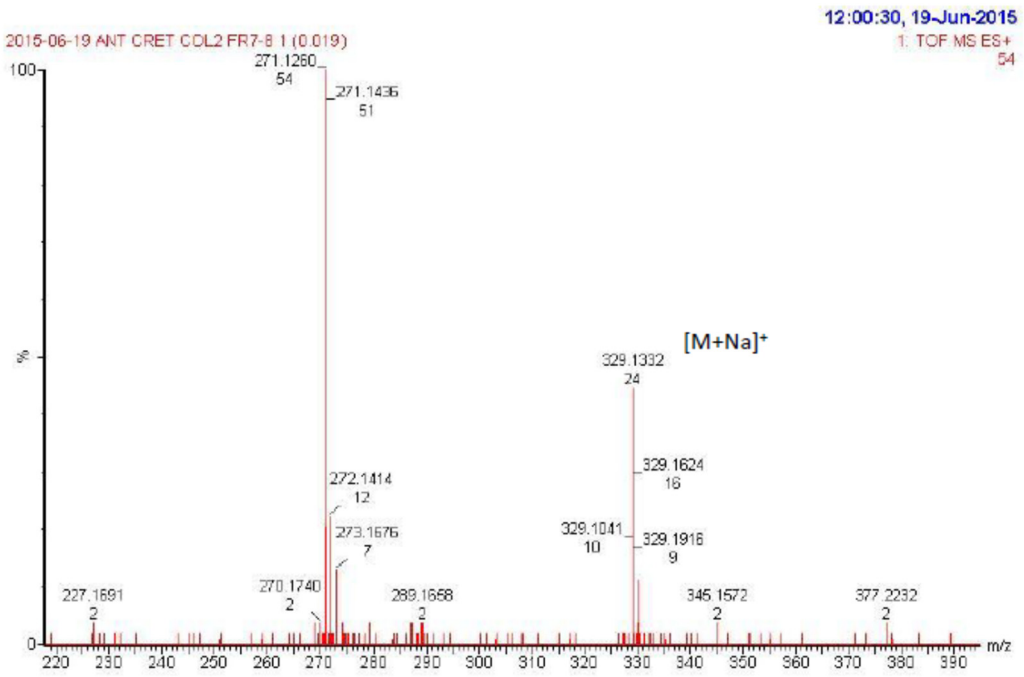
**9-α-acetoxyparthenolide (2):**




Supplementary Figure 5: $^{1}$H NMR spectrum of 9α-acetoxyparthenolide (**2**) in mixture (2:1) with parthenolide (**1**)

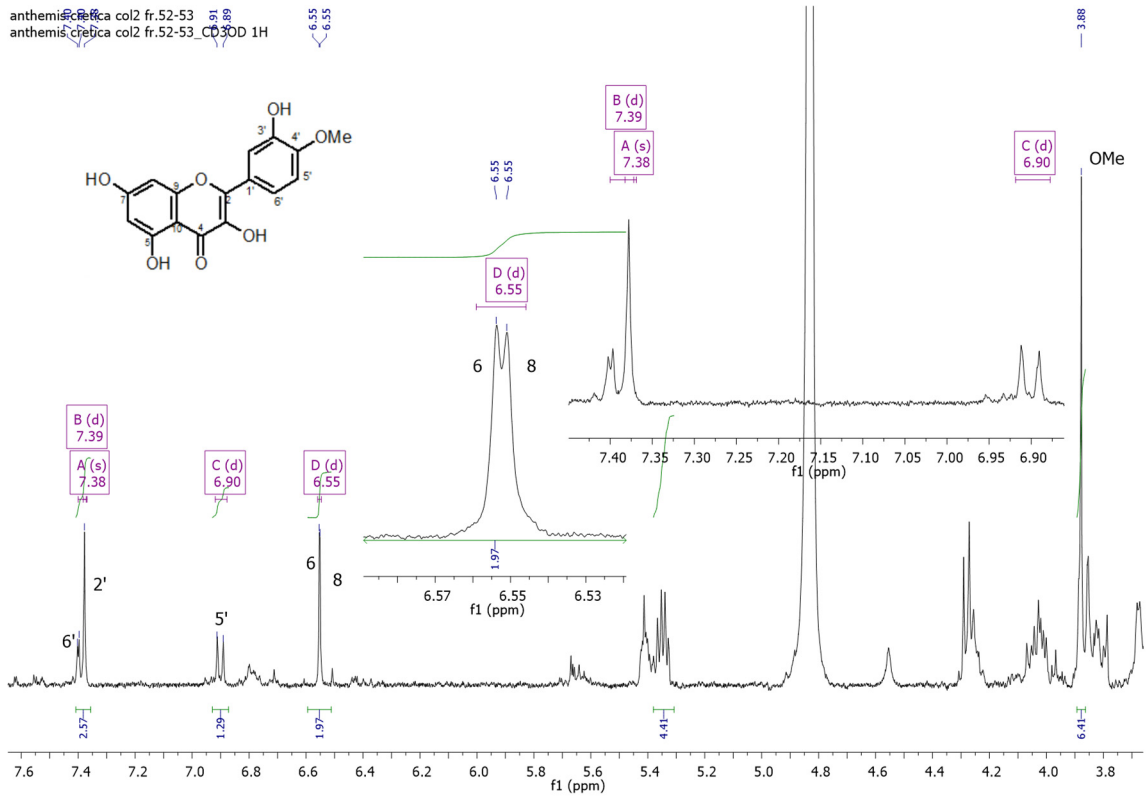



Supplementary Figure 6: $^{13}$C NMR spectrum of 9α-acetoxyparthenolide (**2**) in mixture (2:1) with parthenolide (**1**). Resonances of compound **2** were evidenced.

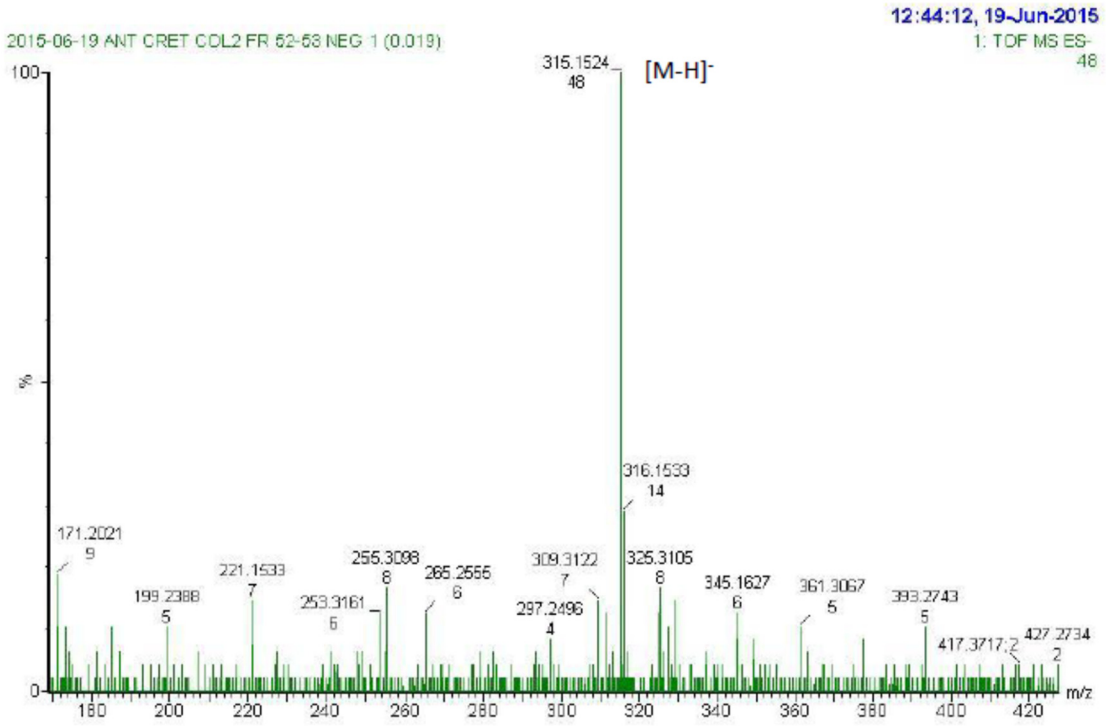



Supplementary Figure 7: MS spectrum of 9α-acetoxyparthenolide (**2**) in mixture with parthenolide (**1**). The sodium adduct of compound **2** is evidenced.

**tamarixetin (3):**

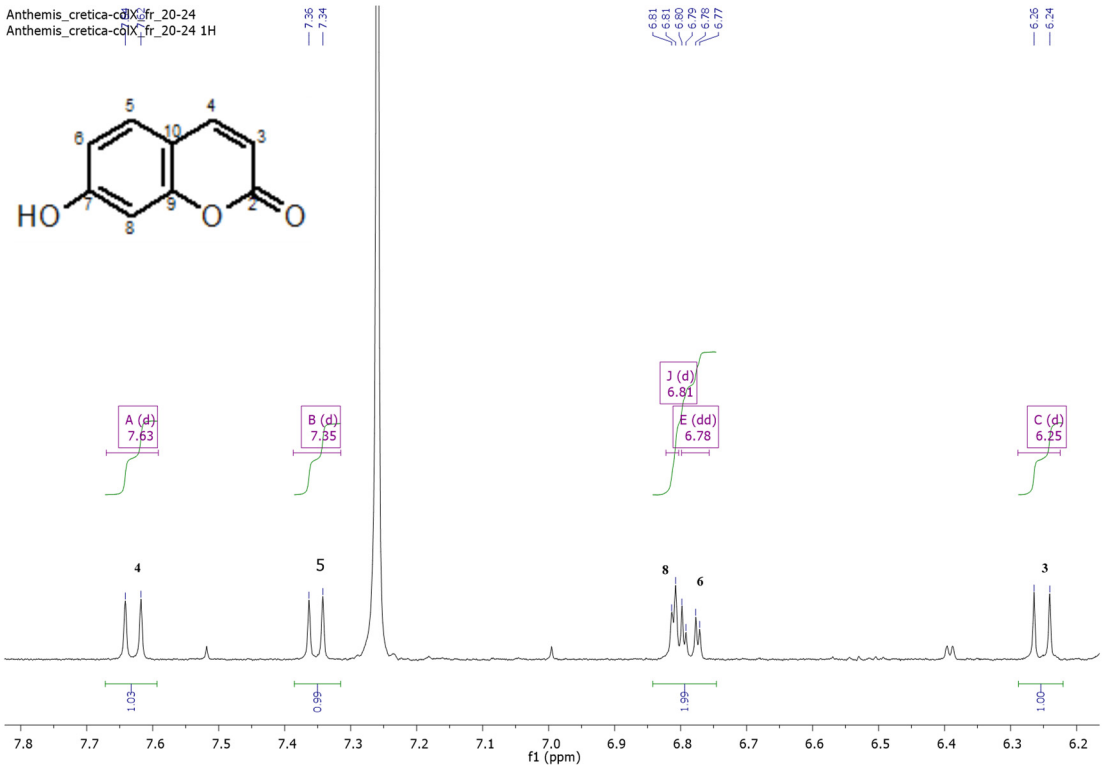



Supplementary Figure 8: $^{1}$H NMR spectrum of tamarixetin (**3**), in mixture with other compounds.

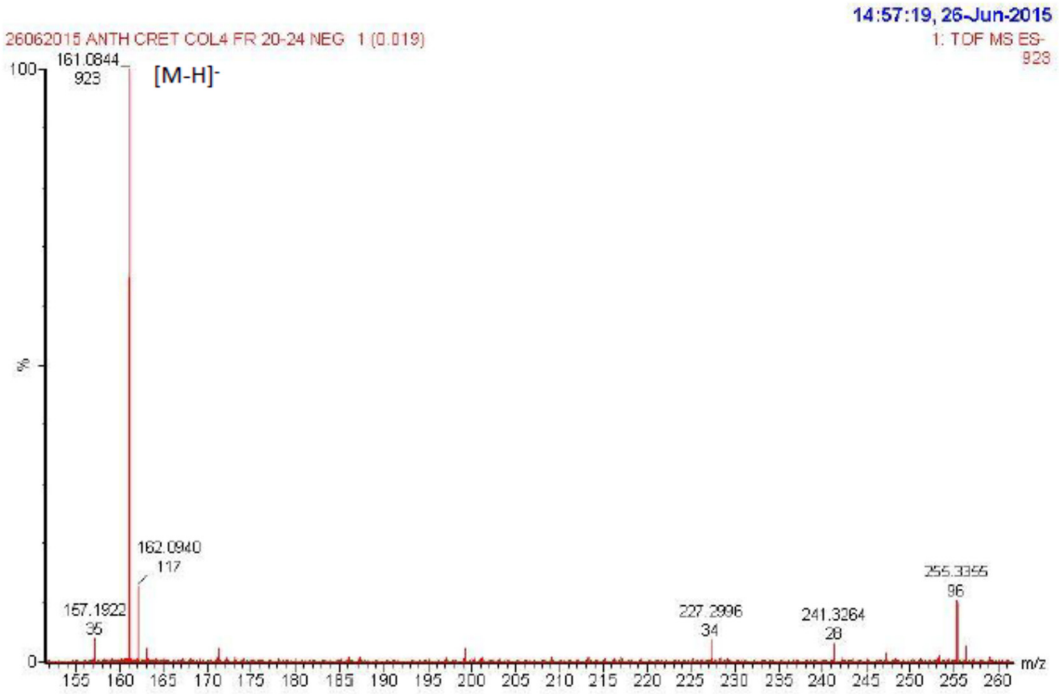



Supplementary Figure 9: MS spectrum of tamarixetin (**3**).

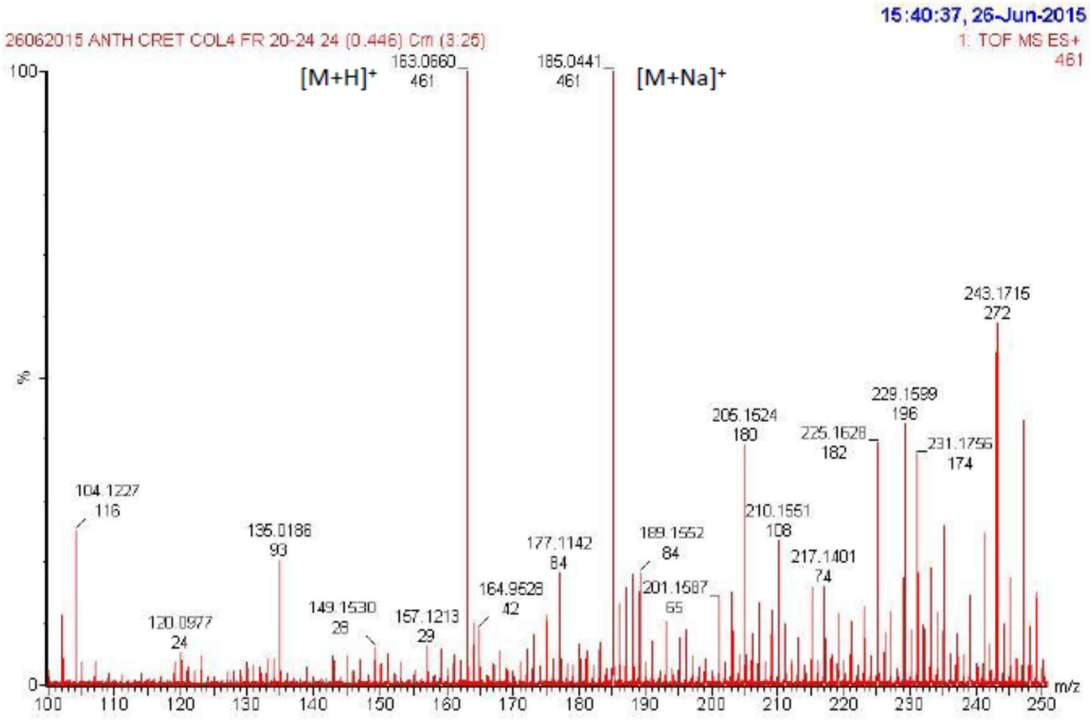
**7-hydroxycoumarin (4)**:




Supplementary Figure 10: $^{1}$H NMR spectrum of 7-hydroxycoumarin (**4**)

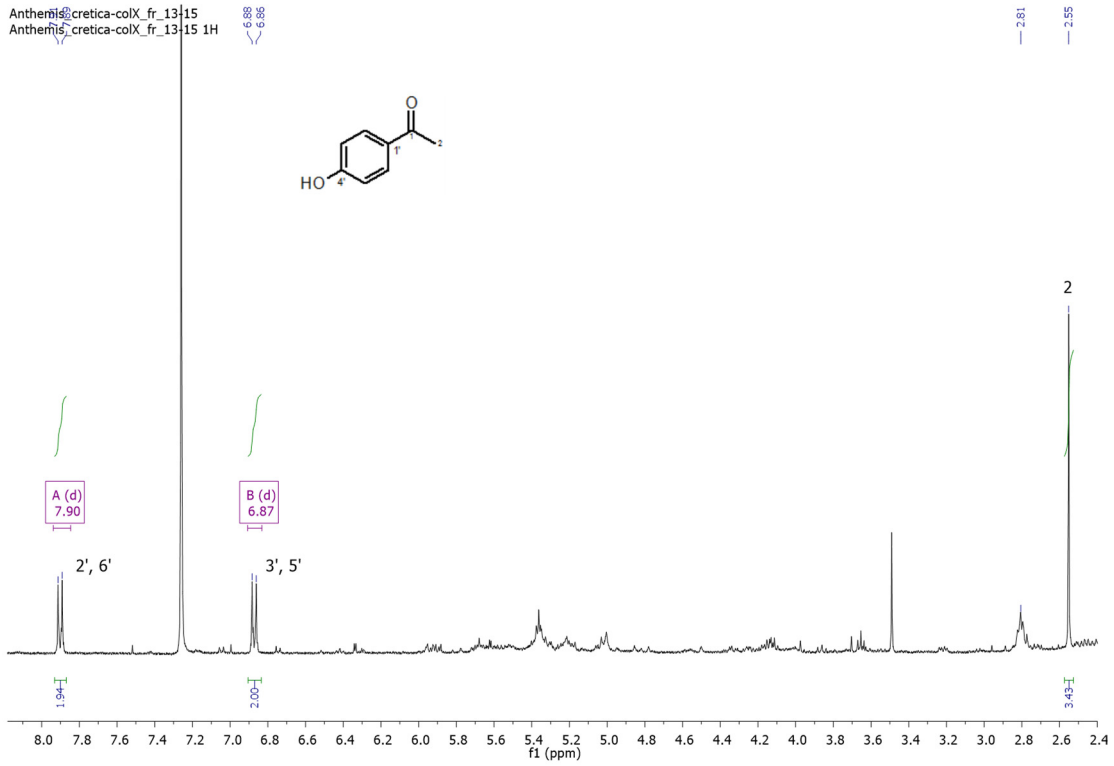



Supplementary Figure 11: MS$^{-}$ spectrum of 7-hydroxycoumarin (**4**)

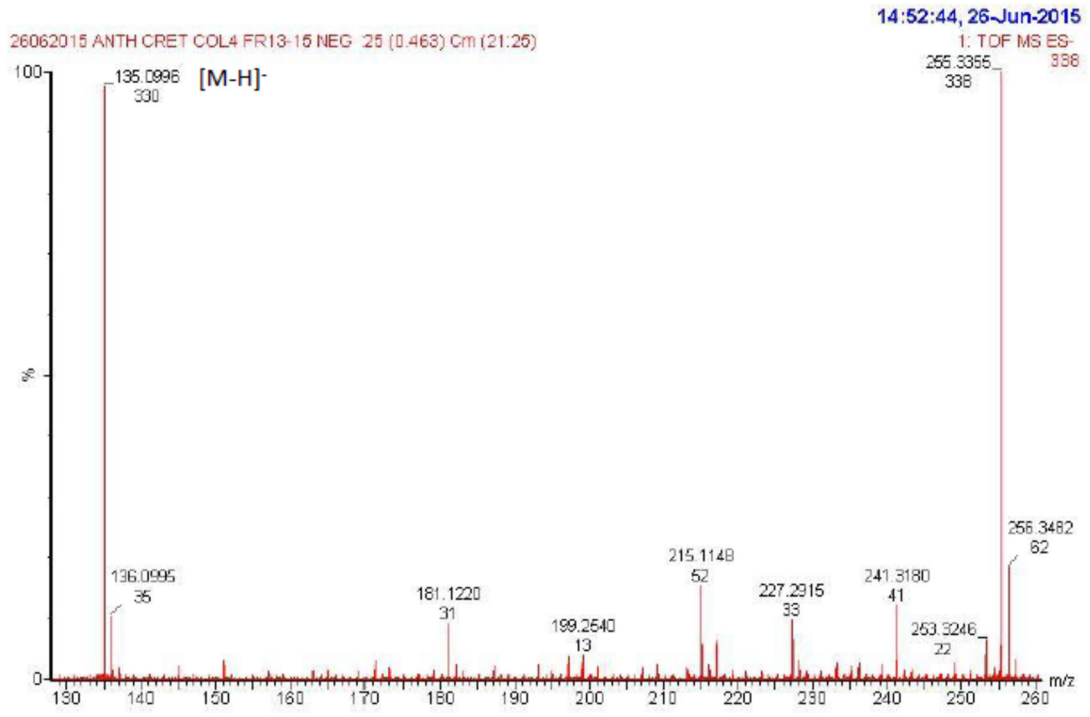



Supplementary Figure 12: $MS^+$ spectrum of 7-hydroxycoumarin (**4**)

**4'-hydroxyacetophenone** (**5**):

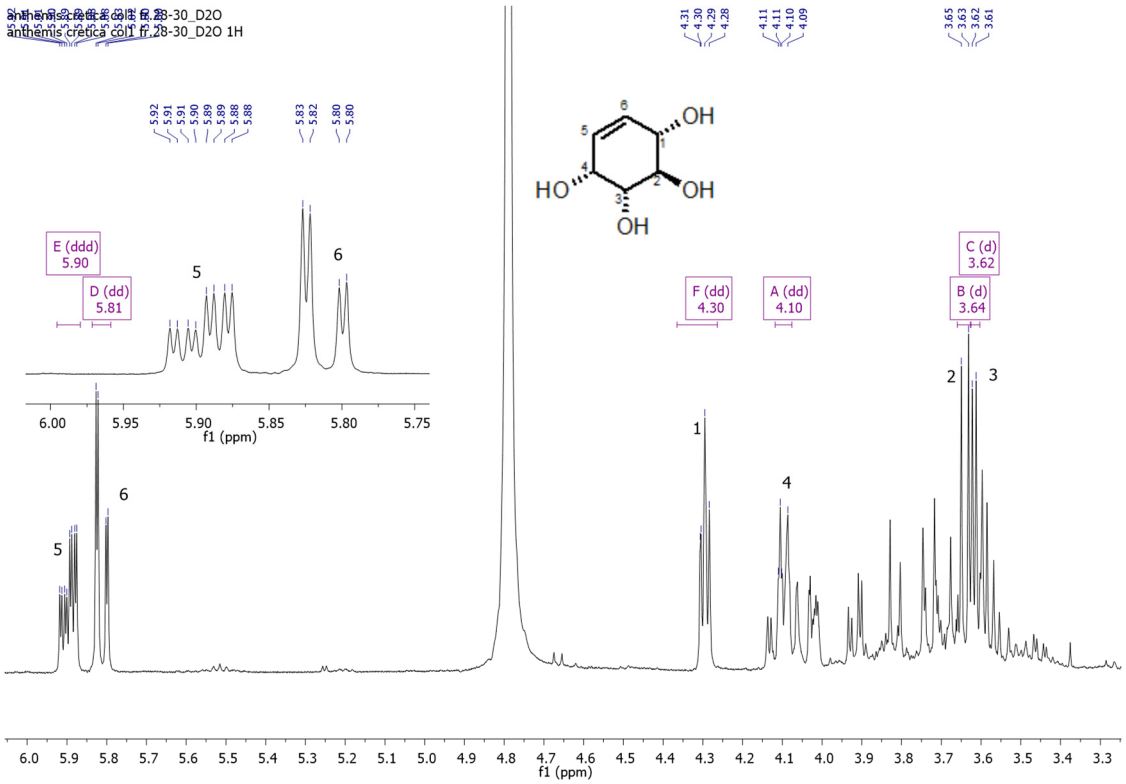



Supplementary Figure 13: $^{1}$H NMR spectrum of 4'-hydroxyacetophenone (**5**)

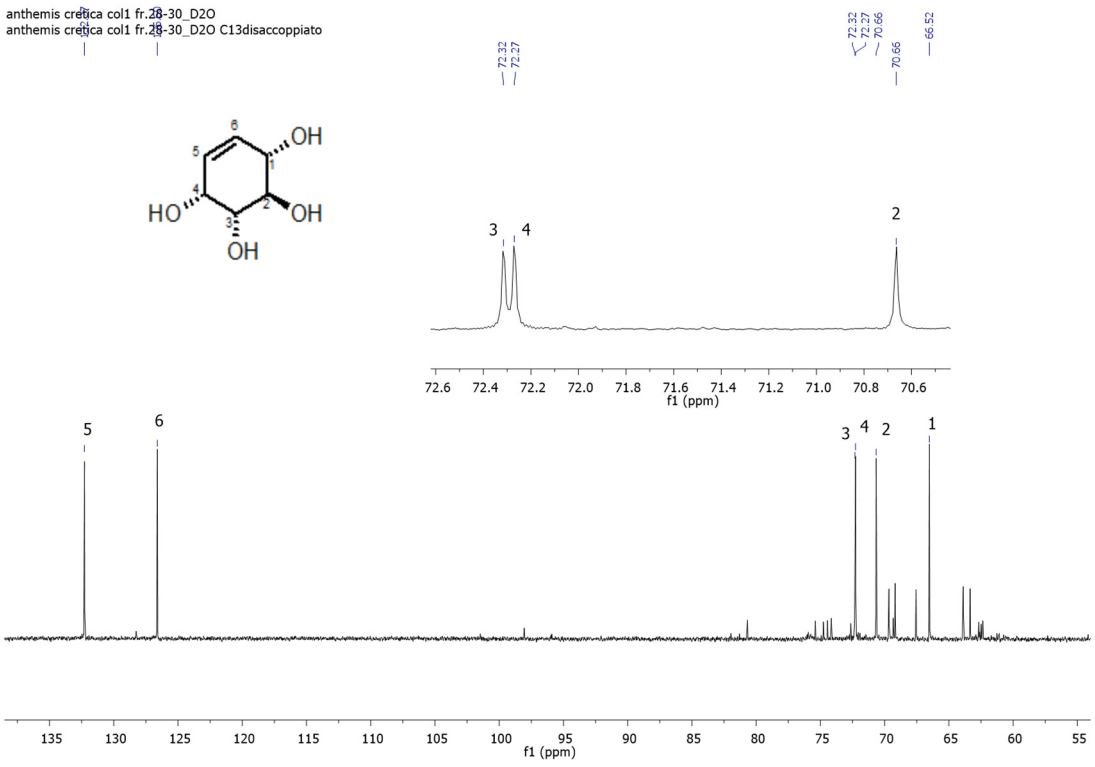



Supplementary Figure 14: MS spectrum of 4'-hydroxyacetophenone (**5**)

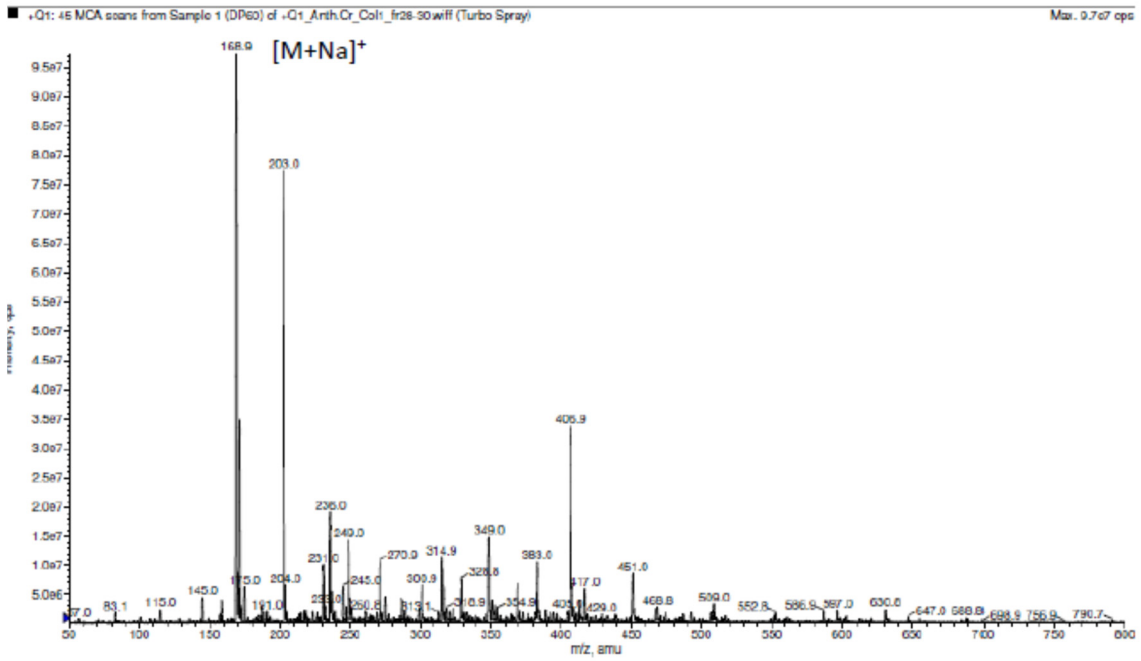
**leucanthemitol (conduritol F) (6)**:



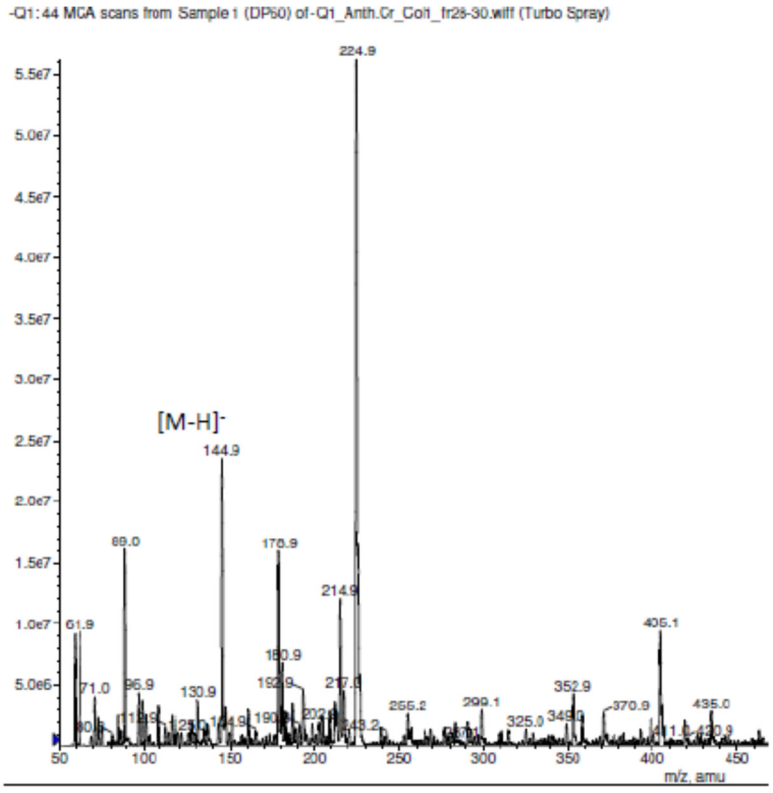

Supplementary Figure 15: $^{1}$H NMR spectrum of leucanthemitol (conduritol F) (**6**)



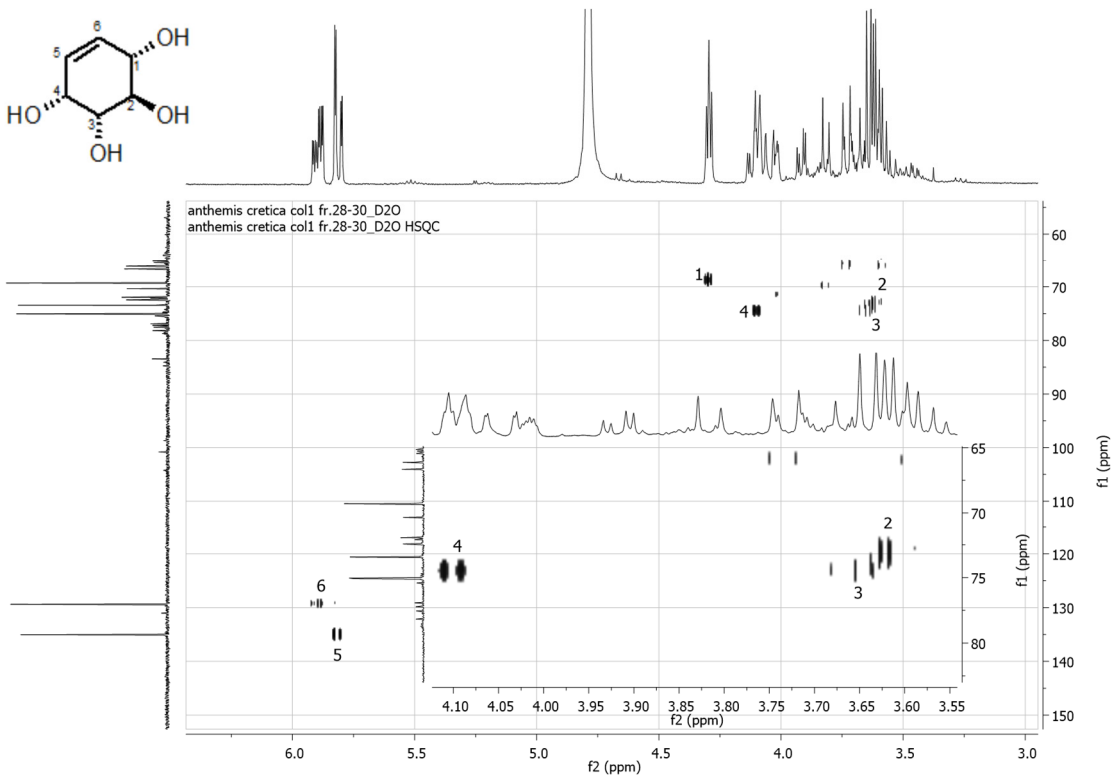

Supplementary Figure 16: $^{13}$C NMR spectrum of leucanthemitol (conduritol F) (**6**)




Supplementary Figure 17: $MS^{+}$ spectrum of leucanthemitol (conduritol F) (**6**)

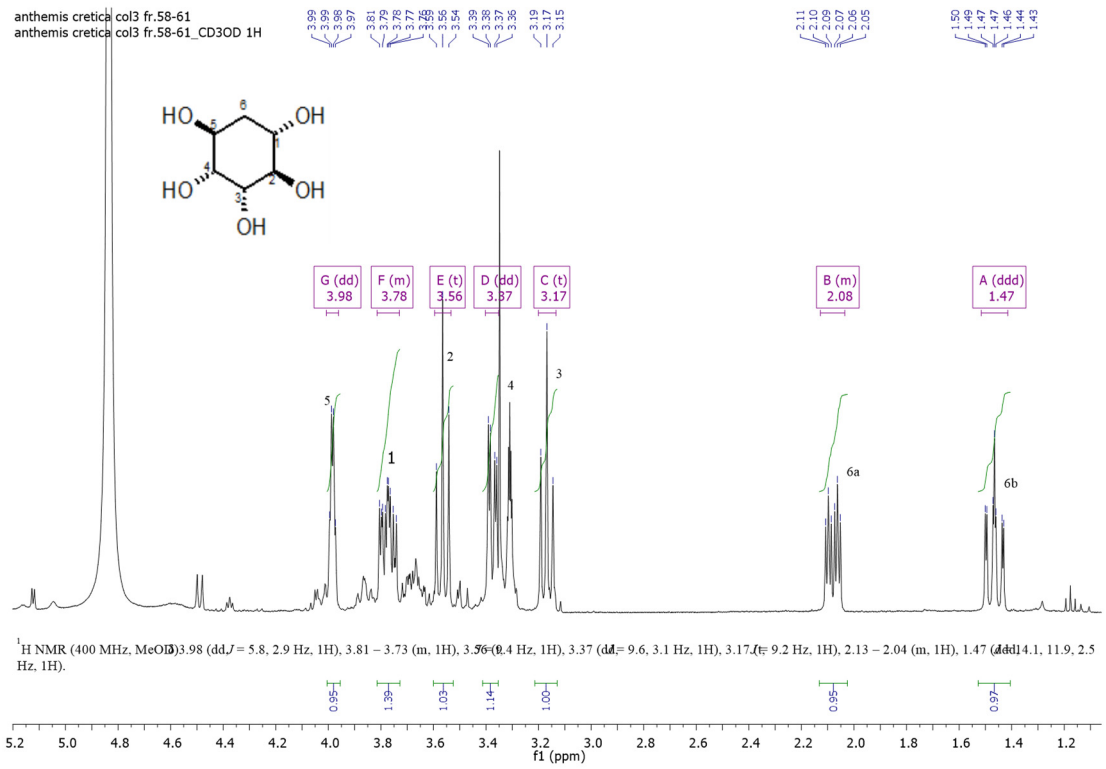



Supplementary Figure 18: $MS^{-}$ spectrum of leucanthemitol (conduritol F) (**6**)



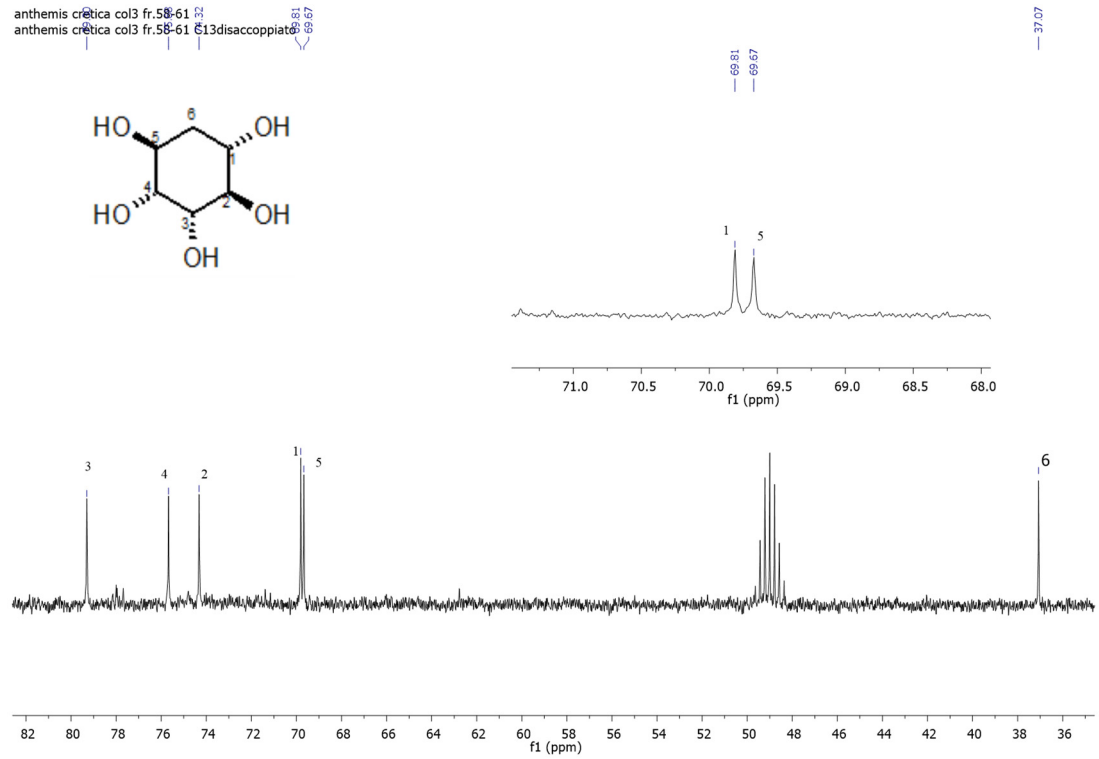

Supplementary Figure 19: HSQC spectrum of leucanthemitol (conduritol F) (**6**)

***Proto*-quercitol (7)**:

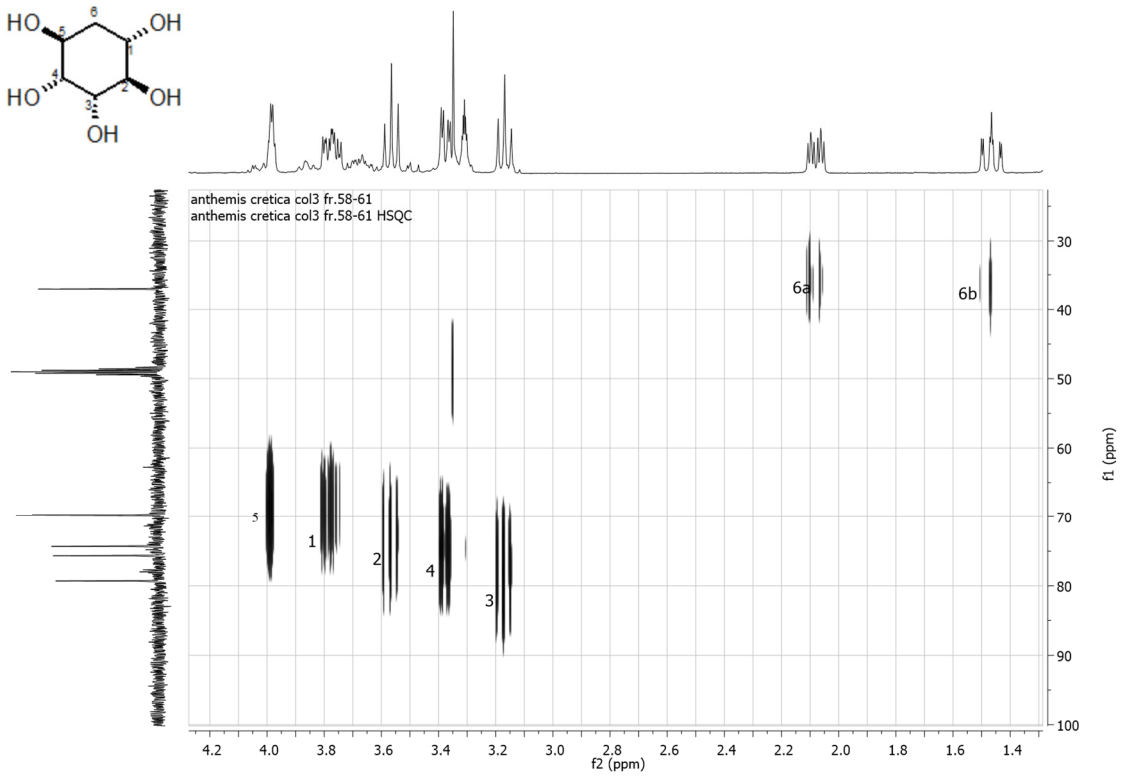



$^{1}$H NMR (400 MHz, MeOD) $\delta$ 3.98 (dd, $J$ = 5.8, 2.9 Hz, 1H), 3.81 – 3.73 (m, 1H), 3.56 (t, $J$ = 9.4 Hz, 1H), 3.37 (dd, $J$ = 9.6, 3.1 Hz, 1H), 3.17 (t, $J$ = 9.2 Hz, 1H), 2.13 – 2.04 (m, 1H), 1.47 (ddd, $J$ = 14.1, 11.9, 2.5 Hz, 1H).

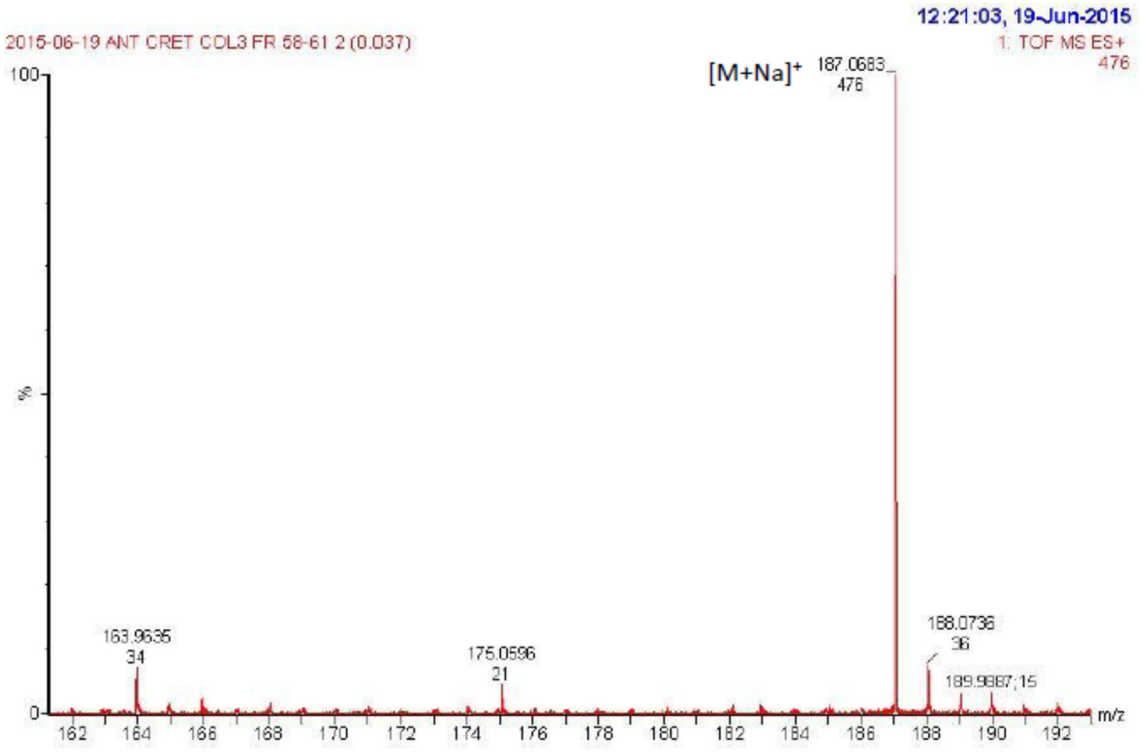
Supplementary Figure 20: $^{1}$H NMR spectrum of *proto*-quercitol (**7**)




Supplementary Figure 21: $^{13}$C NMR spectrum of p*roto*-quercitol (**7**)




Supplementary Figure 22: HSQC spectrum of *proto*-quercitol (**7**).




Supplementary Figure 23: MS spectrum of *proto*-quercitol (**7**)

Experimental NMR data of the isolated compounds compared with literature data.

| Parthenolide (**1**) | | | | |
|---|---|---|---|---|
| | Experimental data (300 MHz, $CDCl_3$), δ, *J* in Hz | Tiumann et al, 2005 (300 MHz, $CDCl_3$), δ, *J* in Hz | Experimental, (75 MHz, $CDCl_3$), δ | Tiumann et al, 2005 (75 MHz, $CDCl_3$), δ |
| | $^1$H NMR | | $^{13}$C NMR | |
| 1 | 5.18 (1H, br d, *J* = 10.7) | 5.21 (dd, *J* = 2.7, 12.0) | 125.29 | 125.3 |
| 2a | 2.33 (1H, d, *J* = 13.0) | 2.32 to 2.49 (m, H-2) | 24.19 | 24.1 |
| 2b | 2.16 (1H, d, *J* = 13.1) | - | | |
| 3 | - | 1.20 to 1.28 (m) | 36.38 | 36.3 |
| 4 | - | - | 61.72 | 61.5 |
| 5 | 2.76 overlap | 2.79 (d, *J* = 9.0) | 66.44 | 66.4 |
| 6 | 3.84 (1H, t, *J* = 8.6) | 3.86 (t, *J* = 8.4) | 82.55 | 82.4 |
| 7 | 2.76 overlap | 2.74 to 2.82 (m) | 47.68 | 47.7 |
| 8 | - | 1.70 to 1.77 (m) | 30.68 | 30.6 |
| 9 | - | 2.32 to2.44 (m) | 41.24 | 41.2 |
| 10 | - | - | 134.67 | 134.6 |
| 11 | - | - | 139.26 | 139.2 |
| 12 | - | - | 169.45 | 169.3 |
| 13a | 6.29 (1H, d, *J* = 3.6) | 6.34 (d, *J* = 3.6) | 121.40 | 121.3 |
| 13b | 5.60 (1H, d, *J* = 3.6) | 5.62 (d, *J* = 3.0) | | |
| 14 | 1.68 (s) | 1.72 (s) | 17.01 | 16.9 |
| 15 | 1.27 (s) | 1.31(s) | 17.33 | 17.3 |

| 9**α**-acetoxyparthenolide (**2**) | | | | |
|---|---|---|---|---|
| | Experimental data (300 MHz, $CDCl_3$), δ, *J* in Hz | Bruno, et al, 1991., (500 MHz, $CDCl_3$), δ, *J* in Hz | Experimental, (75 MHz, $CDCl_3$), δ | Bruno, et al, 1991., (67.9 MHz, $CDCl_3$), δ |
| | $^1$H NMR | | $^{13}$C NMR | |
| 1 | 5.56 (m) | 5.44 (m) | 124.0 | 122.68 |
| 2a | overlapped | 2.47 | 23.0 | 23.52 |
| 2b | Overlapped | 2.25 | | |
| 3 | Overlapped | 1.29 | 34.0 | 35.60 |
| 4 | overlapped | 2.18 | 61.6 | 61.17 |
| 5 | 2.79 - 2.73 overlapped | 2.74 | 66.4 | 66.54 |
| 6 | 3.82 (dd, *J* = 8.6, 8.4) | 3.84 (dd, *J* = 9.0, 7.0) | 82.5 | 82.19 |
| 7 | 2.79 - 2.73 overlapped | 3.39 (m) | 40.3 | 38.72 |
| 8 | overlapped | 2.44-1.98 | 36.3 | 36.19 |
| 9 | 5.19 (dd, *J* = 5.2, 2.0) | 5.20 (dd, *J* = 6.0, 1.0) | 72.9 | 73.20 |
| 10 | - | - | 134.6 | 133.49 |
| 11 | - | - | 139.2 | 139.32 |
| 12 | - | - | 169.4 | 169.46 |
| 13a | 6.26 (d, *J* = 3.0) | 6.32 (d, *J* = 3.5) | 121.3 | 121.30 |
| 13b | 5.64 (d, *J* = 3.0) | 5.56 (d, *J* = 3.5) | | |
| 14 | 1.83 (br s) | 1.77 (br s) | 16.6 | 16.43 |
| 15 | 1.39 (s) | 1.30 (s) | 17.3 | 17.26 |
| Ac | 2.07 (s) | 2.13 (s) | 169.9; 21.2 | 168.83; 21.09 |

| tamarixetin (**3**) | | | |
|---|---|---|---|
| | Experimental (400 MHz, $CD_3OD$), δ, *J* in Hz | Lewin et al., 2010 (DMSO), δ, *J* in Hz | Abdelhady et al, 2015, (300 MHz, DMSO), δ, *J* in Hz |
| 2′ | 7.38 (br s) | 7.61 (m) | 7.75 (d, *J* = 1.8) |
| 6′ | 7.39 (br d, *J* = 8.0) | 7.61 (m) | 7.66 (dd, *J* = 10.0, 1.8) |
| 5′ | 6.91 (d, *J* = 8.0) | 7.04 (d, *J* = 8.8) | 6.93 (d, *J* = 10.0) |
| 6 | 6.56 overlapped signals | 6.18 (d, *J* = 2) | 6.19 (d, *J* = 2.6) |
| 8 | 6.56 overlapped signals | 6.42 (d, *J* = 2) | 6.47 (d, *J* = 2.6) |
| 4′-$OCH_3$ | 3.88 (s) | 3.82 (s) | Not reported |

| 7-hydroxycoumarin (**4**) | | | |
|---|---|---|---|
| | Experimental data (400 MHz, $CDCl_3$), δ, *J* in Hz | Timonen et al, 2011 (400 MHz, DMSO), δ, *J* in Hz | Khalil et al, 2003 (400 MHz, $CD_3OD$), δ, J in Hz |
| 3 | 6.25 (d, *J* = 9.5 Hz) | 6.19 (d, *J* = 9.4 Hz) | 6.19 (d, *J* = 9.5 Hz) |
| 4 | 7.63 (d, *J* = 9.5 Hz) | 7.94 (d, *J* = 9.5) | 7.86 (d, *J* = 9.5 Hz) |
| 5 | 7.35 (d, *J* = 8.4 Hz) | 7.53 (d, *J* = 8.5) | 7.46 (d, *J* = 8.5 Hz) |
| 6 | 6.78 (dd, *J* = 8.4, 2.4 Hz) | 6.80 (dd, *J*= 8.5, 2.2 Hz) | 6.87 (dd, *J* = 8.5, 2.3 Hz |
| 7 | - | - | - |
| 8 | 6.81 (1H, d, *J* = 2.4 Hz) | 6.72 (d, *J* = 2.1 Hz) | 6.78 (d, *J* = 2.3 Hz) |

| *p*-hydroxyacetophenone (**5**) | | | |
|---|---|---|---|
| | Experimental ($CDCl_3$), δ, *J* in Hz | Zhao et al. 2015, (400 MHz, acetone-d6), δ, *J* in Hz | *Dhanuskodi et al. 2005, (*400 MHz, $CDCl_3$), *J* in Hz |
| 2 | 2.55 (3H, s) | 2.47 (s) | 2.65 (s) |
| 2′, 6′ | 7.90 (2H, d, *J* = 8.8) | 7.89 (d, *J* = 8.8) | 7.97 (d, *J* = 8.4) |
| 3′, 5′ | 6.87 (2H, d, *J* = 8.8) | 6.91 (d, *J* = 8.8) | 7.04 (d, *J* = 8.4) |

| Leucanthemitol (**6**) | | | | |
|---|---|---|---|---|
| | $^1$H NMR | | $^{13}$C NMR | |
| | Experimental (400 MHz, $D_2O$), *J* in Hz | Worawalai et al., 2012 (400 MHz, $CD_3OD$) | Experimental (100 MHz, $D_2O$) | Worawalai et al., 2012 (100 MHz, $CD_3OD$) |
| 1 | 4.30, (dd, *J* = 6.5, 2.0) | 4.17 (t, *J* = 4.4) | 66.52 | 68.1 |
| 2 | 3.64, (d, *J* = 6.5) | 3.62 (dd, *J* = 10.4, 7.6) | 70.66 | 72.7 |
| 3 | 3.62, (d, *J* = 3.9) | 3.43 (dd, *J* = 10.4, 4.0) | 72.32 | 74.1 |
| 4 | 4.10, (dd, *J* = 5.0, 3.9) | 3.94 (d, *J* = 7.6) | 72.27 | 73.9 |
| 5 | 5.90, (ddd, *J* = 10.0, 5.0, 2.0) | 5.79 (ddd, *J* = 10.0, 4.8, 2.0) | 132.27 | 133.9 |
| 6 | 5.81, (dd, *J* = 10.0, 2.0) | 5.72 (dd, *J*= 10.0, 2.0) | 126.60 | 128.1 |

| *proto*-quercitol (**7**) | | | | | |
|---|---|---|---|---|---|
| | $^1$H NMR | | $^{13}$C NMR | | |
| | Experimental (400 MHz, $CD_3OD$), *J* in Hz | Wacharasindhu et al. 2009, (400 MHz, $D_2O$), *J* in Hz | Experimental (100 MHz, $CD_3OD$) | Ruangrungsi et al., 1986, (60 MHz, $D_2O$) | Wacharasindhu et al. 2009, (100 MHz, D2O + one drop of acetone-d6) |
| 1 | 3.82 – 3.71 (m) | 3.80 (dd, *J* = 3.2, 2.4) | 69.81 | 68.9 | 68.5 |
| 2 | 3.56 (br t, *J* = 9.4) | 3.57– 3.66 (2H, m) | 74.32 | 74.6 | 71.8 |
| 3 | 3.17 (br t, *J* = 9.2) | 3.44 (dd, *J* = 9.6, 9.2) | 79.30 | 71.0 | 74.2 |
| 4 | 3.37 (dd, *J* = 9.4, 3.1) | Overlapped with H-2 | 75.68 | 72.3 | 70.6 |
| 5 | 3.98 (dd, *J* = 5.9, 3.0) | 3.90 (dd, *J* = 6.4, 3.2) | 69.67 | 68.6 | 68.2 |
| 6a | 2.14 – 2.02 (m) | 1.86 (1H, ddd, *J* = 13.9, 3.2, 3.2) | 37.07 | 33.3 | 32.9 |
| 6b | 1.47 (ddd, *J* = 14.1, 11.9, 2.5) | 1.69 (1H, ddd, *J* = 13.9, 11.6, 2.8) | | | |

## References.

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